RESEARCH

Analyses of fin ray types to detect strontium markers in juvenile blunt-snout bream *Megalobrama amblycephala*

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Abstract

Background Restocking by introducing hatchery-reared fish into wild habitats aids in the restoration of fishery aquatic ecosystems and reefs to increase the abundance of fish resources, restore the ecological balance of water bodies, and enhance ecosystem functioning. Accurately, rapidly, and effectively evaluating the success of restocking using chemical markers (e.g., strontium [Sr]) remains challenging for fisheries management. Consequently, for non-lethal fish sampling, hard tissues, such as fin rays, have received increasing attention as a target for marking method. However, data on the differences in marking different types of fin rays remain limited. Therefore, we exposed juvenile blunt snout bream individuals (*Megalobrama amblycephala*) to 0 (control group) or 800 mg/L of $SrCl_2 \cdot 6H_2O$ (marked group) for 5 days and transferred them into normal aerated water for post-immersion culture. We sampled their pectoral, dorsal, ventral, anal, and caudal fin rays. The Sr marks among the fin types were sampled at 0 and 20 days post-immersion and evaluated using an electron probe micro-analyzer (EPMA) for the five-day Sr/Ca ratios, along with line transect and Sr mapping analyses.

Results Sr marking signatures were observed in all fin types in the marked group, with a success rate of up to 100%. Although marking efficiency varied among the different fin ray types, the highest Sr/Ca ratios were most often detected in the dorsal fin. Cross-sectional Sr concentration maps of all fin rays sampled showed high-Sr domains in the marked group; in contrast, the entire cross-sections of the control group displayed low Sr contents, indicating successful marking efficiency.

Conclusions Fin ray Sr marking is a successful method for juvenile *M. amblycephala*, with the advantages of non-lethality and negligible sampling injuries, facilitating the rapid and effective evaluation of Sr marking in restocking *M. amblycephala*.

Keywords *Megalobrama amblycephala*, Juvenile, Fin ray, Strontium marking, Electron probe micro-analyzer

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Background

Many ecologically adverse factors associated with human activity (e.g., dam construction, environmental damage, water quality deterioration, habitat loss and change, invasive species, and overfishing) have depleted valuable aquatic fishery resources. Several countries and regions have engaged in restoring wild fish populations through a series of corresponding restocking/stocking techniques or stock enhancement by releasing cultured juvenile fish (Warren-Myers et al. 2018a), such as the restocking projects in the Yangtze River that aim to improve fishery sustainability and restore biodiversity (Zhang et al. 2020). Such restocking programs can involve the introduction of hatchery-reared fish (especially juveniles) into wild environments (Bell et al. 2006) in order to increase the abundance of a particular stock (i.e., a genetically and/or morphologically distinguishable subpopulation belonging to a single species (Waldman 2005)), restore the ecological balance of the habitat, and/or enhance ecosystem functioning (Chen et al. 2009; Abelson et al. 2016).

The accurate, rapid, and cost-effective assessment of restocking is a concern in fisheries management and ecology, including ecological surveys using mark–recapture methods (Araki and Schmid 2010; Warren-Myers 2018a; Donadi et al. 2019). The ongoing monitoring of fish populations requires a reliable and reproducible marking technique for released fish to evaluate the relative success of restoration efforts over time (Carriere et al. 2016). In addition, the complexity and cost of the marking method, degree of damage to fish during sampling, and feasibility of detection conditions are important considerations (Pracheil et al. 2014).

The current major conventional internal and external marking methods (e.g., radio transmitters, coded wire tags, passive integrated transponder (PIT) tags, visible implant elastomers, and fluorescent compounds) for fish stocking are limited by multiple factors, including fish size, marking cost, toxicity, lethality, difficulty in large-scale application, and limited amount of adipose tissue available for implantation, many of which affect the normal behavior, growth, and survival of fish, and tag loss/non-detection (Smith and Whitledge 2011; Warren-Myers et al. 2018a). Microsatellite DNA markers cannot distinguish among individual fish within populations, but can yield important information regarding parental identification, allele analysis, and offspring allocation analysis (Smith and Whitledge 2011; Bai et al. 2015).

The trace elements or stable isotope properties of water are deposited in the calcified structures of fish as natural tracers (Campana 1999; Linley et al. 2016; Taddese et al. 2019; David et al. 2019; Fukushima et al. 2019), which can be used to back-trace the environments once inhabited by them. Studies on this topic have focused on the classification of stocked and wild fish and monitoring restocking success (Wolff et al. 2013; Wickström and Sjöberg 2014; Warren-Myers et al. 2018b).

