Mass marking of juvenile *Schizothorax wangchiachii* (Fang) with alizarin red S and evaluation of stock enhancement in the Jinping area of the Yalong River (#20560)

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Mass marking of juvenile *Schizothorax wangchiachii* (Fang) with alizarin red S and evaluation of stock enhancement in the Jinping area of the Yalong River

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Schizothorax wangchiachii is a key fish species in the stock enhancement program of the Yalong River hydropower project. Alizarin red S (ARS) was used to mark large numbers of juvenile *S. wangchiachii* in Jinping Hatchery and later used to evaluate stock enhancement in the Jinping area of the Yalong River. In a small-scale pilot study, 7,000 juveniles of the 2014 cohort were successfully marked by immersion in ARS solution, and no mortality was recorded during the marking process. The ARS mark in the fish otoliths remained visible 20 months later. In the large-scale marking study, approximately 600,000 juveniles of the 2015 cohort were successfully marked. Mortalities of both marked and unmarked juveniles were very low and did not differ significantly. Total length, wet mass and condition factor did not differ significantly between unmarked and marked individuals after three months. On 24 July 2015, about 840,000 Jinping Hatchery-produced young *S. wangchiachii*, including 400,000 marked individuals, were released at two sites in the Jinping area. Recapture surveys showed that 1) marked and unmarked *S. wangchiachii* did not differ significantly in total length, wet mass and condition factor; 2) stocked individuals became an important part of recruitment of the 2015 cohort; 3) instantaneous growth rate of marked individuals tended to slightly increase; and 4) most stocked individuals were distributed along a 10–15 km stretch near the release sites. These results suggest that the ARS method is a cost-efficient way to mass mark juvenile *S. wangchiachii* and that releasing juveniles is an effective means of stock recruitment.

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Abstract

Schizothorax wangchiachii is a key fish species in the stock enhancement program of the Yalong River hydropower project. Alizarin red S (ARS) was used to mark large numbers of juvenile *S. wangchiachii* in Jinping Hatchery and later used to evaluate stock enhancement in the Jinping area of the Yalong River. In a small-scale pilot study, 7,000 juveniles of the 2014 cohort were successfully marked by immersion in ARS solution, and no mortality was recorded during the marking process. The ARS mark in the fish otoliths remained visible 20 months later. In the large-scale marking study, approximately 600,000 juveniles of the 2015 cohort were successfully marked. Mortalities of both marked and unmarked juveniles were very low and did not differ significantly. Total length, wet mass and condition factor did not differ significantly between unmarked and marked individuals after three months. On 24 July 2015, about 840,000 Jinping Hatchery-produced young *S. wangchiachii*, including 400,000 marked individuals, were released at two sites in the Jinping area. Recapture surveys showed that 1) marked and unmarked *S. wangchiachii* did not differ significantly in total length, wet mass and condition factor; 2) stocked individuals became an important part of recruitment of the 2015 cohort; 3) instantaneous growth rate of marked individuals tended to slightly increase; and 4) most stocked individuals were distributed along a 10–15 km stretch near the release sites. These results suggest that the ARS method is a cost-efficient way to mass mark juvenile *S. wangchiachii* and that releasing juveniles is an effective means of stock recruitment.

Key words: *Schizothorax wangchiachii*; stock enhancement; alizarin red S; mass marking; recapture survey; otolith

Introduction

Habitat degradation and over exploitation contribute to the decline of fisheries around the world. In order to bolster fish stocks, stock enhancement by releasing hatchery-produced fish into wild habitats has been widely implemented (Brown & Day, 2002; Taylor et al., 2016; Yang et al., 2013). The effectiveness of stock enhancement can be assessed by mark-release-recapture studies (Blaxter, 2000), which require effective tagging or marking methods. Various marking techniques, such as coded wire tags (Bernard et al., 1998), otolith marking (Volk et al., 1999) and passive integrated transponders (Navarro et al., 2006), have been developed to monitor released fish. Among these techniques, otolith marking is a feasible method that allows long-term identification of small fish (Caraguel et al., 2014; Crook et al., 2009). Otolith marks can be achieved by thermal marking (Volk et al., 1999), isotopic marking (Woodcock et al., 2011) and fluorescent marking (Yang et al., 2016). The most popular marking protocol is to use fluorochromes, which can form chelate complexes with calcium ions that are built into skeletal and otolith structures (Poczyczyński et al., 2011). Fluorescent marks on the otolith are visible under a specific inducing laser because the calcium-fluorochrome complexes emit fluorescent light (Bashey, 2004; Taylor et al., 2005; Yang et al., 2016). Fluorochromes commonly used for otolith marking are oxytetracycline hydrochloride, calcein, alizarin complexone and alizarin red S (ARS). Compared with other fluorochromes, ARS offers better mark quality and lower cost and thus is viewed as a promising dye for mass marking fish at early life stages (Taylor et al., 2005; Yang et al., 2016).

In China, releasing hatchery-reared fish to enhance or restore fish stock abundance and fishery catches has been widely implemented for more than fifty years (Yang et al., 2013). Chinese carps and several other commonly cultured species that do not breed effectively in still waters were selected to conduct early artificial rearing-releasing programs (Wu & Zhong, 1964; Liu, 1965). In recent decades, technical developments and advances in hatchery production have made it possible to breed considerable numbers of endemic and rare fish annually, including Chinese sturgeon *Acipenser sinensis* (Chang & Cao, 1999) and Chinese sucker *Myxocyprinus asiaticus* (Zhou et al., 2002). However, fish stock enhancement programs in China, particularly for freshwater fish species, have focused mainly on artificial propagation techniques and stocking scale, with little attention paid to monitoring and evaluating the success of fish after release (Cheng & Jiang, 2010; Yang et al., 2013; Zhang et al., 2003). Some marking technologies have been tested in different fish species, but rarely has massive marking been used to evaluate stock enhancement and assess the contribution of the stocked fish to the larger population (Zhang et al., 2003; Yang et al., 2013). In most stock enhancement programs, mark-recapture protocols have not been conducted, leaving managers with little information about the released individuals after release. Therefore, it is necessary to carry out post-release evaluation based on mass marking and recapturing.

