

Long-term retention of internal elastomer tags in a wild population of painted crayfish (*Panulirus versicolor* [Latreille]) on the Great Barrier Reef

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Abstract

Many crustaceans are highly exploited fisheries species, but determining the population parameters of these animals via mark-recapture techniques is problematic, primarily due to tag-loss during moulting of the exoskeleton. Recently developed internal elastomer tags may overcome this challenging problem, since they are completely encased in the tissues beneath the exoskeleton. In this study, we evaluated the effectiveness of internal elastomer tags in a wild population of painted crayfish (*Panulirus versicolor*) over an 18-month period. Seventy animals were double-tagged in the abdominal musculature, with individuality obtained using different combinations of tag colour and tag location. Forty individuals were recaptured after 6, 12 and (or) 18 months, giving an overall recapture rate of 57%. Annual tag retention was estimated to be 98%, since only one instance of tag-loss was observed, despite numerous episodes of moulting among tagged individuals. The majority of tags (95%) were easily visible, even after 18 months at liberty. Nonetheless, small reductions in tag condition were observed after the first six months (mostly due to fading and [or] fragmentation), but this did not interfere with overall tag readability. Tag condition did not decline in the following 12 months, nor did it vary with gender or tag location. It is concluded that internal elastomer tags are an effective method for marking wild crayfish over long-term periods. Given the durability of elastomer, and the capacity for individual identification of large numbers of animals, such tags may be suitable for commercial-scale fishery applications.

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1. Introduction

Tagging of wild organisms is an important tool used by fisheries scientists to estimate population size, move-

ment patterns, growth rates (Wydoski and Emery, 1983; Bergman et al., 1992) and the effectiveness of restocking programs (Bannister et al., 1994; Linnane and Mercer, 1998). However, tags are not permanent markers, and it is typical for a significant proportion of tags to become detached from host animals (Ennis, 1986; Muoneke, 1992; Wilkinson and Bester, 1997). Any calculation of demographic parameters from mark-recapture data must therefore account for tag-loss, as failure to do so may result in over-estimation of population size and loss of

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precision (Arnason and Mills, 1981). Despite recognition of this inadequacy some time ago, many tagging studies omit a proper evaluation of tag effectiveness (Bergman et al., 1992).

There are two empirical methods for estimating rates of tag-loss in aquatic animals. One method is to directly observe tagged individuals maintained in captive impoundments (e.g. aquaria or sea-cages), while the other is to attach two independent tags to wild animals and subsequently estimate loss rates from the relative proportions of recaptured animals bearing one versus two tags (Seber, 1973). While providing an absolute measure of tag-loss, the former method may incur bias, since captive conditions can protect tags from factors that would tend to decrease tag retention in the wild. These factors include infection, fouling, entanglement, and tag-removal by other animals (Ennis, 1986; Bergman et al., 1992; Melville-Smith and Chubb, 1997; Rowe and Haedrich, 2001). The latter method, involving wild animals, provides a more realistic estimate of tag-loss, provided that enough individuals can be recaptured.

Unlike other fishery resources, tagging of crustaceans presents a particular challenge, since any tag or mark which is attached to the exoskeleton is lost during ecdysis (moulting). The service-life of paint marks, ablation marks, plastic zip-ties and ultrasonic transmitters is therefore limited to one inter-moult period (i.e. weeks to months for most commercially-important crustaceans) (Mauchline, 1977; Aiken, 1980). Attempts to overcome this problem have resulted in the development of external tags that are sub-skeletally 'anchored' in the musculature (i.e. sphyrion, T-bar and streamer tags). However, even these tags are susceptible to ecdysis, with reports of annual tag-loss rates of 8% for West Australian crayfish (*Panulirus cygnus*, Melville-Smith and Chubb, 1997), 36–40% for American lobster (*Homarus americanus*, Ennis, 1986; Rowe and Haedrich, 2001) and 100% for blue-swimmer crab (*Portunus pelagicus*, McPherson, 2002). Moreover, 'anchor' tags have been associated with internal injury (Scarrat, 1970), infection (Courtney et al., 2001), entanglement (Ennis, 1986) and an increase in the likelihood of predation due to physical hindrance (see Linnane and Mercer, 1998) or tag conspicuousness (Bergman et al., 1992). Tags that interfere with an animal's physiology (e.g. via injury or infection) may also bias estimates of growth rates.

