

Interspecific Variation in Sternohyoideus Muscle Morphology in Clariid Catfishes: Functional Implications for Suction Feeding

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ABSTRACT Depression of the hyoid apparatus plays a crucial role in generating suction, especially in fishes with a dorso-ventrally flattened head shape. It is generally assumed that shortening of the sternohyoideus muscle, which connects the hyoid to the pectoral girdle, contributes to hyoid depression. However, a recent study on the clariid catfish *Clarias gariepinus* has shown that this muscle does not shorten but elongates during this phase through retraction of the pectoral girdle. Here, we test whether this pattern is general among clariid catfish, or if variation in the morphology of the sternohyoideus may result in a different sternohyoideus behavior during hyoid depression. First, sternohyoideus mass, effective cross-sectional area, fiber length and fiber diameter were measured and compared for four clariid species. Next, velocity and magnitude of hyoid depression during prey capture (from high-speed videos), as well as patterns of sternohyoideus strain were analyzed (from high-speed X-ray videos) in these species. While morphology and hyoid depression performance varied considerably among these species, only the species with the most massive sternohyoideus, *Gymnallabes typus*, showed shortening of the sternohyoideus muscle during the initial part of the expansive phase. The data for *Channallabes apus* demonstrate that increasing the magnitude of hyoid depression does not necessarily require a shortening of the m. sternohyoideus, as it shows elongation of this muscle during hyoid depression. *J. Morphol.* 268:232–242, 2007. © 2007 Wiley-Liss, Inc.

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To generate a flow of water into the mouth during suction feeding, aquatic animals rapidly expand the volume of their bucco-pharyngeal cavity. In teleost fishes, this expansive phase of prey capture is characterized by mouth opening, followed by simultaneous abduction (i.e., lateral swing) of the suspensorium and ventral rotation (depression) of the hyoid apparatus (Lauder, 1985). Especially in fishes with a dorso-ventrally flattened head the depression of the hyoid apparatus is assumed to cause a large fraction of the total bucco-pharyngeal volume increase during suction (Alexander, 1970).

Despite the crucial role for hyoid depression in generating suction, relatively little is known about the underlying mechanisms causing the depression of the hyoid apparatus in fishes. The classical view is that the pectoral girdle (cleithrum) rotates caudo-ventrally with respect to the neurocranium by contraction of the hypaxial and by the epaxial muscles causing neurocranial elevation (Fig. 1). The sternohyoideus muscle connects the pectoral girdle to the hyoid, and contracts while being retracted by the pectoral girdle (Fig. 1A). Hence, in this mechanism, the sternohyoideus transmits the force from pectoral girdle rotation and actively increases the magnitude of hyoid depression by shortening (Gosline, 1971; Liem, 1980; Lauder, 1985; Bone and Marshall, 1986; Diogo and Chardon, 2000; Adriaens et al., 2001). As the numerous functional morphological studies on feeding mechanisms in fish have used high-speed video recordings to quantify prey capture kinematics (reviewed by Ferry-Graham and Lauder, 2001) the strain behavior of the sternohyoideus muscle typically cannot be quantified as the pectoral girdle (including the origin of the M-SH) is hidden behind the branchiostegal membranes during most of the expansion phase. To our knowledge, only a single study has actually confirmed the “classical” hyoid-depression mechanism in the Percomorph fish *Micropterus salmoides*, using sonomicrometry (Carroll, 2004).

However, a recent study on the hyoid-depression mechanisms in the African catfish *Clarias gariepi-*

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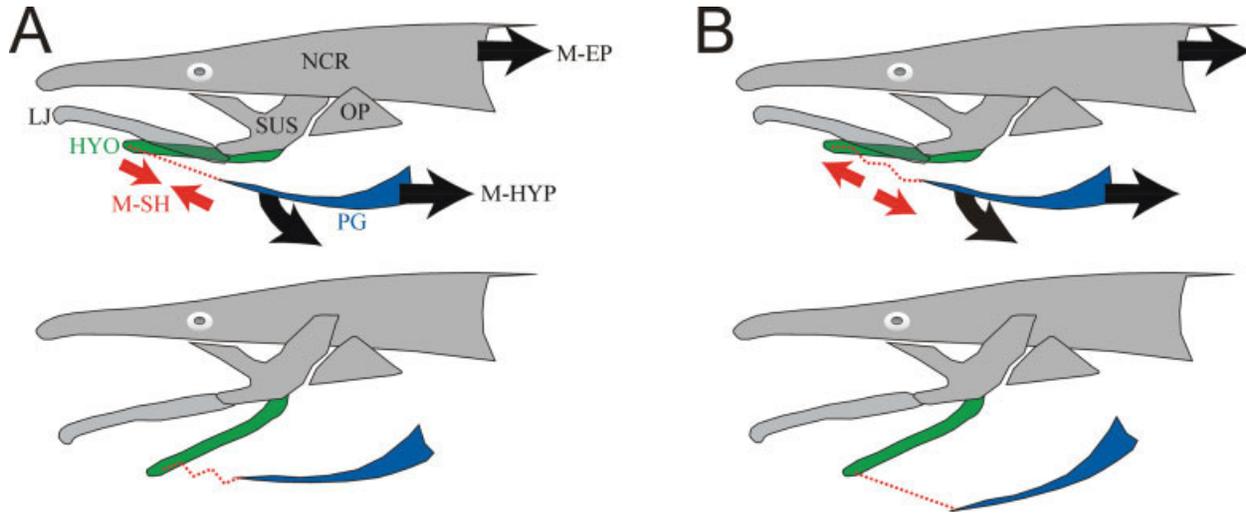


Fig. 1. Schematic illustration of two potential strategies in depressing the hyoid: (A) rotation of the pectoral girdle (blue) as well as shortening of the sternohyoideus muscle (red) contribute simultaneously to the depression of the hyoid (green) and (B) the pattern observed in *Clarias gariepinus* (Van Wassenbergh et al., 2005b): the rotating pectoral girdle retracts and stretches the sternohyoideus that pulls the hyoid. HYO, hyoid; LJ, lower jaw; M-EP, epaxial musculature; M-HYP, hypaxial musculature; M-SH, sternohyoideus muscle; NCR, neurocranium; OP, operculum; PG, pectoral girdle; SUS, suspensorium.

nus did not observe the pattern outlined earlier (Van Wassenbergh et al., 2005b). Although the pectoral girdle does indeed rotate considerably, the sternohyoideus gradually elongates during depression of the hyoid in this species. Consequently, a different strategy has to be used by *C. gariepinus* to depress the hyoid whereby the sternohyoideus transmits the force from pectoral girdle retraction to the hyoid while elongating (Fig. 1B; Van Wassenbergh et al., 2005b). Yet, even if we ignore the passive force that rises when the muscle increases in length, muscle can produce much higher active force in the excentric part of the force–velocity relationship (Hill, 1938). If the sternohyoideus is the least powerful muscular part of the linkage system that causes hyoid depression (also including the epaxial and hypaxial muscles; Fig. 1), the sternohyoideus will therefore inevitably be forced to elongate. This also implies that only relatively large and forceful sternohyoideus muscles will be able to produce isometric forces exceeding the forces exerted on its origin (forces from pectoral girdle retraction) and insertion (forces of resistance to hyoid depression), and therefore be potentially capable of shortening during this phase.

Because relatively little is known about the behavior of the sternohyoideus muscle during hyoid depression in fishes, the generality of each of the two alternative strategies (Fig. 1) is unknown in this large and diverse group of animals. The morphology of the sternohyoideus may determine whether this muscle is able to contribute in powering the buccal expansion by shortening (Fig. 1A) or whether the sternohyoideus only functions as a force transmitter and uses its passive and/or active

excentric force when it is pulled backward by the pectoral girdle.

