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Temporal stability in the otolith Sr:Ca ratio of the yellow croaker, *Larimichthys polyactis* (Actinopterygii, Perciformes, Sciaenidae), from the southern Yellow Sea

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Abstract

Otolith chemical signatures are sufficiently stable across time to allow for accurate stock classification. The classification of the southern Yellow Sea population for *Larimichthys polyactis* (Bleeker, 1877) and its connectivity with others from 1962 is controversial. The study aimed to study the inter-annual variation in otolith strontium:calcium (Sr:Ca) ratios of *L. polyactis* to determine whether otolith natural tags are representative over long periods and can then be used for population structure classification. Spawning *L. polyactis* individuals were captured by stow nets in the same site of the southern Yellow Sea coastal waters during April–May in 2003, 2012, and 2013. EPMA (electron probe microanalysis) was used to determine the Sr:Ca ratios of a total of 25 otolith samples. Mann–Whitney *U*-test was used to test the differences of otolith Sr:Ca ratios from the core to edge for each otolith. One-way ANOVA was performed to compare the mean otolith Sr:Ca values among 2003, 2012, and 2013. Otoliths from 2003, 2012, and 2013 showed similar patterns of Sr:Ca ratios and Sr:Ca ratios could be divided into higher and lower phases in the core and remaining regions, respectively. Inter-annual significant differences for each high or low Sr:Ca phase of otoliths were not observed over short- (between 2012 and 2013) or long-time (between 2003 and 2012, and between 2003 and 2013) scales. Univariate contrasts across the adjacent year and decade classes were statistically similar. The Sr:Ca ratio signatures in the otolith were relatively stable across years and can be used as a reliable natural tag for connectivity assessments and stock identification with little or no genetic differentiation among *L. polyactis* populations. The short- and long-term temporal stability of otolith Sr:Ca ratios also revealed, the existence of stable *L. polyactis* stocks in the southern Yellow Sea, consistent with a previous finding of capture survey.

Keywords

Larimichthys polyactis, southern Yellow Sea, Sr:Ca ratio, temporal stability

Introduction

Otoliths are used as natural tags in fish, because of their capacity to record time-resolved lifetime environmental histories, which provides an opportunity for geolocating individual fish in time and space (Campana et al. 2000; Gillanders 2002). Retrospective determination of the natal source of fish stocks depends on the premise that the otolith chemical signatures are sufficiently stable across time to allow for accurate classification (Rooker et al. 2003; D'Avignon and Rose 2013). Therefore, understanding the temporal scale of otolith chemistry is essential to evaluate the reliability of natural tags among years (Walther and Thorrold 2009). There has been considerable research on the geographical variation of natal geochemical signatures, but limited studies have examined the temporal stability of otolith chemistry. Studies that examined temporal variation in otolith chemical composition found substantial variations within (Thorrold and Shuttleworth 2000; Reis-Santos et al. 2012) and among years (Campana et al. 2000; Gillanders 2002; Walther and Thorrold 2009; Pruell et al. 2012), which could lead to misinterpretation of spatial variability in otolith signatures (Reis-Santos et al. 2012; Avigliano et al. 2018). For instance, in *Platichthys flesus* (Linnaeus, 1758) and *Dicentrarchus labrax* (Linnaeus, 1758), collected from several estuaries along the Portuguese coast in two years and three seasons within a year, significant differences were observed in the majority of otolith elements among estuaries and sampling times, which were likely to be a reflection of the observed variability in environmental conditions, incorporating seasonal variation, which resulted in an 11% increase in the correct classification of individual estuaries (Reis-Santos et al. 2012). There was some overlap in elemental fingerprints among years for different estuaries for juvenile snapper *Pagrus auratus* (Forster, 1801), which was likely to have consequences in the assignment of adult fish to recruitment estuaries, especially if juvenile fish were collected from different estuaries in different years and adult fish were not assigned to recruitment estuaries using elemental fingerprints from the year class of recruits in which they were juveniles (Gillanders 2002). Furthermore, the long-term stability of otolith chemical signatures has been less frequently investigated (Avigliano et al. 2018), suggesting that chemical signatures may serve only as short-term natural tags (1–3 y) (Rooker et al. 2001; Walther and Thorrold 2009; Pruell et al. 2012).

