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The narial musculature of *Alligator mississippiensis*: Can a muscle be its own antagonist?

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Abstract

The crocodilian naris is regulated by smooth muscle. The morphology of this system was investigated using a combination of gross, light microscopic, and micro-CT analyses, while the mechanics of narial regulation were examined using a combination of Hall Effect sensors, narial manometry, and electromyography. *Alligator mississippiensis*, like other crocodilians, routinely switches among multiple ventilatory mechanics and does not occlude the nares during any portion of the ventilatory cycle. In a complex that is unique among vertebrates, a single block of smooth muscle functions in dilation when active, and in constriction when passive. The alligator nares may include one of the best examples of a muscle that functions in "pushing" as well as "pulling." The central muscle for narial regulation, the dilator naris, can legitimately be viewed as its own antagonist.

KEYWORDS
crocodilians, functional integration, nares, smooth muscle

1 | INTRODUCTION

The anterior, or external, nares are a constant feature of tetrapods, functioning in ventilation and olfaction (e.g., Atz, 1952; Bertmar, 1969). In the majority of tetrapods, the olfactory passage remains patent, at least until the nasopharynx (Jankowski, 2013; Parsons, 1971). In some anurans, buccal deformation causes occlusion of a narial valve (Gargaglioni & Milsom, 2007), and diverse vertebrate taxa have independently evolved a variety of more intrinsic means of narial regulation. In a variety of mammals, including the moose (Clifford & Witmer, 2004a), saiga (Clifford & Witmer, 2004b), camel (Eshra & Badawy, 2014), and hippopotamus (Maust-Mohl, Reiss, & Reidenberg, 2019) modified facial skeletal muscle(s) displace a narial plug of connective tissue to occlude the naris or nasal vestibule. Vascular sinuses form a partial or complete narial sphincter in diverse taxa including some salamanders (Bruner, 1901), monitor lizards (Bellairs, 1949), and other reptiles (Bruner, 1907). In many (most?) of these taxa, the vascular sinuses are closely integrated with smooth muscle (see Winokur, 1982 for a description of this integration in turtles). Snakes have a distinct smooth muscle (the subnasal muscle), which is spatially isolated from the vascular tissue found at the periphery of the nostril (Bruner, 1897; Hallermann, 1998; Lillywhite, 2014).

The focus of this study is the third form of narial regulation; smooth muscle surrounding the external naris or nasal vestibule has been described in a diverse group of amphibians (Bruner, 1901) and crocodilians (Bellairs & Shute, 1953). In crocodilians, a dilator muscle attaches to the caudal surface of the nasal vestibule, while a constrictor muscle attaches to the lateral surface of the vestibule and encircles the dilator (Bellairs & Shute, 1953; Bertau, 1935). A similar two-muscle arrangement occurs in some salamanders (Bruner, 1901), while other salamanders have two constrictors (e.g., Wirsig-Wiechmnn & Holliday, 2002). Bertau (1935) offered a functional hypothesis for narial regulation in crocodilians, which was accepted by Bellairs and Shute (1953). What is remarkable about the proposed mechanics is that the narial dilator functions actively to expand the nares, but also functions passively to occlude the nares when it is protracted against the caudal wall of the nasal vestibule by the actively contracting nasal constrictor. As such, the dilator is its own antagonist, depending on its contractile state.

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The present study was undertaken to explore three issues. First, what is the relationship between the ventilatory/airflow patterns and narial closure in alligators? Even among amphibious tetrapods with valvular nares, this relationship is highly variable; the nares are normally kept closed in hippos (Maust-Mohl et al., 2019), open and close cyclically during ventilation in some anurans (Gargaglioni & Milsom, 2007), and do not close during ventilation in some salamanders (Bruner, 1901). Second, to clarify discrepancies among the previous accounts of the narial musculature of crocodilians (Bellairs & Shute, 1953; Bertau, 1935; Bruner, 1897), particularly in regard to the cranial and lateral surfaces of the nares. Third, to test the functional model for this narial complex that was previously put forward (Bertau, 1935) and, in doing so, test for the functional duality of the dilator naris.

2 | MATERIALS AND METHODS

2.1 | Live animals

Four subadult (174–192 cm total length) American alligators, *Alligator mississippiensis* (Daudin, 1802) were obtained from the Louisiana Department of Wildlife and Fisheries. The animals were housed communally in a 29 m^2 facility that featured three submerging ponds, natural light, and artificial lights on a 12:12 cycle. The facility was maintained at 30–33°C, warm water rain showers were provided every 20 min, which helped maintain the facility at >75% relative humidity. The alligators were maintained on a diet of previously frozen adult rats.

2.2 | Morphological analyses

Hatchling (body lengths of 26–29 cm) and subadult (body lengths of 165–183 cm) preserved specimens of *Alligator mississippiensis* from the private collection of BAY were used for this study. Specimens used for histology had been fixed in neutral-buffered formalin; they were decalcified in RDO Rapid Decalcifier (Apex Engineering Products, Aurora, IL) for 24 hr prior to dehydration and paraffin embedding. Sections were cut (at 10 μm), mounted, then stained with either Hematoxylin and Eosin or Masson's trichrome (following Luna, 1968). Microscopic anatomy was documented using a DM 4000B microscope (Leica Microsystems Inc., Buffalo Grove, IL).

Computed tomography (CT) was performed with a clinical 64-detector CT unit (Ingenuity, Philips Medical, Cleveland, OH) using helical acquisition axial 0.67 mm slice thickness, field of view (FOV) 130 mm, 100 kV, 125 mAs, rotation time 500 ms, pitch of 0.391, matrix 512 × 512, sagittally reconstructed. Magnetic resonance imaging (MRI) was performed by placing the specimen in a phased-array surface coil then imaging (T2-weighted, coronal slice 2.7 mm, FOV 100 mm, TR 4050, TE 110, matrix 384 × 320, BW 8.93, Nex 14) with a clinical 0.35-T MRI unit (Ovation, GE Medical Systems, Milwakee, WI). To enhance contrast, the specimen was soaked for 2 days prior to imaging in a 1% solution of Gadolinium in lemon water. The snout of a hatchling specimen was scanned (at 1.5 μm) using a micro-CT (SkySkan 1272, Bruker, Billerica, MA), DiCom images were then used for 3-D reconstruction (CT Vox, Bruker).

