A Marking Method for Kuruma Prawn *Penaeus japonicus*

Toshiaki Miyajima,^{*1} Yuichi Hamanaka,^{*1} and Koji Toyota^{*2}

^{*1}Kyoto Institute of Oceanic and Fishery Science, Odasyukuno, Miyazu, Kyoto 626-0052, Japan

^{*2}Kansai Environmental Engineering Co., Ltd., Miyazu Office, Odasyukuno, Miyazu, Kyoto 626-0052, Japan

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A marking method for Kuruma prawn *Penaeus japonicus*, through recovery of the colored pigment area in uropods, was developed. The method was tested by cutting the uropods of 75 prawns ranging from 21 to 73 mm in body length (*BL*) in the laboratory and 3,733 prawns from 35 to 75 mm in *BL* in the sea off Kyoto Prefecture.

After cutting uropods, the recolored pigment area was visibly reduced though uropods length regrew nearly equal to that of uncut uropods. The degree of pigment reduction increased with the increase in the *BL* of prawn used, and a 50% reduction in comparison with uncut uropods occurred for 60 mm in *BL*. As it was suggested that a 30% reduction of the colored pigment area could be detected by the naked eye as marks, this method is suitable for prawns larger than 30 mm in *BL*. The effective location of cutting uropods was just below the protopodite with no severe effects on survival and growth of marked prawns. From the test in the sea, the method proved successful for application as a long-lasting mark to a large field sample of prawns.

Key words: Kuruma prawn, *Penaeus japonicus*, marking, cutting uropods, pigments

In the ranching project of the Kuruma prawn *Penaeus japonicus*, one of the major problems encountered is the uncertainty of recaptures of prawns released. This is due to the lack of a practical, long-lasting marking method for up to commercial sized prawns after release. Farmer¹⁾ and Kurata and Ishioka^{*3} reviewed the marking methods used for penaeid shrimp, and concluded that no useful methods exist as long-lasting markers and that it is necessary to develop a new method suitable for the purpose of projects.

A number of marking methods has been employed in ranching projects of the Kuruma prawn. These include cutting uropods,²⁾ ribbon tag^{*4,*5} and wire tag.^{3,4)} All of these methods have limitations as long-lasting markers due to biological damage and in terms of practicality for marking large field samples and effectively detecting marked prawns.

In this study, an improved method of cutting uropods was developed for long-lasting marks of Kuruma prawn through the reduction of the dark-red colored pigment area in uropods after cutting. Using this method for a large field sample, the recapture of marked prawns was demonstrated.

Materials and Methods

Two laboratory experiments were designed to determine the effective cutting location in uropods and the body sizes for which the uropod cutting method can be used as marks for Kuruma prawn.

The Kuruma prawns used for the present experiments

were artificially hatched in tanks in July, 1995, and April and July, 1996. Forty prawns hatched in 1995 were used in experiment 1 to determine the effective cutting location in uropods while 60 prawns hatched in 1996 were used in experiment 2 to examine the prawn sizes for which uropods can be cut. The uropod length (*UL*), represented as the length of ramus, and the body length (*BL*), from the orbital margin to the apex of telson, were measured to the nearest 0.1 mm with Vernier calipers. The lateral and medial ramuses on the left side were cut by scissors or knife.

Table 1. The numbers of prawns used (*n*) and the body lengths (*BL*) at initial and final stages in experiments 1 (10 weeks) and 2 (34 weeks)

Group	At initial		At final		Molt/Prawn
	<i>n</i>	<i>BL</i> (mm)	<i>n</i>	<i>BL</i> (mm)	
(Experiment 1)					
Control	10	74.4±4.6	8	92.8±3.0	3–5
G1	10	72.0±3.9	10	92.6±3.6 ^{ns}	3–4
G2	10	72.4±3.9	8	90.0±3.4 ^{ns}	3–4
G3	10	72.5±6.9	10	91.5±6.6 ^{ns}	3–4
(Experiment 2)					
Control	15	21.2±1.3	13	92.5±5.6	7–14
G4	15	21.7±0.7	14	93.9±3.6	10–15
G5	15	29.9±1.2	14	95.0±6.3	9–13
G6	15	40.6±1.1	14	99.8±5.2	8–12

Frequencies of molt during the periods of both experiments are shown as the maximum and minimum among the prawns used.

ns: not significant (*t*-test, *P*>0.05)

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Fig. 1. Diagram showing the cutting locations of left uropod G1: cutting uropod along the line across the middle part colored dark-red, G2: cutting uropod along the line just above the dark-red colored part, G3: cutting whole ramuses.