The yellow croaker, *Larimichthys polyactis* (Bleeker, 1877) (Perciformes: Sciaenidae), is an important fish species endemic to the Northwest Pacific, inhabiting coastal waters across the Yellow, Bohai, and East China seas (Wang et al. 2013; Xiong et al. 2015), and supporting demersal fisheries in China, Korea, and Japan (Lim et al 2010; Xiong et al. 2016). Therefore, it is important to understand the population structure and connectivity patterns between stocks to conserve and manage this species in danger of overexploitation. Among *L. polyactis* populations in China's coastal waters, the classification of the southern Yellow Sea population and its connectivity dynamics from 1962 is controversial (Xiong et al. 2016). Previous fishery ecological studies have suggested four, three, or two groups of *L. polyactis* across the China coast. Some researchers (Liu 1990; Jin 2005) have pointed out the presence of the Bohai Sea and Northern Yellow Sea group, the Middle Yellow Sea group, the Southern Yellow Sea group, and the East China Sea group across the China coast. Others (Zhang et al. 2007; Yan et al. 2014) believe that there were three groups (the Bohai Sea, the Southern Yellow Sea, and the Central Yellow Sea groups) along the coast of China. However, Xu and Chen (2010) suggested that there were two *L. polyactis* groups: the Northern Yellow Sea and Bohai Sea group and the Southern Yellow Sea and the East China Sea group. Therefore, the temporal variability in otolith elemental signatures is essential to determine whether classifications and retroactivity of unknown individuals can be made using baseline data of otolith chemical signatures of this fish species.

The otolith strontium:calcium (Sr:Ca) ratio of concentrations have been applied as a useful scalar to estimate habitat use, migration history, and distinguish population structures of fish (Secor and Rooker 2000; Zimmerman 2005; Yang et al. 2011; Khumbanyiwa et al. 2018), successfully used in marine fish, including the Sciaenidae species *Argyrosomus japonicus* (Temminck et Schlegel, 1843) (see Ferguson et al. 2011) and *Collichthys lucidus* (Richardson, 1844) (see Liu et al. 2015). Recently, the habitat reconstruction and early life history of *L. polyactis* from the southern Yellow Sea was determined, based on the otolith microchemical analysis of the Sr:Ca ratio (Xiong et al. 2014, 2017). The presently reported study aimed to investigate otolith Sr:Ca ratio variability across an adjacent year class and 10-year interval class of *L. polyactis* in the southern Yellow Sea. This is a key step to establishing baseline characteristics of *L. polyactis* otolith Sr:Ca tags, which can then be used for connectivity assessments in the southern Yellow Sea.

Materials and methods

Spawning *L. polyactis* individuals were captured by stow nets in the southern Yellow Sea coastal waters (Fig. 1), as described by Xiong et al. (2017). Sampling was conducted during the late spring spawning run (April–May) in 2003, 2012, and 2013, at the same sampling site (32°05′N, 121°50′E) to determine the otolith Sr:Ca ratio. Eight specimens of *L. polyactis* [standard length (L_S): range, 13.4–18.3 cm; mean ± SD, 15.1 ± 1.7 cm; similarly hereafter] aged 1–2 years were captured in 2003, ten (10.7–15.6 cm, 11.9 ± 1.5 cm) aged 1–2 years in 2012, and seven (12.2–18.6 cm, 14.6 ± 2.0 cm) aged 1–2 years in 2013 (Table 1).

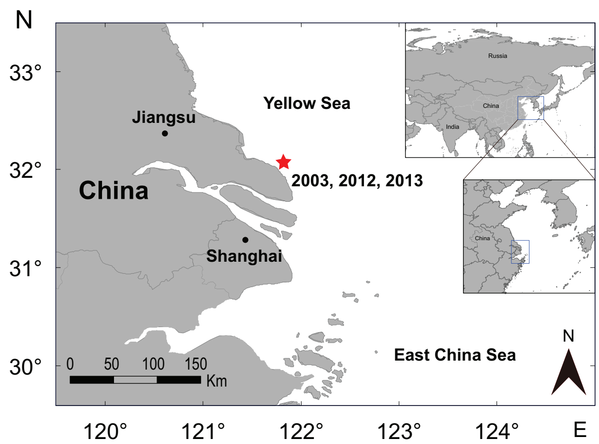

Figure 1. Sampling site for the yellow croaker *Larimichthys polyactis* used in the microchemistry analyses. The sampling site in coastal waters of the southern Yellow Sea in 2003, 2012, and 2013 is indicated by a red star.

