

**Morphological Structures Correspond to the Location of Vertebral Bending During
Suction Feeding in Fishes**

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Abstract

During suction feeding, many fishes use their epaxial muscles to generate a substantial amount of power to produce cranial elevation. When the muscles contract they reduce the angle between the head and body, causing the axial skeleton to bend dorsoventrally. Without axial bending, cranial elevation would be limited and feeding performance would decrease. The goal of this study is to locate where the vertebral column bends during suction feeding and relate this to the axial morphology of different species. We collected and analyzed live feeding data from three species (largemouth bass, pacific staghorn sculpin, and striped surfperch) using 3D animation techniques. CT scans were also analyzed with a focus on the shapes and spacing of the vertebrae, neural spines, and pterygiophores. Each of the three species had unique axial skeletons comprising differently shaped and spaced vertebrae, neural spines, and pterygiophores. We hypothesized that the axis of rotation for each species is located in the anterior-most region of the vertebral column, specifically between the vertebrae with the greatest space between the neural spines and pterygiophores. Our data show that the axis of rotation is consistent for largemouth bass (joints 3 and 4) and striped surfperch (joints 2 and 3), although both species bent at different intervertebral joints. These axes of rotation for both largemouth bass and striped surfperch correspond to the organization of the neural spines and pterygiophores. The staghorn sculpin presented an interesting case where all of its axes of rotation (joints 3, 4, 5, and 6) were not associated with any unique structures. However, cranial elevation increased at the more posterior joints. Given the great deal of interspecific variation of the axial skeleton, it is important to investigate the role of these different morphologies in suction feeding.

Introduction

Suction feeding, the most common form of prey capture in Teleost fishes, is a dynamic behavior powered by complex musculoskeletal couplings (Waltzek & Wainwright 2003; Westneat 2005). To generate suction fish must expand and protrude their jaws rapidly to create a negative pressure within their buccal cavities (Carroll 2004; Higham et al. 2006). The induced pressure drop then draws the prey item into the mouth of the fish. This rapid expansion is facilitated by the synchronized movement of a highly kinetic skull (Motta 1984). Many of these skeletal elements are arranged in linkage systems, wherein movement produced by a muscle at one location can cause movement in a series of more distant bones. For instance, the epaxial muscles elevate the neurocranium, which then pivots the quadratomandibular joints forward and causes the lower jaw to depress (Camp & Brainerd 2015).

In largemouth bass, the epaxial muscles that produce cranial elevation during suction feeding are the same muscles used to power swimming (Camp et. al 2015). Thus, the muscles that produce lateral bending are also primarily responsible for the dorsoventral rotation of the neurocranium. If morphology were to modulate dorsoventral movement about the intervertebral joints and the neurocranium, then it is likely to have a functional impact on feeding mechanics. However, it is not known where the axis of rotation between the neurocranium and the body is located during elevation of the head.

It is also unclear whether fishes have different axes of rotation associated with morphological differences in their vertebral columns.

More interesting, however, is the question of how specific morphologies contribute to bending of the vertebral column during suction feeding. Examples of extreme cranial elevation are not uncommon. Anatomical specializations for extreme head elevation range from well-developed hinge joints between vertebrae (Lesiuk & Linsley 1978) to highly mobile specialized joints (Lauder & Liem 1981). No data are available to examine the relationship between these specializations and their actual contribution to dorsoventral bending during head elevation. We use a combination of data taken from XROMM (X-ray Reconstruction of Moving Morphology) and VROMM (Video Reconstruction of Moving Morphology) to identify the axis of rotation during suction feeding in order to determine where axial bending is centralized and whether different the axial morphologies produce bending at different positions.

Methods

Fish Care and Marking

Leptocottus armatus (n=1) and *Embiotoca lateralis* (n=1) were collected in the San Juan Islands, WA. Fish were kept at Friday Harbor Labs in a flow through system where water quality matched that of their native environment. Fish were fed shrimp daily. To mark the neurocranium and body for video analysis, fish were anesthetized with a non-lethal dose of MS-222 and three to four white plastic beads were attached to the neurocranium and six to eight on the body (Fig. 1).

