Incorporation of Water-Borne Strontium into Otoliths and its Turnover in the Goldfish *Carassius auratus*: Effects of Strontium Concentrations, Temperature, and 17β -Estradiol

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The incorporation and retention of water-borne strontium in otoliths (asterisci and/or lapilli) were studied using the freshwater goldfish *Carassius auratus*. The effects of strontium concentrations, temperature, and 17β -estradiol were examined using atomic absorption spectrophotometry and X-ray analysis. Water-borne strontium (0.1, 1.0, and 10.0 mg l^{-1}) was incorporated into otoliths (asterisci) in a concentration-dependent manner after an 88-day exposure, but a high concentration of 10.0 mg l^{-1} rather depressed the rate of incorporation. Similar results were seen with Sr–Ca ratios in plasma and otoliths. The strontium incorporated into otoliths remained after 92 days in nonstrontium-enriched water, while that in scales and ribs decreased. Otolith (lapillus) Sr–Ca ratios distinctly differed between freshwater ($\text{Sr/Ca} = 2.6 \times 10^{-3}$)- and diluted (1/9, 1/6, and 1/3) seawater (25×10^{-3} on an average)-reared groups, but no difference was found among the seawater groups. These ratios linearly increased with temperatures between 16 and 24°C in asterisci ($r=0.65$). However, they somewhat decreased at 28°C . A single administration of 17β -estradiol resulted in hypercalcemia and decreased strontium incorporation into the lapillus but not in the asteriscus. The lapillus contained 12 times more strontium than the asteriscus.

Key words: otolith, strontium turnover, Sr–Ca ratio, temperature effect, estradiol, *Carassius auratus*

Teleost otoliths mostly consist of calcium carbonate in the aragonite form and grow within the isolated labyrinth environment without any direct contact to cells. Included within the aragonite are other trace metals such as Sr, Fe, Zn, and Al, which have been used for reconstructing the life history of an individual fish. Strontium is readily incorporated into otoliths¹⁾ and its microanalysis has been conducted to provide various information such as the thermal history of fish,^{2,3)} the timing of migration from sea water to fresh water,⁴⁾ the reproductive cycle,⁵⁾ and the progeny between sympatric anadromous and non-anadromous salmonids.⁶⁾ However, these studies were performed for practical goals and relatively little information concerning the physiological aspects of strontium metabolism is available.

Rosenthal⁷⁾ examined the strontium uptake from environmental water and its incorporation into various tissues in three freshwater fish. He showed that these fish had little discrimination in strontium uptake against calcium. However, Ichikawa *et al.*⁸⁾ reported that strontium was discriminated against calcium in branchial uptake in eels. Strontium retention was examined in calcified tissues other than otoliths in Nassau groupers.⁹⁾ Recently, Kalish⁵⁾ showed that otolith Sr–Ca ratios were positively correlated with endolymph Sr–Ca ratios in several marine species, suggesting that these elements in the endolymph were directly incorporated into the otoliths. In spite of these studies, further information on various aspects of strontium metabolism in otoliths is required for a better assessment of strontium data to reconstruct ecological characteristics in the life history of fish.

The present study was undertaken to provide some more

insight into the strontium metabolism in otoliths. The effects of environmental strontium concentrations, temperature, and 17β -estradiol on strontium incorporation and turnover were examined in goldfish otoliths using atomic absorption spectrophotometry. Otolith Sr–Ca ratios were also analysed by X-ray analysis.

Materials and Methods

The goldfish *Carassius auratus* were obtained from a commercial dealer and acclimated to experimental conditions for 2–4 weeks before use. They were maintained in 16 – 28°C depending on the purpose of the experiments and fed carp food pellets once a day throughout the acclimation and experimental periods. These pellets contained calcium and strontium at concentrations of 9.4 mg g^{-1} and $60.6 \text{ } \mu\text{g g}^{-1}$, respectively.

