



Comparing two types of internal tags in juvenile blue crabs

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Abstract

Although methods to tag fish and other vertebrates have been well described, tagging crustaceans, which molt, poses a greater challenge. Tagging very small juveniles, often necessary in population recruitment or stock enhancement studies, presents an ever greater challenge. We compared the success of two tagging techniques in very small (<25 mm carapace width) juvenile blue crabs (*Callinectes sapidus*): (1) microwire (also known as coded wire tags) and (2) elastomer (also known as visual implant fluorescent elastomer (VIFE) tags). Although growth and long-term mortality did not differ between tagging methods, each method had certain advantages. Crabs tagged with elastomer had lower immediate mortality as a result of the tagging process. Tag retention, short- and long-term and as well as field and laboratory, was higher for microwire than elastomer. Moreover, the microwire tagging process is about 70% faster. As a result of the higher tag retention and faster rate of microwire tagging, this method is recommended for very small juvenile blue crabs and other crustaceans. However, success is likely size-dependent, as better sites of elastomer application are accessible in larger blue crabs and probably other crustaceans as well. Due to its survivorship advantages and relative inexpense, elastomer tagging should not yet be ruled out for larger crustaceans or short-term studies of juveniles.

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1. Introduction

The ability to distinguish between groups of individuals within a single species is a valuable tool in the study of population dynamics and fisheries questions, allowing estimates of dispersal, mortality, and population size. Effective, affordable, and highly retained tags have been relatively well described for fishes

(e.g., Russell and Hales, 1992; Henderson-Arzapalo et al., 1999). However, fewer methods have been developed for crustaceans, which discard their carapaces along with any attached devices (but see, e.g., Fitz and Wiegert, 1991; Godin et al., 1996; Linnane and Mercer, 1998). Established methods also tend to favor larger organisms, presenting a challenge for studies of small species or early life-history stages.

Traditionally, crustacean scientists have relied heavily on the use of simple, but impermanent, external tags that are not retained through molts. These include external metal tags (Cronin, 1949; Cargo, 1958), biotelemetry tags (Wolcott and Hines, 1990), and paint marks (Young-Williams, unpubl. data). Though such tags may yield information about individuals within

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life-history stages that molt infrequently, they are impractical when used on frequently molting groups, such as juveniles.

Internal tags are more valuable, allowing long-term tracking of an animal through molts. However, they are invasive and therefore affect the animal more than external tags. For example, dart tags, dye, and ferromagnetic tags can cause mortality (Cargo, 1958; Miller, 1981), either immediately as a result of injury from the tagging process or gradually if, for example, the tag inhibits molting. Internal tags such as spaghetti tags (Fannaly, 1978), anchor tags (Henderson-Arzapalo et al., 1999), PIT tags (Kalvass et al., 1998), and microwire tags (Crook and White, 1995; Kneib and Huggler, 2001) are also associated with high tag loss rate. Tags can be expelled immediately after injection through the entrance wound or over time as the organism grows.

The coded wire tag (microwire) developed by Northwest Marine Technology (NMT) has been the favored internal tag for crustaceans, associated with low mortality, high retention, and quick delivery (van Montfrans et al., 1986; Fitz and Wiegert, 1991). However, this tag had not, until now, been compared with other options such as the elastomer tag in any crab species. Elastomer, a colored polymer developed by NMT (also known as visual implant fluorescent elastomer (VIE or VIFE)) is often used to tag fishes (e.g., Frederick, 1997; Bailey et al., 1998) but has been assessed in only a few crustaceans (crayfish and lobsters: Uglem et al., 1996; Linnane and Mercer, 1998; Willis and Babcock, 1998; Jerry et al., 2001). Elastomer is less invasive and more affordable than microwire and is detectable without expensive equipment. In addition, different colors and placement options allow batches of animals to be distinguished without sacrifice for the wire codes.

The purpose of this study was to determine which of the two tagging methods, microwire or elastomer, was better for juvenile blue crabs (*Callinectes sapidus*), an important fishery species of the US Atlantic and Gulf coasts. We focused on juveniles <25 mm carapace width (CW), the optimal size at which this species has been released in a concurrent experimental stock enhancement program. Advantages and disadvantages of each tag type were assessed in terms of (1) tag-induced mortality (short- and long-term), (2) tag

retention (short- and long-term), (3) inhibition of crab growth, and (4) the time required.

