



ARTIGO | ARTICLE

Gross anatomy of the head lateral line and hearing system of the Ophidiinae, *Genypterus blacodes*, *Raneya brasiliensis* and *Ophidion holbrookii*, of Southern Brazil

Anatomia da linha lateral da cabeça e sistema auditivo em Ophidiinae, Genypterus blacodes, Raneya brasiliensis e Ophidion holbrookii, provenientes do Sudeste do Brasil

Luis Alberto Zavala-Camin¹
Matheus Marcos Rotundo¹

ABSTRACT

Cusk-eels (Ophidiinae) have been studied as fish that produce sound by using their swim bladder, but fish that are well-known for producing sound have a free swim bladder, associated with paired muscle. However, in addition to producing sound, the complex anatomy associated with the swim bladder of cusk-eels suggests that their hearing system is equivalent to or more complex than the Weberian apparatus of the Ostariophysi. The anatomy has been studied in fish caught in commercial shrimp fishery and dissected fresh or after being fixed in formalin; the bones were stained with alizarin; the head lateral lines were observed after skin removal and red syrup was then injected to enhance the lines on the head. The associated anatomy shows specializations in the head lateral line, the center of the swim bladder is fixed on the vertebrae, three specific muscles are associated and there are some exclusive organs, the complexity of which suggests great expertise in the perception of sound/pressure waves. Some hypotheses about their functions are presented in order to stimulate research into this subject.

Key words: Fish. Lateral line. Sound/pressure detectors. Swimbladder.

¹ Universidade Santa Cecília, Curso de Ciências Biológicas. R. Oswaldo Cruz, 277, Boqueirão, 11045-907, Santos, SP, Brasil. Correspondência para/Correspondence to: L.A. ZAVALA-CAMIN. E-mail: <zavalacamin@hotmail.com>.

RESUMO

Os congros (Ophidiinae) têm sido estudados como peixes que produzem som com auxílio da vesícula gasosa, mas peixes bem conhecidos como produtores de som têm a vesícula gasosa livre, associada a músculo par. Entretanto, além de produzir som, a complexa anatomia dos congros sugere que seu aparelho sensorial sonoro seja equivalente ou mais complexo que o aparelho de Weber dos Ostariophysi. A anatomia tem sido estudada em peixes capturados pela pesca comercial de camarão e dissecados em fresco ou após fixados em formalina; os ossos foram corados com alizarina; as linhas laterais da cabeça foram observadas após retirar a pele da cabeça e deixar secar o interior dos canais, para logo injetar xarope vermelho. A anatomia associada mostra especializações na linha lateral da cabeça, fixação nas vértebras do terço médio da vesícula gasosa, três músculos específicos associados e órgãos exclusivos, essa complexidade sugere grande especialização para captar ondas de som/pressão. Algumas hipóteses sobre suas funções são apresentadas com o intuito de estimular pesquisas nesse assunto.

Palavras-chave: Peixes. Linha lateral. Detectores de som/pressão. Vesícula gasosa.

INTRODUCTION

Mechanical waves are detected by hair cells; conventionally, sounds are detected at long distances by the macula, and pressure waves are detected at short distance by the neuromast in the lateral line. Since the hair cell is the receptor in both cases, differences are related to the organ structure.

Some cusk-eels have a large muscle connected to the swim bladder. Due to this structure, they have been studied as producers of sounds (Rose, 1961; Marshal, 1967; Courtenay, 1971; Parmentier *et al.*, 2006; Nguyen *et al.*, 2008). Another feature is the difference between males and females, found in some species, that could be an indicator of a call to females. However, well known sound producing fish, such as Batrachoididae (Demski *et al.*, 1973) and Sciaenidae (Connaughton *et al.*, 2002) only have a drumming muscle (pair) over a free (not attached to the axial skeleton) swim bladder. Therefore, the specialized organs of the cusk-eels seem much more complex than that required to produce sound. The call to females is used for short periods of the year and the announcement of predators could be made by another sensory organ, so that the highly specialized receptors of sound/pressure should be used for a more frequent activity.

The objective of this work is to describe the gross anatomy of the sound-pressure wave receptors

of three Ophidiinae from Southern Brazil and try to associate these characteristics with the search for food, although it is not, as such, a paper about feeding.

