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Full Length Research Paper

The implement of plastic oval tags for mark-recapture in juvenile Japanese flounder (*Paralichthys olivaceus*) on the northeast coast of Shandong Province, China

Hongjian Lv[1], Xiumei Zhang[1]*, Peidong Zhang[1], Wentao Li[1] and Zhenqing Miao[2]

[1]College of Fisheries, Ocean University of China, Qingdao, 266003, PR China.
[2]Fishery College, Zhejiang Ocean University, Zhoushan, 316004, PR China.

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As part of the stock enhancement research project of Shandong Province, China, plastic oval tags (POTs) were used to mark juvenile Japanese flounder for release, *Paralichthys olivaceus* (70 to 133 mm total length, TL), in 2009 and 2010. Optimal tag placement locations, retention, tagging rates, and mortality were initially evaluated. Mark–recapture experiments were carried out in the coastal waters of Weihai City to study their migratory movements: 21,202 individuals in July 2009 at Beihai and 18,350 individuals in July 2010 at Lidao. The number of recaptured individuals were 434 (2.05% recapture rate) in 2009 and 620 (3.38% recapture rate) in 2010. A radiative movement from the release site was observed in the 2009 experiment; however, the tagging experiment showed a predominantly northward dispersal of tagged flounder from the release site in 2010. The mean movement speed of the released fish was calculated as 0.46 km day^{-1} in 2009 and 1.05 km day^{-1} in 2010. Furthermore, in 2009, the average TL and wet mass increments were 36.3 ± 8.4 mm month^{-1} and 27.13 ± 16.09 g month^{-1}, respectively 1 to 6 months after releasing; however, the increments were 14.7 ± 8.8 mm month^{-1} and 5.65 ± 4.17 g month^{-1}, respectively in 2010.

Key words: *Paralichthys olivaceus*, plastic oval tag, mark–recapture, movement, growth.

INTRODUCTION

The Japanese flounder (*Paralichthys olivaceus*) is one of the most important commercial species along the coastal areas of China, southwest of the Korean peninsula, and Japan (Zhu et al., 1963; Cheng and Zheng, 1987; Meng et al., 1995). In recent years, the spawning populations of mature flounder have significantly declined due to over exploitation (Liu et al., 2009). Relying solely on the recovery of natural stocks, the decline has been irreparable. Hatchery-produced juvenile release programs have been conducted in almost all coastal areas around Shandong Province since 1996 to restore wild Japanese flounder resources (Liu et al., 2009). In 2010 alone, approximately 13 million flounder juveniles were released into Shandong inshore waters, and the number has increased every year increased every year. The success of this mass-release program for stock enhancement has yet to be evaluated. Mark-recapture programs are required to assess the effectiveness of the stock enhancement strategies for fisheries management (Brown et al., 2002; Taylor et al., 2005; Baer and RÖsch, 2008).

Since the late 1980s, a variety of tag types have been developed for marking fish, including genetic tags (Perez-Enriquez and Taniguchi, 1999; Saillant et al., 2004; Taniguchi, 2004), chemical and thermal markers (Smoker et al., 2000; Jenkins et al., 2004), internal tags (Davis et al., 2004; Brennan et al., 2005), electronic archival tags (Prentice et al., 1990), and external tags (Stoettrup et al., 2002). Genetic tags and electronic archival tags are not economically suitable for recapture processes because of their high operating costs. The implement of internal tags and chemical marks is not optimal, considering the metamorphosis during the early stage of development and the depressiform body structure of the flounder (Liu et al., 2009). Thermal marks have been widely used for marking hatchery-produced salmon (Hagen et al., 1995;

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*Corresponding author. E-mail: xiumei1227@163.com. Tel: +86 0532 82032076. Fax: +86-0532-82032076.

Abbreviations: POT / POTs, Plastic oval tag/tags.

Volk et al., 1999) because of their lengthy egg incubation period. However, flounders could not be thermally marked because of their relatively short incubation period. Therefore, external tags are the most suitable tools for evaluating large-scale stocks of small juveniles based on ease attachment, cost, and longevity (Brown et al., 2002; Simon and DÖrner, 2005; Baer and RÖsch, 2008). Typically, stock enhancement programs require the application of tags onto many juvenile fishes with minimal harm, yet have high information content and low associated costs (Blankenship and Leber, 1995). The movement and growth of tagged flounder were also discussed. Although, the individual handling of POTs may involve substantial effort, they are external, comparatively benign, and can be used in juvenile fish. POTs are typically clearly visible and have relatively long-term retention rates. Thus, POT-tagged flounders can be quickly identified in catches. Visible external tags with various color and body implant locations can also be used for external identification (Buckley et al., 1994; Frederick, 1997; Willis and Babcock, 1998; Curtis, 2006).

We investigated the application of plastic oval tags (POTs) as external markers for stock enhancement of juvenile Japanese flounder in the inshore waters of Weihai City on the Northeastern coast of Shandong Province by mark–recapture. In the current study, POTs were chosen for external tagging in the experimental treatments, and a series of laboratory-based and field experiments through fishery-dependent and -independent recaptures were conducted to accomplish the following objectives: (1) To develop an efficient tagging system for flounder using POTs that minimize mortality and to streamline the tagging process. (2) To identify optimal tag implant locations for POTs in juvenile flounder, with the highest long-term retention rates and the best underwater visibility. (3) To determine the geographic dispersal of released fish, as well as to analyze the environmental factors affecting their movement and growth.

MATERIALS AND METHODS

Experimental fish

All juvenile Japanese flounder (70 to133 mm total length (TL)) used in this study (both in 2009 and 2010 experiment) were hatchery-reared from a commercial hatchery company located at Beihai of Weihai City. The juveniles were kept in 10 m^3 recirculation tanks (0.45 individuals l^{-1}), and fed twice daily with commercial pellets (Shengsuo, Shandong, China). Water quality was constantly monitored and controlled throughout the rearing and tagging periods, as well as during the recovery period after tagging (temperature, 18 to 20℃; salinity, 31.0 ± 1.0; dissolved oxygen (DO), 5.23 to 5.35 mg l^{-1}; pH, 7.7 to 8.1; photoperiod, 12 L/12 D). Water was changed at a daily rate of 20% of the tank volume.

Plastic oval tags

In the current investigation, one type of POTs (0.1 mm × 5 mm × 10 mm, 0.01 g) was used as an external tag to mark flounder. Two POT colors were used to distinguish the two populations released into different inshore waters and in different years (Figure 1A and B). The green POTs (Figure 1A) were used in the 2009 release experiment at the release site in the inshore waters of Beihai at Weihai City; the yellow POTs (Figure 1B) were used in 2010 at the release site in the inshore waters of Lidao at Weihai City (Table 1). One side of the POTs was marked with "Awarded by Ocean University", and the other side was marked with the phone number of the Fish Behavioural Ecology Laboratory. The POTs were attached using Mark 003 tagging guns (Kingmu Marine Technology, Japan Kingmu Co., Ltd) (Figure 1D). The tagging guns consisted of plastic anchors (10 mm length, 0.02 g weight) (Figure 1C), which were used to attach the POTs to the body of juveniles. The tagging guns were used to insert plastic anchors anterior of the caudal peduncle (through the 1.5 mm diameter hole on the POTs), and the tags were attached on the ocular side of each juvenile Japanese flounder.

