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Marking of tiger shrimp *Penaeus monodon* (Fabricius) juveniles: Comparison among inexpensive tagging options

Jon P. ALTAMIRANO* and Hisashi KUROKURA

Abstract: Three low-cost marking methods (staining with food color, uropod trimming, and T-bar tagging) were tested on 2-mo old juvenile tiger shrimps *Penaeus monodon* with 8.42 ± 0.1 mm mean carapace length (CL) for 8 weeks. Together with an unmarked control group, marker retention and effects on shrimp's survival and growth were monitored. There was no significant difference in specific CL growth rates among treatments ($p>0.05$). Survival was not significantly different among staining (60%), uropod trimming (51%), and control (58%) throughout the experiment, but T-bar tagging showed significantly lower survival from week 6 until 8 (33%) ($p<0.05$). Marker retention was significantly highest for T-bar tags (100%) at the end of 8 weeks, followed by uropod trimming at 65%, while staining was already 0% from week 3. Food color stains are poor shrimp markers because of weak retention. T-bar tags are not effective markers despite having excellent tag retention because of low shrimp survival after 6 weeks. Uropod trimming is a more practical option given that shrimps showed comparable survival with the control group and marker distinction through unique uropod regrowth was relatively high. Further work on modifications on these methods is needed to increase efficiency while maintaining lower cost. This study is viewed to have practical applications for community-based shrimp stock enhancement monitoring.

Keywords: *molting, shrimp tagging, stock enhancement, tag retention*

1. Introduction

World capture fisheries is seen to have become static in the recent years and signs of decline have been reported in some countries (FAO-FIES, 2008; FAO, 2009). In the Philippines, artisanal fish capture production quality and quantity have declined in the past years from various fishing grounds (MINES *et al.*, 1986; LIM *et al.*, 1995; KATON *et al.*, 1998; EVASCO, 2000; FERNANDEZ *et al.*, 2000; PALMA *et al.*, 2002; NEDA, 2005). Specifically for example, the Batan Estuary in the northern Panay Island in central Philippines was known for abundant wild shrimps, including the highly-priced tiger shrimp *Penaeus monodon,* but decreasing catch in the recent decades has become very evident (ALTAMIRANO, 2007; ALTAMIRANO and KUROKURA, 2008). The steady loss and overexploitation of local fishery resources directly affects the livelihood of the already poor fishers.

Shrimp restocking can be an effective tool in enhancing wild stocks (DAVENPORT *et al.*, 1999; WANG *et al.*, 2006) and, at the same time, in alleviating poverty among subsistence fishers through a direct increase in shrimp catches (ALTAMIRANO and KUROKURA, 2008). While the practice of fish stock enhancement has been done since the 1960s, crustacean stock enhancement programs especially for shrimps, have been limited (BELL *et al.*, 2005). For example, shrimp restocking projects were implemented for *Penaeus japonicus* in Japan (HAMASAKI

* Corresponding author:
Department of Global Agricultural Sciences, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan.
Tel.: +81-3-5841-8115; Fax: +81-3-5841-5189; Email: jonalta@gmail.com

Table 1. Some studies on different marking and tagging methods for crustaceans.

Species[1]	Marker or Tag	Setup (Duration)	Size (mm CL)	Survival (%)	Retention (%)	Reference
Neocaridina denticulate (S)	Laboratory	Trypan blue/red	15-30 BL[2]			Niwa *et al.*, 1998
Penaeus monodon (S)	Tank (5-6 mo)	Streamer	15	31-35	100	Benzie *et al.*, 1995
			18	37-70	100	
	Pond (5-6 mo)	Streamer	15	30-44		
			18	44-59		
P. esculentus (S)	Tank (˜2 mo)	Streamer	12-19	˜80		Hill and Wassenberg, 1985
P. plebejus (S)	Pond (2 mo)	Streamer	17-27	35-48		Montgomery and Gray, 1991
P. monodon (S)	Tank (2 mo)	Streamer	16-18	90		Primavera and Caballero, 1992
			18-21	47		
P. esculenlus (S)	Tank (2-3 mo)	Streamer	12-17	75-90		Wassenberg and Kerr, 1990
			15-20	90-93		
P. merguiensis(S)	Tank (2-3 mo)	Streamer	11-17	85-100		
Munida rugosa (L)	Tank (2 mo)	Streamer	<27 to >32	29	100	Claverie and Smith, 2007
		T-bar tags		52	100	
		V.I. Elastomer		95	100	
Macrobrachium rosenbergii (S)	Tank (2.5 mo)	V.I. Elastomer	0.01 BW[3]	100	79	Brown *et al.*, 2003
		V.I. Alpha	<5.5 BW[3]	˜99	60	
Jesus verreauxi (S)	Tank (37 mo)	T-anchor, dart	90-104	43-51	78-82	Montgomery and Brett, 1996
		Uropod trim		47	77	
Homarus gammarus (L)	Trays (3 molts)	Micro wire	5-8	82	97	Linnane and Mercer, 1998
			12-16	97	97	
		V.I. Elastomer	5-8	68	100	
			12-16	97	100	
		Rostrum cut	5-8	80	0	
			12-16	99	0	
		Branding	12-16	57	0	
			16-19	90	0	
		Streamer	16-19	97	100	
P. vannamei (S)	Tanks (3 mo)	V.I. Elastomer	<3.92BW[3]	99-100	91-93	Godin *et al.*, 1996
			21-47BW[3]	100	93	
Litopenaeus vannamei (S)	Tank (˜2 mo)	V.I. Alpha	2.7 BW[3]	81-92	92-98	Arce *et al.*, 2003
			21.5 BW[3]	68-74	80-83	
	Pond (˜4 mo)		2.7 BW[3]	90-93	93-97	
			21.5 BW[3]	80-88	83-85	
Callinectes sapidus (C)	Lab (1.5 mo)	V.I. Elastomer	6-25	˜58-60	˜81-84	Davis *et al.*, 2004
		Micro wire	6-25	˜47-60	˜92-93	
Litopenaeus setiferus (S)	Tank (1 mo)	Micro wire	30-90 BL[2]	81-100	95-100	Kneib and Huggler, 2001
Litopenaeus vannamei (S)	Lab (4 mo)	Uropod cut	13-16	˜98	0	Leano and Liao, 2006
			28-29	˜63	˜88	
		Uropod trim	13-16	˜99	˜91	
			28-29	˜53	100	
Penaeus japonicus (S)	Lab (˜2.5 mo)	Uropod cut	21-73 BL[2]	92	96	Miyajima *et al.*, 1999
	Sea (12 mo)	Uropod cut	35-75 BL[2]		2.4[4]	

