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Functional morphology of gill ventilation of the goosefish, *Lophius americanus* (Lophiiformes: Lophiidae)

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A B S T R A C T

The goosefish, *Lophius americanus*, is a dorso-ventrally compressed marine fish that spends most of its life sitting on the substrate waiting to ambush prey. Species in the genus *Lophius* have some of the slowest ventilatory cycles recorded in fishes, with a typical cycle lasting more than 90 s. They have a large gill chamber, supported by long branchiostegal rays and ending in a siphon-like gill opening positioned underneath and behind the base of the pectoral fin. Our goals were to characterize the kinematics of gill ventilation in *L. americanus* relative to those of more typical ray-finned fishes, address previous assertions about ventilation in this genus, and describe the anatomy of the gill opening. We found that phase 1 of ventilation (during which both the buccal and gill chamber are expanding) is greatly increased in duration relative to that of typical ray-finned fishes (ranging from 62 to 127 s), and during this phase, the branchiostegal rays are slowly expanding. This slow expansion is almost visually imperceptible, especially from a dorsal view. Despite this unusually long phase 1, the pattern of skeletal movements follows that of a typical actinopterygian, refuting previous assertions that *Lophius* does not use its jaws, suspensorium, and operculum during ventilation. When individuals were disturbed from the sediment, they tended to breathe more rapidly by decreasing the duration of phase 1 (to 18–30 s). Dissections of the gill opening revealed a previously undocumented dorsal extension of the adductor hyohyoideus muscle, which passes from between the branchiostegal rays, through the ventro-medial wall of the gill opening, and to the dorsal midline of the body. This morphology of the adductor hyohyoideus shares similarities with that of many Tetraodontiformes, and we suggest that it may be a synapomorphy for Lophiiformes + Tetraodontiformes. The specialized anatomy and function of the gill chamber of *Lophius* represents extreme modifications that provide insight into the potential limits of the actinopterygian gill ventilatory system.

1. Introduction

Active ventilation of the gills is a critical process for fishes, allowing them to exchange gases and ions with the environment and excrete some metabolic wastes. Because ventilation is an energetically expensive process for ray-finned fishes (Actinopterygii) that comprises 5–15% of the total metabolic budget (Cameron and Cech, 1970; Edwards, 1971; Farrell and Steffensen, 1987), fishes have evolved many adaptations for increased respiratory efficiency. These adaptations include maximizing surface area of the gill tissue, minimizing diffusion distance across the gill epithelium, and maximizing the oxygen partial pressure gradient between the blood and water (Hughes, 1966). The latter is achieved through a system of counter-current exchange in which blood in vessels of the gill lamellae flows in the opposite direction of oxygenated water (Hughes and Shelton, 1958). Establishing this unidirectional flow of water over the gills requires coordination of many cranial components. Given the diversity in cranial morphology and metabolic requirements among ray-finned fishes, we can expect to find a great variety of strategies for efficient pumping. However, functional variation in aquatic ventilatory pumps has received little attention.

Gill ventilation in most species of ray-finned fishes relies on changes in pressure driven by pumps in two chambers: the mouth (buccal chamber) and gill chamber. As shown in Fig. 1, these pumps alternate between suction (expansion to create negative pressure, drawing in water) and pressure (compression to create positive pressure, forcing out water; Hughes and Shelton, 1958; Hughes, 1960; Brainerd and Ferry-Graham, 2006). As defined by Hughes and

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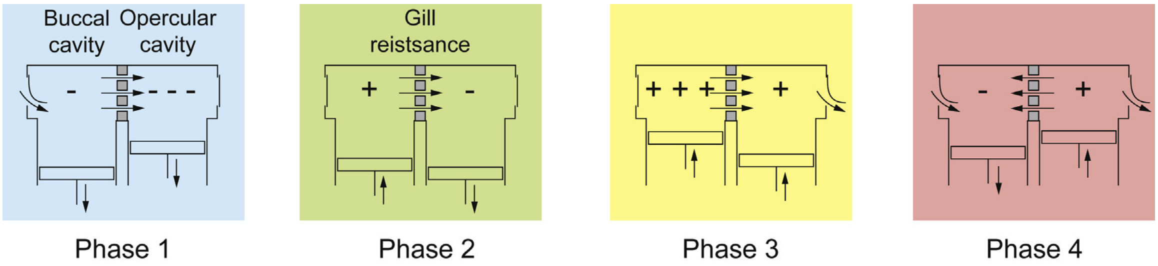

Fig. 1. Four phases of gill ventilation used by actinopterygians. During phase 1, both the buccal and gill chambers are expanding to produce negative pressure and take in water. During phase 2, the buccal chamber begins to compress. During phase 3, both chambers compress to produce positive pressure and force water out of the gill opening. During phase 4, the buccal chamber begins to expand, transitioning back to negative pressure to draw in water. Based on Brainerd and Ferry-Graham (2006), Summers and Ferry-Graham (2002), and Hughes (1960).

Shelton (1958), each ventilatory cycle begins with expansion of the buccal chamber, which draws water into the mouth. The gill chamber then expands, which draws water from the mouth over the gills. The buccal chamber then compresses to force water from the mouth over the gills. Finally, compression of the gill chamber forces water out of the gill opening. The gill tissues introduce resistance between the two chambers, limiting backflow during the transition from pressure back to suction. Changes in buccal chamber pressure are driven by movements of the jaw, suspensorium, and hyoid apparatus, and changes in gill chamber pressure are driven by the opercular bones (opercle, subopercle, and interopercle) and the branchiostegal apparatus (Liem, 1970). The relative timing of these movements and the resulting pressure changes vary considerably among taxa (Hughes, 1960), and there is substantial variation in the anatomy of the skeletal elements and musculature involved, particularly in the branchiostegal apparatus (McAllister, 1968). There is also significant morphological variation in the external openings of the buccal and gill chambers; for example, gill openings can be very large with a wide valve, or they can be restricted to a tiny aperture variably positioned on the head (McAllister, 1968; Farina et al., 2015).

The goosefish, *Lophius americanus*, is a commercially valuable marine fish common in waters off the northeastern coast of North America at depths ranging from just subtidal to over 900 m (Caruso, 2002; Richards et al., 2008). It is dorso-ventrally flattened, and its head is large for its body size (Fig. 2B). As in other anglerfishes (Lophiiformes) such as frogfishes, batfishes, and seadevils, it has an elongated first dorsal spine that has migrated to the front of the head where it supports a fleshy lure known as an esca. Species in the genus *Lophius* differ primarily in pectoral fin ray counts, shape of the esca, and dorsal spine length (Caruso, 1983). All are ambush predators that spend most of their adult life sitting on sandy, muddy, or rocky substrates, using their lure to attract fishes and other prey (Chadwick, 1929; Wilson, 1937; Gudger, 1945). When suction feeding, they rapidly expand the buccal cavity by means of hyoid and jaw depression, combined with a large degree of cranial elevation (Elshoud, 1986). When not feeding, they remain cryptic by matching skin colors to the substrate and using the pectoral fins to create recesses in the sediment (Wilson, 1937; Laurenson et al., 2004).