2.3 | Functional analyses

Individual animals were removed from their enclosure by noosing; their jaws taped shut using vinyl tape, and their forelimbs and hindlimbs taped in a retracted position. The husbandry and use of the live alligators followed all applicable federal guidelines and were

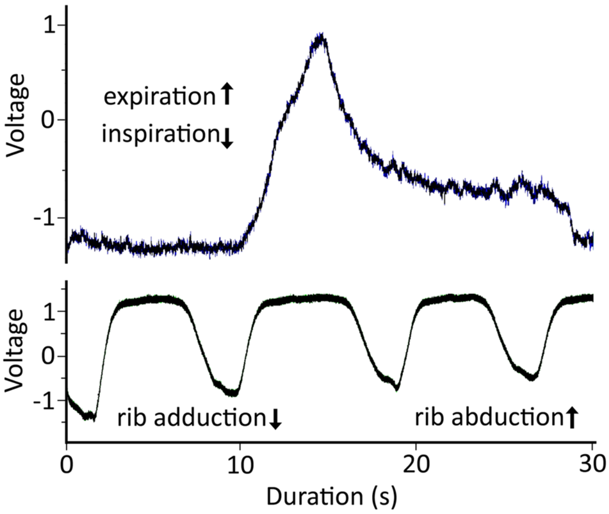

FIGURE 1 Raw 30 s data tracings of (a) narial manometry showing slow exhalatory airflow, and (b) the Hall Effect sensor showing rhythmic displacement of the ribs. Note that the two tracings were not recorded simultaneously

FIGURE 2 *Alligator mississippiensis*, nasal passageway. (a) Photo (courtesy of Kelly Rogers) of a 180 cm alligator showing the dome-like elevation of the external naris. (b) MRI of a similar-sized alligator; the soft tissue of the external naris is in sharp contrast to the bony snout, and the patency of the nasal passageway is clearly evident. Abbreviation: e, external naris

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approved by the IACUC of A.T. Still University (Protocol #215, approved May 20, 2019). The alligator was placed on a stiff board (244 × 28 × 3.8 cm thick), which exceeded the maximum width and length of the alligators used for this study. Heavy-duty straps 2.5 cm wide (Northwest Tarp and Canvas, Bellingham, WA) were used to secure the alligator to the board. The straps secured the alligator to the board but did not interfere with ventilator movements.

Narial manometry was performed using a 4 mm diameter tube of soft latex, the end of which was sealed with cyanoacrylate adhesive. The tube was filled with reptilian Ringers solution (Barfuss & Dantzler, 1976) and then connected to a fluid pressure transducer (P23AC, Statham, Costa Mesa, CA). The transducer was coupled to a preamplifier (P122, GRASS Instruments, West Warwick, RI) and then to the data acquisition system. The narial manometry tube was capable of not only recording the compressive force applied by the narial musculature but also the airflow through the external nares (Figure 1a).

Ventilatory movements were recorded with a Hall Effect sensor. A spreg chip (DRV5053EAQLPG, Texas Instruments, Dallas, TX) was secured on the dorsolateral surface of the alligator using adhesive electrode pads. Small (3 mm diameter) neodymium/iron/boron ceramic magnets (Integrated Magnetics, Culver City, CA) were attached immediately adjacent to (but independent of) the Hall Effect sensor. The total mass of the sensor, cables, magnets, and positioning pads was less than 9 g, which was deemed inconsequential to the movements of the alligator. Output from the Hall Effect sensor was recorded (at 5 kHz) using a MiDAS (Xcitex Incorporated, Woburn, MA) data acquisition system. The Hall Effect sensor was able to cleanly track the displacements of the alligator's ribs (Figure 1b) during ventilation.

Electromyography (EMG) leads were fabricated from insulated 0.05 mm diameter stainless steel wire (California Fine Wire, Grover Beach, CA). Bipolar leads were implanted (using 21 gauge hypodermic

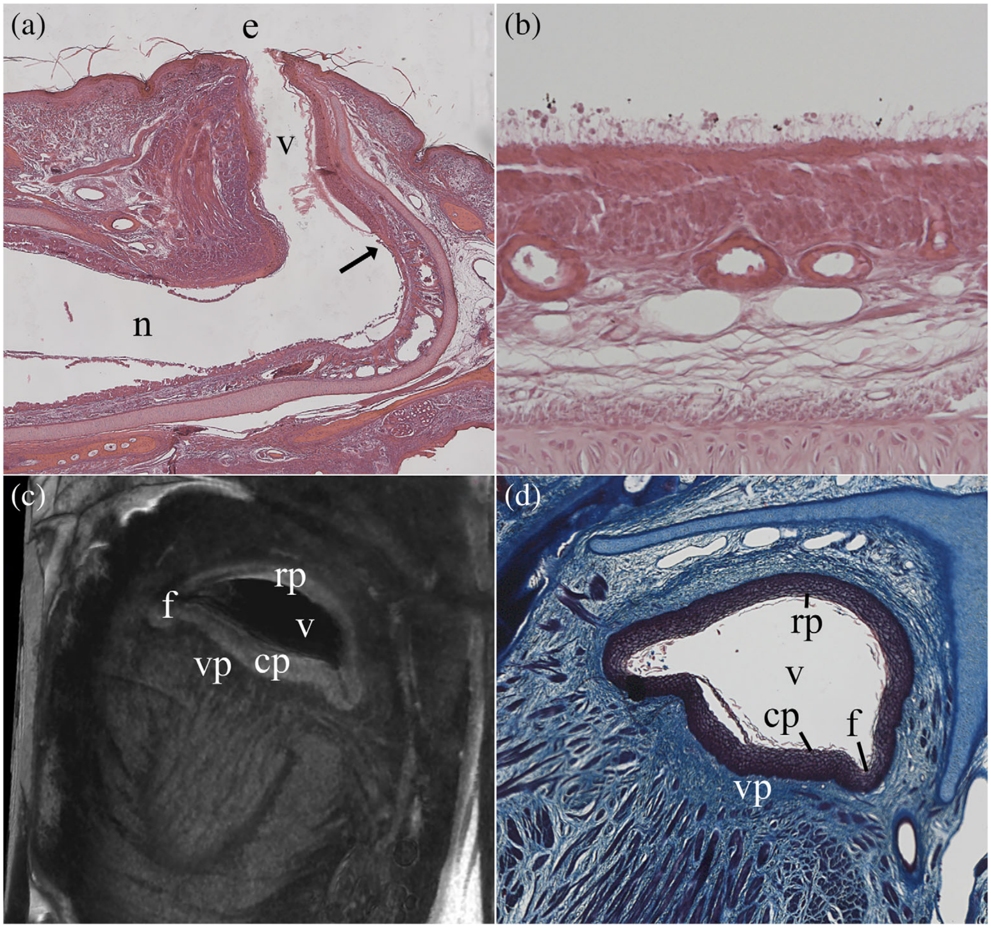

FIGURE 3 *Alligator mississippiensis*, structure of the nasal passageway and vestibule. (a) Sagittal section illustrating the sharp bend between the distal nasal vestibule and the proximal nasopharyngeal duct, the boundary between these two regions (arrow) is marked by an epithelial transition. (b) Sagittal section of respiratory epithelium from the nasopharyngeal duct, which contrasts the stratified squamous epithelium of the nasal vestibule. (c) Micro-CT image through the dorsal portion of the nasal vestibule demonstrating the rostral and caudal vestibular plates that are joined at the epithelial folds, the vestibular pad can be seen on the caudal surface of the caudal vestibular plate. (d) Frontal section through the vestibule; the difference in narial patency is due to a difference in the caudal vestibular plate, while the rostral vestibular plate remains stationary. Abbreviations: cp, caudal vestibular plate; f, epithelial folds; n, nasopharyngeal duct; rp, rostral vestibular plate; v, nasal vestibule; vp, vestibular pad

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needles) into either the constrictor or the dilator portions of the narial musculature. The EMG signals were amplified (QP511, GRASS) and then recorded with the data acquisition system.

