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National Posters (ระดับชาติ)

1. Wannitikul, P. and S. Jitpukdee. 2004. Morphology of sagitta and crystals on sulcus of *Megalops cyprinoides*. *Journal of Microscopy Society of Thailand*.18: 57-60.
2. Krainara, P. and P. Wannitikul. 2004. Sagitta morphology of *Channa* spp. from southern area of Thailand. *Journal of Microscopy Society of Thailand*.18: 61-62.

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1. Wannitikul, P. and S. Jitpukdee. 2004. Crystals on sulcus acusticus of sagitta of *Muraenesox cirreus*. *Proceedings of the 8th Asia-Pacific Conference on Electron Microscopy (8APEM)*, 7-11 June 2004, Kanazawa, Japan, p.870
2. Wannitikul, P. and S. Jitpukdee. 2004. Relationships of some elopomorph fishes inferred from crystal morphology on sulcus of sagittal otolith. *Proceedings of the XIXth International Congress of Zoology*, 23-27 August 2004, Beijing, China, p.468.
3. Jitpukdee, S. and P. Wannitikul. 2004. Sagitta morphology and crystalline structure on sulcus in *Nemipterus* spp. from coastal of Thailand. *Proceedings of the 4th ASEAN Microscopy Conference and the 3rd Vietnam Conference on Electron Microscopy*, 5-6 January 2004, Hanoi, Vietnam, p.127-133.
4. Jitpukdee, S. and P. Wannitikul. 2004. Different morphology of sagittal otoliths of *Cephalopholis* spp. from southern Thailand. *Proceedings of the 8th Asia-Pacific Conference on Electron Microscopy (8APEM)*, 7-11 June 2004, Kanazawa, Japan, p.868.
5. Jitpukdee, S. and P. Wannitikul. 2004. Crystalline structure on sulcus acusticus of some Thai fish sagittae from different habitats. *Handbook and Abstracts: Third International Symposium on Fish Otolith Research and Application*, 11-16 July 2004, Townsville, Queensland, Australia, p.112.
6. Jitpukdee, S. and P. Wannitikul. 2004. Shape of sagitta and sulcus acusticus of Sciaenidae from southern coast of Thailand. *Proceedings of the XIXth International Congress of Zoology*, 23-27 August 2004, Beijing, China, p.455.
7. Jitpukdee, S. and P. Wannitikul. 2004. Sagittal otoliths of Thai marine fishes in order Perciformes. *Abstract of the 9th Biological Science Graduate Congress*, 16-18 December 2004, Chulalongkorn University, Bangkok, Thailand, p.97.
8. Kosavitikul, P. and P. Wannitikul. 2004. Species diversity of terrestrial earthworms in dry evergreen forest of Khao Yai National Park. *Abstract of the 9th Biological Science Graduate Congress*, 16-18 December 2004, Chulalongkorn University, Bangkok, Thailand, p.88.
9. Krainara, P. and P. Wannitikul. 2004. Sagitta morphology of some fishes in family Belontiidae from southern area of Thailand. *Proceedings of the XIXth International Congress of Zoology*, 23-27 August 2004, Beijing, China, p.456.
10. Krainara, P. and P. Wannitikul. 2004. Lagenar otoliths of fishes in order Cypriniformes from southern area of Thailand. *Abstract of the 9th Biological Science Graduate Congress*, 16-18 December 2004, Chulalongkorn University, Bangkok, Thailand, p.98.

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SAGITTA MORPHOLOGY AND CRYSTALLINE STRUCTURE ON SULCUS ACUSTICUS IN *NEMIPTERUS* SPP. FROM COASTAL OF THAILAND

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ABSTRACT

The sagittal otoliths in three fish species of the genus *Nemipterus* (*N. hexodon*, *N. tambuloides* and *N. furcosus*) were collected from fishes along the coastal of Thailand. Morphology and crystalline structure on sulcus acusticus of these sagittae were studied by scanning electron microscope. Morphology of these sagittae was similar, however, distinctive morphology of each species was found, i.e. shape, margin sculpturing, ostium and cauda of sulcus acusticus. Crystals on sulcus acusticus of these sagittae showed different structure. Shape, size, crystalline surface and direction of crystalline arrangement in each species were unique. The differences in sagitta morphology alone could be used as a tool for identification of fish species. However, differences in crystalline structure on sulcus acusticus of sagittae of each species increase the information to confirm the identification of fish these species.

