

Relationship between Sr:Ca Ratios in Otoliths of Grey Mullet *Mugil cephalus* and Ambient Salinity: Validation, Mechanisms, and Applications

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Chih-Wei Chang, Shih-Huan Lin, Yoshiyuki Iizuka and Wann-Nian Tzeng (2004) Relationship between Sr:Ca ratios in otoliths of grey mullet *Mugil cephalus* and ambient salinity: validation, mechanisms, and applications. *Zoological Studies* 43(1): 74-85. To understand the salinity effect on otolith Sr:Ca ratios, Sr and Ca contents of otoliths of juvenile grey mullet *Mugil cephalus* reared for 30 d in 8 salinities (0‰-35‰) were examined. New increments deposited during the rearing period were discriminated by immersing the acclimated mullet in a tetracycline bath (600 µg/ml) for 24 h to create a fluorescent marker in the otoliths. Ca and Sr contents in the rearing water, fish body, and muscle tissues were measured with an atomic absorption spectrophotometer, and those in the otoliths were measured with an electron probe microanalyzer. Both Ca and Sr contents in the rearing water linearly increased with salinities of from 0‰ to 35‰. The increase in Sr:Ca ratios was non-linear; they increased approximately 2-fold from $(7.91 \pm 0.41) \times 10^{-3}$ in 0‰ freshwater to $(15.07 \pm 0.63) \times 10^{-3}$ in 5‰ seawater and remained constant at $(13.95 \pm 0.79) \times 10^{-3}$ in salinities of from 5‰ to 35‰. On the other hand, Ca contents in the new otolith increments deposited in the 30-d rearing period did not change with salinities of 0‰-35‰, averaging $38.56\% \pm 0.43\%$. Sr contents and Sr:Ca ratios in the otoliths increased approximately 2-fold from $0.12\% \pm 0.01\%$ (Sr) and $(3.16 \pm 0.36) \times 10^{-3}$ (Sr:Ca) in 0‰ freshwater to $0.24\% \pm 0.03\%$ (Sr) and $(6.35 \pm 0.70) \times 10^{-3}$ (Sr:Ca) in 5‰-35‰ seawater, which was consistent with changes in Sr:Ca ratios of the rearing water. In addition, Sr:Ca ratios in the otoliths of fish reared in 5‰-35‰ seawater were negatively correlated with the otolith growth rate. These results indicate that Sr:Ca ratios in otoliths can be used to reconstruct an environmental history of the mullet by differentiating when the fish migrated between fresh water and seawater; however Sr:Ca ratios in the otoliths were affected by salinity and the fish growth rate in an interactive manner. <http://www.sinica.edu.tw/zool/zoolstud/43.1/74.pdf>

Key words: Grey mullet, Otolith, Tetracycline marking, Strontium:calcium ratio, Salinity.

Otoliths are found in the membranous labyrinth of the inner ear of teleost fish and function in hearing and balance (Lowenstein 1971). They are composed of calcium carbonate and are deposited rhythmically as aragonite crystals within a protein matrix (Degens et al. 1969, Gauldie and Nelson 1988). They have long been used to determine fish ages (Williams and Bedford 1974, Campana and Neilson 1985). Recently, changes in the strontium (Sr): calcium (Ca) ratios in otoliths have received increasing attention, because these provide a method of reconstructing an environ-

mental history of the fish (Radtko and Shafer 1992). Sr is a calcium analogue sharing a similar crystal ionic radius and can substitute for Ca in the aragonite lattice of otoliths (Amiel et al. 1973). The Sr concentration in seawater is approximately 100-fold greater than that in fresh water (Campana 1999), and a good relationship exists between otolith Sr:Ca ratios and ambient salinities (e.g., Secor et al. 1995, Tzeng 1996). Thus Sr:Ca ratios in otoliths have widely been applied for studying habitat use and migratory behavior of fish between fresh water and seawater (Tzeng et al. 1997

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2002a 2003, Secor and Rooker 2000, Jessop et al. 2002).

Although the Sr:Ca ratio is a powerful tool for studying the environmental history of diadromous fish, information of the validation of Sr:Ca ratios in fish otoliths in relation to ambient salinities is very limited. Two different approaches for validation have been considered. (1) Temporal changes in Sr:Ca ratios in the otoliths can be linked to the entire migratory environmental history of the fish (Secor 1992, Tzeng and Tsai 1994, Tzeng et al. 1997 2002a 2003); or simply Sr:Ca ratios in the otolith edge can be correlated with the recent in situ salinity history of the fish (Limburg et al. 2001, Jessop et al. 2002). (2) Sr:Ca ratios in otoliths can be rigidly corroborated with the salinity gradients traversed and/or different salinity regimes by manipulation in a laboratory-controlled experiment (Secor et al. 1995 1998, Tzeng 1996, Chesney et al. 1998, Kawakami et al. 1998). The advantage of the latter approach is strengthened not only by the direct gathering of evidence of the response of otolith Sr:Ca ratios to manipulated salinities, but also because it can assess potential interactive or confounding factors on otolith Sr:Ca ratios of environmental factors (such as temperature and waterborne ionic composition) and physiological factors (such as age, growth rate, sex, reproduction, hormone, and diet) or a combination of the two (Kalish 1989, Radtke 1989, Sadovy and Severin 1992, Tzeng 1994 1996, Hoff and Fuiman 1995, Limburg 1995, Mugiya and Tanaka 1995, Farrell and Campana 1996, Gallahar and Kingsford 1996, Elsdon and Gillanders 2002).

The grey mullet, *Mugil cephalus* Linnaeus, 1758, is an economically important species for commercial fisheries and aquaculture worldwide (Nash and Shehadeh 1980). The migration of mullet in waters adjacent to Taiwan follows seasonal changes in coastal currents. Mullet of 3-4 yr of age migrate southward with the NE monsoon driven coastal current from the coastal waters of mainland China to the waters off southwestern Taiwan to spawn during the winter (Tung 1981, Chen and Su 1986, Huang and Su 1989). Their eggs and larvae drift with the coastal current from the spawning grounds to estuaries on the western coast of Taiwan where they become juveniles at the age of 1-2 mo post-hatching (Tung 1981, Lee and Kuo 1990, Chang et al. 2000, Chang and Tzeng 2000). Current knowledge of the migratory history of the mullet has been interpreted from fragmentary information based on the spatiotemporal distributions of migrating spawners and estua-

rine recruited juveniles. The entire lifetime migratory history, especially the migration of juveniles from the spawning grounds to estuarine arrival and the migration of adults from the estuary to the feeding grounds and from the feeding grounds to the spawning grounds, is incompletely understood (Tung 1981, Liu 1986, Chen et al. 1989). In addition, the mullet is also believed to be catadromous, migrating from estuarine brackish water to offshore high salinities for breeding (De Silva 1980, Torricelli et al. 1982). However, defining the mullet as being catadromous remains controversial, because it is not certain that they use freshwater habitats (Blaber 1987, McDowall 1988). Thus, using Sr:Ca ratios to determine whether a mullet has entered fresh water before spawning is critical for clarifying the extent of their catadromy.

