Improving the Utility of Artificial Shelters for Monitoring Eastern Hellbender Salamanders (*Cryptobranchus alleganiensis alleganiensis*)

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ACADEMIC ABSTRACT

Improving the Utility of Artificial Shelters for Monitoring Eastern Hellbender Salamanders (*Cryptobranchus alleganiensis alleganiensis*)

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Artificial shelters show great promise as novel, non-invasive tools for studying hellbenders, but their use thus far has faced several challenges. During initial trials in multiple river networks, artificial shelters routinely became blocked by sediment and dislodged during high stream discharge events, and were rarely used by hellbenders. We sought to determine whether these complications could be overcome via alternative shelter design, placement, and maintenance. Between 2013 and 2018, we deployed 438 artificial shelters of two different designs across ten stream reaches and three rivers in the upper Tennessee River Basin. We assessed evidence for several hypotheses, postulating broadly that the availability, stability, and use of artificial shelters by hellbenders would depend on how shelters were constructed, deployed, and/or maintained. We found that maintaining shelters at least once every 40 days limited sediment blockage, and building ~ 40 kg shelters with 3-4 cm thick walls and recessed lids improved their stability during high discharge events. Additionally, we found that hellbenders most frequently occupied and nested in artificial shelters when they were deployed in deeper (~50+ cm) portions of reaches with high adult hellbender densities. Our results suggest that artificial shelters can serve as effective tools for studying hellbenders when designed, deployed, and maintained with these advancements, but also highlight some limitations of their use.

GENERAL AUDIENCE ABSTRACT

Improving the Utility of Artificial Shelters for Monitoring Eastern Hellbender Salamanders (*Cryptobranchus alleganiensis alleganiensis*)

Sky Button

Hellbenders are large, fully-aquatic salamanders that live primarily in cool, rocky, swift-flowing streams in portions of Appalachia and the lower Midwest. They are imperiled across most of their native range due to human-caused habitat degradation, but their declines, conservation needs, and population status have historically been difficult to study due to the fact that they spend the majority of their lives beneath large, often inaccessible boulders. While these boulders are sometimes possible to lift, doing so can disturb critical hellbender habitat. Therefore, alternate, less invasive hellbender sampling methods are necessary in order to improve knowledge about their conservation status and needs. Artificial shelters, which are large, hollow, concrete structures that mimic natural boulder crevices and feature removable lids, show promise as a novel, innovative tool for non-invasively studying hellbenders. However, initial trials of these shelters have yielded mixed results, with shelters often becoming swept away and destroyed during floods, becoming blocked by sand and sediment and thus inaccessible to hellbenders, or simply not being used by hellbenders when accessible. We sought to determine whether these complications could be overcome by optimizing the way that shelters were constructed, deployed, and maintained in streams inhabited by hellbenders. Between 2013 and 2018, we deployed 438 artificial shelters of two different designs across ten stream reaches and three rivers in the upper Tennessee River Basin. Using multiple analyses, we tested one broad overall hypothesis: that the efficacy of using artificial shelters to study hellbenders would depend on how they were constructed, how frequently they were maintained, and where they were placed in the stream. We found that maintaining shelters at least once every 40 days limited sediment blockage, and building ~ 40 kg shelters with 3-4 cm thick walls and recessed lids improved their stability during flood events. Additionally, we found that hellbenders most frequently occupied and nested in artificial shelters when they were deployed in deeper (~50+ cm) portions of reaches with high adult hellbender densities. Our results suggest that artificial shelters are effective tools for studying hellbenders when designed optimally, maintained frequently enough, and placed in appropriate locations. However, exceptions to these findings may exist in certain heavily degraded stream reaches.

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DEDICATION

To my late grandfather Dr. Joseph A. Caruso, who helped foster my interest in science from a young age and was as selfless as he was brilliant.

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I would of course be horribly remiss if I did not also mention my lab mates. I have cherished the friendships we have developed since my arrival in Blacksburg in 2017, and I could not have asked for a better group of people to work with. I will be leaving Blacksburg with many fond memories, and I can honestly say that the vast majority of them are with you all. I also owe you all thanks for the role you played in helping me complete my graduate research, in numerous ways. I would like to thank Jordy Groffen, Brian Case, John Connock, Alex Grimaudo, Clara Frazier, Henry Davie, and Thomas Galligan in particular for their countless hours in the field helping me carry out this research. I am also extremely grateful to each of you, as well as Sydney Hope, Becca O'Brien, Georgia Martin, and Silas Beers for the feedback you all provided me with on my presentations/manuscripts. It has sincerely been a pleasure working with you all.

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ATTRIBUTION

Chapter 1 was co-authored with my advisor, William A. Hopkins, and several current and former members of the Hopkins Lab, including John J. Hallagan, Catherine M.B Jachowski, Brian F. Case, and Jordy Groffen. All co-authors helped edit the chapter and/or helped design the artificial shelters discussed within.

Chapter 2 was co-authored with my advisor, William A. Hopkins, and several current and former members of the Hopkins Lab, including Catherine M.B. Jachowski, Brian F. Case, and Jordy Groffen. All authors provided input on study design and/or helped edit the chapter.

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Introduction

Chapter 1: Weathering the storm: Improving the availability and stability of artificial shelters for hellbender salamanders

Chapter 2: The influence of multiscale variables on artificial shelter use by hellbender salamanders

Introduction

Global biodiversity has declined at an alarming rate in recent decades due to human activities (Barnosky et al., 2011), resulting in the listing of over 97,000 species as threatened or near threatened (IUCN, 2019). Several anthropogenic causes are implicated in these global declines, including climate change (Sala et al., 2000), habitat loss, degradation and fragmentation (Cushman, 2006; Brooks et al., 2002), invasive species (Mack et al., 2000), disease (Scheele et al., 2019), and illegal collection (Smith et al., 2009). Among these threats, habitat loss and degradation pose the most serious risks for many taxa (Brooks et al., 2002), and can also magnify the impacts of other anthropogenic stressors (Grant et al., 2016). While no ecosystem is immune to the damaging effects of habitat loss and degradation, the extent to which these threats endanger biota varies widely among ecosystems (Barnosky et al., 2011).

Species that occur in lotic environments are among those most vulnerable to the effects of anthropogenic habitat degradation (Malmqvist & Rundle, 2002). Over the past several decades, humans have become the world’s primary agent responsible for changes to the structure and function of running waters (Hooke, 2000), leaving few if any watersheds completely intact. However, while the immediate consequences of anthropogenic pressures on lotic ecosystems have been extensively studied, the mechanisms of population declines remain poorly understood for many lotic taxa (Clausen & York, 2008). Moreover, although substantial attention has been given to the study and mitigation of anthropogenic threats posed to sport fishes and other select lotic taxa, the conservation of lotic species that are less charismatic, economically important, and/or detectable has lagged behind considerably (Clarkson et al., 2005).

Amphibian Declines and Detectability. Amphibians are the most imperiled class of vertebrates globally, with over 40% currently listed as either threatened or near-threatened (IUCN, 2019), including over 60% of salamanders. Amphibians are often particularly susceptible to the effects habitat degradation and other anthropogenic stressors (Hopkins, 2007; Cushman, 2006) because they tend to be highly stenotopic, possessing specialized niches (Bonetti & Wiens, 2014), limited distributions (Whitton et al., 2012), and strict physiological requirements (Feder & Burggren, 1992). Moreover, in addition to habitat loss and degradation, imperiled amphibians also face substantial threats from infectious diseases (Scheele et al., 2019), habitat fragmentation (Cushman, 2006), invasive species (Johnson et al., 2011) and other factors. Amphibian declines are often shaped by complex interactions among multiple factors, making it difficult to pinpoint specific human actions that must be addressed in order to enable species recovery (Blaustein et al., 2011).

Assessments of the ultimate drivers of amphibian declines are also inhibited by the low detectability of many declining amphibians (Kéry & Schmidt, 2008). Amphibians can be notoriously difficult to locate, as they often use inaccessible habitats (Bailey et al., 2004; Measey et al., 2003), are cryptically-colored (Rudh & Qvarnström, 2013; Wells, 2010), or are adept at escaping capture (Willson et al., 2005). Given the secretive life histories of many amphibian species, conventional survey techniques often fail to detect imperiled amphibians frequently enough to monitor their populations (Mazerolle et al., 2007). Therefore, it is imperative that novel tools be developed that improve the ease with which poorly difficult to detect, declining amphibians can be studied.

Lotic amphibians are often especially imperiled, as their biology frequently combines features that make amphibians and lotic species individually vulnerable to environmental changes (Calderon et al., 2017; Pui & Das, 2016; Ashton et al., 2006). Unfortunately, as lotic amphibians also tend to use especially inaccessible habitats, they are also often notoriously difficult to locate (Bailey et al., 2004; Maerz et al., 2015; Mazerolle et al., 2007), making inference about their conservation needs challenging. Moreover, existing monitoring tools that are viable for studying amphibians in other ecosystems (e.g., drift fences, crayfish traps, hoop traps) are frequently infeasible in lotic environments (Browne et al., 2011). The need to develop effective protocols that improve amphibian monitoring capabilities is therefore especially urgent among lotic species.

Study System. My thesis focuses on the eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*), a large-bodied lotic amphibian that typifies the tendency of stream salamanders to be both difficult to detect and declining. Hellbenders are extremely sensitive to environmental changes due to their narrow habitat requirements (Jachowski & Hopkins, 2018), and are typically found only in cool, fast-moving, well-oxygenated streams containing moderately deep runs and an abundance of large, crevice-bearing boulders. Though once common throughout their range in Appalachia and portions of the lower Midwest, hellbender populations have declined sharply in recent decades (Briggler et al., 2007; Williams et al., 1981), and the species is now federally or state-listed in nearly every state where it occurs. The declining status of hellbenders appears to be driven in large part by destruction of riparian buffers provided by upstream forest cover (Jachowski & Hopkins, 2018), but the precise mechanisms driving the lack of recruitment within declining hellbender populations are unknown. Historically, hellbenders have only been possible to reliably detect using rock-lifting surveys, which can damage critical habitat and pose risks to both animals and surveyors (Browne et al., 2011). Thus, it is important that improved protocols be developed that make it possible to study hellbenders in a less invasive manner.

Artificial shelters have recently been proposed as a novel potential tool for monitoring hellbenders without damaging their critical habitat (Briggler & Ackerson, 2012). Since hellbenders spend most of their lives in crevices beneath large boulders (Keitzer, 2007), artificial shelters that possess entrances designed to mimic these crevices might be useful for monitoring this species. This logic recently drove the development of “boot design” artificial shelters for hellbenders (Briggler & Ackerson, 2012), which consisted of a single tunnel entrance and an enlarged rear chamber. Importantly, these artificial shelters also possess a removable lid to provide surveyors access to occupying animals. Due to their practical design, boot design shelters have now been deployed to study hellbenders in several states within their range.

Despite their theoretical potential, boot design artificial shelters have so far in practice been largely ineffective for monitoring hellbenders. During initial trials in at least seven states across the hellbender’s range, these shelters have frequently become blocked by sediment or dislodged during high stream discharge events (Messerman, 2014). Thus, hellbender opportunities to use these artificial shelters have often been limited. These challenges have been so pervasive that some groups have abandoned boot design artificial shelters entirely (Mohammed et al., 2016). Moreover, even when hellbenders *have* had the opportunity to occupy and nest in boot design artificial shelters, they have not always done so (Messerman, 2014). Therefore, improving the utility of boot design artificial shelters as tools for monitoring hellbenders requires a two-step process. First, the design, maintenance, and placement of these artificial shelters must be improved so that hellbenders can consistently access them. Second, once artificial shelters are made consistently accessible to hellbenders, the placement of these shelters must ensure that hellbenders will choose to consistently occupy and nest in them. While this process could require considerable trial and error, it is an important endeavor given the current lack of viable alternatives for non-invasively monitoring hellbenders.

My master's research was centered around two major objectives, both related to maximizing the utility of artificial shelters for monitoring hellbenders (Fig. I.1). First, I sought to determine whether shelter design, maintenance and placement influences availability of shelters to hellbenders and stability of these shelters during high stream discharge events. Specifically, I assessed evidence for hypotheses that shelter availability would be driven by where shelters were placed and how often they were maintained, and that shelter stability would depend primarily on how shelters were built. Second, I evaluated where artificial shelters should be placed (once made available and stable) to maximize hellbender occupancy and nesting in these shelters over 5+ years. I predicted that hellbenders would most frequently occupy and nest in shelters that were placed in reaches with high adult/subadult hellbender densities and had been in place for multiple years. Additionally, I predicted that multiscale habitat features would play an important role in shaping hellbender occupancy and nesting in artificial shelters, consistent with the specialized niche of this species.

REFERENCES

Allan, J. D., and Castillo, M. M. (2007). *Stream ecology: structure and function of running waters* Springer Science & Business Media.

Ashton, D. T., Marks, S. B., and Welsh, H. H. (2006). Evidence of continued effects from timber harvesting on lotic amphibians in redwood forests of northwestern California. *Forest Ecology and Management* 221: 183-193.

Bailey, L. L., Simons, T. R., and Pollock, K. H. (2004). Estimating site occupancy and species detection probability parameters for terrestrial salamanders. *Ecological Applications* 14: 692-702.

Barnosky, A. D., Matzke, N., Tomiya, S., Wogan, G. O., Swartz, B., Quental, T. B., Marshall, C., Mcguire, J. L., Lindsey, E. L., and Maguire, K. C. (2011). Has the Earth's sixth mass extinction already arrived? *Nature* 471: 51.

Blaustein, A. R., Han, B. A., Relyea, R. A., Johnson, P. T., Buck, J. C., Gervasi, S. S., and Kats, L. B. (2011). The complexity of amphibian population declines: understanding the role of cofactors in driving amphibian losses. *Annals of the New York Academy of Sciences* 1223: 108-119.

Bonetti, M. F., and Wiens, J. J. (2014). Evolution of climatic niche specialization: a phylogenetic analysis in amphibians. *Proceedings of the Royal Society B: Biological Sciences* 281: 20133229.

Briggler, J., and Ackerson, J. (2012). Construction and use of artificial shelters to supplement habitat for Hellbenders (*Cryptobranchus alleganiensis*). *Herpetological Review* 43: 412.

Briggler, J., Utrup, J., Davidson, C., Humphries, J., Groves, J., Johnson, T., Ettling, J., Wanner, M., Traylor-Holzer, K., and Reed, D. (2007). Hellbender population and habitat viability assessment: final report. IUCN/SSC Conservation Breeding Specialist Group, Apple Valley, MN.

Brooks, T. M., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A., Rylands, A. B., Konstant, W. R., Flick, P., Pilgrim, J., Oldfield, S., and Magin, G. (2002). Habitat loss and extinction in the hotspots of biodiversity. *Conservation Biology* 16: 909-923.

Browne, R., Li, H., Mcginnity, D., Okada, S., Zhenghuan, W., Bodinof, C., Irwin, K., Mcmillan, A., and Briggler, J. (2011). Survey techniques for giant salamanders and other aquatic Caudata. *Amphibian & Reptile Conservation* 5: 1-16.

Calderon, M., Moglia, M., Nievas, R., Colombetti, P., González, S., and Jofré, M. (2017). Assessment of the environmental quality of two urbanized lotic systems using multiple indicators. *River Research and Applications* 33: 1119-1129.

Ceballos, G., Ehrlich, P. R., Barnosky, A. D., García, A., Pringle, R. M., and Palmer, T. M. (2015). Accelerated modern human–induced species losses: Entering the sixth mass extinction. *Science Advances* 1: e1400253.

Clarkson, R. W., Marsh, P. C., Stefferud, S. E., and Stefferud, J. A. (2005). Conflicts between native fish and nonnative sport fish management in the southwestern United States. *Fisheries* 30: 20-27.

Clausen, R., and York, R. (2008). Global biodiversity decline of marine and freshwater fish: a cross-national analysis of economic, demographic, and ecological influences. *Social Science Research* 37: 1310-1320.

Cushman, S. A. (2006). Effects of habitat loss and fragmentation on amphibians: a review and prospectus. *Biological Conservation* 128: 231-240.

Feder, M. E., and Burggren, W. W. (1992). *Environmental physiology of the amphibians* University of chicago Press.

Grant, E. H. C., Miller, D. A., Schmidt, B. R., Adams, M. J., Amburgey, S. M., Chambert, T., Cruickshank, S. S., Fisher, R. N., Green, D. M., and Hossack, B. R. (2016). Quantitative evidence for the effects of multiple drivers on continental-scale amphibian declines. *Scientific Reports* 6: 25625.

Hopkins, W. A. (2007). Amphibians as models for studying environmental change. *Ilar Journal* 48: 270-277.

Hooke, R. L. (2000). On the history of humans as geomorphic agents. *Geology* 28: 843-846.

Jachowski, C. M. B., and Hopkins, W. A. (2018). Loss of catchment-wide riparian forest cover is associated with reduced recruitment in a long-lived amphibian. *Biological Conservation* 220: 215-227.

Johnson, P. T., Mckenzie, V. J., Peterson, A. C., Kerby, J. L., Brown, J., Blaustein, A. R., and Jackson, T. (2011). Regional decline of an iconic amphibian associated with elevation, land-use change, and invasive species. *Conservation Biology* 25: 556-566.

Keitzer, S. C. (2007). Habitat preferences of the eastern hellbender in West Virginia. M.S. Thesis. Marshall University.

Kéry, M., and Schmidt, B. (2008). Imperfect detection and its consequences for monitoring for conservation. *Community Ecology* 9: 207-216.

Mack, R. N., Simberloff, D., Mark Lonsdale, W., Evans, H., Clout, M., and Bazzaz, F. A. (2000). Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications* 10: 689-710.

Maerz, J. C., Barrett, R. K., Cecala, K. K., and Devore, J. L. (2015). Detecting enigmatic declines of a once common salamander in the Coastal Plain of Georgia. *Southeastern naturalist* 14: 771-784.

Malmqvist, B., and Rundle, S. (2002). Threats to the running water ecosystems of the world. *Environmental Conservation* 29: 134-153.

Mazerolle, M. J., Bailey, L. L., Kendall, W. L., Royle, J. A., Converse, S. J., and Nichols, J. D. (2007). Making great leaps forward: accounting for detectability in herpetological field studies. *Journal of Herpetology* 41: 672-690.

Measey, G., Gower, D., Oommen, O., and Wilkinson, M. (2003). Quantitative surveying of endogeic limbless vertebrates—a case study of *Gegeneophis ramaswamii* (Amphibia: Gymnophiona: Caeciliidae) in southern India. *Applied Soil Ecology* 23: 43-53.

Messerman, A. (2014). The Use of Nest Boxes by the Hellbender Salamander in Western North Carolina. M.S. Thesis. Duke University.

Mohammed, M. G., Messerman, A. F., Mayhan, B. D., and Trauth, K. M. (2016). Theory and Practice of the Hydrodynamic Redesign of Artificial Hellbender Habitat. *Herpetological Review* 47: 586-591.

Pui, Y. M., and Das, I. (2016). Streams in Forested Headwaters as Reservoirs of Endemicity in Bornean Amphibians. In*: Naturalists, Explorers and Field Scientists in South-East Asia and Australasia*, pp. 143-155. (Ed.)^(Eds.): Springer.

Rudh, A., and Qvarnström, A. (2013). Adaptive colouration in amphibians. *Seminars in Cell & Developmental Biology* 24: 553-561.

Sala, O. E., Chapin, F. S., Armesto, J. J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L. F., Jackson, R. B., and Kinzig, A. (2000). Global biodiversity scenarios for the year 2100. *Science* 287: 1770-1774.

Scheele, B. C., Pasmans, F., Skerratt, L. F., Berger, L., Martel, A., Beukema, W., Acevedo, A. A., Burrowes, P. A., Carvalho, T., and Catenazzi, A. (2019). Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science* 363: 1459-1463.

Smith, K. F., Behrens, M., Schloegel, L. M., Marano, N., Burgiel, S., and Daszak, P. (2009). Reducing the risks of the wildlife trade. *Science* 324: 594-595.

Wells, K. D. (2010). *The Ecology and Behavior of Amphibians*. Chicago, IL, USA: University of Chicago Press.

Whitton, F. J., Purvis, A., Orme, C. D. L., and Olalla-Tárraga, M. Á. (2012). Understanding global patterns in amphibian geographic range size: does Rapoport rule? *Global Ecology & Biogeography* 21: 179-190.

Williams, R. D., Gates, J. E., Hocutt, C. H., and Taylor, G. J. (1981). The hellbender: a nongame species in need of management. *Wildlife Society Bulletin*: 94-100.

Willson, J. D., Winne, C. T., and Fedewa, L. A. (2005). Unveiling escape and capture rates of aquatic snakes and salamanders (*Siren* spp. and *Amphiuma* means) in commercial funnel traps. *Journal of Freshwater Ecology* 20: 397-403.

FIGURES

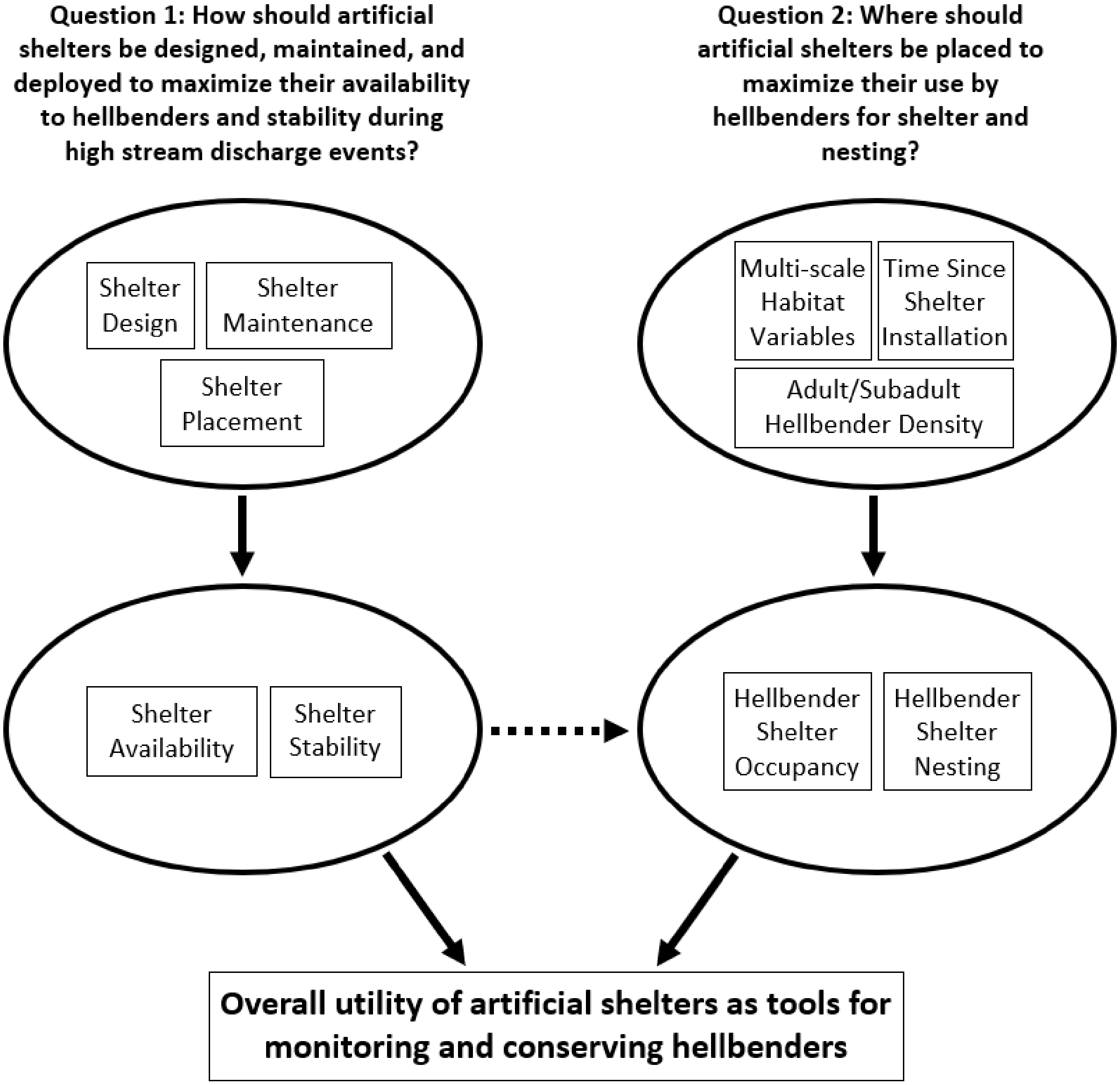

Fig. I.1. A diagram illustrating the proposed influences of artificial shelter design, maintenance, and placement on the utility of artificial shelters for monitoring hellbenders.

CHAPTER 1. Weathering the storm: Improving the availability and stability of artificial shelters for hellbender salamanders

Sky T. Button, John J. Hallagan, Catherine M.B. Jachowski, Brian F. Case, Jordy Groffen, &
William A. Hopkins

ABSTRACT

Artificial shelters show considerable promise as novel tools for studying imperiled hellbender salamanders. However, the full utility of using in-situ artificial shelters to study hellbenders has not yet been fully reached in practice, as during initial trials shelters have often become blocked by sediment or dislodged during high stream discharge events. To determine whether these challenges could be overcome, we deployed 438 artificial shelters of two different designs across ten stream reaches and three rivers in the upper Tennessee River Drainage in 2013-2018. We recorded shelter entrance availability during occupancy and nesting surveys, and recorded which shelters became dislodged following high discharge events. We assessed evidence for two hypotheses: 1) that shelter availability was driven by shelter placement and maintenance frequency and 2) that shelter stability was driven by shelter design and shelter placement. Shelters were available 78.6% of the time on average (range = 0-100%), and 88.6% (388/438) of shelters were stable across all high discharge events experienced. Shelter availability was maximized by clearing sediment from shelter entrances at least once every 40 days (more often in impaired reaches with low upstream forest cover) and after large storm events, situating the shelter with 1 m of at least five boulders, and orienting shelters such that their entrances do not face directly downstream. Shelter stability with our initial shelter design was 77.5% (169/218), but approached 100% (219/220) when shelters were constructed with

recessed lids and a heavier design (~40kg vs. ~25 kg), and when installed in reaches with abundant large boulders. Our findings demonstrate that artificial shelters have the potential to serve as valuable tools for monitoring hellbenders in reaches with modest impairment (i.e., with moderate or high upstream forest cover).

