Shelter Guarding Behavior of the Eastern Hellbender (Cryptobranchus alleganiensis alleganiensis) in North Carolina Streams

Authors: Unger, Shem, Bodinof Jachowski, Catherine M., Diaz, Lauren, and Williams, Lori A.

Source: Southeastern Naturalist, 19(4) : 742-758

Published By: Eagle Hill Institute

URL: https://doi.org/10.1656/058.019.0411
Shelter Guarding Behavior of the Eastern Hellbender 
(Cryptobranchus alleganiensis alleganiensis) in North Carolina Streams

Shem Unger¹²*, Catherine M. Bodinof Jachowski³, Lauren Diaz³, and Lori A. Williams⁴

Abstract - Cryptobranchus alleganiensis alleganiensis (Eastern Hellbender) is a fully aquatic salamander of conservation concern across the southeastern US. Characterization of shelter guarding by males and interspecific behavior during the breeding season in wild populations within the Blue Ridge ecoregion of North Carolina is lacking. To this end, we characterized diurnal video sequences of natural shelter-rock and nest-guarding behavior by male Eastern Hellbenders. We documented several intraspecific behaviors between resident males and nonresident conspecics (males and females) during the breeding season (late summer to early fall) of 2018–2019 using modified action cameras with 4–6 hour battery life deployed in the French Broad River Basin of western North Carolina. Breeding behavior was documented (gravid females entering shelter) in 5 shelters, with 4 nests later confirmed by researchers. Across 21 unique shelters, we documented several examples of aggressive behaviors by resident Eastern Hellbender males toward conspecifics, including defensive posturing, territorial behavior, fights, biting, and bite-holds. All males occupying shelters (residents) spent the majority of time actively guarding the shelter entrance (81.7% of video) followed by maintenance behavior (e.g., modifying the shelter entrance; 5.3% of video), and short bouts (mean = 3.2 minutes) away from the shelter rock (2.5% of video). This study provides the first quantitative report of male breeding-season behavior across multiple natural shelters using non-invasive, affordable, waterproof cameras in North Carolina. We report on the utility of this method for observing behavior in stream systems and its potential application for monitoring of nests and behavior in other diurnal aquatic species.

Introduction

Cryptobranchus alleganiensis alleganiensis Daudin (Eastern Hellbender) is a large, fully aquatic salamander. Surveying for this salamander has historically involved snorkeling and lifting rock shelters, often with the aid of Peavey hooks, which may negatively impact shelters (Nickerson and Krysko 2003). While reproductive activity has been observed in some diurnal populations during the breeding season (Humphries 2007), data on specific reproductive behavior is lacking for this cryptic species, likely due to the difficulty in surveying and documentation in underwater stream habitats. Egg fertilization takes place under large, often mostly embedded shelter rocks where breeding cannot be observed (Smith 1907),

¹Department of Biology, Wingate University, Wingate, NC 28174. ²Carolina Headwaters, LLC, Matthews, NC 28105. ³Forestry and Environmental Conservation, Clemson University, Clemson, SC 29634. ⁴North Carolina Wildlife Resources Commission, 1722 Mail Service Center, Raleigh, NC 27699. *Corresponding author - s.unger@wingate.edu.

Manuscript Editor: Matthew L. Niemiller
yet recent studies have provided preliminary data on breeding behavior. However, reproductive behavior observations have been limited to observations of single nests (Floyd and Unger 2016, Settle et al. 2018a) or behaviors in captive settings (Settle et al. 2018b). Studies have been conducted on parental care in the closely related *Andrias japonicas* (Temminck) (Japanese Giant Salamander), with reproductive behaviors such as tail fanning, breathing, staying still, agitating egg, and even hygienic filial cannibalism characterized (Okada et al. 2015, Takahashi et al. 2017). However, as the Eastern Hellbender is a species of conservation concern in most states throughout its geographic range, further work is needed to document behavioral interactions in wild populations during important temporal conditions, including breeding and potential nest-guarding activities for long-term conservation, monitoring, and management in streams.

