Suckermouth Armored Catfish Resolve the Paradox of Simultaneous Respiration and Suction Attachment: A Kinematic Study of *Pterygoplichthys disjunctivus*

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ABSTRACT

Suckermouth armored catfishes (Loricariidae) use their suckermouth for inspiration, feeding, and attachment to substrates. The sucker consists of a pre-valvular cavity, formed by a modified lip disc, and is separated from the larger post-valvular buccal cavity by a muscular oral valve. The combination of respiration and suction attachment seems paradoxal, as a properly functioning suction device would need a sucker without leakage (yet inspiration must occur via the sucker), and continuous subambient pressure in the sucker cavity (even during expiratory mouth floor elevation). In the loricariid *Pterygoplichthys disjunctivus*, the anatomy of the suckermouth structures was examined, and a kinematic analysis was performed to acquire insights into how respiration and attachment are combined. High-speed external and X-ray recordings show that suckermouth attachment influences respiratory parameters such as decreasing excursion amplitudes of mouth floor elements, and the way water enters the mouth via furrows in the lip disc. Respiration, however, continues during attachment and is not blocked. Our data show that the muscular oral valve actively separates the post-valvular buccal cavity from the pre-valvular sucker cavity. Volume changes of this pre-valvular cavity are opposite to those of the post-valvular cavity and assure sucker function even during expiration. These volume changes are caused by movements of the lower lip, the lower jaws, and the oral valve. The lateral inflow furrow openings, controlled by the maxillary barbels, can occur unilaterally. Morphological and kinematic data also show that the opercle is anatomically and functionally decoupled from the gill opening. *J. Exp. Zool.* 315:121–131, 2011. © 2010 Wiley-Liss, Inc.

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Among craniates, an adhesive suctorial mouth has evolved in hagfishes, lampreys, anuran larvae, and several teleost lineages. It is sometimes accompanied by suctorial devices on the thoraco-pelvic surface and the paired fins, a rough skin surface increasing friction, and/or a streamlined body profile reducing drag. A sucker-like mouth can be regarded as a very complex cranial transformation, as the mouth, turned into a suction cup, still needs to perform tasks in respiration and feeding as well. In the cypriniform suckermouth Gyrinocheilidae, both inspiration and

expiration occur via the gill slits (Smith, '45). In other taxa, inspiration and attachment both using the sucker mouth should occur simultaneously, implying an apparent paradox: inspiration requires a mouth allowing an inflow of water; yet, this "leakage" would seemingly impede the capacity of the suction cup. Moreover, expiratory buccal cavity compression would also impair sucker function. The number of teleost taxa that use their whole mouth as a true sucker is relatively small. In some cyprinids (e.g. *Garra* sp.), only the lower lip acts as a sucker; the papillated ventral mouth of doumeine amphiliids and loaches of the cobitid and balitorid families probably relies mostly on friction (Hora, '30; Diogo et al., 2000). Whole-mouth suction combined with inspiration exists in the mochokid, astroblepid, and loricariid catfishes.

More than 700 species of loricariids exist (Ferraris, 2007), and it is well known that these are able to respire during suction attachment, which many species do during a substantial proportion of their daily activities. Our understanding of the actual attachment mechanism has grown during the last century, although it is not yet fully resolved. Regan (1904) first assumed that flow would be bidirectional via the gill slits (as in gyrinocheilids), and Hora ('30) stated that the lips could not form a true sucker. Alexander ('65), however, documented the continued presence of a water inflow via the lateral portion of the mouth cup, even during the rather powerful suction attachment. Gradwell ('71) discovered a muscle acting on the oral valve, and Vandewalle et al. ('86) tried to explain the observed lip openings during inspiration combined with suction attachment. The internal head anatomy of loricariids has been described in detail, and has previously been linked to the function and evolutionary origin of the suckermouth (e.g. Schaefer and Lauder, '86; Geerinckx et al., 2007a, 2009), but quantitative analyses of the movement patterns of the relevant structures (jaws, hyoid, suspensorium, oral valve, lips, gill covers) are still lacking.

In order to clarify the mechanics of respiration with and without simultaneous suction attachment, we chose to study *Pterygoplichthys disjunctivus*, a relatively large hypostomine suckermouth armored catfish from the upper Madeira tributaries in the southwestern Amazon basin (Weber, '92). We used external and X-ray high-speed video recordings of three individuals to characterize the movements of the cranial structures during attachment and respiration. We did not aim to measure the physiological efficiency of respiration, for which simultaneous oxygen partial pressure recordings would be needed.

We hypothesize that suction attachment will modify the major respiratory movements (e.g. hyoid movements), both in duration and in scale, as the water flow will be restricted, and altered, by the affixation of the mouth to the substrate. In addition, we hypothesize that the unique, muscular oral valve of loricariids can be linked to this respiration, as suggested by Geerinckx et al. (2007a). We provide a kinematic analysis comparing respiration with and without simultaneous suction attachment in *P. disjunctivus* to test the above-mentioned hypotheses.

In this article, the post-valvular cavity is defined as the main portion of the buccal cavity, anteriorly closed by the oral valve; the pre-valvular cavity then refers to the smaller anterior portion of the buccal cavity, dorsocaudally bordered by the valve. The sucker volume is formed by the pre-valvular cavity, and, when the valve is open, is actually increased by the post-valvular cavity, as then both cavities are one continuous volume.

MATERIAL AND METHODS

Three live *Pterygoplichthys disjunctivus* individuals were used for external and X-ray high-speed video recordings (Table 1). One additional 150 mm SL cleared and stained specimen was used for osteological examination and drawing. A fifth 235 mm SL specimen was used for dissection. All specimens were obtained via the commercial aquarium trade.

During the high-speed video recordings in the experimental aquaria, the individuals were either resting on the aquarium floor without use of the suckermouth (respiration mode 1: "normal respiration"), adhering to the floor or vertical wall with their suckermouth (mode 2: "attached respiration"), or adhering more strongly on the floor, being provoked to do so by pulling the tail upward (mode 3: "strongly attached respiration"; Table 1). This third respiratory mode was added to yield more insight in the limits of the combined suction attachment and respiration. As opposed to normally attached respiration (mode 2), which the individuals could maintain during several hours, this last mode probably does not reflect any natural condition, as loricariids typically would resist predators by using the fin spine locking mechanism to anchor themselves in burrows or crevices, or would flee otherwise.

