Examining tagging options and survival estimates for two riffle beetles in a conservation hatchery

by

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Abstract

Small-bodied invertebrates remain underrepresented in ecological research, with major gaps in survival and life history which hinder effective conservation. The Comal Springs Riffle Beetle, *(Heterelmis comalensis)* an endangered, crenic beetle endemic to central Texas, exemplifies these challenges. The beetles' small size complicates recapture and marking, limiting opportunities to estimate survival. This study had two objectives: (1) to synthesize current tagging methodologies for small-bodied organisms through a systematic literature review, and (2) to evaluate the use of said miniaturized tags for riffle beetles under controlled conditions. Based on this synthesis, p-Chips were selected for experimental testing on *H. Comalensis* and a surrogate species, *H. glabra*. Tagged beetles were monitored using an automated detection system across four trials lasting 50-150 days. Two trials yielded sufficient detection data to construct matrices for survival estimation while accounting for incomplete detection. Survival was then compared between tagged and untagged groups to assess potential tagging effects. Results suggest a potential effect of p-Chip tagging on *H. Glabra*, with tagged individuals showing reduced survival relative to controls across one of two experimental trials. No suitable tags currently exist for *H. Comalensis*, and feasible tagging options will continue to be necessary for future conservation, monitoring and management.

Artificial Intelligence (AI) Use Disclosure Statement

In the preparation of this thesis, the following Artificial Intelligence (AI) tools were used: ChatGPT instant (GPT-5.3), and Microsoft Co-pilot 365. These tools were used primarily to aid in the search of additional peer review publications when given relevant prompts, troubleshoot coding errors within R (R Core Team 2025), and aid with advanced google searches when looking for specific items related to research. The author acknowledges full responsibility for the intellectual content of this work and has ensured that all AI-assisted sections have been reviewed and revised for accuracy and appropriate academic style. All AI-generated content was reviewed and validated for relevance, appropriateness, and accuracy before incorporation into the final document to maintain scholarly integrity of this research.

Digital Accessibility Disclosure Statement

In the preparation of this thesis, the following digital accessibility tools were used to ensure this document complies with federal requirements: Microsoft Accessibility Assistant. The author acknowledges full responsibility for the intellectual content of this work and has made a good faith effort to comply with digital accessibility requirements in publishing, wherein the nature of the content does not significantly change in order to do so. Furthermore, all content has been reviewed and revised to meet these requirements prior to final publication.

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General Introduction

Freshwater ecosystems encompass an impressive diversity across taxa, but a myriad of threats has led to an increase in species of conservation concern. Freshwater ecosystems are some of the most biologically diverse environments on Earth supporting around 126,000 species (Balian et al. 2008). Nearly half of all known fish species occur within freshwater (Leveque et al. 2008) and half of all known freshwater species are invertebrates (Leveque 2001). Despite their ecological importance, freshwater habitats are vulnerable to several major threats including pollution, habitat degradation, exploitation, flow modifications, and the introduction of invasive species (Ahmed et al. 2022). These pressures have resulted in serious declines with species facing faster rates of extinction compared to either terrestrial or marine biota (McRae et al. 2017). This becomes especially problematic for species with high levels of endemism (Burlakova et al. 2010) including spring (Nair et al. 2021) and karst-ecosystem obligates (Cibik et al. 2021). Springs are considered biodiversity hotspots and are regarded as insular habitats containing many endemic species (Cantonati et al. 2012, Fernández-Martínez et al. 2024). Correspondingly, regarding organisms belonging to these areas, there have been increased efforts to improve the status assessments (Noss et al. 2021), recovery plans (Boersma et al. 2001), reintroductions (Jordan et al. 2020), and the development of conservation hatcheries for these species (Grant et al. 2017).

Aquatic insects are an often-overlooked taxa that face even greater conservation challenges. Aquatic insects play a significant role in transfer and cycling of nutrients such as efficient processing of fine particulate matter (Vannote et al. 1980). Despite their ecological significance, aquatic insects have large gaps in their taxonomy (Stork 2018) which facilitates our lack of understanding of basic biology and ecology (e.g., life history, reproductive rates,

population size). Although freshwater invertebrates outnumber freshwater fish in terms of described species, they have received only a fraction of the research focus (Strayer 2006). Our lack of basic understanding of aquatic insects poses a significant barrier to their conservation, as species cannot be effectively listed for legal protection without key information on their life history, geographic range, and population abundance (McDonald et al. 2019). Moreover, major gaps in how species respond to threats make it difficult to develop effective recovery plans (Troyer and Gerber 2015). The lack of data availability on many freshwater invertebrates has left many species invisible to traditional conservation efforts.

One species with limited ecological knowledge is the Comal Springs Riffle Beetle (*Heterelmis comalensis*), an endangered aquatic beetle endemic to the Comal and San Marcos springs in central Texas, USA. The Comal Springs Riffle Beetle (CSRB) became a federally endangered species in 1997, primarily due to habitat loss resulting from reduced spring flow, endangering its survival (USFWS 1997). As a crenophillic species, *H. comalensis* is restricted to spring environments, relying heavily on consistent spring flow for its survival (Cooke et al. 2015). The diminutive size of the species, (2mm in length), further complicates conservation efforts because of the challenges associated with developing monitoring and population assessments. Thus, efforts to improve our ecological and husbandry knowledge of the CSRB have been significantly carried out at the San Marcos Aquatic Research Center (SMARC, see below), a facility located in San Marcos, Texas. Unlike other riffle beetles, *H. comalensis* has truncated wings and is flightless, severely limiting its dispersal ability (Bosse et al. 1988). This inability to relocate makes it highly vulnerable to environmental disturbances (Crowe and Sharp 1997). Its dependence on the continued flow of the Comal Springs system leaves it sensitive to habitat disruption (USFWS 2007).

A significant obstacle to the conservation of *H. comalensis* is the lack of effective methods for tagging small-bodied individuals. Individual identification is helpful for estimating key parameters related to population dynamics, survival, and life history traits (Jungwirth et al. 2019). This becomes especially important for captive assurance colonies maintained at conservation facilities (i.e., SMARC) where it becomes difficult to differentiate survival in captivity versus in the wild. Additionally, efforts to establish an appropriate standing stock population for conservation management have been hindered by this limitation (EACHP 2017). In 2015, an N-mixture abundance model was used to estimate the abundance of the wild population of the CSRB (EAA 2015). However, this effort likely underestimated the true population size due to environmental conditions and timeframe of the study (i.e., conducted over a single month during a drought). Brown (1987) found riffle beetle adults receding into the substate in a dried-up creek near Waxahatchee, Texas and it is theorized that *H. Comalensis* also has this receding ability. Thus, it becomes unclear how many beetles escape the drought via moving further in the springs versus actual changes in the population. Other options would be beneficial to accurately quantify population size or track overtime.

Advancements in tagging technology have significantly enhanced ecological research, particularly by improving marking options for smaller organisms. Tags have become essential tools for gathering vital data on population size (Petit and Valiere 2006), growth rate (McQueen et al 2019), survival (Whitlock et al. 2017), and movement patterns (Horton and Letcher 2008). Traditionally, unique identifiers such as external markings (e.g., paint or dyes) or body modifications (e.g., branding or toe clipping) were used, though with mixed success (Silvy et al. 2012). More recently, passive integrated transponder (PIT) tags marked an important breakthrough offering durability, long lifespan, and remote detection capabilities making them

highly effective for long term monitoring (Gibbons and Andrews 2004). For small-bodied organisms, minimally invasive tags such as PIT and Radio Frequency Identification (RFID) have gained favor due to their reliability and reduced risk of deleterious effects (Ousterhout and Semlitsch 2014; Musselman et al. 2017). Elastomer tagging, another method frequently used for small organisms, (Scmidt and Schwarzkopf 2010, Sapsford et al. 2016), provides batch identification opposed to unique identification and has more variable tag retention rates compared to PIT tags (Musselman et al. 2017). Each tagging technique presents specific challenges, including tag size, placement, retention, and potential interference with the organism's natural biology, all of which must be carefully evaluated prior to application (Swarr et al. 2022).

My first study objective is to provide a systematic review of tagging technologies that may prove useful for tagging small aquatic organisms. Because successful tagging studies rely on several assumptions including that the attached tag does not negatively affect the individual's health, survival, or behavior (Swarr et al. 2022). My second objective is to assess mark retention and survival of the CSRB, and a surrogate species housed at SMARC using an automated monitoring system (see below). These objectives will provide informed insights into tagging methodologies and contribute to improved monitoring and conservation efforts for small-bodied species.

Chapter 1. Systematical review of the current miniaturized tagging technologies that may prove useful for tagging small aquatic organisms.

Introduction

Small-bodied organisms are valuable monitoring targets but remain among the most understudied and data-deficient taxa. For example, some small-bodied fishes (e.g., minnows and chubs, *Notropis* spp. and *Semotilus* spp.) are good monitoring targets because they mature early (Wooton et al. 2022), are locally abundant (Hicks and Servos 2017), relatively short-lived (Zak et al. 2021) and can be sensitive to environmental disturbances (Teichert et al. 2018). These characteristics allow for relatively fast feedback from monitoring efforts because it is more difficult to detect ecological problems in longer-lived species that do not reproduce annually (Nagy et al. 2024). Economically important species such as Senegalese sole *Solea senegalensis* (Carballo et al. 2018), freshwater prawn *Macrobrachium rosenbergii* (Dinh et al. 2012), and American lobster *Homarus americanus* (McMahan et al. 2012) have juvenile phases where life-history parameters such as growth and mortality are necessary for accurate stock assessments yet remain difficult to reliably estimate. Thus, many small-bodied organisms remain understudied, with important gaps in their ecological knowledge and life history. This makes it difficult to determine appropriate management strategies that often require data regarding changes in growth, mortality or reproduction (e.g., Western Spadefoot Toad, *Pelobates cultripes,* Renet et al. 2021; Tasmanian crayfish, *Astacopsis gouldi,* Shepard et al. 2011; European Bullhead, *Cottus gobio* Knaepkens et al. 2005). Although numerous obstacles limit our ability to estimate these parameters, one key challenge is the lack of suitable marking or tagging methods for reliably resampling individuals.

Tagging is a fundamental tool in ecological research, but its application has historically been limited to larger organisms due to technical and physiological constraints (Holyoak et al. 2008). A key limitation is tag burden: the maximum load an organism can carry before movement, foraging, or survival are impaired. For example, Kalab et al. (2021) found that field crickets cease movement once burden exceeds a set critical mass. These constraints along with other factors such as tag attachment, retention, and post tagging recovery (O'Donnel and Letcher 2017) continue to inform tagging related efforts. For instance, passive integrative technology (PIT) tags typically require a minimum body size (e.g., Brook Trout > 60mm fork length [FL] and 2.0 g in weight to accommodate a 12mm tag, O'Donnell and Letcher 2017). However, technological advances have led to the development of smaller and lighter tags, expanding tagging capabilities to small-bodied fishes, defined here as species <150mm TL at maturity (Munkitrrick et al. 2010). This includes species such as Creek Chub *Semotilus atromaculatus* (<138mm TL) and Mottled Sculpin *Cottus bairdii* (<88mm TL; Kelly et al. 2017), as well as larval amphibians (Fouillox et al. 2020) and some insects (Blight et al. 2023). These miniaturized tags offer promising opportunities to collect ecological information on survival, larval development, and population dynamics in previously inaccessible organisms. Despite these advancements, important limitations persist, and ongoing innovation in both technology and methodology is necessary to improve effectiveness, minimize handling and enhance our ecological understanding of diminutive species.

Correspondingly, I conducted a systematic review of current tagging technologies and associated characteristics that may be useful for tagging diminutive aquatic organisms (taxa described below). My review identifies the types of tags used for tagging different taxa and provides overviews of tag characteristics as a basis for future tagging opportunities (i.e., different

taxa), the current objectives associated with tag use, and overviews the current uses, strengths, and weaknesses of these tags. I was specifically interested in identifying tags that allowed for individual identification as opposed to batch tagging. Batch tagging provides only presence/absence data of tagged "groups", whereas individual marking allows for fine scale ecological data (e.g., mark-recapture survival analysis, movement ecology, growth assessment). Collectively, the information provided in this review will be useful for determining possible tagging technologies that could be used for a variety of purposes (e.g., survival, abundance) on small-bodied organisms.

Methods

Literature search and study selection

To develop a comprehensive review of tagging methods for small-bodied taxa, (e.g., *H. Comalensis*) I conducted two literature searches using Web of Science (WOS). The first search focused specifically on insects, and the second adopted a broader scope to encompass any organisms involving miniaturized tagging technologies. I began my literature search on insects because "small" is a context-dependent term in the tagging literature, thus I recognized that tags developed for this taxon would typically represent the smallest and lightest options available. Although focusing on tag types used in insect research serves as a useful foundation, I also conducted a broader search to capture miniaturized tags that extend beyond insects but may have broader applicability. Both searches were performed using the WOS Core Collection (WOS: Clarivate Analytics) to retrieve peer-reviewed papers published between 2009 and 2024. This time frame was selected to provide a modern perspective on tagging strategies. Earlier reviews and meta-analysis (which were excluded here; see below) have already addressed tagging within

specific contexts including warmwater fishes (Musselman et al. 2017) coldwater salmonids, (Volset et al. 2020), pollinator insects tagged for mark-recapture and foraging studies (Chapman et al. 2004) and pest insects radio-tracked to locate nests and movement pathways (Kissling et al. 2014). Thus, important gaps remain in understanding whether tagging technologies used in one group are compatible with other small taxa or species with comparable ecological characteristics.

I conducted my review using specific search terms and a variety of peer reviewed documents (Fig. 1, 2). I entered my search terms into WOS to survey article title, abstract, and author-specified key words. The database outputs included books, journals, articles, and government documents. The first of these literature searches (i.e., insects) was completed in January 2024. The search terms used were: (insects OR beetles OR bees OR ants OR wasps) AND (Tags OR Tagging OR RFID tags) NOT (genetic OR recombinant OR protein OR genes). Following this search, I conducted a second literature search in February 2024 (i.e., broader search of taxa). The search terms used for the second search were: ('small fish' OR 'small bodied' OR 'mussels' OR 'crayfish' OR 'amphibian' OR 'larval' OR 'larvae' OR animal) AND ('tags' OR 'tagging' OR Tracking) AND ('survival' OR 'retention' OR 'recapture' OR 'dispersal') NOT ('genetic' OR 'genes'). To ensure my review remained up to date, both searches were repeated in January 2025 to include newly published articles.

In many cases, each search returned many papers unrelated to tagging or ecological studies, thus I established inclusion criteria for both searches to focus on studies involving small tag types that could serve as potential candidates for tagging small organisms. Publications were excluded if they did not involve tag use (i.e., mathematical models, simulations, or algorithms using tag data, n = 75), were focused on genetics (i.e., gene sequence tags, n = 114), tag improvements for other purposes (e.g., cryptography for data security, communication

improvements between pre-existing tags and receivers, robot use, or optimizing receiver designs, n = 12) , or involved tags only suitable for batch tagging (i.e., chemical signaling, aluminum, pesticides (Antimycin-A), fluorescent dyes, or radioactive isotopes, n =18). For papers related to mathematical models, I included the tag if it was not identified in other papers. In most instances, these studies used Radio Frequency Identification (RFID) tags. In cases where manuscripts used tags outside of ecology or biology, I conducted a separate search in Google, as needed, to assess the intended use of the tag (e.g., retail tracking) and relevant tag information (i.e., size, weights, etc.) if not available in the reviewed paper. Lastly, reviews and meta-analysis (n = 11) and duplicates (n = 5) were excluded). For each literature search, I examined all papers for relevant titles, abstracts, and author keywords and retained all papers which met the developed inclusion criteria.

Potential tags for marking small aquatic invertebrates

Following the initial screening, I reviewed the full texts of all remaining papers to ensure they met the established inclusion criteria (see above). From this refined set, I developed and applied three primary requirements to identify tagging technologies suitable for monitoring small aquatic invertebrates. For a tag to be considered viable, it had to: (1) provide unique individual identification (ID), (2) function reliably in aquatic environments, and (3) be sufficiently small (< 3mm). Given that I already filtered studies through the targeted keyword searches (see above), I assumed that all included studies met the first unique ID requirement. Additionally, tags that lacked aquatic compatibility or were excessively large (e.g., > 3mm, may potentially inhibit movement or impose disproportionate weight. For example, field crickets that were tagged with tags that had tag burden of an average of 102% tag burden walked up to 27% slower and traveled

a distance of 41% less than unburden individuals (Kalab et al. 2021). And thus were also not considered. Tags that met all three requirements were considered potential tag candidates.

Study purposes

To better understand findings across all studies included for this review, I assigned each to one or more study purposes based on the authors' research objective(s). Understanding these study purposes are helpful for assessing whether a tag is appropriate for a particular species or research context and useful in understanding its limitations. Thus, I created seven study purposes for classification: survival, retention, behavior, growth, tag burden, recapture and other (Table 1). Studies focusing on survival reported post-tagging mortality, a direct indicator of tag suitability. A survival rate similar to that of an untagged control group indicates that the tagging procedure does not elevate mortality risk and may therefore serve as a useful criterion for evaluating tag suitability (Powell and Proulx 2003). Retention evaluates whether tags remain attached or embedded over the study duration. High tag retention ensures reliable data collection, while low retention may compromise the study's validity (Jepsen et al. 2015). Behavior studies examined whether tagging altered standard behaviors (e.g., changes in locomotion, feeding, social interactions, molting, etc.) when compared to an untagged group. Growth studies tracked changes in body size, mass, or condition to evaluate whether tagging affected physiological development compared to untagged individuals (Baras et al. 2000). Tag burden (i.e., ratio of tag mass to organism mass) focused on the effects of potential overburden. For example, high tag burden could influence survival, growth, and behavior of small-bodied species (Hall et al. 2009; Brown et al. 2010). Recapture studies examine a variety of ecological questions including stock management, population dynamics, movement, and emigration (Hogg et al. 2013; Kallis and

Marschall 2014). Lastly, I created an "Other" category, where I placed studies that were not appropriately classified by other categories. Each study (excluding "other") could be assigned to multiple categories depending on its design and research objectives.

Data extraction and study characteristics

All studies that met the inclusion criteria were systematically reviewed to extract relevant data and prepare it for analysis. I initially assigned each paper to a respective category based on its taxon: fish, amphibian, insect, crustacean, bird, mussel, and reptile. Given the broad taxonomic diversity among reviewed studies, variability in study outcomes becomes more likely. For example, biological traits such as size (Acolas et al. 2007; Soula et al. 2012), morphology (Carballo et al. 2018), and habitat conditions (Long et al. 2023) can influence both tag compatibility and organism response. Next, I extracted the following data when available: survival rate (%), retention rate (%), body mass (g), body length (mm), tag name, tag weight (g), sample size (n), study length (days), tag burden (%), and control group presence. The survival rate is the reported number of surviving organisms post-study divided by the initial total. The retention rate refers to the proportion of individuals that successfully retained their tags for the duration of the study period. Body mass and body length were recorded as key morphometric parameters for each organism prior to tagging and a metric later used to calculate tag burden. The tag name or type (e.g., PIT, p-Chip, etc.) is useful for comparing similarities or differences among research outcomes. Tag weight, defined as the dry weight of the tag, was extracted when reported. If tag weight was not provided, approximate values were obtained from online sources (e.g., Google search) or manufacturer specifications. Sample size was recorded to evaluate the robustness and statistical strength of the reported results. The duration of each experiment was

documented to facilitate standardized comparisons across studies with varying timelines and research objectives. Tag burden, when explicitly reported by the authors, was recorded directly. In studies where this metric was not provided, tag burden was calculated using available data (see below for details).

During data extraction, I recorded information on study design elements, including the presence of control groups, behavioral metrics, study purpose, and the handling of multiple experiments within a single study. The inclusion of a control group was recorded to assess whether a study incorporated a reference point for distinguishing between tagging effects (e.g., survival, growth, and behavior). When present, control groups, (i.e., typically involving untagged individuals), allowed for direct comparison and facilitated interpretation of tagging related responses. However, the use of control groups varied depending on study objectives. Studies designed to evaluate performance often incorporated controls, whereas studies focused on broader ecological (e.g., movement, dispersal, or recapture-based estimates) did not necessarily require them. As a result, the absence of a control group does not imply a limitation in study design, but rather reflects differences in research goals. For studies that assessed behavioral changes, the specific behaviors examined (e.g., mating, resting, grazing) were also recorded. Although recapture-based studies did not typically include control groups, they still provided valuable insight into how tagging methods are applied across ecological contexts. Lastly, some reviewed studies included multiple experiments either involving comparing different tags, species, methodologies, or some combination. In these cases, each experiment was evaluated separately.

I extracted data related to the mass of both the organism to assess the relationship between tag burden and survival. If tag burden was not provided, I calculated it by dividing the

weight of the tag by the organism's mass and then expressing it as a percentage. I recorded NA if a tag burden could not be calculated due to missing body weight information. By standardizing tag burden across studies, I could directly compare how relative tag weight may influence survival across different taxa and body size**s.**

Studies with shared tagging related factors

Before applying tags in field-based research settings, it becomes important to evaluate a tag's suitability for that study design or ecological context. Consequently, information on survival, tag retention, and movement are often key metrics necessary for determining whether a tag is appropriate for a given research objective. However, these outcomes are not always directly comparable across studies because key design features vary among systems. For instance, even when the same tag is tested, differences in taxon, morphology, implantation method, or tagging location can significantly influence both retention and survival outcomes (Musselman et al. 2017). Thus, tag "performance" is often context-dependent rather than universal. A careful synthesis of existing literature may therefore help guide tagging decisions for novel or sensitive systems, particularly when working with endangered species.

Accordingly, I synthesized tagging studies by extracting relevant data and organizing them according to shared tagging-related factors, allowing for a clearer evaluation of tag performance across contexts. Specifically, I grouped studies into four categories based on: taxon, body size, tagging method, and tag comparison. Taxon-specific studies included those that examined the effects of the same tag among species in the same taxon. Body size studies focused on either differences in tagging outcomes across size classes or developmental stages within the same species (e.g., juvenile vs adult). Some studies evaluated how variations in tag applications

(e.g., injection vs surgical implantation) affected a research outcome. This category also included studies assessing tagging experience because of handling variability and tagging precision, which may affect survival and retention rates (Heim et al. 2024). Lastly, tag comparisons where separate groups were tagged with different tag types to compare their relative effects on the organism.

Results

Literature search and study selection

Two systematic literature searches identified 185 articles that satisfied my inclusion criteria; thus forming the foundation of my review. The first WOS search, focused on insects, returned 310 published articles and reports, with 84 articles meeting all my criteria after examining the full text. The second, broader WOS search returned 1,167 results, 101 of which met my inclusion criteria and were not identified by the previous search. One study (i.e., Abbas et al. 2024) did not involve tagging an organism for ecological purposes, instead focusing on creating a mechanical prototype using commercial tags. However, I included this study because it was a novel tag type that could be applicable to future ecological research (i.e., met my inclusion criteria).