Table 1. High and low phases of the strontium:calcium (Sr:Ca) ratio in *Larimichthys polyactis* otoliths. High and low phases of the Sr:Ca ratio in *Larimichthys polyactis* otoliths collected in 2003, 2012, and 2013.

Year	Sample code	Standard length [mm]	Estimated age	High Sr:Ca phase		Low Sr:Ca phase	
				Distance from the core [μm]	Sr:Ca (mean ± SD)	Distance from the core [μm]	Sr:Ca (mean ± SD)
2003	LSPP12	134	1+	0–240	7.57 ± 1.20 [a]	260–3,180	5.77 ± 0.80 [b]
	LSPP13	136	1+	0–140	6.97 ± 0.73 [a]	160–2,180	4.60 ± 0.74 [b]
	LSPP14	148	1+	0–140	7.16 ± 1.51 [a]	160–2,500	4.74 ± 0.63 [b]
	LSPP15	141	1+	0–180	7.10 ± 1.16 [a]	200–2,100	4.71 ± 0.78 [b]
	LSPP18	162	1+	0–40	7.68 ± 1.50 [a]	60–3,040	4.31 ± 0.80 [b]
	LSPP19	161	1+	0–200	6.85 ± 1.24 [a]	220–4,260	4.76 ± 0.99 [b]
	LSPP20	183	2+	0–160	6.90 ± 0.96 [a]	180–1,900	4.88 ± 0.78 [b]
	LSPP21	141	1+	0–300	7.64 ± 0.95 [a]	320–2,320	5.48 ± 0.92 [b]
2012	LSPP01	156	1+	0–200	7.67 ± 1.03[a]	220–3,300	4.31 ± 0.88[b]
	LSPP02	106	1+	0–280	7.31 ± 0.59 [a]	300–2,480	5.18 ± 0.92 [b]
	LSPP03	115	1+	0–80	7.02 ± 2.36 [a]	100–3,280	4.69 ± 1.01 [b]
	LSPP04	117	1+	0–160	6.63 ± 0.58 [a]	180–2,760	4.60 ± 0.96 [b]
	LSPP05	109	1+	0–180	7.04 ± 1.09 [a]	200–2,940	5.33 ± 0.92 [b]
	LSPP06	122	1+	0–60	6.68 ± 0.26 [a]	80–3,140	4.82 ± 0.95 [b]
	LSPP07	107	1+	0–60	7.74 ± 1.46 [a]	80–3,440	4.73 ± 0.86 [b]
	LSPP08	118	1+	0–300	7.58 ± 0.88 [a]	320–3,420	4.36 ± 0.98[b]
	LSPP09	123	1+	0–240	7.14 ± 0.83 [a]	260–3,340	4.85 ± 1.00 [b]
	LSPP10	112	1+	0–380	6.40 ± 0.64 [a]	400–2,780	5.16 ± 1.09[b]
2013	LSLP02	138	1+	0–80	7.62 ± 1.17 [a]	100–3,480	4.28 ± 0.81 [b]
	LSLP04	186	2+	0–220	7.18 ± 1.17 [a]	240–2,520	4.76 ± 0.81 [b]
	LSLP05	152	1+	0–80	7.55 ± 1.94 [a]	100–4,340	4.77 ± 0.90 [b]
	LSLP07	122	1+	0–160	6.79 ± 1.14 [a]	180–2,020	4.57 ± 0.85 [b]
	LSLP08	143	1+	0–80	7.75 ± 1.10 [a]	100–2,760	5.09 ± 0.92 [b]
	LSLP09	133	1+	0–120	7.61 ± 1.30 [a]	140–2,200	4.42 ± 1.02 [b]
	LSLP10	151	1+	0–80	7.34 ± 1.43 [a]	100–1,980	4.48 ± 0.86 [b]

The different superscript lowercase letters (a and b) indicate a significant difference at the 0.01 level for the Mann–Whitney *U*-test, which was used to compare the differences between the high and low Sr:Ca phases of each otolith sample.

