- <u>property/studies/traditional medicine/en/index.html</u>
- 39. Balaram, P., Curr. Sci., 2009, **96**, 1289–1290
- 40. Padma, T. V., Nature, 2005, 436, 486.
- 41. Saper, R. B. et al., JAMA, 2008, 300, 915–923.
- 42. Philippe, O. et al., JAMA, 2003, **290**, 765–772.
- 43. Barnett, R. and Sears, C., Science, 1991, **254**, 188–189.
- 44. Patwardhan, B., Vaidya, A. D. B. and Chopra, A., *Curr. Sci.*, 2003, **84**, 1165–1166.
- 45. Cooper, E. L., Evid. Complement Altern. Med., 2008, 5, 121–122.

- 46. Singh, S., Cell, 2007, 130, 765-768.
- 47. Yang, F., J. Biol. Chem., 2005, **280**, 5892–5901.
- 48. Patwardhan, B. and Mashelkar, R. A., *Drug Discov. Today*, 2009, **14**, 804–811.
- 49. Patwardhan, B. and Gautam, M., *Drug Discov. Today*, 2005, **10**, 495–502.
- Valiathan, M. S., The Legacy of Caraka, Orient Longman, Chennai, India, 2003.

ACKNOWLEDGEMENTS. We thank Drs Rama Vaidya, Kasturba Health Society, Mumbai; Girish Tillu, CDAC, Pune and Darshan Shankar, FRLHT, Bangalore for valuable advice. Special thanks to Dr Alex Hankey, I-AIM, Bangalore for valuable editing.

Bhushan Patwardhan** is in the Interdisciplinary School of Health Sciences, University of Pune, Pune 411 007, India; Ashok D.B. Vaidya, is in the Kasturba Health Society's Medical Research Center, Mumbai 411 021, India

[†]Present address: Manipal Education, 14 HAL Airport Road, Manipal Tower, Bangalore 560 008, India.

*e-mail: bhushan@unipune.ernet.in

Otoliths - the biological CD-ROMs of fish

K. V. Radhakrishnan, W. He, M. Liu and S. Xie

'Otoliths are like biological CD ROMs. They constantly record information about the environment and about how fish lives. The information on them is never lost and you can retrieve potentially any temporal sequence that you like.'

—Suzanne Kingsmill¹

Otoliths are dense calcareous stones found in the inner ear of bony fishes. These fishes have three types of otoliths - sagitta, lapillus and asteriscus; considered to be involved in hearing and balancing functions. They are the first hard part formed in the fish and grow continuously by successive deposition of mineral-rich calcium carbonate (aragonite) and protein-rich layers. Otoliths are metabolically inert, not subject to reabsorption and remodeling by growth and their characteristic shape will not be affected by the mode of fish preservation (excluding acid preservatives). Having these inherent but outstanding qualities, otoliths proved themselves as good recorders of life history of the fish and its surrounding environment. While the otolith morphology is species-specific, the pattern of growth rings in an otolith microstructure reveals the age and temporal growth of the fish in relation to the environmental conditions whereas the elemental composition can answer questions on its preoccupied and current habitat features.

A vast array of research has been conducted on a wide variety of fishes based on otolith analyses, e.g. morphology, microstructure and microchemistry. Gen-

erally the otolith applications can be classified under the following aspects: (1) Age and growth estimation, (2) Early life history recruitment, (3) Habitat shifts and migration, (4) Stock determination and (5) others.

Age and growth estimation

Counting the daily, seasonal and annual increments to estimate age of a fish seems to be the most primary and widest application of otoliths. Otoliths also proved capable of estimating the age of fishes having extreme longevity, those living for more than 80 years, the technology of which has now been used for age estimation in fossil fishes. Radiometric ageing, where the relative abundance of radioisotopes (e.g.: 226Ra and 210Pb, and ²²⁸Th and ²²⁸Ra) at the core of otoliths are measured, promises more accurate age determinations². As bodily (somatic) growth and otolith growth are more often closely correlated, the width of otolith increment will reflect the rate of somatic growth³. Accurate size-at-age and age-at-first maturity estimations assume high significance in management and conservation of exploitable fishery resources, artificial breeding and aquaculture practices.

Early life history and recruitment

Otolith microstructures record life history events and stage transitions of fish

during the early life stages. The deposition of the first otolith increment occurs at the time of hatching, at yolk absorption or at first feeding in most of the fishes. Secondary growth centres are another developmental mark. The onset of metamorphosis is marked in many species by the appearance of transitional zone/bands or settlement mark and/or the formation of secondary growth centre in the otoliths. Otolith growth rates also change at transitions between life history stages². By comparing the hatch date frequency distribution of surviving larvae to the seasonal production of eggs, it is possible to identify larval survival and recruitment success within the season of reproduction⁴. An otolith record of life history events combined with the age and size information contained within them can answer the role of age versus size in the timing of life history transitions.

Habitat shifts and migration

Otolith microchemistry is a developing technique, which finds application in routine migration studies of diadromous fishes. Shifts between marine, freshwater and estuarine habitats can be tracked from predictable variations in strontium-calcium ratio or isotopic concentration within otoliths⁵. Otolith elemental analysis is also applied to understand the influence of physical hindrance on their migration, and to detect short habitat shifts, from the nursery ground to the adult habitat in non-diadromous fishes⁶.