Data extraction and study characteristics

Data extracted from the review revealed several patterns in study design and taxonomic focus. Overall, 32% (60/185) of all studies included a control group as part of their experimental design. Among these studies, 92% (55/60) evaluated survival outcomes, 62% (37/60) assessed growth, and 48% (29/60) investigated behavioral effects. These studies addressed at least one of

these objectives, whereas some tested all three. Fish was the most frequently tagged taxa, compromising 32% of all studies, followed by insects (29%), amphibians (17%), crustaceans (9%), birds (8%), mussels (8%), reptiles (2%) and bats (2%). Further, a closer examination of insect-focused studies revealed that only about 40% (21/53) evaluated tag performance (e.g., survival, retention, or behavioral differences) using statistically significant comparisons between tagged and control groups. The remaining studies primarily addressed broader ecological or behavioral questions such as ant decision-making behavior (Richardson et al. 2018), tracking bumblebees using a protype marker and tracking system (Walter et al. 2021), testing the effects of turbulence on honeybee flight (Crall et al. 2016), or assessing the effects of high frequency radiation on honeybee condition (Darney et al. 2016).

Technical range of miniaturized tagging devices

I identified 14 unique miniaturized tagging technologies, spanning applications in both aquatic and terrestrial ecosystems and across a wide range of taxa from small-bodied amphibians to fish (Table 2). Different kinds of tags were noted such as Radio Frequency Identification (RFID), visual, computer assisted, harmonic radar, and Global Positioning Systems (GPS) tags. Advances in tag miniaturization were achieved with micro-RFID tags (e.g., mic3, 2 × 1.7 mm, 3 mg; USPT Hitachi Chemicals tags, 2.5 × 2.5 × 0.3 mm, 5.4 mg; Dosselli et al. 2016, Olivera et al. 2021), which represent some of the smallest functional RFID devices currently available. Similarly, among the smallest tags were DataDots and p-Chips (≈85 µg, Seheult et al. 2017), each ≈0.5 mm in size. Their extremely small size enabled applications for marking wasps (DataDots, Whitehead and Peakall 2012), and various small-bodied fishes (Faggion et al. 2019, Moore and Brewer 2021, Spooner and Spurgeon 2023). Conversely, the largest tag was a 23-mm

PIT tag (0.6g), that was used for tagging Stonecat *Notorus flavus* (D'Amico et al. 2021). This contrast illustrates the extent of miniaturization in tagging options, as conventional PIT tags are several orders of magnitude heavier than micro-transponder tags (e.g., p-Chips) which weigh only tens of micrograms. Among the most represented taxa, PIT or RFID tags were used in 81% of fisheries studies and 61% of insect studies. However, tag application methods differed with fisheries studies typically employing internal implantation, while the majority insect studies relied on external attachment due to morphological constraints.

Miniaturization of tags has been an important advancement in our technology, however reducing tag size often involves trade-offs in monitoring performance. For example, light-level geolocator tags (GL; Lotek Wireless Inc.), are compact devices (i.e., 0.4 g; 17 × 6.5 × 3 mm) that infer geographic location using ambient light intensity. These tags can provide up to 9 months of battery life and have been used to track migratory songbirds (Zhao et al. 2024) and shorebirds (Weiser et al. 2016). However, while lightweight, geolocators cannot provide real-time location data and typically offer coarser spatial resolution compared to GPS-based technologies. Similar trade-offs are evident within small transmitters designed for insects. For example, tags used to track the Asian hornet *Vespa velutina* (e.g., Pip19/Ag190, 0.22 g, ~375 m range; PicoPip/Ag337, 0.31g, ~800 m range; Kennedy et al. 2018) remain light enough for flight yet provide only limited detection distances. These examples illustrate that while smaller tags are more suited for small-bodied species, they often have reduced detection ranges, lower data resolution, and (when battery powered) have shorter operational life compared to larger alternatives (Dawson et al. 2015; Duret et al. 2022). Tag intrusiveness also varied across methods. For example, photoidentification offered a noninvasive alternative by using natural body markings (e.g., spots, stripes) from photographs, thus eliminating the need for capture or physical tagging.

Photoidentification has been used to assess amphibians (Patel and Das 2020; Faul et al. 2022; Takaya et al. 2023), reptiles (Sreekar et al. 2013, Suriyamongkol and Mali 2018) and insects (Caci et al. 2013). In contrast, relatively intrusive methods (e.g., PIT tag implantation) carried risks associated with injury during insertion (i.e., needle may damage internal organs; Archdeacon et al. 2009) and for amphibians, surgical incisions may increase the likelihood of infection (i.e., disruption of skin microflora; Antwis et al. 2014). These trade-offs highlight a fundamental constraint in miniaturized tagging: smaller tags improve compatibility with small-bodied organisms but often reduce detection range, data resolution or operational lifespan. Therefore, tag selection should be aligned with study objectives and balancing organism safety with the spatial and temporal resolution required for ecological inference.

Study Purposes

Many of the reviewed studies addressed a single aspect of tag-related performance (Table 1). I found that retention (33%) and organism survival (32%) were the most reported study purposes, followed by assessments of growth (21%). Conversely, studies focused on possible changes in organism behavior (17%) and tag burden (16%) were the least frequently studied. Additionally, 42% of all studies reviewed were classified under the recapture study purpose, with a slightly higher percentage of studies (48%) dedicated to assessing at least one type of tag suitability metric. Ten percent of reviewed papers (n = 19 studies) could not be classified under any of the study purposes created and thus were categorized as “Other”. These studies typically focused on experimental or prototype tagging systems or evaluated novel tagging approaches applied in non-traditional contexts. For example, Walter et al. 2021, tested newly developed prototype markers (i.e., lead-sulfide [PbS] dots printed on paper) that use reflected sunlight to

uniquely identify and track honeybees using real-time imaging software. Importantly, this study did not evaluate biological assessments such as tag retention, survival, or behavioral effects under laboratory conditions. Instead, the authors focused on testing the technical feasibility of the system, specifically if the tags could be successfully attached to individuals, detected with available camera equipment, and recognized by the tracking software in outdoor environments. Similarly, other studies also explored similar in-development processes, including testing new software for identification accuracy when using photo-identification methods (Suriyamongkol & Mali 2018, Faul et al. 2022, Caci et al. 2013) or assessing tags for experimental prototypes outside of ecological contexts (Abbas et al. 2024).

Studies with shared tagging related factors

Although many studies had similar objectives, differences in species, body size, tagging techniques, and tag used may create variation in outcomes (Tables 3-6). For example, several studies evaluated how outcomes varied within taxonomic groups, (e.g., small-bodied stream fishes) to assess whether species responded similarly to tagging procedures under comparable conditions (Table 3). In some cases, species exhibited consistent responses. For example, three species of salamanders tagged with p-Chips were reported with overall high survival and retention rates (>97%) (Moore et al. 2024, Table 3). Similarly, six species of PIT tagged (8mm) small-bodied, nongame freshwater fishes had high survival (≥93%, Cary et al. 2017, Table 3). Interestingly, nearly half of all the fish that did not survive (7 of 13) the experiment were smaller (< 60mm total length [TL]) yet only comprised 30% of all tagged individuals. However, differences among species were also prevalent. For example, a laboratory study reported statistically significant survival and retention rates among eight species of 8mm PIT tagged small-bodied fish (Schumann et al. 2020, Table 4). Specifically, Eastern Blacknose Dace

Rhinichthys atratulus, Johnny Darter *Etheostoma nigrum*, and small Creek Chubs *Semotilus atromaculatus*, had 25% to 30% lower survival compared to their untagged control groups, however survival of all other fish was relatively high (>90%) for the remaining species. Retention rates ranged from 57% for Tadpole Madtom *Noturus gyrinus* to 100% for Blacknose Dace. Additionally, Watson et al. (2019) found that Pacific Blue-eye *Pseudomugil signifier* and juvenile Golden Perch *Macquaria ambigua* had 100% mortality within three weeks after being implanted with 8-mm PIT tags. Unfortunately, this assessment did not have control groups and had small sample sizes ($n < 20$), thus it is unclear if this was due to handling (i.e., delayed mortality), experimental conditions (i.e., water quality) or some other factor. These findings highlight the importance of species-specific evaluations and inclusion of controls when relevant.

Tagging outcomes also vary depending on species sensitivity, tag administration methods, or other methodological factors (Table 6). For example, Moore and Brewer (2021) reported that Arkansas River Shiners *Notropis Girardi* (i.e., a federally endangered species) exhibited relatively low survival (53%) following 8-mm PIT tagging; however, tagging similar size species (Emerald Shiner *Notropis atherinoides*) was successful indicating there may have be some differences in morphology or behavior that also affect tagging success (Moore 2020). Similarly, Archdeacon et al. (2009) found survival by 12-mm PIT tagged Rio Grande Minnow *Hybognathus amarus* varied significantly by implantation method (i.e., incision, 87% survival, injection 50% survival, Table 6) and was lower than in the control group (99% survival). In contrast, some studies have reported no significant tagging effects on sensitive species. For example, 12-mm PIT tagging of juvenile *Zingel Asper* (63-90 mm fork length [FL]), had no significant effect on survival (98%) or retention (100%) (Zarvoka et al. 2024). Beyond implantation methods and other differences in behavior or morphology, other methodological

factors (e.g., tagger experience, surgical technique) may also affect organism responses. Heim et al. (2023) demonstrated a significant effect of tagger experience on survival rates in Atlantic salmon *Salmo salar* (Table 6). When tagged by an experienced surgeon, salmon recovery was quicker resulting in higher survival (98%) when compared to fish tagged by a less experienced surgeon (75%). Similarly, Brown Trout *Salmo trutta* (<55mm FL) survival was significantly related to tagger (Richard et al. 2013, Table 6). These examples emphasize how tag approach (e.g., needle size, method, tagger) may affect survival, especially in sensitive taxa. These findings highlight the importance of species-specific evaluations. More broadly, they suggest that tag performance is not universal but instead depends on interactions among species traits, tag characteristics, and methodological choices. As a result, tagging decisions should be guided by a context-dependent framework rather than relying on generalized thresholds.

Using larger tags relative to body size often resulted in lowered survival and lowered tag retention (Table 4, Table 5). Larger tags negatively affected survival in some cases (e.g., Brewer et al. 2016) but not others. For example, Ringed Salamanders *Ambystoma annulatum* tagged with 12-mm PIT tags had significantly lowered survival (44%) compared to those with 8-mm PIT tags (94%) and was similar to control (98%) (Table 6, Ousterhout and Semlitsch 2013). Additionally, PIT tag retention in *Siren intermedia* was influenced by body size where juveniles tagged with 8-mm PIT tags had lower tag retention (55%) compared to adults implanted with 12-mm tags (100%) (Hutton et al. 2024). Similar trends were observed in lamprey: juvenile Pacific Lamprey *Entosphenus tridentatus* tagged with 8-mm PIT tags had reduced survival (38%) compared to CWT-tagged or control fish (100%), whereas larval Sea Lamprey *Petromyzon marinus* (9-mm PIT tags) experienced lower survival (10%) and retention (60%) compared to those tagged with 8-mm PIT tags (94%) (Table 5, Hanson and Barron 2017; Dawson et al. 2015). Size effects were

also evident in other fishes. Chinook Salmon *Oncorhynchus tshawytscha* (80–90--mm FL) had reduced survival (87%) compared to their control (100%) (Table 5, Brown et al. 2010), and White Sands Pupfish *Cyprinodon tularosa* under 1.1 g (≈39--mm TL) exhibited 100% mortality (Peterson et al. 2018). Tagged White Sands Pupfish were 4.73 times more likely to survive for every additional gram in body weight compared to the control. Many other species (e.g., Red-Tail Cutler *Chanodichthys mongolicus*; Lin and Xia 2013; juvenile Sea Bass *Dicentrarchus labrax*, Faggion et al. 2019; Brown Trout, Richard et al. 2013; Red Porgy fingerlings *Pagrus pagrus*, Soula et al. 2011) showed size-dependent survival patterns. However, body size does not always determine tagging outcomes. For example, Tiffan et al. (2015), tested different PIT tag sizes (e.g., 8, 9, and 12-mm) in age-0 Chinook Salmon across three different size classes (40 – 69--mm FL) and found no significant effects on survival (>98%) or tag retention (>93%). Collectively, these findings indicate that tag size and burden alone are insufficient predictors of tagging success. Instead, their effects appear to be mediated by species-specific physiology (e.g., metabolic rate, sensitivity to stress), morphology, and environmental context. This reinforces the need for experimental validation within target systems rather than assuming universal thresholds.

Tag burden may provide important insight into an organism's suitability for tagging, potentially influencing both survival and retention, although much remains unclear. Generally, lower burdens are assumed to be more suitable than higher burdens with evidence suggesting that tagging related mortality increases with tag burden (Spanos et al. 2023). However, this pattern is not always consistent across taxa. For example, Arkansas Darters *Etheostoma cragini* (>48mm TL) tagged with 8mm PIT tags carried a <2% burden and were unaffected (Swarr et al. 2021). In contrast, Sea Lamprey (<120-mm TL) implanted with 8-mm PIT tags also carried <2% burden but experienced high mortality (Dawson et al. 2015, Table 6). Additionally, juvenile

Arctic Lampreys *Lethenteron camtschaticum* tagged with Eel-Lamprey Acoustic Tags (ELAT) (average tag burden of 1.87%) showed significantly higher mortalities than the control group (Haas et al. 2023). Among White Sands pupfish, the lowest survival rates (0-67%) were observed at the highest burdens (2.7 – 3.4%, Table 5, Peterson et al. 2018). These inconsistencies suggest that commonly applied thresholds (e.g., 2% body mass may not be universally appropriate and should be applied cautiously, particularly across taxa with differing morphologies and life histories.

Potential tags for marking small invertebrates

I identified four potential tag candidates with the most potential for marking diminutive aquatic organisms: Datadots (Whitehall and Peakall 2012), p-Chips (Moore and Brewer 2021), computer-aided tag types (Crall et al. 2017, Rosetti et al. 2017), and Coded Wire Tags (CWT) (Catania and McCauley 2014) (Table 8). These options were selected based on their ability to provide individual identification, function in aquatic environments, and be sufficiently small to be applied to a wide range of small invertebrates (e.g., less than 3mm in size). Collectively, these candidate technologies demonstrate that no single tagging solution is optimal across systems. Instead, their suitability depends on trade-offs among tag detectability, retention, and species-specific constraints, reinforcing the need for context-dependent tag selection. Building on this, several key research needs emerge for improving tagging applications in small-bodied organisms.

DataDots

DataDots (DataDot Technology Ltd, datadotusa.com), are microdot identifiers containing unique codes readable under magnification. DataDots were originally developed for security

applications, acting as a discrete way to mark personal vehicles using small (≈0.5mm), individually marked plastic discs. The key advantages of DataDots include our ability to encode up to 26 alphanumeric characters (Brugnola et al. 2013) and their durability in outdoors applications (e.g., tagging jet skis and boats). However, their use in ecological research remains limited, with current applications only limited to Herman's tortoises *Testtudo hermani* (Brugnola et al. 2013) and thynnine wasps *Neozeleboria cryptoides* (Whitehead and Peakall 2012). One potential concern related to DataDots is tag retention. In one study, with limited sample size, 3 of 9 (33%) tortoises lost their tags over 15 months (Brugnola et al. 2013). The same study also noted difficulty in tag legibility on dark backgrounds (i.e., discs are transparent with black-printed codes) that may limit their application. However, adding a white background beneath the tag could increase readability. Additional studies evaluating modified applications of this tag, along with robust sample sizes would be beneficial to broadening the application of DataDots.

Computer-aided tags

Computer-aided tag types use image-based identification software to improve tracking efficiency and reduce observer bias. These methods rely on digital identification techniques, in which a photograph or video frame containing a uniquely patterned tag (i.e., a printed black and white marker) is analyzed to automatically identify individuals. A major advantage of this system is scalability, as tags can be a variety of different sizes while still being analyzed through camera zoom and image processing. Among the two computer-aided tagging systems that I reviewed, BEEtags (Crall et al. 2017) and GRAPHITE (Rosetti et al. 2017), BEEtags appears more suitable for small-scale ecological applications. GRAPHITE requires video input, involves a more complex processing workflow, and reported false positive rates were high (99.4%), limiting their

use in studies if used as is. Conversely, BEEtags have an open-source software, simplified tag printing, and publicly available code, making it more accessible for research applications. Although BEEtags were originally developed for honeybees their application to aquatic systems has proven capable with appropriate modifications (see Appendix C). Here, tags were adapted using waterproof paper to maintain functionality in water, however protective coatings such as non-toxic epoxy could have also been used for similar results. Waterproof paper would provide a way to develop shorter duration studies of hundreds of animals, whereas increasing durability may be needed for longer experiments or harsh, but may limit the tag's use to certain body morphologies (i.e., hard structures).

p-Chips

p-Chips (Pharmaseq, Inc, pharmaseq.com) are small (500 x 500 μm), silicon wafers that transmit a unique identification numerical code when illuminated by laser light. Currently, p-Chips are unavailable for purchase, but may be available after some modification by the manufacturer. p-Chips were first used as a tool to aid in detection and quantification of analytes (e.g., proteins, DNA, antibodies) in lab settings (Rich et al. 2012, Mandecki et al. 2016). Compared to most tags I reviewed, p-Chips offer many advantages (e.g., electronic detectability, resistant tag design, and accessible software interface). Electronic detectability allows for rapid identification of each tag allowing for automated tracking if kept stationary (i.e., stand) thus reducing reliance on manual identification. Further, each tag is constructed from inert silicon material, resulting in no adverse histological effects when inserted in mice tails (Gruda et al. 2010). The tags also have high temperature stability (520 ℃) and are resistant to many aqueous solutions (e.g., water, acids, solvents, etc.) (Mandecki et al. 2018). Alongside its original purpose

in microbiology research, p-Chips have been adopted for use in ecological studies for tagging small animals (e.g., crayfish, Huber et al. 2023; salamanders, Moore et al. 2024; fish, Faggion et al. 2019, Moore and Brewer 2021, Spooner and Spurgeon 2023; and ants, Richardson et al. 2017). Their small size and minimally invasive application make them particularly well-suited choices for tagging small-bodied organisms. However, a key limitation of p-Chips is their detection range as the scanner must be within ≈7mm of the tag for successful identification which may restrict study applications.

Coded Wire Tags

Coded wire tags (CWT, Northwest Marine Technology Inc.) are small stainless-steel wires (~ 1.1 mm in length), that are etched with a unique numeric identifier. These tags have been used extensively in fisheries research, (e.g., salmonids, Courtney et al. 2000, Beacham et al. 2019; anadromous fishes, Fadaee et al. 2006, Kapusta et al. 2015, Hanson and Barron 2017, Lin and Xia 2017) but have also been used on other taxa (e.g., amphibian larvae, Martin et al. 2011; crustaceans, Fullbrook et al. 2016, McMahan et al. 2016). CWTs meet many of the essential criteria for tagging aquatic invertebrates as they are durable (i.e., designed to persist for the full lifespan of an organism), and can be used in aquatic environments (Drenner et al. 2012) However, the application of CWTs outside of conventional fisheries research (e.g., insects or invertebrates) is limited. Only a few studies have used CWTs in insects, notably soft-bodied insect larvae such as yellow mealworms (Schaffler and Isley 2001) and dragonfly nymphs (Catania and McCauley 2014). Typical use of CWTs insertion in either soft tissue or muscle (Nandor et al. 2010). This technique would be difficult to apply for organisms which lack sufficient soft tissue (e.g., adult riffle beetles).

Discussion

This review highlights both the technological progress in miniaturized tagging devices and the persistent methodological and biological challenges that constrain their application. Across 185 studies spanning aquatic and terrestrial ecosystems, tagging technologies have expanded in both diversity and scale. I identified fourteen distinct miniaturized tagging options ranging from non-invasive photo-identification to surgically implanted PIT tags (Table 2). Miniaturized tags have been used in increasingly smaller organisms, including larval or age-0 fish (Cousin et al. 2012, Richard et al. 2013, Lin and Xia et al. 2013, Kapusta et al. 2015, Tiffan et al. 2015, Schuman et al. 2017, O'Donnell and Letcher 2017, Watson et al. 2019), amphibians as small as (0.7g) (Moore et al. 2024, Martin et al. 2011) and insects such as ants (Richardson et al. 2017, Yamanaka et al. 2019), bees (Chen et al. 2012, Colin et al. 2019), beetles (Blight et al. 2023, Boieteau et al. 2011) and dragonfly larvae (Catania and McCauley 2014) thus serving as evidence that technological limitations related to body size are steadily diminishing. My primary objective was to synthesize tagging approaches across taxa and secondly, to evaluate their suitability for size limited organisms, particularly aquatic invertebrates. Among the tags reviewed, four methods (e.g., DataDots, BEEtags, p-Chips, and CWTs) emerged as most promising for marking small-bodied organisms due to their relative suitability, based on characteristics such as size, identification capability and potential for application in aquatic environments. These tags offer various advantages in miniaturization, yet their practical implementation depends on balancing many inherent limitations. Moreover, careful evaluation of size thresholds, detection performance, retention rates, and potential effects on survival or behavior is important for organisms with narrow physiological tolerances. Collectively, these tools have broadened the range of taxa that can be monitored, from small amphibians and

lampreys to insects and migratory birds, however their potential application remains uneven or lacking in both experimental and taxonomic focus.

A central finding of this review is the variation in the incorporation of experimental control groups and in the evaluation of behavioral or fitness-related consequences of tagging. Overall, 32% of studies included a control group, and among insect focused research approximately 40% evaluated tag performance relative to controls. However, the use of control groups was highly dependent on study objectives. Studies designed to evaluate tagging effects or performance were more likely to include controls, whereas studies focused on broader ecological questions (e.g., movement, dispersal, or recapture-based estimates) did not necessarily require them. Within this context, the relatively limited use of control comparisons in studies explicitly assessing tagging effects highlights an important gap in evaluating sublethal or longer-term consequences of tagging, particularly in insect systems (McLean and Fisher 2025). In addition, taxonomic bias and size-dependent discrepancies were evident throughout this review, with fish and insects comprising approximately 60% of all studies, while reptiles, bats, and several invertebrate groups were sparsely represented. Overall, while control groups are not always required, their targeted use in studies evaluating tagging effects is important for improving inference regarding biological responses to tagging and for supporting broader application across taxa.