The external video recordings were made using a Redlake Imaging MotionPro 500 digital high-speed video camera (Redlake, San Diego, CA) set at 100 frames sec^{-1}. The use of a 45° mirror was not possible, given the close contact of the fishes to the aquarium floor or wall; thus, separate sets of movies were recorded in ventral and lateral view. Illumination was provided

Table 1. Numbers of cycles analyzed in this study per individual of *Pterygoplichthys disjunctivus* (*Pd*1-3).

			n.r.	a.r.	s.a.r.
*Pd*1	(287 mm SL,	External	6	12	2
	52 mm HL)	X-ray	4	11	3
*Pd*2	(240 mm SL,	External	14	9	9
	47 mm HL)	X-ray	10	7	0
*Pd*3	(129 mm SL,	External	4	10	0
	25 mm HL)	X-ray	5	6	3

Cycles from external and X-ray sequences are listed separately. SL, standard length; HL, head length (snout tip to level of opercle end); n.r., normal respiration; a.r., attached respiration; s.a.r., (forced) strongly attached respiration.

by four arrays of eight ultra-bright red LEDs. Anatomical points of interest in external view are a medioventral point at the level of the hyoid bar, the lateral edge of the suspensorium, the caudal end of the opercle, the end of the branchiostegal membrane (gill cover), the lip tissue at the lateral furrows, the medioventral end of the lower lip, the medial end of both lower jaws, the contact point between the upper jaws, and the mediocaudal tip of the oral valve.

For the X-ray recordings, small lead markers were inserted in or onto the following structures, using hypodermic needles: the center of the hyoid, the lateral edge of the suspensorium, the caudal end of the opercle, the end of the branchiostegal membrane, the end of the maxillary bones (in the lip tissue bordering the lateral furrows), the medial end of both lower jaws, one upper jaw at the contact point with the other, and the mediocaudal tip of the oral valve. Before implantation of these lead markers, the animals were anesthetized with MS 222 (Sigma Aldrich, St. Louis, MO). High-speed X-ray videos were recorded using a Philips Optimus X-ray generator (Royal Philips Electronics NV, Eindhoven, Netherlands), coupled to a 14 inch image intensifier (set at 10-in zoom function) and a Redlake Motion Pro 2000 camera (1,280 by 1,024 pixels or ca. 0.15 mm/pix; Redlake) set at 100 frames sec^{-1}. All experiments were approved by the animal care and use committee at the University of Antwerp, where experiments were performed.

The external points (non-X-ray recordings) and the internal points (X-ray recordings), thus represented 12 anatomical points of interest and, along with two extra reference points on the skull, were digitized frame-by-frame from the high-speed video sequences using Didge 2.2.0 (Alistair Cullum, Creighton University, Omaha, NE). The coordinates of all landmarks were recalculated to a frame of reference moving with the neurocranium (as determined by the reference points on the skull). After data filtering (fourth order Butterworth zero phase-shift low-pass filter, cut-off frequency of 3 Hz), the movements of all points were analyzed from 66 cycles based on the external video recordings (Pd1: 20 cycles, Pd2: 32 cycles, Pd3: 14 cycles) and 49 respiratory cycles based on the X-ray recordings (individual Pd1: 18 cycles, Pd2: 17 cycles, Pd3: 14 cycles; Table 1). In separate experiments, respiratory water flow was observed using diluted milk slowly injected in the water for flow visualization.

Statistical differences between phase durations and excursion amplitudes in the different respiratory modes were tested using PopTools 3.0 (Monte-Carlo Analysis, 10,000 replicates; Greg Hood, Australia—http://www.cse.csiro.au/poptools/) on Excel 2007. This program uses a randomized permutation procedure, in which the null hypothesis states that the distance (difference) between two groups of variables (phase durations, excursion amplitudes) does not differ from the distance between any pair of groups (e.g. 10,000 replicates) with random inclusion of the variables; the P-value can be calculated from the result (the relative number of times the distance is in the upper 0.975 percentile of replicates; Collyer et al., 2007).

RESULTS

Head Anatomy

The anatomical head features of *P. disjunctivus* most relevant to this study are listed below; for more detailed anatomical descriptions consult Geerinckx et al. (2007a). The suspensorium sutures to the neurocranium and is tightly attached to the armored skin as well (Fig. 1). The hyoid bar is more mobile and very broad; its musculature is also substantially larger than that of the suspensorium. The mobile ventral sucker device is composed of the expanded upper and lower lips, of which the latter is enlarged and folded backward: together they form an almost round suction cup. This configuration also results in the important fact that the loricariid mouth cannot be closed. The
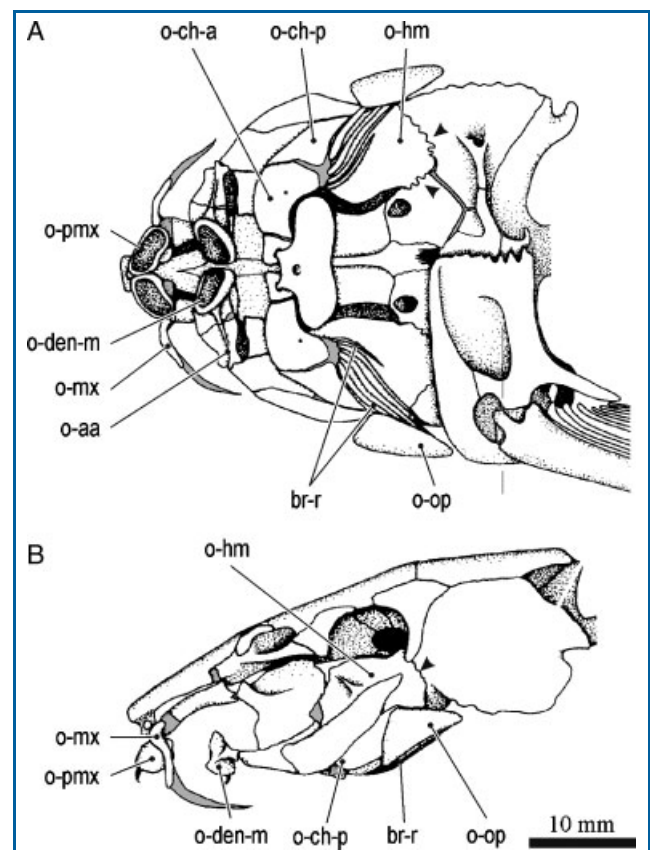

