Evaluating the Opercular Linkage in Suction Feeding Fishes with Video Reconstruction of Moving Morphology (VROMM)

Jeffrey D. Laurence-Chasen[1,2], Elizabeth L. Brainerd[1,2]

Blinks-REU-Beacon Research Fellowship
Summer 2015

[1] Friday Harbor Laboratories, University of Washington, Friday Harbor, WA 98250
[2] Department of Ecology and Evolutionary Biology, Brown University, Providence, RI 02912

Contact information:
J.D. Laurence-Chasen
Brown University
69 Brown Street, Box 2362
Providence, RI 02912
jd_laurence-chasen@brown.edu

Keywords: suction feeding, morphology, operculum, XROMM, VROMM, linkage, high speed video, *Leptocottus armatus, Hemilepidotus hemilepidotus*

Abstract

Suction feeding in ray-finned fishes is a highly kinetic behavior. Musculoskeletal components are arranged in linkages, which move in complex, 3-dimensional ways. In order to precisely measure the 3-D rotations and translations of bones during suction feeding, we developed a new imaging method called Video Reconstruction of Moving Morphology (VROMM). VROMM combines bi-planar high speed video with 3-D mesh models to create highly precise animations of real-life motions. Using VROMM animations, we evaluated the opercular linkage for lower jaw depression by measuring the rotation of the lower jaw and the operculum at the quadratomandibular and operculohyomandibular joints, respectively. We filmed two species of sculpin, *Leptocottus armatus* and *Hemilepidotus hemilepidotus,* during suction feeding, and also filmed a post-mortem manipulation where we pulled straight up on the operculum to simulate the dorsal rotation described by the classic 4-bar linkage. In the manipulated trials, the measured kinetic transmission (KT) for *Leptocottus* and *Hemilepidotus* was 2.3 and 2.0 respectively. The measured KT for the live *Leptocottus* was 4.0, and, notably, the operculum reached maximum rotation significantly before the lower jaw. These data suggest that in *Leptocottus,* operculum rotation is responsible for initial jaw depression, while other linkages subsequently drive the rest of mouth opening.

Introduction

The ray-finned fishes (Class Actinopterygii) consists of 30,000 species, which is half the total of all living vertebrate species (Near et al. 2012). These 30,000 species exhibit a wide range of cranial morphologies, yet the vast majority of them employ suction feeding as their main method of prey capture (Westneat 2006). This poses a challenge for understanding form-function relationships in this system.

The most frequently studied aspect of a suction feeding strike is the rapid expansion of the buccal cavity, caused by hyoid and lower jaw depression, neurocranium elevation, and suspensorium expansion (Lauder 1980; Mueller et al. 1989; Westneat 1990). As pressure drops precipitously in buccal cavity, water, and the prey item, are drawn in. The mechanics of this expansive stage are complex, as the teleost head is a highly kinetic system, with more than 20 moving elements active during suction feeding. **(**Westneat 1990; 2005, Camp and Brainerd 2015**)**.

Researchers have identified several different musculoskeletal linkages responsible for mouth opening and suction generation (Liem 1970; Westneat 1990; Anker 1974; Aerts 1987 Lauder 1980). One of the most frequently studied linkages involves the operculum and the lower jaw, and was first described by Liem (1970). This opercular linkage begins with the contraction of the levator operculi muscles, which powers the dorsal rotation of the operculi. This causes the sub-interoperculum joint to move caudad, putting the interoperculomandibular ligament in tension. This ligament pulls the articular process of the lower jaw caudad, which results in ventral rotation around the quadratomandibular joint, or jaw depression.

The opercular linkage was modeled as a four-bar linkage by Anker (1974). Four-bar systems involve four pin joints connected by four rigid bars, operating a single degree of freedom (DOF). Assuming the bar lengths stay constant, and all remain in one plane, it is then possible to calculate the rotation of all four bars from the measured rotation of just one (Suh and Radcliff 1978). This analysis leads to a kinetic transmission (KT) ratio, or the ratio of input rotation to output rotation. The KT ratio describes the ‘effectiveness’ of a four-bar linkage system, by quantifying the amplification of an input velocity through the system. The KT ratio is used as a metric for comparing the opercular linkage in fishes with different cranial morphologies.