In this study, we attempted (1) to establish a relationship between Sr:Ca ratios in otoliths and ambient salinities, (2) to understand if Sr:Ca ratios in otoliths of the grey mullet can be used to reconstruct the environmental history of fish which migrate between fresh water and seawater, and (3) to determine if this information can be applied to validate larval dispersal from high-salinity offshore spawning grounds to brackish estuarine nursing grounds.

MATERIALS AND METHODS

Experimental design

An anchored bag net was used to collect the juvenile mullet used in this study from the Gongshyuan (GST) Creek estuary of northwestern Taiwan on 21 February 2002. The mean (\pm SD) total length of the juveniles was 29.1 ± 2.4 (range, 21.4-36.5) mm. After acclimation, fish were reared in 8 salinity regimes of 0‰, 5‰, 10‰, 15‰, 20‰, 25‰, 30‰, and 35‰ prepared with fresh water which originated from the Feitsui Reservoir in northern Taiwan, whereas seawater was collected from offshore of northern Taiwan.

The mullet were acclimated in 10‰ seawater, a level close to the 12.3‰ measured in the GST Creek estuary. Fish were acclimated for 1 wk in an aerated aquarium (50 L) and fed to satiation with tubifex worms (*Limnodrilus* sp.). The fish were immersed in a tetracycline (TC) solution of 600 μ g/ml for 24 h to produce a fluorescent TC mark in the otoliths as a time marker (Chang et al. 2000). The fish were gradually transferred to the 8 different salinities by changing the salinity by 5‰

every 30 min. Ten fish were reared in each of 8 salinities for 30 days with 2 replicates, at 19.0-24.5 (mean, 22.5 ± 1.2) °C.

At the end of treatment, fish were sacrificed and fixed in 95% alcohol. Total lengths of the fish were measured to the nearest 0.01 mm with no consideration of the 4% shrinkage of length due to preservation. Sagittal otoliths were extracted for Sr:Ca ratio measurements and growth rate estimation. Otoliths were cleaned, air-dried, embedded in epoxy resin, and polished along the sagittal plane using a grinder-polisher with various grit sandpapers and a 0.05- μm alumina slurry and polishing cloth until the primordium was discernible. The TC mark on the otoliths was examined using a fluorescence microscope with a band-pass filter of 400-440 nm and a long-pass barrier filter of 470 nm. The mean growth rate (G , $\mu\text{m}/\text{d}$) of the otolith of the fish during the experimental period was calculated as follows:

$$G = \Delta OR / t \quad (1)$$

$$\Delta OR = OR_t - OR_0 \quad (2)$$

where ΔOR is the new increment in radius, OR_t is the radius from the primordium to the postrostrum edge of the otolith, OR_0 is the radius from primordium to the TC mark, and t is the number of days reared.

Measurements of Ca and Sr contents in rearing water, fish tissues, and otoliths

Changes in Ca and Sr contents were measured by atomic absorption spectrophotometry (AAS; Z-5000, Hitachi, Japan) using the air-acetylene flame protocol. The rearing water was filtered with a 0.45- μm syringe-filter and diluted with deionized water before measurement. Fish tissues were weighed after drying at 60°C for 48 h, digested with high-purity nitric acid, and diluted with deionized water. Ca and Sr contents in the fish body with the otolith excluded and in muscle tissues were measured alternatively from each of the 2 replicate aquaria. Standard solutions of Ca and Sr at 1000 $\mu\text{g}/\text{ml}$ (Merck, Germany) were diluted with deionized water and/or nitric acid into concentrations of 0, 0.5, 1, 2, and 4 $\mu\text{g}/\text{ml}$ for calibration.

Ca and Sr contents in otoliths of fish reared in the 8 different salinity regimes were measured using a wavelength dispersive spectrometer equipped with an electron probe microanalyzer

(EPMA; JXA-8900R, JEOL, Japan). The EPMA beam conditions were as follows: an accelerating voltage of 15 keV, an accelerating current of 3 nA, and a beam size with a rectangular area of 5x4 μm . The wavelength dispersive strengths of Ca at the $K\alpha$ shell and Sr at the $L\alpha$ shell were evaluated for 20 and 80 s at the peak positions, and 10 and 20 s at the background positions, respectively. Calcite (CaCO_3 ; NMNH 136321) and strontianite (SrCO_3 ; NMNH R10065) were used as standards for calibrating the Ca and Sr contents of the otoliths. The quantitative data were calibrated by the ZAF method (Z, atomic number; A, absorption; and F, fluorescence correction).

Layers of the otolith from the primordium to the TC mark were deposited during larval dispersal from the offshore spawning grounds to the estuarine nursing grounds and during the 1-wk acclimation. New increments beyond the TC mark were deposited during the 30-d experiment. To compare Sr:Ca ratios in otoliths deposited before and during the experimental period, 1 otolith was selected from fish reared in each of the 8 different salinities, and the Ca and Sr contents in those otoliths were measured at intervals of 10 μm from the primordium to the postrostrum edge with 2 or 3 parallel lines. In addition, Sr:Ca ratios in the new increment deposited during the experimental period for each of the surviving fish until the end of experiment were measured to correlate with the rearing salinities.

Data analysis

Differences in mean values among treatments were tested by analysis of variance (ANOVA) or nested-ANOVA and Tukey's multiple comparison. Differences in slope and the adjusted mean of regression lines between treatments were tested by analysis of covariance (ANCOVA) (Sokal and Rohlf 1995).

RESULTS

Somatic and otolith growth rates

Nested ANOVA indicated that the mean (\pm SD) length (L), weight (W), and otolith radius (OR) of fish at the end of the experiment (Table 1) did not significantly differ among the 8 salinity regimes or between the 2 replicates (all $p > 0.05$). Similar results were shown for the L - W and L - OR relationships, irrespective of salinity and aquaria (ANCO-

VA, both $p > 0.05$), as $W = 1.58 \times 10^{-5}L^{4.38}$ ($r = 0.95, n = 89, p < 0.001$) (Fig. 1a) and $OR = 27.70L - 271.91$ ($r = 0.91, n = 89, p < 0.001$), respectively (Fig. 1b).

