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ÑA TAI VA MOT SO PHÖÔNG PHAP XAC ÑÒNH TUOI CA (TONG QUAN)

Ño Höu Hoang
Vien Hai Döông Hoc (Nha Trang)

TOM TAT Xac ñònh tuoi ca la mot ñieu rat quan trong trong viec nghien cöu ca. Gan ñay, ña tai cua ca ñöôc sö dung ngay cang nhieu vì tính chính xac cao trong ñònh tuoi ca, cung nhö öng dung cua chung trong nhöng nghien cöu khac nhö: sö di cö, sinh san, va co the tai lap lai moi tröông ma ca ña trai qua trong cuoc ñôi. Ña tai cua ca ñöôc tao thanh do moi töông tac giöa cac nhan to ben ngoai va ben trong cô the ca, no ghi lai nhöng bien ñoi cua moi tröông ben ngoai nôi ma ca sinh song hoac ñi ngang qua. Vì vay viec phan tích cau truc hien vi cua ña tai, cho phep cac nha khoa hoc tai thiet lap lòch sö ve moi tröông nôi ma ca ña töng song. Tröôc ñay viec dung ña tai ñe ñònh tuoi ca nhiet ñôi la mot viec kho khan bôi vì moi tröông nhiet ñôi khong co mua ro ret nhö ô vung on ñôi. Gan ñay, nhieu cong trình nghien cöu ña chöng minh rang co the dung ña tai cua cac loai ca nhiet ñôi ñe xac ñònh tuoi ca.

FISH OTOLITH AND SOME AGE DETERMINATION METHODS (A REVIEW)

Do Huu Hoang
Institute of Oceanography (Nha Trang)

ABSTRACT Age determination is very important in fisheries researches. Recently, the use of otoliths to determine the fish age has been increasing, due to its accuracy and its various applications for determination of migration, spawning and reconstruction of environment. Fish otolith is formed by the interaction among endogenous and exogenous factors, and it records any changes in the environment where fishes have lived or migrated past. Thus using method of microstructure analysis allows scientists to reconstruct the environmental histories of fish. It is more difficult to determine age of tropical fish based on otolith due to the aseasonal environment, but more and more studies have proved that otoliths of tropical fish can be used for ageing.

I. INTRODUCTION

Age determination is one of the main subjects for studying the dynamics of fish populations. Estimation of a simple variable like growth rate or complex calculations like virtual population analysis, all need the age information (Campana 2001). The most useful biological information is the age structure of the stock and the relationship between fish length and age.

Age data combined with length information, fecundity, spawning season and fishery data, are used to build reliable stock assessments. With age data, fisheries managers can choose from reasonable models to ultimately determine the impact of fishery, and these model predictions are the basis of catch and effort regulations.

There are many methods to estimate age of the fish and aquatic organisms such as length frequency analysis, mark-recapture, or hard part analysis (Casselman 1983, 1987; Campana & Neilson 1985; Campana 2001; King 2001).

Hard part analysis relies on the hard parts of animals such as scales of fish, fish bone, opercula, spines or fin rays, or otoliths, the shells of bivalves and gastropods or the statoliths of squids.

The otolith methods are more and more used by fisheries biologists all over the world because of the following reasons: firstly, for ageing purpose, it is mostly more accurate and precise than other structures in fish (Campana 2001 & 2002); and secondly fish otolith can be applied to various studied aspects such as: life histories, migration, spawning, reconstruction of environmental histories (Campana 1999).

Recently, there are not any publications on fish otolith analysis and its application in Vietnam, although there are many studies on otolith of temperate fish as well as tropical fish for age determination and other applications in the world. With the purpose of developing fisheries management in Vietnam, the application of fish otolith analysis should be considered by fisheries managers and scientists in Vietnam.

This review is going to introduce fish otolith's applications, some methods of preparing fish otolith for age determination on both yearly and daily increments.

II. OTOLITH APPLICATIONS

Otolith formation continues throughout the life of the fish and results in fine increments that other hard structures do not have. This makes age determination relying on otolith is more accurate and precise than other methods.