At the termination of the functional analyses, the EMG leads were removed and the animal returned to the enclosure.

3 | RESULTS

3.1 | General morphology of the nasal complex

The rostral tip of the snout of *Alligator mississippiensis* supports a dome of soft tissue (Figure 2) that Bertau (1935) described as a "muscular cushion" (muskulöses Polster). In the center of this soft tissue dome is the external naris, which leads to the nasal passageway, the distal part of which is vertical. The vertical passageway forms a nearly right-angle bend and then extends caudally within the snout. Along this course, the passageway is devoid of valves, flaps, or infoldings that could occlude the passageway (Figure 2).

The distal portion of the nasal passageway can be divided into two segments (Figure 3a). Following Parsons (1970) the vertical portion, everything distal to the right-angle bend, will be referred to as the nasal vestibule, while the bend and horizontal portions of the passageway will be referred to as the nasopharyngeal duct. The boundary between the vestibule and the nasopharyngeal duct is based on the epithelium. The vestibule is lined by a stratified squamous epithelium, while the nasopharyngeal duct is lined by a ciliated pseudostratified epithelium that includes goblet cells (Figure 3b).

The vestibular epithelium can be divided into two functional components. The rostral vestibular plate forms a semicircle on the rostral side of the vestibule, it is slightly thinner than the caudal counterpart and is adhered to the adjacent cartilaginous nasal capsule by prominent collagenous bands (Figure 3c,d). The rostral vestibular plate is stationary, or nearly so. The caudal vestibular plate is roughly a medial–lateral structure. The basal lamina of the caudal vestibular plate integrates with a prominent block of collagenous fibers, the vestibular pad. The vestibular pad also serves as the insertion site for several of the narial muscles (see below). Accordingly, the contours of the caudal vestibular plate change depending on the contractile state of the narial muscles; most prominently, the middle of the caudal vestibular plate buckles caudally upon contraction of the dilator naris.

The caudal vestibular plate is the mobile portion of the nasal passageway; it approaches the rostral vestibular plate during narial occlusion and is displaced caudally during narial dilation. The mobility of the caudal vestibular plate, relative to its rostral counterpart, is possible due to prominent folds of epithelium that occur on the medial and lateral surfaces of the vestibule. These folds are smallest during maximum caudal displacement of the caudal vestibular plate (narial dilation) and increase in length during narial occlusion (Figure 3c,d).

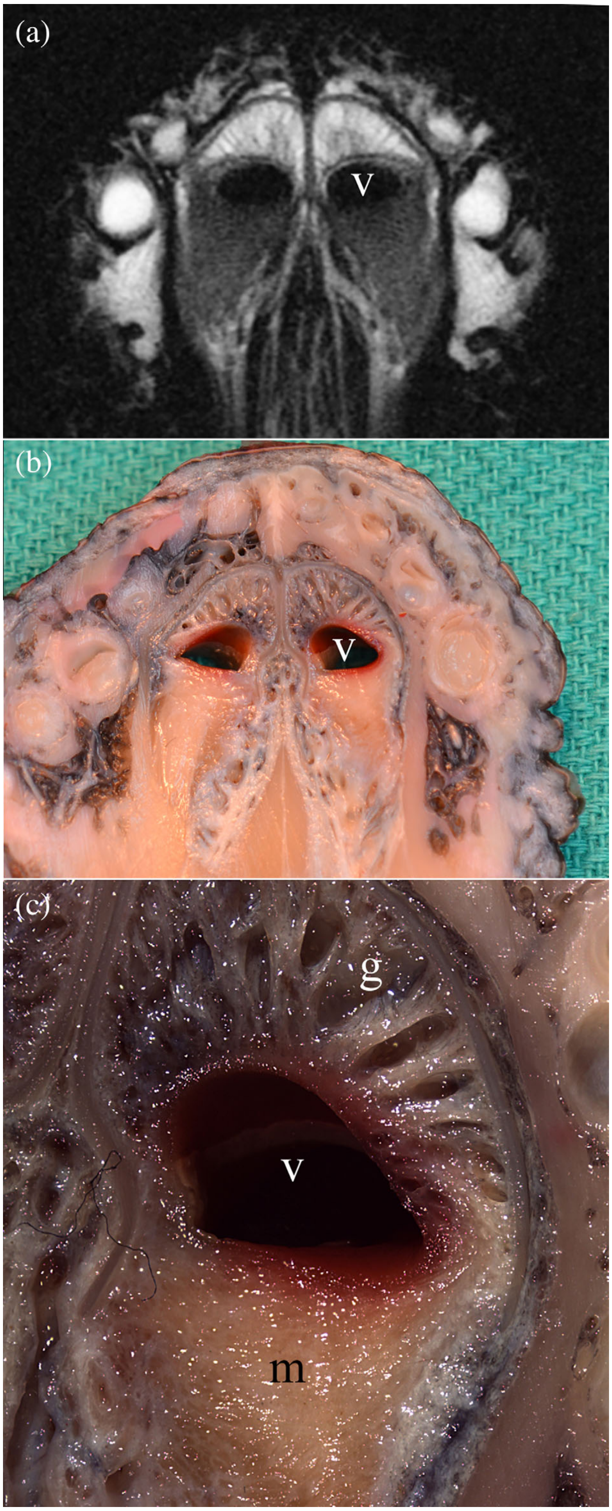

FIGURE 4 *Alligator mississippiensis*, asymmetry of the tissue surrounding the nasal vestibule. (a) Frontal plane weighted-MRI showing the marked difference in tissues on the rostral and caudal surfaces of the nasal vestibule; (b) Thick (1.5 mm) frontal section through the snout and palate showing the nasal vestibules, surrounded by the sectioned teeth; (c) Photo dissection of the right nasal vestibule from (b) demonstrating the nasal gland on the rostral surface of the vestibule and the narial musculature confined to the caudal surface. Abbreviations: g, nasal gland; m, narial musculature; v, nasal vestibule

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The soft tissue surrounding the nasal vestibule is variable, as is clearly illustrated with weighted-MRI (Figure 4a). The soft tissue on the rostral side of the nasal vestibule is dominated by narial glands, which are septate and richly vascularized. The soft tissue on the caudal side of the nasal vestibule is dominated by smooth muscle and the integrated collagen fibers (Figure 4b,c).