INTRODUCTION

Although amphibian population declines have been known for several decades (Scheele et al., 2019; Houlahan et al., 2000; Alford & Richards, 1999), the mechanisms underlying many declines remain speculative or unknown (Blaustein et al., 2011). Forty-nine percent of amphibians (excluding data deficient species), including 68% of salamanders, are currently considered imperiled (IUCN, 2018). This percentage has continued to increase over time despite increasing efforts to determine the causes of amphibian declines (Grant et al., 2016; Hopkins, 2007), due in part to the low detectability of many declining species. Thus, it is important that new techniques be developed for monitoring potentially at-risk amphibians. One of the largest impediments to understanding how environmental factors shape amphibian declines is their secretive life histories (Kellner & Swihart, 2014; Kéry & Schmidt, 2008; Mazerolle et al., 2007). Amphibians are often cryptically colored (Rudh & Qvarnström, 2013; Wells, 2010), adept at escaping capture (Willson et al., 2005), or use habitats that are not easily sampled (Bailey et al., 2004; Measey et al., 2003). All of these factors can limit research and monitoring capabilities, thus it is important that new techniques be developed for monitoring potentially at-risk amphibians.

Some of the most difficult to study yet most at-risk amphibians are those that live in rocky, lotic environments (Kriger & Hero, 2007; Welsh Jr & Ollivier, 1998). Lotic environments harbor an exceptional amount of amphibian diversity (Kriger & Hero, 2007; Olson et al., 2007; Mensing et al., 1998), but are usually difficult to sample, often because optimal amphibian microhabitats within these environments are located under large boulders that are difficult to access. While lifting rocks and debris is sometimes effective for sampling lotic amphibians, doing so often destroys critical microhabitat and is therefore counter to conservation goals

(Nickerson et al., 2003). Additionally, trapping techniques useful in more lentic aquatic environments, such as hoop nets, crayfish traps, and aquatic drift fences, are often either swept away during high stream discharge events in rocky lotic environments, or require nearly continuous maintenance and monitoring (Browne et al., 2011).

One imperiled species that exemplifies the challenges associated with monitoring lotic amphibians is the hellbender (*Cryptobranchus alleganiensis*). Hellbenders are large, fully-aquatic, secretive, long-lived salamanders that tend to thrive in cool, fast-flowing, well-oxygenated streams with moderately deep runs and forested riparian buffers that limit siltation (Briggler et al., 2007; Trauth et al., 1992; Beffa, 1976; Nickerson & Mays, 1973). Once common across Appalachia and the lower Midwest, hellbenders are declining throughout their range, particularly in reaches experiencing a loss of upstream riparian forest cover (Jachowski & Hopkins, 2018). The precise mechanisms underlying hellbender declines are poorly understood, largely because hellbenders are difficult to study, spending the vast majority of their lives hidden under large boulders (Keitzer et al., 2013; Keitzer, 2007; Humphries, 1999; Hillis & Bellis, 1971). Traditional methods for sampling hellbenders have involved manually lifting boulders (Browne et al., 2011), which, although effective, destroys critical hellbender habitat (Nickerson et al., 2003), and is often dangerous for surveyors. Rock lifting surveys are especially harmful between August and April, when male hellbenders are establishing and guarding nests underneath boulders.

Recently, the development of in-situ artificial shelters has presented a less-invasive avenue for researching and monitoring hellbenders. Artificial shelters for hellbenders are commonly built using the “boot design” proposed by Briggler and Ackerson (2012), which was later modified (Jachowski, Briggler, and Hopkins, in press). Boot design shelters are made from concrete and consist of a rectangular-shaped chamber that hellbenders can access through a single tunnel entrance. Assuming artificial shelters remain consistently in place, stay unblocked by sediment, and are used by hellbenders, they have the potential to serve a variety of functions critical for hellbender conservation, including providing eggs for captive rearing, serving as population monitoring tools, improving knowledge about hellbender reproductive biology, and augmenting existing hellbender habitat (Jachowski, Briggler, and Hopkins, in press).

Despite the potential of artificial shelters, their use thus far has faced several challenges. Past attempts to deploy artificial shelters have often been hindered by shelters becoming either unavailable to hellbenders due to sediment blocking their entrance, or dislodged and damaged during high discharge events (Messerman, 2014). Problems associated with shelter blockage and dislodgement have been so pervasive that some have suggested abandoning boot design shelters entirely (Mohammed et al., 2016). However, one advantage of boot design shelters is that they are less expensive to construct (~$30 USD/shelter, not including labor), and easier to transport and deploy than other proposed artificial shelter designs (Jachowski, Briggler, and Hopkins, in press). Still, it remains unclear whether challenges associated with using boot design shelters can be ameliorated to an extent sufficient to validate their continued use. Therefore, in this study we sought to determine whether variables related to the construction and deployment of the boot design shelters could influence their availability (i.e., presence or absence of sediment blocking the tunnel) and stability (i.e., ability to withstand high stream discharge events), and thereby improve their utility. We hypothesized that shelter maintenance frequency would drive shelter availability, that shelter design would drive shelter stability, and that shelter placement would drive both. Our study is the first to evaluate ways to maximize the availability and stability of artificial hellbender shelters, and thus provides important recommendations for improving the effectiveness of these shelters as hellbender monitoring tools.

METHODS

Artificial shelter construction

We built artificial shelters using designs similar to those described by Briggler & Ackerson (2012), containing a single, ~ 24 x 11 x 11 cm tunnel entrance and ~ 40 x 39 x 11 cm rear chamber (Fig. 1.1). We constructed shelters by encasing a boot-shaped frame (composed of hex mesh and chicken wire) in a mixture of sand, Portland cement, and Quikrete (The QUIKRETE Companies; Atlanta, Georgia, USA). From 2013-2015, we followed the design specifications of Briggler and Ackerson (2012) and used only as much concrete as was necessary to build shelters, so that they would be easy to carry into streams. These shelters weighed ~25kg, had 1-2 cm thick walls, and featured raised, custom-fit lids that rested on the dorsal surface of the shelter (Fig. 1.1). Hereafter, we refer to our original shelter design as “Design A”. After several years of use, numerous Design A shelters were dislodged and damaged due to high discharge events. To increase the stability of artificial shelters, in 2016 we modified their design to be heavier (~40kg with ~2-4 cm thick walls), and developed a recessed lid design that could be locked in place using an eye-bolt (Fig. 1.1.). An important feature of our modified design shelters was that we embedded their lids within a fitted, recessed area on the shelter’s dorsal surface. We constructed both lids and dorsal recesses of our modified design shelters using molds, making lids interchangeable between shelters and easy to replace if needed. Hereafter, we refer to our modified design shelters as “Design B”.

Artificial shelter arrays

Between 2013 and 2018, we deployed artificial shelter arrays at 10 reaches (area = 3,090-5,880 m^2; length = 206-376 channel meters) within three rivers in the upper Tennessee River Basin, in the Ridge-and-Valley and Blue Ridge provinces of southwestern Virginia. To prevent the harassment and illegal collection of hellbenders, we refer to our study rivers as Rivers 1, 2, and 3 in this manuscript and do not provide exact localities or a map of our study reaches. We installed shelter arrays within two reaches on River 1, three on River 2, and five on River 3. We spaced adjacent shelter arrays a minimum of 1.5 channel km apart from each other. We installed six arrays in 2013-2016 (one on River 2, five on River 3), which by the end of the study contained a mixture of Design A and B shelters. In 2018, we installed the remaining four arrays (two each on Rivers 1 and 2) using only Design B shelters. Our 10 arrays contained approximately 30 shelters each, yielding a total of ~300 shelters deployed at once. We replaced shelters if they became dislodged following heavy rains or needed to be removed for maintenance, and treated all replacement shelters as separate, new shelters in our analyses. Thus, in total our study included 438 artificial shelters spread across our 10 study reaches (n = 218 for Design A and n = 220 for Design B).

We installed both Design A and Design B shelters in a wide range of microhabitats potentially suitable for hellbenders at each study reach (Table 1.1). To minimize spatial autocorrelation in the availability and stability of adjacent shelters, we spaced shelters an average of 10 linear meters apart (range ≈ 4-30 m). To install shelters, we cleared spaces along the bottom of the stream and embedded shelters within them, deep enough to hold them firmly in place, but shallow enough that shelter tunnel entrances remained unblocked by sediment immediately following installation. Therefore, we typically embedded the bottom 30-50% of the shelter (3-6 cm) into the substrate. So that our artificial shelters mimicked natural crevices, we placed a thin layer (1-2 cm) of sand and gravel inside the tunnel and chamber following installation. We checked artificial shelters every 2-5 days in late summer, and every 2-8 weeks during the rest of the year (Button et al., in prep.), except during winter and high discharge events when fieldwork was unsafe. We recorded whether shelter entrances were available or were partially to completely blocked by accumulation of sediment (i.e., unavailable) on each survey, and immediately cleared all blocked tunnels. Following high discharge events, we recorded which shelters became dislodged.

Data collection

Artificial shelters varied considerably in their surrounding stream features (Table 1.1), making it possible to assess the relationship of multiple stream-related variables with shelter availability to hellbenders and with shelter stability during high stream discharge events. We quantified boulder abundance, throughout each reach and within 1 m of each shelter, as a potential driver of both shelter availability and stability (Table 1.1). To estimate the density of boulders across our study reaches, we walked 10 evenly-spaced transects across a representative 1680 m^2 portion of each reach, and counted the number of boulders that intersected our transects. Since boulders must be relatively large to affect the hydrodynamic properties of an entire reach, we only counted those that were > 40 cm long on their primary axis (“large boulders” hereafter) on our reach-wide transects. For boulders within 1 m of shelters, we included every particle > 256 mm long on the secondary axis in our counts (Wolman, 1954), because nearly all shelter-adjacent boulders tended to influence microcurrents within 1 m of shelters, even if only moderate in size (pers. obs.). We replaced shelter-adjacent boulders that were dislodged during high discharge events as needed, to ensure that the number of boulders within 1 m of each shelter remained roughly constant throughout the study.

We predicted that multiple variables related to in stream conditions and survey frequency would also impact shelter availability. Therefore, we assessed the influence of average days in between surveys, the angle formed between the direction of the tunnel and direction of the current (“tunnel angle” hereafter — where tunnel angle = 0° if the tunnel faces directly downstream and 90° if it faces directly towards the bank), current velocity at the tunnel parallel and perpendicular to the current, presence or absence of a pool-riffle-run transition, steeply cut channel (> 10% incline on both sides), and sand/gravel bar (a patch of > 1 m^2 with > 50% sand/gravel) within 5 m of the shelter, and percent forest cover in the upstream catchment-wide riparian (CWR) area (Jachowski & Hopkins, 2018) on shelter availability (Table 1.1). To ensure that our measurements accurately reflected typical stream conditions, we measured all variables that changed with flow conditions (current velocity at each shelter, shelter depth, and shelter distance from bank) when stream discharges were at their approximate annual medians (Table 1.2). Due to logistical constraints and prolonged high discharge conditions in 2018, we only measured water velocities, water depth, and shelter distance to bank at our six multi-year reaches. To measure tunnel angle, we dropped a plastic bobber attached to a string into the stream, held the string taught above the tunnel at the water’s surface, and measured the resulting angle between the tunnel and string using a protractor. We measured current velocity parallel and perpendicular to the current using a 2D FlowTracker2 Handheld-ADV flow meter (Xylem Inc.; Rye Brook, NY).

We calculated percent forest cover in the upstream CWR area in ArcMap (Environmental Systems Research Institute, Inc.; Redlands, CA), using the 2011 National Land Cover and National Hydrology Dataset (United States Geological Survey, 2011). We deemed the influence of upstream forest cover on shelter availability as potentially important because the loss of riparian forest cover is known to increase sedimentation (Collins et al., 2009; MacKenzie, 2008; Hooke, 2000; Michaelis, 1984), which may in turn increase the rate at which sediment accumulates in, and blocks, shelter tunnels. Additionally, researchers interested in using artificial shelters often plan to deploy them in reaches with declining hellbender populations, which tend to feature low upstream forest cover (Jachowski & Hopkins, 2018).

In addition to variables used as predictors for both shelter availability and stability, we assessed the influence of number of high discharge events experienced, shelter design, and whether or not a shelter was braced by at least one anchor rock (i.e., at least one large, embedded boulder placed firmly against the shelter to keep it in place during high discharge events) on shelter stability. We quantified the number of high discharge events experienced by each shelter using data from the nearest USGS gage within each respective stream (Table 1.2). If the daily discharge at the nearest USGS stream gage was > 4x the annual mean discharge for at least one full day, we considered the stream to have experienced a "high discharge" event that day, because shelters usually only became dislodged when daily discharge exceeded this value (Hopkins unpubl. data).

Data processing and analysis

We used average shelter availability (i.e., times available [*n* = 0-72] divided by times surveyed [*n* = 1-76]) and shelter stability (i.e., stable or dislodged) as response variables in all analyses, and used a multi-step procedure to model our results. After verifying the absence of redundant predictor variables (i.e., verifying that $|r| < 0.6$ for all possible pairs of predictor variables; Appendix A), we used PERMANOVA and betadisper analyses to determine whether our predictor variables were collectively related to shelter availability and stability (Dixon, 2003). PERMANOVA determines whether the average ordinated coordinates of datapoints containing multiple predictor variables are related to a chosen response variable (i.e., shelter availability or stability; analyzed separately), while betadisper analysis determines whether the dispersion of these ordinated coordinates is related to the response variable. PERMANOVA results should generally be viewed with caution when betadisper results are significant (Dixon, 2003). Before ordinating our predictor variables to conduct PERMANOVA and betadisper analyses, we randomly imputed all missing data (~10% of all values in both datasets) using random forest imputations (Stekhoven & Bühlmann, 2011), because PERMANOVA and betadisper analyses are not robust to missing data. We standardized all non-binary predictor variables prior to analysis, used Euclidean distances to construct distance matrices prior to ordination, and carried out all multivariate analyses using the 'vegan' package in R (Dixon et al., 2003; Version 3.3.3, R Core Development Team). We used non-metric multidimensional scaling (NMDS) plots to visualize the results of our PERMANOVA and betadisper analyses (Kruskal, 1964).

After validating our chosen sets of predictor variables using PERMANOVA and betadisper analyses, we used boosted regression trees (BRTs) to assess associations between individual predictor variables (Table 1.1) and shelter availability and stability, using the 'gbm' package in R (Version 3.3.3, R Core Development Team). Boosted regression trees iteratively fit decision trees to a dataset given a specified response variable, and weight the contribution of each tree as a function of how well it predicts the response variable (Elith et al., 2008). The importance and relationship of individual predictor variables with the response is assessed based on its prevalence across all weighted trees and its overall contribution to minimizing the loss function. We treated shelter stability as binomially-distributed and average shelter availability as beta-distributed in all BRT analyses. When using BRTs, no assumptions are necessary about how predictor variables are distributed in order to determine the importance and relationship of those variables with the response. Boosted regression trees were a desirable modeling approach given our study questions, because they tend to be useful for identifying ecological thresholds due to their use of split points (Elith et al., 2008), and perform better than other approaches for datasets that contain spatial structure (Crase et al., 2012).

We modelled shelter availability and stability using two separate BRTs, built initially using all applicable predictor variables, then rebuilt after discarding variables of minimal importance (i.e., < 5% relative influence on the model). Additionally, we discarded variables from our refined models if their inclusion in the model weakened its performance. We used cross validated correlation scores and standard errors to evaluate the performance of our availability BRTs (Elith et al., 2008), and used cross validated AUC scores and standard errors built using k-fold cross validation (Kohavi, 1995) to evaluate the performance of our stability BRTs. We built shelter availability models using tree complexity = 2, learning rate = 0.0005, and bag fraction = 0.5 (De'Ath, 2007), because initial model runs suggested these settings optimized model performance. For shelter stability, we built models using tree complexity = 2, learning rate = 0.01, and bag fraction = 0.75.

To account for the relationship between the number of surveys of a shelter and the expected accuracy of its estimated availability, we weighted shelters in our availability BRTs using a logarithmic scale, based on number of times surveyed (Appendix B). We developed this scale using a simulated binomial distribution, where p = average shelter availability observed across all shelters during our study. We randomly drew i = 1-76 samples (corresponding with the number of surveys conducted at individuals shelters) from this distribution, calculated the mean of these samples (i.e., number of successes divided by i), and calculated the errors of these means (i.e., their difference from the true mean specified earlier). We replicated this process 100,000 times, and used mean results from these simulations to assign a weight to each shelter in future analyses, based on the expected error in our estimate of average shelter availability at that shelter given its number of surveys, relative to if the shelter had been surveyed only once. This allowed us to filter our 6,793 total shelter surveys down to a single average value for each shelter (n = 438) while accounting for the relative amount of uncertainty in our estimate of average shelter availability at these shelters.

We evaluated the influence of individual variables based on their relative influence in our refined models and partial dependence plots, which display model predictions across the range of possible values for 1-2 predictor variables while holding all remaining predictor variables constant at their mean. We fit LOESS regression curves (Cleveland & Devlin, 1988) to all partial dependence plots to allow for generalized interpretation of our results and to serve as a visual aid. To determine whether any pairs of predictor variables had an interactive relationship with shelter availability or stability, we used the procedure described by Elith et al. (2008).

RESULTS

Individual artificial shelters had a mean availability of 78.6% (range = 0-100%), and 388 of 438 shelters (88.6%) were stable during all high discharge events experienced. The two rivers with multi-year arrays (Rivers 2 and 3) experienced respective totals of 24 and 21 high discharge events over the course of the study, while River 1 experienced only a single high discharge event after installing shelters there in May-July of 2018. Design A shelters were more likely to lose their lids than Design B (34.4% [75/218] versus 0% [0/220]), and also became dislodged much more frequently than Design B shelters (22.5% versus 0.5%). Differences in the stability of Design A and Design B increased with time since deployment (Fig. 1.2), and 80% (40/50) of unstable shelters were dislodged within the first 11 high stream discharge events experienced following their deployment.

Our multivariate analyses strongly suggested that shelter characteristics were related to average shelter availability ($F = 5.29$ and $p = 0.001$ for betadisper; $F = 4.66$ and $p = 0.001$ for PERMANOVA) and stability ($F = 59.33$ and $p < 0.001$ for betadisper; $F = 6.28$ and $p < 0.001$ for PERMANOVA). Betadisper analyses provided particularly strong evidence that the dispersion of our ordinated predictor variables was related to shelter availability (r = 0.31 between average shelter availability and distance to median centroid among our ordinated predictor variables) and stability (average distance to centroid = 3.35 NMDS units for stable shelters versus 2.00 for dislodged shelters). However, given the clear results of our betadisper analyses, our PERMANOVA results should be treated with caution. Using $k \geq 4$ dimensions was necessary when building our shelter availability NMDS plots (Appendix C) to ensure that stress < 0.2, while $k = 2$ dimensions were adequate for shelter stability. The influence of shelter availability and stability on datapoint dispersion suggested that our two sets of predictor variables were collectively informative of their respective response variables, and therefore appropriate to use in future BRT models.

Factors influencing shelter availability

After removing unimportant variables, we retained shelter maintenance frequency, number of boulders within 1 m, percent upstream CWR forest cover, and tunnel angle as informative predictors in our final shelter availability BRTs (Fig. 1.3). Shelter availability increased with number of boulders within 1 m, upstream CWR forest cover, and tunnel direction relative to the direction of stream current (i.e., tunnel angle; up to at least 65°), and was inversely related to average number of days between shelter maintenance. Days between shelter maintenance, number of boulders within 1 m, and upstream CWR forest cover contributed the most to the model, and had respective relative influences of 31.3%, 29.2%, and 24.1%. We found no evidence of pairwise interactions among our predictor variables. Model estimates of shelter availability yielded a cross-validated correlation of 0.40 (SE = 0.04), thus our model predictions were 40% correlated with actual average availability values at artificial shelters.

Factors influencing shelter stability

We retained density of reach-wide boulders > 40 cm long, number of high discharge events experienced, number of boulders within 1 m, and shelter design as informative predictors in our final BRTs for shelter stability (Fig. 1.4). Shelter stability was highest when shelters were built using Design B and deployed in reaches with high densities of large boulders (i.e., > 68.5 large boulders encountered on 10 equally-spaced transects across the reach), and dislodged shelters were usually lost within the first 11 high discharge events they experienced. Although shelter stability was related to the number of boulders within 1 m, the relative influence of this predictor was relatively small (10.1%), and the directionality of this relationship was inconsistent (Fig. 1.4). In total, 77.5% of Design A shelters and 99.6% of Design B shelters were stable across all high discharge events experienced. Number of high discharge events experienced (49.2% variable influence) and shelter design (24.3% variable influence) had the highest relative influences in the stability BRT model (Fig 1.5). Although instream variables substantially influenced shelter stability for Design A, there were strong interactions between shelter design and our other predictor variables, and the predicted stability of Design B never dropped below 97.0% (Fig. 1.4). Model predictions of shelter stability were exceptionally accurate (cross-validated AUC = 0.91, SE = 0.02).

DISCUSSION

We sought to determine how artificial shelters should be built and deployed to maximize their availability to hellbenders and stability during high stream discharge events. We found that shelter availability was driven primarily by shelter maintenance frequency, number of boulders within 1 m, percent upstream CWR forest cover, and tunnel angle relative to stream flow. Together, these observations provide evidence that shelters can be made available to hellbenders under circumstances of modest stream impairment if deployed optimally and maintained with sufficient frequency. Further, our modified shelter design (Design B) was more stable than our original design (Design A), demonstrating that the stability of boot design shelters can be greatly enhanced with simple modifications to construction. In light of the problems encountered during the initial years of artificial shelter use for hellbenders (Jachowski, 2016; Messerman, 2014), our solutions to these problems under some field conditions is encouraging.

Artificial shelters have the potential to improve hellbender monitoring capabilities only if their entrances remain unblocked by sediment. Shelter maintenance frequency was an important driver of shelter availability, and the relationships revealed by our availability BRTs (Fig. 1.3) suggested that maintaining shelters every 40 days was usually sufficient to keep their entrances unblocked > 75% of the time. This is less often than the maintenance frequency required for certain other traps and enclosures that are commonly used in streams (Jung et al., 2000; Pauley & Little, 1998; Beachy, 1997). The frequency of shelter maintenance required to keep shelter tunnels unblocked is likely lessened during periods of low or average stream discharge, as we found that many of our shelters only became blocked during high discharge events. Additionally, while most groups using artificial shelters have oriented shelter tunnels directly downstream, our finding that shelter availability increased with tunnel angle relative to current direction (up to at least 65°) suggests that to keep shelter tunnels unblocked, it may be useful to minimize the occurrence of sediment-depositing microcurrents around the tunnel entrance.

Because siltation is the principal cause of shelter blockages, the utility of artificial shelters may be limited in heavily impacted systems where the loss of riparian buffers or other disturbances has increased sediment loading in the stream. Our finding that shelter availability decreased sharply with loss of upstream forest cover is a likely consequence of riparian buffer removal, and is unfortunate given that riparian deforestation, and subsequent siltation, are often highest in watersheds where hellbenders are most at risk of extirpation (Wheeler et al., 2003; Bothner & Gottlieb, 1991; Williams et al., 1981). Thus, a conflict exists between the need for effective hellbender monitoring tools and the practicality of using artificial shelters to fulfil this need in heavily impacted streams. To further illustrate this limitation, we piloted the use of artificial shelters in a silty, heavily impacted reach with low upstream forest cover (57.3%) in the Upper New River Basin. Ninety percent of shelter entrances became blocked by sediment within a week of shelter maintenance under base flow conditions, far more rapidly than at any of our upper Tennessee River Drainage arrays. The rapidity with which shelter tunnels became blocked made shelters impractical to maintain and ultimately forced us to abandon the pilot array, highlighting the limits of artificial shelters in exceptionally silty environments. It remains unclear whether siltation rates and loss of riparian forest cover must reach a threshold before the use of shelters becomes infeasible, especially because other site characteristics such as stream order and gradient likely influence sediment deposition. Thus, it may be useful to pilot the use of a few shelters at impaired hellbender-inhabited stream reaches of interest prior to investing resources towards deploying entire arrays in such reaches.

While the availability of artificial shelters was constrained by environmental conditions such as sediment loading, we found that shelter stability was achievable > 99% of the time by using our modified shelter design (Design B) and following standard shelter installation procedures, such as embedding shelters in the streambed and anchoring them firmly in place. The near-perfect stability of Design B shelters is impressive given that they endured several severe flood events, including multiple tropical depressions and heavy spring thunderstorms that increased stream discharges at nearby USGS gages (Tables 1.3, 1.4) up to ~20x their mean level (U.S. Geological Survey, 2019), and displaced large boulders and substantially altered channel geomorphology in some places (pers. obs.). Despite the strength of flood events during our study, only 0.5% (1/220) of Design B shelters ever became dislodged, in contrast with the 22.5% (49/218) dislodgement observed for Design A (Fig. 1.2). The superiority of Design B is also evident in the fact that their predicted stability never dropped below 97% in our analyses, regardless of the values of all other predictor variables (Fig. 1.4).