MATERIAL AND METHODS

Cusk-eels from southern Brazil are caught secondarily in shrimp fishing; the return journey after the catch took about 20 days and the fish were preserved in ice. 131 specimens of *Ophidion holbrookii* (Putnam, 1874), 23-30cm TL; 84 *Raneya brasiliensis* (Kaup, 1856), 12-27cm TL and 70 *Genypterus blacodes* (Forster, 1801), 59-75cm TL, were collected by the commercial fleet off the southern coast of Brazil (22.5°S - 25°S), at a depth of between 10m and 170m (average 45m) between 2006 and 2009. The scientific nomenclature follows the Eschmeyer Catalog of Fishes, electronic version (2010), of the California Academy of Sciences.

Since some Ophidiinae have sexual dimorphism, the fish were sexed to be sure that both sexes were included. However, most fish of the two smaller species were not able to be sexed via the gonads, so sex was defined in *O. holbrookii* with the help of the sexual dimorphism in the axial skeleton (Rose, 1961) and in *R. blacodes* with the sexual dimorphism at the end of the swim bladder (Robins,

1985). Fresh specimens were selected to study the anatomical features; some were boiled in water for a few minutes to facilitate the axial skeleton dissection. Specimens were fixed in formalin 10% and after 15 days conserved in 70% alcohol; the fish had the muscles on the left side of the body removed to expose the bones, swim bladder and hearing complex, including the associated muscles and were then stained with alizarin (Taylor, 1967) to facilitate the identification of the bones. The skin was removed to identify the lateral lines of the head; the fish were air-dried and red corn syrup was injected. The structures were then immediately drawn since the red color promoted by the syrup disappears within a short time.

RESULTS

General features

Some anatomical features related to sound/pressure wave perception are similar in the three species studied:

Head lateral line: Three classical pair of branches in the lateral line (Figure 1a). Supraorbital branch passing through the skull in a wide groove in the top of the neurocranium (Figure 2a in *G. blacodes*) covered by skin, in the anterior part of the head forming a wider nasal area (nasal bulla) (Figure 1c,d. - in *R. blacodes*) also covered by skin. Infraorbital branch, wider under the lacrimal bone, finds the nasal bulla in the anterior edge. Operculo-mandibular branch starts at the skull, moves towards the preoperculum and then to the lower jawbone. At the beginning of the operculo-mandibular branch, another branch runs toward the equivalent branch on the other side, called the occipital branch (Figure 1a), somewhat like the canal type II in gobioid fish (Takagi, 1988).

Ear: Large otic chamber with large sagitta; skull otic external area with tiny wall formed by part of the prootic, intercalar and basioccipital bones.

Precaudal vertebrae: First neural spine modified and fused with lateral elongated handles (Figures 2f, 3a, 4d) (handles are probably the first parapophysis or the first rib modified) associated with the swim bladder. Large and flattened parapophysis present from the 6th to the last precaudal vertebra.

Muscles (paired): The first one originated on the otic chamber wall and inserted on the swim bladder, called otic-swim bladder muscle (otic-rocker in male *O. holbrookii*). The second one originated in the exoccipital and inserted on the swim bladder anterior face and on the handle, called otic-handle muscle. The third one originated on the supraoccipital and inserted on bones upper side modified from the first neural arch, called skull-neural spine (Figure 2d in *G. blacodes*) (Rose, 1961 for description in *O. holbrookii*).

Barbels: Ophidiidae barbells (Figure 1a) are modified pelvic fins (Nielsen *et al.*, 1999). A medullary nerve from the third or fourth vertebra runs to the pelvic fins crossing over the anterodorsal sides of the swim bladder. From the pelvic fin nerve there is a branch which inserts on the pectoral fin muscles.

Swim bladder: One almost conical main chamber. Two distinct layers, the outer thick and white and the inner one thin and transparent. Extra chambers could be formed, some of only the inner layer. Anterior extreme of the swim bladder associated with muscles and modified bones, a fold suggests contraction and extension. More than half of the central portion of the swim bladder fixed on the centra and parapophysis of several precaudal vertebrae; free posterior portion.

Nasal capsules are without lamellae.

Specific features

Genypterus brasiliensis

Head: Supraorbital branch running in a rift (3.0mm wide on 59cm fish TL) on the top of the neurocranium, cranial channel dorsally opened, covered by skin, with a bone bridge near the frontal bone anterior part (Figure 2a).