As a natural element, strontium (Sr) causes no adverse effects on fish, even when using strontium chloride hexahydrate ($SrCl_2 \cdot 6H_2O$) in marking at a high concentration of 9,000 mg/L (Getchell et al. 2017). In element marking, fish are sacrificed to analyze their otoliths, which is the most common calcified structure for assessing the success rate of fish stocking and determining connectivity among their habitats (Wickström and Sjöberg 2014; Warren-Myers et al. 2018a; Taddese et al. 2019). However, studies focusing on these issues by nonlethally sampling fish fin rays and other hard structures are limited. Unlike the highly calcified otoliths (~98%) (Sweeney et al. 2020), which are composed primarily of calcium carbonate ($CaCO_3$) (Lewis et al. 2022), fin rays are usually much less mineralized (23–29%), consisting of calcium–phosphate hydroxyapatite and containing a large organic component (Tzadik et al. 2017), which is predominantly collagen (Mahamid et al. 2008). Nevertheless, both trace-element and stable isotope analyses can be performed to infer the habitat histories of fish, as their fin rays contain substantial amounts of organic and inorganic components (Tzadik et al. 2017). For example, studies on fin rays have been conducted for a few species (particularly sturgeons) using a single type of fin (often those of the pectoral fin) ray (Smith and Whitledge 2011; Mirali et al. 2017; Loeppky et al. 2020). However, pectoral, dorsal, ventral/pelvic, anal, and caudal fin rays have potential uses in element marking and monitoring restocking success. To our best knowledge, no previous studies have examined the differences among the five types of fins or discerned differences in Sr marking efficiencies among them. Therefore, we conducted a corresponding comprehensive study to address this.
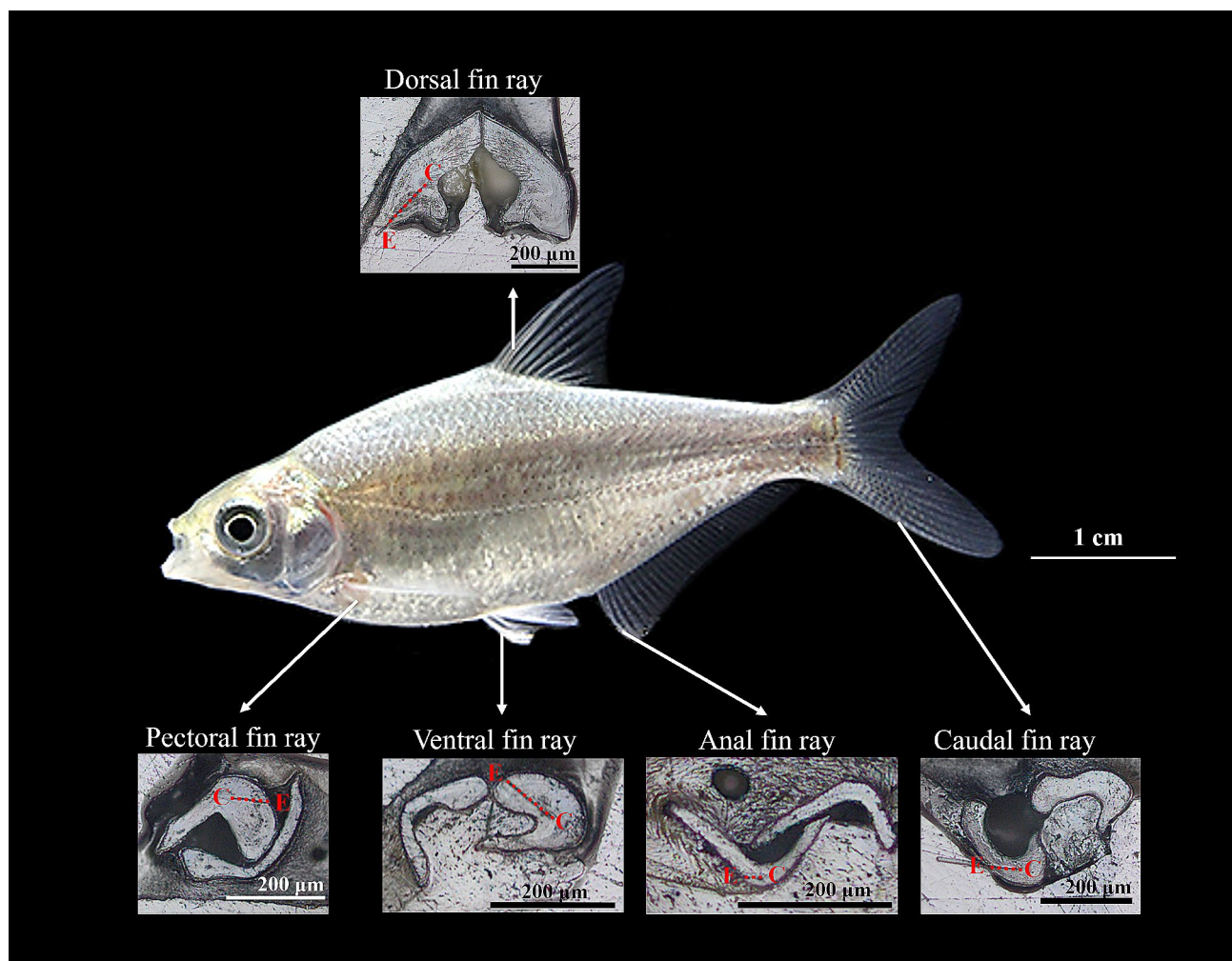
The blunt-snout (or Wuchang) bream (*Megalobrama amblycephala* Yih, 1955) is native to the lakes of the Yangtze River region and widely distributed in China, having high economic value and potential for freshwater aquaculture (Zhou et al. 2008). It is one of the most important species for fish stocking in Chinese freshwater (especially rivers and lakes) bodies. To date, no suitable method for the large-scale marking of the blunt-snout bream has been proposed. Owing to the varying outcomes among different species and studies, the species- or population-specific conditions for hatchery rearing and stocking, and, most importantly, the current status of wild populations and their carrying capacity remain to be fully understood on a case-by-case basis (Araki and Schmid 2010); hence, we used $SrCl_2 \cdot 6H_2O$ for marking. As the removal of otoliths is lethal to fish (Tzadik et al. 2017), we conducted a systematic comparative analysis to assess the effects of Sr marking on five fin types— pectoral, dorsal, ventral, anal, and caudal—from which fin

rays were sampled in vivo after marking for the first time. Moreover, an electron probe microanalyzer (EPMA) is effective in otolith microchemistry research, especially for Sr and Ca (Secor 1992; Mishima et al. 2020). Our previous study demonstrated that EPMA could detect the Sr signal in otoliths, which is consistent with the marking process (Zhu et al. 2022). Additionally, our results indicated that the signal could be measured with accuracy and precision using EPMA (like that described in Campana et al. (1997). Therefore, we used this approach here to detect the concentration of Sr and Ca in different types of fin rays. The objectives of this study were to (1) evaluate whether different types of fin rays exhibit elemental changes consistent with Sr-enriched water in which fish were immersed for labeling with Sr and (2) verify whether the fin rays of the blunt-snout bream could be used for non-lethal tissue biopsies to replace otolith applications in fisheries restocking research.

