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Otolith microchemistry of the conger eel, *Conger myriaster*

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Elemental composition and ontogenic change patterns of Sr and Ca were examined in conger eel, *Conger myriaster*, otoliths. Accumulations of the major elements of seawater, K, Mg, and Na, into otoliths were smaller than the accumulations of 19 other minor elements whose concentrations were determined. Strontium content and Sr:Ca ratios in the otolith fluctuated during the life history transect at 100 to 200 μm from the core of the otolith, indicating a period of metamorphosis. Changes in Sr content and Sr:Ca ratios may be associated with internal physiological factors such as metamorphosis in the early developmental stage in the conger eel.

INTRODUCTION

Most studies of the elemental composition of fish otoliths have reported levels of elements, such as Ca and Sr, in order to reconstruct the migration histories of diadromous fish (Arai & Tsukamoto, 1998; Tzeng et al., 2000; Arai & Miyazaki, 2001), while a few have reported concentrations of other elements (Campana, 1999). The elemental composition of otoliths has been used to discriminate stocks and to examine habitat utilization by fish residing in waters differing in chemical composition because of the natural variability of ambient waters (Campana, 1999) and pollution (Thorrold et al., 1997). Therefore, elemental composition of otoliths has a great potential as a biological tracer such as stock identification as well as an environmental tracer of fish habitats as a record of fish life history.

Conger eel (*Conger myriaster*) is known to be distributed from the East China Sea to the waters off Korea and Japan, and inhabits shallow coastal waters to the edge of the continental shelf (Lindberg & Legeza, 1969). This fish is one of the important commercial species in those areas. However, knowledge regarding the ecological and physiological aspects of, for example, spawning area, metamorphosis, oceanic and inshore migration, population structure, growth, maturation, and reproduction are at only a rudimentary level. Conger eels are one of the higher trophic level organisms in the coastal ecosystem and are distributed widely worldwide; therefore, the species appears suitable for use as a biomonitoring indicator.

The otolith of the conger eel, *Conger myriaster*, was examined in order to accumulate basic information on the elemental composition and ontogenic changes. The results formed the basis of a discussion regarding the metabolic process of the elements in the otolith.

MATERIALS AND METHODS

Conger myriaster were collected using eel pots in Otsuchi Bay, Japan, on 31 January 2001. After measuring the total length (341–637 mm) of each fish, sagittal otoliths were extracted. Both sides of a total of 20 otoliths were used for the present study. The right and the left sides of the otolith in each specimen were examined using inductively coupled plasma mass spectrometry (ICPMS) analysis and using wavelength dispersive X-ray spectrometry by means of an electron microprobe (EPMA), respectively. Otoliths were mechanically cleaned of attached tissue, and then washed in an ultrasonic bath and rinsed with deionized water. After cleaning, the otolith samples were dried at 80°C for 12 h.

In preparation for ICPMS analysis, each whole otolith was weighed to the nearest 0.001 g (mean ± SD: 0.014 ± 0.002 g; range: 0.012–0.016 g) and placed in a PTFE (Teflon) vessel. Otoliths were then digested using microwaves to a transparent solution employing concentrated nitric acid. The resultant solutions were diluted with doubly deionized water and transferred to acid-washed sample tubes. Elemental concentrations were determined using ICPMS (Hewlett-Packard HP-4500). Internal standards were added to all samples, and the standards were calibrated. The concentrations of all elements are reported as $\mu g\, g^{-1}$ on a dry weight basis.

For EPMA analysis, the otoliths were embedded in epoxy resin (Strues, Epofix), mounted on glass slides, and ground to expose the core. After being polished with 6 μm and 1 μm diamond paste on a polishing wheel (Strues, Planopol-V), they were cleaned in an ultrasonic bath, rinsed with deionized water, and carbon coated by high vacuum evaporation. Strontium and Ca concentrations were measured quantitatively using an EPMA (JEOL JXA-8900R), as described by Arai et al. (1997). Calcite ($CaCO_3$) and strontianite ($SrCO_3$) were used as standards. The accelerating voltage and beam current were 15 kV and 1.2×10^{-8} A, respectively. The electron beam was focused on a point about 10 μm in diameter, with spacing measurements of 10 μm intervals. Each datum point represents the average of three measurements (each counting time: 4.0 s).