Key words: Sagittal otolith; Morphology; *Nemipterus*; *Sulcus acusticus*; Scanning electron microscope

Introduction

The otoliths, or earstones, of fish are composed of the aragonite form of calcium carbonate. All teleost fishes (Osteichthyes) have three pairs of otoliths: sagittae, asteriscii and lapilli. The sagittae are the largest pair of otolith and vary tremendously in shape and size in different groups of fishes. Many different factors have been reported to influence sagitta morphology, one factor is the effects of differences in growth rate caused by environmental factors such as water temperature, depth, mineral and food availability.^{1,2,11,13} These morphological variations of otolith have been used for the most widely studies of fish biology. Fisheries biologists have used the morphology of the sagittae for identification of fish species.^{4,5,7,12,14,16,24,25} Palaeontologists have identified species of fishes from otoliths in sediments.³ Biologists can use the otoliths recovered from stomach contents of squids²¹ and marine birds¹⁰ to determine the types of fishes which they have eaten. Otolith ultrastructures (crystal structures) have been also used for identification of fishes in the genus *Coelorinchus*¹⁴.

Three fish species of the genus *Nemipterus* (*N. tambuloides*, *N. hexodon* and *N. furcosus*) are demersal, non-migration and distribute in Indo-west Pacific: Andaman Sea, Philippine, Gulf of Thailand, South China Sea and Indonesia. They inhabit sandy or muddy bottom in depths of 10 to 110 meters. The identification of these species is difficult because external

morphology e.g. dorsal spines, dorsal soft rays, anal spines and anal soft rays are equal in number. Moreover, the colour of these fishes is not usually considered as a character for identification. The purpose of this study is to investigate morphology of the sagittal otoliths in three fish species of *Nemipterus* and crystalline structure on sulcus acusticus of these sagittae in order to use them as a tool for identification of fish species.

Material and Methods

Sagittal otoliths of *N. hexodon*, *N. tambuloides* and *N. furcosus* were collected from fishes along the coastal of Thailand during July 2002 to June 2003. At least ten fishes of each species were collected. The size of fish in adult stage was used as a standard size of each species. The standard length (SL) of each fish species was recorded. Fish were identified according to Bone et al.⁶ and Nelson.¹⁸ The sagittal otoliths were removed from head of fish by vertical method with forceps and washed to remove any residual tissue, gelatin membrane and blood. They were wiped with cloth or paper towel, or rinsed with freshwater, and allowed to dry. The sagittal otoliths were mounted on a brass stub 50 millimeters in diameter and 4 millimeters thick. Mounted otoliths were sputter-coated with a thin layer of gold using a standard coating time of two minutes. Photomicrographs of the whole sagittal otoliths and crystals in ostium of sulcus acusticus were taken with JEOL, JSM-6400 scanning electron microscope. Terminology of parts of sagitta were based on Smale et al.²³ (Fig. 1).