A considerable difference in the morphology of the sternohyoideus between two species of clariid catfishes has been reported (Van Wassenbergh et al., 2004). Remarkably, the species with the largest physiological cross-sectional area of the M-SH (*Clariallabes longicauda*) was also able to generate the largest amount of hyoid depression compared with closely related species (*Clarias gariepinus*). Possibly, the enlarged sternohyoideus muscle in *C. longicauda* is able to reinforce the action of the pectoral girdle on the hyoid by active shortening of its M-SH (Fig. 1A), while the more slender sternohyoideus of *C. gariepinus* does not have enough power even to maintain its initial length when it is pulled backward by the large hypaxial muscle masses causing pectoral girdle rotation and the epaxial muscles causing neurocranial elevation (Fig. 1B). Sternohyoideus shortening instead of elongating during hyoid depression will increase the speed and magnitude of hyoid depression, which would probably enhance suction feeding performance considerably.

To test whether variation in the morphology of the sternohyoideus muscle is associated with different mechanisms of hyoid depression (Fig. 1), or if the pattern observed for *Clarias gariepinus* (Van Wassenbergh et al., 2005b) is general within Clariidae, morphology and function of this muscle during suction feeding is studied in this group of fishes. To do so, we first examine the morphology of the sternohyoideus muscle in four species of Clariidae (Fig. 2) by measuring total mass, physiological cross-sectional area, fiber length and fiber

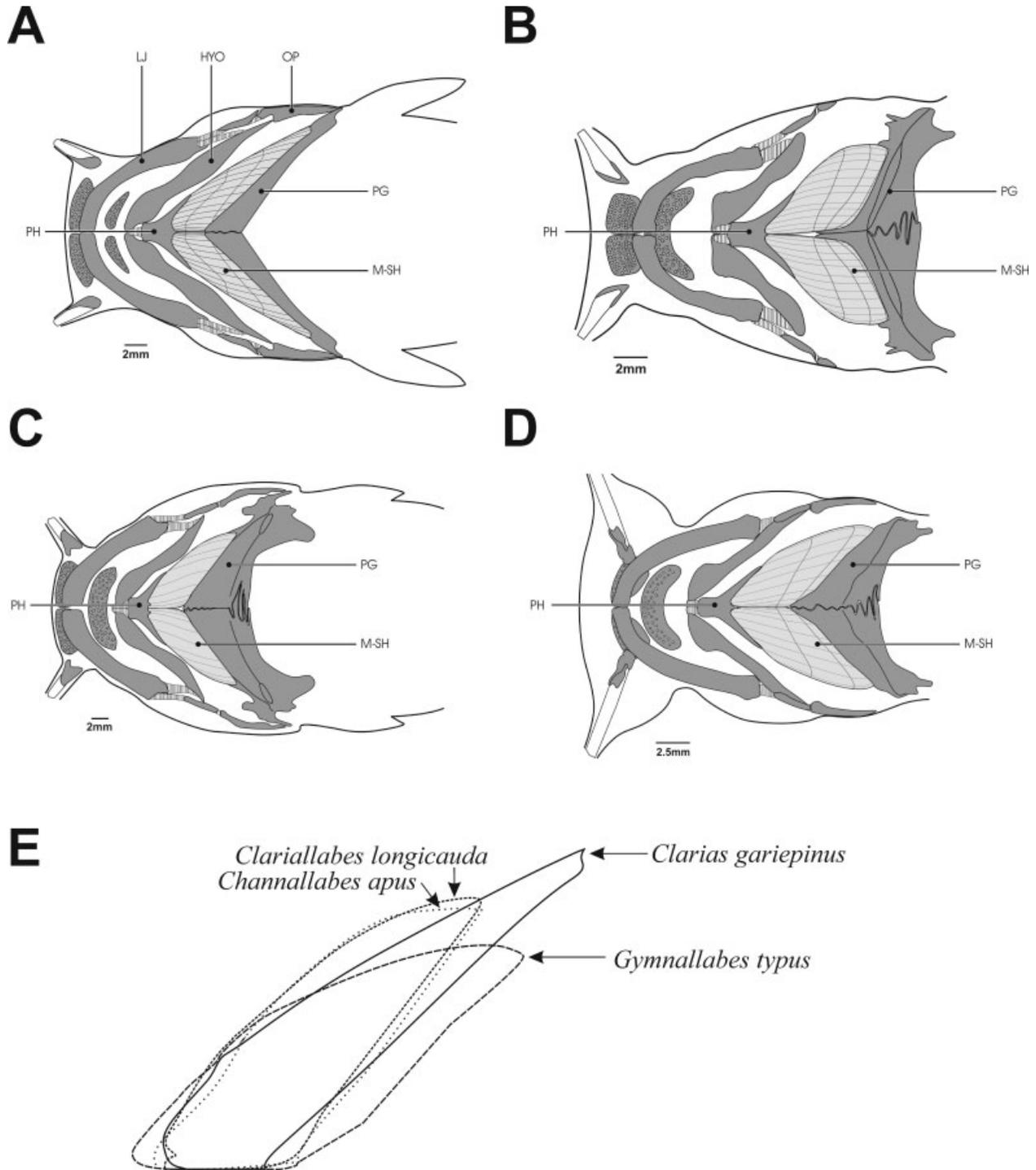


Fig. 2. Ventral view morphology of the head, illustrating the position and shape of the sternohyoideus muscle in four Clariidae: (A) *Clarias gariepinus*, (B) *Channallabes apus*, (C) *Clariallabes longicauda*, and (D) *Gymnallabes typus*. In (E) the left side sternohyoideus of the four species are superimposed, after scaling to the same mediosagittal length between the rostral part of the parurohyal bone and the caudal margin of the pectoral girdle. This drawing illustrates the relatively elongated sternohyoideus shape in *C. gariepinus*, the similarity between the intermediate shapes of *C. apus* and *C. longicauda*, and the relatively broad muscle of *G. typus*. HYO, hyoid; LJ, lower jaw; M-SH, musculus sternohyoideus; OP, operculum; PG, pectoral girdle; PH, os parurohyale.

diameter. Next, we test whether a relationship exists between the morphology and the magnitude and velocity of hyoid depression, as well as the pattern of shortening of the sternohyoideus muscle during prey capture for these species.

MATERIALS AND METHODS

Study Animals

Four species of Clariidae showing morphological differences in the cranial musculature (Cabuy et al., 1999) were included in the analyzes: *Clarias gariepinus*, *Channallabes apus*, *Clariallabes longicauda*, and *Gymnallabes typus*. The *C. gariepinus* specimens were either aquarium-raised specimens of which larval stages were initially obtained from the Laboratory for Ecology and Aquaculture (Catholic University of Leuven, Belgium) or specimens obtained from aquacultural facilities (Fleuren & Nooijen BV, Someren, The Netherlands). Specimens of *C. longicauda* and *C. apus* were caught in Northern Gabon. *Gymnallabes typus* was commercially obtained, originating from Western tropical Africa. The number of individuals used in each of the analyses of the present study is given in Table 1. For each individual, cranial length (i.e., the distance between the rostral tip of the premaxilla and the caudal tip of the occipital process) was measured, using digital calipers and used to quantify fish size. The fishes used for video recordings (see further) were kept in separate, Plexiglas aquaria and were trained to capture food in a narrow, projecting corridor of the aquarium. The thin walls (2 mm) of the corridor minimized the amount of X-ray absorption during cineradiography.

Morphological Data

To measure the properties of the sternohyoideus muscles (Fig. 2), specimens preserved in 70% alcohol were dissected. The inclination of the muscle fibers with respect to the medio-sagittal axis of the head (i.e., the line of action of the sternohyoideus) was determined. The muscles of both sides were removed and weighed, using electronic scales (± 0.0001 g; Mettler MT5, Switzerland). Muscle volume was obtained by dividing the mass by $1,050 \text{ kg m}^{-3}$ (Ward and Lieber, 2005). Next, the muscle bundles were immersed in an HNO_3 30% solution for 24 h to dissolve the connective tissue. Fibers were gently teased apart, using blunt-tipped glass needles, then transferred to and stored in a 50% glycerol solution. The average muscle fiber length was approximated by taking the average of lengths of the most medially positioned fiber, a central muscle fiber and the most laterally situated fiber. The effective physiological cross-sectional area was obtained by multiplying muscle volume by the cosine of the mean fiber inclination and dividing this by the mean fiber length.