RESULTS AND DISCUSSION

The present study showed for the first time the concentrations of various elements in the conger eel, *Conger*

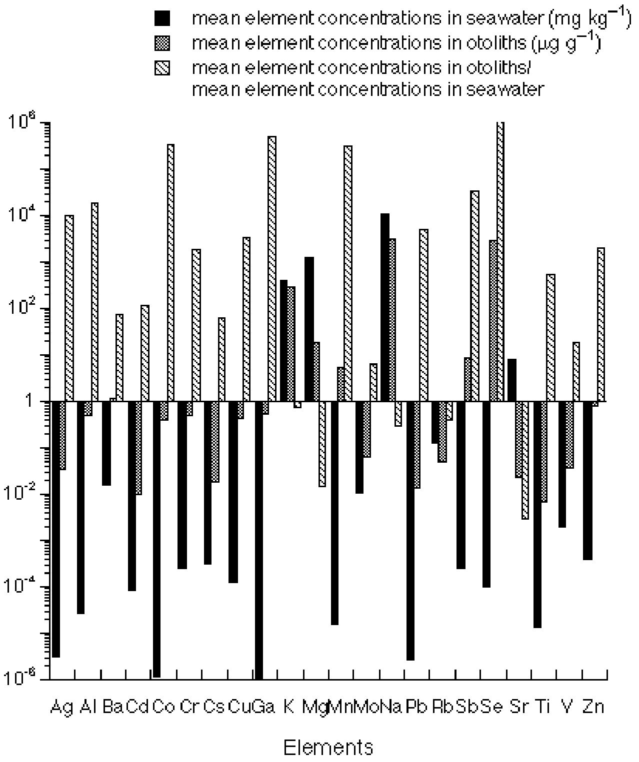

Figure 1. *Conger myriaster*. Mean elemental concentrations in seawater (Nozaki, 1992), elemental composition of otoliths of the conger eel, and concentration factors from seawater to the otolith of the conger eel of each element.

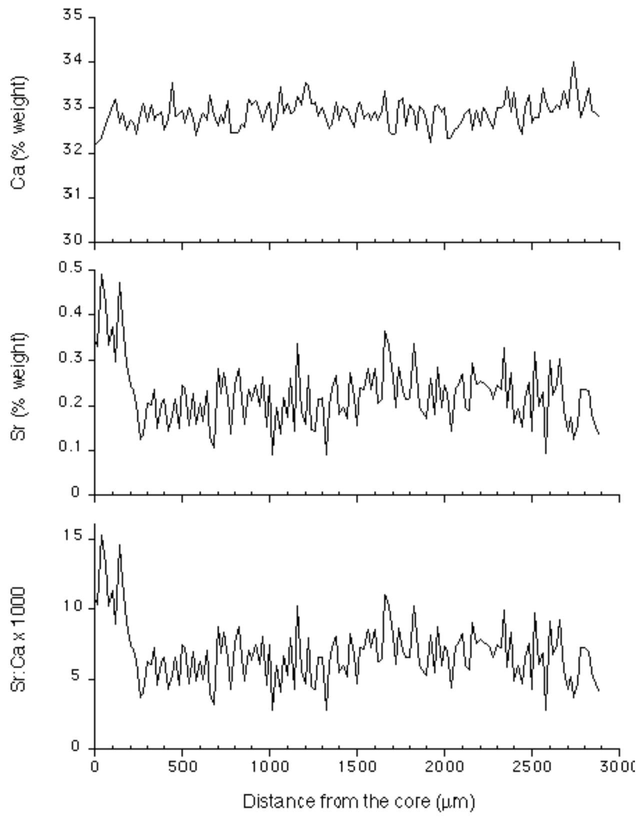

Figure 2. *Conger myriaster*. Profiles of Ca and Sr contents and Sr:Ca ratios from the core to the edge of the otolith of a conger eel (total length: 532 mm) collected in Otsuchi Bay, Japan, on 31 January 2001.