The opercular four-bar linkage has been tested in vivo with high speed videography (Westneat 1990, 1994; Durie and Turingan 2004), but single plane, 2-D video inherently reduces complex 3-D motions into 2-D translations and rotations. However, recent developments in modeling and 3-D reconstruction have already yielded exciting results (Camp et al., 2015). Using bi-planar x-ray videos in a new method called X-ray reconstruction of moving morphology (XROMM) (Brainerd et al. 2010), Camp and Brainerd (2015) created highly precise 3-D animations of suction feeding in largemouth bass (*Micropterus salmoides*) and evaluated the accuracy of the 4-bar opercular linkage model. In agreement with previous testing (Westneat 1990), the model’s predictions did not quantitatively match the observed behavior; the KT ratio observed with XROMM was half the ratio predicted by the model.

In this study, we evaluate the opercular linkage in two species of sculpin: pacific staghorn sculpin (*Leptocottus armatus*), and red Irish lord (*Hemilepidotus hemilepidotus*). Each species employs suction feeding as their main method of prey capture while exhibiting different cranial morphologies. Specifically, based on CT (computed tomography) scan data, *Hemilepidotus* has a significantly longer retroarticular process (Fig. 1). In mechanical linkage terms, this morphological difference represents a change in lever length. Thus far, the effect of changing lever lengths on the effectiveness (KT) of the opercular linkage has not been studied.

We conducted two different opercular linkage experiments. The first was a post-mortem manipulation in which string attached to the dorsocaudal tips of the operculi was pulled vertically. This simulated the classic input link rotation of the opercular linkage. The second experiment was the recording of live feeding events. We predicted that *Leptocottus* would have a greater KT than *Hemilepidotus,* because a shorter articular process would functionally shorten the output link of the 4-bar linkage, increasing the ratio of input to output rotation. Additionally, we predicted that the KT recorded in the