Skull: Long neurocranium (Figure 2d), 29mm height (39%) in a skull 113mm long; large sagitta (15.5 x 8 x 2mm) and large otic chamber.

Vertebrae: 18+53. First to 5th without (or very short) parapophysis. Wide parapophysis appears on the 6th to 14th vertebrae, from the 6th to the 11th or the 12th vertebra where the swim bladder is fixed, the parapophysis are double layer (Figure 2e) with dorsal aorta and the kidney located in the middle; Short swim bladder free portion end. Ribs of the second to 5th vertebrae originated on the centra and associated with the swim bladder; slender second vertebra rib, large third vertebra fixed on the swim bladder, slender 4th and 5th ribs. Following ribs originated on the parapophysis.

Swim bladder: Anterior upper wall of the swim bladder with almost flat front where the handle bone, the second and third modified rib and the otic-swim bladder muscle are inserted. Outer layer inserted on the parapophysis and on the ventral centra of the 6th to 12th vertebrae. The middle swim bladder upper side inserted on the parapophysis and on the ventral sides of the centra of the 6th to 12th vertebrae; the centra can be seen in ventral view (with the swim bladder open) since it is only covered by the tiny transparent inner wall. Seven to nine toe-like extensions externally; internally, the two external toe-like extensions with separate tiny layer and forming small chambers (Figure 2c).

Muscles: Otic-swim bladder muscle originated on the skull basioccipital otic area (Figure 2c, d) and inserted on the swim bladder upper front wall. The otic-handle muscle inserts on the handle and indirectly on the swim bladder.

Stomach contents: Crustaceans (Brachyura) and fishes were the most common food observed.

Raneya brasiliensis

Head: Larger nasal bulla than in the other two species (Figures 1c, d) and associated with an ethmoid anterior extension (used as diagnostic character).

Skull: In a skull 46mm long and 18mm high, sagitta was 8 x 4 x 2mm.

Vertebrae: 15+50. First to 5th vertebrae without parapophysis; free and short second vertebra rib, large third vertebra rib associated with the handle; long 4th and 5th ribs, slender and free; 6th to 15th ribs articulated on the parapophysis distal upper side.

Swim bladder: Cone shape chamber with lateral cavities (externally like toes), and center fixed on vertebrae four to 8. Anterior lateral side attached to the handle bone. Anterior lower half (male and female) with two calcified areas (Figure 3b), bone lower inner side with several concavities (Figure 3c). In the male, swim bladder end without outer layer final end, only the transparent inner layer looking like a hole; both layers are complete in females.

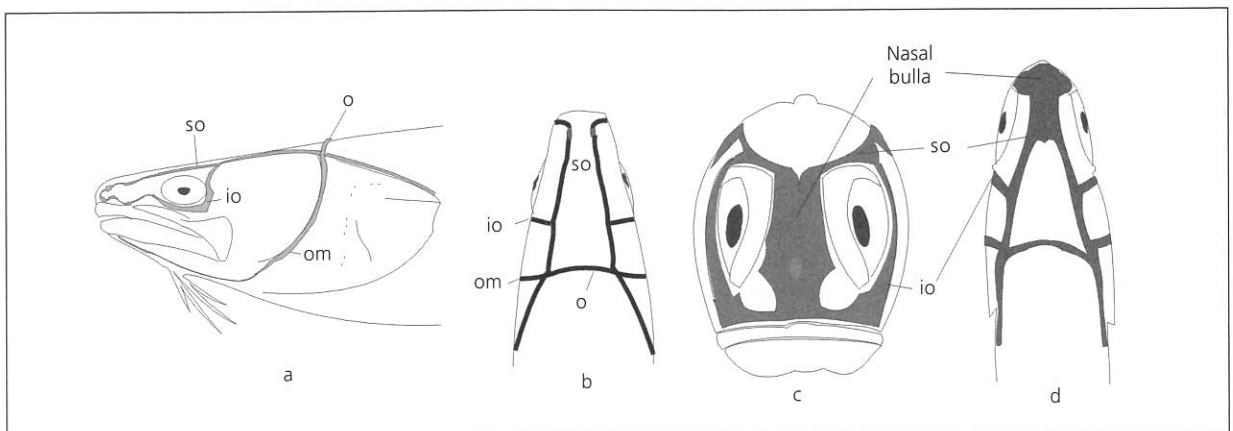


Figure 1. a, b: Lateral and upper views of *G. blacodes*; c, d: Frontal and upper views of *Raneya brasiliensis*. Head lateral lines: supraorbital (so), infraorbital (io), operculo-mandibular (om) and occipital branch (o).

