DIPLOMARBEIT

Titel der Diplomarbeit

Functional morphology of the occipital and neck region and prey capture in gars (*Lepisosteus sp.*)

verfasst von

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Abstract

Lepisosteidae represent an ancient group of actinopterygian fishes (ray finned fishes), which have their origin, according to different sources, in a time span of 75-180 million years before today. They haven't experienced many evolutional changes what gives reason to name them living fossils.

Despite their very inflexible integument built by heavy ganoid scales they are able to quickly overcome their prey by a rapid sideways lunge of the head. Including this point and the fact that there was very little scientific work in this area so far, they represent an interesting research object. It is important to find out, which morphological preconditions are responsible for this fast movement of the head while capturing prey. Bony, cartilaginous and muscular structures in the occipital and neck area have been examined by analyzing computed tomography images which gave a good insight in the anatomy of these animals.

A few structural circumstances could be identified, which eventually support or even allow those movements. Findings in this area include muscular, bony and cartilaginous structures. However, the concrete reasons for their movability are still to find out in further scientific investigations.

Zusammenfassung

Lepisosteidae stellen eine alte Gruppe von Actinopterygiern (Strahlenflossern) dar, welche ihren Ursprung laut unterschiedlichen Quellen in einem Zeitraum von 75-180 Millionen Jahre vor heute haben. Sie haben relativ wenige evolutionäre Veränderungen erfahren, was Anlass dazu gibt, sie als lebende Fossilien zu bezeichnen. Aufgrund der äußerst unflexiblen Körperhülle aus dicken Schmelzschuppen und der doch schnellen Ausführung des Beuteschlages mittels einer schlagartigen Seitwärtsbewegung des Kopfes, stellen die Knochenhechte interessante Forschungsobjekte dar. Aufgrund der bisher unvollständigen wissenschaftlichen Auseinandersetzung auf diesem Gebiet gilt es unter anderem herauszufinden, welche morphologischen Voraussetzungen für die schnelle Bewegungsausführung beim Beuteschlag ausschlaggebend sind. Diese Arbeit untersucht hierbei die Strukturen im Hinterhaupts- und Nackenbereich mittels computertomographischer Aufnahmen, die einen guten Einblick in die knöcherne, knorpelige und muskuläre Anatomie dieser Tiere geben.

Es konnten diverse strukturelle Gegebenheiten identifiziert werden, die eventuell eine solche Bewegung unterstützen oder sogar ermöglichen können. Erkenntnisse dahingehend umfassen sowohl muskuläre, als auch knöcherne und knorpelige Strukturen. Die konkreten Voraussetzungen für die Beweglichkeit gilt es aber in weiteren Studien genauer festzustellen.

1 Introduction

Lepisosteidae as living fossils (Wiley, 1976) represent interesting research objects, which have undergone quite a little amount of changes by evolution and can be traced back to at least 75 million years (Grande, 2010) up to 180 million years (Wiley, 1976) before today. They are usually characterized by their elongated jaws, riddled with spiky teeth, elongated body form and, as a key feature, massive ganoid scales (Grande, 2010; Wiley, 1976). Living *Lepisosteus* consists of four different species*: L. oculatus, L. osseus, L. platostomus an L. platyrhincus.* The survey on hand focuses on individuals of the species *L. oculatus*, but in order to the close relationship in their phylogeny among gars, different species will be discussed. The genus *Atractosteus* is also part of the *Lepisosteidae* and consists of the species *A. tropicus*, *A. spatula* and *A. tristoechus* (Wiley, 1976). Of course also Atractosteus specimen will be included in the research work on prey capture of living gars.

Usually gars can be found in the area of North- and Central America, as well as Cuba and the Isle of youth. The northern limit of their occurance is the southern part of Quebec, the southern limit is located around Costa Rica (Comabella *et al.*, 2006; Alfaro, González and Ferrara 2008; Grande, 2010). In general they fill different habitats like rivers and lakes. Nevertheless they also appear in different water types like freshwater, brackish water as well as marine environments (Grande, 2010).

Due to the thickness of the ganoid scales the assumption exists, that they degrade the swimming performance, especially during fast starts, for example during capturing prey or escape maneuvers (Webb *et al.*, 1992).
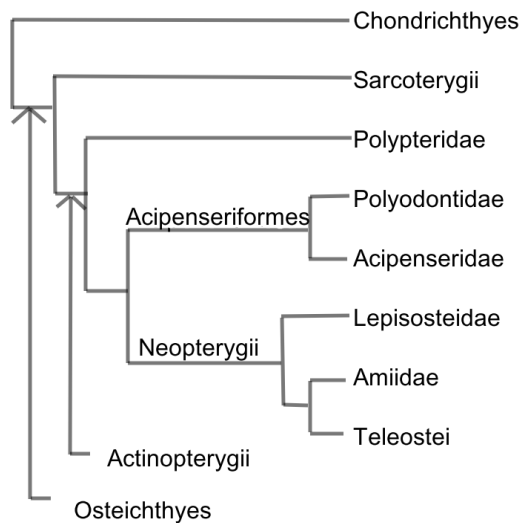
1.1 Phylogeny of living and fossil gars

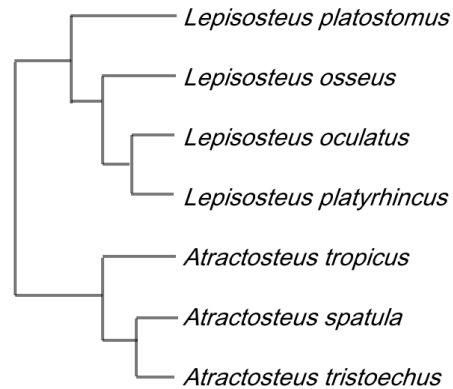Fig.1: Position of the *Lepisosteidae* among other fishes (after Bemis *et al.*, 1997)

Fig.2: Cladogram of recent *Lepisosteidae* (after Wiley, 1976)
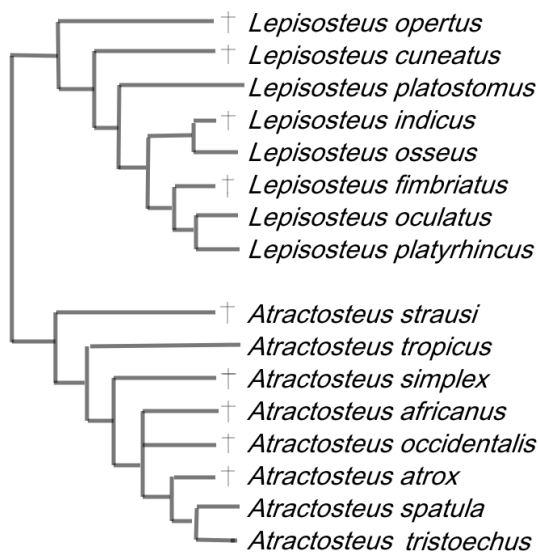
Fig.3 Cladogram of recent and extinct *Lepisosteidae* (after Wiley, 1976)

Figure 1 shows the localization of gars in a phylogenetic tree among other fishes, including all other bony fish and the non-bony ones, as Bemis (1997) declared it. Figures 2 and 3 show cladograms within the group of *Lepisosteidae*, both set up by Wiley (1976). The main difference is, that

Figure 3 additionally demonstrates the extinct species of *Lepisosteus* and *Atractosteus*, marked by a cross.

1.2 Feeding strategies

At first we will take a look at feeding in general, to find out what different feeding-types actually exist. The reader should put a focus on ram-feeding, what will offer us a proper crossover to the usual hunting behavior of *Lepisosteus*, the main objective of this thesis.

Taking a look at the different feeding habits of fishes, three general types of feeding can be distinguished: ram feeding, suction feeding and last but not least manipulation of prey within the orobranchial chamber (Liem, 1980). The following paragraphs will give a short review of how these usages of food supply differ from each other.

Ram feeding as the first named type is a very active way to capture prey. It is normally characterized by a very fast lunge of the whole predator's body towards the prey. This is followed by quickly opening and closing of the jaws on the prey (Wainwright, 1999). That kind of hunting tactics often is found among piscivorous fishes stalking elusive prey or waiting in a stationary position to engulf it such as the pike, *Esox sp.*, sharks or the gar, *Lepisosteus sp.* The forward-lunge often is induced by a so called s-start, where the predator's body forms an s-shape to build up tension within the lateral body musculature and then by releasing the energy, rapidly accelerating (Porter and Motta, 2004; Motta and Huber, 2012).

In suction feeding a pressure differential between the water column and the buccal cavity is generated from which a very fast flow of the circumfluent water into the mouth is the result when the mouth is opened (Wainwright, 1999). Although a rapid body movement to capture the prey is named above as a characteristic for ram feeding, it also can be found in a high number of suction feeding species, just to bridge the last remaining distance to the prey (Wainwright, 1999).

The third type of feeding to mention is manipulation. This part covers a quite broad horizon of taxa, for example many coral reef taxa like surgeonfishes, which feed by benthic-scraping (Wainwright, 1999; Winterbottom and McLennan, 1993). Actually smaller pieces of large prey are bitten off,

respectively the whole prey is detached from the underground by biting jaw movements (Wainwright, 1999).

An interesting fact to note is that a large number of all known fish species use not less than two of the three feeding types in combination (Norton and Brainerd, 1993).

1.3 Fast starts

First of all the different levels of activity are important to understand the classifications of start manouvers. In Beamish (1978) three main categories of activity are stated: (1) sustained (steady), (2) prolonged, and (3) burst swimming. Sustained swimming is characterized by long duration and appears without any muscular exhaustion. Burst swimming is the absolute opposite and is marked by very short duration at high velocity. Prolonged swimming is a category in between and contains a broader range of different speeds. The last two swimming types can result in muscular fatique (Beamish, 1978).