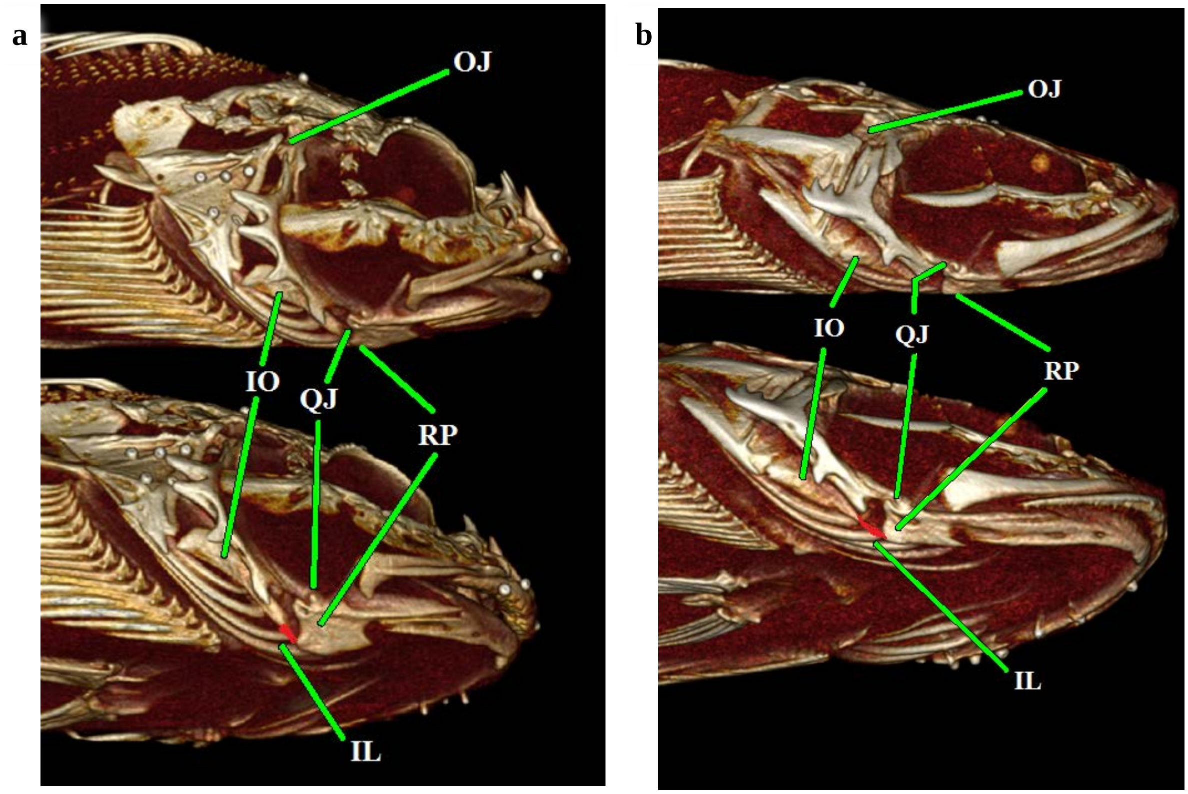

Fig. 1 Lateral view of 3D Volume Renders of *Hemilepidotus hemilepidotus* (a) and *Leptocottus armatus* (b) created from CT scans in Osirix. Note that the larger retroarticular process (RP) in *Hemilepidotus* increases the distance between the quadratomandibular joint (QJ) and the attachment point of the interoperculomandibular ligament (IL). Operculohyomandibular joint, OJ; interoperculum, IO.

live feeding trials would be greater than in the manipulated trials because of other linkages contributing to jaw depression and mouth opening.

In order to test these predictions, we use video reconstruction of moving morphology (VROMM), a new method pairing high-speed videography with 3-dimensional (3-D) bone models to create highly precise animations of bone motion during suction feeding. These reconstructions allow for qualitative as well as quantitative analysis of bone motion in 6 DOF. We measured the KT ratio throughout the strike and the individual rotations and translations of each component in the linkage.

Methods

Specimens

For this study we used two pacific staghorn sculpins (*Leptocottus armatus*) and one red Irish lord (*Hemilepidotus hemilepidotus)*. All fish were obtained by seining at Jackson Beach, San Juan Island**,** WA and housed at the University of Washington, Friday Harbor Labs, WA. All animal care and experimental procedures were approved by the University of Washington IACUC.

Marker Attachment

Each fish was anesthetized with MS-222 and size 15 white plastic beads (markers) were attached to the neurocranium, mandible, operculum, and suspensorium. In order to establish a reference frame for relative motion, it was necessary to establish a 'body plane.' Body plane markers were attached along the dorsal midline of the fish. To provide adequate tracking data, three markers were attached to each bone in a non-linear arrangement.

Data Collection

We filmed the Leptocottus feeding on live prey with three Photron high speed video cameras (Photron USA, Inc., San Diego, CA, USA). The cameras were operated synchronously with a single trigger, and were positioned at different heights and incident angles to maximize marker visibility (Fig. 2).

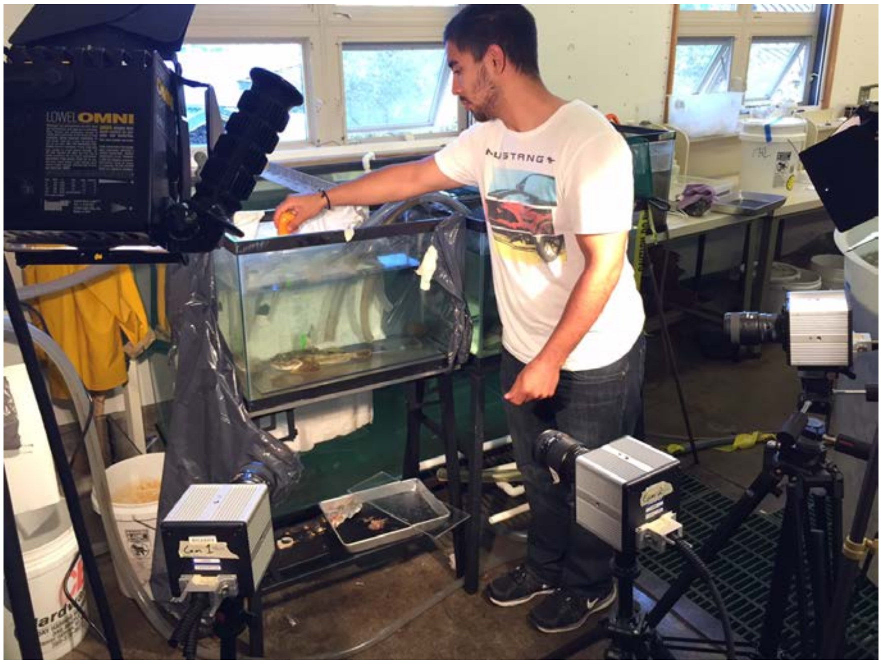

Fig. 2 Data recording setup. Three Photron high-speed cameras synchronously record at 500 frames per second.