Schizothorax wangchiachii (Fang, 1936), which belongs to the subfamily Schizothoracinae, is distributed mainly in the upper Yangtze River and its tributary, the Yalong River (Yue, 2000). This species is adapted to torrential mountain rivers in the southeastern Qinghai-Tibetan Plateau (Yue, 2000). Before the 1990s, *S. wangchiachii* was caught abundantly in many parts of its distribution range. However, its recruitment has declined dramatically since the mid-1990s, likely due to habitat degradation, overfishing and hydropower development (Deng et al., 2000; Duan et al., 1995; Jiang et al., 2007). To improve the health of the *S. wangchiachii* population in the Yalong River, conservation plans, such as building fish hatcheries, have been initiated (Wang et al., 2011).

Jinping Hatchery (28°18′39.09″N, 101°38′50.10″E; Fig. 1) is the first and most important fish hatchery located in the lower Yalong River. The hatchery is used to domesticate and propagate *S. wangchiachii* and many other fish species that are threatened by hydropower development in the Yalong River. Since 2011, annual release of *S. wangchiachii* (total length 40–80 mm) from Jinping Hatchery has been carried out in the Jinping area of the Yalong River (Deng et al., 2016). The objectives of this study were to assess the feasibility of mass marking *S. wangchiachii* using ARS and subsequently to evaluate the effectiveness of stock enhancement by recapturing marked individuals of the 2015 cohort after release.

Materials and Methods

Experimental fish and fluorochrome

Juveniles of *S. wangchiachii* used for marking and stocking in this study were produced at Jinping

Hatchery using an artificial propagation technique. The breeding stocks were native spawners caught from the wild in the Jinping area of the Yalong River in 2011 and 2012. Juveniles were reared in numerous cylindrical tanks in the juvenile rearing room. These tanks are made of fiberglass, have a diameter of 2 m and a height of 1 m, and each has a temperature-controlled water supply (water temperature, 16 ± 1.50 °C; dissolved oxygen concetration, 7.0–7.4 mg L^{-1}; pH, 7.1–7.4) from the communal recirculating aquaculture system.

The fluorochrome ARS ($C_{14}H_7NaO_7S$) used for marking was analytically purified powder. During immersion marking, ARS was dissolved directly in the rearing water according to the experimental design. To optimize marking quality and minimize juvenile mortality, several preliminary experiments were conducted in 2013, and results showed that immersing juvenile *S. wangchiachii* in water containing ARS doses ≤ 100 mg L^{-1} for 24 h resulted in the lowest death rate while producing a mark that could be seen clearly in the otolith.

Small-scale marking pilot study

A small-scale marking pilot study was performed in the rearing tank. Approximately 7,000 40-day-old juvenile *S. wangchiachii* of the 2014 cohort (total length 23.52 ± 1.50 mm, mean ± S.D., n = 20) reared in a tank were selected for immersion marking on 5 May 2014. These juveniles were starved for 24 h prior to the treatment. The inner wall of the tank was carefully cleaned, and the rearing water was completely replaced with about 500 L of clean water. Thirty-five grams of ARS were pre-dissolved in about 10 L of water, which was immediately added to the tank. The juveniles were immersed in the ARS solution (70 mg L^{-1}) for 24 h. During immersion, the solution was aerated continuously and the fish were not fed. After immersion was completed, the ARS solution was discharged into a sewage pool, and at the same time clean water was pumped into the tank to thoroughly rinse out the remnant dye. One day after immersion, dead individuals were counted and removed from the tank. For mark checking, 10 marked fish from the tank were randomly sampled and sacrificed with an overdose of MS-222 (100 mg L^{-1}) on 29 May 2014, 28 December 2014, 4 May 2015 and 24 January 2016. Sampled fish were kept in 100% ethanol until otolith examination.

Large-scale marking application

From late April to early May 2015, five batches of juvenile *S. wangchiachii* (total length 20.85 ± 1.41 mm, mean ± S.D., n = 140) of the 2015 cohort were marked at Jinping Hatchery using the ARS immersion protocol described above. In total, an estimated 600,000 fish were marked. To ensure the safety of the very small juveniles during immersion marking, the concentrations of ARS solution were controlled within a range of 30–50 mg L^{-1}. One day after immersion was completed, dead fish in each tank were counted and recorded. The mortalities of three batches of unmarked fish in the rearing room also were recorded. Five days after marking, both marked and unmarked fish were transferred to four outdoor fishponds in the hatchery. The fish were fed to satiation three times a day with a commercial artificial compound diet.

To assess effects of the marking process on growth, 100 marked and unmarked *S. wangchiachii* at similar daily age were randomly taken from two fishponds on 24 July 2015. These fish were starved for 24 h prior to further treatment. Afterwards, these fish were anaesthetized with MS-222 at a concentration of 100 mg L^{-1}. Their total and body length was measured to the nearest 0.01 mm with a digital caliper, and their wet mass was weighed to the nearest 0.0001g with a precision electronic balance. To assess the mark effectiveness, 400 marked fish also were selected randomly from those in the four fishponds. They were anaesthetized with MS-222 at a concentration of 100 mg L^{-1}, and then stored in 100 % ethanol until otolith examination.

Release and recapture

On 24 July 2015, 840,000 young *S. wangchiachii* of the 2015 cohort, of which 400,000 individuals were marked by ARS, were released at sites 2 and 3 in the Jinping area of the Yalong River (Fig. 1). Site 2 (28°18′47.80″N, 101°38′51.19″E) is located in the wide and deep part of the river (> 5 m maximum depth). Although it was not a suitable habitat for *S. wangchiachii*, site 2 was used because of footsteps toward the river for purpose of release. Site 3 (28°19′41.32″N, 101°38′52.18″E) was near a sand quarry, and the substrate was covered with gravel and small stones: this was an appropriate area for young fish to inhabit. The distance between the two release sites was about 2 km. The fish were first captured from each fishpond with a nylon trawl, and 150 marked individuals were randomly selected for measuring and weighing. Afterwards, fish were transferred to the release sites by a transporter and released into the river using buckets. For comparison of growth, about 10,000 unmarked *S. wangchiachii* were raised as a control group in a fishpond in Jinping Hatchery. They were fed twice daily with commercial feed at a ratio of about 3% of body weight per day.