Feeding & Filming

Fish were filmed feeding on live shrimp and fresh shrimp meat. Three Photron high-speed cameras (model 1024PCI) recorded feeding events from 3 different angles. Feeding events were filmed at various frames rates (250 to 1,000fps). Before each feeding event, cameras were calibrated by placing an object inside the tank where the feeding event would occur. After filming, cameras were calibrated with a moving checkerboard (out of water). The checkerboard calibration served as a means to adjust for the different refraction index between water and air during filming.

VROMM Animation

Fish were euthanized with MS-222 and computed tomography (CT) scans were taken of the specimen with the implanted markers intact at Brown University. Polygonal mesh bone models were then made in OsiriX (version 5.6; 64-bit) and cleaned up in MeshLab. Videos were calibrated and the implanted markers were tracked using XMALab (1.2.12). Rigid body transformations generated in XMALab were imported into Autodesk Maya 2014 to animate the bones in 3D space. To animate a plane representing the motion of the body, a polygon plane was created in Maya, moved to the midline of the body in the CT scan, and then animated with the rigid body transformations from the 6-8 body-marker beads (all treated as one rigid body).

Joint Coordinate System Analysis

The Maya 3D animations contained two rigid bodies: neurocranium and body plane. To identify the axis of rotation during head elevation, joint coordinate systems

(JCSs) were created and aligned with the long axis of the fish (z-axis; Fig. 2). Joint coordinate systems were fixed within the body plane and translated only along the x and y axes. Multiple JCSs were created and placed at the basioccipital joint and each of the intervertebral joints (IVJ). The axis of rotation is defined as the joint where the JCS had the lowest y-translation values (Fig. 3). Axial joints were numbered along the rostrocaudal axis: For example, the basioccipital joint between the base of the neurocranium and the first vertebra was referred to as joint 1 and the intervertebral joint positioned between vertebrae 1 and 2 was referred to as joint 2.

Digital Image Analysis

CT images and anatomical features were analyzed using ImageJ. Distances were measured using an arbitrary, but consistent scale that nonetheless represents the relative lengths and distances of parts within the CT scan. All angles were measured three times and the mean value was used.

Results

Largemouth bass

Morphology

The thoracic vertebrae of largemouth bass can be divided into two regions (Fig. 4). The anterior region consists of the first three vertebrae and is characterized by wide spaces between the neural spines and pterygiophores. The anterior pterygiophores (1-3) have spine bases twice the distance from each other than those of the posterior region (4-7). Similarly, there is a pronounced difference in the distances between a neural spine and

the pterygiophore posterior to it. Neural spine to posterior pterygiophore distance in the anterior region is three times greater than that of the posterior region. Nonetheless, the distances between neural spines and their respective anterior pterygiophores are nearly identical for both regions.

The vertebral centra are generally amphicoelous, with the exception of vertebra 1. Vertebra 1 was rhomboid-shaped with the dorsal portion partially fused with the neurocranium (joint 1). Neural spine 1 was also talon-shaped, with the convex side resting against the neural spine and prezygapophysis of vertebra 2. Although neural spines 1 and 2 were tightly spaced, such was not the case for neural spines 2 and 3 which spread out at a greater angle. With these exceptions, all other structures (neural spines, pterygiophores, pre- and post-zygapophyses) were fairly undeviating in morphology.

Axial Bending

The axis of rotation was located strictly in joints 3 and 4 (Figs. 5 & 6). Bending centered at joint 3 produced an average cranial elevation of 17.2° while at joint 4 resulted in an average elevation of 15.9° (Fig. 7). None of the strikes had their center of bending in the posterior region, nor at joint 1 (partially fused) and joint 2 (neural spine overlay). Instead, center of bending was observed exclusively where neural spines and pterygiophores had the greatest space between them.