General tagging procedure

The Japanese flounder juveniles were starved for 24 h before tagging. They were then harvested in small batches from the source tanks and transported to a second holding tub (about 150 to 200 juveniles per tub) with anesthetized water (80 ppm methane tricane sulfonate; MS-222) and anesthetized for 1 to 3 min. The number of anaesthetized fish was recorded for each source tank and the juveniles were tagged with POTs as previously described. The juveniles were then placed in fresh seawater with 100 ppm of benzylpenicillin sodium for 2 h. After tagging, the juveniles were equally transferred into four separate smooth cement tanks (15 m^3 volume, 0.3 individuals l^{-1}), and reared normally for 3 days. Finally, the tagged juveniles were starved for 24 h before release. The same procedure was repeated in 2009 (n = 21,352, 70 to 116 mm TL) and in 2010 (n =1 8,386, 80 to 133 mm TL).

Tag placement, sampling, and mark analyses

The POTs were attached onto two body locations of Japanese flounder juveniles (Figure 2): (1) location 'a' (n = 10,676, in 2009; n = 9,193, in 2010); (2) location 'b' (n = 10,676, in 2009; n = 9,193, in 2010). Excess parts of the plastic anchors were removed from the insert points to insure that the tags were completely attached on the ocular side of the juveniles. These two attachment locations were chosen to minimize the effect on swimming and feeding behavior; both locations were on the ocular side of the flounder.

In 2009 and 2010, the degree of acute mortality caused by the POTs and the POT retention data were monitored. At the same time, the swimming motion of the tagged flounder was recorded on video. During 3 days post-tagging, 50 individuals per tank (100 individuals per sub-treatment) were randomly sampled each day. From 2009 to 2010, an ANOVA with an independent samples t-test (SPSS 13.0) was conducted to assess the significance of the differences in the survival and initial tag retention based on the data from different tag locations. A 0.05 level of significance was used in the analysis.

Release and recapture studies

Stock enhancement release experiments were performed with Japanese flounder in the inshore waters of Weihai City on 4 July of 2009 (on the beach of Beihai) and on 1 July of 2010 (on the beach of Lidao) (Table 1). July was chosen because fishing by commercial vessels is forbidden during this month.

In the 2009 release experiment, green POTs were attached to location 'a' of 10,676 Japanese flounder juveniles, and 10,676

Table 1. Tag-release information of Japanese flounder tagged in 2009 and 2010 release experiment.

Release date	Release position (GPS data)	Water depth (m)	Color of POTs	Total number released	Number of sampled individual	Initial average total length (mm, mean ± S.D.)	Initial average wet mass (g, mean ± S.D.)
July 4 2009	Beihai of Weihai City (37° 29′N, 121° 55′E)	10	Green	21,202	100	89.2 ± 9.6	5.35 ± 1.89
July 1 2010	Lidao of Weihai City (37° 13′N, 121° 36′E)	13	Yellow	18,350	60	103.6 ± 11.3	11.08 ± 3.57

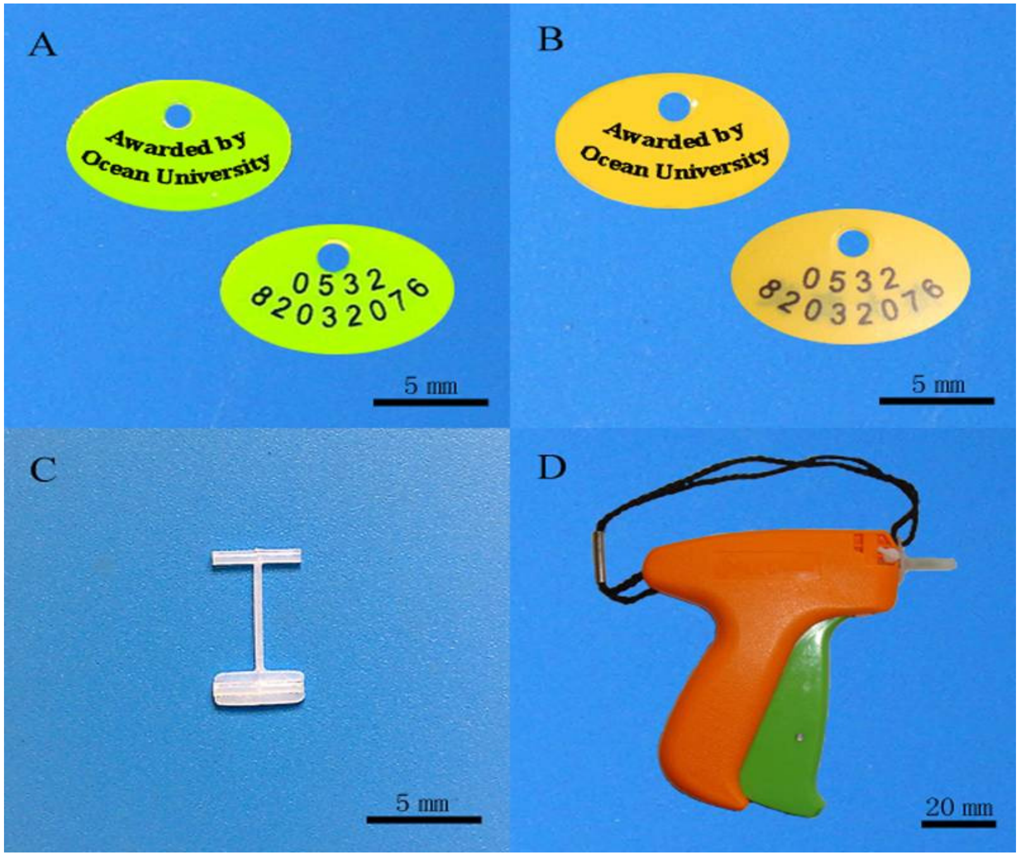

Figure 1. Part of the tools used during tagging procedure: (A) both sides of green plastic oval tag; (B) both sides of yellow plastic oval tag; (C) plastic anchor used to attach POTs on the ocular side of Japanese flounder; (D) Mark 003 tagging guns made by Japan Kingmu CO., Ltd.