Visual Implant (VI) tags are the most recently developed tags for crustaceans. They are completely internal and avoid problems associated with permanent perforation of the integument, such as infection (Bergman et al., 1992). Although several types of VI tag are currently available (e.g. coded wire and alpha-numeric tags), it is

the elastomer form that has demonstrated particular success. The tag itself consists of a biocompatible, liquid-polymer that cures into a solid within hours of hypodermic injection. When placed within the abdominal musculature of shrimp, lobster or crayfish, it forms an apparently permanent mark that is clearly visible through the transparent ventral sclerites (Godin et al., 1996; Linnane and Mercer, 1998; Woods and James, 2003). VI elastomer is also flexible, thus reducing physical hindrance, and it does not require specialized detection equipment (like other VI tags), thus enabling census underwater. Furthermore, the discrete, abdominal positioning of VI elastomer is likely to reduce entanglement and tag-induced predation (Woods and James, 2003).

Preliminary studies with shrimp (*Penaeus vannamei*), European lobster (*Homarus gammarus*) and spiny lobster (*Jasus edwardsii*) have shown that short-term retention rates of VI elastomer are typically 100%, even after multiple moults (Godin et al., 1996; Uglem et al., 1996; Linnane and Mercer, 1998; Woods and James, 2003). However, all of these studies were conducted under laboratory conditions, and none have addressed the issue of tag-loss from wild animals, despite the prospect that captive conditions may favour tag retention (see above). To complete the assessment of VI elastomer as a method for tagging crustaceans, it is therefore necessary to evaluate its performance in a natural system.

The painted crayfish (*Panulirus versicolor*) is a Palinurid (spiny) lobster that is fished throughout coral-reefs of the Indo-Pacific region (Mutagyera, 1978; Kuthalingam et al., 1980; MacDonald, 1982; Munro, 2000), including the Great Barrier Reef (Australia), where it is one of the most individually valuable and popular of all fished species (Kailola et al., 1993). Despite this interest in *P. versicolor*, there is virtually no information on the biology or ecology of this species (Phillips and Kittaka, 2000). As such, this study represents the first phase of a larger investigation into long-term population dynamics of *P. versicolor* on the Great Barrier Reef. The objectives of the present study, therefore, were to (1) evaluate the effectiveness of VI elastomer tags in a wild population of *P. versicolor*, and (2) estimate retention rates of VI elastomer in this species over a long-term period (18 months).

2. Materials and methods

2.1. Study site

The study was conducted on the coral-reef surrounding Northwest Island (23° 18' S, 152° 43' E) which is located in the southern section of the Great Barrier Reef.

The reef at Northwest Island is typical of other reefs in the region (e.g. Done, 1982), with a high diversity and coverage of scleractinian corals and interspersed sand patches. At various depths across the reef, modest numbers of *P. versicolor* inhabit protective coral shelters (dens). Anecdotal observations by local fishermen indicate that individuals of *P. versicolor* show considerable site fidelity, thus making this species ideal for a mark-recapture study. The study site (i.e. search zone) was therefore confined to an area of ca. 60 ha.

2.2. Tag application and evaluation

Crayfish were captured by hand (with the aid of SCUBA) and restrained in a shallow, water-filled box onboard a nearby support vessel. VI elastomer (Northwest Marine Technology, Shaw Island, USA) was prepared as specified by the maker and loaded into a U-100 insulin injector (0.33 × 13 mm; Terumo Medical Corporation, Elkton, USA). The tag was applied by extruding a length of elastomer (15–20 mm) into the abdominal musculature. Care was taken to halt the flow of elastomer before withdrawal of the needle to ensure the tag was completely encased by muscle tissue. Each tag was positioned parallel to (but to one side of) the midline to reduce tag-fragmentation and avoid interference with the abdominal ganglia (Woods and James, 2003). All crayfish were tagged twice, with individuality obtained using combinations of different colours (i.e. black, fluorescent pink and fluorescent green) and different tag locations (i.e. left or right sides in any of the five posterior-most abdominal somites). The outer ramus of a uropod was marked with a paper punch to help indicate animals that shed both tags (in the absence of ecdysis). Other distinguishing features (e.g. missing limbs, carapace injuries, unique colour patterns) were also recorded to aid subsequent identification. Tag-retention rates were then calculated via the double-tagging method described by Seber (1973).