2. Methods

Juvenile blue crabs were raised in aquaculture facilities at the Center of Marine Biotechnology (COMB), University of Maryland Biotechnology Institute as part of a concurrent blue crab stock enhancement program. Cohorts of juveniles were products of three broods produced on 14 February 2002 (Batch A), 13 May 2002 (Batch B), and 30 June 2002 (Batch D), from three females obtained after maturity from the Chesapeake Bay. Crabs were tagged between the ages of 55 and 71 days and from 6 to 25 mm in carapace width.

Elastomer tags, small drops (<0.001 cm³) of orange or red plastic polymer, were administered with Northwest Marine Technology (NMT)'s VIE air injector machines (cost as of 2003: US\$ 6600) powered by a 54-kg air compressor, NMT's VIE hand injector (cost: US\$ 80), and 30 cm³ medical syringes (cost: negligible). Because elastomer must be injected into an area of the crab that is transparent enough to allow detection and muscular enough to allow retention through molting, these tags were injected into the proximal (basal) segment of the swimming leg (fifth pereopod) (Fig. 1). Initial efforts were made to use the paddle (distal segment) of the swimming leg, but the diameter of the needle was often greater than the width of paddle tissue for blue crabs of this size.

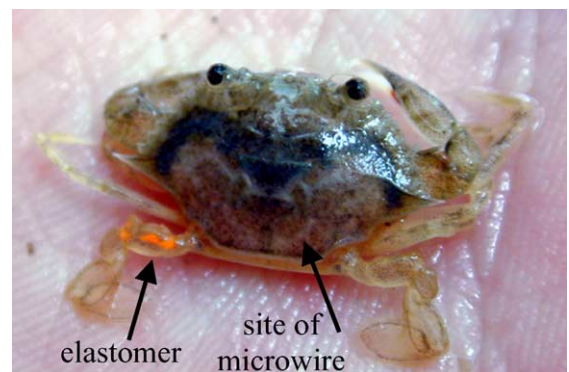


Fig. 1. Diagram of tagging locations for both microwire (M) and elastomer (E) in juvenile blue crabs.

Microwire tags (0.5 mm long, 0.25 mm in diameter) were inserted using automated microwire tagging machines (cost: US\$ 19,150). Because microwire is detected using a metal detector “wand” (cost: US\$ 7100) and transparent tissue is not required, tags can be placed more internally, reducing the risk of tag loss due to limb loss. Microwire tags were injected into the basal muscle of the swimming leg (Fig. 1) by inserting the injector needle between the ventral and dorsal sections of the carapace, as in van Montfrans et al. (1986), Fitz and Wiegert (1991) and Okamoto (1999). All crabs were checked with the wand to ensure successful tagging.

2.1. Timing of the tagging process

The time required to use the four types of injectors (automated microwire, automated elastomer, hand elastomer, syringe elastomer) was compared with timed trials of four people, all of whom used each device for 1 h each. Each person, new to tagging, was allowed several hours to practice with each injector before timed trials began. A repeated measures analysis of variance (ANOVA) was used to compare rates using both tag method and human operator as factors.

2.2. Short- and long-term mortality

Mortality induced by the tagging procedure and by carrying tags long-term was assessed with two experiments. In the first comparison of long-term mortality between tagging methods, we used Batch A crabs that had already been tagged for 8 days and held in the interim in 2.6 m³ cylindrical tanks at COMB. Ten crabs were added to each of seven outdoor flow-through raceways (30 cm in depth and 2 m² in surface area) at the Smithsonian Environmental Research Center (SERC) on the shore of the Chesapeake Bay. Five of these tanks held 3–5 elastomer and 5–7 microwire-tagged crabs (10 in each tank) and two tanks held 10 untagged crabs to serve as controls. Tanks were maintained with aerated, flowing estuarine water (18–29 °C and 10–15 ppt), and crabs were fed pellets and chopped fish. In each tank, pieces of plastic mesh were added to provide structure and decrease aggressive encounters between crabs. Each crab was measured and checked for tag retention weekly for 6 weeks, starting mid-May 2002, until

approximately the 50% mortality level was reached (though it should be noted that this laboratory mortality rate does not necessarily reflect mortality rates in the field). A χ^2 -test was used to compare the number of elastomer- and microwire-tagged crabs alive at the end of the experiment.