We attribute observed differences in the stability of our two shelter designs to the superior structural integrity and recessed lids of Design B. Specifically, Design B shelters have 1-2 cm thicker walls and are ~15 kg heavier than those built using Design A, and have multiple apparent advantages in their lid design. Design B lids have the advantage of being made from a mold, making it possible to replace dislodged lids quickly in the field without having to remove the shelter from the stream to build a new custom-fitting lid. Remarkably, however, we never had to replace the lid of a Design B shelter, despite needing to do so at 34.4% of shelters built using Design A. We believe this occurred because the recessed nature of Design B lids caused them to experience less drag force than Design A, which likely in turn reduced the amount of force exerted onto them by the current (Dey, 2014). Additionally, Design B lids are anchored in place using an eye-bolt and hook, and often become locked in place when the seam between the lid and shelter fills with sand particles, sometimes requiring a sturdy tool (e.g., screwdriver) to pry loose. Design A shelters, by contrast, are held in place with stainless steel brackets that tend to rust and eventually break, and lack the exposed seam between the lid and shelter necessary for accumulating sand particles to lock the lid in place. The superiority of Design B lids has important ramifications for shelter stability, because shelters prone to losing their lids were the ones that became dislodged most often during high discharge events (pers. obs.), suggesting that lid loss increases the odds of shelter dislodgement. Thus, the instability of Design A shelters would likely have been even worse in our study had we not acted to minimize shelter dislodgement immediately following high discharge events by locating and re-attaching lids that had been swept off of Design A shelters.

Although we did not set out to evaluate the structural integrity of the artificial shelters after prolonged deployment in the field, our anecdotal observations suggest that Design B shelters will also be longer-lived than Design A shelters. Even when stable, Design A shelters often developed exposed metal within five years of deployment, and had to be removed due to safety concerns for occupying animals. While we deployed Design B shelters more recently, within the past three years, we do not expect them to deteriorate as quickly as Design A given their thicker walls, because concrete thickness and deterioration rates of instream structures are inversely related (Zhao & Chen, 2001).

Because Design A shelters are already deployed in many watersheds across the hellbender’s geographic range, our findings point to multiple factors that will improve their utility. Our results suggest that the instability of Design A shelters can be partly mitigated by keeping productive (i.e., used, available, and undamaged) shelters that have survived numerous high discharge events in place (Fig. 1.4), since 80% of shelters destined for dislodgement were lost prior to a threshold of number of high discharge events in our study (> 11). In addition, our results suggest that shelter stability improves considerably when shelters are placed in reaches with high densities of large boulders (i.e., > 68.5 large boulders encountered during 10 equally-spaced transects across the reach [16.5% relative influence]), possibly because boulders serve as roughness elements in stream substrate that reduce average current velocity (Ferguson, 2007), which should thereby reduce the amount of force exerted against artificial shelters. For example, we have anecdotally observed decreased shelter stability in reaches that lack these roughness elements and consist mostly of bedrock.

Given the high stability of properly installed Design B shelters, our results suggest that struggles caused by shelter dislodgement in prior studies (Jachowski, 2016; Messerman, 2014) are potentially mitigated by simple adjustments to artificial shelter design and installation. Ongoing efforts to develop entirely new artificial shelter designs, such as the hydrodynamic shelters (Mohammed et al., 2016), will hopefully yield similar promising results. Additional research is needed to determine whether additional design elements can reduce sediment blockage of tunnels, though we suspect that factors related to reach-level sedimentation and microhabitat features at the tunnel entrance will influence tunnel availability regardless of shelter design. Moreover, features of different artificial shelter designs that influence their attractiveness to hellbenders require future assessment.

Our study is the first to quantitatively evaluate how to construct artificial shelters to maximize their availability to hellbenders and stability during high discharge events, and thus their overall utility for monitoring hellbenders. Assuming that hellbenders use them, boot design shelters are practical hellbender monitoring tools when constructed using recessed lids and 3-4 cm thick walls (Design B), maintained at least once every 40 days in reaches with moderate or high upstream forest cover, oriented with a tunnel angle offset by 45-65+° from stream flow, and installed with at least five adjacent boulders (Table 1.3). Future studies should verify the generalizability of our findings in streams with different hydrological and geomorphic characteristics. In addition to improving the feasibility of using artificial shelters to study hellbenders, our study provides a useful starting point for those interested in developing novel techniques to study ecologically similar lotic species. Much like hellbenders, several other large aquatic salamanders, fish, and large crustaceans inhabiting rocky lotic environments are difficult to study using conventional sampling methods (Rice et al., 2018; Ferreira et al., 2016; Comte & Grenouillet, 2013). Future studies should therefore consider assessing the availability and stability of artificial shelters designed for additional aquatic taxa.

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REFERENCES

Alford, R. A., and Richards, S. J. (1999). Global amphibian declines: a problem in applied ecology. *Annual review of Ecology and Systematics* 30: 133-165.

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26: 32-46.

Bailey, L. L., Simons, T. R., and Pollock, K. H. (2004). Estimating site occupancy and species detection probability parameters for terrestrial salamanders. *Ecological Applications* 14: 692-702.

Beachy, C. K. (1997). Effect of predatory larval *Desmognathus quadramaculatus* on growth, survival, and metamorphosis of larval *Eurycea wilderae*. *Copeia*: 131-137.

Beffa, D. A. (1976). Responses of *Cryptobranchus alleganiensis alleganiensis* to different oxygen concentrations, temperatures and photoperiod. Southwest Missouri State University.

Blaustein, A. R., Han, B. A., Relyea, R. A., Johnson, P. T., Buck, J. C., Gervasi, S. S., and Kats, L. B. (2011). The complexity of amphibian population declines: understanding the role of cofactors in driving amphibian losses. *Annals of the New York Academy of Sciences* 1223: 108-119.

Bothner, R. C., and Gottlieb, J. A. (1991). A study of the New York State populations of the Hellbender, *Cryptobranchus alleganiensis alleganiensis*. Proceedings of the Rochester Academy of Science.

Briggler, J., and Ackerson, J. (2012). Construction and use of artificial shelters to supplement habitat for Hellbenders (*Cryptobranchus alleganiensis*). *Herpetological Review* 43: 412.

Briggler, J., Utrup, J., Davidson, C., Humphries, J., Groves, J., Johnson, T., Ettling, J., Wanner, M., Traylor-Holzer, K., and Reed, D. (2007). Hellbender population and habitat viability assessment: final report. IUCN/SSC Conservation Breeding Specialist Group, Apple Valley, MN.

Browne, R., Li, H., Mcginnity, D., Okada, S., Zhenghuan, W., Bodinof, C., Irwin, K., Mcmillan, A., and Briggler, J. (2011). Survey techniques for giant salamanders and other aquatic Caudata. *Amphibian & Reptile Conservation* 5: 1-16.

Cleveland, W. S., and Devlin, S. J. (1988). Locally weighted regression: an approach to regression analysis by local fitting. *Journal of the American Statistical Association* 83: 596-610.

Collins, A., Anthony, S., Hawley, J., and Turner, T. (2009). The potential impact of projected change in farming by 2015 on the importance of the agricultural sector as a sediment source in England and Wales. *Catena* 79: 243-250.

Comte, L., and Grenouillet, G. (2013). Species distribution modeling and imperfect detection: comparing occupancy versus consensus methods. *Diversity & Distributions* 19: 996-1007.

Crase, B., Liedloff, A. C., and Wintle, B. A. (2012). A new method for dealing with residual spatial autocorrelation in species distribution models. *Ecography* 35: 879-888.

De'ath, G. (2007). Boosted trees for ecological modeling and prediction. *Ecology* 88: 243-251.

Dey, S. (2014). *Fluvial Hydrodynamics*. New York, NY: Springer.

Dixon, P. (2003). VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science* 14: 927-930.

Elith, J., Leathwick, J. R., and Hastie, T. (2008). A working guide to boosted regression trees. *Journal of Animal Ecology* 77: 802-813.

Ferguson, R. (2007). Flow resistance equations for gravel-and boulder-bed streams. *Water Resources Research* 43: 1-12.

Ferreira, M., Filipe, A. F., Bardos, D. C., Magalhaes, M. F., and Beja, P. (2016). Modeling stream fish distributions using interval-censored detection times. *Ecology & Evolution* 6: 5530-5541.

Grant, E. H. C., Miller, D. A., Schmidt, B. R., Adams, M. J., Amburgey, S. M., Chambert, T., Cruickshank, S. S., Fisher, R. N., Green, D. M., and Hossack, B. R. (2016). Quantitative evidence for the effects of multiple drivers on continental-scale amphibian declines. *Scientific Reports* 6: 25625.

Hillis, R. E., and Bellis, E. D. (1971). Some aspects of the ecology of the hellbender, *Cryptobranchus alleganiensis alleganiensis*, in a Pennsylvania stream. *Journal of Herpetology*: 121-126.

Hooke, R. L. (2000). On the history of humans as geomorphic agents. *Geology* 28: 843-846.

Hopkins, W. A. (2007). Amphibians as models for studying environmental change. *Ilar Journal* 48: 270-277.

Houlahan, J. E., Findlay, C. S., Schmidt, B. R., Meyer, A. H., and Kuzmin, S. L. (2000). Quantitative evidence for global amphibian population declines. *Nature* 404: 752.

Humphries, W. (1999). Ecology and population demography of the hellbender, *Cryptobranchus alleganiensis.* M.S. Thesis. Marshall University.

Iucn. (2018). The IUCN Red List of Threatened Species.

Jachowski, C. M. B. (2016). Effects of Land Use on Hellbenders (*Cryptobranchus alleganiensis*) at Multiple Levels and Efficacy of Artificial Shelters as a Monitoring Tool. Dissertation. Virginia Polytechnic Institute and State University.

Jachowski, C. M. B., and Hopkins, W. A. (2018). Loss of catchment-wide riparian forest cover is associated with reduced recruitment in a long-lived amphibian. *Biological Conservation* 220: 215-227.

Jung, R. E., Droege, S., Sauer, J. R., and Landy, R. B. (2000). Evaluation of terrestrial and streamside salamander monitoring techniques at Shenandoah National Park. *Environmental Management* 63: 65-79.

Keitzer, S. C. (2007). Habitat preferences of the eastern hellbender in West Virginia. M.S. Thesis. Marshall University.

Keitzer, S. C., Pauley, T. K., and Burcher, C. L. (2013). Stream characteristics associated with site occupancy by the eastern hellbender, *Cryptobranchus alleganiensis alleganiensis*, in southern West Virginia. *Northeastern Naturalist* 20: 666-677.

Kellner, K. F., and Swihart, R. K. (2014). Accounting for imperfect detection in ecology: a quantitative review. *PLoS One* 9: 1-8.

Kéry, M., and Schmidt, B. (2008). Imperfect detection and its consequences for monitoring for conservation. *Community Ecology* 9: 207-216.

Kohavi, R. (1995). A study of cross-validation and bootstrap for accuracy estimation and model selection. International Joint Conference on Artificial Intelligence.

Kriger, K. M., and Hero, J. M. (2007). The chytrid fungus *Batrachochytrium dendrobatidis* is non-randomly distributed across amphibian breeding habitats. *Diversity & Distributions* 13: 781-788.

Kruskal, J. B. (1964). Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. *Psychometrika* 29: 1-27.

Mazerolle, M. J., Bailey, L. L., Kendall, W. L., Royle, J. A., Converse, S. J., and Nichols, J. D. (2007). Making great leaps forward: accounting for detectability in herpetological field studies. *Journal of Herpetology* 41: 672-690.

Measey, G., Gower, D., Oommen, O., and Wilkinson, M. (2003). Quantitative surveying of endogeic limbless vertebrates—a case study of *Gegeneophis ramaswamii* (Amphibia: Gymnophiona: Caeciliidae) in southern India. *Applied Soil Ecology* 23: 43-53.

Mensing, D., Galatowitsch, S., and Tester, J. (1998). Anthropogenic effects on the biodiversity of riparian wetlands of a northern temperate landscape. *Journal of Environmental Management* 53: 349-377.

Messerman, A. (2014). The Use of Nest Boxes by the Hellbender Salamander in Western North Carolina. M.S. Thesis. Duke University.

Michaelis, F. B. (1984). Possible effects of forestry on inland waters of Tasmania: a review. *Environmental Conservation* 11: 331-344.

Mohammed, M. G., Messerman, A. F., Mayhan, B. D., and Trauth, K. M. (2016). Theory and Practice of the Hydrodynamic Redesign of Artificial Hellbender Habitat. *Herpetological Review* 47: 586-591.

Nickerson, M. A., Krysko, K. L., and Owen, R. D. (2003). Habitat differences affecting age class distributions of the hellbender salamander, *Cryptobranchus alleganiensis*. *Southeastern Naturalist* 2: 619-629.

Nickerson, M. A., and Mays, C. E. (1973). The hellbenders: North American" giant salamanders".

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'hara, R., Simpson, G. L., Solymos, P., Stevens, M. H. H., and Wagner, H. J. R. P. V. (2011). vegan: Community ecology package. 117-118.

Olson, D. H., Anderson, P. D., Frissell, C. A., Welsh Jr, H. H., and Bradford, D. F. (2007). Biodiversity management approaches for stream–riparian areas: perspectives for Pacific Northwest headwater forests, microclimates, and amphibians. *Forest Ecology & Management* 246: 81-107.

Pauley, T. K., and Little, M. (1998). A new technique to monitor larval and juvenile salamanders in stream habitats. *Banisteria* 12: 32-36.

Rice, C. J., Larson, E. R., and Taylor, C. A. (2018). Environmental DNA detects a rare large river crayfish but with little relation to local abundance. *Freshwater Biology* 63: 443-455.

Rudh, A., and Qvarnström, A. (2013). Adaptive colouration in amphibians. *Seminars in Cell & Developmental Biology* 24: 553-561.

Scheele, B. C., Pasmans, F., Skerratt, L. F., Berger, L., Martel, A., Beukema, W., Acevedo, A. A., Burrowes, P. A., Carvalho, T., and Catenazzi, A. (2019). Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science* 363: 1459-1463.

Stekhoven, D. J., and Bühlmann, P. (2011). MissForest—non-parametric missing value imputation for mixed-type data. *Bioinformatics* 28: 112-118.

Trauth, S. E., Wilhide, J., and Daniel, P. (1992). Status of the Ozark hellbender, *Cryptobranchus bishopi* (Urodela: Cryptobranchidae), in the Spring River, Fulton County, Arkansas. *Journal of the Arkansas Academy of Science* 46: 83-86.

U.S. Geological Survey, U. S. D. O. I. (2019). USGS Water Data for the Nation.

Wells, K. D. (2010). *The Ecology and Behavior of Amphibians*. Chicago, IL, USA: University of Chicago Press.

Welsh Jr, H. H., and Ollivier, L. M. (1998). Stream amphibians as indicators of ecosystem stress: a case study from California's redwoods. *Ecological Applications* 8: 1118-1132.

Wheeler, B. A., Prosen, E., Mathis, A., and Wilkinson, R. F. (2003). Population declines of a long-lived salamander: a 20+-year study of hellbenders, *Cryptobranchus alleganiensis*. *Biological Conservation* 109: 151-156.

Williams, R. D., Gates, J. E., Hocutt, C. H., and Taylor, G. J. (1981). The hellbender: a nongame species in need of management. *Wildlife Society Bulletin*: 94-100.

Willson, J. D., Winne, C. T., and Fedewa, L. A. (2005). Unveiling escape and capture rates of aquatic snakes and salamanders (Siren spp. and *Amphiuma means*) in commercial funnel traps. *Journal of Freshwater Ecology* 20: 397-403.

Wolman, M. G. (1954). A method of sampling coarse river-bed material. *EOS, Transactions American Geophysical Union* 35: 951-956.

Zhao, Z., and Chen, C. (2001). Concrete bridge deterioration diagnosis using fuzzy inference system. *Advances in Engineering Software* 32: 317-325.

TABLES

Table 1.1. Mean values and ranges for all variables used in our shelter availability and stability analyses. Variables of binary (present/absent) character are coded as 0 or 1, therefore their mean values represent the percentage of shelters where we considered them present. For our assessments of metrics, “visual” = not requiring any specialized tool to measure; “computed” = not directly measured during fieldwork but calculated thereafter; “tape measure” = measured linear distance using a tape measure; “see methods” = described previously in methods section.

Variable	Mean, or Probability of Presence	Range	Variable Type	Analyses Used In	Assessment of Metric
Channel transition status	0.47	0 or 1	Binary	Availability	Visual
Pool-riffle-run transition status	0.15	0 or 1	Binary	Availability	Visual
Sand/gravel bar transition status	0.36	0 or 1	Binary	Availability	Visual
Average days between shelter maintenance	36.93	3.00-112.00	Continuous	Availability	Computed
Distance to bank (m)	3.70	0.10-9.30	Continuous	Availability	Tape Measure
Tunnel angle (degrees)	24.65	0.00-105.00	Continuous	Availability	See Methods
Upstream CWR forest cover (%)	62.6	53.6-70.4	Continuous	Availability	See Methods
Water depth at tunnel (cm)	44.21	19.00-103.00	Continuous	Availability	Meter Stick
Current velocity parallel to current (m/s)	0.28	-0.13-1.10	Continuous	Availability	See Methods
Current velocity perpendicular to current (m/s)	0.13	0.00-0.66	Continuous	Availability	See Methods
Anchor rock status	0.22	0 or 1	Binary	Stability	Visual
Design	Design A: 218 Design B: 220	NA	Category	Stability	NA
High discharge events experienced	7.37	0-24	Count	Stability	See Methods
Boulders within 1 m	5.42	0-11	Count	Both	Visual
Reach-wide large boulder density (count from reach-wide survey)	36.98	14-85	Count	Both	See Methods

Table 1.2. Median, minimum, and maximum daily discharges (in m^3/s) over the period of shelter deployment for each river containing artificial shelter arrays, and ranges of distances to the nearest USGS gage for study reaches in each river. We calculated predictor variables that varied with stream discharge when the discharge of each river was at its approximate annual median. High maximum daily discharges (relative to the median) over the course of the study illustrate the flashy, flood-prone nature of our study rivers. All River 1 and River 2 study reaches were located upstream of the nearest USGS gage. On River 1, two study reaches were located 8.72-12.34 channel km upstream of the nearest USGS gage, and three were located 0.05-17.72 km downstream of the gage.

River	Period of Shelter Deployment	Median	Minimum	Maximum	Channel km Between USGS Gage and Study Reaches (Range)
River 1	June 2018 – Present	2.95	1.64	11.78	23.28-40.77
River 2	June 2014 – Present	3.26	0.85	121.20	15.33-22.75
River 3	May 2013 – Present	2.38	0.65	51.54	0.05-17.72

Table 1.3. Recommendations for artificial shelter placement and post hoc decision-making, given the explicit objective of maximizing shelter availability and stability. We defined the importance of each recommendation qualitatively, based on a combination of the relative influence of each variable in our availability or stability BRTs and the effect size of each variable's influence.

Availability

Variable	Recommendation	Importance
Maintenance Frequency	Check artificial shelters and clear blocked tunnels as often as feasible, but at least every 40 days.	Very High
Adjacent Boulders	Situate shelters within 1 m of at least five large boulders	Very High
Habitat Quality	Pilot the use of a few shelters in impaired reaches with low upstream CWR forest cover and high sediment loads, before committing resources to deploying entire arrays at these reaches. Maintain shelters in impaired reaches more frequently than elsewhere.	Very High
Tunnel Angle	Orient shelters such that tunnel angle is 45-65+° but no more than 90°.	Moderate

Stability

Variable	Recommendation	Importance
Shelter Design	Build shelters with thick walls and recessed lids anchored by an eye-bolt and hook (Design B).	Very High
High Discharge Events Experienced	Do not move productive (i.e. used, available, and undamaged) shelters that have survived > 11 high discharge events.	Very High
Reach-wide Large Boulder Density	If Design A shelters are the only ones available, they may be most stable in reaches with high densities of boulders which likely serve as roughness elements in the stream substrate	High

FIGURES

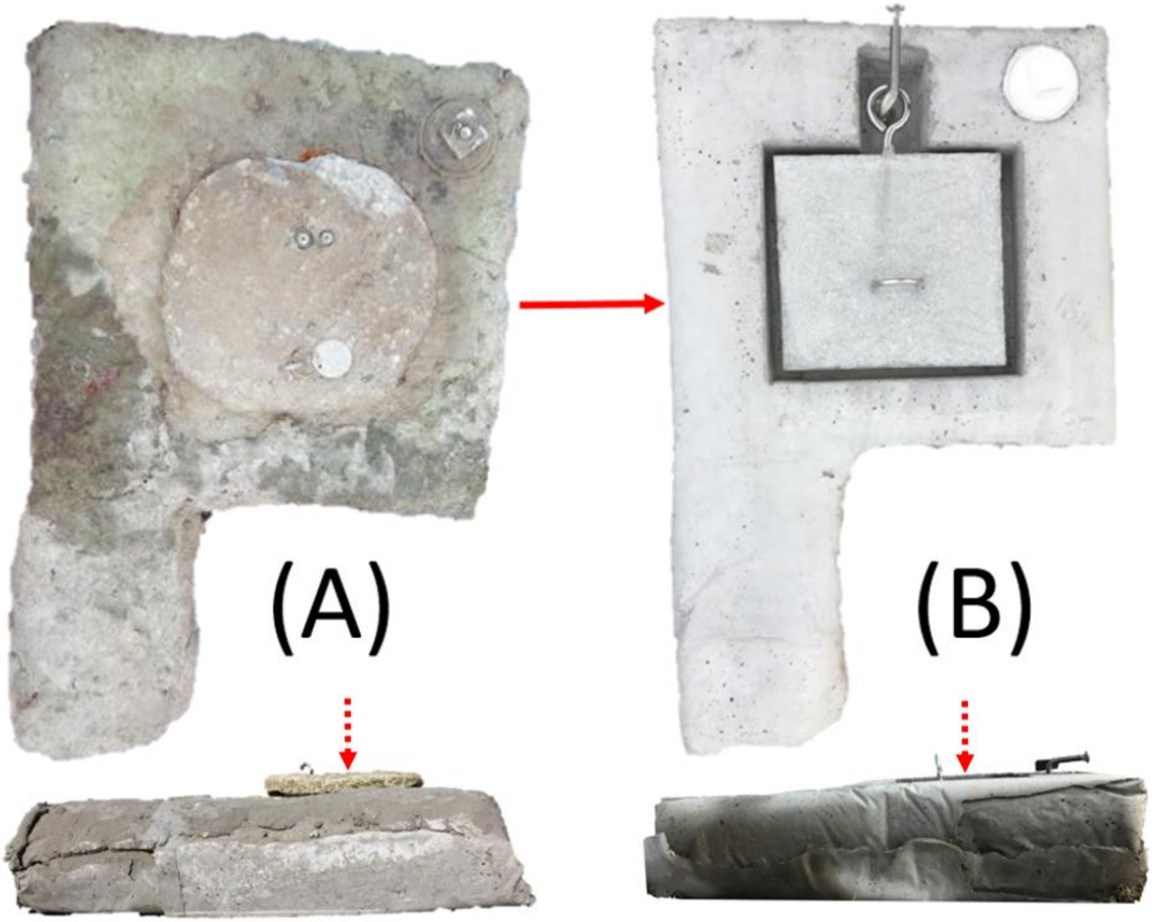


Fig. 1.1. Differences in shelter design between Design A (old design; *n* = 218) and Design B (new design; *n* = 220) artificial shelters deployed to sample hellbenders in the upper Tennessee River Basin.

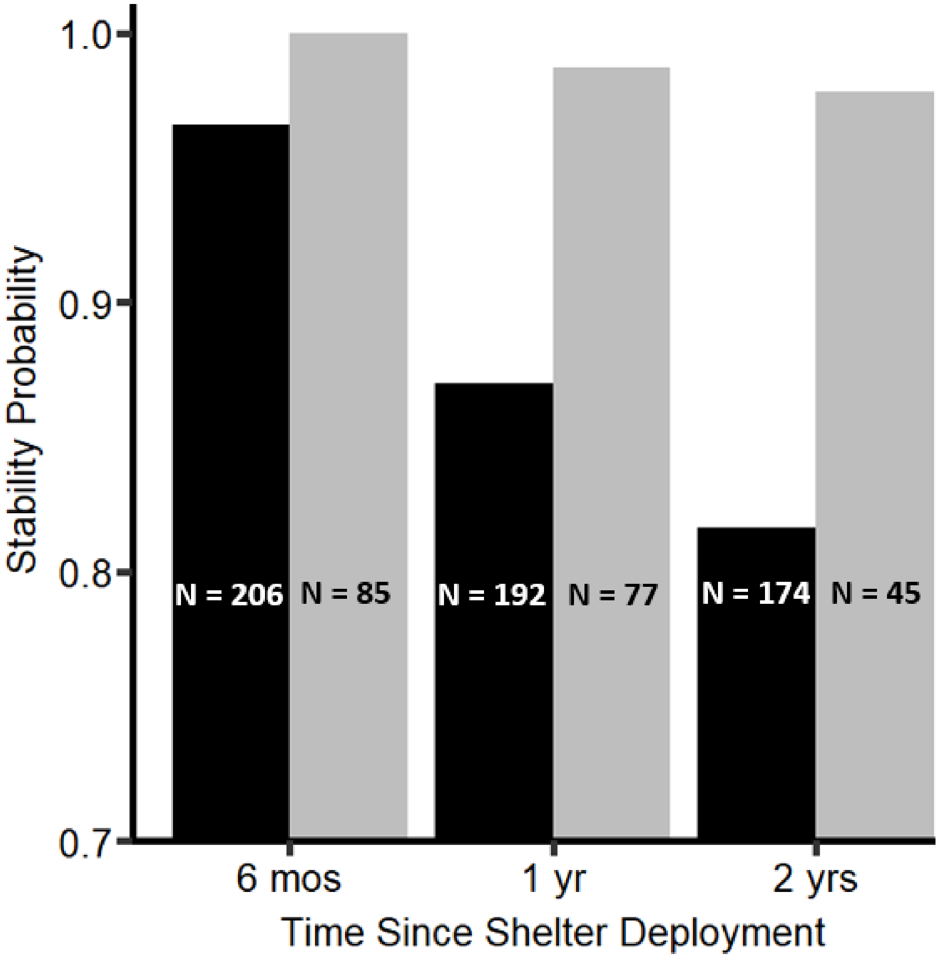


Fig. 1.2. Shelter stability for Design A (black) and Design B (grey) hellbender artificial shelters deployed in the upper Tennessee River Basin, six months, one year, and two years after deployment.