3.2 | Narial musculature

The constrictor naris is a hollow cone of circular smooth muscle, which surrounds the dilator naris. The base of the constrictor naris integrates into the vestibular pad, though some of the most dorsal fibers diverge rostrally to attach to the cartilaginous nasal capsule (Figure 5b). Near the dorsal–ventral midline of the constrictor naris, fibers in the rostral portion of this muscle diverge and form an attachment to the epithelial folds on the lateral and medial surfaces of the nasal vestibule (Figure 5c). Fibers on the ventrolateral margin of this muscle extend over the lateral surface of the nasal vestibule and again attach to the cartilaginous nasal capsule (Figure 5d). Fibers, which are assumed to be portions of the constrictor naris, spread (sparsely) along the rostral surface of the junction between the nasal vestibule and the nasopharyngeal duct (Figure 5d). These fibers often form part of the septae found in this region and, despite their presumed origin from the constrictor naris, would function to maintain the contours of the nasal passageway, rather than constrict it.

In cross-section, there is a clear fascial plane between the deep dilator naris and the surrounding constrictor naris (Figure 6a,b). The dilator naris is a thick strap of smooth muscle, which originates on the caudal wall of the bony naris (formed by the nasal process of the premaxilla) and inserts onto the vestibular pad (Figure 6c,d). The origin of the dilator naris is not surrounded by the constrictor naris, but the rest of the dilator is. Contraction of the dilator naris deforms the vestibular pad (pulling the central portion caudally), increases the width of the epithelial folds in the nasal vestibule, and, ultimately, increases the diameter of the nasal vestibule.

There is a specialized slip of the constrictor naris that herein will be termed the constrictor naris accessorius. This muscle originates along the rostral surface of the constrictor naris. A proliferation of smooth muscle fibers near the ventral midline of the constrictor naris creates a low ridge along the caudal junction between the nasal vestibule and the

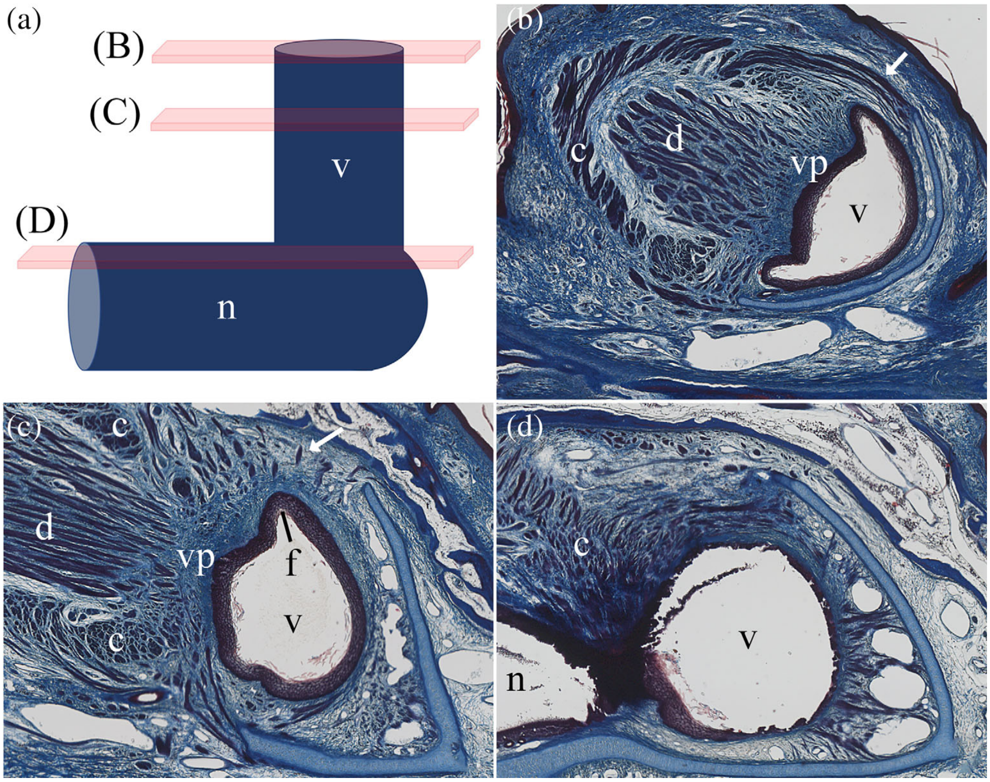

FIGURE 5 *Alligator mississippiensis*, morphology of the constrictor naris. (a) Schematic of the external naris and distal nasal passageway, showing the approximate levels of the three frontal sections. (b) Dorsal section showing the central dilator naris bordered medially and laterally by the constrictor naris, rostral fibers of the constrictor naris diverge around the nasal vestibule to attach to the cartilaginous nasal capsule (arrow); (c) More ventral section showing the clear arrangement between the constrictor and dilator nares, the nature of the vestibular pad, and the more isolated fibers of the constrictor naris that attach near the outer surface of the epithelial folds (arrow); (d) Ventral section near the junction between the nasal vestibule and the nasopharyngeal duct, this section is inferior to the dilator naris and shows the medial–lateral fibers of the constrictor naris, which course deep to the dilator naris. Abbreviations: c, constrictor naris; d, dilator naris; n, nasopharyngeal duct; v, nasal vestibule; vp, vestibular pad

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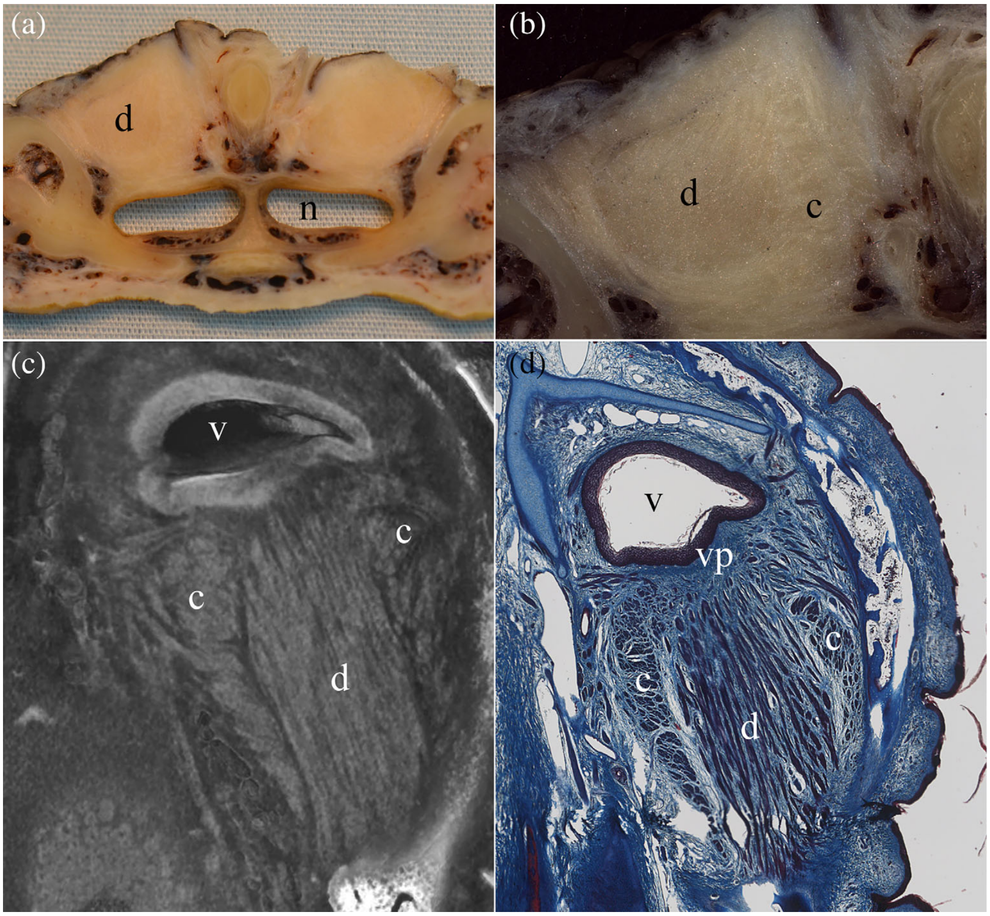