To determine immediate as well as long-term mortality, a second experiment was conducted in which 30 Batch B crabs of each tag treatment (microwire and elastomer) were placed in each of three outdoor tanks at SERC in mid-July 2002 immediately after tagging. After 1 day, 3 days, and weekly for the next 6 weeks, the number of crabs alive and tagged in each tank was counted and all were measured. Short-term mortality, calculated per tank, was compared between tag types using ANOVA ($n = 3$ for each tag type). Long-term mortality was compared with a χ^2 -test. This second outdoor-tank experiment differed from the first in that 180 crabs were used rather than 60; the experiment began immediately rather than 8 days after tagging; and temperatures were 5 °C warmer during the second experiment, resulting in faster growth and higher mortality rates.

2.3. Short- and long-term retention

To assess short-term tag loss, we used data from the second mortality experiment described above. In addition, the proportion of crabs that lost their tags after 8 days was compared between elastomer- and microwire-tagged crabs. Subsamples of a group of several thousand crabs that had been tagged were pulled randomly from two 2.6 m³ holding tanks, one holding only microwire-tagged crabs (six subsamples of 150 crabs) and one holding only elastomer-tagged crabs (two subsamples of 150 crabs). No replication of holding tank was available. The percentage of untagged crabs initially tagged with elastomer was compared using a t -test to that of untagged crabs initially tagged with microwire. Prior to all analyzes, all percentage data were arc-sine square root-transformed.

Longer-term retention was assessed from the two outdoor-tank experiments and by estimating rates of tag loss in the field. On three occasions, several thousand tagged crabs were released into sections of the Rhode River, a small sub-estuary in the upper Chesapeake Bay, as part of a concurrent stock enhancement study (Davis et al., unpubl. data). In each release,

Table 1
Types of tags in three batches of several thousand crabs released into the Chesapeake Bay

Crab batch	Microwire only (%)	Elastomer only (%)	Both tags (%)
A	60	40	0
B	22	0	78
D	14	0	86

Some crabs were tagged with microwire only, some were tagged with elastomer only, and some were tagged with both microwire and elastomer. The three batches of crabs, produced from three different sets of parents, are labeled A, B, and D according to order of birth.

proportions of crabs initially tagged with each tag type were known (Table 1). To determine which tag type was lost more frequently in the field, these initial proportions were compared to proportions of microwire and elastomer-tagged crabs recaptured over the next 14 weeks. In two of the three releases, most crabs were tagged with both tag types (although some were tagged only with microwire), enabling estimates of tag loss for each tag type (Wetherall, 1982). In subsequent resamplings, the rate of microwire tag loss by time (t) (L_M) can be considered from the sum of crabs that had only elastomer (having lost microwire) and crabs that lost both tags:

$$L_M = P_{E(t)} + L_E L_M P_{EM(0)} \quad (1)$$

where $P_{E(t)}$ is the proportion of crabs recaptured with only elastomer at time (t), L_E is the rate of elastomer loss by time (t), $P_{EM(0)}$ is the proportion of crabs initially tagged with both microwire and elastomer, and $(L_E L_M P_{EM(0)})$ is the proportion of double-tagged crabs that had lost both tags by time (t). This equation is rearranged to:

$$L_M = \frac{P_{E(t)}}{1 - L_E P_{EM(0)}} \quad (2)$$

The proportion of elastomer tag loss (L_E) was calculated as

$$L_E = P_{M(t)} - (P_{M(0)} - L_M P_{M(0)}) + L_E L_M P_{EM(0)} \quad (3)$$

where $P_{M(t)}$ is the proportion of crabs recaptured with only microwire at time (t), $P_{M(0)}$ is the proportion of crabs initially released with only microwire, $(L_M P_{M(0)})$ is the proportion of the initially

microwire-only crabs that had subsequently lost their tags. This equation can be rearranged into the form:

$$aL_E^2 + bL_E + c = 0 \quad (4)$$

where

$$a = P_{EM(0)} \quad (5)$$

$$b = P_{EM(0)}(P_{M(0)} + P_{E(t)} - P_{M(t)}) - 1 \quad (6)$$

$$c = P_{M(t)} + P_{E(t)}P_{M(0)} - P_{M(0)} \quad (7)$$

Equations were solved for L_E and L_M . Estimates of L_E and L_M were only possible while both tags were still present in the system, as they are derived from ratios of microwire- to elastomer-tagged crabs. When crabs with elastomer tags were no longer recaptured, L_E was considered to be 100% and no value for L_M could be obtained.