Methods of preparing *L. polyactis* otoliths for use in electron probe microanalysis (EPMA) measurement have been described by Xiong et al. (2017). The sagittal otoliths were extracted, cleaned, and embedded in epoxy resin (Epofix; Struers, Copenhagen, Denmark), mounted on a glass slide, and then ground using a grinding machine (Discoplan-TS; Struers, Copenhagen, Denmark) equipped with a diamond cup-wheel. Each otolith was further polished on an automated polishing wheel (LaboPol-35; Struers, Copenhagen, Denmark), then cleaned in an ultrasonic bath, and rinsed with Milli-Q water. Finally, all otoliths were carbon-coated in a high vacuum evaporator (JEE-420, JEOL Ltd, Tokyo, Japan) for further EPMA measurements.

EPMA was used to study the Sr and Ca concentrations, based on the method described by Xiong et al. (2017) but with a slight modification. Each otolith sample was measured along the longest axis, from the core to the edge, using a wavelength dispersive X-ray electron probe micro-analyzer (JEOL JXA-8100; JEOL Ltd, Tokyo, Japan). Two reference materials, $CaCO_3$ and $SrTiO_3$, were used as standards. The accelerating voltage and electron beam current was set at 15 kV and 2×10^{-8} A, respectively. The electron beam was focused on a point 5 μm in diameter with measurements spaced at 20 μm intervals, and the counting time was 15 s. The Sr X-ray intensity maps were developed from the representative *L. polyactis* otoliths, using the aforementioned electron probe micro-analyzer with the same methods described in our previous study (Xiong et al. 2014; Fig. 2). The beam current was 5×10^{-7} A, counting time was 30 ms, and pixel size was 7 × 7 μm in diameter.

Based on our previous study in 2012, which was the first time *L. polyactis* otoliths from the southern Yellow Sea were analyzed (Xiong et al. 2014), we established the baseline of otolith Sr:Ca ratio (Table 2 and Fig. 2). Otolith Sr:Ca ratios from the core to edge were separated into high Sr:Ca (i.e., (Sr:Ca) × 1000) > 7, reddish regions in X-ray intensity map) and low Sr:Ca (7 ≥ (Sr:Ca) × 1000 ≥ 3, greenish-yellowish regions in X-ray intensity map) regions, which corresponded to a high salinity habitat in early developmental

Table 2. Comparison for high and low strontium:calcium (Sr:Ca) phases in *Larimichthys polyactis* otoliths. One-way ANOVA results for high Sr:Ca and low Sr:Ca phases in *Larimichthys polyactis* otoliths compared in 2003, 2012, and 2013.

		MS	*df*	*F*	*P*	Significance level
High Sr:Ca phase	Inter-groups	0.167	2	1.082	0.356	NS
	Within-groups	0.154	22			
Low Sr:Ca phase	Inter-groups	0.151	2	1.070	0.360	NS
	Within-groups	0.141	22			

NS: Non-significant.