Figure 1. Skull of *Pterygoplichthys disjunctivus* (150 mm SL) in (**A**) ventral view (body armor, branchial basket, and left half of pectoral girdle removed) and (**B**) left lateral view (other half of pectoral girdle also removed), showing positions of the mobile skull elements. Notice the sutures between the suspensorial hyomandibula and the neurocranium (arrowheads). Gray indicates cartilage. br–r, branchiostegal rays; o-aa, os anguloarticulare; o-apal, os autopalatinum; o-ch-a/p, os ceratohyale anterior/posterior; o-den-m, os dento-mentomeckelium; o-hm, os hyomandibulare; o-mx, os maxillare; o-op, os operculare; o-pmx, os praemaxillare.

anterior and posterior intermandibular muscles not only insert on the lower jaw, but have additional separate portions inserting on the lower lip. The maxilla supports the suction cup laterally, bears short maxillary barbels, and is inserted on by the levator tentaculi, a muscle unique for loricariids and related scoloplacids and astroblepids (Geerinckx et al., 2009); its working line indicates it can pull a lateral part of the suction cup off the substrate. The maxilla articulates with the autopalatine, which is inserted by two separate portions of the extensor tentaculi. The lower jaws lack a symphysis or other contact at the midline, point fully medially instead of rostrally, and bear their teeth on the ventral sides. Their dorsoposterior portion supports the anterior part of the floor of the post-valvular cavity, and they are also part of the roof of the pre-valvular cavity (Fig. 2). The teeth of upper and lower jaws point ventrally and can scrape the substrate. The lower jaws and the hyoid are the only mobile elements
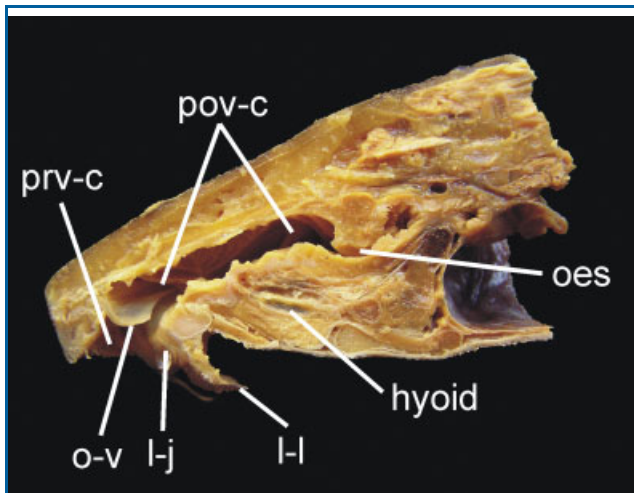

Figure 2. Mediosagittal view of a transsected head of *Pterygoplichthys disjunctivus*. The caudally directed lower lip (l-l) is well visible, as are the right lower jaw (l-j), the oral valve (o-v), the pre-valvular cavity (prv-c) anteroventral to it, the post-valvular cavity (pov-c) posterodorsal to it, and the entrance to the esophagus (oes).

of the mouth floor, as the cleithrum is immovably fixed to the supracleithral part of the compound pterotic bone in the neurocranium. The oral valve is large and is able to completely separate the small pre-valvular cavity from the large post-valvular main buccal cavity (Fig. 2). The pre-valvular cavity is substantially smaller than the post-valvular cavity (which also includes the opercular cavities), and is bordered by the jaw and lip tissues, the oral valve, and (during suction attachment) the substrate. The oral valve is supported medially by a cartilaginous tissue band. A paired retractor veli muscle, originating far dorsocaudally on the hyomandibula, inserts into the valve via a tendon (see also Gradwell, '71). The gill slit is not bordered by the opercle; it is covered by the small branchiostegal membrane only.

Kinematics

Normal Respiration (Mode 1). During respiration, without any form of mouth attachment (thus, without suckermouth function), the average cycle duration without attachment (mean $\pm$ s.d.) is quite variable, being 650 ± 131 msec (see Table 2 for data per individual).

As the suction cup is not attached to the substrate, the pre-valvular cavity does not exist as a separate entity: it is continuously open to the environment. Loricariid lips and jaws cannot close the mouth. The depression phase, during which the buccal floor moves ventrally, increasing the post-valvular buccal cavity, takes as long as the elevation phase (313 ± 49 vs. 311 ± 45 msec, $P = 0.427$). The hyoid moves slightly caudally during elevation and rostrally during depression (Table 3). The cleithrum is immovable (see above) and does not participate at all in buccal or opercular volume changes. The lower jaws move rather synchronously with the hyoid (Fig. 3A; Table 4a and b). Their dorsoventral excursion is much less than that of the hyoid, being $2.75 \pm 0.69\%$ HL, compared with $4.54 \pm 0.86\%$ HL. A rostrocaudal motion also exists, as well as a small mediolateral motion (Table 3). The three directions of movement occur more or less simultaneously (Table 4c and d). Left and right lower jaws typically move in synchrony (Table 4e–g). No motion of the premaxillae is observed during any respiration mode: they are active during feeding only.

The lower lip, not being affixed to the substrate, moves up and down well before the lower jaw and hyoid motions (excursion

Table 2. Some timing and distance values reflecting differences between three individuals of *Pterygoplichthys disjunctivus* (*Pd*1-3).				
		*Pd*1	*Pd*2	*Pd*3
Full cycle duration (ms)	n.r.	670 ± 56 (*10*)	666 ± 154 (*24*)	582 ± 107 (*9*)
	a.r.	787 ± 222 (*23*)	708 ± 149 (*16*)	623 ± 108 (*16*)
	s.a.r.	$1{,}074 \pm 231$ (*5*)	326 ± 72 (*9*)	580 ± 46 (*3*)
Dorsoventral hyoid excursion (mm)	n.r.	5.16 ± 0.74 (*9*)	4.61 ± 0.74 (*22*)	3.50 ± 0.14 (*9*)
	a.r.	2.73 ± 0.59 (*22*)	3.66 ± 0.24 (*12*)	2.34 ± 0.47 (*10*)
	s.a.r.	2.77 ± 0.71 (*5*)	0.79 ± 0.36 (*5*)	–

Average $\pm$ standard deviation (in msec or mm) and *n* are given. n.r., normal respiration; a.r., attached respiration; s.a.r., (forced) strongly attached respiration.