As a type of unsteady swimming, fast-start manouvers contain a quick increasing in speed from a not moving position up to high velocities. Those fast starts are important for both – prey and predator. The muscles produce a very high power that is transferred into hydrodynamic force, which moves the individual rapidly forward. Because of the fact that this acceleration is important for survival, again for prey and predator, fast-starts represent a form of maximum performance leading the individual to its physical limit.

To go a little bit deeper into the topic, some facts about the muscle activity need to be considered. Muscles during certain start variations are activated simultaneously along one body side initiated by the so-called *Mauthner* neurons (a short description see below) (Wakeling, 2001). According to the higher amounts of power produced by muscles in the central part of the fish's body, the vertebral column in this area can be bent faster than the more caudal region. The appearing asymmetrical muscle contraction causes an undulating body movement towards the tail, which generates the hydrodynamic propulsion of the

fish in a forward direction (Wakeling, 2001). So the weaker caudal myotomes have the function to transmit the power generated in the body center by stronger myotomes to the water (Wakeling, 2001).

1.4 Body curvature

Body curvature in fish with fast starts is a common way to describe different start types. Generally two characteristic forms can be distinguished: The C-start, where the body forms a shape similar to the letter C and the S-start where an s-shaped body can be recognized. During the C-start where the body forms a C-shape, it is clearly visible, that the head and the tail are relocated to the same side, what is named the fast-body-bend. This stage is followed by a few motion samples of less extensive muscle contractions on the opposite site that finally lead to the stretching of the tail and with this to a forward propulsion. (Eaton *et al.*, 1977; Webb, 1978). S-starts were for example observed in the strike of *Esox lucius* by Schriefer and Hale (2004). They found out, that the movement patterns in this pre strike behavior is not initiated by the Mauthner neurons like the C-start, but contains a 'more complicated neural circuit' (Schriefer and Hale, 2004).
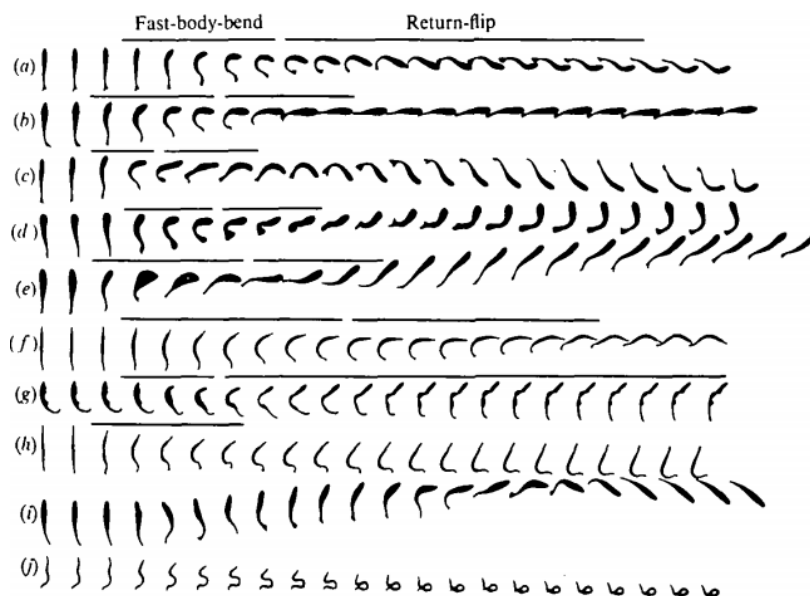

Fig.4: Different body curvatures during fast start manouvers from a dorsal view: (a) trout, (b) goldfish, (c) zebrafish, (d) catfish, (e) marbeled hatchetfish, (f) characid, (g) black ghost, (h) garfish, (i) calico rockfish, (j) spiny eel. (modified after Eaton *et al.*, 1977)

1.5 The *Mauthner* neurons

The *Mauthner* neurons or *Mauthner* cells are two large axons, running along the spinal cord with two special cell types in the anterior part of the white matter of the brain identified and named after Ludwig Mauthner (Mauthner, 1859). In physiological experiments, where the *Mauthner* neurons were excited, J. Diamond found that the stimulation of one of the paired axons causes a trunk- and tail swing to the side of the stimulated cell. Interestingly the stimulation of both neurons at the same time led to no muscular activity at all (Diamond, 1971).

1.6 *Lepisosteid* prey capture

The success in prey capture can be thought to depend on vegetation, but this is just secondarily important. Primarily the prey species, or the area it resides, is determining hunting success. Regardless of vegetation density, the surface of their habitat is the "place to be" for gars, when they are about to watch out for potential victims (Ostrand *et al.*, 2011). The lying-in-wait behavior beneath the surface helps to stay undetected from their prey, on which they can strike with a rapid lateral bending of the head after slowly approaching it, when it´s close enough. Additionally the residence close to the surface seems to serve as an energetically favorable area for foraging gars. (Ostrand *et al.*, 2011).

The partially lung breathing *Lepisosteidae* may also favor the upper area of the water column to avoid hyperventilation after exercise. According to some factors like viscosity and O_2 content, air breathing shows an energetical advantage to water breathing (Farmer & Jackson, 1998; Burleson *et al.*, 1998). Porter and Motta (2004) observed, that *Lepisosteus* shows a special stalking behavior, what is characterized by a very slow approach to the prey. They found out that this pre-strike performance could last quite a long time, up to a few minutes, until the predator positions his head laterally to the prey. From this position the next step is initiated by quickly bending the head and closing the jaws on the prey (Porter & Motta, 2004). The success of a predator's strike

stands in close connection to factors like reducing flight behaviors combined with predator-prey closure times (Webb, 1982).

Undulations of all fins are held very low what involves quite a little velocity. The strike itself takes not more than 40ms but can also occur more rapidly up to 25ms (Webb *et a*l., 1992). Webb *et al.* (1992) claim, that there could be a locomotory limitation in *Lepisosteus* because of the ganoid scales creating a heavy armored skin. In proportion to skin mass, muscle mass is quite little (Long, 1996). It is assumed that *Lepisosteus platyrhincus* is not able to form the s-shape, which would be essential for the production of high velocity in the strike (Porter and Motta 2004).

According to Lauder and Norton (1979) the feeding-process itself can be subdivided in three phases. They do not mention the slow approach as a part of it, but name the strike, the manipulation after capturing the prey and the swallowing, mainly head-first, as main phases. They implanted electrodes in the musculature of the anterior body to measure muscle activity during feeding in the gar. Result of the measurements is, that all observed muscles were active in a bilaterally symmetrical way, except the obliquus superioris (location see Figure 5) which experiences activation about 5-15ms before the other parts of the muscular system, what leads to the lateral bending of the head (Lauder & Norton, 1979).
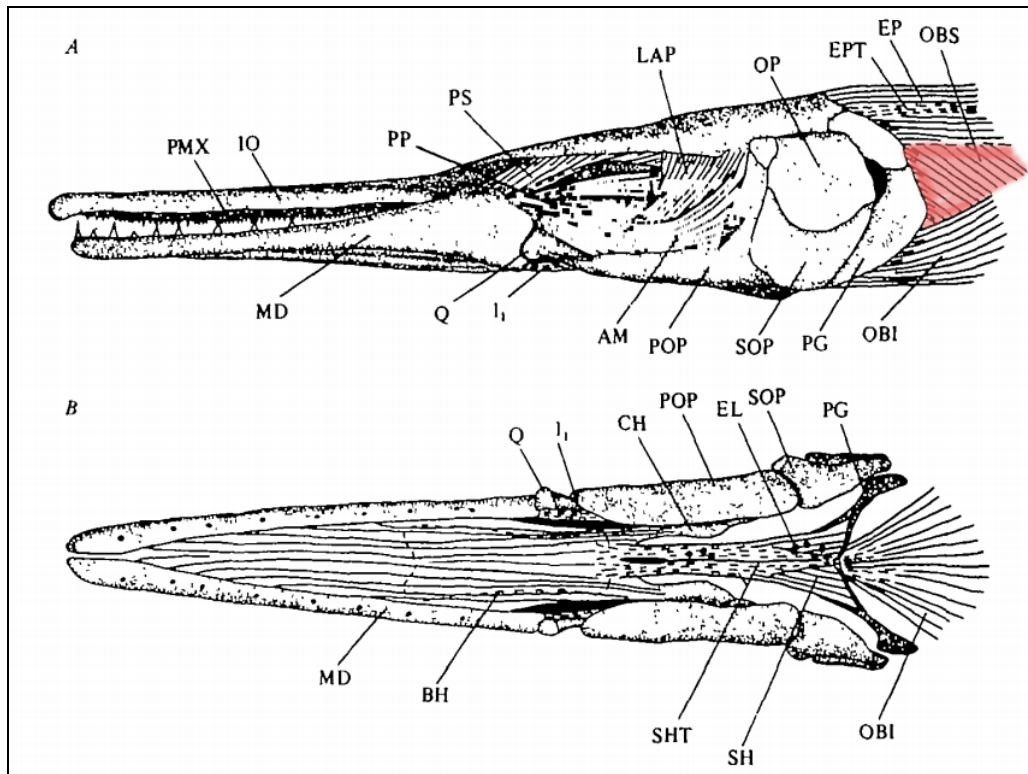
Fig.5: *Lepisosteus oculatus'* head in (a) lateral and (b) ventral view. OBS, obliquus superioris; (modified after: Lauder and Norton 1979)

2 Material and Methods

The specimen used for the present diploma thesis is on the one hand one individual of *Lepisosteus oculatus* at the size of 22cm total body length (from the tip of the snout to the tip of the caudal fin) (Figure 6). It was taken for CT scan and therefore dispatched in clove oil. Afterwards it was put into different concentrations of potassium iodide in ascending order: 30, 40, 50, 60 and 70 percent (laboratory protocol to be found among the supplementary materials).