FIGURE 6 *Alligator mississippiensis*, morphology of the dilator naris. (a) Thick (1.5 mm) transverse section through the snout caudal to the nasal vestibule, the narial musculature can be seen on the dorsal surface of the nasopharyngeal duct; (b) Photo dissection of (a) showing the fascial plane that separates the central (deep) dilator naris from the superficial (peripheral) constrictor naris; (c) Frontal plane micro-CT image showing the strap-like dilator naris, originating from the nasal process of the premaxilla and coursing toward the caudal vestibular plate; (d) Frontal section through the dilator naris showing the fascial plane between the dilator and constrictor nares and the complexity of the vestibular pad between the dilator naris and the caudal vestibular plate. Abbreviations: c, constrictor naris; d, dilator naris; n, nasopharyngeal duct; v, nasal vestibule; vp, vestibular pad

nasopharyngeal duct (Figure 7a). The distal end of this muscle covers the rostral surface of the nasopharyngeal duct, forming a low ridge along the rostral junction between the nasopharyngeal duct and the nasal vestibule (Figure 7b). The constrictor naris accessorius courses over the lateral surface of the junction between the nasal vestibule and the nasopharyngeal duct (Figure 7c,d). There are fibers of the constrictor naris that extend onto the dorsomedial surface of the junction between the nasal vestibule and nasopharyngeal duct (Figure 7c,d), but the smooth muscle on the medial side is not as well developed as on the lateral side.

When the narial musculature is viewed as a functional complex (Figure 8), it is clear that the combination of the circular course of the bulk of the compressor naris, and the limited amount of muscle on the rostral side of the nasal vestibule, is incapable of displacing the rostral vestibular plate. The dense connective tissue of the vestibular pad spans the entire caudal surface of the caudal vestibular plate (Figure 8), presumably ensuring a reasonably uniform displacement of the caudal margins of the nares. Both the dilator naris and the constrictor naris are integrated into the vestibular pad (Figure 8); however, only the dilator naris has a line of action that could alter narial patency. Activation of the constrictor naris would apply radial compression to the dilator naris. Under this compressive force, the dilator naris would function like a diaphragm valve, displacing rostrally, along with the vestibular pad and caudal vestibular plate, to constrict the external nares and nasal vestibule (Figure 9).

3.3 | Patterns of rib displacement and narial airflow

There was no obvious relationship between the rib movements and the pattern of airflow through the nares (Figure 10). Exhalatory airflow was recorded at the external nares during rib abduction, rib adduction, and during periods of no rib movement; likewise, movements of both rib abduction and rib adduction occurred without corresponding ventilatory airflow (Figure 10).

3.4 | Narial movement

At rest, under the influence of anesthesia, and in death, the external nares of *Alligator mississippiensis* have a narrow biconvex shape (Figure 11). During narial constriction, the rostral portion of the naris is static, but the caudal margin displaces rostrally and flattens out (medial-lateral) so the external naris assumes a more planoconvex shape (Figure 11). During narial dilation, the rostral portion of the external naris remains static; as the caudal portion of

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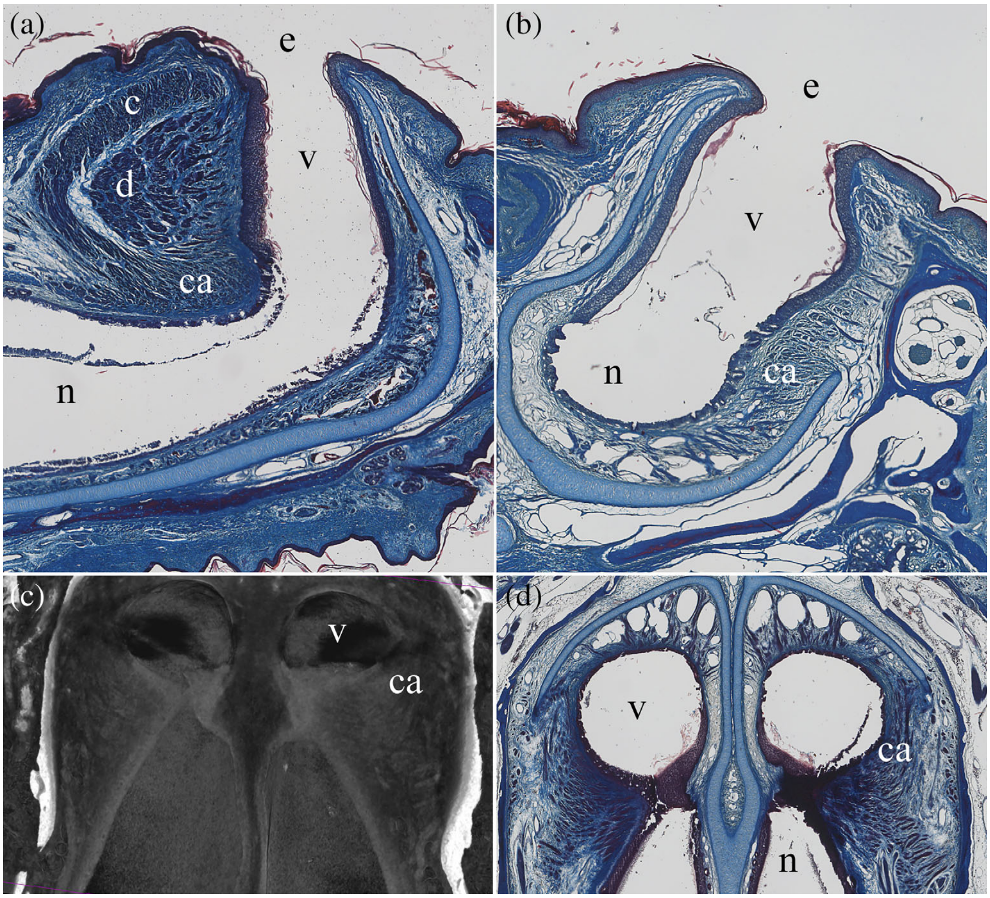