In his behavioral description of *Lophius piscatorius*, Wilson (1937) noted exceptionally slow ventilation, with individuals taking approximately 60–180 s to complete a single ventilatory cycle. Most other species of ray-finned fishes require 0.5–6 s to complete a ventilatory cycle (Hughes, 1960). The slow ventilation of *Lophius* is potentially related to low metabolic demands (evident from the low surface area of their gill lamellae; Hughes, 1966) and a need to remain cryptic as intermittent and opportunistic feeders (Armstrong et al., 1996; Laurenson and Priede, 2005; Fariña et al., 2008; Valentim et al., 2008). They increase their ventilatory rate immediately after feeding events (Wilson, 1937) or when they are disturbed from their sediment recesses. Their ventilatory anatomy consists of a large buccal cavity with a pronounced oral valve, an L-shaped operculum, six elongate branchiostegal rays, and a gill opening ventral and posterior to the pectoral fin (Fig. 2). As in other Lophiiformes, the gill opening is relatively small and siphon-like, forming a tube upon exhalation. The long branchiostegal rays and posterior position of the gill opening create a much larger gill chamber than is typical for actinopterygians.

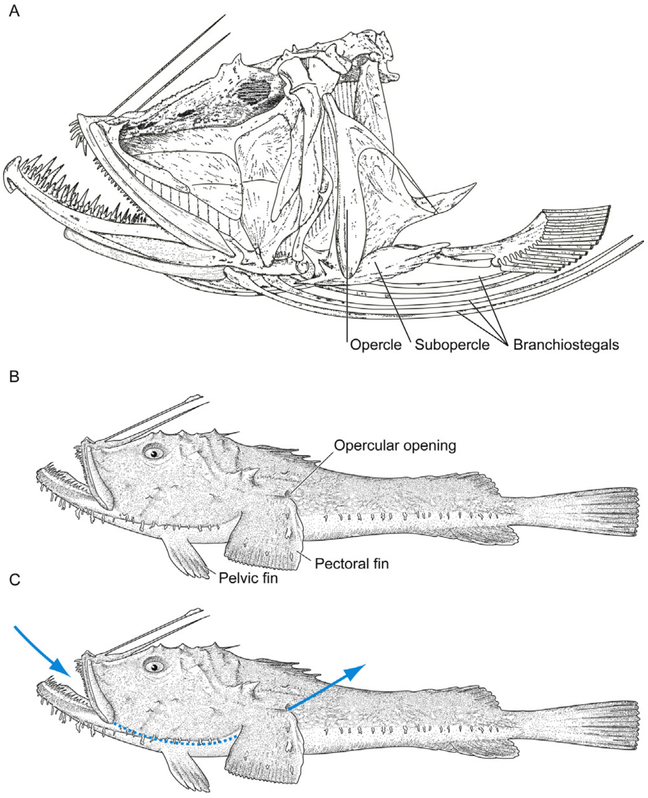

Fig. 2. Cranial skeletal and external anatomy of *Lophius*. (A) The large gill chamber consists of an L-shaped operculum and elongated branchiostegal rays that pass under the pectoral girdle. (B) The gill opening is located in the axillary region, posterior and ventral to the pectoral fins. (C) Water comes in through the mouth and passes over the gills to enter the large gill chamber. It then passes under the pectoral fin and out the gill opening. The illustration of cranial anatomy (A) is redrawn from Gregory (1933) and the illustration of external anatomy is modified from Liem et al. (2001).

L. americanus represents an extreme of fish ventilatory anatomy and function with its exceptionally slow ventilation and enormous gill chamber. It is therefore an important species to investigate when considering limits of the gill ventilation system of ray-finned fishes. Our first goal is to characterize the kinematics of gill ventilation in *L. americanus* and make comparisons to those of more typical ray-finned fishes. We also address some previous assertions and predictions about ventilation in *Lophius*. Our second goal is to describe the anatomy of the gill chamber, specifically the musculature of the gill opening, and discuss our findings in the context of gill ventilatory function and evolution.

2. Materials and methods

2.1. Animals and videography

From June to August 2011, we made observations and video recordings of gill ventilation in five specimens of *L. americanus* collected in the Gulf of Maine, ranging in size from 40 to 55 cm total length. Four individuals obtained by bottom trawl were transported to Shoals Marine Laboratory (SML) and studied for 1–3 weeks in flow-through seawater tanks. Detailed observations and videography at SML commenced after fish were allowed to acclimate to tanks for at least three days. A fifth individual was studied in its exhibit at the Seacoast Science Center (SSC) in Rye, NH. Animal care and use followed protocols approved by Cornell University's Institutional Animal Care and Use Committee (IACUC protocol #2011-0028).

We recorded high definition video at 30 frames per second using a Canon 5D Mark II camera equipped with a 24–70 mm lens. For two individuals housed at SML, we recorded both slow and rapid ventilation. To study "slow ventilation," we minimized any disturbances or human activity in the tank room for at least 1 h prior to videography and only recorded individuals when they were sitting undisturbed in a sand recess. If a fish became alarmed by the presence of a researcher, it would leave its sand recess and swim around the tank. When it ceased swimming and rested above the sand, it would ventilate much more rapidly until it returned to its sand recess or created a new one. We termed this "rapid ventilation" and recorded and analyzed it separately (Table 1). The two additional individuals housed at SML did not create sand recesses and only exhibited rapid ventilation during a week of observation. The individual at the SSC (*L. americanus* 3) had been on exhibit for two months and had been in its sand recess for more than 1 h prior to our videography of slow ventilation. It remained in its recess throughout filming, so we were unable to record its rapid ventilation.

2.2. Kinematic analyses

We analyzed videos of ventilatory cycles using ImageJ (Rasband, 1997–2015) to record the timing of each phase of ventilation through a visual frame-by-frame inspection. Following the scheme shown in Fig. 1, based on the kinematics of ventilatory phases defined in Hughes and Shelton (1958), we used the following movements to denote the start of each of the four phases: phase 1 started with branchiostegal abduction, phase 2 started with mouth closing, phase 3 started with the opening of the gill opening, and phase 4 started with mouth opening. The start of phase 1 was impossible to observe in fishes buried in a sand recess because the branchiostegals were not visible. In these cases, we used the abduction of the opercle (which starts approximately 1 s later than branchiostegal abduction) as the start of phase 1, which may introduce small errors into our estimates of the lengths of phases 1 and 4. For three individuals, we analyzed five complete slow ventilatory cycles, and for a subset of two of these individuals, we additionally analyzed five rapid ventilatory cycles. Table 1 presents the average duration and standard deviation for each phase and for the entire ventilatory cycle.

Using a digitizing tool developed for MatLab (Hedrick, 2008), we tracked two-dimensional movements of points on the head throughout a typical rapid ventilatory cycle (Fig. 3) to demonstrate kinematic patterns seen during ventilation. As markers, we used spots of pigmentation that contrasted with the rest of the skin color to track the lower jaw, opercle, suspensorium, and eye. We subtracted movements of the eye from those of the jaw, opercle, and suspensorium to correct each trace for head movements. Because these bones show minimal lateral movements, only vertical displacement was considered. For the suspensorium and operculum, abduction was observed as an increase in vertical position of the points tracked on these bones, and adduction was observed as a decrease in vertical position. The 0.0 cm position for each trace was set to be the minimum vertical value. We used the same protocol to identify the start and end of each phase as mentioned above. We used the location of the eye relative to the substrate to track cranial elevation during ventilation by measuring vertical displacement on each frame of a video recorded from a lateral view of the fish (Fig. 3). When the fish were in their sand recesses, it was impossible to quantify their movements, because much of the head was obscured and the fish was not optimally positioned for videography. Therefore, we were unable to directly track these movements for slow ventilatory cycles. However, close visual inspection of videos of cycles up to 210 s shows movements of the jaws, suspensorium, and operculum that appear to be very similar to those exhibited in our example sequence (Fig. 3). Additionally, we visualized flow out of the gill opening for one individual by introducing food dye mixed with seawater at the front of the mouth and allowing the fish to inhale and expel the colored water. We repeated the introduction of dye four times until the fish became accustomed to the procedure and held the dye for approximately 30 s before expelling it. We calculated the speed of the resulting jet of water by using ImageJ to track the vertical displacement of the top of the jet during the first 2 s of exhalation.