Other related evaluations of tagging are also cause for concern. Although survival and tag retention were frequently measured, fewer studies assessed potential sublethal effects (e.g., behavior, movement, or growth; Fullbrook et al. 2016, Nightengale et al. 2018). This is interesting given growing evidence that tagging can influence performance in ways that do not immediately manifest as mortality. For example, increased tag mass reduced movement speed

and increased resting frequency in field crickets (Kalab et al. 2021). Similarly, adhesive-based tagging methods may cause physiological effects on health, which could also affect movement (Pope et al. 2015, Toppa et al. 2021). Behavioral changes are also important, as even subtle shifts in environment or experimental conditions can have broader ecological consequences. For example, Semel and Sherman (1985) demonstrated that simply altering the placement of artificial nesting structures changed behavioral patterns and diminished management effectiveness. In small bodied or sensitive taxa, unquantified tagging effects may similarly influence survival, reproductive success, or habitat use following reintroduction. Collectively, these findings indicate that our field has prioritized feasibility and technological deployment over rigorous validation of tag-induced effects.

For studies aimed at assessing dispersal, migration, or survival, accurate interpretation depends strongly on tag retention and failure rates. Even within well-studied taxa such as fishes, tagging outcomes were highly context dependent and vary across species and experimental conditions. Factors including morphology (Haas et al. 2023), life stage (Pillai et al 2009), and tag placement (Hutton et al. 2010) have been shown to significantly influence tag retention and loss. Although some studies report minimal impacts of tagging on growth or survival in small-bodied fishes (e.g., Mountain Galaxias, 80 mm LCF; Allan et al. 2018; Brook Trout, 35 mm FL; O'Donnel and Letcher 2017), others have documented substantial tag loss or failure even under low tag burdens (<0.1%; Soula et al. 2013; Dawson et al. 2015; Lin and Xia 2023; Faggion et al. 2023). These contrasting outcomes highlight that tag retention cannot be reliably predicted using simple metrics alone. Instead, retention outcomes are influenced by a combination of species-specific traits and methodological decisions, limiting the ability to generalize across systems and reinforcing the need for context-dependent evaluation.

Predefined body size thresholds or tag burden ratios are widely used as predictors of tagging success (see also, Lacroix et al. 2004, Chittenden et al. 2009, Brown et al. 2010); however, they do not consistently capture the biological complexity underlying survival and retention. Although larger tags, particularly PIT tags, have been associated with reduced survival or retention in some cases (e.g., <44% survival in juvenile amphibians; Ousterhout and Semlitsch 2013), other studies report high survival (>98%) and retention (>97%) across a range of tag sizes under controlled conditions (e.g., juvenile salmon Tiffan et al. 2015; Lamb et al. 2023; Plains minnow Pennock et al. 2017; Marbled Newts, Chevalier et al. 2017). These contrasting outcomes suggest that tag size alone is not a reliable predictor of success. Instead, survival outcomes appear to reflect interactions survival outcomes reflect interactions among tag characteristics, species biology, and methodological choices. For example, lampreys have exhibited disproportionately high mortality despite relatively low tag burdens (1 -3%), with survival rates reported below 60% (Watson et al. 2019, Dawson et al. 2015, Heim et al. 2023). This is likely due to biological and mechanical constraints, including interference of rigid tags (e.g., PIT) with undulatory locomotion (Breder 1926) and increased difficulty of implantation in elongated body forms thus elevating the risk of organ puncture (Haas et al. 2023).

Methodological factors can further influence survival outcomes. For instance, implantation location may affect internal damage, with PIT tags placed within fat tissue reducing the likelihood of organ injury (Hutton et al. 2024) thereby also influencing tag performance. Further, the origin of individuals may also play a role as wild fish often exhibit lower condition factors and higher pathogen exposure compared to hatchery reared fish (Brown et al. 2010). Even surgical technique can influence tagging outcomes. For example, Stevens et al. (2025) found that the use of barbed sutures in Largemouth Bass improved surgical efficiency (i.e.,

reduced handling time and improved wound closure) compared to smooth sutures. Improved wound closure may reduce tag loss and could influence survival by minimizing tissue damage and inflammation (Wagner et al. 2000, Harms and Lewbart 2011). These findings demonstrate that tagging success cannot be explained by simple body size or tag burden ratios alone. A more comprehensive understanding of how tagging effects interact with species morphology, implantation technique, and environmental contexts will be important to create species-specific frameworks that move beyond generalized rules toward biologically informed tagging guidelines.

Although tag burden and other tagging related effects should be context dependent, early life stages and small-bodied organisms present additional challenges for tagging applications. For example, juvenile organisms are inherently more vulnerable to environmental stressors (e.g., pollution; Leung and McAfee 2019) disease (Ashby and Bruns 2018), in part because a large proportion of their energy is allocated to growth and development (Sibly and Brown 2020). As a result, the additional physiological demands associated with tagging may exacerbate these vulnerabilities. Even when immediate tagging outcomes appear successful, sublethal effects may persist and manifest as longer-term fitness consequences (i.e., carry-over effects) beyond the tagging event (Harrison et al. 2011). Additionally, smaller individuals present various challenges during tag implantation procedures even if the tag (e.g., size, weight) is appropriate. For example, as organism size decreases, the margin for surgical error narrows, thus requiring greater precision to avoid damaging thin body walls, minimal musculature, or a small peritoneal cavity (Hanson and Barron 2017; Wagner et al. 2011). In direct relation to this, surgeon experience has been directly reported to influence injury to the organism specifically time taken, length and depth of incisions, and potential intestinal perforation caused by needle (Cooke et al. 2003, Heim

et al. 2023). Moreover, additional procedural factors can further influence survival during tagging. Complications related to anesthesia (e.g., type, dosage, or exposure time; Archdeacon et al. 2009, Lennox et al. 2025), prolonged handling time (Cooke et al. 2003, Johnson et al. 2016) and water recovery temperature (i.e., warmer temperatures have been associated with higher rates of infection and increased tag expulsion; Robinson et al. 2021) may all contribute to mortality and tag retention during or after tagging.

Technological advances in miniaturized tagging reflect an unavoidable tradeoff between inclusivity and functionality, particularly for studying challenging taxa (e.g., larvae, invertebrates, sensitive species). While reductions in tag size often constrain detection range, data resolution, or real-time tracking capability (Burnett et al. 2013, Kelly et al. 2017, Delcourt et al. 2017), they are typically the only practical option for use in smaller organisms. For example, micro-RFID and p-Chip systems enable identification of extremely small organisms (e.g., insects; Robinson et al. 2009, Kennedy et al. 2018), but typically require close-range scanning, which may limit field applications (i.e., risk water damage to technological equipment; see Moore et al. 2024). Further, stronger wide-range transmitters used in aquatic systems can provide remote detection but are typically heavier since they rely on batteries thus creating trade-offs between power consumption, tag size, and transmission strength (Lennox et al. 2025). Additionally, light-level geolocators can track broad-scale movement patterns but do not provide real-time positioning and generally produce relatively coarse spatial resolution (Zhao et al. 2024). Similarly, Ultra High Frequency (UHF) RFID chips (e.g., NXP UCODE 8), while lightweight (2-5mg), flexible, and offer long-range detection (up to 15 meters) have strong potential for tracking organisms, however, UHF RFID signals do not perform well in natural environments (Li et al. 2012) and have yet to be tested in field-based contexts (Abbas et al.

2024). Collectively, these technologies broaden the range of organisms that can be tagged, however selecting an appropriate tag requires balancing biological feasibility with the level of ecological detail necessary to address specific research or conservation objectives.

Importantly, non-invasive alternatives such as photo-identification and computer assisted visual tags offer promising approaches for taxa sensitive to surgical implantation, although these methods also have limitations. Photo-identification techniques rely on naturally occurring patterns (e.g., spots, stripes, or splotches) to distinguish individuals and can been used in amphibians (Patel and Das 2020, Takaya et al. 2023), reptiles (Sreekar et al 2013, Suriyamongkol and Mali 2018), and insects (Caci et al. 2018). Because these methods do not require internal application, they eliminate risks associated with surgical procedures (e.g., tissue damage or infection, Mazgajska and Mazgajski 2016). However, photo-identification approaches can be labor intensive as photographic matching requires identification skills and thereby susceptible to human errors (Moore et al. 2018, Takaya et al. 2023). Additionally, animals may still require minor handling to obtain usable images (e.g., adjusting orientation or lighting), which can introduce stress (Renet et al. 2021). Hybrid approaches that combine visual tagging with digital image analysis offer further utility in terms of miniaturization. For example, BEEtags, (i.e., small printed QR codes) can be attached externally and automatically recognized using image-processing software (Crall et al. 2017). BEEtags can be scaled to very small sizes with sufficient image resolution, thereby making them suitable for a broader range of small organisms (See Appendix B). Moreover, these technologies can be combined with computer learning (i.e., artificial intelligence, A.I) which can automate pattern recognition and reduce manual processing time with models demonstrating high classification accuracy (e.g., 99.86%, EfficientNetV2; Takaya et al. 2023). However, other software systems have reported high false

positive rates (e.g., 99.2%, GRAPHITE; Rosetti et al. 2021), thus highlighting the need for continued validation before widespread use. Lastly, photo-identification approaches can benefit from integration with smartphone technology and citizen science. For instance, a whale shark monitoring program compiled more than 43,000 images through 3,400 researchers and citizen scientists (Holmberg et al. 2009). Nonetheless, advances in imaging technology and pattern recognition algorithms may expand the potential for applying similar approaches to smaller organisms in the future.

These findings demonstrate that no single tagging technology simultaneously optimizes survival, retention, detection performance, and minimal invasiveness across taxa. Instead, tagging outcomes reflect interactions among multiple factors, including tag design, relative tag burden, implantation method, species morphology, developmental stage, environmental context, and researcher expertise. Although individual studies have identified key contributors to post-tagging survival, comprehensive assessments that integrate these interacting components remain limited. As a result, simplified generalizations such as smaller tags or lower tag burdens often fail to capture species-specific tolerances leaving important knowledge gaps in understanding. For example, where explicitly evaluated, large tag burdens have been linked to reduced movement performance either through impaired mobility (i.e., affecting predator avoidance, Brown et al. 2010; Perry et al. 2013, Hanson and Barron 2017) or interact with tagging wounds (i.e., inflammation; Haas et al. 2023), and influence metabolic demand (i.e., affect changes in growth; Brown et al. 2010, Jepsen et al. 2015, Cary et al. 2017, Nagel et al. 2023). These effects underscore that while tag burden may contribute to survival reductions, however its influence likely depends on how it interacts with multiple factors. Overall, these patterns suggest that tagging success should be approached as a systems-wide problem rather than the result of a

single factor, thus requiring experimental designs that explicitly evaluate interacting drivers rather than relying on generalized rules.

Future research would benefit from three key improvements designed to strengthen inference and improve responsible tag deployment. First, greater use of experimental control groups and standardized reporting of survival, growth, and retention, and behavioral outcomes would substantially improve comparability across tagging studies. For example, Toppa et al. (2020) evaluated the effects of adhesives used to attach RFID tags to the stingless bee *Melipona quadrifasciata*. By incorporating multiple treatments and a control group, including only natural adhesive (i.e., shellac), only synthetic glue, RFID tags combined with each adhesive, and an untreated control group, researchers were able to reveal that bees tagged with RFID chips using synthetic glue showed significantly lower survival than all other groups under controlled laboratory conditions. Additionally, complementary experiments (i.e. field trials comparing bee return with different glue types) reinforced these findings, allowing researchers to attribute differences in survival to the tagging methodology rather than the presence of the tag alone. Conversely, studies lacking control groups make it difficult to interpret outcomes. For example, an exploratory assessment of microPIT tagging in Pacific-Blue eye *Pseudomugil signifier* did not include control treatments, making it unclear whether mortality resulted from the tagging procedure, inherent species sensitivities, or increased handling (Watson et al. 2017). Moreover, by monitoring additional biological outcomes in larval Sea Lampreys, researchers found that individuals tagged with coded wire tags (CWTs) and held in captivity prior to release exhibited reduced growth compared to untagged wild individuals (Johnson et al. 2016). Although the study could not fully disentangle the effects of tagging from handling and temporary laboratory holding, the authors suggested that displacement from natural burrowing habitats during

captivity likely contributed to reduced growth rather than the tag itself. The inclusion of comparative controls and auxiliary response outcomes increases the interpretability of tagging studies and strengthens the reliability of ecological inference.

Second, tagging studies would benefit from explicitly incorporating biological covariates (e.g., body morphology, histology, locomotion mode, implantation location, metabolic rate, life stage, etc.) into experimental design and statistical analyses. Although not exhaustive, these variables likely influence tag tolerance and survival outcomes yet are rarely evaluated directly. For example, Northern Crayfish *Faxonius virilis* were tagged with 8mm PIT tags, and researchers evaluated how carapace length, sex, and three implantation cites (central, pleopod, and lateral) influenced tag responses (Hamilton et al. 2022). Laboratory trials discovered that regardless of sex or size, lower mortality rates were found in the central insertion location compared to the other two, lower mortality was found for females, and in crayfish larger than 20mm. Additionally, Toppa et al. (2020), supplemented laboratory and field experiments with histological examinations of flight muscle tissue in stingless bees (see above). Although no major differences were detected, the inclusion of histological analyses strengthened the study by demonstrating that potential physiological effects were considered alongside behavioral and survival outcomes. Incorporation of these biological covariates would allow researchers to more clearly disentangle whether observed mortality reflects the tag itself (e.g., tag burden, glue toxicity) or from interactions between tag characteristics and species-specific biology. Incorporating more comprehensive biological covariates into tagging experiments will help address current knowledge gaps, improve predictive tag selection, and reduce reliance on generalized tagging thresholds.

Third, emerging mirco-tag technologies should undergo experimental validation under laboratory conditions before large-scale field deployment. Laboratory studies allow researchers to include unmarked control groups, isolate potential sources of mortality or behavior changes (e.g., stress responses, cite) and quantify tag loss (Brown 1977). These controlled experiments provide baseline information for better understanding the mechanisms underlying tagging effects. However, despite the importance of these evaluations, many studies (particularly those involving insects) have deployed tags directly within ecological applications without first assessing their biological effects. For example, the use of photo-luminescence (PL) tags used in honeybees (Walter et al. 2021), DataDots used to identify thynnine wasps (Whitehead and Peakall 2012), and micro-RFID tags used to track Asian hornets (Poidatz et al. 2018), have demonstrated their first ever use in tracking, recapture, and broad scale monitoring of small individuals. However, these technologies were assumed to be suitable for ecological use without fully assessing their potential to introduce bias into study outcomes. I believe this pattern likely reflects broader pressures within ecological research. For example, invasive species (e.g., spotted lanternfly, Asian hornet; Siderhurst et al. 2023, Poidatz et al. 2018), pest insects (e.g., stink bugs; Kirkpatrick et al. 2019) and pollinators (e.g., honeybees; Decourteye et al. 2011, Dosselli et al. 2016) present urgent management concerns due to their influential effects on agriculture and ecosystem functions, thereby creating strong incentives to rapidly deploy management tools. However, urgency should be balanced with careful validation. For taxa such as insects where historical validation is limited (Mclean and Fisher 2025), assuming that tags are harmless may lead to unintended outcomes that complicate ecological inference if tagging effects are not assessed prior to wider use.

In summary, continued advances in technological miniaturization, improved electrical components (e.g., batteries) and novel engineering innovations are likely to expand opportunities for ecological research. These developments have important implications for conservation and management by enabling non-invasive, long-term tagging of organisms that have historically been difficult to study, including small-bodied adults, early life stages (e.g., juvenile, larva), and delicate taxa (e.g., aquatic invertebrates). For example, emerging technologies such as p-Chips may have broader applications, potentially extending to economically important crustacean fisheries (e.g., American lobster *Homarus americanus;* Norway lobster *Nephrops novergicus;* McMahan et al. 2012, Ungfors et al. 2013, Lauria et al. 2015). This is particularly valuable as evidence from Woodland crayfish *Faxonius hylas* demonstrated that internally implanted p-Chips persist through molting cycles (Huber et al. 2018), providing reliable identification for crustaceans (i.e., which lack permanent calcified structures), thereby facilitating repeated growth and survival measurements useful for stock assessment and long-term monitoring (McMahan et al. 2012). Despite this progress, technological innovation has often outpaced experimental standardization, and new approaches will require thorough validation to fully understand their limitations and appropriate biological contexts. However, the effectiveness of these tools will depend on more rigorous experimental validation including the use of control groups and standardized reporting of tagging outcomes, the inclusion of biologically relevant covariates, and preliminary testing under laboratory conditions.

Conclusions

Overall, this review demonstrates that successful tagging of small-bodied organisms depends on interactions among biological traits, tag characteristics, and methodological choices. Tag performance is best understood as a function of organism size, morphology, tag design, and study objectives, rather than as a fixed set of universal rules. This interaction-based framework helps explain variability observed across studies and provides a foundation for selecting appropriate tagging approaches for novel ecosystems.

Tagging small-bodied organisms presents both significant challenges and emerging opportunities as technological advances continue to reduce tag size and expand applicability across taxa. This review demonstrates that tagging success is not governed by universal thresholds, but instead depends on interactions among organism traits, tag characteristics, and methodological choices. As a result, effective tagging requires a context-dependent approach that considers species-specific sensitivity, study objectives, and environmental conditions.

Although miniaturized technologies have expanded the range of organisms that can be studied, trade-offs between tag size, detection capability, and organism impacts remain a central constraint. Addressing these trade-offs through careful study design and continued technological innovation will be essential for improving tagging success in small-bodied systems.

By synthesizing current tagging approaches and identifying key limitations, this review provides a foundation for selecting appropriate tagging methods and advancing ecological research on understudied taxa. These insights will support more effective monitoring, improve understanding of population dynamics, and enhance conservation and management efforts in aquatic ecosystems.

Chapter 2. Assessing mark retention and survival of tagged riffle beetles using an automatic monitoring system

Introduction

The Edwards Aquifer (EA), located within the Balcones Fault Zone of south-central Texas, is one of the most productive karst aquifer systems in North America serving as an important source of groundwater and spring flow for both humans and ecological communities (Lindgren et al. 2005). The Edwards Aquifer supports a diverse assemblage of at least 60 aquatic species, (more than half of which are invertebrates, Longley 1981), and many of which are highly specialized and restricted to spring habitats (Culver and Sket 2000, Krejca and Reddell 2019). Despite its high productivity, the aquifer is highly vulnerable to hydrological and anthropogenic stressors. For example, increasing groundwater extraction, urban development, and contamination can reduce spring discharge and degrade water quality (Chen et al. 2001, Sharp 2019). These pressures are further compounded by climate change, which is expected to alter recharge dynamics and increase frequency of drought. Reductions in spring flow can substantially alter the physical and chemical characteristics of aquatic habitats. For example, lower discharge may increase water temperature (Sharp 2019), elevate concentrations of dissolved solutes (Bouwer 2002), and reduce current velocity, leading to increased sedimentation and habitat degradation. These changes can negatively affect aquatic organisms (e.g., fish eggs and benthic macroinvertebrates), particularly those dependent on stable (i.e., stenothermic) flow conditions. Consequently, species that rely on groundwater fed systems, (e.g., spring-obligate taxa) are particularly vulnerable to changes in flow regime, water quality, and temperature (McKinney and Watkins 1993).

Spring systems are often recognized as biodiversity hotspots, yet they can be inherently insular and fragmented environments that promote the development of specialized biological communities. Among these are spring-obligate invertebrates, which are often confined to individual spring systems or localized groundwater habitats and thus exhibit restricted geographic ranges and high levels of endemism (Nair et al. 2021). These organisms often rely on relatively stable physiochemical conditions tied to groundwater habitats including consistent temperature regimes, reduced dissolved oxygen concentrations, and flow conditions that are not easily replicated outside of spring environments. For example, stygobitic amphipods are typically restricted to specific groundwater systems and exhibit reduced tolerance to changes in temperature, water chemistry, and light compared to surface-dwelling species (Wilhelm 2006). However, despite their potential ecological importance and diversity, life history characteristics (e.g., growth rates, reproductive timing, and longevity), physiological tolerances (e.g., temperature limits and dissolved oxygen variation) and responses to environmental stressors (e.g., reduced flow, habitat disturbance, and water quality degradation) are often either inferred or remain unquantified (Nowlin and Bowles 2023). Additionally, connectivity among spring systems depends on subsurface hydrological pathways, which may become limited under low flow conditions (Steven et al. 2021). This restriction may limit recolonization following local extirpation and further increase vulnerability to environmental change (Strayer and Dudgeon 2010). Collectively, these characteristics make spring-obligate species highly specialized but also particularly sensitive to minor environmental changes.

Among these spring-obligate invertebrate species is *Heterelmis Comalenis*, the Comal Springs Riffle Beetle (CSRB), a species of conservation concern that is closely tied to springflow dynamics. *H. Comalensis* is endemic to the Comal Spring System in central Texas, and was

listed as endangered in 1997 due to increasing groundwater withdrawal and associated reductions in suitable habitat, an important source of concern for beetle persistence (USFWS 1997). As a spring adapted species, *H. comalensis* is likely dependent on microbial food sources (i.e., biofilm) that are associated with the constant flow of spring water (Bowles et al. 2003). Reduced water flow would not only truncate the beetles' habitat but could also limit their food supply, further threatening their survival. Like many spring-obligate species, *H. comalensis* has specific water-quality requirements (i.e., water saturated with dissolved oxygen), and its apparent reliance on sufficient aquifer discharge means that even slight reductions in flow can negatively affect its habitat conditions (Cooke et al. 2015). Their limited dispersal ability and highly specialized habitat use make it unlikely for them to find or colonize new environments if their current habitat is compromised (Cantonati et al. 2012).

Recovery plans are often a central focus of conservation efforts and are used to guide actions aimed at improving the status of vulnerable species. These plans typically establish measurable biological criteria, such as minimum viable population sizes (Shaffer 1981), demographic growth targets (Morris and Doak 2002), and benchmarks for survival and reproduction (Caughley 1994) that help define conditions necessary for ensuring long term persistence. For example, certain recovery strategies for the endangered pearl mussel *Margaritifera laevis* often include maintaining stable or increasing population trends, ensuring successful recruitment over multiple years, and assessing causes of potential recruitment failure (e.g., availability of host fish) (Miura et al. 2024). In addition to demographic targets, recovery plans emphasize the protection and restoration of critical habitat as high quality which is directly linked to increased abundance and reduced mortality. Addressing external stressors is also a key component with efforts often focused on mitigating threats such as habitat fragmentation,

pollution and invasive species. Overall, these elements provide a framework for adaptive management by establishing standardized benchmarks that can be used to evaluate progress and refine conservation strategies over time. Ultimately, recovery plans are useful for promoting self-sustaining populations and preventing further species decline.