Incorporation and Retention of Water-Borne Strontium

Eighteen fish each weighing approximately 1 g were placed in 10-liter tanks containing aerated tap water enriched with strontium at concentrations of 0.02 (control), 0.1, 1.0, or 10 mg l^{-1} . They were reared for 88 days and then half of each group was killed to sample blood, otoliths, scales, and pleural rib bone. The remaining fish were transferred to nonstrontium-enriched tap water and reared for another 92 days. They were killed to examine the retention of incorporated strontium. The ambient water was changed every 4 or 5 days during the metal-exposure period and every 7 days during the subsequent nonexposure period. Water temperature varied between 22 and 25°C during the experiment. At the beginning of the experiment, another

four fish were killed to estimate the initial content of strontium and calcium in the otoliths.

Blood was collected into heparinized capillary tubes by cutting the tail of the fish and centrifuged to obtain plasma. Otoliths (asterisci) were dissected and rinsed several times in distilled water (DW). Several scales were removed from the antero-dorsal trunk and the adhering substances (mucus and epidermis) on the scales were removed by wiping them under a binocular microscope. They were rinsed several times in DW. The fish were then wrapped with aluminum foil and heated at 100°C for 10 min to facilitate the separation of ribs from the soft tissues. Several pleural ribs were dissected and rinsed in DW.

Tissue samples were dried at 105°C overnight, weighed, and ashed at 450°C. Ashed samples were dissolved in a solution of $\text{HNO}_3:\text{H}_2\text{O}_2$ (1:1) and dried on a hot plate. The samples were redissolved in 0.1 N HCl containing 1% La. Strontium and calcium contents were determined by atomic absorption spectrophotometry (Hitachi 170). Plasma strontium and calcium concentrations were determined by the same method using graphite furnace (Hitachi 180) and flame (170) modes, respectively. For the calculation of Sr–Ca ratios, the amounts of strontium and calcium incorporated into the otoliths during the 88-day exposure experiment were defined by subtracting the amounts of these metals which had been deposited on the otoliths before exposure. This correction avoids underestimating the ratios.

Otolith Sr–Ca Ratios in Diluted Sea Water

Twenty-four fish weighing approximately 6 g were given a single intraperitoneal injection of tetracycline–HCl at a dose of 0.1 mg g⁻¹ body weight. Six of these fish were then transferred to a 10-liter tank containing either fresh water (control), 1/9, 1/6, or 1/3 sea water. They were maintained at 25°C for 14 days and then the otoliths (lapilli) were dissected. The ambient water was changed once after 7 days. The otoliths were embedded in epoxy resin, ground with several grades of carborundum paper from the anti-sulcus surface to the nucleus (sagittal plane), and polished with 0.25 µm diamond paste. Otolith sections were ultrasonically cleaned in DW for 30 s and dried at room temperature.

The part of the otoliths formed during the experiment was identified by tetracycline-induced fluorescence under an ultraviolet illuminating microscope. Then the specimens were mounted on a carbon holder with carbon tape and coated with carbon. Several points on the specimens were selected at random and analysed for Sr–Ca ratios at 25 kV by spot analysis using a Horiba EMAX-2000 energy-dispersive X-ray microanalysis system. Calcium and strontium counts were measured at K_α energies of 3.69 and 14.15 keV, respectively. Spectrum collection time was 100 s. All data were normalized to an integrated count after the subtraction of background noise and then averaged.

Temperature Effects on Otolith Sr–Ca Ratios

Groups of 13 fish weighing approximately 8 g were acclimated to different ambient temperatures of 16, 20, 24, or 28 ± 0.4°C for 1 week in 10-liter tanks and then four fish from each group were killed to determine the initial level of otolith (asteriscus) strontium. Strontium was then added to the tanks at a concentration of 1 mg l⁻¹ to improve the accuracy of strontium determination and the rearing was

continued for 14 more days. The ambient water was changed every 2 or 3 days during the experiment. Calcium-45 (NEN) was added to each tank at a concentration of 1.8 × 10⁵ Bq l⁻¹ 3 days before sampling.

The right otolith was dissected, rinsed in DW, and weighed after drying. It was put in a counting vial, solubilized in a mixture of 0.2 ml HClO_4 and 0.2 ml H_2O_2 , and added to Scintizol EX-H (Wako Chem.) for radioactive counting (Beckman, LS 6000). The left otolith was used for strontium determination as previously described.