Daily rings record the age and growth of a fish from the hatching date to the time of death. If we know the dead date, we can calculate the hatching date of the fish and also the spawning season if we have sufficient data (Woodbury et al. 1995).

From the distances among the increments (daily or yearly), the growth of fish in different growing stages can be calculated. The growth can also be checked by marking the otolith and releasing fish to the wild. The incremental distances were also suggested as an indicator to estimate the habitat quality.

There are a lot of age validation

methods based on the otolith like relying on the chemical (natural or unnatural) compositions or counting daily increments.

It is possible to identify populations by otolith shape (Colura & King 1995), or by otolith microchemistry (Otake & Uchida 1998).

Different fish species have different shape of otoliths. In fact, the shapes of fish otoliths have been used to identify fish species that seal and bird ate when analysing stomach contents and droppings. The fish lengths were also estimated from the otolith sizes (Harkonen 1986). It is noted that in some cases the otoliths will be crystalline and show abnormal shapes (FAO 1984), which will cause problems for species identification.

The morphology of fish otolith is also applied in different aspect of fisheries using otolith length, width and weight to distinguish populations of pink salmon; otolith length is useful to discriminate between spawning populations.

Otolith increments are deposited with compositions coming from environment. Indeed, the entire lifetime of the fish is virtually recorded by the otolith. Therefore, with a chemical technique, it is possible to reconstruct temperature, migration, or environmental histories of individual fish based on assays of the otolith growth sequence (Campana et al. 1997; Campana 1999).

The accuracy and precision in age determination and validation, and various applications make otoliths to be studied in many fishery laboratories in the world.

III. PREPARING OTOLITH FOR AGE DETERMINATION

The preparation methods for otolith analysis have been described in many publications (FAO 1981; Chilton & Beamish 1982; FAO 1984; Secor et al. 1992; Ashford et al. 1993; C.A.R.E 2000).

3.1. Otolith preparation for yearly reading

Yearly rings are easier to analyze on the old fish than on young fish (Campana 2001).

3.1.1. Using the whole otolith

After dissecting and cleaning, the otolith is immersed in a clear liquid (commonly water). This way is only suitable if an otolith is thin and translucent (FAO 1981). According to Campana (2001), the annuli are possible to read if the thickness of the otolith is less than 1 mm.

In some species the outer rings are very narrow, because the growth rate of fish is slow down through age. A grinding technique may be used to make outer rings possible to read, but the disadvantages of this technique are that the edge at the concave side still not clear and some outer rings can be removed (FAO 1981).

For some species, ring structures are only readable immediately after the otoliths are removed and cleaned, so it is advisable that the otolith should be viewed when it is fresh (FAO 1981).

3.1.2. Cracking and burning method

The large otoliths can be broken by hand. Then the sections are grinded and burnt with an alcohol burner or candle. Sometimes oil is put on the burning plane to make the dark and light zones clearer, which enhances the annuli. Burning method makes the protein (otolin) reacting with the heat and displaying the

annuli. It is possible for Scanning Electron Microscope (SEM) with annuli studies (Morales-Nin 1992).

Burning method is done on many species, but with Pacific cod otoliths, it makes the annual zones worse, for this species the "thin sectioning" method is better (FAO 1984, fig. 1).

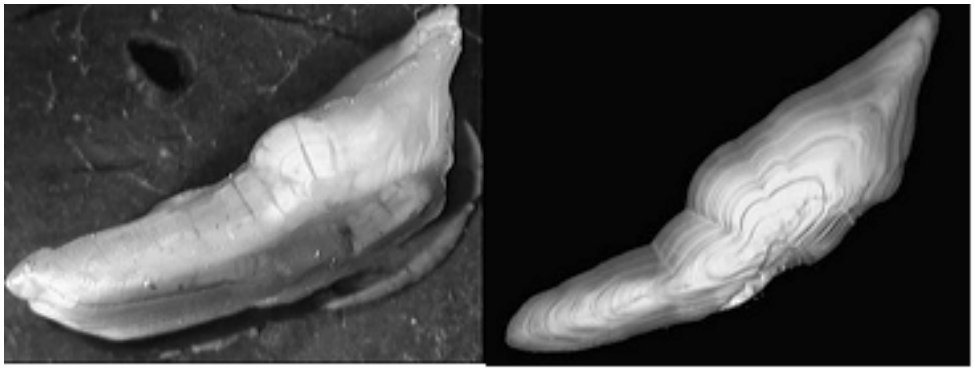

Fig. 1: Pacific cod otolith prepared by breaking and burning method shows the faintness of the annular pattern (left) and by thin sectioning method shows a clear pattern of opaque and translucent zones (by FAO 1984)