Fig.6: (a) Lateral and (b) dorsal views of the freshly obtained specimen and before preparation for potassium iodide-staining.

To examine the specimen without destroying any important structures, it was scanned with the µ-CT of the Department for Paleontology, University of Vienna (Fig.7). The µ-CT-scanner used for recordings was a SkyScan1173 with following settings:

filter	Al 1mm
frame averaging	ON (4)
random movement	ON (20)
rotation step	(deg)=0.200
source voltage	(kV)= 100
source current	(µA)= 80
image pixel size	14.96µm

The principle of computed tomography:

A fixed x-ray source sends its rays focussing on the examined object. Afterwards they arrive at a so-called detector right behind the object in weakened conditions. As already said, the object of interest is positioned in the middle and can be turned in the focus of the x-ray-source on its own axis. The resulting planar pictures are then calculated into single slices (Grillenberger & Frintsch, 2012). The screenshots from the different CT-slices were taken in sagittal, frontal and transversal view with computer software DataViewer 1.5.1.2.

Fig.7: SKYSCAN µCT at the Department for Paleontology, University of Vienna

After the µCT scanning, the individual was cleared in an enzymatic solution and stained with alcian-blue for cartilage and alizarin-red for bones. The procedure followed a mixture of a few different sources how to clear and stain vertebrates, including Dingerkus and Uhler (1977), Taylor and van Dyke (1985) and Song and Parenti (1995). The main difference to all of them was the used enzyme. Instead of Trypsin I used Papain. This enzyme was completely different to handle, because practical methods with Papain in this context actually do not exist, so it

was like trial and error. The cartilage staining did not work for unknown reasons, but it had no influence on the result, mainly because analysis of the cleared and stained individual is not the main goal of this thesis. Nevertheless, the laboratory protocol can be read in the supplementary materials.

The other specimen were held in a 500l freshwater tank (Figure 8) also at the Department of Paleontology, University of Vienna. To observe their prey capture behavior, a camera was positioned right in front of the tank during feeding times. To ensure capture success, the individuals haven't been fed two days before recordings. They got used to be fed with life prey of neon tetra *Paracheirodon innesi* (Figure 9), so a change to other prey didn't seem useful. The neon tetras were purchased from a local commercial supplier of aquarium fish and accessories in Vienna and kept in a 54l freshwater tank.

Fig.8: 500l freshwater tank at the Department for Paleontology, University of Vienna

Fig.9: *Paracheirodon innesi* (image source: http://fishlaboratory.com/fish/neon-tetra-paracheirodon-innesi)

3 Results & Discussion

Main topic of this chapter will be the discussion of the morphological structures by analyzing the µCT slices from different positions, followed by a short description of the observed capturing behavior.

3.1 µCT-analysis

I will start on the left body side at the height of the horizontal septum (horizontal septum see Fig. 10c below, yellow circle). The analysis will move on along the septum towards the vertebral column. Anterior end of the examined section will be approximately in the middle of the individual's gills from dorsal view (see red line in Figure 10a below), the posterior end will be determined by the fourth vertebra, more precisely by the very posterior end of its body (see yellow circle in Figure 10a below). Simultaneously the survey will start on the dorsal top and takes the sagittal direction to the vertebral column. The anterior end, marked by the red line in Figure 10a will also represent one starting point, from which the examination will take the caudal direction.

Abbreviations used to determine different anatomical parts are listed below and partially follow Lauder (1980) and Lauder and Norton (1979):

Bas.Oc., basioccipital

EP., epaxial musculature

Ex.Oc., exoccipital

HA., horizontal arch

HS., horizontal septum

HY, hypaxial musculature

NA., neural arch

NC., neural canal

PS, parasphenoid

V., vertebra

AM., adductor mandibulae

BH., outline of the basihyal

CH., ceratohyal

EPT., lateral tendon of the epaxial musculature

IO., infraorbital bones

LAP., levator arcus palatini

MD., mandible

OBI., obliquus inferioris

OBS., obliquus superioris

OP., operculum

PG., pectoral girdle

PMX., premaxilla

POP., preoperculum

Q., quadrate

SH., sternohyoideus

SHT., median vertical septum, which divides the left and right halves of the sternohyoideus
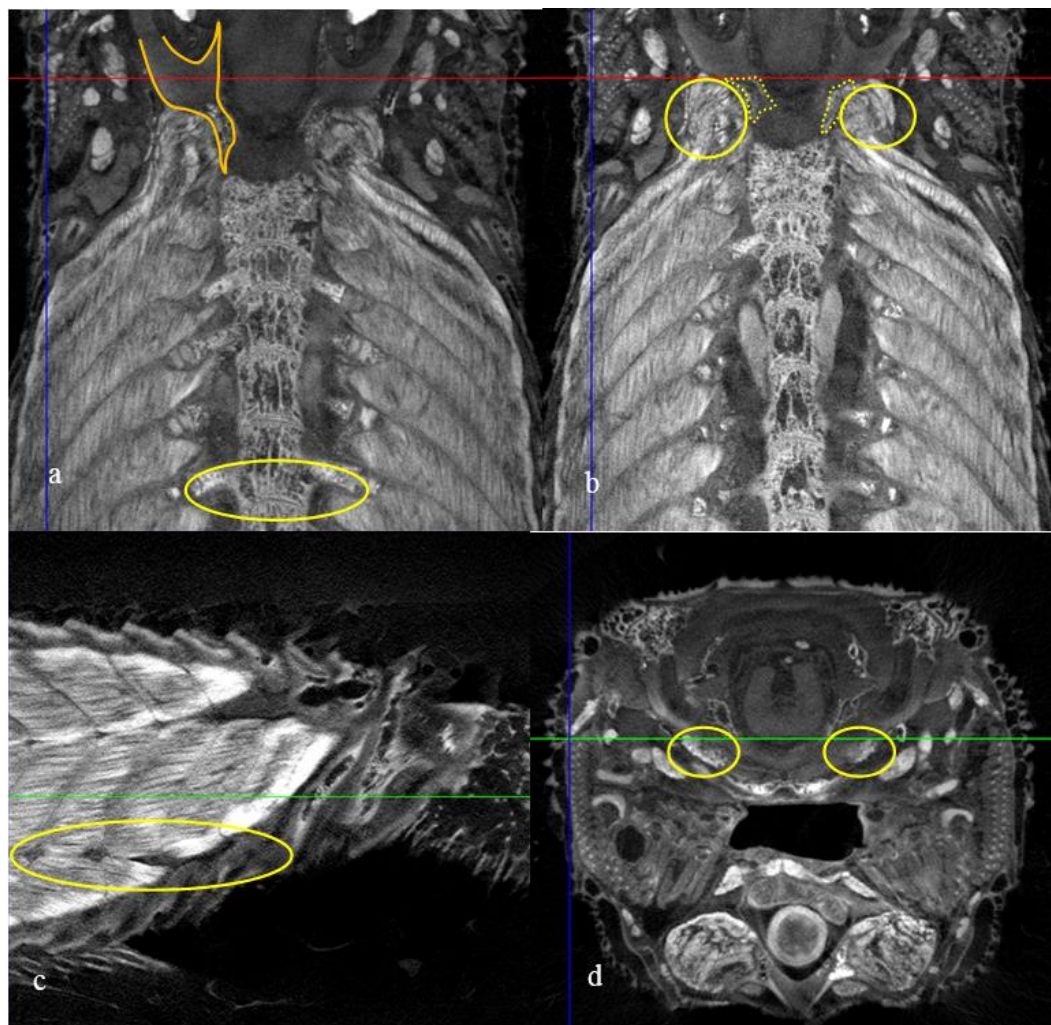
SOP., suboperculum

Fig.10: (a) and (b) dorsal, (c) lateral and (d) frontal view of iodide stained *Lepisosteus oculatus* after µCT scan

Figure 10a shows, that the area at the anterior end of the examined section around the red line has a complete lack of muscular structures (for orientation see Figure 10: identified as muscles are the bright, longitudinally striped structures to the left and the right of the vertebral column). The neurocranial elements in this area are hardly ossified but mainly consist of cartilaginous substance (Figure 10a, continuous orange line).

Patterson (1973) compared a few groups of actinopterygian fish and showed the conforming and the mismatching ossification centers of the neurocranium. As ossified neurocranial segments of the gar-head he mentioned following parts: basioccipital, exoccipital, pterotic, prootic, sphenotic and the orbitosphenoid. Two years later he claimed, that large parts of the braincase of *Lepisosteus* are not bony, but cartilaginous (Patterson, 1975). This fact can easily be recognized in the different computed tomographic images in the paper on hand.

Malcolm Jollie (1984) described the development of the cranial and pectoral girdle bones, what also gives us an interesting insight in the ossification of the gar head. The ossification starts with the basioccipital at a fish size of 25mm, the adult size is mentioned by Jollie to start at a specimens size of 150mm (Jollie, 1984). All this matches with the fact, that the basioccipital of the present individual is the most ossified bone of the braincase.