Data were standardized for the amounts of strontium and calcium deposited on otoliths during the last 3 days. Strontium was calculated by the equation:

$$\text{Net Sr incorporation} = (\text{Sr}_f - \text{Sr}_i) / \text{OW} \times 3/14$$

where Sr_i and Sr_f signify the amount of otolith strontium at the initial and final times of the experiment, respectively. OW is otolith weight. The amount of calcium deposited on otoliths was calculated using the specific activity ($\text{Ca}/^{45}\text{Ca}$) in plasma.

Estrogen Effects on Strontium Incorporation into Otoliths

Ten immature fish weighing approximately 15 g were given a single intraperitoneal injection of 17β-estradiol (Nutritional Biochem. Co.) dissolved in propylene glycol at a dose of 5 mg kg⁻¹ body weight. Another 10 fish received only the solvent and served as the control. They were reared in separate tanks (50 l each) containing tap water (20°C) enriched with strontium (1 mg l⁻¹). They were killed 10 days after injection to collect blood and otoliths (asteriscus and lapillus). Plasma and otoliths were analysed for calcium and strontium, respectively, by atomic absorption spectrophotometry.

Statistical Analysis

A one-way ANOVA (Fisher) was used for statistical evaluation. Significance was accepted for $p < 0.05$.

Results

Incorporation and Retention of Water-Borne Strontium

The experimental fish on the average gained from an initial level of 1.1 to 7.9 g on day 180 through 3.5 g on day 88. Otolith (asteriscus) weight was 1.8, 2.2, and 3.4 mg on day 0 (initial), 88, and 180, respectively. No significant difference was found in somatic and otolith growth between the control and strontium-treated groups.

Ambient water-borne strontium was incorporated into otoliths, scales, and rib bones in a concentration dependent manner (Fig. 1). However, the rates of incorporation were not linear to the ambient concentrations but were reduced for a high concentration of strontium. These relationships will be described later.

The amount of strontium incorporated was the highest in scales, followed by ribs and then otoliths on day 88 in the strontium-enriched water (Fig. 1). The strontium incorporated into the scales and ribs decreased significantly after a subsequent rearing of 92 days in nonstrontium-enriched environment, while it increased in the otoliths, especially in the group of 10 mg Sr l⁻¹ (Fig. 1).

The concentration-dependent incorporation of strontium into plasma and otoliths (asterisci) was a nonlinear function

Fig. 1. Strontium incorporation into otoliths (asterisci), scales, and ribs and its retention in goldfish.

Fish were kept in the different concentrations of ambient strontium for 88 days and half of them were examined for strontium incorporation. The rest were transferred to nonstrontium-enriched water and killed to examine strontium retention after 92 days. Vertical bars represent the SE of mean for six to nine fish.

* $p < 0.05$ and ** $p < 0.01$ for control.

of actual concentrations (0.02, 0.08, 0.58, and 7.58 mg l^{-1} on an average) of ambient strontium (Fig. 2). Similar relationships occurred in terms of Sr–Ca ratios (Fig. 3). The relationship between plasma and otolith Sr–Ca ratios was also nonlinear (Fig. 4), suggesting that the rate of strontium incorporation into otoliths is reduced at a high concentration of plasma strontium.

Strontium content was compared between the asteriscus and the lapillus in the control group. The lapillus contained approximately 12 times higher strontium than the asteriscus on a weight basis (Table 1). Calcium content was almost the same between the two types of otoliths, resulting in a higher Sr–Ca ratio in the lapillus.

Otolith Sr–Ca Ratios in Diluted Sea Water

This experiment was designed to investigate which of the Sr–Ca ratios or absolute strontium concentrations in the ambient water affects the Sr–Ca ratio of otoliths. Actual concentrations of strontium (calcium) were 0.02 (7.7), 0.9

Fig. 2. Concentration-dependent incorporation of ambient strontium into plasma and otoliths (asterisci) in goldfish.

Fish were kept in the strontium-enriched water for 88 days. Plotted values signify the mean $\pm$ SE of six to nine fish.