Experiment 1

The mean *BL* of 40 prawns used was 72.4 ± 4.8 mm (mean $\pm$ S.D.) and the prawns were separated equally into four groups (Table 1); G1: uropods cut along the line across the dark-red middle part (Fig. 1), G2: uropods cut just above the dark-red colored part, and G3: uropods of which the whole ramus is cut. To assess the potential effect of such cutting on growth (molt frequency) and survival of the above three groups, 10 prawns with uncut uropods (72.4 ± 4.6 mm *BL*) were used as control under the same culture conditions as G1, G2 and G3. Each group of 10 prawns was isolated in a 270 l tank with flowing filtered sea water (270–540 l/h). The water temperature was 21–25°C throughout the experiment. The prawns were fed pelleted diets, 2–3% of their weight, once daily. As it was anticipated that molting prawns might be eaten by the others, 10 plastic net enclosures were individually installed in each tank to protect newly molted prawn.

Prior to cutting the left uropods, the *BL* and *UL* were measured. During a ten-week test period, the measurements were made biweekly and the molting frequency was individually recorded. Whenever *UL* was measured, uropods of both sides were photographed to compare the recolored pigment area (pixel numbers) of cut mesial ramus with that of the right uncut one by image processor (NIH Image).

Experiment 2

Three groups based on *BL* class of Kuruma prawn; G4: 21.7 ± 0.7 mm, G5: 29.9 ± 1.2 mm, G6: 40.6 ± 1.1 mm, were used in experiment 2 (Table 1). To assess the effect of cutting uropods on *BL* growth and survival among the three groups, 15 uncut prawns (21.0 ± 1.3 mm *BL*) were treated as controls. A total of 60 prawns (15 prawns per group) were isolated individually in a $26 \times 13 \times 19$ cm³ plastic tank for 34 weeks after cutting their left uropods.

Both ramuses of left uropod were cut off by scissors or knife shown by G3 in Fig. 1. The water in the tanks was filled with flowing water (2–3 l/h) and the water temperature was kept at 24–25°C throughout the experiment. Pellet food was supplied in the same manner as in experiment 1. The increase in *BL* and *UL* were measured in monthly. In order to minimize handling damage to the small prawns such as G4, only at the final stage of the experiment were photographs taken on uropods on both

sides of all prawn for estimating the recolored pigment area by the same image processor as in experiment 1.

The regrowth ratios of uropod length (*R*) were calculated as $R = (\text{length of left uropod} / \text{length of right uropod}) \times 100$ for both ramuses. Length reduction ratios can be given as $100 - R$. Recolored ratios of pigment area in mesial ramus (*S*) were estimated by $S = (\text{pixel numbers in cut uropod} / \text{pixel numbers on uncut uropod}) \times 100$. Pigment reduction ratios can be given as $100 - S$. Survival rates of prawns in both experiments were calculated by comparing the number of prawns remaining at the final stage of the experiments with those at the initial stage. The differences in growth between prawns with cut uropods and those with uncut uropods were compared by the mean *BL* in each group at the final stage of the experiments.

The same uropod cutting procedure as in the laboratory experiments was employed in the field experiment during the studies of the Kuruma prawn population in Kunda Bay, Kyoto Prefecture. Three thousand seven hundred thirty-three prawns hatched artificially, ranging from 35 to 75 mm in *BL*, were marked by cutting left uropods as shown by G3 in Fig. 1 and were released in August 1996. Recapture analysis was used to evaluate the utility of this method for marking a large field sample.

Results

In experiments 1 and 2, there was no significant difference in the survival of Kuruma prawn between cut and uncut groups (Table 1).

During a 10-week period in experiment 1 and a 34-week period in experiment 2, there were no significant differences in the molt frequency and/or growth (*BL*) between cut and uncut groups (*t*-test, $P > 0.05$). However, the cut uropods did not completely regenerate compared to uncut uropods (Table 2). For example, the mean length reduction ratio ($100 - R$) of G1 and G3 was 9.6–13.7% for mesial ramus and 10.8–17.9% for lateral ramus (G3). In experiment 2, which lasted 34 weeks, there did not appear to be large differences between cut and uncut uropods, 2.7–8.6% on average for mesial ramus and 2.5–8.1% on average for lateral ramus (Table 3). This indicated that the effect of cutting uropods on their regeneration in the *UL* diminished with time after cutting.