2.3. Anatomy of the gill opening

We dissected eight specimens of *L. americanus* collected by the Northeast Fisheries Science Center (NEFSC) in Woods Hole, MA, on annual bottom trawl surveys between 2008 and 2012. Muscles of the gill opening were exposed by carefully removing the loose, scaleless skin covering the branchiostegals and trunk. Dissections of three specimens were photographed using a Canon 5D Mark II camera equipped with a 24–70 mm lens. We removed the entire branchiostegal apparatus of a particularly large specimen (30 kg, 1.3 m total length) and photographed it on a light table. We also removed a square of tissue from the dorsal extension of the adductor hyohyoideus (*hyohyoidei adductores*; Winterbottom, 1973) muscle and examined it using an Olympus SZX12 stereozoom microscope. Additionally, we dissected the gill opening region of two other Lophiiformes (one individual each): *Histrio histrio* (CUMV 79429) and *Chaunax pictus* (CUMV 43866).

3. Results

The average length of a ventilatory cycle for the three studied individuals of *L. americanus* was 91.5 s (SE: ± 16.1 s; $n = 3$), or approximately 0.011 cycles per second, during slow ventilation. The longest ventilatory cycle was measured in the individual at the SSC and lasted 210.5 s. Phase 1 was the longest phase of ventilation, and it varied among individuals (Table 1). When the fish were out of their recesses, rapid ventilation occurred primarily through the reduction of phase 1. Phase 3 was also shorter during rapid ventilation, but phases 2 and 4 remained approximately the same length in both slow and rapid ventilation. The duration of phase 2 (the transition from suction to pressure) was consistently very short (Table 1), regardless of the individual or the conditions.

Table 1
Relative timing of each phase of gill ventilation in each individual. The mean ventilatory rate is presented, followed by mean duration and standard deviation (in parentheses) for each phase.

	Mean ventilation rate (Hz)	Phase 1 (s)	Phase 2 (s)	Phase 3 (s)	Phase 4 (s)
L. americanus 1 (cryptic)	0.013	74.05 (6.19)	0.14 (0.03)	2.79 (0.09)	0.51 (0.08)
L. americanus 1 (rapid)	0.037	24.88 (1.87)	0.16 (0.06)	1.77 (0.09)	0.39 (0.03)
L. americanus 2 (cryptic)	0.014	70.36 (2.93)	0.13 (0.05)	2.03 (0.33)	0.91 (0.16)
L. americanus 2 (rapid)	0.041	20.86 (0.70)	0.21 (0.02)	1.8 (0.23)	1.45 (0.34)
L. americanus 3 (cryptic)	0.008	120.04 (24.28)	0.69 (0.13)	2.41 (0.12)	0.41 (0.08)

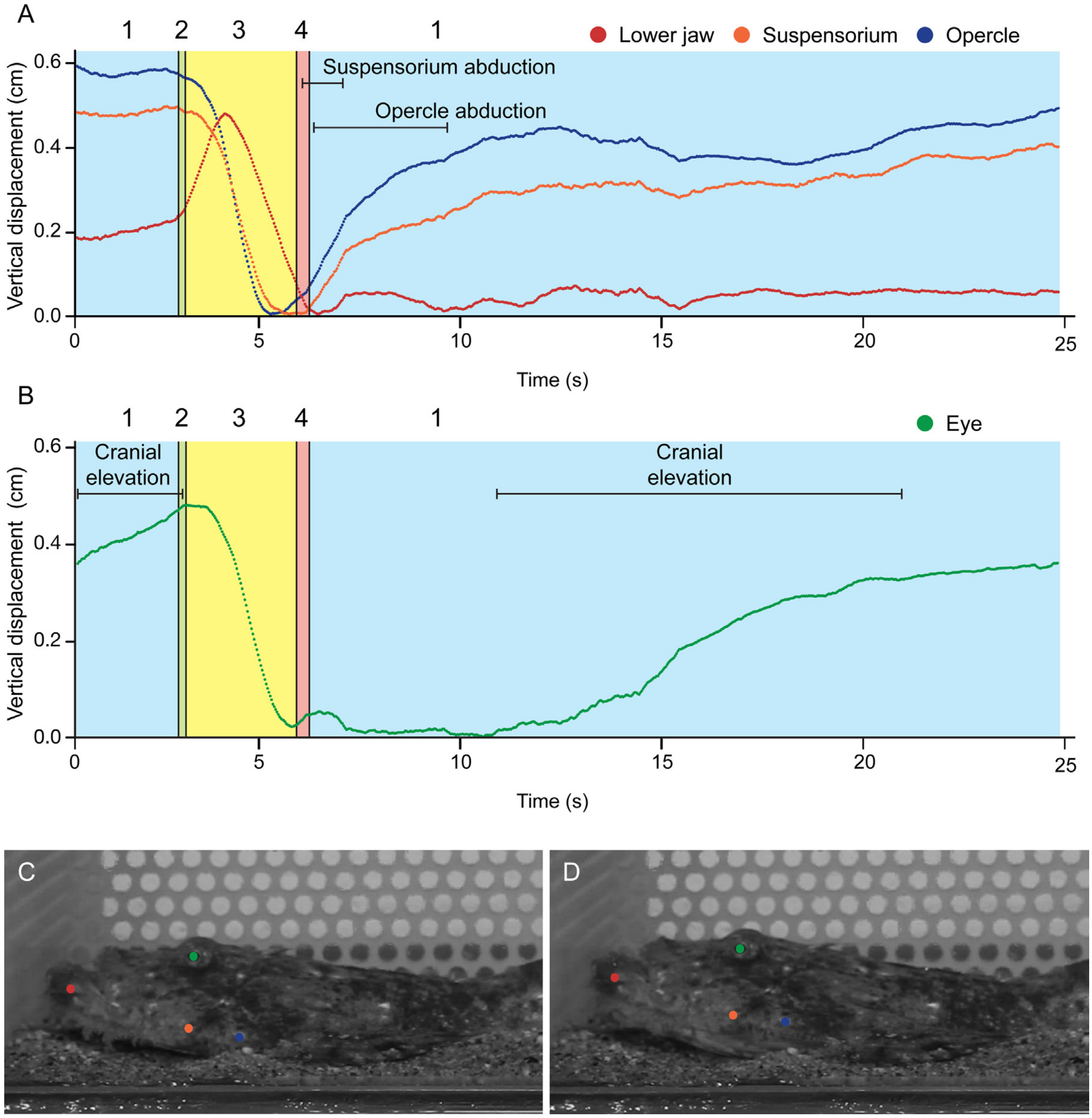

Fig. 3. An example ventilatory cycle of *Lophius americanus*. (A) Digitized vertical movements of the jaw, suspensorium, operculum, and eye show that *L. americanus* pumps water throughout the ventilatory cycle. The position of the eye was subtracted from each of the vertical movements of the jaws, suspensorium, and operculum to show movement independent of head position. (B) Eye movement is plotted separately to demonstrate when cranial elevation occurs. (C and D) Stills from the analyzed video show the position of the fish at the start of phase 1 (C) and at the start of phase 3 (D), with colored markers indicating the locations of points tracked for kinematic plots shown in (A) and (B).