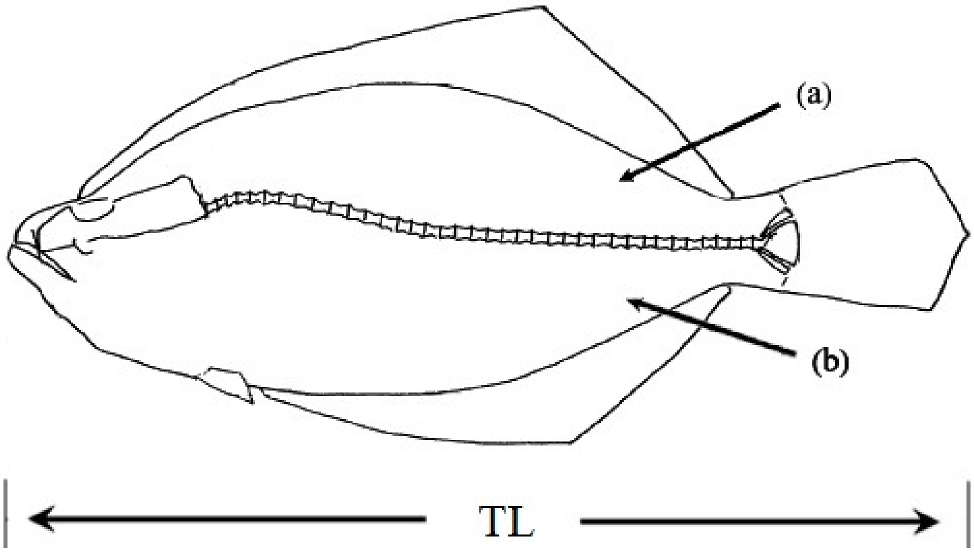

Figure 2. Schematic of POTs attached locations (indicated by arrows): (a) anterior direction of the caudal peduncle, and near the middle of dorsal fin and vertebra, (b) anterior direction of the caudal peduncle, and near the middle of anal fin and vertebra.

individuals were tagged on location ‘b’. The tagged juveniles were then held for 4 days until release. The final number of released juveniles was 21,202 (Table 1). On July 4th, the fish were packed into plastic bags with 10 l of seawater at a density of about 20 individuals l^{-1}. The bags were packed into 50 l-styrofoam boxes and transported to the release site by truck and boat. The boxes were individually unpacked, and about 10 l of seawater was added to the bags to allow the fish to acclimate prior to release (final volume of 20 l per bag). After 5 to 10 min, the fish in the opened plastic bags were transported to an artificial reef area (37° 29′ N, 121° 55′ E) and released into the open sea of Shuangdao Bay in Weihai City, which was 3 km off the coast of Beihai (Table 1). The water depth at the release site was about 10 m.

In the 2010 release experiment, all 9,193 juveniles were tagged with yellow POTs on the two locations, and the final number of released juveniles was 18,350. On July 1st, the same release procedure was followed after four days of holding, and the release site was changed to an artificial reef area (37° 13′ N, 121° 36′ E) in Lidao of Weihai City, which was 2 km off the coast (Table 1). The water depth at the release site was about 13 m.

From July 2009 to February 2011, recapture studies were carried out about 20 days after the stock enhancement release. Posters offering reward for tag returns with the date and recapture data (size of the fish, and place and date of capture, etc.) were distributed to local fish markets, fishing companies, and fishermen. Other recapture methods were applied as follows: (1) Interviewing local fishermen who have set nets around the release sites. (2) Visiting commercial fishery ports with trawlers near the release sites. (3) Using laboratory trawlers to recapture the released juveniles around the release sites. (4) Feedback from fishermen through phone calls. Overall, four recapture methods for assessing the stocking of hatchery-raised flounder were applied in this study. The geographic dispersal of the released fish was also investigated based on the recapture locations, and environmental factors affecting the migration were initially discussed.

Growth

Up to 100 juveniles in 2009 and 60 in 2010 (Table 1) were randomly selected per experiment before the date of release to examine the growth of Japanese flounder juveniles in the field. The TL (mean ± S.D.) and wet mass (W, mean ± S.D.) of all selected and recaptured juveniles were measured to the nearest 0.1 mm and 0.01 g, respectively. Their age (in months) was calculated from the date of release to the date of recapture:

Age = (catch date – release date)/30

The mean growth rates per month (mean ± S.D.) were used to evaluate growth according to Isabel et al. (1992):

Mean growth rates of TL = (final TL – initial TL)/age
Mean growth rates of W = (final W – initial W)/age

The initial mean TL averaged 89.2 ± 9.6 mm and W averaged 5.35 ± 1.89 g in the 2009 release experiment. In 2010, the TL averaged 103.6 ± 11.3 mm and W averaged 11.08 ± 3.57 g.

RESULTS

Initial POT retention, tagging rates, and mortality

Initial tag placement studies showed that 3 days after POT tagging, the retention was good for both tested locations (above 98.7% tags retained), with little change in the retention results during the 3 days both in 2009 and 2010 (Table 2). On the third day, POT retention was still high; in 2009, a slight POT loss was observed from the middle of the dorsal fin and vertebra (99.3%), and the middle of the anal fin and vertebra (98.7%); in 2010, there was no POT loss from the middle of the dorsal fin and vertebra (100%), and a slight loss from the middle of the anal fin and vertebra (99.3%). In both years, the POT tags in 39,738 juvenile Japanese flounder had a high overall retention of 98.7% after 3 days.

Table 2. Sampling results of initial tag retention trials for plastic oval tags (POTs).

Experimental time	Insert location	Total number of sampling specimen	Tag retention (day after tagging)		
			1	2	3
2009	Location a	300	100	100	99.3
	Location b	300	100	99.7	98.7
2010	Location a	300	100	100	100
	Location b	300	100	99.7	99.3

In both 2009 and 2010, an independent sample t-test showed no significant difference in POT retention between locations ‘a’ and ‘b’ during the 3-day experiment ($p > 0.05$ for the 2 location treatments, both in 2009 and 2010).

The maximum tagging rate for the tagging operations was 250 to 300 fish h^{-1} operator^{-1} in 2009. In 2010, the tagging rate was much higher, which is 300 to 350 fish h^{-1} operator^{-1}. For both years, about ten researchers were present for POT tagging. The tagging mortality ranged from 0.2% (in 2010) to 0.7% (in 2009), and there was no significant difference ($p > 0.05$). Although, the effects of handling, anesthetizing, and tagging collectively caused mortality, the harm caused by tagging was the most common and obvious cause of mortality. In addition, MS-222 overexposure should not be underestimated.

Recapture data

For the 2009 and 2010 release studies, the recaptured fish were identified, and the POTs were clearly visible (Figure 3). In the 2009 release study, a total of 434 tagged flounder with green POTs were recaptured until the end of February 2011, resulting in a recapture rate of 2.05% (434 out of 21202; 87 tagged Japanese flounder were returned to the laboratory, whereas only tags or information were collected from 347) (Table 3). However, the number of recaptures with yellow POT was 620 up until the end of February 2011 in the 2010 experiment, and the recapture rate increased to 3.38% (620 out of 18350; 310 tagged Japanese flounder were returned to the laboratory, whereas only tags or information collected from 310) (Table 3). There was a significant difference in recapture rate between 2009 and 2010 ($p < 0.05$).

Most of the recaptures occurred 3 months after the release. Recaptures became less frequent over time: these were regularly reported up to the fourth month, and recaptures became very scarce after the fifth month. During the first month after releasing, tagged flounder were primarily recaptured by set nets in the same locations where they were released. Moreover, more recaptures began to appear offshore from the release sites, and were caught by gears other than set nets (principally trawlers and feedback from fishermen) during the second and third months (Table 3). Thus, Japanese flounder were recaptured as soon as they were released into the coasts, where they were targeted by inshore fisheries or as by-catch of commercial fishing. This trend was confirmed during the period corresponding to the decrease in the number of recaptures between the second and sixth months after release, both in the 2009 and 2010 release studies, because tagged flounder were only caught by inshore fisheries in coastal summer feeding areas.

