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COMPARISON OF TAGGING TECHNIQUES FOR TRACKING FRESHWATER TROPICAL SHRIMP: ASSESSMENT OF SURVIVAL AND BEHAVIOUR AS A FUNCTION OF TAG TYPE

BY

LAUREN J. KABAT1,2,6), TODD A. CROWL2), ÁNGEL S. ESTRUCHE-SANTOS3), JACK CHESHIRE4), W. RYAN JAMES1,2,5) and ROLANDO O. SANTOS1,2)

1) Department of Biological Sciences, Florida International University, 3000 NE 151st Street, North Miami, FL 33181, U.S.A.

2) Institute of Environment, Florida International University, 11200 SW 8th Street, Miami, FL 33199, U.S.A.

3) Department of Environmental Sciences, University of Puerto Rico at Río Piedras, 14 Avenue Universidad, San Juan, PR 00925, U.S.A.

4) Department of Biological Sciences, North Carolina State University, 112 Derieux Place, Raleigh, NC 27607, U.S.A.

5) Department of Earth and Environment, Florida International University, 11200 SW 8th Street, Miami, FL 33199, U.S.A.

ORCID iDs: Kabat: 0009-0009-8051-897X; Crowl: 0000-0002-4712-8701;
Estruche-Santos: 0009-0003-8514-3288; Cheshire: 0009-0007-4110-5967;
Ryan James: 0000-0002-4829-7742; Santos: 0000-0002-3885-9406

ABSTRACT

Freshwater shrimps are abundant in several rivers and streams of Puerto Rico. These species provide many ecosystem services, including cycling nutrients, connecting coastal and montane ecosystems, and serving as food for other organisms. Tracking the movements of individual shrimp is necessary to understand their responses to environmental and anthropogenic disturbances. To explore methods of measuring movement, we conducted two types of lab experiments. The first measured the acute and chronic responses of *Xiphocaris elongata* (Guérin-Méneville, 1855) and *Atya lanipes* (Holthuis, 1963) to visual implant tags. Daily observations showed 100% survival, and video analysis of movement metrics (percent active time, total distance moved, and mean velocity) revealed no significant differences between tagged and control shrimp. The second experiment assessed the response of *A. lanipes* to passive integrated transponder tags. These shrimp showed low survival, independent of size. This study supports the use of visual implant tags but recommends further study before utilizing passive integrated transponders to mark these small shrimp species.

Key words. — aquatic invertebrates, animal movement, passive integrated transponder, visual implant tag

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6) Corresponding author; e-mail: kabat.lauren@gmail.com

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INTRODUCTION

Capture-mark-recapture (CMR) is a valuable method for studying ecological processes. It involves capturing, marking, releasing, and later recapturing individuals to create histories that reveal patterns in the study population. Early CMR models focused on estimating population size (Jolly, 1965; Seber, 1965), growth rate, and recruitment (Pradel, 1996). Marking individuals across demographic categories such as sex, age, and/or size adds to the life history and community research that managers use to assess and conserve populations of interest. CMR methodologies are dependent on the research questions being asked and evolve as new technologies emerge (Lindberg, 2012). Additionally, advances such as miniaturization of tags and remote sensing expand tracking capabilities to better understand movement patterns including migrations and interspecific interactions (Cooke et al., 2013; Kays et al., 2015; Lennox et al., 2017). Because these applications depend on reliably linking each capture event to the correct animal, accurate identification of individuals is fundamental to the integrity of CMR studies (Heemeyer et al., 2007; Lindberg, 2012). This drives the development and selection of diverse marking and tagging methods tailored to species and study objectives (Osbourn et al., 2011; Cooke et al., 2013; Kays et al., 2015; Vandenabeele et al., 2015).

Selecting appropriate marking techniques and understanding the study animal's life history and responses to marking and handling are paramount when designing effective CMR studies. In some larger aquatic fauna, photo identification can be used to distinguish individuals by their unique natural features, such as the shape and markings on the dorsal fins of dolphin species (Maglietta et al., 2022). However, for animals difficult to visually differentiate, unique markings must be applied. To collect ethical and accurate tracking data, tag type, application methods, readability, weight, and retention of the tag must be suitable for the size and physiology of the tracked animal (Kays et al., 2015; Vandenabeele et al., 2015). Even similar tags in similar sized individuals can have different effects depending on the species' physiology, life stage, habitat, and movement modality. Thus, tagging an animal presents the inherent risk of altering the health or behaviour of the individual (Vandenabeele et al., 2015), especially considering the weight and attachment methods (Kays et al., 2015). While larger tags can collect more data (e.g. quantity, type, and complexity of data), smaller tags are less invasive but may limit the duration of the period of observation. However, smaller tags may be necessary to reduce interference with the animals' natural behaviour. Tagging methods vary widely from external physical tags to internal implants, and must balance factors such as tag size, retention, readability, and potential impacts on the animal to ensure ethical and effective monitoring (Kays et al., 2015; Vandenabeele et al., 2015). Selecting an appropriate tagging strategy is critical to maximize data quality while minimizing adverse effects on study organisms.

Tag-related effects, such as mortality and behavioural changes, can impact both the health of the animal and the quality of collected data. Mortality is a primary concern associated with tagging and may result from injury, impaired movement, or increased vulnerability to predation (Theuerkauf et al., 2007; Vandenabeele et al., 2015). Mortality rates also vary depending on species, tag size, implantation site, and handling protocols, highlighting the need for species-specific trials before field deployment (Heemeyer et al., 2007; Hamilton et al., 2022). In addition to mortality, tagging can induce behavioural changes such as reduced activity levels, altered movement patterns, or shifts in feeding and habitat use (Black, 2010; Vandenabeele et al., 2015; Batsleer et al., 2020). These behavioural alterations have the potential to harm the animal and influence study results, highlighting the importance of selecting tagging methods that minimize such impacts. Researchers must carefully weigh the trade-offs among available tag options and conduct controlled tests on their study species to determine the most suitable methodology (Heemeyer et al., 2007; Cooke et al., 2013).

Concerns related to tagging are especially pronounced in small-bodied organisms, where even minimally invasive tags can represent a significant proportional burden (Batsleer et al., 2020). The added weight or volume of a tag may alter locomotion, feeding efficiency, or predator avoidance (Vandenabeele et al., 2015), while implantation procedures can increase handling stress and lead to higher post-tagging mortality (Hamilton et al., 2022). In some invertebrates, additional challenges arise from moulting, which can cause tag loss or displacement (Godin et al., 1996; Hein & Crowl, 2010; Haddaway et al., 2011). Despite increasing awareness of these issues, the effects of tagging on invertebrates remain underexplored compared to vertebrate systems (Batsleer et al., 2020).

