The determination of high retention flag marking methods with yellowfin bream (*Acanthopagrus latus*) fry

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Research Article

Keywords: Mass marking methods, Mark retention rate, Survival rate, Yellowfin bream, Acanthopagrus latus

Posted Date: August 9th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-762950/v1

Abstract

With the decline in coastal fishery resources, hatchery-reared fry of yellowfin bream (*Acanthopagrus latus*) have been commonly mass released to the surrounding waters in China in recent years. Although the release plan has been underway in China for many years, the releasing effects were not often assessed. Therefore, it is necessary to study several suitable mass marking methods that have a high mark retention rate for fry. From October to November 2020, tetracycline was immersed to mark calcified hard tissue (MI), with tagging cut tail fin (TC) and the control, and the species of yellowfin bream by live fish were examined. Moreover, a double marking method, i.e., mark calcified hard tissue (MI) after tag fish for two weeks, was examined to determine any increase in the mark retention rate for yellowfin bream in November 2020. The dual marking and tagging method combining MI and TC is effective and is suitable for yellowfin bream. Both MI of 0.25% treatment and MI of 0.5% treatment also have lower costs, and more individuals can be marked.

Introduction

The decrease in global marine fish populations has rekindled interest in marine fish enhancement. The latest technological advances in fish marking and marine fish farming are successful hatchery-based marine improvements and are the main matters in sea ranching[1,2]. Among them, the population size, distribution, movement, and demographic parameters of a wild population were usually obtained by marked and recaptured fry[3]. Therefore, the mark retention rate of sample fish was the key factor for the marking method[4]. Since the 1950s in China, hatchery-reared fry have usually been released to enhance natural fisheries stocks[5]. The total number of fish released from cultivated fisheries in China may be the largest in the world. The establishment of an accurate assessment method of the resource benefits of fishery release is also the attitude of fulfilling social responsibility, which is gradually being valued. However, the release effect of fry has rarely been evaluated because the size of legally released fry was approximately 5 cm, which were too small to be easily marked separately in the past. Moreover, several tens of thousands to hundreds of thousands of released fry are too many for marking, which is not easy to complete in a short time. In addition, migratory fish are not easily captured and recovered[6].

Techniques exist for specific individual markings, for example, fin clipping[7], branding[8,9], tattooing[10], and coded tags[11]. Fluorescent labeling is a practical and usable marking technique that can be used for large-scale marking of fish raised in hatcheries. To date, more than 200 fish species have been successfully marked by this marking technique[12]. In addition, some studies can improve the mark retention and recovery rate of sample fish by the double marking method[13,14]. However, there are still few studies of fish using the double marking method. These techniques are usually expensive, labor intensive and may damage fish bodies due to handling[15]. Other methods, for example, immersing the fish body into a water solution containing comestible pigment to dye the body surface[16] or tetracycline to mark calcified hard tissue[17], feeding fish mash containing tetracycline to mark calcified hard tissue[18], and spraying a harmless pigment with fluoresce to mark skin[19], may be suitable for mass marking of fry.

The mortality of fish in their early life stage in a natural environment usually influences the year-class strength of the adult population[20,21]. For some species, such as chum salmon (*Oncorhynchus keta*), coho salmon (*Oncorhynchus kisutch*), and pink salmon (*Oncorhynchus gorbuscha*), the mortality was highest in the initial days after being released[22,23,24,25] and depended on whether people adopted suitable marking methods.

The ordinary trade fry size among aquaculture farmers is approximately 5 cm for many species in China. Therefore, the economic fish size was adopted as the legal size released in recent years by the government. This was despite not having much assessment of the releasing programs in process for many years in China. Therefore, it is necessary to find several suitable mass fry marking methods with a high mark retention rate, time saving operations, stress avoidance due to handling, low expense, and a labor-saving procedures to assess the effects of the release programs.

Yellowfin bream are a warm water bottom fish that live in shallow waters. They commonly inhabit coastal waters and estuaries and prefer rocky areas. Generally, yellowfin bream do not travel long distances[26]. They have strong adaptability, as they can live in a water temperature of 4–35°C and adapt to rapid changes in salinity, living normally in freshwater, brackish water and sea water[27]. Yellowfin bream are widely distributed in the Red Sea, the Arabian Sea and the waters in and around India, Indonesia, North Korea, Japan, the Philippines, and the southeastern coast of China[26]. Yellowfin bream are considered a marine fish species of economic importance and the most important species for stock enhancement on the coast of Fujian Province in China[26,28,29,30]. Generally, *A. latus* attain maturity ca. 24.5 cm, and their maximum body length and weight can reach 35.0 cm and 350 g[31,32,33].