Incomplete cranial ossification seems to be not unique in *Lepisosteidae*, other basal actinopterygian fishes also show the same pattern. Ramsay Traquair wrote about the, as he calls it 'primordial', cranium of *Polypterus*, which shows large cartilaginous parts. Especially the otic region is one of the non-ossified centers of the cranium (Traquair, 1870). Interestingly this region plays an important role in cranial elevation in *Lepisosteus*, because the occipital tendon causes the cranial movement by transferring muscular contraction forces to its attachment area at the epiotic (Gemballa and Röder, 2004). Patterson (1973) also did not mention the epiotic as an ossification center, so similarities between *Lepisosteus* and *Polypterus* in this field do exist.

Further information about the work of the occipital tendon and its importance for prey capture in *Lepisosteus* read conclusion 5.

Going a few slices further in caudal direction the first epaxial muscular structures are visible (see Figure 10b and d, yellow circles), attaching to the exoccipital region of the neurocranium. The epaxial represents the muscles above the horizontal septum, the muscles below are represented by the hypaxial. In this area the neurocranial elements are getting ossified. However the neurocranial ossification at this position is quite hard to recognize. In Figure 10b on the left side of the right yellow circle a thin bright area running caudally along the first muscle portion represents this first ossifications equally with a small brighter part in the right upper corner besides the left circle (ossifications highlighted by orange dotted lines).

Taking a look at the anatomy from the very dorsal view, epaxial muscles are covering the whole area on top of the occipital region with longitudinally running myomeres (highlighted by the orange dotted line in Figure 11, representatively for all myomeres in this region).
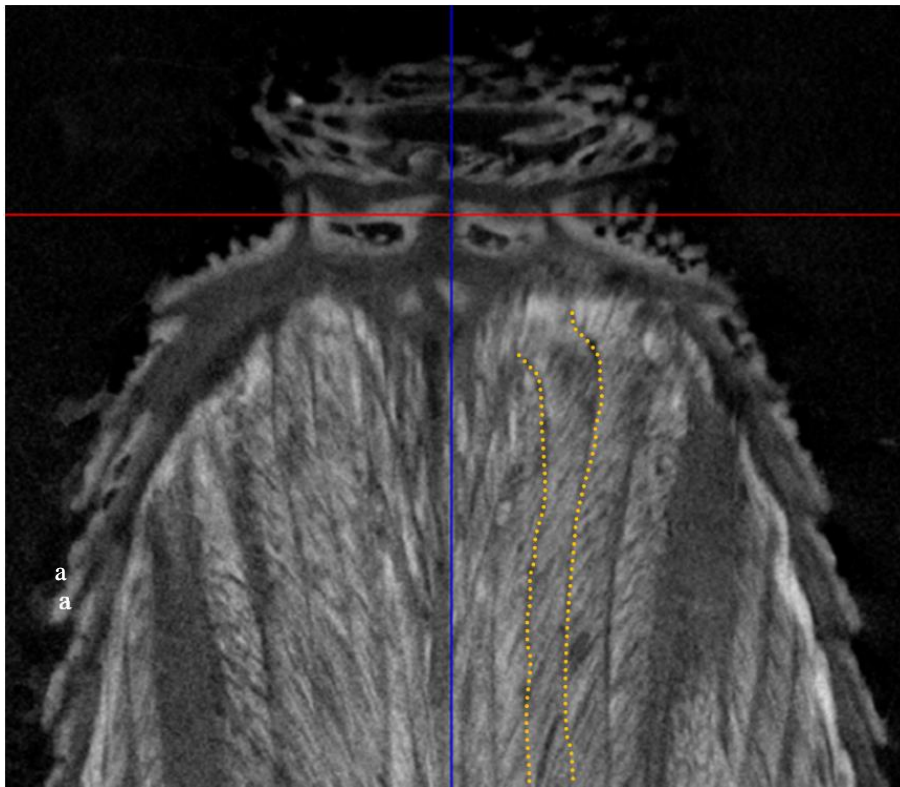

Fig.11: Dorsal view, starting at the very top of the body

Going deeper to the height of the neural canal (NC), the epaxial musculature is changing its arrangement into transversally running myomeres. Only a small portion is still longitudinally orientated (see Figure 12, yellow circles). The ossifications of the occipital region at this slice position are much higher than several slices before (orange dotted lines).
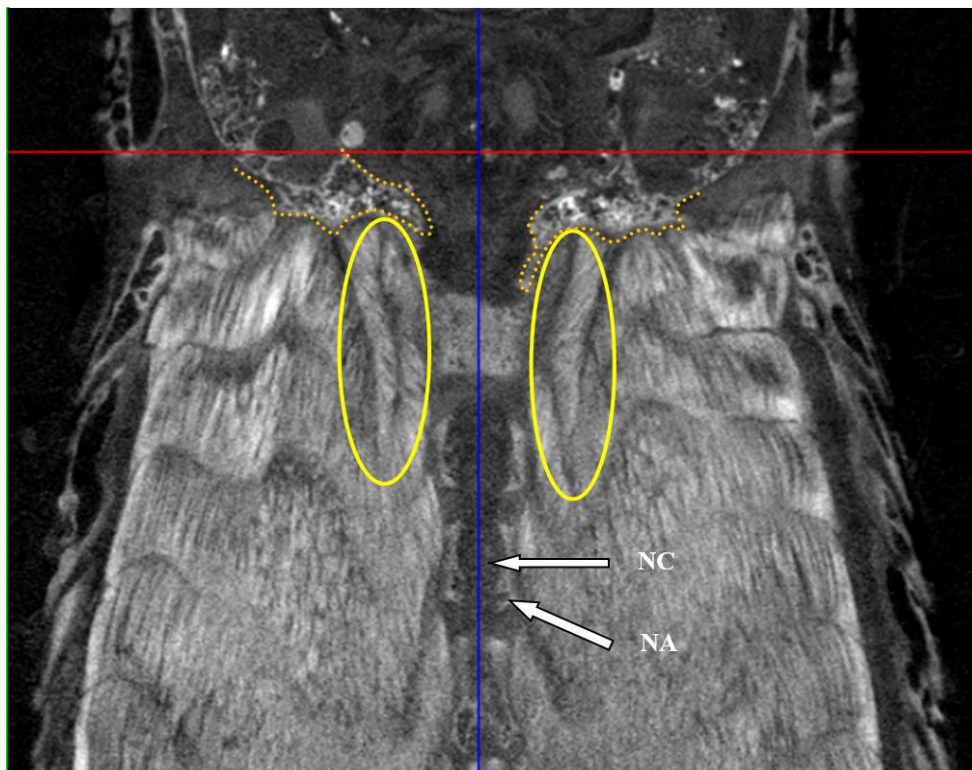

Fig. 12: Remaining longitudinally running myomeres; higher ossification of the occipital region

Those longitudinally running muscle portions are present downwards close to the broadest position in diameter of the neural canal. To this point they continuously get smaller until they totally disappear and only the large transversal myomeres are visible (see Figure 13).
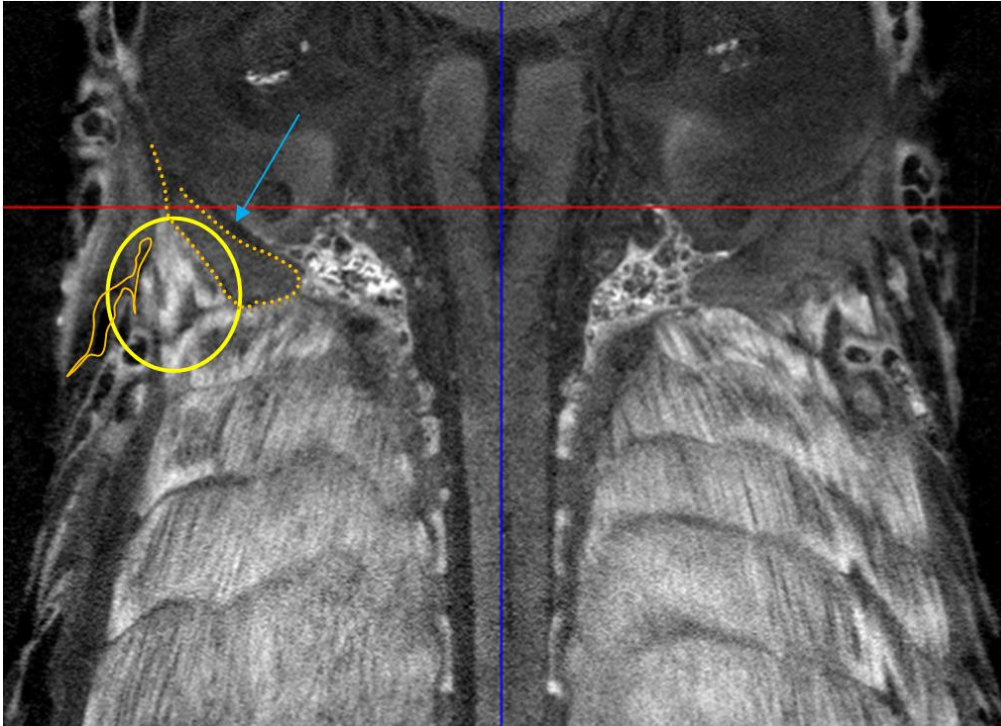
Fig. 13: Forward reaching muscles of the epaxial behind the scale joint; remarkable gap between cartilage
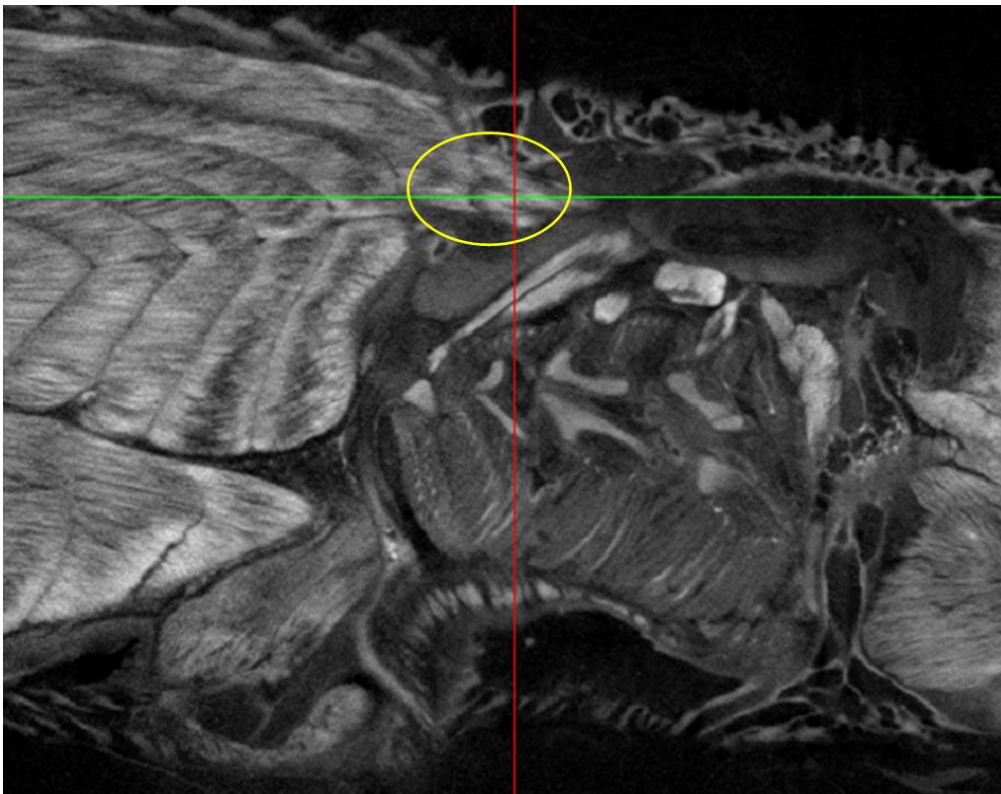