Inter- and intra-specific variation of the sternohyoideus muscle fiber diameter was evaluated for all the species (*Clarias gariepinus*, *Channallabes apus*, *Clariallabes longicauda*, and *Gymnallabes typus*), using serial histological sections (5 μm , Technovit 7100

embedding). Five sections were obtained, using a Leica Polycut microtome. To test whether fiber diameter is subjected to ontogenetic scaling, two specimens of *C. gariepinus* were analyzed (cranial lengths of 7.6 and 17.0 mm). For each specimen, a section in the middle of the sternohyoideus was selected on which the diameters were measured, using digital images obtained through an Olympus 52×9 stereomicroscope, equipped with Colorview 8 CCD-camera. A total of 400 fibers were measured in the left sternohyoideus (only 273 fibers could be measured in the smallest *C. gariepinus* specimen). As fibers were cut slightly obliquely, always the minimal fiber diameter was used.

High-Speed Video

High-speed video recordings ($250 \text{ frames s}^{-1}$) of prey capturing catfish were made from a lateral view, using a digital Redlake Imaging Motionscope (640×480 pixels) or MotionPro ($1,280 \times 1,024$ pixels) camera. Two *Clarias gariepinus* individuals of larger than 94-mm cranial length were recorded at $100 \text{ frames s}^{-1}$ with a JVC GR-DVL9800 camera, while we used a Panasonic F15 (50 frames s^{-1}) for the 210-mm cranial length individual. As the duration of a prey capture event is considerable longer in these large individuals, the lower recording speeds do not result in an increased experimental error when determining velocity and maximal displacement of the hyoid (see further). Two floodlights (600 W) provided the necessary illumination. Only those prey capture sequences that were approximately perpendicular to the camera lens were selected and retained for further analysis. Two prey types were used: 1) pieces of cod fillet (*Gadus morhua*) and 2) North Sea shrimps (*Pandalus borealis*). Both prey types were attached to a thin, plastic coated steel wire. Ten prey capture sequences were analyzed for each individual, generally including five fish and five shrimp captures. For 17 out of the 23 individuals of the species *C. gariepinus*, only the first prey type (fish) was used (Van Wassenbergh et al., 2005a). However, as the magnitude and speed of hyoid depression do not differ between the two prey types for this species, this does not influence the results (Van Wassenbergh et al., 2006).

The XY-position of the eye and the tip of the hyoid (Fig. 3) were digitized frame-by-frame using Didge (version 2.2.0, Alstair Cullum, Creighton University, Omaha, NE). The distance between these two points was plotted against time. The difference between the minimal and maximal value was taken as a measure of the total hyoid depression for a given prey capture sequence. After data filtering (4th order Butterworth zero phase-shift low-pass filter) and differentiation versus time, linear velocities were calculated. As we are mainly interested in maximal performance, the maximal value per individual (i.e., the largest excursion of the hyoid tip, highest peak velocity) was used in the comparative analyzes.

High-Speed Cineradiography

First, small metal markers were inserted subcutaneously at specific positions of interest, using hypodermic needles. Prior to

TABLE 1. Number of individuals analyzed

	Sternohyoideus dissection	Serial section	High-speed video	High-speed X-ray video
<i>Clarias gariepinus</i>	23 ^{a,b}	2	17 ^c	3 ^d
<i>Channallabes apus</i>	6	1	4	2
<i>Clariallabes longicauda</i>	5 ^b	1	4 ^b	0
<i>Gymnallabes typus</i>	4	1	3	1

Data from

^aHerrel et al., 2005.

^bVan Wassenbergh et al., 2004 (3 individuals).

^cVan Wassenbergh et al., 2005a.

^dVan Wassenbergh et al., 2005b.

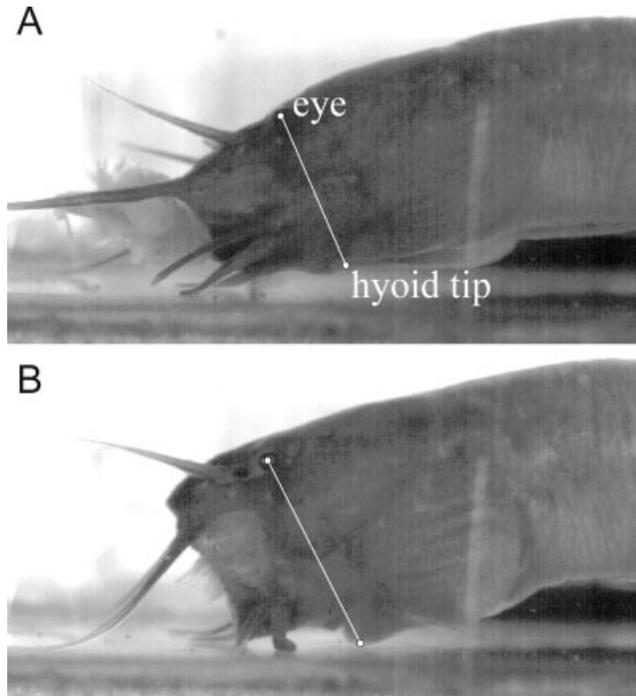


Fig. 3. High-speed video frames (*Channallabes apus* feeding on a piece of fish) showing the two anatomical landmarks used to determine the magnitude of hyoid depression (distance between both landmarks) at the start of the sequence (A) and at the time of maximal hyoid depression (B). The corresponding video is given in the Supplementary Materials section.

implantation of these radio-opaque markers, the animals were anesthetized with MS222 (Sigma Chemical Company, St. Louis, MO). Next, X-ray videos and photographs were recorded, using a Philips Optimus X-ray generator coupled to a 14-inch image intensifier and a Redlake Motion Pro camera (1,280 × 1,024 pixels). Videos were made in lateral view and were recorded at 250 frames s⁻¹, using the 6-inch zoom function. Three prey types were used: 1) pieces of cod fillet and 2) unpeeled North Sea shrimps attached to a plastic-coated steel wire, and 3) small, spherical pieces of shrimp meat, which were loosely attached to the tip of a needle. For *Clarias gariepinus* 20 recordings were analyzed per individual (cranial lengths of 70.2, 74.5, and 94.1 mm; 5, 5, and 10 sequences, respectively for each prey type). For both *Channallabes apus* individuals (cranial lengths of 24.5 and 26.0 mm) and the *Gymnallabes typus* individual (cranial length of 22.0 mm) only the third prey type was used, with respectively 13, 14, and 6 sequences analyzed. No high-speed X-ray videos could be recorded for *Clariallabes longicauda*, as no living specimens could be obtained of this species before the start of the X-ray video recordings.

In this way, it was possible to track the movements of two markers: one at the rostral tip (i.e., near the symphysis) of the hyoid and one at the tip of the cleithrum (pectoral girdle). The distance between these two points is spanned by the sternohyoideus muscle, and may also include part of the short parurohyale bone and parurohyalo-hypophyale ligament (Fig. 2). This variable is used to determine whether or not the sternohyoideus muscle is shortening. Note, however, that the amount of bone or ligament included in this hyoid-to-cleithrum distance may vary slightly between individuals. Consequently, relative muscle shortening velocities could only be obtained by approximation. An example of an X-ray video sequence of prey capture in *Clarias gariepinus* accompanies this article on the journal's website. In this video, the markers at the tip of the cleithrum and the tip of the hyoid are highlighted by white and black dots, respectively.