Phase 1 of a typical ventilatory cycle (Fig. 3A) began with a slow abduction of both the opercle and the branchiostegals, along with continuing movements of the jaw and suspensorium that began in phase 4. Approximately 3 s after the start of phase 1, the operculum reached maximum abduction (Fig. 3A). However, the branchiostegals continued to expand throughout phase 1. The fish also slowly elevated its head using its pectoral and pelvic fins throughout this phase to accommodate the expansion of the branchiostegal apparatus (Fig. 3B). The oral valve remained open for most of this phase, although it would occasionally partially close. Phase 2 began with

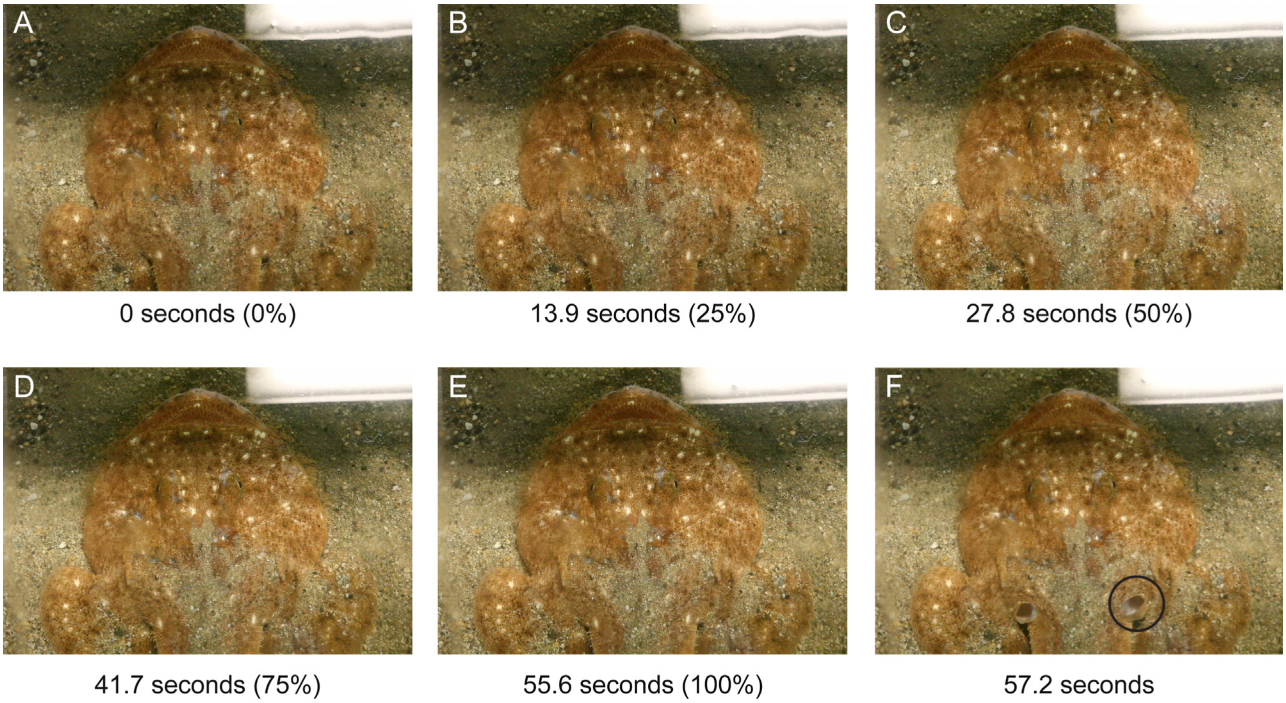

Fig. 4. Dorsal view of *Lophius americanus* during slow ventilation. (A–E) Phase 1 at 0% (A), 25% (B), 50% (C), 75% (D), and 100% (E) of the phase. From a dorsal perspective, motion of *L. americanus* is almost imperceptible, except during exhalation (F).

raising the lower jaw and hyoid apparatus, followed shortly thereafter by the start of suspensorial adduction, compressing the buccal chamber. Phase 3 began almost immediately after the start of phase 2, and it was signaled by the opening of the valve of the gill chamber and adduction of the operculum and branchiostegals. Near the end of phase 3, the operculum ceased adducting and began to relax back to a neutral position, resulting in an increase in vertical displacement at the end of phase 3 and throughout phase 4 until it actively began to abduct in phase 1. Phase 4 began with depression of the lower jaw and hyoid while the gill chamber valve was still open and while adduction of the branchiostegals was still occurring. Phase 4 was also very short relative to the total ventilatory cycle.

Movement occurred throughout the cycle (Fig. 3), despite being visually imperceptible during most of phase 1 (Fig. 4A–E; supplementary Video S1 in the online Appendix). This continual movement was evident when the speed of the video was increased considerably, as shown in supplementary Video S2, which is 20 times the true speed. Exhalation was far more obvious, and the gill opening could be seen from above to open into a siphon-like aperture (Fig. 4F; supplementary Video S3). During this exhalation (phases 3 and 4), water was ejected out the gill opening in a large, dorsally-directed jet (Fig. 5). In one individual, this jet traveled upward at a speed of approximately 0.1 m/s (0.21 body lengths per second). The position of the pectoral fins relative to the substrate influences water flow out of the gill opening; when an individual is sitting in its sand recess, the pectoral fins are extended laterally, holding the opening in its typical siphon shape (Figs. 4F and 6A). However, when the fish is above the substrate or housed on a glass substrate, it uses its pectoral fins to prop itself up, producing a flap-like gill opening shape (Fig. 6B).

The gill opening is ventral and posterior to the pectoral fin base (Fig. 7A) and is formed by several tissues, including skin, muscles, fascia, and two large masses of connective tissue (CT in Figs. 7 and 8). The thick connective tissue masses provide a lining for the occluding parts of the valve. The dorsal lip of the valve sits between the pectoral fin and the trunk and is supported by one of the connective tissue masses (CT1 in Fig. 7). The ventral lip begins at the anterior base of the pectoral fin and ends at the posterior margin of the dorsal lip, where it is supported by a second connective tissue mass (CT2 in Fig. 7). The tips of three branchiostegals (rays 3–5) sit in the gill opening and raise the ventral lip during exhalation. In dissections of the gill opening muscles, we observed that the adductor hyohyoideus, a muscle that spans the branchiostegals to pull them together during exhalation, extends posteriorly from between each branchiostegal pair to surround the medial and ventral walls of the gill opening. There is also a large dorsal extension of this muscle; we followed these dorsally-extending muscle fibers from their origin between branchiostegals 2–5, where they form a cross-hatching pattern with the muscle fibers that transversely join neighboring branchiostegals (Figs. 8C and D). These muscle fibers pass through the medial surface of the gill opening, pass over one of the connective tissue masses (CT2) and continue dorsally up the side of the body. A narrow band of this extension of the adductor hyohyoideus

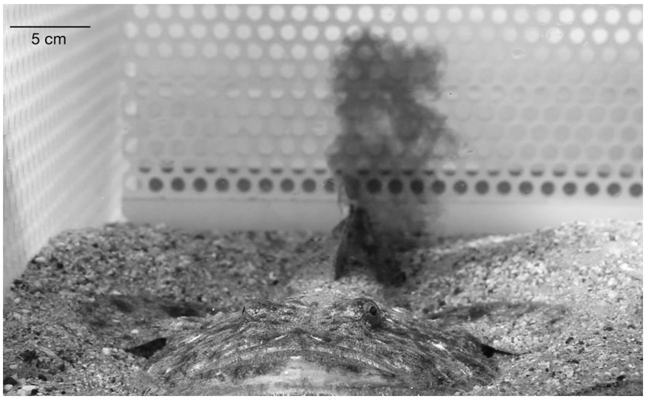

Fig. 5. Visualization of flow from the gill openings during exhalation. The ventilatory current leaving the gill opening is forced upward, as shown by water seeded with dye expelled from the opening. The resulting jet traveled upward at approximately 0.1 m/s.