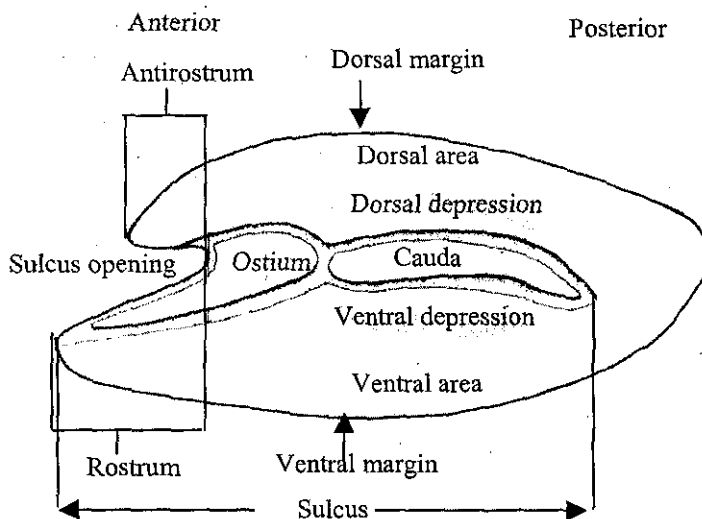


Fig. 1 Diagram of right otolith surface

Results

Otolith morphology

The sagittal otoliths in three species of the genus *Nemipterus* from coastal of Thailand show distinctive morphology that is species specific, i.e. shape, ostium and cauda of sulcus acusticus and margin sculpturing. The shapes of otoliths have 2 types: elliptic and ovate. The sulcus acusticus is heterosulcoid with ostial opening. The margins sculpturing have 3

characters: crenate, sinuate and lobe. Ostium is oval or narrow and elongate. Cauda is straight, flexed near tip ending closed to margin. Other morphology of the sagittal otoliths of *Nemipterus* e.g. rostrum, antirostrum, dorsal rim, ventral rim, dorsal area, ventral area, dorsal depressor, ventral depressor is also species specific. These anatomical characters are unique to each species.

The shape of sagittal otolith of *N. hexodon* is elliptic, while they are ovate in *N. tambuloides* and *N. furcosus* (Fig. 2). The posterior area is higher than the anterior area in all species. However, the posterior part of sagittal otolith in *N. furcosus* is smaller and narrower than in other two species. The anterior part of *N. hexodon* and *N. furcosus* is a well defined difference in the rostrum and antirostrum, while it is poorly defined in *N. tambuloides*. The sulcus acusticus of these three species is heterosulcoid but ostium and cauda are different. The ostium and rostrum is wide and oval in sagittal otolith of *N. tambuloides* but narrow and elongate in *N. hexodon* and *N. furcosus*. The cauda of sulcus acusticus of *N. hexodon* and *N. tambuloides* are similar, straight and gently flexed along its length, while in *N. furcosus* is strongly flexed tip and have ventral groove. The sculpturing on ventral margin of *N. hexodon* and *N. furcosus* are sinuate but of *N. tambuloides* is crenate. The sculpturing on dorsal margin of *N. hexodon* is sinuate whereas of *N. furcosus* and *N. tambuloides* are lobe (Fig. 2.)

Differences in other morphology of these sagittal otoliths are also found. The rostrum is wide and oval in *N. tambuloides* but narrow and elongate in *N. hexodon* and *N. furcosus*. The antirostrum of *N. tambuloides* is minute and round, while in other two species is large. The dorsal margin and dorsal area are more rough than ventral in all species. The dome-shaped on dorsal margin is found in sagittal otolith of *N. tambuloides*. The dorsal area of

sagittal otolith in *N. tambuloides* is smoother than in *N. hexodon* and *N. furcosus*. In contrast, the ventral margin and ventral area of these sagittal otoliths are not different. The dorsal depression of the sagittal otolith in *N. hexodon* and *N. furcosus* are shallow, oval-shaped but it is extend to posterior cauda in *N. hexodon*, while it is deep and narrow over anterior cauda in *N. tambuloides*.