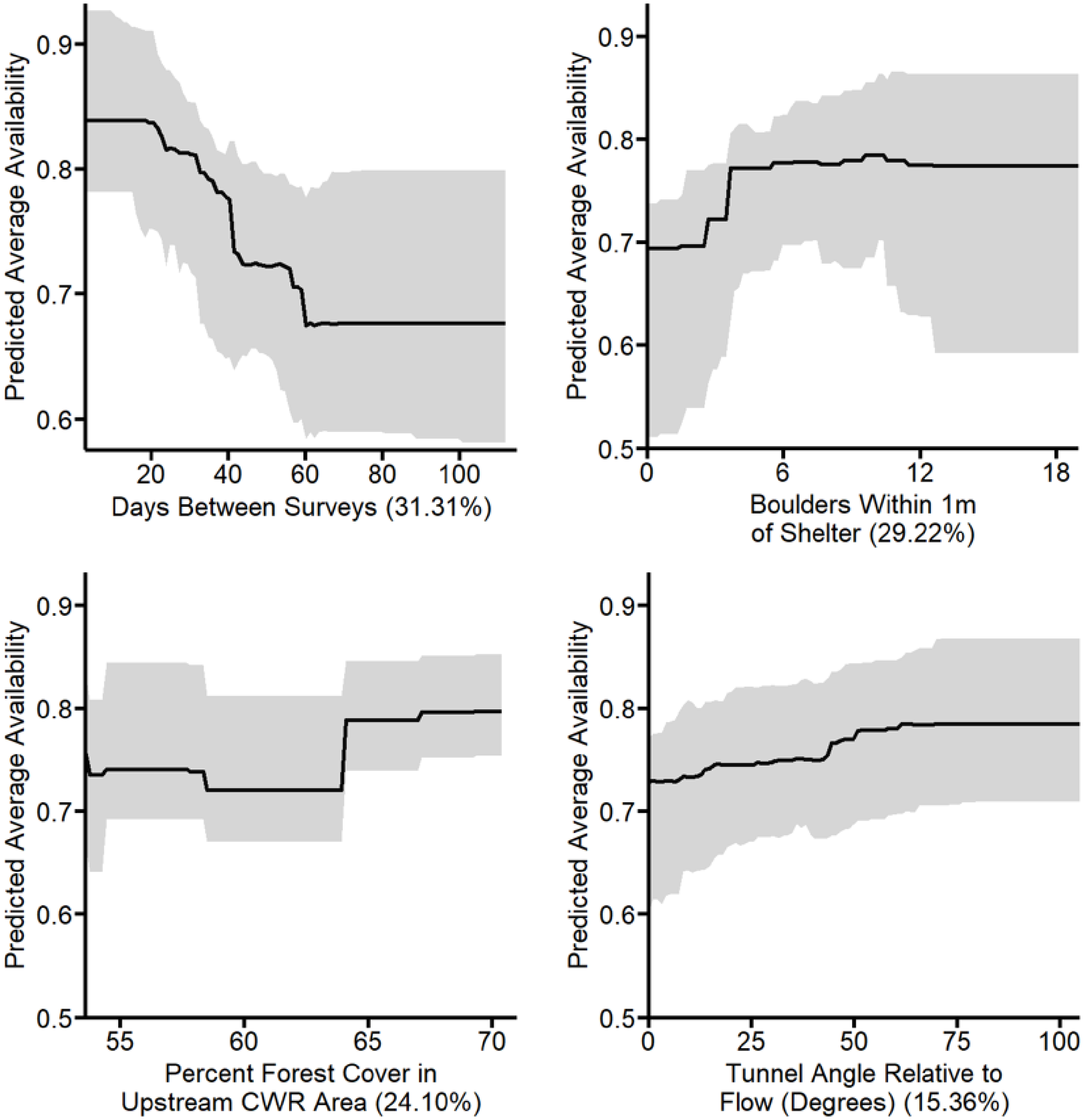


Fig. 1.3. Partial dependence plots with back-transformed shelter availability predictions for important predictor variables retained in the final version of our shelter availability model. Percentages shown on the x-axis represent relative variable influence. Solid black lines show fitted functions, while shaded areas represent 95% percentile-based confidence intervals built using 200 bootstraps.

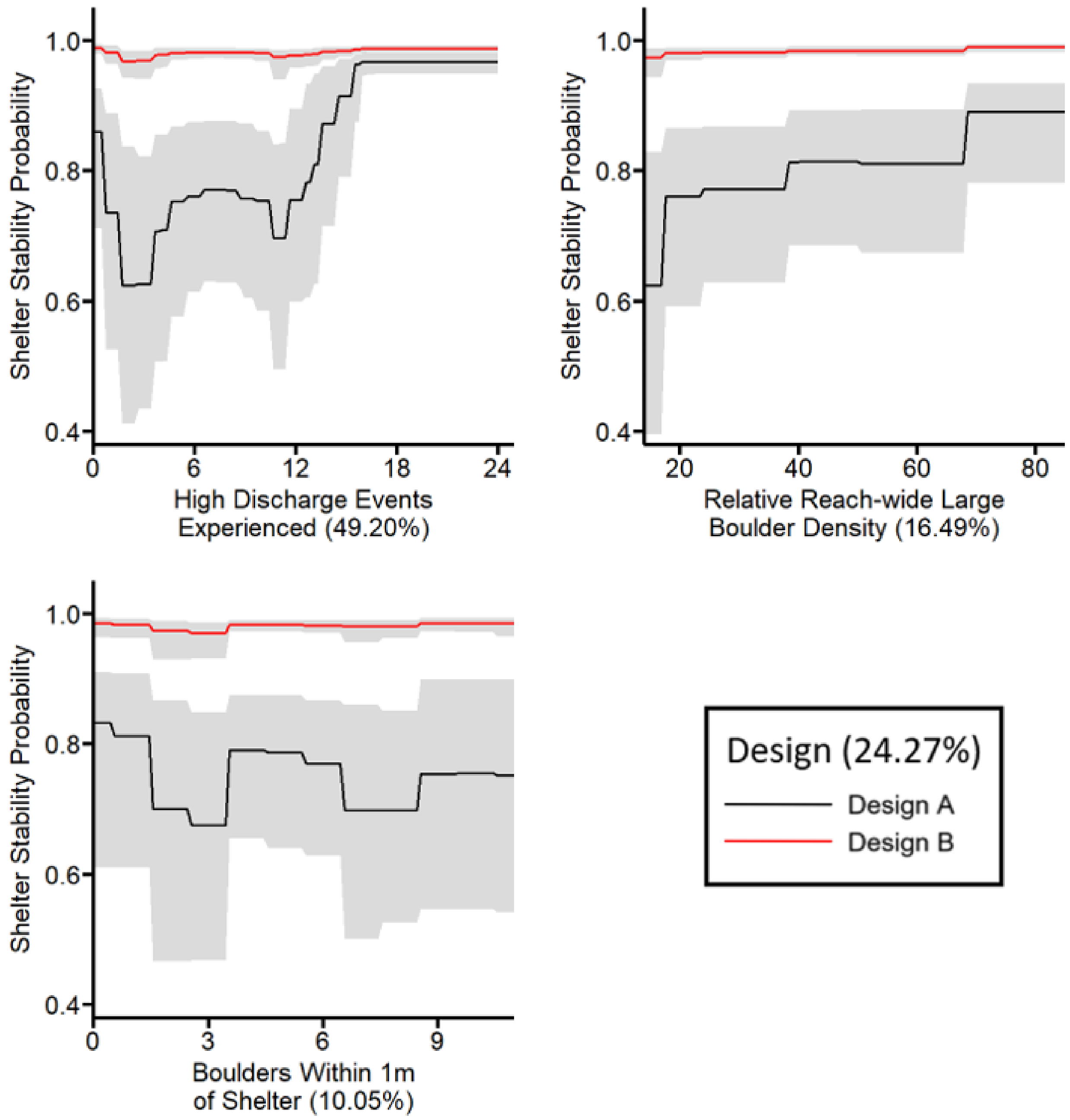


Fig. 1.4. Plots of the interactive relationships of shelter design and all other relevant predictor variables with shelter stability. Percentages shown on the x-axis represent relative variable influence. Solid lines show fitted functions, while shaded areas represent 95% percentile-based confidence intervals built using 200 bootstraps. Predicted shelter stability was always at least

97% for Design B, but increased with number of high discharge events survived and reach-wide large boulder density for Design A.

APPENDIX A: CORRELATION MATRICES

	Freq	PRRtrans	SandGrav	Channel	Angle	Depth	ParVel	PerpVel	DistBank	AnchorRock	USForCov	RWBould	AdjBould
Freq	1	0.19	-0.06	0.04	0.05	0.13	0.06	0.11	0.05	0.10	-0.05	-0.04	-0.14
PRRtrans	0.19	1	0.01	0.12	0.04	0.17	0.06	0.10	0.01	-0.04	-0.25	-0.15	0.05
SandGrav	-0.06	0.01	1	0.25	0.13	0.07	-0.18	-0.1	-0.02	0.01	-0.19	-0.10	-0.08
Channel	0.04	0.12	0.25	1	0.16	0.47	-0.01	0.01	-0.20	0.02	-0.28	0.05	0.08
Angle	0.05	0.04	0.13	0.16	1	0.16	0.04	-0.04	-0.12	0.13	0.01	-0.09	-0.18
Depth	0.13	0.17	0.07	0.47	0.16	1	0.22	0.23	0.00	0.03	-0.59	-0.10	0.18
ParVel	0.06	0.06	-0.18	-0.01	0.04	0.22	1	0.52	0.08	0.01	-0.21	0.23	0.01
PerpVel	0.11	0.10	-0.10	0.01	-0.04	0.23	0.52	1	0.02	0.01	-0.10	0.26	-0.03
DistBank	0.05	0.01	-0.02	-0.2	-0.12	0.00	0.08	0.02	1	0.16	0.17	-0.13	-0.02
AnchorRock	0.10	-0.04	0.01	0.02	0.13	0.03	0.01	0.01	0.16	1	0.15	0.02	-0.32
USForCov	-0.05	-0.25	-0.19	-0.28	0.01	-0.59	-0.21	-0.10	0.17	0.15	1	0.12	-0.15
RWBould	-0.04	-0.15	-0.10	0.05	-0.09	-0.10	0.23	0.26	-0.13	0.02	0.12	1	0.05
AdjBould	-0.14	0.05	-0.08	0.08	-0.18	0.18	0.01	-0.03	-0.02	-0.32	-0.15	0.05	1

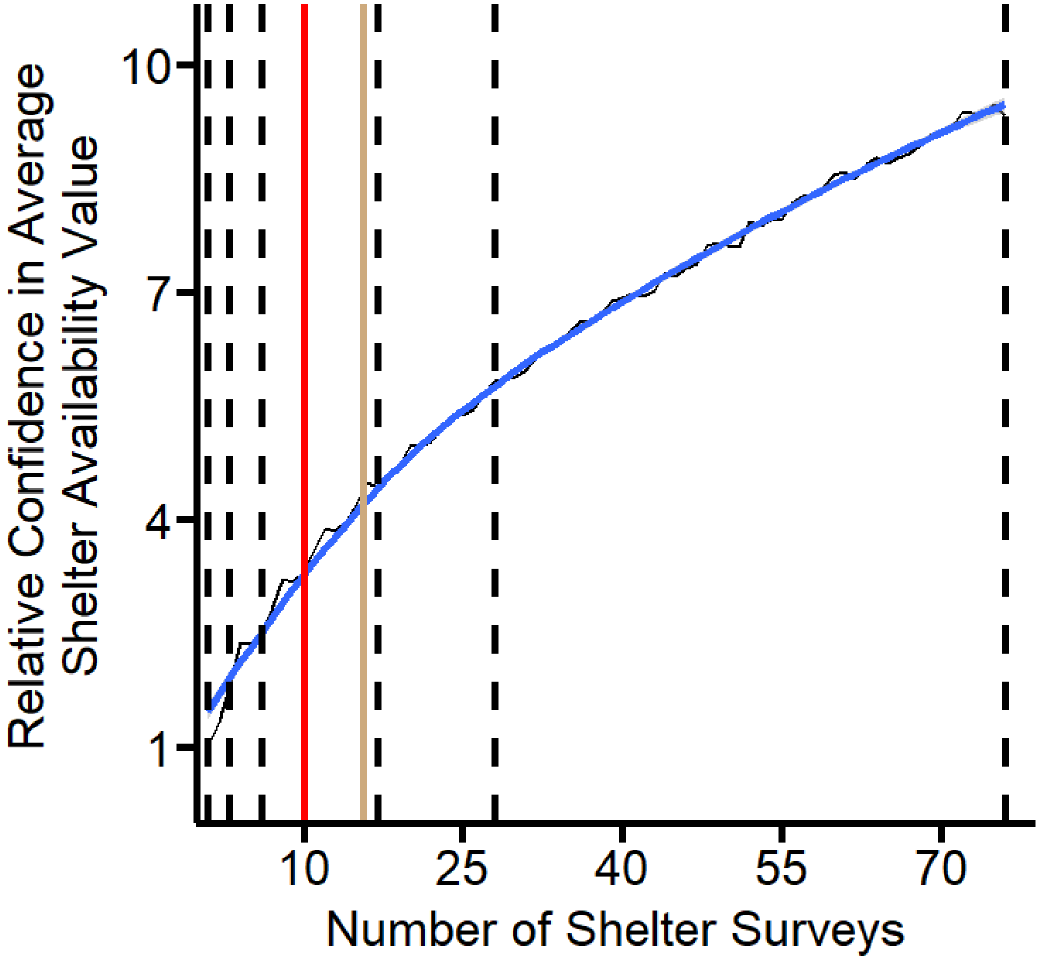
A1. A correlation matrix of variables used to model shelter availability. All binary variables were coded as 1 (success) or 0 (failure). Freq = average days in between shelter maintenance; PRRtrans = presence/absence of a pool-riffle-run transition within 5 m; SandGrav =

presence/absence of a sand/gravel bar within 5 m; Channel = presence/absence of steeply cut channel within 5 m; Angle = tunnel angle (defined earlier); Depth = water depth at the tunnel; ParVel = current velocity at the tunnel, parallel to the current; PerpVel = current velocity at the tunnel, perpendicular with the current; DistBank = shelter distance to bank; AnchorRock = presence/absence of an anchor rock; USForCov = percent upstream CWR forest cover; RWBould = reach-wide density of large (> 40 cm long) boulders; AdjBould = boulders within 1 m of the shelter. No two predictor variables were strongly correlated with each other (i.e., $|r| > 0.6$), so we did not discard any prior to running our NMDS and BRT models.

	HFEvents	**AnchorRock**	**RWBould**	**AdjBould**
HFEvents	1	0.35	0.08	-0.28
AnchorRock	0.35	1	-0.02	-0.36
RWBould	0.08	-0.02	1	0.13
AdjBould	-0.28	-0.36	0.13	1

A2. A correlation matrix of variables used to model shelter stability. All binary variables were coded as 1 (success) or 0 (failure). HFEvents = number of high discharge events experienced; AnchorRock = presence/absence of an anchor rock; RWBould = reach-wide density of large (> 40 cm long) boulders; AdjBould = number of boulders within 1 m of the shelter. Shelter design was not included in this matrix, because it is a categorical variable. No two predictor variables were strongly correlated with each other (i.e., |r| > 0.6), so we did not discard any prior to running our NMDS and BRT models.

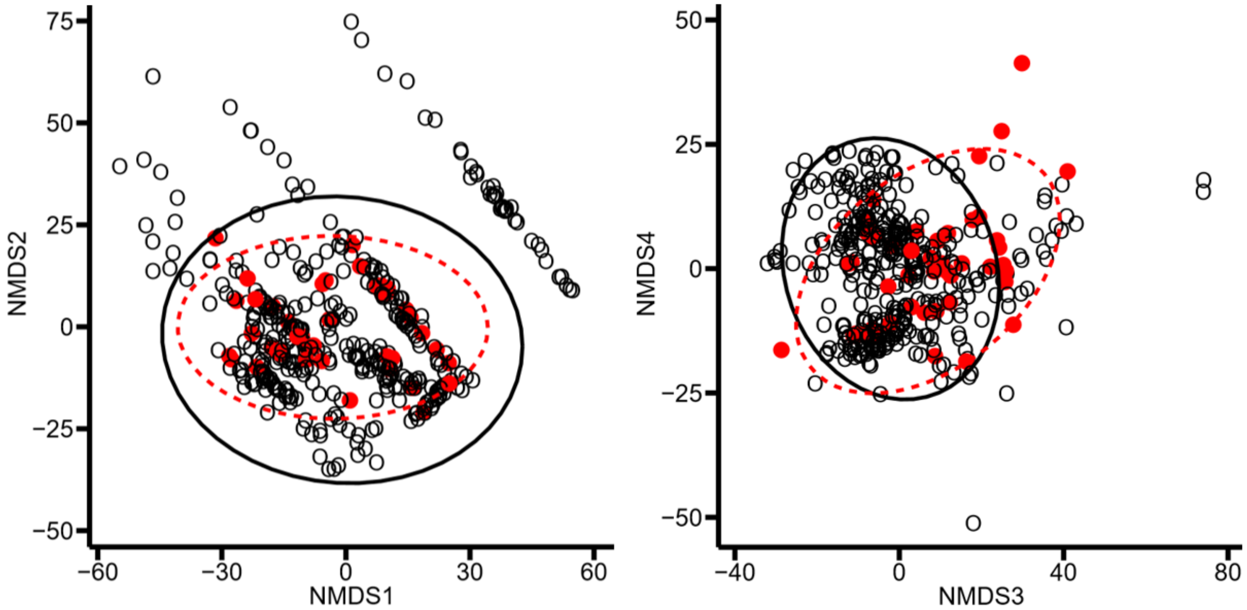
APPENDIX B: WEIGHTING SCALE USED IN SHELTER AVAILABILITY MODELS



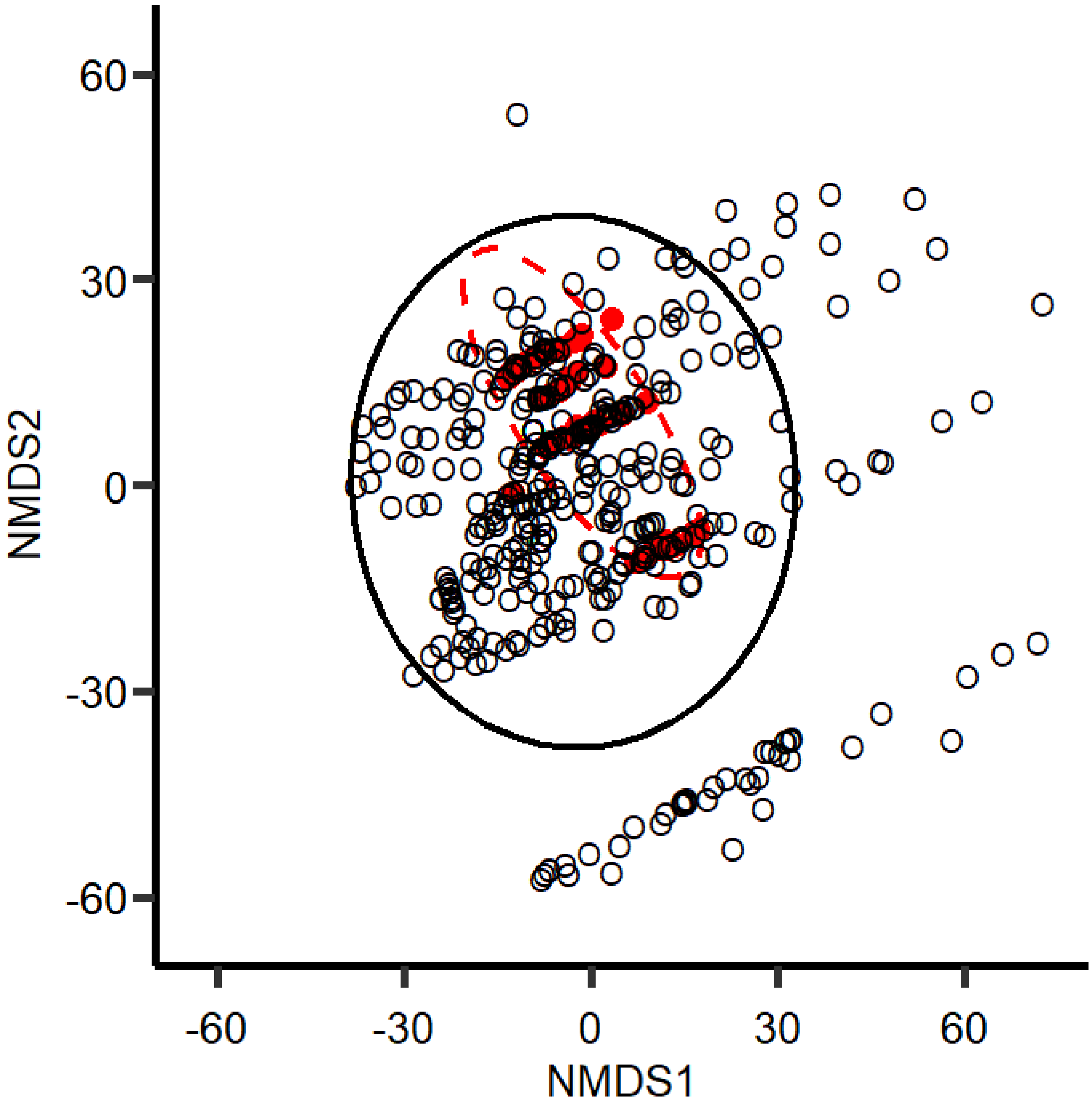
B1. The simulated relationship between number of shelters surveys and the accuracy of average shelter availability estimates obtained at each shelter. Predicted accuracy for each x-axis value was computed relative to the predicted estimate accuracy if a shelter was surveyed only once. Predicted values are indicated by a thin, solid black line, while a LOESS-smoothed regression line is shown in blue. The simulated accuracy of availability estimates at artificial shelters increased logarithmically and ranged between 1 (for a shelter surveyed once) and 9.52 (for a shelter surveyed 76 times). Vertical black, dotted lines correspond with the 0, 20, 40, 60, 80 and 100th percentiles for the number of surveys conducted at our artificial shelters (range = 1-76).

Median and mean number of shelter surveys are represented by red and brown solid vertical lines respectively.

APPENDIX C: SHELTER AVAILABILITY AND STABILITY NMDS PLOTS



C1. NMDS plots with 95% confidence ellipses artificial shelters found available on > 50% of all surveys versus on < 50% of all surveys. Shelters that were usually available or usually unavailable are shown by hollow black circles and solid red circles, respectively. Confidence ellipses are shown as a solid black line for shelters that were usually available and a dashed red line for shelters that were not. Shelters that were usually unavailable had a smaller 95% confidence ellipse than those that were usually available for the two most important NMDS components (NMDS1 and NMDS2), suggesting that they were characterized by a narrower range of conditions.



C2. NMDS plot with 95% confidence ellipses for stable and dislodged artificial shelters. Stable and dislodged shelters are represented by hollow black and solid red circles, respectively. Confidence ellipses are shown as a solid black line for stable shelters and a dashed red line for dislodged shelters. Dislodged shelters had a smaller 95% confidence ellipse than stable shelters, suggesting that they were characterized by a narrower range of conditions.

CHAPTER 2. The influence of multiscale habitat variables and population density on artificial shelter use by hellbenders

Sky T. Button, Catherine M.B. Jachowski, Brian F. Case, Jordy Groffen, William A. Hopkins

ABSTRACT

Recently, artificial shelters have been proposed as a novel tool for monitoring imperiled hellbender salamanders (*Cryptobranchus alleganiensis*). Factors influencing shelter use by hellbenders have not been identified, but are important to maximize their utility. To identify these factors, in 2013-2018 we deployed 438 artificial shelters across ten stream reaches within three rivers in the upper Tennessee River Basin, in locations representing a wide range of instream conditions. We monitored hellbender shelter occupancy every 2-8 weeks, and surveyed shelters for nests every 2-5 days during the hellbender breeding season (August 15-September 20). We hypothesized that occupancy and nesting would depend on shelter placement, and would be highest in reaches with high adult/subadult hellbender densities (i.e., > 1.5 individuals per 100 m^2). Hellbenders occupied 46% (203/438) of all artificial shelters, and nested in 17% (61/369) of artificial shelters that were in place for at least one breeding season. Hellbenders were most likely to occupy and nest in shelters placed in 50+ cm deep portions of reaches with high adult/subadult hellbender densities. Population density was the most important factor influencing hellbender shelter occupancy. Nesting was most influenced by water depth, and was influenced more evenly by hellbender density and time since shelter installation than occupancy. Both occupancy and nesting increased for 2-3 years following shelter deployment, suggesting that shelter use might be improved by relocating shelters not used by hellbenders within 2 years of deployment. For optimally-placed shelters, predicted occupancy and use for nesting reached 67%

and 24%, providing encouraging evidence that artificial shelters constitute efficient tools in some streams for monitoring the occurrence and reproduction of hellbenders.