When the recolored ratio in mesial ramus was more than 30%, it was possible to judge visually the marked prawns and the differences among individuals can be detected. Reduction of more than 30% was found in groups G2 and G3 (Fig. 2 and Table 2), while prawns in control showed no reduction. In group G1, midline of the colored area, the mesial ramuses were recolored $S = 95\%$ for the mean of 10 prawns, and it was hard to judge then as marked prawns. Therefore, the cutting locations of G2 and G3 were selected as suitable sites for cutting the uropods and, as shown in Fig. 2, recolor ratios of G2 seemed to reach a stable state and these of G3 showed no considerable increase after eight to ten weeks.

In experiment 2, a total of 55 prawns survived. Based on the recolored ratio (*S*) of their left mesial ramus, usable prawn size (*BL*) was evaluated as G5 and G6, as shown in Table 3. *S* value decreased with an increase of *BL*, namely, a recolored ratio over 30% in cut mesial ramus was found

Table 2. Regrowth ratios of uropods (mesial and lateral ramuses) lengths (R) are given as $R = 100 \times (\text{regrown length of left uropods}) / (\text{length of right uncut uropod})$ on average of prawns used

Group	Regrowth Ratio: R (%)	100- R (%)	Recolor Ratio: S (%)	100- S (%)
(Mesial Ramus)				
Control	98.7(95.7–101.0)	1.3	103(98–106)	
G1	86.3(78.5–92.9)	13.7	95(84–103)	5
G2	89.0(80.6–101.0)	11.0	36(11–48)	64
G3	90.4(77.5–100.0)	9.6	42(10–60)	58
(Lateral Ramus)				
Control	96.9(94.3–100.0)	3.1	—	
G1	89.2(82.3–93.3)	10.8	—	
G2	86.3(75.6–99.2)	13.7	—	
G3	82.1(59.1–98.5)	17.9	—	

Numbers in parentheses show the maximum and minimum R and S among prawns at the final stage of experiment 1.

Length reproduction ratio: 100- R

Pigment reduction ratio: 100- S

Table 3. Regrowth ratio of uropod (mesial and lateral ramuses) lengths (R) are given as $R = 100 \times (\text{regrown length of left uropod}) / (\text{length of right uncut uropod})$ on average of prawns used

Group	Regrowth Ratio: R (%)	100- R (%)	Recolor Ratio: S (%)	100- S (%)
(Mesial Ramus)				
Control	98.5(95.9–103.9)	1.5	97(92–105)	
G4	97.3(91.2–102.9)	2.7	78(65–100)	22
G5	91.4(85.6–99.0)	8.6	70(57–88)	30
G6	96.0(91.3–102.4)	4.0	64(43–82)	36
(Lateral Ramus)				
Control	99.8(97.8–102.0)	0.2	—	
G4	97.5(93.2–102.1)	2.5	—	
G5	91.9(86.6–97.5)	8.1	—	
G6	93.5(84.6–100.9)	6.5	—	

Numbers in parentheses show the maximum and minimum R and S among prawns at the final stage of experiment 2.

Length reproduction ratio: 100- R

Pigment reduction ratio: 100- S

Fig. 2. Recolor ratio (%) in left mesial ramus with elapsed time after cutting, comparing to uncut right one.

(●) shows the mean ratios for cut mesial ramus by three cutting locations G1, G2 and G3 as shown in Fig. 1, (○) for uncut one as control.

for the prawns of mean $BL = 29.9$ mm (70% recolored on average) and 40.6 mm (64% recolored), while a rather small reduction of 22% on average was estimated for a mean BL of 21.7 mm (G4). Some of the prawns in G4 showed a 100% recolored pigment area in mesial ramus. Therefore, experiment 2 suggested that the large reduction of the recolored pigment area in cut uropods occurred for prawns larger than 29.9 mm (G4) in BL .

When it was assumed that considerable regeneration of the colored pigment area in group G3 did not occur beyond 42% on average such as G2 (Fig. 2), a relationship between BL and S could be given for the range of BL from about 20 to 70 mm, as shown in Fig. 3. From the regression line ($r = 0.998$), 30% and 50% reductions of the recolored pigment area were estimated approximately for prawns of 30 mm and 60 mm BL , respectively.