In response to growing pressures on the Comal Springs complex, conservation efforts have included the establishment of captive populations of *H. comalensis* at facilities such as the San Marcos Aquatic Research Center (SMARC). These captive assurance colonies are intended to supplement wild populations and provide a safeguard against population declines associated with environmental disturbances (e.g., reduced spring flow or drought). For species at risk of extinction, captive breeding programs may play an important role in increasing population size while preserving genetic diversity, which is closely related to overall fitness and long-term viability (Reed and Frankham 2003; Leus 2011). However, maintaining this diversity requires capturing a sufficiently large representative group of founders to avoid genetic bottlenecks in captivity (Willoughby et al. 2015). Captive propagation is particularly important for species with restricted distributions and limited dispersal ability, and natural recolonization following population loss in unlikely (Fraser 2008). The success of captive propagation programs is often influenced by the availability of species-specific biological information, which can affect the ability to fully optimize survival and reproductive outcomes under controlled conditions (Lees and Wilcken 2009). For *H. Comalensis*, rearing techniques have improved substantially over time, and successful breeding has been achieved in laboratory conditions (Huston and Gibson 2015). These advances represent important progress in the development of effective conservation strategies. As with many conservation programs involving specialized taxa, continued refinement of husbandry techniques and knowledge surrounding environmental conditions can help increase

consistency in survival and reproductive output. An improved understanding of how *Heterelmis* beetles survive, behave, and reproduce in captivity will directly support more effective propagation and long-term recovery efforts.

Tagging organisms provides a powerful tool for improving estimates of survival and abundance in both natural and captive populations. By uniquely identifying individuals and tracking them over time, researchers can directly estimate survival, detect mortality events, and distinguish between true mortality and non-detection, often an important limitation in studies of unmarked populations. For *H. Comalensis*, previous attempts to estimate abundance was using non-marking approaches (e.g., N-mixture models, Bio-west 2015), however were influenced by environmental conditions, (i.e., reduced spring flow) which limited detectability and resulted in abundance estimates lower than observed in previous periods. Mark-recapture approaches using tagged individuals can improve these estimates by accounting for imperfect detections and providing more reliable measures of population size and demographic rates (Pollock 1982, Lebreton et al. 1992). Additionally, repeated observations of marked individuals can provide insight into survival, movement, and habitat use (Powell et al. 2000). These data are particularly important in the context of conservation planning, where understanding population dynamics is important to evaluate the sustainability of management actions. For species maintained in captive assurance colonies, (e.g., *H. Comalensis*), accurate demographic information is necessary for balancing conservation goals. Maintaining genetic diversity requires the inclusion of individuals from wild populations, yet uncertainty in wild population size and survival limits the ability to determine whether such removals are sustainable (EAA 2015). Improved estimates of survival and abundance in wild populations are thus important for informing founder selection and ensuring that conservation actions do not negatively impact population persistence.

Addressing these knowledge gaps will strengthen population estimates, improve evaluation of both captive and wild management strategies, and support more informed decision making aimed at sustaining and recovery for macroinvertebrates populations. Building on the synthesis of tagging populations presented in my Chapter 1, this study evaluates the application of tagging for *Heterelmis* beetles. Specifically, my objectives were to (1) assess tag retention and (2) evaluate survival of tagged beetles within a controlled experiment chamber relative to an untagged control group.

Methods

Study organisms

I used two species of riffle beetle to determine survival and tag retention: the Comal Springs riffle beetle (CSRB; *Heterelmis comalensis*), and a surrogate species (described below). The CSRB is a federally endangered species housed at SMARC. This species is endemic to the Comal (Comal County) and San Marcos (Hays County) springs located in central Texas, USA, which are fed by the Edwards Aquifer (Cooke et al. 2015). CSRB's restricted range and growing pressures limiting water availability and quality caused this species to be listed as endangered in 1997 (USFWS 1997). Like most riffle beetles, the CSRB spends its entire life history within water using a plastron, a surface film of air that functions as a gill allowing the beetle to breathe when submerged in water (Elliot 2008). Under controlled conditions, the beetles display positive rheotaxis, showing a propensity to move toward current (EAA 2002). Adult beetles are small (≈2mm in length) and have nonfunctional flight wings (Bosse et al. 1988). These traits make the species suitable for captivity, as it seems to require little space and has access to aquifer sourced well water.

Given the endangered status of the CSRB which limits its availability for research, a closely related species, *Heterelmis glabra*, was used as a surrogate for some of the experimental trials. *H. glabra* is morphologically similar to *H. Comalensis*, but is more widespread, found in streams, rivers, and springs across the southwestern USA, Mexico, and Central America (Brown and Barr 1988). Due to an ongoing drought, availability of *H. Comalensis* is relatively low; thus, I will use *H. glabra* as a surrogate in some of our trials to increase the number of beetles in the experiments. *H. glabra* were mainly collected from Finnegan Springs along the upper Devils River, Val Verde County, Texas. Compared to the CSRB, *H. glabra* occupies a broader geographical range, has fully developed wings, and demonstrates a greater dispersal ability (Brown 1972, 1987). These differences suggest that physical and ecological constraints may restrict the distribution of *H. Comalensis*. However, the similarities in body size, overlapping ranges, and spring-adapted nature also make this species a reasonable surrogate species for my laboratory experiments. I assumed that the two species would perform similarly enough when within trials.

The beetles used in our experiments were raised in a hatchery or collected from the wild by USFWS biologists. Adult CSRB or *H. glabra* were collected by USWFS staff using poly cotton lures following Gibson et al. (2008) and Hutson et al. (2015). Since 2000, a captive assurance colony of the CSRB has been housed at SMARC (Mays et al 2021). The adult beetles were housed at SMARC in custom aquatic holding units and provided established media with biofilm for food sources.

Study Area

This experimental portion of my study was conducted in a laboratory located at the SMARC, a facility operated by United States Fish and Wildlife Service (USWFS). SMARC maintains refugia populations for 22 endangered species associated with the Comal Springs area under the Edwards Aquifer Habitat Conservation Plan (EAHCP) (EAHCP 2017). A central goal of the EACHP is to sustain captive refuge populations that can safeguard against native population losses. At SMARC, staff conduct research on captive populations and propagate species for long-term conservation.

Comal Springs, located in New Braunfels, Texas, is a spring system at considerable risk of water quality and quantity changes. Springs are located on public lands in south-central Texas (Brune 1981). The source of the springs is the Edwards (Balcones Fault Zone) Aquifer, an extensive karstic aquifer that is also the sole supplier of water for the city of San Antonio (Maclay 1995). The Edwards Aquifer is susceptible to many hydrogeological effects including increasing water demands, changes in groundwater recharge, altering porosity, and other human effects. (Chen et al 2001). In more recent years, human demand of water from the aquifer has increased as the population in south-central Texas continues to grow. These effects are expected to be exacerbated over time, particularly due to climate change and population growth (Loaiciga and Schofield 2019).

p-Chips

My study used p-Chips, laser-activated transponder tags measuring 500 x 500 µm, as the primary method for beetle tagging. Each tag carries a unique nine-digit code that is read by a handheld wand connected to a computer. When activated by the laser, the p-Chip transmits its identification number, which is displayed by tracking software and can be exported to external

programs (e.g., Microsoft excel) for data logging and analysis. p-Chips provide several advantages, including rapid and consistent reading and compatibility with standard data management software. A limitation of this technology is its short read range, (≤ 7mm; Pharmaseq Inc. 2012). The ID reader can be mounted on a stand to enable continuous tag reading in controlled environments. This technology has been successfully applied to a range of taxa, including ants (Robinson et al. 2009), crayfish (Huber et al. 2023), honeybees (Tenczar et al. 2014), mice (Gruda et al. 2010) and fish (Moore and Brewer 2021). These examples highlight the potential applicability of p-Chips for diverse ecological and biological studies.

Construction of Treatment and Control Chambers

Treatment chambers

I modified the design of the chambers that USFWS used to house my experimental beetles and to enable p-Chip detection as individuals moved between connected tubes (Fig. 3, Table 9) I designed the chambers by expanding the design of USWFS so that 1) the direction of water flow could be reversed at regular intervals and 2) I could connect the reader and allow the beetles to be scanned continuously. My modification used the loop portion of Velcro as a walking medium for the beetles that moved through a narrow tube connected to two larger chambers. The two larger chambers had water inflow/outflow capabilities on each end such that the water flow direction could be controlled. This design relied on the assumption that rheotactic beetles would orient toward the current (Bio-West 2014), thus passing through the scanning area and allowing their tags to be read (Fig. 5).

First, I constructed the chamber centerpiece by joining the two schedule 40 polyvinyl chloride (PVC) reducing bushings (5.08cm x 1.27cm) using the schedule 40 female adapter

fitting (5.08cm) on one end (was not glued yet, see next paragraph). I then inserted a 12.7cm section of thin polycarbonate tubing into the 1.27cm opening of one bushing. I tightly wedged two O-rings (1.746cm OD x 1.429 cm ID x 0.159cm) around the polycarbonate tube using a toothpick, ensuring a tight fit between the tube and PVC bushing. Next, I oriented the PVC structure vertically and applied polyurethane adhesive (The Gorilla Glue Company) around the polycarbonate tube. Gravity allowed the adhesive to seep into the joint, after which I applied a second bead of glue to reinforce the seal. I left the assembly to dry in the vertical position for 12 h. This process was repeated at the opposite end so that both sides of the chamber were constructed symmetrically. Lastly, a thin piece (≈ 3-4mm) of white (contrasts with the dark beetles), loop Velcro was placed on the inside of the polycarbonate tube and ran into each of the larger chambers for a continuous walking surface. I removed the adhesive backing only from the portion attached to the polycarbonate tube leaving both ends unadhered with ≈14mm tag extensions.

To complete the chamber assembly, I attached the two clear PVC schedule 80 threaded tubes (5-cm diameter 10.16-cm long, hereafter "clear PVC") to the center piece. There was a 5cm-wide strip of white loop Velcro (Velcro Brand) along the bottom of each clear PVC tube. Before attaching the Velcro, I cut a narrow slit near the end closest to the polycarbonate tube. The thin Velcro tag end extending from the polycarbonate tube was threaded through this silt and secured beneath the wider Velcro strip. I removed the adhesive backing and the remaining portion of the Velcro was pressed firmly along the bottom of the clear PVC. This step created a continuous strip of Velcro running from one clear PVC tube, through the polycarbonate connector, and into the opposite tube. After the Velcro was secured, I attached the PVC bushing and adapter using PVC primer and cement. Cementing was performed only after the Velcro tag

ends were positioned beneath the wider strips, which prevented twisting when the clear PVC tubes were screwed into the bushings.

Lastly, I constructed the end pieces of the chamber to serve as water inlets and outlets. I compressed a piece of steel wire mesh (0.35mm) between a schedule 40 PVC reducing bushing (5.08cm x 1.27cm) and a PVC adapter to measure the amount of mesh needed for each fitting. I cut around the rigid indentation of wire and inserted the trimmed piece between the PVC pieces. The PVC adapter and bushing were then cemented together. This process was repeated for the opposite end of the chamber. Finally, I screwed two Nylon barb fittings onto each end, to allow water to enter and exit the chamber.

Control chambers

I constructed control chambers to isolate tagging effects from environmental influences my experimental chambers (Table 10). Although it would be ideal to have the same chambers for the control and treatment, this was not reasonable because 1) I wanted to minimize handling of the beetles (i.e., chambers needed to be opened and beetles counted on a tray) and 2) USWFS biologists would need to count the control beetles each week and this modified design allowed for relatively simple checks while minimizing any handling. Each control chamber consisted of a 1.84-L clear, plastic container (Rubbermaid), fitted with two PVC bulkhead water tank adapters (Fig 4.). First, I drilled two 3.81 cm holes on the shorter ends of each chamber, one near the top and one closer to the bottom to improve water circulation. The bulkheads were installed in these openings. Next, to prevent beetle escape, I placed a piece of wire mesh (see above) inside the female end of each bulkhead. I drilled a 2.54-cm hole into the sealed male end of each bulkhead to allow water flow, and the male end was screwed into the female portion to secure the mesh. For bulkhead installation, the flat flange and gasket of the bulkhead were positioned on the

outside of the container, while the gasket and male end were placed inside. This arrangement minimized potential hiding places for beetles and facilitated weekly checks. Finally, I wrapped a barbed polypropylene male hose fitting (12.7mm x 6.35mm, Proline series) in Teflon tape (as with the treatment chambers) and attached them to the external end of each bulkhead to connect to the water inflow and outflow. I affixed two strips of white loop Velcro (5 cm wide x 15 cm long) to the inside of the chambers to provide a non-slick substrate for beetles, the same as I did for the treatment chambers (i.e., same substrate for biofilm to develop).

Experimental Design

p-Chip experiment

My experiment was designed to assess feasibility of p-Chip tags by estimating riffle beetle survival in a controlled laboratory setting. For each trial, I used 3-4 experimental chambers consisting of both treatment and control chambers that were externally marked for identification (T1 – T4). Each chamber housed ≈15 tagged beetles, a conservative density below the recommended maximum of 20 beetles per chamber of similar size (Bio-West 2017). I randomly assigned beetles to chambers, after which water was supplied through an inflow hose, filling the chambers, and then discharged into a collection tank below. I mounted a p-Chip reader on a stand at the middle of each chamber with the laser centered on the polycarbonate tube aligned with the Velcro strip to intersect the beetles' path. To avoid potential burn from prolonged laser exposure, the reader's position was slightly adjusted each week. I included control chambers in each trial. Initially, each control chamber contained 10 beetles, but this number later increased to 15 based on survival outcomes. Throughout the course of the study, the

number of chambers, species tested (endangered or surrogate), study duration, and beetle density varied by trial (Table 11, Table 12).

Tag Attachment

Cold exposure beetles

I first evaluated cold exposure as a method to temporarily slow beetle movement during tagging. Cold anesthesia is commonly used to temporarily immobilize insects (e.g., honeybees, flies) during experimental treatments (Nilson et al. 2006, Gooley and Gooley 2021). Previous studies have shown that beetles exposed to cold environments exhibit reduced activity (Overgaard and MacMillian 2017) thus, facilitating beetle tagging and allow the glue to dry more effectively. To test whether chilling affected survival in *H. comalensis*, I tagged 12 beetles: six were placed in a freezer for ≈2-min prior to tagging and six were not chilled. Mortality did not differ between treatments after 52 days (only two chilled beetles and one unchilled beetle died). Based on these results, I moved forward with cold exposure as a standard method to slow beetles prior to tagging.

Experimental beetles

I tagged beetles with p-Chips to evaluate both tag retention and estimate survival. I first tagged the surrogate species *H. glabra,* before proceeding to *H. comalensis*. I removed several *H. glabra* from the flow-through colony tube at SMARC and transferred them to a sterilized container filled to one-third capacity with well water. This container was placed in an ice-water bath to maintain a temperature of 15-20 °C, which I monitored with a digital thermometer. I

randomly selected individual beetles using soft-tip entomology forceps (wide tip 107.95mm, DR Instruments) or brushes and placed them onto a small receptacle lined with cotton cloth moistened with well water to prevent desiccation under the microscope light. I examined each beetle under a Nikon SMZ18 Research Stereo Microscope (0.75-13.5x) to confirm it was healthy and mobile. Next, I took the receptacle containing *H. glabra* and placed it into a freezer (-18 C) for ≈2min to cold-anesthetize the beetle. After freezer removal, beetles were checked under the microscope to confirm reduced mobility. If the beetle was still active, the receptacle was placed back into freezer for another 30 s (because we wanted to ensure that the beetles did not get their legs in the glue). If the beetle remained active after 30 more sec, a different beetle was randomly selected to avoid excessive stress.

I tagged each treatment beetle, verified the tag for readability, and placed each in a recovery receptacle. First, I removed excess moisture from the elytra of *H. glabra* using a Kim Wipe. I added a small amount of super glue (cyanoacrylate glue) to the elytra of the beetle (slightly offset center) using a pre-cut piece of metal wire (18-gauge). Next, I used a wooden applicator (i.e., skewer) with a p-Chip pre-affixed on the tip using water soluble CMC glue (See Appendix B) to position the tag directly onto the glue droplet with the readable side upwards. I held the tag firmly in place for 2-10 sec to ensure adhesion. After placement, I rinsed the beetle gently with well water to dissolve the CMC glue, leaving the p-Chip secured. I checked each beetle for mobility and proper tag adherence, then scanned each to conform tag readability. Tagged beetles were transferred to a recovery container cooled with ice water, where they remained until recovered and then placed into their respective experimental chamber.

Statistical Analysis

Multi-state model for experimental chambers

To account for incomplete detection and estimate survival probabilities when monitoring beetles in experimental chambers, I used a robust design multi-state Hidden Markov Model (HMM) with latent imputation. This approach was based upon a state-space formulation of the Cormack-Jolly Seber (CJS) framework, extended for imperfect detection and unobserved deaths (Cormack 1964). Unlike the traditional CJS framework, which estimates survival and detection in a single "alive" state, multistate models allow individuals to transition stochastically among multiple observable or latent states, (e.g., breeding vs. non-breeding, alive vs dead) (Boulanger et al. 2013, Breed et al. 2016). This flexibility improves biological interpretation and provides a more robust treatment in situations of uncertainty (Johns et al. 2018). State-space models allow for disentangling population processes by separating the underlying state (e.g., survival status) from their observation process (e.g., detectability) (Gimenez et al. 2007).

I used passive detections of beetles collected via p-Chip scanners to develop a Bayesian multistate model in JAGS. Each detection was automatically timestamped, enabling the construction of individual detection histories across my experimental trials. I used these capture histories to form the basis for estimating survival in captive populations where continued detection indicated survival and detection lapses required probabilistic inference. I chose a multistate framework as it was necessary to account for imperfect detection, an inherent limitation of passive monitoring systems (Kery and Schmidt 2008, Kellner and Swihart 2014). Here, individuals could remain alive yet undetected due to behavioral avoidance, limited scanner coverage, or intermittent tag non-functionality. This modeling approach is especially valuable when direct observation is limited, as non-detection cannot be assumed to indicate mortality (Pradel 2005). Understanding survival patterns in *Heterlemis* beetles, particularly in response to

tagging, is important for assessing how tagging may influence individual viability and for informing conservation strategies for this endangered species.

I modeled experimental beetle survival using a two-state HMM framework in which each individual occupied one of two latent biological states at each closed sampling occasion: (1) alive or (2) dead. My model was implemented with three closed sampling occasions, producing two estimable survival intervals between consecutive closed periods (see below). Because known survival recapture data were available, cumulative survival at day 50 was calculated separately but included in the overall survival curve as a final endpoint. My model required only three inputs: the number of individuals, the number of sampling occasions, and a binary detection matrix. By combining a biologically defined state process with an explicit observation model for imperfect detection, the framework probabilistically reconstructed individual survival histories across sampling periods, even when detections were intermittent.

The state process describes transitions between the latent biological states (e.g., alive and dead) which represent the true but unobservable condition of individuals (Gimenez et al. 2007, Wash et al. 2018). Transitions were governed by a survival parameter (ϕ), defined as the probability that an individual alive at one closed sampling period remained alive at the next closed period (Pradel et al. 2008, Kendall et al. 2013). Death was modeled as an absorbing state, meaning individuals classified as dead could not return to the alive state. The observation process linked these latent states to the binary detection data (i.e., 0 = not detected, 1 = detected). Detection was modeled conditional on being alive through a detection probability parameter (p), while dead individuals had detection probability fixed at zero. Observations were constrained so that detections could only occur when an individual was alive. I treated detection at a sampling occasion as definitive evidence of life, thus directly constraining the corresponding latent state to

the alive condition. I deterministically assigned all beetles to the alive state at the first sampling occasion, reflecting the experimental design in which beetles were known to be alive at the time of placement. By explicitly separating the biological survival process from imperfect detection, my model reconstructs individual survival histories probabilistically while maintaining a simplified and identifiable structure appropriate for my spare detection data.

To align my model structure with the biological and observational processes of the study, I placed individual detections histories into temporal blocks of unequal duration. Because detections occurred intermittently and lacked consistent daily patterns, I defined block lengths by grouping days with similar observation intensity, resulting in intervals that varied in length (Figs. 6, 7). I implemented a robust design framework consisting of 6 (i.e., 3 closed, 3 open) blocks spanning 50 days, arranged in an alternating closed-open structure, beginning with a closed block (Kendall et al., 1997; Kendall and Bjorkland, 2001). Closed blocks represented periods during which chamber conditions were assumed stable and detections could occur. During these periods, the latent biological state was not assumed to change, allowing detection probability to be estimated (p) independently of survival (Kendall et al. 1997). This separation reduces bias arising from temporary detection heterogeneity (Pollock 1982). In contrast, open blocks represented intervals, during which no detections were recorded, and individuals were allowed to transition between alive and dead states according to the survival parameter (ϕ). Survival is thus estimated over the open period. This block-based structure follows the assumptions of multistate robust design models, which rely on clearly defined temporal boundaries between periods of demographic change and observation.

The general assumptions underlying standard Cormack-Jolly-Seber (CJS) models extend to multistate frameworks, with additional considerations to account for multiple latent states. As

in traditional mark-recapture models, it is assumed that tags are retained throughout the study, remain readable upon detection, and that individuals are correctly identified (Arnason 1981, Pollock 1990). Survival and detection processes are assumed to be independent across individuals (Sun and Burton 2024). In a multistate context, these assumptions further require that each individual occupies a single, defined state during each sampling period (i.e., not in multiple states) and that all transitions between states follow the probabilistic rules defined by the model (Hestbeck et al. 1991, Brownie et al. 1993).

During construction of my detection histories, I encountered instances where individuals did not fall within my assigned closed period but were found nearby temporally within an open period. To maintain and support the framework required by the robust design, I reassigned detections to the nearest closest period if the detection was ≤7 days of that interval. Similar data-alignment strategies have been applied in other multi-state capture-recapture analyses to address incomplete detection histories (Melynchuk et al. 2017, Coulter et al. 2018a). This approach ensured that transient detections did not artificially inflate my mortality estimates and maintained the temporal resolution necessary for reliable inference on latent survival states.

The survival analysis was implemented as a two-state hidden Markov model composed as a state transition process and an observation process. The joint state-observation likelihood propagates individual survival states forward in time through the transition matrix and generates observations conditional on those states (Gimenez et al. 2007, Lebreton et al. 2009). The observation process was modeled as a Bernoulli likelihood applied at each closed sampling period. Let $y_{i,t}$denote whether individual was detected or not detected (1) or not detected (0) during closed period t. Conditional on being alive, detections occurred with probability pt, while dead individuals had a probability of zero. Thus,

Let $z_{i,t} \in \{1,2\}$ denote the latent state of individual i at closed sampling occasions t, where $z_{i,t} = 1$ indicates alive and $z_{i,t} = 2$ indicates dead. Let $y_{i,t} \in \{0,1\}$denote the observed detection outcome, where $y_{i,t} = 1$indicates that the individual was detected at least once during closed period t.

