

# Stock Enhancement of Portunid Crabs in Japan

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## Abstract

Portunid crabs such as swimming crab *Portunus trituberculatus*, blue swimming crab *p. pelagicus* and mud crab species *Scylla paramamosain* and *S. serrata* are one of the most important fishery resources in Japanese coastal waters. The annual catch of the Portunid crabs have fluctuated between ~2,300 and 5,600 tons in Japan. In order to sustain and/or increase the Portunid crabs stock, a number of 30 million hatchery-produced juveniles have been released annually since the late 1980s. Estimating recapture rates of stocked crabs is indispensable to evaluate the effectiveness of stock enhancement programs, so that it is necessary to develop marking methods that distinguish between wild and hatchery-released individuals. The stocking effectiveness of Portunid crabs have been difficult to estimate because there have been no appropriate methods to mark small body sized juveniles which frequently molt. Therefore, recently, we developed a technique to mark juveniles and eventually estimated the contribution rates of released crabs to the total catch of the mud crab and swimming crab. The mixture rate of released juveniles in the total catch of the mud crab is estimated by the method of genetic stock identification, to be 5-19.7%. The contribution of released juveniles to the total catch was estimated to be about 0.5-1 metric ton. The recapture rate of released juveniles of the swimming crab is estimated by the marking technique of clipping swimming leg dactylus. Estimated contribution rate of marked crabs to the landings was about 3.0%. Effective marking methods and potential of stock enhancement programs for the Portunid crabs is discussed.

**Keywords:** juvenile release, marking, recapture rate, stocking effectiveness

## Introduction

Portunid crabs such as swimming crab *Portunus trituberculatus*, blue swimming crab *P. pelagicus* and mud crab species *Scylla paramamosain* and *S. serrata* are among the most important fishery resources in Japan. The annual catch of the Portunid crabs in Japan have fluctuated between ~2,300 and 5,600 tons. In order to sustain and/or increase the Portunid crabs stock, about 30 million hatchery-produced juveniles have been released annually since the late 1980s.

Estimating the recapture rates of stocked crabs is indispensable to evaluate the effectiveness of stock enhancement programs. A technique was

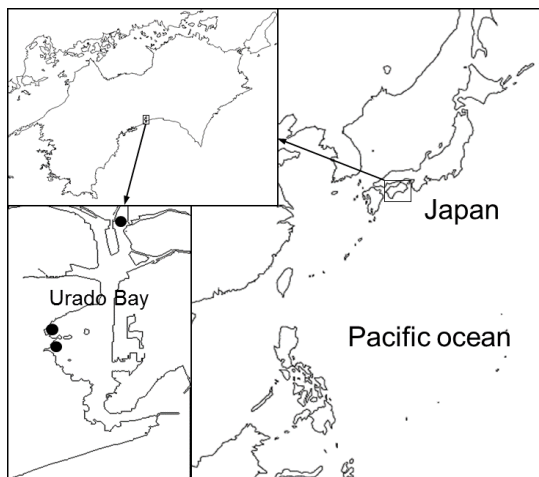
developed in Japan to mark the juveniles and eventually the contribution rates of released crabs to the total catch of the mud crab and swimming crab could be estimated.

The recapture rate of released juveniles of the swimming crab was estimated based on the marking technique of clipping the swimming leg dactylus. Thus, the estimated contribution rate of marked crabs to the landings was about 3.0%. Effective marking methods and potentials of stock enhancement programs for the Portunid crabs are discussed in this paper.

## Mud crab (*Scylla paramamosain*)

In Japan, more than 1.0 MT of mud crab could be captured every year from three areas, namely: Lake Hamana, Urado Bay and Ryukyu Islands. In Lake Hamana and Urado Bay, the dominant species are *S. paramamosain* and in Ryukyu Islands is *S. serrata*. A study on stock enhancement of crabs was carried out in Urado Bay, Kochi Prefecture in Japan (Fig. 1).

Considering that estimating the recapture rates is indispensable in order to evaluate the effectiveness of stock enhancement programs, it has become necessary to develop marking methods in order to distinguish between wild and reared individuals. At the early stages of the program, the stocking effectiveness of mud crabs was difficult to estimate because of lack of effective marking methods.