Fig.14: Lateral view of the forward reaching epaxial structure

The small muscular structure on the left body side (Figure 13, yellow circle) is orientated much more forward than the rest of the epaxial portions, seen in this figure from a dorsal view. The orientation of the muscle bands and associated with that the direction of contraction can´t be identified clearly. For a better identification a slice from lateral view is given in Figure 14. This figure shows, as well as Figure 13, that the muscle package is directed anteriorly, but now the orientation of the muscle bands is also visible. They run just like the other epaxial fibres longitudinally to the body axis. Combined with the flexible cartilage structures in this region (see Figure 13, orange dotted line) and the positioning directly behind the scale joint (see Figure 13, continuous orange line) a contraction of this muscle portion could probably support the sideways-upwards movement of the head. In Figure 13 there is also a gap between two cartilaginous portions (blue arrow) visible, what could act like a mobile joint and may foster a lateral-upward bending of the head. Of course this extended muscle portion is present symmetrically on both body sides.

The question remains open whether the gap is large enough to serve as a mobile joint and the muscle package is strong enough to move the head in a certain direction on its own or if it just supports some movements.
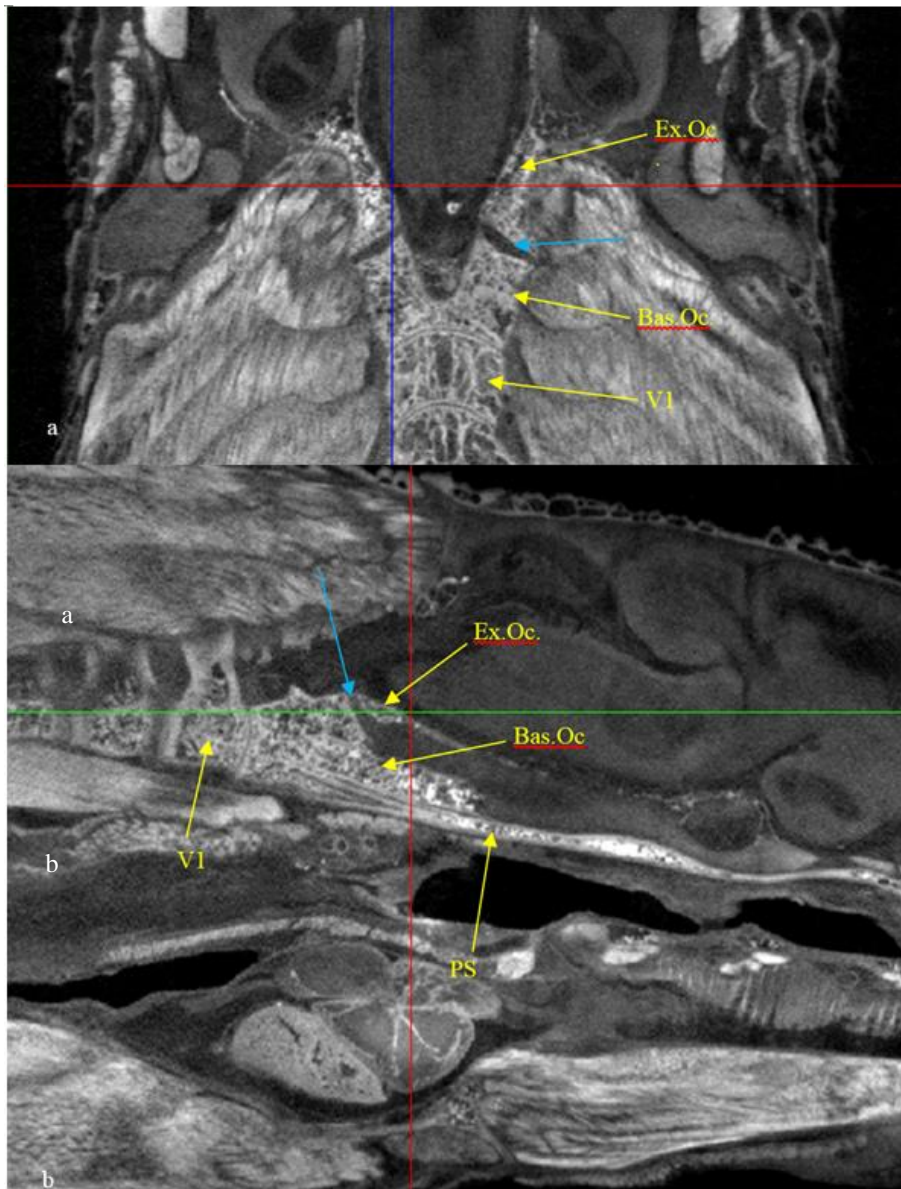
Fig.15: (a) dorsal and (b) lateral view of occipital and neck region

Figure 15a shows the dorsal view of a frontal cut through the specimen's body at the height of the vertebral centra. At this slice it's visible that the highly ossified basioccipital is immovably fixed to the very anterior end of the first vertebra with its posterior end, what logically would reduce mobility of the head and cause a stiff neck area. Figure 15b shows the lateral view of a sagittal cut through the body along the blue line in figure 15a. The ossification of the exoccipital region is not clear when taking a look at the section from dorsally, but the lateral cut offers a view which shows the only partly ossified exoccipital part of the neurocranium. The most interesting fact to see in

those pictures is the gap between the basioccipital and the exoccipital, marked by the blue arrow in both slices. It's not clear, if this is a complete gap or if cartilaginous material is located in between. However, if cartilaginous or not, this gap or connection could play a role in different movements and increase the mobility of the head.

There could even be another theory of the composition of the material between those two bones: Claeson and Hagadorn (2008) write about a soft tissue which encloses the occipital neural arch of the ropefish *Erpetoichthys calabaricus*, a basal Actinopterygian fish belonging to the *Polypteridae*. This tissue is not cartilaginous, but it's also not visible at computed tomography images. They claim, the neural arch is kind of freely floating in this matrix of soft tissue (Claeson and Hagadorn, 2008). So the gap between basicoccipital and exoccipital bone could also consist of this material what would create preconditions for higher movability.

The slices (Figure 17 a-d) should underline this theory by showing the continuous interruption between the exoccipital and the basioccipital bone beginning laterally on the left body side moving sidewards to the body center, marked by the blue arrow.

Moving further, the basioccipital is getting more massive. The ossified material is forming a solid body, which is posteriorly completely fixed to the convex anterior end of the first vertebra, the anterior end of the basioccipital has still no bony connection to the exoccipital (see Figure 18a). In addition, a few parts of the haemal arches are visible. The question remains, if there is any connection between the two endocranial bones at all.

