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Short Communication

Enamel-based mark performance for marking Chinese mystery snail *Bellamya chinensis*

Alec Wong[1]*, Craig R. Allen[2], Noelle M. Hart[3], Danielle M. Haak[3], Kevin L. Pope[2], Nicholas A. Smeenk[3], Bruce J. Stephen[1] and Daniel R. Uden[3]

1 *School of Natural Resources, Hardin Hall 3310 Holdrege Street, University of Nebraska-Lincoln, Lincoln, NE 68583, USA*
2 *U.S. Geological Survey, Nebraska Cooperative Fish and Wildlife Research Unit, School of Natural Resources, Hardin Hall 3310 Holdrege Street, University of Nebraska, Lincoln, Lincoln, NE 68583, USA*
3 *Nebraska Cooperative Fish and Wildlife Research Unit, School of Natural Resources, Hardin Hall 3310 Holdrege Street, University of Nebraska, Lincoln, Lincoln, NE 68583, USA*

E-mail: alec.wong2013@huskers.unl.edu (AW)

**Corresponding author*

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Abstract

The exoskeleton of gastropods provides a convenient surface for carrying marks, and in the interest of improving future marking methods our laboratory assessed the performance of an enamel paint. The endurance of the paint was also compared to other marking methods assessed in the past. We marked the shells of 30 adult Chinese mystery snails *Bellamya chinensis* and held them in an aquarium for 181 days. We observed no complete degradation of any enamel-paint mark during the 181 days. The enamel-paint mark was superior to a nail-polish mark, which lasted a median of 100 days. Enamel-paint marks also have a lower rate of loss (0.00 month^{-1} 181 days) than plastic bee tags (0.01 month^{-1}, 57 days), gouache paint (0.07 month^{-1}, 18.5 days), or car body paint from studies found in scientific literature. Legibility of enamel-paint marks had a median lifetime of 102 days. The use of enamel paint on the shells of gastropods is a viable option for studies lasting up to 6 months. Furthermore, visits to a capture-mark-recapture site 1 year after application of enamel-paint marks on *B. chinensis* shells produced several individuals on which the enamel paint was still visible, although further testing is required to clarify durability over longer periods.

Key words: capture-mark-recapture; freshwater invasive snail; population monitoring; Kaplan-Meier survival estimate; Viviparidae

Introduction

Marking individuals for future recognition is essential for assessing somatic growth (Shigamiya and Kato 2001), movement (Kareiva 1983), mortality (Buckland 1982), and other time-dependent life-history characteristics of organisms in the field. Recapture of marked individuals can also be used to estimate population densities (Boulanger et al. 2004) and growth rates (Pradel 1996). Although commonly used for many taxa, the application of this practice is variable. For example, capture-mark-recapture techniques are used 10 times more frequently for terrestrial vertebrates than for aquatic gastropods (Henry and Jarne 2007), despite the hard exoskeleton of many gastropods that provides a surface for carrying marks with few adverse effects on life-history (Gosselin 1993). Adequate marking requires minimal disturbance of the animal, persistence against natural weathering, and minimal effect on the animal's behavioural, energetic, or survival capabilities (Henry and Jarne 2007). Marks inevitably degrade over time, and researchers should take additional care to correct for mark loss with a sample of double-marked individuals when the rate of mark loss is nontrivial (Williams et al. 2002).

A range of common marking varieties exist for use on gastropod shells, including drilling or scoring of the exoskeleton, PIT tagging, and application of paints, plastics, or other materials. Enamel paint produces a durable, conspicuous mark that can remain identifiable for four months (Goater et al. 1989, laboratory: *Helisoma anceps*; Boulding and van Alstyne 1993, field: *Littorina* sp.) and longer (Boulding et al. 1993; Johnson and Black 1998, *in situ*: *Bembicium vittatum*; Tablado et al. 1994, field: *Siphonaria lessoni*) on freshwater and marine gastropod shells. Unfortunately, precise observations of mark loss over time do not exist. Goater (1989) only reports maximum duration of enamel paint in a laboratory setting, without any comment on rate of degradation. Boulding et al. (1993) report personal observations of mark endurance > 1 year, but there is no consideration of mark retention in the field. Tablado et al. (1994) report an average monthly loss rate over 270 days of 0.03 marks per month in a field study of the marine limpet *Siphonaria lessoni* (Blainville); however, the majority of their observations (53% and 96% in two respective samples) were censored. This study aims to clarify the performance of enamel paint on Chinese mystery snail *Bellamya chinensis* (Gray, 1834) shells in a laboratory setting.