To assess changes in the condition of VI elastomer tags over time, the ventral abdominal region was photo-

graphed (before and after recapture) and tag condition was ranked as either ‘poor’ (rank 1; tag faintly visible due to fragmentation, migration, over-growth or fading), ‘good’ (rank 2; tag visible but incomplete or showing signs of minor alteration), or ‘excellent’ (rank 3; tag obvious and in excellent condition). Carapace length (L_c ; between the supra-orbital ridge and the posterior, dorsal edge of the carapace) was determined using vernier calipers and the frequency of moulting inferred from modal growth increments accrued during the inter-census period (Mauchline, 1977). The entire tag-and-measure process consumed approximately 5 min after which each crayfish was manually returned to its den. The location of all dens was recorded by global positioning system (GPS) to aid subsequent recapture.

2.3. Census periods

The study site was censused over ten consecutive days on four occasions — December 2003, June 2004, December 2004, and June 2005. During each census, all newly-encountered crayfish were tagged and released. All recaptured crayfish were processed (for L_c and tag condition) and released again, thus making it possible for multiple recaptures of the same individual at six month intervals. In each case, time-at-liberty was dependent upon the date of initial capture and tagging. For example, crayfish recaptured in June 2005 were at liberty for six months if tagged in December 2004, 12 months if tagged in June 2004, or 18 months if tagged in December 2003.

2.4. Data analyses

Samples with the same time-at-liberty (i.e. 6, 12 or 18 months) were pooled across censuses and the Wilcoxon paired-samples test (Zar, 1999) employed to identify differences in tag condition before release and after recapture. Next, each treatment group was made independent by excluding all previous (i.e. repeated) measures of individuals common to more than one

Table 1
Rates of recapture and tag retention for wild *Panulirus versicolor* during the period December 2003 to June 2005

Tag date	Number tagged	Recapture date	Time at liberty (mo)	Number recaptured ^a	Recapture rate (%)	Retention rate (%)
Dec. 2003	17	June 2004	6	10	59	100
June 2004	42	Dec. 2004	6	17	40	100
Dec. 2004	11	June 2005	6	5	45	100
Dec. 2003	17	Dec. 2004	12	8	47	100
June 2004	42	June 2005	12	13	31	96
Dec. 2003	17	June 2005	18	9	53	100

^a Values sharing the same ‘tag date’ are not mutually exclusive.

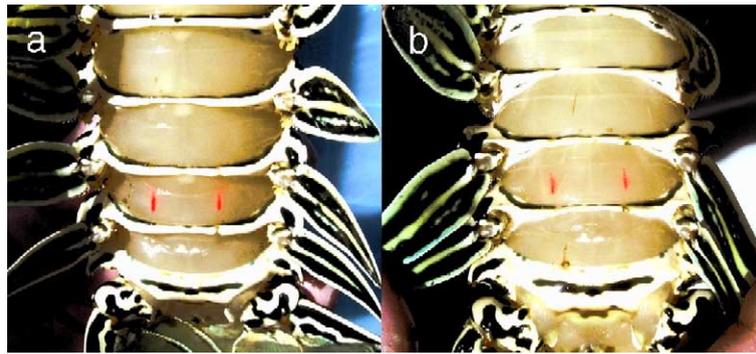


Fig. 1. Photographs showing the condition of VI elastomer tags in the fifth abdominal somite of *Panulirus versicolor* before release (a) and after 18 months at liberty (b) on the Great Barrier Reef. Tag condition in this individual was rated as ‘excellent’ (rank 3) before release and ‘good’ (rank 2) after recapture (see Materials and methods section for further information on tag condition).

group. A Kruskal–Wallis test (Zar, 1999) was then used to test for differences in tag condition between groups (i.e. to test for effects of time-at-liberty). Homogeneity of initial tag condition was checked *a priori* using the same statistical test.

Crayfish at liberty for 12 months were pooled across censuses and grouped according to moult frequency (i.e. 0, 1 or ≥ 2), gender (i.e. male or female), and tag location (i.e. anterior abdomen [somites 2 and 3] or posterior abdomen [somites 4 to 6]). As before, treatment groups were made independent and initial tag condition was checked for homogeneity. The effects of ecdysis, tag location, and gender on tag condition were then assessed using a Mann–Whitney ‘U’ or Kruskal–Wallis test (Zar, 1999). Significant differences were considered to occur if $p < 0.05$.

3. Results

3.1. Capture and recapture of crayfish

A total of 70 *P. versicolor* were tagged between December 2003 and December 2004 (Table 1). This included 32 males (L_c 93–148 mm) and 38 females (L_c 94–156 mm). Forty individuals were recaptured after 6, 12 and (or) 18 months, giving an overall recapture rate of 57%.

