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Influence of Visible Implant Fluorescent Elastomer (VIE) Tagging on Growth, Molting and Survival of the Eastern White River Crayfish, *Procambarus acutus acutus* (Girard, 1852)

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Abstract: Tag retention, growth, molting, and survival of crayfish, *Procambarus acutus acutus*, 35-37 mm in total length were evaluated between tagged and untagged groups. There were no significant differences in molting and survival between the tagged and untagged groups (P > 0.05). Tag retention was 100% for crayfish kept individually in plastic containers for the 60-day experimental period. The tagged group had a mean growth of 7.0 mm (molts only) while the untagged group had a mean growth of 4.0 mm (molts only). Molting rates were similar between the tagged and untagged groups. Nine molts were observed each in the tagged (n = 30) and untagged (n = 30) groups, respectively. Survival rates were 96.6% for both groups. Although the tags did not affect molting or survival, they did significantly affect the growth of crayfish in the short term (P < 0.05).

Key Words: Crayfish, growth, molting, survival

Gözle Görülebilir Fluorescent Elastomer (VIE) Markalamanın Tatlı Su İstakozu'nun, *Procambarus acutus acutus* (Girard, 1852), Büyüme, Kabuk Değişimi ve Yaşama Oranı Üzerine Etkisi

Özet: Bu çalışmada, 35-37 mm boyundaki kerevitlerde, *Procamborus acutus acutus,* markalamanın büyüme, kabuk değişimi ve yaşama oranı üzerine etkisi araştırılmıştır. Markalanan ve markalanmayan kerevitler, kabuk değiştirme ve yaşama oranı bakımından istatistiksel olarak bir fark göstermemiştir (P > 0,05). Plastik küvetlerde bireysel olarak 60 gün süresince bakılan kerevitlerde marka tutum oranı % 100 olarak bulunmuştur. Markalanan kerevitlerde büyüme oranı 7,0 mm iken bu oran markalanmamış grupta 4,0 mm olarak bulunmuştur. Markalanmış ve markalanmamış grubundaki kerevitler kabuk değişimi oranı bakımından benzer olup, her iki grupta da 9'şar adet kerevitin kabuk değiştirdiği gözlenmiştir. Yaşama oranı ise, markalanmış ve markalanmamış grupta % 96,6 olarak bulunmuştur. Kısa süreli calışmalarda, markalamanın, kabuk değiştirme ve yaşama oranı üzerine etkili olmamasına rağmen, büyüme üzerine etkili olduğu görülmüştür (P < 0,05).

Anahtar Sözcükler: Kerevit, büyüme, kabuk değişimi, yaşama oranı

Introduction

Tags are useful tools for studies on the growth, density, migration, and dynamics of populations and the management of many organisms, including crayfish. Slack (1955) and Bryan and Ney (1994) successfully utilized an injection technique with ink for crayfish and fish. An alternative marker is visible implant fluorescent elastomer (VIE) tags (North-west Marine Technology) injected transversely into the ventral surface of the first or second abdominal segment of the crayfish by the same technique (Brandt and Schreck, 1975). The tags were visible upon inspection.

This technique is often used to tag large numbers of individual identification markers to be generated by applying various combinations of 4 fluorescent color markers (yellow, green, orange, and red) to different

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locations on the crustacean and fish body. Examples of tags used are as follows: (1) external tags, (2) pleural clips, (3) branding, (4) removal of appendages, (5) ink injection, (6) painting, (7) radioactive tags, and (8) fluorescent pigments. For successful tagging, the tags should be easily recognizable and should remain in place. The VIE tag that has been successfully applied to very small fish with high retention and survival rates (Frederick, 1997; Olsen and Vollestad, 2001). VIE tags can vary among species, and previous studies have had difficulty with poor results for tag retention after molting (Price and Payre, 1984; Hurly et al., 1990), and survival decreased in the 100 days after application (Haines et al., 1998). This tagging system also may be applied in aquaculture programs, including selection and stock improvement trials. The advantages of visible implant tags are that they are simple and inexpensive to apply and visible for short-term studies.

The long-term tagging of crustaceans is difficult because the tags are generally lost within 2 to 3 molts and sometimes are not read easily to identify the crayfish (Haines et al., 1998).

Another type of tag, such as the internal binary coded wire tag (Jefferts et al., 1963), was used on marine crustaceans and yielded high tag retention and had little effect on the growth and survival of tagged individuals. This tag has been successfully used to mark *Pandales platyceros* and *Homarus gammarus* crustaceans (Prentice and Rensel, 1977; Uglem and Grimsen, 1995).

The aim of the present study was to investigate the effect of tagging on growth, molting, and survival of crayfish over a 60-day period.

Materials and Methods

A laboratory study was conducted to examine: (1) tag retention, (2) tag retention after molting, (3) number of molts, (4) survival, and (5) growth.

Tag Description

The VIE tagging system utilizes a specially developed, biocompatible, 2-part elastomer material that contains fluorescent coloring. After mixing, the elastomer is a liquid, which can be injected transversely into the ventral surface of the first or second abdominal segment with a hypodermic syringe. Within 24 h, this material cures into a pliable solid at room temperature. In order to cure properly, the elastomer and curing agent must be mixed at a 10:1 ratio. Once mixed, the tags were carefully injected between the third and fourth periopods. The animals were then placed in their respective containers.