Materials And Methods

Over the past 20 years, *A. latus* have been one of approximately twenty coastal demersal hatchery-reared species commonly released into the Chinese coastal waters[34]. To study the mark retention rates and survival rates of three marking treatments, fish bodies were immersed in a water solution containing tetracycline to mark calcified hard tissue, and the control samples were examined from October to November 2020. In addition, a double marking method, i.e., spraying harmless pigment with fluorescence to mark skin and tagging cut tail fin (TC), and marking hard tissue by immersing (MI) for two weeks, was examined to see if it could increase the mark retention rate for yellowfin bream by live fish in November 2020.

All the fry in this study came from an aquaculture hatchery in southern China. The initial mean length of MI was 5.8 ± 0.8 cm, that of TC was 5.7 ± 0.7 cm, and that of the control was 5.8 ± 1.2 cm for yellowfin bream. The initial mean length of the double marking treatment was 6.2 ± 1.3 cm, and that of the control was 6.2 ± 1.0 cm for yellowfin bream. The initial mean weight of MI was 2.3 ± 0.3 g, that of TC was 2.1 ± 0.7 g, and that of the control was 2.1 ± 0.1 g for yellowfin bream. The initial mean weight of the double marking was 2.5 ± 0.4g, and that of the control was 2.4 ± 0.3 (Table 1).

Table 1
Initial mean length (cm) and mean weight (g) or fry for yellowfin bream.

Treatment	yellowfin bream	
	Length	Weight
TC	5.7 ± 0.7	2.1 ± 0.7
MI	5.8 ± 0.8	2.3 ± 0.3
Control	5.8 ± 1.2	2.1 ± 0.1
Double marking	6.2 ± 1.3	2.5 ± 0.4
Control	6.2 ± 1.0	2.4 ± 0.3
MI: Mark hard tissue by immersing.		
TC: tagging cut tail fin.		
Double marking:		

The pigment used in MI was lipid soluble, blue in color and comestible. The fry were immersed different treatments 0.2, 0.25, 0.5, 0.75% pigment solution for 180 minutes in a plastic container[16] to let the pigment adhere to the body surface. The body surface pigment can be examined by the naked eye.

The agent used in MI is an antibiotic allowed in aquaculture in China[35] and is often used in bone-tissue markers[17]. The fry was immersed in a 0.5% tetracycline solution for 120 minutes for yellowfin bream and 30 minutes for yellowfin bream in a plastic container[17] to let the tetracycline combine with calcium-containing tissues, e.g., vertebrae, scales, spines, and otoliths. The marks can be examined in a darkened room with UV light.

In the double marking treatment, yellowfin bream fry were cut part of tail fin and immediately immersed in 0.5 g of tetracycline per liter[18]. The yellow-green fluorescence marks on hard tissue can also be examined in a darkened room with UV light. Each experiment had a control group. Each treatment had 2 replicates, each with 50 individuals. All specimens were kept in a rectangular concrete pond (10 m × 2.5 m × 150 cm) in an aquaculture hatchery located in Zhangzhou, southern Xiamen City. The specimens of each treatment, which were separated into round shaped nylon floating frames with a 50 cm radius, were fed two times a day at room temperature with running water and sufficient air pumped in. The water temperature, salinity, dissolved oxygen, and pH values were measured each time.

The mark retention rates and survival rates of each treatment were calculated for two weeks every three days for yellowfin bream, every two days for yellowfin bream and every three days for yellowfin bream in the double marking experiment.

The survival rate (S_i) and mark retention rate (R_i) were defined as follows.

$$S_i\ (\%) = n_i/n \times 100 \ldots\ldots (1)$$

$$R_i\ (\%) = n_i^{*}/ni \times 100 \ldots\ldots (2)$$

where i is the sampling time every two or three days, n_i is the number of living fries on the i-th day, n is the initial number of fries, and $n_i{}^*$ is the number of living fries with marks on the i-th day.