State Process:

Survival dynamics modeled as a first-order Markov process. Conditional on being alive at occasion t, an individual survival to the next closed period occasion t + 1with probability ϕ_t:

$$P\big(z_{i,t+1} = 1 \mid z_{i,t} = 1\big) = \phi_t,$$

$$P(z_{i,t+1} = 2 \mid z_{i,t} = 1) = 1 - \phi_t.$$

Death was treated as an absorbing state:

$$P(z_{i,t+1} = 2 \mid z_{i,t} = 2) = 1.$$

All individuals were initialized in the alive state at the first closed sampling occasion: Zi,1 = 1

Observation Process:

Conditional on the latent state, detections were modeled using a Bernoulli observation model. If an individual was alive at occasion t, it was detected with probability pt.

$$P(y_{i,t} = 1 \mid z_{i,t} = 1) = p_t.$$

Dead individuals could not be detected:

$$P(y_{i,t} = 1 \mid z_{i,t} = 2) = 0.$$

Thus, detections provided definitive evidence that an individual was alive at that occasion.

Joint Likelihood:

The full likelihood is defined as the product of state transition and conditional observation probabilities across individuals and sampling locations. Because latent state Zi,t is

unobserved, inference proceeds by integrating over all possible latent state histories which is performed implicitly within the Bayesian MCMC framework. This formulation explicitly separates the biological survival process from the observation process, allowing unbiased estimation of survival probabilities in the presence of imperfect detection.

I conducted inference using Markov Chain Monte Carlo (MCMC) sampling in JAGS called from the R package 'jagsUI' (Kellner 2024). I specified broad, uninformative priors to reflect our limited knowledge of survival and detection (Hobbs and Hooten 2015). Specifically, I assigned independent Beta (1,1) priors to survival (ϕ) and detection (p) probabilities corresponding to uniform distributions on the probability scale which allowed the data to dominate posterior inference. Posterior distributions were estimated using four parallel MCMC chains, each run for 10,000 iterations with a burn-in of 5,000 iterations and thinning every five samples. Model convergence was assessed through visual inspection of trace plots, posterior density overlays, and the Gelman Rubin statistic (R). Convergence was considered adequate when chains mixed well, density plots overlapped across chains, and all R values were below 1.1 (Gelman and Rubin 1992).

I evaluated my multistate HMM model to ensure adequacy of survival estimates using posterior predictive diagnostics, thus supporting my validity of comparisons. First, I performed posterior predictive checks (PPCs) by comparing observed detection frequencies within closed sampling blocks to replicate datasets simulated from the posterior predictive distribution (Kery and Schaub 2012). For each trial and sampling occasion, replicated detection totals closely matched observed value and Bayesian p-values were centered around 0.5 indicating that the fitted model adequately reproduced key features of the observed detection data (Gelman et al. 2013, Conn et al. 2018). I also assessed model calibration using randomized Probability Integral

Transform (PIT) residuals from the Bernoulli observation model (Yang 2024). PIT residuals were evaluated for uniformity (i.e., means near 0.5), variances that were consistent with a uniform (0,1) distribution, no systematic tail accumulation (i.e., close to the expected 5%), and non-significant Kolmogorov–Smirnov tests (Nelson 2012, Yang 2024).

Hazard model for control chambers

To estimate mortality within control chambers, I used a piecewise-constant hazard (PCH) model implemented within a Bayesian framework. In survival analysis, a common interest focuses on the individuals' lifespans, including their associated hazard rate (i.e., the instantaneous risk of death at time *t* conditional on survival), and this trend over time (Zens and Peart 2003; Rinne 2014). Many standard hazard models assume a constant hazard through time; however, extensions exist that allow the hazard to vary across predefined intervals. This structure is known as a piecewise interval-specific constant hazard model, allowing for the hazard to change at distinct time points, but constraining the hazard to be constant within each interval, often resulting in an improved fit to the survival data (He et al. 2013, Goodman et al. 2025). I chose this modeling structure as it proved to be well suited for my control chamber data as my interval boundaries could be aligned with the onset of open periods used in the experimental trials thus facilitating comparisons between experimental and control survival processes.

Hazard based modeling was appropriate for my control data because mortality was observed as a time-to-event process rather than through recaptures (i.e., automatic detections) that dealt with imperfect detection. Through the coordination of United States Fish and Wildlife (USFWS), they recorded deaths within short monitoring intervals (i.e., ≈ a week) and I treated individuals that remained alive at the end of the 50-day study period as right-censored (Ergon et

al. 2018, Barrajon and Barrajon 2020). Hazard based modeling is effective as it is compatible with right-censored survival data and allows direct estimation of hazard rates within set intervals (e.g., open and closed periods). Importantly, hazard rates can be converted to survival probabilities through a standard transformation (Ergon et al. 2018), enabling direct comparison with survival estimates derived from the experimental multi-state model. Within each interval, hazards were assumed constant but were allowed to vary across intervals to accommodate temporal heterogeneity in mortality risk (Cooney and White 2021). Control beetles were monitored over the same 50-day period as the experimental beetles to ensure comparability of cumulative survival estimates. Mortality was recorded when individuals exhibited prolonged immobility or inverted posture indicative of death. Because mortality checks were conducted weekly (in accordance with USFWS scheduling) the exact day of death was unknown, thus deaths were assigned to the week in which they were observed. Although this introduces some temporal uncertainty, the observation process remained more informative than that of experimental chambers, supporting the use of a hazard-based survival model for the control group.

In applied time-to-event analysis, flexible parametric approaches such as modeling the hazard rate as a piecewise constant function of time, are beneficial for a variety of additional data structure or statistical reasons. First, this framework is well suited to mortality data that are sparse and temporally clustered, (e.g., small sample ecological studies) by avoiding overfitting and stabilizing inference (Laird and Oliver 1981). Second, by constraining the hazard to be constant within predefined intervals, the model stabilizes parameter estimation even under low event counts while still allowing temporal variation in mortality risk (Rosenbaum et al. 2024). Third, piecewise constant hazard models naturally produce monotonic survival curves without

imposing restrictive parametric assumptions on survival shape, because survival is expressed as the exponential of a non-decreasing cumulative hazard function (Gelfand and Mallick 1995). For each trial and interval, deaths were modeled as Poisson events, with the expected number of deaths defined as the product of the interval-specific hazard rate and the total exposure time at risk during that interval. The cumulative hazard was calculated as the sum of interval specific hazards weighted by interval duration and survival probabilities were derived as the exponential of the negative cumulative hazard (Emad-Eldin et al. 1994). This formulation allows survival or hazard to be estimated reasonably within a single modeling framework.

I conducted inference for my piecewise hazard model within a Bayesian framework using Markov chain Mote Carlo (MCMC) sampling implemented in JAGS. For each interval *g*, hazard rates *hg* was assigned with weakly informative Gamma priors with shape and rate parameters ah = 0.5 and bh = 0.5. I chose this prior as it placed substantial probability mass near zero while allowing moderate to large hazard values, thus avoiding degenerate estimates without strongly constraining the posterior distribution (Lunn et al. 2012). Because hazard rates are constrained to be non-negative, the Gamma prior provides a computationally stable specification (Jackson 2011, Arjas and Gasbarra 2021). I obtained posterior survival curves by transforming sampled hazard to survival probabilities (ensuring uncertainty was propagated into survival estimates) using a computational formula (see Appendix C). These Bayesian survival curves for control chambers served as baseline references for comparison with experimental survival trajectories. All comparative analyses were conducted within the same Bayesian framework, allowing survival differences to be evaluated using posterior distributions rather than point estimates alone. For visualization and interpretation, posterior mean survival curves were plotted with corresponding credible intervals to represent uncertainty.

I evaluated the adequacy of my piecewise constant hazard model using posterior predictive checks (PPCs) to assess whether the model reproduced key features of the observed control mortality data (Kery and Schaub 2012, Conn et al. 2018). For each trial, I generated replicate datasets from the posterior distribution by simulating interval-specific death counts under the estimated hazard rates and observed exposure times. I evaluated model fit at two complementary levels: (1) interval specific comparisons between observed and replicated numbers of deaths within each predefined time interval and (2) global comparisons of total deaths across the full 50-day study period using both total count and discrepancy-based test statistics (Forbes et al. 2022). Because mortality in control chambers was sparse, several interval-level Bayesian p-values were near one (i.e., within intervals containing zero deaths). Under low event rates, interval level-PPCs are less informative, and thus model adequacy was evaluated primarily using global PPC which integrate information across all intervals (Royle and Dorazio 2008). Global Bayesian p-values for total deaths and discrepancy measures were centered near 0.5 for both trials, indicating no systematic under or under-prediction of mortality and supporting the overall fit of the piecewise hazard model (Gelman et al. 2013).

Survival comparison

Because control and experimental datasets differed in structure and observation process, I conducted both analyses within a unified Bayesian framework so that survival estimates could be expressed on a common probabilistic scale. In control chambers, where mortality was observed as a time-to-event process and detection was mainly unambiguous, I estimated survival using a piecewise constant hazard model. In experimental chambers, beetles required a state-space modeling approach that involved imperfect detection for estimating survival. To facilitate direct

comparisons, control survival probabilities were evaluated at the same time points used in the experimental analyses (e.g., shared interval boundaries and cumulative survival). This temporal alignment allowed survival trajectories to be compared without altering the structure or assumptions of either model. Since the piecewise model produces hazard rates, I made sure to convert hazards into their respective survival probabilities so that estimates produced by the multistate model were directly comparable (see above).

Survival in experimental and control chambers was compared at two levels: (1) interval-specific survival evaluated at the endpoints of open periods and (2) cumulative survival over the full 50-day study duration. I quantified group differences using posterior distributions of survival contrasts defined as experimental survival minus control survival at each time point. For each comparison, I calculated the posterior mean difference and summarized uncertainty using 90% Bayesian credible intervals. I selected this interval width to balance precision and interpretability in the context of moderate sample sizes and to align with common practice in Bayesian ecological analyses (White et al. 2020). Posterior contrasts were computed by drawing pairs from each model and forming a derived distribution of differences, thereby propagating uncertainty from both models into the comparison. In addition to interval estimates, I calculated directional evidence using the posterior probability ($Pr(Diff < 0)$), defined as the proportion of posterior draws in which experimental survival was lower than control survival. These measures provide standardized summaries of posterior distributions while remaining consistent with the interpretation of Bayesian probability statements (Clark 2005). Unlike frequentist p-values, which describe long-run sampling expectations, Bayesian credible intervals and posterior probabilities represent conditional probability statements about parameters directly given the observed data (Ellison 2004). When 90% credible intervals excluded zero and posterior mass

was concentrated on one side of zero, this was interpreted as strong directional support for a survival difference (White et al. 2020). Posterior summaries and contrasts were computed using the R package "posterior" (Burkner et al. 2025) and posterior distributions were further summarized using the R package "bayestestR" (Makowski et al. 2019), which provides standardized indices of probability of direction and highest-density intervals (HDIs).

Results

Tag retention and chamber comparison outcomes

Tag retention and chamber effects were evaluated to better assess tagging efficacy and to ensure that observed mortality was not confounded by differences in chamber design. Tag retention varied across trials and may have been closely related with adhesive type used. Because attachment methods changed over the course of the study, retention is reported here by glue type rather than by trial. Early trials used cyanoacrylate glue (i.e., superglue) to attach p-Chips, resulting in an overall retention rate of 82%. In later trials, aquarium-grade adhesive was used instead and retention was 91%.

Because both experimental and control chambers differed by design it was important to evaluate whether chamber type could influence mortality and to distinguish tagging or tagging related effects from any survival differences attributable to the chambers alone. To address this, in Trial 4, I used an interspersed design with approximately equal numbers of tagged and untagged beetle distributed across three experimental chambers and three control chambers (Table 11). However interpretation of this comparison was limited because I had to rely on an already captive stock of *H. Comalensis* that was likely older than ideal for this study. As a result,

mortality was high in both chamber types and was likely influenced by natural senescence rather than tagging or chamber effects. Consequently, the chamber comparison attempt was inconclusive. Regardless, this trial represented an important effort to evaluate whether chamber design itself may have contributed to observed survival patterns.

Survival comparison

I assessed tag suitability by comparing survival of tagged beetles housed in experimental chambers to that of untagged beetles maintained in control chambers over a 50-day period. Survival estimates were used to construct survivorship curves for both groups. Due to limited detections in some trials, not all trial datasets were suitable for analysis. For example, Trial 1 yielded only 8 detections across 51 tagged individuals, and Trial 4 yielded a single detection across 31 tagged individuals. Both trials involved the endangered species, and the low number of detections prevented reliable separation of mortality from non-detection for use in the HMM model. As a result, only trials with sufficient detections were retained for analysis. For these trials, survival was estimated separately for experimental and control groups and expressed on a common scale to allow direct comparison. Across analyses, survivorship curves were generated for each group and compared through time to evaluate differences between tagged and untagged beetles.

Trial 2

Survival declined in both groups, with differences remaining relatively similar through time until the end of the study (Fig 8). At day 21 (first open interval), the posterior mean survival difference was -0.05 (90% CrI -0.34 to 0.27), with Pr(diff <0) = 0.61, indicating no evidence that

experimental survival was lower than control survival at this stage. Detection during the preceding closed period was moderate ($p = 0.44$), with interval survival estimated at $\phi = 0.62$. By day 44 (second open interval), the posterior mean difference was -0.21 (90% CrI -0.52 to 0.14), with $Pr(diff<0) = 0.85$, again suggesting little evidence of reduced survival. Detection remained similar during the second closed period ($p = 0.48$) with interval survival estimated at $\phi = 0.49$. By day 50, cumulative survival diverged between groups. The posterior mean difference was -0.40 (90% CrI -0.63 to -0.17) and $Pr(diff <0) = 1.00$, indicating lower survival in the experimental group at the end of the study. Overall results from Trial 2 indicated survival was not different until approximately day 50 where there was evidence of reduced survival in the treatment chambers (i.e., tagging effect).

Trial 3

In Trial 3, control beetles exhibited consistently high survival, where experimental beetles experienced earlier and more obvious declines in survival (Fig. 9). On day 19, (first open period), the posterior mean survival difference was -0.38 (90% CrI -0.61 to -0.09) with $Pr(diff < 0) = 0.99$, indicating statistical evidence that beetles in the experimental survival had reduced survival compared to the control group lower than control survival. Detection toward the preceding closed period was moderate ($p = 0.44$) with interval survival estimated at $\phi = 0.55$. By day 43 (second open interval), separation between groups was more pronounced, with a posterior mean difference of -0.58 (90% CrI -0.81 to -0.26) and $Pr(diff <0) = 0.997$. Detection remained similar during the second closed period ($p = 0.53$), with interval survival estimated at $\phi = 0.52$. By day 50, cumulative survival differences remained distinct. The posterior mean difference was -0.55 (90% CrI -0.70 to -0.37) and $Pr(diff <0) = 1.00$, indicating lower survival in

experimental chambers relative to controls. Overall, Trial 3 indicated that survival under experimental conditions displayed stronger divergence than controls when evaluated across all time points.

Collectively, my results show that posterior distributions of survival differences increasingly shifted below zero at later time points, with 90% credible intervals occasionally excluding zero and indicating lower survival in the experimental group. However, the magnitude and certainty of these differences varied across trials and intervals. The support from my two experimental analyses suggests that a tagging-related survival effect is plausible, but the evidence remains mixed and does not support a strong or consistent conclusion. These inferences should be interpreted within the context of the modeling framework, including assumptions related to model structure, interval alignment, and imperfect detection. Notably, observed differences in survival tended to increase over time rather than reflecting an immediate mortality response, suggesting that any potential tagging effect may be delayed or cumulative in nature.

Discussion

My results indicate that p-Chip tagging may reduce survival in riffle beetles, although retention rates were generally high and the technology shows promise for marking small-bodied invertebrates. Studies evaluating tag suitability in insects remain limited, and we are unaware of previous research testing p-Chips in riffle beetles. Across three comparative analyses (e.g., two empirical trials and a simulated trial), although there is uncertainty, we see reduced survival in the treatment compared to control chamber beetles. These patterns were supported by interval-

specific and cumulative survival comparisons conducted within a Bayesian hidden Markov modeling framework with differences becoming more pronounced over the 50-day study period. These results indicate that the tagging procedure or the addition of a p-Chip may reduce short-term survival in beetles under the conditions tested. However, p-Chips meet several desirable criteria for marking small invertebrates, including compact size, the ability to uniquely identify individuals, and offer minimal external obstruction relative to alternative tagging approaches (Blight et al. 2013, McLean and Fisher 2025). Retention rates varied across trials, and adhesive used but were generally high (82% - 91%). Because carapaces were exposed to flowing water post-mortem, physical degradation may have increased the likelihood of tag loss after death, suggesting retention rates could be higher than I observed. Further investigation into the use of p-Chips may prove advantageous if some of the unknown factors related to survival over time are better understood (e.g., glue, tag burden, biological stress).

Imperfect detection of tagged beetles introduced uncertainty into survival estimates and represents an area that could be improved with design modification. Even in Trials 2 and 3, which produced sufficient detections to inform the multistate model, only about half of the tagged individuals were detected at least once (Trial 2: 29 of 60; Trial 3: 24 of 45). Increasing detections would reduce the uncertainty associated with distinguishing mortality from non-detection. For example, in fish-telemetry studies, increased detections often occur through active sampling that occurs in conjunction with the use of acoustic or radio passive arrays (Gannon 2008, Mann et al. 2008). Riffle beetles exhibit variable movement, and while their rheotactic behavior (i.e., water flow direction, Bio-west 2017) was used to encourage desired movement along a set path, individuals could still be missed. Improved designs could trim the path walkway to be narrower or make use of multiple readers to increase the probability of detection.

Alternatively, a subset of manual scans could be performed. Although this could increase stress to the beetles, it may be less stressful than the regular counts that they already undergo during monitoring efforts. However, because I anticipated imperfect detection, survival was analyzed using a Bayesian multistate model that allowed for survival to be estimated while accounting for incomplete detection.

Mortality patterns may also reflect inherent physiological sensitivity of spring-adapted riffle beetles rather than tagging effects alone. Both *Heterelmis glabra* and *Heterlemis comalensis* inhabit thermally stable spring systems and are adapted to relatively constant temperature and specific flow regimes (Nowlin et al. 2017, Nair et al. 2023). Previous experimental studies show that both spring-associated species (e.g., *H. comalensis, H. glabra*) exhibit elevated metabolic responses to temperature fluctuations compared to other more widespread, surface-water dwelling, elmid beetle species (e.g., *H. vulnerata, M. pusillus*), thus indicating limited tolerance to natural variation (Nowlin et al. 2017). Although water temperature, flow, and levels of dissolved gases (e.g., DO and C02) were carefully maintained by USFWS staff, laboratory systems may not fully replicate the exact intricates of natural spring systems (Beracko and Revajova 2019, Sun et al. 2020). Even under carefully controlled laboratory conditions, baseline survival of stenothermal riffle beetles varied even in our controlled chambers. For example, in my study, untagged *H. Glabra* exhibited varying survival curves between Trials 2 and 3 despite consistent housing conditions, suggesting that captive survival can vary even under standardized setups (Figs. 8, 9). These patterns indicate that baseline mortality in captivity may reflect physiological sensitivity to their natural rather than experimental manipulation alone. The physiological sensitivity may not be related to laboratory environment but could vary due to age or other factors.

In addition to environmental sensitivity, natural senescence likely contributed to observed mortality representing a key limitation of using wild-caught individuals of unknown age. For example, most riffle beetles, including the CSRB and *H. Glabra* have an estimated adult lifespan of approximately one year (Bio-west 2018), and individuals were of unknown age at the time of experimentation (i.e., no reliable morphological markers exist for determining age in adult beetles). Given these biological constraints, some observed mortality is expected from natural senescence. Laboratory studies have reported mortality rates of up to 25% per month in *H. Comalensis* (Nowlin et al. 2017) and up to 50% mortality for 6 months under similar captive conditions (Bio-west 2019). Moreover, adult beetles were collected from the field and held for one or two months prior to tagging (per USFWS scheduling), increasing the likelihood that some individuals may have been older than optimal for the study. In Trial 4, several beetles appeared sluggish via observation indicative of physiological decline. Thus, preexisting senescence may have increased vulnerability to additional stressors, including handling or tagging. Combined stressors are commonly known to be problematic for aquatic organisms (Heugens et al. 2011). Consequently, future efforts would benefit from focusing on reducing some of these uncertainties to better examine tagging opportunities for these beetles.

Although tagging inevitably introduces stress, most potential stressors were anticipated and mitigated through careful handling procedures. The beetles were exposed to several potential stressors including direct handling (Biowest 2014), temporary interruption of respiration via water removal (Thorpe and Crisp 1949), exposure to bright light (Cooke et al. 2015), thermal fluctuations (Nowlin and Schwartz 2013), short-term freezing (Groening et al. 2018) and adhesive application. Some stress was minimized by using soft-tip forceps and fine brushes during transfers (i.e., between containers and experimental chambers) and beetles were placed on

moistened cloth to maintain humidity. Tagging was typically completed in under two minutes reducing oxygen deprivation. To further limit thermal stress, beetles were held in cool water baths maintained at 21°C, replicating natural aquifer conditions (Bio-west 2015). Previous studies indicate that CSRB can tolerate short exposures (5-10 minutes) of rapid temperature increases (up to 45 °C) and low dissolved oxygen (0 mg/L), without lasting effects (Biowest 2015), suggesting that these short handling events were unlikely to cause significant physiological stress. Because certain cyanoacrylate glues may negatively affect beetle survival (Boiteau et al. 2009), I selected a non-toxic aquarium-grade adhesive (Woldoclean Professional), commonly used in aquatic systems as a safer alternative. Although these precautions likely reduced some of the concerns related to cumulative stress, some examinations of these individual stressors have not been well examined. Moreover, there were notable differences in the behaviors of the two beetle species, suggesting some concern related to using *H. glabra* as a suitable surrogate species.

Miniaturized tagging technologies offer significant potential to expand research on small-bodied invertebrates, a taxonomic group for which reliable marking methods remain limited. In aquatic macroinvertebrates, most tagging efforts have relied on batch marking approaches such as epoxy mixtures (Neves 1979), glitter (Jackson et al. 1999), or fluorescent powder (Brusven 1970), which do not permit individual identification (except in batches). True individual marking among insects is comparatively rarer and has only been achieved in a few study systems, including numbered tags in stonefly larvae *Plecoptera spp*. (Frelich 1989) and miniature radio transmitters in dobsonfly larvae *Megaloptera spp*. (Hayashi and Nakane 1989). For organisms near or below 2-3mm, body length represents the primary barrier to individual tagging. Tag burden is often considered a secondary constraint, yet emerging evidence suggests that certain

beetles can tolerate relatively high proportional burdens. For example, the seed beetle *Mimosetes cephalotes*, similar in size to *H. Comalensis* (≈2 mm; 0.61 mg; Canto et al. 2019) would experience an approximate tag burden of 14% when fitted with an ≈85 µg p-Chip (Sheult et al. 2024). This magnitude falls within ranges reported for other beetles without significant impairment. For example, RFID tags comprising 23-33% of body mass did not impair flights in Colorado potato beetles *Leptinotarsa decemlineata* (Boiteau and Colpitts 2015), and glued PIT tags representing 20-33% of body mass did not substantially reduce movement in ground beetles *Carabus nemoralis* (Testud et al. 2019). These studies suggest that beetles may tolerate tag burdens that would be prohibited in other taxa. This research provides one of the first evaluations of p-Chip technology in macroinvertebrates and contributes to a growing body of work aimed at overcoming size-related constraints in macroinvertebrate research.