Documentation of in situ life history and breeding ecology remains a challenge for aquatic species and environments. Quantification of animal behavior in terrestrial communities often relies on the use of stationary trail cameras (Rollack et al. 2013). Presently, there are limited options available for similar affordable waterproof cameras that offer an acceptable balance of battery life, portability, and affordability for use in the field. Reproductive behavior of freshwater vertebrates has been readily observed in aquarium setups or under laboratory conditions (Cavraro et al. 2013, Degidio et al. 2017). Widely available underwater video cameras have provided a valuable method for surveying fish assemblages and obtaining behavioral breeding data of aquatic organisms, mostly fish (Cronnon et al. 2019, Hartl and Simpson 2019, Schmid et al. 2016). Baited remote underwater systems are frequently used in marine ecosystems, yet seldom applied to studies in freshwater ecosystems (Witmarsh et al. 2017). Subsequently, these waterproof action cameras have only more recently been utilized sparingly for herpetofauna (Schmid and Giarrizzo 2019). Advantages of deploying stand-alone video cameras in aquatic habitats include both documenting animal behaviors that may occur quickly and in rapid succession and serving to minimize observer bias (Branconi et al. 2019, Claassens and Hodgson 2017). Moreover, any salamander behavioral study requires interaction between the observer and the study animal (Marsh and Hanlon 2004), which can present challenges if video can only be recorded while an observer is present. However, action cameras are often constrained to relatively short periods (e.g., ~80–120 minutes) due to limited battery life in aquatic systems (King et al. 2017, Li et al. 2019, Struthers et al. 2015). Few studies have focused on validating the utility of action cameras for documenting stream amphibians or determining if cameras can provide prolonged periods of observations.

Herein, we assess the utility of modified action cameras to record and quantify Eastern Hellbender behavior during the breeding season and thus advance our knowledge of breeding behaviors near occupied, underwater shelter rocks in North Carolina. We also report on reproductive, guarding, maintenance, and aggressive behaviors of this species. Lastly, we relate our findings to those from captive settings or natural nests to highlight the potential application of our approach for use in aquatic environments across the southeastern US.
Materials and Methods

Study species

Eastern Hellbenders are fully aquatic salamanders found throughout clear, cool streams of the midwestern, northeastern, and southeastern portions of the US (Petranka 1998). Within the southeastern portion of their geographic range, western North Carolina, eastern Tennessee, and northern Georgia may represent some of the more stable populations for the species. Across their range, however, the species is facing numerous threats including increased sedimentation and habitat destruction (Mayasich et al. 2003) and overcollection and exploitation (Nickerson and Briggler 2007), with declines observed for many states (Burgmeier et al. 2011a, Foster et al. 2009). Adult Eastern Hellbenders typically occupy large rocks with flat bottoms within North Carolina (Rossell et al. 2013). They normally breed late summer to early fall, with males excavating cavities under large shelter rocks in which they remain, guarding offspring, following successful breeding (Nickerson and Mays 1973).

Study site

Based on previous surveys (L.A. Williams, unpubl. data), we anticipated the onset of breeding in our study area to occur between mid-August and mid-September. Thus, we constrained all of our surveys to occur during this time frame to identify potential shelter rocks with males present for deployment of cameras. We deployed cameras in 5 tributaries of the French Broad River Basin in Pisgah National Forest, western North Carolina (specific stream reach information on file with the North Carolina Wildlife Resources Commission and withheld to protect specific location).

Field surveys

We identified potential breeding and nesting shelters (rocks > 60 cm diameter; Burgmeier et al. 2011b) by conducting snorkeling surveys at each stream location prior to camera deployment. Before each camera was deployed, we used passive snorkel surveys (i.e., hands-off, no habitat or animal disturbance) aided by dive lights to detect resident Eastern Hellbenders near the entrance of natural rock shelters. When we determined that an individual Eastern Hellbender was present, we positioned waterproof cameras directly in front of the (single) shelter entrance. We assigned a unique identifier to each shelter to permit deployment and monitoring during future breeding seasons.