3.2. Tag retention

Tag retention was estimated to be 100% after completion of all but one of the recapture surveys (Table 1). Only one instance of tag-loss was observed, so overall tag retention was estimated to be 98% after 12 months at liberty. Untagged crayfish caught during the study did not show any evidence of previous capture, such as uropod ablation. Nor did any of these untagged animals

possess colour patterns or injuries that resembled those of previously tagged crayfish.

3.3. Tag condition

The majority of tags (38/40) were easily visible, even after 18 months (Fig. 1). Tag condition in recaptured crayfish was rated as ‘good’ (rank 2) or ‘excellent’ (rank 3) in all but two cases, although significant declines in tag condition were observed after 6, 12 and 18 months at liberty (Table 2). Degradation in tag condition was primarily due to fading and (or) fortification of the surrounding abdominal tissues after tag implantation. A few tags fragmented, although in most cases the constituent pieces remained in the same area as the original tag and thus did not interfere with tag interpretation. On two occasions, very small pieces of elastomer (relative to the main body of the tag) migrated into nearby abdominal somites.

Despite differences in tag condition before release and after recapture (i.e. within groups), there was no significant difference in tag condition (after recapture)

Table 2

The effect of time-at-liberty on tag condition in *Panulirus versicolor* on the Great Barrier Reef

Time at liberty (mo)	Number of samples ^a	Tag condition before release	Tag condition after recapture	Significance ^b (<i>p</i>)
6	32	3	2	<0.001
12	21	3	2	0.006
18	9	3	2	0.02

Tag condition is expressed as the median rank (rank 1 = tag faintly visible due to fragmentation, migration, over-growth or fading; rank 2 = tag visible but incomplete or showing signs of minor alteration; rank 3 = tag obvious and in excellent condition).

^a Values are not mutually exclusive.

^b Wilcoxon paired-sample test (one-tailed).

Table 3

The effect of moult frequency, gender and tag location on tag condition in *Panulirus versicolor* at liberty for 12 months on the Great Barrier Reef

	Moult frequency ^a			Gender		Tag location	
	0	1	≥2	Male	Female	Anterior ^b	Posterior ^c
Tag condition before release	3	3	3	3	3	3	3
Tag condition after recapture	2	2	2	2	2	2	2
Number of samples	7	6	8	10	11	10	8

Tag condition is expressed as the median rank (rank 1 = tag faintly visible due to fragmentation, migration, over-growth or fading; rank 2 = tag visible but incomplete or showing signs of minor alteration; rank 3 = tag obvious and in excellent condition).

^a Inferred from modal growth increments.

^b Abdominal somites 2–3.

^c Abdominal somites 4–6.

between groups (Kruskal–Wallis test; $p=0.87$). In other words, tag condition declined after initial implantation (i.e. during the first six months at liberty), but there were no further declines in tag condition after this time.

Sixty-five percent (26/40) of all recaptured crayfish increased in size (L_c) during the inter-census period. Modal growth increments were observed at 2–3, 5–6, and 8–9 mm. These increments were inferred to represent one, two and three moults respectively. After 12 months at liberty, the condition of tags in non-moulted crayfish was not significantly different to the condition of tags in crayfish that moulted one or more times (Table 3) (Kruskal–Wallis test; $p=0.89$). In other words, moult frequency did not significantly influence tag condition.

Tag condition was not influenced by gender or tag location. After 12 months, tag condition in male crayfish was not significantly different to that in female crayfish (Table 3) (Mann–Whitney ‘U’ test; $p=0.69$). Similarly, the condition of tags placed in anterior abdominal somites was not significantly different to the condition of tags placed in posterior abdominal somites (Table 3) (Mann–Whitney ‘U’ test; $p=0.88$).

4. Discussion

The results presented here support previous findings that VI elastomer is an effective method for tagging crustaceans (Godin et al., 1996; Jerry et al., 2001; Woods and James, 2003). However, the present study makes two important contributions which are additional to those of other studies. Firstly, VI elastomer is shown to be an effective method for tagging crustaceans in the wild. This finding is significant because rates of tag retention in the wild can be different to those in captivity (due to factors such as increased predation and infection). Secondly, VI elastomer is retained for long-term periods (i.e. up to 18 months). Previous to this study the

maximum reported longevity of VI elastomer tags in crustaceans was only six months (Jerry et al., 2001; Woods and James, 2003).