3.1.3. Sectioning method

For the thick otolith it is necessary to do the cross-section. For a new studied species it is recommended that the cross-section is checked first to see if the narrow rings exist before deciding to view the whole otolith (FAO 1981). The cross-section can be transverse, frontal, or diagonal (Fig. 2). Each section has to be across the centre of the nucleus of the otolith, if not it will cause the bias of estimation (FAO 1981; Secor et al. 1992). How to decide the cutting plane was discussed in detail by Secor et al. (1992), he concluded that the best section depends on how the otolith grows. The growths of many otoliths are asymmetrical. In sagitta, the predominant growth is in the internal or sulcal phase, so the transverse and diagonal cross sections are recommended. In otolith with preferential longitudinal growth, the transverse may contain many checks, so the frontal section is used (Morales-Nin 1992).

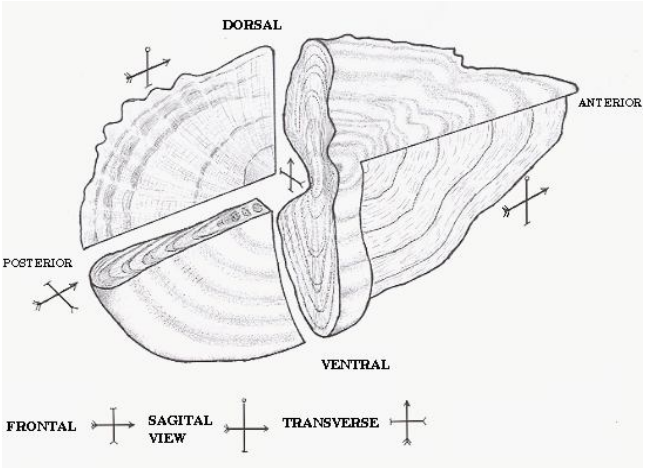

Fig. 2: Three different cross-sectional views demonstrating the concentric nature (core) of the otolith. For use of analysis in the lab, otoliths are polished to yield a cross-section similar to the top sagitta view above. (Image from Pannella 1980)

a. Embedding:

The otoliths are placed in an embedded mould and a small amount of viscosity glue is used to keep them located on the mould with the section plane parallel with the cutting lines (Bedfor 1977; Rauck 1975, cited from Morales-Nin 1992) (Fig. 3).

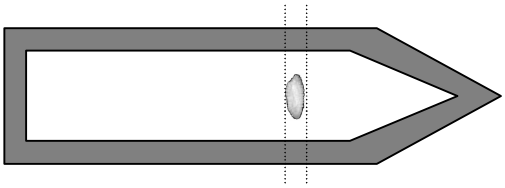

Fig. 3: The otolith was situated on the pattern, the section was cut through discontinuous lines (modified from Secor et al. 1992)

b. Sectioning:

The embedded block is then cut with a precise saw through the predefined section. The otolith's thin sections (2 mm) can be created by a double blade saw. The purpose of sectioning is to cut away the excess plastic adjacent to the otolith core (nucleus), and obtain a flat surface to the defined section plane (Fig. 2).

c. Polishing:

The purpose of polishing is to enhance all the increments in the studied plane. This is a good method because one or both surfaces are polished (Fig. 4). It is easy to measure and analyze the images that the breaking and burning technique cannot do (Campana 1984).

Whole otolith readings give maximum age estimation of 30 years, while sections could be estimated up to 80 years (Wright 1998). Due to the fact that outer rings are more condensed than interior rings. The section techniques are better than whole otolith reading, particularly for fish with great age and growing slowly. Reading whole otolith is applied for young fish and sections for larger fish, which establishes good results (Morales-Nin 1992).