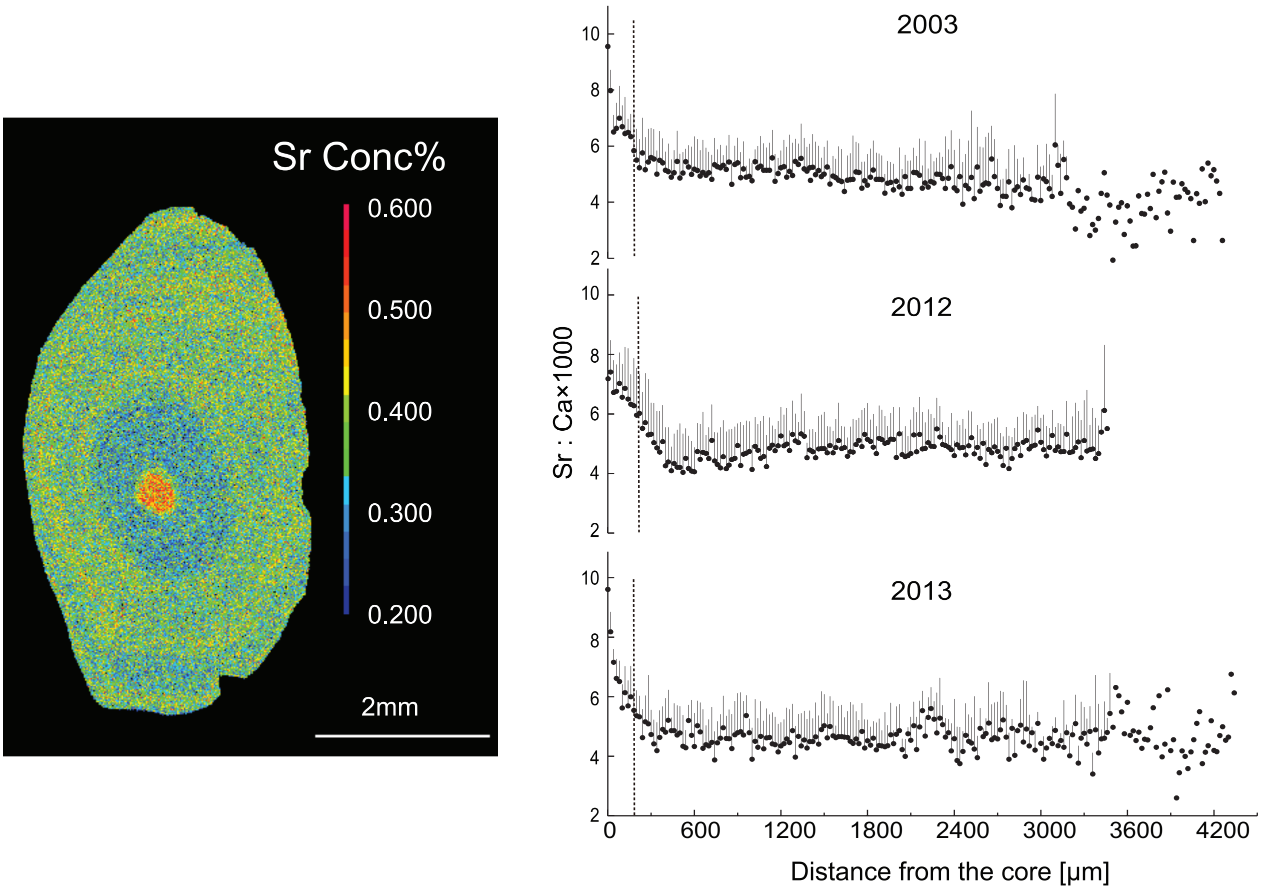

Figure 2. X-ray intensity map of strontium (Sr) content and strontium:calcium (Sr:Ca) ratios fluctuation in *Larimichthys polyactis* otolith. X-ray intensity map of the Sr content in *Larimichthys polyactis* otoliths collected in 2012 from the southern Yellow Sea (left). This pattern is representative of with overall low Sr levels (greenish and yellowish color), except for higher Sr contents in the core (reddish color). Mean Sr:Ca ratios fluctuated (with positive SD values) along the transect from the core (0 μm) to the edge of otoliths (right). The figure in 2012 has been referenced from Xiong et al. (2014). The dashed line represents the boundary between the high and low Sr phases; the high phases are on the left and the low phases are on the right.

stages, and middle salinity habitat in later developmental stages, respectively. The X-ray intensity map corresponds to 16 color map patterns of Sr content from blue (lowest) through green and yellow to red (highest).

According to *L. polyactis* baseline data from 2012 (Xiong et al. 2014), otolith Sr:Ca ratios in 2003 and 2013 from the core to edge were separated into high and low regions, respectively. The Mann–Whitney *U*-test is a non-parametric statistical technique, which is used to analyze differences between the medians of two data sets. This study presents the Mann–Whitney *U*-test as a statistical technique to examine differences between two independent groups on a continuous scale, namely, the differences in otolith Sr:Ca ratios between high and low phases of each otolith sample as described in previous studies (Yang et al. 2006; Liu et al. 2015; Jiang et al. 2019; Sokta et al. 2020). The Mann–Whitney *U*-test was defined as follows:

$$W_{XY} = \sum_{i=1}^{m} \sum_{j=1}^{n} U_{ij}$$

where X and Y represent two samples (high and low phases) in this study, and m and n are their sample sizes, respectively.