Staghorn sculpin

Morphology

The axial skeleton of the staghorn sculpin (Fig. 8) did not exhibit a distinctive

regionalization of neural spine and pterygiophore architecture like that of the largemouth bass. Instead of having widely spaced pterygiophores, there was a large space between the neurocranium and the first neural spine (found on V2). Vertebrae 2 through 4 had relatively small prezygapophyses that were nearly parallel to the long axis of the fish. However, starting at vertebra 5, the prezygapophyses became larger and formed wider angles. From V2 to V4, mean prezygapophysis angle was 15° whereas from V5 to V10 it increased to 36° . Increased prezygapophysis angles were also accompanied with an increase in intervertebral joint size starting at joints after V5.

Axial Bending

The axis of rotation for the five strikes of the staghorn sculpin were located at joints 3, 4, 5, and 6 (Fig. 8). Only one strike was detected for all joints except joint 4, which had a mean cranial elevation of 14° (Fig. 7). It should be noted that one of the two strikes with an axis of rotation at joint 4 had relatively little cranial elevation (7°), and this is because the fish struck at a downward angle toward the food item during that particular feeding event. Although this strike was unlike the other strikes for *Leptocottus*, we chose to include the feeding event in this study because it was a successful strike that revealed cranial elevation happens even for downward strikes. Finally, only the one strike at joint 6 was associated with the wide-angled prezygapophysis of V6.

Striped surfperch

Morphology

The structure of the deep-bodied surfperch consisted of long neural spines and pterygiophores running parallel to each other (Fig. 9). The anterior vertebral column, from the neurocranium to V2 had a slight downward bend. The V1 centrum was irregularly shaped, and rest of the vertebrae were amphicoelous. The V1 also had a neural spine that was positioned closely to the neurocranium with its anterior base forming a groove that matched to the corresponding protrusion on the posterior of the neurocranium. The fourth pterygiophore was long and extended down to make contact with the posterior side of neural spine 2, where it appears neural spine 2 and 3 form a wide angle. Most axial structures posterior to V4 are similar.

Axial Bending

Striped surfperch bending occurred strictly at joints 2 and 3 for all five strikes (Fig. 6). Mean cranial elevation when the motion was centered at joint 2 was 8.75° and 10.3° at joint 3 (Fig. 7).

Discussion

The vertebral morphology of these fishes corresponds to position of the axis of rotation during cranial elevation. For example, V1 and V2 of largemouth bass had robust neural spines where neural spine 1 appeared to curve and rest onto the bottom half of neural spine 2. However, space was available for bending at the IVJs between vertebrae 2 and 4 (Fig. 4).

In the case of the staghorn sculpin, the axis of rotation did not correspond to any unique spacing between neural spines and pterygiophores. Staghorn sculpin had a fascinating change in the overall structure and orientation of the prezygapophyses posterior to V5 and these changes were also accompanied by the widened intervertebral joints. Although only 1 strike was associated with any of these structures, their peculiarity makes them worth describing. It is unclear what contributions may be made by the larger and wider-angled prezygapophyses, however, personal observations made from feeding events and descriptive dissections do confirm that these do indeed contribute to whole body flexibility (both lateral and dorsoventral bending). It should also be made clear that an axis of rotation denotes the center of bending happens close to (but not at) these specialized prezygapophyses, so it is possible that bending occurs at these joints. But it still remains unclear what other role they may play, especially in locomotion.

The surfperch vertebral column had a subtle bend from the neurocranium to V2, making it difficult to quantitatively capture morphology with measurements of neural spine angle. All axes of rotation were located in this specific region, and it is possible that the vertebral column goes from a resting bent shape to an actively straightened shape during cranial elevation.

Our analysis also revealed that there are interesting structures present in axial skeleton of fishes that can facilitate dorsoventral bending during cranial elevation. However, there are multiple ways to acquire this flexibility including changes in the morphology of neural spine and pterygiophore structure, prezygapophysis orientation, and overall shape of the vertebral column. Still, much is to be learned about these

structures as they contribute to feeding, locomotion, and body form in general. Thus, a thorough understanding of these structures requires morphological investigation that is keenly aware of the multifunctional potential of these systems.