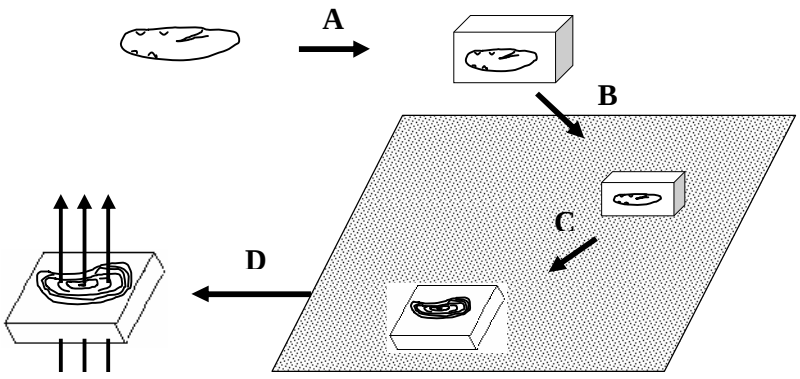

Fig. 4: The polishing procedure for SEM (modified from Secor et al. 1992)

III.2. Preparing the otoliths for daily analysis

The preparation for daily increment analysis requires more careful procedures than that for annuli. The techniques vary with the age and size of the otolith, increment width and clarity, degree of resolution required, available equipment and application.

3.2.1. Preparing for light microscope

All the otoliths except the small larval otoliths must be in thin polished sections where increments can be read directly (magnifications of 400-1000x) (Morales-Nin 1992).

The sections are washed in water, dried under gentle heat and mounted on a slice. The growth increments will be clearer after 2-3 weeks in the mounting medium (Morales-Nin 1992).

3.2.2. Preparing for Scanning Electron Microscope (SEM)

After embedding, sectioning and polishing, otolith sections are then etched with acid, washed, cleaned and sputtered coat with gold. SEM reading is not affected by the thickness of the section (Morales-Nin 1992), and the polishing can be at one or two surfaces (Morales-Nin 1992; Secor et al. 1992). The single polish is also good for SEM and for annuli (Morales-Nin 1992).

a. Etching:

Etching process is to enhance the contrast between discontinuous zones and incremental zones (Fig. 5), which allows reading to daily increments (Campana & Neilson 1985). Chemicals, concentrations and time of etching depend on the size, species and SEM purpose. Secor et al. (1992) mentioned that most people used HCl, tri-sodium ethylenediaminetetra acetate (EDTA) and aqueous EM grade glutaraldehyde (GA) with different concentrations. The solution he used was: HCl (0.1-2%, pH= 2.0-5.0), (EDTA) (5-7%, pH=7.2-7.6), and GA (2%, pH=7.2-7.6). The combination of EDTA-GA can be effective. Morales - Nin (1992) suggested that the low concentration and short time must be tried first for each studied case before defining the longer time and higher concentration of chemicals.

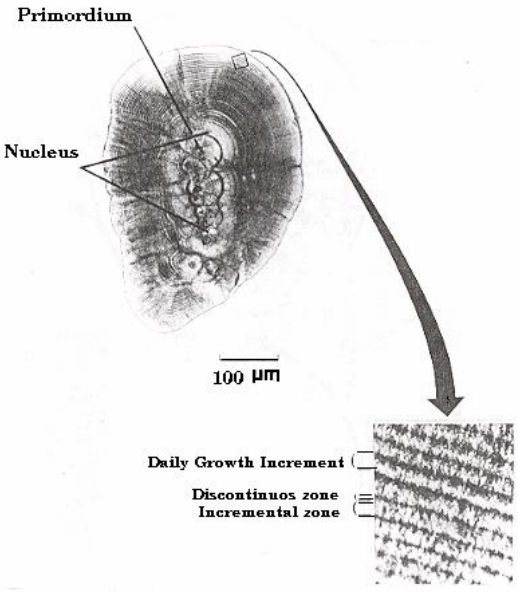

Fig. 5: Incremental growth of chinook salmon otolith was viewed with transmitted light (by Campana & Neilson 1985)

The pH ranges are dependent on species, development stages (larvae, juveniles, adults), otolith types, and section types, so the suitable pH and the time of exposition will be determined by trials (Secor et al. 1992).