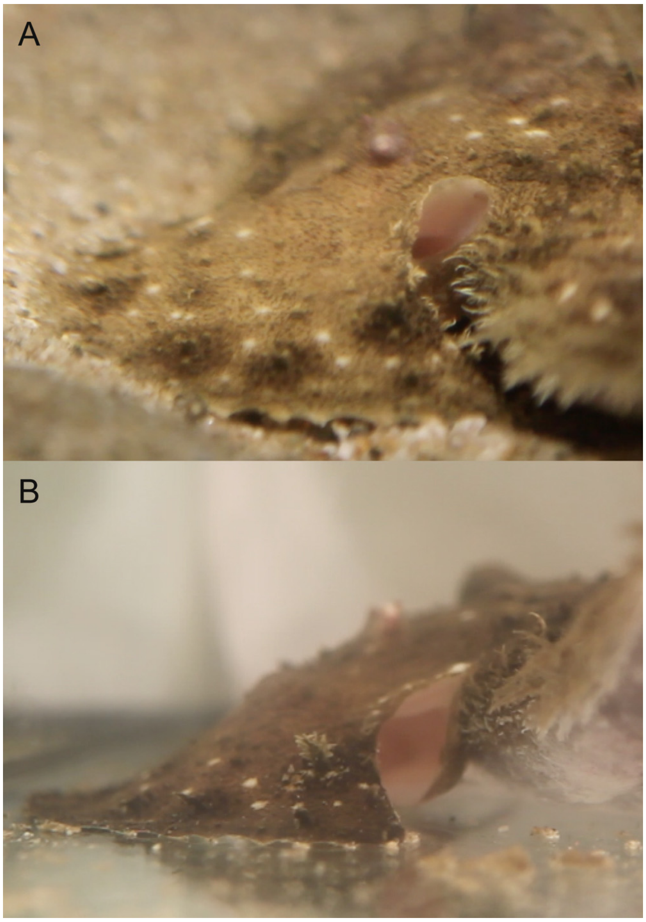

Fig. 6. The role of fin positioning in gill opening aperture shape. Posterior views of the gill opening during phase 3 show that the positioning of the fins is impacted by the substrate and changes the shape of the gill opening. (A) When the fish is in its sand recess, its pectoral fins extend laterally from the body, and the gill opening forms a vertically directed siphon-like shape. (B) When the same individual is removed from the substrate, the pectoral fins extend laterally and ventrally, causing the gill opening to have a flap-like shape directed posteriorly.

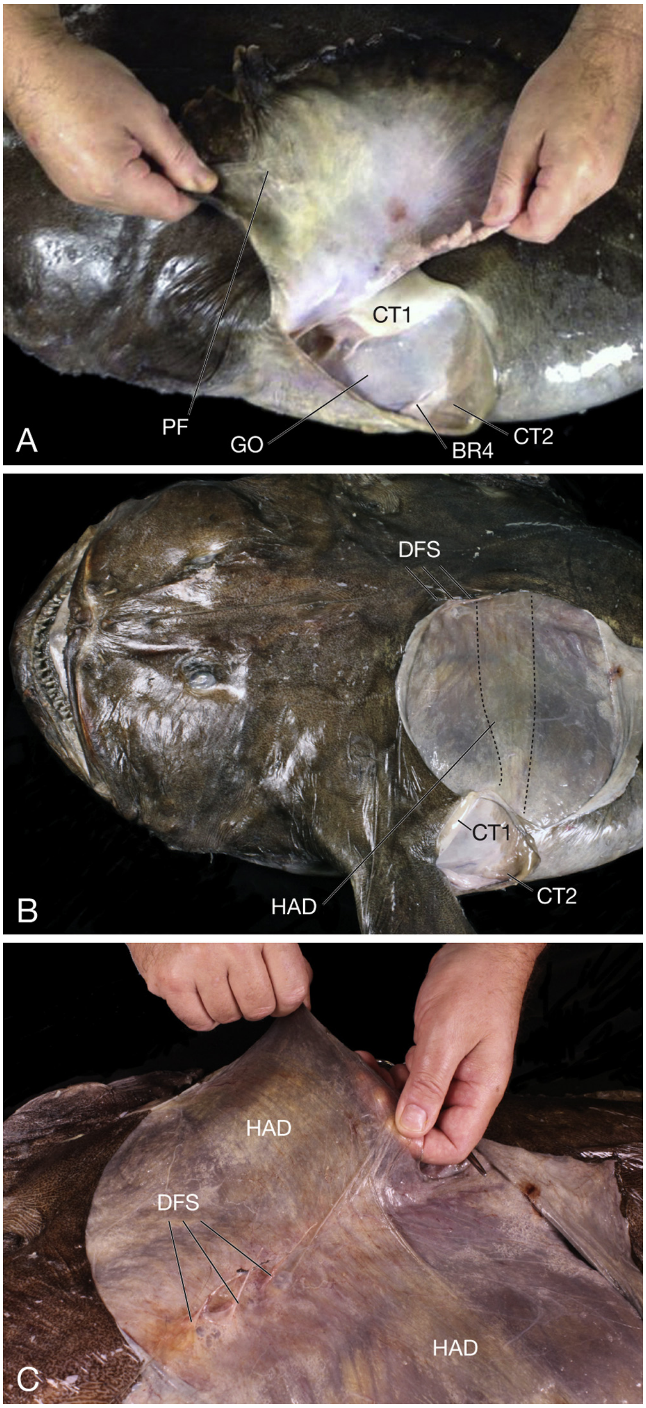

Fig. 7. Position of the gill opening and associated musculature. (A) The gill opening is positioned ventrally and posteriorly to the pectoral fin, and two connective tissue masses that we term lips form the occluding margins of the opening. (B) When the skin is removed from the dorsal surface of the trunk, a dorsal extension of the adductor hyohyoideus is visible as a thin band of muscle embedded in the fascia of the trunk. (C) This adductor hyohyoideus extends dorsally from the gill openings to meet its antimere at the dorsal midline behind the dorsal spines. Abbreviations: BR, branchiostegal ray; CT, connective tissue mass; DFS, dorsal fin spines; GO, gill opening; HAD, adductor hyohyoideus; PF, pectoral fin.

muscle sits just lateral to the trunk and abdominal muscles in a thin sheet of fascia (Figs. 7, 8B and C). The left and right extensions meet at the dorsal midline of the trunk, just posterior to the dorsal fin spines (Fig. 8C). We examined the tissue of this narrow band using microscopy and found conclusively that it consists of muscle fibers. We also noted expansions of the adductor hyohyoideus muscles of *Chaunax pictus* and *Histrio histrio* in dissections of the branchiostegals and gill opening region. In both species, muscle fibers of the adductor hyohyoideus extended beyond the branchiostegals to surround the gill opening. In *C. pictus*, these muscle fibers also extended dorsally into the fascia overlying the trunk, but they did not extend all the way to the dorsal midline as in *L. americanus*.