FIGURE 7 *Alligator mississippiensis*, morphology of the constrictor naris accessorius. (a) Parasagittal section through the nasal passageway showing the origin of the constrictor naris accessorius on the cranio-ventral surface of the constrictor nares, the abundance of smooth muscle fibers results in a bulge or ridge on the inner surface of the nasal passageway; (b) Parasagittal section through the nasal passageway, lateral to the section in (a), showing the insertion of the constrictor naris accessorius on the cranio-lateral surface of the nasal vestibule; (c) Frontal plane micro-CT image near the junction between the nasal vestibule and the nasopharyngeal duct, showing the fibers of the constrictor naris accessorius on the lateral surface of the nasal passageway; (d) frontal section near the junction between the nasal vestibule and the nasopharyngeal duct, showing the constrictor naris accessorius and the more isolated smooth muscle elements within the nasal gland on the rostral side of the nasal vestibule. Abbreviations: c, constrictor naris; ca, constrictor naris accessorius; d, dilator naris; e, external naris; n, nasopharyngeal duct; v, nasal vestibule

the external naris displaces caudally (increasing the diameter of the biconvex narial opening) a deformation forms in the middle of the caudal vestibular plate (Figure 11). During dilation, the caudal portion of the external naris depresses slightly relative to the rostral portion.

The external nares did not undergo cyclic changes during the ventilatory cycle. The nares were typically maintained in a partially patent position (i.e., neither occluded nor fully patent), throughout the ventilatory cycle, and during the latency period between breaths.

3.5 | Electrical activity of the narial musculature

The lack of regular changes in the narial patency, coupled with the variation in the narial airflow patterns (Figure 12), complicated the interpretation of the EMG data. The EMG leads were implanted in two locations, the center of the narial musculature (which is the dilator naris) and superficially on the medial side of the narial musculature (where the constrictor naris is thickest). There are only two muscles in these regions of the alligator's head; since the two EMG placement sites yielded two different temporal patterns of electrical activity relative to narial airflow, the identification of the implanted muscle was straightforward. The dilator naris was active prior to the onset of exhalatory airflow and typically remained active during at least a portion of the exhalatory airflow (Figure 12). The constrictor naris became active during the inhalatory airflow and was maintained after the cessation of airflow (Figure 12).

4 | DISCUSSION

Reference to the narial musculature of crocodilians can be found in Hoffman (1890), Röse (1893), Martin and Bellairs (1977), and

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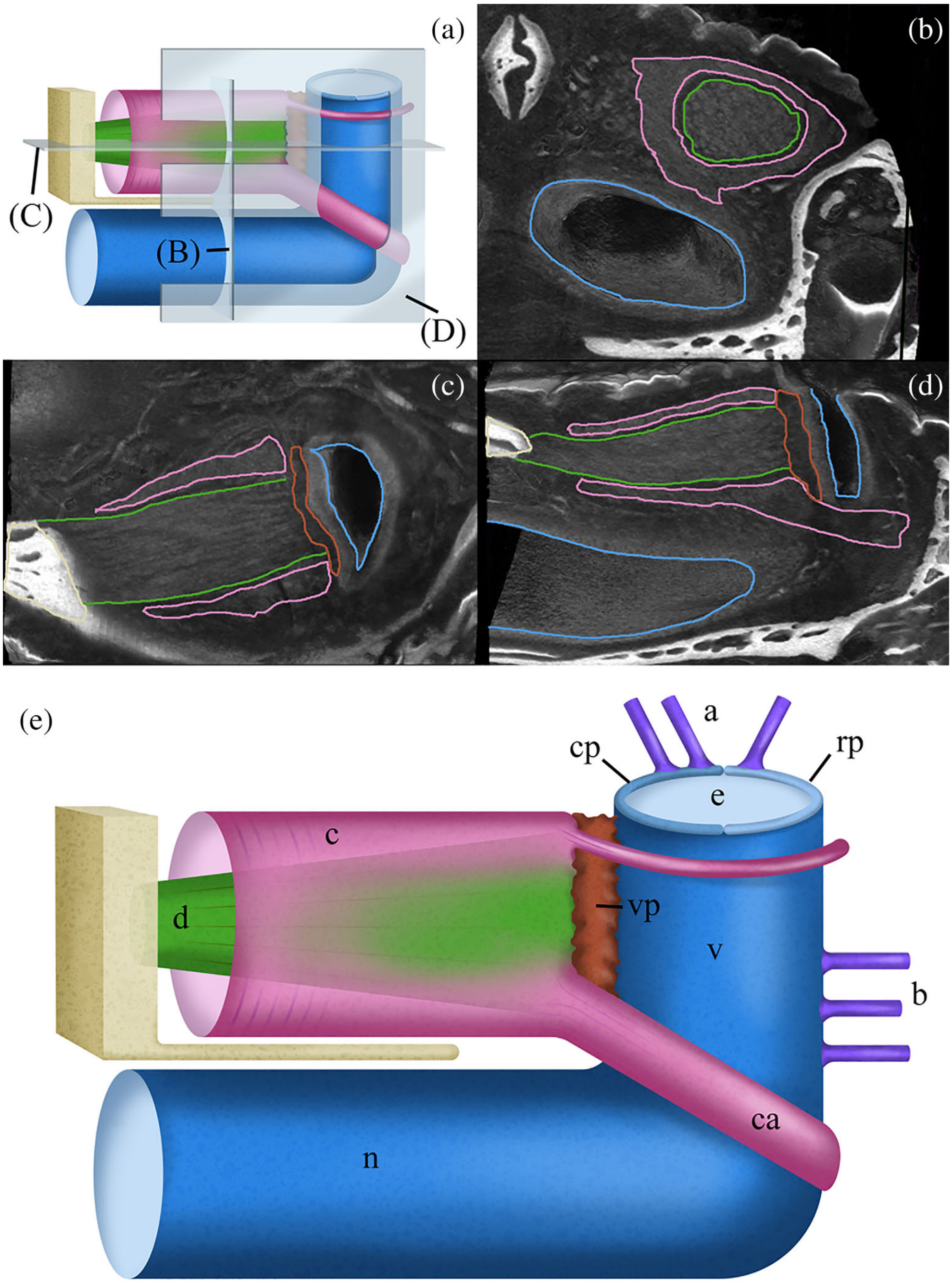

FIGURE 8 Summary of the narial musculature of *Alligator mississippiensis*. (a) Reference schematic; (b) transverse, (c) frontal, and (d) parasagittal sections through a micro-CT based 3-D reconstruction of the external naris, which has been colorized for visualization. (e) Graphic illustration of this morphology (courtesy of Jamie Carroll). Abbreviations: a, isolated fibers attaching near the epithelial fold; b, isolated fibers attaching on the rostral margin of the nasal vestibule; c, constrictor naris; ca, constrictor naris accessorius; cp, caudal vestibular plate; d, dilator naris; e, external naris; n, nasopharyngeal duct; rp, rostral vestibular plate; v, nasal vestibule; vp, vestibular pad. Colors: blue, nasal vestibule and nasopharyngeal duct; brown, vestibular pad; green, dilator naris; pink, constrictor naris and constrictor naris accessorius; yellow, premaxilla

Klembara (1991); a detailed description of these narial muscles can be found in three earlier studies (Bellairs & Shute, 1953; Bertau, 1935 and Bruner, 1897). The three detailed studies all agree on the presence of a central dilator naris surrounded by a constrictor naris. Only Bertau (1935) accurately described what is herein termed the constrictor naris accessorius, and the dorsal and lateral radiation of the constrictor naris muscle. None of the previous studies accurately represented the cranial attachments of the constrictor naris.