Among small-bodied organisms, visible implant (VI) tags are frequently used where external or rigid tags are not feasible. Visible implant elastomer (VIE) is a coloured polymer injected as a liquid beneath translucent tissue that cures into a flexible, biocompatible mark. Researchers can customize the colour, placement, and amount of elastomer to suit the animal's size and pigmentation, allowing identification by capture region, population, or batch. While the visibility of these tags relies on translucent and evenly pigmented tissue, fluorescence under ultraviolet light aids in recognizing the marks. VIE tags have been used successfully on a variety of species, including octopus (Brewer & Norcross, 2012), sea stars (Martinez et al., 2013), and earthworms (Butt & Lowe, 2007). VIE has effectively stayed with tadpoles through metamorphosis (Fouilloux et al., 2020), and in decapod species elastomer tags persist through moulting and do not negatively affect growth, gender ratio, or predation rates in whiteleg shrimp (Godin et al., 1996), or Chinese white shrimp (Xiaopeng et al., 2017).

However, as the colours of VIE limit the number of distinct combinations (Osbourn et al., 2011), an alternative or additional marking is needed for accurate detection of individuals. Visible implant alphanumeric tags (VIA) are each comprised of a unique series of letters and numbers to distinctly mark individuals. Studies show that VIA tags are effective for marking small amphibians like juvenile salamanders, with fluorescence improving readability (Osbourn et al., 2011), and have shown good short-term retention and clarity in some juvenile fishes (Turek et al., 2014), demonstrating their value for marking small-bodied species. VIA tags have minimal effects on the growth and survival of decapods and are retained internally for several years (Isely & Stockett, 2001; Pérez-Reyes et al., 2015).

Passive integrated transponder (PIT) tags are another minimally invasive internal tagging option, and an excellent tool for tracking species in situ that are difficult to catch using other methods. PIT tags are also effective in species where the anatomy of the study species doesn't allow for the detection of VI tags due to pigmentation or shape. This tag type is encoded with a unique series of letters and numbers and is encapsulated in glass. It is implanted into the animal's tissue by a needle and read by a specialized scanner which eliminates the need for the tag to remain visible through the animal's tissues. PIT tags have been successfully used in small fish (Jørgensen et al., 2017; Nyqvist et al., 2024), birds (Farr et al., 2021), and shrimp (Foote et al., 2018; Dunn et al., 2021).

While this miniaturization of tags allows ecologists to successfully investigate smaller animals and animals at younger life stages (Hussey et al., 2015; Lennox et al., 2017), the size and invasive application of PIT tags can cause challenges. Mortality related to PIT tagging in estuarine fish (Kimball & Mace, 2020) and decapods (Black, 2010) is typically acute and occurs mainly within the first 10 days post-injection. In sea urchins that retain PIT tags, tag movement or tissue overgrowth can make tag detection inconsistent (Lauzon-Guay & Scheibling, 2008). Survival after PIT tagging varies by species, with juvenile white shrimp demonstrating high survival at carapace lengths (CL) of 25-35 mm (Dunn et al., 2021), northern crayfish showing high survival at CL >20 mm (Hamilton et al., 2022), and approximately 80% survival rate for woodland crayfish with >23 mm CL (Westhoff & Sievert, 2013). Although size and survival are not directly correlated across all size ranges, these studies indicate species-specific minimum sizes for low mortality.

It is important to choose a PIT tag insertion location compatible with the physiology of the study species. For example, in northern crayfish, Hamilton et al. (2022) found low mortality when tagged in the centre of their ventral side. Other studies tested PIT insertion and orientation among various locations along the main body axis of the dorsal side in white shrimp (Dunn et al., 2021) and black tiger shrimp (Foote et al., 2018), and found survival rates similar to control groups. Together, these studies demonstrate that PIT tag insertion sites and detection reliability should be thoroughly evaluated in preliminary trials prior to their use in field studies involving small or sensitive species.

Previous CMR studies on crustaceans have primarily focused on aquaculture species and larger individuals in field settings (Webb & Kneib, 2004; Mace & Rozas, 2015), with little information on the effects of various tag types on small tropical freshwater shrimp. Our study focuses on the freshwater shrimps *Xiphocaris elongata* (Guérin-Méneville, 1855) and *Atya lanipes* (Holthuis, 1963). These species are abundant and ecologically important in several Caribbean nations' rivers and streams (Covich & McDowell, 1996; Crowl et al., 2001). They provide many ecosystem services such as nutrient cycling (Crowl et al., 2001), algae control (March et al., 2002) and connecting coastal and montane ecosystems through their amphidromous lifecycles (March et al., 1998; Kikkert et al., 2009). In Puerto Rican headwater streams native decapods dominate the aquatic food web (Covich & McDowell, 1996; March et al., 2002; Greathouse & Pringle, 2005; Hein & Crowl, 2010) and serve as a traditional food source to local communities (González-Cabán & Loomis, 1997; Kartchner, 2003; Ortiz-Zayas & Scatena, 2004; Santiago & Loomis, 2009; Scalley, 2012). *A. lanipes* are filter feeders and grazers, using their cheliped fans to filter particles (Covich, 1988). Atyids are predicted to live a minimum life span of 8 years and reach an orbital carapace length between 20-25 mm with males having a larger average size than females (Cross et al., 2008). They have a mottled coloration ranging from tan to dark brown. *X. elongata* are translucent shrimp that forage on leaves, algae, insects, and dead animals (Covich & McDowell, 1996; March & Pringle, 2003; Cross et al., 2008). In headwater streams, *X. elongata* live a minimum of 5 years and grow to an estimated 14 mm CL with most of their growth in the first 2-3 years of their life (Cross et al., 2008).

Based on the life history data of these species, *X. elongata* (max. 14 mm CL) are too small for PIT tag implantation, whereas larger *A. lanipes* (25 mm CL; Cross et al., 2008) may be suitable. Both species share traits such as small size, frequent moulting, and limb regeneration (Vogt, 2014) that influence tag retention, though they differ in physiology, maximum size, sexual dimorphism, and coloration (Martínez-Mayén et al., 2000). Establishing survival thresholds through controlled testing for tagged *X. elongata* and *A. lanipes* is essential for determining the best marking approach for these species.