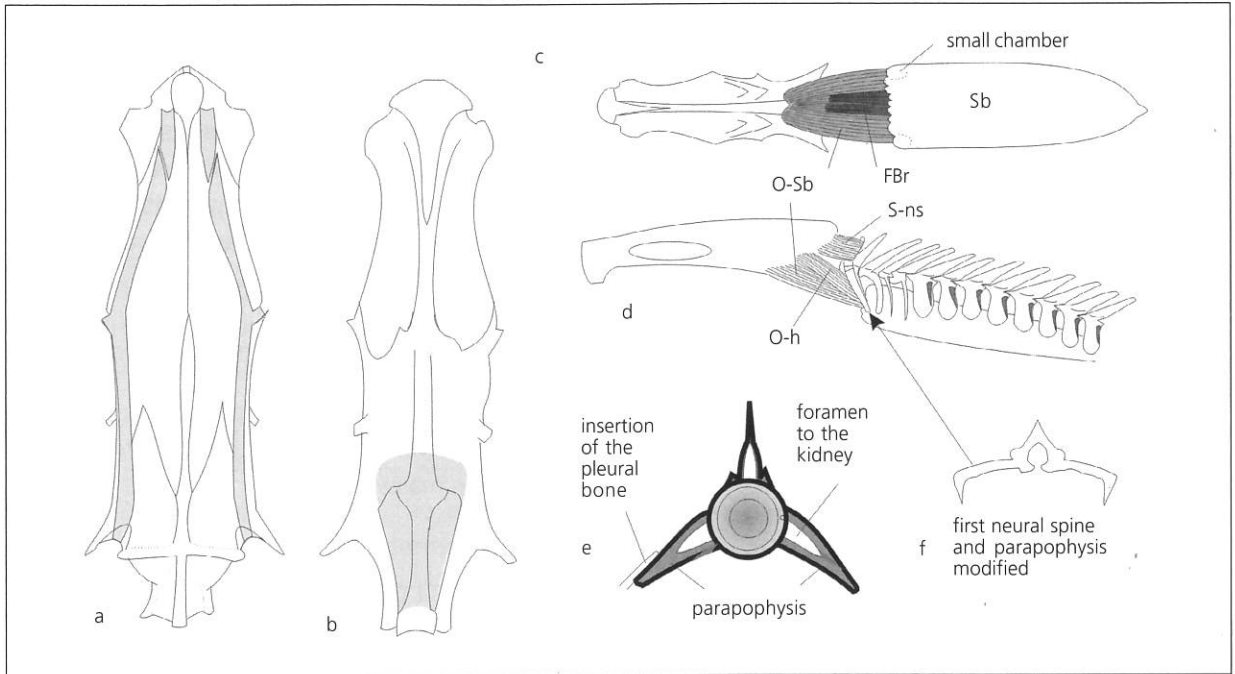


Figure 2. *G. blacodes* a) Cranium dorsal view; in grey: grooves to supraorbital branches; b) Cranium ventral view; in grey: otic area; c) Otic-swim bladder muscles ventral view (O-Sb); Sb: swim bladder; posterior portion of pharyngo-branchial muscles (FBr); d) Lateral view of the muscles related to the swim bladder and cranium (O-h: otic-handle muscle; S-ns: skull-neural spine muscle); e) 7th vertebra showing foramens on the parapophysis; f) Neural spine and parapophysis (or rib) modified from the first vertebra.