These hyoid-to-cleithrum distances will be presented as a percentage of the average hyoid-to-cleithrum distance observed for each individual. To account for strike-to-strike variability and to avoid the potential confounding effects on kinematic means, the time axis of each prey capture sequence was scaled to the total duration of hyoid depression of that sequence. Consequently, time will be expressed as a percentage of hyoid depression duration, with 0% being the start of hyoid depression and 100% the time of maximal hyoid depression. Linear interpolations are used to extract data at 2% intervals on this new, relative time scale. In this way, also the effects of differences in body size on the speed of prey capture kinematics (Van Wassenbergh et al., 2005a) are removed, which thus simplifies the comparison between individuals.

Statistics

Kolmogorov–Smirnov tests were used to test the normality of the distributions and, if necessary, the data were log₁₀ transformed. As probably the most important source of variation between individuals is body size, one-way covariance analyses (ANCOVA) were performed to test whether species (independent variable) differ, using cranial length as a covariate. Only if an overall significant difference between species ($P < 0.05$) is indicated by the ANCOVA, further post hoc tests were performed to statistically compare the means for the species used in this study. Given the variation in the number of individuals per species included in the analyses (Table 1), Spjotvoll–Stoline tests (Tukey honest significant difference tests for unequal N) were used for this purpose. To eliminate scaling effects in these post hoc tests, the data were adjusted according to the scaling relationship found for *Clarias gariepinus*, the species for which data are available for a broad range of individuals of different sizes (Herrel et al., 2005; Van Wassenbergh et al., 2005a). To do so, each value was divided by the power of cranial length by which this specific variable scales in *C. gariepinus*. This approach is analogous to an analysis of residuals, but has the advantage of using a real scaling relationship, rather than the artificial scaling relationship obtained by pooling all species to calculate the residuals. In both analyses (ANCOVA and post-hoc tests) it was thus assumed that the scaling relationships measured for *C. gariepinus* (Herrel et al., 2005; Van Wassenbergh et al., 2005a) also apply to the other species. Although unlikely to occur because of the close phylogenetic relatedness of the species, it should be mentioned that the validity of the results would be restricted to individuals of *Clariallabes longicauda*, *Gymnallabes typus*, and *Channallabes apus* within the size-range of the individuals used for each species in the experiments in case of interspecific differences in scaling relationships.

RESULTS

Cross-Sectional Area

The effective physiological cross-sectional area of the sternohyoideus muscle differed significantly between species (ANCOVA, $P = 0.042$). On average, *Clarias gariepinus* had the most slender sternohyoideus muscle, closely followed by *Channallabes apus* (Fig. 4A; Table 2). The largest values were measured for *Gymnallabes typus*, which was the only species for which a significantly larger cross-sectional area compared with *C. gariepinus* could be demonstrated (Spjotvoll–Stoline test, $P = 0.002$). The average difference between these two species was considerable, with *G. typus* more than doubling the effective cross-sectional area of the sternohyoideus of *C. gariepinus* for animals of the same head size (Table 2). *Gymnallabes typus* also

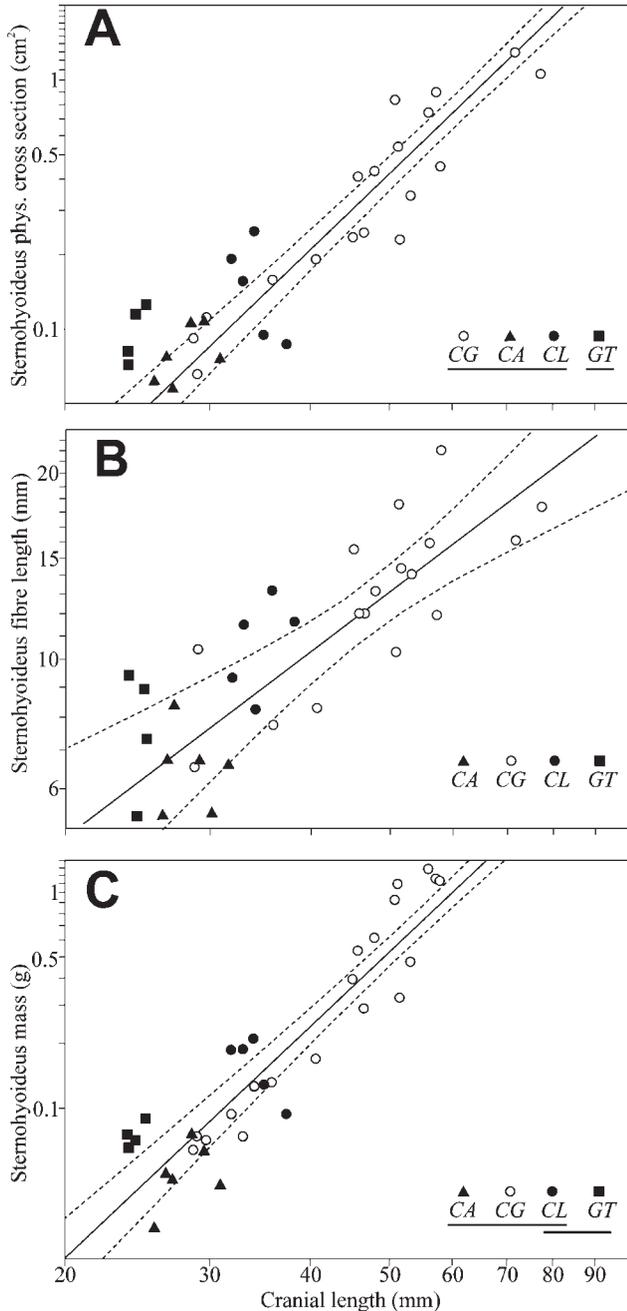


Fig. 4. Logarithmic plot of the effective physiological cross-sectional area (A), the fiber length (B) and the mass (C) of the sternohyoideus muscle in *Clarias gariepinus* (CG), *Channallabes apus* (CA), *Clariallabes longicauda* (CL), and *Gymnallabes typus* (GT). Symbol legends and post hoc test results (Spjotvoll–Stoline) are given at the lower right part of the graph. No significant differences in fiber length between species were found. The regression line (with 95% confidence limits) shows the scaling relationship for *Clarias gariepinus*. All regressions are highly significant ($P < 0.0001$) and slopes are 3.15 (A: $R^2 = 0.96$), 1.08 (B: $R^2 = 0.65$), and 3.56 (C: $R^2 = 0.94$).

differed significantly from *Channallabes apus* and *Clariallabes longicauda* (Spjotvoll–Stoline test, respectively $P = 0.008$ and $P = 0.012$). The effective

physiological cross-sectional area of the sternohyoideus in the latter two species was similar compared with *C. gariepinus* (Spjotvoll–Stoline test, respectively $P = 0.93$ and $P = 0.88$).

Fiber Length

Although the average length of the sternohyoideus fibers tended to increase with increasing average cross-sectional area of this muscle (Table 2), the species did not differ significantly in fiber length (ANCOVA, $P = 0.075$; Fig. 4B).

Sternohyoideus Mass

Like the cross-sectional area of the sternohyoideus, the total mass of the muscle varied significantly in the Clariidae (ANCOVA, $P = 0.0003$). After correcting for size, the largest masses were observed for *Gymnallabes typus*, followed by *Clariallabes longicauda*, *Clarias gariepinus*, and *Channallabes apus* (Fig. 4C; Table 2). *Gymnallabes typus* was the only species for which the sternohyoideus masses were significantly larger compared with *C. gariepinus* (Spjotvoll–Stoline test, $P = 0.018$; Fig. 4C). The scaled average sternohyoideus mass for *G. typus* was almost double that of *C. gariepinus* (Table 2).