Field video recording

At each shelter, we deployed a GoPro® 3+ waterproof action camera (GoPro, San Mateo, CA) on a tripod attached to weights (Fig. 1). Each camera was outfitted with Digipower re-fuel™ 12-hour action pack batteries for GoPro®, which is a 4800-mAh lithium-ion battery pack that attaches directly to the back of a GoPro® camera and provides a waterproof seal. Prior to deployment of the cameras, we verified that each battery was fully charged and that day and time were correctly programmed, to ensure consistent video recording across locations. We used the following camera
settings: continuous video recording mode, wide field of view, 720 video resolution, 30 frames per second, auto low light feature on, WIFI off, and default on all other settings. Under these settings, the extended batteries allowed for continuous recording with 64 GB micro SD cards at 720p60 video frame for up to 9 consecutive hours.

We deployed a camera in each location in less than 5 minutes. We positioned cameras approximately 0.5–0.6 m from the shelter entrance and allowed cameras to record unattended (all researchers outside of the stream) to minimize bias. Video recordings consisted of ~20-minute videos saved in succession. After cameras were collected, we removed microSD cards, backed up video files on external hard drives, and sorted files according to date, stream location, and shelter identity. One of the authors (S. Unger) reviewed and coded video files for observed behaviors times according to our ethogram (Table 1) in VLC media viewer (VideoLan, Paris, France).

**Defining behavioral observations and data analysis**

After we reviewed the video footage, we developed an ethogram (an exhaustive list of behavioral patterns) for behavior and action patterns observed, which also incorporated observed behaviors from previous studies on salamanders, including

![Figure 1. Action camera setup with extended battery, weight, and tripod showing (A) resident male biting conspecific non-resident and (B) resident male actively guarding while conspecific non-resident is in front of shelter.](https://bioone.org/journals/Southeastern-Naturalist)
general, aggressive or agonistic, locomotory, and social behaviors (Table 1; Davis 2002, Okada et al 2015, Settle et al. 2018b, Verrell and Donovan 1991). These action patterns recorded during video observations allow for quantification of time allocated to each behavior while males were present at shelters. We described the dominant behavior(s) observed when reviewing video, in chronological order, for each unique camera deployment event. For example, a typical behavior may have involved a resident male engaged in active guarding of the rock shelter, then defensive posturing or attacking as a conspecific individual Eastern Hellbender approached, followed by a bite from the guarding male and the conspecific non-resident fleeing. We carefully reviewed individuals in frame to determine sex based on cloacal morphology. For each video deployment, we confirmed as males individuals that had readily visible and clearly evident cloacal swelling, a diagnostic physical trait present in males during the breeding season (Nickerson and Mays

Table 1. Ethogram of Cryptobranchus a. alleganiensis (Eastern Hellbender) unique behaviors observed during daylight in tributaries of the French Broad River in North Carolina, 2018–2019. We define “resident” as male occupying shelter.