All treatments were compared by one-way ANOVA and by Duncan's new multiple range tests for pairwise treatment means ($\alpha = 0.05$).

Results And Discussion

Dead fries were observed for yellowfin bream among the three treatments and the control on the 3rd day; thereafter, the survival rates of all treatments gradually decreased with time. The survival rates of MI, TC and the control group were not significantly different in the whole period ($p < 0.05$), and the two marking methods did not raise the death rate compared with the control. However, the survival rate of MI was lower than that of the above three treatments throughout the whole period ($p < 0.05$) (Fig. 1).

The mark retention rate of marking skin by spraying fluorescent pigment (MS) can reach above 80% in salmon[19], while it is under 60% in this study, which perhaps can be accounted for by inadequate spray gun air pressure[19,36]. But this method is not friendly to fish, therefore, this study decided not to use this method. The ineffective MI in this experiment may come from many unsuitable processes, such as solution concentration, immersion time, or the dye used. Similar low mark retention rates were observed in earlier studies[16,37]. Most likely, the TC is unsuitable for mass Tagging fry. Although the survival rate was the lowest, the mark retention rate was the highest among all treatments in this study. This study suggested that the immersion time of MI may be so long that the physiological function of the fry may be damaged[38,39]. In other words, MI can be a good and suitable marking method under a shorter immersion time for yellowfin bream.

For yellowfin bream, the survival rates of all treatments and the control were above 80%, except for MI of 0.2% treatment and the control on the 14th day of the whole period. The survival rates of MI of 0.2% treatment, 0.25% and the control were not significantly different except MI between the 2nd and 12th days ($p > 0.05$), so the two marking methods did not increase the death rate compared with the control. That of MI of 0.75% treatment was the highest, and TC was the lowest on the MI of 0.2% treatment 14th day ($p < 0.05$) (Fig. 2).

The mark retention rate of MI of 0.75% treatment was the highest, MI of 0.5% treatment was next, and both MI of 0.2% treatment and the control were lowest in the entire period ($p < 0.05$). Among them, MI of 0.25% treatment and 0.2% were not significantly different from the 2nd to 6th days ($p > 0.05$), while the former was higher than the latter between the 8th and 14th days ($p < 0.05$).

The high mark retention rates of MI of 0.75% treatment and 0.5% (above 80%) indicate that both methods were effective for the entire period. The medium initial mark retention rate of MI of 0.75% treatment, which survival rates dropped to near 0% during the experimental period, indicates that the method was unsuitable for mass marking even though it is inexpensive and easy to complete[16] (Fig. 2).

The survival rates of MI were above 95% and higher than those of the other treatments and the control throughout the whole period ($p < 0.05$). This study suggests that tetracycline is an antibiotic that could enhance the fish resistance and increase survival rates.

The mark retention rate of TC was the highest among all the treatments and stayed at almost 100.0% in the whole period, that of MI was quickly down to 0% from the 3rd to the 15th day (Fig. 3). It is obvious that the marking method of TC was effective, and MI could be used but not well enough.

The mark retention rate of MS is above 80% and similar to earlier studies of salmon[19,18]. This similarity of high mark retention rates could come from yellowfin bream, which, like salmon, belong to small-scale species that are under the same spray gun air pressure. The survival rate of MI of 0.75% treatment was the lowest among the three treatments and the control in the whole period ($p < 0.05$). This study suggests that the immersion time may be so long that the physiological function of the fry may be impeded or influenced by yellowfin bream. The low mark retention rate of MD is similar to earlier studies[16,37]. Most likely, MI of 0.75% treatment is unsuitable for mass marking of fry in this study.

No dead fries were observed in either double marking or the control from the 1st to the 7th day ($p > 0.05$); thereafter, the survival rates of double marking were kept above 80% and higher than that of the control on the 9th and 15th days ($p < 0.05$) (Fig. 3). This study concludes that double marking by combining MI and TC would not increase the death rate of the samples compared with the control. The reason may come from the use of tetracycline, which can enhance the fry resistance.

Both tag and mark retention rates of TC and MI were both 100%, while the marker retention rate of the control period was 0% (Fig. 3). The TC and MI markers of all fry can be detected at the same time. In other words, the double marking method combining TC and MI is effective and is suitable for yellowfin bream fry.