Fiber Diameter

The average fiber diameter of the sternohyoideus muscle differed significantly between species (ANOVA, $P < 0.001$; Table 2). Similar to the total muscle's cross-sectional area, *Clarias gariepinus* had the most slender muscle fibers, while fibers were on average twice as thick in *Gymnallabes typus* (Fig. 6, Table 2). The values for *C. gariepinus* were significantly lower than those for all other species (Spjotvoll–Stoline test, all $P < 0.001$). *Channallabes apus* and *Clariallabes longicauda* were intermediate between *C. gariepinus* and *G. typus* and differed significantly from these species (Spjotvoll–Stoline test, all $P < 0.001$). The maximal fiber diameters followed the same trend as the averages (Fig. 5). The results for two *C. gariepinus* individuals of different head size (7.6 and 17.0 mm) show that M-SH fiber diameters in this species were independent of head size for the range of sizes used in this study (Fig. 5).

Maximal Hyoid Depression

The maximal amount of depression of the tip of the hyoid apparatus during prey capture (see Fig. 3) differed significantly between species (ANCOVA, $P < 0.001$). Apparently, also for this hyoid depression magnitude, *Clarias gariepinus* showed the least depression of the four species (Fig. 6A; Table 2). For a given head size, *Channallabes apus* and

TABLE 2. Scaled characteristics of the sternohyoideus and the hyoid depression in Clariidae (mean \pm standard error)

	Sternohyoideus PCSA, cm ² (10 ³ CL ^{-3.15})	Sternohyoideus FL, mm (10 ² CL ^{-1.08})	Sternohyoideus mass, g (10 ⁷ CL ^{-3.56})	Sternohyoideus fiber diameter, (μ m)	Max. hyoid depression, mm (CL ^{-0.87})	Peak speed hyoid depression, mm s ⁻¹ (CL ^{0.09})
<i>Clarias gariepinus</i>	5.5 \pm 0.4	3.4 \pm 0.2	5.0 \pm 0.4	15.4 \pm 0.3	130 \pm 8	134 \pm 6
<i>Channallabes apus</i>	6.3 \pm 0.8	3.1 \pm 0.2	3.6 \pm 0.4	25.7 \pm 1.3	190 \pm 10	170 \pm 25
<i>Clariallabes longicauda</i>	6.6 \pm 1.6	4.1 \pm 0.3	5.8 \pm 1.1	27.1 \pm 1.4	185 \pm 9	259 \pm 19
<i>Gymnallabes typus</i>	12.0 \pm 1.9	4.5 \pm 0.7	8.7 \pm 0.4	31.1 \pm 1.6	208 \pm 14	148 \pm 18

CL, cranial length (in m); PCSA, effective physiological cross-sectional area; FL, fiber length.

Gymnallabes typus performed significantly larger hyoid depressions while suction feeding when compared with *C. gariepinus* (Spjøtvoll–Stoline test, respectively $P = 0.045$ and $P = 0.020$). On average, *C. apus* was able to lower the hyoid tip 46% more than an equally-sized *C. gariepinus* individual (Table 2). For *G. typus*, this difference was even larger, with an average increase of 59% in total hyoid depression compared with *C. gariepinus*. Also the amount of hyoid depression measured for *Clariallabes longicauda* was more similar to *C. apus* and *G. typus* than it was to *C. gariepinus* (Spjøtvoll–Stoline tests, respectively $P = 0.99$, $P = 0.79$, and $P = 0.069$).

Speed of Hyoid Depression

The maximal speed of depression of the tip of the hyoid apparatus during suction feeding (see Fig. 3) also differed significantly between species (ANCOVA, $P < 0.001$; Fig. 6B; Table 2). However, only one species, *Clariallabes longicauda*, reached hyoid depression speeds that were significantly higher than those of *Clarias gariepinus* (Spjøtvoll–Stoline test, $P < 0.001$), with on average almost twice the peak hyoid depression velocity of the latter species. On the other hand, the speed of hyoid depression for *Channallabes apus* and *Gymnallabes typus* could not be discerned statistically from *C. gariepinus* (Spjøtvoll–Stoline test, respectively $P = 0.22$ and $P = 0.92$).

Patterns of Sternohyoideus Strain

Changes in the length of the sternohyoideus muscle during prey capture were measured (Fig. 7) for *Channallabes apus* and *Gymnallabes typus* and could thus be compared with the pattern observed for *Clarias gariepinus* (Van Wassenbergh et al., 2005b). The strain pattern of this muscle during prey capture in *C. apus* was roughly similar to the pattern in *C. gariepinus* (Figs. 7A,B). During the first half of the hyoid depression phase, the sternohyoideus approximately maintained a constant length (on average during the first 36 and 44% of hyoid depression for, respectively, *C. gariepinus* and *C. apus*) after which the muscle started elongating. This M-SH elongation then continued during the entire second

half of hyoid depression. In contrast to *C. apus*, a limited amount of elongation of the sternohyoideus muscle could still be observed after the maximal hyoid depression was reached in *C. gariepinus* (Fig. 7A,B).

For *Gymnallabes typus*, however, a different pattern of sternohyoideus length changes during prey capture was observed (Fig. 7C). In contrast to the other species (Fig. 7A,B), the sternohyoideus shortened considerably in *G. typus* (~17% of the muscle's mean length) during the first half of the hyoid depression phase. Yet, muscle shortening did not continue until the end of the hyoid depression. Similarly to the other Clariids, the second half of the hyoid depression phase was characterized by elongation of the sternohyoideus. However, at the instant of maximal hyoid depression, the length of the sternohyoideus was still shorter than at the start of the hyoid depression in *G. typus* (Fig. 7C).

DISCUSSION

As suggested by previous work (Van Wassenbergh et al., 2004), interspecific variation in the morphology of the sternohyoideus muscle exists within the catfish family Clariidae. More specifically, significant differences in the mass, cross-sectional area and fiber diameter of the sternohyoideus are observed (Figs. 4A,C and 5) while sternohyoideus fiber length is similar for all species studied (Fig. 4B). The sternohyoideus of the species with the larger cross-sectional area of this muscle also contains fibers with larger diameters (Table 2). As the average fiber diameter is generally associated with the percentage of fast glycolytic (Type IIb) fibers, a relatively large-fibered muscle is probably better in performing rapid muscle contractions compared with a smaller-fibered muscle of the same dimensions. Consequently, the combination of an increased effective cross-sectional area and larger fibers (Fig. 5), as for example observed in *Gymnallabes typus* with respect to *Clarias gariepinus* (Figs. 4A and 5), may therefore result in a hyoid depression system, in which the function of the sternohyoideus is no longer limited to force transmission (as in *C. gariepinus*; Fig. 1B), but also includes powering of the hyoid expansion by shortening while the muscle is retracted by the pectoral girdle (Fig. 1A).

