

Behavior and conservation: hellbender reproductive behavioral responses to poor water quality and the value of private lands research for conservation outreach

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ABSTRACT

The behavior of humans and animals is an important component of conservation. The way people behave can cause environmental harm, but it also can be a solution to conservation challenges. Similarly, wildlife can respond to changing environments in ways that are detrimental to their survival, but they can also in some cases adapt or even thrive in human-impacted environments. In this dissertation, I discuss the ways in which human and hellbender behaviors relate to conservation.

First, using survey and interview data, I explore the reasons why private landowners allow access to their property for research. I find that landowners are more likely to allow research on their property if they have previously allowed research, if they are interested in learning about the research taking place, if they have larger properties, and if they had positive attitudes towards conservation. Conversely, they are less likely to allow access to their property if they are concerned that doing so might restrict free use of their property. Other possible predictors that I investigate, such as landowner demographics, landowner trust in science, and landowner attitudes towards hellbenders are not significant predictors of allowing research.

I next explore the effects of engaging landowners in research either via citizen science or "traditional" private lands research where scientists work on the landowner's property, but the landowner is not involved in data collection. I assess the effects of these types of engagement on landowner conservation knowledge, attitudes, awareness, and behavioral intentions and compare the effects across these two treatment groups and a control. I find similar changes in both the traditional and citizen science treatment groups, with involvement in the research leading to greater conservation knowledge, increased awareness of conservation concerns, more positive attitudes towards conservation, and/or more conservation behavioral intentions. However, I observe limited behavior change in any treatment group, and I identify a lack of efficacy and a hesitancy to accept responsibility for water quality degradation as possible barriers to behavior change.

My investigations of animal behavior utilize custom-built underwater infrared camera technology to study hellbender reproductive behavior. I first add to current understanding of hellbender breeding behavior by describing the basic hellbender mating sequence, identifying the presence of potential alternative mating tactics, and describing two novel behaviors, including possible signal production via wave-based communication and a unique egg laying behavior by female hellbenders. I then measure the extent to which hellbender males (which provide the parental care) may help protect their offspring from low oxygen and high siltation, and I assess the possibility of trade-offs between parental care behaviors that benefit the eggs and self-maintenance behaviors that benefit the parent. I find that guarding male hellbenders increase both their parental care behaviors and their self-maintenance behaviors in response to low dissolved oxygen,

but that they also show evidence of a trade-off between the two, with males ceasing parental care in favor of self-maintenance at sufficiently low levels of dissolved oxygen. Males show no parental response to high levels of silt.

My findings add to our understanding of human and animal behavior and highlight the importance of behavioral flexibility to conservation. I find that hellbenders can to some extent modify their behaviors to compensate for the impacts of poor stream management on their own and their offspring's health, and I find that engaging landowners in research can encourage changes in awareness, attitudes, and behavioral intentions regarding conservation.

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GENERAL AUDIENCE ABSTRACT

The behavior of humans and animals is an important component of conservation. The way people behave can cause environmental harm, but it also can be a solution to conservation challenges. Similarly, wildlife can respond to changing environments in ways that are detrimental to their survival, but they can also in some cases change to survive or even thrive in human-impacted environments. In this dissertation, I discuss the ways in which human and hellbender behaviors relate to conservation.

First, using survey and interview data, I explore the reasons why private landowners allow access to their property for research. I find that landowners are more likely to allow research on their property if they have previously allowed research, if they are interested in learning about the research taking place, if they have larger properties, and if they had positive attitudes towards conservation. Conversely, they are less likely to allow access to their property if they are concerned that doing so might restrict free use of their property. Other possible predictors that I investigate, such as landowner age and sex, landowner trust in science, and landowner attitudes towards hellbenders are not significant predictors of allowing research.

I next explore the effects of engaging landowners in research either via citizen science, where landowners help collect data, or "traditional" private lands research where scientists work on the landowner's property, but the landowner is not involved in data collection. I assess the effects of these types of engagement on landowner conservation knowledge, attitudes, awareness, and planned actions and compare the effects across these two treatment groups and a control. I find similar changes in both the traditional and citizen science treatment groups, with involvement in the research leading to greater conservation knowledge, increased awareness of conservation concerns, more positive attitudes towards conservation, and/or more conservation planned actions. However, I observe limited behavior change in any treatment group, and I identify a lack of perceived ability to make a difference among landowners and a hesitancy to accept responsibility for water quality decline as possible barriers to behavior change.

My investigations of animal behavior use custom-built underwater night-vision cameras to study hellbender baby-making. I first add to current understanding of hellbender breeding behavior by describing basic hellbender mating activities, identifying the possibility that some males sneak into other male's nests to mate, and describing two new behaviors, including possible signal production via sound or vibration and a unique egg laying behavior by female hellbenders. I then measure how much hellbender males (which provide the parental care) may help protect their eggs from low oxygen and high dissolved dirt, and I assess the possibility of trade-offs between parental care behaviors that benefit the eggs and self-maintenance behaviors that benefit the parent. I find that guarding male hellbenders increase both their parental care behaviors and their self-maintenance behaviors in response to low dissolved oxygen, but that they also show evidence of a trade-off between the two, with males stopping parental care in favor of

self-maintenance at low levels of dissolved oxygen. Males show no parental response to high levels of dissolved dirt.

My findings add to our understanding of human and animal behavior and highlight the importance of behavioral flexibility to conservation. I find that hellbenders can to some extent change their behaviors to compensate for the impacts of poor stream management on their own and their children's health, and I find that engaging landowners in research can encourage changes in awareness, attitudes, and planned behaviors regarding conservation.

## DEDICATION

To my parents, who taught me to love wild things.

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## ATTRIBUTION

Chapter 1 was coauthored by Ashley Dayer and William Hopkins who assisted in procuring funding, designing, and implementing the study. All coauthors provided comments and approved the manuscript.

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## INTRODUCTION

Behavior is both a driver of and a solution to environmental degradation. This includes behavior by both humans and animals. Human behaviors can exploit and degrade the natural environment (Ceballos et al. 2015), but they can also protect and restore natural systems and support species persistence (e.g., Hobbs 2012). Animals, in turn can respond to environmental degradation in ways that are adaptive and help them to survive in a changing environment (e.g., Angkaew et al. 2019), or they can fail to respond adaptively or even adopt detrimental behaviors that harm their own and other species (Schlaepfer et al. 2002). Understanding what causes these different responses, and how to encourage behaviors that promote sustainable ecosystems is an important task for conservation researchers.

One area where human and animal behavior are tightly linked to conservation outcomes is on private land. Although public protected lands are valuable because they provide contiguous habitat with relatively low levels of disturbance, they alone are not enough to protect the earth's resources. The limitations of public land are two-fold: First, public lands are limited in size. Over half the United States is privately owned (USGS GAP, 2022), and a large portion of habitat for at-risk species is located on private property (US GAO, 1995). Second, public lands are limited in scope. Public lands tend to protect steep, high-elevation areas with lower than average soil productivity that are farther from human infrastructure (Scott et al. 2001; Joppa and Pfaff 2009). This means that many species, and in some cases almost entire ecosystems, have limited protection on public land (Scott et al. 2001). Additionally, species and ecosystems (such as freshwater systems; Hermoso et al. 2015; Abell et al. 2007), frequently cross between jurisdictions, and thus require an approach that spans multiple levels of land ownership.

However, there are barriers to private lands conservation that must be addressed if its potential is to be realized. One such barrier is the lack of research on private lands. There is much less peer-reviewed literature based on private land conservation research than on public (Hilty and Merenlender 2003), and as a result there are fewer empirical resources to guide conservation management and decision making in this context. One reason for this imbalance is the additional challenges working on private lands poses for

researchers. These challenges begin with the process of gaining access to the properties (Hargiss and Dekeyser 2014a). In contrast to public lands, where there is often a clear protocol for gaining permission to undertake research, performing studies on private lands is a more nuanced and often complicated process. An improved understanding of how to approach gaining access to private lands could be valuable for encouraging private lands research among conservation scientists.

An additional challenge to private lands conservation is that it requires support from private landowners. With a few exceptions, most private lands conservation is entirely under the control of the landowner and subject to their individual situations and interests (Daley et al. 2004; Poudyal and Hodges 2009). Promisingly, the increasing prevalence of private lands research has the potential to also increase support among landowners for private lands conservation efforts by influencing their attitudes and behaviors regarding conservation (Lutter et al. 2018), although this possibility has seen little attention. By expanding knowledge of how to effectively undertake private lands research and studying its effects on landowner behavior, researchers may be able to encourage behaviors that support conservation efforts.

The wildlife found on private lands are strongly influenced by land management decisions, and in response to human activities, wildlife may also behave in ways that are beneficial or detrimental to their fitness. One category of behavior that may be particularly important to individual fitness is reproductive behavior, or behavior concerned with the production and survival of offspring. Reproduction is a critical component of species persistence, and thus understanding reproductive behavior and how it may vary in response to anthropogenic threats is a valuable conservation goal. For example, understanding breeding behaviors and requirements (such as requisite habitat conditions) can be valuable in both a captive settings, where it can enable more effective breeding programs (e.g., Martin-Wintle et al. 2015) and in the wild where it can inform necessary management actions (e.g., Oviedo and Solís 2008; Hagen et al. 2015).

For species with parental care, this too can be a critical component of reproductive success. Juveniles are often sensitive to environmental stressors and suffer high levels of mortality (Coulson et al. 2001). Parental care can help shield juveniles from environmental factors and ensure their survival into

older, more self-sufficient life stages (Klug and Bonsall 2014). Importantly, parents may modify their care behaviors in response to the environment (Ghalambor and Martin 2002; Jones and Reynolds 1999; Delia et al. 2013; Torricelli et al. 1985; Potts et al. 1988; St. Mary et al. 2001; Durant et al. 2019; Kight and Swaddle 2011; Dybala et al. 2013; Lissåker et al. 2003; Doody et al. 2006; Hale et al. 2003). Such parental flexibility may be increasingly important as human activities alter habitats. Rapid anthropogenic change can outpace genetic adaptation, making it difficult for species to persist. This is particularly true for species with slow life history strategies and long generation times (Peterson 1988; Taber et al. 1975). However, if parents are able to alter their care behaviors in ways that buffer juveniles against the ill effects of human activity, this may enable continued stable recruitment despite environmental change, and it could buy time for more slow-developing genetic changes to occur (Hendry et al. 2008).

In this dissertation, I explore the role of behavior in conservation through a study of human behavior regarding private lands research and a study of the reproductive behaviors of eastern hellbender salamanders (*Cryptobranchus alleganiensis alleganiensis*). I focus on why landowners in a Virginia stream's watershed allow access to their property for research and the potential for private lands research to serve as a means of encouraging conservation behaviors, such as stream best management practices. For my work on hellbender reproductive behavior, I provide a novel insight into hellbender breeding behavior and describe how hellbenders may modify or fail to modify their parental care behaviors in response to poor water quality that can be caused by poor stream management.

### *Biological science study site and species*

Eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*) are long-lived, giant, aquatic salamanders, and the clear, fast-flowing streams on which they depend (Nickerson and Mays 1973b) often flow through private property. This makes their fate intimately linked with private land management. Although previously abundant across a broad range, populations of this species have declined throughout their range (Wheeler et al. 2003; Foster et al. 2009), and existing populations have shifted from being

demographically well-distributed, to consisting primarily of large, older individuals (Jachowski and Hopkins 2018; Burgmeier et al. 2011; Foster et al. 2009; Wheeler et al. 2003). The reproductive period has been identified as a primary concern in these declines (Hopkins et al. 2023), making hellbenders a valuable species in which to study the interplay between reproductive behavior and environmental degradation.

Hellbender population declines and shifts in population demographic structure have been attributed to a variety of human-caused factors. Among these are changes in dissolved oxygen concentration and sediment levels that have resulted from land management practices (Mayasich and Phillips 2008). Hellbender eggs, larvae, and adults all depend on high dissolved oxygen concentrations to survive (Nickerson and Mays 1973a) and changes in water temperature due to deforestation, excessive nutrient loading, and other factors can decrease available oxygen (Kalff 2002). Deforestation can also contribute to erosion and increased sediment levels which can smother eggs (Kemp et al. 2011; Potts et al. 1988) and fill the interstitial spaces on which hellbender larvae depend (Nickerson et al. 2003). It also may contribute to biogeochemical conditions that further deoxygenate the nest cavity (Atkinson et al. 2008).

Given that reproductive failure is a major concern for hellbenders, I have focused my work on this period of life history including breeding and parental care behaviors. Prior to my research, the hellbender mating system was known to be a resource defense polygyny in which males fight to defend a nesting cavity that is visited by one to several females (Nickerson and Mays 1973b). After laying eggs, the females leave the male, called the “den master,” (Takahashi et al. 2017) to defend and care for the young on his own (Smith 1907). Den masters perform a number of care behaviors for their offspring, and some of these care behaviors may vary in direct response to water quality. For example, one egg care behavior is tail fanning, where males move their tails back and forth across the eggs (Settle et al. 2018a). This serves to oxygenate the eggs and the intensity of fanning may be modified in response to changing levels of dissolved oxygen as has been shown in other species (Jones and Reynolds 1999; Reeb et al. 1984). Another behavior is nest cleaning, where males use their legs, tails, and snouts to stir up sediment and push it out of the nest (Terry et al. 2018). This may reduce the levels of fine silt in the shelter.

This study took place in a third-order stream in southwestern Virginia, USA. The stream is the site of an ongoing hellbender research project and supports a comparatively healthy population of hellbenders (Jachowski and Hopkins 2018). The study sites have an average of 67% forest cover (Yang et al., 2018), average fall conductivity of  $0.19 \pm 0.01 \mu\text{S}/\text{cm}$ , average fall total dissolved solid levels of  $0.13 \pm 0.008 \text{ mg}/\text{L}$ , average fall pH of  $7.7 \pm 0.09$ , and an average dissolved oxygen concentration of  $10.3 \pm 0.66 \text{ mg}/\text{L}$ . This makes the stream comparatively more forested and less basic with lower conductivity and less total dissolved solids than many Virginia streams with hellbender populations (Bodinof Jachowski et al., 2016; O'Brien, unpublished data.)

### *Social science study site and population*

Copper Creek is a tributary of the Clinch River that runs through Scott and Russell counties in southwest Virginia. The creek's watershed suffers from high sedimentation, substrate instability, and channel scour (Martin 2019) and has been identified as a watershed of high conservation value due to the large number of threatened and endangered species in the creek (VDGIF, 2015). Copper Creek's watershed covers roughly 85,000 acres and is almost entirely privately-owned, meaning that landowners play an important role in determining stream management. The land that is not forested in the watershed is used primarily for agricultural purposes (39% of catchment-wide land area is agricultural and 52% is forested [USGS GAP, 2022]), and creekside property size ranges from less than an acre to more than 200 acres with an average size of 24.5 acres (VGIN, 2018).

Residents of Scott and Russell counties are 98% white, with a roughly equal male to female ratio (U.S. Census Bureau, 2019), and individuals over the age of 65 make up the most strongly represented age group (22% of the population). Seventy-nine percent of residents have a high school diploma or higher, 13% have received a bachelor's degree or higher, and the median income is \$39,404 (U.S. Census Bureau, 2019). This population is slightly older, less educated, and less wealthy than average residents of Virginia counties containing hellbender populations (Dickenson, Floyd, Giles, Grayson, Lee, Montgomery, Pulaski,

Russell, Scott, Smyth, Tazewell, Washington, Wythe, and Radford counties). The latter two traits make them an important population to target for outreach, as previous studies suggest that individuals with lower levels of education and lower income are less likely to adopt conservation activities/practices (Baumgart-Getz et al. 2012; Prokopy et al. 2008).

### *Objectives*

This dissertation addresses conservation through the lens of behavior through a focus on Copper Creek landowners and eastern hellbenders. In chapters one and two, I focus on the human side of conservation. I address why landowners allow conservation research on their property (Chapter 1) and explore the effect that engaging landowners in research has on their conservation knowledge attitudes, awareness, and behavioral intentions (Chapter 2). In chapters three and four, I focus on hellbenders. I describe hellbender breeding (Chapter 3) and parental care behaviors (Chapter 4) in greater detail than has previously been possible and seek to determine the extent to which hellbender parental care behaviors may buffer hellbender eggs and larvae from anthropogenic stressors (Chapter 4). Through this research I seek to shed light on how behavior can support conservation goals.

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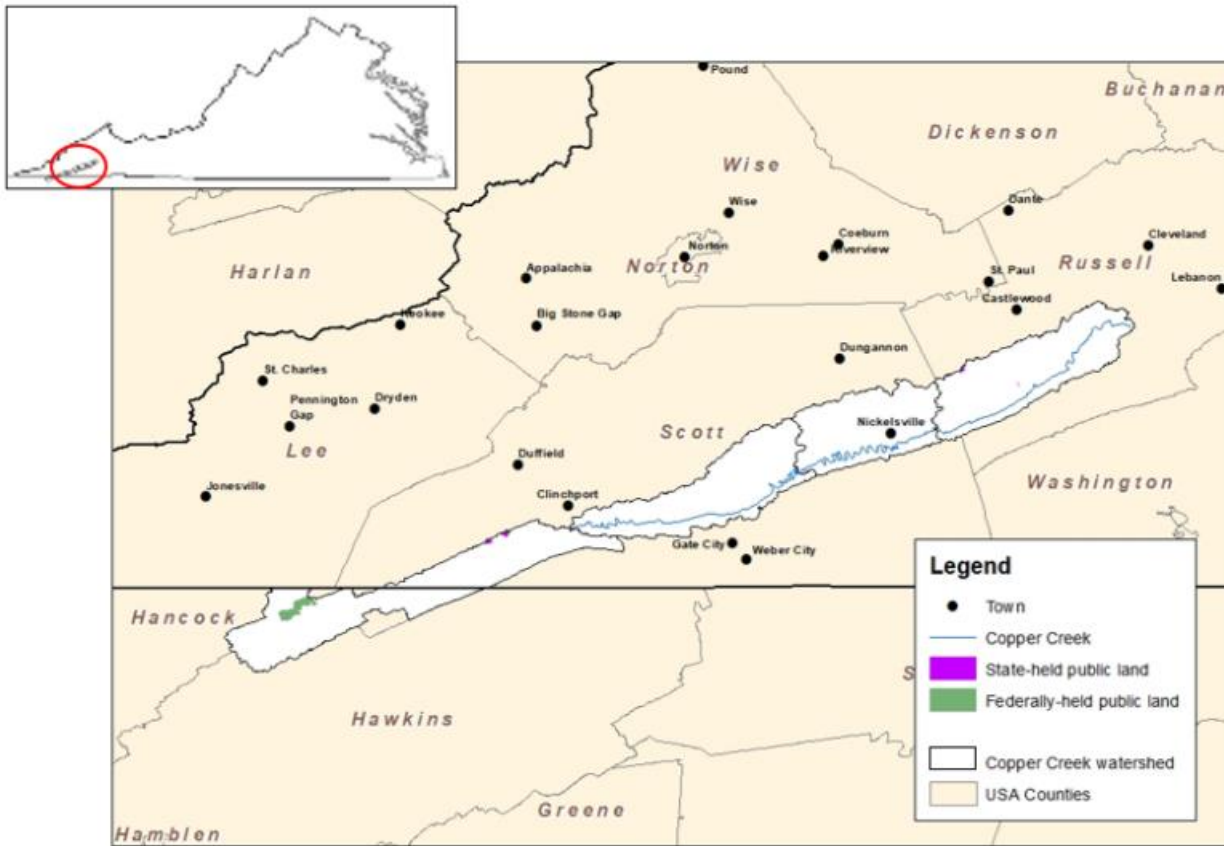
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**Figure I.1** My social science research took place in the Copper Creek watershed in southwest Virginia, which is almost entirely privately owned.

## CHAPTER 1:

### THE EFFECT OF ENGAGEMENT IN PRIVATE LANDS RESEARCH ON LANDOWNER CONSERVATION KNOWLEDGE ATTITUDES, AWARENESS, AND BEHAVIORAL INTENTIONS

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#### ABSTRACT

Private landowners and scientists often interact during conservation research projects on private lands, creating the opportunity for impactful outreach efforts. However, this potential has received little attention in the literature. This lack of attention is particularly true for traditional private lands research where landowners interact with researchers, but do not actively participate in data collection (as they do in citizen science projects). In this paper, we explore and compare the effects of engaging landowners in a traditional private lands research project and engaging landowners in citizen science research on landowner conservation knowledge attitudes, awareness, and behavioral intentions. We found similar effects across both the traditional and citizen science treatment groups, with involvement in the research leading to greater conservation knowledge, increased awareness of conservation concerns, more positive attitudes towards conservation, and/or more conservation behavioral intentions in 1/3 or more of the participants. However, landowners reported limited tangible behavior change during our study. Our results suggest that engaging with landowners while undertaking private lands research may be valuable to conservation goals, although further research on how to optimize these interactions within the constraints of data collection are needed.