Figure 1. Marker placement on the body of *Embiotoca lateralis*

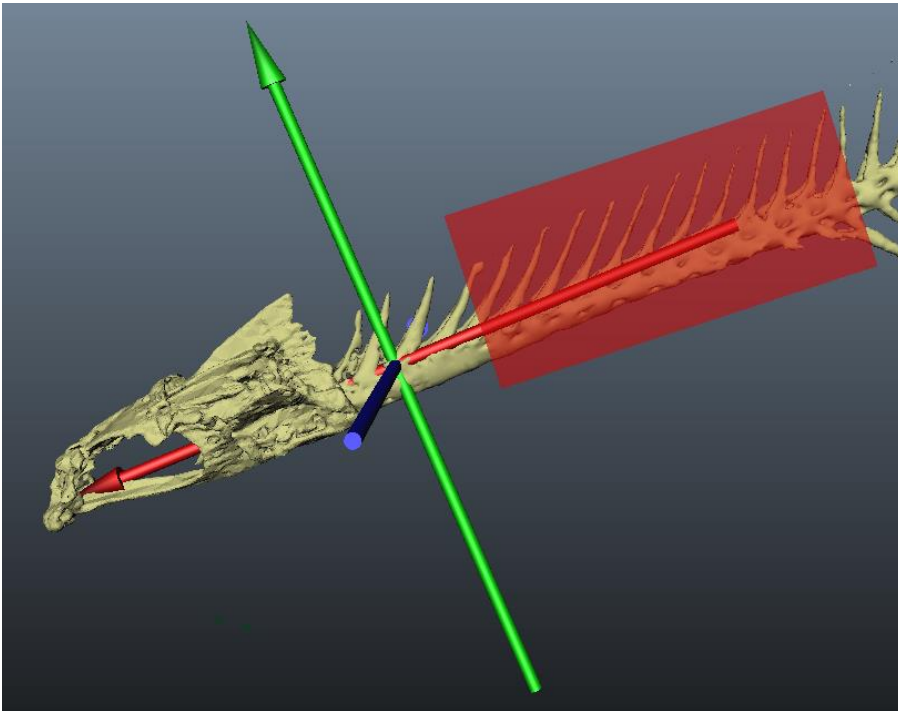


Figure 2. Joint coordinate system (JCS) placed between the neurocranium and body plane (red rectangle) of *Micropterus salmoides*. Although the vertebral column itself is not

animated, it is shown here for reference. X (red), Y (green), and Z axes (blue) are all shown.

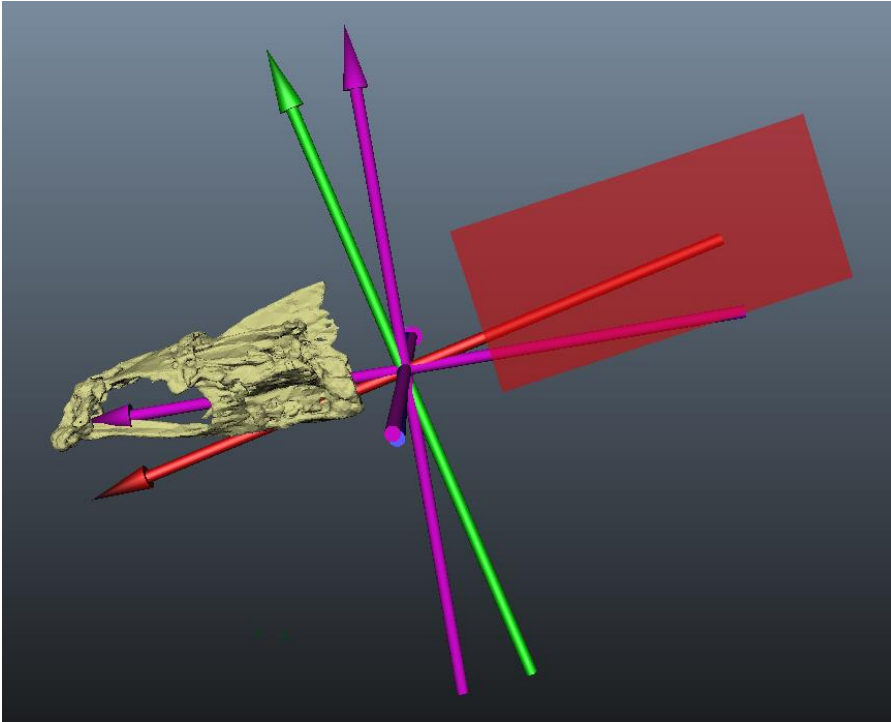


Figure 3. During cranial elevation, one coordinate system (red, green, and blue) remains fixed to the body plane. A separate coordinate system (pink) follows the motion of the neurocranium during head elevation. Displacement of these 2 coordinate systems from each other is quantified in the form of rotation and translation.

