ELSEVIER

Journal of Experimental Marine Biology and Ecology,
238 (1999) 21–27

JOURNAL OF EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

Two-dimensional X-ray mapping of otoliths, a high-resolution technique for determining amphidromy in the tropical goby *Lentipes concolor* (Gill)

R.L. Radtke[a,*], D.W. Townsend[b], R.A. Kinzie III[c], D. Fey[d]

[a]*School of Ocean and Earth Sciences and Technology, University of Hawaii, Honolulu, HI 96822, USA*
[b]*School of Marine Sciences, University of Maine, Orono, ME, USA*
[c]*Department of Zoology, University of Hawaii, Honolulu, HI 96822, USA*
[d]*Department of Oceanography, Sea Fisheries Institute, ul. Kollataja 1, 81-332 Gdynia, Poland*

Received 13 May 1997; received in revised form 3 September 1998; accepted 4 September 1998

Abstract

High-resolution X-ray maps of Sr/Ca ratio concentrations in otoliths from a diadromous tropical goby, *Lentipes concolor* (Gill), were produced that distinguish elemental variations on a scale considerably less than 1 μm^2. X-ray maps displayed distinct patterns of strontium incorporation and, combined with the position of daily microincrements, permitted delineation of the timing of experimentally manipulated salinity changes on a daily basis. This technique may have important applications for studying diadromous fishes and in providing evidence for movement between water masses of different salinity over short time periods.

Keywords: Diadromy; Mapping; Otolith chemistry; X-ray analyses

1. Introduction

Hawaiian freshwater gobies have an amphidromous life cycle, where demersal eggs are laid in the adult freshwater stream habitat, and newly hatched young are swept out to sea where they undergo a period of development (Radtke et al., 1988). After the marine larval period, post-larvae return to a stream, where they settle, metamorphose into juveniles and begin their upstream migration to the adult habitat (Maciolek, 1977; Kinzie, 1990). The nature and timing of recruitment to the streams may have important

*Corresponding author.
E-mail address: radtke@hawaii.edu (R.L. Radtke)

0022-0981/99/$ – see front matter
PII: S0022-0981(98)00160-9

implications for the survival and population dynamics of these fish, however little is understood about this life history event.

Changes in water chemistry are reflected by changes in the elemental composition of fish otoliths (Radtke et al., 1988; Reiman et al., 1994). Strontium/calcium concentration ratios in fish otoliths have been shown to be directly related to ambient salinity at the time of otolith precipitation (Casselman, 1982; Radtke et al., 1988; Secor, 1992; Radtke et al., 1996). This may be due to the fact that strontium concentrations in freshwater are considerably lower (0.07 ppm) than in seawater (8 ppm) (Angino et al., 1966; Mackenzie and Garrels, 1966; Rosenthal, 1981; Bowen, 1988). Because otolith material is inert once deposited, the correlation between Sr/Ca concentration ratio and salinity has been used successfully to determine migrational histories of individual fish (e.g., Radtke et al., 1988; Secor, 1992; Radtke et al., 1996). Most studies utilized a wavelength dispersive electron microprobe (WDEM) to measure strontium and calcium concentration differences along a narrow transect of individual points across the otolith. By analyzing the Sr/Ca concentration ratio profile along the transect, environmental salinity history was reconstructed. The WDEM provides accurate quantitative results with measurement errors of less than 3–5% by weight of the elements measured (Radtke et al., 1996, 1998).

WDEM analyses have limited spatial resolution due to the diameter of the focused electron beam (Gunn et al., 1992). To obtain accurate quantitative results and avoid errors caused by electron beam-induced sample damage, the beam must be adjusted to no smaller than a minimum diameter of 1–5 μm^2 (Radtke, 1989). This results in a minimum spatial resolution which may correspond to 1–5 daily increments on the otolith's surface, depending on the species and otolith growth rate (Townsend et al., 1992). The technique described here permits analysis on a much smaller spatial scale. A narrow beam (1 μm diameter) is moved in small increments (0.1 μm) resulting in a map of X-ray intensity values. This technique provides quantitative data on elemental concentrations with 0.1 μm resolution and reveals variations recorded in the otolith.