A yellowish mark in the otoliths of TC-immersed juvenile mullets was discernible with the fluorescent microscope (Fig. 2a). The TC mark became a deep check after etching with EDTA (Fig. 2b, c). Approximately 30 growth increments were found in the otoliths between the TC mark and the otolith edge, indicating that the increments were deposited daily (Fig. 2c). The otolith growth rates in the experimental period varied greatly among individuals, ranging from 0.17 to 6.67 $\mu\text{m}/\text{d}$ with a mean (\pm SD) of $2.67 \pm 1.40 \mu\text{m}/\text{d}$ (Fig. 3). Nested-ANOVA indicated that mean otolith growth rates did not significantly differ among the 8 salinity regimes and the 2 replicates ($p > 0.05$). In addition, otolith growth rates during the experimental period were not affected by the initial size of the otolith for any salinity treatment, because there was no significant correlation between otolith radius before treatment and otolith growth rates during the experimental period (Fig. 4).

On the other hand, the daily incremental widths of otoliths of juvenile mullet increased from 2-3 μm in the core to approximately 10 μm on the 20th day of the larval stage and reached an asymptote when they arrived at the estuary as juveniles (Fig. 5). This indicates that otolith growth rates of the mullet change with developmental stage.

Table 1. Mean (\pm SD) fish length, weight and otolith radius of juvenile grey mullet after 30 days rearing in salinities ranging from 0-35‰. Data from two aquaria were combined at each salinity level

Salinity (‰)	No. of fish initial	No. of fish end	Length (mm)	Weight (mg)	Otolith radius (μm)
0	20	6	34.5 \pm 3.6	83.8 \pm 38.1	668.3 \pm 112.0
5	20	15	32.2 \pm 2.5	61.3 \pm 21.9	609.3 \pm 72.7
10	20	13	31.4 \pm 3.6	61.3 \pm 31.3	601.5 \pm 94.6
15	20	17	31.3 \pm 3.1	59.8 \pm 26.6	602.4 \pm 117.8
20	20	16	31.3 \pm 2.7	60.7 \pm 22.2	596.3 \pm 78.9
25	20	13	32.4 \pm 1.7	68.3 \pm 19.1	629.2 \pm 59.9
30	20	7	31.8 \pm 2.1	69.0 \pm 21.9	617.1 \pm 71.6
35	20	2	34.9 \pm 3.1	87.6 \pm 31.7	660.0 \pm 14.1
Total	160	89	32.0 \pm 2.9	64.6 \pm 25.5	613.1 \pm 87.1

Variations in Ca and Sr contents and Sr:Ca ratios

1. Variations in Ca and Sr contents of the rearing water

Ca contents in the rearing water were linearly correlated with salinity, increasing approximately 65-fold from 8.28 $\mu\text{g}/\text{ml}$ in 0‰ fresh water to 539.56 $\mu\text{g}/\text{ml}$ in 35‰ seawater. The relationship between Ca contents and salinity (S) of the rearing water was calculated as $[\text{Ca}]_{\text{water}} = 15.50S - 5.56$ ($r = 0.998, n = 24, p < 0.001$) (Fig. 6a). Sr contents were also linearly correlated with salinity with an approximate 108-fold difference between 0‰ fresh water (0.07 $\mu\text{g}/\text{ml}$) and 35‰ seawater (7.14 $\mu\text{g}/\text{ml}$). The regression of Sr on salinity was calculated as $[\text{Sr}]_{\text{water}} = 0.21S + 0.03$ ($r = 0.999, n = 24,$

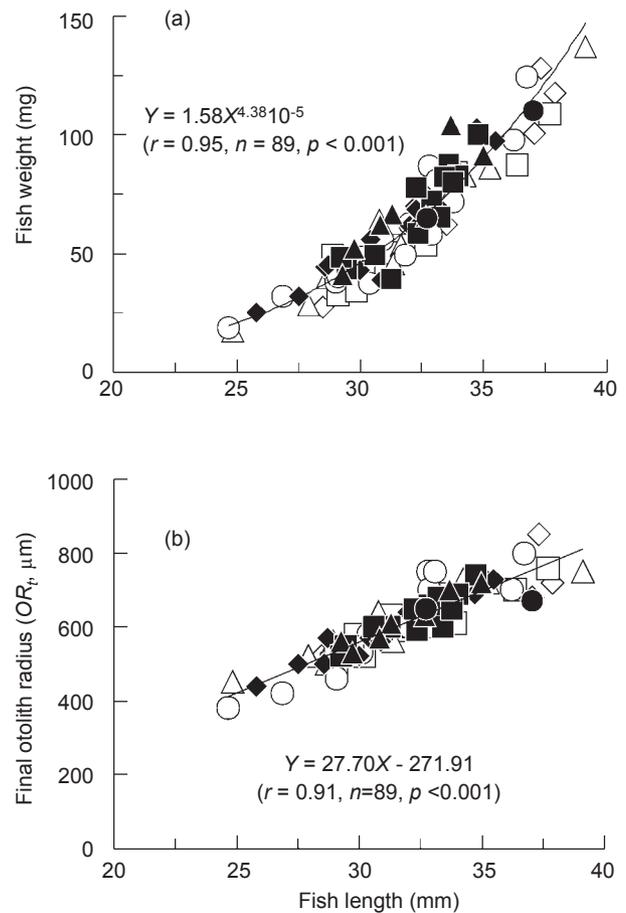


Fig. 1. The relationships between fish length and weight (a) and between fish length and final otolith radius (b) of juvenile grey mullet reared in salinities of 0‰ (\diamond), 5‰ (\square), 10‰ (\triangle), 15‰ (\circ), 20‰ (\blacklozenge), 25‰ (\blacksquare), 30‰ (\blacktriangle), and 35‰ (\bullet).

$p < 0.001$) (Fig. 6b). Sr:Ca ratios increased 2-fold from $(7.91 \pm 0.41) \times 10^{-3}$ in fresh water to $(15.07 \pm 0.63) \times 10^{-3}$ in a salinity of 5‰ but remained constant at $(13.95 \pm 0.79) \times 10^{-3}$ in the salinities of 5‰-35‰ (Fig. 6c). The relative contents of Sr were obviously less in fresh water than in seawater as were the Sr:Ca ratios as well.