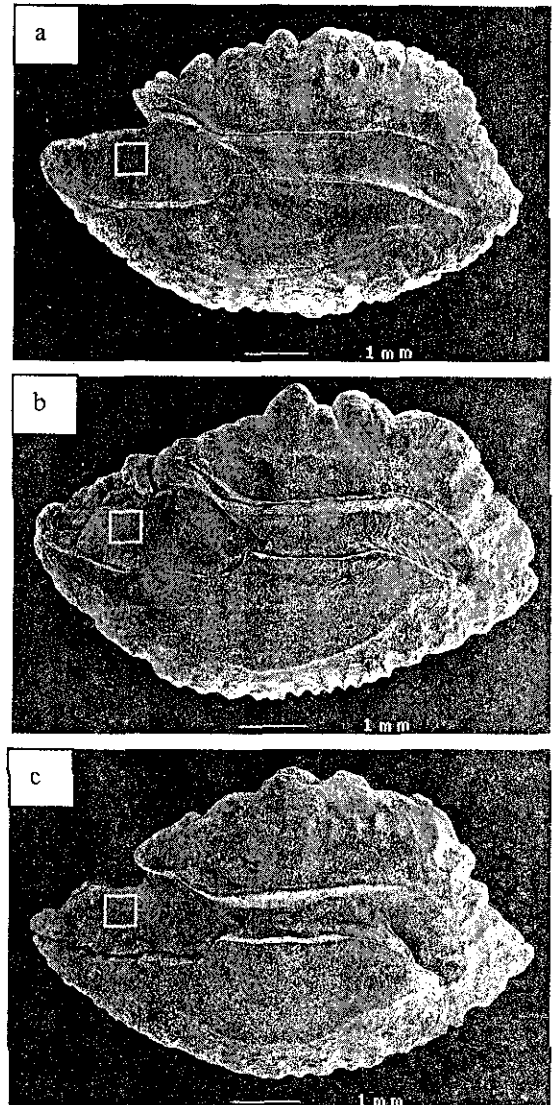


Fig. 2. The sagittal otoliths of (a) *Nemipterus hexodon*, (b) *N. tambuloides*, and (c) *N. furcosus*. Scale bar = 1 mm. The square (□) showing the area of ostium where crystals were enlarged and investigated.

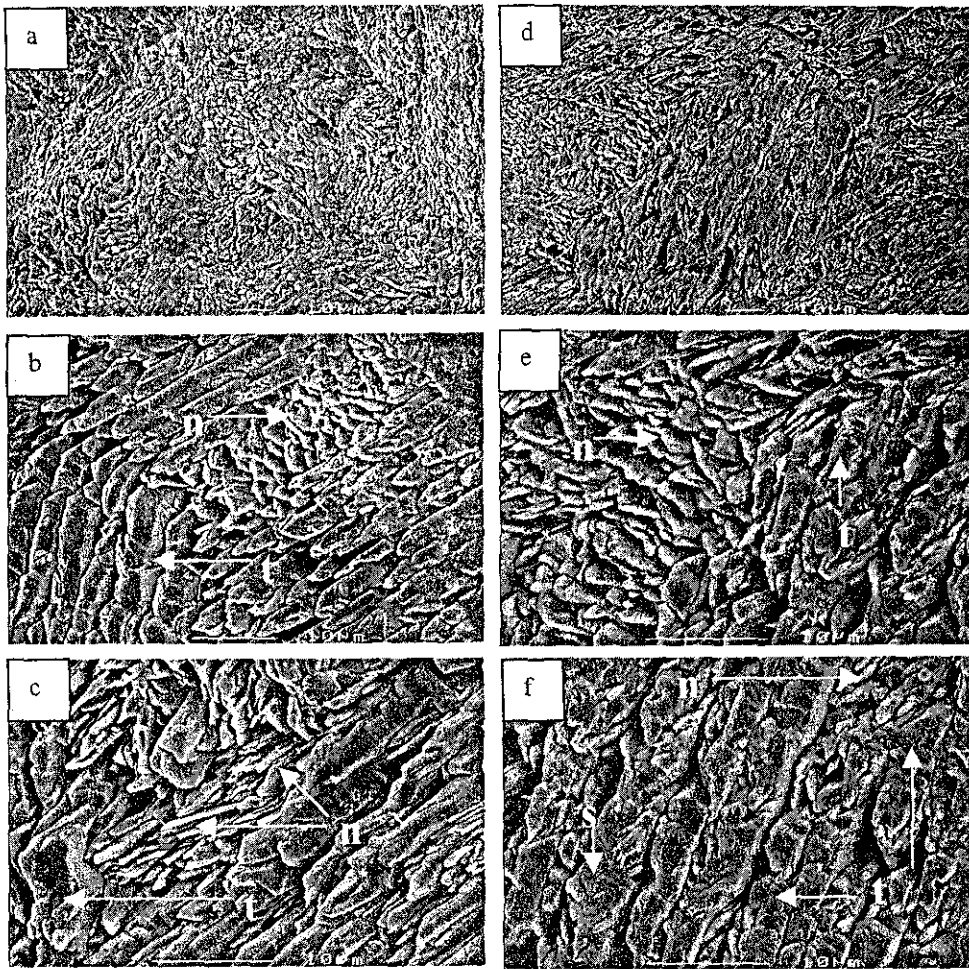


Fig. 3 Crystals on sulcus acusticus of (a-c) *N. hexodon* and (d-f) *N. tambuloides*, (a, d) crystalline arrangement, (b, c, e, f) enlarged crystals in (a) and (d) showing thick (t) and thin (n) crystals and fused thin crystals (s). Scale bar = 10um.

