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REGULAR ARTICLE

EVALUATION OF COSTS ASSOCIATED WITH EXTERNALLY AFFIXING PIT TAGS TO FRESHWATER MUSSELS USING THREE COMMONLY EMPLOYED ADHESIVES

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ABSTRACT

Despite the increasing use of passive integrated transponder (PIT) tags in freshwater mussel research and conservation, there has been no evaluation of the trade-offs in cost and effort between commonly used adhesive types. These factors could be important to consider if tag retention rates do not vary by adhesive, the effects of handling are large, or resources are limited. We modeled and evaluated how material costs and effort function over a range of sample sizes by using field data from the relocation of 3,749 PIT-tagged Clubshell (*Pleurobema clava*) and Northern Riffleshell (*Epioblasma rangiana*) in Illinois, 261 Eastern Elliptio (*Elliptio complanata*) in Maryland, and the release of 99 Cumberland Combshell (*Epioblasma brevidens*) in Virginia. Each study used externally affixed 12.5-mm, 134.2-kHz PIT tags, but used a different adhesive to encapsulate tags (Illinois, underwater epoxy resin; Maryland, surface-insensitive gel cyanoacrylate; and Virginia, dental cement). We determined the total cost-per-tag-effort (CPTE) after parameterizing cost, quantity required, application time, and time for each adhesive. After accounting for standardized costs of staff time and adhesive, cyanoacrylate was the least costly adhesive to affix, encapsulate, and cure PIT tags on a per mussel basis. Differences in CPTE were small when the number of mussels tagged was low, but they increased by US$2–6 mussel^{-1}. A primary goal in mussel projects is reduced stress from aerial exposure. Using underwater epoxy, which requires time above water to cure, can negate this goal and increase costs as it requires more handling effort than cyanoacrylate or dental cement. Nevertheless, more resource-intensive adhesives may still be an appropriate choice when the number of study animals is low. Further study is warranted to understand how our model may vary by adhesive brand, application rate, staffing level, and environmental factors.

KEY WORDS: relocation, translocation, tagging, mark–recapture, monitoring, sensors

INTRODUCTION

Relocation and reintroduction is a common conservation strategy to address the national decline in populations of freshwater mussels (Haag and Williams 2014; FMCS 2016). Understanding survival and demographic rates of mussel populations is imperative to assess conservation and management actions, which necessitates tracking a sufficient number of individual animals or cohorts over time. Studies that seek to monitor and assess the success of freshwater mussel conservation actions (e.g., translocation, relocation, and reintroduction) typically use sampling designs that require individually marked animals (e.g., capture–recapture, Villela

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et al. 2004). The resulting models of demographics and vital rates are based on the probability of detecting a marked animal in subsequent surveys (Burnham et al. 1987). Although mostly sessile, mussels exhibit imperfect detection that can vary by species, size, environmental factors, sampling design, survey method, and observer (Metcalfe-Smith et al. 2000; Meador et al. 2011; Stodola et al. 2017). Consequently, evaluating mussel conservation actions has been hampered by low rates of recapture (Cope and Waller 1995; Cope et al. 2003), leaving the fate of many mussels unknown. An inability to recapture a sufficient number of marked animals may cause data to be deficient, imprecise, or possibly even biased and has implications for conservation (Wisniewski et al. 2013; Hua et al. 2015).

Passive integrated transponder (PIT) tags are relatively inexpensive means of uniquely marking animals that has been widely used to track populations of large and small terrestrial vertebrates (Gibbons and Andrews 2004). As PIT tag technology has advanced, the reduced size of microchips and waterproof tag readers have allowed them to be used with small-bodied aquatic vertebrates and invertebrates, including fishes (Roussel et al. 2000; Cooke et al. 2011; Pennock et al. 2016), crayfishes (Black et al. 2010), and bivalve mollusks (Kurth et al. 2007; Hamilton and Connel 2009; Hale et al. 2012). More recently, this technology has been used to study freshwater mussel movement and behavior (Peck et al. 2007; Gough et al. 2012; Newton et al. 2015) and the survival of released endangered species (wild, Fernandez 2013; hatchery produced, Hua et al. 2015). In the first evaluation of PIT tag use for mussel translocation monitoring, Kurth et al. (2007) observed recapture rates were twice as high as rates observed using visual surveys. Hua et al. (2015) found near complete detection of hatchery-stocked mussels during seven monitoring events over a 2-yr period. Tiemann et al. (2016) recovered 83% of PIT-tagged mussels during 17 monitoring events over 3 yr following a short-distance relocation.