#### INTRODUCTION

Increasingly creative and expansive approaches to conservation have emphasized the importance of private lands to achieving conservation goals (Cortés Capano et al. 2019; Kremen and Merenlender 2018; Stolton et al. 2014). Means of encouraging private lands conservation are diverse and can range from regulatory measures, such as those found in the Endangered Species Act, to supporting voluntary action like providing resources to landowners interested in native prairie restoration (Kamal et al. 2015). Voluntary

conservation measures are in many ways preferable, as they can, among other benefits, enhance intrinsic motivation (DeCaro and Stokes 2008) and encourage the persistence of conservation behaviors after formal commitments have ended (Dayer et al. 2018). However, encouraging voluntary action can be challenging, as, among other barriers, landowners must recognize the need for action, accept that they can be part of the solution, and have the knowledge and resources to address the problem (Schwartz 1997).

Personal contact is often identified as the most effective means of encouraging conservation behaviors (Ryan 2009; Toman et al. 2006; Shindler et al. 2009), but cost and time restrictions limit the extent to which it can be employed. For this reason, identifying and capitalizing on pre-existing opportunities for contact could be valuable to conservation goals. One such opportunity is conservation research on private property (Green et al. 2022; Lutter et al. 2018). Research on private lands is increasingly common (Smith, 2022) and will likely continue to grow in frequency to support private land conservation efforts (Dreiss and Malcom 2022; Burger et al. 2019). The resultant interactions between researchers and landowners may influence landowner cognitions and behaviors regarding conservation.

Substantial previous research on engagement of the public in research has focused on citizen, or participatory, science projects where members of the public volunteer their time to participate in scientific research through data collection and/or analysis (Green et al. 2022; Toomey and Domroese 2013; Evans 2005; Crall et al. 2013; Forrester et al. 2017a). These studies suggest that involvement in research can increase participants' knowledge, encourage positive attitudes towards conservation, and/or inspire more conservation-oriented behaviors (Green et al. 2022; Toomey and Domroese 2013; Evans 2005; Crall et al. 2013; Forrester et al. 2017). These effects can also extend beyond participants themselves, through their interpersonal sharing with others within their social network (Green et al. 2022; Forrester et al. 2017). However, most prior research on citizen science has involved volunteers seeking out participation in the project. These strongly conservation-oriented participants may have had little room for change or may have been predisposed towards environmental behavior (e.g., Crall et al. 2013; Overdeest et al. 2019; Brossard et al. 2005; Forrester et al. 2017).

Another common format for private lands research that has, in contrast, seen very little research attention is a more traditional model of private lands research where scientists collect data on private property and interact with the landowners, but the landowners themselves are not involved in data collection. In this study, explore the effect of both citizen science and participation in traditional private lands research on the cognitions (knowledge, awareness, and attitudes), behaviors, and behavioral intentions of landowners actively recruited from a rural watershed in southwestern Virginia.

## METHODS

Between 2019 and 2022, we explored the effects of engagement in research on landowners while undertaking an ecology research project focused on eastern hellbender salamanders (*Cryptobranchus alleganiensis alleganiensis*) in a rural southwest Virginia creek. The average resident of the counties where this study occurred is over the age of 65, educated to the completion of high school or receipt of a GED, and makes below the national median income (U.S. Census Bureau 2019). Our study had three stages including initial interviews with landowners to gain a baseline understanding of their perspectives on conservation, three years of engaging those landowners to varying degrees in research, and then follow-up interviews to assess what, if any, effect the involvement might have had on their behaviors and cognitions.

The initial round of interviews took place in the spring of 2019 and involved 37 landowners. These landowners came from a pool of 108 individuals who agreed to be contacted on a previously administered survey (O'Brien et al. 2021). All interviews were conducted in person by the lead author save one, which was done over the phone with a landowner who was unable to meet in person. Interviews were semi-structured, and our questions focused on the landowners' attitudes towards conservation and the creek on their property (Appendix 2.B). We also asked landowners about any conservation activities they were doing or planned to do on their property.

We divided the interviewed landowners into three groups (Figure 2.1): a control group, a traditional science group, and a citizen science group. We attempted to ensure, to the greatest extent possible, that each

treatment group had similar gender ratios and age distributions. Additionally, factors such as property suitability for the hellbender research project influenced to which treatment group landowners were assigned. Our first treatment group (hereafter the “traditional science group”) consisted of ten landowners who were engaged in a research project where scientists came onto their property to study the aquatic eastern hellbender salamander (*Cryptobranchus alleganiensis alleganiensis*). We interacted with these individuals several times per year through phone/email contacts to let them know we were coming to their property (mean=6.2 phone conversations, range=2-9) and had in-person conversations with them while conducting our ecology research (mean=5 conversations, range=1-12). During these conversations, we made small talk and also discussed our research whenever it fit naturally into the conversation. We also invited these landowners to join us in the creek to see our work. The second group (hereafter referred to as the “citizen science group”) consisted of ten landowners who were engaged in a citizen science project collecting water quality data from a stream on their property to inform the hellbender project. To represent the most common citizen science experience (which often as limited engagement with scientists, Sullivan et al. 2009), we did not interact in-person with the citizen science group at all beyond an initial meeting with them, but we mailed them packets three times a year containing materials and instructions to sample the water quality in the creek on their property. A final group of seventeen landowners (hereafter referred to as the “control group”) were interviewed prior to and after the three-year project, but they were not otherwise engaged in research or contacted by the researchers in any way.

We sent holiday cards each winter to both the citizen science and traditional science group thanking them for their participation, and, during a year-long period when we were unable to meet in-person with landowners due to COVID-19 restrictions, we sent landowners in the traditional science treatment group a short photo essay showing what we had been working on and updating them on our progress. This was not sent to the citizen science group, as their engagement was not impacted by COVID-19. At the conclusion of the project, we sent a mailing to both the traditional science and the citizen science groups detailing the

results of our findings from both projects and suggesting related conservation actions landowners could undertake.

At least one month (4-14 weeks) after the final mailing was sent out, we contacted landowners in all three treatment groups to request follow-up interviews. The interviews were conducted by an individual not involved in the ecological research to limit the effect that the interviewer's presence had on landowner responses. We interviewed a total of 20 individuals, including seven individuals in the traditional science group, eight individuals in the control group, and five individuals in the citizen science group. Three of our initial interviewees passed away between the initial and follow-up interviews (one in each treatment group), eight could not be reached, and six declined to participate (four from the control group and one from each of the other two groups). During the interviews, we asked landowners open-ended questions about their perspectives on conservation, any changes in their perspectives that had occurred over the past three years, and if there were any conservation behaviors they had done or planned to do (Appendix 2.A). We also asked landowners in the traditional and citizen science groups if they had learned anything from participating in the project and if they had talked to friends or neighbors about the ecological study.

The lead author first inductively, then deductively (using the theory of planned behavior, Ajzen 1991, and norm activation theory, Schwartz et al. 1997) coded landowner responses to the initial interview (Table 1). Relevant codes from these findings were then used to code the second round of interviews, and we added additional codes to reflect the concepts newly assessed in the follow-up interviews. We compared responses across treatment groups to assess the effect of different levels of engagement on landowner knowledge, attitudes towards conservation, awareness of conservation issues, behaviors, and behavioral intentions. We used the pre-interviews to confirm that landowner perspectives had or had not changed when it was not clear from the post-engagement interview content itself. For the landowners in the traditional science group, we also took notes on comments they made during their interactions with researchers to capture the effect of the engagement. These comments were inductively coded.

## RESULTS

We found similar changes in cognitions and behavioral intentions in both the traditional and citizen science landowners and limited change in the control group, suggesting that the two forms of engagement influence landowners and do so in a similar way. Landowner conservation behaviors showed little change in any group during the period of engagement. Quantitative summaries of interview findings can be found in Appendix 2.C.

One of the few changes that occurred across all three groups of landowners was an increase in knowledge. This occurred both as a result of participation in the project (for the traditional and citizen science groups which discussed learning from interactions with researchers and mailed project summaries) and through learning more on their own (for all three groups), particularly via internet searches. Landowners who learned more on their own primarily did so at the start of the project or immediately following the initial interview. Landowners in the control group discussed learning more about hellbenders, while landowners in the citizen science and traditional science groups discussed learning about a broader array of topics including hellbenders (primarily in the traditional science group), water quality, scientific techniques, and stream management (Table 2).

Moving beyond simple knowledge acquisition, landowners also explicitly discussed a change in their awareness of and attitude towards water quality concerns as a result of participation in the project. As a landowner from the citizen science treatment group said of his growing awareness “Before it didn't really bother me to see a stream and somebody had trashed it, but now I think ... they're hurting people and animals, and that's a very bad thing... I notice the environment more and particularly the streams and the water.”

For landowners in the traditional and citizen science group, the change in perspective that came with participation inspired an intention to change their behavior. For example, a landowner in the citizen science group said, “I have more respect for this little stream on my property. Before I didn't think a lot about it, but now I want to protect it, just like a field that I would care for... I'm protective of that little stream as long as

I'm able get down there.” A landowner in the control group also discussed new conservation behavioral intentions that were not clear from the initial interview. Nearly all change discussed by any treatment group was aspirational, although one landowner in the traditional science group mentioned that she had asked the person who leases her land not to spray the bushes next to the creek because she was concerned that the chemicals could harm the hellbenders.

Landowners, also discussed a lack of perceived behavioral control in the face of environmental challenges to the creek (Ajzen 1991). One citizen science landowner said, “I don't like the way things is done, but I can't change it. I mean I don't like pollutants in the water. There's just a lot of things that's going on that I don't agree with, but I don't have no control over that.” In some cases, this barrier to action appeared to have grown with increased awareness of the water quality issues in the creek. Some landowners also showed a reluctance to acknowledge that they might have a role in the poor water quality in the watershed, focusing on a nearby wastewater treatment plant or neighbors' land management when issues of water quality came up.

In addition to personal impacts from the research, landowners also discussed sharing, or were observed sharing, their experience participating in the research and the results of the research with their social network. For example, a landowner who was well connected in the community said “I would go to the library and make copies of [the results mailings] and send them to [one of my neighbors]. I made sure that they could read it.” The same individual invited neighbors to come observe the research taking place on her property and mentioned talking to friends about the results of the study. In some instances, this sharing through the social network led to changes in the perspectives of individuals not actively involved in the research. For example, one individual in the control group reported discussing the research with a neighbor who was in the traditional research group. The control group individual mentioned that learning about the research through his neighbor had increased his (the control individual's) trust in science, saying:

I always actually believed in [science], but I was more impressed when you guys came over and started doing stuff .... And not only that, but my neighbors, I talk to a lot of my neighbors and stuff

and, you know, they're pretty much in agreement [about science] ... especially because everything that you guys are trying to do.

## DISCUSSION

This study represents one of only a few studies of the effects of engaging landowners in more traditional private lands research (e.g., Lutter et al. 2018; Green et al. 2022b) and is the first to our knowledge to compare traditional engagement to engagement in citizen science. Our findings suggest that biological conservation research on private lands may be a valuable means of influencing conservation knowledge, attitudes, awareness, and behavioral intentions among private landowners. Our findings also suggest that these effects may extend beyond the directly engaged landowners to their social networks. These effects we detected appear to be similar whether the type of engagement is citizen science or more traditional research. However, it is possible that the limitations to our engagement caused by the COVID-19 pandemic impacted our ability to effectively test for differences in the effect of the two treatments since it impacted our engagement with landowners. More research is necessary to clarify and expand on these effects.

One of the most consistent changes we observed was an increase in knowledge that resulted from participation in the project, which aligns with findings from other citizen science research (e.g., Evans 2005) and extends them to more traditional science engagement as well. Landowners from both treatment groups discussed learning about a wide breadth of topics including scientific methods, land management, water quality, and hellbenders. Our finding that many landowners did independent research very early on in the project indicates that long-term engagement is not always required to inspire this behavior.

While some landowners reported increased concern about conservation issues and intentions to shift their behaviors following participation in the project, participants tended to avoid taking personal responsibility for stream degradation. Leading behavioral theories (e.g., Norm Activation Theory, Schwartz et al., 1997, and the Theory of Planned Behavior, Ajzen 1991) emphasize the importance of several factors

to behavioral intentions including attitude towards a behavior, awareness of the consequences of the behavior, beliefs about personal responsibility, perceived ability to effect a desired outcome, and perceptions of how others would perceive their actions. Our results suggest that for landowners that exhibited new conservation behavioral intentions as a result of the research, changes in attitudes towards the behavior and increased awareness of consequences may have had a role. However, we found that even when researchers share actionable conservation steps with landowners, participation in conservation research does not always encourage feelings of behavioral control or acceptance of responsibility. Our study did not provide insight on the effects of participation on perceptions of how others would perceive landowner actions.

We found that landowners shared their experience of participating in the research as well as the results of the research with their social network. This finding suggests that engaging landowners in research may have a larger than expected impact on a community, particularly since peer-to-peer communication has been identified for its importance to conservation action (Schubert & Mayer 2012). Sharing in this way has been identified in previous studies of private lands research (Lutter et al. 2018; Green et al. 2022b), suggesting that it may be a consistent aspect of private lands research.

Although our study indicates that there are benefits to be gained from engaging landowners in private lands research, there is much that remains to be learned about the current state of private lands research engagement and its effects. For example, it would be valuable to explore the effects of engagement on perceptions of how others would perceive conservation behavior since our study did not clarify this (Schwartz et al. 1997; Ajzen 1991). Additionally, it would be useful to know more about what current traditional science engagement looks like and the extent to which this engagement may also influence the researchers' own perspectives on private landowners and private lands conservation. A larger sample size would lend more confidence to our findings.

The push for increased private lands conservation has shone a spotlight on the need for more peer-reviewed conservation research on private lands, and the potential to harness the resultant engagement with

landowners to encourage conservation could be greatly beneficial. Such engagement could be as minimal as calling landowners prior to each visit to inform them of the impending visit. This contact provides an opportunity to share updates, build relationships, and it is a chance for the landowners to ask questions and express concerns. In a world that requires increasingly creative approaches to conservation, opportunities that capitalize on pre-existing occasions for conservation outreach should not be overlooked.

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**Table 2.1** Codes used in interview analysis. Not all codes were relevant to both the initial and follow-up interviews, so each code is classified as utilized in coding the initial interview (I), follow-up interview (F) or both (B).

Source	Parent code	Child code 1	Child code 2	Description	Interviews coded
Deductive (Schwartz 1977)	Norm activation theory	Awareness of consequences	Yes	Awareness that the behavior of landowners living in the watershed influences water quality in the creek	B
			No	Lack of awareness that landowner behavior influences water quality in the creek	B
		Change	Change in awareness of the consequences of landowner behavior	F	
		Acceptance of responsibility	Yes	Recognition that their personal behavior influenced the water quality of Coper Creek	B
	No		Lack of recognition that their personal behavior influenced the water quality of Coper Creek	B	
	Change		Change in recognition that their personal behavior influenced the water quality of Coper Creek	F	
	Personal norms		Personal norms	Internalized sense of conservation's value	I
Deductive (Ajzen, 1991)	Theory of planned behavior	Attitude	Positive	Positive attitude towards conservation	B
			Negative	Negative attitude towards conservation	B

			Change in attitude	Change in attitude towards conservation	F
			Subjective norms	How those important to landowner perceived conservation	I
		Perceived behavioral control	Efficacy	Perceived ability to make a difference in Copper Creek's water quality	B
			Lack of efficacy	Perceived lack of ability to make a difference in Copper Creek's water quality	B
			Control	Perceived ability to undertake conservation behavior and perception that improving water quality was "up to them"	B
			Lack of control	Lack of perceived ability to undertake conservation behavior and perception that improving water quality was "up to them"	B
Inductive	Learning	Hellbenders	Hellbenders	New knowledge about hellbenders	F
		Water quality	Water quality	New knowledge about water quality (e.g., stream chemistry)	F
		Scientific techniques	Scientific techniques	New knowledge about scientific techniques (e.g., water quality testing, hellbender population monitoring)	F
		Stream management	Stream management	New knowledge of stream management (e.g., the value of riparian vegetation)	F
Inductive	Sharing research			Sharing research with family, friends, or neighbors	F
Inductive	Conservation Behaviors	Behavioral intentions	Yes	Plans to undertake conservation behavior in the future	B

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Behaviors	No	No plans to undertake conservation behavior in the future	B
	Yes	New conservation behaviors as a result of participation	B
	No	No new conservation behaviors as a result of participation	B

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**Table 2.2** Types of knowledge gained during the study, relevant treatment groups, and example quotes.

Subject learned	Group	Example quote
Hellbenders	Citizen Science, Traditional, and Control	[Hellbenders] require very clean water and, you know, it'd be a good tip off to us as to the ... water purity, I guess, if we had mud puppies. -Control participant
Water quality	Citizen Science and Traditional	I didn't I had no idea that there were that many I guess nitrates, or whatever else there was in the water. -Citizen science participant
Scientific techniques	Citizen Science and Traditional	I learned how to check the nitrates and the pH and everything -Citizen science participant
Stream management	Citizen Science and Traditional	I got to learn about the water quality and about the need also to not trim your trees and stuff back for erosion because the sediment, it hurts the water quality and it helps warm the water and it helps actually discourages the hellbenders from coming out... -Traditional science participant



**Control group**

- Not contacted between initial and concluding interview



**Traditional science group**

- Invited to accompany researchers working on hellbender research project on their property
- Contacted via phone or email prior to each visit
- Mailed progress update during COVID-19
- Mailed holiday cards each winter
- Mailed summary of both citizen science and traditional science projects results at project's conclusion



**Citizen science group**

- Collected data on water quality to inform the hellbender research project
- Mailed a citizen science packet three times a year
- Mailed holiday cards each winter
- Mailed summary of both citizen science and traditional science projects results at project's conclusion

**Figure 2.1** We divided landowners into three treatment groups including a control group, a traditional science group, and a citizen science group.

## CHAPTER 3:

### PRE-BREEDING, COURTSHIP, AND MATING BEHAVIORS OF WILD EASTERN HELLBENDERS (*CRYPTOBRANCHUS ALLEGANIENSIS ALLEGANIENSIS*)

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#### ABSTRACT

Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*) are an evolutionarily unique species of salamander that is of conservation concern. Despite growing interest in their breeding behavior, significant gaps remain in our understanding of hellbender reproduction, particularly the behavior occurring immediately prior to and during breeding, as these activities typically occur within the nesting cavity and out of view. In this study, we used custom-built infrared cameras installed in artificial breeding shelters to record pre-breeding behaviors, complete mating sequences, and failed mating attempts in eleven shelters. Using these recordings, we describe the basic mating sequence, the presence of potential alternative mating tactics, and two novel behaviors, including possible signal production via wave-based communication and unique egg laying behavior by female hellbenders. These findings will add to our understanding of hellbenders' life history as well as informing conservation efforts in both captive and wild environments.

## INTRODUCTION

UNDERSTANDING a species' reproductive behavior can contribute to advancements in evolutionary ecology as well as more applied conservation efforts. For example, knowledge of breeding behavior can elucidate the evolutionary history of a species by revealing evidence of past or current selective pressures (e.g., Pfennig and Pfennig 2009) and can reveal when reproductive behaviors arose in a clade's evolution (e.g., Ron 2008). From a more applied perspective, improved understanding of reproduction has enabled more effective captive breeding of giant pandas (Martin-Wintle et al. 2015), informed the protection of breeding habitat for humpback whales (Oviedo and Solís 2008), and highlighted the risk posed by increasing light pollution for fireflies (Hagen et al. 2015). There is also overlap between conservation and evolutionary history, with improved understanding of evolutionary history informing conservation priorities and approaches. For example, evolutionary uniqueness has been suggested as a potential means for determining conservation priorities (Faith 1992; Redding et al. 2014), and it can inform which, if any, species may be appropriate surrogate species for determining conservation actions.