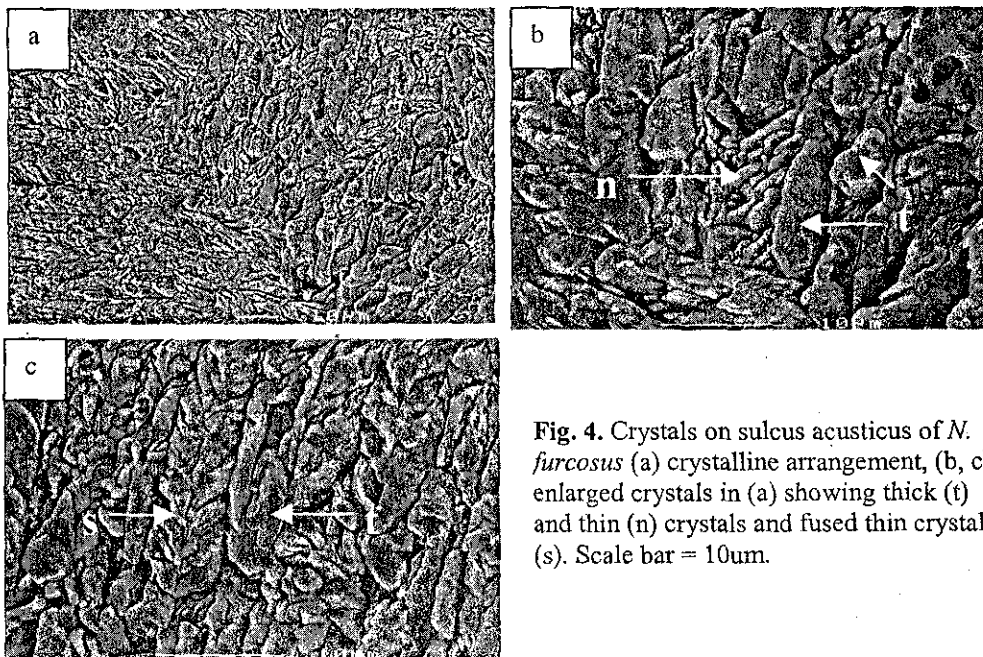


Fig. 4. Crystals on sulcus acusticus of *N. furcosus* (a) crystalline arrangement, (b, c) enlarged crystals in (a) showing thick (t) and thin (n) crystals and fused thin crystals (s). Scale bar = 10um.

Crystalline structure on sulcus acusticus

The crystals on sulcus acusticus of *Nemipterus* are separated into 2 types: thick and thin crystals. The thick crystals are long, rectangular-shaped and arranged in vertical plane, the thin crystals are flat, rectangular-shaped and stacked in horizontal plane (Fig.3 and 4). Differences of thick crystals among three species are found. The thick crystals of *N. hexodon* are longer and smoother than of *N. furcosus* and *N. tambuloides* and some of them are arranged in oblique direction. (Fig. 3a and 3b). The thick crystals of *N. furcosus* are shorter and thicker than of *N. tambuloides* (Fig. 3e and 4b-c). The thick crystals in some area of *N. tambuloides* and *N. furcosus* look like fusion of many thin crystals (Fig. 3e-f and 4b-c), especially in *N. furcosus* (Fig. 4c), which is not found in *N. hexodon*. Generally, the arrangement of thick and thin crystals on sulcus acusticus in *N. tambuloides* and *N. furcosus* are similar (Fig.3d and 4a), the differences are shown in *N. hexodon* (Fig.3a). The thin and thick crystals of *N. hexodon* are arranged 3 directions: vertical, oblique and horizontal planes (Fig. 3). In *N. tambuloides* and *N. furcosus* the thick crystals are grouped together in vertical plane and are surrounded by thin crystals which are stacked in horizontal plane as layers. The group of thick crystals can be clearly separated from the layers of thin crystals (Fig. 3d and 4a), this character is not found in *N. hexodon* (Fig. 3a). However, in some areas of these three species sagittae the thin crystals are grouped as a small clump and are surrounded by the thick crystals (Fig. 3b, 3e and 4a-b).