The PIT tags are located subcutaneously in vertebrates and larger invertebrates because their body mass is large relative to the tag size. Internal insertion is generally avoided for freshwater mussels in favor of external affixation because it can result in premature tag rejection or animal mortality (Kurth et al. 2007). Although mussels have been tagged internally (e.g., Layzer and Heinricher 2004), external placement of shellfish tags is the predominant method used to mark mussels in capture–recapture studies (Lemarie et al. 2000; Villela et al. 2004), especially when using PIT tags (Kurth et al. 2007; Peck et al. 2007) and sensors (Hauser 2015; Hartman et al. 2016a, 2016b). Cyanoacrylate and epoxy resin adhesives have been primarily used to externally affix PIT tags to mussel shells, and they have variable curing times, costs, and chemical compositions, in addition to bond strength and longevity. These types of adhesives have shown low rates of mortality and high rates of PIT tag retention in laboratory and in situ settings (Young and Isley 2008). A third, less commonly used adhesive (dental cement) has shown similar performance (Kurth et al. 2007; Hua et al. 2015).

Despite their rapidly increasing use in mussel research and conservation, there has been just a few studies on the effects of external adhesion on mussel behavior, movement, growth, and survival (e.g., Wilson et al. 2011; Peck et al. 2014; Hartmann et al. 2016a; Hua et al. 2016). Furthermore, there has been no evaluation of the trade-offs in material cost and effort (i.e., application and curing time) between the three most widely implemented adhesive types. These could be important factors to consider when developing a conservation plan or ecological study that incorporates PIT tags if the effects of handling or transportation may already be large or if resources are limited. Our objective was to model and evaluate how these factors function over a range of tagging sample sizes for epoxy resin, cyanoacrylate, and dental cement adhesives.

METHODS

We used data from three case studies that represent field applications of externally affixed PIT tags by using three adhesive types with four freshwater mussel species that have been monitored for $\geq$2 yr.

Illinois Case Study

Natural resource agencies in Illinois PIT tagged 1,766 Clubshell (*Pleurobema clava*) and 1,983 Northern Riffleshell (*Epioblasma rangiana*) translocated from the Allegheny River beneath the existing U.S. Highway 62 Bridge, Forest County, Pennsylvania, between 2012 and 2014. Clubshell ranged in length from 23 to 62 mm ($\mu = 45.2$ mm), whereas Northern Riffleshell varied from 26 to 78 mm ($\mu = 53.1$ mm). Mussels were shipped in coolers from Pennsylvania to Illinois ($\sim$10 h out of water) and then placed in quarantine holding tanks at the Illinois Natural History Survey Aquatic Research Facility in Champaign-Urbana, Illinois. Each tank provided continuous ground water (temperature ranged from 20 to 22°C), lacked substrate, and was aerated using air pumps. The 2012 cohort was held in quarantine for 14 d, whereas the 2013 and 2014 classes were quarantined for 4–5 d before being released.

While in quarantine, individual mussels were externally affixed with 12.5-mm, 134.2-kHz PIT tags (BioMark, Inc., Boise, ID) by using Devcon 11800 marine grade epoxy resin (Devcon, Danvers, MA). Batches of up to 50 individuals were scrubbed to removed debris (e.g., algae and caddisfly cases), towel dried, and affixed with a PIT tag on the right valve and a uniquely numbered, vinyl shellfish tag (Hallprint, Hindmarsh Valley, South Australia) on the left valve. To affix both PIT and shellfish tags, technicians placed a small bead of cyanoacrylate to hold a tag in place; the brand of cyanoacrylate varied and no accelerant was applied to the glue (Fig. 1a). Once dried, PIT tags were completely encased in epoxy, whereas shellfish tags were encased in cyanoacrylate (Fig. 1b). Individuals were then databased (i.e., recorded species, sex, length, tag numbers, and other information) before being returned to the holding tanks. Out-of-water time averaged 30 min mussel^{-1}. Animals were held at least 24 h for the epoxy to fully cure before being hand planted at eight sites in the Vermilion River basin (Wabash River drainage).