The presence of the invasive freshwater *B. chinensis* has been documented by the U.S. Geological Survey (Kipp et al. 2012) in the water systems of Nebraska, U.S.A. The first known introduction of *B. chinensis* was in 1890 by sailors returning from Yokohama, Japan (Jokinen 1982). Although populations are well-established and considered part of the permanent fauna in the northeastern United States of America (Jokinen 1982), there is very little information regarding the impacts of this species on invaded ecosystems (Solomon 2010). Jokinen (1982) indicates that the species has been found in Chinese markets and are traded as food items (Wood 1892). Karatayev et al. (2009) explain that the aquarium and ornamental trade is another major vector for introduction. Indeed, they state that this is the main vector for introduction of the Chinese mystery snail. Unfortunately, the literature quantifying the incidence of introduction through these means is lacking. Solomon et al. (2010) indicates that the snail can reach extremely high densities (0.16 – 4.00 snails/m^2) in lakes of the Northern Highlands Lake District of Wisconsin. Chaine et al. (2012) provides evidence that snails occur in densities up to 5.2 snails/m^2 in reservoirs in Nebraska. The occurrences of *B. chinensis* are associated with low densities of some native species, but the overall species assemblage is not significantly affected (Soloman et al. 2010). *Bellamya chinensis* is a competitor to native snails in experimental mesocosms (*Physa gyrina* Say, 1821*, Lymnaea stagnalis* Linnaeus, 1758) (Johnson 2009). Johnson (2009) also demonstrates that *B. chinensis* physiology promotes an increase in surrounding N:P molar ratios, which can alter algae growth. Effectively marking these snails could improve future monitoring and control efforts of this potentially competitive invasive.

Materials and methods

Bellamya chinensis specimens were collected from Wild Plum Lake (40°36'52" N, 096°54'09" W), about 7 km east of Crete, Nebraska, and were transported back to the Aquatic Ecology Laboratory at the University of Nebraska–Lincoln. On 29 August 2011, individuals (n = 30) received an application of general hardware store grade multipurpose oil-based enamel paint (ACE brand Indoor/outdoor Oil-based Enamel - Red) on the penultimate whorl of the shell. The shell markings were unique letters or numbers. After allowing the marks to dry for 15 minutes, the marked snails were placed in a 30×59×42-cm aquarium filled with 18°C de-chlorinated tap water and sand substrate. The snails were fed romaine lettuce supplemented with goldfish flakes three times per week. A 50% water exchange was conducted each week, refilled with de-chlorinated tap water. De-chlorination was accomplished using approximately 8 g of crystalline sodium thiosulfate per 80 L of water, or by off-gassing the same volume of water for at least 1 week.

From 29 August 2011 to 26 February 2012, exclusive of December 2011, snails were observed three times per week. During observations, individual snails were extracted from their aquarium and inspected. Any degradation of the markings was noted, and the legibility of marks was assessed. Assessments were made by a single individual throughout the experiment. Mark endurance was estimated using Prism statistical software (Graphpad Software 2008), with estimates following the procedure of Kaplan and Meier (1958). A log-rank test was performed in Prism to discern significant differences between our marking material and other experimental results.

We determined the duration of marks according to their legibility and visibility. The retention of legible marks is necessary in situations where individuals need to be distinguished from one another and symbolic characters are used. Our second analysis determined endurance of marks regardless of individual mark definition, considering marks to be lost when no colour was present. This is more relevant to population studies where identification relies on only the presence or absence of a mark. Marked snails that experienced death (n = 7) during the experiment were censored from the analyses and immediately removed from the experimental tank; all marks on censored snails were legible at time of death.