In addition to ensuring survival, we must be confident that tag application does not alter our study species' normal movement behaviour. *X. elongata* and *A. lanipes* swim and crawl to forage for food as well as optimize their habitat by travelling along the stream reach (Crowl et al., 2001). Additionally, as small shrimp species serve as prey to larger prawns, tagged individuals must maintain their ability to both move at their natural speed to seek refuge, and then remain still when avoiding capture as well as withstand the flashy discharge of their stream environment (Crowl & Covich, 1994; Ocasio-Torres et al., 2021). These behavioural demands highlight the need to evaluate whether our tagging methods influence the natural movement behaviour of our study species. Several metrics can be used to analyse the movement of small organisms. Percent active time provides a measure of time spent moving versus remaining stationary and is particularly relevant for assessing behaviours related to predator avoidance and refuge use. Total distance moved is commonly used in various behavioural studies with ambulatory organisms in natural settings such as in billbugs (Gireesh & Joseph, 2022), and in toxicology research of both terrestrial (Kulbeth et al., 2021) and aquatic (Szep et al., 2023) species. Likewise, mean velocity is appropriate to pair with the total distance moved metric to further assess mobility function, as demonstrated in studies of fish and insects (Agrafioti et al., 2021; Xia et al., 2021). Together, these metrics provide a framework for evaluating whether tagging influences natural movement behaviour in our study species.

Therefore, we addressed two research questions: (1) Do VI or PIT tags affect the survival of *A. lanipes* or *X. elongata*? (2) Do VI or PIT tags affect the movement behaviour (percent active time, total distance moved, and mean velocity) of *A. lanipes* or *X. elongata*? For VI tags, we hypothesized that individuals of both shrimp species would survive the initial stress of tagging and the duration of the experiment, and that VI tags would not affect the movement in either species due to their small size and minimally invasive application. For PIT tags, we tagged only *A. lanipes* and hypothesised size-dependent survival, with larger individuals surviving and retaining the tag the longest. We expected no adverse effects on surviving shrimp during feeding or moulting. We also expected surviving individuals with PIT tags to exhibit normal movement, while those that did not survive might show altered activity patterns prior to mortality. Reduced activity could indicate recovery from the tagging wound, whereas increased activity might reflect stress or attempts to expel the foreign body.

MATERIALS AND METHODS

Sampling and experimental set-up

We conducted two trials. The first trial ran from 10 June to 4 July 2022. This trial included 20 *X. elongata* (10 control, 10 VI) and 30 *A. lanipes* (10 control, 10 VI and 10 PIT). The second trial ran from 14 July to 3 August 2022 and contained 27 *A. lanipes* (13 control and 14 PIT).

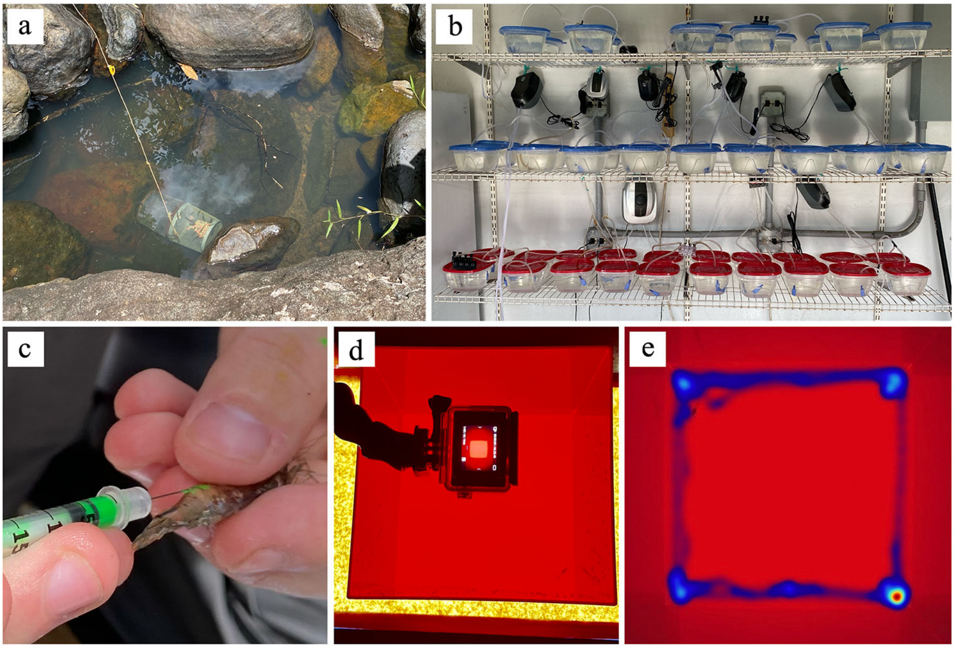

Fig. 1. Overview of collection, housing, tagging, and behavioural analysis methods: a, *Atya lanipes* (Holthuis, 1963) and *Xiphocaris elongata* (Guérin-Méneville, 1855) collected from Quebrada Prieta using baited G-4 wire minnow traps; b, randomly arranged, aerated enclosures containing shrimp from various treatment groups and size classes; c, injection of green elastomer into an *X. elongata* assigned to the visible implant treatment; d, behavioural arena constructed from a red acrylic 22 × 22 × 22 cm cube filled with 1 litre of stream water, with shrimp placed inside and recorded using a GoPro positioned above the arena; e, heat map depicting 5 min of *X. elongata* movements.

We sourced our study shrimp from Quebrada Prieta, a high-gradient, second-order stream in the Luquillo Experimental Forest, part of El Yunque National Forest in northeastern Puerto Rico. The stream is lined with numerous boulders that create a matrix of connected and disconnected pools (Scalley et al., 2009). From these pools we collected our shrimp using baited Gee G-4 wire minnow traps (Cuba Specialty Manufacturing, Filmore, NY, U.S.A.) (fig. 1a).

For the first trial, we captured an excess of shrimp and selected the largest 20 *X. elongata* and 30 *A. lanipes* for use in the experiment. We assessed the health of individuals, noting injuries and activity level, and measured each shrimp from the apex of the rostrum to the posterior median edge of the carapace to the nearest 0.1 mm. We placed each animal in a separate aerated container filled with approximately 1 litre of stream water with temperature and pH comparable to the shrimp capture location. We randomly selected 10 *X. elongata* of various sizes and assigned them to the VI experimental group and assigned 10 to the control group. Similarly, we distributed *A. lanipes* into three groups of 10: VI, PIT and control groups. We arranged the enclosures such that the treatments and sizes were distributed randomly to prevent environmental variables in the lab such as lighting from influencing results (fig. 1b). After shrimp were assigned to their treatments and placed in individual enclosures, they were given two days to acclimate to their new environments before any further handling took place. During all trials, we fed all shrimp aquarium fish flakes and measured water pH and temperature every other day. We replaced the water with fresh stream water approximately every five days with all shrimp receiving the same treatments on the same day.