To reduce fish stress and predict on-camera feeding performance, the fish were moved into the filming tanks (12.5x15x24 inches) several days before the marker attachment. Videos were recorded at 500 frames per second with a 1/1000 shutter speed. No fewer than 10 strikes on dead shrimp were recorded for each individual. After feeding trials were completed, computed tomography (CT) scans were taken of each fish to reconstruct the bones and markers. The scans were taken with an Animage Fidex veterinary CT scanner, with a resolution of 0.17 mm/pixel, and 3D mesh models of the bones and markers were created in Osirix (Pixmeo, Geneva, Switzerland) and refined in Meshlab.

VROMM Analysis

The markers were tracked using XMALab (available from xromm.org) in each of the three camera views. When a marker could not be seen in one camera, the tracking data from the other two cameras were used for 3-D reconstruction. The 3-D space was calibrated from a Lego calibration object (Fig. 3) taken of a checkerboard pattern manually moved towards and away from the cameras at different angles. The XYZ coordinates of the markers were found from the mesh models and were imported into XMALab to calculate the rigid body transformations of each bone. These transformations

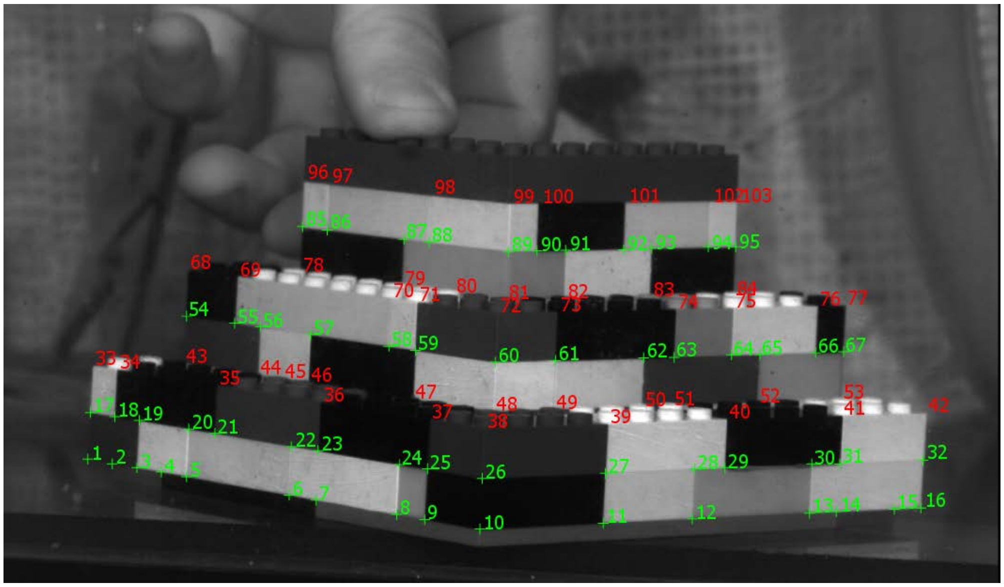

Fig. 3 Calibration object used to compute the 3-D camera positions. Computation done with XMALab software.

were then applied to the bone mesh models to create animations of the bone movements during a strike.

Anatomical and Joint Coordinate Systems

We used joint coordinate systems (JCSs) to measure the motion of animated bones in six DOF. A JCS is composed of two anatomical coordinate systems (ACSs), one attached to the proximal element and one to the distal element of a joint. The relative motion of the two ACSs gives the 6 DOF motion of the JCS. We used JCSs to measure motion of the operculum and lower jaw relative to the suspensorium (Fig. 4 & 5)