The retention rate of VI elastomer tags in wild *P. versicolor* was estimated to be 98% after one year. This is much higher than the retention rates reported for T-bar (Melville-Smith and Chubb, 1997), streamer (Rowe and Haedrich, 2001) and sphyron tags (Ennis, 1986), all of which are percutaneous (i.e. they permanently perforate the integument). Although some degradation in the condition of VI elastomer tags was observed, there were no differences in the condition of tags among crayfish at liberty for 6, 12 or 18 months. The most likely explanation for this pattern is that reductions in tag readability (due to either fading, fragmentation or tissue modification) occur shortly after tag insertion (i.e. within the first six months), but do not continue after this time. In this regard, readable VI elastomer tags may be retained for many years, at least in *P. versicolor*.

Tag condition was not affected by moult frequency, gender or tag location. This result supports the notion that VI elastomer tags are resistant to moulting (Uglem et al., 1996; Jerry et al., 2001), and confirms the proposition that VI elastomer tags can be placed in different somites (to increase the number of unique tag combinations) without affecting tag retention or condition (Uglem et al., 1996).

Despite qualitative reductions in tag condition after six months, the majority of tags were easily discernible in daylight, either onboard the vessel or underwater. The most visible colour was pink, primarily because it obtained maximum contrast with surrounding tissues. Black, on the other hand, was the least visible because it matched the appearance of intramuscular parasites. If recapture operations occur at night or under low visibility conditions, fluorescent colours and an ultra ultraviolet light can be used to further enhance tag visibility (Frederick, 1997).

The visibility of each VI elastomer tag was also dependent upon tag size and internal positioning. In particular, the most visible tags were as long as possible and parallel to (but just under) the abdominal sclerites (i.e. shallow in depth). Other techniques for improving tag condition include orientating tags parallel to surrounding muscle fibres (Woods and James, 2003) and suppressing abdominal movements (i.e. tail flicking) immediately after tag implantation (to reduce tag fragmentation). We also recommend that females are tagged in the anterior-most abdominal somite to avoid the subsequent concealment of tags if the animal becomes gravid.

Most studies to date have utilized VI elastomer for batch tagging — similarly coloured, single tags have been inserted into equivalent somites of different experimental animals (Linnane and Mercer, 1998; Jerry et al., 2001; Woods and James, 2003). One disadvantage of such batch tagging is the loss of information relating to individual animals (e.g. growth rate). Here we used combinations of different colours and tag locations to distinguish individuals. Up to 405 animals could have been identified with the use of two tags and a choice of three colours. Incidentally, if three tags are used and there is a choice of five colours, the number of possible combinations increases to 15 000. The number of tagged crayfish could be doubled by re-using the same combinations in both males and females (gender in *P. versicolor* is readily distinguishable). VI elastomer tags would thus appear to provide enough individuality to accommodate a commercial-scale tagging program.

Apart from having excellent retention and good visibility, the ideal tag does not alter behaviour, survival, or the potential for recapture (Bergman et al., 1992). The characteristics of VI elastomer tags are such that they are likely to satisfy all of these criteria. Firstly, VI elastomer tags are small (in comparison to the overall size of the host) and flexible, thus minimizing physical hindrance. Secondly, they are internally compartmentalized, thus reducing the likelihood of infections common to other tag types that permanently perforate the integument. Thirdly, VI elastomer tags can be discretely positioned on the ventral side of the host — an aspect which would tend to reduce tag conspicuousness among potential predators. It is also unlikely that VI elastomer tags interfere with the host's metabolism, since elastomer is medically-approved and non-toxic to humans (Northwest Marine Technology, Shaw Island, USA). This is an important consideration for tagging animals that may be consumed.

Despite the abovementioned benefits, one disadvantage of using such small, internal tags is that potential hosts must be captured and handled to search for tags.

This may prove unacceptable for some applications (e.g. broodstock identification for aquaculture), but irrelevant to others (e.g. trawl fisheries). Another disadvantage is that ready-to-use (i.e. pre-mixed) elastomer has a short shelf-life at ambient temperature, especially in the tropics (ca. 1 h). One consequence is that VI elastomer tags must be continuously prepared. However, this disadvantage is at least partially offset by the ease of application and cost efficiency of VI elastomer tags.

It is concluded that VI elastomer tags are a highly effective method for marking *P. versicolor* over long-term periods in the wild. In particular, VI elastomer is highly durable and clearly visible, even after multiple moults. VI elastomer also accommodates the need for individual identification, the recovery of information without sacrifice, and the capacity for visual recovery without the use of specialized equipment (cf. coded microwire tags, Sharp et al., 2000). It is therefore predicted that VI elastomer tags will be useful for tagging a range of Palinurid lobsters and other crustaceans.

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