2. Variations in Ca and Sr contents of fish body and muscle tissues

Ca contents of neither fish body and muscle tissue significantly differed among salinities

(ANOVA, both $p > 0.05$). The mean Ca content was significantly higher in the fish body ($44.56 \pm 5.75 \mu\text{g}/\text{mg}$) than in muscle tissue ($11.35 \pm 6.62 \mu\text{g}/\text{mg}$) ($p < 0.01$) (Fig. 7a). This may have been because the fish body is comprised of calcified tissues such as bone and scales but muscle is not. The Sr content of the fish body differed significantly among salinities, but no pattern was found in the relationship ($p < 0.05$). The Sr content of muscle tissues fluctuated greatly among individuals and thus did not differ significantly among salinities ($p > 0.05$). The mean Sr content was significantly lower in the fish body ($0.06 \pm 0.01 \mu\text{g}/\text{mg}$) than in muscle tissue ($0.10 \pm 0.05 \mu\text{g}/\text{mg}$) ($p < 0.01$) (Fig. 7b). Mean Sr:Ca ratios in both fish body and muscle tissues differed significantly among salinities, but no pattern was found in the relationship (both $p < 0.05$). Mean Sr:Ca ratios were significantly

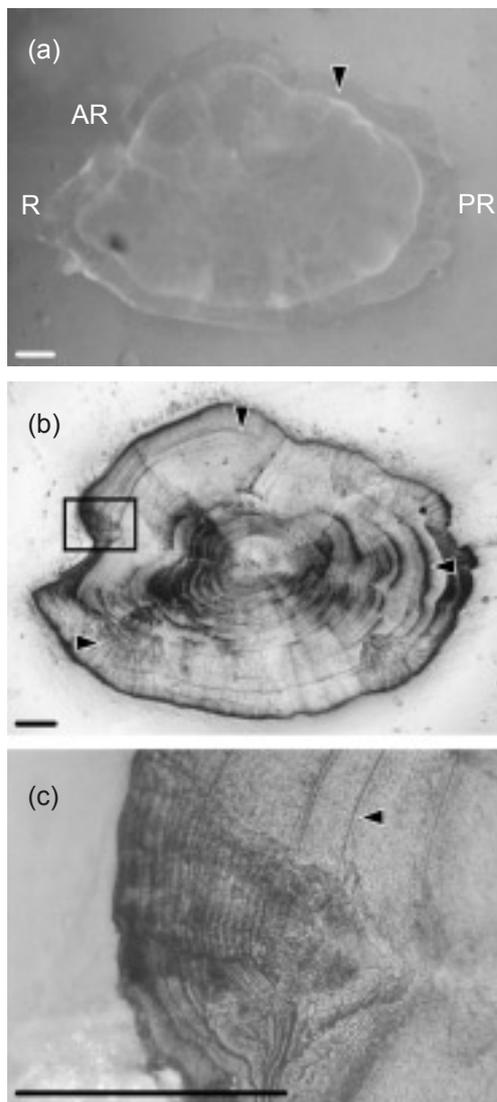


Fig. 2. Tetracycline (TC) mark and daily growth increments (DGIs) in a sagittal-sectioned juvenile grey mullet otolith photographed by fluorescent light (a) and reflected light microscope (b, c). (c) DGIs between TC mark and otolith edge magnified from (b). AR, antirostrum; R, rostrum; PR, postrostrum. Triangle, TC mark; Scale bar = 100 μm .

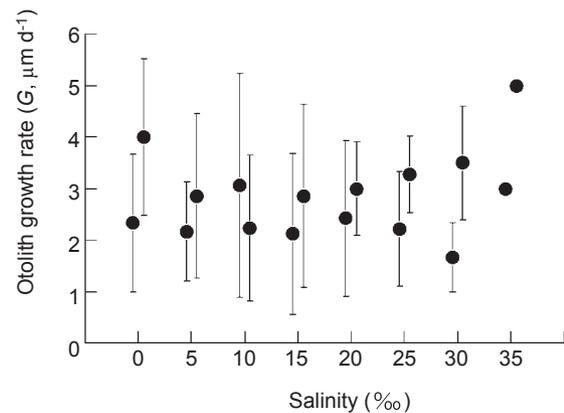


Fig. 3. Comparison of the mean otolith growth rates of juvenile grey mullet among 8 different salinities with 2 replicates.

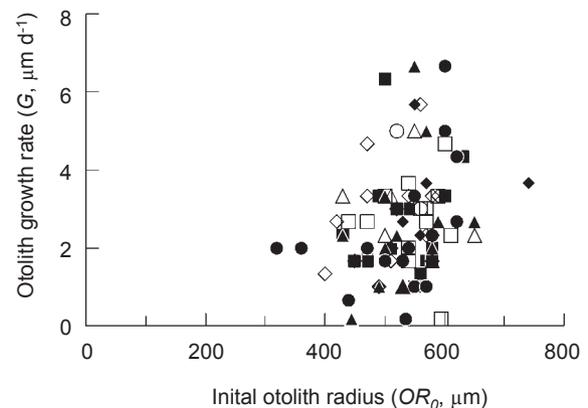


Fig. 4. The relationship between initial otolith radius and growth rate of new increments of the otoliths of juvenile grey mullet reared in salinities of 0‰ (\diamond), 5‰ (\square), 10‰ (\triangle), 15‰ (\circ), 20‰ (\blacklozenge), 25‰ (\blacksquare), 30‰ (\blacktriangle), and 35‰ (\bullet).

lower in the body at $(1.29 \pm 0.09) \times 10^{-3}$ than in muscle tissue at $(9.42 \pm 3.72) \times 10^{-3}$ ($p < 0.01$) (Fig. 7c).

3. Variations in Ca and Sr contents of otoliths

In total, 19 microprobe transects of Sr:Ca ratios of the 8 otoliths which were selected from juvenile mullet reared in the 8 different salinities were scanned from the primordium to the postrostrum edge, including the wild, acclimation, and experimental periods (Fig. 8). The regression of Sr:Ca ratios on the radius of otoliths of juvenile mullets in the wild period was negatively significant ($r = -0.32$ to -0.60 , all $p < 0.01$). The mean (\pm SD) Sr:Ca ratios in otoliths significantly decreased from $(10.05 \pm 0.71) \times 10^{-3}$ in the core to $(5.89 \pm 0.92) \times 10^{-3}$ at the end point of the wild period before acclimation ($p < 0.01$).