After trial 1 was complete, we conducted trial 2 to increase the sample size of PIT tagged *A. lanipes* and strengthen our dataset. Trial 2 was set up similarly, this time with 14 *A. lanipes* in the PIT tag treatment and 13 *A. lanipes* in the control treatment. All individuals in trial 2 were captured, assessed, and cared for using the methodology above.

Tagging

On tagging day for each trial, considered experiment day 0, all shrimp started alive and active with no observable problems. All shrimp within the VI treatment received both VIE and VIA tags injected according to instructions from the manufacturer (Northwest Marine Technology, Anacordes, WA, U.S.A.). A small amount of green VIE was injected in a posterior to anterior direction by passing a medical-grade hypodermic needle into the muscle tissue between abdominal segments 3 and 4 on the shrimp's left side (fig. 1c). VIA tags measuring 1.2 mm by 2.7 mm were inserted using the VI Alpha Injector between abdominal segments 3 and 4 on the shrimp's right side. This injection site allowed the three-digit series to be visible from underneath abdominal segments 2 and/or 3 depending on the shrimp's size.

For the PIT tag treatment, we used MiniHPT8 tags that weighed 30 mg and measured 8.4 mm long by 1.4 mm diameter (Biomark. Boise, ID, U.S.A.). In our study, PIT tags were inserted with a spring assisted syringe implanter between segments 4 and 5 for small *A. lanipes* and between 3 and 4 for larger *A. lanipes*. These insertion sites were selected such that the PIT tag lay in the muscle tissue underneath segments 1, 2 and/or 3 (depending on the size of the shrimp) aligned along the main body axis. Based on the small size of our species, care was taken to insert the PIT tag such that it lay dorsally in the thickest part of the animal's abdomen but did not puncture internal organs or the gill chamber. The handling time for each tagged shrimp was recorded and an average handling time was calculated. Control shrimps were handled out of the water for this average time to simulate the stress of the tagging process but without injections of any tag.

Survival

We conducted two trial periods during which we recorded daily observations on each shrimp. Each day, shrimp were categorized as either alive or dead, and observations were made about each shrimp's health condition, including missing legs, antennae, uropods, moulting status, and healing of the tag insertion location. Tag readability was assessed by visually inspecting VI tags and using a scanner for PIT tagged shrimp. All daily health observations were made without handling the shrimp.

Behaviour

Movement behaviour was analysed using video recordings taken at 3 timepoints in each trial. To complete this, we brought each shrimp into a dark room and placed it in a red acrylic 22 × 22 × 22 cm cube with 1 litre of stream water. Each shrimp was given 5 min to explore the new container and to adjust after the transfer. Following this acclimation period, the container was placed on a dim light table for an additional 2 min before recording movements. Because long wavelength light is least disruptive to shrimp and they are most active at night (Johnson & Covich, 2000), we used a container and lighting that simulated nighttime conditions while still providing enough illumination for video recording. For each shrimp, we recorded a 6-min video with a GoPro camera (GoPro, San Mateo, CA, U.S.A.) positioned directly above the container (fig. 1d). During this filming process, the shrimp was left undisturbed in a quiet environment to not influence its behaviour. The videos were recorded at a GoPro setting of 48 frames per second, 1140 pixel resolution, and low light. As recommended by Henry et al. (2019), these settings produce the maximum quality video that can be analysed with the movement software used in this analysis. Throughout the process, each shrimp was accompanied by a card with its identification number to ensure that all videos were labelled correctly. Videos were taken by order of shrimp identification number which alternated between treatments. Therefore, each treatment had individuals recorded at various timepoints throughout the day. The first video time point took place after the acclimation period but before tagging. In trial 1, all 20 *X. elongata* were videoed on 11 June and all 30 *A. lanipes* were videoed on 12 June.

All shrimp in trial 1 were tagged on 13 June (day 0). *X. elongata* were videoed again on 16 June (day 3) and 24 June (day 11). *A. lanipes* were videoed on 17 June (day 4) and 25 June (day 12). These videos followed the same methodology described above. For trial 2, *A. lanipes* were first videoed on 12 July, tagged on 14 July (day 0), and videoed again on 18 July (day 4) and 25 July (day 11).

We used EthoVisionXT (Noldus Software, Leesburg, VA, U.S.A.) to generate movement values and heat maps for each video (fig. 1e). The software tracked the shrimp using center point detection focused on the dark organs in the head of the shrimp. The period of analysis was the 5 min between minutes 1 and 6 of the videos. We did not use the first minute of video in the analysis as shrimp behaviour during this time may have been influenced by the researcher moving around the lab space to turn on the camera. For our experiment, we analysed percent active time and two related movement metrics, total distance moved (mm) and its time-standardized counterpart, mean velocity (mm/min).

On 12 June, following taking the videos of *A. lanipes*, the full lab was moved because of a Covid19 outbreak within the field station. This unplanned move resulted in a lab setting with air conditioning that changed the water temperature within the shrimp enclosures from the original 26°C to a much cooler 18°C. As all 50 shrimp in the first trial were subjected to similar stress from this move, we were still able to compare control and treatment shrimp within each species and video timepoint. During water changes, stream water was kept aerated and allowed to cool to the lab's ambient temperature before being added to the shrimp's enclosure.

Statistical analyses

Survival

We used a Cox proportional hazards model in the R package survival (Therneau, 2022) to analyse the survivorship of shrimp across trials and treatments. The model estimates the hazard, or risk of death over time, as a function of one or more covariates (Cox, 1972). In our analysis, we included trial (trial 1 or 2), treatment (control or PIT), and cephalothorax length as fixed effects, with survival time defined as the number of days until death. This allowed us to assess whether survivorship differed by trial or treatment, and whether shrimp size was associated with survival, while accounting for the effects of the other variables. We assumed proportional hazards across the study period, meaning we expected the relative differences in risk between treatments to remain constant over time.

Behaviour

We analysed *X. elongata* and *A. lanipes* movement in each video for each timepoint using three behavioural metrics calculated by Noldus EthoVisionXT software: percent active time, total distance moved (mm), and mean velocity (mm/min). Percent active time was calculated as the proportion of time each shrimp was moving during the video trial. Total distance moved and mean velocity were included as complementary measures of locomotor activity, with total distance reflecting cumulative activity during the trial and velocity representing the rate of movement. For behavioural response, we fit models with timepoint, treatment, and their interaction as fixed effects, and included shrimp ID as a random effect to account for repeated measures of individuals (Behavioural response ∼ timepoint * treatment (1| shrimp ID)).