Jollie (1989) describes the development of the exoccipital in gars where he mentions, that it first appears at a specimen's size of 25mm. From about 38mm total length a synchondrosis, a cartilaginous connection, with the basiocipital is visible, which separates the bony parts of the basioccipital and the exoccipital. He claims that in the adult gar, from about 150mm on, this cartilaginous junction does not exist anymore, but a continuous suture between the two bones establishes and the two sides of the exoccipital get together above the spinal cord and build the

foramen magnum (Jollie, 1984). This is also visible in Figure 16 but the non-bony connection in this individual remains (between the two orange dotted lines), in contrast to the description of Jollie. Malcolm Jollie used specimen of *Lepisosteus platostomus*, this paper used computed tomographic images of *Lepisoteus oculatus*. If this fact is decisive for the differences, it has to be cleared in further investigations.
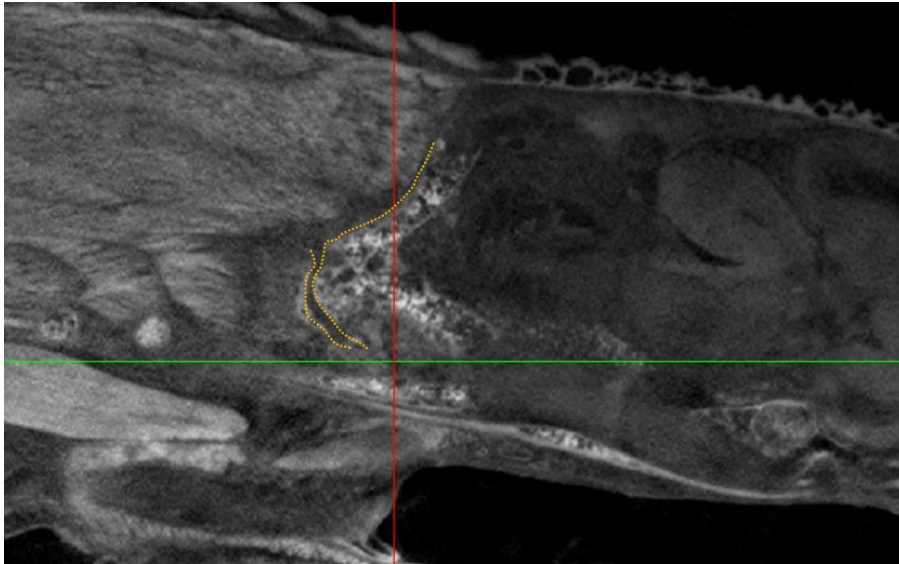

Fig.16: Foramen magnum from dorsal view

Furthermore Figure 18 shows a frontal view, which gives an insight of how the muscles are arranged left and right of the basioccipital (yellow circles). This arrangement can be found along the complete length of the fish's vertebral column. The tip of the anterior cone of the rearward myomere extends into the posterior end of the preceding myomere like the key and lock principle. This arrangement persists continuously from the basioccipital to the end of the vertebral column. Figure 19 a-d shows the muscular transition between the first and the second vertebra, representatively for all following myomeres (orange dotted line).
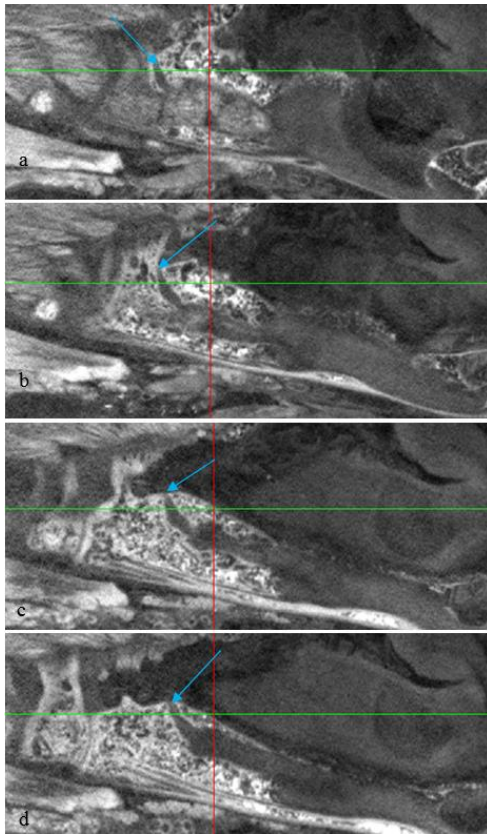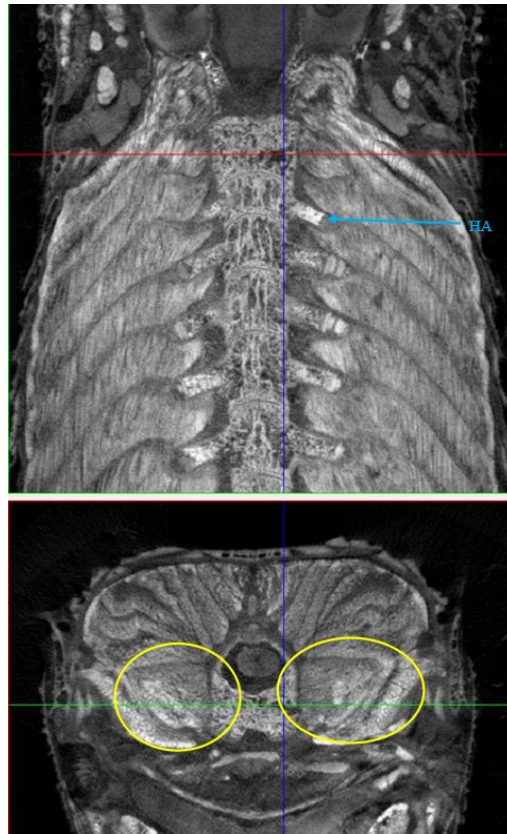
Fig.17: Interruption Ex.Oc. and Bas.Oc

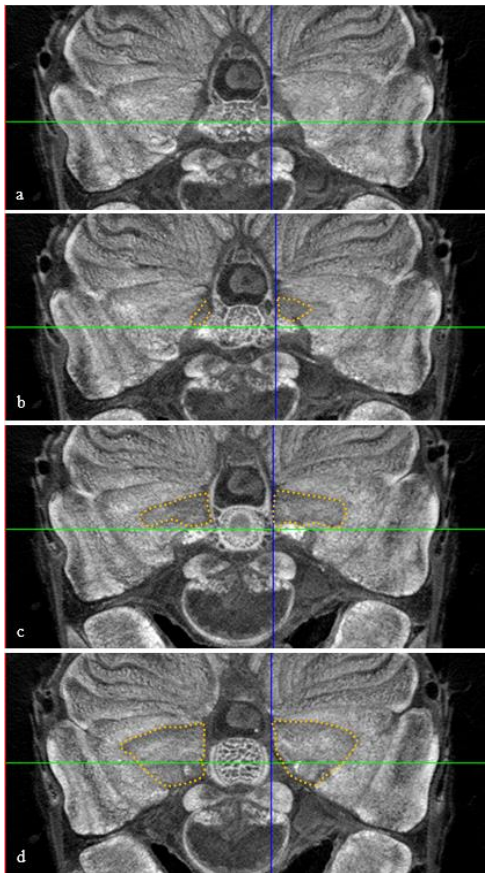Fig.18: HA and Bas.Oc.-accompanying muscles

Fig.19: Muscular transition

Moving on through the next slices, the bony and muscular architecture doesn't change and follows the same patterns as before: partly ossified epaxial, completely ossified basioccipital and vertebrae. The myomeres of the hypaxial still run in a transversal direction. The next anatomical change is visible a few slices below the bottom of the vertebral column, where the horizontal septum appears, which divides the trunk musculature into the upper epaxial and the lower hypaxial (see Figure 20 a-f). Interestingly Gemballa *et al.* (2003) write in their paper in which they compare the structure and evolution of the horizontal septum of different fishes, that *Lepisosteus* completely lacks a horizontal septum (Gemballa *et al.*, 2003). To ensure that this hypothesis was correct, I examined the cleared and stained individual via microdissection in 100 percent ethanol. I do not agree with Gemballa's and his colleague's theory of the not existing horizontal septum in *Lepisosteus*, due to my own observations.
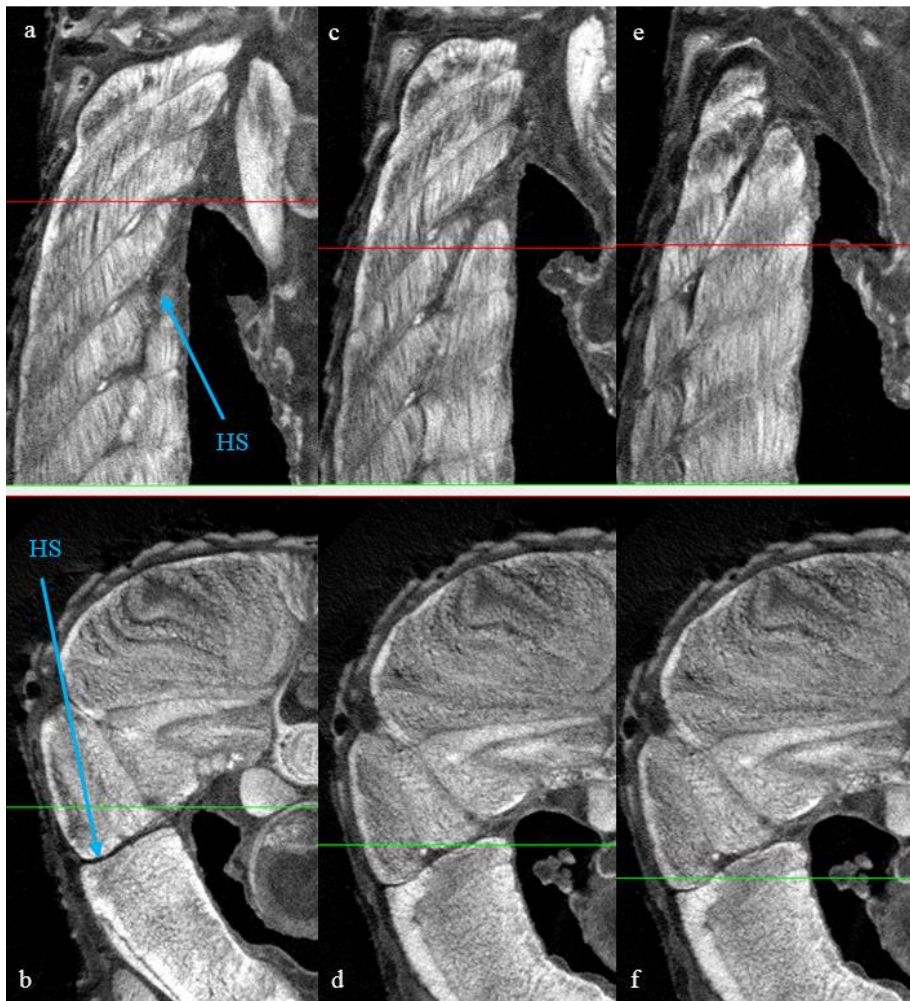