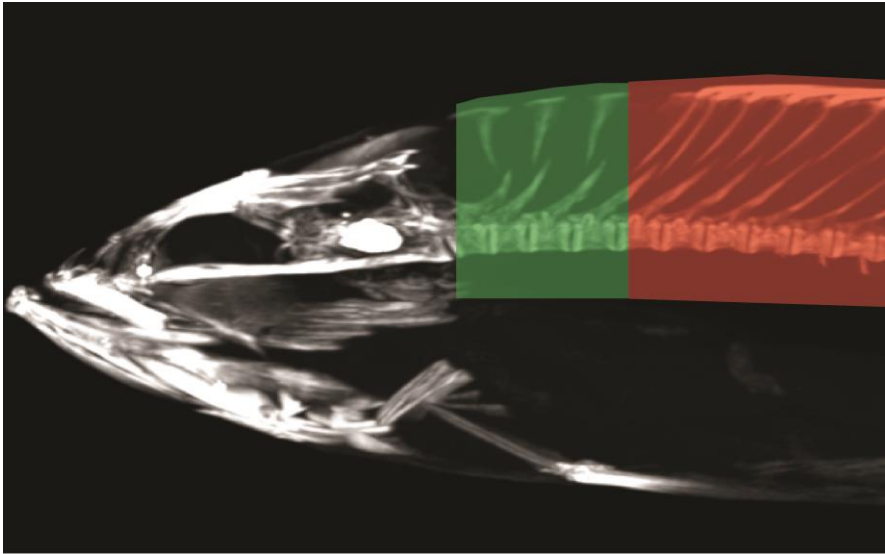


Figure 4. CT scan showing a lateral view of the skeletal morphology in largemouth bass (*Micropterus salmoides*). Thoracic vertebrae can be divided into anterior and posterior regions based on the differences of spacing of neural spines and pterygiophores. The anterior region (green) is characterized by the large spaces between pterygiophores, whereas the posterior region (red) has consistent and narrow spacing between the axial features.

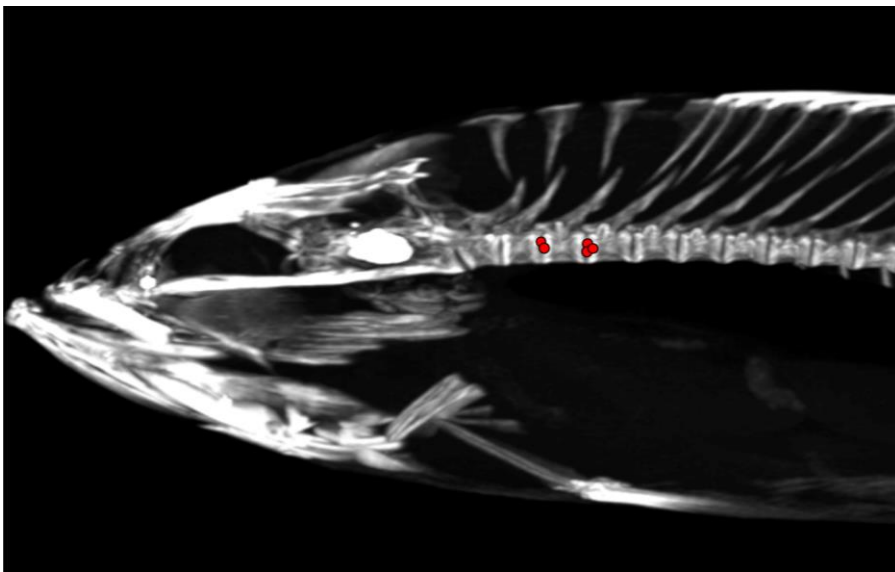


Figure 5. The same view of the CT scan showing where along the vertebral column the axes of rotation were identified (*Micropterus salmoides*). The exact points were drawn

in after in place of the joint coordinate systems for simplicity. They positions are accurate to the joint at which they occurred.