The increment of otoliths deposited in the acclimation period was approximately 7/30 of the radius from the TC mark to the edge. Sr:Ca ratios in otoliths deposited in the acclimation period varied from $(3.27$ to $9.28) \times 10^{-3}$ with a mean of $(6.05 \pm 1.35) \times 10^{-3}$. Beyond the TC mark, the Sr:Ca ratios in otoliths deposited in the experimental period differed significantly among rearing salinities. Mean otolith Sr:Ca ratios in the new increments deposited when the fish were reared in 10‰ salinity were similar to those acclimated in the same salinity (Fig. 8c). However, mean (\pm SD) Sr:Ca ratios in the new increments of otoliths of fish reared in 0‰ fresh water significantly decreased to $(3.39 \pm 0.58) \times 10^{-3}$ ($p < 0.05$) (Fig. 8a). On the other hand, otolith Sr:Ca ratios of fish reared in salinities of 5‰ and 15‰-35‰ averaged $(5.80 \pm 1.23) \times 10^{-3}$ (range, $(2.64$ - $8.82) \times 10^{-3}$) (Fig. 8b, d-h), which did not significantly differ from

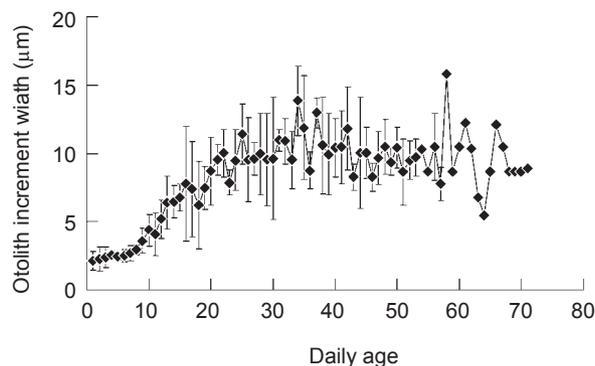


Fig. 5. Temporal changes in the mean daily increment widths in the otoliths of 4 juvenile grey mullets collected in the Gongshytan Creek estuary. Vertical line, SD.

those of fish acclimated at 10‰ salinity (ANOVA, $p > 0.05$). This may indicate that, except for fish reared in fresh water, it is difficult to discriminate among differences in Sr:Ca ratios in otoliths of fish reared in salinities of from 5‰ to 35‰.

To more-precisely identify the salinity effect on otolith Sr:Ca ratios, the 864 measurements of Ca and Sr contents in the otoliths deposited in the experimental period for the 89 fishes that survived were examined (Fig. 9). Ca contents in otoliths (range, 37.46%-39.58%; mean \pm SD, $38.56\% \pm 0.43\%$) did not significantly differ among the 8 different salinities for each of the 2 replicates (nested-ANOVA, $p > 0.05$) (Fig. 9a). However, Sr contents and Sr:Ca ratios of otoliths both significantly increased from a low level in 0‰ fresh water (Sr, 0.11%-0.14%, $0.12\% \pm 0.01\%$; Sr:Ca ratios, $(2.68$ - $3.52) \times 10^{-3}$, $(3.16 \pm 0.36) \times 10^{-3}$) to a high level in 5‰-35‰ seawater (Sr, 0.18%-0.31%, $0.24\% \pm 0.03\%$; Sr:Ca ratios, $(4.72$ - $8.02) \times 10^{-3}$, $(6.35 \pm 0.70) \times 10^{-3}$) (both $p < 0.01$) (Fig. 9b, c), and the

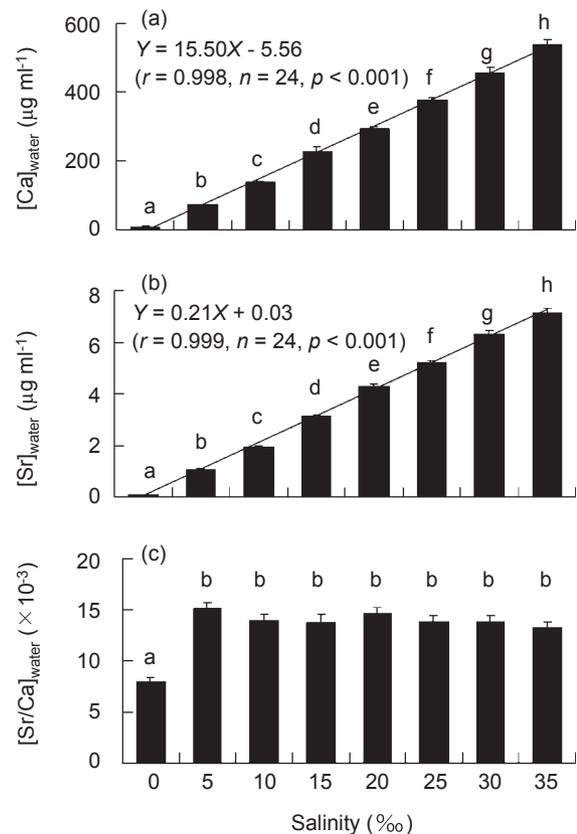


Fig. 6. Comparison of mean Ca contents (a), Sr contents (b) and Sr:Ca ratios (c) among rearing water of salinities from 0‰ to 35‰. Similar letters indicate homogenous groups; Vertical line, SD.

difference in Sr:Ca ratios was approximately 2-fold, which was similar to that of the Sr:Ca ratios in the rearing water (Fig. 6c). A significant positive correlation existed between otolith Sr:Ca ratios and salinities ($r = 0.28$, $p < 0.05$), but the correlation was not significant when the 0‰ freshwater data were excluded ($r = 0.10$, $p > 0.05$).

A significant negative correlation between the otolith growth rate and Sr:Ca ratios was found for fish reared in 5‰-35‰ seawater, but it was not significant for those reared in 0‰ fresh water. The regression of otolith Sr:Ca ratios on growth rate (G) for fish reared in 5‰-35‰ seawater was calculated as $[\text{Sr}/\text{Ca}]_{\text{otolith}} \times 10^3 = -0.23G + 6.95$ ($r = -0.45$, $n = 83$, $p < 0.001$) (Fig. 10).

Correlations of Ca and Sr contents and Sr:Ca ratios among the rearing water, fish tissues, and otoliths

Correlation coefficients of Ca and Sr contents and Sr:Ca ratios among the rearing water, fish body, and muscle tissues, and otoliths were calculated to understand the changes in Ca and Sr con-

tents from ambient water through the fish body to the otoliths (Table 2). The Ca content in otoliths was not correlated with that in the rearing water ($p > 0.05$), but was highly correlated with that of body tissue ($r = 0.50$, $p < 0.01$). Otolith Sr content was positively correlated with that of the rearing water ($r = 0.29$, $p < 0.01$), and the otolith Sr:Ca ratio was correlated with those of the rearing water and body tissue ($r = 0.76$ and 0.44 , both $p < 0.01$). However, no correlations in Ca and Sr contents or the Sr:Ca ratio were found between muscle tissue and otoliths. This may indicate that the otolith Sr:Ca ratio was primarily influenced by the Sr:Ca ratio of the ambient water, and that the fish body may also play an important role in regulating the relative Ca and Sr contents when the ions were assimilated from ambient water to the fish otolith.