Fig.20: Horizontal septum

The last change that happens in the structural morphology of the muscular apparatus takes place near the bottom of the fish's body, in the lowest quarter when looking at the cross sectioned body from a frontal position. This portion is no longer part of the hypaxial and is probably part of the head musculature, but could, due to its location at the bottom of the head, as well as partly laterally to and below the hypaxial, play a role in sideways movement of the head to a greater or lesser extent. Visualization of this muscular segment follows in Figure 21 a-c and 22 a-d, highlighted by the yellow circles.
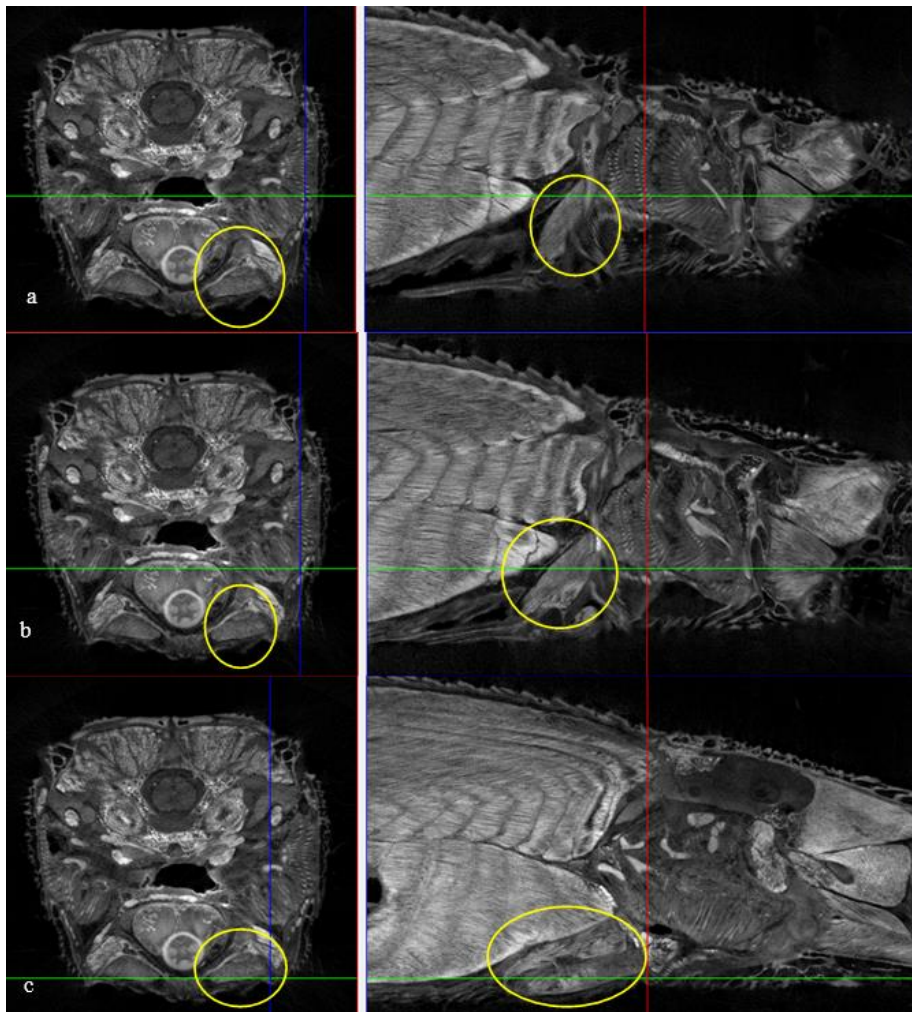

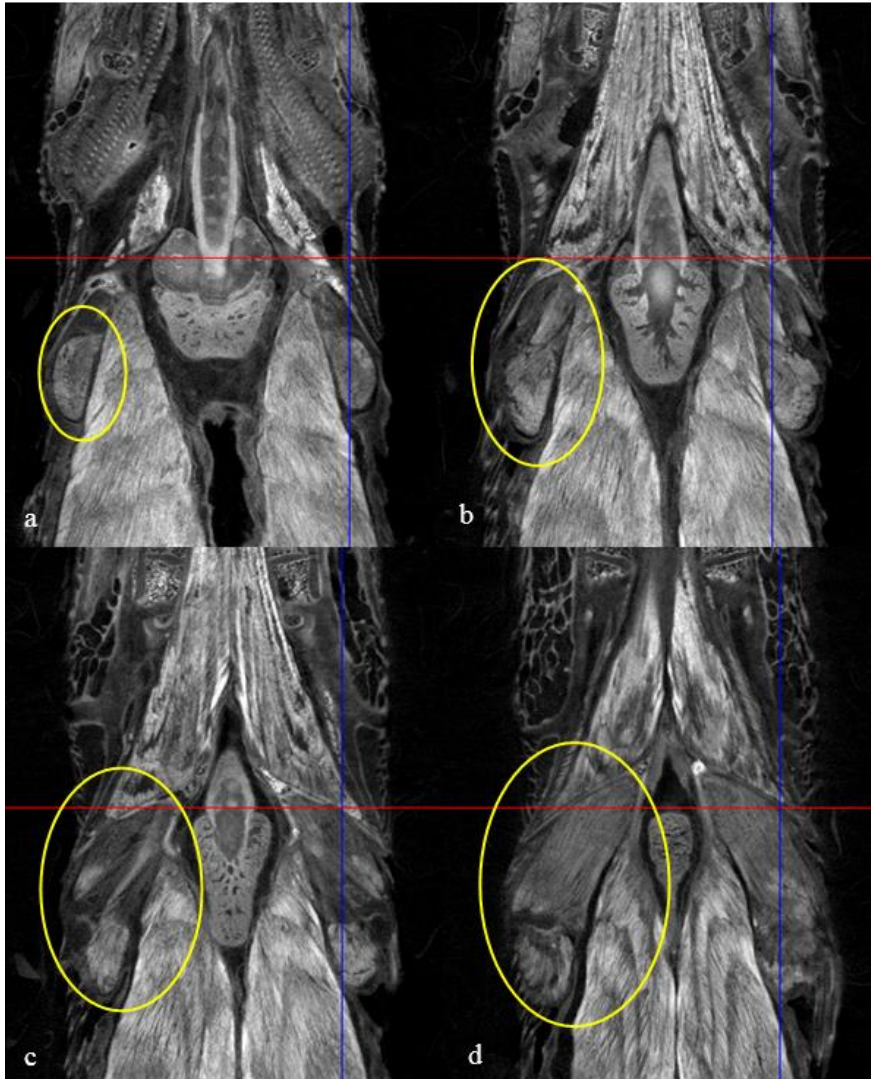Fig.21: Frontal and lateral views of bottom-located muscular portion

Fig.22: Dorsal view of bottom-located muscular portion

3.2 Film sequences

This short topic serves as a comparison to other observations of the process of capturing prey. Videos were taken with a camera resolution of 1280x720 pixels at 30fps. Results are shown in Figure 23.

In Figure 23, slice a-c show the slow approach of *Lepisosteus* towards its prey, characterized by minimum speed and very small fin undulations. Prior to this stage, the predator was stalking its prey for several minutes. Slice c shows the moment when he positions its head laterally to the prey's body, opens the jaws and initiates the rapid lateral bend of the head, shown in slice d. This slices shows a relatively intense curvature of the neck area but nearly no curvature in the caudal part of the trunk. Slice e shows the gar holding its prey orthogonally to
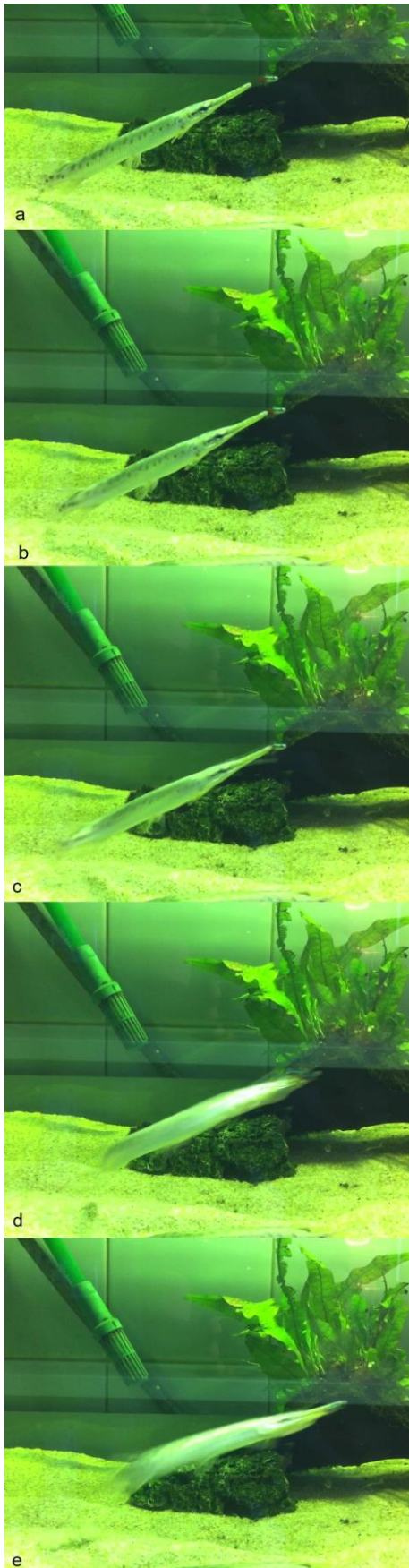
Fig.23: Capturing process

its jaws. Not pictured here are the following stages, where the predator manipulates the prey between the jaws to position it headfirst and then swallows it.