The Eastern Hellbender, *Cryptobranchus alleganiensis alleganiensis*, is a species of giant, fully aquatic salamander that is both evolutionarily unique (Larson and Dimmick 1993) and of conservation concern (Wheeler et al. 2003; Burgmeier et al. 2011; Graham et al. 2011), yet its reproductive behavior remains poorly understood. Eastern Hellbenders are one of five species in the family Cryptobranchidae that, along with Hynobiidae, shares a basal node with the rest of urodeles, possibly excluding Sirenidae (Larson and Dimmick 1993; Pyron and Wiens, 2011). Populations of Eastern

Hellbenders, as well as the closely related to the Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) are declining rapidly throughout their range in the United States (Wheeler et al. 2003; Foster et al. 2009; Burgmeier et al. 2011; Graham et al. 2011), and recent work has indicated that failure to produce viable offspring is contributing to population declines (Hopkins et al., 2023). This emphasizes the need to better understand hellbender reproductive biology. Additionally, there are several captive breeding programs currently underway to support hellbender recovery (e.g., Ettling et al. 2013; Civiello et al. 2019; McGinnity et al. 2022), and there remain challenges in encouraging breeding in captive environments and ensuring egg viability (Ettling et al. 2013; Civiello et al. 2019).

Until recently, understanding of hellbender breeding behavior was limited to anecdotal accounts (e.g., Bishop 1941; Peterson 1988; Foster et al. 2009), studies focused on the timing of breeding (e.g., Peterson 1988; Peterson et al. 1989), and one observational study of two captive breeding events (Smith 1907). However, recent research has employed improved technologies to study reproductive behavior in both captive (Settle et al. 2018b) and natural (Unger et al. 2020) environments. Research to date shows that breeding males (hereafter referred to as occupying males) begin defending nesting cavities several weeks prior to the commencement of breeding. These cavities are visited by one to several females and can receive multiple clutches which are fertilized externally. The females leave the nest following oviposition, but the male remains with the eggs until the larvae emerge from the nest in early spring. Several mating behaviors have been observed outside the nest cavity prior to breeding including guarding and patrolling of the nest chamber by the male, nose-to-nose contact between

individuals, biting of conspecifics, cleaning of the nest cavity, cloacal sniffing, swimming in tight circles, and tail swishing (Unger et al. 2020; Settle et al. 2018b; Bishop 1941). However, critical gaps remain in our understanding of the mating process. Most significantly, all previous studies of hellbender breeding have been restricted to interactions visible from outside of the nesting cavity. The small, enclosed nature of most hellbender breeding cavities means that observing or filming the behavior occurring within them is difficult, so the details of the actual breeding event, and the mating behavior immediately preceding it, have not been recorded. Additionally, previous observations of breeding behavior have been limited in duration, with observations averaging 10 nocturnal hours in captive environments (Settle et al. 2018b) or 3.95 diurnal hours in natural environments (Unger et al. 2020). This means that full breeding interactions were not always captured (Settle et al. 2018b).

In this study, we sought to build on previously identified mating season behaviors by capturing uninterrupted footage of the behaviors that occur within the nesting cavity, including the activities of occupying males while alone and his interactions with visiting hellbenders. Using novel infrared camera technology and artificial breeding shelters in a natural stream, we were able to record pre-breeding activities in eleven occupying males as well as six complete breeding sequences and multiple failed mating attempts.

## METHODS

### *Study System*

This research took place across four distinct reaches of a stream in southwestern Virginia. The stream supports a comparatively healthy population of hellbenders and has

relatively high forest cover (Yang et al., 2018) compared to many streams with hellbenders in Virginia (Bodinof Jachowski et al., 2016; O'Brien, unpublished data) making it a good location to assess typical behavior in a comparatively healthy population.

The approximately 100 – 200 m long reaches are dispersed across 27.3 channel km, and for the past 10 years they have been part of an ongoing research effort utilizing fully submerged concrete shelters. These shelters consist of a rectangular chamber with a single tunnel entrance and are installed in arrays of at least 30 shelters per reach. Hellbenders readily utilize the shelters for both cover and nesting (Bodinof Jachowski et al. 2019; Hopkins et al., 2023), and males that nest in the shelters are comparable to those utilizing wild nests in mass and snout-vent length (R O'Brien personal observation). For this project, we added a total of 19 shelters over the span of two breeding seasons that were modified to accommodate a camera to provide an aerial view of the interior of the shelter (Figure 3.1). Eleven of these shelters were occupied by males during the period of study. Males that successfully procured eggs were captured, weighed, and measured for total length within 4 days of oviposition.

### *Video Recording*

We recorded footage in our modified shelters using a microcomputer (Raspberry pi Foundation; Cambridge, UK) attached to a Sainsmart infrared camera (Lenexa, KS, USA). We programmed the cameras to operate for 48-hour cycles and then shut down so that we maximized the battery life but avoided forced shutdowns that could damage the computer over time (Supplementary material). The recording was continuous except for

pauses to exchange batteries and data storage devices or when circumstances, such as very high flows, prevented access to the stream or otherwise prevented data collection. The cameras saved video footage in 15-minute segments at a frame rate of 15 frames per second and a resolution of 1640×1232 pixels.

Our study took place in the fall breeding seasons of 2020 and 2021. We recorded some footage in late July that was not analyzed but used to test the cameras, and then formally installed the cameras at the beginning of nesting, between 29 August and 10 September (Table 1). Based on a decade of monitoring in these streams, we know that hellbenders within the same stream reach breed relatively synchronously, so after a week of checking shelters in a reach without finding any new nests, we determined that breeding in that reach was complete and stopped filming. All of the shelter arrays with camera-equipped shelters had high occupancy (54.6–68.9%), so we had multiple nests in each reach to indicate the cessation of breeding. Additionally, we continued to check for eggs in the shelters that had not produced nests for two weeks following the end of breeding, so we were able to confirm that all shelters were accurately categorized as either having or not having received eggs. The total duration of our recordings per shelter ranged from 18.5 to 521.9 hours (i.e., <1 to 21.7 days, Table 1) depending on how soon after installation, if at all, the shelter received eggs.

### *Behavioral Coding and analysis*

We used ELAN 6.4 software (Brugman and Russel, 2004) to code focal samples (Altmann 1974) of the behaviors of occupying males and other visiting hellbenders within each shelter. When occupying males were alone in the shelter, we analyzed 15 min

of behavior every four hours to gain an understanding of their basic behaviors in the shelter. When a visiting hellbender (an animal other than the occupying male) entered the shelter, we analyzed the footage continuously from the start of the segment when the visiting animal entered the shelter through the end of the segment in which the visiting animal left the shelter. We coded the behavior of all animals, including the occupying male and any other individuals in the shelter. Once the visiting animal left, we returned to the reduced sampling scheme until another visiting hellbender entered. We measured the duration of each visiting animal's stay in the shelter and coded behavioral data using the ethogram in Table 2. Our ethogram was based on previously identified breeding behaviors (Guimond and Hutchison 1973; Terry et al. 2018; Unger et al. 2020; Settle et al. 2018b) as well as novel behaviors we identified that occurred across more than one individual and appeared significant to the breeding process. To enable a more easily interpretable description of breeding behaviors, we allowed for overlapping behavioral codes. We also recorded mutually exclusive data on the position of the occupying male in the box. The lead author did all coding.

We confirmed the sex of the occupying male through passive integrated transponder tags or, for untagged animals, through the observation of successful breeding events. Gravid females who did not breed were sexed based on their appearance and behavior: they were swollen with eggs, moved sluggishly while in the shelter, and elicited attentive behavior from the resident male. Individuals who entered and were not obviously gravid females were classified as intruders and not assigned a sex. When possible, we used distinguishing characteristics such as scars, spotting patterns, or

physical deformities to identify when the same animal entered the shelter more than once (Fig. 2).

### *Analysis*

We calculated time budgets for the occupying male's position in the shelter when alone and when visited by a female, and we described the basic sequence of events for breeding interactions. We also used t-tests to compare behaviors between successful and unsuccessful males as well as between females on ovipositing and non-ovipositing visits. All statistical analyses were done using R 3.6.2 (R Core Team 2022).

## RESULTS

We were able to capture the breeding season behavior of 11 shelter-guarding males as well as 38 gravid females and 29 individuals whose sex could not be definitively identified. From our recordings, we determined how males spent their time alone in the shelter prior to receiving eggs as well as how they and visiting hellbenders behaved when together. Six of the males that we recorded were successful in procuring eggs, and we were able to record and describe the breeding event in its entirety from the female's entry into the shelter through her exit after depositing eggs.

Our positional time budget revealed that males spend the majority of their time guarding the tunnel of the shelter when alone, but once a female enters the shelter, they switch to spending the majority of their time in the chamber (Table 3). After oviposition begins, they resume some guarding, but still return to the chamber to a greater extent than they do when alone. Males only left the shelter when no female was present.

While alone in the shelter and prior to receiving their first clutch, occupying males primarily spent their time rocking (mean percent time =  $7.0 \pm 1.2$  SE) and cleaning (mean percent time =  $2.4 \pm 0.7$  SE). Cleaning behaviors included pushing sediment using the nose or feet, as well as rapid movement of the tail and hind legs, and occurred both in the main chamber and at the entrance to the tunnel (Supplemental video S1, available online). Based on footage captured before the study began (not formally analyzed), males began cleaning as many as seven weeks prior to breeding. Other behaviors that males engaged in while alone (each for less than one percent of the total recorded time) included striking at targets outside the shelter, twitching their legs, eating shed skin, scratching their faces, rolling, biting at material in the water, opening and closing their mouths, clenching their jaws, flicking their tails, and fighting with unseen animals in the tunnel. Hellbenders also spent time resting in the chamber.

Occupying males (both successful and unsuccessful) had one to six gravid females enter their shelters throughout the course of the filming, with these interactions occurring during both day and night. Ovipositing females spent between 2.02 and 4.21 hours in the shelter during a visit, with an average time of  $3.01 \pm 0.26$  hours. For females that did not oviposit, the time spent in the shelter was far less than ovipositing females, ranging from 1.6 min to 50.8 min with an average stay of  $14.6 \pm 2.0$  min. Regardless of whether they oviposited or not, females always left of their own volition. However, they were often nipped on the tail by the occupying male as they left.

Individuals whose sex could not be definitively identified (hereafter “intruders”) also frequently entered the shelters, and six of the eight breeding events had at least one intruder present during breeding and/or oviposition. Intruders were always smaller than

the occupying male and did not appear to have abdomens distended with eggs. They rarely (9.7% of the time in the shelter) were present in the shelter without a female. Intruders were present more often when ovipositing females were in the shelter ( $94.4\% \pm 2.8$  of the time they were in the shelter) as compared to when non-ovipositing females were present ( $13.1\% \pm 8.9$  of the time they were in the shelter.) Intruders typically remained motionless in the shelter after entry except for short bursts of rapid movement. The occupying male usually ignored the intruders, particularly when a female was present, but did periodically attempt to drive them out by biting them. Intruders never behaved aggressively toward the occupying male, suggesting they were not seeking to displace him, but at least one intruder did appear to release sperm over a clutch of eggs while in the shelter (indicated by sudden cloudiness of the water). Several times, multiple intruders were present in the shelter simultaneously, with one shelter containing four intruders at once in addition to the occupying male and ovipositing female. The same intruder often came and went from a shelter several times.

During interactions between hellbenders, we observed a previously undescribed “quivering” behavior, characterized by rapid contractions of the hellbender’s sides (Supplemental video S2, available online). Although this behavior was much more common among females, we also observed quivering in occupying males and intruders. Occupying males showed evidence of responding to female quivering by changing their position in the shelter. We witnessed males responding to quivering in several shelters, but it was particularly striking in one shelter (shelter A), where the occupying male spent 76% of his time guarding the tunnel while the ovipositing female was present. This male periodically returned to the interior chamber and approached the female. After a short

period, the female would begin to quiver, the male would return to the tunnel, and the female would continue her ovipositing activities in the shelter. Non-ovipositing females spent a greater percentage of their time in the shelter quivering prior to oviposition than did ovipositing females throughout their visit (Table 5).

Interactions between occupying males and visiting females followed a similar pattern prior to oviposition across all recorded breeding pairs (Fig. 3) as well as pairs that did not ultimately breed. Females entered the shelter slowly when males did not interfere, but the males sometimes bit the females' heads before they completely entered and pulled them in (Supplemental video S3, available online). While the female was entering, males performed a tail swish in 59.5% of all interactions (Supplemental video S4, available online), and they touched noses in 25% of all interactions (Supplemental video S5, available online). Females began quivering in some cases the moment they became visible on the camera, and continued to quiver off-and-on throughout their interactions with the occupying male.

Occupying males typically bit visiting females one to several times after entry, most commonly on the head (56% of bites) but also on the tail, leg, and side. Tail bites typically occurred as the female was leaving the shelter after the breeding event was completed. Bites on the leg, side, and tail were typically fairly brief (an average hold time of  $9.5 \pm 5.8$ ,  $1.9 \pm 0.5$ , and  $1.6 \pm 0.2$  seconds respectively), but head bites were often held for prolonged periods with an average of  $6.3 \pm 1.3$  min). One occupying male held a continuous head bite for 38 min. This bite was, in practical terms, even longer, as it immediately followed a 15.5-minute head bite with only 32 seconds of rest in between. Of the eight successful breeding events we captured on film, three did not include the

male biting the female prior to laying. However, two of these three breeding events took place after the male had already received one clutch of eggs. When not holding a bite, the male climbed over and around the visiting female, often nudging her side and cloaca and crawling under her belly and resting there. Occupying males also frequently rested under the female's chin. Females remained relatively passive during these interactions, moving lethargically and submitting to male bites.

Females began to lay eggs an average of  $41.8 \pm 17.1$  min after entering the shelter. Throughout the period of oviposition, all the recorded females undertook a previously undescribed behavior (hereafter referred to as "threading") in which they wove in and out of the egg mass while ovipositing, effectively tying the eggs in a large knot (Supplemental video S6, available online). This threading behavior appeared to continue even after the female had finished laying eggs. After the ovipositing female had left, we observed a few occupying males also performing this threading behavior, although in their case it did not affect clutch morphology. Ovipositing females increased rocking behavior throughout the course of breeding, spending  $1.8 \pm 1.1$  percent of the time rocking before and  $11.3 \pm 2.7$  percent of the time after oviposition began, rocking.

Once females began to oviposit, occupying males across all eight shelters remained in the chamber for a few min (average  $3.1 \pm 0.7$  min) before moving to the tunnel to guard the chamber (although two males began guarding before oviposition began, Fig. 3). After guarding commenced, the male would periodically return to the chamber to crawl over and around the female and the eggs, which the female often responded to with quivering. These returns to the chamber happened 5 to 16 times throughout the course of egg laying, or an average of once every 8.9 min. It was unclear

if males were depositing sperm during this time, as sperm release was not always clearly visible on the recordings due to disturbed sediment, skin secretions, and/or the presence of pre-existing sperm clouding the water.

We found no significant difference between successful and unsuccessful occupying males in terms of percent time biting visiting females or percent time spent cleaning the nest which were the most prominent breeding season behaviors of probable interest to females (Table 4). However, successful males did spend longer holding head bites than unsuccessful males, and further investigation is warranted (Table 4). The behavior of females that laid their eggs vs. those that did not differed more notably than successful and unsuccessful male behaviors did. Prior to oviposition, females that laid their eggs spent a significantly smaller percentage of their time in the shelter quivering than did non-breeders during the duration of their visit (Table 6). Ovipositing females also entered the shelter more slowly than non-ovipositing females.

In three shelters, the ovipositing female entered the shelter, interacted with the occupying male and left, only to later return and lay eggs. The time difference between the first and second entry varied between these three shelters with times of 3.42, 10.00, and 49.65 hours between the initial non-breeding and the later ovipositing visits.

Although in one shelter the occupying male did not bite the female during either visit, in the other two, the occupying male bit more and held the bites for longer on the female's initial visit than he did on the second visit where oviposition occurred (Table 6). Females also behaved differently between visits depending on whether or not they laid eggs during the visit. They spent more time quivering and did so more continuously on their initial visit where they did not oviposit than on the later visit where they did. Females

also took less time to enter the shelter on their initial visit than they did at oviposition, although this may be due in part to males biting females and pulling them into the shelter on their initial visit rather than allowing them to enter at their own pace at oviposition. In two of the three shelters, the same intruder individuals entered with the female during both of her visits.

While most of the shelters we recorded received only one clutch of eggs, we recorded one shelter that received three clutches of eggs from three distinct females over a period of three days. Ovipositing females entered this shelter at 20:17 hours on September 5<sup>th</sup>, 10:32 on September 6<sup>th</sup>, and 08:26 on September 8<sup>th</sup> with no overlap between visits. This shelter also had two females enter but not lay eggs after the occupying male had received the first clutch (a circumstance we also observed in other shelters). While the occupying male bit and held the head of the first visiting female for 3.52 min, he did not bite the second female at all, and bit the third only on the tail as she left the shelter. The females who visited when eggs were already present cannibalized a small number of the previously laid eggs both before and during their own oviposition without any apparent response from the male. Each subsequent female spent less time in the shelter (252.22, 185.51, and 164.52 min. present for the first, second, and third ovipositing female, respectively) and spent less time quivering (32.5, 40.4, 5.9 seconds quivering for the first, second, and third ovipositing female, respectively) than the previous ovipositing female. The occupying male in this shelter was larger than the other males in our study in both mass and total length (Table 1).

## DISCUSSION

This research represents one of only a few studies of hellbender breeding behavior, and it is the first to make observations within a nesting cavity rather than outside of it. Our results confirmed some previous observations about hellbender breeding behavior and identified novel behaviors and mating strategies that had not been previously described. These findings shed light on the evolution of hellbender life history and offer considerations for future conservation management of the species.

One of the new behaviors that we identified in our filming was the behavior we called threading where during, and in some cases continuing after, oviposition, females wove in and out of the clutch, resulting in a knotted mass of eggs. Although clutch morphology in *Andrias* has not been explicitly described, accounts of the females rotating (Luo et al. 2018) or spinning (Kuwabara et al. 1989) during oviposition suggest that threading may be a characteristic shared across Cryptobranchidae. We were unable to find any reports of similar clutch morphology in other species of urodeles, suggesting that it may be unique to cryptobranchids.

There are several possible explanations for threading. One possible explanation is that the knotted clutch enables more effective management of the eggs during parental care or reduces the likelihood that loose strands caught by the stream's current pull the clutch out of the nest cavity. This would represent a novel solution to clutch management and an alternative to attaching the eggs to substrate as is seen in many other aquatic amphibians (Altig and McDiarmid 2007). It is also possible that threading improves fertilization success. Hellbenders fertilize externally, so weaving in and out of the clutch may help disperse milt and ensure that the entire clutch is fertilized.

Another possibility is that this behavior spreads anti-microbial skin bacteria or skin secretions on the eggs. Skin peptides from hellbenders have been found to inhibit bacterial growth (Pereira et al. 2023) and other species have also been found to produce anti-fungal compounds and/or support bacteria that could reduce egg infection (Austin 2000; Banning et al. 2008; Lauer et al. 2007). Weaving in and out of the eggs may spread these beneficial compounds/bacteria. This has implications for captive rearing, as in-vitro fertilization (IVF) would deprive eggs of these compounds and could adversely affect offspring survival and health. This may be one reason why eggs collected for IVF have a lower survival rate than eggs that have been raised with the den-master (D. McGinnity, personal communication; McGinnity et al. 2021), although there are additional parental care behaviors that likely also play a role in the reduced survival (Settle et al. 2018a; Unger et al. 2020). Additionally, if threading spreads beneficial bacteria, this would suggest that ongoing efforts to maintain the skin microbiome in captive hellbender breeding stock are highly valuable (Hernández-Gómez et al., 2019; Kenison et al., 2020, D. McGinnity, personal communication). Another novel behavior that we observed was the quivering motion, which is likely some form of signal given the response it elicited from occupying males in some shelters. The sensory mode that quivering appeals to is difficult to determine without further study. It may be that quivering produces a wave-based signal (because of the lack of clarity in distinguishing vibrational and acoustic communication (Hill 2008), we use wave-based to encompass both possibilities). Hellbenders have been found to sense vibrations via their jaws and lateral lines, but little is known about their signal production (Nickerson and Mays 1973). Wave-based communication and the sensing of vibrations via the lateral line has been

identified in Hynobiidae (Park et al. 1996; Tanaka 1989; Usuda 1995; Tanaka 1986; Kim et al. 2009; Park et al. 2008). However, these waves are produced through body undulations or tail waving rather than the quivering that we observed. Acoustic communication has also been identified in the closely related *Andrias davidianus* (Evans and Ding 2005), but how the sound is produced is unclear. Evans and Ding (2005) noted that it is sometimes accompanied by bubble release, suggesting that the lungs may be involved. We did not ever observe bubble production during quivering in our recordings, but the potential involvement of the lungs in this signaling is intriguing, as hellbenders primarily respire cutaneously and our understanding of the function of their lungs remains poor (Guimond and Hutchison 1973). An interesting possibility is that hellbenders are drumming against their lungs in a manner similar to the way fish drum against the swim bladder to produce sound (Jones and Marshall 1953). However, this possibility remains highly speculative and would need further investigation.