DISCUSSION

Do Sr:Ca ratios in otoliths reflect those in the rearing water?

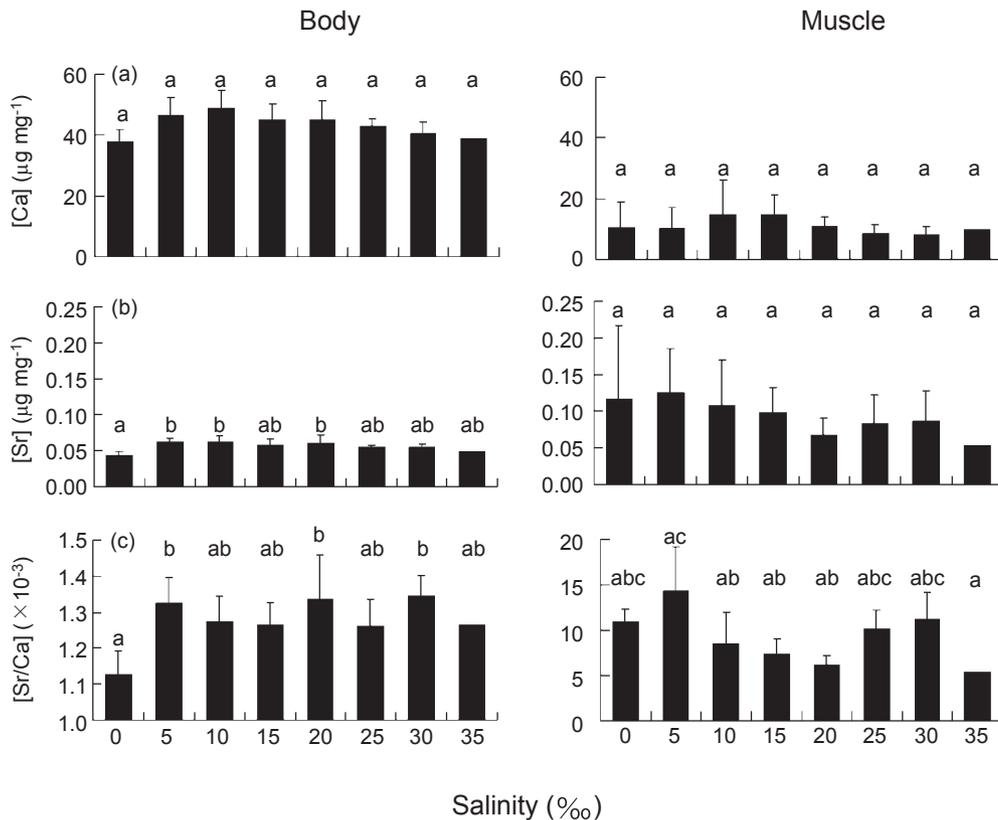


Fig. 7. Comparisons of mean Ca contents (a), Sr contents (b) and Sr:Ca ratios (c) in the body and muscle tissues of juvenile grey mullet of salinities from 0‰ to 35‰. Similar letters indicate homogenous groups; Vertical line, SD.

Sr:Ca ratios in fish otoliths have been found to be positively correlated with ambient salinity in many studies, but the results are not consistent among species. Secor et al. (1995) established a positive linear regression of otolith Sr:Ca ratios on salinity for striped bass *Morone saxatilis* juveniles reared in 6 experimental salinities of 0‰, 5‰, 10‰, 15‰, 20‰, 25‰, and 30‰. Tzeng (1996) found that Sr:Ca ratios in otoliths of Japanese eel *Anguilla japonica* elvers reared in the 4 salinities of 0‰, 10‰, 25‰, and 35‰ were divided into 2 homogeneous groups of salinities of 0‰, 10‰, and 25‰ and of 25‰ and 35‰, and he also established a positive linear regression for the otolith Sr:Ca ratio on salinity of $[\text{Sr}/\text{Ca}]_{\text{otolith}} \times 10^3 = 0.14S + 3.797$. Kawakami et al. (1998) also found that Sr:Ca ratios in otoliths of Japanese eel elvers significantly differed among 4 rearing salinities (0‰, 11.3‰, 22.7‰, and 34‰), but the difference in the mean ratios was not significant for the latter 2 salinities. Secor et al. (1998) established a predictive linear regression for back-calculating salinity from otolith Sr:Ca ratios of Japanese sea bass *Lateolabrax japonicus* juveniles reared in 4 salinities (0‰, 10‰, 20‰, and 30‰), and found that Sr:Ca ratios only significantly differed between 0‰ fresh water and 10‰-30‰ seawater. In this study, Sr:Ca ratios in otoliths of grey mullet juveniles significantly increased from 0‰ fresh water to 5‰ seawater, but remained constant for salinities of 5‰-35‰. The linear regression of otolith Sr:Ca ratios on salinity was not significant when the 0‰ fresh water data were excluded (Fig. 9). This is very similar to data for the Japanese sea bass. However, the mean (\pm SD) Sr:Ca ratios in otoliths of mullet reared in 0‰ fresh water were $(3.16 \pm 0.36) \times 10^{-3}$, which were lower than those of

Japanese eel at $(4.20-5.0) \times 10^{-3}$ and sea bass at 4.26×10^{-3} ; whereas Sr:Ca ratios for those reared in 5‰-35‰ seawater were $(6.35 \pm 0.70) \times 10^{-3}$, which were higher than those of Japanese sea bass at $(4.91-5.56) \times 10^{-3}$ but similar to those of Japanese eel at $(4.99-9.27) \times 10^{-3}$. These demonstrate that the relation between otolith Sr:Ca ratios and ambient salinity is species-specific, and that the regression of the otolith Sr:Ca ratio on salinity is not universal in different species.