Figure 1. Marking of Northern Riffleshell (*Epioblasma rangiana*) and Clubshell (*Pleurobema clava*) by (a) attaching passive integrated transponder (PIT) tags to shells with cyanoacrylate and (b) encapsulating PIT tags in epoxy resin; Eastern Elliptio (*Elliptio complanata*) by using cyanoacrylate by (c) attaching PIT tags to shell and (d) encapsulating the PIT tag in cyanoacrylate; and Cumberland Combshell (*Epioblasma brevidens*) by (e) attaching a PIT tag to the shell with cyanoacrylate and (f) encapsulating the PIT tag in dental cement.

Animals have since been monitored to estimate the survival and gauge the success of the project (Stodola et al. in review). Of the 3,749 animals tagged and relocated, 3,371 (90%) have been encountered at least once during subsequent recapture monitoring by using a portable submersible PIT tag antennae.

Maryland Case Study

Maryland Department of Natural Resource biologists relocated 2,345 Eastern Elliptio (*Elliptio complanata*) in 2014 from the direct and indirect impact zones of a stream bank stabilization project along Route 24 in Deer Creek, Harford County, Maryland. Particular attention was paid to the effort required to remove, process, and relocate mussels because this was the first large relocation in the state. As a result, an additional 541 mussels were collected in preremoval surveys to assess the potential effects of relocation via capture–recapture monitoring (Ashton et al. 2016). In total, 427 of the 2,866 mussels collected in the removal and preremoval surveys were externally PIT tagged. These mussels have been monitored at five relocation sites and three control sites that received no relocated mussels annually since 2014. This has resulted in an additional 149 (2015) and 112 (2016) naive (i.e., unmarked) mussels being PIT tagged. The Eastern Elliptio PIT tagged ranged in length from 19 to 86 mm ($\mu = 57.3$ mm).

Mussels collected in preremoval, removal, and monitoring surveys were held on site in flowthrough containers or aerated coolers that received frequent changes of river water before processing. After being cleaned of debris, the shell length (millimeters) of each mussel was measured, and each valve was marked with a Hallprint tag adhered using a surface-insensitive, cyanoacrylate gel. Eastern Elliptio <50 mm in shell length and every fifth naive mussel were externally affixed with a 12.5-mm, 134.2-kHz PIT tag. PIT tags were held in place on the shell in a small bead of cyanoacrylate gel (Fig. 1c). Using a separate tube of cyanoacrylate without an application tip, PIT tags were then encapsulated on all sides with additional adhesive (Fig. 1d). In 2014, PIT tags were affixed and encapsulated with LOCTITE gel control (Henkel Corp., Rocky Hill, CT). In 2015 and 2016, Turbo Fuse gel (Palm Labs Adhesives, DeBary, FL) was used to attach tags. Total time to measure and tag was maintained at 2 min mussel^{-1} to minimize aerial exposure by using one or two sprays of a cyanoacrylate curing accelerant (Turbo Set I, Palm Labs Adhesives) in all years. After processing was complete, mussels were kept in flowthrough or aerated holding containers of river water before being hand planted into the substrate. Of the 576 animals PIT tagged in 2014 and 2015, approximately 25% have been relocated through visual survey methods at least once in subsequent monitoring (M.J. Ashton et al., unpublished data).

Virginia Case Study

Ninety-nine Cumberland Combshell (*Epioblasma brevidens*) were propagated at the Freshwater Mollusk Conservation

Table 1. Comparison of adhesives to attach and encapsulate passive integrated transponder tags to freshwater mussels.

Study	Adhesive	Adhesive Type	Approximate Time to Apply (min)	Cure Time (min)	Cost (US$ g^{-1})	Adhesive (g·mussel^{-1})
Illinois	Devcon 11800	Epoxy resin	5	1,440[a]	0.14	0.72
Maryland	Palm Labs 440 Turbo Fuse Gel	Cyanoacrylate	1	1	0.35	0.54
Virginia	Fuji Glass Ionomer Luting Cement	Dental cement	1	1	2.54	0.94

[a] We estimated that 2% of the total cure time (30 min) involved costs associated with effort (e.g., transfer of mussels to holding tanks, arrangement within tank, collection for transport).

