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Short communication

# A comparison of the effect of three common tagging methods on the survival of the galatheid *Munida rugosa* (Fabricius, 1775)

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#### Abstract

Three tagging methods – T-bar tags, streamer tags and visual implant elastomer (VIE) – were tested on *Munida rugosa* and survival was monitored for 8 weeks. The VIE technique appeared not to affect survival (95% survival after 60 d). T-bar tags did not significantly affect short-term survival either, but there was a gradual increase in mortality (52% after 60 d) associated with the presence of black necrotic tissue around the tag wound, suggesting delayed mortality due to infection. Streamer tags as applied in the study are not suitable for this species, since short-term mortality was high (38% survival after 10 d). In conclusion, VIE was found to be the best type of tag to use on *M. rugosa*, although it is not as visible to the casual observer as the other types, which could be an important consideration if recapture reports from fishers are an important element of a mark-recapture study.

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#### 1. Introduction

The long-clawed squat lobster, Munida rugosa, is a common galatheid in Scottish waters, with populations that can reach sufficient densities to support targeted commercial fishing (Howard, 1981). However, little is known about the biology of *M. rugosa*, including fundamental life history parameters that would be necessary for fisheries stock assessment, such as natural mortality, intermoult duration, moult increment and mobility of animals. Commonly, crustacean growth, survival or mobility are measured using capture-recapture techniques (e.g. Smith et al., 2001; Ulmestrand and Håkan, 2001). For such studies, the primary concern is to mark animals individually with a tag that is retained through subsequent moults and does not substantially affect survival, growth or behaviour. There appear to be no published tagging studies of galatheids, but numerous techniques have been used to tag decapod crustaceans such as crabs or lobsters. However, variation in the effectiveness of tagging methods among species and the particular morphology of squat lobsters compared with crabs and lobsters (i.e. abdomen flexed under the cephalothorax and dorso-ventral flattening of the body) sug-

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gested the need to evaluate the efficiency of different tagging methods in *M. rugosa*.

The main problem in long-term tagging studies of crustaceans is that they shed any tags externally attached to the exoskeleton at ecydsis. Alternative techniques consist of inserting internal or external tags that are retained after subsequent moults. Various tagging and marking techniques exist for crustaceans (Caceci et al., 1999; Davis et al., 2004; Linnane and Mercer, 1998; Montgomery and Brett, 1996). Three effective, convenient and cheap techniques are commonly used for decapods: T-bar and streamer tags are considered as standard techniques for some species (Dubula et al., 2005; Ulmestrand and Håkan, 2001), and visual implant elastomer (VIE) has become more widely used in recent years for tagging crustaceans, as well as other taxa (Malone et al., 1999; Purcell et al., 2006). Streamer tags and T-bar tags can be lost in variable proportions and have different effects on growth and survival, depending on the species marked, individual size or moult stage (Benzie et al., 1995; Dubula et al., 2005; Linnane and Mercer, 1998; Montgomery et al., 1995; Montgomery and Brett, 1996). Moreover, T-bar and streamer tags may affect risk of predation by making marked individuals more conspicuous (Benzie et al., 1995; Linnane and Mercer, 1998). VIE however appears to have consistent effects among the species studied so far, with nearly no tag loss and no effect on mortality or growth (Davis et al., 2004; Godin et al., 1996;

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Jerry et al., 2001; Linnane and Mercer, 1998; Uglem et al., 1996; Woods and James, 2003). A study of VIE-tagged fish showed no effects on predator attraction (Malone et al., 1999). VIE may become fragmented, or migrate within tissue and become obscured by muscle or the exoskeleton during growth (Davis et al., 2004; Linnane and Mercer, 1998; Woods and James, 2003), but the availability of fluorescent pigments enhances the visibility of partially obscured VIE tags, particularly under ultraviolet illumination (Godin et al., 1996). VIE is better suited to batch tagging, but permutations of positions and colours can be applied to allow individual tagging (Uglem et al., 1996). Alphanumeric internal tags are available for individual tagging, but appear to be less visible (Jerry et al., 2001).

There have been numerous previous studies of survival or tag retention in crustaceans for the VIE, T-bar and streamer methods separately, but there have been no direct comparisons of these three techniques, which appear to be the dominant methods used nowadays. Consequently, the present paper compares T-bar, streamer and VIE tagging techniques in *M. rugosa*.

# 2. Materials and methods

## 2.1. Sampling and maintenance

*M. rugosa* were sampled by beam trawling (2 m wide and 70 mm mesh size) in the Clyde Sea Area in early August 2004. This fishing technique is considered to result in significant post-capture mortality (Bergmann et al., 2001), so animals were kept alive in tanks for up to 2 weeks before starting experiments, to avoid mortality related to fishing stress.