3. Results

3.1. Growth

No differences in growth of the elastomer-tagged, microwire tagged, and untagged crabs were measured in either laboratory experiment (ANCOVA, comparison of slopes of the lines, $P \gg 0.05$) (Fig. 2).

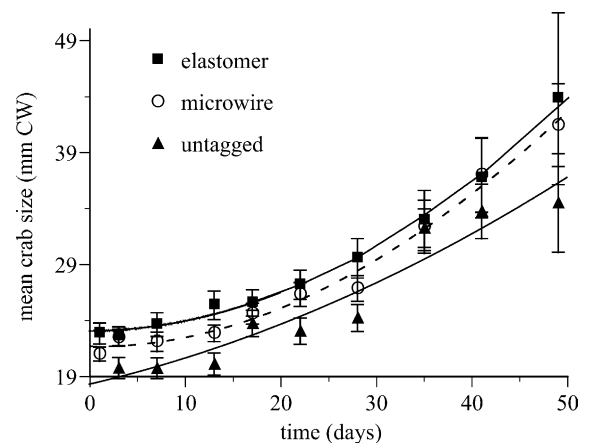


Fig. 2. Growth over 48 days of microwire-tagged, elastomer-tagged, and untagged crabs held at SERC. No differences in slope (ANCOVA, $P \gg 0.05$) were measured between tag treatments. Growth in the second experiment was similarly not different between tag types and therefore data are not shown.

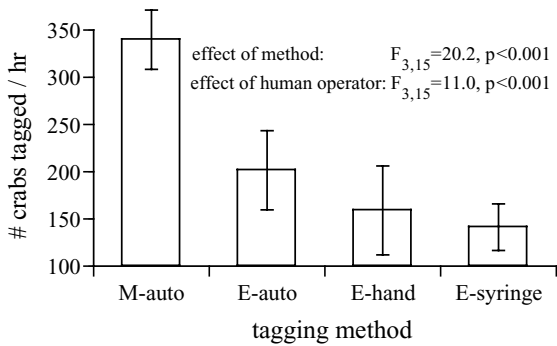


Fig. 3. Differences in the time requirement of each tagging method. M: microwire, E: elastomer. Repeated measures ANOVA statistics are listed.

3.2. Timing of the tagging process

Crabs were more quickly tagged with automated microwire machines (340 crabs/h) than with any elastomer method: air injector (202 crabs/h), hand injector (159 crabs/h), or syringe (141 crabs/h) (Fig. 3). Within the elastomer methods, the US\$ 6600 automated air injector, the US\$ 80 hand injector, and syringes did not significantly differ in rate of tag delivery.

3.3. Short- and long-term mortality

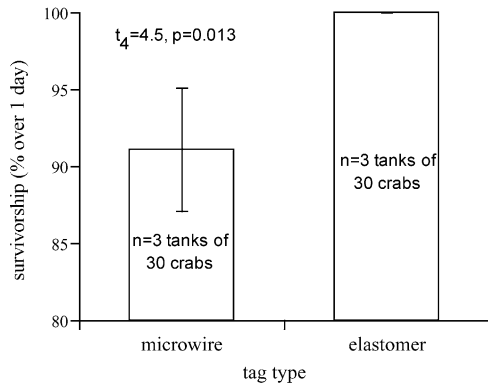
Immediate survivorship within 1 day of tagging was significantly lower for microwire-tagged (91%) than elastomer-tagged crabs (100%) (Fig. 4A). Longer-term survivorship, assessed until the 50% mortality level over the next 35 days, did not differ between the tag types (Fig. 4B). An additional experiment covering the period from 8 to 48 days after tagging (to the 50% mortality level) also revealed no differences in survivorship between microwire-tagged, elastomer-tagged, and untagged crabs (Fig. 4B). Differences in time to the 50% mortality level between the two experiments may be attributed to lower crab densities and lower temperatures of the first experiment.

3.4. Short- and long-term retention

Elastomer tags were lost immediately after tagging (within 8 days) more frequently (9.2±1.9% for 8 days) than microwire tags (1.8±1.2% for 8 days) when held

in high densities in 2.6m³ holding tanks (Fig. 5A). Rather than expulsion of the elastomer out through the tagging wound, most elastomer tag loss appeared to result from loss of the tagged limb by autotomy, defensive self-amputation of a limb common in blue crabs. Of the elastomer-tagged crabs that had lost tags, 65.4% were missing at least one swimming limb. Of crabs that lost microwire tags, only 16.7% were missing a swimming limb. Tag loss appeared to be related to conditions in which crabs were kept. In the outdoor tank experiment, where crab densities (and therefore probably aggressive encounters leading to autotomy) were lower than in the 2.6 m³ holding tanks, immediate tag loss did not differ between elastomer- and microwire-tagging (Fig. 5B).