INTRODUCTION

Artificial shelters have substantially improved research and conservation of birds, mammals, and several other animal taxa (Harley, 2006; Libois et al., 2012; Madikiza et al., 2010; Rohrbaugh Jr & Yahner, 1997), but have so far only occasionally been used to study amphibians (Shoo et al., 2011). Since amphibians are declining globally and are often difficult to detect using existing survey methods, the development of novel protocols that make them less challenging to monitor is critical for the conservation of many secretive and declining amphibian species. Stream-associated salamanders exemplify this need, as they are declining at an especially alarming rate (Calderon et al., 2017; Pui & Das, 2016; Ashton et al., 2006; Lecis & Norris, 2003) and can be notoriously difficult to detect (Browne et al., 2011; Lecis & Norris, 2003). Because they mimic features of natural instream habitat that are otherwise difficult to access, artificial shelters show considerable promise as a novel tool for improving the monitoring of certain stream-associated salamanders (Jachowski, 2016; Briggler & Ackerson, 2012). Given that artificial shelters may improve monitoring capabilities for multiple imperiled stream-associated salamanders, their development and use warrants assessment.

One imperiled salamander that may be monitored using artificial shelters is the hellbender (*Cryptobranchus alleganiensis*). Hellbenders are large (up to 68 cm TL), fully-aquatic salamanders found across Appalachia and portions of the lower Midwest (Nickerson & Mays, 1973), primarily in cool, fast-moving, well-oxygenated streams. Hellbenders are declining rapidly across much of their range (Briggler et al., 2007; Williams et al., 1981; Jachowski & Hopkins, 2018), particularly in impaired watersheds with degraded upstream forest cover (Jachowski & Hopkins, 2018). Ultimate causes of hellbender declines are poorly understood, largely because hellbenders spend most of their lives beneath large boulders and are therefore difficult to detect. Researchers have usually studied hellbenders using rock-lifting surveys, which involve dislodging and overturning instream boulders. While often effective for detecting hellbenders, rock-lifting surveys are dangerous for both animal and surveyor, can damage critical instream habitat (Browne et al., 2011), and are ill-advised between August and April, when hellbenders nests could be destroyed. Less invasive alternatives to rock-lifting surveys are therefore needed in order to effectively monitor hellbenders without exerting undue survey-related pressures on their remaining populations.

Recently, the advent of artificial shelters has presented a potential non-invasive alternative to rock-lifting surveys for studying hellbenders (Briggler & Ackerson, 2012). Though less destructive than rock-lifting surveys, the utility of artificial shelters for monitoring hellbenders has thus far yielded mixed results in practice, in part because hellbenders have often not used them (Messerman, 2014). Moreover, while improvements have recently been made to increase stability and availability of artificial shelters (Button et al., in review), it remains unclear whether characteristics of shelter placement within streams influence shelter use by hellbenders. We therefore sought to determine whether several, multiscale instream variables of potential biological relevance influenced hellbender occupancy and nesting in artificial shelters. Our study is the first to evaluate patterns of artificial shelter use by hellbenders over several (5+) years, and therefore provides unique guidance for determining where to place artificial shelters to maximize their utility for monitoring this species.

METHODS

Study Reaches

We deployed ten artificial shelter arrays across three rivers in the upper Tennessee River Drainage in southwest Virginia. For simplicity, hereafter we refer to the extent of artificial shelter arrays (range = 206-376 channel meters) as “reaches”. We cannot provide maps of precise study locations to prevent the possible collection and harassment of hellbenders, and refer to our study rivers as Rivers 1, 2, and 3. All three rivers were of fourth-order magnitude at our study reaches, though these reaches varied widely in their upstream catchment size (range = 131-309 km^2). When river discharges were at their annual medians (Table 2.1), Rivers 1 and 3 were 14-20 m wide, while River 2 was 6-18 m wide. Our study reaches varied considerably in their level of impairment, as measured by percent upstream forest cover in the catchment-wide riparian (CWR) area (range = 54-70%; Jachowski and Hopkins, 2018). The amount of suitable hellbender habitat (i.e., large unembedded boulders with suitable crevices) also varied widely among our study reaches (Table 2.2), making the placement of our artificial shelter arrays useful for evaluating the influence of reach-scale habitat variables on hellbender shelter use. Within each river, we spaced consecutive study reaches an average of 5.5 channel km apart from each other (range = 1.5-14.3 channel km).

Hellbender Demographics

Since population density is a known determinant of hellbender shelter occupancy (Jachowski et al., 2016), we also deployed artificial shelter arrays in reaches that varied considerably in their hellbender density and demographic structure. Most study reaches in River 3 contained moderate to high adult/subadult hellbender densities (0.65-3.04 individuals/100 m^2), and exhibited a relatively stable population age-structure and successful recruitment (Jachowski and Hopkins, 2018). By contrast, the most downstream study reach in River 3, and all study reaches on Rivers 1 and 2 featured low to moderate (0.36-0.64 individuals/100 m^2), or unknown adult/subadult hellbender densities (Jachowski, 2018; Appendix A). Low-density hellbender populations within our study reaches tended to exhibit a geriatric age structure (Jachowski and Hopkins, 2018), suggesting a likely lack of recruitment and/or successful reproduction.

Artificial Shelters

We constructed artificial shelters using the designs described by Button et. al (in prep.) and earlier by Briggler & Ackerson (2012), and deployed approximately 30 shelters within each study reach (n = 300 shelters deployed at once) in April-July of 2013-2018. We replaced damaged or dislodged shelters as necessary, and therefore used 438 shelters in total over the course of the study. Typically, we deployed replacement shelters in different locations and orientations from the shelters that they replaced. We anchored all shelters firmly into the stream substrate during installation, in microhabitats representing a range of conditions potentially-suitable for hellbenders (Table 2.2). We deposited a thin layer of sand and gravel into shelter tunnels so that they mimicked natural crevices, and spaced adjacent shelters an average of 10 channel meters apart from each other (range ≈ 4-20 m) within each reach.

Data Collection

We monitored shelter occupancy throughout each year of the study, and recorded nesting in shelters during each hellbender breeding season (15 August – 20 September). We conducted occupancy surveys every 2-8 weeks during the non-breeding season (21 September – 14 August), except when unfavorable conditions made surveys infeasible. To determine if shelters were occupied, we removed the lid and manually probed the interior. We surveyed shelters for nests every 2-5 days during the breeding season, and simultaneously recorded shelter occupancy in doing so.

Since reach scale habitat variables sometimes constrain fine-scale resource use by aquatic animals (Anderson et al., 2009; Thompson et al., 2001), we collected habitat data across three spatial scales: reach-wide, within 5 m of each shelter, and at or within 1 m of each shelter (Table 2.2). We used hellbender movement behaviors to guide the selection of spatial scales used in our analyses. Given their low vagility (Blais, 1996; Topping & Peterson, 1985), stream reaches are perhaps the largest scale that hellbenders select resources at during their lifetimes, while the area enclosed by 5 m radii around artificial shelters is similar to the area of core habitat use within a hellbender home range (Blais, 1996). Within the area of core habitat use, hellbenders occupying artificial shelters likely use microhabitats immediately adjacent to (i.e., at or within 1 m of) those shelters most often, since they exhibit high shelter (Hopkins unpubl. data) and boulder (Bodinof et al., 2012; Blais, 1996) fidelity. In addition to our multiscale habitat variables, we also assessed the influence of reach-wide hellbender density and average time since shelter installation across all surveys or breeding seasons on both shelter occupancy and nesting. Hellbender density and time since shelter installation positively influence shelter occupancy during the first two years following installation (Jachowski, 2016), but their influence on shelter use for reproduction and on occupancy beyond this timeframe is thus far unknown.

Reach scale. We assessed the influence of three reach-level habitat variables on shelter occupancy and nesting: upstream catchment size, percent forest cover in the upstream CWR area, and the reach-wide density of large (i.e., > 40 cm on the primary axis) boulders with crevices suitable for hellbenders. Upstream catchment size is known to influence community structure and, by extension, species realized fine-scale niches (Bis et al., 2000), while upstream forest cover is an important mediator of stream impairment and habitat quality (Jachowski & Hopkins, 2018; Collins et al., 2009; Hooke, 2000). We calculated both upstream catchment size and percent upstream CWR forest cover in ArcMap (Environmental Systems Research Institute, Inc.; Redlands, CA) using the 2011 National Land Cover and National Hydrography Datasets (United States Geological Survey, 2011).

The density of suitable boulder habitat within a reach is a potentially important factor influencing shelter use. Previous hellbender work in our study system quantified physical hellbender habitat as the proportion of a reach that consisted of boulders/bedrock (Jachowski, 2016), but did not consider whether these substrate features were available to hellbenders by way of at least one suitable crevice. Therefore, we built upon this foundation and quantified hellbender habitat at each of our study reaches based on the reach-wide density of boulders that specifically bore crevices suitable for hellbenders. We only counted boulders that were > 40 cm long on their primary axis, because 95% (1002/1056) of hellbender captures from natural crevices in our study system came from boulders this size or larger (Hopkins unpubl. data). We considered these large boulders suitable for hellbenders if they met all of the following criteria: the boulder did not move when nudged, an observer could slide their hand into a crevice on the boulder up to at least their second knuckle (i.e., it was not fully embedded and had a crevice), the crevice was not packed with debris (i.e., sticks and leaves, which are indicative of boulders that are perched high and collect debris), and the crevice lacked apparent connections to other crevices (i.e., it was not possible for an observer to touch their hands together when reaching under separate crevices). To estimate the density of boulders that were both large and suitable at each study reach, we walked 10 evenly spaced transects across a representative 1680 m^2 portion of the reach and measured every boulder that intersected these transects. Reach-wide densities of large, suitable boulders explained 80% of the variation in hellbender densities across our study reaches.

<u>5 m scale.</u> The area enclosed within a 5 m radius around each shelter (78.54 m^2) is similar to the size of the core area within a typical hellbender home range (Bodinof et al., 2012; Burgmeier et al., 2011; Blais, 1996). Therefore, we evaluated the potential influence of three 5 m-scale variables on shelter occupancy and nesting. Five meter-scale variables included the presence or absence of a steeply cut channel (> 10% incline on both sides), sand/gravel bar (> 1 m^2), and pool-riffle-run transition within 5 m of each shelter. We hypothesized that these three variables would be related to hellbender shelter use due to their potential influence on the amount of resource complementarity provided by local habitat. We assessed all three 5 m-scale variables visually, and used the same criteria as Jachowski (2016) to demarcate pools, riffles, and runs. If it was visually unclear whether a variable was present within 5 m of a shelter, we used a tape measure to determine its proximity.

<u>Microhabitat scale.</u> Microhabitat features are often the most important mediator of resource use in non-vagile species (Welsh Jr & Ollivier, 1998). Therefore, we assessed the influence of several microhabitat-scale variables (i.e., at or within 1 m of the shelter) on both occupancy and nesting. We measured the angle formed between the direction of the shelter tunnel and direction of the current (“tunnel angle” hereafter), current velocity at the tunnel parallel to and perpendicular with the current (referred to as “downstream current velocity” and “bank-to-bank current velocity” hereafter), water depth at the tunnel, shelter distance to the bank, percent canopy cover above the shelter, vertical distance to canopy (where applicable), and the number of crevice-bearing boulders within 1 m of the shelter. To determine tunnel angle, we attached a fishing bobber to the end of a 50 cm-long string, held the opposing end of the string at the

water's surface above the base of the tunnel, and measured the angle formed between the string and the tunnel using a protractor. We used a 2D FlowTracker2 Handheld-ADV flow meter (Xylem Inc.; Rye Brook, NY) to assess downstream and bank-to-bank current velocity, and measured water depth at the tunnel with a meter stick. Using a tape measure, we calculated distance to bank, then combined this measurement where applicable with a clinometer-based angle to above-shelter canopy taken from the bank to determine vertical distance to canopy. To estimate percent canopy cover, we photographed the canopy above each shelter using a fisheye lens (GoPro Inc.; San Mateo, CA), digitally overlaid a densiometer-style grid of 96 dots onto each photo, and multiplied the number of dots that intersected canopy by 1.04 (Lemmon, 1956). We considered boulders (Wolman, 1954) within 1 m of shelters to be crevice-bearing using the same criteria as for reach-wide boulders. To ensure that our measurements accurately represented average instream conditions, we measured all discharge-dependent variables once, when discharge at the nearest USGS gage (Table 2.1) was at its approximate annual median.

Time since installation and hellbender density. In addition to being influenced by multiscale habitat variables, we predicted that shelter occupancy and nesting would increase over several years following shelter installation and increase concomitant with adult/subadult hellbender density. We used average months since shelter installation across all surveys to model shelter occupancy, and used number of breeding seasons in place to model shelter nesting. To evaluate the influence of hellbender density on shelter use, we used existing density estimates from five of our six multi-year reaches (Hopkins and Jachowski, 2018), and estimated density at the sixth using a single season Huggins closed capture model (Huggins, 1989) in 2018 (Appendix A).

Shelter design. Button et al. (in review) found evidence that artificial shelters should be constructed with thick walls, heavy frames, and inset lids to improve their stability during high stream discharge events. Therefore, we assessed whether hellbenders occupied and nested in shelters built using a heavy, sturdy design (*n* = 220) as often as in less stable shelters (*n* = 218) built using the original, more lightweight design (Briggler and Ackerson, 2012). In doing so, we sought to verify that no tradeoff existed between shelter stability and shelter use.

Data Processing and Analyses

Response units. We used average occupancy and nesting at individual artificial shelters as our response variables in all analyses. To estimate average hellbender occupancy at each artificial shelter, we divided the number of surveys in which we found each shelter occupied (*n* = 0-64) by our total number of surveys of the shelter (*n* = 1-76). To calculate average shelter nesting, we divided the number of breeding seasons in which hellbenders nested in each shelter (*n* = 0-4) by the total number of breeding seasons that each shelter was in place (*n* = 1-6).

Combining habitat and density. We predicted that hellbender occupancy and nesting in artificial shelters would be highest in reaches that contained limited natural hellbender habitat relative to their adult/subadult hellbender population density, since there is likely an interaction between the way these two variables influence shelter use (Jachowski, 2016). Therefore, to evaluate the influence of suitable habitat relative to adult/subadult hellbender density on shelter use, we developed a standardized “habitat surplus” metric, which we calculated by subtracting standardized adult/subadult hellbender density estimates from standardized large suitable boulder density estimates at each study reach.

Non-metric multidimensional scaling. We used the same two-step analytical approach as Button et al. (in review). First, we verified that our predictor variables were collectively informative of shelter use using PERMANOVA and betadisper analyses, which determine whether the location and dispersion of collectively-ordinated predictor variables are related to a chosen response variable (i.e., average shelter occupancy or nesting; Dixon, 2003). PERMANOVA results should be viewed with caution when betadisper analysis yields significant results. Prior to conducting all multivariate analyses, we used random forest imputations (Stekhoven & Bühlmann, 2011) to randomly generate values for missing data (~10% of both datasets), because PERMANOVA and betadisper are not robust to missing values. We excluded data from single-year reaches that lacked hellbender density estimates from both NMDS analyses, because their inclusion would have required an untenably high level of imputation. We standardized all non-binary predictor variables, constructed distance matrices for both datasets using Euclidean distances (Lele & Richtsmeier, 1991), and carried out all multivariate analyses using the 'vegan' package in R (Version 3.3.3; R Core Development Team).

We used NMDS plots to visualize the approximate relationships identified by PERMANOVA and betadisper analyses (Appendix D). Non-metric multidimensional scaling uses distance matrices to collapse datapoints containing several variables into a specified number of dimensions (Kruskal, 1964). We carried out NMDS ordinations for average shelter occupancy and nesting using the minimum number of dimensions where stress < 0.2 (Anderson, 2001).

Boosted regression trees. After verifying that our predictor variables were informative of shelter use, we used boosted regression trees (BRTs) to determine the influence of individual predictor variables on occupancy and nesting, using the 'gbm' package in R (Version 3.3.3, R Core Development Team). Boosted regression trees use iterative decision trees to model the influence of predictor variables on a chosen response (i.e., shelter occupancy or nesting), and weight each tree based on how much its inclusion in the model minimizes the loss function (Elith et al., 2008). The influence and importance of individual predictor variables is subsequently determined based on their prevalence and average influence across the weighted set of decision trees. Boosted regression trees tend to be useful for identifying ecological thresholds due to their use of split points (Elith et al., 2008), and often outperform other modeling approaches for datasets that contain spatial structure (Crase et al, 2012). We modelled shelter occupancy and nesting using two different sets of BRTs, and used average shelter occupancy across all surveys, or average nesting across all breeding seasons, as our unit of replication. We treated both response variables as beta-distributed in all BRT analyses. To account for the differing uncertainty associated with average occupancy and nesting estimates calculated for shelters surveyed or available for nesting differing numbers of times, we assigned shelters weights in our models based on their number of times we surveyed them or number of breeding seasons they experienced, using the same approach as Button et al. (2019) to develop a weighting scale (Appendix B), making it possible to use individual shelters rather than individual shelter surveys as our unit of replication.

We included data from all study reaches in our BRTs, including single-year reaches that lacked hellbender population density estimates (two on River 1, and two on River 2). Shelter use and habitat data from single-year reaches were valuable even in the absence of density estimates, because we collected these data 5-90 days after shelter installation in these reaches (*n* = 318 occupancy and 59 nesting datapoints), during the months most critical for shaping long-term shelter use patterns, and when differences in shelter occupancy among reaches increase most rapidly (Jachowski, 2016). Model performance corroborated the utility of including these single-year reaches in our models (Table 2.3), as expected given that BRTs exclude missing values when fitting tree nodes, thus preventing missing data from substantially influencing the shape and slope of modeled relationships. However, we excluded both study reaches in River 1 from our nesting BRTs, because we were unable to survey these reaches for nests due to continuously high stream discharge during the 2018 hellbender breeding season.

We evaluated BRT performance based on the correlation of model predictions with observed occupancy and nesting values (i.e., cross-validated correlation) using k-fold cross validation with five folds (Kohavi, 1995). After constructing initial models, we dropped all variables with < 5% contributions, reran these models, and repeated this process until all variables contributed at least 5% to the model, to avoid overfitting. We also dropped additional variables from our refined models if their inclusion in the model worsened its performance. We built all models using tree complexity = 2, learning rate = 0.0005, and bag fraction = 0.5 (Elith et al., 2008), because these values maximized model performance during preliminary model building. We evaluated the influence of individual predictor variables on shelter occupancy and nesting using partial dependence plots, which make predictions by varying a single predictor variable while holding the others constant at their mean, and relative variable influence for predictor variables retained in our top-performing models.

To determine whether reach-scale variables constrained the influence of finer-scale habitat variables on shelter use, we compared the performance of BRTs that excluded 5 m and/or reach-scale predictors to those that included predictor variables from all spatial scales. To ensure that the inclusion of data from single-year reaches did not weaken model performance, we also reran and evaluated the performance of our top model from the above set using only data from our six multi-year study reaches with density estimates. Additionally, we reran our top occupancy and nesting model with shelter design as an added predictor variable, to determine whether a tradeoff existed between shelter stability and use.

RESULTS

Hellbenders consistently occupied our artificial shelters, but shelter use varied widely across reaches. In total, hellbenders occupied artificial shelters on 2518 of 6793 possible occasions (37%), with reach-wide occupancy averaging 22% (range = 0-58%) across all surveys. Average occupancy since shelter installation peaked at 26% approximately two years after shelter deployment, and plateaued thereafter (Fig. 2.1). Shelter occupancy increased most rapidly after shelter deployment in reaches containing high densities of adult/subadult hellbenders (> 1.5 individuals/100m^2), but plateaued after two years regardless of hellbender density.

We also observed considerable hellbender nesting in our artificial shelters. Hellbenders established nests in shelters on 95 of 925 nesting opportunities (10%), and reach-wide nest initiation averaged 8% (range = 0-18%) across all breeding seasons. These 95 nests were established in 61 different shelters, by 54 unique males. Prior nesting at a shelter substantially increased the probability of future nesting in that shelter (Fig. 2.2); males that nested in a shelter in a given year had a 59% chance of nesting in that same shelter the following year if the shelter was still in place (n = 34). Importantly, we found no evidence that shelter design influenced occupancy or nesting frequency.

Relationship between collective habitat variables and shelter use

Our PERMANOVA and betadisper analyses revealed that the collective character of our two sets of predictor variables was related to both shelter occupancy (F = 4.20 and p = 0.001 for betadisper; F = 13.08 and p = 0.001 for PERMANOVA) and shelter nesting (F = 2.38 and p = 0.016 for betadisper; F = 2.29 and p = 0.015 for PERMANOVA). Moreover, while our PERMANOVA results should be viewed with caution due to the significance of our betadisper results, the betadisper results provided clear evidence of at least a moderate relationship between shelter use and the dispersion of ordinated predictor variables (Appendix D; r = -0.25 between the response variable and the average distance of predictor variables from the overall centroid for average shelter occupancy, and r = -0.48 for average shelter nesting). Therefore, we deemed our two sets of predictor variables appropriate for modeling the relationship between individual predictor variables and shelter use in subsequent BRTs.

Factors influencing shelter occupancy

Our model built using variables from all three spatial scales, prior to dropping unimportant variables, had the highest performance of all models in the set (CV correlation = 0.658, SE = 0.043; Model A in Table 2.3, Appendix C.1), and suggested that shelter occupancy depended primarily on adult/subadult hellbender density, and secondarily on average months since installation and water depth at the tunnel (Fig. 2.1). Other models in the set performed similarly, and tended to retain a similar set of predictor variables. Shelter occupancy increased sharply with adult/subadult hellbender density in our top model, and was > 4 times as influential as any other predictor variable in the model (69% relative influence; Fig. 2.1). Average months since installation and water depth were also positively associated with shelter occupancy to a lesser degree, and had relative influences of 16% and 15% on the model respectively. However, average shelter occupancy was only positively associated with time since shelter installation during the first two years of shelter deployment, and increased most rapidly in reaches with high adult/subadult hellbender densities (Fig. 2.3). The low contribution of shelter design when added to our top model (0.5%) suggested that it did not influence shelter occupancy. When all variables were optimized (Table 2.4), predicted average occupancy reached 67%.

Factors influencing shelter nesting

We constructed our top nesting model (cv correlation = 0.278, SE = 0.042; Model A in Table 2.3) using predictor variables from all three spatial scales prior to dropping variables with minimal contributions. The top model explained up to 36% more variation in nesting than other models in the set (Table 2.3, Appendix C.2), and retained water depth (44% influence), adult/subadult hellbender density (34% influence), and breeding seasons in place (22% influence) as important predictor variables. Predicted nesting frequency was highest given water depths of approximately 50-60 cm, and increased consistently with increasing adult/subadult hellbender density (Fig. 2.4). Average nesting frequency increased during the first three breeding seasons following shelter deployment, and plateaued thereafter. Unlike shelter occupancy, the rate of increase in shelter nesting over time following shelter deployment was unrelated to adult/subadult hellbender density (Fig. 2.3). The relative influence of shelter design was negligible (2.4%) when added to our top model, suggesting that it did not substantially influence nesting frequency. When all variables were optimized (Table 2.4), predicted average nesting frequency reached 24%.

DISCUSSION

We sought to identify factors influencing the use of artificial shelters by hellbenders in order to improve their use as tools for research and monitoring. Encouragingly, we observed far more consistent hellbender occupancy and nesting in our shelters than has previously been documented during certain initial trials (Messerman, 2014), highlighting the utility of artificial shelters for studying hellbenders in some streams. Moreover, since we only detected an influence of three different variables on occupancy and nesting, our results suggest that within our study system, optimizing shelter placement is a relatively simple process. Expected occupancy and nesting frequency in optimally-placed artificial shelters reached 67% and 24% respectively, suggesting that artificial shelters are a potentially powerful tool for monitoring hellbender populations when placed under optimal conditions.

Shelter occupancy increased sharply and consistently with increasing adult/subadult hellbender density (Fig. 2.4) and was positively influenced to a lesser extent by water depth and time since installation, suggesting that the utility of artificial shelters for monitoring hellbenders is influenced by shelter placement. Despite the overarching influence of adult/subadult hellbender density, shelter occupancy still exceeded 25% in low density reaches (i.e., < 1 hellbender per 100 m^2) within two years of shelter deployment when shelters were deployed in locations > 50 cm deep (Table 1). This finding suggests that deeply-placed artificial shelters may be effective for monitoring hellbenders regardless of population density, possibly because hellbenders are seasonally reliant upon deep runs (Green, 1934).

Regardless of where artificial shelters were placed, their occupancy by hellbenders tended to increase for two years before plateauing. We interpret this being the result of their low vagility (Bodinof et al., 2012; Blais, 1996; Peterson, 1987; Topping & Peterson, 1985), and thus gradual discovery of the augmented habitat provided by artificial shelters. Studies of nest box use by birds and mammals have produced similar results, and have often documented periods of rapidly increasing box use following box installation, which eventually levels off or declines later on (Lindenmayer et al., 2009; Katzner et al., 2005; McCamant & Bolen, 1979). Given that our results suggest most discovery of shelters by hellbenders occurs within two years of deployment, we recommend relocating shelters that are never occupied during two consecutive years to improve their likelihood of future occupancy. Relocating consistently unused shelters may yield especially rapid payoff in reaches with high adult/subadult hellbender densities, because shelter occupancy increased far more rapidly in these reaches than elsewhere (Fig. 2.3).