Methods

Experimental process

The experiment was conducted at the Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, Wuxi, Jiangsu Province. All procedures were performed at room temperature (23–30 ℃) and under regular lighting with no photoperiod control. The experimental juveniles (3 months old, total length of approximately 47 mm) were bred from broodstock fish cultured in the reproduction base of our center (Fig. 1). A preparatory experiment was conducted to optimize the Sr dosage and immersion duration. This study included 20 fish equally divided into control (0 mg/L $SrCl_2 \cdot 6H_2O$) and marked (800 mg/L $SrCl_2 \cdot 6H_2O$) groups, which were placed in the same glass aquarium (100 cm length × 45 cm width × 50 cm height) filled with aerated tap water for temporary feeding. Before immersion, the fish were starved for 1 d and immersed in tanks (30 cm length ×

Fig. 1 Blunt-snout bream *M. amblycephala*, corresponding positions of pectoral, dorsal, ventral, anal, and caudal fin rays, and their locations after embedding. Red dotted lines show the linear transect for EPMA analysis of Sr and Ca concentrations on the longest axis from the core (C) to the edge (E) of each fin ray sample

20 cm width × 30 cm height) containing 0 or 800 mg/L SrCl$_2$·6H$_2$O solutions prepared using aerated tap water for 5 days; the marking solution was prepared in advance. The marked and control groups were not fed throughout the immersion process. After the immersion was completed, the fish were rinsed in tap water for 10 min and transferred to different plastic basins containing clean water. The above operation was repeated three times to ensure that no marking solution residue remained on the fish scales. The fish were transferred to clean glass aquaria (100 cm length × 45 cm width × 50 cm height) containing clean aerated tap water for culturing after the marking was done (Fig. 2).
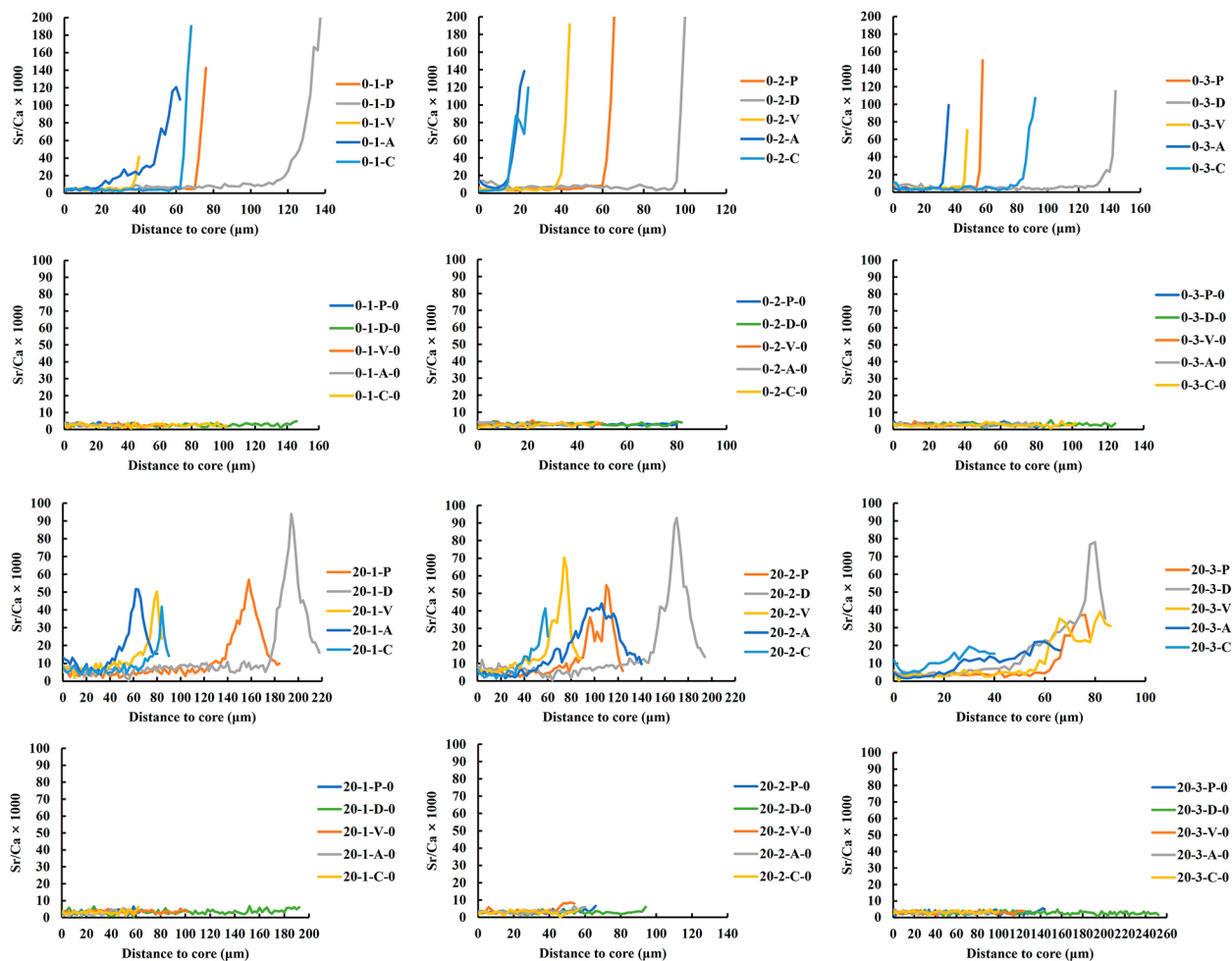
Five fish samples were collected from days 0–20 after the end of the immersion period. Their body weight (g) and total length (mm) were measured and the samples were frozen quickly at −20 °C until examination. The procedures were conducted according to the Standard

of Laboratory Animals—Guidelines for Ethical Review of Animal Welfare (GB/T 35892–2018) and Standard of Laboratory Animals—General Code of Animal Welfare (GB/T 42011–2022) and followed those approved by the Ethics Committee of the Freshwater Fisheries Research Center, Chinese Academy of Fisheries Sciences (Protocol Code 2011AA1004020012) and the American Fisheries Society guidelines (Use of Fishes in Research Committee 2014).

During each day of culture after marking, the fish were fed to satiation with commercial pellets of Fish Formula Feed 126 (Wuxi Tongwei Biological Technology Co., Ltd., Wuxi, China) twice per day; fecal excreta were cleaned and the water was changed once per day.