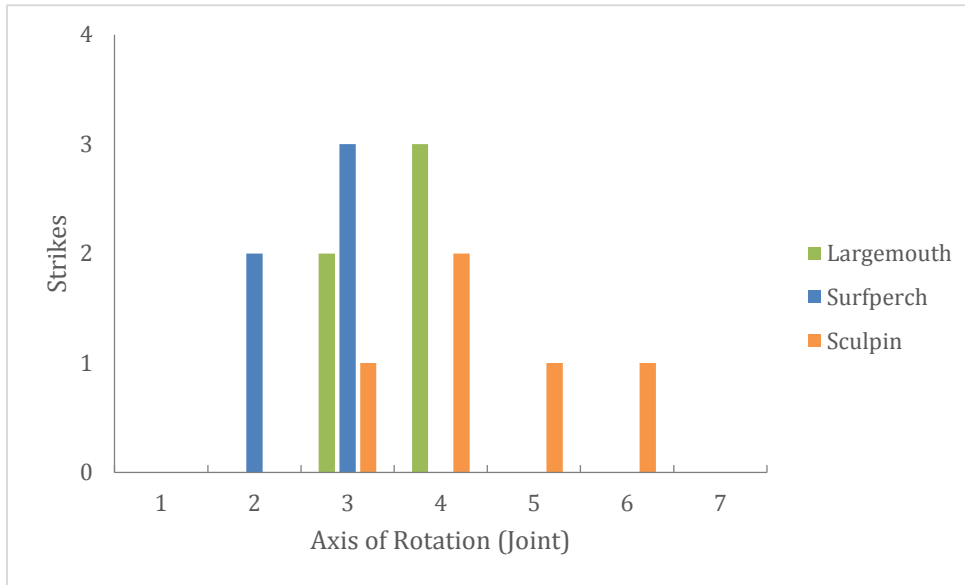


Figure 6. On the x-axis is the axis of rotation (center of bending) for each joint. Joint 1 is the basioccipital joint (neurocranium and vertebra 1) for each fish, joint 2 is the joint between vertebrae 1 and 2, and so forth. On the y-axis is the number of strikes observed for a particular axis of rotation.

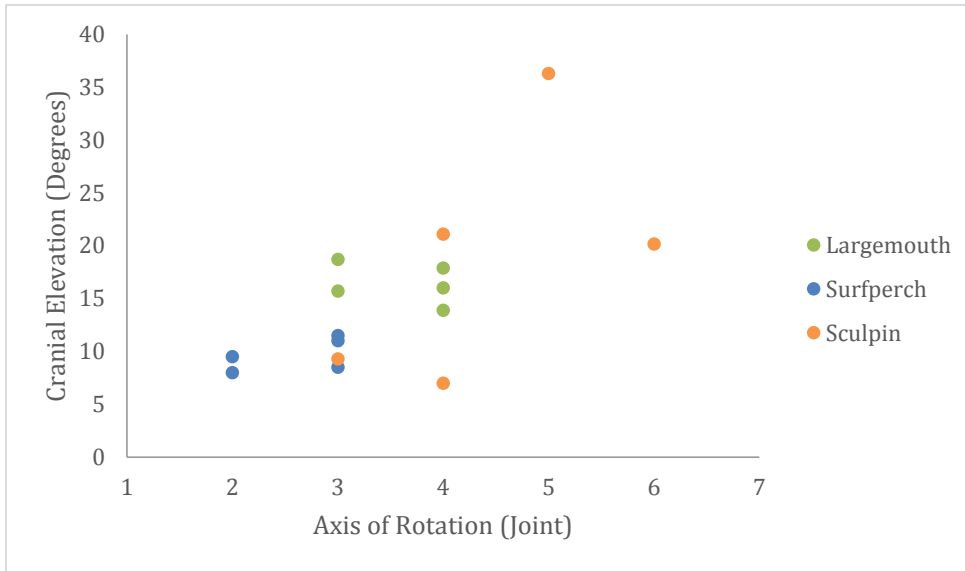


Figure 7. The axis of rotation as it relates to the amount of cranial elevation for each species.