The morphology presented herein suggests the following functional hypothesis for narial regulation. Dilation of the narial passageway is primarily achieved by the dilator naris causing caudal displacement of the vestibular pad and caudal vestibular plate (Figures 6 and 8), this action produces a clear deformation in the caudal margin of the external nares (Figure 11), and a slight depression of the caudal margin due to the more ventral origin of the dilator naris. The dilation of the nasal vestibule also involves shorter muscle bundles on the lateral and medial surfaces of the nasal vestibule; these fibers attach onto the outer surfaces of the vestibular epithelial folds (Figures 5 and 8) and help maintain the medial–lateral patency of the external nares. Lastly, scattered smooth muscle fibers on the cranial surface of the nasal passageway, near the ventral margin of the nasal vestibule and the junction between the nasal vestibule and the nasopharyngeal duct, function to maintain the patency of the ventral portion of the nasal vestibule.

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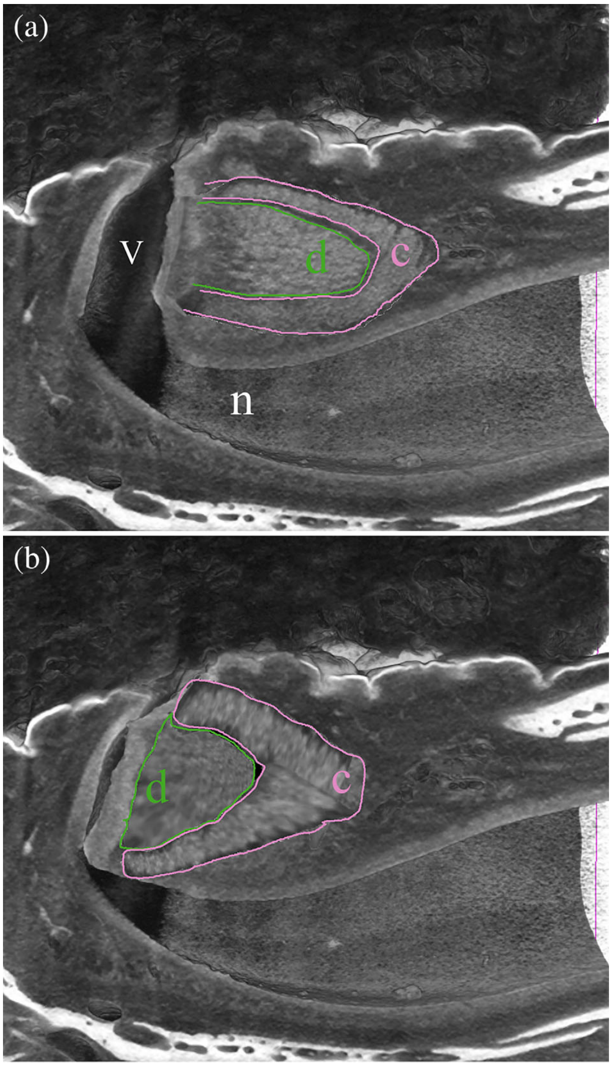

FIGURE 9 Graphical depiction of the dilated (a) and constricted (b) nares of *Alligator mississippiensis*. Note that these are graphically manipulated micro-CT sections designed to illustrate the hypothesized closing mechanics, not actual anatomical images. Abbreviations: c, constrictor naris (indicated in pink); d, dilator naris (indicated in green); n, nasopharyngeal duct; v, nasal vestibule

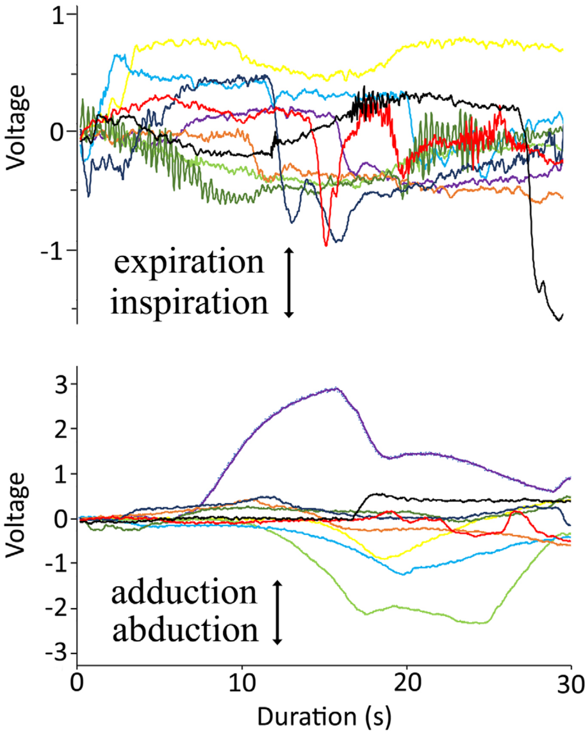

FIGURE 10 Nine 30 s data traces, all taken from the same alligator; the upper traces are narial constriction/airflow while the bottom traces are rib displacement. Each trial is color coded. Note that there is no consistent pattern of either airflow or rib displacement, nor is there a correlation between the two data curves

Constriction of the external nares also involves three distinct components. The primary constriction of the nasal vestibule is accomplished when the circular fibers of the constrictor naris cause a cranial displacement of the dilator naris that, in turn, causes a cranial displacement of the caudal vestibular plate (Figures 5, 8, and 9). Constriction of the external naris is enhanced by smooth muscle fibers that diverge from the dorsal surface of the constrictor naris, extend along the medial and lateral surfaces of the nasal vestibule, and attach to the cartilaginous nasal capsule (Figures 5 and 8). These diverging fibers help keep the bulk of the constrictor naris anchored cranially, elevate the caudal vestibular plate, and help shape the epithelial folds that mark the lateral and medial surfaces of the constricted external naris (Figure 3). Lastly, the fibers of the constrictor naris accessorius wrap around the lateral surface of the junction between the nasal vestibule and the nasopharyngeal duct; contraction of these fibers would oppose the two low ridges on the inner surface of the nasal passageway at this level (Figures 7 and 8).