Table 1. Strontium and calcium contents, and the Sr–Ca ratios of otoliths (asteriscus and lapillus) in goldfish

	Sr (ng/mg)	Ca ($\mu\text{g/mg}$)	Sr/Ca ($\times 10^{-3}$)
Asteriscus (n=4)	$60.0 \pm 23.1^*$	384.7 ± 6.4	0.16
Lapillus (n=5)	714.1 ± 9.8	368.8 ± 4.2	1.94

* Mean $\pm$ SE.

(31.7), 1.6 (64.4), and $2.9 (123.6) \mu\text{g l}^{-1}$ in fresh water, 1/9, 1/6, and 1/3 sea water, respectively. Strontium–calcium ratios were 2.6, 28, 25, and 23×10^{-3} in the same order.

The Sr–Ca ratio of control otoliths (freshwater lapilli) was 0.73×10^{-3} on the average (Fig. 5). Compared with this ratio, significantly higher ratios were obtained in the diluted seawater groups ($p < 0.05$). No significant difference occurred in the ratios among the seawater groups.

Temperature Effects on Otolith Sr–Ca Ratios

Strontium incorporation into otoliths (asterisci) increased linearly with temperatures between 16 and 24°C ($r = 0.70$) (Fig. 6). However, the rate of increase decreased to some extent at 28°C . The rate of calcium incorporation into otoliths may properly reflect the rate of somatic growth in

Fig. 3. Relationships in Sr-Ca ratios between the ambient water and plasma and between the ambient water and otoliths (asterisci) in goldfish.

Fish were kept in the strontium-enriched water for 88 days. Plotted values signify the mean $\pm$ SE of six to nine fish.

Fig. 4. Relationship in Sr-Ca ratios between plasma and otoliths (asterisci) in goldfish.

Fish were kept in the strontium-enriched water for 88 days. Plotted values signify the mean $\pm$ SE of six to nine fish.

this experiment. The rate of incorporation was fit by a linear increase ($r=0.63$) with temperatures between 16 and 24°C (Fig. 6). It increased even further between 24 and 28°C, indicative of a difference from strontium incorpora-

Fig. 5. Strontium-calcium ratios of otoliths (lapilli) in the goldfish kept in differently diluted sea water for 14 days.

The ratios were determined by X-ray analysis. Plotted values are the mean $\pm$ SE of five or six fish.

* $p < 0.05$ and ** $p < 0.01$ for control (FW).

Fig. 6. Effects of temperature on the incorporation of strontium (dotted line) and calcium (solid line) into otoliths (asterisci) in goldfish.

Fish were kept at four different temperatures for 14 days and the incorporation rate of these elements was calculated for the last 3 days in terms of mean $\pm$ SE of eight or nine fish.

* $p < 0.05$ for 16°C.

tion.

Strontium-calcium ratios of otoliths increased with temperatures up to 24°C, showing an excellent fit to the regression,

$$\text{Sr/Ca} = (1.21 \text{ temperature} - 7.05) \times 10^{-4} (r=0.65) \text{ (Fig. 7).}$$

On the contrary, this ratio decreased at 28°C, resulting in a negative correlation between 24 and 28°C. The Sr-Ca ratio at 28°C was still significantly higher than that at 16°C ($p < 0.05$).

Estradiol Effects on Strontium Incorporation into Otoliths

A single injection of estradiol induced hypercalcemia (6.1 mM plasma Ca) 10 days after injection, while the control calcium level remained at 2.7 mM. Lapilli contained much

Fig. 7. Effects of temperature on the Sr-Ca ratios of otoliths (asterisci) in goldfish.

Fish were kept at four different temperatures for 14 days and the ratios were calculated for the last 3 days in terms of mean $\pm$ SE for eight or nine fish.

* $p < 0.05$ and ** $p < 0.01$ for 16°C.

Fig. 8. Effects of 17 β -estradiol on the incorporation of strontium into otoliths (asteriscus and lapillus) in goldfish.

Fish were given a single intraperitoneal injection of 17 β -estradiol and kept in the strontium-enriched water for 14 days. Vertical bars represent the SE of mean for 10 fish.

* $p < 0.05$ for control.

higher strontium than asterisci (Fig. 8), consistent with the results shown in Table 1. The strontium content of asterisci was not affected by estrogenization, probably because of their small metal content. However, the incorporation of strontium into lapilli was reduced significantly ($p < 0.05$) in the estradiol-treated group (Fig. 8).