1 (S) =shrimps, (L) =lobster, (C) =crab; 2 BL=Body Length (mm) ; 3BW=Body Weight (g) ; 4 recaptured

and KITADA, 2006), *Penaeus chinensis* in China (WANG *et al.*, 2006), *Penaeus monodon* in Taiwan (SU and LIAO, 1999) and Sri Lanka (DAVENPORT *et al.*, 1999), and *Penaeus esculentus* in Australia (LONERAGAN *et al.*, 2006). Although various methods and technology have been applied, the problems in monitoring still remain to be a challenge, even for a financially-capable community.

To evaluate the success or failure of shrimp

restocking initiatives, an efficient method of marking shrimps is important in sorting recaptured stocks from wild catch. The main challenge for shrimp tagging is the difficulty imposed by molting. A number of marking options for shrimps have been tested and improved since the late 1950s, to include various modes of staining, internal or external tags, and cutting of body parts (NEAL, 1969; FARMER, 1981). Table 1 summarizes some of the studies on marking and tagging of crustaceans, especially shrimps. Most of these studies use sub-adults and larger juveniles, requiring longer pre-release culture that increases cost. On the other hand, other mass shrimp restocking programs use post-larvae shrimps that cannot be marked because of their very small size. Therefore, shrimps that are to be released should be big enough to be tagged but should not require long culture periods. In this study, juvenile shrimps of about 10 mm CL or about 60 days old were marked.

The main concern in choosing a marking method or tag is its efficiency in terms of retention and minimal effects on the commodity's growth, movement and survival. It has been recommended that visual implant elastomers (VIE) and micro wire tags are the more viable options for crustaceans (GODIN *et al.*, 1996; LINNANE and MERCER, 1998; DAVIS *et al.*, 2004); however, the main limitation for these tags is the cost required for its use. Except those involving ablation of body parts, majority of shrimp marking and tagging studies use specialized branded materials that are expensive.

In addition, stock-enhancement programs are also viewed to have better efficiency when implemented and managed by its direct beneficiaries — the local communities (GARAWAY *et al.*, 2006). Although community-based stock enhancement may have positive prospects, its implementation is mostly restricted financially. Therefore, for local stock enhancement programs, the availability and cost of tags and methods are also very important considerations.

In this study, we attempted to use and compare low-cost and readily available options for tagging and marking small juvenile shrimps *P. monodon*. Instead of specialized stains, we opted to use ordinary natural food color (McCormick and Company, Inc., Maryland) that has also been described in some reports and applied to stain other taxa like nematodes (THIES *et al.*, 2002). Ordinary plastic T-bar tags (Bano'k, Japan), commonly used in tagging clothing merchandise, were also tested as a possible substitute for streamer tags and specialized T-bar tags (Hallprint Pty Ltd., Australia). Uropod trimming, discussed by various authors (MIYAJIMA *et al.*, 1999; TOYOTA *et al.*, 2003; LEANO and LIAO, 2006), was also tested because it involves no additional costs for materials.

2. Materials and Methods

This experiment was a part of a series of studies on the stock enhancement of tiger shrimp *Penaeus monodon* in the Batan Estuary, Aklan, Philippines led by the Laboratory of Global Fisheries Science, the University of Tokyo, Japan. This study was conducted at the Brackishwater Aquaculture Station of the College of Fisheries and Marine Science (CFMS), Aklan State University, New Washington, Aklan, Philippines.