Sample treatment and analytical measurements
Three fish were randomly selected on days 0 and 20 from the five-fish sample, and their pectoral, dorsal, ventral,

Fig. 2 Pattern of Sr/Ca ratios along line transects from the core (0 mm) to the edge in the five (pectoral, dorsal, ventral, anal, and caudal) types of fin rays for control and marked groups of juvenile *M. amblycephala* at days 0 and 20 of the post-immersion culture. 0 (or 20)-1 (or 2, 3)-P (or D, V, A, C)-0: First "0" or "20" indicates at day 0 or 20 of post-immersion culture; 1, 2, or 3 indicates the first, second, or third sampled fish at day 0 or 20; P or D, V, A, and C indicates pectoral, dorsal, ventral, anal, and caudal fin ray, respectively; and "-0" indicates the control group

anal, and caudal fin rays were collected after thawing (Fig. 1). The first ray of each fin type was selected as close to the fin's base position as possible during sampling. The fin rays were washed with clean water to remove impurities and dried with anhydrous ethanol of ≥99.5% purity (Analytical Grade Reagent; Sinopharm Chemical Reagent Co., Ltd., Shanghai, China). The fin rays were then embedded in epoxy resin (Struers, Denmark); after the resin had solidified, a 500-μm sheet (Jiang et al. 2021) was cut and pasted with epoxy adhesive (Bond Quick 30, Konishi Chemical Ind. Co., Ltd., Wakayama, Japan) on a glass slide, ground with silicon carbide grinding paper (grit 2000, grit 4000, Struers, Denmark), and polished with non-drying fumed silica suspension (0.25 μm, OP-S NonDry, Struers, Denmark) to maintain a scratch-free surface. Finally, after washing the sample in an ultrasonic washer, it was rinsed with Milli-Q water (resistivity 18.2 MΩ·cm) and completely dried (Fig. 1). The fin rays were coated with carbon (36 A, 25 s) using a vacuum coating machine (2×10^{-4} Pa, JEE-420, JEOL Co., Ltd., Tokyo, Japan). Linear transect analysis of the Sr and Ca concentrations (wt%) on the longest axis from the core to the edge of each fin ray sample was conducted using EPMA (JXA-8100, JEOL Co., Ltd., Tokyo, Japan) (Fig. 1). The accelerating voltage and beam current were 15 kV and 2×10^{-8} A, respectively. The electron beam was focused on a point that was 2 μm in diameter, with measurements spaced at intervals of 4 μm. X-ray intensity maps of the Sr contents (wt%) were measured for each fin ray with EPMA using an accelerating voltage of 15 kV, beam current of 5×10^{-7} A, counting time of 30 mS, and pixel size of 6×6 μm. The electron beam was focused on a point with a diameter of 5 μm. Measurement quality was verified using calcium carbonate ($CaCO_3$) and strontium titanate ($SrTiO_3$) standards purchased from the Chinese Academy of Geological Sciences, Beijing, China, with recovery rates of 99.88% and 99.10%, and analytical errors of 1.22% and 0.91% for Sr and Ca, respectively (presented in Supplementary Material).

Data analysis

As the Sr content is much lower than that of Ca, the conventional Sr/Ca ratio refers to the standardized ratio of $(Sr/Ca) \times 10^3$ (Yang et al. 2011). The experimental data and images were processed using Microsoft Office 2016. A Mann–Whitney U test (SPSS 26.0, IBM SPSS Statistics Inc., Chicago, IL, USA) was used to compare the Sr/Ca ratios in different fin rays at the same ratio phases for fish sampled at days 0 and 20 post-immersion culture, as well as in the pre-marking and marking phases of the rays of the different fins for the marked fish group. Bonferroni correction was performed to correct all *p*-values after the test. In this study, the time period during which the Sr/Ca ratios increased is referred to as the marking phase, while the phase before the Sr/Ca ratios increased is referred to as the pre-marking phase.

In the results from the quantitative line transect analysis, the pre-marking and marking phases were divided based on the Sr/Ca ratio pattern transition curve for the fin rays. Specifically, the sequential *t*-test analysis of regime shifts (STARS) method was used to analyze regime shifts. If more than one continuous point significantly changed (*p*-value based on data variance and *t*-test), it was then regarded as a 'shift' and generated a new moving average. Therefore, we set the cut-off length to 10, the Huber's weight parameter to 1, and the significance level *p* to 0.1 for the trend conversion of the Sr/Ca ratio of the fin ray to differentiate between the pre-marking and marking phases. Growth indicators were evaluated using the same tests. The level of significance was set at $p \leq 0.05$ (Marques de Sá 2007).

Different colors in the X-ray intensity maps indicated the Sr contents in the fin rays via an increasing gradual change of 16 color patterns from red (highest) through yellow and green to blue and black (lowest). The fin ray Sr/Ca ratios at different phases are expressed as the mean±standard deviation (SD).

Results

Effects of Sr marking on the survival and growth of juvenile blunt-snout bream

During the 5-d immersion of juvenile blunt-snout bream in Sr, no deaths occurred in either group, indicating that Sr immersion had no acute toxicity to the fish; additionally, no deaths occurred after day 20 in the immersion culture. There were no significant differences in the total lengths (*n*=5) or body weights (*n*=5) between the two groups (*p*>0.05) of samples at day 0 (control group: total length, 47.84±6.48 mm and body weight, 1.07±0.50 g; marked group: total length, 49.94±4.85 mm and body weight, 1.21±0.34 g) and at day 20 (control group: total length, 55.26±11.75 mm and body weight, 1.69±1.17 g; marked group: total length, 54.35±11.19 mm and body weight, 1.63±0.85 g), indicating that Sr marking had no significant effects on the growth indicators of marked fish at the dose used in this experiment.