Of the 3,733 marked prawns, a total of 89 individuals (2.4% recapture ratio) were recaptured by fishermen in one year (October 1996 to September 1997) after releasing. The BL of recaptured prawns ranged from 120 to 190 mm (Fig. 4). Though there appeared to be a variety of recolored pigment areas in uropods among individuals as shown in laboratory experiments (Tables 2 and 3), fishermen could detect the marked prawns when removing prawns from a gill net on board.

Fig. 3. Plots of mean recolor ratios in cut whole mesial ramus against the three sizes of Kuruma prawns. The ratio of G3 (●) was at 10 weeks after cutting and the others (○; G4, G5 and G6) were at 34 weeks.

The four plots show that the reductions of recolored pigment area increased with the increase of prawn size ($r=0.998$) ranging from 20 to 70 mm BL.

Discussion

For a large field sample of Kuruma prawn, the marking method must be much easier to use and much more economical than the trace element method,¹⁾ staining method⁵⁾ and other internal marker method.^{6,7)}

Moreover, external marks (clipping⁶⁾ and cutting some parts of shrimp and prawn) and tags (Petersen and Atkins types) should be long-lasting after repeated molting, and should be visually detectable on board or at prawn landing places. Recently, in Kuruma prawn ranching projects, gold and stainless wire tags^{3,4)} and ribbon tag^{*4,*5)} are used in field studies. The former tag, however, cannot be visually detected; rather it is detected by a soft X-ray detector, and so is not suitable for a large field sample. Moreover it is considered unsuitable for edible species such as Kuruma prawns which are caught for human consumption. The latter tag type unavoidably causes morphological and behavioral damage during molt. Also, it was reported that these tags are lost due to frequent molting of prawns of up to commercial size.⁷⁾

Kurata and Ishioka^{*3)} and Kurata²⁾ found that the UL of cut uropods of Kuruma prawn regrew mostly to the length of uncut uropods after three or four successive molts in a month and concluded that the length of regenerated uropods (UL) after cutting could not be used as a long-lasting mark. Furthermore, they focused not on the reduction of the colored pigment area in uropods because the BL of

Fig. 4. Frequency distributions of the body lengths (BL) of Kuruma prawns released in August 1996 and recaptured during the period from October 1996 to September 1997.

All the prawns were marked by cutting left uropods as shown by G3 in Fig. 1. The number of prawns measured was 100 of 3,373 individuals at release and 35 of 85 individuals at recapture, respectively.

prawns used was too small to detect the reduction. As a temporary measure for visually detecting marked prawns, however, rather short uropods viewed along side uncut ones might be useful. The marking method by cutting uropods allows thousands of prawns to be marked and released to fishing grounds, but is not useful for identifying individual prawns. Recently in ranching projects of Kuruma prawn, prawns larger than 30 mm in BL have been used in the hope that the released prawns can recruit into a catchable stock.^{8,9)} Therefore, this method for prawns of BL > 30 mm is suitable for field studies on population size, density and dispersal or migration after releasing, as severe adverse effects on the survival of marked prawns were not observed. Care must be taken, however, if this method is used for a large amount of small prawns of BL < 30 mm, because severe effects on the survival of marked prawns are likely to occur due to the handling roughness caused by hand and eye fatigue at cutting of small uropods, and the difficulty of detecting the recolored pigment area.

The releasing experiment in August 1996 and recaptures during the period from October 1996 to September 1997 confirmed the usefulness of using this marking method for a large field sample. However the recapture ratio should be recollected based on the recolor ratios of each BL class. Namely, prawns smaller than BL = 40 mm can recover 64% dark-red pigment in cut mesial ramus and probably prawns larger than BL = 60 mm show recolor ratios lower

than 50%. Therefore, it seems that there is higher detectability in released large prawns than in small ones.

As shown in Fig. 3, when prawns of about $BL=60$ mm are released after cutting the uropods, a 50% reduction of the colored pigment area is expected. In July 1995, a total of 1,680 prawns of mean $BL=65$ mm were released using the uropods cutting method as shown in G3 (unpublished). Eight prawns ranging from 180 to 197 mm for males and from 203 to 240 mm for females were recaptured by fishermen during the period from April to June 1997, around 2 years later. This provides strong proof that the reduction of colored pigment area by cutting uropods is one of the most desirable methods as a long-lasting mark for Kuruma prawn. As might be suspected, in addition to the prawns recaptured after two years, further recaptures of marked prawns released in 1995 are expected.

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