2. Materials and methods

Twenty-one newly recruited *Lentipes concolor* (Gill) from Hanakapiai, Kaua'i were held in an aquarium maintained at 21–22°C with a 12 h light/dark cycle, and reared through a series of different salinities (Table 1). Following the salinity experiment, two fish were randomly selected and the sagittal otoliths were dissected and mounted on 2.5 cm glass disks with heat setting resin. Mounted samples were ground using 600 grit

Table 1
Experimental salinity treatment

Day number	Duration (days)	Salinity (ppt)
1	1	0
2–15	14	18
16	1	27
17–28	12	36
29–50	21	0

grinding paper, and polished with 0.3 and 0.05 μm alumina paste. The resulting surface was a highly polished sagittal section through the core of the otolith (determined by reflected light microscopy). In preparation for analysis on the microprobe, samples were carbon coated to a thickness of 250–300 Å.

Analyses were made with a Cameca Camebax-SX50 wavelength dispersive electron microprobe, which has the capability of scanning a beam over small areas and recording the results as maps of the distribution of X-ray intensities keyed to specific elemental spectra. Scans used a focused (1 μm) electron beam with an accelerating voltage of 15 kV and a beam current of 12 nA. Strontium and calcium were measured using TAP and PET crystals, respectively, and were standardized using strontianite and calcite, respectively. The beam was moved in steps of 0.1 μm for a duration of 50 ms per step. The scanned area was a square 51.2 μm on a size. A complete scan took 60 s and provided 512×512 resolution. The square was scanned 256 times per sample and the data accumulated. The total scan time was greater than 4 h. These long scanning times were necessary to accumulate enough 'hits' per pixel to raise counts to reliable levels, especially for Sr, which was in low concentration.

The scanning procedure produced an image of the elemental spatial distribution on a cathode-ray tube. Each brightened pixel of the resultant image was produced by the arrival of an X-ray to the spectrometers calibrated for strontium and calcium respectively (Fig. 1). More numerous or dense brightened pixels correspond to higher strontium concentrations. Luminosity analysis of the image provides the strontium concentration of each computer pixel after standardization. The resulting X-ray intensity data were converted into conventional image file format for analysis using *Image* v. 1.59, National Institutes of Health. Sr/Ca ratios were calculated from numerical intensity data and presented as a black and white image.

3. Results

Mathematical composites of X-rays characteristic for strontium/calcium ratios over selected portions of otoliths from two different individuals are presented in Figs. 1 and 2. Fig. 1 illustrates an X-ray intensity map derived from the portion of the otolith deposited during oceanic larval life (bottom right corner), recruitment to freshwater and freshwater existence (dark band), capture and immersion in 18 then 36 ppt seawater (upper left corner), then return to freshwater (far upper left corner). Fig. 2 illustrates a map of the portion of an otolith deposited during experimental changes in environmental salinity from 0 ppt (left dark band), 18–36 ppt (central light band), and 0 ppt (right dark band). Changes in otolith elemental composition corresponding to changes in environmental salinity were illustrated by changes in the optical density of the scans. Light areas correspond to higher Sr/Ca values.

4. Discussion

Analyses of fish otoliths can document periods of perturbation or stress (Pannella, 1980; Radtke and Shafer, 1992; Radtke et al., 1996). Moreover, changes in Sr/Ca

