Evaluation of the New Fluorescent Internal Tag (Soft Visible Implant Alphanumeric Tag) in the Freshwater Prawn, *Macrobrachium rosenbergii*

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Abstract

The new version of the polyester visible implant alphanumeric tag (VI Alpha tag; Northwest Marine Technology Inc., Shaw Island, Washington, USA) was evaluated in the giant river prawn (*Macrobrachium rosenbergii*) under laboratory conditions. The new VI Alpha tag is soft and fluorescent with an alphanumeric code designed to identify individual specimens. Two tag sizes - standard (1.0 x 2.5 mm) and large (1.5 x 3.5 mm) - were tested on juveniles (standard size), subadults (standard size), and adults (large size) for 10 weeks. Retention, readability, and effects on growth and survival were evaluated. Final mean tag retention was 71.25±3.3% in juvenile prawns, 91.3%±2.73 in subadult prawns, and 59.0%±1.65 in adult prawns. Final tag readability was 100.0% in juveniles, 88.6±5.3 in subadults, and 77.8±4.8 in adults. Average daily growth (mg/day) and SGR (%/day) of tagged juvenile prawns (22.4±0.23 and 0.81±0.02, respectively) did not significantly differ (p>0.05) from that of the untagged control (20.5±1.47 and 0.83±0.04). Similar results were observed in subadult and adult *M. rosenbergii.* Mean final survival did not significantly differ (p>0.05) between tagged and untagged prawns. Results indicate that tagging *M. rosenbergii* with VI Alpha tags has no adverse effect on survival or growth and can therefore be effectively used to identify individuals in selective breeding programs.

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Introduction

The giant freshwater prawn *Macrobrachium rosenbergii* is widely cultured in many tropical and subtropical countries, especially in South and Southeast Asia. Aquaculture production of this species has dramatically increased from 19,035 tons in 1994 to 205,000 tons in 2005 (FAO, 2007). Despite the potential for increased production, the sustainability of freshwater prawn farming is currently threatened by low production efficiency and vulnerability of farmed stocks to disease, as is the case in marine shrimp production (Vijayan et al., 2005; New et. al., 2008). Thus, there is renewed interest in selective breeding programs to improve growth rates and disease resistance in this species.

A reliable tagging method is necessary for selective breeding programs. Tagging crustaceans is difficult because of the periodic shedding of their exoskeleton (molting). Different types of internal tags have been evaluated in crustaceans with varying degrees of success (Nielsen, 1992). The polyester visible implant (VI) tag developed by Northwest Marine Technology Inc. (Shaw Island, Washington, USA) is a tagging system with individual alphanumeric characters that can be implanted in juvenile aquatic species (Haw et al., 1990). The soft VI Alpha tag is a new version of the older polyester VI tag, developed using a medical grade elastomer that is pliable and pigmented with non-toxic fluorescent material (Arce et al., 2003). The advantage of the soft VI Alpha tag is that it is fluorescent, which makes it much easier to detect and read than the earlier polyester tag. As the old version was not fluorescent, it occluded with the pigmentation of prawn tissues and was difficult to read. Further, the old polyester tag was hard and its sharp edges cut into prawn tissues, leading to shedding of the tag and injury to the animal.

Various colors and codes allow over 40,000 individuals to be marked. The tags are implanted into transparent tissues and are readable externally. The soft VI Alpha tags were successfully used in decapod crustaceans such as juvenile and subadult shrimp *Litopenaeus vannamei* (Arce et al., 2003), juvenile freshwater prawn *M. rosenbergii* (Brown et al., 2003), and crayfish *Procambarus clarkii* (Isely and Stockett, 2001; Jerry et al., 2001). Prior to initiating a selective breeding program in *M. rosenbergii*, we evaluated the efficacy of the new soft fluorescent VI Alpha tagging system in juveniles, subadults, and adults by observing tag retention, tag visibility, growth, and survival over a 10-week period.

Materials and Methods

Live juveniles, subadults, and adults of *M. rosenbergii* were collected from the nursery and growout farm facility of the Central Institute of Freshwater Aquaculture in Orissa, India, brought to the laboratory, and acclimated for one week in ferro-cement tanks containing filtered fresh water. The prawns were stocked at 30/m^2 in the case of juveniles and 10/m^2 in the cases of subadults and adults. The tanks were provided with continuous aeration from an air blower. Water quality was maintained at optimum levels by exchanging 50% of the water with filtered well-aerated fresh water every other day. During acclimatization, the prawns were fed a commercial prawn feed (30% crude protein) twice daily at 6% of their body weight/day.