The reliance of plastron respiration for elmids (i.e., mechanism for gas exchange) may make them sensitive to habitat changes and raise concerns with how external tags might interfere with survival (Brown 1987), however, evidence suggests they may remain resilient enough for external tagging. Because tag attachment requires adhesive on the elytra, there is potential for interference with plastron function or with grooming behaviors that prevent hair clumping as both are important for maintaining the air layer necessary for respiration (Thorpe and Crisp 1949). However, survival trials suggest that immediate tagging effects are not lethal. Tagged beetles displayed normal locomotion and self-righting behaviors, and several *H. glabra* individuals survived for extended periods (e.g., 86 to 150 days; Table 12). Both CSRB and *H. glabra* tolerated extreme conditions in short-term trials with loss of response only at very low oxygen levels (mean 0.48mg/L; 11/16 individuals affected) and at elevated temperatures of 45–50 °C (Nowlin and Schwartz 2014). This becomes significant given that normal DO

environmental conditions at both Comal Springs and Devils River range from 5 -7 mg/L. Although tagging may introduce short-term stress, plastron function remains sufficiently robust to support survival, thus reinforcing the broader feasibility of p-Chip tagging in aquatic invertebrates.

Accurately monitoring endangered CSRB populations is a central challenge for conservation efforts in the Comal Springs region, where the Edwards Aquifer Authority (EAA) seeks to balance aquifer management with species protection. This task is complicated by habitat degradation linked to climate change and increased groundwater abstraction which reduces spring flows from the Edwards Aquifer (Bio-west 2015). Reduced flow not only shrinks available surface habitat but may also drive individuals deeper into subsurface aquifer systems (Boulton and Foster 1998; Bio-west 2014), making it difficult to distinguish true population declines from reduced detectability. The adoption of p-Chip tagging technology or another viable alternative (see Chapter 1) could help overcome these challenges by enabling individual-level identification. Importantly, p-Chips make it possible to distinguish between previously captured individuals, something analogous current monitoring strategies cannot effectively achieve. These advancements would allow for recapture-based population estimates and facilitate demographic studies of survival (Bradshaw et al. 2007, Skalski et al. 2009), movement (Wheeler and Marlow 2016), and habitat use (Inoue et al. 2014, Freedman et al. 2016) over time. After additional laboratory evaluation, future efforts may be conducted in field-based tagging approaches, thus reducing stress from handling and transport.

These findings have important implications for conservation and management of the endangered Comal Springs Riffle Beetle and other spring-obligate invertebrates. Reliable estimates of survival and abundance are important for evaluating population status, guiding

captive propagation efforts, and informing recovery strategies. Although p-Chip tagging may impose short-term survival costs under laboratory conditions, it remains one of the few (but see Chapter 1) viable approaches for uniquely marking individuals of this size, offering a potential pathway to improve demographic monitoring. Careful refinement of the tagging procedures, including minimizing handling stress and optimizing attachment methods, may help reduce the negative effects while retaining the benefits of individual identification. In addition, incorporating tagging into both captive and field-based studies could improve estimates of survival, movement, and population size, thereby supporting more informed decisions regarding habitat management, water resource allocation, and species recovery planning. More broadly, this work highlights the need to balance methodological innovation with organism welfare when developing monitoring tools for sensitive and endangered taxa.

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Tables and Figures

Table 1. Classification definitions for articles published on miniaturized tags for organisms that were included in our review

Study Purpose	Description
Survival (S)	Study reported and measured the survival rate of the organism after tagging occurred
Retention (R)	Study reported and measured how well the tag was retained
Behavior (B)	Study reported any interactions in feeding or foraging (i.e., lack, increase)
Growth (M)	Study was interested in basic growth and body changes over time, (i.e., length, weight)
Recapture (RE)	Study reported if an organism was recaptured
Tag Burden (TB)	Study measured the organisms' tag to mass ratio and investigated the effects of burden on previous factors
Other (O)	Does not fit any other category

Table 2. Summary of the various types of tags used in this review. Provided is a short description of each tag under "Description", the "Advantages" and "Disadvantages" of each tag labeled respectively, the estimated cost for a singular tag or unit under "Est. Cost", and the relative "Size" and "Weight" of each tag respectively, finally the number of associated studies labeled under "Associated Studies".

Tag Type	Description	Advantages	Disadvantages	Cost	Size	Weight	Associated Studies
Passive Integrative Transponders (PIT)	Electronic microchips encased in glass	Provides detailed and long-term observation	Detection distance decreases with size	$4-5	8-23mm	27mg-102mg	75
Radio Frequency Identification (RFID)	Transmits data using specific frequencies to a reader	Small size, can track movement accurately	Short detection distance	$0.45	8mm x 1.4mm	30 mg	36
Coded Wire Tag (CWT)	Steel wire tags with unique imprinted codes	Small, and non-invasive	Can only be read post-mortem	$0.12	1.1mm x 0.25mm	3mg	8
Visible Implant Alpha (VIA)	Coded tag inserted beneath clear tissue	Can be easily applied	Tags are prone to loss/migration	$0.92	2.69 x 255.19 mm	1.13g	10
Harmonic Radar	Wire tags detected using microwaves	Small size, tag is easy to construct	Can not produce unique signals	$0.10	9mm x 0.16mm diameter	3mg	12

p-Chips	Micro-tags that are read when activated by light	Minimal effects of tag burden, unique ID	ID can only be read within a small range by reader	$3.00	0.5 x 0.5mm	0.085mg	7
T-bar tag	External tags attached to the organism	Easily read, and cost effective	Effective on larger animals	$1.25	5-7cm	1g	10
Acoustic Tags	Tags which transmit sound pulses through water	Suitable for long term passive monitoring	Signal interference in noisy or complex environments	$40 - $135	12 – 36mm	6 -7g	3
Photo-Luminescent tags (PL)	Paper coated with lead	Can be used to track distances >1000 ft	Vegetation obscures detections	$0.10	5mm	12.5mg	1
DataDots	Microdot identifiers with unique alphanumeric ID	Requires no complex tools, ready to mark	Cannot be read when attached to a dark background	$0.02	0.5 x 0.5mm	12.5mg	2
Geolocator tags	Uses recorded light levels to estimate location later	Long battery life (months to years)	Not suitable for fine scale data, accuracy affected shading, weather	$250 - $300	18 x 8 x 6mm	0.65 – 1.5g	2

Computer-aided identification	Digital identification using computer software	Non-intrusive and can correctly identify patterns	May require extensive data processing time	$0.00	NA	NA	8
GPS Tags	Receives signals from satellites for location	Provides fine scale movement, can transmit real time data	Heavier and requires batteries	$1,000 - $3000	21 x 13 x 9mm	1.1g	5

1. Portz and Workman 2014
2. Harrison et al. 2022
3. Hammer and Blankenship 2001
4. Merbeek et al. 2013
5. Lee et al. 2013
6. Spooner and Spurgeon 2023
7. Hamel et al. 2012
8. McMicheal et al. 2010
9. Walter et al. 2021
10. Whitehead and Peakall 2018
11. Weiser et al. 2016
12. Crall et al. 2019
13. Bridge et al. 2013

Table 3. Study outcomes of interest survival (S) and retention (R) across different species (Common Name and scientific name provided) tagged using a variety of microtransponder tags (Type). Organisms were tagged with either passive integrative transponders (PIT) or p-Chips with tag mass (TM) provided. All PIT tags used were 8 mm unless indicated with a footnote. The animals examined had different body sizes (BS) which was expressed either in mass or total length. Sampe size (n), study duration (SD) and tag-to-mass (T2M) ratios are provided. (Tag-to-mass ratio) is the percentage of an organism's body in relation to the tag mass. NA indicates that a particular measurement was not provided in the study.

Common Name	Scientific Name	n	BS (g or TL)	Type	TM (g)	SD (days)	T2M (%)	S (%)	R (%)
Barton springs salamander[1]	*Eurycea sosorum*	32	2.0 ± 0.7g	p-Chip	6×10^{-5}	240	0.003	97	97
Red-legged salamander[1]	*Plethodon shermani*	43	0.7 ± 0.1g	p-Chip	6×10^{-5}	240	0.009	100	98
Great-crested newt[1]	*Triturus cristatus*	38	3.4 ± 1.7g	p-Chip	6×10^{-5}	240	0.002	99	100
Creek chub[2]	*Semotilus atromaculatus*	30	2.2 ± 0.7g	[1]PIT	0.03	42	1.9	83*	90
Creek chub[2]	*Semotilus atromaculatus*	30	9.2 ± 1.1g	PIT	0.03	42	0.61	100	100
Johnny darter[2]	*Etheostoma nigrum*	30	1.5 ± 0.3g	PIT	0.03	42	3	63*	70
Plains topminnow[2]	*Fundulus sciadicus*	30	1.8 ± 0.4g	PIT	0.03	42	2.3	100	97
Sand shiner[2]	*Notropis stramineus*	30	2.8 ± 0.5g	PIT	0.03	42	1.5	90	77
Eastern blacknose dace[2]	*Rhinichthys atratulus*	30	6.3 ± 0.6g	PIT	0.03	42	0.94	73*	100
Common shiner[2]	*Luxilus cornutus*	30	7.2 ± 0.7g	PIT	0.03	42	1	100	60
Tadpole madtom[2]	*Notorus gyrinus*	30	2.6 ± 0.8g	PIT	0.03	42	2.2	100	57
White sucker[2]	*Catostomus commersonii*	30	2.2 ± 0.9g	PIT	0.03	42	0.87	90	90
Pacific blue-eye[3]	*Pseudomugil signifier*	10	0.52 ± 0.1g	PIT	0.03	21	5.9	0*	100

Crimson spotted rainbow fish[3]	*Melanotaenia duboulayi*	10	2.9 ± 1.8g	PIT	0.03	21	1.2	60	100
Empire gudgeon[3]	*Hypseleotris compressa*	10	2.8 ± 1.8g	PIT	0.03	21	1.6	60	100
Agassiz's glass fish[3]	*Ambassis agassizii*	10	1.9 ± 0.5g	PIT	0.03	21	1.95	70	100
Southern pygmy perch[3]	*Nannoperca australis*	10	1.8 ± 0.6g	PIT	0.03	21	2.65	83	100
Australian bass[3]	*Percalates novemaculeata*	10	1.7 ± 0.5g	PIT	0.03	21	1.1	100	100
Golden perch[3]	*Macquaria ambigua*	10	1.6 ± 0.5g	PIT	0.03	21	1.8	0*	100
Murray cod[3]	*Maccullochella peelii*	10	3 ± 0.7g	PIT	0.03	21	0.55	100	100
Silver perch[3]	*Bidyanus bidyanus*	10	6 ± 1.4g	PIT	0.03	21	0.75	100	100
Eel tailed catfish[3]	*Tandanus tandanus*	10	4.8 ± 1.9g	PIT	0.03	21	2.05	100	100
Bluehead chub[4]	*Nocomis leptocephalus*	144	40 - 100 mm TL	PIT	0.03	7	NA	97	96
Creek chub[4]	*Semotilus atromaculatus*	51	39 - 101 mm TL	PIT	0.03	7	NA	98	98
Mottled Sculpin[4]	*Cottus bairdii*	43	50 - 80 mm TL	PIT	0.03	7	NA	98	98
Yellowfin Shiner[4]	*Notropis lutipinnis*	83	51 - 87 mm TL	PIT	0.03	7	NA	93	92

* indicate outcomes that were significantly different than the control group ($p < 0.05$)

Used 8.5mm PIT tag

1. Moore et al. 2024

2. Schumann et al. 2020

3. Watson et al. 2019

4. Cary et al. 2017

Table 4. Survival (S) and retention (R) outcomes for studies tagging the same species (with Common name and scientific name provided) allowing comparisons across different body sizes (expressed as either mass or total length). Organisms were tagged with a variety of tags, including passive integrative transponders (PIT; 6mm, 8mm, or 12, see footnote), visible implant alphanumeric (VIA; 1 x 2.5mm) tags, or Coded Wire Tags (CWT). Name of tag is listed under (Type) with tag mass (TM) reported. Sample size (n), study duration (SD), and tag-to-mass ratio (T2M) are also provided. The (tag-to-mass ratio) is the percentage of an organism's body mass in relation to the tag mass. NA indicates missing or unavailable data.

Common Name	Scientific Name	n	BS (g or TL)	Type	TM (g)	SD (days)	T2M (%)	S (%)	R (%)
Chinook salmon[1]	*Onocorhynchus tshaytscha*	149	80-99 mm	PIT[a]	0.74	30	11.5	88	91
Chinook salmon[1]	*Onocorhynchus tshaytscha*	147	90-99 mm	PIT[a]	0.74	30	8	89	99
Chinook salmon[1]	*Onocorhynchus tshaytscha*	150	100-109 mm	PIT[a]	0.74	30	5	97	97
Senegalese sole[2]	*Solea senegalensis*	25	0.33 ± 0.07g	PIT[b]	7 x 10-3	57	2.19	96	92
Senegalese sole[2]	*Solea senegalensis*	150	0.86 ± 0.28g	PIT[b]	7 x 10-3	57	0.84	97	90
Senegalese sole[2]	*Solea senegalensis*	50	2.07 ± 0.96g	PIT[b]	7 x 10-3	57	0.35	98	98
White Sands Pupfish[3]	*Cyprinodon tularosa*	139[a]	35.45 ± 0.35 mm (TL)	PIT[c]	0.027	75	3.38	0	NA
White Sands Pupfish[3]	*Cyprinodon tularosa*	NA	36.9 ± 0.36 mm (TL)	PIT[c]	0.027	75	3	0	NA
White Sands Pupfish[3]	*Cyprinodon tularosa*	NA	38.14 ± 0.39 mm (TL)	PIT[c]	0.027	75	2.7	67	NA
White Sands Pupfish[3]	*Cyprinodon tularosa*	NA	39.29 ± 0.41 mm (TL)	PIT[c]	0.027	75	2.45	75	NA

White Sands Pupfish[3]	*Cyprinodon tularosa*	NA	40.78 ± 0.46 mm (TL)	PIT[c]	0.027	75	2.16	86	NA
White Sands Pupfish[3]	*Cyprinodon tularosa*	NA	43.31 ± 0.55 mm (TL)	PIT[c]	0.027	75	1.18	95	NA
White Sands Pupfish[3]	*Cyprinodon tularosa*	NA	47.4 ± 0.73 mm (TL)	PIT[c]	0.027	75	1.35	99	NA
Giant freshwater prawn[4]	*Macrobrachium rosenbergii*	120	2.1 ± 0.35g	VIA	4×10^{-3}	70	0.2	93.3	71
Giant freshwater prawn[4]	*Macrobrachium rosenbergii*	120	14.9 ± 2.69g	VIA	4×10^{-3}	70	0.028	95	91
Giant freshwater prawn[4]	*Macrobrachium rosenbergii*	120	27.4 ± 1.37g	VIA	4×10^{-3}	70	0.015	90	59
Atlantic sturgeon[5]	*Acipenser oxyrinchus oxyrinchus*	32	7.3 ± 2.2g	CWT	5×10^{-4}	56	0.007	100	90
Atlantic sturgeon[5]	*Acipenser oxyrinchus oxyrinchus*	30	35 ± 10g	CWT	5×10^{-4}	56	0.001	90.6	100

[a]Sample sizes were pooled

[a]used a 12mm PIT tag

[b]used a 6mm PIT tag

[c]used a 8mm PIT tag

1. Brown et al. 2010

2. Carballo et al. 2018

3. Peterson et al. 2018

4. Pillai et al. 2009

5. Kapusta et al. 2015

Table 5. Survival (S) and retention (R) outcomes across studies evaluating different tags on the same species (listed under Common name and scientific name) to compare responses in tag variation. Several types of tags were evaluated, including passive integrated transponders (PIT; 8mm, 9mm, 12mm; see footnote), coded wire tags (CWT), visible implant alphanumeric tags (VIA), radio frequency identification (RFID; 9.6mm, 11mm, 15mm; see footnotes), and eel lamprey acoustic transmitter (ELAT; 12mm). For each tag, the table reports its classification (Type) and tag mass (TM). Body size (BS) of organisms is expressed as either mass or total length. Sample size (n), study duration (SD), and tag-to-mass ratio (T2M) are provided. The tag-to-mass ratio represents the percentage of an organism's body mass relative to the tag mass. NA indicates missing or unavailable data.

Common Name	Scientific Name	n	BS	Type	TM (g)	SD (days)	T2M (%)	S (%)	R (%)
Ringed salamander[1]	*Ambystoma annulatum*	9	1.58 ± 0.14g	PIT[a]	0.1	42	7.28	44	100
Ringed salamander[1]	*Ambystoma annulatum*	9	1.57 ± 0.16g	PIT[c]	0.03	42	2.09	94	100
Pacific Lamprey[2]	*Entosphenus tridentatus*	24	0.59 ± 0.09g	PIT[c]	0.03	137	5.08	38	89
Pacific Lamprey[2]	*Entosphenus tridentatus*	19	0.43 ± 0.13g	CWT	5×10^{-4}	137	0.12	100	64
Sea Lamprey[3]	*Petromyzon marinus*	50	1.0 - 7.3g	PIT[c]	0.03	28	0.96	40	95
Sea Lamprey[3]	*Petromyzon marinus*	50	1.2 - 7.7g	PIT[b]	0.1	28	4	10	60
Stonecat[4]	*Noturus flavus*	41	71 - 150mm TL	RFID[f]	0.1	840	0.28	90	85
Stonecat[4]	*Noturus flavus*	45	71 - 150mm TL	RFID[f]	0.1	840	0.28	93	96
Stonecat[4]	*Noturus flavus*	71	150 – 213mm TL	RFID[d]	0.6	840	1.7	98	89

Artic lamprey[5]	*Lethenteron camtschaticum*	36	71 – 286.5g	RFID[e]	1.5	98	1.25	93	100
Artic lamprey[5]	*Lethenteron camtschaticum*	36	71 – 286.5g	RFID[g]	0.57	98	0.5	94	100
Artic lamprey[5]	*Lethenteron camtschaticum*	36	71 – 286.5g	RFID[h]	0.3	98	0.25	97	96
Artic lamprey[5]	*Lethenteron camtschaticum*	36	71 – 286.5g	T-bar	0.22	98	NA	93	96
White-clawed crayfish[6]	*Austropotamobius pallipes*	20	34.8 ± 1.768g	PIT[c]	0.027	360	0.08	93	100
White-clawed crayfish[6]	*Austropotamobius pallipes*	20	39.9 ± 3.283g	PIT[a]	0.1	360	0.25	100	100
Texas blind salamander[7]	*Eurycea rathbuni*	11	1.6g	PIT[c]	0.03	365	2.06	100	55
Texas blind salamander[7]	*Eurycea rathbuni*	20	1.6g	VIA	4×10^{-3}	365	0.62	100	90
European eel[8]	*Anguilla Anguilla*	100	18.2 ± 4.4g	ELAT	0.08	75	0.44	88	99
European eel[8]	*Anguilla Anguilla*	100	17.4 ± 4.1g	PIT[a]	0.1	75	0.57	97	100
Chinook salmon[9]	*Oncorhynchus tshawytscha*	21	5.5 ± 0.8g	PIT[a]	0.1	14	1.81	100	100
Chinook salmon[9]	*Oncorhynchus tshawytscha*	21	5.5 ± 0.8g	PIT[c]	0.03	14	0.55	95	90

[a]Used a 12mm PIT tag

[b]Used a 9mm PIT tag

[c]Used a 8mm PIT tag

[d]Used a 23mm RFID tag

[e]Used a 15mm RFID tag

[f]Used a 12mm RFID tag

[g]Used a 11mm RFID tag

[h]Used a 9.6mm RFID tag

1. Ousterhout and Semlitsch 2013
2. Hanson and Barron 2017
3. Dawson et al. 2015
4. D'Amico et al. 2021
5. Spanos et al. 2023
6. Nightingale et al. 2018
7. Moon et al. 2022
8. Jepsen et al. 2022
9. Conrad et al. 2016

Table 6. Survival (S) and retention (R) outcomes across reviewed studies when compared with respect to variation in tagging methods. The methodology, which describes the tag application techniques or other tagging procedure factors, is listed under (Method) for each species (common and scientific provided). Animals were tagged with either passive integrated transponders (PIT; 12mm) or very high frequency (VHF) radio tags with each tag noted under (Type) and its Tag Mass (TM) reported. Body size (BS) of each organism is expressed either as mass or total length. Sample size (n), study duration (SD) and tag-to-mass ratio (T2M) ratios are provided. The (tag-to-mass ratio) is the percentage of an organism's body mass in relation to the tag mass. NA indicates missing or unavailable data.

Common Name	Scientific Name	n	BS (g or TL)	Type	TM (g)	Method	SD (days)	S (%)	R (%)
Italian riffle dace[1]	*Telestes muticellus*	58	73-82 mm	PIT	0.1	Injection	49	95	97
Italian riffle dace[1]	*Telestes muticellus*	58	72-81 mm	PIT	0.1	Incision	49	100	100
Atlantic salmon[2]	*Salmo salar*	98	48.25 ± 9.25g	VHF Radio	1.5	Surgeon A	8	98	NA
Atlantic salmon[2]	*Salmo salar*	97	52.25 ± 9.25g	VHF Radio	1.5	Surgeon B	8	75	NA
Brown Trout[3]	*Salmo trutta*	29	1.49 ± 0.13g	PIT 12 mm	0.1	Injector A	60	76	86
Brown Trout[3]	*Salmo trutta*	28	1.56 ± 0.13g	PIT 12 mm	0.1	Injector B	60	86	92
Brown Trout[3]	*Salmo trutta*	30	1.56 ± 0.14g	PIT 12 mm	0.1	Injector A	60	67	55
Brown Trout[3]	*Salmo trutta*	27	1.61 ± 0.18g	PIT 12 mm	0.1	Injector B	60	96	82

1. Schiavon et al. 2017

2. Heim et al. 2023

3. Richard et al. 2013

Table 8. Summary of reviewed studies evaluating potential tags for marking small invertebrates. The name of the tag is labelled under "Tag Type", the approximate size of each under "Size", tag reading method under "Identification Method", the likely durability of each tag under "Durability", and the corresponding "Advantages" and "Limitations" of each tag.