Line transect Sr/Ca analysis across the five blunt-snout bream fin rays

Following immersion, three blunt-snout breams from the post-immersion culture were collected on days 0 and 20, and their pectoral, dorsal, ventral, anal, and caudal fin rays (*n*=3 for each type of fin ray) were analyzed (Fig. 1). The 800 mg/L treatment with $SrCl_2 \cdot 6H_2O$ resulted in Sr marking on the fin rays in the marked group, while no Sr marking was detected in the corresponding fin rays of fish in the control group (Fig. 2). Moreover, in the marked groups, for samples collected on days 0 and 20, the Sr/Ca

Table 1 Changes in Sr/Ca ratios in the fin rays of juvenile blunt-snout bream at 0 d post-immersion in the marked group

Sample code	N	Pre-marking phase			Marking phase			Difference
		Distance from core (μm)	Detected points	Sr/Ca ratio (mean ± SD)	Distance from core (μm)	Detected points	Sr/Ca ratio (mean ± SD)	
20-1-P	1	0–124	63	4.96 ± 1.63	126–184	30	24.60 ± 14.45	*
20-2-P	1	0–80	41	5.23 ± 1.78	82–124	22	24.82 ± 13.45	*
20-3-P	1	0–62	32	3.81 ± 0.95	64–78	8	25.81 ± 9.73	*
20-1-D	1	0–176	89	7.00 ± 2.05	178–218	21	45.27 ± 23.16	*
20-2-D	1	0–146	74	7.20 ± 2.56	148–194	24	42.95 ± 24.08	*
20-3-D	1	0–50	26	5.58 ± 1.87	52–84	17	35.09 ± 18.57	*
20-1-V	1	0–58	30	7.71 ± 2.02	60–84	13	25.26 ± 12.47	*
20-2-V	1	0–58	30	8.68 ± 3.22	60–88	15	34.53 ± 17.96	*
20-3-V	1	0–56	29	4.00 ± 1.35	58–86	15	26.13 ± 8.30	*
20-1-A	1	0–44	23	8.67 ± 3.05	46–80	18	28.29 ± 12.72	*
20-2-A	1	0–50	26	4.00 ± 1.35	52–140	45	24.21 ± 11.73	*
20-3-A	1	0–20	11	2.99 ± 1.17	22–66	23	14.10 ± 4.60	*
20-1-C	1	0–68	35	6.29 ± 1.66	70–90	11	19.12 ± 8.90	*
20-2-C	1	0–32	17	3.85 ± 1.36	34–60	14	19.62 ± 10.00	*
20-3-C	1	0–12	7	6.56 ± 2.60	14–40	14	14.23 ± 3.22	*

Note: In the sample code, "0" represents the number of post-immersion culture days, "1," "2," and "3" represent the order of the fish sample, and "P," "D," "V," "A," and "C" represent the pectoral, dorsal, ventral, anal, and caudal fin rays, respectively. * indicates a significant difference between pre-marking and marking phases for juvenile blunt-snout bream (Mann–Whitney U test, $p < 0.05$)

Table 2 Changes in the Sr/Ca ratio in the fin rays of juvenile blunt-snout bream at 20 d post-immersion in the marked group

Sample code	N	Pre-marking phase			Marking phase			Difference
		Distance from core (μm)	Detected points	Sr/Ca ratio (mean ± SD)	Distance from core (μm)	Detected points	Sr/Ca ratio (mean ± SD)	
20-1-P	1	0–124	63	4.96 ± 1.63	126–184	30	24.60 ± 14.45	*
20-2-P	1	0–80	41	5.23 ± 1.78	82–124	22	24.82 ± 13.45	*
20-3-P	1	0–62	32	3.81 ± 0.95	64–78	8	25.81 ± 9.73	*
20-1-D	1	0–176	89	7.00 ± 2.05	178–218	21	45.27 ± 23.16	*
20-2-D	1	0–146	74	7.20 ± 2.56	148–194	24	42.95 ± 24.08	*
20-3-D	1	0–50	26	5.58 ± 1.87	52–84	17	35.09 ± 18.57	*
20-1-V	1	0–58	30	7.71 ± 2.02	60–84	13	25.26 ± 12.47	*
20-2-V	1	0–58	30	8.68 ± 3.22	60–88	15	34.53 ± 17.96	*
20-3-V	1	0–56	29	4.00 ± 1.35	58–86	15	26.13 ± 8.30	*
20-1-A	1	0–44	23	8.67 ± 3.05	46–80	18	28.29 ± 12.72	*
20-2-A	1	0–50	26	4.00 ± 1.35	52–140	45	24.21 ± 11.73	*
20-3-A	1	0–20	11	2.99 ± 1.17	22–66	23	14.10 ± 4.60	*
20-1-C	1	0–68	35	6.29 ± 1.66	70–90	11	19.12 ± 8.90	*
20-2-C	1	0–32	17	3.85 ± 1.36	34–60	14	19.62 ± 10.00	*
20-3-C	1	0–12	7	6.56 ± 2.60	14–40	14	14.23 ± 3.22	*

Note: In the sample code, "20" represents the number of post-immersion culture days, "1," "2," and "3" represent the order of the fish sample, and "P," "D," "V," "A," and "C" represent the pectoral, dorsal, ventral, anal, and caudal fin rays, respectively. * indicates a significant difference between the pre-marking and marking phases for juvenile blunt-snout bream (Mann–Whitney U-test, $p < 0.05$)

ratio of the pre-marking phase of each fin ray (mean of 3.1–8.7 at day 0 and 2.9–8.6 at 20 days) was significantly lower than that of the marking phase (mean of 28–147 at day 0 and 14–45 at day 20) ($p < 0.05$) (Tables 1 and 2). In contrast, in the control group, the Sr/Ca ratio for all types of fin rays was low, averaging 2.5–3.0 and 2.8–4.1 at days 0 and 20, respectively (Tables 3 and 4). Sr was not completely deposited in the fin rays in the samples collected on day 0, and the Sr/Ca ratio showed an increasing trend with time (Fig. 2). The maximum Sr marking values for the pectoral and dorsal fin rays were greater than those for the ventral, anal, and caudal fin rays. At day 20, the samples showed a significant downward trend in the maximum Sr/Ca ratio compared with that on day 0 (Tables 1 and 3).