(A) Short-term (1 day after tagging)



(B) Long-term

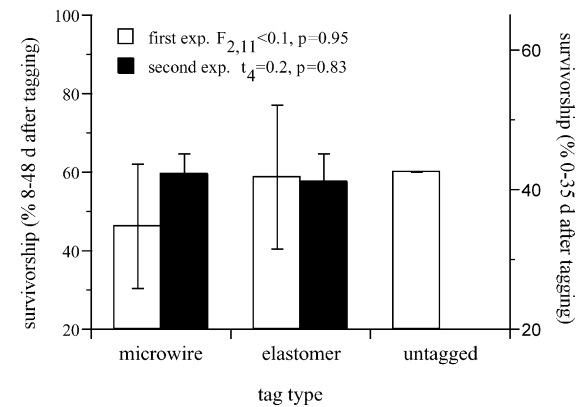


Fig. 4. Survivorship by tag type treatment (A) immediately (within 1 day) after tagging ($t_4 = 4.3$, $P = 0.013$) and (B) after 50% of the crabs had died in two separate experiments. t -Test and ANOVA statistics are listed.

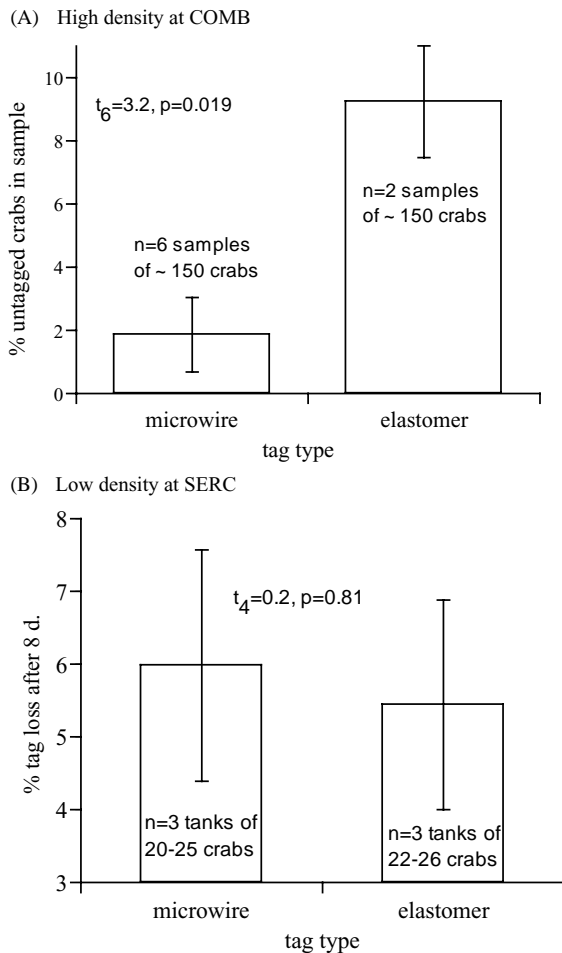


Fig. 5. Immediate tag retention (A) 8 days after tagging, held at high densities at COMB, (B) 8 days after tagging, held at low densities at SERC (second experiment). t -Test statistics are listed.

In laboratory experiments (covering the period from 8 to 48 and 0 to 35 days post-tagging) long-term tag loss was higher for elastomer than for microwire (Figs. 6 and 7), especially in the second experiment during which, due to faster growth, crabs attained a larger size (73 ± 4 mm CW) than in the first experiment (52 ± 6 mm CW). Elastomer became increasingly difficult to detect as crabs exceeded 50–60 mm and the exoskeleton increased in calcification (Fig. 7A). Microwire tag loss was lower and linear (Fig. 7A). Based on these relationships, 100% tag loss is predicted after 75 mm for elastomer and after 175 mm for microwire.