After acclimatization, 120 juveniles and the same number of subadult and adult prawns were selected. Prawns were anesthetized before tagging using clove oil (10 mg/l; Coyle et al., 2005). Two sizes of the soft visible implant alphanumeric tag or VI Alpha tag were used - the standard size (1.0 x 2.5 mm) in juvenile and subadult prawns and the large size (1.5 x 3.5 mm) in adult prawns. Both sizes included a fluorescent red format with black alphanumeric characters. Tags were implanted using the injector supplied by the manufacturer and inserted horizontally parallel to the body axis in the epidermal layer on the lateral side of the second abdominal segment just beneath the cuticle. Sixty prawns of each size group were tagged and 60 were untagged as a control.

The experiment was carried out in eighteen 3600-l ferro-cement tanks containing 1800 liters of filtered well-aerated fresh water (3 replicates of 20 prawns/tank for each treatment). The prawns were fed a commercial prawn feed (30% crude protein) twice daily at 6% of their body weight/day.

Tanks were cleaned daily and 50% of the water was exchanged every other day. Water temperature and dissolved oxygen were measured twice daily with a digital DO meter (Eutech Instruments, Singapore). Every other day, prior to water exchange, pH was measured with an electronic pH meter (Thermo Orion, USA) and ammonia by the phenol hypochlorite method of Solorzano (1969).

Prawns were measured (total length in mm) and weighed (to the nearest 0.1 g) at the beginning of the experiment and at monthly intervals. Tagged prawns were checked once every week to determine retention and readability. Retention was calculated as the percent of surviving prawns with tags at each tag check (Arce et al., 2003). Readability was calculated as the percentage of readable tags among the retained tags. Growth and survival data were analyzed using the *t* test. Tag retention and readability data were analyzed using one-way ANOVA. Significance was established at $p<0.05$.

Results

The mean water quality parameters did not differ between treatments and were: temperature 29.6°C, pH 7.8, dissolved oxygen 6.3 mg/l, and total ammonia nitrogen 0.032 mg/l.

Growth and survival of tagged and untagged juveniles, subadults, and adults are presented in Table 1. The average daily growth and SGR of tagged prawns did not significantly differ ($p>0.05$) from those of the untagged control. Likewise, mean final survival did not differ between tagged and untagged prawns.

Tags were lost during the first and second week in juveniles, during the first week in subadults, and through the ninth week in adults (Fig 1). Final mean tag retention significantly differed ($p<0.05$) between size groups. It was highest in subadult prawns (91.3±2.73) and lowest in adult prawns (59.0±1.65).

All tags in juvenile prawns were readable until the end of the experiment (Fig 2). In subadults, readability declined after the fourth and sixth weeks, then remained steady until the end of the experiment. In adult prawns, readability declined until the second week,

Table 1. Growth and survival of control and VI Alpha-tagged juvenile, subadult, and adult *Macrobrachium rosenbergii.*

	Juveniles (76 days)		*Subadults (70 days)*		*Adults (70 days)*	
	Control	*Tagged*	*Control*	*Tagged*	*Control*	*Tagged*
Initial mean wt (g)	1.7±0.11	2.1±0.35	12.9±0.40	14.9±2.69	26.7±3.24	27.4±1.37
Final mean wt (g)	3.2±0.22	3.8±0.31	15.1±0.36	17.8±3.21	29.5±3.19	30.2±0.59
Growth (mg/d)	20.5±1.47	22.4±0.23	31.4±0.88	41.5±1.9	40.6±1.4	40.0±0.92
SGR (%)	0.83±0.04	0.81±0.02	0.22±0.02	0.25±0.03	0.14±0.02	0.14±0.03
Survival (%)	95.8±5.9	93.3±11.5	93.4±2.5	95.0±5.0	92.8±9.5	90±17.3
Retention (%)	-	71.25±3.30	-	91.3±2.73	-	59.0±1.65
Readability (%)	-	100	-	88.6±5.3	-	77.8±4.8

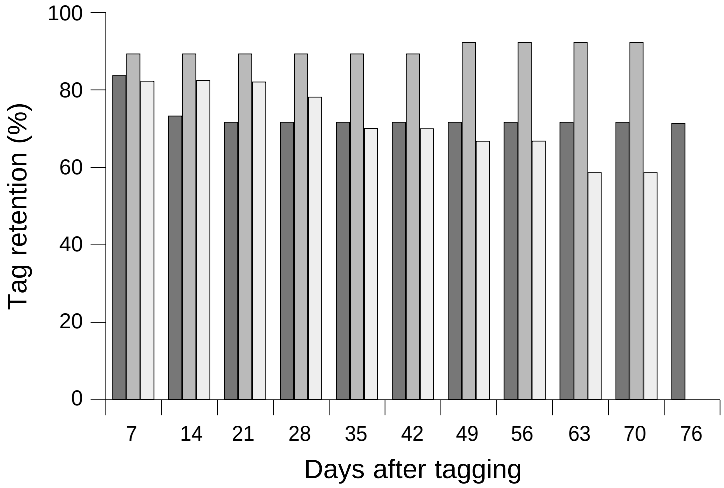

Fig. 1. Retention (%) of VI Alpha tags in juvenile (■), subadult (■), and adult (□) *Macrobrachium rosenbergii*.

then remained steady. Final tag readability was highest in juveniles (100%), followed by subadults (88.6±5.3) and adults (77.8±4.8).