Tag Type	Size	Identification Method	Durability	Advantages	Limitations
Datadots[1]	0.5mm diameter	Visual inspection under magnification	Highly durable, withstands outdoor environments	Only requires magnification	Susceptible to individual recording error
Computer-aided identification (BEEtags)[2]	Can be scaled to any size	Uses picture of printed out tags and identified using MATLAB software	Moderate, paper like durability	Low cost, low chance of false positives	Requires computational identification time
p-Chips[3]	0.5 x 0.5mm	Read via laser identification and p-Chip software	Highly durable, can withstand harsh conditions	Allows for data storage, used in multiple taxa	Requires a reader, and tags can only be read within a limited distance
Coded wire tags (CWT)[4]	0.5 mm length	Visual Inspection under magnification	Highly durable, meant to last the lifespan of the organism	Only requires magnification, reported across many studies	Most protocols require the tag to be inserted rather than attached

1. Whitehall and Peakall 2012

2. Crall et al. 2017

3. Moore et al. 2024, Huber et al. 2023, Spooner and Spurgeon 2023, Faggion et al. 2019, Moore and Brewer 2021

Table 9. The supplies needed to construct the treatment chambers for evaluating tag retention and survival of tagged beetles. We provided the use of each piece (Description), the dimensions, quantity needed for each chamber, and the location (Purchased) and cost at the time that we purchased.

Supply	Description	Dimensions	Amount needed for 1 chamber	Purchased	Cost (USD)
Stainless Steel wire mesh screen	Allows water flow but prevents beetle escape	40 mesh size (0.45 mm opening)	(2) 6cm sheets	AggFencer (Amazon)	$9.99
Nylon barb fitting	Allows for hose to be inserted into chamber allowing for water flow	6.35 x 12.7mm	2	Proline Series (Lowes)	$2.78
Sch[1] 40 PVC female adapter fitting	Allows for connections between bushing and Sch 80 tube	5.08cm	4	Charlotte Pipe (Lowes)	$3.12
Sch 40 PVC[2] reducing bushing	Connects to poly carbonate rigid tubing	5.08 x 1.27cm	2	Charlotte Pipe (Lowes)	$4.70

Sch 40 PVC reducing bushing	Is connected to adapter and nylon barb	5.08 x 2.54cm	2	Charlotte Pipe (Lowes)	$3.65
Poly carbonate rigid tubing	Clear plastic tube allowing for laser to pass through	1.91 OD x 1.59 ID x 0.159 cm L= 12.7 cm	1	Small Parts (Amazon)	$17.02
PVC Sch 80, clear threaded tube	PVC pipe that is between 2 PVC adapters	5 cm diameter 10.16 cm long	2	Alsco Industrial Products	$42.33
White Velcro	Is placed inside rigid tubing	5 cm wide	NA	Velcro Brand (Walmart)	$8.99
Original Gorilla Glue	To connect clear polytube between the two subchambers	NA	NA	Gorilla Brand (Lowes)	$6.98
# 35 O-ring	Provides a waterproof seal around tube and tighter fit	1.746 OD x 1.428 ID x 0.159 cm	4	Danco (Lowes)	$3.13
Wire brush	A wire brush used for	NA	NA	Lavaxon	$8.49

	cleaning the mesh vents			(Amazon)	
PVC Purple Primer	8 fluid oz can of PVC primer	NA	NA	Oatey (Lowes)	$9.38
Medium Clear PVC Cement	8 fluid oz can of PVC cement used for PVC pipe	NA	NA	Oatey (Lowes)	$8.18

1. Sch – Schedule
2. PVC – Polyvinyl chloride

Table 10. The supplies needed to construct the control chambers where survival of control beetles will be evaluated. We provided the use of each piece (Description), the dimensions, quantity for each chamber, and the location (Purchased) and cost at the time that we purchased.

Supply	Description	Dimensions	Amount needed for 1 chamber	Purchased	Cost (USD)
Rubbermaid Brilliance plastic container	Container where control beetles will be kept	1.84 L	1	Newell Brands (Amazon)	$18.99
PVC Bulkhead water tank connector	Keeps mesh screen in place & connects to hose fitting	12.7mm female, 12.7 mm male, 38mm diameter	2	QuQuyi (Amazon)	$11.99
NPT barbed hose fitting	Polypropylene fitting that connects to hose	12.7 x 6.35mm	2	Banjo Corporation (Amazon)	$6.40
Teflon tape	Prevent water leaking	NA	NA	VOTMELL (Amazon)	$5.99
White Velcro	Placed inside container	5 cm wide	2 strips	Velcro Brand	$8.99

Table 11. Overview of beetle allocations across four experimental trials, detailing distribution of *H. Comalensis* and *H. glabra* among experimental and control chambers. (T) is shorthand for tagged and (U) is shorthand for untagged. Prior to Trial 4, all beetles placed in experimental chambers (T1-T4) were tagged beetles, and all beetles placed in control chambers were untagged beetles. "NA" was placed if those types of beetles were not used in that trial.

	Trial 1	Trial 2	Trial 3	Trial 4
H. comalensis	Chamber T1 = 12 Chamber T3 = 9 Control 1 = 15	NA	NA	Chamber T2 = 6 (T), 5 (U) Chamber T3 = 6 (T), 5 (U) Chamber T4 = 6 (T), 1 (U) Control 1: 6 (T), 5 (U) Control 2: 5 (T), 5 (U) Control 3: 5 (T), 5 (U)
H. glabra	Chamber T2 = 14 Chamber T4 = 16 Control 2 = 15 Control 3 = 15	Chamber T1 = 15 Chamber T2 = 15 Chamber T3 = 15 Chamber T4 = 15 Control = 10	Chamber T2 = 15 Chamber T3 = 15 Chamber T4 =15 Control = 15	Chamber T4 = 2 (U)

Total	n = 96	n = 70	n = 60	n = 62

Table 12. Summary of detection success and survival outcomes of beetles across trial outcomes. The duration of each trial measured in days is found under "Study Length". The chamber type used is found under "Type". The total number of beetles placed in each chamber under "n", the number of unique individuals scanned under "Detection", the number of "individuals remaining", and the percentage of beetles that survived under "Survival". "NA" was used for missing or unavailable data)

Trial	Study Length	Type	n	Detection	Individuals remaining	Survival (%)
Trial 1	86 days	T1	12	0	0	0
		T2	14	5	4	29
		T3	9	0	0	0
		T4	16	3	3	19
		Control 1	15	NA	10	67
		Control 2 & 3*	30	NA	14	47
		Freezer Control	12	NA	7	58
Trial 2	150 days	T1	15	11	1	7
		T2	15	10	1	7
		T3	15	2	0	0
		T4	15	11	0	0
		Control	10	NA	4	40
Trial 3	50 days	T1	15	11	5	33
		T2	15	8	2	13

		T4	15	9	2	13
		Control	15	NA	13	87
Trial 4	50 days	T1	11	1	1	9
		T3	11	0	5	45
		T4	9	0	1	9
		Control 1	11	NA	4	36
		Control 2	10	NA	0	0
		Control 3	10	NA	0	0

*Due to an oversight in keeping track in matching labels to each chamber, survival outcomes were pooled at the end of the trial

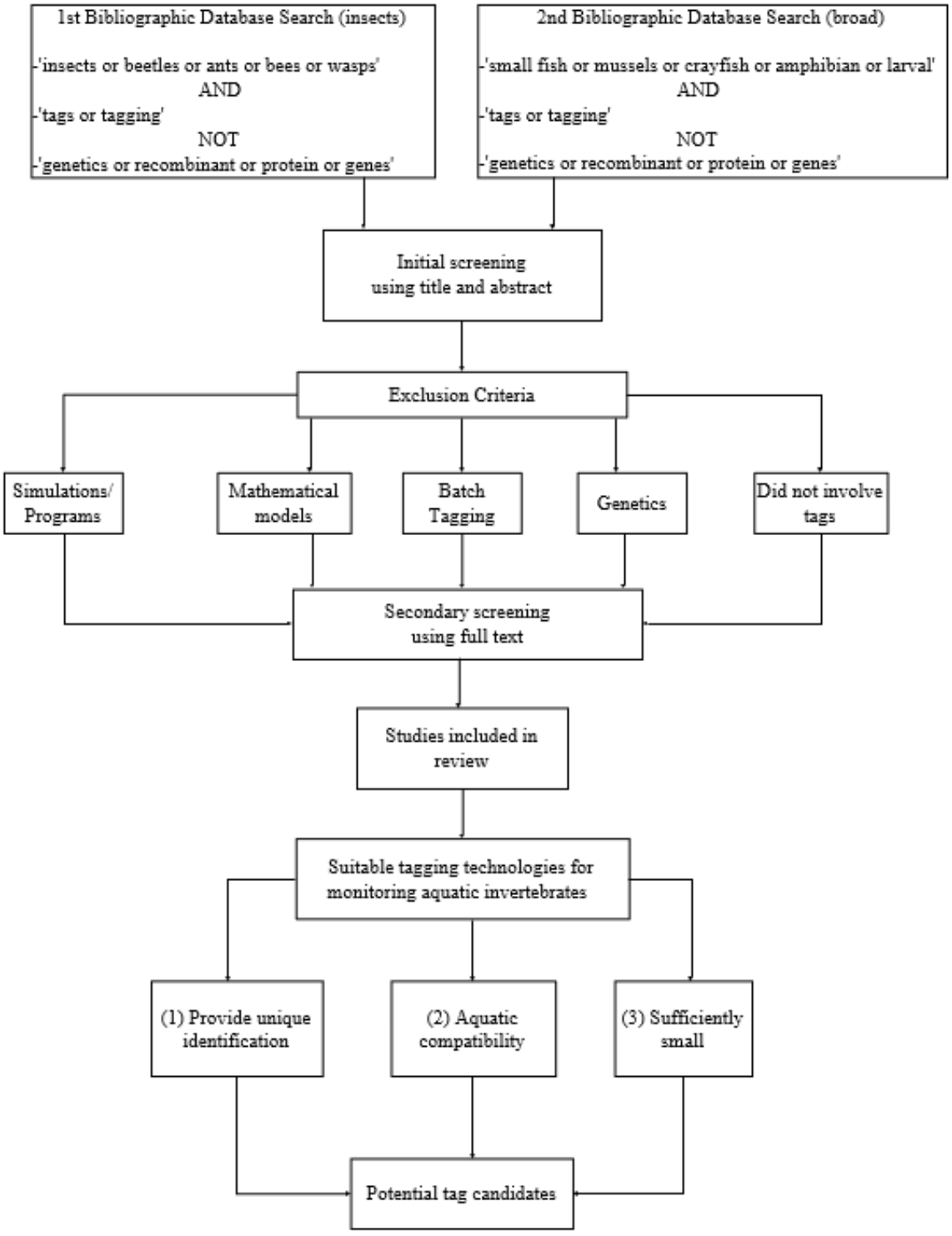

Figure 1. Flow chart describing the systematic review process for identifying potential tags suitable for monitoring freshwater invertebrates. This review was constructed using two bibliographic searches: one focused on insects and another targeted a broader range of taxa.

Studies were refined through two levels of screening (primary and secondary) with exclusion criteria applied to narrow the literature. After screening, I pooled study results and established tag requirements. Tags that met all three requirements were classified as potential candidates.

Alternative Text: Flowchart of a systematic literature review process showing two database searches, screening steps, exclusion criteria, and final selection of tagging technologies based on identification, aquatic compatibility, and size requirements

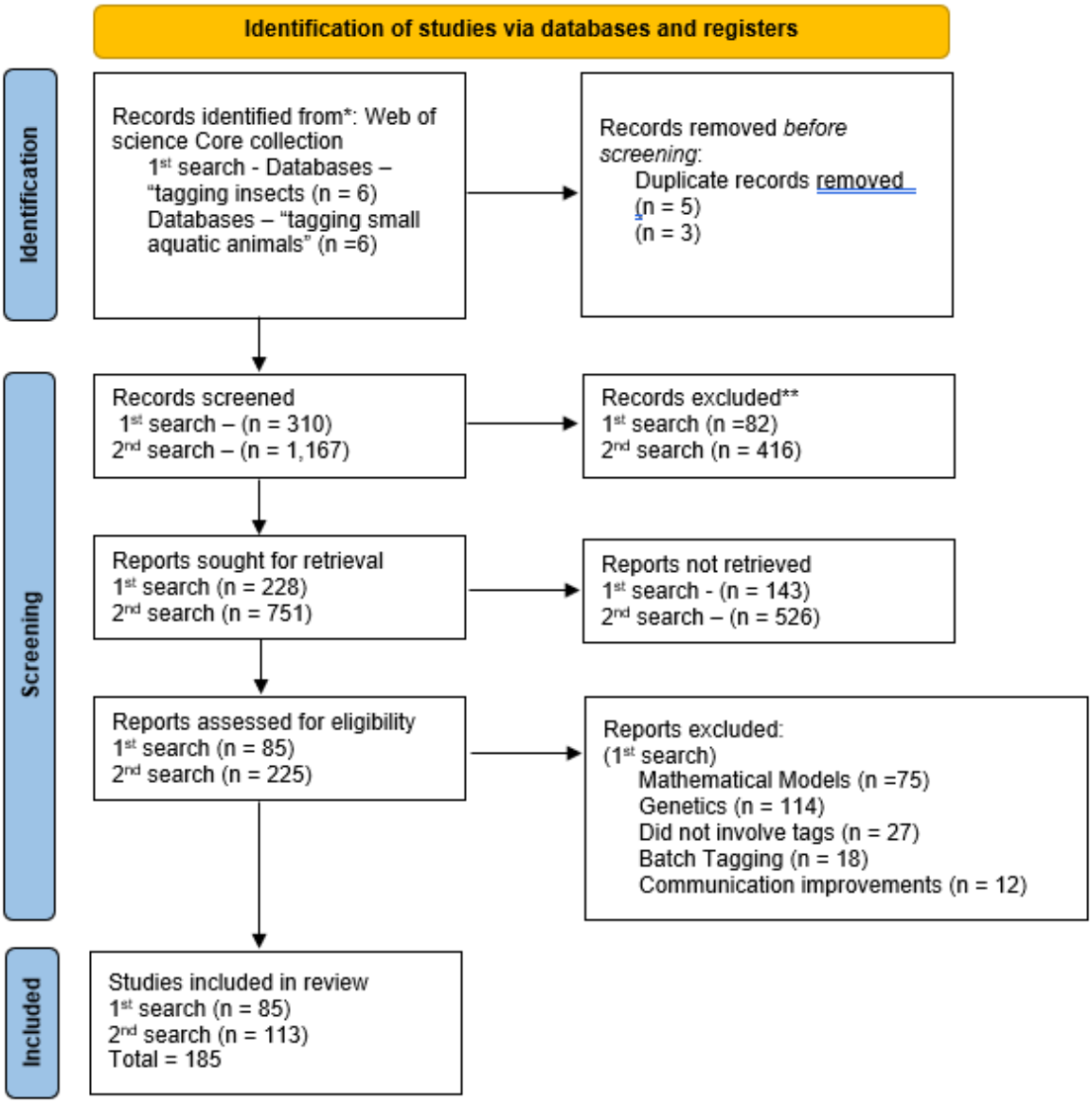

Figure 2. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). Records were identified from two Web of Science searches focusing on insects and small aquatic animals. After removing duplicates, studies were screened by title and abstract, with many excluded. A total of 185 studies were ultimately included in the review."

Alternative text: PRISMA-style flow diagram showing study identification, screening, exclusion, and final inclusion, resulting in 185 studies included in the review

Figure 3: p-Chip scanner in between each of the control chambers which was used to continuously detect passing tagged individuals as they passed through each of the chambers.

Alternative Text: Close-up image of an experimental flow-through chamber system showing a handheld laser scanner positioned over a transparent central tube connecting two PVC chambers. The scanner is mounted above the tube to read p-Chip tags as organisms pass through the detection zone

Figure 4: Treatment chambers where tagged beetles were housed during experiments evaluating tag retention and survival of tagged Comal Spring riffle beetles. The system allows organisms to move between chambers, with the central section serving as the detection zone for tag scanning.
Alternative Text: Image of a dual-chamber setup consisting of two PVC flow-through tubes connected by a central transparent section.

Figure 4: Control chamber where untagged beetles will be placed during trials. The chamber design allows for a 360^0 view for an accessible view of beetles when inside. The lid can be easily removed to further visualize beetles from above.

Alternative Text: Clear plastic control chamber with a lid, fitted with inlet and outlet ports on opposite sides. The transparent design allows direct visual observation of organisms during the experiment.

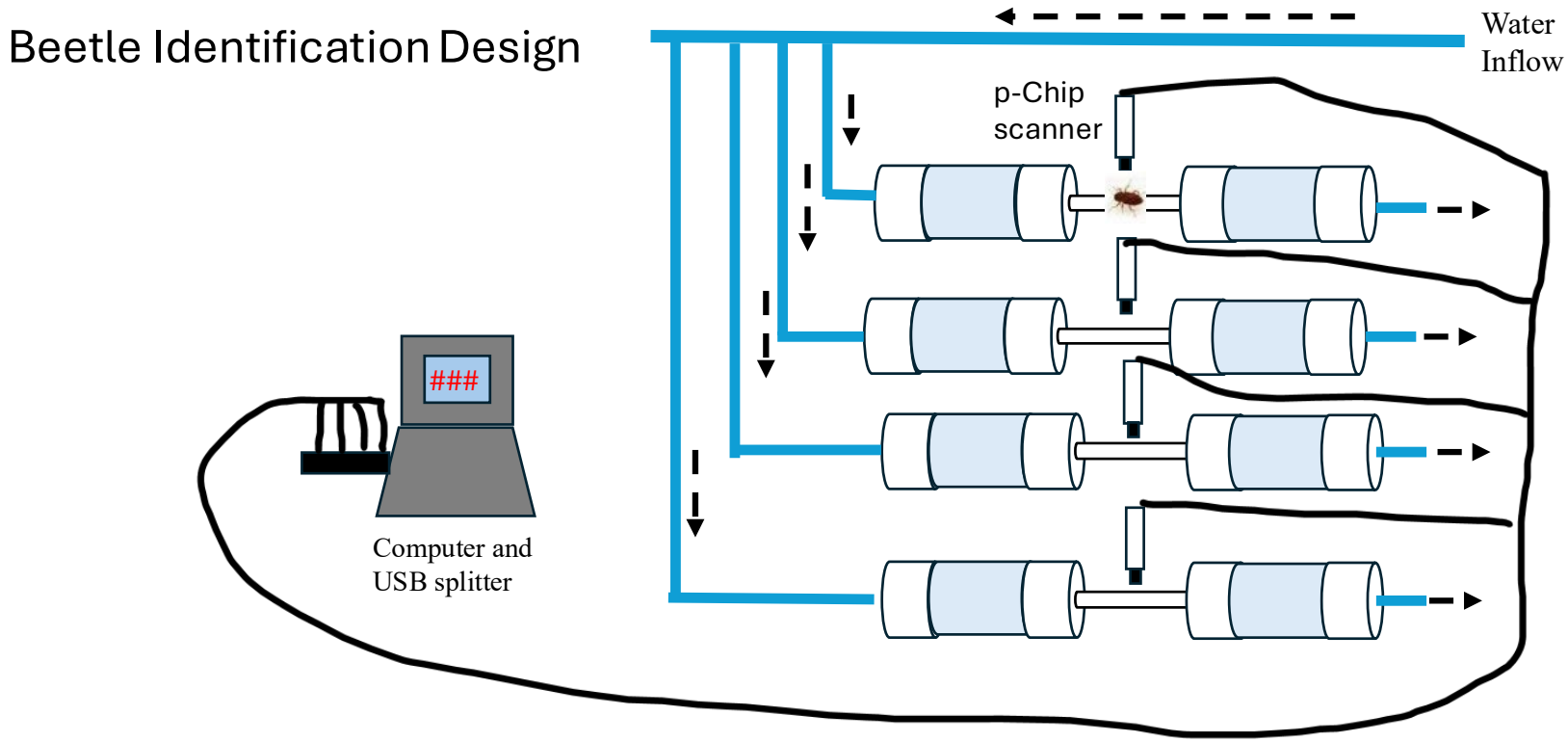

Figure 5. Visual diagram for my beetle identification system, displays how tagged beetles are connected by water inflow and connected via scanners. Water inflow is distributed to each chamber, and beetles move between paired chambers through a central tube where a p-Chip scanner detects tagged individuals. The system is connected to a computer for data recording.

Alternative text: Schematic diagram of a beetle identification system showing multiple paired flow-through chambers connected by tubing.

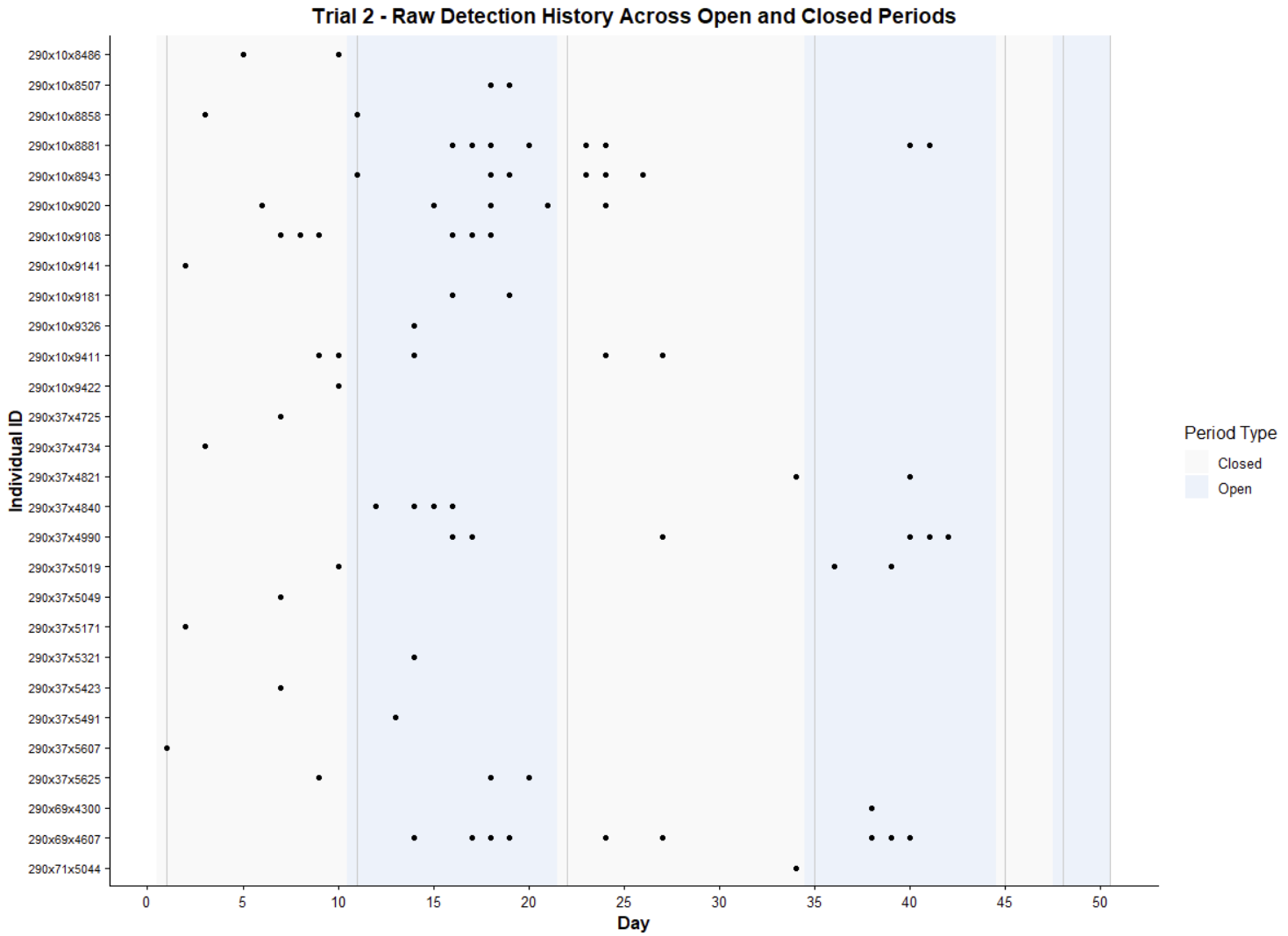

Figure 6. Detection history of individual beetles during Trial 2 over a 50-day period. Each point represents a detection event for a given individual per day. Shaded vertical bands indicate alternating closed and open periods.