Table 3. Distance averages of some movements for the three respiration modes in three individuals of *Pterygoplichthys disjunctivus*.

	n.r.	a.r.	s.a.r.
Dorsoventral hyoid excursion	4.54 ± 0.86 (*38*)	2.90 ± 0.69 (*47*)	1.78 ± 1.17 (*10*)
Rostrocaudal hyoid excursion	0.80 ± 0.34 (*38*)	0.52 ± 0.50 (*47*)	0.77 ± 0.09 (*2*)
Mediolateral suspensorium excursion	0.50 ± 0.15 (*10*)	0.27 ± 0.12 (*21*)	0.54 ± 0.10 (*3*)
Mediolateral opercular excursion	0.71 ± 0.11 (*13*)	0.40 ± 0.10 (*12*)	0.51 ± 0.10 (*7*)
Dorsoventral lower jaw excursion	2.75 ± 0.69 (*28*)	1.68 ± 0.58 (*23*)	–
Rostrocaudal lower jaw excursion	2.12 ± 0.77 (*83*)	2.13 ± 0.88 (*119*)	1.85 ± 1.20 (*16*)
Mediolateral lower jaw excursion	1.03 ± 0.63 (*41*)	1.21 ± 0.54 (*68*)	0.77 ± 0.13 (*4*)
Dorsoventral oral valve excursion*	10.24 ± 0.25 (*4*)	7.80 ± 1.22 (*13*)	5.85 ± 0.13 (*2*)
Rostrocaudal oral valve excursion*	4.32 ± 0.42 (*11*)	4.17 ± 1.21 (*25*)	6.41 ± 0.02 (*2*)

*Individual *Pd*1 only. Average ± standard deviation (in mm) and *n* are given. n.r., normal respiration; a.r., attached respiration; s.a.r., (forced) strongly attached respiration.

peaks occur on average 149 ± 43 msec before peak hyoid movements, Table 4h, i). The dorsoventral excursion of the caudal tip of the lower lip seems to be almost universally larger than the hyoid excursion.

In lateral view, the oral valve moves in an elliptic path, both rostrocaudally and dorsoventrally (Fig. 3A). It starts with a sudden dorsocaudal motion around the onset of hyoid elevation, just after the onset of gill slit opening, closing the post-valvular cavity anteriorly while water is expelled posteriorly. Then, at gill slit closure and around maximal hyoid elevation, it starts moving rostroventrally. During this phase, the valve seems to be more relaxed, as suggested by its loosely flapping caudal edge. Functionally, rostrocaudal motions open and close the post-valvular cavity, whereas the dorsoventral motions can be related to the volume of the pre- and post-valvular cavities. The dorsoventral valve movements are extensive, averaging 10.24 ± 0.25% HL (data of *Pd*1, for which lateral X-ray data of the valve are available; Table 3).

Suspensorial movements occur more or less in synchrony with the hyoid movements (Table 4j), but the abduction and adduction amplitudes of each suspensorium are significantly smaller than the hyoid elevation and depression movements ($P < 0.001$; Table 3). The opercle moves with or slightly after the suspensorium (Table 4k), with abduction and adduction amplitudes not differing significantly from those of the suspensorium ($P > 0.999$; Table 3). Table 4l, m and Figure 4 show that opercular abduction and adduction peaks are never concurrent with the maximal gill slit openings or closures by the branchiostegal membrane.

Attached Respiration (Mode 2). When respiration is combined with suckermouth attachment onto a horizontal or inclined substrate, the contact between the suction cup and the substrate creates the pre-valvular cavity, a chamber that is not present during normal respiration.

Compared with normal respiration, the total respiratory cycle time does not significantly change (716 ± 185 msec; $P = 0.978$).

The hyoid depression phase, however, now is significantly longer than the elevation phase (430 ± 155 vs. 327 ± 62 msec, $P < 0.001$), and the amplitude of the hyoid and lower jaw elevation and depression movements is significantly smaller than during normal respiration ($P < 0.001$; Fig. 3; Table 3). As opposed to normal respiration (without attachment), the dorsal movement of the oral valve is delayed with respect to the hyoid and lower jaw elevation (Table 4n–q).

As the lower lip edge continuously touches the substrate, only the central part of the lower lip pad is seen moving up and down (Fig. 5A and B). Its upward movement (or elevation) increases the volume of the pre-valvular cavity, whereas its downward movement (or depression) decreases it. As in normal respiration, lip pad elevation occurs well before hyoid and lower jaw elevation (although now depression occurs almost synchronously; Table 4i): elevation reaches its maximum 109 ± 49 msec before the maximal lower jaw elevation, whereas the depression of the lip pad occurs only 15 ± 19 msec before lower jaw depression.

Inspiration during suction-generated attachment is allowed by the elevation of lip tissue at the lateral sides of the suction cup, where the maxillary barbel projects from the upper and lower lip connection (Fig. 5F). The very small inflow furrows thus created exist during depression of the buccal floor, when the oral valve is open and are narrowed or completely closed well before the water expulsion through the gill slits (Fig. 6; Table 4r–v). This is confirmed by flow visualization experiments (using diluted milk). Maximal furrow opening is observed 166 ± 32 msec after maximal hyoid elevation and 225 ± 19 msec before maximal hyoid depression (Table 4u, v). During normal respiration, without any contact to a substrate, some up-and-down movements of this lip tissue were often but not always observed (along with the lower lip movements).