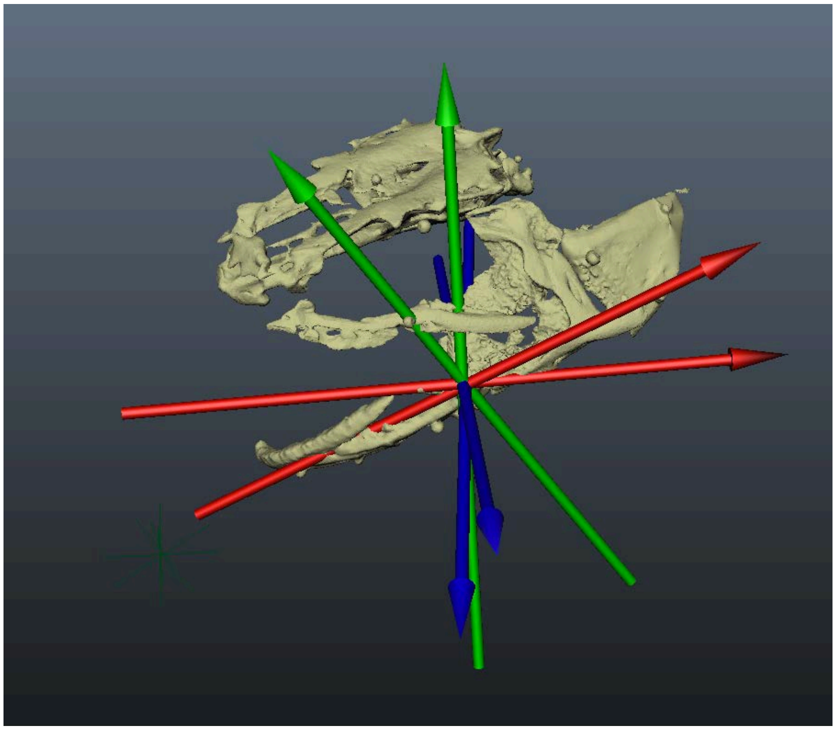

Fig. 4 JCS measuring the rotation of the lower jaw about the quadratomandibular joint. The JCS consists of two ACSs parented to the suspensorium, as a reference, and the lower jaw.

When measuring opercular motion, an ACS was placed on the operculohyomandibular joint. The Z-axis was oriented medio-laterally, Y-axis was oriented rostrocaudally, and the X-axis was orthogonal to the Z- and Y-axes, roughly dorsoventrally (Fig. 1). The lower jaw ACS was placed at the quadratomandibular joint, and the same axes orientations were used (Fig. 2). To measure the kinetic transmission ratio of the opercular linkage, we used the Z axis (dorsoventral) rotations of the operculum and lower jaw, relative to the suspensorium. For the two manipulated individuals, the KT ratio was measured as an average taken over 10 frames during peak gape.

Results

We used VROMM animations to measure dorsoventral rotation of the operculum and the lower jaw of the three individuals. The operculum of the live Leptocottus rotated to a maximum 9.10 ± .40°, and the lower jaw rotated to a maximum of 36.80 ± 5.35°