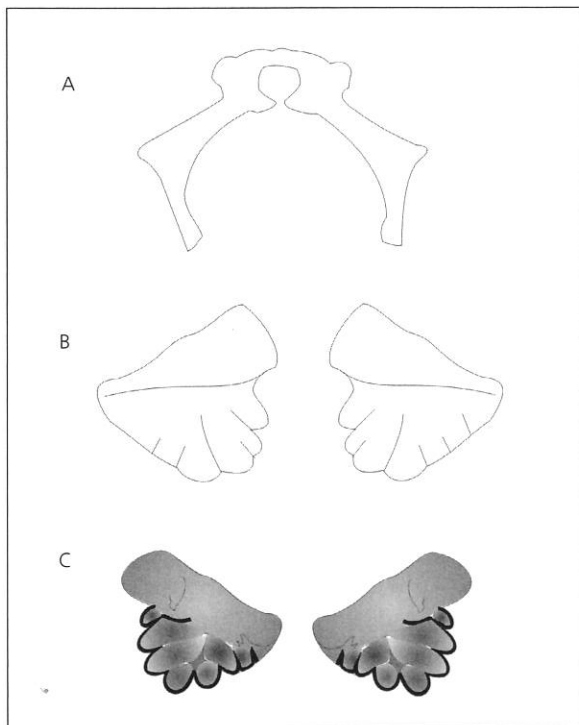


Figure 3. *Raneya brasiliensis*. A: First neural spine and parapophysis modified; B, C: Bones of the swim bladder anterior face; B front side; C inner side.

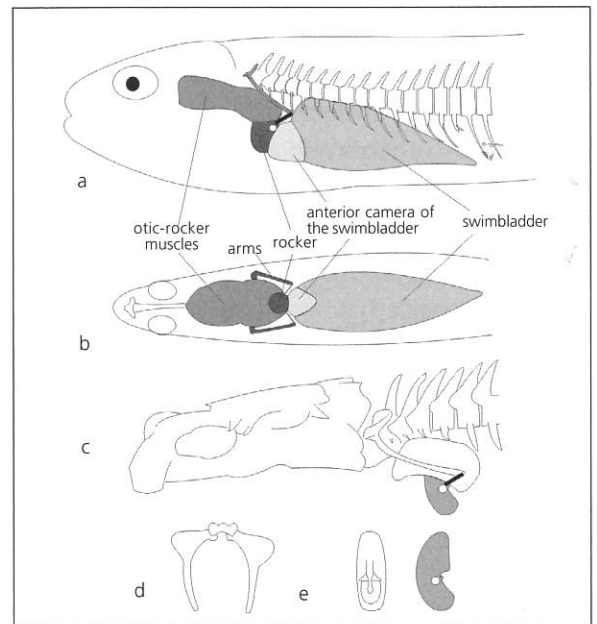


Figure 4. Lateral and ventral view of rocker associated structures in the male *Ophidium holbrookii*. a) Lateral view (without bone plates); b) Ventral view; c) Rocker position on the anterior axial skeleton (with bone plates); d) First neural spine and parapophysis modified, e) Posterior and lateral view of the rocker.

Stomach contents: Crustaceans (Brachyura), bivalve mollusks and fish were observed, including a small *Porichthys porosissimus*, a fish that is accustomed to burying itself in the sand.

In one sample of 48 specimens, 47 were males and only one was female.

Ophidion holbrookii

This is a species with sexual dimorphism represented by differences between the vertebral column and the presence of an organ called a rocker, only in the male (Rose, 1961).

Skull: In a 45mm length and 17mm height, sagitta was 8mm high, 5mm wide and 2.5mm thick, representing almost 50% of neurocranium height; very tiny skull external bone wall over the otic chamber permitting the observation of sagitta. In a 25cmTL fish, magnum foramen dimensions were 3.5mm and 2.5mm wide (locking more height than necessary to the spinal cord passage).

Vertebrae: 16+50. In the male, parapophysis transformed in plates from the second to the 5th vertebra, where rocker is situated; small plates in the female and holding the swim bladder upper anterior part. Ribs articulated on the centra from the second to 5th vertebra and above the parapophysis from the 6th to the 16th vertebra.

Rocker: specialized organ, human kidney shape (Figure 4e), with dorsal half enclosed in a cavity formed by modified parapophysis; cavity with a thick gelatinous layer probably working as lubricant, to absorb and mitigate movements. Convex anterior face and posterior face with different shapes, otic-rocker muscle inserted on the anterior superior half, and in the lateral and middle side there is a point inserting ligaments from handle bones; center has lateral grooves where swim bladder projects and forms two lateral small bulla (Figure 4e - in lateral view) covered with a layer with a similar texture to that of the swim bladder wall. Lower posterior facet is found freely in swim bladder tiny ventral chamber.

Swim bladder: Conical shape; in the male it is fixed on the 6th to 10th vertebrae (Figure 4a), the

posterior part projects to the posterior coelomic cavity. An extra, conically-shaped chamber formed only by one tiny inner layer located in the anterior lower extreme (Figure 4a, b), which inserts freely on the lower half of the rocker posterior. Part of the upper front fixed on the modified parapophysis of the first vertebra and a small fold on the sides.