4. Discussion

L. americanus has an extremely slow ventilatory cycle, which exceeds the duration of a typical actinopterygian ventilatory cycle by at least 30 times. This is accomplished with a long phase 1 (Fig. 3), during which the large gill chambers allow *L. americanus* to slowly take in a large volume of water. While the operculum in most fishes contributes substantially to the expansion of the gill chamber, in the case of *L. americanus* the operculum only abducts for approximately 3 s at the beginning of phase 1 (Fig. 3). However, the branchiostegal rays expand throughout phase 1, and, for most of the ventilatory cycle, they are the only structures involved in driving the ventilatory current. Even after the operculum reaches maximum abduction, the branchiostegals continue to expand, drawing water from the mouth over the gills. The oral valve also stays open throughout phase 1, allowing water to enter the mouth. Continual expansion of the gill chamber by the branchiostegals combined with a continuously open oral valve is strong evidence that *L. amer-*

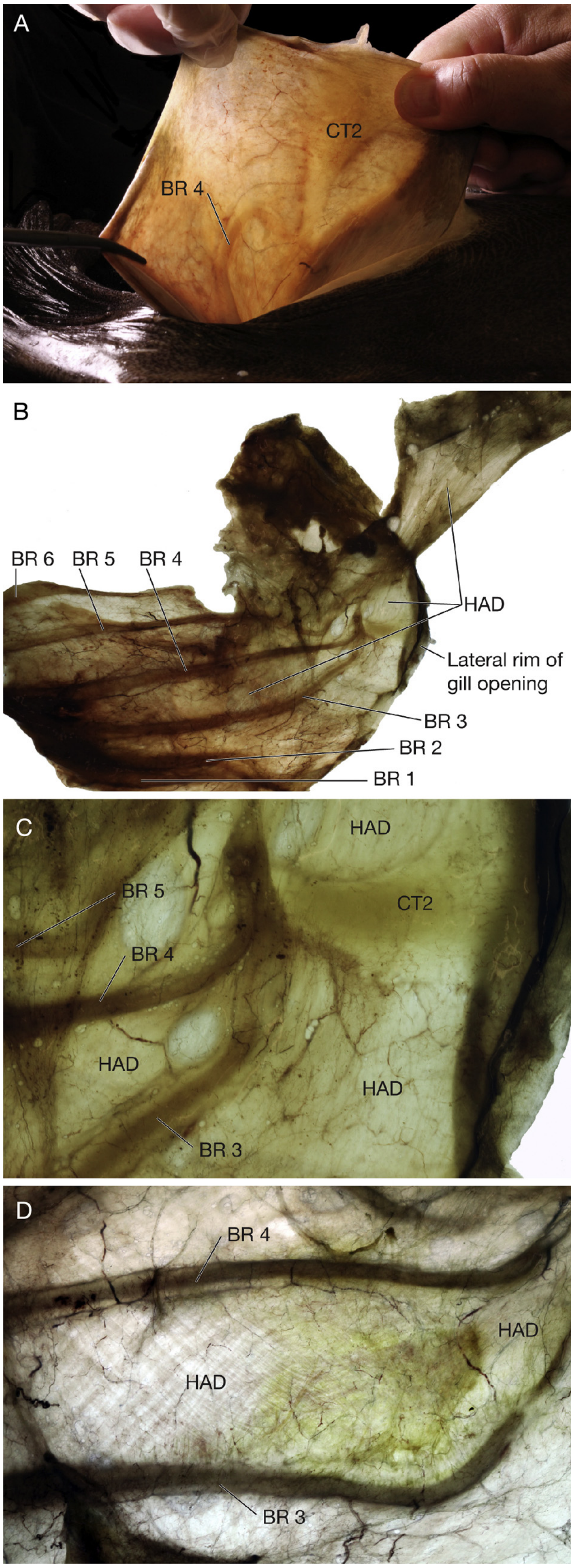

Fig. 8. Anatomy of the gill opening. (A) The ventral wall of the gill opening is supported by a branchiostegal and a connective tissue mass. (B–D) We removed the gill chamber (branchiostegal rays and gill opening) from the fish and placed it on a light table. The gill opening is lined with adductor hyohyoideus muscle (B), which extends across the second connective tissue mass before extending dorsally up the body (C). The adductor hyohyoideus stretches transversely (anteriorly) or obliquely (posteriorly) between the branchiostegal rays until the tips of the rays, at which point the muscle fibers form a cross-hatching pattern as they meet and continue posteriorly beyond the branchiostegals (D). Abbreviations: BR, branchiostegal ray; CT, connective tissue mass; HAD, adductor hyohyoideus.

icanus maintains a constant flow of water over the gills despite its slow pumping.

Although this extremely slow expansion of the branchiostegals represents a deviation from typical actinopterygian ventilation, the rest of the ventilatory movements of *L. americanus* follow the same general pattern as in other actinopterygians, with all of the same phases of buccal and gill chamber expansion and compression occurring (Hughes and Shelton, 1958; Hughes, 1960) and the same skeletal structures actuating the chambers. In a dissertation on the feeding of *L. piscatorius*, a species that is nearly identical to *L. americanus* except in escal and dorsal spine morphology (Caruso, 1983), Elshoud (1986) referred to the enlarged gill chamber as a "ventro-caudally situated muscular respiratory sack" and suggested that this "sack" operates independently of feeding-associated structures (jaws and suspensorium) during gill ventilation. He went so far as to state: "*Lophius* has invented a new respiratory system." He proposed that *Lophius* reduces or eliminates abduction of the suspensorium during ventilation, because such abduction would produce cranial elevation that would lift the fish from the sediment and compromise its concealment due to its extremely dorso-ventrally flattened head. Likewise, in a study of feeding anatomy, Field (1966) noted the relatively small muscles actuating the suspensorium and operculum of *Lophius* and inferred that they were "fixation muscles" with no role in ventilation. However, we observed suspensorial adduction during phases 2 and 3 and suspensorial abduction during phase 4 and at the beginning of phase 1 (Fig. 3A). We also observed opercular adduction during phases 3 and abduction at the beginning of phase 1 (Fig. 3A). We noted that most of this abduction and adduction resulted in vertical displacement of the suspensorium and operculum, in contrast to more typical teleosts, which primarily exhibit lateral displacement of these bones during ventilation. This is likely due to the dorso-ventral flattening of the head, which results in an unusually horizontal orientation of the suspensorium and opercle. The head also elevated during phase 1 (Fig. 3B), which did not appear to compromise concealment of the fish. While Field (1966) and Elshoud (1986) were correct about the importance of the branchiostegal apparatus, the ventilatory movements of *Lophius* are more similar to those of typical ray-finned fishes than these authors suggested.

The slow ventilation of *Lophius* is likely an adaptation related to crypsis and a low metabolic rate. Lophiiformes generally have many features that contribute to their cryptic feeding strategy, which involves ambushing prey that is attracted to the deceptive lure on their dorsal spine. *Lophius* species in particular are highly cryptic, possessing a flattened skull, tags of skin around the margin of the head and body that break up their outline, and the ability to match their skin color to the substrate (Wilson, 1937). Their slow ventilatory movements during phase 1 are likely visually imperceptible to predators and prey, especially in dorsal view (Fig. 4 A–E; supplementary Video S1). Exhalation is much more obvious but is brief and infrequent. This strategy is almost certainly impossible in more active fishes, because the low rate of water passing over the gills during inhalation would not provide enough oxygen to support high levels of activity. Additionally, the slow rate of ventilation may reduce the metabolic demands of ventilatory movements, allowing for a reduction in energy budget. *Lophius* has been noted for having a much smaller total gill surface area relative to body weight (Hughes, 1966), which indicates that its metabolic demands are low. The relationship between crypsis and low metabolic rate is complex, but terrestrial ambush predators, which are often cryptic, generally also have lower metabolic rates (e.g., for lizards, Bennett and Gorman, 1979; for rattlesnakes, Secor and Nagy, 1994). This is postulated to be due to the unpredictability of their food supply and a lack of sustained locomotion required for active foraging and hunting. It is difficult to determine if the slow ventilation of *Lophius* evolved in response to a need for crypsis or to low metabolism, although we predict that it is likely advantageous for both.