2.1. Set-up and monitoring

Hatchery-bred tiger shrimp *P. monodon* porstlarvae (PL15) were acquired (approx. 1000 pcs) from a local hatchery (wild broodstock were sourced locally). These were reared until 2 mo old in hapa nets inside an open pond with half-opened gates to allow regular water change from a nearby creek. In a separate experiment, 2-month old juveniles were observed to be the optimal age of shrimps for release in the immediate local water conditions of the Batan Estuary. At this age, the study showed that *P. monodon* juveniles have a carapace length of about 8-10 mm. From the reared stocks, shrimp juveniles measuring 8.42 $\pm$ 0.1 (s.e.) mm mean carapace length (CL) and weighing 3.4 $\pm$ 0.02 (s.e.) mg mean body weight (BW) were used in the experiment. A total of 12 aquaria (140 L) were prepared with washed fine sand as substrate (2 cm deep) then filled with filtered water (120 L) from the same rearing pond and creek. Water from each aquarium was drained by 10% daily using a

siphon tube. Water was refilled through individual distribution pipes connected to a central holding tank where the filtered source water was collected daily, aided by a submersible electric pump. Constant aeration was supplied using airstones powered by an electric blower. There were three replicate aquaria for each marking treatment, namely: staining, uropod trimming, and T-bar tagging. Another three aquaria were designated as unmarked control replicates. These 12 aquaria were placed adjacent to a wide window in the laboratory to allow natural diel cycles, arranged in one row and alternating among treatments.

Prior to the actual experiment, tagging trials were done as practice runs for each tagging method to achieve minimal time in marking and to reduce stress of shrimps while out of the water. In the final run, twenty shrimp juveniles were tagged/marked accordingly (as described below) and placed in each aquarium for a day to acclimate. The same number was also stocked untagged in each control aquaria. Few dead shrimps, as a possible result of tagging shock or acclimation after about 24 h was noted as immediate mortality and removed. Those that survived were further randomly reduced to 15 individuals per aquarium as the final number used in this study for the next 8 weeks. Visual observations were carried out daily. Monitoring for growth was conducted weekly in terms of carapace length measured with a digital caliper, and wet body weight using a digital top loading scale (0.01 g sensitivity). Shrimp's survival and mortality were assessed from weekly total count. Examination of marker retention and/or visibility was also done every week. The experiment lasted for 8 weeks, from 20 April to 15 June, 2008.

Finely chopped fish meat was provided as feed daily (1800 H) *ad libitum*. Uneaten food and unwanted particulates in the aquaria were siphoned-out daily during every water change. Water quality data (salinity, temperature, DO, pH) were monitored daily (0900–1000 H) using a Horiba UX-21 multi-parameter probe. A data logger (ACT-HR, Alec Electronics, Japan) was also used to monitor a detailed (10 min interval) trend in salinity and temperature.

2.2. Staining

Biological staining has been used to mark shrimps since the 1950s (MENZEL, 1955; NEAL, 1969) and has evolved into using some expensive patented materials. In this experiment, however, the cost of staining agent must be affordable and locally available. Hence, the common natural food color (MCCORMICK) was tested. Using a small (6.35 mm) sterile tuberculin syringe and a 30-gauge needle, a small amount (about 0.01 ml) of red food color was injected ventrally through the articular membrane of the shrimp between the 1st and 2nd abdominal segments. Stained shrimps were placed in a holding pan with aeration for about 20 min. Stains spread beneath the exoskeleton and mostly accumulate in the gills. Only those that appeared stable and survived the initial shock of staining were replaced in each aquarium (20 shrimps aquarium^{-1}) for a day of acclimation. Then, only the final number of 15 individuals was retained per aquarium for monitoring throughout the experiment.

2.3. Uropod trimming

Trimming of shrimp's uropod was chosen rather than complete removal of uropod. Trimmed uropod usually creates unique regrowths after molting that can be used as a marking indicator (LEANO and LIAO, 2006). Trimming was done using a pair of small fine-tip surgical scissors. Half of the left outer uropod was cut, adapting the methods described by TOYOTA *et al.* (2003) and LEANO and LIAO (2006). Trimmed shrimps were placed in an aerated collection pan for a few minutes before replacing in their respective aquarium. After given about 24 h to stabilize and acclimate, the number of individuals in each aquarium was reduced to 15, setting day zero of the experiment. Observations and monitoring proceeded as described above.

2.4. T-bar tagging

Physically-attached tags have been extensively used for stock enhancement monitoring purposes and a number of these have been utilized for tagging crustaceans, including shrimps (NEAL, 1969; HOWE and HOYT, 1982; HILL and WASSENBERG, 1985; TEBOUL, 1993;

LINNANE and MERCER, 1998; ARCE *et al.*, 2003; BROWN *et al.*, 2003; CLAVERIE and SMITH, 2007). Most of these special tags however, require considerable capital to acquire, making it impractical for low-budget studies. In this experiment, ordinary T-bar tags, commonly used in clothing's price label, were used. The plastic T-bar tags were about 0.3 mm thick and 11 mm long, giving enough room for shrimp's growth until marketable size. Specifically, the Bano'k 303XL T-bar injector gun was chosen because of its thin and long injector needle. This injector gun drives the T-bar tags from a set of cartridges.