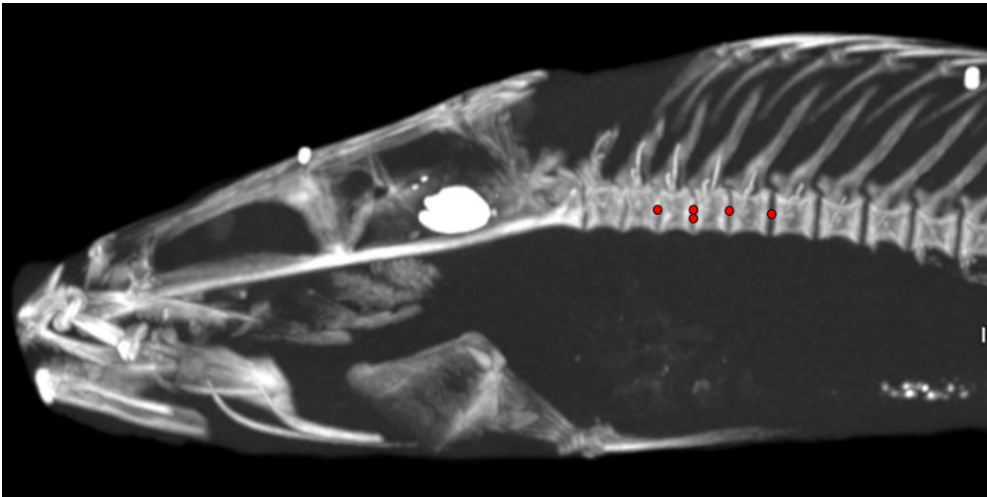


Figure 8. CT scan showing a lateral view of staghorn sculpin (*Leptocottus armatus*) showing the anatomical position where the axes of rotation were identified.

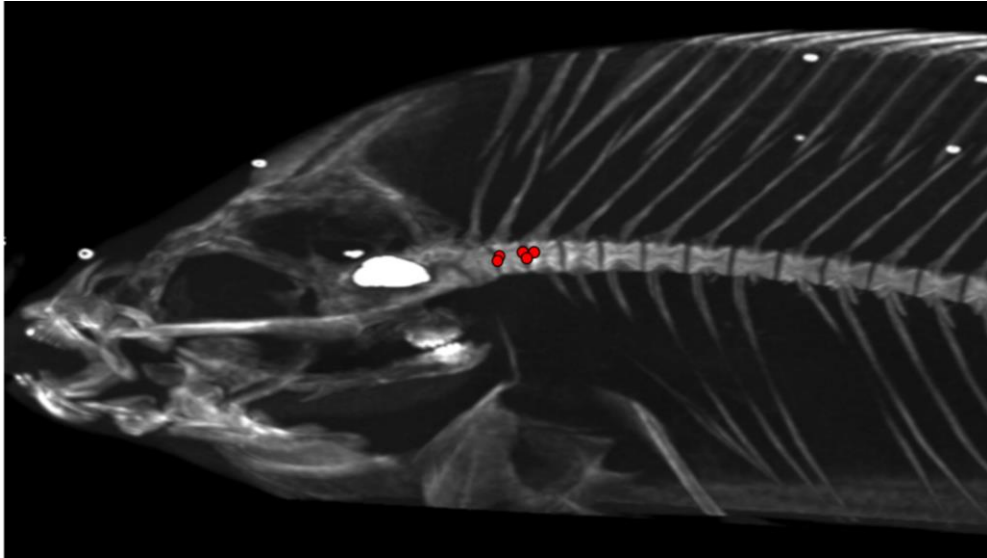


Figure 9. CT scan showing a lateral view of striped surfperch (*Embiotoca lateralis*) showing the anatomical position of where the axes of rotation were identified.

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