Discussion

Strontium (ion radius, 1.12 Å) is well-known to behave like calcium (0.99 Å), being incorporated into skeletal tissues such as bone and scales. The previous¹⁾ and present studies showed that this metal was relatively easy to incorporate into otoliths. The rate of incorporation was concentration dependent but not linear to the concentrations. A high

concentration of environmental strontium decreased its incorporation into plasma and otoliths. The same was the case with Sr-Ca ratios between the plasma and the otoliths as well as between the ambient water and the plasma. These results suggest that cells concerning strontium transport discriminate strontium against calcium at both levels of strontium uptake from the ambient water and of strontium transport from plasma to otoliths. Although we have no knowledge about cells relevant to strontium uptake, chloride cells in the gill are a candidate for an uptake site, because strontium can pass through calcium channels¹⁰⁾ and calcium is taken up by chloride cells.¹¹⁾ However, fish do not seem to have an active system for strontium regulation, because it is a nonessential biological metal.

Calcium transport from blood to otoliths was studied using an isolated preparation of the otolith-containing sacculus in rainbow trout.¹²⁾ Ca²⁺-ATPase¹²⁾ and a Na-Ca exchanger¹³⁾ seem responsible for the transcellular transport of calcium. If strontium behaves like calcium at the labyrinth level, it may be transported to otoliths through the intracellular route of epithelial cells constituting the labyrinth. Mannitol, a marker of paracellular transport, did not diffuse into the inside (endolymph) of the labyrinth,¹³⁾ excluding the possibility of the paracellular transport of strontium and calcium. However, since Ca²⁺-ATPase was not activated by strontium in coral calcification,¹⁴⁾ different mechanisms may be involved between strontium and calcium transport, resulting in the biological discrimination of these two metals.

The present results of strontium retention were very similar to the results of calcium retention.¹⁵⁾ The strontium incorporated into scales and ribs decreased significantly after 92 days in nonstrontium-enriched water, while that of otoliths increased at least in the highest strontium-treated group. These different profiles of strontium retention reflect different turnover rates in these tissues. Otoliths are highly calcified acellular tissues and grow without any direct contact to cellular components in the isolated environment, the labyrinth. Their calcium turnover is very inert after at least a few hours of crystallization.^{15,16)} Scales and ribs are less calcified than otoliths and grow in contact with cells with well-supplied blood capillaries. Therefore, the mineral deposited on these tissues are physiologically labile and can mobilize to blood, especially in scales.^{17,18)} The inert turnover of otolith minerals makes the otolith a qualified recorder for reading stored information.

The present study revealed that the small (slow-growing) otolith, the lapillus, contained much higher strontium than the large (fast-growing) one, the asteriscus. Similarly, the sulcus acusticus, the slowest-growing part of otoliths showed a high strontium content in grenadiers.¹⁹⁾ The groove of scales, the less-calcified slow-growing depression, was also reported to contain very high strontium compared with the other calcified parts of scales in red sea bream.²⁰⁾ A causative relationship between the strontium content and growth activity of calcified tissues should be studied.

If Sr-Ca ratios of otoliths differ between freshwater and seawater environments, migratory information on anadromous and catadromous fish is expected from the microchemistry of otoliths. Radtke *et al.*⁴⁾ indicated a dramatic drop in otolith Sr-Ca ratios, reflecting a movement from seawater to freshwater environment in gobies.

Kalish⁶⁾ successfully applied these ratios to distinguishing the progeny of sympatric anadromous and non-anadromous salmonids. The present experiment succeeded in distinguishing the freshwater- and diluted seawater-reared groups using these ratios. However, in spite of the different concentrations ($0.9\text{--}2.9\text{ mg l}^{-1}$) of strontium, no significant difference occurred among the three groups reared in the diluted sea water ($\text{Sr}/\text{Ca}=25 \times 10^{-3}$ on an average). These results suggest that otolith Sr–Ca ratios are more sensitive to Sr–Ca ratios than to the absolute concentration of strontium in the ambient environment.