Squat lobsters were measured and maintained in a 660-litre fibreglass tank with groups of large (>32 mm carapace length (CL)), medium (between 27 and 32 mm CL), and small (<27 mm CL) animals and males and females separated by partitions (high density polyethylene 8-mm mesh on wooden frames) to limit aggressive interactions. The bottom of the tank was covered with fine sand and there was a continuous flow through of unfiltered sea water that allowed animals to feed as deposit feeders on organic material settling from the water (Zainal, 1990). Since *M. rugosa* also feed on macroscopic food, they were fed every 5 d *ad libitum* with pieces of frozen fish (Zainal, 1990). Aeration was provided to maintain sufficient dissolved oxygen levels.

# 2.2. Tagging procedure

#### 2.2.1. Visible implant fluorescent elastomer (VIE) tags

Squat lobsters were tagged ventrally in different segments of the abdomen (for unique coding; Fig. 1) by injecting red elastomer (Northwest Marine Technology, Shaw Island, Washington) under the transparent cuticle of the abdomen with a hypodermic syringe. The needle was inserted into the abdominal cuticle at its border with the pleura and advanced towards the mid-line and the elastomer was injected as the needle was withdrawn. Exposure to air was minimized to reduce stress.

Unique codes were created by marking up to six different segments on the right or left side of the animal (Fig. 1). By adding up to three marks of the same colour it would be possi-



Fig. 1. Ventral view of the abdomen of a male *Munida rugosa*, showing positions in which visual implant elastomer was injected to create unique codes.

ble to produce 268 individual codes, or 2048 if two colours are used (10 colours are available and six are fluorescent, Northwest Marine Technology). The strategic application of an additional elastomer mark to the left or right side of the 5th thoracic segment (which also has a transparent ventral exoskeleton) aided recognition of individuals in the event of tag migration or fragmentation. Elastomer marks were usually visible under normal illumination, but ultraviolet light was also used to check for partially obscured marks.

## 2.2.2. Polyethylene streamer tags

Polyethylene streamer tags (Hallprint Pty Ltd., 90 mm  $\log \times 3$ -mm wide with a narrowed central section  $20 \text{ mm} \times 2 \text{ mm}$ ) were used. A stainless steel needle attached to one side of the tag was used to insert the tag dorsally between the cephalothorax and the first abdominal segment. The morphology of this species required the tag to be passed through the abdomen ventral to the dorsal artery and the intestine, but dorsal to the nerve cord.

#### 2.2.3. Plastic T-bar internal anchor tags

T-bar tags (Hallprint Pty Ltd., 30-mm long with a 5-mm wide anchor) were inserted into the dorsal musculature with an appropriate injection gun equipped with a short split needle. The needle was inserted between the cephalothorax and the first abdominal segment and slightly to one side of the mid-line, to avoid puncturing the heart or intestine. Then the tag was injected and the needle gently removed, taking care not to withdraw the tag.

#### 2.3. Statistical design and analysis

A total of 84 squat lobsters were used for this experiment: 21 each of controls (not tagged), VIE tagged, streamer tagged, and



Fig. 2. Percentage survival over 60 d of controls (solid), T-bar (long dash), streamer (short dash) and VIE (dotted) tagged squat lobsters (*Munida rugosa*).

T-bar tagged. In each of the six compartments containing squat lobsters grouped by size and sex, individuals were randomly allocated to one of the three tagging techniques or the untagged control group. Tagged and untagged squat lobsters were kept in the same compartments in the tank to allow interactions between them and note any relevant damage that could occur in nature. No more than six animals tagged by the same method were kept in the same compartment. CL measurement allowed individual identification in cases of tag loss.

Survival and tag retention was recorded daily. For analysis, tagging mortality has been considered in terms of short-term mortality due to the trauma of handling and injury (e.g. haemolymph loss) and longer-term mortality, due to slower-acting agents, such as infection, reduced food intake and altered behavioural interactions (Armstrong et al., 1981). Differences in mortality among tagging methods were assessed by comparing the proportions surviving after 10 d for short-term mortality and 60 d for long-term mortality by a  $\chi^2$  test (Zar, 1999). Multiple comparisons of the proportion surviving in each tag type group with that of the control group were made with a one-tailed Dunnett-type test (Zar, 1999).

### 3. Results

There was significant heterogeneity in the proportion of squat lobsters surviving (Fig. 2) among the control and tag type groups after 10 d ( $\chi^2 = 28.57$ ; df = 3; P < 0.05) and 60 d ( $\chi^2 = 26.28$ ; df = 3; P < 0.05). VIE marking was the most successful, with 100% survival after 10 d and 95% after 60 d. The VIE tag survival rate was not significantly different from the controls after 10 d or after 60 d (Table 1). Survival of T-bar-tagged animals

Table 1

Dunnett-type multiple comparison of the proportion surviving 10 d and 60 d after tagging by each of the three methods with untagged controls

Tagging method	10 d		60 d	
	Survival (%)	$q_4'$	Survival (%)	$q_4'$
VIE	100	1.54	95	0.98
T-bar	86	0.44	52	2.32*
Streamer	38	3.66**	29	3.85**

q' is the one-tailed Dunnett test statistic. \*P < 0.05, \*\*P < 0.01.

was also not significantly different from controls after 10 d, but a significantly lower proportion (52% versus 86%) were alive after 60 d (Fig. 2; Table 1). In most cases of mortality, necrotic tissue was observed around the insertion point of T-bar tags. With streamer tags, the survival rate was significantly less than the controls after 10 d and 60 d (Table 1), and survival was only 38% after 10 d and 29% after 60 d.