An alternative possibility is that quivering is related to pheromone production. Previous research has suggested that olfactory signals may be utilized by hellbenders during breeding season (Peterson 1988), and in the aquatic newt, *Cynops pyrrhogaster*, males release a pheromone and then waft it towards the female using tail vibration (Kikuyama and Toyoda 1999). Something similar could be occurring in hellbenders with the quivering serving to release or propel the chemical signal away from the skin. It is unlikely that the quivering is purely a tactile signal, as it was often produced without physical contact between animals.

Although multiple males have previously been observed entering nests during breeding (Smith 1907; Unger et al. 2020), this is the first study to provide evidence of

possible alternative reproductive tactics in hellbenders. In most breeding events, we observed the presence of one to several intruding individuals in the shelter during mating. Although we did not capture any of the intruders to confirm their sex, we have, in previous field seasons, captured up to four males with swollen cloacae (indicating sexual readiness) in a single shelter with an ovipositing female. Additionally, the observations of intruder behavior enumerated in our results suggest the intruding individuals are likely males employing an alternative mating tactic. These individuals likely represent a satellite mating strategy which has been documented in Japanese giant salamanders (Kuwabara et al. 1989; Kawamichi and Ueda 1998; Okada et al. 2015 and untranslated citations therein) as well as in some territory-guarding species of Hynobiidae (Hasumi 2015).

Satellite males are submissive individuals that, rather than defending a territory and/or attracting females on their own, parasitize the mating events of larger males by sneaking fertilizations (Dunbar 1982; Taborsky et al. 1987). Consistent with a satellite mating strategy, the intruders we observed were typically much smaller than the occupying male. Given the strong relationship between age and size (Taber et al. 1975), it is likely that satellites are young individuals who may not yet be large enough to defend their own nesting cavity. However, we also observed some successful males that were quite small, so what determines whether a male becomes a satellite or an occupying male may be a matter of nest cavity availability. The tolerance that occupying males displayed towards intruders suggests that the risk of lost fertilization opportunities as a result of intruders does not justify the cost of defending the nest. Given the likely young age of the intruders and our limited observations of obvious sperm release by them, fertilization

by intruders may not always occur. Additionally, there may be a high cost to the occupying male of driving the intruders out such as physical injury, energy loss, and/or disruption of mating. Taborsky (2001) identifies three characteristics that support the evolution of satellite mating in fish, including indeterminate growth, the prevalence of external fertilization, and paternal investment, all three of which are present in Cryptobranchidae.

The criteria that females are using for mate choice was not clear from our observations, and we did not find evidence of highly stereotyped courtship rituals. However, there were some behaviors that individuals exhibited across nearly all breeding events. For example, in all breeding events, the occupying male crawled under and around the visiting female and nudged her belly in a way similar to that described in *Andrias* (Kuwabara et al. 1989). Periodically during this crawling, the occupying male rested under the female's chin and torso while she quivered, which may have been an opportunity for him to more strongly sense her signaling (Nickerson and Mays 1973). We also observed bite-and-hold behaviors in nearly all breeding interactions. Biting of gravid females by males has previously been seen in *Andrias* (Kuwabara et al. 1989; Luo et al. 2018). However, previous hellbender research has identified aggressive interactions primarily between males (Unger et al. 2020) or between females (Settle et al. 2018b) rather than between sexes, likely because these recordings were taken outside of the nest. Successful males held the bites for longer on average than unsuccessful males, so it is possible that biting plays some role in sexual selection, but further research is still needed to determine if hold time plays a role in female choice. We observed touching noses in about a quarter of interactions between occupying males and females, but neither this

behavior, nor tail swishing which occurred in over half of interactions, seemed related to mating success. Still, it cannot be ruled out given our small sample size.

Males cleaned their nests in all recorded shelters, but the exact purpose of this behavior and whether it was subject to sexual selection remained unclear. Terry et al. (2018) suggested that this behavior in Japanese giant salamanders might serve primarily to enlarge and shape the nesting cavity or to reduce the threat of water mold to eggs by minimizing the amount of other substrate for growth. Both of these possibilities would be expected to make clean nests more attractive to potential mates, but we did not detect any relationship between time spent cleaning and mating success with our small sample sizes. However, if cavity enlargement due to cleaning is a factor in mate choice, the artificial nature of our nesting cavities could remove this variable from female choice, meaning we would only be able to detect this effect in natural cavities. It could also suggest a possible unintended effect of artificial shelter installation, particularly if nest maintenance is an indicator of other aspects of male quality. An alternative explanation is that the piles of sediment that accumulate outside of the tunnel as a result of cleaning might help deter intruders by narrowing the nest entrance and making it easier to defend (Svensson and Kvarnemo 2003; Unger et al. 2020). Given how far in advance of breeding we recorded nest cleaning in anecdotal observations, it is also possible that this behavior does not have a purpose related to mating. Future video recordings at other times of year could help eliminate this possibility.

Our observations suggest that hellbenders potentially engage in mate choice copying and comparative mate evaluation (Bateson and Healy 2005; Pruett-Jones 1992). In the shelter where we recorded multiple clutches being deposited, each subsequent

ovipositing female spent less time in the nest and spent a smaller percentage of the time present quivering. The occupying male also only substantially bit the first female to enter. These differences in behavior suggest that the females may have been relying in part on the presence of eggs to enable a faster assessment of the male's quality. Another finding was that in several nests we recorded females entering the same nest multiple times before ovipositing. Although re-entry into a nesting cavity by the same female has been observed in *Andrias* (Kawamichi and Ueda 1998), this study was, to our knowledge, the first confirmation that re-entry by females also occurs in hellbenders. Since in some instances we began recording only shortly before oviposition began, it is possible that this behavior also occurs more frequently than we recorded. The existence of re-entry suggests that the females were evaluating potential mates and possibly comparing them to alternative choices. When deciding between males that vary in more than one characteristic, the available choices can influence which characteristics the female emphasizes in making her selection (Sedikides et al. 1999). If comparative evaluation is taking place in hellbenders, the choices that females make may vary depending on the quality and quantity of alternative males (Bateson and Healy 2005). This may highlight a new implication for declining hellbender populations and health. Females may not be able to make many comparisons if populations are too low, or they may be comparing males that differ in ways that are not typical of a healthy population. This could cause changes in how females evaluate potential mates or which characteristics they emphasize in making a choice (Bateson and Healy 2005), and suggests there may be a minimum populations size at which optimal mate choice decisions can be made. In captive populations, comparative mate choice suggests that providing females with several

potential mates (as done by Settle et al. 2018) is beneficial and that care should be taken in selecting these potential mates.

### *Future directions*

Although this study has deepened our understanding of hellbender behavior, there is much that remains to be learned. Further investigations into the significance of threading and quivering, as well as the specific characteristics used in sexual selection would be exciting next steps that would further our understanding of hellbender natural history and promote conservation efforts such as captive rearing. Confirmation of our observations in a natural nest to remove any self-selection bias (Webster and Rutz 2020) would also be an exciting, although challenging future study. By continuing to advance our knowledge of hellbender breeding ecology, we can improve our understanding both of their evolutionary history and the future conservation of this remarkable species.

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**TABLE 3.1** Date, duration, and location of camera installation and removal from underwater shelters as well as occupying male mass and total length. Only successfully breeding male hellbenders were captured and measured. Shelter G-K did not receive eggs and so the oviposition date, time, mass, and total length are not applicable, or N/A.

Shelter	Stream reach	Date installed	Date removed	Total filmed hours	Oviposition Initiation date	Oviposition Initiation time EST	Male mass (g) at oviposition	Male total length (cm) at oviposition
A	1	9/4/2020	9/5/2020	21	9/5/2020	10:37	590	45.4
B	2	9/1/2020	9/10/2020	207	9/10/2020	5:54	440	42.2
C	2	9/9/2020	9/10/2020	19	9/10/2020	12:20	475	44.9
D	3	8/31/2020	9/11/2020	267	9/11/2020	14:20	480	44.4
E	4	9/4/2021	9/13/2021	224	9/13/2021	16:14	720	47.8
F	2	8/29/2021	9/8/2021	237	9/6/2021 9/6/2021 9/8/2021	2:51 10:42 <sup>a</sup> 8:27 <sup>a</sup>	870	50.9
G	4	9/1/2020	9/15/2020	336	N/A	N/A	N/A	N/A
H	3	8/29/2021	9/20/2021	522	N/A	N/A	N/A	N/A
I	3	9/4/2021	9/20/2021	383	N/A	N/A	N/A	N/A
J	4	9/10/2021	9/20/2021	231	N/A	N/A	N/A	N/A
K	1	8/31/2020	9/16/2020	391	N/A	N/A	N/A	N/A

<sup>a</sup> The exact initiation of oviposition could not be determined due to the presence of previously laid eggs, so the reported times are when the female entered the shelter on her ovipositing visit.

**Table 3.2** Ethogram of hellbender behaviors coded during video analysis. Cleaning, rocking, and guarding behaviors were defined based on Guimond & Hutchison 1973, Terry et al. 2018, and Unger et al. 2020 respectively. Nose-to-nose and tail swishing were based on Settle et al., 2018. Entering, threading, and quivering were all newly described from our observations.

Behavior	Description
Nest cleaning	Movement of loose substrate using the hind legs in a kicking motion or shoveling with the nose. Sometimes very fast undulation of hind legs and tail to achieve the same effect.
Conspecific biting	Biting of the head, tail, side, or leg of another animal.
Tail swish	Male sways hips to move tail in a lateral waving motion.
Nose-to-nose	Occupying male and visiting female touch or nearly touch noses.
Rocking	Lateral back and forth movement of the body while all four feet remain grounded.
Entering	The time between when a female's nose first became visible in the shelter chamber until her hind legs entered the chamber.
Threading	Weaving in and out of the egg mass.
Quivering	A rapid contraction of the animal's side
Other	Eating shed skin, resting in chamber, striking at or running towards targets outside of shelter tunnel, struggling with unseen subject in the tunnel, flicking tail, biting at material floating in water, rolling, yawning, scratching face, shaking leg, opening and closing mouth, resting.

Position	Description
Nest guarding	Male's face or entire body is in the tunnel.
Absent	Male is absent from the camera's field of view after exiting the tunnel. Subsequent re-entry is face-first.
Chamber	Male is in the chamber without his face in the tunnel.

**Table 3.3** Positional time budgets for occupying male hellbenders. Some behaviors are only applicable to successful or unsuccessful males, and the alternative is therefore marked as not applicable, or N/A.

Circumstance	Position	Percent time spent in position		
		All	Successful males	Unsuccessful males
Alone	Guarding	77.6 ± 3.7	79.2 ± 5.0	74.8 ± 5.7
	Absent	1.8 ± 0.5	2.2 ± 0.6	1.1 ± 0.5
	Chamber	20.6 ± 3.8	18.6 ± 5.0	24.1 ± 6.0
Female that oviposited present	Guarding	9.9 ± 7.0	2.0 ± 2.0	17.9 ± 20.2
	Absent	0 ± 0	0.0 ± 0.0	0.0 ± 0.0
	Chamber	90.1 ± 7.0	98.0 ± 2.0	82.1 ± 13.5
Female that oviposited present (prior to oviposition)	Guarding		0.5 ± 0.3	
	Absent	N/A	0.0 ± 0.0	N/A
	Chamber		99.5 ± 0.3	
Female that oviposited present (after oviposition begins)	Guarding		32.8 ± 2.9	
	Absent	N/A	0.0 ± 0.0	N/A
	Chamber		67.2 ± 2.9	

**Table 3.4** We did t-test comparisons of the percent time cleaning and average time biting each visiting female hellbender's head (the coded behaviors most likely to influence mating success) between successful and unsuccessful males. There were no significant differences.

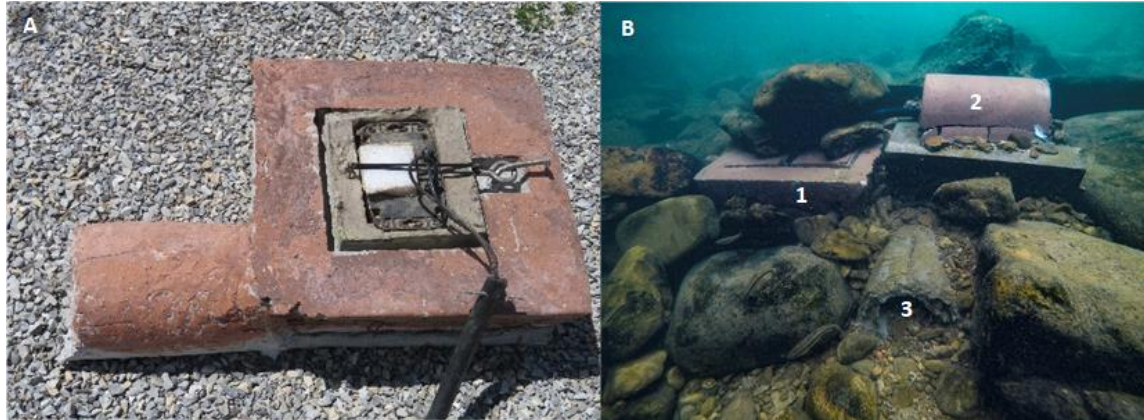
Behavior	Circumstance	Percent time or total min. spent on behavior.			T	df	p
		All	Successful shelter	Unsuccessful shelter			
Percent time cleaning	Alone	2.4 ± 0.7	2.1 ± 1.0	2.9 ± 0.8	0.57	8.96	0.58
Total time holding head bite of female (min)	Female present prior to oviposition	6.3±1.3	7.2 ± 2.1	5.4 ± 1.5	-0.71	33.49	0.49

**Table 3.5** We did t-test comparisons of behaviors of female hellbenders that oviposited on a given visit and those that did not. Ovipositing females spent significantly longer in the box and quivered significantly less prior to oviposition than non-ovipositing females did during the duration of their visit.

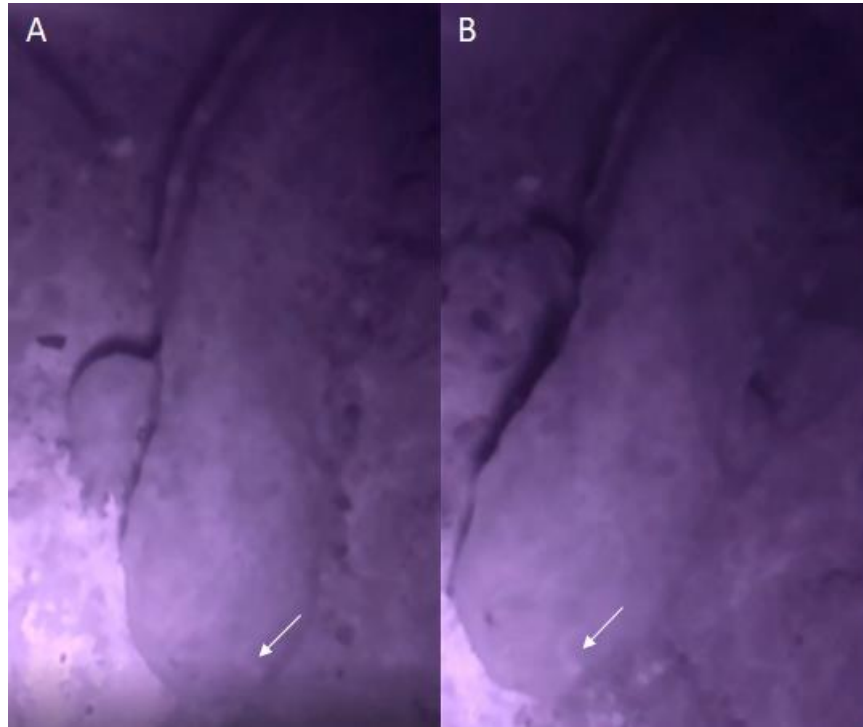
Behavioral variable	Percent or total time spent on behavior.					
	All	Female that oviposited	Female that did not oviposit	T	df	p
Time to enter (s)	75.9±16.67	154.1±69.6	60.3±13.6	-1.32	5.39	0.24
Total time present	47.8±11.2	180.7±15.4	14.6±2.0	-10.66	7.25	<0.001
Time present prior to oviposition (for ovipositing females) or exit (for non-ovipositing females) (min)	18.6±3.6	46.1±16.4	13±1.8	-2.00	5.12	0.10
Percent time quivering prior to oviposition (for ovipositing females) or exit (for non-ovipositing females)	29.0 ± 3.7	15.3±3.8	31.8±4.2	2.91	20.05	0.01

Behavior	Average duration of behavior (min)		Average total time spent on behavior (min)	
	Initial visit	Oviposition	Initial visit	Oviposition
Quivering	0.65 ± 0.18	0.18 ± 0.03	5.45 ± 3.16	4.59 ± 2.90
Entering	N/A	N/A	0.39 ± 0.15	3.62 ± 2.30
Biting	3.79 ± 2.39	1.00 ± 0.47	7.58 ± 2.46	4.01 ± 1.73

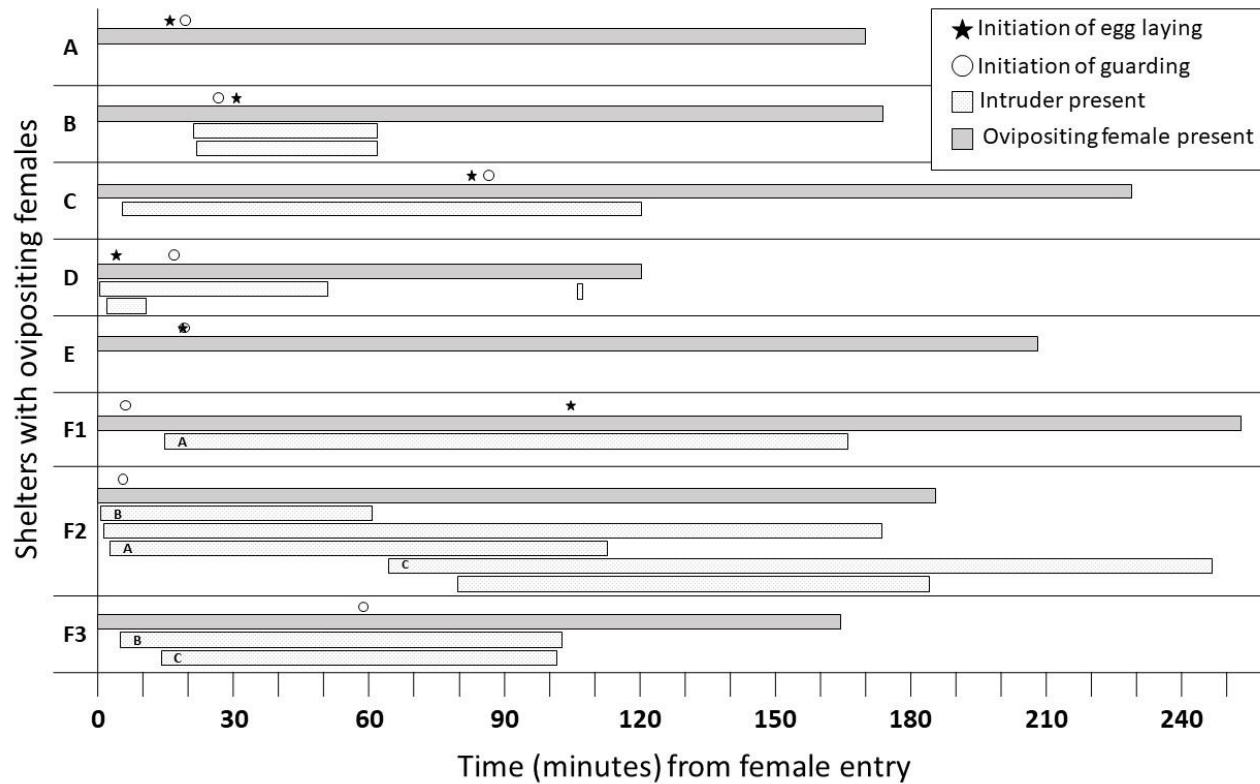
**Table 3.6** Average duration of behaviors (bouts of quivering or hold times for bites—not applicable to entering) and average total time spent on behaviors for shelters where ovipositing females entered multiple times. Behaviors were only measured prior to oviposition.