A number of tagging locations have been used for physical tags, such as a dorso-ventral insertion of T-bar tags between the cephalothrax and 1st abdominal segment for squat lobsters *M. rugosa* (CLAVERIE and SMITH, 2007), and horizontal tagging of streamer tags between abdominal segments on *P. monodon* (BENZIE *et al.*, 1995) primarily to minimize difficulty of shedding exuviae during molting. However, the present study adapted the methods for streamer tags on *P. monodon* by WASSENBERG and KERR (1990) and PRIMAVERA and CABALLERO (1992) where the tag was inserted laterally through the left side of the middle of the 2nd abdominal segment, exiting and of the body. This tagging location provided the least resistance for movement of the shrimps. WASSENBERG and KERR (1990) also found that this location has the fastest wound healing effect. The T-bar tag was injected through the guide needle with a single squeeze of the injector gun, carefully avoiding further tissue damage, and was slowly removed after the tag was locked in place. Same as the other treatments, T-bar tagged shrimps were held in aerated pans before replacing in the aquaria. The final number of 15 individuals was maintained a day after tagging and monitored throughout the experiment.

2.5. Data analysis

Water quality data (water pH, DO, temperature and salinity) were subjected to two-way analysis of variance (ANOVA) with marking treatment and measurement week as fixed factors.

Initial and weekly growth parameters in terms of CL and BW, as well as total count of surviving shrimps, were recorded to determine growth and survival rates, respectively. Weekly specific growth rates (SGR) were computed for CL and BW using the formula adapted from YE *et al.* (2009):

$$\text{SGR } (\% \text{ d}^{-1}) = 100 \times (\ln x_t - \ln xi) / d ,$$

where SGR is Specific Growth Rate for CL (%CL d^{-1}) or BW (%BW d^{-1}), xi is initial measurement for CL or BW, x_t= measurement for CL or BW at a given time t, and d = number of days between measurements. A similar measure for SGR was also used by PRIMAVERA and CABALLERO (1992) to present CL and BW growth rates of *P. monodon* marked with streamers tags.

Survival was represented as a percentage of remaining shrimps from the initial 15 individuals during each weekly monitoring. Marker retention and visibility was examined and recorded as a percentage from among the surviving shrimps per monitoring week.

Data were initially tested for normality using the Shapiro-Wilk test, and for homoscedasticity using Levene's test. Percentage data were further transformed to arcsine prior analyses to improve homogeneity of variances (ZAR, 1999). Then, data analysis proceeded with ANOVA. When significant differences were detected, further analyses with Tukey's Honestly Significant Difference (HSD) multiple comparison tests were performed. Comparisons were made to determine differences among treatments and control on the survival and growth of shrimps, as well as on the tag retention. Significance was established at $p<0.05$. Statistical analyses were performed with SPSS statistical software version 14 (SPSS Inc., Chicago, IL).

3. Results

3.1. Water condition

Water pH, dissolved oxygen (DO), salinity and temperature were constantly monitored and showed no significant differences among treatments on every monitoring week (two-way ANOVA, treatment* week, $p>0.05$). Average measurements (mean ± s.d.) of these are as follows: pH, 7.77±0.89; DO, 3.83±0.66 mg

L^{-1}; salinity, 18.80 ± 1.77; and temperature, 26.96 ± 2.59 ℃. Since aeration was sustained and water exchange was maintained, DO was relatively stable throughout the experiment, as well as pH. Some fluctuations in temperature and salinity were recorded by the data logger especially during the sustained rains in May but no drastic changes occurred that may have affected the shrimps.

3.2. Shrimp's growth

Specific growth rates in terms of % CL d^{-1} showed no significant differences among treatments and control within the 8-week period (ANOVA, $p>0.05$) (Fig. 1).

The trend in CL changes between monitoring weeks suggests two molting stages within 8 weeks (Fig. 1). Results confirmed rapid molting and growth within the first 2 weeks after marking, reaching an overall average CL change of 0.75 ± 0.03 %CL d^{-1} after week 1 and 1.29 ± 0.14 %CL d^{-1} on week 2. Observations also indicated that about 75% of the experimental shrimps molted within these first two weeks. On week 3, zero growth rate was recorded which was significantly lower (ANOVA, Tukey's HSD, $p<0.05$) than the previous week indicative of intermolt period (Fig. 1). After week 3, an increasing growth rate was again manifested until week 6 (0.69 ± 0.11 %CL d^{-1}), highlighting the second molt stage. Although less pronounced and statistically not significant (ANOVA, Tukey's HSD, $p>0.05$), another carapace rigidity stage followed on week 7 as denoted by another zero average specific growth rate (Fig. 1). A slight increase ini-