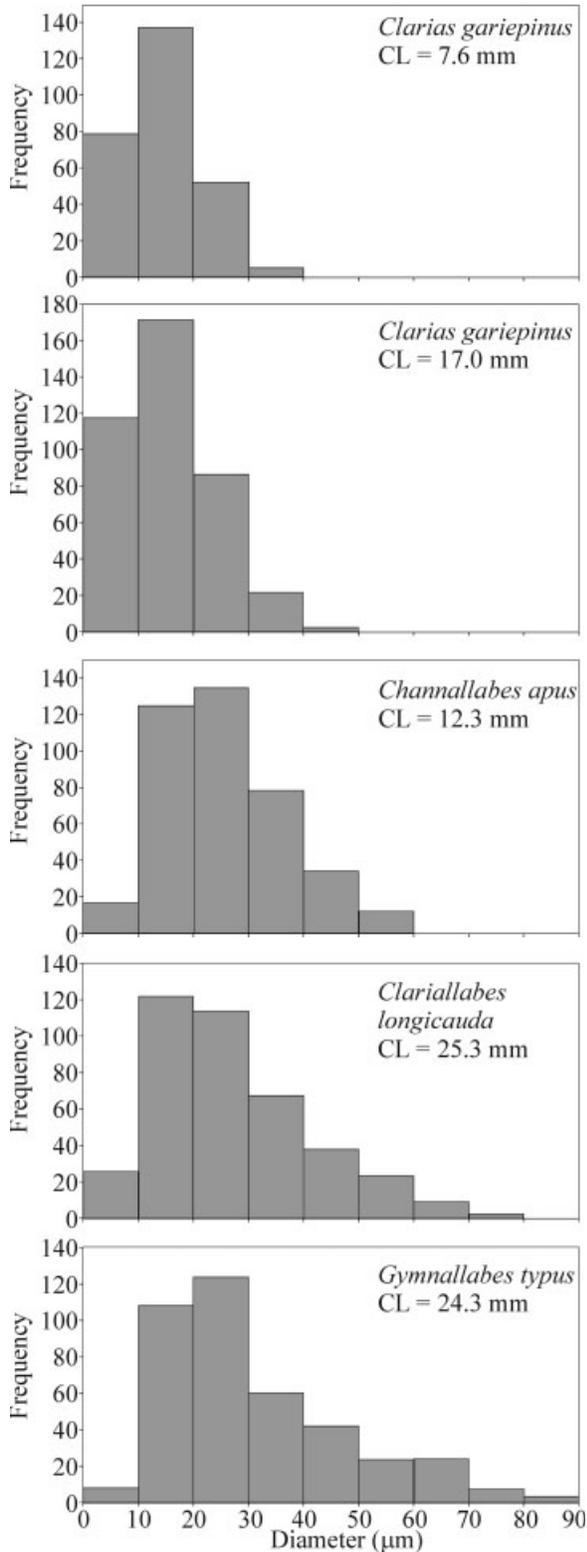


Fig. 5. Histograms of sternohyoideus fiber diameter in the species studied. The upper two graphs indicate that no scaling effects existed for *Clarias gariepinus* within the range of head sizes presented here. $N = 400$ except for the uppermost graph ($N = 273$). The average fiber diameter differed significantly among species. Further information is given in the text and in Table 2.

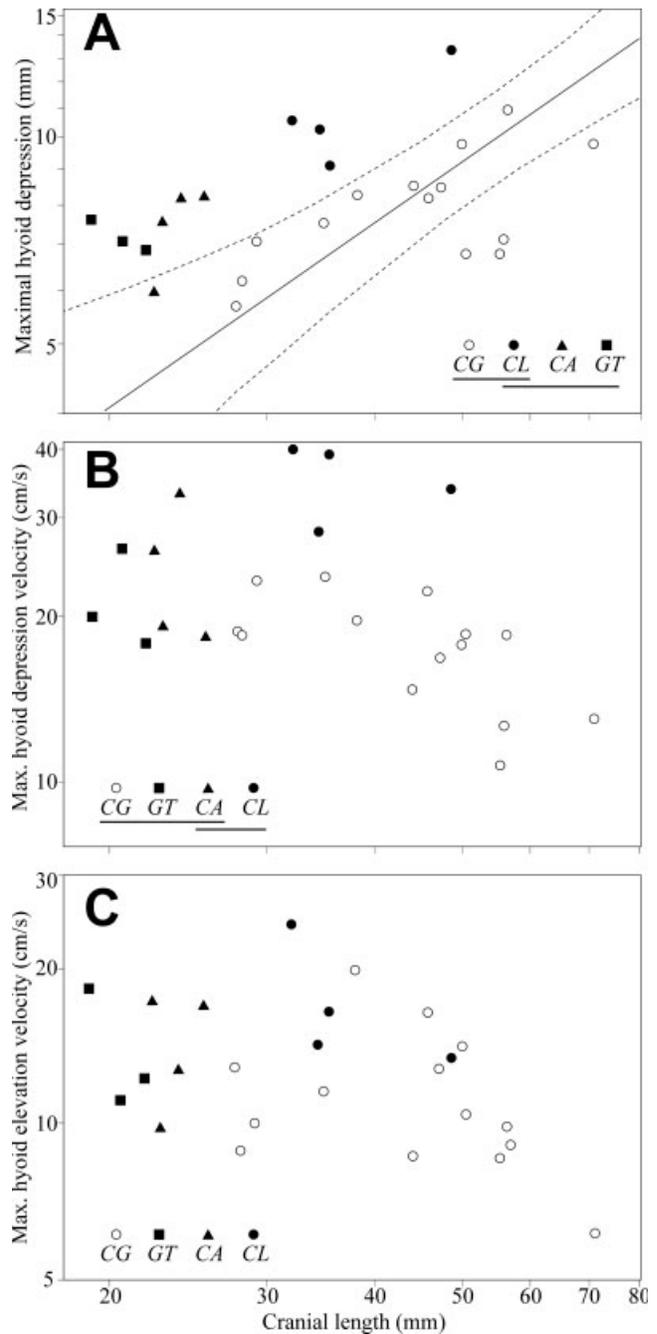


Fig. 6. Logarithmic plot of the maximal hyoid depression (A), the maximal peak velocity of hyoid depression (B) and the maximal peak velocity of hyoid elevation (C) during prey capture in *Clarias gariepinus* (CG), *Channallabes apus* (CA), *Clariallabes longicauda* (CL), and *Gymnallabes typus* (GT). Symbol legends and post hoc test results (Sjotvoll–Stoline) are given at the lower part of the graph. Note that no significant differences in hyoid elevation velocity between the species were found (C). The regression line (with 95% confidence limits) shows the scaling relationship for *Clarias gariepinus*. Note that only maximal hyoid depression (A) increased significantly with size in this species (slope = 0.87; $R^2 = 0.78$; $P < 0.0001$).

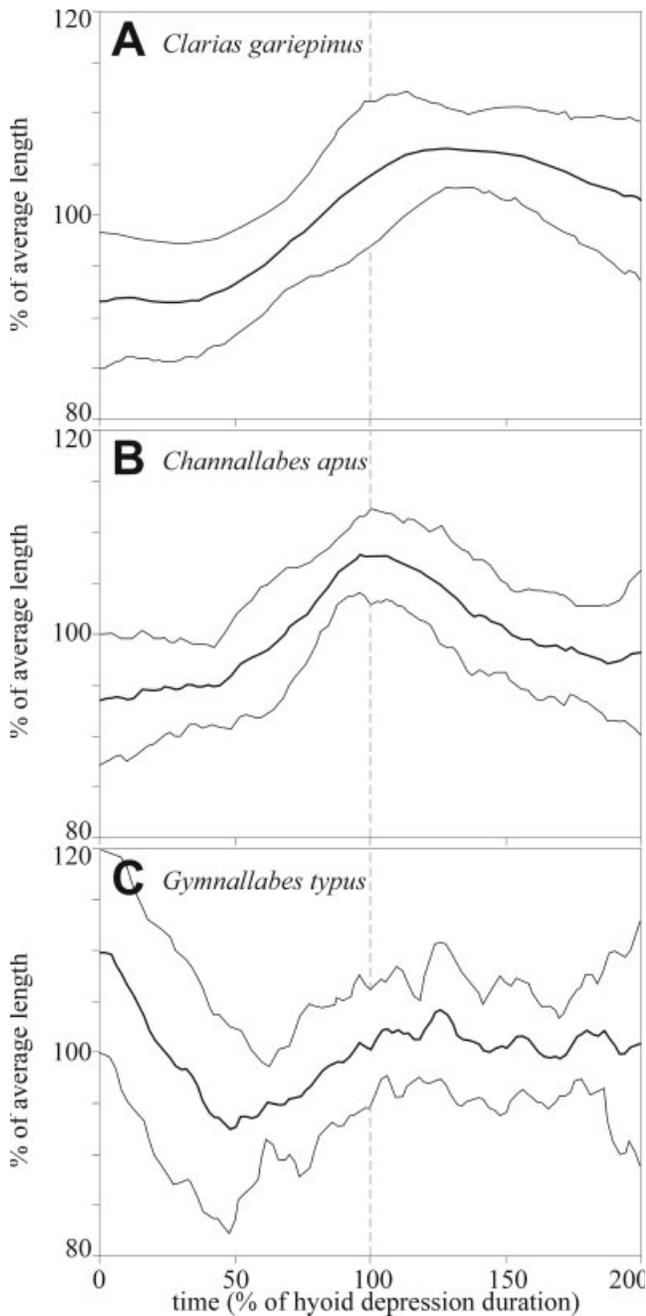


Fig. 7. Mean profiles of sternohyoideus length changes for the period of hyoid depression (0–100% time) and elevation (100–200% time) during prey capture in *Clarias gariepinus* (A), *Channallabes apus* (B) and *Gymnallabes typus* (C). The lines accompanying above and below each curve represent standard errors. See text for further information.