**Figure 3.1** Modified hellbender shelters ready to be installed (A) and newly installed (B) in a stream. The camera is located under the white foam on top of the shelter in image A. In image B, an external battery enclosure (1) is located next to the installed shelter, and a domed protective concrete cover (2) has been installed in the right images to protect the camera housing. The tunnel (3) is pointed downstream.



**Figure 3.2** To determine instances of repeated entry by the same individual, we took note of features such as spotting patterns, scars, or other physical deformities. This image shows a breeding female at first (A) and second (B) entry into a shelter. The carved scar on her face highlighted by the arrow, as well as her spotting pattern helped identify her as the same female.



**Figure 3.3** Sequence of events taking place in hellbender nests of males that successfully received eggs. The initiation of egg laying for the second and third females in shelter F could not be determined due to the large number of eggs already in the shelter. Letters in intruder bars denote the same individual returning multiple times with different females. One intruder in shelter D entered twice while the same female was present.

## CHAPTER 4:

### MODULATION OF SELF-MAINTENANCE AND PARENTAL CARE BEHAVIORS IN RESPONSE TO MICROHABITAT CONDITIONS BY EASTERN HELLBENDERS (*CRYPTOBRANCHUS ALLEGANIENSIS*)

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#### ABSTRACT

The rapid environmental changes associated with the Anthropocene mean that flexible behavioral responses may be a critical component of species adaptation, particularly for species with long generation times. One type of behavior that exemplifies this potentially important flexibility is parental care. Eggs and juvenile animals are sensitive to environmental stressors, and the ability of parents to adjust care behaviors to buffer their offspring from rapidly changing conditions may be critical to successful reproduction. In this study, we explore the role of parental care in buffering juveniles from anthropogenic stressors in the long-lived, fully aquatic eastern hellbender salamander. Using custom-designed infrared cameras installed in artificial shelters in a natural stream, we describe hellbender parental care behaviors in greater detail than has previously been possible, and we assess the ability of parental care behaviors to buffer hellbender eggs and larvae from increasing levels of silt and decreasing concentrations of dissolved oxygen in nesting cavities. We found that while hellbender parents buffer their offspring from low dissolved oxygen concentrations by increasing parental care, this response is moderated by an increase in self-maintenance behavior at the expense of parental care behavior. Additionally, parents did not show evidence of buffering their offspring from the effects of increasing silt or organic material in their nest cavities. Our study indicates that hellbender parents may be able to reduce the impacts to their

offspring caused by declines in dissolved oxygen concentration to a limited extent, but they may be unable to protect offspring from increasing silt.

## INTRODUCTION

Eggs and juvenile animals are often sensitive to environmental stressors and suffer high levels of mortality as a result (Coulson et al. 2001; Oro et al. 2010). As detrimental human activities exacerbate the challenges that wildlife face, early life stages are at particular risk. One means of improving the survival of young animals is parental care, which can help shield (or “buffer”) them from environmental factors until they reach older, more self-sufficient life stages (Klug and Bonsall 2014; Dybala et al. 2013). Parents have been shown to modify their behavior in response to changing environmental conditions (Jones and Reynolds 1999; Delia et al. 2013; e.g., Torricelli et al. 1985; Potts et al. 1988; Durant et al. 2019; Kight and Swaddle 2011; Doody et al. 2006), but this modification is not without tradeoffs to the parents (Jones and Reynolds 1999). Such tradeoffs are in line with evolutionary theory which suggests that parents often balance the success of current and future reproductive events, or balance their own survival and the survival of their offspring (Stearns, 2000).

Understanding how parents respond to environmental challenges, and any limitations to this response including tradeoff assessment, is increasingly important to informing prioritization of conservation efforts and understanding how species may respond to the mounting stressors of the Anthropocene. For species with slow life history strategies and long generation times, behavioral modifications in response to novel stressors may be particularly important (Taber et al. 1975; Peterson et al. 1988) In these

species, rapid anthropogenic change can outpace their capacity for genetic adaptation (Chevin et al. 2010).

Freshwater streams represent excellent, yet understudied systems for studying the effects of anthropogenic change on parental care behaviors. One emerging threat to these systems is declining dissolved oxygen which can lead to developmental delays, and mortality in eggs and young aquatic animals (Jacob et al. 1984; Pollock et al. 2007; Mills and Barnhart 1999). Although hypoxic conditions, including parental responses to such conditions, have been well-studied in marine and lake systems, the importance of hypoxia in freshwater streams is understudied (Blaszczak et al. 2023). Because oxygen concentrations in freshwater streams are typically much higher than in marine systems (Blaszczak et al. 2023), even mild changes in dissolved oxygen may have an effect. Research on freshwater stream oxygen concentrations is limited, but existing data suggests hypoxia is likely to be an increasing concern due to increasing stream temperatures from global climate change (Rajesh and Rehana 2022) and stream bank clearing (Davies and Nelson 1994), as well as increased nutrient loading (Smith 2003). Another growing threat to freshwater systems is siltation. Deforestation (Davies and Nelson 1994) and increasingly heavy rainfall due to climate change (Trenberth 2011) are all exacerbating stream siltation. Silt can, among other concerns, smother eggs and larvae (Kemp et al. 2011; Potts et al. 1988) and fill in the interstitial spaces upon which young animals may depend (Nickerson et al. 2003). It also may contribute to biogeochemical conditions that influence deoxygenation (Atkinson et al. 2008).

We tested the ability of parental care to buffer offspring from the effects of increased siltation and decreasing oxygen using eastern hellbender salamanders

(*Cryptobranchus alleganiensis alleganiensis*, Figure 4.1) as our model organism. Eastern hellbenders, and closely related Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*), are long-lived, fully aquatic giant salamanders with a relatively prolonged period of paternal parental care (Taber et al. 1975; Peterson et al. 1988; Nickerson and Mays 1973b). In wild conditions, hellbenders lay their eggs in narrow cavities under large boulders in fast-flowing streams (Nickerson and Mays 1973b). Breeding males (hereafter referred to as “den masters”; Takahashi et al. 2017) remain with the eggs from the time they are laid in early fall, through hatching, and into the spring when the larvae emerge from the nest (at least 8 months following oviposition; Hopkins et al., 2023). Previous research has shown that, during this time, den masters guard the eggs from predators, clean the nest, and oxygenate the eggs through a tail fanning behavior where they wave their tails over the clutch (Settle et al. 2018b; Unger et al. 2020). However, these studies have been limited to behavior that is visible through the narrow entrance of the nesting cavity, and as a result they may not have been able to fully observe care behaviors (Unger et al. 2020; Settle et al. 2018b). Additionally, the extent to which parents may modify their behavior in response to changing stream conditions is unknown. Hellbender populations have declined throughout their range (Foster et al. 2009; Wheeler et al. 2003; US Fish & Wildlife Service 2018; Jachowski and Hopkins 2018) and reproductive failure during the parental care period has been implicated in these declines (Hopkins et al. 2023), so fully understanding how hellbenders modulate their parental care in response to environmental conditions is a critical knowledge gap.

In this study, we expand current knowledge of hellbender parental care behaviors and explore the ability of these behaviors to help buffer hellbender eggs from variations

in water quality and siltation. Using recordings from custom-designed underwater infrared cameras installed in artificial nesting cavities in a natural stream, we overcame prior limitations to observing hellbender behavior by collecting continuous footage from inside the nesting cavity, enabling us to provide a more comprehensive understanding of hellbender parental care. We described hellbender parental care behaviors, assessed nocturnal and diurnal patterns of behavior, and determined how, if at all, the dissolved oxygen concentrations and silt levels in the nest relate to oxygenating (Settle et al. 2018b; Okada et al. 2015) and nest cleaning behaviors (Terry et al. 2018; Unger and Williams 2018) provided by the den master. We also document whether dissolved oxygen conditions within the nest cavity trigger a self-maintenance, rocking behavior which increases the flow of water over the adult's own skin (Harlan and Wilkinson 1981), and whether there are tradeoffs between self-maintenance and parental care behaviors.

## METHODS

### *Study site*

This study took place in a third-order stream in southwestern Virginia, USA. The stream is the site of an ongoing hellbender research project and supports a comparatively healthy population of hellbenders (Jachowski and Hopkins 2018). The study sites have an average of 67% forest cover (Yang et al., 2018), average fall conductivity of  $0.19 \pm 0.01 \mu\text{S}/\text{cm}$ , average fall total dissolved solid levels of  $0.13 \pm 0.008 \text{ mg}/\text{L}$ , average fall pH of  $7.7 \pm 0.09$ , and an average dissolved oxygen concentration of  $10.3 \pm 0.66 \text{ mg}/\text{L}$ . Although the water quality is not as good as some sites where hellbenders are found (Keitzer et al., 2013), the stream is still comparatively more forested and less basic with lower conductivity and less total dissolved solids than many Virginia streams with

hellbender populations (Bodinof Jachowski et al., 2016; O'Brien, unpublished data. The moderate water quality in the stream enabled us to identify both relatively low quality microhabitats as well as high quality microhabitats within a relatively small stretch of a single stream (measuring 13027 river meters from the lowest to the highest site).

### *Video capture*

We filmed den master behavior in concrete underwater artificial shelters consisting of a rectangular chamber with a single tunnel entrance. These shelters were similar to a traditional design (Briggler and Ackerson 2012) that has been in use by our research team since 2012, but they were modified to improve stability in the stream (Button et al. 2020b) and to accommodate a microcomputer (Raspberry pi Foundation; Cambridge, UK) attached to a Sainsmart infrared camera (Lenexa, KS, USA) in the shelter lid. We programmed the cameras to record at a frame rate of 15-frames-per-second and a resolution of 1640x1232 pixels. The videos were saved to a data storage device every 15 minutes, and the computer shut down after 48 hours to avoid a hard shutdown from battery drainage that could damage the computer. Filming was semi-continuous with short pauses in filming to allow us to exchange batteries before the 48-hour programmed shutdown, replace data storage devices, and restart computers. Longer pauses occurred when circumstances, such as unmanageably high flows, prevented the scheduled exchange. The cameras were designed such that the data storage devices and batteries were external to the shelter, enabling us to make exchanges without disturbing the animals.

Prior to the 2020 breeding season, we installed ten modified shelters (hereafter referred to as camera shelters) across a range of microhabitats in a stream in southwestern

Virginia, USA. The shelters were installed in pre-existing arrays of traditional hellbender shelters (Jachowski and Hopkins 2018). Four of these shelters received clutches in 2020. In 2021, we installed an additional five shelters, and we received another two clutches from unique hellbenders that year. To increase our sample size, in 2020 we also exchanged four traditional shelters that received nests with camera shelters one month after oviposition had occurred. Pilot work conducted in 2019 prior to the initiation of the study demonstrated that we could exchange a den master's shelter without inducing abandonment ( $n = 3$  with no abandonment) and that the den masters resumed parental care in a manner similar to that seen in undisturbed shelters (unpublished video data). To exchange a shelter, we removed the den master and his eggs and held them in cold stream water for approximately one hour. We placed a camera shelter that had been previously weathered in the stream in the same benthic footprint as the original traditional shelter, and we transferred the substrate (i.e., silt, gravel, leaf litter) from within the traditional shelter into the newly placed camera shelter. We then returned the male and his eggs to the shelter, and all den masters resumed parental care. Comparisons of behavior prior to and following the disturbance indicated minimal change in behavior, but we did not utilize behavior data for twelve hours following the disruption to ensure that it did not influence our results.

In total, we obtained 9,109 hours of paternal care video footage on 10 nests guarded by 10 unique den masters across two breeding seasons ( $n = 8$  and 2 nests for 2020 and 2021 respectively). An eleventh nest was included in our study originally, but the nest failed when the den master pulled a strand of eggs out of the tunnel while exiting the shelter. The stream current subsequently dragged the entire clutch out of the shelter

and downstream. We had minimal footage before this occurrence, so this nest was eliminated from our study. We recorded behavior nearly continuously from 30 days (+/- two days) after oviposition through 60 days (+/- two days) after oviposition (hereafter these periods will be referred to as mid-gestation and hatching, respectively). In the event of a nest failure (the nest contained no viable eggs or larvae), we ceased our behavioral analysis when the nest was definitively identified as having no eggs or larvae by a snorkeler.

At both mid-gestation and hatching, we captured the den masters to collect data on their body condition, measure their clutch size, and to collect blood samples for a separate project. We allowed the animals twelve hours to recover from this intrusion before we resumed analyzing their behavior from the videos.

#### *Water quality measurements*

We measured dissolved oxygen concentrations in each shelter using a miniDOT dissolved oxygen logger (Precision Measurement Engineering) affixed to the ceiling of each shelter's nest chamber. We set the loggers to take a reading every minute, and we calculated the average hourly dissolved oxygen every four hours (to reflect our behavior sampling frequency described below). Times when the shelter was open, such as during animal processing, were eliminated from our data set.

To assess the level of silt and organic material in each shelter, two researchers scored each shelter on these parameters (silt and organic material independently) using a qualitative scale from zero to four with zero being minimal silt or organic material and four being heavy silt or organic material. We did this at mid-gestation and hatching, and

the scores were averaged across the two reviewers. To assure the veracity of our fine sediment scores, we also collected two sediment cores from each shelter. The sediment was sieved using a 63  $\mu\text{m}$  sieve to separate out fine silt from coarse, and the two silt types were each dried and weighed. We then calculated the percent fine silt in each core. Our qualitative scores were positively correlated with our quantitatively measured fine silt levels ( $r^2 = 0.37$ ,  $p = 0.02$ ), so we used our qualitative scores in all statistical models.

### *Behavioral coding*

We used a focal sampling approach and coded the first fifteen minutes of den master behavior every four hours using ELAN software (Max Planck Institute for Psycholinguistics, 2023). This resulted in a cumulative 311 hours of analyzed footage. A panel of reviewers who were blind to the den master identification coded the videos, and they went through several rounds of training with the lead author before their analysis was considered reliable. Additionally, ten percent of all videos were crosschecked between reviewers to ensure consistency, and the lead author reviewed the codes for errors (e.g., improperly interpreted behaviors).

Our ethogram (Table 1) was based on previously identified parental behaviors in hellbenders and in closely related *Andrias* species (Settle et al. 2018b; Okada et al. 2015; Luo et al. 2018; Terry et al. 2018) as well as novel behaviors that we observed while coding. To assess the effects of shelter water quality on parental care, we focused on fanning (Supplementary video 1), which serves to oxygenate the eggs; rocking (supplementary video 2), which is a self-maintenance behavior of adult hellbenders in conditions of low dissolved oxygen and/or high energetic demands; and cleaning

(supplementary video 3) which serves to agitate and remove sediment from the shelter. Behaviors sometimes occurred simultaneously, and for the purposes of this study, we allowed overlapping codes. An exception to this was when recording the den master's position in the shelter which was exclusively coded as in the tunnel, in the chamber, or absent from the shelter to enable time budget analysis.

### *Analyses*

We qualitatively summarized our observations of hellbender parental care and calculated time budgets for the den master's position in the shelter (tunnel, chamber, or absent.) Non-nesting hellbenders are known to exhibit diel and seasonal differences in the timing of their observable activity outside of boulder cover and bedrock crevices (Noeske and Nickerson 2018; Humphries 2007). However, nothing is known about diel patterns of behavior of nesting hellbenders. Thus, we also used t-tests to compare nocturnal and diurnal levels of noteworthy individual behaviors including standing in the tunnel, rocking, cleaning, fanning, charging, leaving the shelter, and striking at targets outside the tunnel. For simplicity, we considered any behavior between 7:00 and 19:00 to be diurnal and anything between 19:00 and 7:00 to be nocturnal.

Using R 4.2.1 (for this and all other statistical analysis, R Core Team 2022), we tested the effects of dissolved oxygen concentration on the percent time that hellbenders spent fanning their eggs or rocking using zero-inflated linear mixed models with beta distributions, and we included the specific shelter (shelter ID) as a random effect in the models. We found no effect of coder on our results ( $p \geq 0.20$  for all tested behavior codes), so we did not include this as a factor in our models. We first tested the effect of

dissolved oxygen on percent time spent rocking to confirm that there was an effect of oxygen on this behavior. Upon finding an effect, we included percent time spent rocking as well as average hourly dissolved oxygen as fixed effects in our model of fanning behavior. This enabled us to test both if fanning increased in response to lower dissolved oxygen levels and also if rocking moderated this response.

To determine whether den masters cleaned more in shelters with more silt and/or organic material, we used a zero-inflated negative binomial model. We included both silt and organic material as fixed effects and included shelter as a random effect. Additionally, because sediment conditions in shelters can change rapidly, we used only the five days of behavior prior to or following the silt or organic material scoring (depending on the sampling date) to analyze the effect of shelter sediment on parental care.

## RESULTS

We were able to code between 9.25 and 42.25 hours of behavior per shelter with an average of 31.3 hours per individual. This variability in codable video footage was due to nest failure and conditions such as rain events making recording impossible. One of our ten nests failed by one month following oviposition and three failed by hatching, which is consistent with expectations based on a large-scale study of hellbender nesting ecology (Hopkins et al., 2023). These failed nests did not have rotten eggs or larvae, but rather had no eggs or larvae present at the time of failure. Beyond the nest that had the clutch swept downstream and was eliminated from the study, we were unable to confirm the exact cause of nest failure in any of our nests. One shelter seems to have lost its

clutch during a high flow rain event, as the eggs were visible prior to but not after a rain event in which the water became very turbid. The others were likely the result of cannibalism (as described in Hopkins et al., 2023) because cannibalism was observed on their videos, but we cannot definitively assert this as the cause of complete nest failure.

### *General observations of behavior*

Positionally, males primarily spent their time in the tunnel of the shelter (mean = 76.3%) which put them between their clutch of eggs and the only entrance to the nest cavity. On several occasions, we observed den masters apparently struggling with unseen targets while in the tunnel in potential shelter guarding behavior. Of their remaining time, males spent most of it in the chamber (mean = 19.6% of their time) and they left the shelter only rarely, spending an average of 4.2% of the analyzed time out of the shelter (range = 0-16.5% of analyzed video time). Only three males were absent for more than 2% of the analyzed time, and two of those three nests failed. The average amount of time a male was absent from the shelter was also significantly correlated with the average number of bouts of cannibalism ( $\beta = 1.19410$ ,  $SE = 0.24$ ,  $T = 4.95$ ,  $p = 0.001$ ) suggesting that these males may have been less invested in parental care.

We definitively identified filial cannibalism of healthy eggs by five of the ten den masters, including males that had both successful ( $n = 3$ ) and unsuccessful ( $n = 2$ ) nests. These instances consisted of the male eating one to a few eggs at a time which he bit and jerked free from the clutch. For all five of these males, we had sufficient visibility to clearly see the eggs that the den master ate and visually assess that they were developing properly at the time of consumption (as opposed to dead embryos which are visually distinct). We also were able to observe swallowing motions. In one of these shelters, we

additionally confirmed filial cannibalism of an opaque, obviously rotten egg, suggesting hygienic cannibalism also occurs (supplementary video 4). In three shelters where we could not definitively identify cannibalism, we nonetheless observed what was very likely egg consumption. In these cases, we observed body and clutch movements indicative of cannibalism, and in most instances saw the male with an egg strand in his mouth. However, due to limits in the camera's field of view or obstructions to the line of sight, such as other eggs, we were not able to clearly see the egg being consumed to classify the cannibalistic event as hygienic or otherwise. In these cases, we also were not always able to confirm that the egg was swallowed. We observed several instances of hellbenders taking eggs into their mouths and spitting them out, so although the animal's behavior may have suggested cannibalism, we refrained from classifying them as cannibals due to this uncertainty.

In addition to males biting the eggs and spitting them back out, we also observed other non-cannibalistic interactions with the eggs. For example, we recorded den masters nibbling at the eggs and the enveloping jelly connecting eggs without any apparent attempt to eat them. Males also frequently crawled under and through the clutch and nuzzled the eggs with their snout. At times, males physically moved the clutch's position in the shelter by pushing it with the side of their torso (supplementary video 6). We also observed males agitating the clutch by biting it and shaking it back and forth. This behavior was not an immediate precursor to cannibalism, but rather an independent behavior. Although its purpose remains unclear (it has been suggested as a behavior to prevent yolk adhesion (Okada et al. 2015), we did observe silt being shaken free from the clutch during some periods of agitation suggesting that it may serve to remove silt from

the clutch. We did not find a clear nocturnal/diurnal bias in behavior in the den masters we recorded. The only significant difference in behavior we found was a small difference in the amount of time the den master spent in the tunnel, with males spending significantly more time in the tunnel at night than during the day ( $\bar{x}_{\text{night}} = 33.43$  min,  $\bar{x}_{\text{day}} = 25.2$  min,  $p < 0.001$ , Figure 4.2).