Brother et al. (1976) stated that discontinuous zones were more etched with HCl because it contained higher amount of calcium carbonate (cited from Mugiya et al. 1981). In contrast, some studies showed that discontinuous zones had less calcium than incremental zones (Pannella 1971; Mugiya et al. 1981).

b. Mounting:

After etching the sample section is placed into embedding or mounting media. Carefully removing the section from the slide and attaching it to a SEM stub with thermoplastic glue or carborundum paint.

c. Sputtering coat of the section with gold:

This is usual in SEM technique for increasing conductivity (Morales-Nin 1992).

Ashford et al. (1993) described a method of preparing a large number of otolith sections for SEM.

IV. AGING METHODS

1. Reading Annuli

Annuli are possible to read under a light microscope with reflected light. Slow growth rings (winter rings) appear dark and the fast growth rings appear light under reflected light and dark background. For clarity, the otoliths can be immersed in oil, paraffin or glycerine in a dark container (Morales-Nin 1992).

Rings can be read by following progression of the rings formed on the edge of the otolith.

2. Reading daily rings

Daily rings are possible to read by a light microscope or by SEM. The magnifications of light microscope (400 – 1000x) depend on the increment thickness. Many species have very fine increments, which are impossible to detect by the light microscope (Morales-Nin 1989, cited from Morales-Nin 1992). The SEM can detect any increments, no matter how fine (Morales-Nin 1992).

Under the light microscope, the increments appear as concentric rings consisting of alternately light and dark zones. Each pair forms a daily growth increment. Under the SEM, increments appear as crest and sutures because of the difference of the response to acid (Morales-Nin 1992).

Morales - Nin (1992) recommended that firstly locating the radius with most clear increments at low magnification then moving the preparation until the nucleus remains in the centre of the visual field. Using the suitable magnification for reading, beginning the count following the predefined radius.

For SEM the working distance, the degree of inclination and the voltage are important. Low voltages (15 KV) are recommended to avoid the problems with specimen conductivity (Morales-Nin 1992).

In measurement, the surface must be placed horizontally to the electronic face. Increments can be measured in photographs or on the screen using acetate grid paper (Morales-Nin 1992).

V. OTOLITH OF TROPICAL FISH

Previously, it is thought that tropical fish could not be aged from otolith, because tropical environment does not have clear seasons (aseasonal). In many tropical fishes, it has not been possible to show the existence of annual zones (Harkonen 1986). But growth and checked zones in bones and otoliths of tropical fish were reported (Pantulu 1962, 1963; Eziuzo 1963; Poinsard & Troadec 1966, cited from Pannella 1974).

After showing the presence of daily pattern on otolith of temperate fish (Pannella 1971), Pannella continued to find the criteria for age determination in tropical fish (Pannella 1974). He concluded that there were no winter checks or seasonal patterns on the otolith of tropical fish, but the daily, fortnight, monthly and spawning checks were found. This is one of the fundamental studies for determining age of tropical fish.

Since 1980s the quantity of methods of ageing tropical fish have been increasing (Fowler 1995); and many published papers showed that the age of tropical fish can be determined (FAO 1984; Brothers 1992; Morales-Nin 1992; Zhang & Moksness 1993; Ponton et al. 2001).

Fowler (1995) showed that he could prepare and read the otolith of many species of tropical fish; Yosef & Casselman (1995) estimated the age of Tilapia (Oreochromis nilocus) relying on otolith and giving the results that this species has biannual reproduction and growth cycles. The authors said that this method could be applied to any species that shows biannual growth and reproductive cycles. Most of the studies of tropical fish otolith were done on microstructure analysis (Secor et al. 1992; Fowler 1995; Yosef & Casselman 1995); late 1990s some studies based on annual growth of coral reef fish applying light microscopy were presented (Fowler 1995). Usually the annuli were validated by counting daily increments (Fowler 1990; Joyeux et al. 2001), the daily validation was also conducted by using chemical (Bush et al. 1996; Hernaman et al. 2000).