Movement

In both the 2009 and 2010 studies, Japanese flounder juveniles were released into artificial reef areas, and flounder were frequently found around neighboring reefs from the first to the fourth month after release (Table 3). This could mean that fish stayed for at least 4 months on the release grounds, which made it possible to recapture the released fish that survived as long as possible over the reefs.

The movement patterns were investigated based on depth analyses of the locations where the tagged flounder were recaptured and the behavioral data; the migratory paths of the released fish in 2009 (Figure 4) and 2010 (Figure 5) were then projected. Results indicate a radiative movement from the release site in the 2009 release experiments. However, the tagged flounder dispersed predominantly northward (78.5%, 487 out of 620) from the release site in 2010. The longest mark–recapture duration was 496 days, with a distance of up to 215 km from the release site in the 2009 experiment. Moreover, the mean speed was calculated as approximately 0.46 km day^{-1} in 2009 and 1.05 km day^{-1} in 2010, and there were significant differences ($p < 0.05$).

Depth profiles suggested that all juveniles were captured at depths of 2 to 60 m in both years. The tagged juveniles spent most of the first 3 months between 3 and 17 m on the edge of land (≤17 km from the release site), which was followed by movement into 20 to 60 m depths (approximately 18 to 215 km away from the release site) (Tables 5 and 6). Moreover, the movement patterns showed a seasonal shift to deeper waters over time toward winter, both in 2009 and 2010.

Table 3. The number of Japanese flounder recaptured by four methods in 2009 (green POTs) release experiment and in 2010 (yellow POTs) release experiment. The four recapture methods: (1) Set-nets; (2) commercial Fishery ports; (3) trawlers by laboratory; (4) feedback from fishermen.

Date	Number of recapture							
	2009 release experiment				2010 release experiment			
	Set-net	Commercial fishery port	Trawler by laboratory	Feedback from fishermen	Set-net	Commercial fishery port	Trawler by laboratory	Feedback from fishermen
July 2009	$2^a + 29^b$	0	0	0				
August 2009	$3^a + 151^b$	$2^a + 41^b$	0	$1^a + 18^b$				
September 2009	$59^a + 20^b$	$3^a + 10^b$	0	$3^a + 13^b$				
October 2009	$6^a + 12^b$	$2^a + 17^b$	0	8^b				
November 2009	$1^a + 4^b$	$2^a + 12^b$	0	2^b				
December 2009	2^a	0	0	5^b				
January-June 2010	0	2^b	0	1^b				
July 2010	0	0	0	0	$103^a + 57^b$	$50^a + 94^b$	0	2^b
August 2010	0	0	0	0	8^b	$108^a + 137^b$	0	$2^a + 4^b$
September 2010	0	0	0	2^b	10^a	$19^a + 4^b$	0	4^a
October 2010	0	0	0	0	$1^a + 2^b$	$10^a + 1^b$	0	1^a
November 2010	0	1^a	0	0	0	0	0	$1^a + 1^b$
December 2010	0	0	0	0	0	0	0	1^a
January 2011	0	0	0	0	0	0	0	0
February 2011	0	0	0	0	0	0	0	1^a
Total number	$73^a + 216^b$	$10^a + 82^b$	0	$4^a + 49^b$	$114^a + 67^b$	$187^a + 236^b$	0	$9^a + 7^b$
Total number of recaptures	434 ($87^a + 347^b$)				620 ($310^a + 310^b$)			
Total recapture rate (%)	2.05%				3.38%			

[a]Tagged Japanese flounder, which had been returned to the laboratory; [b]Only tag or information (no fish) collected.

Growth

The growth of the released flounder in the field from release to recapture was computed for each recaptured individual, and the mean growth rates per month were calculated for all recaptures (only including the fish returned to the laboratory; sex combined). The flounder were recaptured at an age of 1 to 17 months with a TL of 119.5 to 445.0 mm and a W of 13.70 to 880.00 g for 87 individuals in the 2009 release experiment. In contrast, the flounder were recaptured at an age of 1 to 8 months with a TL of 109.5 to 272.0 mm and a W of 12.01 to 196.85 g for 310 individuals in the 2010 release experiment (Table 4). The biggest flounder recaptured was 445.0 mm TL and 880.00 g W at an age of 17 months from release in the 2009 release experiment.

The mean growth rates per month in the 2009 release experiment ranged from about 36.3 mm TL and 11.65 g W at an age of one month, to 20.9

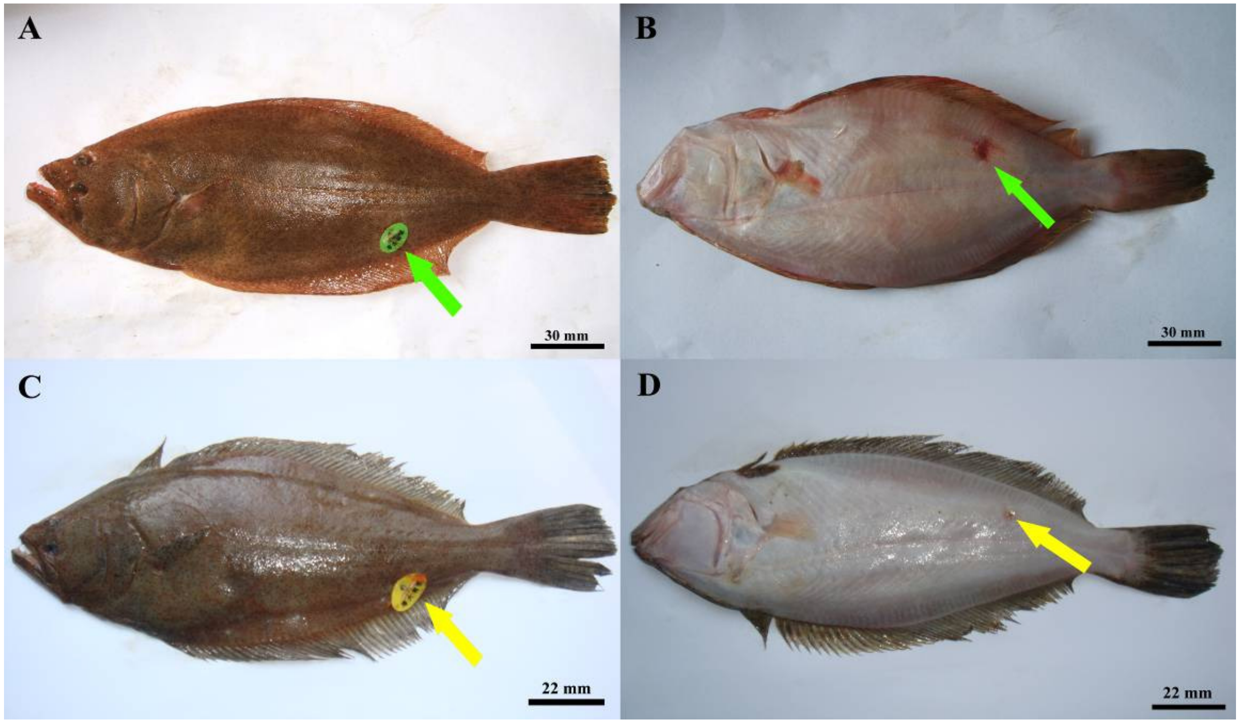

Figure 3. Photographs of ocular and anocular side of Japanese flounder recaptured. Fish were recaptured on the 71st day after releasing in 2009 (A, B). Fish were recaptured on the 125th day after releasing in 2010 (C, D). The arrows indicate tag placement of POTs.