We observed between zero and four strikes at targets outside the tunnel by individuals during the course of filming and between zero and seven charges (we did not observe striking in five individuals or charging in two individuals). Several of the strike events were accompanied by biting and swallowing, suggesting that they were successful attempts at capturing prey. However, the prey was always consumed in the tunnel and out of the camera's field of view, so we were unable to confirm its identity. In field palpations we were often able to feel crayfish exoskeletons and in one instance recorded an individual vomiting crayfish claws, so this was likely a common target. Charging occurred more frequently than striking and was never immediately followed by striking, suggesting that it was either a defensive behavior and/or a hunting behavior that was often not successful.

Several rain events occurred during our period of filming, and these events caused some shelters to accrue sediment and leaves. However, the extent to which this occurred varied across shelters. We did not detect any change in nest cleaning behavior following rain events, suggesting that males did not modulate their care in response to the increased sediment and organic material. During the rain events males typically remained in the tunnel of the shelter.

### *Effects of water quality on parental care*

The 4-hour averages of dissolved oxygen concentrations within shelters ranged from 6.34 to 11.45 mg/L (66.20 to 108.28 percent saturated) with an average concentration of 9.06 mg/L. Our sediment scores ranged from 1.5 to 3.75 for both silt and organic materials. We did not find a significant relationship between a shelter's average dissolved oxygen and either its average organic material score ( $\beta = 0.19$ , SE = 0.13,  $p = 0.19$ ) or its average silt score ( $\beta = -0.14$ , SE = 0.20,  $p = 0.51$ ).

We found that dissolved oxygen influenced both rocking (a self-maintenance behavior) and fanning (a parental care behavior). We also found a negative relationship between the two behaviors, suggested a trade-off may exist in the time males allocate to each behavior (Table 2). Males were more likely to both rock and fan in conditions of lower dissolved oxygen, with the model showing a two-fold increase in the probability of rocking and an 86-fold decrease in the probability of fanning between oxygen concentrations of six and twelve mg/L (figures 4.3a and 4.3b). Males were also likely to spend a greater percentage of their time fanning and rocking in conditions of low dissolved oxygen, with the model showing a more than five-fold decrease in the percent time rocking and 430-fold increase in the percent time fanning between oxygen concentrations six and twelve mg/L (Figures 4.2c and 4.2d). The relationship between rocking and fanning was somewhat nuanced. The more time males spent rocking, the less likely they were to fan at all. However, the amount of time that males spent rocking did not influence the amount of time that they fanned (Table 2). We did not find any relationship between the amount of organic material or silt in the shelter and cleaning behavior (Table 2).

## DISCUSSION

This study provides the most detailed observation to date of hellbender parental care behavior, and it suggests that parental care may help hellbender offspring cope with some anthropogenic stressors. However, our findings suggest that such behavioral buffering is not without limits, as males only responded to some stressors through increased parental care, and they showed evidence of trade-offs between parental care (tail fanning) and self-maintenance (rocking) as the intensity of stressors increased. Recent research suggests that these buffering limits may be critical, as once den masters reach the limits of their ability to buffer offspring, they may fully cannibalize their clutch (Hopkins et al. 2023), terminating parental care and resorting to the ultimate self-maintenance strategy.

By collecting continuous footage of parental care behavior across both night and day, we were able to confirm previous findings and provide new insights into basic parental care behavior in hellbenders. For example, consistent with previous observations (Unger et al. 2020; Settle et al. 2018b), we found that den masters primarily spend their time in the tunnel of the shelter and rarely leave, and we observed den masters cleaning the shelter and agitating the clutch. When hellbenders agitated their eggs, we at times observed sediment being shaken free from the eggs. We also observed several novel parental care behaviors that occurred within the chamber including crawling over and through the clutch, moving the clutch's position in the shelter, biting at the eggs without obvious intent to pierce them, and taking eggs into their mouths and spitting them out. One possible explanation for these latter two behaviors is that they

represent egg grooming behavior, which serves to remove pathogens and reduce infection by mechanically removing fungal spores and/or applying antimicrobial compounds through transferred saliva (Costa 2006). Although not previously described in salamanders, such behaviors have been identified in other taxa such as insects and fish (Knouft et al. 2003; Smith-Grayton and Keenleyside 1978), and given the threat of water mold to hellbender eggs (Smith 1907; Civiello et al. 2019), such behavior may be beneficial to hellbenders.

Previous work on diel patterns of hellbender activity have focused on activity outside of shelters during the non-breeding season and have identified primarily nocturnal behavior (Noeske and Nickerson 2018). In contrast to this, we found little difference between nocturnal and diurnal behavior patterns during the period of parental care, including in the time that males spent away from the shelter. This difference may be due to differences in diel patterns between the period of parental care and other periods, differences in the age or sex of individuals observed (it is possible prior studies did not observe breeding males), or our more extensive period of recording could have identified results previously difficult to detect. It is also possible that diel patterns are specific to certain streams or drainages. For example, hellbenders are known to be diurnally active outside of shelter in a stream in North Carolina (Humphries 2007), yet more nocturnal in other streams (Noeske and Nickerson 2018).

We were unable to definitively identify the cause of nest failure in any shelters except one, whose eggs were lost to the stream current immediately after recording began. However, having the clutch swept downstream was likely the cause of failure in another nest whose eggs were present prior to a high flow event but not after. Preventing

clutch loss may be one reason den masters stand in the tunnel during rain events rather than secluding themselves in the chamber. We have also observed den masters saving their eggs from being pulled downstream by biting an escaping clutch and pulling it back into the shelter, suggesting that preventing clutch loss of this sort may be a critical parental care behavior. As high flow events continue to increase in intensity as a result of climate change (Tabari 2020) and deforestation (Bradshaw et al. 2007), and as siltation reduces nest cavity size (Hopkins and DuRant 2011) this behavior may be critical for helping hellbenders cope with anthropogenic environmental changes.

Our finding that cannibalism occurs in the majority of nests, including successful ones, supports recent suggestions that some level of filial cannibalism may be a normal behavior for hellbenders (Hopkins et al. 2023). Although we were able to definitively identify some cannibalized eggs as rotten, all cannibalistic males also appeared to eat at least some healthy eggs, suggesting that the behavior is not entirely hygienic. Based on studies in other species, filial cannibalism may occur in hellbenders for a variety of reasons. One that deserves particular attention is the possibility that filial cannibalism serves to remove eggs that have not been fertilized or have been fertilized by other males (Neff 2003; e.g., Neff and Gross 2001). Either of these two conditions is possible in hellbenders. Den masters are known to experience nest parasitism by smaller males who attempt to fertilize the eggs (O'Brien et al, in press), so multiple paternity of some clutches is likely. Additionally, hellbenders are facing increasingly high stream salinity as a result of human activity (Kaushal et al. 2018), which is known to influence fertilization success in brook trout (Bonisławska et al. 2015) and may influence hellbenders as well. Furthermore, recent research has linked full clutch cannibalism to

reduced forest cover (Hopkins et al. 2023), consistent with the possibility that degraded stream water chemistry and possible low fertilization success is contributing to nest failure.

We did not find a relationship between the amount of time that males spent cleaning the shelter and the amount of silt or organic material in the nest. This suggests that males do not behaviorally buffer their offspring from negative effects of increasing siltation. Our findings are in contrast to the one study we were able to find of parental response to siltation in marine systems, which found a behavioral response to increased siltation (Potts et al. 1988). It is possible that there are other behaviors hellbenders use to remove silt from the eggs, such as agitation, but further investigation is needed to confirm this. Additionally, because our modified shelters were relatively large compared to a natural nest cavity or a traditional artificial shelter, the effects of siltation may have been modified. Deforestation has the potential to increase the amount silt in hellbender nesting cavities and if den masters fail to respond, these greater silt levels may smother their eggs and larvae. Increased siltation may also have more extensive effects, such as promoting biogeochemical conditions that favor deoxygenation in the nest cavity (Atkinson et al. 2008) and creating inhospitable microhabitat conditions for the eggs and den masters. These findings emphasize the importance of reducing siltation rates in freshwater streams.

In contrast to cleaning, we found that den masters did buffer their offspring from the effects of low dissolved oxygen by increasing their tail fanning behavior. However, the ability of den masters to respond to declining oxygen levels by fanning their eggs was tempered by an increase in rocking behavior which serves to increase the oxygen

available to the den master himself. The aerating effects of rocking and fanning have not been compared. However, rocking is not thought to be a parental care behavior (Okada et al. 2015) and is likely less effective at clutch oxygenation than fanning, although work to confirm this is needed. Our findings suggest that there is a trade-off between parental care and self-maintenance behavior, and that although parents may be able to buffer their offspring from low dissolved oxygen to some extent, this ability is not without limits. Energy expenditure may be an important aspect of this limitation. Although we demonstrated that hellbenders can simultaneously rock and fan, this is a highly exaggerated movement that is likely energetically expensive. In common gobies (*Pomatoschistus microps*) increased fanning has been shown to cause greater weight loss in the care-taking parent and increased probability of future nest abandonment (Jones and Reynolds 1999). There may be similar costs in hellbenders that limit their ability to fully compensate for declining oxygen concentrations for their own and their offsprings' benefit.

The prioritization of self-maintenance behaviors over parental care behaviors that we observed may be influenced by hellbender life-history. Hellbenders can breed every year and live more than 25 years. This longevity and fecundity may inspire an evolutionary strategy to invest in future reproduction at the expense of current reproduction under difficult environmental conditions (Stearns, 2000). If water quality continues to decline, this strategy of prioritizing future reproduction may have grave consequences for species survival. However, prioritization of future reproduction has found limited supported in previous hellbender research (Hopkins et al., 2023) and needs further investigation.

By shedding light on the basics of hellbender parental care and uncovering from which stressors den masters may be able to shield their offspring and from which they may not, our study provides foundational insights into hellbender behavior and reproduction. Additionally, it represents one of the only studies of parental response to environmental stressors in freshwater streams. Our results suggest that increasing siltation may be of greater immediate concern than decreasing levels of dissolved oxygen for young hellbenders under parental care. However, the increased energetic costs of parental buffering behaviors may induce tradeoffs which needs further investigation. Additionally, the extent to which other freshwater species also show a greater ability to buffer their offspring from oxygen stress than siltation is unknown and may complicate management prioritization. Our study was additionally conducted in a stream with relatively good water quality and a fairly narrow range of environmental conditions. Future work across a greater range in siltation levels and oxygenation conditions may reveal additional shifts in behaviors and thresholds that induce these shifts. A better understanding of how parents shield their offspring from anthropogenic stressors, as well as better understanding of limitations on their ability to do so, will enable us to better predict how animals respond to their changing world.

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**Table 4.1** Our ethogram of den master behaviors was based on previously identified parental care behaviors in hellbenders and closely related species as well as novel behaviors that we identified while coding.

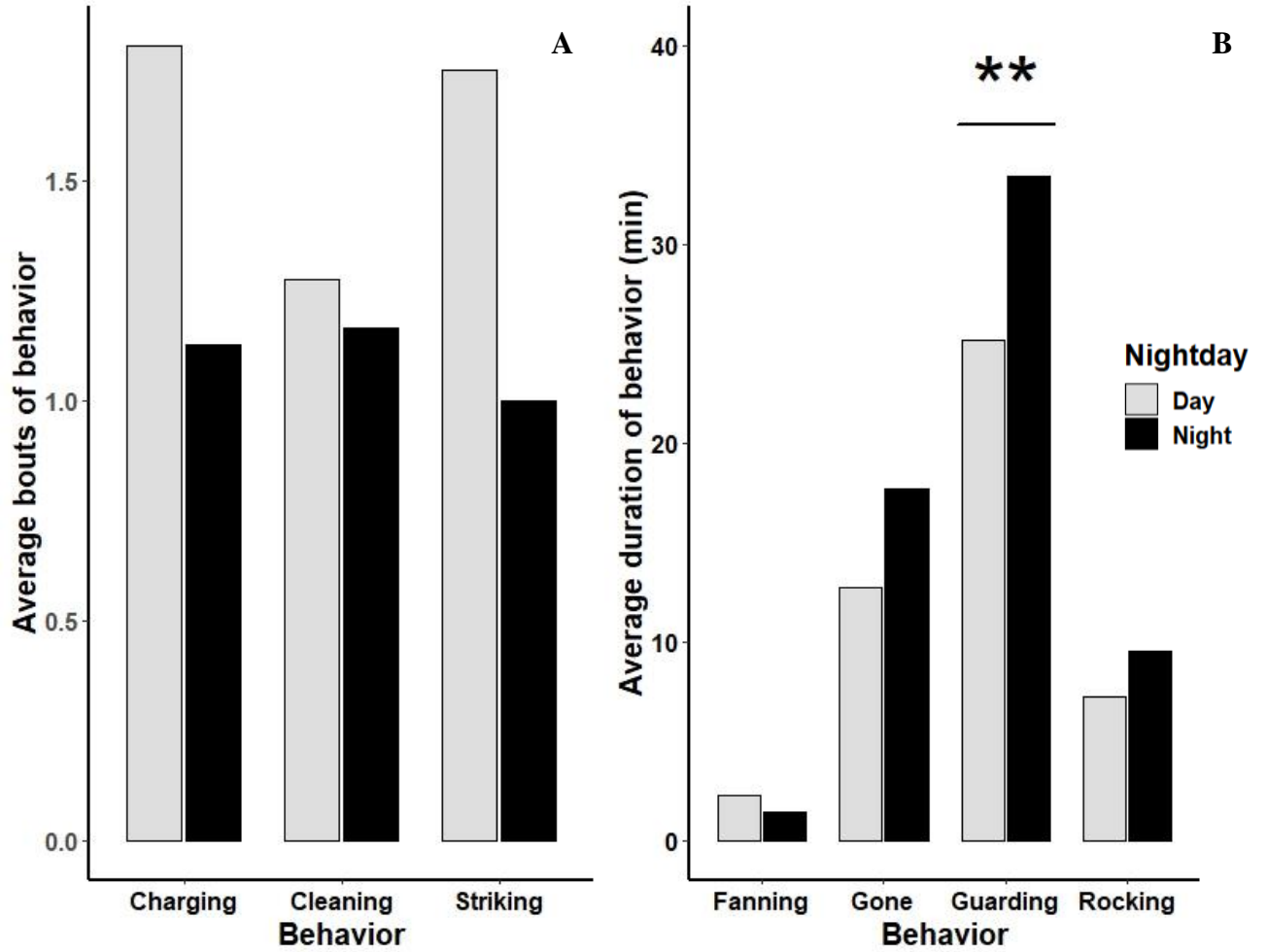
<b>Behavior</b>	<b>Description</b>
Fanning	A lateral undulation of the tail
Cleaning	Movement of loose substrate using the hind legs in a kicking motion or movement of loose substrate using the nose
Rocking	Lateral and vertical movement of the body while all limbs remain grounded
Striking	Rapid forward lunge while all four feet remain grounded
Charging	Rapid movement towards and through the tunnel
Absent	Male is absent from the camera's field of view after exiting the tunnel and his subsequent return is face first
Chamber	Male is in the chamber without his face in the tunnel
Tunnel	Male's face or entire body is in the tunnel
Agitating	Vigorous movement of the clutch using the head, typically by biting clutch.
Cannibalism	Confirmed consumption of apparently healthy eggs
Hygienic cannibalism	Confirmed consumption of apparently unhealthy eggs
Other behaviors	Rolling on to side or rolling completely over Stretching towards shelter ceiling, at times accompanied by falling over backwards Opening and closing mouth Clenching jaws Yawning Vomiting crayfish claws Biting at objects floating in water Struggling with unseen object in tunnel Twitching legs Nibbling at eggs Moving eggs with body

**Table 4.2** Models predicting den master behavior. The zero-inflated component of the model can be thought of as a binomial model where greater positive correlation indicates a greater chance of a behavior *not* happening, while the conditional model is similar to a traditional regression.

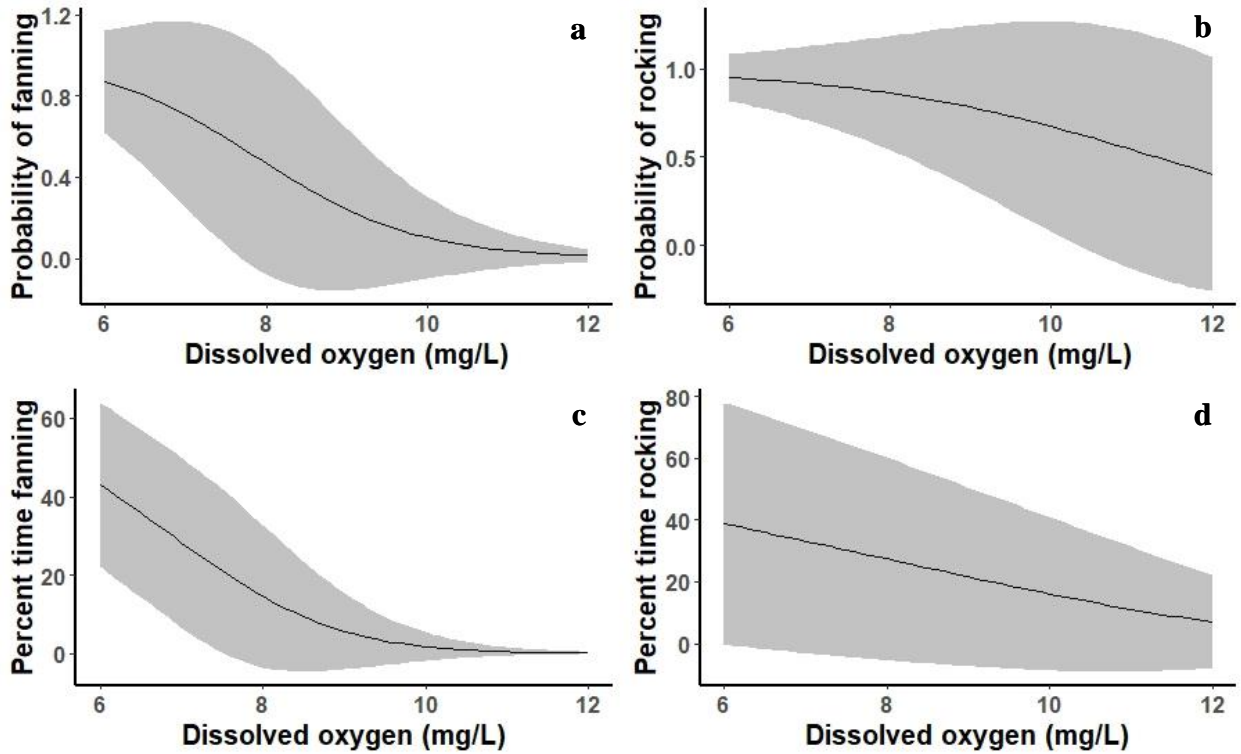
Dependent variable	Model	Parameter	Intercept	$\beta$	SE	p
Percent time spent rocking	Conditional	Dissolved oxygen (mg/L)	0.81	-0.20	0.51	<0.001
	Zero-inflated	Dissolved oxygen (mg/L)	-6.25	0.55	0.09	<0.001
Percent time fanning	Conditional	Dissolved oxygen (mg/L)	2.21	-0.38	0.08	<0.001
		Percent time spent rocking		0.32	0.29	0.28
	Zero-inflated	Dissolved oxygen (mg/L)	-8.17	0.10	0.10	<0.001
		Percent time spent rocking		0.34	0.34	0.004
Cleaning Bouts	Conditional	Silt	-0.90	-0.16	0.16	0.31
		Organic material		-0.24	0.19	0.20
	Zero-inflated	Silt	-3.02	0.82	0.74	0.27
		Organic material		0.67	0.71	0.34
		Eating shed skin				
		Etc.				



**Figure 4.1** Hellbender clutches consist of long strands of eggs connected by an enveloping jelly. The eggs are typically tied into a knotted form (O'Brien et al., *in review*). This figure shows a den master saving an escaping clutch from being swept downstream. Such behavior may be an important parental care behavior.