Unlike the Mann–Whitney *U*-test, which compares median values between two groups, one-way ANOVA compares values among multiple sets of groups. In this study, one-way ANOVA was used to determine whether the otolith Sr:Ca values varied with time for three comparisons across an adjacent year (2012 vs. 2013) and decade (2003 vs. 2012, and 2003 vs. 2013), calculated from the high and low Sr:Ca phases, respectively. The assumption of normality and homogeneity of variance for each variable was examined using Kolmogorov–Smirnov (KS) and Levene's tests, and the variables were log-transformed if necessary. Variables that did not pass the normality and homogeneity tests were excluded from further analysis. Statistical analysis was performed using IBM SPSS (version 19.0; IBM Corp., Armonk, NY, USA).

One-way ANOVA was defined as:

$$SS_B = \sum_{i=1}^{k} n_i \left(\bar{x}_i - \bar{x} \right)^2$$

$$\mathrm{MS_B} = \frac{\mathrm{SS_B}}{V_\mathrm{B}}$$

$$\mathrm{SS_W} = \sum_{I=1}^{K}\left\{\sum_{j=1}^{n}\left(x_{ij} - \bar{x}_i\right)^2\right\}$$

$$\mathrm{MS_W} = \frac{\mathrm{SS_W}}{V_\mathrm{W}}$$

where $\mathrm{SS_B}$ represents the sum of squares between groups, $\mathrm{SS_W}$ represents the sum of squares within-group, $\mathrm{MS_B}$ represents the mean square between groups, $\mathrm{MS_W}$ represents the mean square within-group, V_B represents the degrees of freedom between-groups ($V_\mathrm{B} = 2$), V_W represents the degrees of freedom within-group ($V_\mathrm{W} = 22$), *i* represents three groups (2003, 2012, and 2013), and *j* represents the total number of samples ($n = 25$) minus *K* (3 groups), namely 22.

The age of each sample of the presently reported study was estimated with the body length-at-age growth equation

$$\left(L_t = 240.6 \times \left[1 - e^{-0.56\times(t+0.25)}\right]\right)$$

reported by Zhang et al. (2010), because the growth rings were indistinguishable in both scales and otoliths (Table 1).

Results

Otoliths from 2003, 2012, and 2013 showed similar patterns of Sr:Ca ratios (Table 1, Fig. 2). All otolith samples had Sr-rich cores (regions < 380 µm radius from the core), whereas the remaining regions were characterized by relatively lower and more stable Sr patterns. Despite the individual variation, the corresponding results from the life history transects of the otolith Sr:Ca ratios could be divided into higher and lower phases in the core and remaining regions, respectively (Mann–Whitney *U*-test, $P < 0.01$) (Table 1).

The mean Sr:Ca ratios of the regions in the high Sr:Ca phase ranged from 6.79 to 7.68 in 2003, 2013, and 2012, whereas the corresponding ratios in the low Sr:Ca phase varied substantially in the remaining stages (Fig. 2).

Inter-annual differences in otolith chemistry were not observed over short- (between 2012 and 2013) or long-time (between 2003 and 2012, and between 2003 and 2013) scales (Table 2). Univariate contrasts for each high or low Sr:Ca phase across the adjacent year and decade classes were statistically similar (ANOVA, $P > 0.05$) (Table 2).