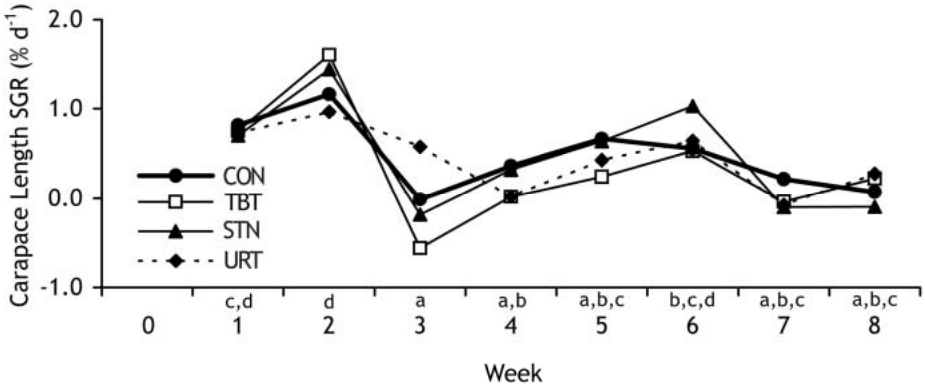

Fig. 1. Specific growth rates (SGR) in terms of carapace length (%CL d^{-1}) of 2-mo old *Penaeus monodon* juveniles marked through staining (STN), T-bar tagging (TBT), and uropod trimming (URT) together with untagged control (CON) within 8 weeks. Molting periods are shown, terminating during the carapace rigidity stages (zero growth) at weeks 3, then week 7. Similar superscripts among weeks are not significantly different (ANOVA, $p>0.05$). Also, values among marking methods were not found to be significantly different.

Table 2. Specific growth rate (SGR) in terms of BW (mean ± s.e., %BW d^{-1}) of 2-mo old *Penaeus monodon* juveniles marked through staining (STN), T-bar tagging (TBT), and uropod trimming (URT), including untagged control (CON) within 8 weeks.

Week	CON	STN	URT	TB T
1	3.45 ± 0.38	3.72 ± 0.73	3.57 ± 0.34	3.14 ± 0.52
2	1.80 ± 0.24	0.58 ± 0.45*	3.41 ± 0.26	1.43 ± 0.22
3	2.75 ± 0.35	2.28 ± 0.29	1.44 ± 0.22	2.07 ± 0.38
4	1.48 ± 0.27	0.58 ± 0.49	2.23 ± 0.72	2.50 ± 0.28
5	1.37 ± 0.30	0.82 ± 1.12	0.35 ± 0.57	1.01 ± 0.68
6	0.42 ± 0.46	−0.51 ± 0.73	1.17 ± 0.92	1.50 ± 0.18
7	0.73 ± 0.46	−0.79 ± 1.00	0.83 ± 0.33	−0.49 ± 0.45
8	0.75 ± 0.33	1.02 ± 0.47	−0.08 ± 1.04	0.82 ± 0.23

* significant difference ($p<0.05$) from among treatments on the same week.

tiated again on week 8 (0.12 ± 0.08 %CL d^{-1}) that may have been the start of the 3rd molting cycle.

Specific growth rates in terms of %BW d^{-1} showed a generally decreasing trend, from an overall mean of 3.47 ± 0.12 %BW d^{-1} during the first week to only 0.63 ± 0.24 %BW d^{-1} during the last week (Table 2). Generally, no significant differences in BW-SGR were found among treatments and control throughout the study period, except only for staining at week 2.

3.3. Shrimp's survival

The small initial size (mean CL±s.e., 8.42 ± 0.1 mm; mean BW±s.e., 3.4 ± 0.02 mg) of shrimp juveniles was a challenge for tagging. As a result, high immediate mortality was observed especially for staining and T-bar tagging during the practice runs as a direct effect of mishandling. However, tagging efficiency greatly increased after familiarization of the tagging procedure. During the actual experiment, immediate mortality (<1 hr after marking) was very low even for T-bar tagging (1 to 3 individuals).

Survival steadily declined and was not significantly different (ANOVA, $p>0.05$) among treatments up to week 5 (control, 84%; staining, 73%; uropod trimming, 71%) (Fig. 2). Although similarly not significantly different from other treatments, T-bar tagging showed a lower survival of 67% after 5 weeks. The decrease in survival continued with similar trend until week 8 for control (58%), staining (60%), and uropod trimming (51%). However, T-bar tagging showed significantly lower (ANOVA, Tukey's HSD, $p<0.05$) survival rates of shrimps from week 6 (47%) until week 8 (33%).

3.4. Marker retention

Marker retention from survived shrimps was significantly highest (ANOVA, Tukey's HSD, $P<0.05$) up to the last week for T-bar tags, where 100% were retained throughout the 8-week experiment (Fig. 3). However, visibility of trimmed uropods steadily declined until 65% at the end of 8 weeks, while only 14% of stained shrimps were identified as having been marked after the first week and none showed signs of staining from week 3.