4 Conclusions

The investigations on the morphological conditions that possibly lead to the special sideways lunge of the head of gars resulted in a few conclusions which are now listed below to give a brief overview:

Conclusion 1:

Figure 13 and 14 show a small muscle portion that reaches much further than the rest of the epaxial muscular system around. Because of the anterior onset of the muscle package, it could eventually support a sideways-upward movement of the head. Due to its small mass, an exclusive function of movement in the mentioned direction can be excluded. A second explanation for this part of the body as an area of higher, respectively specialized movement is the positioning of this muscle portion right behind the scale joint. This section decreases the drag of the very stiff body and allows mobility to a certain degree.

The third movement-supporting fact could be the gap between the two cartilaginous portions of the neurocranium, highlighted in Figure 13, lying on the opposite side of the scale joint. This gap is located between two cartilaginous portions of which one is situated directly behind the small, lengthened muscle package.

Conclusion 2:

A continuous gap between the basioccipital and the exoccipital, shown in Figure 15 and 17, gives cause to think, this segment could support the movement of the head. As already described, it's not clear whether the gap is filled with cartilaginous material or not or even with the soft tissue described by Claeson and Hagadorn (2008), but in all cases the mobility of the head can be increased because there's no stiff connection to prevent higher range movements.

Conclusion 3:

The third main conclusion, which could help understanding the fast lateral lunge of the head of *Lepisosteus sp.* and *Atractosteus sp.* is shown in Figures 21 and 22. A muscle package at the bottom of the body on the left and right side, covering an area including parts of the head and the trunk gives reason to claim it meets requirements to move the head quickly sideways-downwards. A further interesting characteristic of this muscular portion is, that it's not directly connected to the hypaxial.

Conclusion 4:

This conclusion is already mentioned in the work of Lauder and Norton (1979), though indispensable to take into the conclusions of this paper. As already said on the first pages of this paper, they implanted electrodes for muscular activity measurements while feeding, what resulted with the finding of a separate activity of the obliquus superioris e few milliseconds before the other observed muscles in the anterior part of the body, so at least a supporting function can't be denied. They even claim, that the fibers of this muscle, which attach to the pterotic, the posterolateral margin of the skull, definitely lead to the lateral bending of the head. (Lauder and Norton, 1979).

Conclusion 5:

This point hasn't been worked out completely in this paper before, but it's necessary to include it in the process of understanding, how the functional morphology of gars while capturing prey works.

Gemballa and Röder (2004) worked on the myoseptal system of basal Actinopterygians. Myosepta consist of not extensible overlapping fibers of collagen, which form kind of a meshwork that allows deformation but no elongation and serves as an attachment area for muscle fibers (Bone and Moore, 2008). The group of specimen used for their research consisted of *Polypterus, Acipenser, Lepisosteus* and *Amia*. In a direct comparison via 3D reconstructions of the posterior part of the head and parts of the vertebral column of *Polypterus palmas* and *Lepisosteus*

platyrhincus they compared the length of the so called occipital tendon. This comparison shows the much more elongated tendon of *Lepisosteus*, reaching from the epiotic region to the sixth vertebra, in contrast to the occipital tendon of *Polypterus*, which reaches the area between the third and fourth vertebra. Regarding to the fact, that neurocranial elevation worked out by forces of the epaxial muscles is essential for the feeding mechanisms they claim, that the occipital tendon, able to gather forces from a higher amount of muscle fibers, transfers those forces to the neurocranium, its attachment point (Gemballa and Röder, 2004). Westneat (2006) also showed the very early activity of the epaxial muscles attaching to the neurocranium, which causes the neurocranial elevation, based on the results of EMG (electromyography) data. He also demonstrated the essential difference to biting fish species like for example piranhas, which show little or no activity of the epaxial muscles (Westneat, 2006). When Lauder and Norton (1979) examined the asymmetrical muscle activity during feeding in the spotted gar, *Lepisosteus oculatus*, they found two tendons like Gemballa and Röder (2004) did, but named them lateral tendons of the epaxial muscles, see Figure 5 – abbreviation EPT and concluded the attachment area near the articulation between dermopterotic and supracleithrum. They claim that the tendons effect movements caused by epaxial contractions. In contrast Gemballa and Röder (2004) located the insertion of the tendons at the epioticum. It's not clear whether they are writing about the same tendons but found differing attachement areas, or if the tendons are two different types.

As already said, Lauder and Norton (1979) worked on the asymmetrical muscle activity and concluded that in epaxial muscles there is no asymmetrical activity during feeding, what would mean, that epaxial musculature is only responsible for movements like cranial elevation but not for lateral movements.

Conclusion 6:

Innervation. The innervation of the different muscles plays an essential role after Lauder and Norton (1979). They claim that the asymmetrical active muscle obliquus superioris is responsible for the lateral head bending. All observed muscles which act asymmetrically are innervated by occipital or spinal nerves, muscles innervated by cranial nerves never showed kinds of asymmetrical activity (Lauder and Norton 1979). This point can't be worked out in this paper and requires further investigations.

5 Acknowledgements

6 References

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7 Supplementary Materials

Laboratory protocol: potassium iodide staining of *Lepisosteus oculatus*

Date	Step
05.08.2015	- euthanizing specimen in clove oil - ventral dissection and removal of the guts - start of fixation in 25% ethanol
07.08.2015	- put into fresh 25% ethanol solution
13.08.2015	- raise concentration to 50% ethanol
14.08.2015	- put into fresh 50% ethanol solution
01.09.2015	- raise concentration to 75% ethanol
15.09.2015	- raise concentration to 100% ethanol
30.09.2015	- put into 30% potassium iodide solution
14.10.2015	- put into 40% potassium iodide solution
22.10.2015	- put into 50% potassium iodide solution
31.10.2015	- put into 60% potassium iodide solution
10.11.2015	- put into 70% potassium iodide solution

Laboratory protocol: clearing and staining of *Lepisosteus oculatus*

Date	Step
20.01.2015	- removal of the skin and storage in aqua dest.
05.02.2015	- put into 100% ethanol
06.02.2015	- put into 70% ethanol
12.02.2015	- put into 50% ethanol
17.02.2015	- put into 25% ethanol
24.02.2015	- put into aqua dest.
25.02.2015	- cartilage staining in a solution consisting to 80% of 100% ethanol and to 20% of 100% acetic acid. This solutions contains 10mg Alcian-blue on 100ml solution.
27.02.2015	- put into 70% ethanol - flushed in a few changes of 100% ethanol
02.03.2015	- put into 50% ethanol
03.03.2015	- put into 25% ethanol
04.03.2015	- put into aqua dest.
05.03.2015	- put into a solution consisting to 80% of 0.5% KOH and to 20% of 3% H_2O_2 - flushed in a solution consisting to 6.5% of Borax which was dissolved in hot aqua dest.
05.03.2015	- put into enzyme solution consisting to 70% of aqua dest. and 30% of the Borax solution described above. 6.5 grams of Papain were resolved in 2 liters of this solution.

13.03.2015	- put into the same enzyme solution, but 7 grams of Papain instead of 6.5g
20.03.2015	- put into fresh enzyme solution with 7 grams of resolved Papain
26.03.2015	- bone staining: Basic solution consisting of 0.1g Alizarin-red in 100ml aqua dest. Staining solution consisting of 2ml basic solution in 200ml of 0.5% KOH
30.03.2015	Destain: 0.5% KOH for about 20 Minutes Dehydration: alcohol solutions with concentrations in ascending order (30%, 50%, 70% - each for 30 minutes)
30.03.2015	Storage: Glycerin in ascending concentrations (50%, 85%, 99%)

8 Curriculum Vitae

Michael Herke

Academic studies and career

since 2009	Biology and Russian studies at the University of Vienna
since 11/2013	security worker at fashion store C&A
2011-2012	employee at „Reefness Meerwasseraquaristik/ F.S. Aquaristik Services" for aquaristic mainte-nance service
2008- 2009	squad leader and instructor at the caserne Bruck-neudorf/Austria
2006- 2008	Education at the Military Academy of Wiener Neu-stadt
2005-2006	preparation course for the military academy
2005	school leaving examination at BORG Eisenstadt
2003-2005	highschool BORG Eisenstadt
2001-2003	military highschool Wiener Neustadt
1997-2001	highschool BG/BRG/BORG Eisenstadt (with a focus on Croatian language)
1993-1997	elementary school (Vienna and Eisenstadt)

Language Skills:	German (native language)
	English (excellent command)
	Russian (upper intermediate)
	Croatian (basic translation skills)