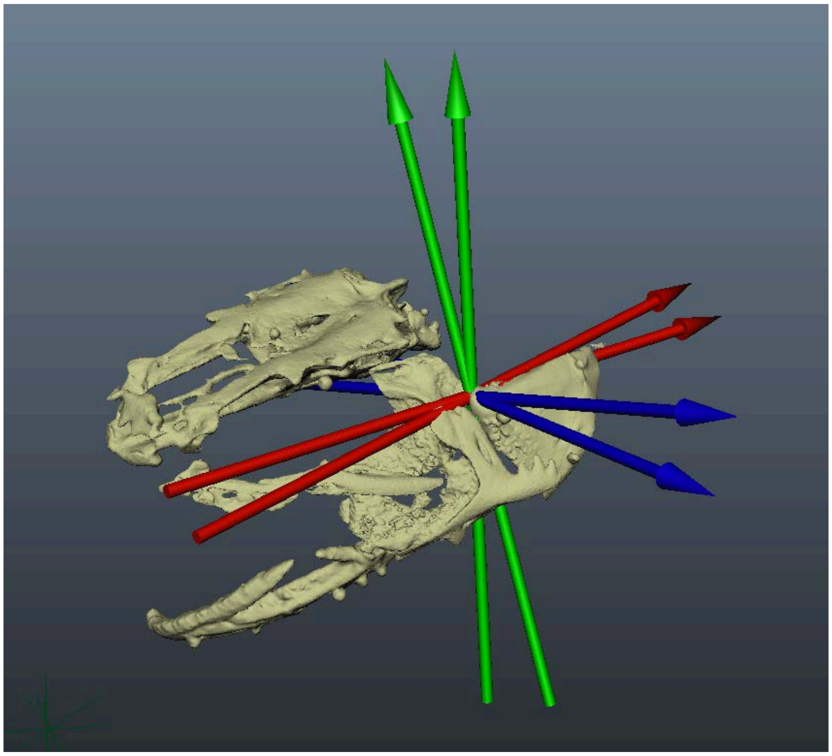

Fig. 5 JCS measuring the rotation of the operculum about the operculohyomandibular joint. The JCS consists of two ACSs parented to the suspensorium, as a reference, and the operculum.

(Fig. 6). The KT ratio was consistently 4.0 in the three strikes analyzed so far. The operculum of the manipulated *Hemilepidotus* rotated to a maximum of 12.50°, and the lower jaw rotated to a maximum of 24.75° (Fig. 7a). The KT ratio was 2.0. The operculum of the manipulated *Leptocottus* rotated to a maximum of 6.30°, and the lower jaw rotated to a maximum of 15.00° (Fig. 7b). The KT ratio was 2.3.

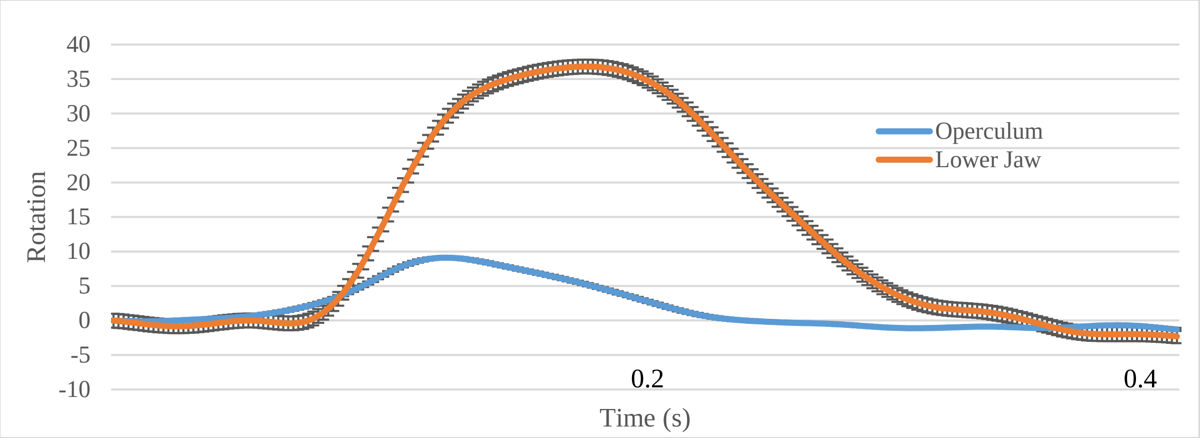

Fig. 6 Operculum and lower jaw rotations measured from VROMM animations of a live Leptocottus feeding on dead shrimp. n=3 strike. Error bars are s.e.

Discussion

We conducted manipulation and live feeding trials to observe the differences in KT ratio. Our predictions were confirmed; in the manipulation experiment the *Leptocottus* had a slightly greater KT ratio than *Hemilepidotus*, and the live *Leptocottus* had a greater KT ratio than both manipulated individuals.

Manipulated vs. Live

The lower jaw depression in the live *Leptocottus* was substantially greater than the depression in the manipulated *Leptocottus*, and the KT was larger in the live

*Leptocottus.*These results are best explained by the contribution of other musculoskeletal linkages. It is likely that hyoid depression and lateral suspensorial expansion both contribute to mouth opening and suction generation in a live fish. The manipulation experiments specifically actuated just the operculum. Additionally, the peak operculum rotation occurred before the peak jaw rotation in the live Leptocottus. This suggests that these other linkages are most active *after* initial dorsal operculum rotation.