Before the recapture surveys were conducted, appropriate recapture sites along the Jinping area of the Yalong River were chosen. Criteria included ease of fishing, suitable habitats for young fish and the distances from the release sites. Seven sites, including the two release sites, along an about 60 km stretch of the Jinping area of the Yalong River ultimately were selected for the recapture surveys (Fig. 1). Site 1 (28°17′46.38″N, 101°38′39.19″E) was a shallow riffle area (< 0.5 m in depth) with a substrate of gravel and small stones located about 1.5 km upstream of site 2 and 7 km downstream of Jinping Dam Ⅱ. Site 1 was an important nursery ground for *Schizothorax* fish at early life stages. Site 4 (28°20′50.68″N, 101°39′18.04″E), site 5 (28°24′10.28″N, 101°43′24.86″E), site 6 (28°27′41.38″N, 101°44′49.65″E) and site 7 (28°36′58.04″N, 101°55′56.04″E) were located about 3, 15, 20 and 50 km downstream of site 3, respectively, and had a similar substrate of small stones and occasional boulders.

Recapture surveys were carried out at an interval of three months (in October 2015, January 2016 and April 2016) using roughly the same method and sampling effort each time. At each site, three 9-m long fishing pots with 6-mm mesh were used for recapturing fish for four successive days, and catches in each pot were removed once a day. The fishing pot used in these surveys was a trap-type stationary fishing device that was especially suitable for catching small fish with total length < 20 cm. Electrofishing permitted by the Sichuan Municipal Bureau of Aquatic Products was performed only one time at each site, and it involved using a 30-cm-diameter anode and a 6-mm mesh landing net to sample for 40 min along the river. Specimens of *S. wangchiachii* assumed to be from the 2015 cohort based on personal experience were sacrificed with an overdose of MS-222 and measured and weighed, whereas other fish were released. Meanwhile, 50 individuals sampled from the control group also were measured. All sampled fish were stored in 100 % ethanol for further processing.

Otolith removal and examination

Three pairs of otoliths were removed from all fish sampled, and the left three otoliths were mounted on glass slides using neutral balsam. To check the ARS mark and to read age, otoliths were observed under an Olympus BX40 fluorescence microscope fitted with a Q-Imaging MicroPublisher 5.0 RTV digital camera using the green laser and normal transmitted light (Yang et al., 2016).

Data analysis

In this study, instantaneous growth rate of mean total length (G_l) was calculated following Ricker (1975) as:

$$G_l = (\ln l_2 - \ln l_1)/3$$

where l_1 is the mean total length in millimeters of *S. wangchiachii* of at a given time point, l_2 is the corresponding mean total length three months later, and 3 is the sampling interval of three months.

Instantaneous growth rate of mean wet mass (G_w) was calculated as:

$$G_w = (\ln w_2 - \ln w_1)/3$$

Where w_1 is the mean wet mass in grams of *S. wangchiachii* at a given time, w_2 is the corresponding mean wet mass three months later, and 3 is the sampling interval for three months.

The proportion (P_i) of marked individuals out of all marked *S. wangchiachii* for each recapture survey was calculated as:

$$P_i = n_i/N_t$$

where n_i is the number of marked fish at site i of the given recapture date and N_t is the total number of marked fish of the given recapture.

The condition factor of *S. wangchiachii* was calculated following Fulton (1904) as:

$$\text{condition factor} = 10^6 \times w/l^3$$

where w is the wet mass in grams of *S. wangchiachii* at a given time point and l is the total length in millimeters of *S. wangchiachii* at the same time.

Statistical analysis for all data was performed with SPSS 19.0 software. The significance level was set as $P < 0.05$.

Results

Small-scale marking pilot study

One day after immersion was completed, no marked fish had died. Twenty-three days after marking, all sampled individuals showed a visible red-orange mark in their otoliths (Fig. 2a). Without polishing, visible marks also were easily identified in the otoliths of marked individuals sampled on 28 December 2014 (Fig. 2b), 4 May 2015 (Fig. 2c) and 24 January 2016 (Fig. 2d). More than one year after immersion, the ARS mark still remained visible, and there was no evidence that the mark was significantly decaying.

Large-scale marking application

In the large-scale immersion marking of juvenile *S. wangchiachii*, mortalities in each batch were very low (≤ 0.50%), and no significant difference in the mortality between marked and unmarked batches was detected (Table 1; independent t-test, $P = 0.836$). Three months after being reared in outdoor fishponds, samples of marked and unmarked individuals showed no significant difference in total length ($P = 0.936$), wet mass ($P = 0.629$) and condition factor ($P = 0.244$) (Table 1; independent t-test). A visible red-orange mark in the otoliths could be identified under green laser in all marked fish sampled.

Recapture and evaluation

Otolith checking confirmed that a total of 852 *S. wangchiachii* of the 2015 cohort were caught during the three recapture surveys. Of these fish, 262 individuals had a clear ARS mark in their otoliths, and 590 individuals had no mark (Fig. 3; Table 2). The proportions of marked individuals in each recapture survey were 32.73% in October 2015, 26.36% in January 2016 and 30.53% in April 2016, respectively.

In the October 2015 recapture effort, total length, wet mass and condition factor of both marked and unmarked *S. wangchiachii* were significantly lower than those of the control group (Table 2;

one-way-ANOVA with *post hoc* Games-Howell, $P < 0.001$). The condition factor of marked individuals at the time point when they were recaptured was also significantly lower than at the time point when they were released (Table 2; independent t-test, $P < 0.001$). Between marked and unmarked individuals, there was no significant difference in wet mass ($P = 0.134$) and condition factor ($P = 0.735$), whereas slight difference in total length was detected ($P = 0.030$). In addition, the total length, wet mass and condition factor differed significantly among samples caught from different recapture sites (Table 3; two-way-ANOVA test without the data for site 7, $P < 0.001$), but a significant difference was not observed between marked and unmarked individuals ($P > 0.05$). Recapture site and ARS mark had interaction effects on wet mass ($P = 0.006$).