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REFERENCES

1. Ashford J. R., K. Ronbinson, M. G. White, 1993. A method for preparing large numbers of otolith sections for viewing by scanning electron microscope. ICES Journal of Marine Science, 50: 227-229.
2. Brothers E. B., 1992. Final mission report. Preliminary estimates of growth parameters for

commercially valuable species of Trinidad and Tobago. FAO, Port of Spain, Trinidad, 14 pp.

3. Bush P. G., G. C. Ebanks, E. D. Lane 1996. Corporate. Validation of ageing technique for Nassau grouper (Epinephelus striatus) in the Cayman Islands. In: Arreguin-Sanchez F., J. L. Munro, M. C. Balgos, D. Pauly (eds). Author International Cent. for Living Aquatic Resources Management. ICLARM conference proceedings no. 48: 150-158.
4. Campana S. E. 1984. Comparison of Age Determination methods for Starry Flounder. Transactions of American Fisheries Society, 113: 365-369.
5. Campana S. E. 1999. Chemistry and composition of fish otoliths: Pathways, mechanisms and applications. Marine Ecology Progress Series, 188: 263-297.
6. Campana S. E. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology, 59: 197-242.
7. Campana S. E., J. D. Neilson 1985. Microstructure of fish otoliths. Canadian Journal of Fisheries and Aquatic Science, 42: 1014-1032.
8. Campana S. E., S. R. Thorrold, C. M. Jones, D. Günther, M. Tubrett, H. Longerich, S. Jackson, N. M. Halden, J. M. Kalish, P. Piccoli, H. de Pontual, H. Troadec, J. Panfili, D. H. Secor, K. P. Severin, S. H. Sie, R. Thresher, W. J. Teesdale, J. L. Campbell 1997. Comparison of accuracy, precision, and sensitivity in elemental assays of fish otoliths using the electron microprobe, proton-induced X-ray emission, and laser ablation inductively coupled plasma mass spectrometry. Canadian Journal of Fisheries and Aquatic Sciences, 54: 2068-2079.
9. Committee of Age Reading Experts (CARE), May 2000. Manual on Generalized Age Determination Procedures For Groundfish. Prepared by: CARE - Pacific Coast Groundfish Ageing Technicians. Under the Sponsorship of Pacific States Marine Fisheries Commission. For: The Technical Subcommittee of The Canada/U.S. Groundfish Committee.
10. Casselman J. M., 1983. Age and growth assessment of fish from their calcified structures - techniques and tools. U. S. Dept. Comm., NOAA Tech. Rep., NMFS 8: 1-17.
11. Casselman J. M., 1987. Determination of age and growth. In: Weatherley A. H., H. S. Gill (eds), The Biology of Fish Growth. Academic Press, London, 209-242.
12. Chilton D. E., R. J. Beamish, 1982. Age Determination Methods for Fish Studied by the Groundfish Program at the Pacific Biological Station. Canadian Special Publication of Fisheries and Aquatic Science, Ottawa, 60, 102 pp.
13. Colura R. L., T. L. King 1995. Using Scale and Otolith Morphologies to Separate Spotted Seatrout (Cynoscion nebulosus) Collected from Two Areas Within Galveston Bay. In: Secor D. H., J. M. Dean, S. E. Campana (eds), Recent Developments in Fish Otolith Research. University of South

Carolina Press, Columbia, p. 617-628.