**Fig. 1.** Map showing the location of Urado Bay where marking surveys for *S. paramamosain* were conducted (black circles indicate the release points)

Due to their small size, the carapace width (CW) is about 10 mm, external tags could not be used on released juvenile mud crabs. Therefore, the use of genetic tags using mtDNA D-loop region was adopted. The haplotypes of mtDNA D-loop region were determined for a sample based on the methods detailed in Imai *et al.* (2002). The mtDNA D-loop region was amplified by PCR. The PCR product was digested by three restriction enzymes (Hinf I, PshB I and Pac I). The RFLP profiles were visualized by agarose gel electrophoresis, where haplotypes were discriminated.

A total of 98-149 broodstock females (CW 143 mm) were collected using gill nets in Urado Bay around December each year. A minor haplotype was selected as the genetic tag for released juveniles. About 3-5 million larvae from selected crabs were reared in a 200-kg indoor tank at Tamano Station, out of which between 72 thousand and 149 thousand juveniles were released during mid-May to early-June of each year from 1997 to 2001. The mean carapace width was between 8.5 to 9.9 mm, and each year, haplotypes of the released crabs differed.

From 1997 to 2002, 145 to 567 live *S. paramamosain* were collected, with a carapace width exceeding the market-size of 11 cm, from fishers during the main fishing season from August to December. The mean carapace width of collected crabs was 13.6 cm. The percentage of released juveniles (mixed populations) in the total catch was estimated using the maximum likelihood method. The log likelihood function for the samples is given by this equation (Obata *et al.*, 2006). In this case, the log likelihood function is maximized using the estimates of haplotype frequency.

$$\log L(\theta | n_i^{(0)}, n_i^{(1)}, n_i^{(2)}) = \sum_{i=1}^m n_i^{(0)} \log(\theta p_i^{(1)} + (1-\theta)p_i^{(2)}) + \sum_{j=1}^2 \sum_{i=1}^m n_i^{(j)} \log p_i^{(j)} + \dots + \sum_{m=1}^m n_m^{(j)} \log p_m^{(j)}$$

$\theta$  : the mixing rate of stocked crabs

$m$  : the frequency of haplotype  $i$  in baseline population  $j$

$n_i^{(0)}$  : the number of individuals that haplotype  $i$  in the sample drawn from the mixed population

$n_i^{(j)}$  : the number of individuals that haplotype  $i$  in the samples drawn from baseline population  $j$

$p_i^{(j)} = \frac{n_i^{(j)}}{n}$  : the estimates of haplotype frequency

Optimization was performed to the quantity of released juveniles (mixed populations), using the solver of Microsoft Excel. The 95% confidence intervals were estimated by the bootstrap method, using 10 thousand times re-sampling of the sample with replacement. The life span of this crab is about 2 years and newly settled crabs were considered to reach market-size in October. Assuming that the crab's life span is two years, the mixed population was divided into 2 periods, where the period from October in the release year to September the following year was taken as the first year after release while the period from October the following year of release to June in the year after the next was considered as the second year after release. The frequency of haplotypes in the 1997 release group increased from 15.4% to 30.5% after release. While the 1998 release group also increased from 9.8% to 16.1%, but the 1999 release group did not increase after release. However, the 2000 release group increased from 2.7% to 4.4%, and likewise, the 2001 release group also increased from 0.9% to 7.5% (**Table 1**).

In the 1997 release group, the weight and number of catch were estimated at 915 kg and 2,226 individuals, respectively. The 1998 release group had 1145 individuals and weighed 471 kg, the 2000 release group, the weight was estimated at 77 kg with 188 individuals, and the 2001 release group had 588 individuals and weighed about 242 kg. The return rates of each release group were calculated as 2.26%, 1.53%, 0.23% and 0.39%, respectively. The unit cost of released juveniles was about five Japanese Yen. The mean market price of mud crabs was about 2,500 Yen/kg. The economic efficiency was therefore calculated as 4.7, 3.1, 0.5 and 0.8, respectively, for each release groups. As a result, the *S. paramamosain* stock enhancement program has been considered as economically viable in two cases.

**Table 1.** Changes in the haplotype frequency (%) which was selected as a genetic tag for each release group of *S. paramamosain*

Year of release	Haplotype used for the tag	Previous year of each release		First year after release <sup>1</sup>		Second year after release <sup>2</sup>	
		Haplotype (%)	<i>n</i>	Haplotype (%)	<i>n</i>	Haplotype (%)	<i>n</i>
1997	2	15.4	149	30.5	213	25.9	143
1998	3, 9	9.8 <sup>3</sup>	213	16.1	143	10.9	403
1999	4	7.7	143	3.7	403	6.1	521
2000	6	2.7	403	4.0	521	3.2	158
2001	18	0.8	521	7.0	158	2.0	247

<sup>1</sup> October in release year to September in the following year.