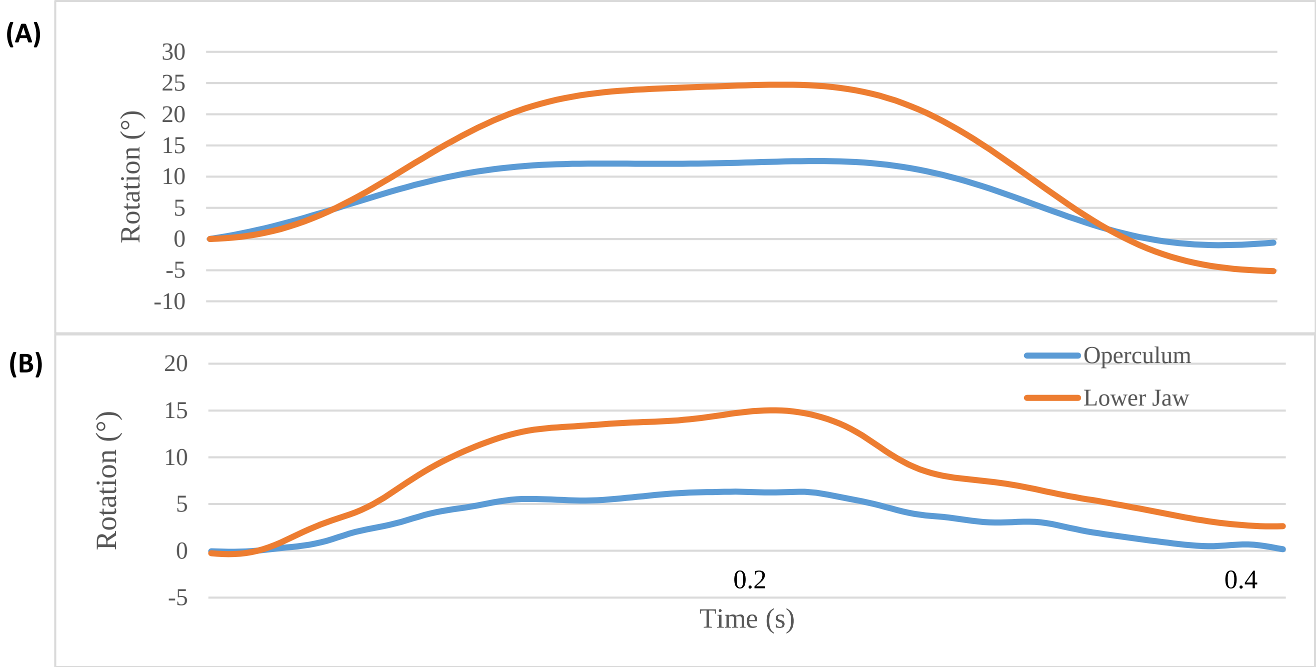

Fig. 7 Operculum and lower jaw rotation of **(A)** Hemilepidotus and **(B)** Leptocottus during manipulation trial. Each graph shows data from a single 'pull'.

Species Differences

The manipulated *Leptocottus* had a greater KT ratio than the *Hemilepidotus*. This can be explained by morphological differences at the quadratomandibular joint. When examining the CT scan of each individual, we noticed a significantly shorter retroarticular process in the Leptocottus. If described as a 4-bar linkage, this would, by definition, shorten the output-link of the opercular linkage. A shorter output link would result in a greater KT ratio.

VROMM

VROMM has allowed us to measure the rotations of individual bones during suction feeding. These results are an exciting first step in validating a new method of 3-D reconstruction. Applicable to far more than just suction feeding, VROMM can be used to evaluate a variety of biological systems that have thus far lacked a means of precisely and accurately quantifying component and holistic motion.

References

Aerts, P. (1987). Model of Jaw Depression During Feeding in Astatotilapia. *Journal of Morphology, 109*.