myriaster (Figure 1). It is noteworthy that significant differences in accumulations from seawater to otolith are found in each element (Figure 1). Potassium, Mg, and Na are major elements ($>100\,mg\,kg^{-1}$) in seawater; however, uptake from seawater into the otolith of those elements is lower than other minor elements ($<100\,mg\ kg^{-1}$) in seawater (Figure 1). This suggests that elemental discrimination might occur during the movement from ambient water to the otolith. The basic pathway of the bulk of inorganic elements in the otolith is from the water into the blood plasma via the gills or intestine, then into the endolymph, and finally into the crystallizing otolith. Water passing over the gills (branchial uptake) is the primary source of most elements in freshwater fish, while the continual drinking of marine fish is the main source of water-borne elements for assimilation via the intestine (Olsson et al., 1998). A small but unknown proportion of elements is undoubtedly assimilated from food sources. Farrel & Campana (1996) suggested that some of the Sr in the diet was incorporated into the otolith. However, other experiments suggested that the otolith uptake of elements from food was minimal (Hoff & Fuiman, 1995). Therefore, the majority of the inorganic otolith elements are probably derived from ambient water. Campana (1999) suggested that the biggest barriers to elemental uptake occurred at the intestine–water interface in marine fish, where osmoregulation controlled the movement of water-borne ions into the fish, while minor elements such as Ba, Mn, Pb, Sr, and Zn were consistent with an environmental effect. These considerations all lead to the conclusion that in the otolith marked elemental discriminations regarding K, Mg, and Na are due to strict osmoregulatory control in seawater, while other minor elements reflect the elemental composition of the ambient water.

Elemental discrimination also occurs during the movement from plasma to endolymph (Campana, 1999), resulting in an endolymph composition which is depleted in all major ions other than K (Kalish, 1991; Gauldie & Romanek, 1998). In general, the composition of the endolymph appears to be closer to the composition of the otolith than is the water or blood plasma. The transfer of calcium and other ions into the endolymph may occur via a transcellular route, although the factors influencing endolymph composition remain poorly understood. Therefore, ensuring significant regulation of both the selection of elements and their concentrations in the endolymph (Mugiya & Yoshida, 1995). Recent experiments by Payan et al. (1998) indicate that the elemental composition of the endolymph is less affected by starvation than is the plasma.

Otolith Sr content and Sr:Ca ratios fluctuated strongly during the life history transect in accordance with the developmental stage (Figure 2). Strontium content increased outwards to a maximum level of about 0.43% at 100–200 μm from the core, and thereafter decreased rapidly at 150–230 m from the core to 0.13%. Subsequently, no marked fluctuation was apparent toward the edge of the otolith (0.21%), although slight fluctuations were apparent. Calcium content was almost constant, averaging 32.9%, throughout the otolith (Figure 2). Sr:Ca ratios changed in a similar manner to Sr content, reaching a maximum level, averaging 13.2×10^{-3} some 100–200 μm from the core (Figure 2). The higher level continued until 150–230 μm from the core, followed by a rapid drop, averaging 3.9×10^{-3}. These marked fluctuations in the Sr and Sr:Ca ratios of the otolith corresponded to the period during leptocephalus and glass eel stages (Otake et al., 1997). Changes in otolith Sr:Ca ratios have been considered related to environmental factors such as water temperature and salinity. In the early developmental stage of the conger eel, however, Sr content or Sr:Ca ratios did not seem to be significantly affected by environmental factors. Because the leptocephali apparently spend their lives in a water mass, undergoing only mild variations in environmental conditions, it is suggested that the increase in otolith Sr content or Sr:Ca ratios during the leptocephalus stage was the result of some endogenous factor(s) rather than environmental factors. The high Sr content in the central core region during the leptocephalus stage may be derived from the large amounts of gelatinous extracellular matrix that fill their bodies until metamorphosis. This material is composed of sulphated glycosaminoglycans (GAG) which is converted into other compounds during metamorphosis (Pfeiler, 1984). The drastic decrease in Sr may occur because these sulphated polysaccharides have an affinity for alkali earth elements, and are particularly high in Sr, suggesting that a high Sr content in the body has a significant influence on otolith Sr content through the saccular epithelium in the inner ear, and the sudden loss of Sr-rich GAG during metamorphosis probably results in the lower Sr concentration in otoliths after metamorphosis (Arai et al., 1997).

Outside of the high Sr and Sr:Ca around the core region, there was observed a slight range of Sr and Sr:Ca values (Figure 2). Changes in otolith Sr:Ca ratios have been considered to be related to environmental factors such as water temperature (Campana, 1999) and salinity or Sr concentration in ambient water (Tzeng et al., 2000; Arai & Tsukamoto, 1998; Arai & Miyazaki, 2001). It seemed that a slight change in Sr and Sr:Ca ratios outside the core region correlated with abiotic factors such as ambient water temperature and salinity rather than with biotic factors. These considerations all lead to the conclusion that both biotic and abiotic factors affect otolith microchemistry in the life history of the conger eel.

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