Discussion

Growth and survival of juvenile, subadult, and adult *M. rosenbergii* tagged with soft VI Alpha tags did not significantly differ from that of untagged control prawns, indicating that soft VI Alpha tags have no significant negative effects on either growth or survival in these prawns.

Previous workers who examined the effect of VI Alpha tagging on survival of decapod crustaceans also reported high survival rates in tagged animals. There were no significant differences in growth or survival between VI Alpha tagged and untagged red swamp crayfish *P. clarkii* (Isely and Stockett, 2001). Survival after 42 days of rearing in an indoor microcosm laboratory was 90% in juvenile shrimp *L. vannamei* tagged with standard sized VI Alpha tags and 80.9% in those tagged with large sized VI Alpha tags; however, survival in untagged shrimp (96.7%) was significantly higher (Arce et al., 2003). After 75 days of rearing, survival was 93.3% in subadult *L. vannamei* tagged with standard VI Alpha tags and 90% in those tagged with large VI Alpha tags (Arce et al., 2003). There were no significant differences in final weight between tagged and untagged juvenile or subadult *L. vannamei* (Arce et al., 2003). Growth rates of tagged and control *M. rosenbergii* postlarvae were similar (Brown et al., 2003).

In the present study, the tags were retained in juvenile and subadult *M. rosenbergii* within the ranges reported by previous researchers (Isley and Stockett, 2001; Arce et al., 2003). Tag loss in juvenile prawns occured within the first two weeks and in subadult prawns within the first week. The initial tag loss may be due to handling and tagging stress. In addition, tag loss could be caused by handling after implantation where insertion wounds have not properly healed (Kincaid and Calkins, 1992). Tag loss in adults continued through the ninth week. Tag retention in adult *M. rosenbergii* was lower than reported for *L. vannamei* (Arce et al., 2003) and crayfish (Isley and Stockett, 2001). The low tag retention was probably a result of improper tagging due to the increased difficulty encountered in tagging the thicker exoskeleton of adult prawns.

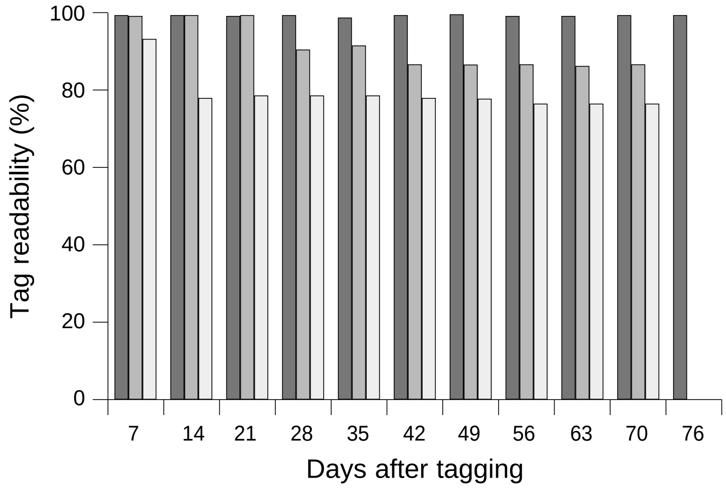

Fig. 2. Readability of VI Alpha tags in juvenile (■), subadult (□), and adult (□) *Macrobrachium rosenbergii*.

All tags in juveniles were readable until completion of the experiment. High tag readability in juvenile prawns is probably due to the relatively soft and translucent nature of the exoskeleton. Readability in subadult prawns was also high (>80%). The fluorescence of the tags helped to detect and read the tag easily. VI Alpha tags in juvenile red swamp crayfish were 100% readable 150 days post tagging (Isley and Stockett, 2001) while readability in juvenile yabby *Cherax destructor* was 96% (Jerry et al., 2001). Readability was 82% in juvenile *L. vannamei* with a mean weight gain of 10.5 g after 42 days (Arce et al., 2003). However, in juvenile *M. rosenbergii*, only 64% showed good visibility 20 days post tagging (Brown et al., 2003). Tag readability in adult prawns was significantly lower due to the pigmented nature and increased thickness of the exoskeleton. There was melanization of tissues surrounding the tag in some individuals of all size groups with a higher incidence of melanization in adults.

The present study shows that the new soft VI Alpha tags are suitable for tagging juvenile and subadult *M. rosenbergii* due to the relatively high retention and visibility of the tags without compromising survival or growth rate. The use of these tags will increase the efficiency of selective breeding programs as they will allow collective rearing of different selected lines under the same environmental conditions. This will also reduce the number of tanks required as well as eliminate possible differences in growth among families due to environmental effects.

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