Most of the studies were based on elements such as Ca, Sr, Na, K, Mg, Ba, P, S and Cl, whereas a few are on trace elements, such as Cu, Zn, Fe and Sn. Typical transitional zone, in the form of a slow growing protein-rich area with special microstructure features, is often formed in the otoliths of some fishes during habitat shifts and migrations.

Stock determination

Microstructure. microchemistry and morphology of otoliths can be used for stock determination. Protracted spawning season, differences in hatching time and varied pattern of growth of individuals belonging to separate stocks can be understood from increment pattern and counts. Variation in elemental and isotopic compositions in otoliths showed that they can also act as natural markers or tags from different geographical stocks², and has been used to determine nativity of a given stock, natal homing rates of marine fish, connect the juvenile and adult, or larvae and juvenile populations at the instances of stock-mixing and also to discriminate fish stocks and nursery areas⁷. Differential growth rate produces corresponding variation in otolith shape, whereby fish stocks that maintain distinct distributions can be distinguished from otolith morphology. In addition to external measurements, shape of internal features such as annulus has been also used in recent stock discrimination studies³. Stock determination and characterization are the most important prerequisites for sustainable exploitation and conservation of rare fish stocks.

Other applications

Otolith has many applications in miscellaneous fields also. Right-left asymmetry (fluctuating) of otolith length, weight, width, growth rate, as well as right-left otolith shape differences, are being used as measures of developmental instability (DI), arising from human induced or natural disturbances⁸. It is also proved many a time that the increment deposi-

tion in otoliths is also affected by environmental stress, thus ably qualifying them to be used as stress analysers or indicators. Storm, hydrological fluctuations and meteorological changes also can induce variations in the increment pattern⁹. Since otoliths incorporate various chemical elements present in the environment (biomineralization) as they grow, they can also be used as good tracers of chemical pollution¹⁰. High sensitivity of otolith characteristics to temperature changes was used to induce permanent, recognizable 'thermal marking' on otoliths by exposing fish to short periods of relatively cold water¹¹. Otolith tagging by immersion in solutions of fluorescent substances (Alizarin complex or Calsein or tetracycline hydrochloride) has proved to be highly economical and successful, and has been extensively used in many marine and freshwater diadromous fishes¹². Fish otoliths recovered from gastrointestinal contents of piscivorous fish, marine mammals and birds are used to understand their diet $composition ^{13} \\$

Fishery scientists all over the world have now realized the importance of otolith based analyses due to their accuracy, precision and versatile applications. Adoption of such techniques can make remarkable contributions to Indian Ichthyology as well. Age and growth, life history and stock structure studies of majority of the commercially important and exploited fishery resources in India are just emerging. Effective biodiversity conservation plans are difficult to implement due to the lack of authentic data on habitat shifts and migrations especially in the case of threatened fish species. No serious attention has been paid to the declining populations of large migratory fishes; due to dam constructions, pollution and over-exploitation. Otolith microstructure and morphological analyses give well-defined information on the age, growth and stock structure of fishes. Thus, features of fishery management importance such as recruitment, age at first maturity, size at age relationships and decisive environmental parameters to fish growth, etc. can be elucidated. As otolith elemental analyses give an exact idea on the migration behaviour of the fishes, environmental stress due to pollution and other factors, such data can be incorporated into the fishery management plans and appropriate conservation strategies can be put forth for those fishes facing serious threat of extinction.

- 1. Kingsmill, S., Science, 1993, **260**, 1233–1234
- Campana, S. E. and Neilson, J. D., Can. J. Fish. Aquat. Sci., 1985, 42, 1014– 1032.
- Xie, S., Watanabe, Y., Saruwatari, T., Masuda, R., Yamashita, Y., Sassa, C. and Konishi, Y., J. Fish Biol., 2005, 66, 1704–1719.
- 4. Searcy, S. P. and Sponaugle, S., Mar. Ecol. Prog. Ser., 2000, 206, 213–226.
- Tzeng, W. N., Severin, K. P. and Wickström, H., *Mar. Ecol. Prog. Ser.*, 1997, 149, 73–81.
- 6. Gillanders, B. M. and Kingsford, M. J., *Mar. Ecol. Prog. Ser.*, 1996, **2**, 13–20.
- Gagliano, M. and McCormick, M. I., Mar. Ecol. Prog. Ser., 2004, 278, 291– 296
- 8. Wilson Jr, R. R., Copeia, 1985, 4, 1011– 1017.
- MacLellan, S. E. and Saunders, M. W., Recent Developments in Fish Otolith Research, Univ. South Carolina Press, Columbia, SC, 1995, pp. 567–580.
- Brothers, E. B., Spec. Publ. Am. Soc. Ichthyol. Herpathol., 1984, 1, 50–57.
- Volk, E. C., Schroder, S. L., Grimm, J. J. and Ackley, H. S., *Trans. Am. Fish. Soc.*, 1994, **123**, 811–816.
- Oliver, J. D., Holeton, G. F. and Chua, K. E., *Trans. Am. Fish. Soc.*, 1979, 108, 130–136.
- Bowen, W. D., Can. J. Fish. Aquat. Sci., 2000, 57, 898–905.

K. V. Radhakrishnan, W. He, M. Liu and S. Xie* are in Key Laboratory of Biodiversity and Conservation of Aquatic Organisms, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei 430072, P. R. China; W. He is also in Graduate School of the Chinese Academy of Sciences, Beijing, 100049, P. R. China. *e-mail: xiesg@ihb.ac.cn