Alternative Text: Scatterplot showing detection histories of multiple individual beetles across 50 days. The x-axis represents time (days 0-50), and the y-axis lists individual beetle IDs. Each black dot indicates a detection event for a specific beetle on a given day. The background is divided into alternating vertical bands representing closed periods (light gray) and open periods (light blue). Detection events are distributed unevenly across individuals and time, with some beetles detected frequently and others only a few times. Most detections cluster around certain time intervals, and other individuals show spare or no detections in later periods.

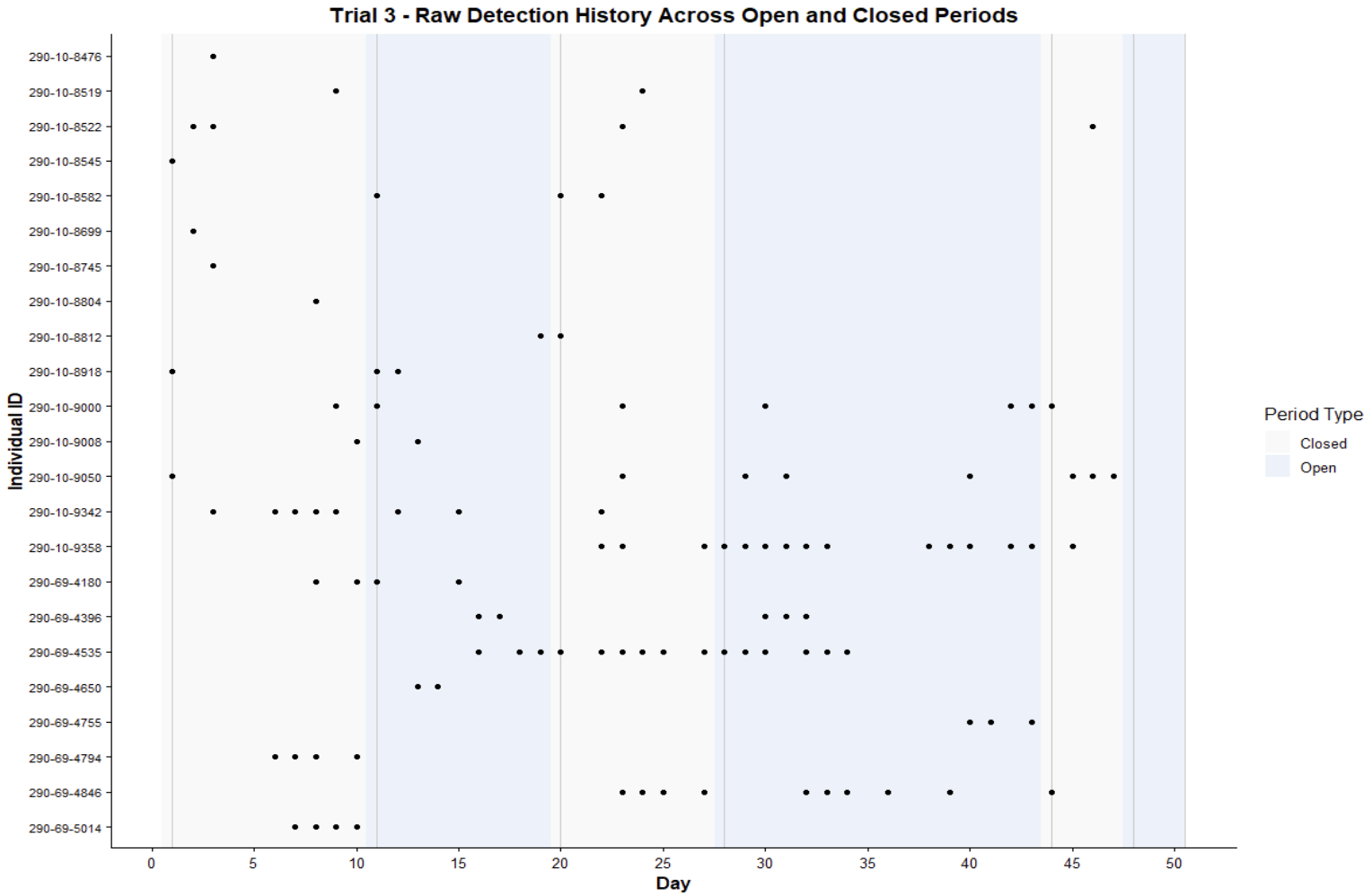

Figure 7. Detection history for Trial 3 detailing open and closed periods for detected beetles over a period of 50 days. The detection matrix is divided into bins with detection frequency of individual beetles at each bin.

Alternative Text: Scatterplot showing detection histories of multiple individual beetles across 50 days. The x-axis represents time (days 0-50), and the y-axis lists individual beetle IDs. Each black dot indicates a detection event for a specific beetle on a given day.

The background is divided into alternating vertical bands representing closed periods (light gray) and open periods (light blue). Detection events are distributed unevenly across individuals and time, with some beetles detected frequently and others only a few times. Most detections cluster around certain time intervals, and other individuals show spare or no detections in later periods.

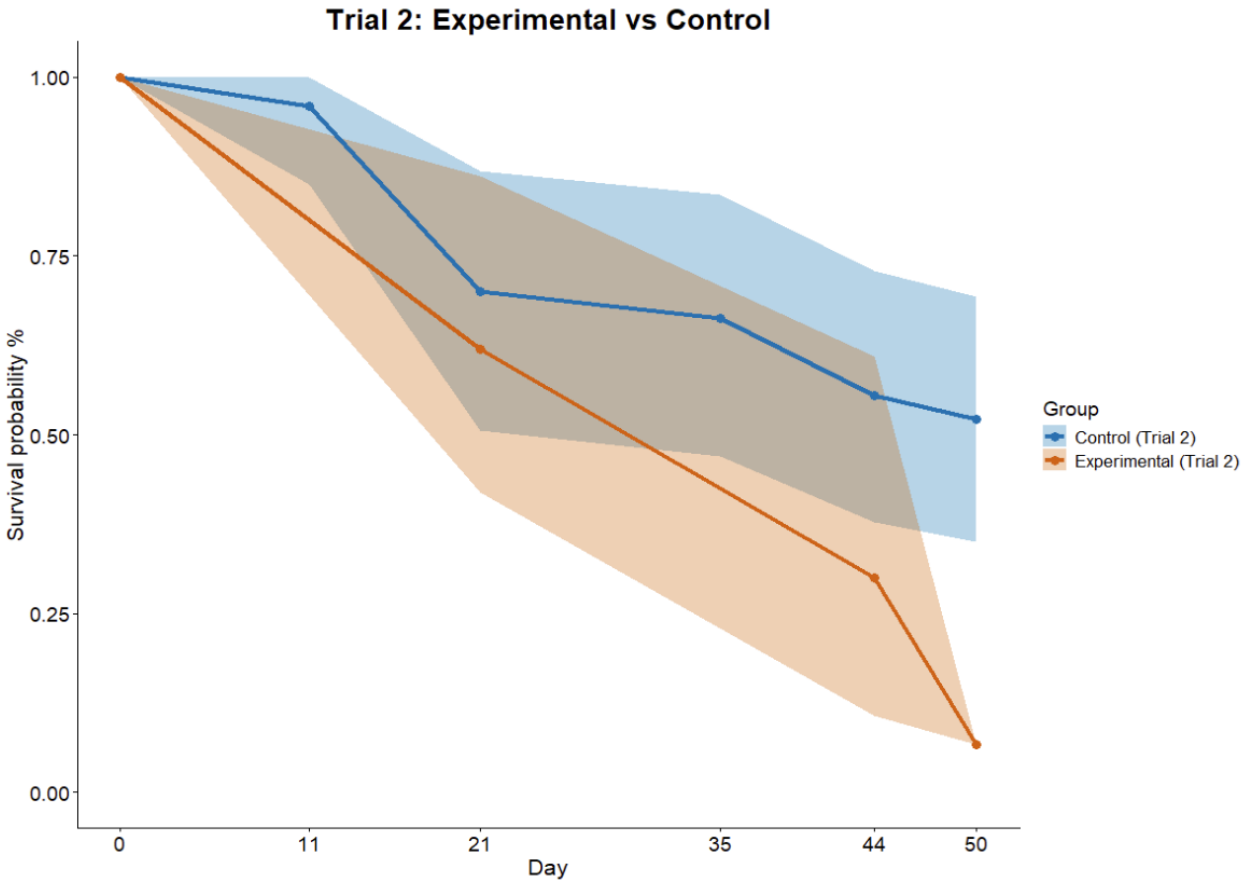

Figure 8. Comparison curves between experimentally tagged beetles and untagged control chamber survival outcomes for Trial 2. Both curves were constructed with a similar Bayesian framework to facilitate comparison. Control group curves were constructed using a Piecewise constant hazard model and experimental curves were created using a multistate hidden Markov model.

Alternative Text: Line graph showing survival probability over time for control and experimental groups in Trial 2. Both groups begin with similar survival, with moderate overlap in uncertainty intervals during early and middle time periods. Over time, the experimental group shows a moderate decline in survival compared to the control, with increasing separation between groups toward the end of the study.

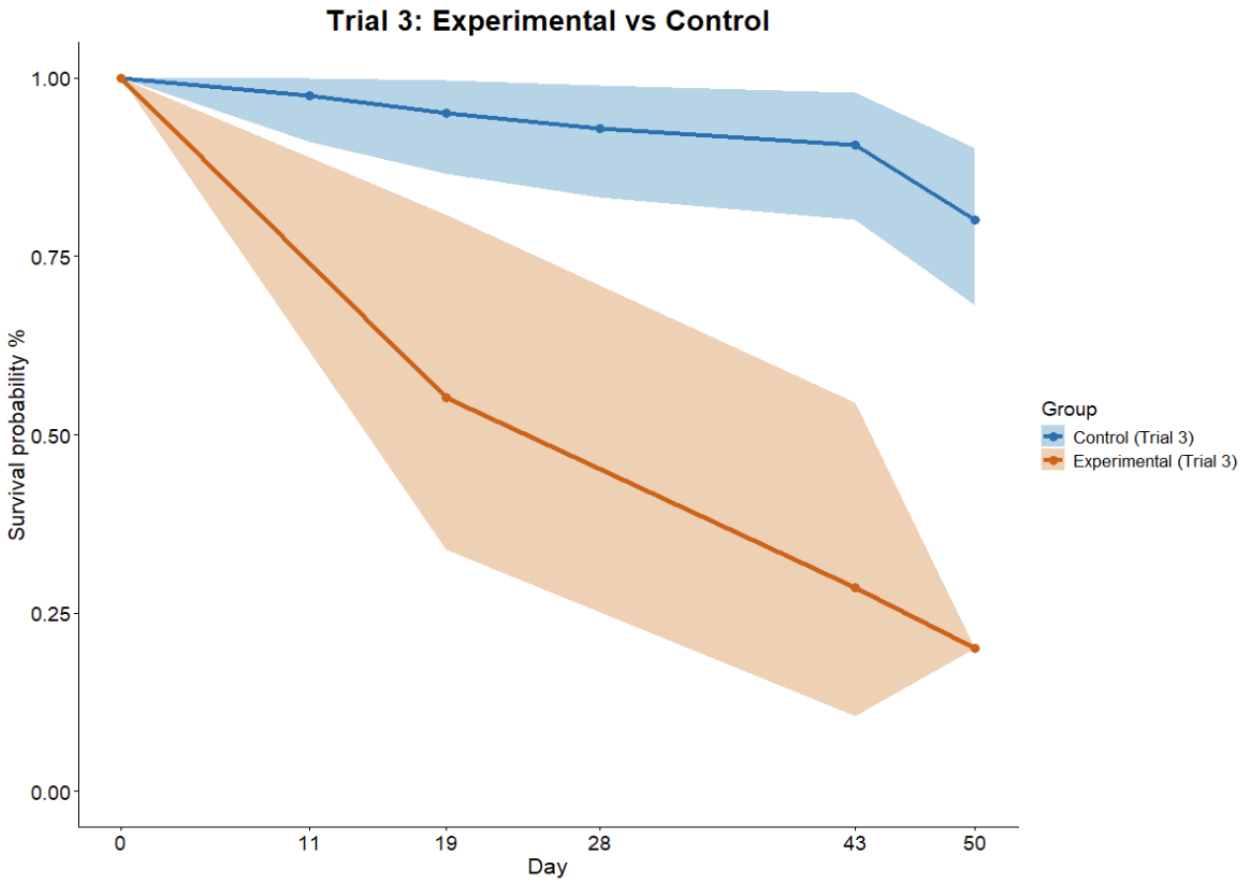

Figure 9. Comparison curves between experimentally tagged beetles and untagged control chamber survival outcomes for Trial 3. Both curves were constructed with a similar Bayesian framework to facilitate comparison. Control group curves were constructed using a Piecewise constant hazard model and experimental curves were created using a multistate hidden Markov model.

Alternative Text: Line graph showing survival probability over time for control and experimental groups in Trial 3. The control group maintains relatively high survival throughout the study, while the experimental group shows a consistent decline beginning early. The two groups display clear and increasing separation over time, with minimal overlap in uncertainty intervals in later stages.

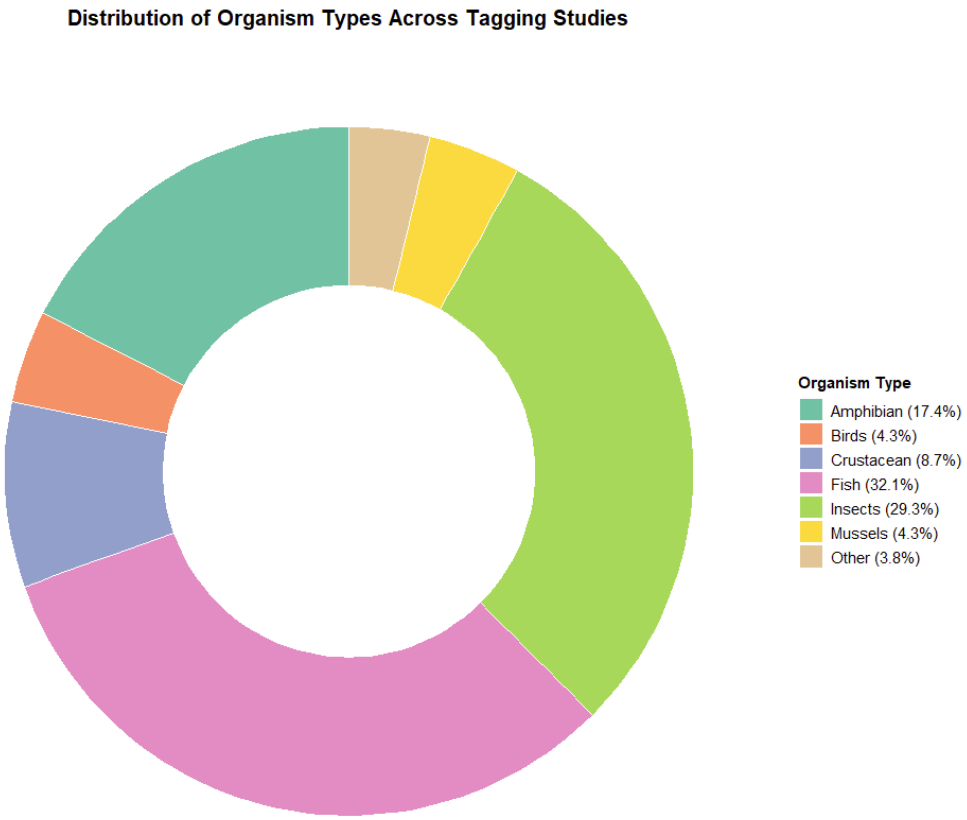

Figure 11. Donut chart showing the distribution of organism types across tagging studies. Fish and insects represent the largest proportions, each accounting for roughly 30 percent of studies. Amphibians make up about 17 percent, while crustaceans account for around 9 percent. Birds, mussels, and other groups each represent small proportions of approximately 4 percent or less. Overall, the chart indicates a taxonomic bias toward fish and insect studies

Alternative Text: Donut chart showing organism types in tagging studies, with fish and insects dominating, followed by amphibians and crustaceans, and smaller contributions from birds, mussels, and other groups.

Appendix A

Table A1. Components needed to make water soluble glue (CMC glue) for attaching a tag to a stick for later placement on the organism. The glue is designed to come off the tag when in contact with water

Chemical/ equipment	Description	Dimensions	Amount needed	Purchased	Cost (USD)
CMC[1]	Chemical component	N/A	2.5 g	Millipore sigma	73.20 (1x 100g)
Water[2]	Chemical component	N/A	48 ml	Thermo Fisher Scientific	118.00 (1x 1000ml)
Glycerol[3]	Chemical Component	N/A	2 ml	Millipore sigma	49.50 (1x 500ml)
Beaker	Container used for mixing components	250 mL	1	N/A	N/A
Centrifuge tube	Stores CMC glue after mixing	50 mL	1	N/A	N/A
Graduated cylinder	Used for measuring water	N/A	1	N/A	N/A
Digital hot plate/stirrer	Used for stirring mixture	N/A	1	N/A	N/A
Spatula	Measuring and breaking up clumps	N/A	1	N/A	N/A

Magnetic stir bar	Mixes components	NA	1	N/A	N/A
Weigh boat	For separating components into parts	N/A	8	N/A	N/A
Mixer/tube roller[4]	Roller used for continual mixing of glue	NA	NA	BT Lab Systems	666.00

1. CMC - Sodium carboxymethyl cellulose, Cat #419303-100G
2. Water – Invitrogen nuclease free water, Cat #AM9932
3. Glycerol – Cat #G7757-500ML
4. Mixer, tube roller, Cat #BT914

Procedure for making CMC adhesive

1. Ensure that the beaker, spatula, and stir bars to be used are sterilized before use. Make sure to wear gloves when preparing adhesive or handling reagents and glassware.
2. Using a 50ml graduated cylinder measure 48 ml of water and pour into the 250 mL beaker, containing a magnetic stir bar.
3. Set the stirrer to 450 rpm.
4. Divide the total CMC quantity (2.5g) to 8 parts, each of 0.3125g
5. Measure 8 near parts of 0.3125g in 8 separate weigh boats
6. Add each part slowly into the beaker at equal time intervals of 7.5 minutes.
7. Monitor the solution for clumps and use a spatula to dissolve the clumps during the above step.
8. Add 2ml of glycerol into the beaker and keep stirring the solution for an additional 20 minutes.
9. Pour the contents of the beaker into a 50ml centrifuge tube and rotate the tube on the roller mixer at 20rpm for 24hr, before moving at 4C for long term storage.
10. The solution at this stage is ready to be used.

Appendix B

Posterior survival curves were obtained by transforming posterior hazard samples into survival probabilities using:

$$H(t) = \sum_{k=1}^{t} h_k \cdot \Delta t_k$$

where:

- h_k = hazard in interval k
- Δt_k = length of interval k
- $H(t) =$ cumulative hazard

Appendix C

The BEhavioral Ecology tag (BEEtag) is an open source, image-based tracking system used in MATLAB (Matrix Laboratory, MathWorks) that allows unique identification of printed binary tags (Crall et al. 2015). Each tag consists of a 5x5 matrix of black and white pixels, forming a unique visual code that can be decoded from digital images. Tags can be scaled to different sizes and are printed on lightweight materials, making them a potential alternative to microtransponder tags for small aquatic invertebrates. I evaluated the use of BEEtags for tagging Comal Springs Riffle Beetles by modifying tag size to meet the small body size of the species. I printed tags on waterproof tear resistant synthetic paper (Duracopy Waterproof Printer Sheets, JL Darling) using a high resolution (1200 dpi) laser printer. Each sheet contained approximately 100 unique tags, drawn from a larger set of ≈1,800 possible codes. Initial tags were printed following the original design, after which tag size was reduced (≈1.2 mm) to better match beetle dimensions (see below).

To further improve resolution at smaller sizes, I reconstructed BEEtags manually at the pixel level using Piskel (https://www.piskelapp.com). Tags were recreated by copying known binary patterns into a high-resolution pixel grid, exported as PNG files, and printed at reduced

scale. Individual tags were then cut under a dissecting microscope using a razor blade. Tag identification was performed in MATLAB using the BEEtag software. Images were converted to binary format, and the software identified candidate tag regions based on shape and contrast. Pixel patterns within each detected region were then matched to a library of valid tag codes, allowing individual identification.

Evaluation of BEEtags was conducted over a 50-day period alongside Trial 3 of the p-Chip study. Survival of tagged beetles was low: only 2 of 10 tagged individuals remained alive at the end of the experiment. Similarly, only 3 of 15 untagged control beetles survived, indicating potential tag-related effects. Mortality among tagged individuals was evident within the first week, and tag loss was observed in multiple individuals during early observations.

I hypothesize that tag size (≈1.2 mm) contributed to reduced survival due to increased hydrodynamic drag and interference with movement in flowing water. Tag geometry (e.g., square edges) may have further exacerbated these effects. Reducing tag size (≈0.7 mm) and modifying shape (e.g., rounded edges) may improve performance, although smaller tags may reduce detection reliability. To address this, I performed a validation test to assess identification success at smaller tag sizes and confirmed no significant reduction in accuracy. Overall, these findings indicate that BEEtags can effectively mark individual aquatic macroinvertebrates, and offer benefits in cost, scalability, and a lightweight design. However, their application to small aquatic beetles may be limited by physical and hydrodynamic constraints. Further optimization of tag dimensions, and continued assessment of survival in laboratory settings will be required before implementation in future studies.

Figure C1. An example of a riffle beetle with a BEEtag attached

Alternative text: Image of a riffle beetle tagged with a paper QR code based tag.