As the most important function of the sternohyoideus muscle during suction feeding in Clariidae is causing a ventral depression the hyoid (Fig. 1), the potential increase in power and speed of the more voluminous and thicker fibered sternohyoideus could result in an increased hyoid-depression performance. Compared with *Clarias*

gariepinus, such significantly increased muscle and fiber cross-sectional diameter of the sternohyoideus can be found in one species: *Gymnallabes typus* (Fig. 6; Table 2). As expected, this species also showed larger hyoid depressions compared with *C. gariepinus*. However, such increased total hyoid depression was also observed for *Channallabes apus*, a species with similar mass, fiber length and cross-sectional area of the sternohyoideus compared with *C. gariepinus* (Table 2). Furthermore, only *Clariallabes longicauda*, and not the species with the most massive sternohyoideus muscle (*G. typus*) has gained significantly in peak velocity of hyoid depression (Fig. 6B). Therefore, no simple relationship exists between the morphology of the sternohyoideus and hyoid depression performance in Clariidae.

Observing a larger or faster lowering of the bottom of the mouth cavity is indicative of a more powerful system causing hyoid depression. Indeed, any increase in the volume of the mouth cavity during suction feeding by means of hyoid depression requires an input of force exerted on the hyoid. The sternohyoideus has an essential role in the hyoid-depression system as all force has to be transmitted to the hyoid through this muscle (Fig. 1). Yet, not only the sternohyoideus but also two other muscles contribute in producing this force: the hypaxial and the epaxial muscles. In fact, the sternohyoideus is only a single part of the entire system causing hyoid depression: it couples the retraction of the pectoral girdle to the hyoid (Fig. 1). Consequently, changes in the magnitude or speed of hyoid depression do not necessarily imply a shift in function of the sternohyoideus: the observed hyoid depression also depends on how the origin of the sternohyoideus is retracted by the pectoral girdle and on the morphology of the hyoid itself.

The strength of the sternohyoideus after activation will determine its behavior when it is being retracted by the pectoral girdle. If the retraction of the muscle's origin is too powerful, the muscle may not be able of shortening during hyoid depression. This situation seems to occur in *Clarias gariepinus*, the species with the slenderest sternohyoideus in our study (Fig. 7A; Van Wassenbergh et al., 2005b) and also for *Channallabes apus*, a species with a sternohyoideus of similar mass and effective cross-sectional area, but with larger fiber diameters compared with *C. gariepinus* (Fig. 7B). Consequently, the increased magnitude of hyoid depression observed in *C. apus* (Fig. 6) with respect to *C. gariepinus* is apparently not caused by a shift in the strain pattern of the sternohyoideus.

The data for *Channallabes apus* therefore exemplify that an increased retraction and depression of the anterior tip of the pectoral girdle can cause an increased hyoid depression without altering the strain behavior of the sternohyoideus. If we compare the displacement patterns of the hyoid and

the pectoral girdle between different feeding sequences of a single individual, variation in the magnitude of hyoid depression is typically associated with similar variation in the amount of pectoral girdle rotation (Fig. 8). Unfortunately, despite its apparent crucial role in generating suction in fish, very few studies exist on the functional morphology or mechanics of the pectoral girdle (e.g., Oliveira et al., 2001).

Gymnallabes typus also shows considerably greater hyoid depressions when compared with *Clarias gariepinus*, but unlike *Channallabes apus*, this species has a sternohyoideus with almost twice the mass and more than twice the effective cross-sectional area and average fiber diameter of *C. gariepinus* (Table 2). The distinct sternohyoideus morphology of *G. typus* appears to be reflected in a different pattern of shortening during hyoid depression, with a period of shortening during the initial stages of hyoid depression (Fig. 7C). Yet, even this species does not show the “classical” mechanism of hyoid depression, in which shortening of the sternohyoideus is assumed to continue throughout the entire hyoid depression phase (Fig. 1A): after a short period of shortening, the sternohyoideus of *G. typus* starts elongating as was observed in *C. gariepinus* and *C. apus*.

In *Clarias gariepinus* and *Channallabes apus*, the sternohyoideus is nearly maximally elongated at the moment of maximal hyoid depression (Fig. 7A,C). This means that, compared with the situation in which the sternohyoideus would shorten during hyoid depression, the cleithrum (pectoral girdle) has to rotate over a larger angle to cause an equal amount of hyoid depression (see Fig. 1B compared with Fig. 1A). This could have significant consequences for the recovery phase, i.e., when the hyoid and cleithrum are elevated and the buccal cavity is compressed: it may require a longer time to reposition the cleithrum unless shortening of the sternohyoideus during the recovery phase may actually help in protracting the cleithrum. This may be important, as our observations have shown that in case of an unsuccessful suction-feeding attempt, the catfish were able to perform a subsequent prey-capture attempt shortly after. Being able to quickly reposition the cleithrum is probably an important aspect of the capability to perform consecutive prey captures. In this respect, it would be interesting to investigate how the cleithrum recovery time differs between *C. gariepinus* or *C. apus* and *Gymnallabes typus*, the single species that does not have a fully elongated sternohyoideus at the start of the recovery phase. Although we did not include unsuccessful prey captures in our analysis (which would particularly motivate fish to recover quickly), both *C. gariepinus* and *C. apus* showed M-SH shortening during the recovery phase (Fig. 7A,B), while *G. typus* did not (Fig. 7C). Given that hyoid elevation velocity (and thus the speed in which the rostral end of the

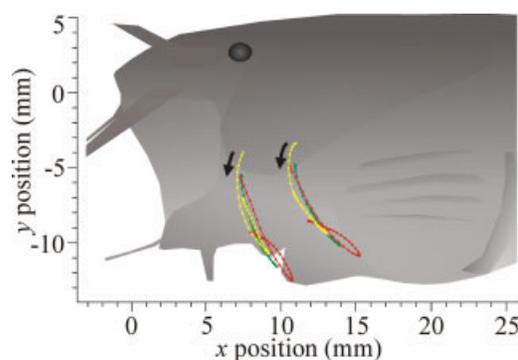


Fig. 8. Displacement profiles of the hyoid tip (anterior points) and cleithrum tip (posterior points) relative to roof of the buccal cavity during three feeding sequences of *Channallabes apus* with different hyoid depression magnitudes (yellow < green < red) imposed on a schematic illustration of the head of *C. apus* at the moment of maximal hyoid expansion. This figure illustrates the tightly coupled kinematics of both elements and thereby demonstrates that the amount of hyoid depression was modulated predominantly through the amount of rotation of the pectoral girdle with respect to the neurocranium. A similar pattern was also observed in the other clariid species.

sternohyoideus is protracted during this phase) does not differ significantly between the species studied (Fig. 6C), this indeed suggests that the sternohyoideus contributes (through active contractile force and/or passive elasticity) in elevating the cleithrum and reducing the recovery time by shortening in the course of the compressive phase in these species. In this way, the potential delay due to repositioning the cleithrum after this element has stretched the sternohyoideus during the expansion phase is compensated for by M-SH shortening that helps in protracting the cleithrum afterwards.