Strontium distribution patterns in the five different blunt-snout bream fin rays

Marked areas with enriched Sr concentrations were detected in all fin rays on days 0 and 20 (i.e.,

Table 3 Changes in the Sr/Ca ratio in the fin rays of juvenile blunt-snout bream at 0 d post-immersion culture in the control group

Sample code	N	Distance from the core (μm)	Detected points	Sr/Ca ratio (mean ± SD)
0-1-P-0	1	0–48	25	2.70 ± 0.90
0-2-P-0	1	0–80	41	2.74 ± 0.70
0-3-P-0	1	0–82	42	2.97 ± 0.81
0-1-D-0	1	0–146	74	2.67 ± 0.78
0-2-D-0	1	0–82	42	3.03 ± 0.83
0-3-D-0	1	0–124	63	2.88 ± 0.82
0-1-V-0	1	0–62	32	2.51 ± 0.80
0-2-V-0	1	0–50	26	3.03 ± 0.72
0-3-V-0	1	0–52	27	2.79 ± 0.69
0-1-A-0	1	0–36	19	2.73 ± 0.57
0-2-A-0	1	0–36	19	2.74 ± 0.93
0-3-A-0	1	0–82	42	2.70 ± 0.74
0-1-C-0	1	0–102	52	2.51 ± 0.90
0-2-C-0	1	0–48	25	2.50 ± 1.02
0-3-C-0	1	0–102	52	2.61 ± 1.84

Note: In the sample code, "0" represents the post-immersion culture days, "1," "2," or "3" represents the order of the fish sample, and "P," "D," "V," "A," and "C" represent the pectoral, dorsal, ventral, anal, and caudal fin rays, respectively

Table 4 Changes in the Sr/Ca ratio in the fin rays of juvenile blunt-snout bream at 20 d post-immersion culture in the control group

Sample code	N	Distance from the core (μm)	Detected points	Sr/Ca ratio (mean ± SD)
20-1-P-0	1	0–68	35	3.41 ± 1.18
20-2-P-0	1	0–66	34	3.38 ± 1.03
20-3-P-0	1	0–144	73	3.06 ± 0.95
20-1-D-0	1	0–192	97	3.46 ± 1.26
20-2-D-0	1	0–94	48	2.87 ± 0.85
20-3-D-0	1	0–252	127	2.78 ± 0.87
20-1-V-0	1	0–102	52	3.44 ± 0.76
20-2-V-0	1	0–54	28	4.13 ± 2.00
20-3-V-0	1	0–124	63	2.99 ± 0.81
20-1-A-0	1	0–54	28	2.69 ± 1.38
20-2-A-0	1	0–60	31	3.32 ± 1.12
20-3-A-0	1	0–92	47	3.06 ± 0.63
20-1-C-0	1	0–62	32	3.16 ± 1.16
20-2-C-0	1	0–56	29	2.84 ± 1.06
20-3-C-0	1	0–100	51	3.26 ± 0.71

Note: In the sample code, "20" represents the post-immersion culture days, "1," "2," or "3" represents the order of the fish sample, and "P," "D," "V," "A," and "C" represent the pectoral, dorsal, ventral, anal, and caudal fin rays, respectively

yellow–green–red patterns in EPMA Sr distribution maps), while the fin rays of the control group displayed low and constant Sr concentrations (i.e., blue-colored EPMA Sr distribution maps).

The low-Sr blue map pattern of the fin rays observed in the marked group was comparable to the same blue color pattern of the pre-marking phase when the fish were reared in the water with a normal low Sr concentration

before marking. However, for the fish sampled at day 0 after immersion, yellow, green, or red marked areas were detected on the pectoral, dorsal, ventral, anal, and caudal fin rays. Conversely, when sampled on day 20, a complete high-brightness color ring was formed on all fin rays. Yellow–green areas were detected outside the red Sr-marked areas with the highest Sr concentration in the pectoral, dorsal, ventral, anal, and caudal fin rays. For the control group, the mapping results of the five fin ray types for days 0 and 20 appeared blue, indicating low and unchanged Sr contents (Fig. 3).
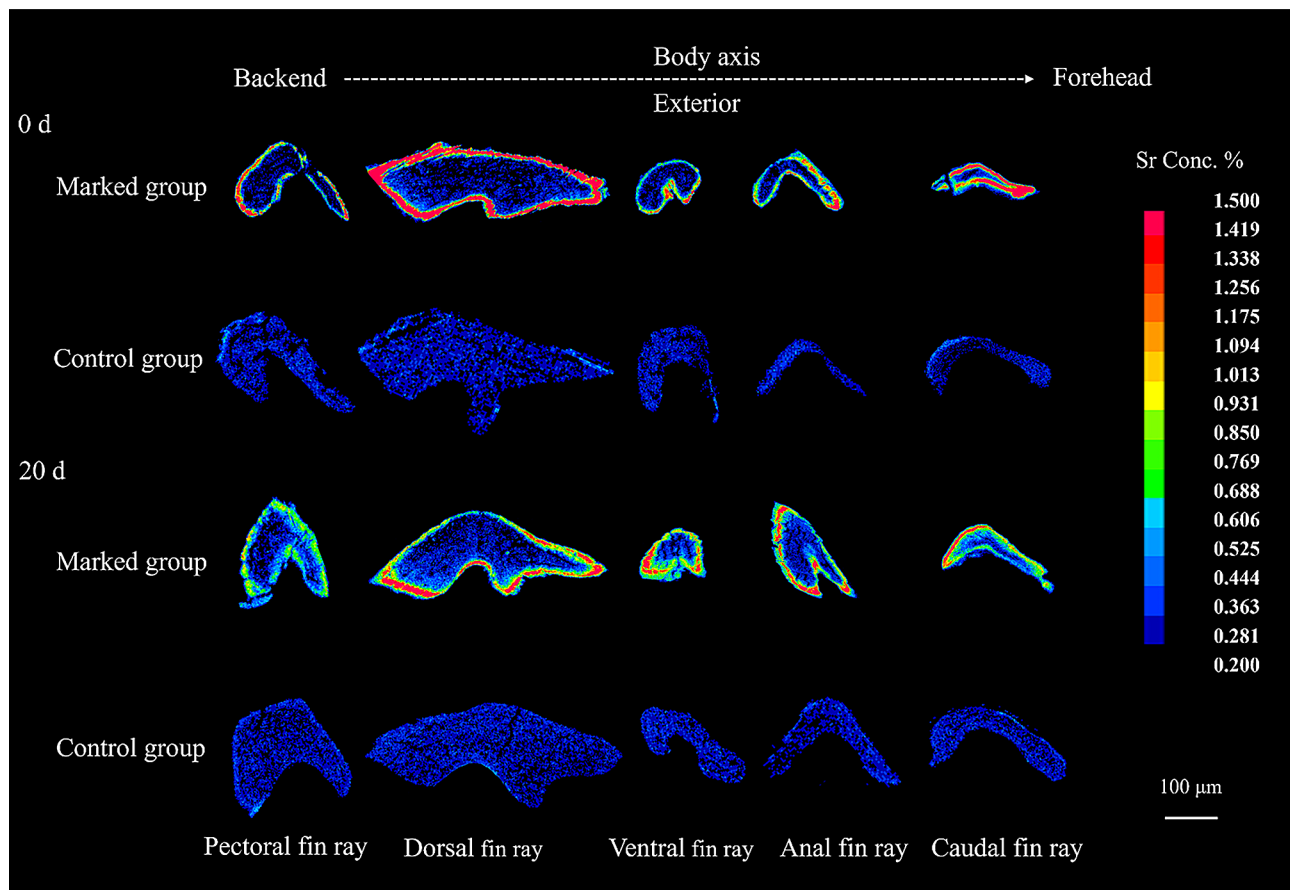
Discussion