All the MI of 0.2% treatment, 0.25%, and 0.5% survival rates of yellowfin bream were higher. The reasons can be attributed to improved techniques, including the immersion time of MI. The TC, and MI survival rates of yellowfin bream is relatively high. The reasons can be attributed to technical improvements, including the reduction of the MI soaking time from 120 minutes to 30 minutes. The conclusion of this study is that the soaking time of MI may have an optimal value; otherwise, too short a time will result in low mark retention of fry, and too long will affect the osmotic adjustment of fry[38].

The retention of almost 100% of MI markers in yellowfin bream indicates that this method is suitable for fry. The double marking method applied to yellowfin bream in this study includes two fluorescent marking and tagging methods, which can be applied to fry population marking at the same time. In other words, the fluorescent dual marking method was used for the first time in fry marking research. In the future, when marked fry are released to increase fishery resources, even if the marking is lost, they can still be found and restored. In addition, the loss rate of each mark can be estimated[4,40].

Conclusion

The cost is approximately US$ 0.07 for TC, US$ 0.002 for MI of 0.75% treatment, US$ 0.007 for MI of 0.5% treatment, and US$ 0.00005 for MI of 0.2% treatment per fry. The 60 samples were used in MC in approximately 3 hours, so the average marking time was approximately 0.3 samples per minute. The MI of 60 samples lasted approximately 2 hours, so the average marking time was approximately 0.5 samples per minute. TC took approximately 20 minutes for 30 samples, so the average marking time was approximately 350–360 samples per minute. Among them, the cost of TC and MI is lower, and there are more individual fry that can be relatively marked.

All of the aforementioned marking methods focused on the legally released fry size of 5 cm, and other fry sizes were not considered in this study. Furthermore, the high mortality of marked fry after release usually occurs in the initial stage[22,25], which is why this study focused on only a two-week period. In the future, appropriate marking methods for various species that are generally released into the waters surrounding China should be studied.

Declarations

Acknowledgements

The study was conducted within the framework of Evaluation of the release effect of yellowfin bream in Xiamen Bay (No. S20166) for Institute of Ocean and Fisheries in Xiamen. We are grateful for the assistance of the Da-Sheng Aquaculture Hatchery.

Funding information

This research was partially supported by Institute of Ocean and Fisheries in Xiamen.

Competing interests The authors declare no competing interests.

Ethics declarations

Ethical approval

All applicable national and institutional (Faculty Ethical Research Committee No 28 JIMEI 01/01/2020) guidelines for the care and use of animals were followed by the authors.

Consent to participate

All the people involved in this work gave their consent to participate.

Consent for publication

All the people mentioned in this paper gave their consent to be co-author.

Competing interests

The authors declare no competing interests.

Code availability

Not applicable.

Availability of data and material

Data are available upon request to the corresponding author.

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Writing – review & editing: P.C.C., C.H.S. and L.M.H.