As in normal respiration, opercular abduction and adduction peaks are not simultaneous with maximal gill slit openings or
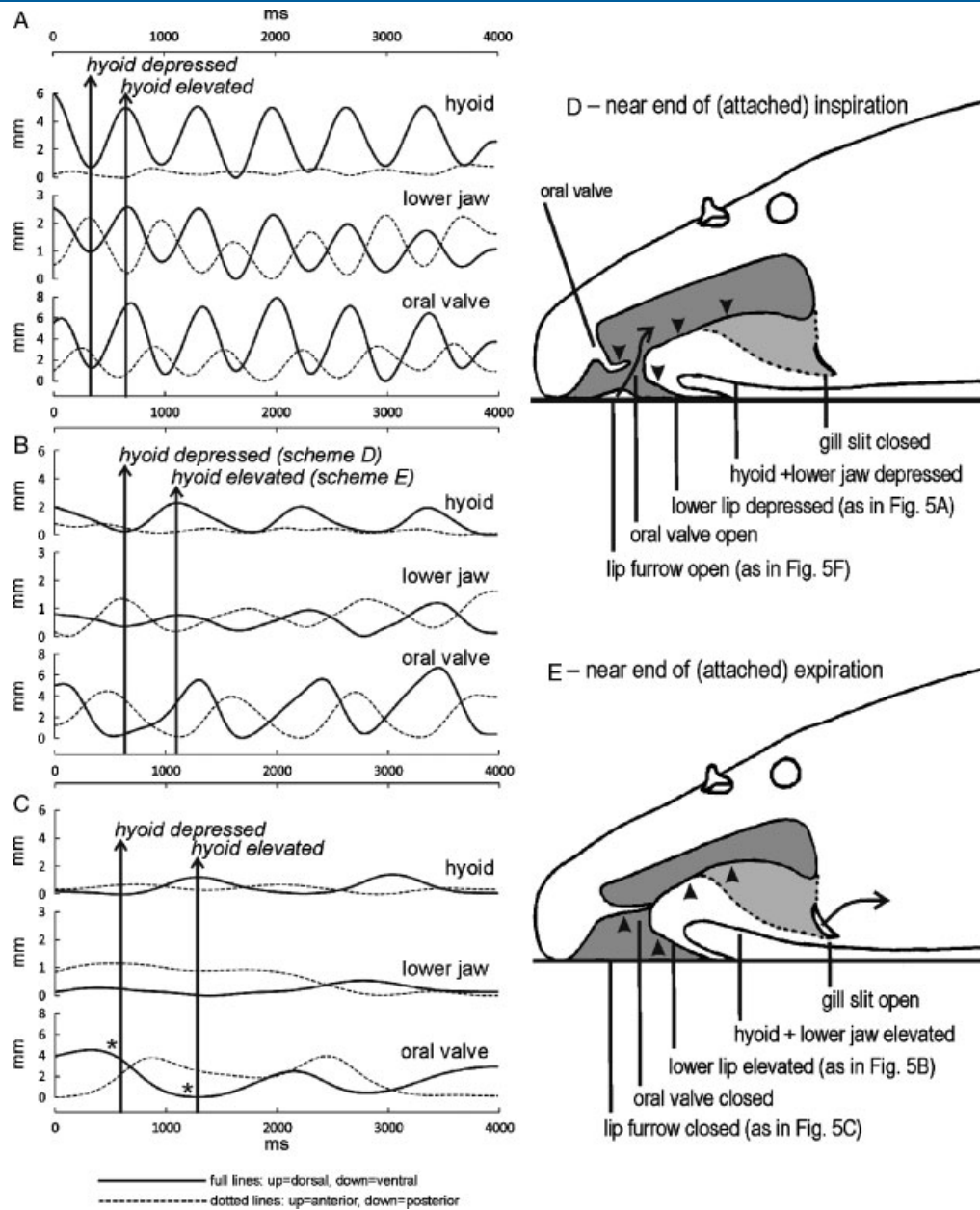
Figure 3. Graph showing hyoid, lower jaw, and oral valve movements during (A) normal respiration, (B) attached respiration, and (C) strongly attached respiration of *Pterygoplichthys disjunctivus* (*Pd*1). Data obtained from three 4,000 ms lateral X-ray high-speed video sequences. Full lines: dorsoventral movements, dotted lines: rostrocaudal movements. The oral valve is positioned caudally (and thus is *closed*) during hyoid elevation, which typically coincides with the gill slit *opening* and vice versa. With attachment added to respiration, excursion distances decrease. During strongly attached respiration, this trend persists, and the dorsoventral oral valve movements (indicated by asterisk) are opposite to the hyoid and lower jaw movements. (D) and (E) are schemes representing the attached respiration cycle. In (D), inspiration is almost completed: one or both lateral lip furrows are open, the (larger) post-valvular cavity is expanded while the pre-valvular cavity is compressed (arrowheads), the oral valve is relaxed, and the gill slits are closed. In (E), expiration is almost completed: the lateral lip furrows are closed, the post-valvular cavity compresses (arrowheads), with water being expelled through the opened gill slits, and the oral valve closes the expanding pre-valvular cavity. Pre- and post-valvular cavities as seen in mediosagittal section are colored dark gray; the lateral (opercular) portion of the post-valvular cavity is colored light gray. Arrows indicate water inflow and outflow; arrowheads indicate movements of mouth floor, lower lip, and oral valve.

Table 4. Relative timing averages of relevant movements for the three respiration modes in three individuals of *Pterygoplichthys disjunctivus*.

	n.r.	a.r.	s.a.r.
Timing of lower jaw elevation maximum after hyoid elevation maximum[a]	1 ± 20 (*28*)	16 ± 30 (*49*)	-2 ± 5 (*5*)
Timing of lower jaw depression maximum after hyoid depression maximum[b]	-9 ± 22 (*32*)	15 ± 20 (*47*)	2 ± 16 (*6*)
Timing of lower jaw dorsoventral maxima after lower jaw rostrocaudal maxima[c]	9 ± 18 (*34*)	0 ± 25 (*27*)	–
Timing of lower jaw mediolateral maxima after lower jaw rostrocaudal maxima[d]	10 ± 41 (*57*)	10 ± 19 (*96*)	–
Timing of right lower jaw dorsoventral maxima after left lower jaw dorsoventral maxima[e]	-2 ± 11 (*17*)	-12 ± 19 (*12*)	–
Timing of right lower jaw rostrocaudal maxima after left lower jaw rostrocaudal maxima[f]	5 ± 18 (*44*)	6 ± 21 (*69*)	-9 ± 24 (*14*)
Timing of right lower jaw mediolateral maxima after left lower jaw mediolateral maxima[g]	0 ± 61 (*22*)	9 ± 26 (*35*)	–
Timing of lower lip elevation maximum after hyoid elevation maximum[h]	-153 ± 43 (*12*)	-109 ± 49 (*12*)	–
Timing of lower lip depression maximum after hyoid depression maximum[i]	-145 ± 44 (*13*)	-15 ± 22 (*12*)	–
Timing of suspensorium mediolateral motion maxima after hyoid Dorsoventral maxima[j]	3 ± 32 (*23*)	10 ± 38 (*21*)	-8 ± 5 (*4*)
timing of opercle mediolateral maxima after suspensorium mediolateral maxima[k]	37 ± 21 (*14*)	36 ± 53 (*21*)	6 ± 13 (*4*)
Timing of maximal gill slit opening after opercle abduction maximum[l]	140 ± 13 (*7*)	129 ± 73 (*9*)	10 (*1*)
Timing of maximal gill slit closing after opercle adduction maximum[m]	129 ± 30 (*7*)	145 ± 45 (*11*)	–
Timing of oral valve ventral maximum after hyoid depression maximum (*Pd*1 only)[n]	0 ± 26 (*3*)	-49 ± 94 (*8*)	210 ± 99 (*2*)
Timing of oral valve dorsal maximum after hyoid elevation maximum (*Pd*1 only)[o]	35 ± 7 (*2*)	135 ± 89 (*8*)	780 (*1*)
Timing of oral valve rostral maximum after hyoid depression maximum[p]	-29 ± 41 (*19*)	-41 ± 59 (*26*)	60 ± 93 (*8*)
Timing of oral valve caudal maximum after hyoid elevation maximum[q]	-35 ± 35 (*17*)	-26 ± 38 (*28*)	104 ± 230 (*8*)
Timing of onset of branchiostegal gill slit opening after hyoid depression maximum[r]	-16 ± 24 (*24*)	14 ± 42 (*30*)	89 ± 29 (*13*)
Timing of onset of branchiostegal gill slit closing after hyoid elevation maximum[s]	-77 ± 11 (*25*)	-82 ± 13 (*29*)	-28 ± 42 (*12*)
Timing of completion of branchiostegal gill slit closing after hyoid elevation maximum[t]	21 ± 22 (*25*)	29 ± 25 (*31*)	15 ± 33 (*22*)
Timing of lateral lip furrow opening maximum after hyoid elevation maximum[u]	–	166 ± 32 (*5*)	–
Timing of lateral lip furrow opening maximum after hyoid depression maximum[v]	–	-225 ± 19 (*5*)	–