**Figure 4.2** Comparison of nocturnal and diurnal behaviors in eastern hellbender den masters during the parental care period, Panel A depicts the average bouts for behaviors with very short durations and Panel B depicts the average durations for longer-lasting behaviors. \*\* denotes significant a difference between diurnal and nocturnal behavior.



**Figure 4.3** Parental response of eastern hellbender den masters to reduced dissolved oxygen concentrations in terms of both parental care behaviors (fanning) and self-maintenance behaviors (rocking). We found that average dissolved oxygen concentration had a significant effect on the probability of fanning (a) and the percent time spent fanning (c) as well as the probability of rocking (b) and the percent time spent rocking (d). For the purposes of visualization, confidence intervals are displayed using a normal distribution.

## CONCLUSION

Human and animal behavior is critical to successful wildlife conservation both for its flexibility and its predictability. For wildlife, behavioral flexibility is a valuable asset as it provides a potentially important buffer against the environmental changes of the Anthropocene. Unlike genetic adaptations that can be slow to develop within populations, behavior is a more plastic response that can enable time for other adaptive changes to occur (Wong and Candolin 2015). For humans, behavior is at the core of what has caused most anthropogenic environmental degradation, and its flexibility means that humanity has the capacity to reduce or even reverse some of its undesirable effects on the environment (e.g., Steffen and Patt 2022). Importantly, behavior can also be to some extent be predicted, enabling an improved ability to understand and respond to conservation challenges.

One area where behavior plays a critical role is on private lands. Private landowners can heavily influence ecosystem conditions on their property in ways that are beneficial (Hobbs 2012; Burger et al. 2019) or detrimental (Randolph 2012) to conservation goals, and this, in turn, has a strong effect on the species that depend on private lands as habitat. In this dissertation, I studied landowner decision making regarding allowing conservation research on their property, and I explored the effects of that research on landowner conservation knowledge, attitudes, awareness, and behaviors. In particular, I focused on conservation behaviors in the context of the watershed that was almost entirely privately owned. As a result, landowner management decisions have a strong influence on water quality parameters such as siltation levels and dissolved oxygen concentrations. I also studied how eastern hellbender salamanders, which are heavily

influenced by landowner management decisions, respond to reduced water quality. My hellbender research focused on their breeding and parental care behaviors because reproductive success has been identified as a primary contributor to population declines in this species (Hopkins et al. 2023). I described hellbender parental care and breeding behaviors, and I analyzed the ability of parental care to buffer larvae against anthropogenic stream degradation, specifically low dissolved oxygen concentrations and high siltation levels.

## CONTRIBUTIONS TO THE FIELD

Private lands research has previously been recognized for its importance to conservation (Hilty and Merenlender 2003b), yet there were few resources to guide researchers in this endeavor. I addressed some of this knowledge gap through a study focused on how to effectively gain access to private lands for research. Using a combination of surveys and interviews, I found that many landowners were motivated to allow research on their property out of a desire to be helpful. Additionally, they were more likely to allow research on their property if they were interested in learning about the research taking place, if they had previously allowed research on their property, if they had a positive attitude towards conservation, and if they had larger properties. Conversely, landowners were less likely to allow research if they were concerned about the research restricting free use of their property.

I then built on these ideas by exploring the potential for private lands research to influence landowner perspectives on conservation. Although there has been significant research regarding the effects of citizen science on landowner behavior (e.g., Jordan et al.

2011; Crall et al. 2013; Toomey and Domroese 2013; Evans 2005), there was little looking at engagement in more traditional scientist-landowner interactions. Additionally, many of the prior studies suffer from ceiling effects due to participants already being strongly conservation-oriented (Call et al., 2013; Overdevest et al., 2004; Scott and Willits, 1994; Brossard et al, 2005). I addressed these limitations by comparing the effects of engagement in citizen science and traditional science on landowners and by attempting to recruit participants from groups less commonly associated with conservation behavior. From this study, I found that landowners were similarly influenced by engagement in citizen science and in traditional research, and that engagement increased landowner knowledge as well as increasing their awareness of conservation issues and encouraging a positive attitude towards conservation. I also found that the effects of engagement extend beyond the landowners engaged in citizen science or traditional science research to their social networks, giving landowner involvement in science an outsized impact in the community.

One species of wildlife that is strongly influenced by private landowner behavior is the eastern hellbender. Hellbender populations are declining throughout their range (Wheeler et al. 2003; Foster et al. 2009; Burgmeier et al. 2011; Graham et al. 2011), and land management is a primary factor driving these declines (Jachowski and Hopkins 2018). In order to fully understand how hellbenders will respond to anthropogenic stressors, it is important to understand both the fundamental aspects of hellbender behavior, and the ways in which hellbenders may behaviorally respond to human impacts on stream water quality.

Nest failure is a primary concern for hellbenders living in impacted habitat (Hopkins et al. 2023), and although there has been growing interest in hellbender reproductive biology (Unger et al. 2020; Settle et al. 2018c,a), there is much that remains to be learned about the topic. In particular, prior to my research, there was only very limited understanding of what occurred in nesting cavities during breeding and parental care. Natural cavities and traditional artificial shelters are too small to enable observation or filming of behavior, so all previous studies were based on the limited view provided by external observation. To overcome this limitation, I modified pre-existing artificial shelter designs to accommodate a camera in the lid and increased the size of the shelter to provide sufficient field-of-view. Additionally, I custom-designed and built underwater, infrared cameras capable of continuously recording hellbender behavior with minimal disturbance.

The camera technology that I developed provided the most detailed observations of pre-breeding behaviors, complete mating sequences, failed mating attempts, and breeding behaviors that have been documented. From the recordings, I was able to describe the sequence of hellbender mating behaviors including possible signal production via wave-based communication and a unique egg laying behavior by female hellbenders. Additionally, I also identified the potential for alternative mating tactics in the species and identified some patterns in the characteristics of successful vs. failed breeding attempts such as longer periods of time spent biting potential mates by the males. I also used the cameras to provide the first published description of hellbender parental care behavior including full observation of behaviors occurring inside the nest cavity. In addition, I compared nocturnal and diurnal patterns of behavior, and assessed

parental ability to buffer hellbender larvae against the effects of anthropogenic stream degradation. Prior to this study, there was little research exploring how animals might respond to multiple stressors through parental care, and few field studies in aquatic systems. This study showed that while parents may modify their behaviors to help buffer their offspring against anthropogenic stressors, they do not respond to all stressors, and even when they do respond, the buffering is moderated by self-maintenance behaviors.

## IMPLICATIONS FOR CONSERVATION

My wish for my dissertation is that it will, in at least some small way, contribute to conservation of the natural world. I hope that my social science research will empower and encourage conservation researchers to pursue work on private lands and to view their interactions with landowners as an opportunity to magnify the impact of their work. In this way, private lands research will not only increase the knowledge base needed for conservation, but will also encourage conservation behaviors on private lands.

Conservation requires an inclusive approach that takes advantage of every available opportunity, and actions that capitalize on pre-existing activities, such as private lands research, are critical to its success.

Additionally, I hope that findings from my studies of hellbender reproductive biology will inform efforts at captive rearing and clarify the needs of hellbenders for successful breeding in natural environments. I also hope that the studies' reach will extend beyond hellbender reproductive biology to provide insight into ways that animals that exhibit parental care may be able to buffer their offspring against the impacts of anthropogenic stressors. A better understanding of how species respond to anthropogenic

stressors through behavior change will enable a more informed approach to conservation prioritization. For example, if some stressors can be mitigated through behavior change and others cannot, conservation efforts could prioritize those that species are least able to respond to behaviorally.

## FUTURE DIRECTIONS

Research often provides more questions than it does answers, and this dissertation is no exception. Learning to let some questions go for future researchers to pursue is one of the most difficult and humbling lessons a scientist can learn. I hope that future studies will seek to address at least some of the many questions I have not had the time or ability to answer during my time at Virginia Tech.

For landowner research, I provided evidence that engagement in research influences landowner knowledge and attitudes towards conservation, but further empirical investigation is needed to clarify and confirm this relationship. In particular, it would be interesting to explore what, if any, differences exist in the effects of citizen science vs. traditional science on the type of learning that occurs with each. In that same vein, it would be useful to know what aspects of allowing or participating in research have the greatest significance to landowners (e.g., Is it access to expert knowledge for consultation? A sense of belonging inspired by participation? A specific aspect of the research experience that appeals to landowner values or interests?). A better understanding of this could inform what types of research might most influence

landowner perspectives and which aspects of the engagement are important to emphasize during interactions with landowners.

It also would be valuable to know to what extent engagement in research reaches a unique segment of the population compared to those who typically participate in conservation programs. This could be with regards to the traits of the participants (e.g., property size, income, ethnicity, etc.), the focus of recruitment efforts (do scientists and those promoting conservation programs target similar groups?), or simply geographical location. This would clarify the value of engagement in research for encouraging conservation activities as well as for reaching under-represented groups in conservation outreach. Based on my results, there is some evidence that there may be overlap in the factors that predict who participates in conservation programs and who allows research on their property, but this would require further study. A similar question could also be extended to the types of individuals reached through citizen science vs. traditional science engagement.

Finally, it would be interesting to learn how private lands research influences the scientists doing the research. Influence is a two-way-street, and changes to researchers' attitudes and behaviors could be every bit as important as those to landowners' attitudes. This could include how it influences researchers' approach to conservation, how it influences their attitudes and behaviors towards landowners, how it changes their attitudes towards and understanding of their subject of study, or how it affects the types of questions they pursue. There is also possibly a positive feedback effect where changes in the attitudes and behaviors of researchers might make them more successful at gaining access to and conducting research on private lands in the future.

In terms of hellbender breeding behavior, there is a need to explore the potential for alternative mating tactics in greater detail. In particular, an area ripe for future research is the minimal conflict I observed between the (probable) sneaker males and the den masters they parasitize. If the individuals in the shelter are sexually mature males capable of fertilizing eggs, den masters would be expected to be motivated to drive them from the shelter, yet I rarely observed this behavior. Among the possible causes that could be investigated for this apparent lack of conflict are that sneakers are too immature to have much fertilization success (Watanabe and Maekawa 2010), that the sneakers are genetically related to the den master and therefore pose a reduced risk to the den master passing on his genes (Hamilton 1964), or that the sneakers are in some way masking their identity from the den master (Dominey 1981). Another area of future research regarding sneakers is the potential for males to recognize eggs that have been fertilized by an external source. This has been observed in other species (e.g., Neff and Gross 2001), and could explain some of the partial clutch cannibalism of apparently healthy eggs that occurs in successful nests.

Sexual selection in hellbenders is another area of their reproductive biology that is relatively untouched, and further investigation in this area would not only advance basic knowledge of the species, but could also be valuable for captive breeding efforts (Meghan S. Martin-Wintle et al. 2015). For example, it is unclear what traits the female is assessing in deciding where to lay her eggs, including whether she is relying on an assessment of the male or of the nesting cavity to make her decision. Relatedly, although my research has provided some evidence to suggest that mate choice copying can occur, this would need empirical confirmation.

The characteristics that males use in selecting their nest sites is also unclear. Although nest site choice has been studied to some extent through a thorough investigation by Button et al. (2020), the metrics used in this study were all taken outside of the shelter. It would be valuable to do a similar study using characteristics inside the shelter such as siltation level and dissolved oxygen concentration. The success of camera shelters in attracting nests also suggests that shelter size may play some role in cavity choice. However, camera boxes were intentionally placed in deeper runs which are preferred nesting microhabitats for hellbenders (Button et al., 2020), so the relative influence of shelter size and water depth would need to be teased apart.

Perhaps one of the questions I am most loath to leave behind regards the potential for wave-based communication in hellbenders. In my study of hellbender breeding, I identified a quivering behavior among both male and female hellbenders that may represent a means of communication. Confirming that this behavior is signaling, identifying its modality, and investigating its role in mating behaviors through field and lab studies would be a fun and fascinating line of inquiry that could shed some light on signal evolution in salamanders.

Finally, there are a several questions that remain in terms of parental care behavior. For my research regarding den master responses to low dissolved oxygen, a critical remaining question is what, if any, difference exists in the effectiveness of rocking vs. fanning in oxygenating eggs. The evidence that I found of a trade-off between parental care and self-maintenance is contingent on rocking being a less effective means of oxygenating eggs, but this has not been confirmed. It is also still

unclear what drives egg agitation behavior by den masters and the extent to which nest cleaning is a parental care behavior, mating behavior, both, or neither remains unclear.

## CLOSING

Behavior is fascinating for both its predictability and its flexibility, and both of these components are critical to behavior's role in conservation. The predictability of behavior enables conservationists to understand what drives certain decisions, and to use those predictors to guide or respond to the behavior in order to support conservation goals. Its flexibility enables animals to more rapidly adapt to anthropogenic changes to their environment, and, perhaps most importantly, behavioral flexibility means that for humanity there is always the potential for change.

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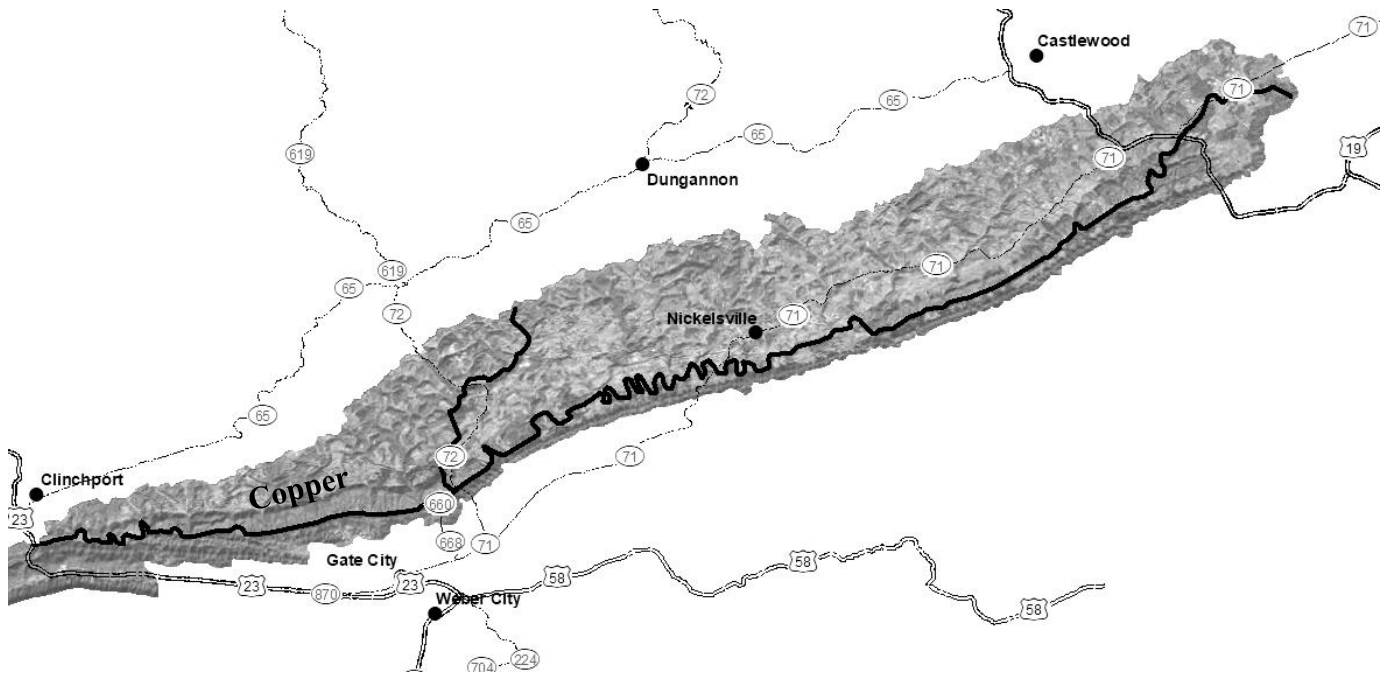
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# Landowner Perspectives on Nature and Wildlife in the Copper Creek Area



Fall 2018



***Thank you for taking our survey! Please answer the following questions regarding the property you own in Scott and/or Russell County, Virginia. First, we'd like to know a little about the creeks on your property. If Copper Creek is on or alongside your property, please answer regarding that creek. If your property is not on Copper Creek, please answer regarding the widest creek on or alongside your property.***

**1. Is there a creek on or running alongside your property in Scott and/or Russell County?**

- Yes, Copper Creek is on or alongside my property
- Yes, another creek is on or alongside my property
- No (if no, skip to question 4)

**2. Between May and August of this year, approximately how frequently did you spend time in or around the widest creek on your property (for example, fishing, wading, or relaxing)?**

- Daily
- Weekly
- Monthly
- Never

**3. How would you rank the health of the widest creek on your property?**

- Very healthy
- Moderately healthy
- Neutral
- Moderately unhealthy
- Very unhealthy

**4. Below are pairs of differing opinions people may have about conserving nature on their property. For each question, please circle the number that best matches where you fall between the two opinions. For example, for the first question, if you thought conserving nature was neither important, nor unimportant, you would circle 3.**

**I would describe conserving nature on my property as... (Please circle only one number per row.)**

Important	1	2	3	4	5	Unimportant
Beneficial	1	2	3	4	5	Harmful
Unaffordable	1	2	3	4	5	Affordable
Interesting	1	2	3	4	5	Uninteresting

**5. Now we'd like to know a bit about your thoughts regarding nature in general. To what extent do you agree or disagree with the following statements? (Please circle only one option per row.)**

	Strongly Disagree	Moderately Disagree	Neither Agree nor Disagree	Moderately Agree	Strongly Agree
The so-called "ecological crisis" facing humankind has been greatly exaggerated	1	2	3	4	5
If things continue on their present course, we will experience an ecological catastrophe	1	2	3	4	5
The balance of nature is <u>not</u> easily upset by human activities	1	2	3	4	5
Humans are severely abusing the environment	1	2	3	4	5

**6. We'd like to know about how you see your and other landowners' roles in conserving nature. Please indicate the extent to which you agree or disagree with the following statements. (Please circle only one option per row.)**

	Strongly Disagree	Moderately Disagree	Neither Agree nor Disagree	Moderately Agree	Strongly Agree
The overall health of nature is <u>not</u> landowners' responsibility	1	2	3	4	5
How I manage my property has very little influence on the health of nature	1	2	3	4	5
There are actions that landowners can take to reduce their impact on nature	1	2	3	4	5
I have a personal obligation to take care of the nature on my property	1	2	3	4	5
I am capable of reducing my impact on nature	1	2	3	4	5
Conserving nature on my property is <u>not</u> important to me	1	2	3	4	5
The people I care about expect me to conserve nature on my property	1	2	3	4	5
The people I care about conserve nature <u>on their own</u> property	1	2	3	4	5

7. We'd like to know about how much people in your community look to you for advice regarding land management. By management we mean how you use and care for your property. Please indicate the extent to which you agree or disagree with the following statements. (Please circle only one option per row.)

	Strongly Disagree	Moderately Disagree	Neither Agree nor Disagree	Moderately Agree	Strongly Agree
Other landowners come to me for advice about how to manage their property	1	2	3	4	5
I often influence other landowners' opinions on how to manage their property	1	2	3	4	5
Other landowners do not listen to my advice on managing their property	1	2	3	4	5

8. We are interested in learning about your trust of science. Please indicate the extent to which you agree or disagree with the following statements. (Please circle only one option per row.)