For uropod trimming, marker retention was determined through abnormal regrowth of the trimmed portion (Fig. 4). The decline in marker visibility for this method was caused by normal regeneration of uropods after molting on some shrimps. In weeks 2 and 3, after the first molt, 88% of the survived shrimps (14 out of 15 individuals) showed identifiable abnormal uropod regrowths; while from week 4 until week 7 (after the second molt), only 75% of the 10 surviving shrimps from each aquarium retained abnormal uropod regrowth. Lastly, at week 8, only 65% were recognizable as having had a trimmed uropod. For this method, it is important that the observer be properly trained to differentiate an untrimmed

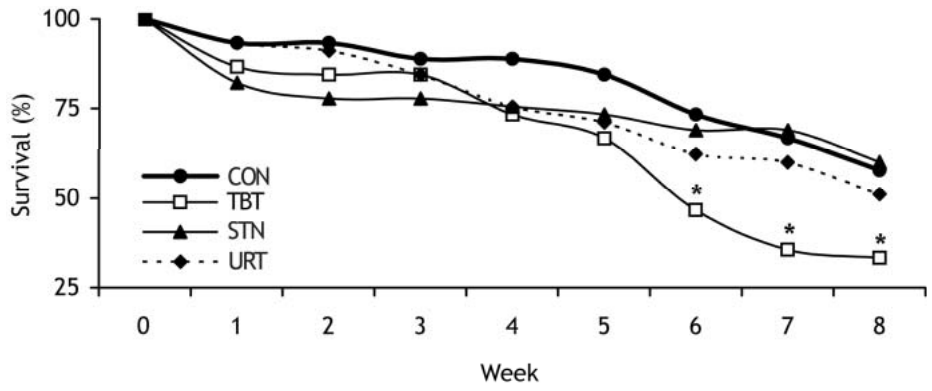

Fig. 2. Survival (%) of *Penaeus monodon* juveniles marked through staining (STN), T-bar tagging (TBT), and uropod trimming (URT), including untagged control (CON) within 8 weeks. Asterisks (*) denote significant difference from among other treatments in the same monitoring week (ANOVA, $p<0.05$).

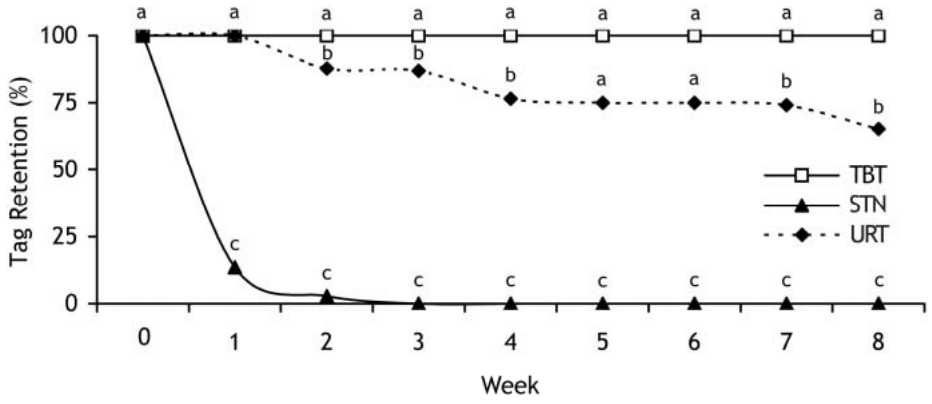

Fig. 3. Tag retention/visibility (%) from survived *Penaeus monodon* juveniles marked through staining (STN), T-bar tagging (TBT), and uropod trimming (URT), including untagged control (CON) within 8 weeks of laboratory experiment. Similar superscripts denote no significant difference among treatments in the same monitoring week (ANOVA, $p>0.05$).

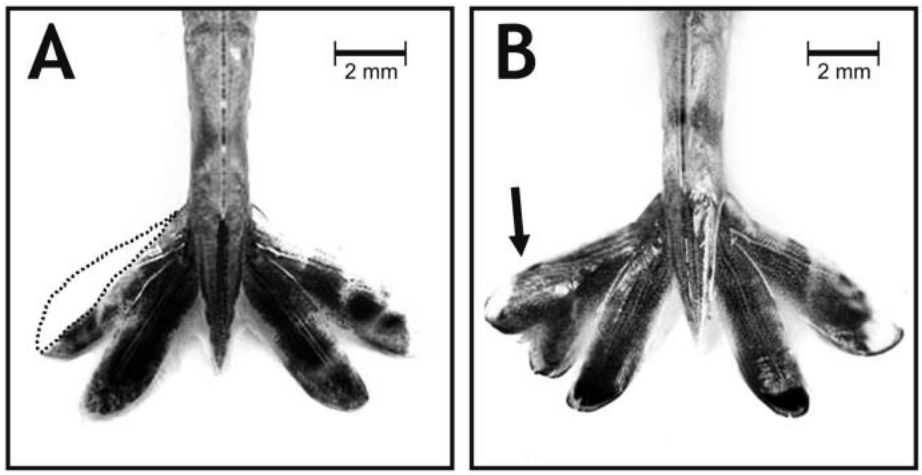

Fig. 4. (A) *P. monodon* juvenile marked by uropod trimming (trimmed portion represented by dotted line). (B) After molting, abnormal regrowth from the trimmed uropod can be observed (arrow).

normal uropod and that of the regenerated uropod and that careful scrutiny is necessary during monitoring.