Table 4. The mean growth rates per month (mean±S.D., sex combined) of recaptured Japanese flounder both in 2009 and 2010 release experiment.
TL, total length; W, wet mass; n, number of recaptured flounder. Different superscripts denote statistically significant differences(one-way ANOVA and paired comparisons post hoc test (*p*<0.05)).

Parameter	TL at release (mm)	W at release (g)	Age (month)	n	TL range at Recapture (mm)	Growth-TL (mm month^{-1})	W range at recapture (g)	Growth-W (g month^{-1})
2009 release experiment	89.2 ± 9.6	5.35±1.89	1	2*	119.5-131.5	36.3 ± 8.5^{a}	13.70-20.30	11.65 ± 4.67^{a}
			2	6*	142.5-198.5	41.9 ± 10.2^{a}	22.90-72.00	21.09 ± 9.24^{ab}
			3	65*	122.0-257	35.1 ± 8.3^{a}	14.00-154.00	22.85 ± 9.85^{ab}
			4	8*	224.0-265.3	39.4 ± 3.8^{a}	115.10-217.6	40.02 ± 10.02^{bc}
			5	3*	266.6-330.6	43.5 ± 7.1^{a}	219.70-374.00	59.84 ± 15.66^{cde}
			6	2*	349.3-388.0	46.6 ± 4.6^{a}	478.00-580.00	87.28 ± 12.02^{d}
			17	1*	445.0	20.9 ± 0.0^{b}	880.00	51.45 ± 0.00^{e}

Table 4. Contd.

p						<0.05		<0.05
Average growth rates in the first six months						36.3±8.4		27.13±16.09
Total				87*				
2010 release experiment	103.6 ± 11.3	11.08 ± 3.57	1	153*	109.5-140.5	12.6 ± 9.2^{a}	12.12-17.03	3.76 ± 2.12^{a}
			2	110*	110.7-165.2	10.3 ± 7.4^{a}	12.01-45.00	8.71 ± 6.98^{ab}
			3	33*	130.9-192.2	21.6 ± 6.2^{abc}	14.94-67.01	9.7 ± 4.87^{ab}
			4	12*	183.9-249.0	27.9 ± 5.5^{c}	58.13-143.91	20.42 ± 7.28^{bc}
			5	1*	272.0	33.68 ± 0.0^{d}	178.98	33.58 ± 0.00^{d}
			8	1*	271.8	21.03 ± 0.0^{b}	196.85	23.22 ± 0.00^{c}
p						<0.05		<0.05
Average growth rates in the first six months						14.7±8.8		5.65 ± 4.17
Total				310*				

* Tagged Japanese flounder, which had been returned to the laboratory.

mm TL and 51.45 g W at an age of seventeen months; and ranged from about 12.6 mm TL and 3.76 g W at an age of one month to 33.68 mm TL and 33.58 g W at an age of five months in the 2010 release experiment (Table 4). Moreover, in 2009, at an age of 1 to 6 months, the average TL and W increments (month^{-1}) were 36.3 ± 8.4 mm and 27.13 ± 16.09 g, respectively. In the 2010 release experiment, the average TL and W increments (month^{-1}) were 14.7 ± 8.8 mm and 5.65 ± 4.17 g, respectively, and there were significant differences ($p < 0.05$) between the mean growth rates of the two experiments in the first six months (Table 4). The growth rates in terms of TL-at-age and W-at-age (during 1 to 6 months after releasing) for the 2009 and 2010 experiments are shown in Figure 6.

DISCUSSION

Initial marking quality and application

The current study shows that tag placement location and tagging techniques did not influence normal swimming behavior based on observation and on the recorded video. Tagging in the two locations was logistically simple and achieved high tag retention both in the 2009 and 2010 experiments. For POTs, the period after releasing and some algae which grew on the tags significantly influenced the visibility of the markings based on the recaptures. This study also showed that juveniles (>70 mm, TL) could be successfully marked and non-lethally sampled. These results further agree with several studies (Sauer et al., 2000; Carmen et al., 2007; Fritsch et al., 2007) showing that the use of POTs as external tags are effective for marking, which can be widely used in mark–recapture research and evaluation of stock enhancement programs.

The tagging rates achieved in our large-scale tagging operations were similar to those reported in other studies (Dewey and Zigler, 1996; Bailey et al., 1998; Astorga et al., 2005; Brennan et al., 2005). With one POT applied to each fish but on two locations, our tagging rates were still 200 to 350 fish h^{-1} operator^{-1}. In our experiments, there was slight mortality during the tagging procedure and the temporary culturing period (mortality ranged from 0.2% in 2010 to 0.7% in 2009). The mortality was primarily caused by the harm of tagging and overexposure to MS-222. In other