In the January 2016 recapture effort, the total length and wet mass of both marked and unmarked *S. wangchiachii* were still significantly lower than thoes of the control group (Table 2; one-way-ANOVA with *post hoc* Games-Howell, $P < 0.001$), but the difference in condition factor was not significant ($P = 0.304$). Between marked and unmarked individuals, no significant differences in total length ($P = 0.828$) and wet mass ($P = 0.816$) were detected. The total length, wet mass and condition factor of *S. wangchiachii* differed significantly among samples caught from different recapture site (Table 3; two-way-ANOVA test without the data for sites 1 and 7, $P < 0.001$), but ARS mark and the interaction between it and recapture site did not have significant effects on the three indexes ($P > 0.05$).

In the April 2016 recapture effort, the control group was not sampled because of fishpond cleaning. There was no significant difference in total length, wet mass or condition factor between unmarked and marked *S. wangchiachii* (Table 2; independent t-test, $P > 0.05$). The total length, wet mass and condition factor of *S. wangchiachii* differed significantly among samples caught from different recapture sites (Table 3; two-way-ANOVA test, including only data for sites 3, 4 and 6, $P < 0.001$). A significant difference in condition factor (Table 3; $P = 0.009$) was observed between marked and unmarked individuals, but a significant difference was not observed for total length ($P = 0.181$) or wet mass ($P = 0.07$). The interaction effects of recapture site and ARS mark were not significant ($P > 0.05$).

The G_l and Gw of marked individuals tended to slowly increase after release. In the first trimester after release, the G_l and Gw values of marked individuals were 0.0566 and 0.1065, respectively, which were lower than those of the control group (0.1430 and 0.4964, respectively). In the second trimester, both G_l (0.0683) and Gw (0.2832) of marked individuals had increased slightly. At the time point, the Gw of marked individuals was slightly higher than that of the control group (0.2569), whereas the G_l of marked individuals was still lower than that of the control group (0.1080). In the third trimester, the G_l of marked individuals had increased to 0.0878, whereas the Gw (0.2815) remained almost the same.

After being released, the hatchery-produced *S. wangchiachii* began to move away from the release area. In October 2015, marked individuals were recaptured at all recapture sites upstream and downstream of the release sites (Table 2). Site 2 was not a suitable a nursery ground for *S. wangchiachii*, but P_2 (18.90%) was still a high proportion for this recapture (Fig. 4). In the subsequent recaptures, catches at site 2 were very small because of unsuitable habitat (Table 2). In the three surveys, mean P_3 (28.69 ± 1.03%) and P_4 (31.62 ± 25.98%) were much higher than that of P_5 (11.49 ± 7.80%), P_6 (8.58 ± 4.38%) and P_7 (4.17 ± 3.23%). P_i significantly decreased with distances from the release sites. P_i for the distant sites 6 and 7 in the latter two recaptures increased slightly compared to that in the first recapture, but the values were still much lower than those of sites 3–5 (Fig. 4). This implies that stocked fish were mainly distributed over a 10–15 km long stretch around the release sites.

Discussion

Feasibility of ARS mass marking

Previous studies of marking different fish species demonstrated that ARS treatment produces excellent mark quality and has no significant harmful effects on the fish body (Baer & Rösch, 2008; Bashey, 2004; Caraguel et al., 2014; Liu et al., 2009). However, faced with sustained pressure to produce enough fish seed to achieve the annual goals of release programs, managers of many hatcheries continue to worry that mass marking using the ARS method will cause high mortality, and this concern has negative impact on the use of marking to evaluate fish stock enhancement. In this study, marking juvenile *S. wangchiachii* (mean total length 23.52 ± 1.50 mm) by immersion in 70 mg L^{-1} ARS solution for 24 h did not cause death. In the following large-scale marking application, the mortality of five marked batches and three unmarked ones was negligible (≤ 0.50%), and no significant difference in mortality between marked and unmarked fish was detected. After rearing for three months in outdoor fishponds, no significant differences in total length, body mass, or condition factor between marked and unmarked groups were detected. Because juvenile *S. wangchiachii* experienced natural mortality, the extremely low mortalities that occurred during the immersion marking process might not have been due to ARS solution. In addition, immersion marking was carried out directly in the rearing tanks, which avoided manipulations of fish, reduced stress, and increased security.

The ARS mark that develops in the otolith remains highly readable for several years, whether fish are reared in the laboratory or in the field (Champigneulle & Cachera, 2003; Nagiec et al., 1995; Partridge et al., 2009; Poczyczyński et al., 2011). Because sunlight and turnover of skeletal calcium can cause fluorescent marks to fade, external fluorescent marks on scales and fin rays cannot be readily detected over time (Bashey, 2004; Elle et al., 2010). In contrast, otoliths are protected by the skull and previously deposited otolith materials are not resorbed (Campana & Neilson, 1985), which prolongs the lifetime of the mark. In the 2014 marking effort, *S. wangchiachii* marked with 70 mg L^{-1} ARS retained highly visible marks on otoliths after rearing for about 20 months in an indoor tank, and they did not present clear signs of significant fading. In the mass marking of 2015, fish marked with 30–50 mg L^{-1} ARS were transferred to outdoor fishponds and reared for about three months. Jinping Hatchery is located in the arid river valley region of the western Sichuan Province, where sunshine is very strong all year long (Yuan et al., 2013). Nevertheless, ARS marks of fish sampled from each marking batch were highly visible. After release, all ARS marks on the otoliths of fish recaptured were as clear as they were at release. However, because otoliths continuously grow and thicken, over time the mark can be covered by otolith materials, and marks can become faint and difficult to detect unless exposed by grinding and polishing the otoliths (Baer & Rösch 2008; Sánchez-Lamadrid, 2001; Taylor et al., 2005). In this study, although it was not experimentally tested, the final retention time of the ARS mark in the otoliths of *S. wangchiachii* was long enough to monitor the released individuals to evaluate stocking effectiveness.