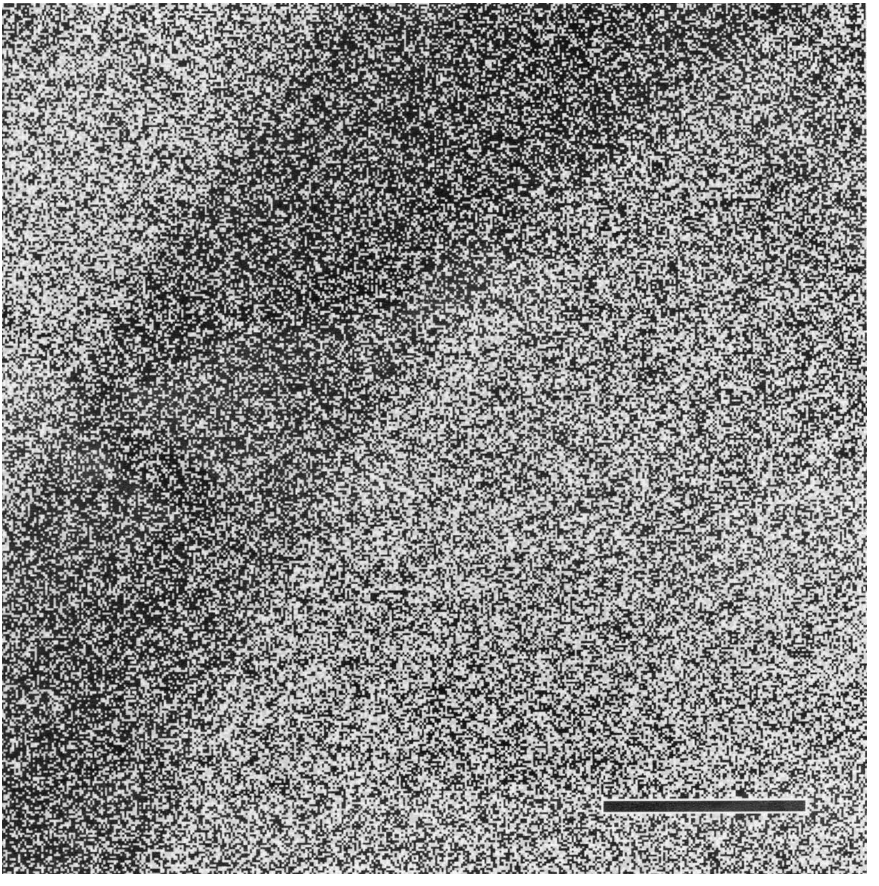

Fig. 1. X-ray intensity map delineating Sr/Ca concentration ratios in sections of a *Lentipes concolor* otolith formed during oceanic larval life (bottom right corner), recruitment to freshwater and freshwater existence (dark band), capture and immersion in 18 then 36 ppt seawater (upper left corner), then return to freshwater (far upper left corner). Lighter areas indicate higher Sr/Ca values, characteristic of higher salinity water. Scale bar = 20 μm.

concentration ratios in otoliths can document changes in ontogenetic factors, salinity, growth rate, food or nutrients, and temperature (Radtke and Shafer, 1992; Mugiya and Yoshida, 1995). Pinpointing the timing and causation of checks and elemental concentration change found in otoliths may provide important information about the life history of fish.

The scanning procedure described in this paper can produce maps of elemental composition at sub-micron level resolution. Pixel intensity can be assigned numerical values and used to provide elemental concentrations or ratios. This can provide a detailed two-dimensional map of changes in otolith chemistry (Sadovy and Severin, 1992; Otake et al., 1994; present study). Maps of elemental composition can be related