Center, Department of Fish and Wildlife Conservation, Virginia Tech in Blacksburg, Virginia. Over a 2-yr period, mussels were released from hatchery or in situ culture systems after they reached a minimum length of 20 mm into the Powell River, Claiborne County, Tennessee. Tagged Cumberland Combshell ranged in length from 17.8 to 22.9 mm ($\mu = 19.3$ mm).

While in culture, subadult Cumberland Combshell were marked with a bee tag (The Bee Works, Ontario, Canada) or vinyl shellfish tag by using cyanoacrylate. A three-step process was used to externally affix PIT tags in the field. After being cleaned and dried, PIT tags were held with LOCTITE gel control cyanoacrylate (Fig. 1e). Tags were then completely encapsulated in Fuji Glass Ionomer Luting Cement (Fig. 1f; GC Fuji Luting, Tokyo, Japan). A hypodermic needle was used to mix the dental cement powder and liquid on a manufacturer's supplied application pad and apply the mixed cement onto the PIT tag via syringe. To reduce negative effects of exposure, the PIT tagging process was conducted in the field under shade and took 2 min mussel^{-1}. Mussels were hand planted into the substrate at the monitoring site after tagging was complete. The released mussels were monitored using a portable submersible PIT tag antennae to assess individual heterogeneity of demographic rates (Hua et al. 2015). Of the 99 animals tagged and released, 97 (98%) have been encountered at least once during subsequent recapture monitoring (Hua et al. 2015).

Evaluation

We evaluated the total cost to externally affix PIT tags to freshwater mussels by parameterizing the cost (US$ g^{-1}) of each primary adhesive (A), quantity of adhesive (qA) used in each case study (g mussel^{-1}), time (min mussel^{-1}) needed to apply the adhesive and PIT tag (tA), and time (min mussel^{-1}) actively engaged with tagged mussels during the adhesive curing process (cA) (Table 1). Costs of adhesives per unit were calculated from purchase records kept in each case study. We did not include the cost of PIT tags and adhesive used to attach the tag as they were similar among studies. We also did not include adhesive use and tag application data from the 2014 portion of the Maryland case study because it was discovered that a relatively large amount of adhesive remained inside the applicator even after it appeared exhausted.

The quantity of adhesive used per mussel was determined by dividing the number of mussels tagged in each study by the quantity of adhesive consumed. We used the average hourly salary rate published by the General Services Administration's Contract-Awarded Labor Category for project scientists in the environmental services schedule with a Bachelor's or higher education level to determine a constant cost in staff time (US\$96.00 h^{-1}) to affix PIT tags (GSA 2016). Cost in time spent to cure adhesive type was calculated in the same manor, but for epoxy the time was estimated at 30 min for batches of 50 mussels instead of for an individual mussel. The parameters of cost were then totaled and extrapolated on a per mussel tagged basis (cost-per-tag-effort; CPTE in $US) for cyanoacrylate and dental cement as follows:

$$\begin{aligned}\mathrm{CPTE} = {} & [(A \times \mathrm{q}A) \times \mathrm{N_{mussels}}] \\ & + \left[\left(\$96.00 \cdot \mathrm{h}^{-1} \times (\mathrm{t}A \times \mathrm{N_{mussels}})\right]/60\,\mathrm{min}\right. \\ & + \left(\$96.00 \cdot \mathrm{h}^{-1} \times (\mathrm{c}A \times \mathrm{N_{mussels}}))\right]/60\,\mathrm{min}. \qquad (1)\end{aligned}$$

For epoxy, CPTE was calculated as follows:

$$\begin{aligned}\mathrm{CPTE} = {} & [(A \times \mathrm{q}A \times \mathrm{N_{mussels}})] \\ & + \left[(\$96.00 \cdot \mathrm{h}^{-1} \times (\mathrm{t}A \times \mathrm{N_{mussels}}\right]/60\,\mathrm{min} \\ & + \left(\$96.00 \cdot \mathrm{h}^{-1} \times (\mathrm{c}A \times \mathrm{N_{mussels}}/50))\right]/60\,\mathrm{min}. \qquad (2)\end{aligned}$$

To generate a predictive equation for the relationship between CPTE and number of mussels tagged, we constructed ordinary least squares regression models for each adhesive type by using the lmList function in R package nlme (Pinheiro et al. 2016). A linear method was chosen as opposed to fitting the extrapolated parameter values against other distributions because parameters of CPTE increase at a constant rate mussel per mussel (equation 1) or batch per batch (equation 2). We used the lm method of the geom_smooth function in R package ggplot 2 (Wickham 2009) to visualize these relationships.