Our findings build upon a recent two-year study that we also conducted in southwest Virginia (Jachowski, 2016). Similar to that study, we found that shelter occupancy increased concomitant with adult/subadult hellbender density and improved for two years following shelter deployment, which highlights the clear importance of these relationships. However, a fundamental difference between the two studies relates to how we assessed natural habitat (boulder) availability and its influence on hellbender use of artificial shelters (Appendix E). Jachowski (2016) considered all boulders (particles > 25.6 cm on the secondary axis) and bedrock to be available habitat for hellbenders, and found that boulder/bedrock density negatively influenced hellbender occupancy in artificial shelters. In contrast, we only included large (> 40 cm long on the primary axis) boulders that bore suitable crevices in our estimates of *suitable* hellbender habitat. Unlike the prior study, the metric used in the current study removes all bedrock and boulder lacking suitable crevices and thus not used by hellbenders. As a result of this more stringent classification of habitat, and possibly other differences in study design (i.e., inclusion/exclusion of different study reaches), we did not detect a negative influence of suitable habitat on artificial shelter use by hellbenders. In fact, we found the opposite; natural shelter density (i.e., reach-wide density of large suitable boulders) was positively correlated and redundant with adult/subadult hellbender density (*r* = 0.8), and would have actually had a positive impact on occupancy (i.e., the opposite result from Jachowski, 2016) had we included it in our final models. A visual comparison of boulder characteristics from study sites shared by Jachowski, 2016 and the current study further emphasizes this difference. Clearly, additional study is needed to understand the complex interplay among natural habitat availability, population density, and shelter use by hellbenders.

Shelter nesting was related to similar factors as shelter occupancy, but the relative influence of these factors was far more evenly-partitioned (Fig 2.4). Nesting was highest in shelters located in moderately deep (50-60 cm) portions of the stream. Water depth was also nearly three times as important as breeding seasons since shelter deployment for predicting nesting frequency (Fig. 2.4). Hellbenders may have perceived moderately deep runs suitable for nesting because these areas featured cooler, better-oxygenated water than shallower areas (Kramer, 1987). Alternatively, hellbenders might have perceived these deeper areas as being better protected from certain predators (e.g., wading birds) than shallow areas. Since shelter nesting was more than twice as high in moderately deep (50-60 cm) areas than in shallow (20-40 cm deep) areas, monitoring hellbender reproduction using artificial shelters may be viable even in reaches with declining populations if shelters are placed deeply enough.

Our study is the first to quantitatively evaluate patterns of artificial shelter use by hellbenders over several (5+) years, and suggests that optimal shelter placement greatly improves the probability of hellbender occupancy and nesting in them.. Our finding that hellbenders occupied individual shelters 22% of the time and created 95 nests in artificial shelters during the study is promising, and presents the opportunity to study numerous aspects of the biology and conservation needs of hellbenders that are currently unknown. Future studies should consider evaluating the applicability of our results to other hellbender lineages across the species’ range, and should also consider examining whether artificial shelters built using alternative designs (Mohammed et al., 2016) are equally likely to be used by hellbenders. If applicable in other watersheds, our results provide compelling evidence that artificial shelters deployed in optimal locations can serve as novel, valuable tools for monitoring and conserving hellbenders. Additionally, our study provides a logical starting point for using artificial shelters to study other crevice-associated aquatic species (e.g., certain other aquatic salamanders, fishes, and large crustaceans) that are secretive and/or of conservation concern.

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REFERENCES

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26: 32-46.

Anderson, T. J., Syms, C., Roberts, D. A., Howard, D. F., and Ecology. (2009). Multi-scale fish–habitat associations and the use of habitat surrogates to predict the organisation and abundance of deep-water fish assemblages. *Journal of Experimental Marine Biology* 379: 34-42.

Ashton, D. T., Marks, S. B., and Welsh, H. H. (2006). Evidence of continued effects from timber harvesting on lotic amphibians in redwood forests of northwestern California. *Forest Ecology and Management* 221: 183-193.

Bis, B., Zdanowicz, A., and Zalewski, M. (2000). Effects of catchment properties on hydrochemistry, habitat complexity and invertebrate community structure in a lowland river. In*: Assessing the Ecological Integrity of Running Waters*, pp. 369-387. (Ed.)^(Eds.): Springer.

Blais, D. P. (1996). Movement, home range, and other aspects of the biology of the eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*): a radio telemetric study. State University of New York at Binghamton, Biological Sciences Department.

Bodinof, C. M., Briggler, J. T., Junge, R. E., Beringer, J., Wanner, M. D., Schuette, C. D., Ettling, J., Gitzen, R. A., and Millspaugh, J. J. (2012). Postrelease movements of captive-reared Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*). *Herpetologica* 68: 160-173.

Briggler, J., and Ackerson, J. (2012). Construction and use of artificial shelters to supplement habitat for Hellbenders (*Cryptobranchus alleganiensis*). *Herpetological Review* 43: 412.

Briggler, J., Utrup, J., Davidson, C., Humphries, J., Groves, J., Johnson, T., Ettling, J., Wanner, M., Traylor-Holzer, K., and Reed, D. (2007). Hellbender population and habitat viability assessment: final report. IUCN/SSC Conservation Breeding Specialist Group, Apple Valley, MN.

Browne, R., Li, H., Mcginnity, D., Okada, S., Zhenghuan, W., Bodinof, C., Irwin, K., Mcmillan, A., and Briggler, J. (2011). Survey techniques for giant salamanders and other aquatic Caudata. *Amphibian & Reptile Conservation* 5: 1-16.

Burgmeier, N. G., Sutton, T. M., and Williams, R. N. (2011). Spatial ecology of the Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) in Indiana. *Herpetologica* 67: 135-145.

Calderon, M., Moglia, M., Nievas, R., Colombetti, P., González, S., and Jofré, M. (2017). Assessment of the environmental quality of two urbanized lotic systems using multiple indicators. *River Research and Applications* 33: 1119-1129.

Collins, A., Anthony, S., Hawley, J., and Turner, T. (2009). The potential impact of projected change in farming by 2015 on the importance of the agricultural sector as a sediment source in England and Wales. *Catena* 79: 243-250.

Crase, B., Liedloff, A. C., and Wintle, B. A. (2012). A new method for dealing with residual spatial autocorrelation in species distribution models. *Ecography* 35: 879-888.

Dixon, P. (2003). VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science* 14: 927-930.

Elith, J., Leathwick, J. R., and Hastie, T. (2008). A working guide to boosted regression trees. *Journal of Animal Ecology* 77: 802-813.

Green, B. N. (1934). *Cryptobranchus alleganiensis* in West Virginia. *Proceedings of the West Virginia Academy of Science* 7: 28-30.

Harley, D. K. (2006). A role for nest boxes in the conservation of Leadbeater's possum (*Gymnobelideus leadbeateri*). *Wildlife Research* 33: 385-395.

Hooke, R. L. (2000). On the history of humans as geomorphic agents. *Geology* 28: 843-846.

Huggins, R. J. B. (1989). On the statistical analysis of capture experiments. 76: 133-140.

Jachowski, B., Catherine, M., Millspaugh, J. J., and Hopkins, W. A. (2016). Current land use is a poor predictor of hellbender occurrence: why assumptions matter when predicting distributions of data-deficient species. *Diversity and Distributions* 22: 865-880.

Jachowski, C. M. B. (2016). Effects of Land Use on Hellbenders (*Cryptobranchus alleganiensis*) at Multiple Levels and Efficacy of Artificial Shelters as a Monitoring Tool. Dissertation. Virginia Polytechnic Institute and State University.

Jachowski, C. M. B., and Hopkins, W. A. (2018). Loss of catchment-wide riparian forest cover is associated with reduced recruitment in a long-lived amphibian. *Biological Conservation* 220: 215-227.

Katzner, T., Robertson, S., Robertson, B., Klucsarits, J., Mccarty, K., and Bildstein, K. L. (2005). Results from a long-term nest-box program for American Kestrels: implications for improved population monitoring and conservation. *Journal of Field Ornithology* 76: 217-227.

Kohavi, R. (1995). A study of cross-validation and bootstrap for accuracy estimation and model selection. Ijcai.

Kramer, D. L. (1987). Dissolved oxygen and fish behavior. *Experimental Biology of Fishes* 18: 81-92.

Kruskal, J. B. (1964). Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. *Psychometrika* 29: 1-27.

Lecis, R., and Norris, K. (2003). Geographical distribution of the endemic Sardinian brook salamander, *Euproctus platycephalus,* and implications for its conservation. *Herpetological Journal* 13: 125-133.

Lele, S., and Richtsmeier, J. T. (1991). Euclidean distance matrix analysis: A coordinate-free approach for comparing biological shapes using landmark data. *Journal of Physical Anthropology* 86: 415-427.

Lemmon, P. E. (1956). A spherical densiometer for estimating forest overstory density. *Forest Science* 2: 314-320.

Libois, E., Gimenez, O., Oro, D., Mínguez, E., Pradel, R., and Sanz-Aguilar, A. (2012). Nest boxes: A successful management tool for the conservation of an endangered seabird. *Biological Conservation* 155: 39-43.

Lindenmayer, D. B., Welsh, A., Donnelly, C., Crane, M., Michael, D., Macgregor, C., Mcburney, L., Montague-Drake, R., and Gibbons, P. (2009). Are nest boxes a viable alternative source of cavities for hollow-dependent animals? Long-term monitoring of nest box occupancy, pest use and attrition. *Biological Conservation* 142: 33-42.

Madikiza, Z. J., Bertolino, S., Baxter, R. M., and San, E. D. L. (2010). Nest box use by woodland dormice (*Graphiurus murinus*): the influence of life cycle and nest box placement. *European journal of wildlife research* 56: 735-743.

Mccamant, R. E., and Bolen, E. G. (1979). A 12-year study of nest box utilization by Black-bellied Whistling Ducks. *Journal of Wildlife Management*: 936-943.

Messerman, A. (2014). The Use of Nest Boxes by the Hellbender Salamander in Western North Carolina. M.S. Thesis. Duke University.

Mohammed, M. G., Messerman, A. F., Mayhan, B. D., and Trauth, K. M. (2016). Theory and Practice of the Hydrodynamic Redesign of Artificial Hellbender Habitat. *Herpetological Review* 47: 586-591.

Nickerson, M. A., and Mays, C. E. (1973). The Hellbenders: North American "Giant Salamanders". Milwaukee, Milwaukee Public Museum.

Peterson, C. L. (1987). Movement and catchability of the hellbender, *Cryptobranchus alleganiensis*. *Journal of Herpetology*: 197-204.

Pui, Y. M., and Das, I. (2016). Streams in Forested Headwaters as Reservoirs of Endemicity in Bornean Amphibians. In*: Naturalists, Explorers and Field Scientists in South-East Asia and Australasia*, pp. 143-155. (Ed.)^(Eds.): Springer.

Rohrbaugh Jr, R. W., and Yahner, R. H. (1997). Effects of macrohabitat and microhabitat on nest-box use and nesting success of American Kestrels. *The Wilson Bulletin*: 410-423.

Shoo, L. P., Olson, D. H., Mcmenamin, S. K., Murray, K. A., Van Sluys, M., Donnelly, M. A., Stratford, D., Terhivuo, J., Merino-Viteri, A., and Herbert, S. M. (2011). Engineering a future for amphibians under climate change. *Journal of Applied Ecology* 48: 487-492.

Stekhoven, D. J., and Bühlmann, P. J. B. (2011). MissForest—non-parametric missing value imputation for mixed-type data. 28: 112-118.

Thompson, A. R., Petty, J. T., and Grossman, G. D. (2001). Multi-scale effects of resource patchiness on foraging behaviour and habitat use by longnose dace, *Rhinichthys cataractae*. *Freshwater Biology* 46: 145-160.

Topping, M., and Peterson, C. (1985). Movement in the hellbender, *Cryptobranchus alleganiensis*. *Transactions of the Missouri Academy of Science* 19: 121.

Welsh Jr, H. H., and Ollivier, L. M. J. E. A. (1998). Stream amphibians as indicators of ecosystem stress: a case study from California's redwoods. 8: 1118-1132.

Williams, R. D., Gates, J. E., Hocutt, C. H., and Taylor, G. J. (1981). The hellbender: a nongame species in need of management. *Wildlife Society Bulletin*: 94-100.

Wolman, M. G. (1954). A method of sampling coarse river-bed material. *EOS, Transactions American Geophysical Union* 35: 951-956.

TABLES

Table 2.1. Median, minimum, and maximum daily discharges (in m^3/s) over the period of shelter deployment for each river containing artificial shelter arrays. We calculated predictor variables that varied with stream discharge when the discharge of each river was at its approximate annual median. High maximum daily discharges (relative to the median) over the course of the study illustrate the flashy, flood-prone nature of our study rivers. All River 1 and River 2 study reaches were located upstream of the nearest USGS gage. On River 1, two study reaches were located 8.72-12.34 channel km upstream of the nearest USGS gage, and three were located 0.05-17.72 km downstream of the gage.

River	Period of Shelter Deployment	Median	Minimum	Maximum	Channel km Between USGS Gage and Study Reaches (Range)
River 1	June 2018 – Present	2.95	1.64	11.78	23.28-40.77
River 2	June 2014 – Present	3.26	0.85	121.20	15.33-22.75
River 3	May 2013 – Present	2.38	0.65	51.54	0.05-17.72

Table 2.2. Mean values and ranges for all variables used in our shelter availability and stability analyses. Variables of binary (present/absent) character are coded as 0 or 1, therefore their mean values represent the percentage of shelters where we considered them variables present.

Variable	Mean, or Probability of Presence	Range	Variable Type	Scale
Adult/subadult hellbender density (individuals/1680 m^2)	23.05	6-51	Continuous	Reach
Habitat surplus (continuous density)	0.05	-0.28-0.45	Continuous	Reach
Reach-wide density of large suitable boulders	6.38	3-14	Count	Reach
Upstream catchment size (km^2)	197.24	131.31-309.00	Continuous	Reach
Upstream CWR forest cover (%)	63	54-70	Continuous	Reach
Channel transition status	0.47	0 or 1	Binary	5 m
Pool-riffle-run transition status	0.15	0 or 1	Binary	5 m
Sand/gravel bar transition status	0.36	0 or 1	Binary	5 m
Bank-to-bank current velocity (m/s)	0.13	0-0.66	Continuous	Microhabitat
Distance to bank (m)	3.70	0.1-9.30	Continuous	Microhabitat
Downstream current velocity (m/s)	0.28	-0.13-1.10	Continuous	Microhabitat
Tunnel angle (degrees)	24.65	0.00-105.00	Continuous	Microhabitat

Table 2.2 (Cont.). Mean values and ranges for all variables used in our shelter availability and stability analyses. Variables of binary (present/absent) character are coded as 0 or 1, therefore their mean values represent the percentage of shelters where we considered them variables present.

Variable	Mean, or Probability of Presence	Range	Variable Type	Scale
Percent canopy cover	70	0-100	Continuous	Microhabitat
Vertical distance to canopy (m)	3.01	0.19-16.05	Continuous	Microhabitat
Water depth at tunnel (cm)	44.21	19.00-103.00	Continuous	Microhabitat
Crevice-bearing boulders within 1 m	3.38	0-11	Count	Microhabitat
Total breeding seasons since installation (nesting)	2.56	1-6	Count	Temporal
Average months since installation (occupancy)	10.06	1-31	Count	Temporal

Table 2.3. Scores of every occupancy and nesting model. Model A = habitat variables from all three spatial scales used during initial model construction; Model B = built with reach-scale predictors excluded during initial model construction; Model C = reach and 5 m-scale variables excluded during initial model construction; Model D = time since shelter installation and reach-wide hellbender density as the only predictor variables; Model E = same predictor variables as the top model, but built using data from our six original study locations only. The top performing model (Model A in both analyses) is bolded.

Occupancy

Model	CV Correlation	CV Standard Error
A	**0.658**	**0.043**
B	0.655	0.024
C	0.655	0.024
D	0.651	0.049
E	0.649	0.039

Nesting

Model	CV Correlation	CV Standard Error
A	**0.278**	**0.042**
B	0.205	0.062
C	0.205	0.062
D	0.256	0.047
E	0.215	0.056

Table 2.4. Recommendations for artificial shelter placement and post hoc decision-making, given the explicit objectives of maximizing shelter occupancy and nesting. We defined the importance of each recommendation qualitatively, based on a combination of the relative influence of each variable in our availability or stability BRTs and the effect size of each variable's influence.

Occupancy		
Variable	**Recommendation**	**Importance**
Adult/Subadult Hellbender Density	Expect lower than average hellbender occupancy in shelters deployed in reaches with < 1 adult/subadult hellbender per 100 m^2	Very High
Time Since Installation	Move shelters that are not found occupied within two years of their deployment	Moderate
Water Depth	Deploy artificial shelters in places where the stream is at least 50 cm deep during median discharge	Moderate

Nesting		
Variable	**Recommendation**	**Importance**
Water Depth	Deploy artificial shelters in places where the stream is at least 50 cm deep during median discharge	Very High
Adult/Subadult Hellbender Density	Expect lower than average hellbender nesting in shelters deployed in reaches with < 1 adult/subadult hellbender per 100 m^2	High

FIGURES

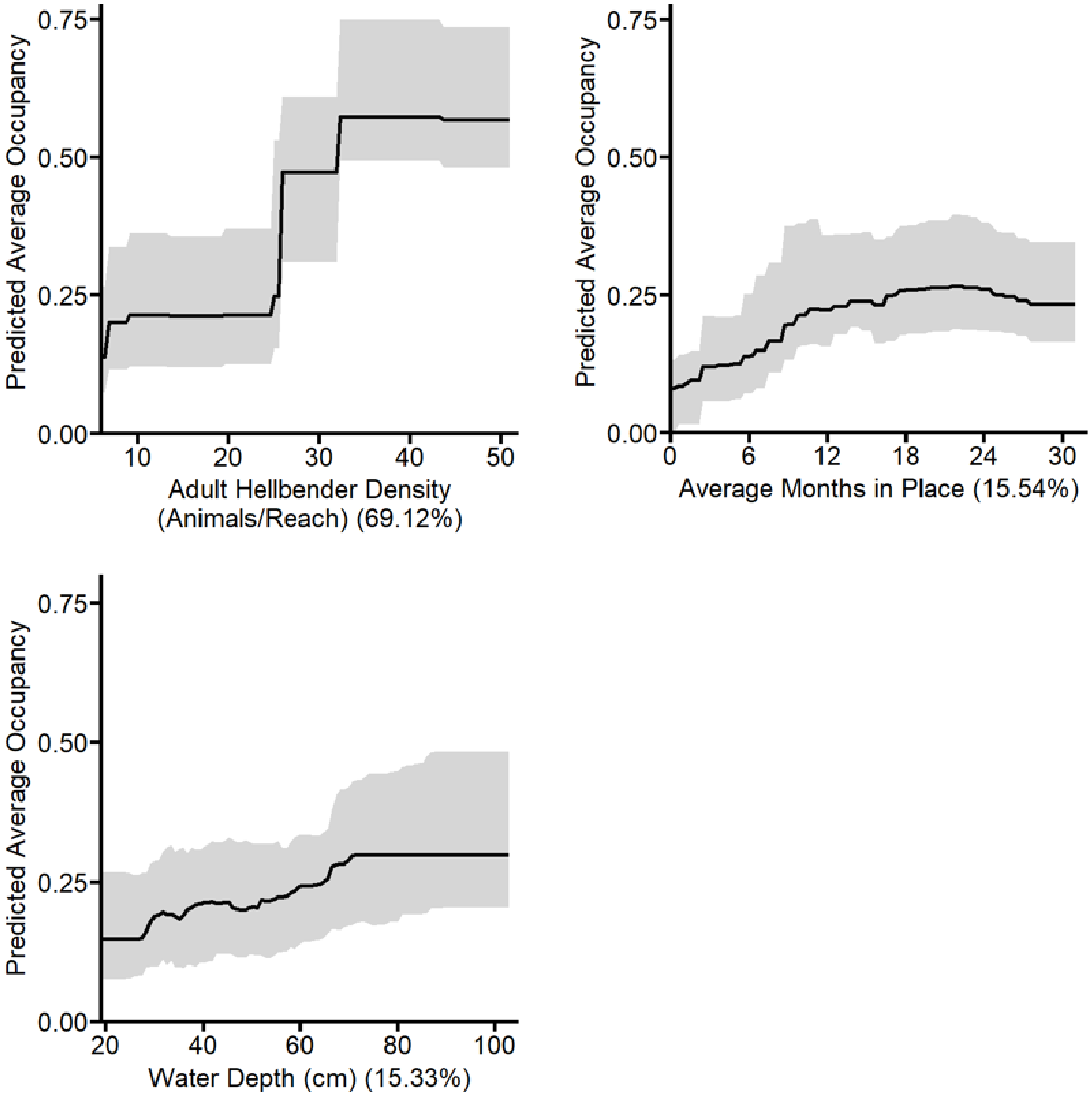


Fig. 2.1. Partial dependence plots for the most important predictor variables retained in the final version of the selected model of shelter occupancy. Solid black lines show fitted functions, while shaded areas represent 95% percentile-based confidence intervals built using 200 bootstraps.

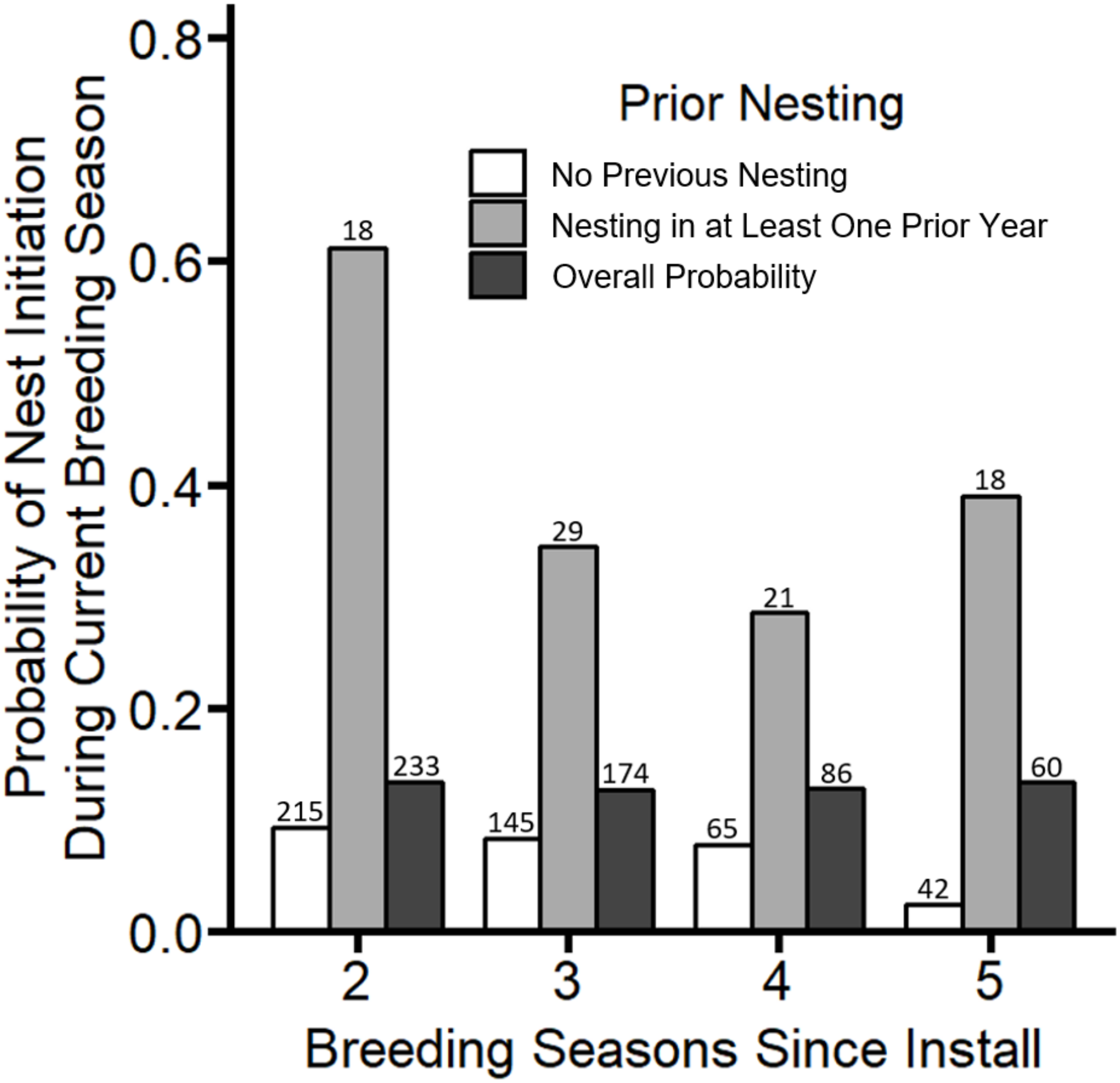


Fig. 2.2. The influence of breeding seasons since install and nesting during prior breeding seasons on nest probability during the current breeding season. Shelter nest initiation increased dramatically if a shelter was nested in during at least one prior breeding season, while overall nesting remained roughly constant for shelters in place for multiple years.