Brainerd, E. L., Baier, D. B., Gatesy, S. M., Hedrick, T. L., Metzger, K. a, Gilbert, S. L., & Crisco, J. J. (2010). X-ray reconstruction of moving morphology (XROMM): precision, accuracy and applications in comparative biomechanics research. *Journal of Experimental Zoology. Part A, Ecological Genetics and Physiology*, *313*(5), 262–279. http://doi.org/10.1002/jez.589

Camp, a. L., & Brainerd, E. L. (2015). Reevaluating Musculoskeletal Linkages in Suction-Feeding Fishes with X-Ray Reconstruction of Moving Morphology (XROMM). *Integrative and Comparative Biology*, *55*(1), 36–47. http://doi.org/10.1093/icb/icv034

Camp, A. L., & Brainerd, E. L. (2014). Role of axial muscles in powering mouth expansion during suction feeding in largemouth bass (Micropterus salmoides). *The Journal of Experimental Biology*, *217*(Pt 8), 1333–45. http://doi.org/10.1242/jeb.095810

Camp, A. L., Roberts, T. J., & Brainerd, E. L. (2015). Swimming muscles power suction feeding in largemouth bass. *Proceedings of the National Academy of Sciences*, *2015*, 201508055. http://doi.org/10.1073/pnas.1508055112

Carroll, A. M., & Wainwright, P. C. (2003). Functional morphology of prey capture in the sturgeon, Scaphirhynchus albus. *Journal of Morphology*, *256*(3), 270–284. http://doi.org/10.1002/jmor.10095

Carroll, A. M., Wainwright, P. C., Huskey, S. H., Collar, D. C., & Turingan, R. G. (2004). Morphology predicts suction feeding performance in centrarchid fishes. *The Journal of Experimental Biology*, *207*(Pt 22), 3873–3881. http://doi.org/10.1242/jeb.01227

Durie CJ, Turingan RG. 2004. The effects of opercular linkage disruption on prey-capture kinematics in the teleost fish Sarotherodon melanotheron. J Exp Zool 301:642–53.

Lauder, B. Y. G. V, & Clark, B. D. (1984). Water Flow Patterns During Prey Capture By Teleost Fishes, *150*, 143–150.

Lauder, G. V. (2010). Form and Function : Structural Analysis in Evolutionary Morphology Form and function : structural analysis in evolutionary morphology. *Paleobiology*, *7*(4), 430–442.

Lauder, G. V. (1980). Evolution of the feeding mechanism in primitive actionopterygian fishes: A functional anatomical analysis of *Polypterus, Lepisosteus,* and *Amia*. *Journal of Morphology*, *163*(3), 283–317. http://doi.org/10.1002/jmor.1051630305

Lauder, G. V. (1980). The suction feeding mechanism in sunfishes (Lepomis): An experimental analysis. *Journal of Experimental Biology*, *88*, 49–72.

Lauder, G. V., & Liem, K. F. (1981). Prey capture by Luciocephalus pulcher: implications for models of jaw protrusion in teleost fishes. *Environmental Biology of Fishes*, *6*(3-4), 257–268. http://doi.org/10.1007/BF00005755

Liem, K. F. (1970). Comparative Functional Anatomy of the Nandidae (Pices: Teleostei). *Field Museum of Natural History*, *56*.

Muller, M. (1989). A quantitative theory of expected volume changes of the mouth during feeding in teleost fishes. *Journal of Zoology, London*, *217*, 639–662.

Wainwright, P., Carroll, A. M., Collar, D. C., Day, S. W., Higham, T. E., & Holzman, R. a. (2007). Suction feeding mechanics, performance, and diversity in fishes. *Integrative and Comparative Biology*, *47*(1), 96–106. http://doi.org/10.1093/icb/icm032

Westneat, M. W. (1990). Feeding mechanics of teleost fishes (Labridae; Perciformes): A test of four-bar linkage models. *Journal of Morphology*, *205*(3), 269–295. http://doi.org/10.1002/jmor.1052050304

Westneat, M. W. (1991). Linkage Biomechanics and evolution of the unique feeding mechanism of Epibulus Insidiator (Labridae: Teleostei). *Journal of Experimental Biology*, *159*, 165–184.

Westneat M. 1994. Transmission of force and velocity in the feeding mechanisms of labrid fishes (Teleostei, Perciformes). Zoomorph 114:103–18.Westneat, M. W. (2004). Evolution of levers and linkages in the feeding mechanisms of fishes. *Integrative and Comparative Biology*, *44*(5), 378–389. http://doi.org/10.1093/icb/44.5.378

Westneat, M. W. (2005). Skull Biomechanics and Suction Feeding in Fishes. *Fish Physiology*, *23*(C), 29–75. http://doi.org/10.1016/S1546-5098(05)23002-9