RESULTS

The PIT tagging of 3,749 Clubshell and Northern Riffleshell consumed approximately six 454-g epoxy adhesives over the 3-yr period. Tagging of 149 Eastern Elliptio in 2015 and 112 individuals in 2016 consumed four and three 20-g cyanoacrylate adhesives, respectively. Three 35-g dental cement adhesives were used to tag 99 Cumberlandian Combshell in 2009 and 2010. The quantity of adhesive used

Table 2. Costs of materials and effort incurred during the adhesion and curing of passive integrated transponder (PIT) tags to freshwater mussels per mussel and extrapolated per 100 individuals by adhesive type.[a]

No. Mussels Tagged	Dental cement (US$)				Cyanoacrylate (US$)				Epoxy (US$)			
	Adhesive (q*A*)	Application (t*A*)	Cure (c*A*)	Cost (CPTE)	Adhesive (q*A*)	Application (t*A*)	Cure (c*A*)	Cost (CPTE)	Adhesive (q*A*)	Application (t*A*)	Cure (c*A*)	Cost (CPTE)
1	2.40	1.60	1.60	5.60	0.22	1.60	1.60	3.42	0.10	8.00	48.00	56.10
100	239.76	160.00	160.00	559.76	22.46	160.00	160.00	342.46	10.30	800.00	96.00	906.30
200	479.51	320.00	320.00	1,119.51	44.92	320.00	320.00	684.92	20.60	1,600.00	192.00	1,812.60
300	719.27	480.00	480.00	1,679.27	67.38	480.00	480.00	1,027.38	30.90	2,400.00	288.00	2,718.90
400	959.02	640.00	640.00	2,239.02	89.84	640.00	640.00	1,369.94	41.19	3,200.00	384.00	3,625.19
500	1,198.78	800.00	800.00	2,798.78	112.31	800.00	800.00	1,712.31	51.49	4,000.00	480.00	4,531.49
600	1,438.53	960.00	960.00	3,358.53	134.77	960.00	960.00	2,054.77	61.79	4,800.00	576.00	5,437.79
700	1,678.29	1,120.00	1,120.00	3,918.29	157.23	1,120.00	1,120.00	2,397.23	72.09	5,600.00	672.00	6,344.09
800	1,918.04	1,280.00	1,280.00	4,478.04	179.69	1,280.00	1,280.00	2,739.69	82.39	6,400.00	768.00	7,250.39
900	2,157.80	1,440.00	1,440.00	5,037.80	202.15	1,440.00	1,440.00	3,082.15	92.69	7,200.00	864.00	8,156.69
1,000	2,397.55	1,600.00	1,600.00	5,597.55	224.61	1,600.00	1,600.00	3,424.61	102.99	8,000.00	960.00	9,062.99

[a] q*A*, quantity of adhesive used in each case study (g mussel^{-1}); t*A*, time (min mussel^{-1}) needed to apply the adhesive and PIT tag; c*A*, time (min mussel^{-1}) actively engaged with tagged mussels during the adhesive curing process; CPTE, cost-per-tag-effort.

to PIT tag these mussels was similar across years by adhesive type.

Parameters of adhesive consumption, application, and curing effort varied by adhesive type (Table 1). Cyanoacrylate required 24% less adhesive to affix a PIT tag to an individual mussel than the epoxy and 43% less than dental cement. In contrast, epoxy was 2.5 times less costly per gram than cyanoacrylate and 18 times less costly than dental cement. Epoxy required 5 times more effort to apply and encapsulate a PIT tag than both dental cement and cyanoacrylate. Total cure time for epoxy was considerably greater than other adhesives, yet little of this time was spent handling mussels. Consequently, less effort associated with the process of adhesive curing accumulated as more mussels were tagged with epoxy than with cyanoacrylate and dental cement by handling mussels in batches of 50 (e.g., 100 mussels cured in 60 min vs. 60 mussels in 60 min).