The gill openings of *Lophius* are positioned in the axillary region behind and underneath the pectoral fin (Fig. 7A), which is an atypical position for the gill opening of a ray-finned fish. Typical gill openings are positioned immediately behind the opercular bones and thus often are called "opercular openings". The unusual L-shaped opercle and subopercle of *Lophius* (Fig. 2A) are far anterior to the gill opening. Instead, there is a close association between the pectoral fin and the gill opening, and the shape of the opening changes based on the position of the pectoral fins. When the fish is in its sand recess (Fig. 6A), its pectoral fins extend from the body laterally, resting on the edge of the recess, and the gill opening has a siphon-like shape. However, when the same individual is moved to a tank without substrate (Fig. 6B), its pectoral fins extend laterally and ventrally to support the body by fanning out along the bottom, and the gill opening has a flap-like shape. The siphon-like shape allows the fish to exhale in a dorsally-directed jet (Fig. 5A; Wilson, 1937) that does not disturb the sediment and pushes the fish down further into its recess, increasing its concealment. While the fish were acclimating to tanks and digging their recesses, they would occasionally exhale forcefully, pushing themselves further into the sand. Additionally, the position of the gill opening allows for a short transition between the suction and pressure phase (phase 2; Table 1), allowing the fish to quickly begin exhalation. The posteriorly positioned gill openings can be seen to open almost immediately after the lower jaw begins to close, so that the buccal and gill chambers can be emptied nearly simultaneously.

Previous studies of the cranial anatomy of *Lophius* primarily focused on the enlarged buccal skeleton that allows them to rapidly engulf large prey, but several authors also comment on the specialized skeletal anatomy of the gill chamber (Gregory, 1933; Field, 1966; Elshoud, 1986), including the large branchiostegals. Regarding the musculature of *Lophius*, Field (1966) and Winterbottom (1973) described the enlarged hyohyoideus muscles that actuate the branchiostegal rays. The abductor hyohyoideus in *Lophius* consists of thick transverse bands of muscle that originate on the fascia of the ventral midline of the head and insert onto the first two branchiostegals (Field, 1966). The inferior hyohyoideus consists of much thinner strips of obliquely positioned muscle that originate on the ceratohyal and insert on the first four branchiostegal rays (Field, 1966 Winterbottom, 1973). Together, these two muscles control the expansion of the branchiostegals during inhalation (Field, 1966). The adductor hyohyoideus consists of thin sheets of muscle positioned transversely or obliquely between each of the branchiostegals and between the sixth branchiostegal and the subopercle (Field, 1966 Winterbottom, 1973). These muscles are responsible for the contraction of the gill chamber during exhalation. Given the length of the branchiostegal rays, these muscles have tremendous surface area. We found that these muscles extend posteriorly from branchiostegal rays 2–5 to form a complex musculature that helps to control the gill opening (Fig. 8). There is a large and previously undocumented extension of this muscle, with muscle fibers extending dorsally through the medial surface of the gill opening and continuing up the side of the body in the thin sheet of fascia that covers the trunk musculature (Fig. 7). These muscles only appear to be active during exhalation (phases 3 and 4), and therefore likely function to pull the gill opening into a siphon shape when the fish exhales and the pectoral fins are positioned normally (Fig. 6A).

All Lophiiformes lack a close association between the gill opening and the opercular bones and show a restriction of the gill opening to a small aperture. In most Lophiiformes, the gill opening is posterior and ventral to the pectoral fin base, sitting in the axillary region of the fin, as in *Lophius*, although in some cases it is positioned much farther back on the body, including in Chaunacidae, *Antennarius analis* and *Antennarius duescus* (Pietsch and Grobecker, 1987). Antennariid frogfishes use their siphon-like restricted gill opening to produce a slow jetting behavior (Fish, 1987; Pietsch and Grobecker, 1987). We found extensions of the adductor hyohyoideus muscles beyond the branchiostegal rays in *Chaunax pictus* and *Histrio histrio*. In *C. pictus*, these muscles surround the gill opening before passing dorsally into the fascia covering the trunk musculature. In *H. histrio*, these muscles extend from between the branchiostegals and surround the tiny gill opening. In his studies of teleost muscles, Winterbottom (1973, 1974) noted that the adductor hyohyoideus frequently extends dorsally to the opercular bones, but in Tetraodontiformes, these muscles are often greatly enlarged, lining the lateral and sometimes ventro-medial surfaces of the gill chamber, muscularizing the gill opening valves. In some tetraodontiforms, the adductor hyohyoideus also extends to the pectoral girdle and the epibranchials (Winterbottom, 1974). These expansions of the adductor hyohyoideus beyond the branchiostegal rays and the medial surfaces of the opercular bones may be homologous with the expansion of the adductor hyohyoideus we observed in Lophiiformes studied here. Lophiiformes and Tetraodontiformes have been proposed as closely related clades (e.g., Miya et al., 2003; Alfaro et al., 2009; Betancur et al., 2013), and were resolved as sister taxa by Near et al. (2013). We propose that this expansion of the adductor hyohyoideus as well as the presence of restricted gill openings (Chanet et al., 2013; Farina et al., 2015) may be a synapomorphy for Tetraodontiformes + Lophiiformes. However, further investigation into the musculature of related taxa is needed.

5. Conclusions

The specialized gill chamber anatomy of *L. americanus* facilitates slow gill ventilation. However, even with these specializations, *L. americanus* maintains a basic pattern of pumping similar to that of other actinopterygians, primarily differing in the duration of the phases, particularly phase 1. *Lophius* is therefore an excellent example of the robustness of the actinopterygian ventilatory pattern, while showcasing potential for evolutionary modulation of this system. Understanding the diversity and extremes of gill ventilation can be expected to provide new insights into actinopterygian evolution.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.zool.2016.01.006.

References

Alfaro, M.E., Santini, F., Brock, C., Alamillo, H., Dornburg, A., Rabosky, D.L., Carnevale, G., Harmon, L.J., 2009. Nine exceptional radiations plus high turnover explain species diversity in jawed vertebrates. Proc. Acad. Nat. Sci. Philadelphia 106, 13410–13414.

Armstrong, M.P., Musick, J.A., Colvocoresses, J.A., 1996. Food and ontogenetic shifts in feeding of the goosefish, *Lophius americanus*. J. Northwest Atl. Fish. Sci. 18, 99–103.

Bennett, A.F., Gorman, G.C., 1979. Population density and energetics of lizards on a tropical island. Oecologia 42, 339–358.

Betancur, R.R., Broughton, R.E., Wiley, E.O., Carpenter, K., López, J.A., Li, C., Holcroft, N.I., Arcila, D., Sanciangco, M., Cureton, J.C., Zhang, F., Buser, T., Campbell, M.A., Ballesteros, J.A., Adela Roa-Varon, A., Willis, S., Borden, W.C., Rowley, T., Reneau, P.C., Hough, D.J., Lu, G., Grande, T., Arratia, G., Ortí, G., 2013. The tree of life and a new classification of bony fishes. PLoS Curr., http://dx.doi.org/10.1371/currents.tol.53ba26640df0ccaee75bb165c8c26288, Tree of Life Edition 1.