Muscles: Otic-swim bladder muscle of female similar to the other two species studied, but in the male it is inserted in the rocker (otic-rocker muscle).

Stomach contents: Snails and crustaceans (Brachyura) were the most common food items observed in males.

DISCUSSION

Anatomical features of Ophidiinae species suggest a highly specialized activity in terms of sound/pressure detection, although it could also be used for production of sound.

The well developed lateral lines of the head; large sagittae; bones developed in the swim bladder front; almost half of swim bladder length fixed on the centra and large parapophysis with free anterior part to move; three specific muscles associated with the swim bladder and modified bones are indicative of a highly complex apparatus developed for detecting low frequency waves that can be produced by the movement of small animals under the sand and therefore, it could be a device to enable the search for food.

Since anatomy represents physiology, some hypotheses on how this complex apparatus works can be conducted according to the experience of other works.

The wide lateral lines on the head seem suitable for capturing low frequency sound/pressure waves. In the head lateral lines, wide channels mean selectivity to low frequency sounds (Montgomery *et al.*, 1995; Janssen *et al.*, 1999). Fish which search for food under the sand usually have large otolith organs. Large mass otoliths increase the sensitivity to low frequency sounds (Lychakov & Rebane, 2002).

The first five parapophyses are modified or slender allowing the vibration of the swim bladder anterior portion; the following parapophysis (6th to 10th) being wide and strong and permits a strong vertebrae fixation. Since well known sound producing fish have a free swim bladder, it means that the fixation should be used in more complex activities, likely to control the vibration produced in the swim bladder by the sound/pressure waves. The three muscles can be used to select, control and guide the sound.

The otic-swim bladder muscle is much larger than is required to bring the swim bladder or the rocker forward; comparatively the pharyngeal retractor muscle is small considering its heavy workload (Figure 2c, in *G. blacodes*). When the otic-swim bladder muscle contracts, it pulls on the free anterior part of the swim bladder (which is folded), thereby increasing the volume and diminishing gas density, becoming more sensitive to the vibrating sound. Therefore, the sound could be guided to the otic chamber wall by the otic-swim bladder muscle (like the Weberian ossicles). The presence of other muscle, the otic-handle muscle, almost in the same place, denotes another kind of control over the swim bladder vibration, which has an influence on the handle, associated with the muscle skull-neural spine, that could guide sound over the foramen magnum almost directly to the otic chamber. A similar type of muscle is observed in Pimelodidae (Ladich, 2001), a small muscle called tensor tripodis, with the job of controlling the vibration in the swim bladder. The presence of extra chambers in the swim bladder, some formed only by the inner wall, the ossified front in *Raneya brasiliensis* associated with the male swim bladder end only with the inner tiny layer, and the rocker presence in males of *Ophidion holbrookii*, indicates diverse, special, mechanical performances.

In searching for food under the sand (mollusks, crustaceans), fish probably use the barbels as a taste detector and head lateral lines as sound detector. The movement of prey in the sand should be enough to be detected with the specialized lateral lines of the head, low frequency vibrations transmitted to the swim bladder could be enhanced reducing

the threshold. The pelvic nerve running along the front of the swim bladder seems an obvious pathway, but could suggest the possibility of associating taste information with sound/pressure of the prey; something similar to the laterophysic connection in the chaetodontidae butterflyfish (Webb & Smith, 2000).

It is also possible to speculate that the fish could produce special sounds to hurt or disturb prey and thus identify their location by the head lateral line.

G. blacodes, both male and female, have similar sound pressure specialization, but the question is, why are the males of *R. blacodes* and *O. holbrookii* different from females? The call to the female could be a plausible answer, but if the search for food is the aim of specialization, why are males more specialized? The answer could be from the sex ratio in a given area; since only seven of 124 specimens of *O. holbrookii* and, one of *R. brasiliensis* in one sample of 48 were females, it is likely that species have segregated sexual distribution and males search for food in different places. Selective research on this issue could provide the answer.

The non-lamellar nasal chamber may indicate that odor is not very important since they have highly specialized sound/pressure detectors.