To analyse percent active time, we fit a generalized linear mixed model (GLMM) with a beta error distribution and a logit link using the package glmmTMB (Brooks et al., 2017). We used a beta distribution as it is preferred for continuous values between two endpoints (Zuur & Ieno, 2016). As the beta distribution assumes values between 0 and 1, percent active time was transformed using the following formula:

$$y = \frac{x(n-1)+0.5}{n}$$

where x is the proportion of active time and n is the number of observations (Smithson & Verkuilen, 2006; Cribari-Neto & Zeileis, 2010). For total distance moved and mean velocity, we fit separate linear mixed-effects models (LMM) with timepoint, treatment, and their interaction as fixed effects using the lme4 package in R (Bates et al., 2015).

Model selection was performed using likelihood ratio (χ^2) tests to compare nested models and assess the significance of fixed effects with the anova() function (test = "LRT"). Non-significant terms were removed sequentially to obtain a parsimonious final model. Model assumptions were evaluated (normality of residuals, collinearity, homogeneity of variance, and normality of random effects) with the check model function from the performance package (Lüdecke et al., 2023).

For the final model, we obtained Type III Wald χ^2 tests for each fixed effect using the Anova() function from the car package (Fox & Weisberg, 2019), allowing us to evaluate the relative contribution of predictors while accounting for model structure. Estimated marginal means and pairwise contrasts for significant predictors were then calculated using the emmeans package (Lenth, 2021). For LMMs, coefficients were directly interpretable on the original measurement scale. For GLMMs, logit coefficients were back-transformed to proportions and expressed as odds ratios to aid interpretation. All analyses were conducted in R version 4.5.1 (R Core Team, 2025).

RESULTS

Survival

X. elongata had 100% survival in both the VI ($n = 10$) and control ($n = 10$) treatments during their 21-day period of observation. Five individuals from the control treatment, and 6 from the VI treatment each moulted once during the experiment, and all tags remained in place and visible. During daily health inspections, 2 *X. elongata* in the control treatment (1 missing a leg and 1 missing a uropod) and 1 in the VI treatment (missing a leg) were observed with minor injuries.

A. lanipes had 100% survival in both the VI ($n = 10$) and control ($n = 10$) treatments during the 21-day duration of trial 1. The only shrimp to moult in this trial was in the VI treatment, and moulting did not affect the placement or legibility of the tags. During daily health inspections, 2 *A. lanipes* in the control treatment (1 missing an antenna and 1 missing a leg) and 2 in the VI treatment (2 missing a leg) were observed with minor injuries occurring during the study period. The PIT tag group ($n = 10$) had 30% survival, with all mortality events occurring within the first 7 days of the 21-day monitoring period. The cephalothorax lengths of PIT tagged *A. lanipes* ranged from 10.1-18.7 mm with a median of 14.3 mm and mean of 14.2 mm. Out of the 3 surviving PIT tagged *A. lanipes*, 1 lost a leg.

Trial 2 contained only PIT ($n = 14$) and control ($n = 13$) treatments and was monitored for 20 days. However, excessive heat and loss of power on experimental days 17 and 18 caused enclosure water to rise to this species' upper water temperature tolerance of 28°C, causing mortalities in both control and PIT groups. Therefore, for trial 2 analyses we considered only mortality data from the first 16 days of monitoring. In these 16 days, 5 *A. lanipes* moulted, 3 in the control group and 2 in the PIT tag group. These 2 shrimp in the PIT tag treatment extruded their tags during moulting on day 7 and day 9 (fig. 2), reducing the percentage of shrimp that both survived and retained their PIT tags. Over the trial period, 43% (6/14) of *A. lanipes* in the PIT tag group survived and retained their PIT tags and 85% (11/13) in the control group survived. The cephalothorax lengths of PIT tagged *A. lanipes* in trial 2 ranged from 14.6-19.8 mm with both the median and mean equal to 17.3 mm. Mortality and PIT tag extrusion was spread throughout the 16 days and not constrained to the first week as seen in trial 1. Minor injuries were observed in surviving shrimp in both the control treatment (2 missing a leg and 1 missing an antenna) and the PIT tag treatment (1 missing a leg and 1 missing a uropod).

The Cox proportional hazards model ($n = 47$, events $= 17$) showed that PIT tagged individuals were almost 12 times as likely to die at any given time compared to the control treatment (hazard ratio = 11.74, 95% CI: 2.65-51.99, $p<0.01$) even after accounting for cephalothorax length (fig. 3). Survival did not differ significantly between trials after adjusting for treatment and body size (hazard ratio = 0.78, 95% CI: 0.25-2.45, $p = 0.67$). It is important to note that this model assumed proportional hazards across trials and did not test for interaction between trial and treatment. *A. lanipes* cephalothorax length was not a significant predictor of survival in this model (hazard ratio = 1.04, 95% CI: 0.82-1.33, $p = 0.75$). Thus,

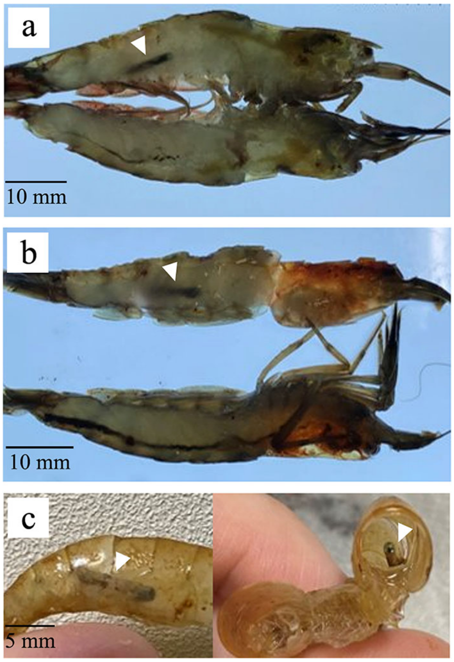

Fig. 2. *Atya lanipes* (Holthuis, 1963) in the PIT tag treatment of trial 2. White arrow denotes PIT tag presence. a, Individual with cephalothorax length of 19.1 mm died 1 day after tagging; b, individual with cephalothorax length of 19.8 mm survived the 16 days of the trial. Note that while the size and PIT tag location in shrimp a and b were similar, their survival varied; c, moult recovered from an *A. lanipes* enclosure with PIT tag extruded during the moulting process.