Sr marking in five fin rays of blunt-snout bream

A previous study on smallmouth bass (*Micropterus dolomieu*) reported that the different Sr/Ca ratios in various water habitats formed corresponding Sr/Ca ratio markers in their pectoral fin rays (Smith and Whitledge 2010). In this study, the blunt-snout bream was immersed in 800 mg/L of $SrCl_2 \cdot 6H_2O$ for 5 days, and its five fin ray types were investigated using EPMA. The Sr marking signal was analyzed in all five types of fin rays in the marked group, and the ideal marking effect was achieved in all five types of fin rays, with a marking success rate of 100%. In contrast, no Sr marking signal was found in the corresponding fin rays of fish in the control group (Fig. 3). The average Sr/Ca ratios were higher in the pectoral and especially dorsal fin rays (Tables 1 and 2).

The dorsal, anal, and caudal fins of fish are single, while their pectoral and ventral (pelvic) fins are paired (Larouche et al. 2017). Therefore, considering the more detectable marked fin ray materials and the stronger Sr marking signal, pectoral and dorsal fin rays are the better choice for assessing the marking effect from a practical perspective, as single rays can be removed from fish without killing them or impeding their swimming. Nevertheless, the reasons for the differences in the Sr marking signal and optimum immersion concentration of the five fin rays in this study remain unclear.

Inter-fin ray variation in Sr marking of blunt-snout bream

With the increase in sampling time during the post-immersion culture, the Sr/Ca ratio peak of the various types of fin rays detected on day 20 exhibited a downward trend compared with that of the samples on day 0. During the marking phase, the average Sr/Ca ratio in the pectoral fin ray on day 20 was nearly four times lower than that on day 0, while those for the dorsal, ventral, anal, and caudal fin rays decreased by nearly two times on day 20 compared with those on day 0. That is, with the increase in sampling time, the maximum Sr/Ca ratio of the fin rays gradually decreased, with different rates of decline for the different types of fin rays, indicating an absorption and metabolism mechanism of the elements in each fin ray;

Fig. 3 Mapping of Sr content of the five types of fin rays for blunt-snout bream juvenile *M. amblycephala* at days 0 and 20 post-immersion. Different colors in X-ray intensity maps from blue (lowest) to green, yellow, and red (highest) indicate Sr concentrations

this requires further investigation. Considering the dorsal fin rays at 20 d post-immersion, combined with the 0-d line distribution data, the entire immersion marking process achieved marking during both the immersion and post-immersion culture phases (Figs. 2 and 3). The Sr content in the dorsal fin ray margin began to decrease at 20 d in post-immersion culture and was close to the pre-immersion level. Based on the duration required for the formation of a complete marking ring in this study, we recommend that more than 20 d are needed for post-immersion culture in order to gain a better insight into the process of marking and objectively and accurately assess marking effectiveness.

Unlike $CaCO_3$ depositions in otoliths, which are inert and comprise almost exclusively inorganic material (Campana 1999), fin rays have a unique growth pattern relative to endoskeletal bones (Tzadik et al. 2017). Osteogenesis occurs in the same manner as that in other bones, except that new layers in fin rays encapsulate the old ones; hence, a cross-section of the ray reveals annuli with the oldest layers at the core (i.e., hatching/birth) and the newest layers at the outer edge (Beamish and Chilton 1977; Rossiter et al. 1995). Importantly, multiple

biological barriers exist during the movement of ions from the environment into mineralized tissues (Campana 1999). These barriers can be both ion-specific and tissue-specific, resulting in the accumulation and fractionation of elements in the organism (Campana 1999; Loewen et al. 2016). In comparison with other mineralized structures in the body, fin rays appear to have little to no tissue turnover; in part, this may be due to their pattern of additive growth that encapsulates old layers, instead of the continuous growth of a single layer (Tzadik et al. 2017). In contrast, fish species with higher metabolic demands and larger individual ranges (e.g., billfish and tuna) are much more difficult to age due to the effects of resorption (Beamish 1981). Carriere et al. (2016) observed that the Sr isotope marking success of the fin rays of the lake sturgeon (*Acipenser fulvescens*) was determined by the duration of immersion in the marker and concentration of the marker introduced to the water.

Whether elements deposited in fin rays are gradually utilized as the time to post-immersion culture increases, thus decreasing to the level in the fish itself, and whether the stability of the marking signal changes

over time require verification in subsequent long-term experiments.

Advantages for selection of fin rays to detect Sr markers

The difficulty in implementation of the marker method, the advantages and disadvantages of its effect, the simplicity of sampling materials, and the lethality to fish in the sampling process should be considered when evaluating the effect of marking for restocking. In the chemical marking of fish, hard tissues for detection mainly include otoliths, vertebrae, fin rays, fin spines, and scales. Notably, as three pairs of otoliths are located in the inner ears of fish (Payan et al. 2004), removing one or more of the otoliths is lethal, as is removing the vertebrae. To improve testing feasibility, using external fish organs reduces the impact on the fish and testing costs. As an external detection material, scales are often preferred due to their convenient sampling, but they can easily fall off and regenerate after loss during growth (Hammond and Savage 2009), which affects the final marking effect and creates errors in the evaluation results. However, fin rays rarely fall off or regenerate. In a previous study that investigated fin ray micro-chemical characteristics, the fin rays of adult fish older than 2 years old were found to have a natural habitat tag (Jiang et al. 2021). The fin rays are composed of a hard tissue that records chemical information during growth, are easy to sample, and sampling them is non-lethal to fish (Zymonas and McMahon 2006). Upon experimental removal or loss due to injuries, fin rays can regenerate, allowing the fish to possibly regrow damaged fins (Goss and Stagg 1957).