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Figures

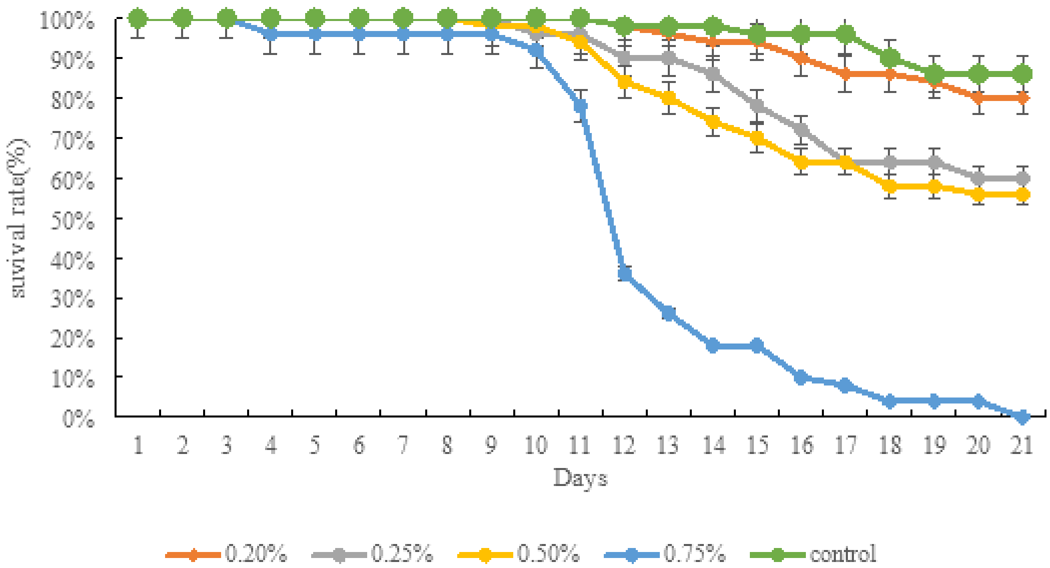

Figure 1

Survival rates (%) of marking body surface by different treatment and control for Acanthopagrus latus.

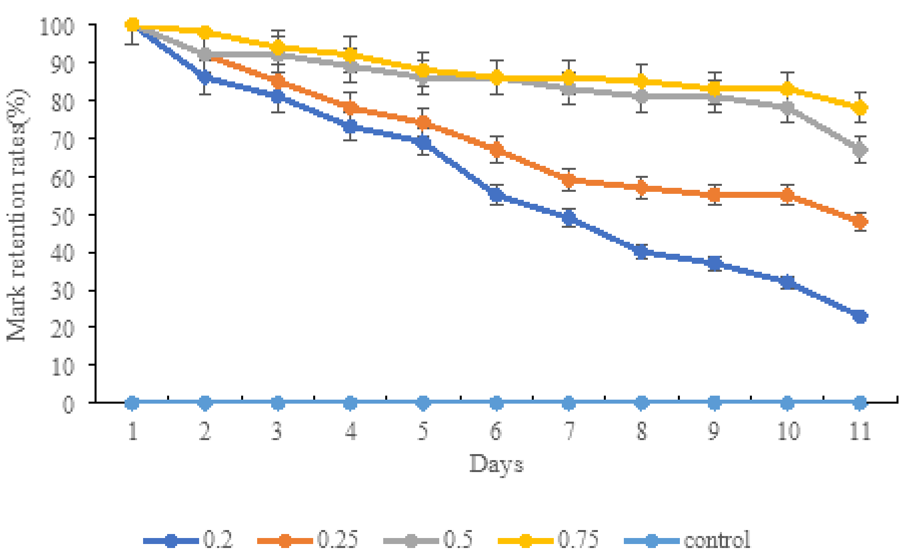

Figure 2

Mark retention rates (%) for marking different treatment and control for Acanthopagrus latus.

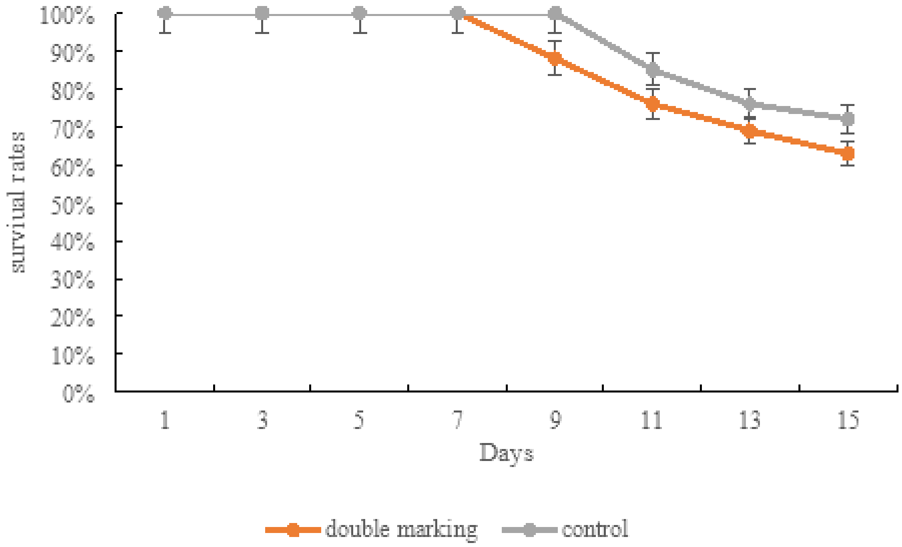

Figure 3

Double marking yellowfin bream fry were cut part of tail fin (TC) and immediately immersed in 0.5 g of tetracycline per liter (MI) and the control for yellowfin bream.