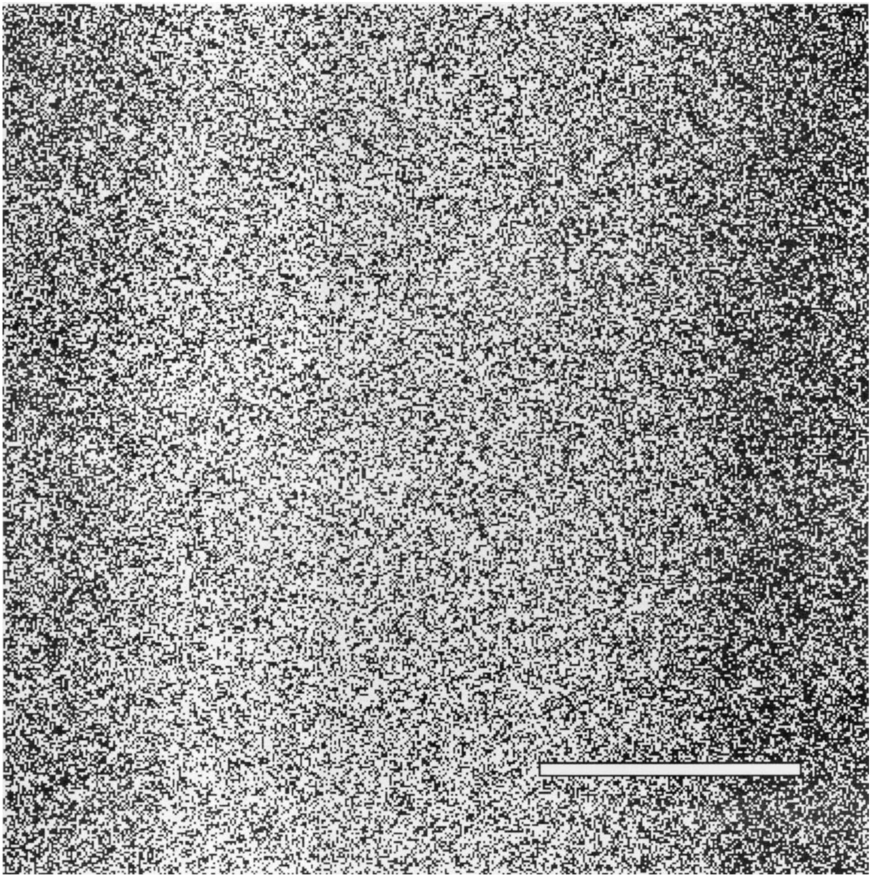

Fig. 2. X-ray intensity map delineating Sr/Ca concentration ratios in sections of a *Lentipes concolor* otolith formed during experimental changes in environmental salinity from 0 ppt (left dark band), 18–36 ppt (central light band), and finally 0 ppt (right dark band). Lighter areas indicate higher Sr/Ca values, characteristic of higher salinity water. Scale bar = 10 μm.

to chronological (daily or annual) features of the otolith, and hence calibrated to a temporal scale. It is possible to have 20 or more pixels per primary (or 'daily') otolith increment, allowing environmental conditions to be deciphered on a sub-daily level (Fig. 1). In the present study diadromy changes are demonstrated and can be calculated as to dates, ages and transition of freshwater recruitment.

Many electron microprobes can be adjusted to scan an electron beam across the surface of a sample to obtain X-ray intensity maps like those presented here (e.g., Sadovy and Severin, 1992; Otake et al., 1994). This procedure has the advantage that the image can be monitored during data acquisition and there is little sample damage caused by the electron beam because the beam remains at each point on the otolith's surface for only a fraction of a second.

This technique allows visualization of changes in trace element deposition in otoliths with sub-daily resolution. Such resolution may provide valuable insights into the life history dynamics of diadromous fishes.

Acknowledgements

Thanks are due to D. Malone, C. Chong, and T. Hulsebosch for their assistance in this study. This study was funded, in part, by National Science Foundation grants DEB-93-22618, OPP 95-30081, and OCE 95-31995. This is Hawaii Institute of Geophysics and Planetology contribution No. 1040 and SOEST contribution No. 4771.

References

Angino, E.E., Billings, G.K., Andersen, N., 1966. Observed variations in the strontium concentration of sea water. Chem. Geol. 1, 145–153.

Bowen, R., 1988. Isotopes in the Earth Sciences. Elsevier Applied Science, London.

Casselman, J.M., 1982. Chemical analyses of the optically different zones in eel otoliths. In: Loftus, K.H. (Ed), Proceedings of the 1980 North American Eel Conference, Ontario Fisheries Technical Report No. 4, pp. 74–82.