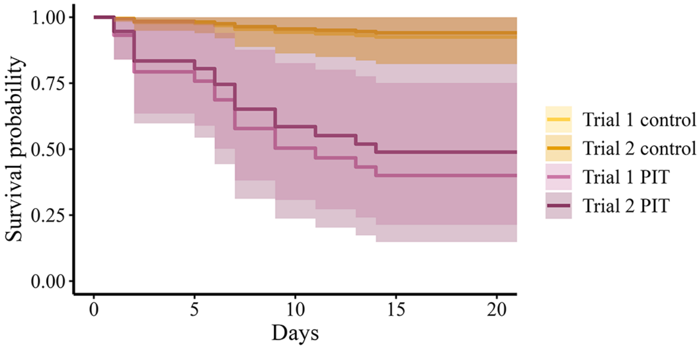

Fig. 3. *Atya lanipes* (Holthuis, 1963) survival by trial and treatment based on a Cox proportional hazards model. Treatment (control vs. PIT) was a significant predictor of survival, whereas trial and cephalothorax length were not significant. Trial 1 lasted 21 days and trial 2 lasted 16 days. Shrimp surviving to the end of each trial were treated as right-censored.

the observed differences in survival were unlikely to be confounded by individual size differences.

The overall model fit was strong, with a concordance index of 0.77, indicating accurate ranking of survival times in about 77% of pairwise comparisons. Global tests of model significance including the likelihood ratio test ($p<0.01$), the Wald test ($p = 0.01$), and the log-rank test ($p<0.01$), confirmed that the model provided a significantly better fit to the data than a null model with no predictors.

Behaviour

Behavioural analyses focused on comparing the movement parameters of *X. elongata* and *A. lanipes* in the VI treatment to their control treatment. All *X. elongata* and *A. lanipes* in the control and in the VI treatment survived the length of the experiment and were videoed at each of the three planned timepoints, before tagging, a few days after tagging, and a week and a half after tagging. While *A. lanipes* in the PIT tag treatment were videoed as well, high mortality occurred in this group for both trials, leaving too small of a sample size of surviving *A. lanipes* to reach any robust statistical conclusions regarding their behaviour. Therefore, EthovisionXT behavioural analyses focused on only the control and the VI tagged treatments in trial 1.

For *X. elongata* percent active time, including the interaction between timepoint and treatment did not improve model fit ($\chi^2 = 2.29$, df = 2, $p = 0.32$), nor did the addition of treatment ($\chi^2 = 0.70$, df = 1, $p = 0.40$). Timepoint had a significant effect on percent active time ($\chi^2 = 9.86$, df = 2, $p<0.01$). Therefore, the final model included timepoint as a fixed effect and shrimp individual as a random effect. Percent active time differed significantly among timepoints (Type III Wald $\chi^2 = 11.09$, df = 2, $p<0.01$). Model estimates indicated a decline in activity over time (fig. 4a), with shrimp spending an average of 35 ± 5% (SE) of the video moving before tagging, 22 ± 4% on day 3, and 17 ± 3% on day 11. Pairwise comparisons indicated a significant decrease in activity from before tagging to day 11 ($p<0.01$), while differences between other pairs were not significant ($p>0.05$).

Total distance moved and mean velocity for *X. elongata* showed no effects of timepoint, treatment or their interaction (all $p>0.3$), so the null models with individual as a random effect were retained. Diagnostic plots showed minor unevenness in variance, likely reflecting natural variation in movement rather than model issues. The random intercept variance for individual was estimated at zero for these models, indicating little among-individual variation, but was retained for consistency across analyses. Overall, total distance moved and mean velocity were similar across timepoints and treatment (fig. 4b-c).

In *A. lanipes*, including the interaction between timepoint and treatment did not improve model fit for percent active time ($\chi^2 = 1.84$, df = 2, $p = 0.40$), nor did

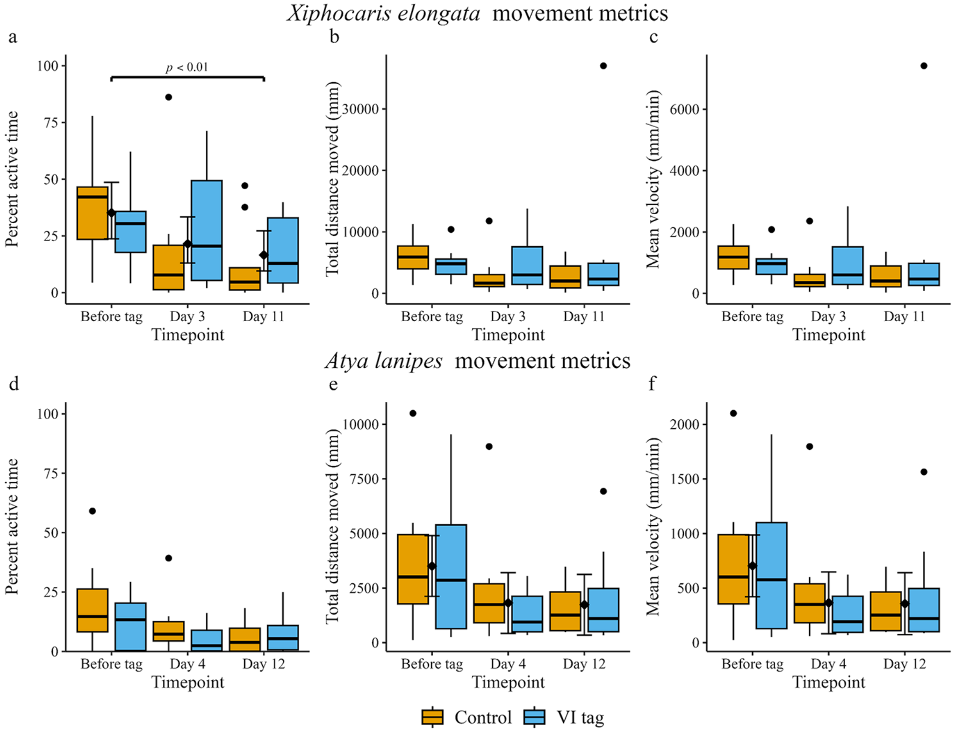

Fig. 4. a-c, *Xiphocaris elongata* (Guerin-Méneville, 1855) movement metrics; d-f, *Atya lanipes* (Holthuis, 1963) movement metrics by treatment and timepoint. Boxplots show the distribution of individual shrimp movement data for each treatment and timepoint; black diamonds in a, e, f, indicate estimated marginal means (±95% confidence intervals) derived from the statistical model, shown only for metrics with significant effects; bracket and p-value in plot a indicate a significant pairwise comparison.

the addition of treatment ($\chi^2 = 1.40$, df = 1, $p = 0.24$). The model including only timepoint provided the best fit, although the effect of timepoint was not statistically significant ($\chi^2 = 3.92$, df = 2, $p = 0.14$). Overall, the percent active time remained similar across treatments and timepoints (fig. 4d).