Linear models of total cost (US$) per PIT-tagged mussel based on our cost and consumption parameters illustrated that cyanoacrylate ($\text{CPTE} = \$3.42 \times N_{\text{mussels}} - 1.23^{-10}$) was less costly than dental cement ($\text{CPTE} = \$5.60 \times N_{\text{mussels}} - 2.52^{-13}$) or epoxy ($\text{CPTE} = \$9.04 \times N_{\text{mussels}} + \$14.96$) (Table 2 and Fig. 2a). Costs associated with adhesive consumption increased at a greater rate for dental cement and cyanoacrylate than epoxy (Fig. 2b). The rate at which CPTE increased as the number of mussels tagged increased was higher for epoxy than cyanoacrylate and dental cement due to higher costs associated with adhesive application effort (Fig. 2c). An initial investment of effort to cure the first batch of 50 mussels led to higher upfront costs (i.e., larger y-intercept) for epoxy, but ultimately resulted in lower costs in comparison with cyanoacrylate and dental cement as the number of mussels tagged increased (Fig. 2d).

DISCUSSION

External attachment of PIT tags is a marking technique that can increase detection rates of freshwater mussels (Kurth et al. 2007) and improve the accuracy of survival and demographic rates (Hua et al. 2015; Tiemann et al. 2016). For this reason, PIT tags seem especially suited for use in mussel relocation and conservation monitoring due to historically low recapture rates (Cope et al. 1995, 2003). A primary goal in studies that employ recapture sampling is reduced stress from handling, especially out of water time (Dunn et al. 2000). Aerial exposure to apply and adhere tags to freshwater mussels by using cyanoacrylate was generally <15 min mussel^{-1} (Lemarie et al. 2000; Villella et al. 2004), yet this can be reduced to 2 min mussel^{-1} by using a curing accelerant. Dental cement has a similar curing time. Using underwater epoxy to affix PIT tags can negate the reduced handling time goal as it requires more handling and total curing time than cyanoacrylate (Table 1 and Fig. 2c).

In this evaluation of the materials and staff time needed to affix and encapsulate PIT tags to freshwater mussels from three studies, cyanoacrylate was overall less costly than dental cement and epoxy on a per mussel basis. Absolute differences in total cost compared to cyanoacrylate are relatively small when the number of mussels tagged is low, but they increased by more than $2 mussel^{-1} for dental cement and almost $6 mussel^{-1} for epoxy. We suggest that dental cement and waterproof epoxy resin may be an appropriate choice of adhesive for transmitters when the number of study animals is low. In this scenario, differences in costs among adhesive types will be negligible, and dental cement or epoxy may be better suited to protect PIT tags from damage should even minimal tag loss affect the statistical power to detect a change in population size or condition. A quicker, more controlled method of applying epoxy warrants investigation as the effort