Laboratory Experiment

The 60 animals used in this study were collected from the culture pond at Clemson Aquaculture Research Facility in January 2001. After each collection, they were transported to the Clemson University Shellfish Laboratory. They were separated into 2 groups, large and small. Only the small ones were analyzed for this experiment. All specimens, 30 tagged and 30 untagged, were individually placed in 25-ml plastic containers. Total length (TL, tip of rostrum to the telson) of the crayfish was measured to the nearest millimeter and then weighed (wet weight, WWT) to the nearest 0.01 g and recorded. The juvenile crayfish ranged from 35 to 37 mm in total length (mean tagged length 35.4 mm ± 1.61 mm, mean untagged length 36.2 mm ± 1.95 mm). The specimens were tagged once and returned to their proper containers. All specimens were handled using the same procedure to prevent different treatments between tagged and untagged specimens.

The crayfish were given a pellet high protein commercial fish feed (45% protein) at a rate of 3% of body weight per day 7 days a week over 60 days. Water quality was maintained through frequent water changing after feeding. Feces and waste feed were removed from the containers daily before feeding. The animals were examined after water exchange for evidence of molting, tag retention, and mortality. The crayfish were counted, measured, and examined under a UV light for tag retention at the end of the experiment. Growth of the crayfish was determined from the growth of the molted specimens.

Data Analysis

Data were analyzed with the Micro-SAS Statistical Software System Version 8 (SAS, 1999). A t-test was used to compare length differences between the tagged and untagged groups. All testing was done at a level of significance of $P < 0.05$.

Results

Tag retention was observed among all 30 tagged specimens for the entire experiment. All tags were retained after all molts. Each group molted 9 times, which indicates that tagging these specimens had no effect on molting and shows specifically which specimens molted with corresponding growth. Total mortality for the study was 2 (3.3%), i.e. 1 from the tagged group and 1 from the untagged group. In the present study, no significant difference was found in survival between the tagged and untagged groups (Table). Mortality between the groups was equal. Average growth measured from TL for tagged crayfish was about 7 mm (16.5%) at the end of the experiment (n = 30, mean = 42.4 mm ± 2.22), whereas untagged crayfish had average growth of 4 mm (9.9%) (n = 30, mean = 40.2 mm ± 3.10) (Table). The results of the t-test at 0.05 level of significance indicated that there was a significant difference when comparing the growth rate of the tagged and untagged crayfish.

Discussion

It would seem that the tag is suitable for short-term studies and can be used to identify individuals or groups of crayfish. It would not appear that the tag had any negative effects on survival or tag retention, but did affect growth. It was also noted that tag loss did not occur after molting. Survival rates were the same (96.6%) for the 2 groups. This rate is favorable compared with the retention rate of 65% for crayfish marked with dyes (Brandt and Schreck, 1975) and 12%-55% for crayfish marked with internal anchor tags (Paret et al., 1988). In addition, Slack (1955) reported a relative study tag retention of 12.7% in 566 crayfish, and in another study it was 17.5% in 273 crayfish (Nielson, 1992) over 1 year. The long-term tagging of crustaceans has been problematic because of losing the tag and because it was not easy to identify tagged individuals. Another reason for tag loss would be an incorrect application technique, which may have resulted in elastomer leakage from the point of insertion (Buckly et al., 1994). Frederick (1997) suggested that a subcutaneous injection too deep might hide the tag, especially when the growth rate is rapid, although missing tags were not detected using UV light. In studies on juvenile lobster *Homarus* spp. tagged with coded wire tags, retention rates of 80% - 99% (Wickins et al., 1986), and 92%-98% (Uglem and Grimsen, 1995) have been reported.

In our study all the tags were retained over the 60-day experimental period and they held up well during the molting process. Nine of the specimens molted and the VIE tags were retained and visible until the end of the experiment.

Cannibalism is an important mortality factor (Lowery, 1988), particularly when crayfish are frequently molting as were the rapidly growing crayfish, but in this study, due to individual stocking in each container, no differences in mortality were detected between the tagged and untagged groups.

In summary, the tags appear to be retained throughout the study and did not appear to affect retention or the survival of the animals. However, it affected positively the growth of the crayfish. Paret et al.

Table. Mean and standard deviation of the size and growth (TL in mm) tagged and untagged crayfish for 60 days.

Parameter	N	Tagged	N	Untagged
Initial TL (mm)	30	35.4 ± 1.61	30	36.2 ± 1.95
Final TL (mm)	29	42.4 ± 2.22	29	40.2 ± 3.10
Growth TL (mm)	9	7.00 ± 0.95	9	4.00 ± 1.62
Survival (%)	29	96.6%	29	96.6%
Tag retention (%)	30*	100%	30	100%

* The specimen that died had retained the tag until molting occurred.

(1988) indicated that the total increase in length for untagged crayfish was about 9.9 mm (16.4%), whereas in tagged crayfish it was 16.6 mm (27.5%). However, Paret et al. (1988) did not explain why tags affected growth. Our study indicated that the tag was retained in *P. a. acutus.* This is probably because the crayfish often molts and the tag could increase the molting frequency (meaning growth) due to stress. Therefore, the VIE tagging system seems to be appropriate for short-term studies. VIE tags may be a valuable tool for future studies (crayfish stock density, survival, ecology, culture, and migration) on crayfish.

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