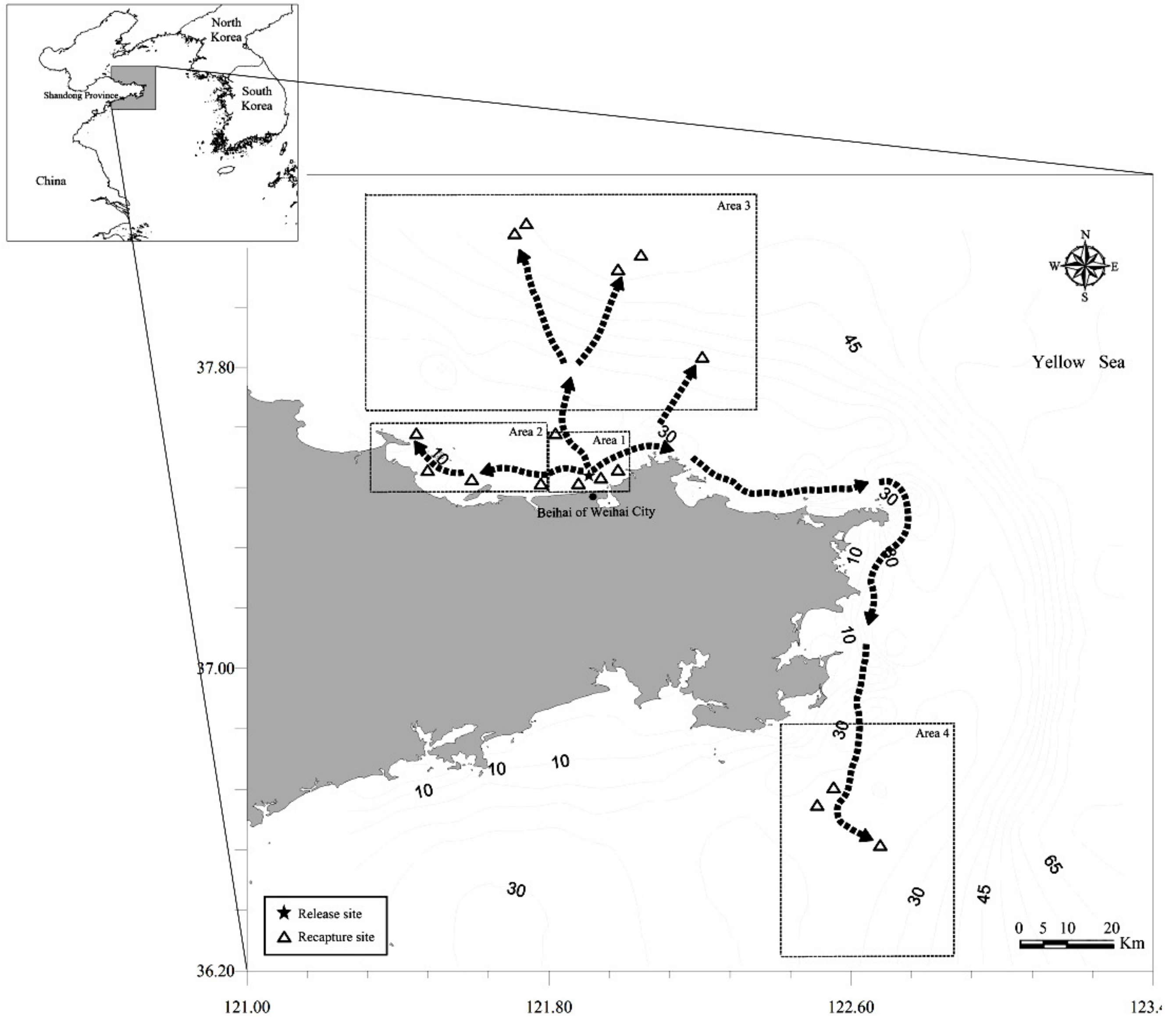

Figure 4. Results of releasing 21,202 Japanese flounder juveniles tagged with green plastic oval tags (POTs) from July 2009 to February 2011 (the number of △ does not represent the number of recaptures). Dotted lines represent migratory paths of released Japanese flounder, which are surmised. Depth contour is indicated by dashed curvy line.

studies (Astorga et al., 2005; Nathan et al., 2007), similar reasons for mortality were observed.

The maximum POT retention time in the present investigation was 496 days based on the recaptures in the 2009 experiment. However, a longer retention time for similar external marks has been demonstrated (Duggan and Miller, 2001; John, 2003; Robert et al., 2007). Thus, mark retention may not be a problem in marking flounder with POTs. Individual metabolic differences and different environmental factors may cause different reten-tion times. In addition, marking of fish with external tags involves a compromise between materials, color, shape, size, attachment locations, mortality, growing condition, and retention time to produce the best mark (Jakobsson, 1970). In the present investigation, their light weight, low cost, bright color, good visibility, long retention, and quick application increased the feasibility of using POTs, and they appear to be a promising technique for large-scale marking programs in assessing stock enhancements. As a result, POTs were found to be useful for stock enhancement studies on juvenile Japanese flounder.

Mark-recapture analysis

Mark–recapture studies of Japanese flounder in the

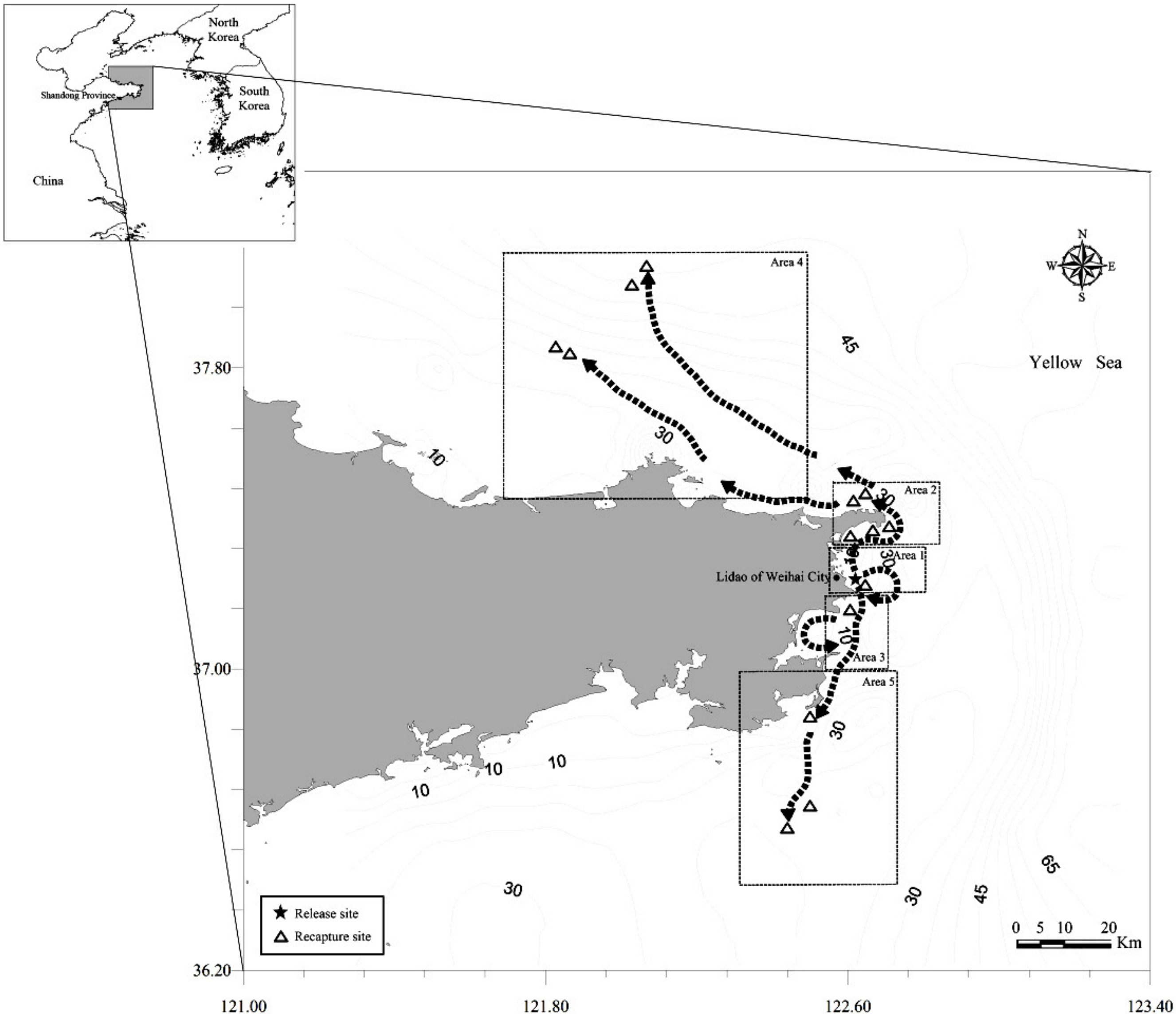

Figure 5. Results of releasing 18,350 Japanese flounder juveniles tagged with yellow plastic oval tags (POTs) from July 2010 to February 2011 (the number of △ does not represent the number of recaptures). Dotted lines represent migratory paths of released Japanese flounder, which are surmised. Depth contour is indicated by dashed curvy line.

inshore waters of Weihai City indicated low recapture rates (2.05% in 2009 and 3.38% in 2010), which were lower than in other studies (Fujita et al., 1993; Tominaga and Watanabe, 1998; Tanaka et al., 2005). The relatively low recapture rates may be partially attributed to the limited geographic scope of our publicity campaign to fishermen and to the lack of an economic reward for the return of tagged individuals (Carmen et al., 2007). In addition, the lower catch-ability caused by behavior or mortality of the tagged fish, and the tag loss in bigger fishes should not be underestimated (Stewart, 2003).