Average $\pm$ standard deviation (in msec) and *n* are given (for the forced attachment experiments, sometimes only one value is available). n.r., normal respiration; a.r., attached respiration; s.a.r., (forced) strongly attached respiration.

closures (Fig. 4; Table 4l, m). The flow visualization experiments demonstrated the water expulsion during post-valvular compression, without any sign of backflow.

Strongly Attached Respiration (Mode 3). This third mode, as mentioned, was provoked to test how the seemingly paradoxal combination of the respiration and suction function might fail. With strong attachment, the duration averages 561 ± 349 msec but a full cycle may take substantially longer (*Pd*1: 1074 ± 231 msec) or shorter (*Pd*2: 326 ± 72 msec), according to the individual. Large standard deviations indicate the extensive variation in timings, even in a single individual (Table 2). The depression phase averages longer than the elevation phase, but highly variably so (480 ± 338 vs. 298 ± 189 msec; $P=0.147$).

During these provoked experiments, the outer rim of the suction cup is the only body part in contact with the substrate. Because of the manipulation (pulling), this part and the enclosed pre-valvular cavity change shape with changing body posture relative to the substrate (Fig. 5C–F). During strong attachment and rarely during normal attachment, the creation of the lateral inspiration furrows can be unilateral; at the "inactive" side, the suction disc remains immobile, usually affixed closely to the substrate at all times. The opening of the branchiostegal slits is delayed and their closing is advanced, when compared with the previous two respiration modes (Table 4r–t): as such the moment that they are open is significally shortened.

During strong attachment, a substantial difference exists in the oral valve movements. The dorsoventral movements vary: they may follow the hyoid movements or may almost be opposite (Fig. 3C). The only constant feature is the caudal movement during, or somewhat before, the hyoid elevation (ensuring the closure of the post-valvular cavity during expiration). Sometimes, a cyclic valve movement was observed without any hyoid or other motion occurring (Fig. 6).

DISCUSSION

Effect of Attachment on Respiration—The Paradox Resolved

As hypothesized, sucker attachment modifies the respiratory pattern in duration and in scale. Compared with normal respiration, in attached respiration the hyoid and suspensorium excursions become significantly smaller (Table 3), although overall cycle duration does not increase significantly during attached respiration (Table 2), and water outflow is still cyclically
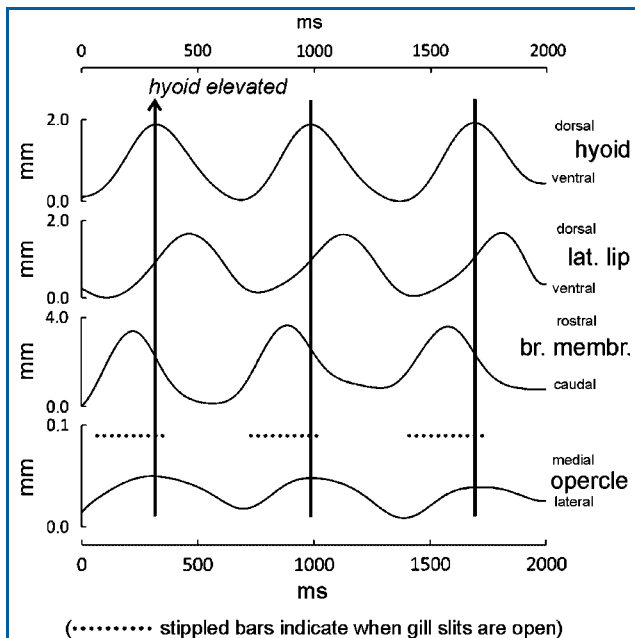
Figure 4. Graph illustrating movements of the inflow and outflow structures during attached respiration of *Pterygoplichthys disjunctivus* (*Pd*1). Data obtained from a 2,000 ms lateral X-ray high-speed video sequence. Respiratory cycle is represented by the hyoid dorsoventral movements (vertical lines indicate elevation peaks). The lateral lip tissue is elevated most during the depression phase of the hyoid (cf. Fig. 3D), creating the lip furrow. The branchiostegal membrane abducts ventrally during the elevation phase of the hyoid (cf. Fig. 3E); stippled bars indicate periods when the gill slit is open. The cyclic nature of the opercular movements (laterally visible as slight rostrocaudal motion) is similar to that of the hyoid and not to that of the branchiostegal membrane or gill slit.

observed through the opening gill slits; normal suckermouth attachment apparently does not block respiration.