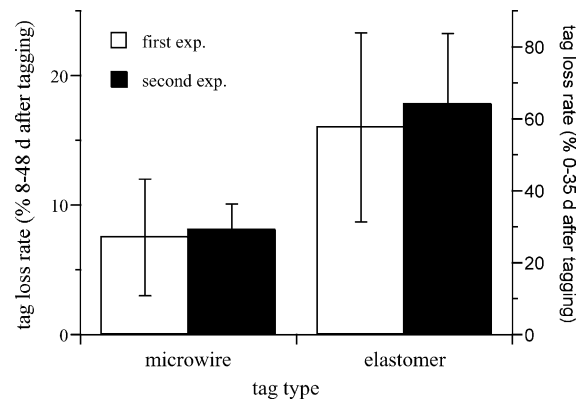


Fig. 6. Longer-term tag loss after 48 days (first experiment, $\chi^2 = 1.5$, d.f. = 1, $P > 0.10$), and after 35 days post-tagging held at SERC (second experiment, $\chi^2 = 4.6$, d.f. = 1, $P < 0.02$).

Results of the field release experiments similarly suggest that elastomer loss (and/or detection failure) was higher than that of microwire. Field loss rates based on the multiple-tag field releases were generally consistent with laboratory tag loss rates (Fig. 7B). No elastomer-tagged crabs larger than 80 mm were recaptured. In contrast, microwire-tagged crabs as large as 128 mm were recaptured (Fig. 8). At mid-summer growth rates, detection of elastomer was predicted to end after 6 weeks. Microwire detection was predicted to last 20 weeks. In crabs of the fourth release, which occurred in September 2002 during a time of slower growth rates, elastomer was visible for much longer, persisting through the 2002–2003 overwintering period until crabs began to grow again. Results from this slower-growing batch emphasize that crab growth, rather than time, is the limiting factor in elastomer retention/detection. Although deviations of the elastomer:microwire recapture ratio from the release ratio (Fig. 8) cannot be attributed unequivocally to higher elastomer tag loss, the only other mechanism, lower survivorship of elastomer crabs in the field, was not supported by laboratory experiments.

3.5. Predictions based on mortality and tag loss

In the short-term, elastomer yielded a higher proportion of tagged crabs than microwire. Of 573 crabs that were tagged with elastomer and recounted 8 days later, 84.5% were alive and still tagged. Of 1919 crabs tagged with microwire, 73.1% were alive and still

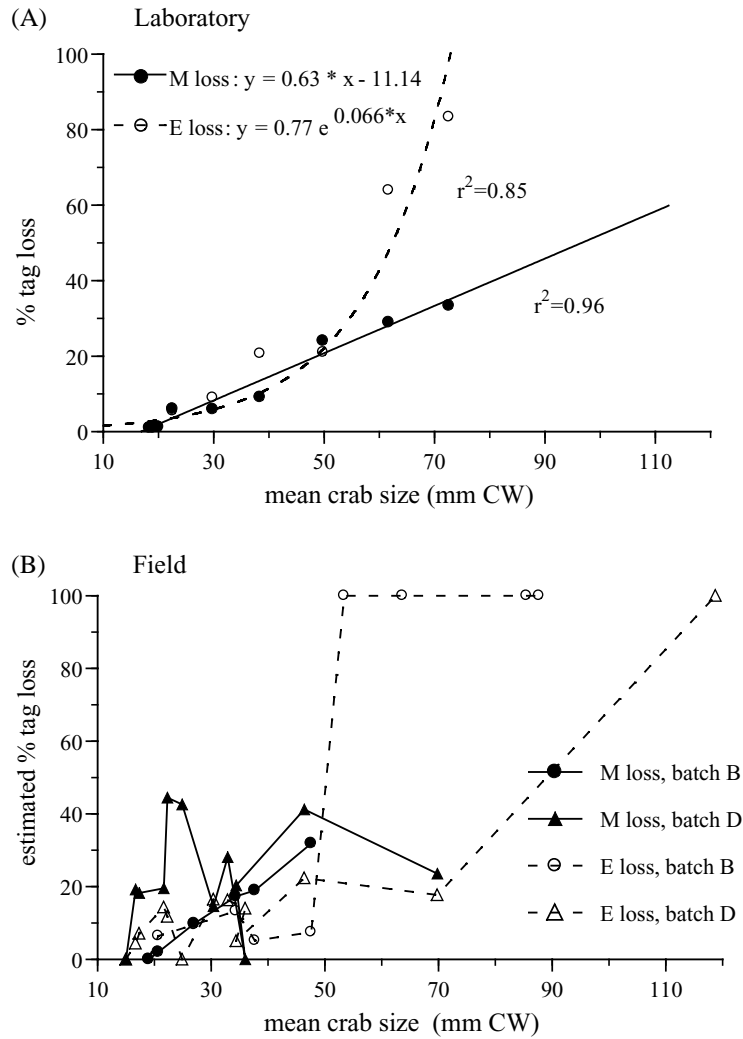


Fig. 7. (A) Tag loss rates based on laboratory experiments. (B) Tag loss estimates based on releases and recaptures of double-tagged crabs in the field. M: microwire, E: elastomer.