The recapture rate in 2010 (3.38%) was significantly higher ($p < 0.05$) than that in 2009 (2.05%). The different recapture rates may be caused by a variety of factors. The tagging study by Nihira (1987) reported that the recapture rate of flounder was high and decreased with decreasing size at the release (>13 cm TL at release). In the Fukushima Prefecture, the recapture rates of released small-group (80 mm TL) and large-group (100 mm TL) flounder juveniles were estimated to be 19.4% and 30.9%, respectively (Fujita et al., 1993). Moreover, Yamashita et al. (1994) released flounder with alizarin complexone-marked otoliths, ranging from 4 to 15 cm TL, and examined the size-at-release of subsequently recaptured flounder by fishing survey and commercial catch. The results demonstrated that back-calculated release size distributions gradually shift to larger values as time progresses and that the negative size-selective mortality

Table 5. The recapture details of 2009 (green POTs) release experiment. Number of release fish, release site, release date, number of recaptures, recapture area, GPS data, depth of recapture location, time since release (days), distance from release site (Km) are illustrated.

Release site	Number of released fish	Release date	Recapture area	GPS data	Number of recapture	Depth of recapture location (m)	Time since release (days)	Distance from release site (km)
Beihai of Weihai City	21202	July 4 2009	Area 1	37.47°-37.57°N 121.81°-122.01°E	71[a] + 279[b]	4-10	27-143	0-11
			Area 2	37.48°-37.63°N 121.33°-121.81°E	6[a] + 26[b]	3-18	42-125	18-49
			Area 3	37.70°-38.23°N 121.30°-122.20°E	9[a] + 40[b]	20-60	65-198	20-72
			Area 4	36.23°-36.85°N 122.25°-122.95°E	1[a] + 2[b]	17-40	312-496	175-215
Total					87[a] + 347[b]			

[a] means tagged Japanese flounder, which had been returned to the laboratory; [b] means only tag or information (no fish) recaptured.

Table 6. The recapture details of 2010 (yellow POTs) release experiment. Number of release fish, release site, release date, number of recaptures, recapture area, GPS data, depth of recapture location, time since release (days), distance from release site (km) are illustrated.

Release site	Number of released fish	Release date	Recapture area	GPS data	Number of recapture	Depth of recapture location (m)	Time since release (days)	Distance from release site (km)
Lidao of Weihai City	18,350	July 1 2010	Area 1	37.22°-37.32°N 122.55°-122.80°E	38[a] + 55[b]	3-17	7-102	0-7
			Area 2	37.33°-37.55°N 122.50°-122.86°E	233[a] + 207[b]	3-20	16-119	8-35
			Area 3	37.00°-37.20°N 122.47°-122.70°E	16[a] + 30[b]	2-19	43-87	8-40
			Area 4	37.45°-38.10°N 121.70v-122.50°E	22[a] + 17[b]	15-45	41-122	44-124
			Area 5	36.47°-37.00°N 122.30°-122.74°E	1[a] + 1[b]	10-40	127-130	45-90
Total					310[a] + 310[b]			

[a] means tagged Japanese flounder, which had been returned to the laboratory; [b] means only tag or information (no fish) recaptured.

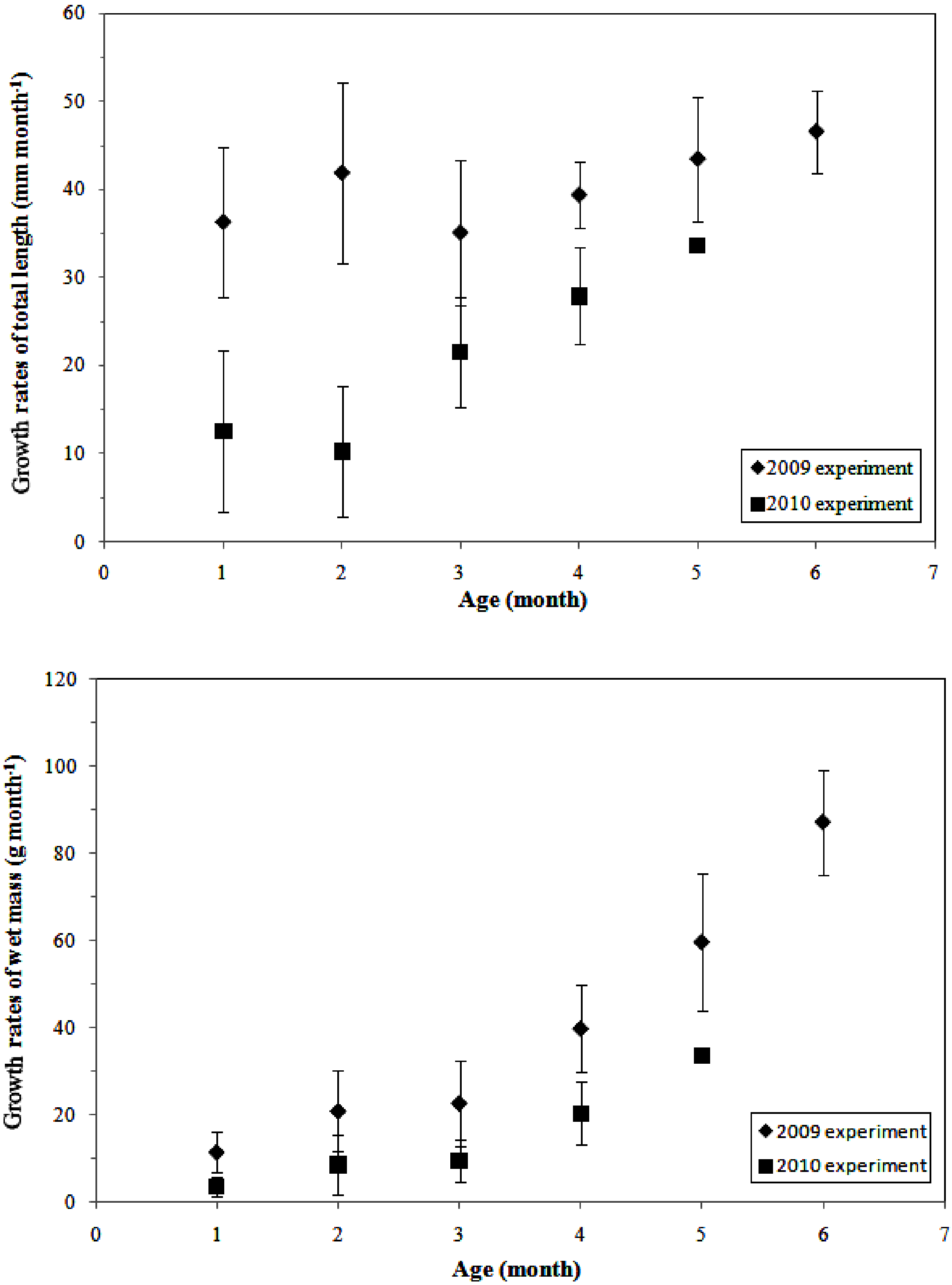

Figure 6. Comparison of the growth rates of total length-at-age and wet mass-at-age (between 1 and 6 months after release) in 2009 and 2010 experiment. For number of individuals for each year see Table 4.