Only during attached respiration, the pre-valvular cavity exists as a separate entity: during buccal compression it is also closed posteriorly by the oral valve. It seems that the pre-valvular cavity is the key to understanding the seemingly paradoxal continuous suckermouth function even during the expiration phase, and on a perfectly smooth, glass surface, deemed impossible by the earliest investigators (Regan, 1904; Hora, '30). During expiration (Fig. 3E), the lower jaws and hyoid of the mouth floor elevate, whereas the oral valve and lip furrows are closed: gill slits open and water is expelled. As shown in Figure 3E, the elevation of the lower jaws, but also of the central lower lip and even the oral valve, now increase the volume of the pre-valvular cavity, apparently ensuring the function of the suction cup. During inspiration, pre-valvular and post-valvular cavity is connected, as the oral valve is open. The pre-valvular cavity volume decreases, but the much larger post-valvular cavity

volume increases: water is sucked through the opened, but small, lip furrows and the function of the suction cup is maintained (Fig. 3D). The lower jaws thus not only, in conjunction with the hyoid, drive the buccal pressure pump, but also serve to change the pre-valvular cavity volume. In teleosts without a sucker-mouth (and thus without a distinct pre-valvular cavity), the lower jaws only have the former respiratory function (Hughes and Shelton, '58).

Hora ('30) assigned the loricariid attachment to a combination of capillary forces between the lip and the substrate, and negative pressure created by increased water flow between the substrate and the closely held body of the fish (Bernouilli's law). In several, but not all recorded occasions, pelvic fin movements generate a slight flow under the body. This can probably only partially explain the adhesion of a relatively large fish on a vertical wall in a tank without flow. Moreover, the powerful, forced suction attachment sequences prove that Hora's hypothesis cannot explain the strong attachment as observed in *P. disjunctivus*. In these sequences, the body of the fish is pulled far away from the substrate (thus, no flow-created underpressure), and most of the lip disc (except for the outer rim) is not in contact with the substrate (resulting in limited or no capillary force). These experiments show what usually seems to cause the ultimate failure of the attachment in the experimental conditions: the soft lower lip tissue folds inward, likely owing to the subambient pressure (Fig. 5D), causing the sucker to loose its grip. The rough lip surface, bearing papillae, probably aids in attachment, but is far less important than the respiratory sucker action and is probably most beneficial during weak attachment onto rough substrates or where continuous (river) flow speeds are present (Gerstner, 2007). Presumably the papillae, bearing many unicellular projections, also play a role in the scraping of food off substrates (Ono, '80; Geerinckx et al., 2007b).

During attached respiration, the early onset of the upward movement of the lower lip (with respect to the lower jaw and hyoid movements) advances the onset of expansion of the pre-valvular cavity, preventing the occurrence of pre-valvular sucker overpressure around the onset of buccal compression. With or without attachment, the elevation of the lip invariably occurs sooner than that of the jaw and hyoid, and without attachment the ventral lower lip movement is also initiated earlier (thus, apparently lip "depression" is delayed during attached respiration; see Table 4i). This early onset implies a separate muscular action starting before the jaw and hyoid elevation/depression musculature. Both the anterior and the posterior intermandibular muscles have "labial" bundles inserting on the lower lips, separate from the bundles inserting on the lower jaws (Geerinckx and Adriaens, 2007c). It can be hypothesized that an action of the former labial bundles, ahead of that of the latter, can likely best explain the early motions of the lower lip. As such, the essential function of the labial bundle of the posterior intermandibular muscle seems to be the expansion of the pre-valvular cavity,
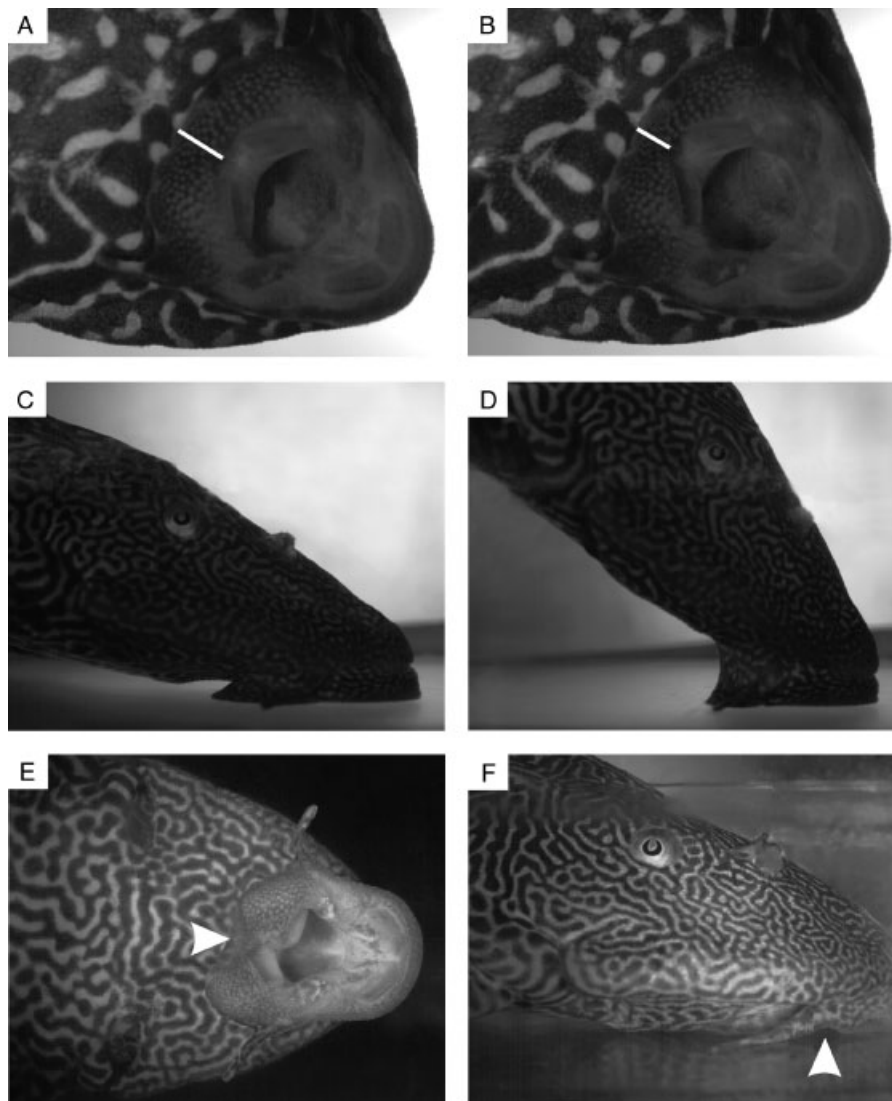
Figure 5. (A, B) Video frames illustrating the change in pre-valvular cavity volume in *Pterygoplichthys disjunctivus* at (A) maximal hyoid depression (or maximal post-valvular buccal cavity volume) and (B) maximal hyoid elevation (or minimal post-valvular buccal cavity volume), during almost head-down attachment on a vertical glass wall. In (A), the pre-valvular cavity is small, and in (B), it is enlarged by elevation of the lower lip and lower jaws; the oral valve is elevated and closes the post-valvular cavity entrance. Line lengths reflect the angle of the lower lip to the substrate, and thus the up-and-down movements of the central lower lip pad (see also Fig. 3D–E). The outer rim of the lower lip maintains close contact during both phases. (C, D) Video frames illustrating the suction cup changing shape with provoked changing body posture relative to the substrate (the tail was slowly being pulled upward by the investigators). Two milliseconds after the frame (D) was shot, suction attachment failed because of leakage and/or impeded respiration. (E) Video frame showing the infolding lower lip (arrowhead) during strong sucker attachment. The asymmetrical lower jaw positions are unusual and apparently passive (the body being pulled upward). (F) Video frame showing the lateral lip furrow (arrowhead) mediated by movements of the maxillary barbel.