	Strongly Disagree	Moderately Disagree	Neither Agree nor Disagree	Moderately Agree	Strongly Agree
The rules and procedures of research ensure good science	1	2	3	4	5
The outcomes of science are generally beneficial	1	2	3	4	5
The people I care about are generally distrusting of science	1	2	3	4	5
Scientists are genuinely good people trying to do their best	1	2	3	4	5
I am generally a trusting person	1	2	3	4	5
Science has made the world a better place	1	2	3	4	5
I <u>do not</u> feel I have much in common with scientists	1	2	3	4	5
There are standard procedures to ensure that science is conducted honestly	1	2	3	4	5



*Pictured to the left is the eastern hellbender. Hellbenders are large, brown salamanders that average 1.5 feet long as adults. They live in creeks their whole lives and often hide under large rocks. Unlike some similar-looking salamanders, hellbenders do not have gills (resembling feathers) on their neck.*

*You may know this animal by one of its many other names such as water dog, mudpuppy, grampus, snot otter, or Allegheny alligator. To reduce confusion, we will refer to them as “hellbenders” throughout this survey.*

**9. Had you heard of a hellbender prior to this survey?**

- Yes    No

**10. Have you ever seen a hellbender in the wild?**

- Yes    No    Unsure

**11. When was the last time you, or someone you know saw a hellbender on your property?**

- Less than one year ago  
 Approximately \_\_\_\_\_ years ago (*please write the number of years*)  
 As far as I know, neither I nor anyone I know has seen one

**12. Based on your experience, would you say that there are more hellbenders, fewer hellbenders, or the same number of hellbenders now compared to how many there were 15 years ago?**

- More    Fewer    The same number    Do not know

**13. Different people have different thoughts about hellbenders. Below are pairs of opposing qualities that could be used to describe hellbenders. For each question, please circle the number between the two descriptors that best matches your thoughts about hellbenders. For example, for the first question, if you felt they are neither disgusting, nor appealing, you would circle 3.**

**I would describe hellbenders as...** (*Please circle only one number per row.*)

Disgusting	1	2	3	4	5	Appealing
Good	1	2	3	4	5	Bad
Harmful	1	2	3	4	5	Beneficial
Interesting	1	2	3	4	5	Uninteresting



**17. Sometimes landowners participate in the research projects taking place on their property beyond just them to occur. Examples of participation could include observing researchers collecting data or assisting with data collection. Have you previously participated in research on property you own (in Scott and/or Russell County or elsewhere)?**

- Yes    No    Research has never taken place on my property

**18. Have you previously participated in a scientist’s research on property you do not own (in Scott and/or Russell County or elsewhere)?**

- Yes    No

**19. If scientists were to perform research on your property, please circle how much you would agree or disagree with the following statements regarding your participation. Examples of participation could include observing researchers or assisting with data collection. (Please circle only one option per row.)**

	Strongly Disagree	Moderately Disagree	Neither Agree nor Disagree	Moderately Agree	Strongly Agree
Participating in research on my property would help me maintain control over what takes place	1	2	3	4	5
Participating in research on my property would make me feel unskilled	1	2	3	4	5
I would enjoy learning about the research taking place on my property	1	2	3	4	5
I would feel awkward interacting with researchers on my property	1	2	3	4	5
Participating in research on my property would make me feel like part of a community	1	2	3	4	5
Even if I participated in research I would not have any power to make decisions	1	2	3	4	5

**20. We are also interested in your general values. Please indicate how important or unimportant you find each of the following as a guiding principle in your life. (Please circle only one option per row.)**

	Extremely Unimportant	Moderately Unimportant	Neutral	Moderately Important	Extremely Important
Being influential	1	2	3	4	5
Helping others	1	2	3	4	5
Remaining curious	1	2	3	4	5
Promoting equality	1	2	3	4	5
Respecting parents and elders	1	2	3	4	5
Ensuring family security	1	2	3	4	5
Having authority	1	2	3	4	5
Gaining wealth	1	2	3	4	5
Leading a varied life	1	2	3	4	5
Respecting the earth	1	2	3	4	5
Working to end injustice	1	2	3	4	5
Living in unity with nature	1	2	3	4	5
Leading an exciting life	1	2	3	4	5
Being self-disciplined	1	2	3	4	5
Protecting the environment	1	2	3	4	5

***Finally, please answer the following questions to help us better understand the local landowners.***

**21. Approximately how many acres of property do you (or your spouse) own or rent in Scott and/or Russell County, Virginia? (Insert number of acres to closest whole number.)**

\_\_\_\_\_ acres owned

\_\_\_\_\_ acres rented

*(continued on next page)*

22. Do you intend to sell all of your property, or the part of your property that contains the largest creek, in the next 5 years?

- Yes    No    Unsure    Neither I nor my spouse owns the property

23. Approximately how many years have you (or your spouse) lived on the property in Scott and/or Russell County, Virginia? *(Insert number of years to closest whole number or check a box.)*

- \_\_\_\_\_ years       Neither I nor my spouse currently live on the property  
 Neither I nor my spouse have ever lived on the property

24. What year were you born? \_\_\_\_\_

25. Are you?

- Male    Female

26. What is the highest level of education you have attained? *(Please check only one box.)*

- Less than high school diploma/G.E.D.  
 High school diploma/G.E.D.  
 Associate's degree (AS, AA, etc.)  
 College undergraduate degree (BA, BS, etc.)  
 Graduate or professional degree (MS, PhD, MD, etc.)

***Researchers from Virginia Tech may follow up with some landowners to discuss research related to Copper Creek and nearby creeks.***

27. Are you willing to be contacted by Virginia Tech to discuss research on your property? Checking yes will not obligate you to participate and does not mean you will be contacted. Other factors, such as property location, will play into determining which landowners are contacted.

- Yes    No *(please skip #28 and return the survey)*

28. If you checked "yes", please provide the full name and phone number of whom we should contact. This information will be kept confidential and will not be kept in the same file as your other responses.

Name \_\_\_\_\_ Phone number \_\_\_\_\_ - \_\_\_\_\_ - \_\_\_\_\_

***Thank you very much for completing this survey. We realize that there are many demands on your time and it means a lot to us that you took the time to help us. The survey can be returned using the pre-addressed stamped envelope included with this survey or it can be mailed to:***

C/O Rebecca O'Brien  
Department of Fish and Wildlife Conservation  
Cheatham Hall, RM 101 (MC0321)  
310 West Campus Drive  
Blacksburg, Virginia 24061

***All answers will be kept strictly confidential. If you chose to provide your name and contact information, it will be stored separately from your answers in the database.***

***If you have any additional thoughts or comments, please feel free to write them in the space below:***

## APPENDIX 2.A

### Initial Interview (pre-engagement)

I'd like to first get to know a bit about you and your property and your experience in the Copper Creek area.

- How long have you lived in the Copper Creek area?
  - Here whole time?
  - How often visit?
- Can you describe your property to me?
  - What do you use the land for?
  - Interact with Copper Creek?
- What do you do/did you do for work?
- Are you active in the local community? How?

You might remember from the survey, one thing that we are interested in is what folks think about conservation. This includes both how they think about conservation and their opinion about it.

- First, could you describe what nature conservation means to you?
  - *Clarification:* what comes to mind when you think about conservation?
- Some landowners do conservation activities on their land and some don't or don't have time. Have you done any conservation activities on your property? There's no right or wrong answer.
  - Yes→
    - What have you done? Why?
      - Have you done any work specific to Copper Creek?
      - Some landowners in this area have done tree planting or fencing around creeks on their own or working with the soil and water district. Have you done any of those things?
    - Is there any conservation work you plan or hope to do in the next two years, but haven't yet?
    - Is there anything preventing you from doing the conservation work that you'd like to do?
  - No→
    - Is there any conservation work you plan or hope to do in the next two years, but haven't yet?
      - Some landowners in this area have done tree planting or fencing around creeks on their own or working with the soil and water district. Have you done any of those things?
    - Is there anything preventing you from doing the conservation work that you'd like to do?
    - Do you feel like there is conservation work to be done on your property?

We're also interested in your thoughts on science.

- Science is a really broad topic, so first, I'm just curious what sorts of things you think about when you think about science?

People have differing opinions about science. Some people have positive opinions about science, some people have negative opinions, some have no opinion. Where do you lie on that spectrum?

- Why do you feel that way?
- Do you trust science?
  - Why?
  - Changed over the past ten years?
- One aspect of science is science like what we're doing here where scientists work with landowners on their property. If you had a scientist at your command are there any questions about their property that you would like them to study?
  - *Clarification:* Are there any questions you have about your property that you would like science to answer?
- Have you ever allowed science research to take place on your property?
  - *Yes:*
    - Why did you allow it?
    - Tell me a bit about the research. What was it like?
    - What did you like about having research take place on your property? OR did you feel like you were benefiting at all from allowing this research to take place?
    - What did you dislike?
  - *No:*
    - When we talked over the phone, prior to this interview, you said you would be interested in participating in this research project. Why did you say yes?
    - What do you think you would like about having research take place on your property?
    - What do you think you would dislike?
- We talked over the phone a bit about our research, but to remind you, We're looking at how stream health, specifically the amount of sediment or dirt in the water and the amount of oxygen in the water effects eastern hellbenders and we also hoping to put in nest boxes to help support hellbender populations. What about the research specifically that caught your attention?
  - *If respond regarding participating in research in general:* Is there anything about this project in particular that you find interesting?
- When was the last time you saw a hellbender on your property?
- Do you have any thoughts that you want to share with me that we didn't cover in the survey?

## APPENDIX 2.B

### Traditional science Group

Before we begin, I want to clarify that there are no right or wrong answers to these questions. We would greatly appreciate your honest thoughts, so please feel free to speak openly including both positive and negative feedback. I personally didn't have any involvement in the hellbender project.

First, I'd like to hear about your experience with the hellbender research project.

- Can you describe the research that took place on your property?
  - What did the project involve?
  - Do you remember what the researchers were trying to learn?
  - Do you remember what they discovered?
- Did you interact with the researchers at all while they were on your property?
  - Was there a reason why you did/did not?
  - Can you tell me about your experience accompanying researchers? What was that like?
- We're interested in what you may have liked or disliked about having the research on your property.
  - We'll start out with what you liked: What did you like about having research take place on your property?
  - What did the research team do well?
- What did you dislike about having research take place on your property?
  - Is there anything you wish that the research team had done differently?
- Did you feel like you personally learned anything from participating in the project?
  - What did you learn?
  - How did you learn about this (pamphlets in the mail, accompanying research team, etc.)?
  - Can you tell me about your experience receiving the results of the research?
    - What did you think about the results?

Some people find that participating in research changes their perspectives on things and some people don't. Did you find that participating in the hellbender project changed how you thought about anything?

- Did participating in the hellbender project inspire you to learn more about any topics on your own?
- Have you talked to any friends, neighbors, or family about participating in the project or about what you have learned?

Have you ever participated in research projects on your property other than the hellbender research project?

→Yes:

- What were the research projects?
- Did you participate in them before or during the hellbender project?

- Were there things you liked better about either the hellbender project or the other project(s) you participated in?
  - *(If participated after/during hellbender project)* Did participating in the hellbender project play a role in you deciding to participate in the other research project?
- No: Okay!

The research project focused on hellbenders and water quality in the Copper Creek area. Some people like hellbenders and some people don't or don't have an opinion about them. What is your opinion about hellbenders?

- Has your perspective on hellbenders changed at all over the past three years?
  - Yes: Can you describe how it has changed?
  - What caused this change?
  - Did this change have anything to do with the research on your property?
- Has your perspective on Copper Creek and where you live changed at all over the past three years?
  - Yes: Can you describe how it has changed?
  - What caused this change?
  - Did this change have anything to do with the research on your property?

We also are interested in is what folks think about conservation. First, could you describe what nature conservation means to you?

- *Clarification:* what do you think of as conservation?
- Has your perspective on conservation changed at all over the past three years?
  - Yes: Can you describe how it has changed?
  - What caused this change?
  - Did this change have anything to do with the research on your property?
- Some landowners do conservation activities on their land and others don't or don't have time. Have you done any conservation activities on your property? There's no right or wrong answer.
  - Yes
    - What have you done?
      - How long have you been doing these activities?
      - What inspired you to do the conservation activity?
        - Did this have anything to do with the research on your property?
      - Just to double check you aren't doing something you might not be thinking of: Some landowners in this area have done tree planting or fencing around creeks on their own or working with the soil and water district. Have you done any of those things?
    - Is there any conservation work you plan or hope to do in the next three years, but haven't yet?
  - No
    - Is there any conservation work you plan or hope to do in the next three years, but haven't yet?

- Do you feel like there is conservation work to be done on your property?
- Just to double check you aren't doing something you might not be thinking of: Some landowners in this area have done tree planting or fencing around creeks on their own or working with the soil and water district. Have you done any of those things?

We're also interested in your thoughts on science. People have differing opinions about science. Some people have positive opinions about science, some people have negative opinions, and some have no opinion. Where do you lie on that spectrum?

- Why do you feel that way?
- Has your perspective on science or scientists changed at all over the past three years?
  - Yes: Can you describe how it has changed?
  - What caused this change?
  - Did this change have anything to do with the research on your property?
- Do you trust science?
  - Yes: What makes you trust it?
  - No: What makes you not trust it?
  - Has that trust changed over the past three years?
    - Yes: Can you describe how it has changed?
      - What caused this change?
      - Did this have anything to do with the research on your property?

Finally, we're also interested in your perspectives on the great blue herons in the area. Like hellbenders, many landowners have strong opinions about herons in the area and we'd like to better understand your perspective.

- What do you know about herons?
- Have you had any experiences with herons?
  - Yes: What were those experiences like?
  - No: Do you know anyone who has? What the experience was like for them?
- Some people like herons and others don't or don't have much of an opinion about them. Do you have any opinions on whether herons are good or bad?
  - Why do you feel that way?
- Has your perspective on herons changed in the past 3 years?

Do you have any thoughts that you want to share with me that we didn't cover in the interview?

## Control Group

Before we begin, I want to clarify that there are no right or wrong answers to these questions. We would greatly appreciate your honest thoughts, so please feel free to speak openly.

- Have you participated in any research projects on your property?
  - Yes:
    - What were the research projects?
    - When did you participate in them?
    - What did you like about participating in the project?
      - What did the research team do well?
    - What did you dislike about having research take place on your property?
      - Is there anything you wish that the research team had done differently?
  - No: Okay!
- Are you familiar with hellbenders or water dogs?
  - No: They are giant salamanders that live in creeks and streams.
  - Some people like hellbenders and some people don't or don't have an opinion about them. What is your opinion about hellbenders?
  - Has this changed at all over the past three years?
  - Yes: What caused this change?
  - Has your perspective on the stream on your property and where you live changed at in the past three years?
  - Yes: What caused this change?

We also are interested in is what folks think about conservation. First, could you describe what nature conservation means to you?

- *Clarification:* what do you think of as conservation?
- Some landowners do conservation activities on their land and others don't or don't have time. Have you done any conservation activities on your property? There's no right or wrong answer.
  - Yes
    - What have you done?
      - How long have you been doing these activities?
      - What inspired you to do the conservation activity?
        - Did this have anything to do with the research on your property?
      - Just to double check you aren't doing something you aren't thinking of: Some landowners in this area have done tree planting or fencing around creeks on their own or working with the soil and water district. Have you done any of those things?
    - Is there any conservation work you plan or hope to do in the next three years, but haven't yet?
  - No

- Is there any conservation work you plan or hope to do in the next three years, but haven't yet?
- Do you feel like there is conservation work to be done on your property?
- Just to double check you aren't doing something you aren't thinking of: Some landowners in this area have done tree planting or fencing around creeks on their own or working with the soil and water district. Have you done any of those things?
- Has your perspective on conservation changed at all over the past three years?
- Yes: What caused this change?

We're also interested in your thoughts on science. People have differing opinions about science. Some people have positive opinions about science, some people have negative opinions, some have no opinion. Where do you lie on that spectrum?

- Why do you feel that way?
- Has your perspective on science or scientists changed over the past three years?
  - Yes: How so? What caused this change?
- Do you trust science?
  - Why?
  - Has that trust changed over the past three years?
- Yes: What caused it to change?

Finally, we're also interested in your perspectives on the great blue herons in the area.

- What do you know about herons?
- Have you had any experiences with herons?
  - No: Do you know anyone who has? What the experience was like for them?
- Some people like herons and others don't or don't have much of an opinion about them. Do you have any opinions on whether herons are good or bad?
  - What made you think this way?
- Has your perspective on herons changed in the past 4 years?
- Do you have any thoughts that you want to share with me that we didn't cover in the interview?

## Citizen Science Group

Before we begin, I want to clarify that there are no right or wrong answers to these questions. We would greatly appreciate your honest thoughts, so please feel free to speak openly including both positive and negative feedback. I personally didn't have any involvement in the citizen science project.

First, I'd like to hear about your experience with the citizen science project.

- Can you describe the research that took place on your property?
  - What did the project involve?
  - Do you remember what the researchers were trying to learn?
  - Do you remember what they discovered?
- Did you collect data when you received citizen science packets in the mail?
  - Was there a reason why you did/did not?
  - Can you tell me about your experience collecting data? What was that like?
- We're interested in what you may have liked or disliked about having the research on your property.
  - We'll start out with what you liked: What did you like about having research take place on your property?
  - What did the research team do well?
- What did you dislike about having research take place on your property?
  - Is there anything you wish that the research team had done differently?
- Did you feel like you personally learned anything from participating in the project?
  - What did you learn?
  - How did you learn about this (pamphlets in the mail, accompanying research team, etc.)?
  - Can you tell me about your experience receiving the results of the research?
    - What did you think about the results?

Some people find that participating in research changes their perspectives on things and some people don't. Did you find that participating in the citizen science project changed how you thought about anything?

- Did participating in the citizen science project inspire you to learn more about any topics on your own?
- Have you talked to any friends, neighbors, or family about participating in the project or about what you have learned?

Have you ever participated in research projects on your property other than the citizen science project?

→Yes:

- What were the research projects?
- Did you participate in them before or during the citizen science project?
- Were there things you liked better about either the citizen science project or the other project(s) you participated in?

- (If participated after/during citizen science project) Did participating in the citizen science project play a role in you deciding to participate in the other research project?
- No: Okay!

The research project focused on hellbenders and water quality in the Copper Creek area. Some people like hellbenders and some people don't or don't have an opinion about them. What is your opinion about hellbenders?

- Has your perspective on hellbenders changed at all over the past three years?
  - Yes: Can you describe how it has changed?
  - What caused this change?
  - Did this change have anything to do with the research on your property?
- Has your perspective on the creek on your property or where you live changed at all over the past three years?
  - Yes: Can you describe how it has changed?
  - What caused this change?
  - Did this change have anything to do with the research on your property?

We also are interested in is what folks think about conservation. First, could you describe what nature conservation means to you?

- *Clarification:* what do you think of as conservation?
- Has your perspective on conservation changed at all over the past three years?
  - Yes: Can you describe how it has changed?
  - What caused this change?
  - Did this change have anything to do with the research on your property?
- Some landowners do conservation activities on their land and others don't or don't have time. Have you done any conservation activities on your property? There's no right or wrong answer.

→ Yes

- What have you done?
  - How long have you been doing these activities?
  - What inspired you to do the conservation activity?
    - Did this have anything to do with the research on your property?
  - Just to double check you aren't doing something you might not be thinking of: Some landowners in this area have done tree planting or fencing around creeks on their own or working with the soil and water district. Have you done any of those things?
- Is there any conservation work you plan or hope to do in the next three years, but haven't yet?

→ No

- Is there any conservation work you plan or hope to do in the next three years, but haven't yet?
- Do you feel like there is conservation work to be done on your property?

- Just to double check you aren't doing something you might not be thinking of: Some landowners in this area have done tree planting or fencing around creeks on their own or working with the soil and water district. Have you done any of those things?

We're also interested in your thoughts on science. People have differing opinions about science. Some people have positive opinions about science, some people have negative opinions, and some have no opinion. Where do you lie on that spectrum?

- Why do you feel that way?
- Has your perspective on science or scientists changed at all over the past three years?
  - Yes: Can you describe how it has changed?
  - What caused this change?
  - Did this change have anything to do with the research on your property?
- Do you trust science?
  - Yes: What makes you trust it?
  - No: What makes you not trust it?
  - Has that trust changed over the past three years?
    - Yes: Can you describe how it has changed?
      - What caused this change?
      - Did this have anything to do with the research on your property?

Finally, we're also interested in your perspectives on the great blue herons in the area. Like hellbenders, many landowners have strong opinions about herons in the area and we'd like to better understand your perspective.

- What do you know about herons?
- Have you had any experiences with herons?
  - Yes: What were those experiences like?
  - No: Do you know anyone who has? What the experience was like for them?
- Some people like herons and others don't or don't have much of an opinion about them. Do you have any opinions on whether herons are good or bad?
  - Why do you feel that way?
- Has your perspective on herons changed in the past 3 years?

Do you have any thoughts that you want to share with me that we didn't cover in the interview?

## APPENDIX 2.C

	Citizen science (n=5)	Traditional science (n=7)	Control (n=8)
Increase in knowledge	4	5	2
Increased awareness of conservation concerns	3	3	0
Behavioral intentions regarding conservation	2	2	1
Lack of perceived behavioral control	4	1	1
Shared experience participating in the research or findings from the research	2	6	N/A

**Table 2.C** The number of individuals who discussed each observed outcome from participation in their respective group (citizen science, traditional science or control). We do not report the number of individuals in the control group who shared their experience of participating in research or the result of the research with their social network because we did not ask about this in their interviews.

And that's that.