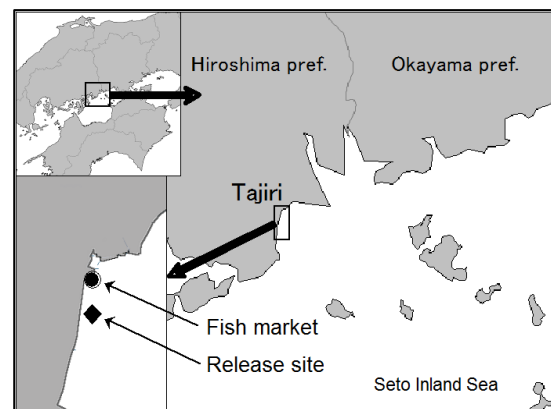
<sup>2</sup> October in the following year of release to June in the year after next.

<sup>3</sup> Haplotype 3 and 9 were combined.

### Swimming crab (*Portunus trituberculatus*)

A marking method used for swimming crab juveniles was by clipping the dactylus of the swimming leg from the central tip, with small scissors. Three types of malformed dactylus were found and the frequency of crabs with malformed swimming leg dactyli reached about 70% with vertical slit of about 60% of the dactylus length. Further, the size of juveniles for clipping dactylus should be at fifth crab stage with 20 mm carapace width. Mark-recapture experiments were carried out at Tajiri, Hiroshima Prefecture in Seto Inland Sea (Fig. 2). A total of 11,900 and 13,000 hatchery-produced juveniles with clipped swimming leg dactyli were released at the artificial tideland in 2006 and 2007, with mean carapace widths of 28.4 mm and 29.9 mm, respectively. The juveniles were transported from the hatchery to the release site for 2 hours by truck. The swimming crabs were captured using a small set net and were landed at Tajiri Fish Market. Sampling surveys for estimating the mixed populations of marked crab to commercial catches were carried out at the said market. Total catch during the sampling survey was 2.7 metric

tons in both years. The ratios of observed number of crabs in the total catches were 28% in 2006 and 21% in 2007. Numbers of crabs surveyed were 3874 individuals in 2006 and 2407 individuals in 2007. Numbers of marked crabs in the catches were estimated at 130 individuals in 2006 and 57 individuals in 2007. The quantity of marked crabs in the total catches was estimated at 3.36% in 2006 and 2.37% in 2007 (Table 2).



**Fig. 2.** Map showing the location of Tajiri in Seto Inland Sea, Hiroshima Prefecture, Japan

**Table 2.** Estimates of weight and number of recaptures, and recapture rate of released crabs

Year released	Number of juveniles (individual)	Total catch (kg)	Mixture rate (%)	Estimates for recaptures			
				Weight (kg)	Number (individual)	Ratio (%)	YPR (g/ individual)
2006	11,900	2,516	3.36	81	473	4.0	6.8
2007	13,000	2,722	2.37	59	288	2.2	4.5

In the 2006 releases, the weight and number of recaptures and recapture rate of released crabs were 81 kg, 473 individuals and 4.0%, respectively, and 59 kg, 288 individuals and 2.2%, respectively in the 2007 releases. Yields from released individuals (YPR) were estimated at 6.8 g in 2006 and 4.5 g in 2007. YPR was estimated at 33.6 g for *P. trituberculatus* using regression analysis between annual number of C2 juveniles released and annual catches in Seto Inland Sea. Results should that the YPR estimates of 4.5–6.8 g from marking surveys

### Recommendations

Marking method for swimming crab, using mtDNA control region sequence and microsatellite DNA markers had been developed. The stocking effectiveness of swimming crab in Ariake Bay has been investigated using this

### References

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were lower than that value. This could be due to the estimations carried in marking surveys that depend on catches from small set nets. After recruitment, swimming crabs are also caught by gill nets and small trawls, so the YPR in the marking surveys could be underestimated. The manner of clipping the dactylus might have also affected the behavior of released juveniles resulting in low survival rate or the quality of seed juveniles might have reduced due to loss of legs during the nursery culture before release.

method together with release techniques such as size, time and site at release. However, it is also necessary to estimate not only the stocking effectiveness but also reproduction by the released crabs.

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