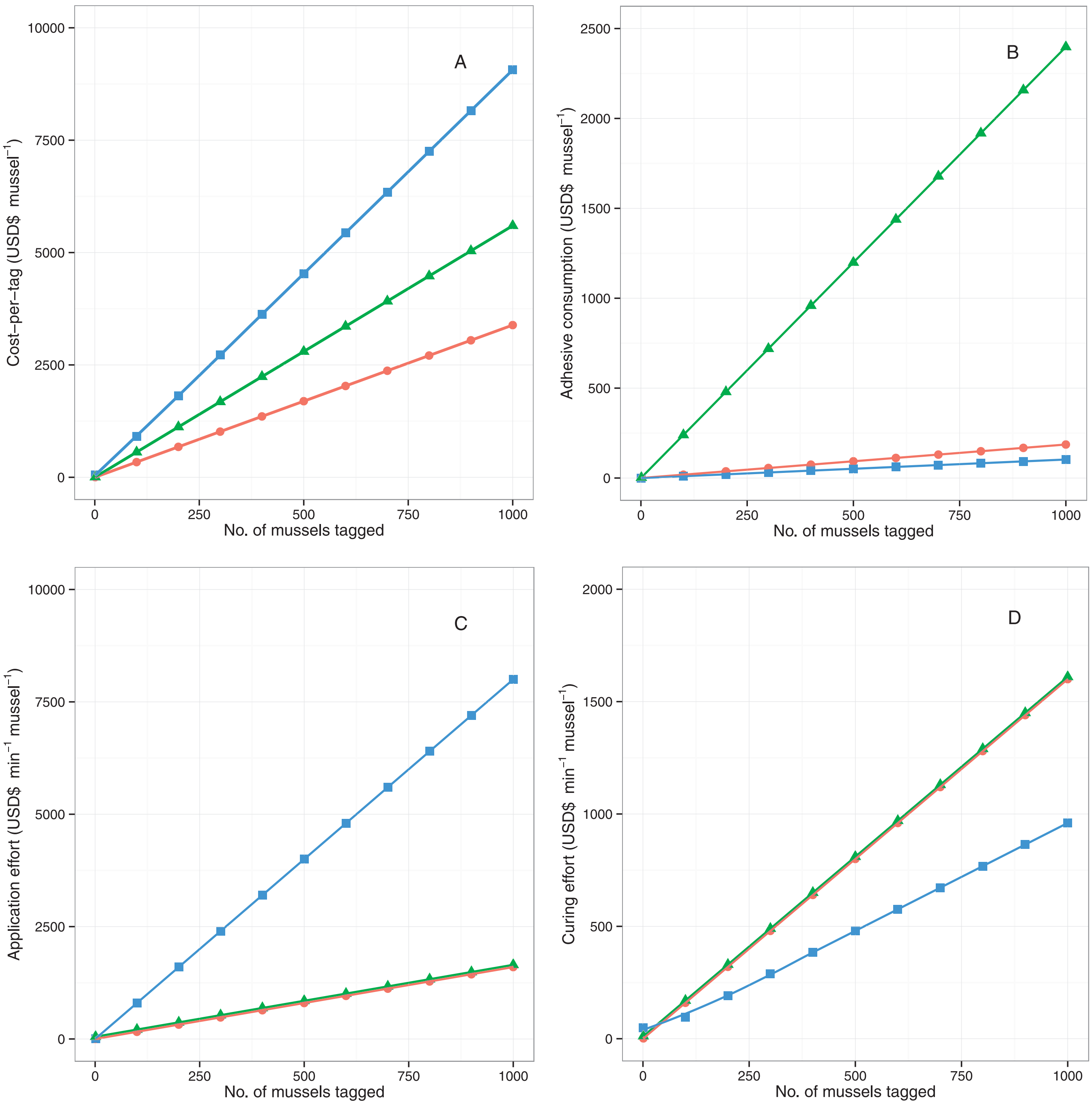

Figure 2. Linear models for epoxy resin (blue squares), cyanoacrylate (red circles), and dental cement (green triangles). Relationships between (a) cost-per-tagged mussel versus number of mussels with externally affixed PIT tags and individual cost-per-tag-effort (CPTE) parameters of (b) adhesive consumption, (c) application time, and (d) curing time versus number of mussels tagged.

associated with its application evaluated in this study was 5 times more than that of cyanoacrylate or dental cement. This difference in effort drove CPTE higher for epoxy (Fig. 2a, c), even though the cost of adhesive consumption per tag was less and curing in batches may reduce and even reverse any cost advantage achieved from using a faster curing adhesive (Fig. 2b, d). A more controlled applicator could also reduce the quantity of epoxy consumed per tag, thus realizing additional savings in materials. Because application and curing times were similar for cyanoacrylate and dental cement, differences in CPTE could be mitigated by more conservative cement application or a less costly formula.

Prices of adhesives can vary widely, especially when considering the advent of online shopping, buying in bulk, or discounts some groups receive (e.g., governmental agencies). The difference in adhesive cost per unit may in part be because the epoxy evaluated in this study is sold in a greater quantity per standard package than both dental cement and cyanoacrylate. On average, 600 individuals could be affixed with PIT tags by using a 454-g package of epoxy. In contrast, about 30 individuals could be tagged using a 35-g package of dental cement. Other factors to consider are the ability to rapidly procure adhesive, surcharges when not ordering in bulk, or unintended curing of unused product. For example, acquiring dental cement can be challenging because its intended use is in a regulated industry. Also, unexpected demand for additional adhesive (e.g., tagging more mussels than expected or more liberal adhesive application) requires the need for impromptu purchasing. We have observed prices varying by 10–30% among major retailers for the same cyanoacrylate adhesive. Cyanoacrylate adhesives and accelerants are often sold in cases of 10 or 12 and have a suggested shelf life of a year. There are often surcharges to purchase units less than a case, which would increase cost per unit parameters if a relatively small number of mussels are to be tagged. With adequate planning time, comparison shopping should help keep actual costs comparable to our studies; however, we noted a 30% increase in the price of epoxy since the last purchase from the same vendor.