tagged 8 days later. Our loss of tagged crabs to death and/or tag loss was 26.9% for microwire and 15.5% for elastomer. Based on tag retention and mortality estimates, we predict that loss of tagged crabs in the case of microwire was due mainly to death, and in the case of elastomer was due more to tag loss.

In the long-term, however, releasing equal amounts of microwire- and elastomer-tagged juvenile crabs into the field will yield higher recaptures of microwire-tagged crabs. If 100 crabs are each tagged with elastomer and microwire, after 1 week (using our

estimates of short-term retention and survivorship), 91 microwire-tagged crabs and 98 elastomer-tagged crabs would remain. Based on our field recapture ratio, after another 3 weeks, even if all 91 microwire-tagged crabs survived and were detected, only 27 of the elastomer-tagged crabs would be detectable. Applying our laboratory rate of juvenile microwire-tagged blue crab mortality over this period (18%), a more realistic estimate of 75 microwire-tagged crabs and 22 elastomer-tagged crabs would be present. After another period of growth no elastomer would be

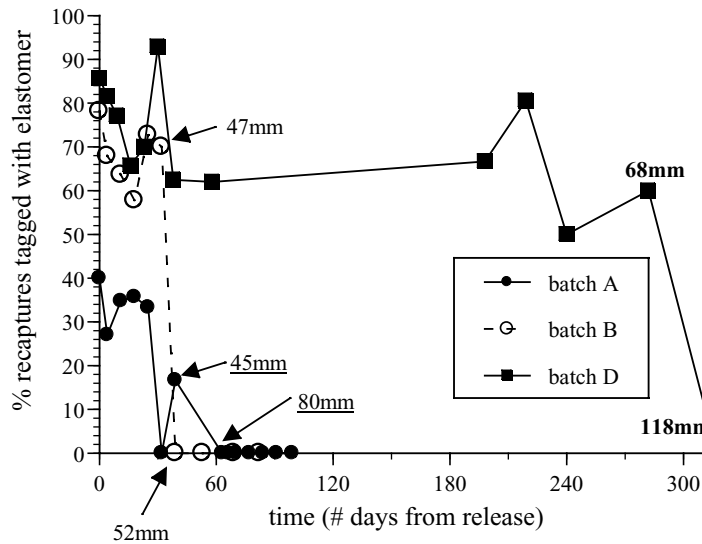


Fig. 8. Persistence of elastomer in the field over time for three batches of crabs. Values on day 0 are the initial percentages of elastomer-tagged crabs (batch A: 40%, batch B: 78%, batch D: 85%). Average size of crabs for the first day on which elastomer-tagged crabs were no longer recaptured and the day prior are noted for each batch, indicating the crab size range at which elastomer disappears.

detectable due to increased calcification of the swimming leg.

4. Discussion

Although microwire tagging resulted in higher immediate mortality than elastomer, other advantages made it a better tagging method in small (<20 mm CW) blue crabs: (1) Injection of microwire tags is quicker, an important factor if large numbers are to be tagged. (2) Long-term mortality did not differ between tag types (or untagged control crabs). (3) Microwire was more detectable in the field as crabs grew to >65 mm CW, leading to more accurate population estimates. These results differ from those of the few other studies comparing elastomer and microwire, in which differences between the tag types were not observed in brown and rainbow trout (Hale and Gray, 1998) or juvenile lobsters (Linnane and Mercer, 1998). The disparity between these and our study may be attributed to differences in characteristics of our study species.

Although elastomer was easily detected in smaller crabs, three factors contributed to its loss over time. First, autotomy of the tagged swimming leg was com-

mon. Second, elastomer often migrated over time up into the more calcified body, making detection difficult (also noted in lobster, Linnane and Mercer, 1998). Third, the proximal segment of the swimming leg calcifies and becomes less transparent as the crab grows, making elastomer difficult to detect. Difficulty of elastomer detection has also been reported for fish (Close, 2000). The last two factors are less problematic in larger blue crabs (>30 mm CW), as the distal segment (the paddle) can be used (Young-Williams, unpubl. data).