Discussion

The sagittal otoliths in three species of *Nemipterus* show different morphology, which is species specific. The morphology of *N. hexodon* sagittal otolith is clearly separated from *N. tambuloides* and *N. furcosus*. The shape of sagitta of *N. furcosus* shows the prominent posterior part which is smaller and narrower than of *N. tambuloides* and cauda of sulcus acusticus in *N. furcosus* is different from in *N. tambuloides*, it is strongly flexed tip and processes ventral groove (Fig. 2c).

The crystals on sulcus acusticus of sagittae in three species of *Nemipterus* are different in shape, size and arrangement. The crystalline structure in *N. hexodon* is clearly different from *N. tambuloides* and *N. furcosus*. The crystalline structure of *N. furcosus* can be distinguished from *N. tambuloides* in the area where thin crystals fuse or melt or pack together and become thick crystals as shown in Fig. 3f and 4c. This character can be seen clearly in *N. furcosus* in Fig. 4c.

The differences in sagitta morphology and crystalline structure on sulcus acusticus of *N. hexodon*, *N. tambuloides* and *N. furcosus* might relate to many different factors such as environmental factors,^{1,2,11,13,24,26} biological factors,^{9,17,19,25} etc. *N. hexodon* are found most abundant in depth range 20-50 meters, in tropical, 25° N- 26° S, Whereas *N. tambuloides* are found in depth range 50-70 meters, in tropical, 18° N- 8° S and *N. furcosus* are found in depth range 8-110 meters, in tropical, 34° N- 25° S.⁸ The differences in environmental factors, i.e. latitude and depth range might cause these fishes to adapt to their environment which might effect the pattern of crystallization and shape of otoliths.^{15, 20,22,26}

The findings of this study show that the sagittal otolith morphology alone can be used as a tool to identify *N. hexodon*, *N. tambuloides* and *N. furcosus*. However, the information from

crystalline structure on sulcus acusticus will confirm the identification of these three fish species.

This paper presents the preliminary results of work documenting interspecific differences in sagitta morphology and crystalline structure on sulcus acusticus among three fish species of the genus *Nemipterus* from coastal of Thailand. Further study using many more fish species will be needed to compile the information of the sagittae of Thai marine fishes.

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References

1. Aguirre, H. and Lombarte, A. (1999). Ecomorphological comparisons of sagittae in *Mulus barbatus* and *M. surmuletus*. *J. Fish. Biol.* 55: 105-114.
2. Arellano, R. V., Hamerlynck, O., Vinex, M., Mees, J., Hostens, K. and Gijssels, W. (1995). Changes in the ratio of the sulcus acusticus area to the sagitta area of *Pomatoschistus minutus* and *P. lozanoi* (Pisces, Gobiidae). *Mar. Biol.* 122: 355-360.
3. Beech, M. and Salem, M. (1999). Modelling the ancient seasonal exploitation of marine resources in the Southern Arabian Gulf: a study of archeological otoliths and modern fisheries data. Department of Archaeology, The King's Monor, University of York, York Yoi.
4. Begg, G. A., Overholtz, W. J. and Munroe, N. J. (2001). The use of internal otolith morphometrics for identification of haddock (*Melanogrammus aeglefinus*) stocks on Georges Bank. *Fish. Bull.* 99: 1-14.
5. Bolles, K.L. and Begg, G. A. (2002). Distinction between silver hake (*Merluccius bilinearis*) stocks in U.S. waters of the northwest Atlantic based on whole otolith morphometrics. *Fish. Bull.* 98: 451-462
6. Bone, Q., Marshall, N.B. and Blaxter, J.H.S. (1995). *Biology of Fishes* (2nd ed.). New York: Chapman & Hall.
7. Friedland, K. D. and Reddin, D. G. (1994). Use of otolith morphology in stock discrimination of Atlantic Salmon (*Salmo salar*). *Can. J. Fishes. Aquat. Sci.* 51: 91-98.
8. *Fish base*. (2003). [On-line]. Available: <http://www.fishbase.org/Summary/SpeciesSummary.cfm?id=5843>
9. Gauldie, R. W. and Crampton, J. S. (2000). An eco-morphological explanation of individual variability in the shape of fish otolith: comparison of the otolith of *Hoplostethus atlanticus* with other species by depth. *J. Fish. Biol.* 60: 1204-1221.
10. Harvey, J. T., Loughlin, T. R., Perez, M. A. and Oxman, D. S. (2000). Relationship between fishes size and otolith length for 63 species of fishes from the Eastern North Pacific Ocean. NOAA Technical Report NMFS150, U.S. Department of Commerce, Seattle, Washington.
11. Lombarte, A. (1992). Changes in otolith area: sensory area ratio with body size and depth. *Environ. Biol. Fish.* 33: 405-410.