Strontium–calcium ratios of otoliths have been expected to provide information on the thermal history of an individual fish, because the ratios change depending on the temperature at which aragonite crystals are precipitated. Radtke²⁾ first reported a negative correlation of otolith Sr–Ca ratios to ambient temperature and reconstructed the thermal history of juvenile killifish. Similar results were obtained in herring.³⁾ Sadovy and Severin²¹⁾ found that otolith Sr–Ca ratios were inversely correlated with log body growth rate in white grunts. On the other hand, Kalish²²⁾ has emphasized that the interpretation of otolith Sr/Ca cannot be based on temperature data alone and that the careful consideration of various environmental and physiological factors are necessary. These studies were conducted using marine fish species.

In the present study, we tested the otolith Sr–Ca ratio–temperature relationship using the freshwater goldfish. The results were expressed by two different regressions. A positive correlation of Sr–Ca ratios to temperature occurred between 16 and 24°C. However, a negative correlation was the case with the higher temperatures between 24 and 28°C. Although a definite interpretation of these results is difficult to be explained so far, a temperature of 28°C was probably too high for goldfish physiology. The optimal upper limit of temperature for this species may be around 25°C, because the maximum frequency of opercular movement was reported at 25°C in the goldfish reared in our local area (northern Japan).²³⁾ The present result that otolith Sr–Ca ratios were positively correlated with physiological temperature is opposite to the previously mentioned studies. Since seawater and freshwater fish regulate their plasma ion concentrations in an opposite way,²⁴⁾ a difference in environmental salinity might result in the opposite results in temperature-dependent Sr–Ca ratios in otoliths between freshwater and seawater fish species. Such a hypothesis should be tested using other freshwater fish.

Estradiol induces the synthesis of vitellogenin, a calcium-binding protein, resulting in hypercalcemia. In this case, the level of protein-bound calcium increases significantly, while that of ionized calcium remains almost unchanged. These sequences did not essentially affect the calcification of otoliths in goldfish.²⁵⁾ However, in the present study, estradiol reduced strontium incorporation into otoliths (lapilli). This may be explained by the competitive binding of strontium *versus* calcium to vitellogenin, resulting in a low level of plasma strontium available for incorporation into otoliths. Kalish^{5,22)} stated that the development of ovaries affected otolith Sr–Ca ratios likely through the selective binding of calcium to vitellogenin over strontium. The binding properties of these metals to vitellogenin should be studied to provide more insight into

the effect of gonadal maturation on Sr–Ca ratios in fish otoliths.