This hypothesized model for narial regulation was tested using a combination of bipolar electromyography and narial nanometry. This was complicated due to the lack of a regular pattern in either narial regulation or airflow (see below). Nevertheless, two very different patterns of electric activity were observed (Figure 12). The dilator naris was active prior to the expansion of the external nares and the associated airflow, while the constrictor naris was activated during airflow and had electrical activity maintained after the termination of airflow and occlusion of the nares.

The rostral surface of the nasal vestibule supports glandular tissue interspersed with collagenous septa and strands of smooth muscle (Figure 4). Bruner (1901) detailed multiple patterns of interaction between the nasal glands and narial musculature in amphibians; including forms in which the nasal gland may play a role in narial occlusion. More commonly in reptiles, it is the interaction between smooth muscle and vascular sinuses that functions in narial occlusion (e.g., Winokur, 1982). We agree with the conclusion of Bellairs and Shute (1953) that the glandular tissue in *Alligator* does not function in narial occlusion. Herein, the isolated smooth muscle present on the rostral surface of the nasal vestibule (Figure 5) is thought to function in maintaining patency; this does

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FIGURE 11 *Alligator mississippiensis*, narial displacement. These photographs of the external naris (rostral is to the left) from the same *Alligator mississippiensis* show the different positions of the caudal margin of the external nares during rest (middle), dilation (bottom), and narial occlusion (top)

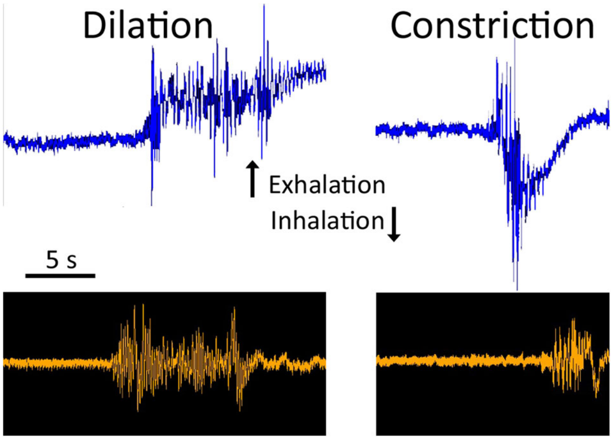

FIGURE 12 Concurrently recorded raw data of narial airflow (upper traces) and EMG activity (lower traces) from the same specimen of *Alligator mississippiensis*. The bipolar EMG leads were placed either deep in the center of the narial muscles (dilator naris, left) or superficially on the medial side (constrictor naris, right); the two placements resulted in a different temporal pattern relative to the recorded airflows

not preclude that they may also function in promoting glandular discharge.

Simultaneous recording of costal displacement and narial airflow revealed no discernable relationship between thoracic volume and narial airflow (Figure 10). Earlier studies of ventilation in *Alligator* (e.g., Farmer & Carrier, 2000; Gans & Clark, 1976) have noted that *Alligator* has multiple means of ventilation, of which the hepatic pump may be the most regular. During these experiments, the alligators did extensive periods of breath-holding (apnea) rather than rhythmic ventilation and generally exhibited more abdominal than thoracic movement. The lack of a clear relationship between rib kinematics and ventilatory airflow may also reflect that during the present study the animals were in a sprawling posture, which likely places more restriction on thoracic than abdominal expansion.

The ventilatory cycles we observed in *Alligator* were not associated with cyclic changes in narial patency. As Bertau (1935) noted, alligators do not close their nares during ventilation. Bruner (1901) described a similar pattern of sustained narial patency in salamanders, though cyclic narial closure occurs in some vertebrates (Gargaglioni & Milsom, 2007). The episodes of changes in narial patency that we did observe were associated more with defensive airflow (snorting and narial hissing) than routine ventilation.

Our results suggest that the specialized narial musculature of *Alligator* is unlikely to play a role in creating or maintaining a ventilatory pattern; though as Farmer and Carrier (2000) noted, alligators exhibit different ventilatory patterns during rest, exercise, and recovery. Crocodilians produce a variety of intraspecific and interspecific vocal communications (Vergne, Pritz, & Mathevon, 2009; Young, Mathevon, & Tang, 2013), but this vocalization is regulated by skeletal muscle in the glottis (Riede, Li, Tokuda, & Farmer, 2015; Riede, Tokuda, & Farmer, 2011) rather than the nares. Crocodilians hold the nares closed when submerged (e.g., Bellairs & Shute, 1953; Ferguson, 1981; Heard, 2010 and Johnson, 1973) suggesting that the sympathetic innervation of the narial muscles is coupled to receptors in the narial epithelium. A similar dive reflex has been described in other vertebrates with and without valvular nostrils (e.g., Andersen, 1963;

Angell-James & De Burgh-Daly, 1973; Dutschmann & Paton, 2002; Furilla & Jones, 1986).

Bruner (1897) provided the first detailed description of the crocodilian narial muscles, recognizing the unusual pattern of the constrictor naris surrounding the dilator naris. In his subsequent comparative study of the amphibian nose, Bruner (1901) noted the superficial similarities between the crocodilian narial muscles and those found in some salamanders. There are two important differences; in the salamanders, the constrictor naris is dorsal to the dilator nares (instead of encircling it), and the constrictor naris courses around the rostral surface of the nasal vestibule in salamanders (Bruner, 1901). More detailed reconstructions of the narial muscles in the tiger salamander (*Ambystoma tigrinum*) revealed spatial segregation between the narial dilator and constrictor muscles (Wirsig-Wiechmnn & Holliday, 2002). As detailed above, in *Alligator* the dorsal portion of the constrictor naris still has some attachment rostral to the nares (Figure 5). Nevertheless, compared to other vertebrates the crocodilian constrictor naris has made a functional transition from constricting the naris to constricting the dilator naris. This transition may have been associated with an expansion in cross-sectional area of the dilator naris, such that the dilator naris effectively split an initially dorsal constrictor naris. Alternatively, reduction in soft tissue and increasing rigidity on the rostral surface of the nasal vestibule may have reduced the mechanical efficiency of the constrictor naris, favoring a mechanically beneficial switch in the locus of constriction to the adjacent dilator muscle.

The functional transition of the constrictor naris from direct to indirect constriction of the external naris and nasal vestibule had an important consequence. The dilator naris became functionally co-opted by the surrounding constrictor naris and now plays a key role (as a passive block of dense tissue) as a form of diaphragm valve to reduce the narial patency. In a practical sense, the dilator nares functions by both pulling (dilating) and pushing (constricting) on the caudal vestibular plate; by switching between these active (dilating) and passive (constriction) roles, the dilator naris is, essentially, its own antagonist.

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AUTHOR CONTRIBUTIONS

Matthew Klassen: Conceptualization; investigation; methodology; writing-review and editing. **James Adams:** Formal analysis; investigation; writing-review and editing. **Michael Cramberg:** Formal analysis; methodology; writing-review and editing. **Lucas Knoche:** Formal analysis; investigation; methodology; writing-review and editing. **Bruce Young:** Conceptualization; data curation; formal analysis; investigation; methodology; supervision; writing-original draft; writing-review and editing.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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