The rearing water of different salinities in this experiment was prepared by mixing 0‰ fresh water and natural seawater. Although the absolute Ca and Sr contents of the rearing water were positively proportional to salinity, the Sr:Ca ratios were distinctly divided into 2 ranks of 0‰ fresh water and 5‰-35‰ seawater. In other words, Sr:Ca ratios were constant in the seawater irrespective of its dilution with fresh water. This phenomenon fits the law of relative proportions or the constancy of the composition of seawater, i.e., regardless of the absolute values of the major constituents, their relative abundances remain constant (Riley and Chester 1971). The Sr:Ca ratios in fresh water at $(7.91 \pm 0.41) \times 10^{-3}$ were approximately 2-fold lower than in seawater at $(13.95 \pm 0.79) \times 10^{-3}$ (Fig. 6c). This difference was consistent with differences in Sr:Ca ratios of otoliths of mullet reared in fresh water at $(3.16 \pm 0.36) \times 10^{-3}$ and seawater at $(6.35 \pm 0.70) \times 10^{-3}$ (Fig. 9c). Sr:Ca ratios in mullet otoliths corresponded to Sr:Ca ratios rather than to the absolute Sr contents in the rearing water. This phenomenon has also been found in sockeye salmon *Oncorhynchus nerka* (Rieman et al. 1994), tilapia *Oreochromis niloticus* (Farrell and Campana 1996), and spot *Leiostomus xanthurus* (Bath et al. 2000). Thus, the incorporation of Ca and Sr into fish otoliths from the environment reflects the proportion of elements in the water.

Table 2. Correlation coefficients of the Ca and Sr contents and Sr:Ca ratios of juvenile grey mullet otoliths, body and muscle tissue reared at salinities ranging from 0-35‰. *: $p < 0.05$, **: $p < 0.01$

	Correlation coefficient		
	[Ca]	[Sr]	[Sr/Ca]
Water vs Body	0.03	0.06	0.31*
Water vs Muscle	0.23	0.29	0.01
Water vs Otolith	0.01	0.29**	0.76**
Body vs Otolith	0.50**	-0.16	0.44**
Muscle vs Otolith	-0.04	0.26	-0.02

Regulation of otolith Sr:Ca ratios by biological processes

The incorporation of elements into otoliths is a complicated biogeochemical process. In addition to environmental variables such as salinity, temperature, and elemental composition, biological processes such as developmental stage, growth rate, and assimilation by fish may also play important roles in regulating the deposition of Sr:Ca ratios in otoliths of fish (Kalish 1989, Radtke and Shafer 1992, Sadovy and Severin 1992, Farrell and Campana 1996, Gallahar and Kingsford 1996).

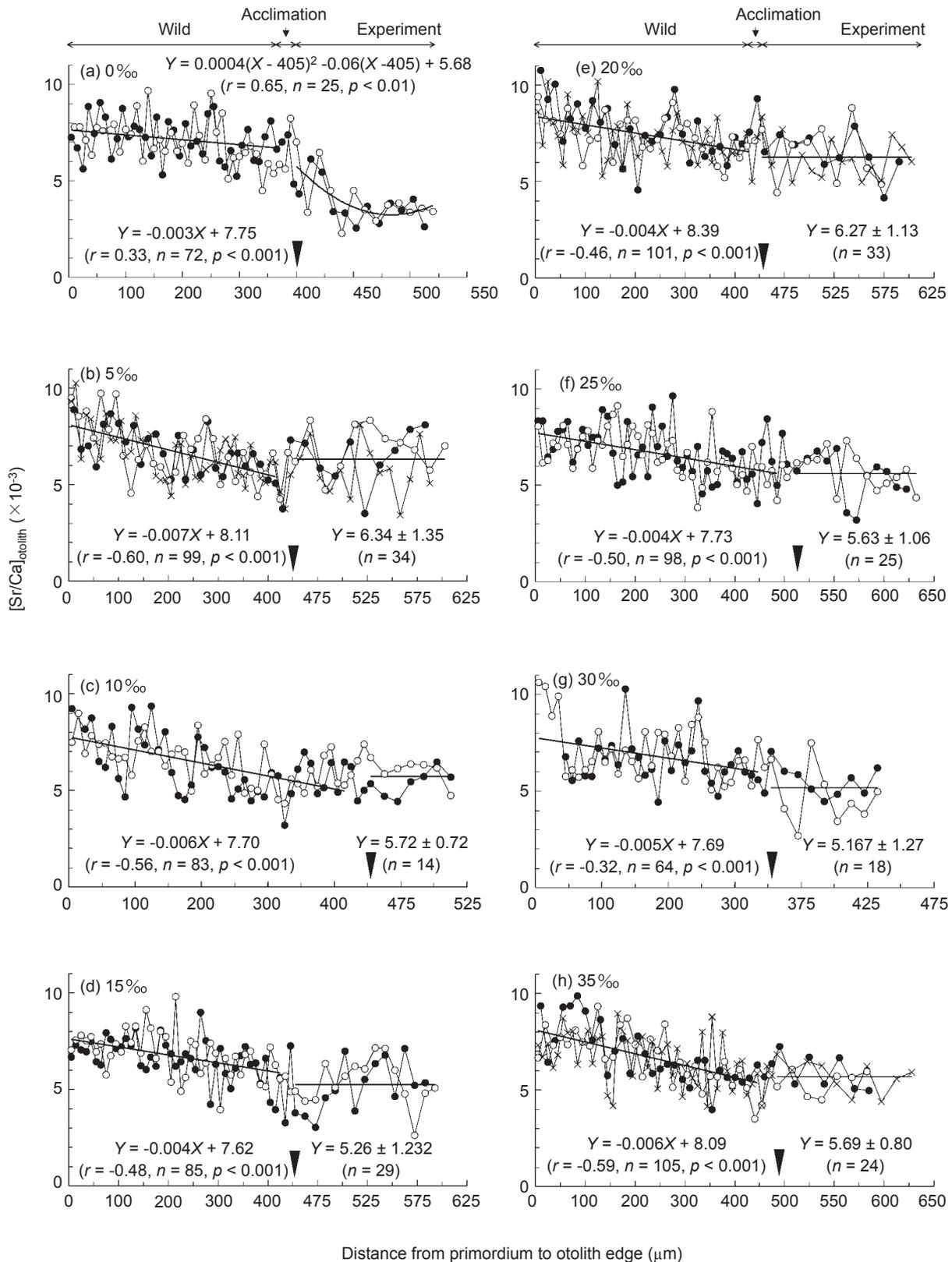


Fig. 8. Changes in the Sr:Ca ratios from the primordium to the otolith edge for juvenile grey mullet in the wild, during the acclimation period and when reared in salinities from 0‰ to 35‰ during the experimental period (a-h). Triangle, the location of TC mark. Scales beyond TC mark are magnified 2x.