CONCLUSION

The hearing system gross anatomy of *G. blacodes*, *Raneya brasiliensis* and *Ophidion holbrookii* suggests a similar or more complex system than the weberian ossicles of the Ostariophysi. Special features in the anatomy of the three species included a groove on the neurocranium where the supraorbital branch of the head lateral line is situated, and an extra branch on the head posterior-superior side, the occipital branch. The three species have the swim bladder central portion fixed on the vertebrae, and in *G. blacodes*, the parapophysis where the swim bladder is fixed is double layered. Some hypotheses on how the organs work are presented with the aim of prompting research on the subject.

REFERENCES

- Connaughton, M.A.; Fine, M.L. & Taylor, M.H. (2002). Weakfish sonic muscle: influence of size, temperature and season. *Journal of Experimental Biology*, 205(PT5):2183-8.
- Courtenay, W.R. Jr. (1971). Sexual dimorphism of the sound producing mechanism of the striped cusk-eel, *Risola marginata* (Pisces: Ophidiidae). *Copeia*, 1971(2):259-68.
- Demski, L.S.; Gerald, J.W. & Popper, A.N. (1973). Central and peripheral mechanisms of teleost sound production. *American Zoologist*, 13(4):41-67.
- Eschmayer, W.N. (Ed.) (2010). (Catalog of Fishes, electronic version 2010. Available from: <<http://research.calacademy.org/ichthyology/catalog/fishcatmain.asp>>. (cited: 2 Jul. 2010).
- Janssen, J.; Sideleva, V. & Biga H. (1999). Use of the lateral line for feeding in two Lake Baikal sculpins. *Journal of Fish Biology*, 54(2):404-16.
- Ladich, F. (2001). Sound-generating and detecting motor system in catfish: design of swimbladder muscles in doradids and pimelodids. *The Anatomical Record*, 263(3):297-306.
- Lychakov, D.V. & Rebane, Y.T. (2002). Otoliths and modeling ear function. *Bioacoustics*, 12(2/3):125-8.
- Marshall, N.B. (1967). Sound producing mechanism and the biology of the deep-sea fishes. In: Tavalga, W.N. *Marine bio-acoustics*. Oxford: Pergamon Press. v.2, p.123-133.
- Montgomery, J.; Coombs, S. & Halstead, M. (1995). Biology of the mechanosensory lateral line in fishes. *Review of Fish Biology and Fisheries*, 5:399-416.
- Nguyen, T.K., Lin, H., Parmentier, E. & Fine, M.L. (2008). Seasonal variation in sonic muscle in the fawn cusk-eel *Lepophidium profundorum*. *Biological Letters*, 4(6):707-10.
- Nielsen, J.G.; Cohen, D.M.; Markle, D.F. & Robins, C.R. (1999). Ophidiiform fishes of the world (Order Ophidiiformes). An annotated and illustrated catalogue of pearlfish, cusk-eels, brotulas and other ophidiiform fish known to date. Rome: FAO. v. 18. (FAO Fisheries Synopsis 125).
- Parmentier, E.; Lagardère, J-P.; Braquegnier, J.B.; Vandewalle, P. & Fine, M.L. (2006). Sound production mechanism in carapid fish: first example with a slow sonic muscle. *The Journal of Experimental Biology*, 209:2952-60.
- Robins, C.R. (1985). The status of the Ophidiid fish *Ophidium brasiliense* Kaup. *Japanese Journal of Ichthyology*, 31(4):441-3.
- Rose, J.A. (1961). Anatomy and sexual dimorphism of the swim bladder and vertebral column in *Ophidion holbrooki* (Pisces, Ophidiidae). *Bulletin of Marine Science of the Gulf and Caribbean*, 11(2):280-308.
- Takagi, K. (1988). Cephalic sensory canal system of the gobioid fishes of Japan: comparative morphology with special reference to phylogenetic significance. *Journal of the Tokyo University of Fisheries*, 75(2):499-568.
- Taylor, W.R. (1967). An enzyme method of cleaning and staining small vertebrates. Proceedings of the U.S. National Museum. *Smithsonian Institute*, 122(3596):1-17.
- Webb, J.F. & Smith, W.L. (2000) The laterophysic connection in chaetodontid butterflyfish: morphological variation and speculation on sensory function. *Philosophical Transactions of the Royal Society of London*, 355B:1125-1129.

Received on: 3/12/2009

Final version resubmitted on: 10/5/2010

Approved on: 24/6/2010