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References

- 1) Y. Mugiya, T. Hakomori, and K. Hatsutori: Trace metal incorporation into otoliths and scales in the goldfish, *Carassius auratus*. *Comp. Biochem. Physiol.*, **99C**, 327–331 (1991).
- 2) R. L. Radtke: Strontium–calcium concentration ratios in fish otoliths as environmental indicators. *Comp. Biochem. Physiol.*, **92A**, 189–193 (1989).
- 3) R. L. Radtke, D. W. Townsend, S. D. Folsom, and M. A. Morrison: Strontium: calcium concentration ratios in otoliths of herring larvae as indicators of environmental histories. *Env. Biol. Fish.*, **27**, 51–61 (1990).
- 4) R. L. Radtke, R. A. Kinzie, and S. D. Folsom: Age at recruitment of Hawaiian freshwater gobies. *Env. Biol. Fish.*, **23**, 205–213 (1988).
- 5) J. M. Kalish: Otolith microchemistry: validation of the effects of physiology, age and environment on otolith composition. *J. Exp. Mar. Biol. Ecol.*, **132**, 151–178 (1989).
- 6) J. M. Kalish: Use of otolith microchemistry to distinguish the progeny of sympatric anadromous and non-anadromous salmonids. *Fish. Bull. U.S.*, **88**, 657–666 (1990).
- 7) H. L. Rosenthal: Uptake, turnover and transport of bone seeking elements in fishes. *Ann. N.Y. Acad. Sci.*, **109**, 278–293 (1963).
- 8) R. Ichikawa, M. Oguri, and N. Fukuda: Metabolism of radionuclide in fish-II. Strontium–calcium discrimination in gill excretion. *Nippon Suisan Gakkaishi*, **27**, 990–995 (1961).
- 9) W. P. Norris, W. Chavin, and L. S. Lombard: Studies of calcification in a marine teleost. *Ann. N.Y. Acad. Sci.*, **109**, 312–336 (1963).
- 10) K. Kitamura and H. Kuriyama: Calcium channels; its properties and functions. *Metab. Dis.*, **22**, 307–316 (1985) (in Japanese).
- 11) W. S. Marshall, S. E. Bryson, and C. M. Wood: Calcium transport by isolated skin of rainbow trout. *J. Exp. Biol.*, **166**, 297–316 (1992).
- 12) Y. Mugiya: Effects of calmodulin inhibitors and other metabolic modulators on *in vitro* otolith formation in the rainbow trout, *Salmo gairdnerii*. *Comp. Biochem. Physiol.*, **84A**, 57–60 (1986).
- 13) M. Yoshida: A pharmacological study on calcium transport to isolated otoliths in the rainbow trout, *Oncorhynchus mykiss*. Master's thesis, Faculty of Fisheries, Hokkaido University, Hakodate, 1994, pp. 1–62.
- 14) Y. K. Ip and A. L. L. Lim: Are calcium and strontium transported by the same mechanism in the hermatypic coral *Galaxea fascicularis*? *J. Exp. Biol.*, **159**, 507–513 (1991).
- 15) T. Ichii and Y. Mugiya: Comparative aspects of calcium dynamics in calcified tissues in the goldfish, *Carassius auratus*. *Nippon Suisan Gakkaishi*, **49**, 1039–1044 (1983).
- 16) P. J. Wright, C. Talbot, and J. E. Thorpe: Otolith calcification in Atlantic salmon parr, *Salmo salar* L. and its relation to photoperiod and calcium metabolism. *J. Fish Biol.*, **40**, 779–790 (1992).
- 17) Y. Mugiya and N. Watabe: Studies on fish scale formation and resorption-II. Effect of estradiol on calcium homeostasis and skeletal tissue resorption in the goldfish, *Carassius auratus*, and killifish, *Fundulus heteroclitus*. *Comp. Biochem. Physiol.*, **57A**, 197–202 (1977).
- 18) J. F. Carragher and J. P. Sumpter: The mobilization of calcium from calcified tissues of rainbow trout (*Oncorhynchus mykiss*) induced to synthesize vitellogenin. *Comp. Biochem. Physiol.*, **99A**, 169–172 (1991).
- 19) R. W. Gauldie, G. Coote, K. P. Mulligan, I. F. West, and N. R. Merrett: Otoliths of deep water fishes: Structure, chemistry and chemically-coded life histories. *Comp. Biochem. Physiol.*, **100A**, 1–31 (1991).
- 20) M. Ishikawa, A. Iida, T. Ishii, S. Hayakawa, and K. Okoshi: Elemental distribution on the scale of the red sea bream *Chrysophrys*

- major scanned by a synchrotron monochromatized X-ray microbeam. *Nippon Suisan Gakkaishi*, **57**, 1813–1819 (1991).
- 21) Y. Sadovy and K. P. Severin: 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 (1992).
- 22) J. M. Kalish: Determinants of otolith chemistry: seasonal variation in the composition of blood plasma, endolymph and otoliths of bearded rock cod *Pseudophycis barbatus*. *Mar. Ecol. Prog. Ser.*, **74**, 137–159 (1991).
- 23) S. Kawamoto: The physiology of fishes, in “General Aquaculture” (ed. by K. Kawamoto), Kouseisha-Kouseikaku, Tokyo, 1967, pp. 55–138 (in Japanese).
- 24) S. Utida and T. Hirano: Effects of changes in environmental salinity on salt and water movement in the intestine and gills of the eel, *Anguilla japonica*, in “Responses of Fish to Environmental Changes” (ed. by W. Chavin), Thomas Publisher, Springfield, 1973, pp. 240–269.
- 25) Y. Mugiya: Calcium metabolism in estrogenized fish, in “Comparative Endocrinology of Calcium Regulation” (ed. by C. Oguro and P. K. T. Pang), Japan Scientific Societies Press, Tokyo, 1982, pp. 109–114.