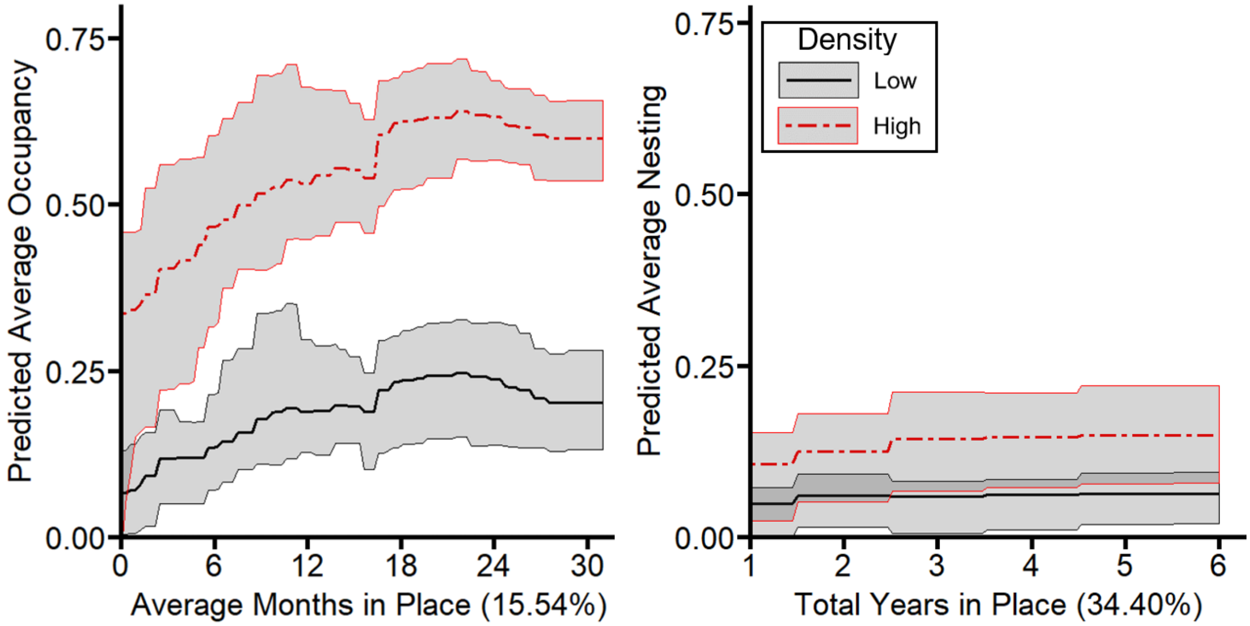


Fig. 2.3. The influence of adult/subadult hellbender density on the relationship between time in place and shelter use. We considered density “high” in reaches with > 1.5 adult and subadult hellbenders per 100 m^2 or “low” otherwise. We considered classified hellbenders as adult or subadult during surveys if their total length was at least 29 cm. Shelter occupancy increased more rapidly in high density reaches than in low density reaches. Shelter nesting increased at a roughly equal rate in high and low density reaches, and remained much lower than shelter occupancy throughout the study.

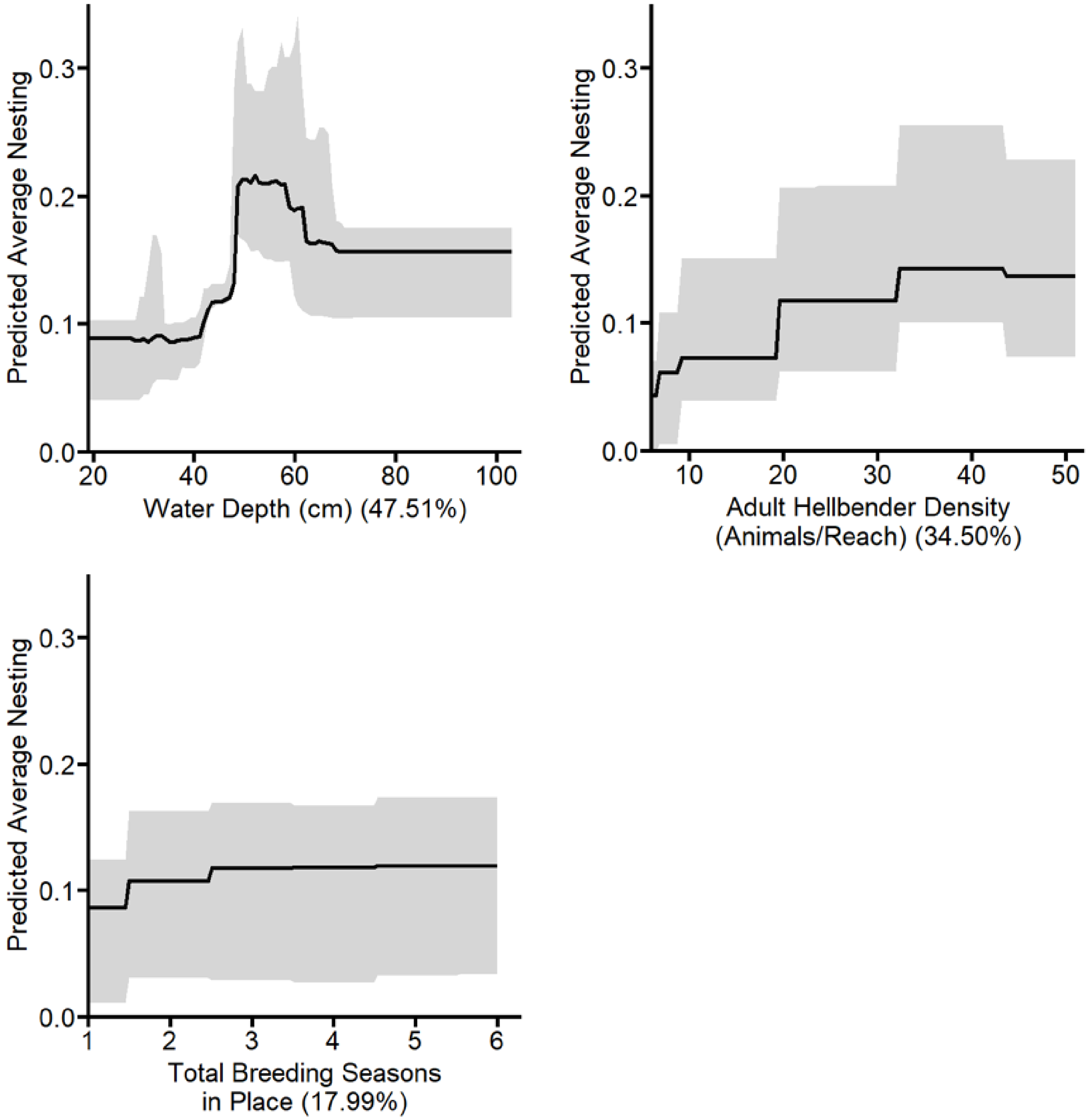
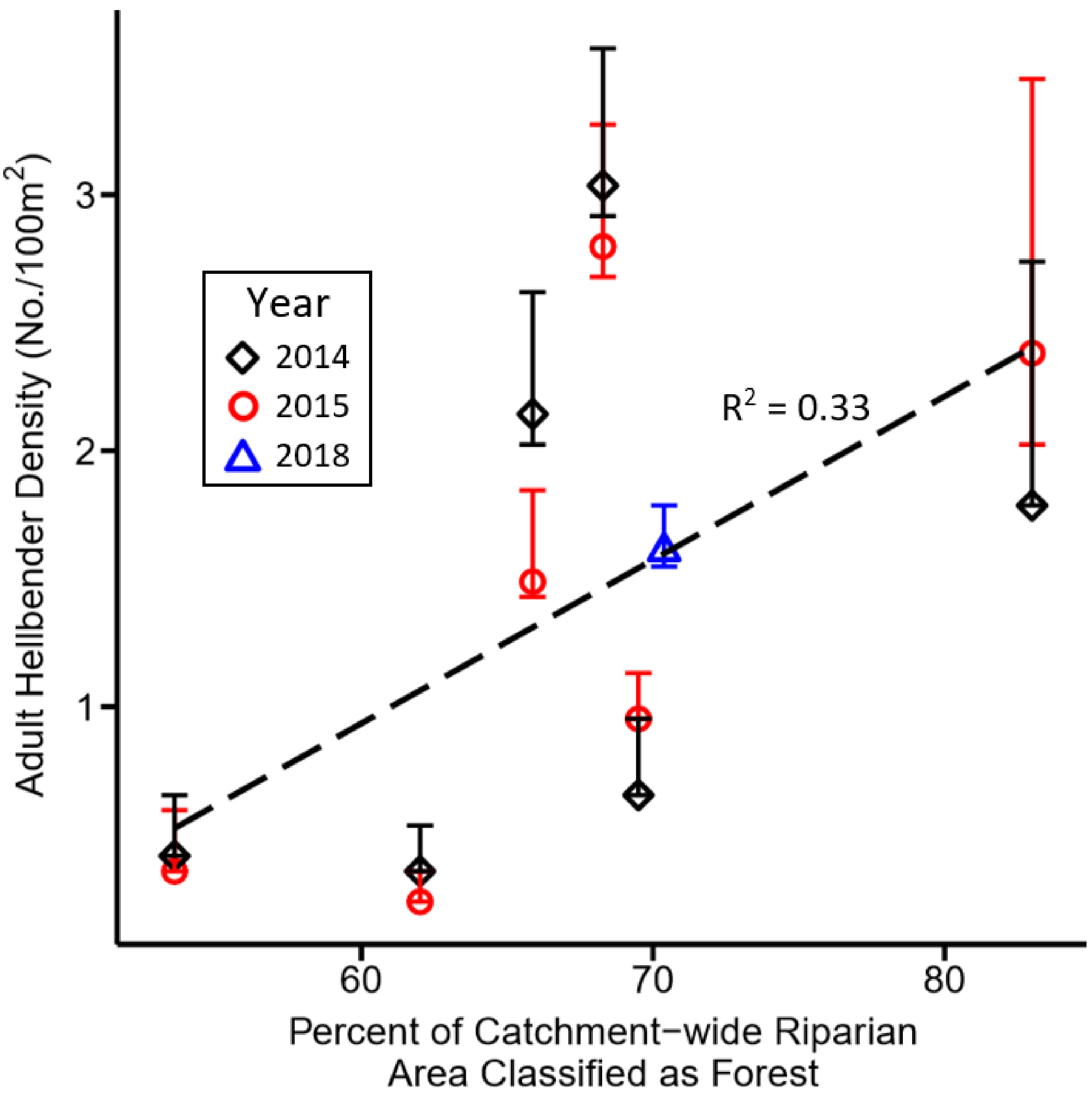


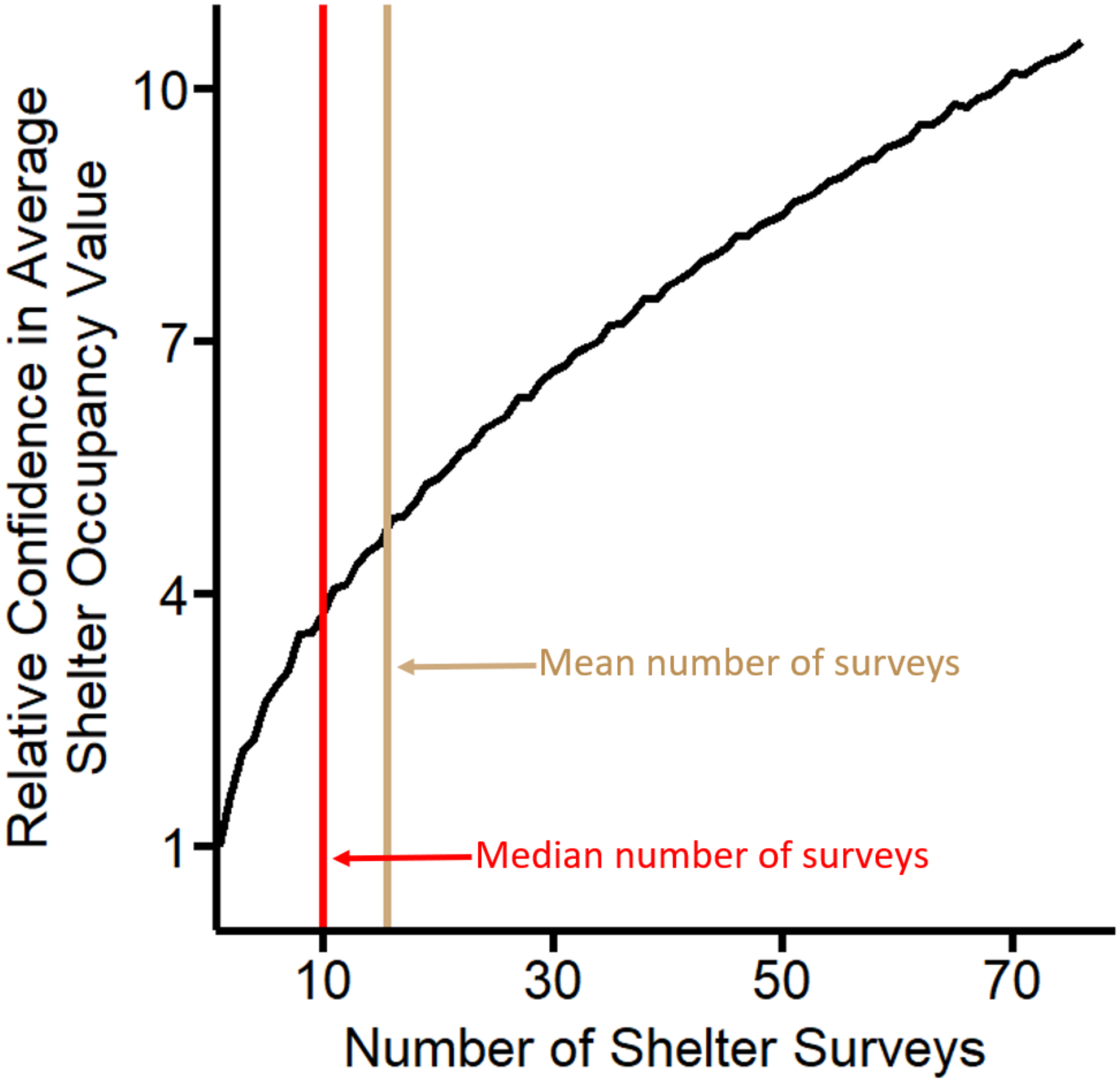
Fig. 2.4. Partial dependence plots for the most important predictor variables retained in the final version of the top performing nest initiation model in the set. Solid lines show fitted functions, while shaded areas represent 95% percentile-based confidence intervals built using 200 bootstraps.

APPENDIX A: ADULT/SUBADULT HELLBENDER DENSITIES

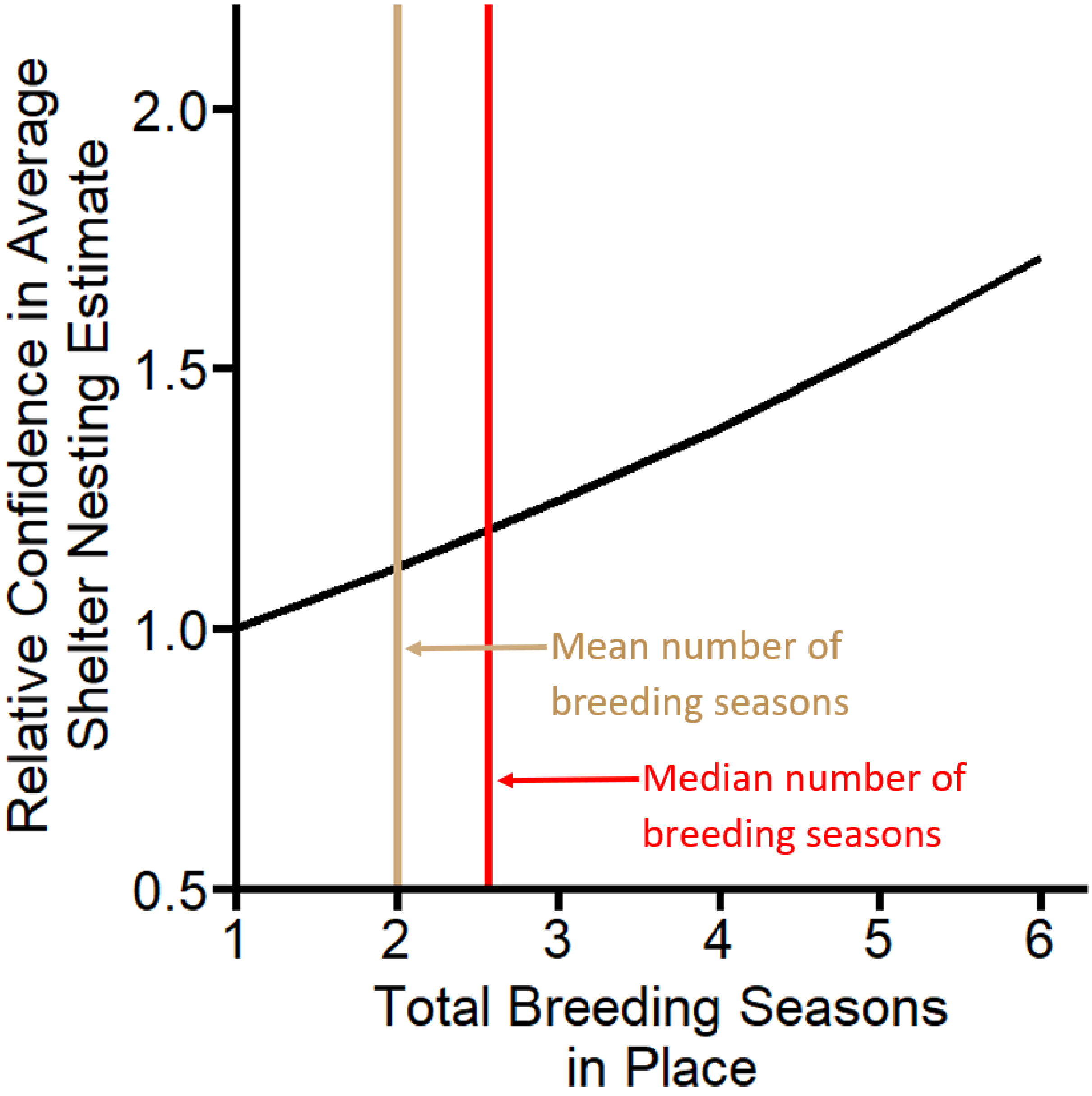


A1. The relationship between the amount of percent forest cover in the upstream catchment-wide riparian area are our six multi-year study reaches and adult/subadult hellbender density estimates from those reaches. Density surveys were conducted in 2014, 2015, and 2018 by flipping all crevice-bearing boulders within a representative 1,680 m^2 section of the reach. Density estimates were determined using Huggins closed capture models (Huggins, 1989).

APPENDIX B: WEIGHTING SCALE USED IN MODELS

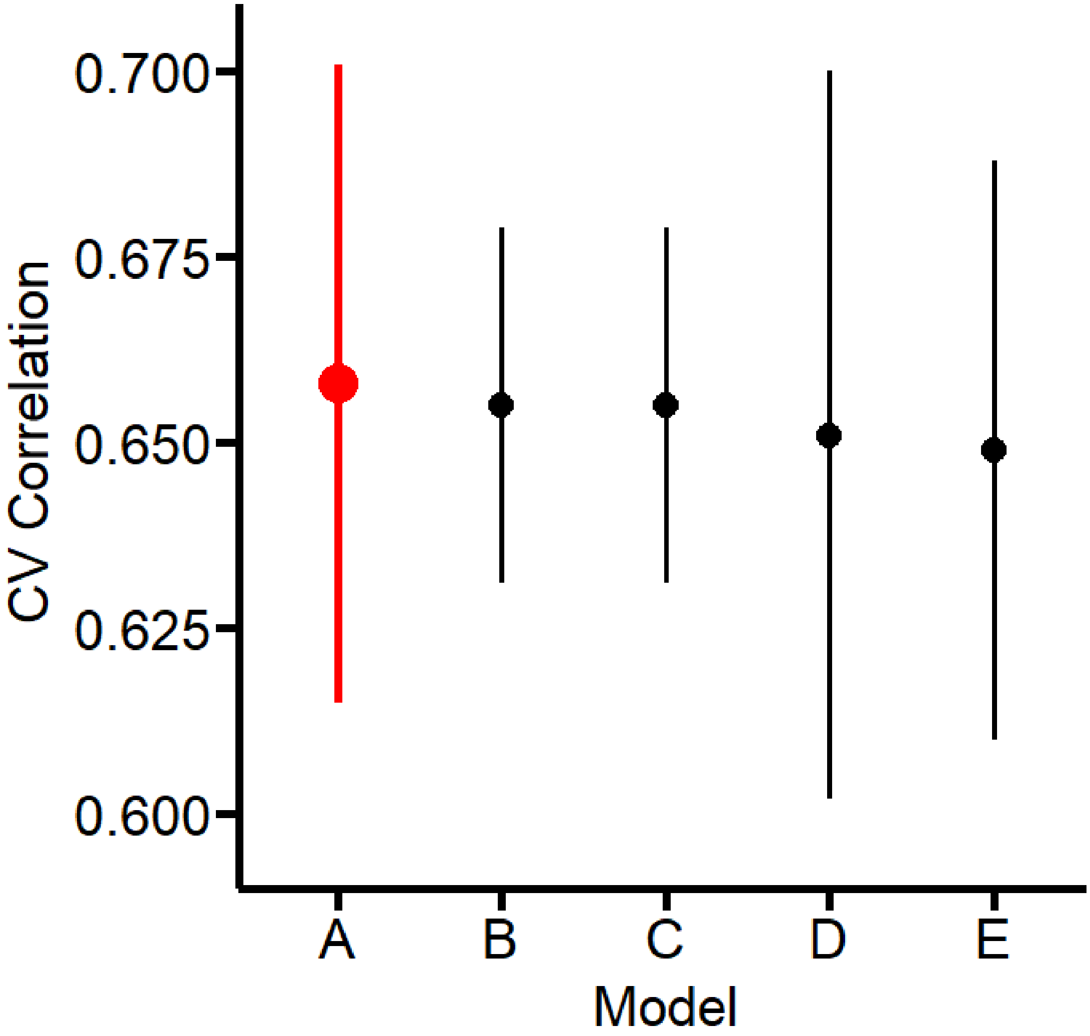


B1. The simulated relationship between the number of surveys of a shelter and the expected accuracy of average occupancy estimates obtained at that shelter. Predicted accuracy for each x-axis value was computed relative to the predicted estimate accuracy if a shelter was surveyed only once. Predicted values are indicated by a solid black line. The simulated accuracy of shelter occupancy estimates increased logarithmically with number of surveys, and ranged between 1.00 (for a shelter surveyed once) and 9.52 (for a shelter surveyed 76 times).

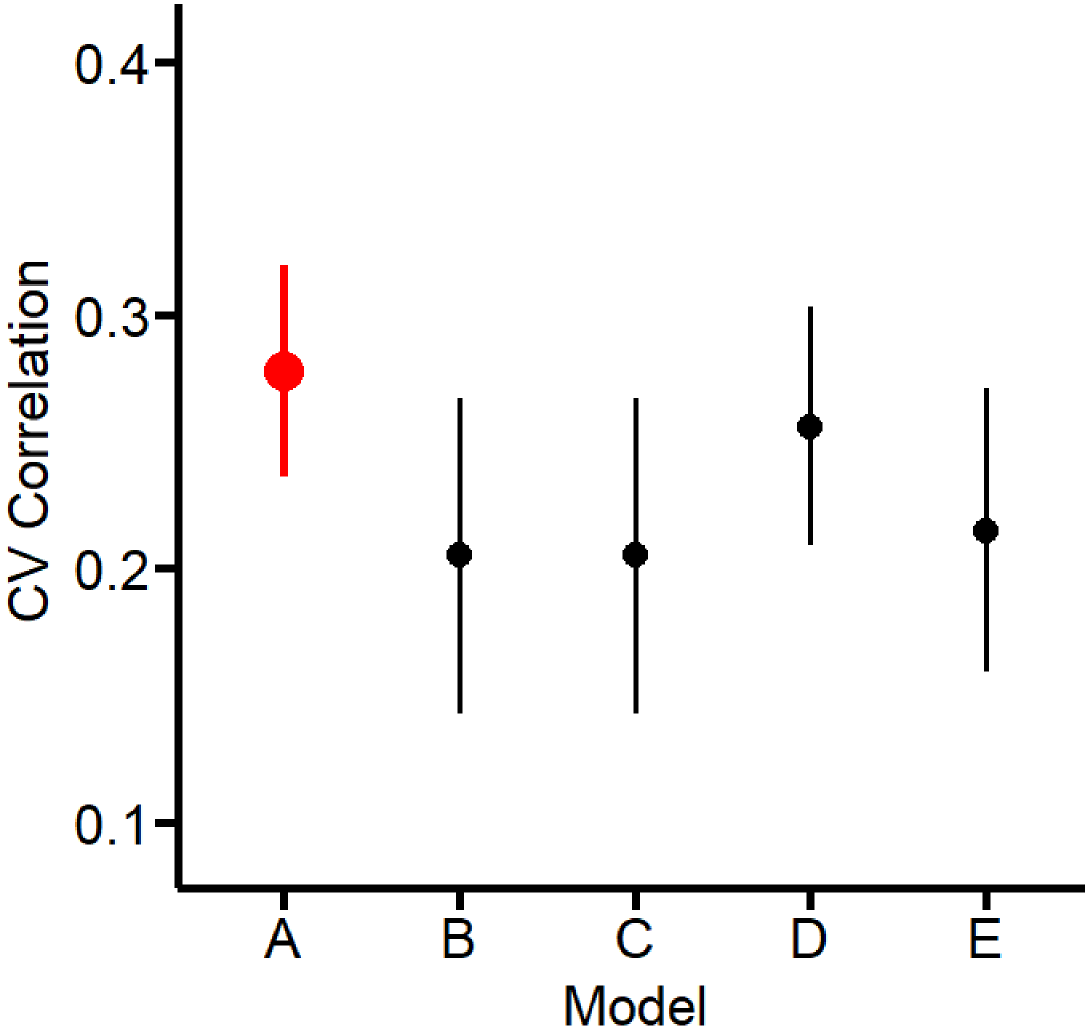


B2. The simulated relationship between the number of breeding seasons a shelter has been in place for and the expected accuracy of overall nesting estimates obtained at that shelter. Predicted accuracy for each x-axis value was computed relative to the predicted estimate accuracy if a shelter was in place for only one breeding season. Predicted values are indicated by a solid black line. The simulated accuracy of shelter nesting estimates increased logarithmically with number of breeding seasons in place, and ranged between 1.00 (for a shelter in place during one breeding season) and 1.68 (for a shelter in place during six breeding seasons).