Microwire tag loss was also probably affected by several factors. Tags may have been expelled over time. In addition, the wand used to detect microwire may not be 100% effective (Uglen and Grimsen, 1995).

High short-term retention for microwire has been noted for other species (Buckmeier, 2001), but reports of long-term retention rates for both tag types vary greatly depending on species and size at tagging. For example, long-term retention of a microwire-like tag was only 10% in crabs (*Portunus trituberculatus*) that were 10–20 mm CW at the time of tagging, but 70% in crabs over 20 mm CW (Okamoto, 1999). Our rates of microwire retention were much lower than those reported for larger juvenile blue crabs by

Fitz and Wiegert (1991) and van Montfrans et al. (1986), who found rates of 97% over 80 days (for crabs tagged when 29–67 mm CW) and 88% over 51 days (for crabs tagged when 21–39 mm CW), respectively. Our estimated rates for those periods were 60 and 35%.

Reports of long-term (on the order of one to several months) microwire retention for other species range from 98% for striped bass (Buckmeier, 2001), 99% for rainbow trout (Oven and Blankenship, 1993), and 100% for crayfish (Isely and Eversole, 1998) to 35% for white shrimp (Kneib and Huggler, 2001), 29% for a galaxid fish (Crook and White, 1995), and 23% for paddlefish (Guy et al., 1996). Estimates of long-term (one to several months) elastomer retention rates include 60% in squawfish (Haines et al., 1998), 92–100% in lobster (Uglem et al., 1996; Linnane and Mercer, 1998), and 100% in shrimp (Godin et al., 1996). Factors contributing to variability include site of tag insertion (Kneib and Huggler, 2001), experience of people operating the tagging equipment (Bailey et al., 1998; Close, 2000), and conditions in which animals are kept (Guy et al., 1996). In the present study, short-term elastomer loss was higher when crabs were kept for 8 days in high-density than low-density conditions.

Reports of mortality rates are equally wide-ranging, but can also depend on animal size for both elastomer and microwire (Frederick, 1997; Linnane and Mercer, 1998). Although tagging can have an immediate mortality effect (Uglem and Grimsen, 1995, this study), most studies, like ours, do not report a tag effect on long-term mortality for either microwire (e.g., van Montfrans et al., 1986; Fitz and Wiegert, 1991; Uglem and Grimsen, 1995) or elastomer (Bailey et al., 1998; Malone et al., 1999; Davis and Ovaska, 2001). As in the present study, most also reported no effects on animal growth of either microwire (e.g., Russell and Hales, 1992; Kneib and Huggler, 2001) or elastomer (Malone et al., 1999; Davis and Ovaska, 2001). The presence of tags did not interfere with the molting process in blue crabs in a way that affected overall growth (this study, Fitz and Wiegert, 1991) or the step increase in size per molt (van Montfrans et al., 1986). However, in the latter study, microwire-tagged crabs molted less often than untagged crabs. In our study, we observed that crabs tagged with elastomer in advanced pre-molt stages of the molting cycle left the tagged

leg behind during ecdysis; however, this impediment did not appear to affect growth or survivorship.

Tagging rate depended on human operator, but overall, automated microwire machines were faster than elastomer. A hand-held microwire device does exist, but has low rates (120 crabs/h, Young-Williams and Carrier, unpubl. data). Within the three methods of elastomer delivery, the relatively expensive automated and hand injectors did not provide a significant speed advantage over inexpensive medical syringes. Microwire tagging rates in the present study (260–400 crabs/h) were comparable to those reported for lobster (240/h, Wickins et al., 1986) and fish (e.g., 389–583/h, Buckmeier, 2001). Elastomer rates (92–325 crabs/h) were also comparable to those reported in other studies (e.g., 130–140 fish/h, Haines et al., 1998).

Both methods of tagging have potential to contribute greatly to such fields as population recruitment and stock enhancement. Coupled with higher long-term retention, faster tagging makes microwire the recommended choice for enhancement studies of juvenile blue crabs and other crustaceans posing similar tagging limitations. However, the use of elastomer as a second tag can provide valuable additional information, allowing easier distinguishing among batches of animals, for a shorter time period.

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