Effectiveness of stocking enhancement

After release, trimonthly recapture surveys confirmed that some of the stocked *S. wangchiachii* had survived. Assuming that the proportions of marked (47.62%) and unmarked (52.38%) *S. wangchiachii* remained unchanged in the stocked cohort, the percentages of catches that originated from stock enhancement were estimated to be 68.73 % in October 2015, 55.35% in January 2016 and 64.11% in April 2016, respectively. This demonstrated that stocked *S. wangchiachii* constituted an important part of the young fish with a mean level of 62.73% recruitment of the 2015 cohort. In previous successful stock enhancements, such as those for vendace *Coregonus albula* (Poczyczyński et al., 2011), brown trout *Salmo trutta* (Caudron & Champigneulle, 2009), Japanese Spanish mackerel *Scomberomorus niphonius* (Obata et al., 2008), and red sea bream *Pagrus major* and Japanese flounder *Paralichthys olivaceus* (Kitada & Kishino, 2006), hatchery-produced fish contributed considerably to population recruitment, and stocking successes were often attributable to appropriate release sizes and environmental conditions at release sites. In this study, the relatively high proportions of released *S. wangchiachii* might be explained by the fact that young fish for release were fully covered with scales, which would protect the skin against mechanical injury and bacteria and parasites (Yan et al., 2014). In addition, the negligible fishing pressure on young *S. wangchiachii* under age 2 and few predators, such as *Percocypris pingi* and *Silurus asotu*s, might have had positive effects.

However, the comparative analysis of recapture data showed that total length, wet mass, and condition factor of recaptured *S. wangchiachii* differed significantly among different recapture sites (Table 3). The body sizes of fish recaptured at release sites were often "smaller" than those at other sites for both marked and unmarked fish (Table 2). This finding suggests that fish at release sites did not live as well as fish at other sites. Pebbly nursery grounds in shallow waters are essential for *Schizothorax* fish at early life stages. In the Jinping area of the Yalong River, water flow sharply decreases (at a maximum percentage of about 95%) due to the upstream dam of Jinping Dam Ⅱ, which leads to marked physical habitat degradation (Wang et al., 2007). Excavation of sand in the river, which take place frequently at five sites along the 60 km long survey area, further destroyed the habitats. This reduction of essential habitats could have a significant negative effect on the river's carrying capacity for *Schizothorax* fishes. It is likely that the released fish moved very slowly so that nine months after release most of them still were distributed over a 10–15 km long stretch near the release sites, although a few marked fish were caught about 50 km downstream three months after release. The relatively slow migration speed would maintain a high density of fish in the release area, which would result in both released and wild fish having to compete intensely with each other for resources.

Wild fish can be replaced with hatchery-reared fish when the latter are released in numbers that exceed the carrying capacity, but it is difficult to verify the extent to which they replace the wild ones (Kitada & Kishino, 2006). The surveys conducted in this study showed that, there are some spawning grounds for *Schizothorax* fishes in the Jinping area of the Yalong River, where some naturally born juveniles were caught in April 2014. This study showed that natural recruitment still accounted for a sizeable proportion (approximate 40%) of *S. wangchiachii* recruitment of the 2015 cohort. Additionally, the non-significant difference in total length, wet mass and condition factor between the marked and unmarked fish indicated that ARS marking did not have significant harmful effects. This finding suggests that the stocked *S. wangchiachii* should live at the similar degree as the naturally born fish in the Jinping area of the Yalong River. Releases at the scale used in this study might have not exceeded the spare carrying capacity of release sites or replaced wild fish, however, it might nearly saturate the habitat due to annually large scale release. To maintain the fish population at sustainable levels, new release sites around site 7 should be added (Fig. 1). Moreover, sand excavation in the river must be stopped immediately, and nursery habitats must be restored to expand the carrying capacity of the river.

Wild *S. wangchiachii* live in rapid flowing river water and consume adherent alga (Bacillariophyta) using their sharp outer horny sheath on the lower jaw to scrape it off the substrate.

In contrast, the hatchery-reared fish are reared in still water ponds and fed on commercial diets. It is well known that environmental differences between hatchery-reared and wild fish can influence their behavior, especially foraging behavior and avoidance of predators, which may subsequently affect post-release success (Hervas et al., 2010; Le Vay et al., 2007; Johnsson et al., 2014). On first release into the wild, hatchery-reared fish must not only avoid predators but also adapt to a new food supply (Blaxter, 2000). In this study, condition factors of the stocked *S. wangchiachii* recaptured three months after release were significantly worse than those of the control group as well as themselves at release (Table 2). This suggests that these hatchery-produced fish might need to be acclimatized to the wild habitat, as their growth was negatively affected by the change of environmental conditions. Perhaps, hatchery-reared fish suffered a high level of short-term post-release mortality during the first trimester after release. However, it was difficult to precisely estimate this mortality due to lack of historical data on fisheries and prior investigation in the area. In the subsequent recaptures, the condition factor of stocked fish had returned to a level as good as that of the control group. In addition, the G_l and Gw of marked fish displayed a slowly increasing trend (Tables 2 and 4). Therefore, survival of hatchery-produced *S. wangchiachii* suggests that they gradually adapted to the wild habitat, and they exhibited favorable growth six months after release.

Conclusions

This study offers fishery administrators a cost-efficient method of mass marking juvenile *S. wangchiachii* with ARS. The marking process did not cause significant mortality or affect fish growth in this study. Release-recapture surveys indicated that the present stock enhancement might make a considerable contribution to the recruitment of young *S. wangchiachii* in the Jinping area of the Yalong River. Results of this study will be instrumental in promoting application of mass marking techniques and applying responsible approaches to the development of stock enhancement in China. However, the present study presents the exploratory stage, and much information about stock enhancement remains unknown, including the post-release mortalities of stocked fish, their contribution to the spawning population, and their genetic impact on the wild population. Therefore, in order to improve stocking strategy and better protect *S. wangchiachii* and other fish species in the Yalong River, long-term monitoring and further studies of the released fish should be conducted.

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Table 1(on next page)

The mortalities of juvenile *S. wangchiachii* unmarked and marked by immersion in late April to early May 2015, and total length, wet mass and condition factor of these fish three months later.

Mortality (mean ± S.D.) of three batches of unmarked juvenile *S. wangchiachii* and five batches marked by immersion in 30–50 mg L^{-1} ARS solution for 24 h in late April to early May 2015, was very low and did not differ significantly (independent t-test, $P = 0.836$) between treatmens. After being reared for three months in outdoor fishponds, total length ($P = 0.936$), wet mass ($P = 0.629$) and condition factor ($P = 0.244$) (mean ± S.D.) of marked and unmarked *S. wangchiachii* did not differ significantly (independent t-test).