For *A. lanipes* total distance moved, including the interaction between timepoint and treatment did not improve model fit ($\chi^2 = 1.13$, df = 2, $p = 0.57$), nor did the addition of treatment ($\chi^2 = 0.26$, df = 1, $p = 0.61$). The model including only timepoint provided the best fit ($\chi^2 = 7.65$, df = 2, $p = 0.02$). Shrimp moved substantially less distance after tagging (fig. 4e), averaging 3505 ± 509 mm before tagging, 1818 ± 509 mm on day 4, and 1736 ± 509 mm on day 12. Movement was marginally lower on day 12 compared to before tagging ($p = 0.05$), while differences among other timepoints were not significant ($p > 0.05$).

For the mean velocity of *A. lanipes*, including the interaction between timepoint and treatment did not improve model fit ($\chi^2 = 1.25$, df = 2, $p = 0.54$), nor did

TABLE I

Results of Type III Wald χ^2 tests from best-fit mixed-effects models examining behavioural responses of *Xiphocaris elongata* (Guérin-Méneville, 1855) and *Atya lanipes* (Holthuis, 1963) to tagging treatments

Species	Response variable	Fixed effect	χ^2	df	p value	$p<0.05$
X. elongata	Percent active time	Timepoint	11.09	2	<0.01	*
X. elongata	Total distance moved	–	–	–	–	
X. elongata	Mean velocity	–	–	–	–	
A. lanipes	Percent active time	Timepoint	3.92	2	0.14	
A. lanipes	Total distance moved	Timepoint	7.65	2	0.02	*
A. lanipes	Mean velocity	Timepoint	7.25	2	0.03	*

Percent active time analysed using generalized linear mixed models, total distance moved and mean velocity analysed using linear mixed models.

the addition of treatment ($\chi^2 = 0.19$, df = 1, $p = 0.66$). The model including only timepoint provided the best fit ($\chi^2 = 7.25$, df = 2, $p = 0.03$). Mean velocity declined following tagging (fig. 4f), with shrimp moving at an average of 703 ± 104 mm/min before tagging, 365 ± 104 mm/min on day 4, and 357 ± 104 mm/min on day 12. Pairwise contrasts indicated marginal decreases in velocity between before tagging and both post-tagging timepoints ($p = 0.06$).

Across all analyses for both species, including treatment (VI tagged vs. control) or its interaction with timepoint did not improve model fit, and models containing only timepoint, or null models, provided the best fit across all behavioural metrics (table I). Although differences were not statistically significant in many comparisons, movement generally declined after tagging across both species and treatments. This reduction in movement was likely a result of decrease in water temperature from the unexpected experimental logistics of changing lab spaces. As our primary analyses focused on the movement values between treatments within each timepoint, this drop in water temperature did not interfere with investigating our research question.

DISCUSSION

Population surveys have been essential for understanding the ecology of small freshwater shrimps such as *X. elongata* and *A. lanipes*. Tracking the movements and behaviours of individual shrimp in the field is the next step to continue learning about how these species respond to their changing environment. Applying CMR methodology can help us determine mortality estimates, stream connectivity, residence time within pools, and life history traits such as growth, life span, and fecundity. However, without thorough studies on tagging methodologies, it can be difficult to determine if mortality or changed behaviour of uncaptured individuals reflect natural processes, tag effects or tag loss. To address these potential confounding factors and support effective long-term monitoring, we assessed the performance of two minimally invasive tagging methods, VI and PIT tags, in the abundant and ecologically important shrimp species *A. lanipes* and *X. elongata*. Through controlled laboratory trials that tracked survival and behaviour, our goal was to determine which methods are appropriate for these small-bodied shrimp and to identify any species-specific constraints that may limit the effectiveness of these tagging techniques.

Overall, we found that survival varied across the two tag types. For VI tags, both *A. lanipes* and *X. elongata* showed 100% survival across the study. However, PIT tagged *A. lanipes* showed acute impacts with most mortality occurring within a week of PIT tag insertion. The results from both PIT tag trials showed a higher mortality (62.5%) than other PIT tag field and lab studies conducted in large-bodied crayfish where mortality rates were 10.6% (Hamilton et al., 2022) and 33% (Westhoff & Sievert, 2013). Despite selecting a range of adult *A. lanipes* for our study (10.1-19.8 mm CL, mean 16 mm, median 16.5 mm), these shrimp were still smaller than the threshold presented in similar studies including PIT tagging slender crayfish >22 mm CL (Black et al., 2010), northern crayfish >20 mm CL (Hamilton et al., 2022), and woodland crayfish >22 mm CL (Westhoff & Sievert, 2013). As CL was not a significant predictor of survival in our analyses, we could not determine a PIT tagging length threshold, suggesting that the adult size of this species is likely below the threshold needed for consistent survival with this tagging method. With a PIT tag mass of 30 mg and adult female *A. lanipes* weighing 1.2-9.4 g (Cruz-Soltero & Alston, 1990), the tag represents only 0.32-2.5% of body weight, below the 4% threshold noted for some animals, such as rodents (Theuerkauf et al., 2007). Therefore, the size of the PIT tag, rather than its weight, most likely drives the observed mortality in *A. lanipes*.

Future studies could restrict PIT tagging to only the largest *A. lanipes*, but doing so would restrain results to a narrow portion of the population. For example, Atyid shrimp show sexual dimorphism (Martínez-Mayén et al., 2000; Sánchez Palacios et al., 2008), where gravid female individuals from our study site measured between 5-18 mm CL, and males can grow to 20-25 mm CL (Pérez-Reyes et al., 2015). Therefore, if future PIT tagging studies on freshwater shrimp do show size dependence with more success in larger individuals as they do in crayfish (Black, 2010), researchers should be careful to consider the implications of tagging larger animals that may only represent one sex.

We did not observe any moulting events in the PIT tag group where an *A. lanipes* retained its PIT tag. Two shrimp in trial 2 fully moulted and survived but only after expelling their PIT tag during the moult. After this process, both shrimps appeared lively and survived the remainder of the trial, though each displayed a perforation wound on the lateral carapace where the tag had exited. In juvenile white shrimp, Dunn et al., 2021 found no significant PIT tag loss; however, as those shrimp were larger, their PIT tags lay in the first abdominal segment. In contrast, due to the smaller abdomen of *A. lanipes*, the 8 mm PIT tag rested underneath at least 2, and sometimes 3 abdominal segments potentially restricting the degree of abdominal flexion required for moulting. Daily observations of PIT-tagged individuals showed impaired movement and partial or complete inability to flex the abdomen. The remaining shrimp presented PIT tag migration, incomplete healing, or carapace deformation near the insertion site. Because decapods rely on abdominal flexion and muscular contractions to moult (Longmuir, 1983), the rigidity of the tag may have limited these fine movements, preventing successful moulting and ultimately causing mortality. Therefore, as none of our study shrimp in the PIT tag treatment moulted and retained their PIT tag, survival in this group may have been tied to each individual's position in its moulting cycle rather than to size or exact tag placement.