mortality of the flounder occurs between release and capture at commercial size. In the present study, the recapture rate of large flounder (mean TL ± S.D. = 103.6 ± 11.3 mm, in 2010) was about 1.64 times higher than that of small-group (mean TL ± S.D. = 89.2 ± 9.6 mm, in 2009). Although, the recapture rates of each size differed between studies, the release size was obviously an important factor that influenced the recapture rate in the current study. Furthermore, the number of fishing boats working on the coast of Lidao was much higher than

than in Beihai during the recapture period. Therefore, the differences were also likely to be associated with the difference in the fishing effort.

Robert et al. (2007) reported that for red abalone, *Haliotis rufescens*, the smaller size-class had increased mortality. Predation is thought to be the major cause of the early mortality of released Japanese flounder (Sudo et al., 1992; Furuta et al., 1992), and their size-dependent mortality is probably related to predation (Tominaga and Watanabe, 1998). For the two experiments in the current study, the different recapture rates were considered to be associated with the release size, which might influence the mortality in the field. Overall, body size at the release time appeared to have a major effect on recapture rate; however, other factors may be present, such as the fishing effort from the release station, the time of release, the mortality in the field.

The number of recaptured flounder per month decreased rapidly during the first few months after release (Table 3) using four recapture methods. The same trend in recapture number after release has also been reported in Japan (Furuta, 1988; Tominaga et al., 1994). The results presented here showed that the four recapture methods (Table 3) were effective for use in recapture surveys, which is significant because of their comprehensiveness, economy, and quickness. However, the recapture rate was still relatively low because of factors such as the coverage of publicity and the feedback activeness of the fishermen.

Differences in the movement patterns between the 2009 and 2010 release experiments

The movement patterns of the released flounder were similar to those observed by Sauer et al. (1997). In the 2009 release experiment, the movement of tagged flounder indicated a radiative pattern; these daily movements may be associated with the presence of predators in the area and foraging dispersions (Sauer and Lipinski, 1991). However, the results of the 2010 release experiment showed that tagged flounder dispersed predominantly northward (78.5%) from the release site, yet some of the tagged flounder moved southward about 2 months after their release. Simultaneously, the water temperature on the Southern coast (approximately 19.2–22.8℃) is much higher than on the Northern coast (approximately 17.6 to 19.7℃) of Lidao in the Yellow sea in July (Zhang, unpublished data), which is followed by the northward-delayed seasonal water temperature increment over time. Minami (1997) and Kuwahara and Suzuki (1982) reported that the most appropriate growth temperature for flounder was between 13 and 18℃. Thus, the northward movement of the tagged flounder might be associated with seasonal changes in the coastal water temperature; these results are similar to the results of Kiyono and Hayashi (1977). The same southward migration, which started at the time when coastal water temperature decreased, has also been reported for Japanese flounder released off the coast of Japan (Kato et al., 1987; Tominaga et al., 1994; Takeno and Hamanaka, 1994).

The mean speed was calculated based on the migratory paths of the released flounder in 2009 (about 0.46 km day^{-1}, Table 5) and 2010 (about 1.05 km day^{-1}, Table 6), and there were significant differences between the mean speeds in 2009 and 2010 ($p < 0.05$). Furthermore, the results of the 2010 release experiment showed that the movement of the tagged flounder was against the coastal current. The main ocean current along the coast of Lidao (2010 release site), a coastal branch of the ocean circulation in the Yellow sea, flows southward in July, but almost no ocean current exists in July (Yuan, 1998) along the coast of Beihai (2009 release site) except for the tide. Some tagging experiments in the coastal area of the Japan sea have also shown that the movement of the tagged flounder was against the current, like the Tsushima current (Kato et al., 1987; Tominaga et al., 1994). In summary, the different movement patterns and different dispersion speeds were probably the results of differences in the water temperature and ocean current.

Growth conditions of the released flounder

The present study also showed significant differences ($p < 0.05$) between the growth rates in terms of TL-at-age and W-at-age (during 1 to 6 months after release) for the 2009 and 2010 experiments (Figure 6). Reports have shown that growth is associated with both the water temperature and food availability in some flatfishes, such as plaice (*Pleuronectes platessa*) (Karakiri et al., 1989), sole (*Solea solea*) (Marchand, 1991), and winter flounder (*Pseudopleuronectes americanus*) (Sogard and Able, 1992). Japanese flounder juveniles commonly exhibit a simple feeding chronology, initially feeding on mysids and switching to fish with growth (Kato, 1996; Fujii and Noguchi, 1996). Water temperature is one of the factors that influence the density of mysids (Mauchline, 1980). Tanaka et al. (1997) reported that the peak mysid density occurs in May to June, and the density decreases markedly when the water temperature exceeds 20 to 21 ℃ in the Southern part of the Sea of Japan. Furthermore, Furuta (1996) demonstrated that the abundance of mysids, which is influenced by water temperature, is important for post-release feeding and growth of released juveniles. Tanaka et al. (2006) reported that the growth of the early release group was slower than that of the late release group, probably because the ambient water temperature during the late release was 4 to 5℃ higher than that during the early release. Japanese flounder reportedly grows fast at high water temperatures (Iwata et al., 1994; Tanaka et al., 1997). In this study, the experiment was conducted in July at 20.5℃ (on the coast of Beihai, 2009) and 18.8℃ (on the coast of Lidao, 2010) (Zhang, unpublished data). Therefore, the faster growth of the released Japanese flounder in 2009 (compared with that in 2010) was obviously because of the higher food availability caused by the higher water temperature. Moreover, the different release size (Fujita et al., 1993; Yamashita et al., 1994) was obviously an important factor that influenced the growth in this study.

These results show that because survival, movement, and growth were related to water temperature and food availability, the release time and release size of hatchery-reared Japanese flounder should be comprehensively considered and evaluated before mass release. This type of in situ ecological experiment was conducted to develop release techniques for restocking the flounder population, and could also have great potential for understanding the recruitment mechanism in the wild population (Tanaka et al., 2006). In the current study, POT was useful for tagging Japanese flounder; the results indicate that the POT in the Japanese flounder were successfully developed and can be applied for stock enhancement research projects, especially for short-term (<12 months) movement and behavioral studies. Furthermore, POT can be a powerful tool for fishery managers in assessing the effectiveness of stock enhancements.

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