Fin rays are approximately 23–29% mineralized and consist of calcium–phosphate hydroxyapatite and contain a large organic component (Tzadik et al. 2017). Both the Sr and Ca concentrations (wt%) in the bulk fin ray samples (consisting of bioapatite and organic matter) were detected using EMPA. Similar analytical conditions were used to analyze the fin rays from the pre-marking and marking phases, allowing the detection of significant differences and demonstration of the Sr marking efficiency. The present study demonstrated that pectoral, dorsal, ventral, anal, and caudal fin rays offer non-lethal alternatives to otoliths.

To the best of our knowledge, this study is the first to examine the differences in Sr marking efficiencies among the five types of fin ray, here using 3-month-old juvenile (life history stage most suitable for mass marking) bluntsnout bream. The Sr/Ca ratios in the pre-marking phases were slightly elevated compared with those of the control group. Although the underlying mechanisms remain unknown, one possible reason for this observation is that the resorption of fin rays may occur following the initial deposition due to vascularization (Drew et al. 2006; Sweeney et al. 2020). The sample fish used in this study were juveniles; hence, the fin rays might not yet be fully mineralized. Therefore, bone remodeling might have redistributed some Sr from Sr-marked bones into these pre-existing bones. Data on the long-term stability of such chemical labeling in fish are lacking. In our previous study, based on similar hard tissues from field anadromous estuarine tapertail anchovy (*Coilia nasus*; ca. 2 years old), we reported that the fluctuation in the Sr/Ca ratio and Sr concentration maps of pectoral fin rays examined using the same EPMA approach coincided well with those of otoliths, demonstrating a high similarity of microchemical "fingerprints" between the hard tissues of fin rays and otoliths (Jiang et al. 2021). Similarly, the Sr levels were strongly correlated between otoliths and fin rays for Arctic grayling (*Thymallus arcticus*) (Clarke et al. 2007). Therefore, Sr labeling in fin rays may retain long-term and stable high intensity, supporting the practice that both trace-element and stable isotope analyses can be performed to infer the habitat histories of fishes (Tzadik et al. 2017). Our findings provided evidence that marked groups immersed in water with 800 mg/L of $SrCl_2 \cdot 6H_2O$ for 5 d could be easily identified and measured using EPMA. The fin ray-based Sr EPMA approach has the advantage of acquiring precise and quantitative Sr and Ca analyses at even 1-µm spot sizes with corresponding elemental color maps. Corresponding element Sr levels and Sr/Ca ratios could also be investigated using alternative analysis approaches, such as laser ablation–inductively coupled plasma mass spectrometry (LA-ICPMS) (Rude et al. 2014) and synchrotron radiation-induced X-ray fluorescence (XRF) (Limburg et al. 2007). Comparative studies between the alternative approaches are warranted to confirm our findings. A comparison of EPMA and other analysis approaches on the same specimens will help to determine the most practical and efficient approach to measure Sr marking.

Besides Sr and its Sr/Ca ratio profiles, the isotopic approach of $^{87}Sr/^{86}Sr$ in hard fish tissue as a natural tracer can be used to assess the migration/provenance of fish (Wolff et al. 2013), distinguishing wild and hatchery-reared fish (Zitek et al. 2023), and evaluating restocking success without additional effects from temperature, salinity, or biological factors (Bakhshalizadeh et al. 2021). The application of this isotopic approach should be considered in future studies.

Conclusions

In this study, the initial use of 800 mg/L of $SrCl_2 \cdot 6H_2O$ for up to 5 d of marking in juvenile blunt-snout bream was demonstrated to be feasible. Selecting the fin rays for detection marking is a viable option that can be conducted without sacrificing the fish. Our quantitative line analysis and element (i.e., Sr) distribution maps results showed marking signals on the pectoral, dorsal, ventral,

anal, and caudal fin rays of the blunt-snout bream, with 100% success. There were slight differences in the marking signals among the different types of fin rays used. The maximum Sr/Ca ratio for the dorsal fin ray marking was the highest, and the ratios of the remaining rays for the four other fins reflected a similar, though somewhat reduced, marking signature. Blunt-snout bream fin rays are an advantageous bony hard tissue that reliably records in vivo applied Sr marking, enabling non-lethal sampling and facilitating mark detection using high-resolution in situ analytical techniques, such as EPMA. Our study facilitates the establishment and optimization of the marking technology scheme for the stocking of the blunt-snout bream and other species, which is useful for the long-term monitoring and assessment of released fish.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13717-024-00501-z.

Supplementary Material 1

Author contributions
Yahua Zhu: Conceptualization, Resources, Investigation, Methodology, Data Curation, Visualization, Writing—Original Draft, Funding acquisition. Tao Jiang: Conceptualization, Resources, Investigation, Methodology, Visualization, Writing—Original Draft, Funding Acquisition. Xiubao Chen: Resources, Investigation, Methodology, Writing—Original Draft. Hongbo Liu: Resources, Investigation, Methodology, Writing—Original Draft. Quinton Phelps: Conceptualization, Methodology, Writing—Review and Editing. Jian Yang: Conceptualization, Resources, Methodology, Visualization, Writing—Review and Editing, Supervision.

Data availability
The data and materials used in this study are available in the Supplementary Material.

Declarations

Ethics approval and consent to participate
The animal study protocol was approved by the Ethics Committee of the Freshwater Fisheries Research Center, Chinese Academy of Fisheries Sciences (Protocol Code 2011AA1004020012), and conducted according to the Standard of Laboratory Animal—Guideline for Ethical Review of Animal Welfare (GB/T 35892–2018), Standard of Laboratory Animals—General Code of Animal Welfare (GB/T 42011–2022), and Guidelines for the Use of Fishes in Research (American Fisheries Society 2014).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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