Although we focused our effort on resources required to affix PIT tags, the cost of tags can also vary depending on the quantity, size, and manufacturer. For the data evaluated in our models, tag cost would have been constant because large quantities were procured from the same vendor at or about the same time. However, over the course of these studies tag price has fluctuated year to year and vendor to vendor by (+) 150 to (–) 250% (e.g., prices have ranged from \$2 to \$5 per tag). Other costs we did not measure and account for in our evaluation should also be considered when choosing an adhesive type for PIT tagging of freshwater mussels. For example, the curing time associated with underwater epoxies could reduce the number of mussels that can be tagged and returned to a stream in a day or require travel between study sites and laboratory facilities thus extending the number of field days. Specialized facilities and equipment may also be necessary to hold mussels in captivity during the curing time, whereas mussels can be immediately returned to the stream after cyanoacrylate and dental cures. Tiemann et al. (2016) speculated that prolonged handling and exposure may have contributed to the initial mortality observed following relocation. Factors other than cost may also warrant consideration, including the presence of potentially harmful compounds, adhesive durability, and ability to reapply in the field. For example, Hartmann et al. (2016a) chose not to adhere sensors to Duck Mussel (*Anodonta anatina*) with epoxy resin due to its complex application and presence of bisphenol-A. Environmental factors (e.g., air temperature and relative humidity) can also affect adhesive viscosity and curing time.

We propose that PIT tag retention is generally not an important factor in choosing an adhesive as previous studies have shown that retention rates do not seem to vary substantially by adhesive type (e.g., Young and Isley 2008). However, PIT tag attachment may fail regardless of adhesive type if debris causes the bond between shell and adhesive or adhesive and tag to break. Insufficient PIT tag encapsulation could cause them to be damaged if mussels become dislodged or struck with coarse particles during high flow events. Still, externally affixed PIT tag loss appears to be low over 1–2-yr periods and comparable to retention rates of vinyl shellfish tags (e.g., Lemarie et al. 2000). For example, Ashton et al. (2016) observed the loss or failure of eight (2%) cyanoacrylate-affixed PIT tags 12 mo after relocation on Eastern Elliptio that were recovered 650 to 1,500 m downstream of the point of their relocation in a coarse substrate stream. Similar levels of tag damage due to cyanoacrylate erosion were observed after 18 mo by Young and Isely (2008), but they observed no tag damage due to adhesive loss for underwater epoxy. Tiemann et al. (2016) reported one (1%) tag failure during their assessment of short-distance mussel relocation with epoxy encapsulated PIT tags. Hua et al. (2016) observed no failure of tags embedded in dental cement. We are unaware of any published studies that have evaluated PIT tag retention beyond 3 yr so we cannot speculate whether a particular type is more suited for long-term (>10-yr) study.

The findings of our evaluation are likely limited in their scope to the adhesives we evaluated (gel cyanoacrylate, dental cement, and 24-h curing waterproof epoxy resin); however, the assumptions used to parameterize our model are flexible to other costs and adhesive properties. Accordingly, the costs incurred from applying and handling with the epoxy used in this study would have been likely similar if a quicker curing formula was used based on observations of others (e.g., Young and Isley 2008). For this reason, we expect that epoxy resin would sustain higher total costs per mussel tagged without reductions in application time while also maintaining a minimal level of effort during the curing process. Further limitations in our findings may arise from a lack of quantified variation within each case study and by adhesive type. Variation when applying model parameters could arise from fluctuations in adhesive costs, level of adhesive applicator experience, and staffing. For example, actual staff costs incurred in the Illinois and Maryland case studies may have been lower than our model because some tag applicators were volunteers. However, a relocation or reintroduction involving a federally listed, cryptic species may necessitate primary investigators with specialized experience, which could lead to higher salary rates. Added variation could result from adhesive brand and environmental factors, including air temperature and relative humidity. We believe a more thorough comparison of commercially available adhesives used to externally PIT tag mussels is warranted.

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