APPENDIX C: MODEL COMPARISON GRAPHS

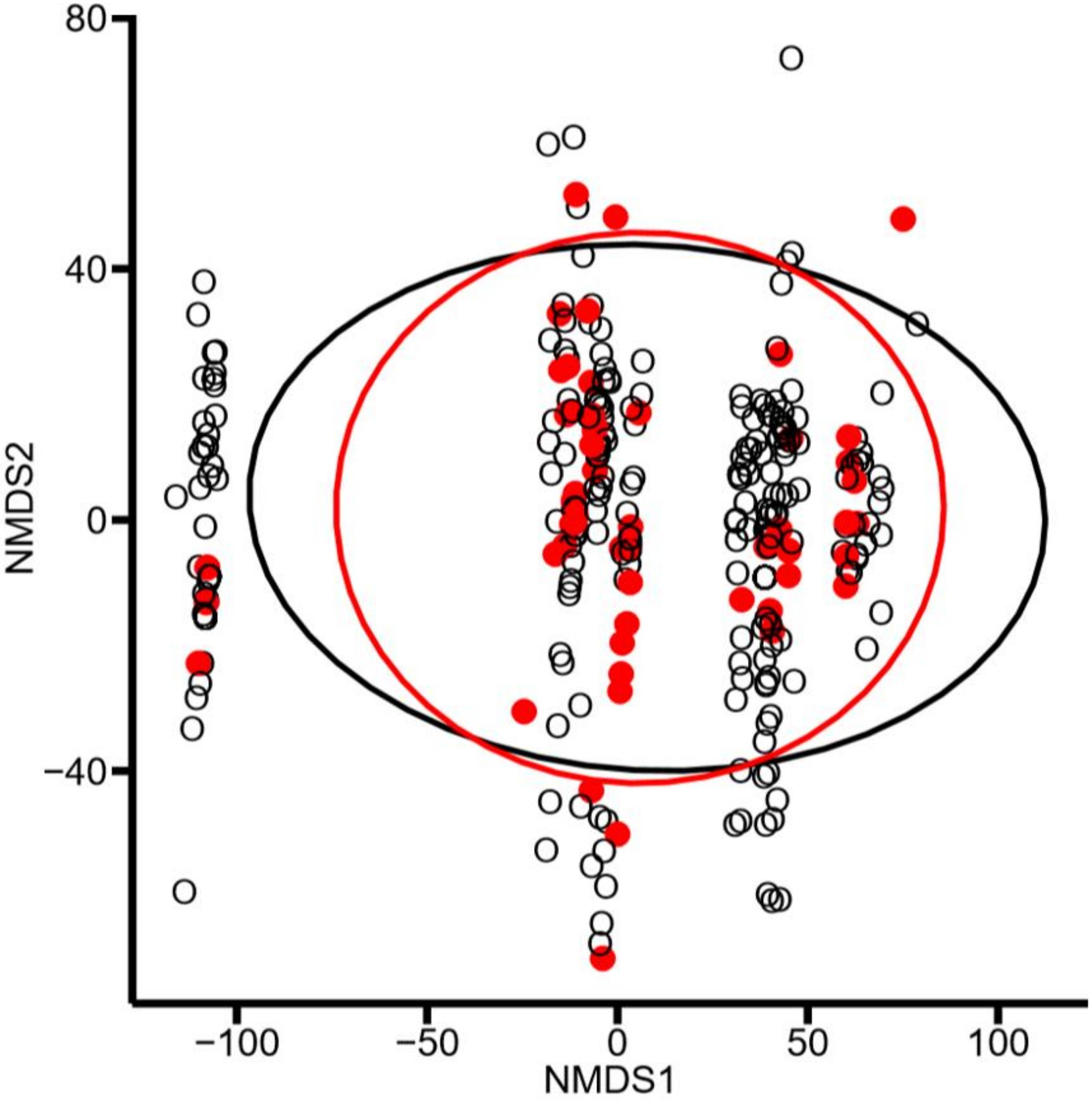


C1. Cross validated correlation scores of all models of shelter occupancy. Error bars represent cross-validated standard errors. I used model A (thickened/red; CV correlation = 0.658; SE = 0.043) to build partial dependency plots and estimate the relationship between habitat variables and nest initiation at artificial shelters, because this model had the highest CV correlation of all models in the set. Model B = a model built with reach-scale predictors excluded during initial model construction; Model C = reach and 5 m-scale variables excluded during initial model construction; Model D = time since shelter installation and reach-wide hellbender density used as sole predictor variables; Model E = constructed with same variables as top model (A), using data from six original study locations only. All models in the set were similar in their performance.



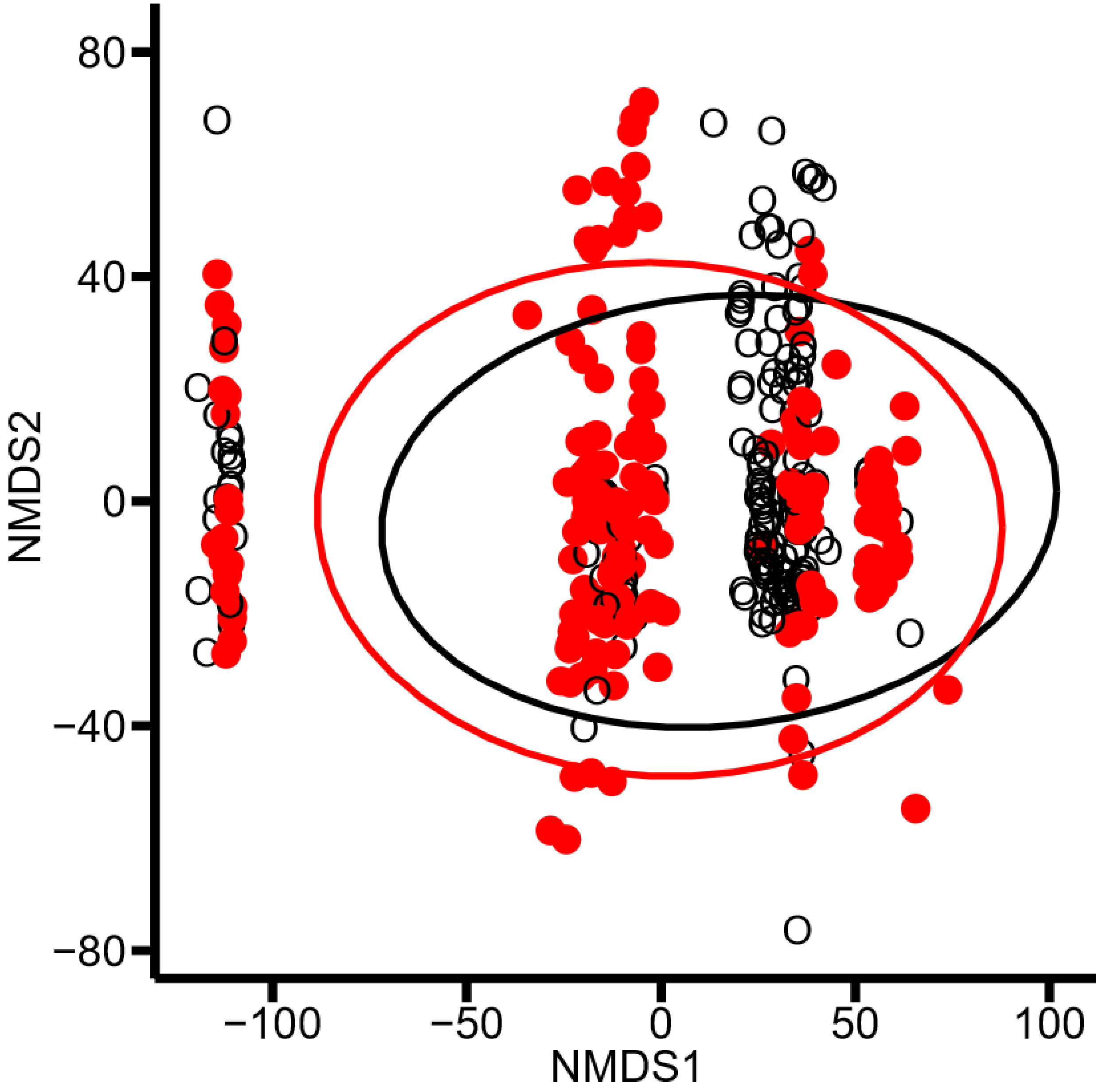
C2. Cross validated correlation scores for all nest initiation models. Error bars represent cross-validated standard errors. I used the top-performing model (Model A, shown thickened and in red, and built using predictor variables from all spatial scales before dropping uninformative variables) to build partial dependence plots and estimate the relationship between predictor variables and average nest initiation at artificial shelters. Model A (best model) = predictor variables from all scales used in initial model construction; Model B = reach-scale predictors excluded during initial model construction; Model C = reach and 5 m-scale variables excluded during initial model construction; Model D = average breeding seasons since shelter installation and reach-wide hellbender density used as sole predictor variables; Model E = constructed with same variables as the top model (A), using data from only our six multi-year study reaches that have density estimates.

APPENDIX D: SHELTER AVAILABILITY AND STABILITY NMDS PLOTS



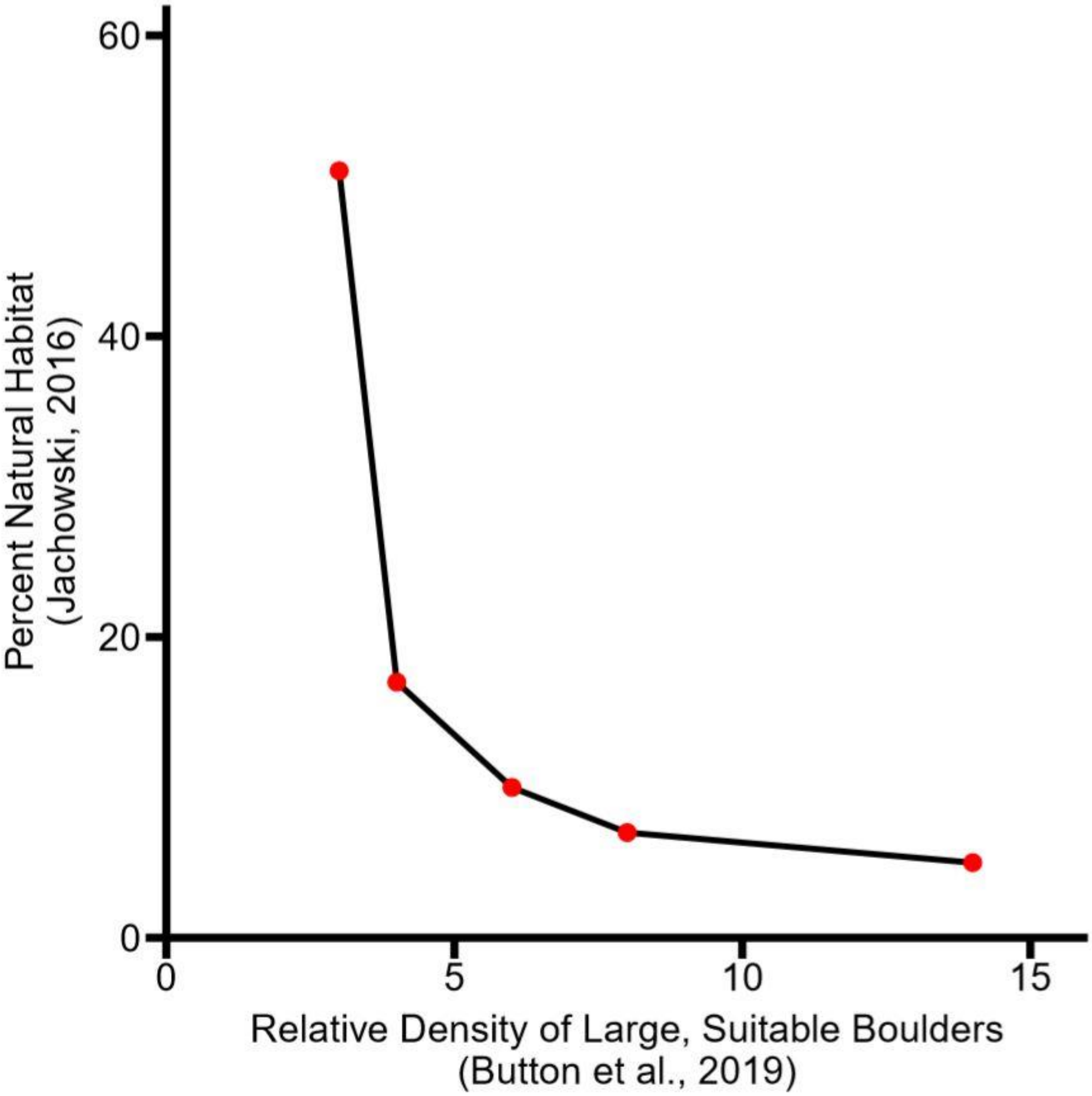
D1. NMDS plots with 95% confidence ellipses artificial shelters occupied on > 50% of all surveys versus on < 50% of all surveys. Shelters that were usually occupied or usually unoccupied are shown by hollow black circles and solid red circles, respectively. Confidence ellipses are shown as a black line for shelters that were usually occupied and a red line for shelters that were usually not. Shelters that were usually unoccupied had a smaller 95%

confidence ellipse than those that were usually occupied, suggesting that they were characterized by a narrower range of conditions. The relationship between shelter occupancy and datapoint dispersion is not fully represented by this plot, since shelter occupancy is a continuous beta-distributed variable but had to be broken into categories in order to make the plot.



D2. NMDS plots with 95% confidence ellipses artificial shelters nested in at least once versus never nested in. Shelters that were nested in or not nested in are shown by hollow black circles and solid red circles, respectively. Confidence ellipses are shown as a black line for shelters that were nested in at least once and a red line for shelters that were not. Shelters that were nested in at least once had a smaller 95% confidence ellipse than those that were not, suggesting that they were characterized by a narrower range of conditions. The relationship between shelter nesting and datapoint dispersion is not fully represented by this plot, since shelter nesting is a continuous beta-distributed variable but had to be broken into categories in order to make the plot.

APPENDIX E: DIFFERENCES IN ESTIMATED NATURAL HELLBENDER HABITAT BETWEEN THIS STUDY AND A PREVIOUS ONE



E1. The relationship between the amount of natural habitat quantified at five reaches on River 3 by this study versus by Jachowski (2016). We treated the relative density of large suitable boulders among these reaches as a suitable proxy for the amount of existing hellbender habitat. We assessed this metric by walking ten equally-spaced transects across a representative 1680m^2 portion of each study reach and counting the number of large (> 40 cm long) boulders with crevices suitable for hellbenders on these transects. Jachowski (2016) conducted 20 equally-spaced transects over the same portion of each of these reaches, but considered all boulders (> 25.6 cm on the secondary axis) and all bedrock suitable for hellbenders regardless of whether the substrate harbored suitable crevices. Additionally, instead of assessing the relative density of suitable habitat between reaches, Jachowski (2016) used the percentage of all measured particles that were classified as boulder/bedrock to predict hellbender shelter occupancy. We suspect that these differences in methodology used to estimate natural hellbender habitat explain differences in the predicted relationship between amount of reach-wide natural habitat and hellbender occupancy in artificial shelters between the two studies. While Jachowski (2016) found evidence of a negative relationship between percent “natural habitat” and hellbender shelter occupancy, our study revealed that shelter occupancy and the relative reach-wide density of large, suitable boulders were positively related.

Discussion & Conclusion

Although threats to lotic ecosystems have been well-documented for multiple decades (Malmqvist & Rundle, 2002; Hooke, 2000), the ultimate causes of lotic species declines remain largely enigmatic. Declining lotic amphibians exemplify this knowledge gap, since the frequent inability of current monitoring techniques to detect them makes it challenging to determine precise drivers of their declines. While artificial shelters may represent useful, novel tools for improving lotic amphibian monitoring capabilities, the successful application of artificial shelters for studying lotic amphibians has been severely limited thus far, and has faced numerous obstacles during initial attempts.

Hellbenders are one lotic amphibian species for which artificial shelters have been proposed as a monitoring tool. However, initial attempts at using artificial shelters to study hellbenders in the wild have had mixed success in practice (e.g., Messerman, 2014). I therefore sought to assess whether the utility of artificial shelters for monitoring hellbenders could be improved by optimizing shelter design, maintenance, and placement. To evaluate the potential utility of artificial shelters for monitoring hellbenders, I used a two-step analytical framework. First, I evaluated the influence of shelter design, maintenance frequency, and placement on the availability of artificial shelters to hellbenders, and on the stability of artificial shelters during high discharge events (Chapter 1). Next, I assessed how multiscale factors related to shelter placement influenced occupancy and nesting by hellbenders in artificial shelters (Chapter 2).

Factors Influencing Shelter Availability and Stability. My research demonstrated that shelters could be made available to hellbenders under many circumstances and stable during high discharge events, by optimizing their design, maintenance, and placement. In reaches with modest impairment, shelters were consistently available to hellbenders when maintained at least once every 40 days (and after high discharge events), situated within 1 m of five or more boulders, and angled such that their entrances did not point directly downstream. Remarkably, shelters were also stable more than 99% of the time when built using our modified shelter design (Design B), which weighed ~40 kg and featured a standardized, recessed lid, held in place using an eye-bolt and hook.

While artificial shelters were generally useful for monitoring hellbenders in our system, there were occasional exceptions to this finding. For example, artificial shelters deployed in a highly impaired, sedimented pilot reach in the upper New River Basin became rapidly blocked by sediment regardless of their placement. Therefore, artificial shelters of the "hydrodynamic" design proposed by Mohammed et al. (2016) have the potential to offer advantages over boot design shelters for monitoring hellbenders in exceptionally impaired reaches, if the streamlined design of these shelters prevents sediment accumulation at their entrances. However, use of hydrodynamic shelters will not yield improvements to shelter stability, because our modified boot design artificial shelters (Design B) were already stable > 99% of the time, and withstood several severe high discharge events including multiple named tropical storms.

Factors Influencing Hellbender Occupancy and Nesting in Shelters. When given the opportunity, hellbenders most frequently occupied and nested in artificial shelters located in moderately deep portions of reaches with high adult/subadult hellbender densities. Occupancy was also further improved when shelters had been in place for two or more years. Encouragingly, predicted hellbender occupancy and nesting peaked at 67% and 24% respectively, representing a substantial increase over what has previously been documented. Therefore, artificial shelters were effective tools for monitoring hellbenders in our study system when their placement was optimized. However, due to the overarching influence of adult/subadult hellbender density on shelter use, it is likely necessary to deploy more artificial shelters in sparsely populated reaches than elsewhere in order to effectively monitor hellbenders in these reaches.

Future Directions. The applicability of our results should be reviewed across different portions of the hellbender's range, with different hellbender lineages, especially in streams with a diversity of flow and sedimentation characteristics. Genetic evidence supports the existence of five major hellbender lineages (Hime, 2017); only one of which occurred within our primary study system. Given that allopatric lineages of hellbenders may occur in streams with different geomorphic properties and biotic communities than at our study reaches, these lineages may also exhibit ecological differences in their use of artificial shelters. Additionally, since flow and sediment transport regimes vary widely across physiographic regions (Allan & Castillo, 2007), the feasibility of keeping shelters available to hellbenders might also vary across such regions. The replication of our study across different physiographic regions should therefore be carried out in order to determine how broadly applicable our findings are.

If applicable in other watersheds, our results create the opportunity to answer numerous questions about the biology and conservation needs of hellbenders that have previously been untestable. For example, it so far remains unclear whether different upstream agricultural land uses (e.g., production of different types of crops, cattle ranching, horses, etc.) have differing impacts on hellbender populations. Since the dominant form of agriculture strongly influences the occurrence of many aquatic and semi-aquatic amphibians (Waddle et al., 2013), it seems likely that this factor might also impact hellbenders. Additionally, future research should determine whether deploying artificial shelters in impaired reaches with limited natural habitat can improve long-term hellbender population trajectories in these reaches by augmenting the existing habitat, and whether artificial shelters can be used effectively to soft-release captive-reared animals in order to improve their post-release survival (Crane & Mathis, 2011). These are a few of many questions that may be possible to answer using artificial shelters, given their consistent use by hellbenders.

In addition to being useful for monitoring hellbenders, artificial shelters might also improve monitoring capabilities for a variety of other lotic taxa. Salamanders in the genera *Necturus*, *Andrias, Siren*, *Proteus*, and *Dicamptodon*, for example, may become easier to detect using artificial shelters than with more conventional sampling methods, because some species within these genera are broadly ecologically similar to hellbenders, occurring beneath boulders (Sugg et al., 1988; Ashton, 1985; Johnston, 1999; Sket, 1997) and in rivers (Petranka, 1998). Additionally, most members of these genera, like hellbenders, are of medium or large body size and are often difficult to detect using existing survey methods (Browne et al., 2011), making it equally important that their monitoring be improved. Finally, artificial shelters might also be useful for sampling lotic fishes that are ecologically similar to hellbenders; particularly for imperiled species that cannot be monitored using electrofishing without unwanted accidental mortalities (Nielsen, 1998).

Conclusion. Although lotic amphibians are among the most imperiled organisms globally, the precise causes of their declines remain poorly understood due to their secretive life histories and low detectability using existing survey techniques. In consequence, efforts to conserve lotic amphibians have often struggled in the absence of information about appropriate management actions. Hellbenders represent an archetypal example of this problem due to their rapid declines and the limited utility of traditional methods for monitoring them. Moreover, while artificial shelters are a potentially useful, novel tool for monitoring hellbenders, factors that impact their utility for monitoring hellbenders have been previously unexplored. My assessment of how artificial shelters should be designed, maintained, and deployed to overcome previous practical hurdles and improve the use of these shelters by hellbenders therefore offers important recommendations for improving hellbender monitoring techniques. Moreover, since the precise conservation needs of hellbenders can only be determined and implemented if the species can be effectively monitored, my findings also have the potential to aid in the development of long-term strategies for hellbender population recovery.

REFERENCES

Allan, J. D., and Castillo, M. M. (2007). *Stream ecology: structure and function of running waters* Springer Science & Business Media.

Ashton, R. (1985). Field and laboratory observations on the microhabitat selection, movements, and home range of *Necturus lewisi*. *Brimleyana*: 83-106.

Browne, R., Li, H., Mcginnity, D., Okada, S., Zhenghuan, W., Bodinof, C., Irwin, K., Mcmillan, A., and Briggler, J. (2011). Survey techniques for giant salamanders and other aquatic Caudata. *Amphibian & Reptile Conservation* 5: 1-16.

Crane, A. L., and Mathis, A. (2011). Predator-recognition training: a conservation strategy to increase postrelease survival of hellbenders in head-starting programs. *Zoo Biology* 30: 611-622.

Hime, P. M. (2017). Genomic Perspectives on Amphibian Evolution Across Multiple Phylogenetic Scales.

Hooke, R. L. (2000). On the history of humans as geomorphic agents. *Geology* 28: 843-846.

Johnston, B. (1999). Terrestrial Pacific Giant Salamanders (*Dicamptodon tenebrosus*): natural history and their response to forest practices. University of British Columbia.

Malmqvist, B., and Rundle, S. (2002). Threats to the running water ecosystems of the world. *Environmental Conservation* 29: 134-153.

Messerman, A. (2014). The Use of Nest Boxes by the Hellbender Salamander in Western North Carolina. M.S. Thesis. Duke University.

Mohammed, M. G., Messerman, A. F., Mayhan, B. D., and Trauth, K. M. (2016). Theory and Practice of the Hydrodynamic Redesign of Artificial Hellbender Habitat. *Herpetological Review* 47: 586-591.

Nielsen, J. L. (1998). Scientific sampling effects: electrofishing California's endangered fish populations. *Fisheries* 23: 6-12.

Petranka, J. W. (1998). *Salamanders of the United States and Canada* Smithsonian Institution Press.

Sket, B. (1997). Distribution of *Proteus* (Amphibia: Urodela: Proteidae) and its possible explanation. *Journal of Biogeography* 24: 263-280.

Sugg, D. W., Karlin, A. A., Preston, C. R., and Heath, D. R. (1988). Morphological variation in a population of the salamander, *Siren intermedia nettingi*. *Journal of Herpetology* 22: 243-247.

Waddle, J. H., Glorioso, B. M., and Faulkner, S. P. (2013). A quantitative assessment of the conservation benefits of the Wetlands Reserve Program to amphibians. *Restoration Ecology* 21: 200-206.