Samples	N	Acute mortality (% ± S.D.)	Total length (mm ± S.D.)	Wet mass (g ± S.D.)	Conditon factor (% ± S.D.)
Marked batches	5	0.16 ± 0.20			
Unmarked batches	3	0.19 ± 0.20			
Marked individuals	100		49.17 ± 9.76	1.1460 ± 0.6563	1.715 ± 0.175
Unmarked individuals	100		49.07 ± 7.16	1.1052 ± 0.5288	1.748 ± 0.212

1 N, number of samples

Table 2(on next page)

Mean total length, wet mass and condition factor of marked and unmarked *S. wangchiachii* of 2015 cohort obtained from initial release and recapture surveys in the Jinping area of the Yalong River.

Source	Marked fish				Unmarked fish			
	N.	Total length (mm)	Wet mass (g)	Condition factor	N	Total length (mm)	Wet mass (g)	Condition factor
Jul 2015								
Initial releasing	150	44.94 ± 6.67	0.8225 ± 03332	1.762 ± 0.373				
Control group					50	41.54 ± 4.91	0.6740 ± 0.2786	1.893 ± 0.762
Oct 2015								
Control group					50	63.79 ± 11.26	2.9886 ± 1.8327	1.995 ± 0.431
Site 1	45	52.21 ± 7.34	1.0341 ± 0.4257	1.430 ± 0.170	69	54.61 ± 6.72	1.1551 ± 0.4236	1.447 ± 0.193
Site 2	31	53.02 ± 5.61	1.0889 ± 0.4352	1.472 ± 0.115	51	57.27 ± 9.87	1.5174 ± 1.0875	1.518 ± 0.133
Site 3	48	54.03 ± 7.11	1.1733 ± 0.5013	1.405 ± 0.187	118	55.88 ± 6.96	1.2401 ± 0.4663	1.349 ± 0.151
Site 4	6	59.30 ± 6.51	1.5886 ± 0.6685	1.400 ± 0.075	20	58.85 ± 6.36	1.4423 ± 0.4303	1.392 ± 0.179
Site 5	27	51.04 ± 6.32	0.9882 ± 0.3255	1.482 ± 0.184	68	51.54 ± 5.61	1.0309 ± 0.3654	1.481 ± 0.195
Site 6	6	59.88 ± 13.19	1.8959 ± 1.3197	1.511 ± 0.158	9	52.46 ± 5.83	1.1436 ± 0.3253	1.570 ± 0.153
Site 7	1	55.36	1.4478	1.835	2	53.58 ± 3.76	1.3499 ± 0.3667	1.618 ± 0.047
Total recaptures	164	53.26 ± 7.23	1.1320 ± 0.5263	1.443 ± 0.169	337	55.03 ± 7.38	1.2325 ± 0.5939	1.431 ± 0.182

Jan 2016								
Control group					50	88.20 ± 7.77	6.4593 ± 1.5860	1.770 ± 0.261
Site 1	–				–			
Site 2	0				2	67.13 ± 17.12	2.2926 ± 1.7039	1.604 ± 0.265
Site 3	17	53.57 ± 8.82	1.3193 ± 0.7693	1.648 ± 0.186	36	56.87 ± 7.22	1.5691 ± 0.7476	1.676 ± 0.157
Site 4	21	67.51 ± 15.57	2.9274 ± 2.1312	1.622 ± 0.212	77	67.70 ± 14.15	2.9843 ± 2.1267	1.705 ± 0.150
Site 5	9	70.02 ± 8.99	3.1357 ± 1.2962	1.867 ± 0.145	17	74.55 ± 9.04	4.1380 ± 1.6331	1.989 ± 0.245
Site 6	7	75.37 ± 9.24	3.5619 ± 1.4448	1.749 ± 0.118	18	69.63 ± 8.20	2.9381 ± 0.9454	1.823 ± 0.199
Site 7	4	76.28 ± 3.93	4.1186 ± 0.7443	1.996 ± 0.209	12	72.64 ± 9.62	3.4644 ± 1.6936	1.814 ± 0.162
Total recaptures	58	65.37 ± 13.98	2.6471 ± 1.7583	1.709 ± 0.214	162	66.59 ± 12.70	2.8128 ± 1.8472	1.748 ± 0.194
Apr 2016								
Site 1	–				–			
Site 2	0				4	65.62 ± 12.48	2.7153 ± 1.7478	1.688 ± 0.104
Site 3	11	75.28 ± 10.18	3.8435 ± 1.4643	1.553 ± 0.094	20	77.58 ± 14.38	4.1291 ± 3.2624	1.450 ± 0.173
Site 4	22	85.22 ± 17.52	6.3145 ± 4.1856	1.653 ± 0.125	45	79.16 ± 9.76	4.5388 ± 2.0997	1.628 ± 0.116
Site 5	1	96.71	9.1327	1.934	11	99.50 ± 15.42	10.1536 ± 4.5667	1.805 ± 0.143
Site 6	4	104.02 ± 5.54	10.2389 ±	1.764 ± 0.126	11	94.99 ± 13.61	7.7649 ±	1.627 ± 0.164

			1.4235				3.4934	
Site 7	2	93.32 ± 13.11	7.5525 ± 3.2873	1.671 ± 0.076	0			
Total recaptures	40	85.06 ± 16.42	6.1598 ± 3.7384	1.644 ±0.134	91	82.59 ± 14.84	5.4373 ± 3.5597	1.613 ± 0.171

–, no fish were recaptured because we were unable to get to the site

N, number of fish

Table 3 (on next page)

The results of Two-way ANOVA test on total length, wet mass and condition factor of *S. wangchiachii* of 2015 cohort recaptured in the Jinping area of the Yalong River.