14. FAO, 1981. Methods of Collecting and Analyzing Size and Age Data for Fish Stock Assessment. Fao Fisheries Circular, No. 736, 104 pp.
15. FAO, 1984. Ageing Tropical Fish by Growth Rings on the Otoliths. Prepared by: Gjрs„ter J., P. Dayaratne, O. A. Bergstad, H. Gjrрs„ter, M. I. Sousa, I. M. Beck. Fao Fisheries Circular, No. 776, 54 pp.
16. Fowler A. J., 1990. Validation of annual growth increments in the otolith of a small, tropical coral reef fish. Marine Ecology Progress Series, 64: 25-38.
17. Fowler A. J., 1995. Annulus Formation in Otoliths of Coral Reef Fish – A review. In: Secor D. H., J. M. Dean, S. E. Campana (eds), Recent Developments in Fish Otolith Research. University of South Carolina Press, Columbia, p. 45-63
18. Harkonen T., 1986. Guide to the otolith of the bonny fishes of the Northeast Atlantic. Danbiu ApS. Biological Consultants, Hellerup, Denmark. Printed in Sweden, 256 pp.
19. Hernaman V., P. L. Munday, M. L. Schlappy, 2000. Validation of otolith growth - increment periodicity in tropical gobies. Marine Biology, 137: 715-726.
20. Joyeux J., C. Aliaume, A. Zerbi, 2001. An alternative to validation of otolith microincrementation. Journal of Fish Biology, 58: 873-879.
21. King M., 2001. Fisheries biology, Assessment and management. Fishing new book, Blackwell Science Ltd. 341 pp.
22. Morales-Nin, B. 1992. Determination of growth in bony fishes from otolith microstructure. FAO Fisheries Technical Paper, No. 322, 51 pp.
23. Mugiya Y., N. Watabe, J. Yamada, J. M. Dean, D. G. Dunkelberger, M. Shimuzu, 1981. Diurnal Rhythm in Otolith Formation in the Goldfish. Carassius auratus. - Comparative Biochemistry and Physiology, 68A: 659-662.
24. Otake T., K. Uchida, 1998. Application of otolith microchemistry for distinguishing between amphidromous and non-amphidromous stocked ayu, Plecoglossus altivelis. Fisheries Science, 64: 517-521.
25. Pannella G., 1971. Fish otoliths: daily growth layers and periodical patterns. Science, 173: 1124-1127.
26. Pannella G., 1974. Otolith growth patterns: an aid in age determination in temperate and tropical fishes. In: Bagenal T. B. (ed), Ageing of Fish. The Proceeding of an International Symposium on The Ageing of Fish. Unwin Brothers Ltd, The Gresham Pess, England, p. 28-39
27. Pannella G., 1980. Growth patterns in Fish Sagittae. In: Rhoads D. C. & R.A. Lutz (eds), Skeletal Growth of Aquatic Organisms. Biological Records of Environmental Change. Plenum Press, New York, p. 519-560
28. Ponton D., J. H. Mol, J. Panfili, 2001. Use of otolith microincrements for estimating the age and growth of young armoured catfish Hoplosternum littorale.

Journal of Fish Biology, 58: 1274-1285.

29. Secor D. H., J. M. Dean, S. E. Campana, E. H. Laban, 1992. Otolith removal and preparation for microstructure examination. In: Stevenson D. K., S. E. Campana (eds), Otolith Microstructure Examination and analysis. Canadian Special Publication of Fisheries and Aquatic Sciences, 117: 19-57.
30. Woodbury D., A. B. Hollowed, J. A. Pearce, 1995. Interannual variation in Growth Rate and Back-calculated Spawn Dates of Juveniles Pacific Hake (Merluccius productus). In: Secor, D. H., J. M. Dean, S. E. Campana (eds), Recent Developments in Fish Otolith Research. University of South Carolina Press, Columbia, 481-496.
31. Wright P. J., 1998. The Present Status of Otolith Research and Applications. Proceedings of a workshop, held at ORSTOM, Brest, France 27-29 May 1997. Marine Laboratory Aberdeen PO Box 101. Aberdeen AB9 8DB, Scotland. EFAN Report 1-98.
32. Yosef T. G., J. Casselman, 1995. A Procedure for Increasing the Precision of Otolith age Determination of Tropical fish by Differentiating Biannual Recruitment. In: Secor D. H., J. M. Dean, S. E. Campana (eds), Recent Developments in Fish Otolith Research. University of South Carolina Press, Columbia, 247-269.
33. Zhang Z., E. Moksness, 1993. A chemical way of thinning otoliths of adult Atlantic herring (Clupea harengus) to expose the microstructure in the nucleus region. ICES Journal of Marine Science, London, 50: 213-217.