preventing the occurrence of pre-valvular or sucker overpressure around the onset of buccal compression. The loricariid posterior intermandibular muscle is homologous to the anterior portion of the protractor hyoidei of other fishes (Geerinckx and Adriaens, 2007c). The transverse labial bundle of the anterior intermandibularis may well function to narrow the lower lip, and thus reduce the pre-valvular volume. This is analogous to the assumed functions of the separate lip portion of the protractor hyoidei and the anterior intermandibularis of the mental disc of the cyprinid *Garra mullya* (Saxena and Chandy, '66). The
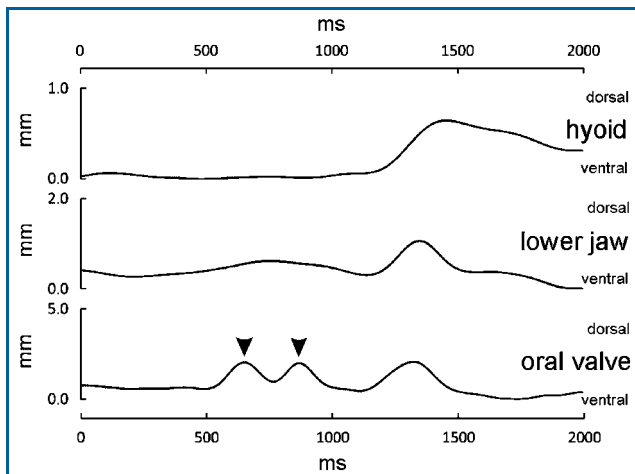
Figure 6. Graph showing dorsoventral hyoid, lower jaw, and oral valve movements during strongly attached respiration of *Pterygoplichthys disjunctivus* (*Pd*3). Data obtained from a 2,000 ms ventral external high-speed video sequence. Oral valve movements occur without any simultaneous movement of the hyoid, the lower jaws, or other structures (arrowheads).

functional hypotheses can be tested by emg recordings and controlled stimulation experiments.

An Active Role of the Muscular Oral Valve

During attached respiration, the oral valve is opened simultaneous with the lateral lip furrows. During normal respiration, the oral valve functions in closing the post-valvular cavity entrance during expiration, as the mouth itself cannot be closed. To close the entrance of the post-valvular cavity during hyoid elevation, the valve is stretched in a dorsocaudal direction (Fig. 3); based on assumed pressure differences only, one would rather expect a ventral and not dorsal movement. The paired retractor veli muscle connects the valve to a more dorsocaudal origin on the hyomandibula (Geerinckx et al., 2009); this configuration suggests it is able to close the oral valve by pulling it in a dorsocaudal direction. Gradwell ('71) experimentally demonstrated that denervating both muscles or severing them at their insertions made valve closure impossible, resulting in backflow leakage: now being fully passive, the valve cannot completely close. This corroborates the active role of the valve. During the provoked strong attachment experiments, the retractor veli apparently only succeeds in pulling the valve caudally (closing it, so separating the cavities), but its dorsal effect is annihilated, probably by the assumed larger pressure differences: dorsoventral valve movements are now almost opposite to hyoid and jaw movements (Fig. 3C). Last, the observed separate cyclical valve movements (without simultaneous hyoid or jaw movements; Fig. 6), also support the hypothesis of an actively controlled, muscular oral valve.

Water Intake During Attachment

Based on limited external kinematic data in *Hypostomus punctatus*, Vandewalle et al. ('86) assumed that the lower jaw movements creates the opening of the lateral lip furrow, through which inspiratory water then enters during attachment. Our results, however, give strong support for the hypothesis of Alexander ('65) that the maxillary barbels are responsible for the creation of the furrows. During unilateral furrow opening, the lower jaws show no motional asymmetry. Unilateral furrow opening is rather rare and could be observed in tanks without flow, when visual or other disturbance caused the fishes to attach more tightly or when strong attachment was provoked in the experiments. This observation seems to be the result of asymmetrical muscle action, most probably on the maxillary bone. Geerinckx et al. (2009) describe the levator tentaculi, a muscle inserting on the maxillary, nonhomologous to the retractor tentaculi, found only in the suckermouth loricariids and astroblepids. The extensor tentaculi, acting indirectly on the maxillary, consists of two separate bundles in loricariids (Gosline, '75; Geerinckx et al., 2009).

The Opercle and the Gill Opening

There is no synchronization between the opercular movements and the opening and closing of the gill slit by the branchiostegal membrane (this invariably occurs later; Table 4l and m). As shown by the current kinematic and morphological results and corroborated by the earlier findings by Geerinckx and Adriaens (2006) on *Ancistrus* sp., the opercle has been anatomically and functionally decoupled from the gill slit mechanism.

CONCLUSION

The present results clearly demonstrate that substantial differences exist between respiration with and without mouth–substrate contact. Our data show that buccal, post-valvular compression and expansion actions significantly differ, that a pre-valvular sucker cavity is formed, and that water inflow is limited and controlled by lateral lip furrows during attachment. The lip furrow and oral valve movements add control in how and when water is inspired, such that the function of the suction cup is maintained. As hypothesized, sucker attachment clearly modifies, but not blocks, the respiratory pattern. The results are also a strong indication that evolutionary changes in the morphology of the cranial structures in the loricarioid clade, as presented by Schaefer and Lauder ('86) and Geerinckx et al. (2009), have had an adaptive advantage for life in habitats where station holding is important.

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