Studies using electromyography (EMG) have demonstrated repeatedly that the sternohyoideus is active during the expansive phase of suction feeding in a variety of teleost species (e.g., Lauder, 1983; Alfaro et al., 2001; Wainwright, 2002; Carroll, 2004). As suction-feeding motor patterns are considered to be evolutionary conservative in fishes (Wainwright, 2002), it is likely that also clariid catfishes activate their sternohyoideus during hyoid depression. In this way, the pattern observed in *Clarias gariepinus* and *Channallabes apus* (Fig. 7A,B) would imply active, eccentric force exerted by the sternohyoideus on the hyoid during buccal expansion, while M-SH contraction during recovery would result from the elasticity build up by the previous elongation. Yet, the observed sternohyoideus strain pattern in these two species leaves the possibility that the sternohyoideus is used as a passive force transmitter during expansion, and is only activated to power cleithrum protraction during the recovery phase. If the latter situation occurs, this would not only imply a different “strategy” of the sternohyoideus to depress the hyoid, but even an alternative “function” compared with

other fishes (see Carroll, 2004). Unfortunately, no EMG data are available for prey capturing clariid catfishes to distinguish between these two possibilities in the timing of M-SH activation. However, the data on *Gymnallabes typus* showing an early M-SH shortening period (Fig. 7C) are indicative of the traditional M-SH activation during the expansive phase of suction.

Although a mechanistic explanation was given for the diverging M-SH strain pattern of *Gymnallabes typus* compared with the other clariid species (i.e., the considerably larger M-SH mass and cross-sectional area enable this muscle to depress the hyoid by shortening during the first instants of buccal expansion), this does not explain why this species has developed this alternative pattern (Fig. 7). Recent results showed that *G. typus* has a highly constrained lateral expansion (suspensorium abduction) compared with the other species (Van Wassenbergh et al., 2007). Consequently, the more massive sternohyoideus (Fig. 4) and the large maximal hyoid depression in *G. typus* (Fig. 6) may be needed to compensate for this reduced amount of suspensorial abduction. In turn, this compensation may be required to maintain an efficient suction-feeding ability.

In conclusion, the species with the largest cross-sectional area of the sternohyoideus muscle also tend to show an increased magnitude of hyoid depression and (with exception of *Gymnallabes typus*) a higher maximal speed of hyoid depression (Table 2). This suggests that a thicker sternohyoideus is able to transmit higher forces to the hyoid during suction feeding. Still, only the species with the largest cross-sectional area and mass of the sternohyoideus of the four species studied (*G. typus*) was able to shorten its sternohyoideus during part of the expansive phase. Consequently, the sternohyoideus shortening pattern for *Clarias gariepinus* observed in a previous study (Van Wassenbergh et al., 2005b) is not an exception among catfishes. Increasing the magnitude of hyoid depression compared with *C. gariepinus* does, however, not necessarily require an alternative pattern of sternohyoideus strain, as the force transmission from the pectoral girdle to the hyoid can apparently be performed through an excentric contraction as in *Chan-nallabes apus* or a partly concentric contraction as in *G. typus*. Still, none of the species studied was able to show a continuing sternohyoideus shortening during hyoid depression as often hypothesized to be the general mechanism of hyoid depression in teleost fishes (Lauder, 1985; Bone and Marshall, 1986). Our data emphasize the importance of the postcranial muscles (epaxial and hypaxial musculature) for powering suction feeding in clariid catfishes.

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LITERATURE CITED

- Adriaens D, Aerts P, Verraes W. 2001. Ontogenetic shift in mouth opening mechanisms in a catfish (Clariidae, Siluriformes): A response to increasing functional demands. *J Morphol* 247:197–216.
- Alexander RMcN. 1970. Mechanics of the feeding of various teleost fishes. *J Zool (Lond)* 162:145–156.
- Alfaro ME, Janovetz J, Westneat MW. 2001. Motor control across trophic strategies: Muscle activity of biting and suction feeding fishes. *Amer Zool* 41:1266–1279.
- Bone Q, Marshall NB. 1986. Food and feeding, chapter seven. In: Bone Q, Marshall NB, editors. *Biology of Fishes*. Glasgow: Blackie. pp 130–147.
- Cabuy E, Adriaens D, Verraes W, Teugels GG. 1999. Comparative study on the cranial morphology of *Gymnallabes typus* (Siluriformes: Clariidae) and their less anguilliform relatives, *Clariallabes melas* and *Clarias gariepinus*. *J Morphol* 240: 169–194.
- Carroll AM. 2004. Muscle activation and strain during suction feeding in the largemouth bass *Micropterus salmoides*. *J Exp Biol* 207:983–991.
- Diogo R, Chardon M. 2000. Anatomie et fonction des structures céphaliques associées à la prise de nourriture chez le genre *Chrysichthys* (Téléostei: Siluriformes). *Belg J Zool* 130:21–37.
- Ferry-Graham LA, Lauder GV. 2001. Aquatic prey capture in Ray-finned fishes: A century of progress and new directions. *J Morphol* 248:99–119.
- Gosline WA. 1971. *Functional Morphology and Classification of Teleostean Fishes*. Honolulu: University of Hawaii Press.
- Herrel A, Van Wassenbergh S, Wouters S, Adriaens D, Aerts P. 2005. A functional morphological approach to the scaling of the feeding system in the African catfish, *Clarias gariepinus*. *J Exp Biol* 208:2091–2102.
- Hill AV. 1938. The heat of shortening and dynamic constants of muscle. *Proc R Soc B* 126:136–195.
- Lauder GV. 1983. Food capture. In: Webb PW, Weihs D, editors. *Fish Biomechanics*. New York: Praeger. pp 280–311.
- Lauder GV. 1985. Aquatic feeding in lower vertebrates. In: Hildebrand M, Bramble DM, Liem KF, Wake DB, editors. *Functional Vertebrate Morphology*. Cambridge, MA: Harvard University Press. pp 210–229.
- Liem KF. 1980. Adaptive significance of intra- and inter-specific differences in the feeding repertoires of Cichlid fishes. *Amer Zool* 20:295–314.
- Oliveira C, Diogo R, Vandewalle P, Chardon M. 2001. Osteology and myology of the cephalic region and pectoral girdle of *Plotosus lineatus*, with comments on Plotosidae (Teleostei: Siluriformes) autapomorphies. *J Fish Biol* 59:243–266.
- Van Wassenbergh S, Herrel A, Adriaens D, Aerts P. 2004. Effects of jaw adductor hypertrophy on buccal expansions during feeding of air breathing catfishes (Teleostei, Clariidae). *Zoomorphology* 123:81–93.
- Van Wassenbergh S, Aerts P, Herrel A. 2005a. Scaling of suction-feeding kinematics and dynamics in the African catfish, *Clarias gariepinus*. *J Exp Biol* 208:2103–2114.
- Van Wassenbergh S, Herrel A, Adriaens D, Aerts P. 2005b. A test of mouth-opening and hyoid-depression mechanisms during prey capture in a catfish using high-speed cineradiography. *J Exp Biol* 208:4627–4639.
- Van Wassenbergh S, Herrel A, Adriaens D, Aerts P. 2006. Modulation and variability of prey capture kinematics in clariid catfishes. *J Exp Zool* 05A:559–569.
- Van Wassenbergh S, Herrel A, Adriaens D, Aerts P. 2007. No trade-off between biting and suction feeding performance in clariid catfishes. *J Exp Biol* 210:27–36.
- Wainwright PC. 2002. The evolution of feeding motor patterns in vertebrates. *Curr Opin Neurobiol* 12:691–695.
- Ward SR, Lieber RL. 2005. Density and hydration of fresh and fixed human skeletal muscle. *J Biomech* 38:2317–2320.