Brainerd, E.L., Ferry-Graham, L.A., 2006. Mechanics of respiratory pumps. In: Shadwick, R.E., Lauder, G.V. (Eds.), Fish Physiology, vol. 23. Elsevier Academic Press, San Diego, pp. 1–28.

Cameron, J.N., Cech, J.J., 1970. Notes on the energy cost of gill ventilation in teleosts. Comp. Biochem. Physiol. 34, 447–455.

Caruso, J.H., 1983. The systematics and distribution of the lophiid anglerfishes. II. Revisions of the genera *Lophiomus* and *Lophius*. Copeia 1983, 11–30.

Caruso, J.H., 2002. Goosefishes or monkfishes. Family Lophiidae. In: Collette, B.B., Klein-MacPhee, G. (Eds.), Bigelow and Schroeder's Fishes of the Gulf of Maine. , 3rd ed. Smithsonian Institution Press, Washington, pp. 264–270.

Chadwick, H.C., 1929. Feeding habits of the angler-fish, *Lophius piscatorius*. Nature 124, 337.

Chanet, B., Guintard, C., Betti, E., Gallut, C., Dettaï, A., Lecointre, G., 2013. Evidence for a close phylogenetic relationship between the teleost orders Tetraodontiformes and Lophiiformes based on an analysis of soft anatomy. Cybium Int. J. Ichthyol. 37, 179–198.

Edwards, R.R.C., 1971. An assessment of the energy cost of gill ventilation in the plaice (*Pleuronectes platessa* L.). Comp. Biochem. Physiol. A 40, 391–398.

Elshoud, G.C.A., 1986. Fish and Chips. Computer Models and Functional Morphology of Fishes. Ph.D. Thesis. Rijksuniversiteit Leiden, The Netherlands.

Farina, S.C., Near, T.J., Bemis, W.E., 2015. Evolution of the branchiostegal membrane and restricted gill openings in actinopterygian fishes. J. Morphol. 276, 681–694.

Fariña, A.C., Azevedo, M., Landa, J., Duarte, R., Sampedro, P., Costas, G., Torres, M.A., Cañás, L., 2008. *Lophius* in the world: a synthesis of the common features and life strategies. ICES J. Mar. Sci. 65, 1272–1280.

Farrell, A.P., Steffensen, J.F., 1987. An analysis of the energetic cost of the branchial and cardiac pumps during sustained swimming in trout. Fish Physiol. Biochem. 4, 73–79.

Field, J.G., 1966. Contributions to the functional morphology of fishes. Part II. The feeding mechanism of the angler-fish, *Lophius piscatorius* Linnaeus. Zool. Afr. 2, 45–67.

Fish, F.E., 1987. Kinematics and power output of jet propulsion by the frogfish genus *Antennarius* (Lophiiformes: Antennariidae). Copeia 1987, 1046–1048.

Gregory, W.K., 1933. Fish skulls: a study of the evolution of natural mechanisms. Trans. Am. Phil. Soc. 23, 1–481.

Gudger, E.W., 1945. The angler-fishes *Lophius piscatorius* et *americanus*, use the lure in fishing. Am. Nat. 79, 542–548.

Hedrick, T., 2008. Software techniques for two- and three-dimensional kinematic measurements of biological and biomimetic systems. Bioinspir. Biomim. 3, 034001.

Hughes, G.M., 1960. A comparative study of gill ventilation in marine teleosts. J. Exp. Biol. 37, 28–45.

Hughes, G.M., 1966. The dimensions of fish gills in relation to their function. J. Exp. Biol. 45, 177–195.

Hughes, G.M., Shelton, G., 1958. The mechanism of gill ventilation in three freshwater teleosts. J. Exp. Biol. 35, 807–823.

Laurenson, C.H., Priede, I.G., 2005. The diet and trophic ecology of anglerfish *Lophius piscatorius* at the Shetland Islands. UK. J. Mar. Biol. Assoc. UK 85, 419–424.

Laurenson, C.H., Hudson, I.R., Jones, D.O.B., Priede, I.G., 2004. Deep water observations of *Lophius piscatorius* in the north-eastern Atlantic Ocean by means of a remotely operated vehicle. J. Fish Biol. 65, 947–960.

Liem, K.F., 1970. Comparative functional anatomy of the Nandidae (Pisces: Teleostei). Fieldiana Zool. 50, 1–175.

Liem, K.F., Bemis, W.E., Walker, W.F., Grande, L., 2001. Functional Anatomy of the Vertebrates, 3rd ed. Thomson/Brooks Cole, Belmont, CA.

McAllister, D.E., 1968. Evolution of branchiostegals and classification of teleostome fishes. Bull. Natl. Mus. Canada 221, 1–237.

Miya, M., Takeshima, H., Endo, H., Ishiguro, N.B., Inoue, J.G., Mukai, T., Satoh, T.P., Yamaguchi, M., Kawaguchi, A., Mabuchi, K., Shirai, S., Nishida, M., 2003. Major patterns of higher teleostean phylogenies: a new perspective based on 100 complete mitochondrial DNA sequences. Mol. Phylogenet. Evol. 26, 121–138.

Near, T.J., Dornburg, A., Eytan, R.I., Keck, B.P., Smith, W.L., Kuhn, K.L., Moore, J.A., Price, S.A., Burbrink, F.T., Friedman, M., Wainwright, P.C., 2013. Phylogeny and tempo of diversification in the superradiation of spiny-rayed fishes. Proc. Natl. Acad. Sci. U.S.A. 110, 12738–12743.

Pietsch, T.W., Grobecker, D.B., 1987. Frogfishes of the World: Systematics, Zoogeography, and Behavioral Ecology. Stanford University Press, Stanford, CA.

Rasband W.S., 1997–2015, Image J, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/.

Richards, R.A., Nitschke, P.C., Sosebee, K.A., 2008. Population biology of monkfish *Lophius americanus*. ICES J. Mar. Sci. 65, 1291–1305.

Secor, S.M., Nagy, K.A., 1994. Bioenergetic correlates of foraging mode for the snakes *Crotalus cerastes* and *Masticophis flagellum*. Ecology 75, 1600–1614.

Summers, A.P., Ferry-Graham, L.A., 2002. Respiration in elasmobranchs: new models of aquatic ventilation. In: Bels, V.L., Gasc, J.P., Casinos, A. (Eds.), Vertebrate Biomechanics and Evolution. Bios Scientific Publishers Ltd., Oxford, pp. 87–100.

Valentim, M.F.M., Caramaschi, E.P., Vianna, M., 2008. Feeding ecology of monkfish *Lophius gastrophysus* in the south-western Atlantic Ocean. J. Mar. Biol. Assoc. U.K. 88, 205–212.

Wilson, D.P., 1937. The habits of the angler-fish *Lophius piscatorius* L., in the Plymouth aquarium. J. Mar. Biol. Assoc. U.K. 21, 477–496.

Winterbottom, R., 1973. A descriptive synonymy of the striated muscles of the Teleostei. Proc. Acad. Nat. Sci. Philadelphia 1973, 225–317.

Winterbottom, R., 1974. The familial phylogeny of the Tetraodontiformes (Acanthopterygii: Pisces) as evidenced by their comparative myology. Smithson. Contr. Zool. 155, 1–201.