Discussion

In the presently reported study, the *L. polyactis* otolith Sr:Ca ratios were the highest and most variable near the otolith core in 2003, 2012, and 2013, which was possibly influenced by ontogenetic physiology and ambient chemistry. High Sr in the adult stage is more likely to reflect physiological state, particularly reproduction, than to reflect waterborne sources (Sturrock et al. 2015). However, the edge of high Sr region in the otolith core of larval *L. polyactis* is corresponding to the age of around 5–7 days and the first feeding ring is formed on the second day after hatching, which is demonstrated in Xiong et al. (2017). Moreover, for other sciaenid species *C. lucidus*, the Sr-richest regions (radius < 90 µm regions from the core) were attributed to the brachyhaline sea water brought by large flood tides (Liu et al. 2015). So the high Sr otolith core cannot be explained as the influence of the maternal yolk. Coastal areas are characterized by greater chemical heterogeneity owing to upwelling and fluvial (Sturrock et al. 2012). Furthermore, at the sampling site of the presently reported study, upwelling enriched trace elements (e.g., Sr) occurs in the coastal waters of the Lüsi near the Yangtze River estuary (Zhu et al. 2004; Lü et al. 2006). In March, the mixed water masses of the East China Sea and the Yellow Sea with low temperature and high salinity appeared in the coastal waters of the southern Yellow Sea (Su et al. 1983). Therefore, high Sr:Ca values and fluctuations during the early development of *L. polyactis*, which more likely correlated with waterborne sources in coastal areas of the southern Yellow Sea. These spawning fish were almost 1-year-old during this study, and fish of this age would probably have experienced drastic environmental changes in the western seawater of Jeju Island in which *L. polyactis* migrate for both overwintering and spawning (Xiong et al. 2016) because temperatures there have been observed to range from 26.1°C in November to 12.2°C in March (Hu 2013). However, such remarkable variations in water temperature did not appear to influence the otolith Sr:Ca ratios of *L. polyactis*, because the remaining development showed stable Sr:Ca ratios. Therefore, the otolith Sr contents or Sr:Ca ratios of *L. polyactis* in the presently reported study appeared to correspond to the salinity habitat. Although Sr uptake in otoliths of diadromous fish may be influenced to some degree by temperature, growth rate, age, diet, and stress (Yang et al. 2011), temperature and other factors did not seem to be the major factors (Howland et al. 2001; Yang et al. 2011). Additionally, the salinity of the ambient water is the most consistent and prominent factor influencing Sr uptake and may mask the effects of other factors (Yang et al. 2006).

In *L. polyactis* later developmental stages, the otolith Sr:Ca ratios remained stable and showed no significant differences between short- and long-time, suggesting that this ontogenetic stage experienced a relatively uniform physicochemical environment, which is supported by the fact that Ca and Sr exhibit quasi-conservative distributions resulting in comparatively stable Sr:Ca levels (Brown and Severin 2009) or near-constant Sr:Ca ratios in marine ecosystems (Secor et al. 1995; Steele et al. 2009).

The Sr:Ca ratio was selected to infer migration through habitats with different salinities in otolith microchemistry studies. Otolith Sr:Ca from *Pangasius krempfi* Fang et Chaux, 1949 varied between 1999 and 2017, possibly owing to changes in water environmental conditions with the development of hydropower dams along the Mekong

River (Tran et al. 2019). Otherwise, the inter-annual variability of elemental composition (including Sr:Ca) in the otolith nuclei of *Coilia nasus* Temminck et Schlegel, 1846 at each site was minimal, indicating that the elemental signatures at a given site might be maintained at comparable levels over a period of 3 years, resulting in a characteristic marker for that stock (Dou et al. 2012). In the presently reported study, the *L. polyactis* otolith Sr:Ca results in adjacent year and decade intervals may be indicative of consistent environmental conditions along the migration route across years. The tendency of temporal variation across years in Sr:Ca for *L. polyactis* was similar to that reported by Liu et al. (2015) for the Sciaenidae species *C. lucidus* from 2003 and 2010, although the prior study did not compare interannual variation.

Furthermore, we documented directly, for the first time, the uniform migratory history demonstrated by stable Sr:Ca over short- and long-term scales, which could suggest the existence of a stable *L. polyactis* stock in the southern Yellow Sea. This corroborates the findings of a previous fishery investigation during 2003 and 2013 that showed a similar migration distribution for *L. polyactis* in the southern Yellow Sea (Xiong et al. 2016). Up to now, genetic studies have failed to provide compelling evidence for the existence of a single stock of *L. polyactis* in the southern Yellow Sea, despite the development of numerous discriminatory tools and genetic markers (Li et al. 2013; Zhang et al. 2020). Compared to molecular approaches, the Sr:Ca ratio of otoliths, especially the inter-annual stability of the Sr:Ca ratio, might provide an alternative method for stock identification when little or no genetic differentiation exists among *L. polyactis* populations.

In conclusion, the *L. polyactis* otolith Sr:Ca signatures were found to be stable across years in the southern Yellow Sea, and therefore we suggest that further studies on *L. polyactis* should focus on the connectivity of spawning groups and overwintering groups, and their population structure in China seawater by analyzing the otolith microchemistry.

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