Source	Dependent variable	SS	*df*	*F*	*P*
Oct 2015					
Recapture site	Total length	1362.827	5	272.565	< 0.001
	Wet mass	8.040	5	1.608	< 0.001
	Condition factor	1.091	5	0.218	< 0.001
ARS mark	Total length	1.948	1	1.948	0.844
	Wet mass	0.086	1	0.086	0.596
	Condition factor	0.005	1	0.005	0.683
R × A	Total length	487.183	5	97.437	0.088
	Wet mass	5.087	5	1.017	0.006
	Condition factor	0.168	5	0.034	0.316
Error	Total length	24574.918	486		
	Wet mass	149.255	486		
	Condition factor	13.776	486		
Jan 2016					
Recapture site	Total length	8178.913	4	15.296	< 0.001
	Wet mass	118.057	4	10.572	< 0.001
	Condition factor	1.860	4	15.275	< 0.001
ARS Mark	Total length	2.128	1	0.016	0.900
	Wet mass	0.001	1	< 0.001	0.984
	Condition factor	0.019	1	0.612	0.435
R × A	Total length	433.993	4	0.812	0.519
	Wet mass	9.423	4	0.844	0.499
	Condition factor	0.218	4	1.792	0.132
Error	Total length	27805.420	208		
	Wet mass	580.665	208		
	Condition factor	6.331	208		
Apr 2016					
Recapture site	Total length	4442.714	2	13.549	< 0.001
	Wet mass	208.859	2	12.230	< 0.001
	Condition factor	0.476	2	13.454	< 0.001
ARS Mark	Total length	297.492	1	1.815	0.181
	Wet mass	28.595	1	3.349	0.070
	Condition factor	0.126	1	7.134	0.009
R × A	Total length	418.750	2	1.277	0.283
	Wet mass	25.205	2	1.476	0.233
	Condition factor	0.049	2	1.388	0.254

Error	Total length	17542.681	107
	Wet mass	913.667	107
	Condition factor	1.895	107

Figure 1

Map of the Jinping area of the Yalong River showing the locations of Jinping Hatchery and the sites where the stocked *S. wangchiachii* were released and recaptured.

Fish were released at sites 2 and 3 in July 2015, recaptures were conducted at sites 1–7 from October 2015 to April 2016.

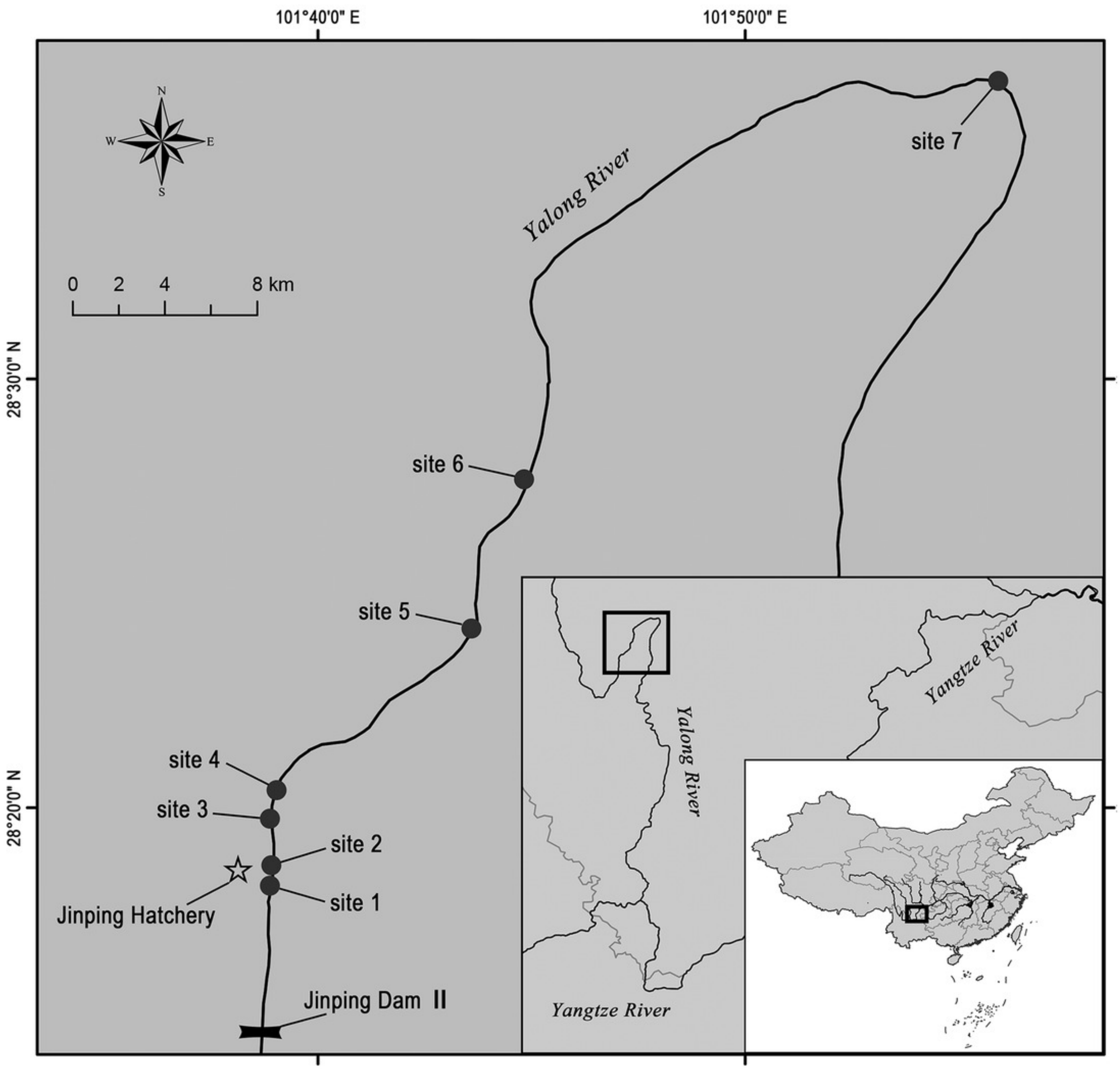

Figure 2

Photographs of lapillus otoliths of ARS marked *S. wangchiachii* of the 2014 cohort sampled on 29 May 2014 (a), 28 December 2014 (b), 4 May 2015 (c) and 24 January 2016 (d).

These fish were marked through immersing in 70 mg L^{-1} ARS for 24 h on 5 May 2014. Photographs were taken under green laser and × 40 magnification. White arrows showed the ARS marks.

**Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.*

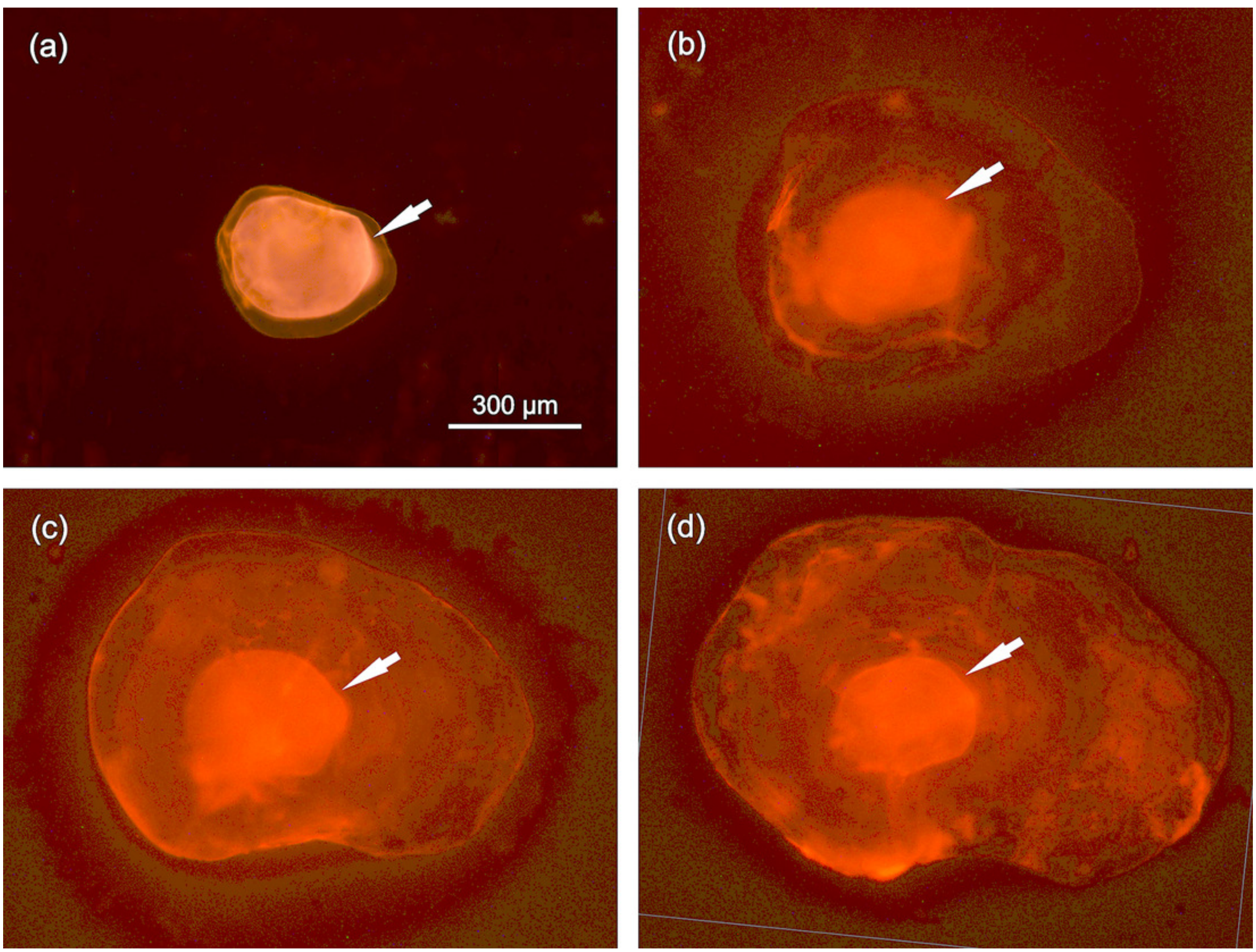

Figure 3

Photographs of ARS marked (a) and unmarked (b) lapillus otolith of *S. wangchiachii* of the 2015 cohort recaptured in the Jinping area of the Yalong River.

Photographs were taken under green laser and ×40 magnification. White arrows showed the ARS marks.

**Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.*

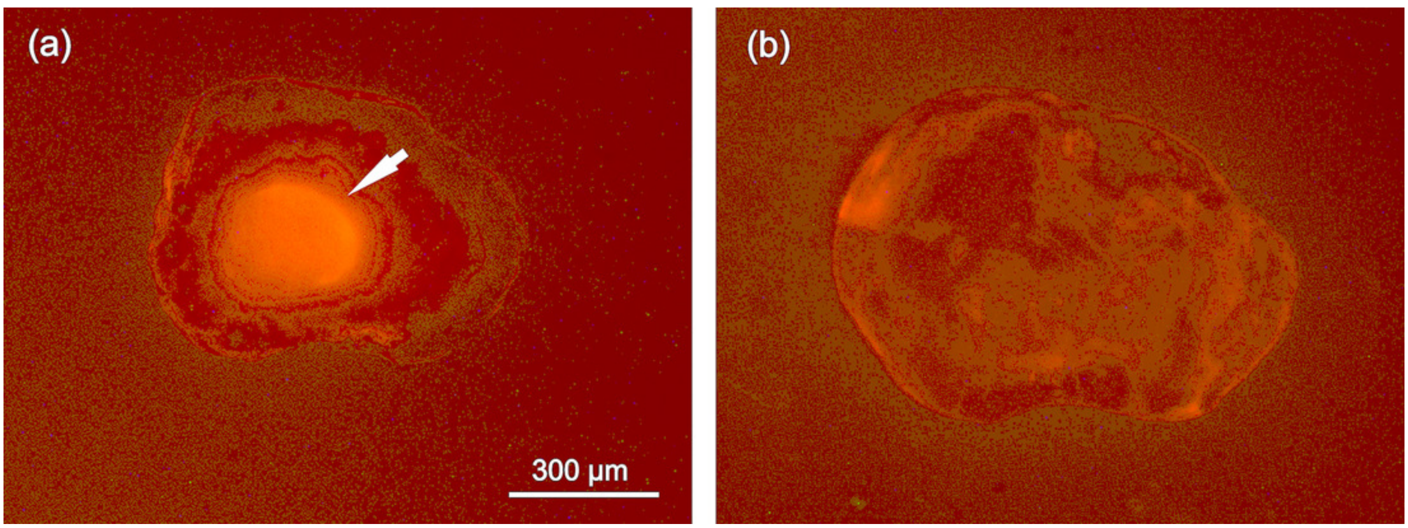

Figure 4

Proportions of marked individuals of each site in all marked *S. wangchiachii* for three recapture surveys.

(×, no recapture survey was conducted; *, the proportion was 0%).