The mullet used in this study were collected from the same estuary at the same time, were similar in total length, were reared under identical temperature fluctuations, and were fed with the same feed, but the Sr:Ca ratios in the otoliths were inversely correlated with their growth rate (Fig. 10), as was also found for other species (Sadovy and Severin 1992, Secor et al. 1995). This indicates that growth rate may play an important role in determining the deposition of Sr:Ca ratios in fish otoliths. Ca is not a limiting factor. It was very rich in the rearing water; however, Sr was a trace element in the rearing water, and its content was very low in comparison with Ca (Fig. 6). The uptake of Sr is relatively less than that of Ca when fish grow faster and the requirements for both Ca and Sr for otolith growth simultaneously increase. Perhaps this is the reason that Sr:Ca ratios in otoliths decrease as fish grow faster.

On the other hand, the distribution coefficients of Sr:Ca ratios (D_{Sr}), $D_{Sr} = [Sr/Ca]_{otolith} / [Sr/Ca]_{water}$,

in otoliths of mullet were approximately 0.40 when fish were reared in 0‰ fresh water and 0.46 in 5‰-35‰ seawater. Both of these values were less than 1.0, indicating that Sr and Ca ions in the water pass through 3 main interfaces (brachial uptake and/or intestine assimilation, cellular transport, and crystallization) may be selected by the fish before they are incorporated into the otolith (Campana 1999). The presence of these interfaces, which may concentrate or dilute elements, ensures that otoliths do not necessarily directly reflect the relative abundance of the element in the surrounding fresh water or seawater (Elsdon and Gillanders 2002).

Ecological implications of otolith Sr:Ca ratios

Most grey mullet are believed to migrate in seawater but some immature mullets may stay in fresh water until the spawning migration (McDowall 1988). Sr:Ca ratios in otoliths of mullet reared in fresh water were significantly lower than those reared in seawater. This provides a powerful method for differentiating mullet resident in fresh water and seawater. Sr:Ca ratios in otoliths combined with age data (Ibáñez-Aguirre and Gallardo-Cabello 1996, Chang et al. 2000) can be further used to determine habitat utilization rates and migratory behavior of the fish. In addition, if the Sr contents and other elemental composition differ among rivers, they may also be imprinted on the otoliths when fish migrate to those rivers (Rieman et al. 1994, Limburg 1995). Accordingly, based on

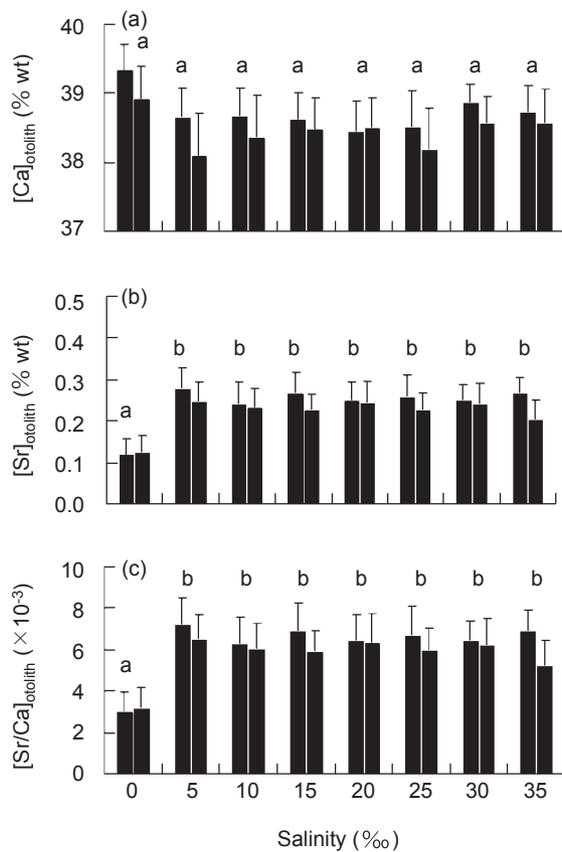


Fig. 9. Comparison of the mean Ca contents (a), Sr contents (b) and Sr:Ca ratios (c) in the otoliths of juvenile grey mullet reared in salinities from 0‰ to 35‰. Similar letters indicate homogenous groups; Vertical line, SD.

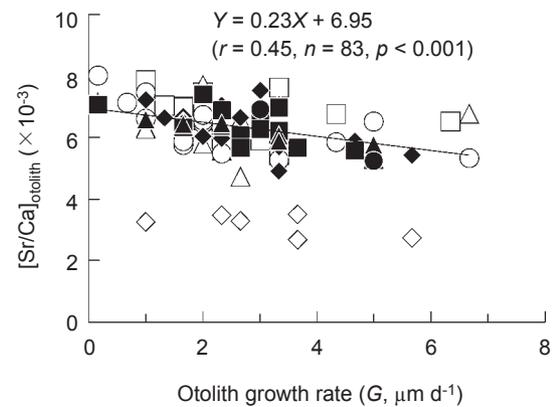


Fig. 10. The relationship between growth rate and Sr:Ca ratios of the otoliths of juvenile grey mullet reared in salinities of 0‰ (◇), 5‰ (□), 10‰ (△), 15‰ (○), 20‰ (◆), 25‰ (■), 30‰ (▲) and 35‰ (●). The regression of Sr:Ca ratios on salinity was calculated excluding 0‰.

the relationship between water and otolith elemental composition established from different rivers and otoliths of mullet, the potential exists to discriminate unit stocks originating from different rivers.

Sr:Ca ratios in otoliths decrease from the primordium to the otolith edge of juvenile mullet when larvae disperse from offshore spawning grounds and develop into juveniles in estuarine nursing grounds (Tung 1981, Chang et al. 2000). The decrease in Sr:Ca ratios in otoliths may be expected to reflect the salinity decrease from 34.0‰-34.8‰ in the spawning grounds off southwestern Taiwan (Su and Jane 1974) to 23.5‰ ± 9.6‰ in the nursing grounds in estuaries on the western coast of Taiwan (Tzeng et al. 2002b). However, our experiment indicated that Sr:Ca ratios in otoliths of mullet did not significantly differ in the salinity range of 5‰-35‰ (Fig. 9c). Thus, the decrease in otolith Sr:Ca ratios from the primordium to the otolith edge of juvenile mullet may be mediated by biological processes such as the growth rate and ontogenetic changes rather than solely by a salinity effect if larvae disperse within the same water mass of similar Sr:Ca ratios from offshore spawning grounds to low-salinity estuarine nursing grounds. Accordingly, when otolith Sr:Ca ratios are applied to reconstruct the environmental history of the fish, not only the environmental variables but also the biological processes of the fish should be considered together.

In conclusion, Sr:Ca ratios in mullet otoliths can be used to discriminate their migration between fresh water and seawater, but the Sr:Ca ratios in mullet otoliths are modified by biological processes such as the growth rate and ontogenetic changes.

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