The T-bar method was the fastest technique, with an average time of 46 s to tag an animal. Marking with VIE took 82 s per individual, on average, but since the elastomer product cures slowly over several hours, the injection time increased during marking sessions (from 1 to 2 min). The streamer tag technique was the slowest, with an average of 148 s per animal.

There was no tag loss from individuals that did not moult. Few animals moulted during the course of the experiment. One animal with a T-bar tag and four tagged with VIE moulted. All of the tags were retained during the moulting process, but two recently moulted animals (one of each tag type) were discovered dead and partially cannibalized. In three animals tagged with VIE, some tag migration (movement of elastomer to an adjacent segment) was observed.

# 4. Discussion

The high survival rate found with VIE in the present study is consistent with previous studies (Linnane and Mercer, 1998; Woods and James, 2003). There was 100% tag retention for each method, including the four VIE-tagged animals and one T-bar tagged animal that moulted. These observations and the levels of tag retention found in previous studies (Godin et al., 1996; Linnane and Mercer, 1998; Uglem et al., 1996; Woods and James, 2003) suggest that there would be low levels of VIE tag loss after subsequent moulting. Some migration and fragmentation of elastomer has been seen in other studies (Jerry et al., 2001; Linnane and Mercer, 1998; Woods and James, 2003) and migration was observed in the present experiment, which could be a problem for individual identification. An additional mark on the left or right side of the 5th thoracic segment and the use of ultraviolet light to read tags aided individual recognition in cases of elastomer migration (100% of individual recognition was possible at the end of the experiment). Moreover, marking with VIE is not thought to affect predation rate, since the tag is applied to the ventral surface of the abdomen and is therefore concealed from predators. However, even though the VIE is readily visible to humans, particularly if red elastomer is used (Godin et al., 1996), the tag remains hidden underneath the folded abdomen, and only persons looking for a tag would see it. Consequently, the return rates of tagged animals recaptured by fishermen would probably be low, owing to the low visibility of the tag. Moreover, even though VIE is non-toxic to humans (Godin et al., 1996), it might affect the commercial value of animals, since the tag remains inside the animal and can be fluorescent.

T-bar tagging does not appear to cause significant immediate mortality in *M. rugosa*, but the present findings indicate that there are adverse effects on longer-term survival, as seen in other species (Dubula et al., 2005; Montgomery and Brett, 1996). A gradual increase in mortality in the longer-term (>10 d) for T-

bar tagged animals could have been caused by infection, since black necrotic tissue around the wound was observed. Dubula et al. (2005) noted similar problems in southern rock lobster (*Jasus lalandii*) tagged with T-bar tags, which had lower survival and growth rates than untagged controls, particularly when tagged in premoult as opposed to intermoult.

Survival with streamer tags decreased soon after tagging (38% after 10 d), consequently mortality is attributed to stress or injury induced by the tagging procedure. Survival of streamer-tagged individuals was also much lower than previous findings for other species (Benzie et al., 1995; Linnane and Mercer, 1998; Montgomery et al., 1995). Streamer tags as applied in the present study are not suitable for *M. rugosa*. Trials of inserting streamer tags dorsally to the intestine were unsuccessful, owing to the thinness (<3 mm) of the tissue and a posterior extension of the cephalothorax limiting access to the tagging position. Moreover, these tags could increase predation (Benzie et al., 1995; Linnane and Mercer, 1998).

Compared with completely internal marks, such as VIE, tags such as T-bar and streamer tags, are prone to being continuously moved or pulled by friction on rocks when squat lobsters move in and out of narrow crevices (Nickell and Sayer, 1998). This may prevent the tag wound from healing, leading to continued haemolymph loss and infection by bacteria or ciliates (Armstrong et al., 1981; Johnson, 1976). The effects of tagging were not assessed in the field in the present study. A study of king prawns (*Penaeus plebejus*) using streamer tags (Montgomery et al., 1995) suggested no differences in tag loss between laboratory and field.

In conclusion, VIE appears to be the best compromise in terms of survival, tag retention (Godin et al., 1996; Linnane and Mercer, 1998; Uglem et al., 1996; Woods and James, 2003), predator attraction (Benzie et al., 1995; Linnane and Mercer, 1998; Malone et al., 1999) and tag readability for marking *M. rugosa* and perhaps other galatheids, compared to T-bar and streamer tags.

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