On the other hand, staining showed very significantly lower (ANOVA, Tukey's HSD, $P<0.01$) marker retention rate than the other methods even on the first week where only 14% of survivors were able to retain the red food color stain, mostly in the gills. At week 2, only 3% were observed to have a tinge of red in their body. Then, from week 3 onwards, no identifiable stain marks were observable from the experimental subjects.

4. Discussion

Although some studies already recommended the use of more expensive visual implant elastomers (VIE) and micro wire tags for crustaceans (Godin *et al.*, 1996; Linnane and Mercer, 1998; Davis *et al.*, 2004; Lebata *et al.*, 2009), the current study on the other hand, evaluated the utility of more economical options for marking shrimps. Three marking categories were tested in this experiment: staining, tagging with physical tags, and ablation of body parts. The use of common food color as a staining agent and tagging with

ordinary plastic T-bar tags have never been mentioned in published literature, especially for small shrimp juveniles.

Various stains such as trypan blue (Hartman-Leddon Company, Philadelphia) and similar others have been injected to accumulate in the gills of shrimps such as *Neocaridina denticula* (NIWA *et al.*, 1998). Literature search of current studies on shrimp marking by staining returned no results. In a review by NEAL (1969), he mentioned that stains appeared to have less utility as these tend to fade after a few days although, in some cases, it can last for about a week. The results in the current study also confirmed this observation. Only 14% of shrimps retained their stain after one week and were completely faded starting the third week. However, stains have practical applications for laboratory studies of a few days. The current experiment also showed that even ordinary food color can have the same short-term effectiveness as that of more expensive stains although a direct comparative experiment is needed to really prove this. In addition, proper staining with natural food color seemed to have very minimal effect on shrimp survival and growth, where current results for staining were not significantly different from the control group (Figs. 1, 2).

Physical cutting or modifying body parts such as rostrum ablation in lobsters were tested by LINNANE and MERCER (1998) but complete regeneration was only as short as three molts. Trimming of uropods in shrimps, however, showed higher retention rates since molting often resulted in distinct abnormal growths from cut or trimmed uropod in *Litopenaeus vannamei and Penaeus japonicus* (MIYAJIMA *et al.*, 1999; TOYOTA *et al.*, 2003; LEANO and LIAO, 2006). Similar results were found in the present study for *P. monodon* juveniles. Abnormal regrowths were noted, often characterized by a branched protruding extension from the trimmed uropod (Fig. 4). The first molt and regeneration was observed within 1 week after trimming which was similar with that of *L. vannamei* (LEANO and LIAO, 2006). Aside from unique regrowth, MIYAJIMA *et al.* (1999) also mentioned noticeable differences on coloration of the regenerated uropod of bigger (around 60 mm BL) *P. japonicus* but this was not clearly observed in our samples, probably because of relatively smaller sizes. Survival of shrimps for this method was high, reaching 98% for juvenile *L. vannamei* after 4 mo (LEANO and LIAO, 2006). In the present study, survival for uropod trimming (51%) was not significantly different from the control group (58%) after the 8-week experiment. This suggests that the relatively low survival rate may be attributed to external factors such as water quality and natural mortality, rather than a direct effect of the marking method. One noticeable immediate effect of uropod trimming on shrimps was the initial difficulty in swimming. Shrimps were observed to swim sideways because of the unbalanced trimmed uropods. However, after a few hours, some were able to correct their balance and swim upright. It is therefore recommended to allow the uropod-trimmed shrimps to stabilize for a few hours or days before actual release in the open to minimize possible susceptibility from predation.

Through-the-body external markers were widely used, especially the streamer tags, noting that larger shrimps have lower short-term or immediate mortality rate than smaller juveniles (HILL and WASSENBERG, 1985; WASSENBERG and KERR, 1990; MONTGOMERY and GRAY, 1991; PRIMAVERA and CABALLERO, 1992; BENZIE *et al.*, 1995). Anchor tags or T-bar tags are smaller; hence, short-term mortality was significantly lower than streamer tags as used in squat lobster *Munida rugosa* (CLAVERIE and SMITH, 2007) and *Jesus verreauxi* (MONTGOMERY and BRETT, 1996). The main advantages of external tags are high retention rates and ease in tag detection. In the present study, observations for marker retention and/or visibility were easiest for T-bar tags because of the externally protruding plastic were easy to spot. Marker retention for this method was 100% from the survived shrimps in the present study. This high retention (100%) of the similar T-bar or T-anchor tags was also true for *M. rugosa* after 2 mo (CLAVERIE and SMITH, 2007), while 78–82% was retained for shrimp *J. verreauxi* after a long-term study of 37 mo (MONTGOMERY and BRETT, 1996).