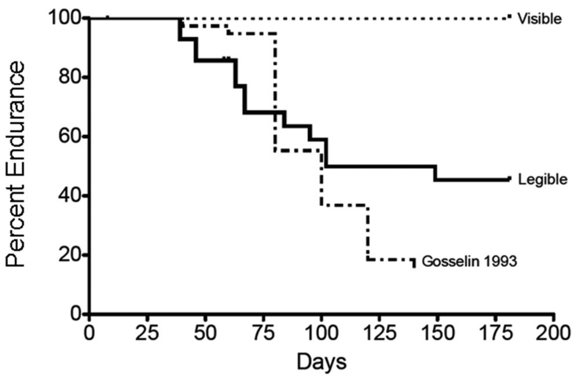

Figure 1. Endurance of enamel-paint batch marks (visible; this study), nail-polish batch marks (Gosselin 1993), and enamel-paint individual marks (legible; this study). Censored events (i.e. snail mortality) occurred on days 9 (1 event), 60 (1 event), 63 (3 events) and 67 (1 event) for this study.

Approximately one month into the experiment, native *Physa gyrina* (Say, 1821) pulmonate fresh-water snails inadvertently entered the experimental aquarium and established a population (n > 200). Interactions between the two species included movement of *P. gyrina* over the shells of *B. chinensis*, egg deposition by *P. gyrina* on *B. chinensis* shells, and inter- and intraspecific rasping on shells. The effects of these interactions are unknown and were not accounted.

A separate study at Wild Plum Lake that estimated Chinese mystery snail movement patterns made marks with a similar enamel paint (white) on adult snails (n = 494) on 8 September 2011. These marks were a solitary "X" on the penultimate whorl of the shell. We used recapture data to estimate a maximum endurance for enamel paint *in situ*.

Results

All marks (n = 23) in our main study persisted to the end of the experiment (i.e., there was no complete loss of marking). Ninety-two percent of the marks were legible 46 days into the experiment. The median life time for mark legibility was approximately 101 days, and 40% of the marks were legible at the conclusion of the experiment (Figure 1). Additionally, some of the marked (enamel paint) snails released in Wild Plum Lake as part of the separate movement pattern study were recovered with visible marks 339 days after release.

Discussion

We compared these results with other studies of marking shelled gastropods. Henry and Jarne (2007) provided a general literature review of some available marking methods and associated mark performance standardized for a 1-month duration with a constant rate of mark loss. They catalogued estimates of mark loss rates for plastic bee tags (0.01 month^{-1}, 57 days), gouache paint (0.07 month^{-1}, 18.5 days), car body paint (0.03 month^{-1}, 57 days), and nail polish (0.03 month^{-1} in laboratory, 0.16 month^{-1} in field, 120 days). Using the same methods, enamel-paint marks had a loss rate of 0.00 month^{-1}, as none of our marks had disappeared by the conclusion of the experiment.

The use of symbolic markings introduces risk of misinterpretation, and therefore, should be avoided whenever possible. The procedure suggested by Gosselin (1993) presents an efficient method for marking individuals using combinations of colour, instead of symbolic marks, for individual recognition. Using 6 colours, Gosselin's system allows for individual identification of 186 unique combinations by applying ≤ 3 dots of paint. Caution is warranted when using multiple colours because loss rates for gouache-paint marks vary with colour (Henry and Jarne 2007). It is unknown whether there is any dependency on colour for longevity of enamel-paint marks; our laboratory study used red colour enamel paint.

Nail polish appears frequently as a method of marking gastropods in scientific literature (Iyengar 2002; Muzii and Skinner 1966; Maltz 2003; Tolley-Jordan and Owen 2008; Gosselin 1993; Shigemaya et al. 2001), and appears to be the simplest and most cost-effective technique for marking shelled gastropods.Visibility of enamel-paint marks is maintained longer than that of nail-polish marks. Severns (2009) describes another possible marking media, metallic marking pens manufactured by Pilot™, used on terrestrial snails (*Vespericola* sp.). Metallic silver and gold marks lasted up to 1.5 years, and the conspicuous markings were not associated with higher predation rates. The metallic pens are superior to enamel paint in that ink can be applied and cured within 1 minute, obviating the relatively long drying time required for setting enamel paint and the associated desiccation of vulnerable aquatic gastropods. However, Severns (2009) described the metallic ink as easily removed by light abrasion after being immersed in water for 3 months, making its reliability on aquatic gastropods dubitable.

We conclude that enamel-based paint can be used reliably for marking of *B. chinensis* shells for durations up to 6 months. Given the maximum observed lifetime of enamel-paint marks (1 year), it is clear that subsequent testing is required to clarify the detailed mark retention over longer periods. The application of enamel paint on terrestrial snails also appears to have utility.

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