Gunn, J.S., Harrowfield, I.R., Proctor, C.H., Thresher, R.E., 1992. Electron probe microanalysis of calcified tissues in fishes – evaluation of techniques for studying age and stock discrimination. J. Exp. Mar. Biol. Ecol. 158, 1–36.

Kinzie III, R.A., 1990. Species profiles: life histories and environmental requirements of coastal vertebrates, Pacific Ocean Region: Report 3, Amphidromous macrofauna of island streams. Technical report EL-89-10, US Army Engineer Waterways Experiment Station, Vicksburg, 28 pp.

Mackenzie, F.T., Garrels, R.M., 1966. Chemical mass balance between rivers and oceans. Am. J. Sci. 264, 507–525.

Maciolek, J.A., 1977. Taxonomic status, biology, and distribution of Hawaiian Lentipes, a diadromous goby. Pacif. Sci. 31, 355–362.

Mugiya, Y., Yoshida, M., 1995. Effects of calcium antagonists and other metabolic modulators on in vitro calcium deposition on otoliths in the rainbow trout *Oncorhynchus mykiss*. Fish. Sci. 61, 1026–1030.

Otake, T., Ishii, T., Nakamura, M., Nakamura, R., 1994. Drastic changes in otolith strontium/calcium ratios in leptocephali and glass eels of Japanese eel *Anguilla japonica*. Mar. Ecol. Prog. Ser. 112, 189–193.

Pannella, G., 1980. Growth patterns in fish sagittae. In: Rhoads, D.C., Lutz, R.A. (Eds), Skeletal Growth of Aquatic Organisms. Plenum Press, New York, pp. 519–560.

Radtke, R.L., Kinzie III, R.A., Folsom, S.D., 1988. Age at recruitment of Hawaiian freshwater gobies. Environ. Biol. Fishes 23, 205–213.

Radtke, R.L., 1989. Larval fish age, growth and body shrinkage: information available from otoliths. Can. J. Fish. Aquat. Sci. 46, 1884–1894.

Radtke, R.L., Shafer, D.J., 1992. Environmental sensitivity of fish otolith microchemistry. Aust. J. Mar. Freshwater Res. 43, 935–951.

Radtke, R.L., Svenning, M., Malone, D., Klementsen, A., Ruzicka, J., Fey, D., 1996. Migrations in an extreme northern population of the Arctic charr, *Salvelinus alpinus* (L.): insights from otolith microchemistry. Mar. Ecol. Prog. Ser. 136, 13–25.

Radtke, R.L., Dempson, J.B., Ruzicka, J., 1998. Microprobe analyses of anadromous Arctic charr, *Salvelinus alpinus*, otoliths to infer life history migration events. Polar Biol. 19 (1), 1–8.

Reiman, B.E., Myers, D.L., Nielsen, R.L., 1994. Use of otolith microchemistry to discriminate *Oncorhynchus nerka* of resident and anadromous origin. Can. J. Fish. Aquat. Sci. 51, 68–77.

Rosenthal, H.L., 1981. Content of stable strontium in man and animal biota. In: Skoryna, S.C. (Ed), Handbook of Stable Strontium. Plenum Press, New York, pp. 503–514.

Sadovy, Y., Severin, K.P., 1992. Trace elements in biogenic aragonite: correlation of body growth rate and strontium levels in the otoliths of the white grunt, *Haemulon plumieri* (Pisces: Haemulidae). Bull. Mar. Sci. 50, 237–257.

Secor, D.H., 1992. Application of otolith microchemistry analysis to investigate anadromy in Chesapeake Bay striped bass *Morone saxatilis*. U.S. Fish. Bull. 90, 798–806.

Townsend, D.W., Radtke, R.L., Corwin, S., Libby, D.A., 1992. Strontium:calcium ratios in juvenile Atlantic herring *Clupea harengus* L. otoliths as a function of water temperature. J. Exp. Mar. Biol. Ecol. 160, 131–140.