12. *Lombarte, A and Castellon, A.* (1991). Interspecific and intraspecific otolith variability in the genus *Merluccius* as determined image by analysis. *Can. J. Zool.* 69: 2442-2449.
13. *Lombarte, A. and Lleonart, J.* (1993). Otolith size changes related with body growth, habitat depth and temperature. *Environ. Biol. Fish.* 37: 297-306.
14. *Lombarte, A. and Morales-Nin, B.* (1995). Morphology and ultrastructure of saccular otolith from five species of the genus *Coelorinchus* (Gadiformes Macrouridae): from the southern Atlantic. *J. Morphol.* 225: 179-192.
15. *Lychakov, D. V. and Rebane, Y. T.* (2000). Otolith regularities. *Hear. Res.* 143: 83-102.
16. *Miller, T. J., Herra, T. and Leggett, W. C.* (1999). The relation between otolith size and larval size at hatching for Atlantic cod, *Gadus morhua*. *Fish. Bull.* 97: 294-305.
17. *Morales-Nin, B.* (2000). Review of the growth regulation processes of otolith daily increment formation. *Fish. Res.* 46: 53-67.
18. *Nelson, J.S.* (1994). *Fishes of the World.* (3 rd ed). New York: Wiley & Son.
19. *Parmentier, E. Vandewalle, P. and Lagardere, F.* (2001). Morpho-anatomy of the otic region in carapid fishes: eco-morphological study of their otoliths. *J. Fish. Biol.* 58: 1046-1061
20. *Paxton, R.J.* (2000). Fish otoliths: do sizes correlate with taxonomic group, habitat and/or luminescence?. *Phil. Trans. R. Soc. Lond. B.* 355:1299-1303.
21. *Phillips, K. L., Jackson, G. D. and Nichols, P. D.* (2001). Predation on myctophids by the squid *Moroteuthis ingens* around Macquarie and Heard Island: stomach contents and fatty acid analyses. *Mar. Ecol. Prog. Ser.* 215: 179-189.
22. *Popper, A. N. and Lu, Z.* (2000). Structure-function relationships in fish otolith organs. *Fish. Res.* 46: 15-25.
23. *Smale, M. J., Watson, G. and Hecht, T.* (1995). Otolith Atlas of Southern African Marine fishes. Ichthyological monographs of the J. L. B. Smith Institute of Ichthyology, No. 1, 253 p.
24. *Torres, G. J., Lombarte, A. and Morales-Nin, B.* (2000a). Sagitta otolith size and shape variability to identify geographical intraspecific differences in three species of the genus *Merluccius*. *Mar. Biol. Ass. U. K.* 80: 333-342.
25. *Torres, G. J., Lombarte, A. and Morales-Nin, B.* (2000b). Variability of the sulcus acusticus in the sagittal otolith of the genus *Merluccius* (Merlucciidae). *Fish. Res.* 46: 5-13.
26. *Volpedo, A. and Echeverria, D. D.* (200). Ecomorphological patterns of the sagitta in fish on the continental shelf off Argentine. *Fish. Res.* 1462: 1-10.