As a potential caveat, we also considered whether changing water temperature associated with moving between laboratory spaces may have influenced survival outcomes. Water temperature varied across time points in trial 1 with stream water measuring 25°C at collection and dropping to 18°C due to air conditioning. In trial 2, water temperature increased to 26°C due to hot summer air temperatures. These values are at the edges of the species' typical annual temperature range of 20-24°C (Pérez-Reyes et al., 2015). While water temperature may influence moulting and growth (Pérez-Reyes et al., 2015), our data showed no evidence that temperature fluctuations directly caused mortality in any treatment group during the analysed time-period. Therefore, although temperature varied between timepoints, mortality patterns were best explained by tagging effect rather than environmental conditions.

Across all behavioural metrics, percent active time, mean velocity, and total distance moved, we found no statistical differences between the behaviours of control shrimp and VI tagged shrimp for either species. These findings indicate that the insertion and presence of VIE and VIA tags do not impair the ability of *X. elongata* or *A. lanipes* to remain active or move similarly to untagged shrimp under the same environmental conditions. These results are consistent with previous studies testing the use of VIE on freshwater decapods as seen in white-clawed crayfish (Haddaway et al., 2011; McFarlane et al., 2019). Because our laboratory capacity limited sample sizes, future work incorporating larger and more size-diverse groups would allow for more robust statistical analyses. Activity was slower in cooler water compared to warmer conditions, which likely explains the reduced movement observed for *X. elongata* between the before tag and day 11 timepoints. This pattern occurred in both VI and control treatments, indicating that it was driven by temperature differences rather than tagging

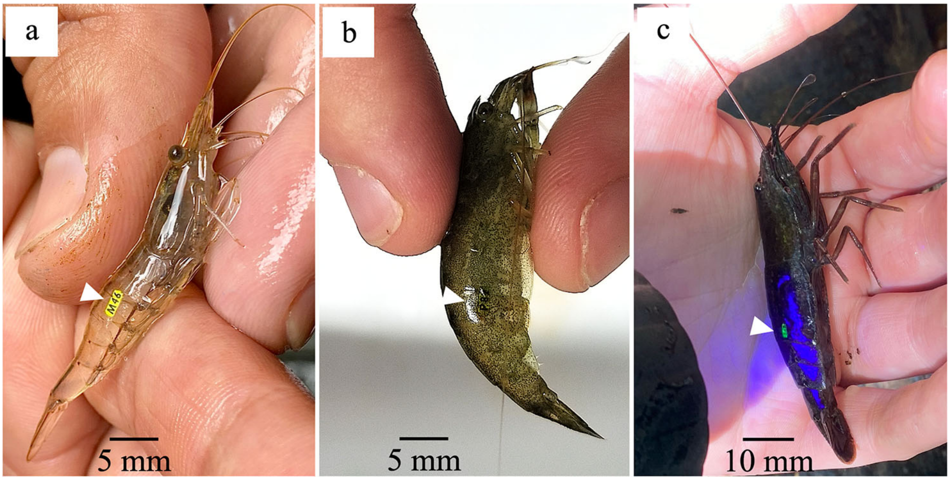

Fig. 5. Shrimp tagged with fluorescent visible implant alphanumeric tag; white arrow indicates tag location. a, Clear readability in *Xiphocaris elongata* (Guérin-Méneville, 1855) due to its translucent carapace; b, reduced readability in *Atya lanipes* (Holthuis, 1963) due to mottled pigmentation; c, ultraviolet illumination aiding in detection and readability in darkly pigmented *A. lanipes*.

effects. Examining additional behaviours such as maximum speed, refuge use or grooming, may provide further insight whether VI tags influence more complex or context-dependent behaviours. Additionally, immediate post-tagging behavioural monitoring as done in McFarlane et al., 2019, would also be valuable for assessing how VI tagging influences shrimp that are released in the field shortly after tag application.

We observed 100% VI tag retention in both species which is consistent with previous research reporting high retention of VIE (Godin et al., 1996), and VIA tags (Xiaopeng et al., 2017). VIA tags have been used to track *X. elongata* shrimp via CMR spanning more than a decade (Pérez-Reyes et al., 2015) and their translucent carapace, along with tag retention, facilitates easy identification and reading these tags (fig. 5a). In *A. lanipes*, VIA tags were also retained, but often difficult to read (fig. 5b). By using an ultraviolet light, we found that the fluorescent colours of VIE and VIA were visible through the carapace of darkly pigmented *A. lanipes* but mottled pigmentation could obscure the three-digit VIA series partially or entirely (fig. 5c). Therefore, although VI tags pose no threat to the survival and behaviours of *A. lanipes*, a different tag type that is more easily identified could minimize errors in field data collection, especially in darkly pigmented adult males.

Overall, our study shows that VI tags are a practical and low impact marking option for both *X. elongata* and *A. lanipes*, as all shrimp survived, retained their tags, and exhibited behaviours similar to controls. In contrast, PIT tags caused mortality and moulting problems for *A. lanipes*, suggesting that this method is not well suited for this species. Based on our combined survival and behavioural findings, we recommend VI tags for field studies on freshwater shrimp and do not recommend PIT tagging for these smaller species. As new tagging technologies and behavioural tracking tools develop over time, continued exploration into freshwater shrimp marking options will aid in the efforts to understand the movement ecology of these important species.

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AUTHOR CONTRIBUTIONS

LJK conceptualized the project, ran experiments, analysed data including statistical analyses, and wrote the manuscript. TAC conceptualized the project and provided background knowledge on the study species and methods. AES and JC ran experiments and analysed data. WRJ provided leadership on statistical analyses and manuscript revisions. ROS conceptualized the project, supervised project progress, and provided comments on manuscript drafts and analyses. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

CONFLICT OF INTEREST

The authors state no conflict of interest.

DATA AVAILABILITY

The datasets and code generated during the current study are available on Github at https://github.com/CoastalFishScience/Kabat_etal_shrimptag_Crustaceana and in the Zenodo repository at https://doi.org/10.5281/zenodo.18790925.

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