One concern in having tags inserted through the body is the negative effect during molting. LINNANE and MERCER (1998) observed that cast exoskeleton frequently became entangled on the streamer tags during molting of *Homarus gammarus* and that tagged shrimps needed more flicks to discard old exoskeleton on *P. esculentus* (BENZIE *et al.*, 1995). However, this effect seems to be only minimal. BENZIE *et al.* (1995) also showed that time to complete molting was not affected, while molting and general growth rate was also not significantly different between streamer-tagged and untagged shrimps (HILL and WASSENBERG, 1985; BROWN *et al.*, 2003). The same observations were also noted in the present study for *P. monodon* juveniles, where exuviae sometimes get entwined with the T-bar tag; however, growth rates and intermolt periods were still not significantly different than the control group or other marking methods (Fig. 1, Table 2.). *P. monodon* has been known to molt at an average of every 5 days during post-larval stage and about every 14 days for sub-adults (KIBRIA, 1993). For this reason, longer studies and actual release experiments employing through-the-body markers like T-bar tags may eventually have adverse effects on survival after these multiple moltings. As shown in the current study, the main drawback in using T-bar tags was in the survival of shrimps. A significantly lower survival (47%) was recorded for T-bar tagging after 6 weeks compared with other treatments (62–73%) (Fig. 2). This lower survival was estimated to roughly coincide with the second molting of the shrimps at about 6 weeks although no further confirmation was conducted. Other studies using a specialized T-bar or T-anchor tags produced the same results, such as for *M. rugosa* with 52% survival after 2 mo (CLAVERIE and SMITH, 2007), and *J. verreauxi* with 43–51% after 37 mo (MONTGOMERY and BRETT, 1996). These high mortalities, especially after a month or so can be the effects of delayed infection and some studies applying antibiotics did not seem to have any major advantage (NEAL, 1969). WASSENBERG and KERR (1990) also observed that wounds inflicted by the inserted tags and its constant movement hinders the quick healing of the injuries and thereby increasing the chances for irritation and infection. High mortalities were also recorded during tagging of smaller shrimps (>10 mm CL) in our experiment during the practice tagging runs. During the actual experiment; however, survival rates were comparable with the control group in the first 5 weeks. For *P. monodon,* the minimum size of juveniles for T-bar tagging was established to be about 9–10 mm CL in this study but smaller shrimps where also successfully tagged. WASSENBERG and KERR (1990) even estimated a higher critical size limit for streamer tags for *P. esculentus* (18 mm CL) and *P. merguiensis* (17 mm CL). This means that T-bar tags, streamer tags, and similar through-the-body tags may only be practically used on larger juveniles or sub-adults unless significant modifications on the tag size or applicator device can be developed. However, T-bar tags have practical applications for restocking studies in smaller areas that expect full recapture within a month or so.

Although the present study was done in a laboratory, natural water conditions were attempted to be simulated. Hence, only creek water was used with no other treatments made aside from filtration. In effect, changes in natural water conditions were also manifested in the aquaria through daily water exchanges. Water condition is also an important factor that affects shrimps growth and survival. At 30℃, JACKSON and WANG (1998) showed that *P. monodon* can grow double in weight than that at 20℃ within 180 d. Abrupt changes in salinity also affects mortality although younger prawns (< 40 days post-metamorphosis) can tolerate short acclimation periods of 6 h to 3 days and can survive in low salinity water better than older animals (CAWTHORNE *et al.*, 1983). Some fluctuations in both temperature and salinity were observed during the study but these were still within tolerable ranges and were not observed to have drastic effects on the shrimps. Dissolved oxygen was also far from the lethal level of 0.9 mg L^{-1} (ALLAN and MAGUIRE, 1991), as this was maintained at about 4 mg L^{-1} with aeration. Other biological and chemical factors may also have affected the growth and natural mortality of

the shrimps in this study. However, limited by resources, no measurements were done on these aspects. Nevertheless, all treatments were subjected to the same water condition, hence, comparisons among marking treatments and control can still be done, negating other external causes.

Generally, results in the present study showed comparable outcomes with those made with conventional yet more expensive tagging materials, as discussed above. Of course, direct comparisons among different studies cannot be accurately made because of differences in laboratory setup, shrimp's initial age and size, stocking density and water condition. It is interesting to see that shrimp marking activities do not necessarily need proprietary tags and sophisticated equipment to implement. In terms of cost, the most expensive equipment used in our experiment was perhaps the Bano'k 303XL injector gun, purchased for about US$25. Of course, a similar local brand would have even cost less. Other materials like food color and syringe for staining, T-bar cartridges, and small scissors for uropod trimming can be easily and cheaply acquired.

5. Conclusion

Staining is not an effective marker for shrimps because marker retention only lasts for a few days or at most a week or two, limiting its use for very short-term studies. Externally visible tags like T-bar tags have very high retention rates but there is a significant decline on shrimps' survival after about 6 weeks. This maybe used for studies requiring high marker retention but also only limited for about a month. For initial restocking trials in the current site, the Batan Estuary, this method can be applied because tag distinction is fairly easy even for untrained local fishers. Also with the current status of fishing pressure in this small area (ALTAMIRANO, 2007), recapture of released stocks can be expected to be in the order of a few weeks only. On the other hand, the more practical method may as well be the trimming of uropods as this provides both good retention and survival of shrimps even for longer tagging experiments. However, more study is recommended on finer modifications of these methods for applications on specific restocking sites to primarily increase efficiency but with equal consideration on cost of materials and methods for community-based stock enhancement programs.

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