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breeding</td>
<td>One conspecific female enters nest shelter and resident male breeds with female</td>
</tr>
<tr>
<td>Approach</td>
<td>One conspecific individual approaches another resident individual, in &gt;5 seconds</td>
</tr>
<tr>
<td>Nose-to-nose</td>
<td>One conspecific individual presses nose with nose of another individual</td>
</tr>
<tr>
<td>Biting</td>
<td>One resident individual bites at another conspecific individual quickly, under 1 second</td>
</tr>
<tr>
<td>Bite-hold</td>
<td>One individual continues biting longer than 1 second or “seize”</td>
</tr>
<tr>
<td>Open mouth gape</td>
<td>One individual opens mouth temporarily</td>
</tr>
<tr>
<td>Skin consumption</td>
<td>One individual consumes his own shed skin</td>
</tr>
<tr>
<td>Rocking</td>
<td>One individual moves laterally side to side within rock</td>
</tr>
<tr>
<td>Attacking/Chasing</td>
<td>One resident individual charges or moves rapidly towards another conspecific non-resident individual, often leaving resident shelter</td>
</tr>
<tr>
<td>Territorial</td>
<td>One resident moving around immediate area in front of entrance, in response to presence of a conspecific non-resident individual</td>
</tr>
<tr>
<td>Active guarding</td>
<td>One individual actively guarding rock entrance, head facing out</td>
</tr>
<tr>
<td>Maintenance</td>
<td>One individual modifying sediment around shelter entrance to widen it, usually with head, or rear legs and tail, or sediment visibly exiting shelter entrance from resident under shelter; may include temporary hovering immediately outside shelter</td>
</tr>
<tr>
<td>Absent from rock</td>
<td>One individual confirmed outside of shelter, unknown location</td>
</tr>
<tr>
<td>Defensive posturing</td>
<td>Resident individual placing upper half of body out of shelter, often while facing a potential intruder, conspecific individual, or potential predatory fish sensed visually or by chemosensory detection</td>
</tr>
<tr>
<td>Absent from view</td>
<td>One individual not in camera view but confirmed under shelter</td>
</tr>
</tbody>
</table>
To efficiently review large amounts of video footage, only 1 behavior was defined per time interval (1 second). After all behaviors observed during a deployment were determined, we calculated the total time spent performing each behavior, separately, for each camera deployment.

In addition to characterizing dominant behaviors observed on video, we calculated the number of interactions between Eastern Hellbenders within the field of view and general vicinity of the focal shelter (approximately 0.5–0.75 m from the shelter). We recorded the sex of conspecifics involved in the interactions when possible and recorded the species of potential egg predators observed near focal shelters, including fish and mammals (e.g., *Lontra canadensis* (Schreber) [North American River Otter]). We calculated the number of occasions when the resident Eastern Hellbender left the shelter and the mean and median time (in minutes) that residents spent away from a shelter per camera deployment. Since many of the recorded behaviors occurred while the resident male was either actively guarding or under a shelter (not visible on camera but known to be in shelter by reviewing enter and leave times in video), we report on the overall time (in minutes) resident males spent under shelter (e.g., when time spent outside of a shelter rock was calculated). We validated data for consistency by ensuring the subtotals of time spent per behavior summed to the total recording time, separately for each deployment. We pooled data across deployments prior to data analysis. We used descriptive statistics (e.g., means, median scores) to compare time spent by individuals performing specific behaviors described in our ethogram across video deployments.

**Results**

**Field video recording**

We deployed and recorded video across 36 observation events focused on 21 shelters (9 repeated observations from the same shelters) representing 21 individual Eastern Hellbenders. Deployments occurred from 1 September to 9 September in 2018 (*n* = 16 deployments) and 31 August to 9 September in 2019 (*n* = 20 deployments) (Table 2). Deployment locations were characterized by relatively shallow water (~0.75–1 m) and excellent water clarity. All deployments were initiated between 1000 and 1600 h. We confirmed that all resident Eastern Hellbenders (positioned in shelter entrances) were male based on visual evidence of an enlarged cloaca (Nickerson and Mays 1973) in video recordings. In total, we deployed 1–5 cameras daily over 12 total days and recorded a total of 141.9 hours of video. Average deployment time for shelters reviewed was 3.95 hrs.

**Behavioral observations**

The most frequently observed behaviors included active guarding by resident males (81.69% of cumulative recording time) (Fig. 1), followed by maintenance behavior (5.31% of time). In total, resident hellbenders were either inside their shelter (out of view) or visible near the entrance 97.5% of the time, and away from the rock 2.50% of the time. Resident males exhibited defensive posturing behavior 2.45% of the time (Fig. 2), usually in response to potential intruders (either conspecific...
hellbenders or fish). Breeding behavior (female entering resident male shelter) was only observed on 5 occasions and made up 1.59% of cumulative deployment time. In 4 out of the 5 breeding behavior events observed, we confirmed the presence of fertilized eggs under the same shelter at a later date (Unger et al. 2020). Resident males were absent from view 3.64% of the time, and in all cases presumed under shelter (as evidenced by reviewing video before and after being absent from view). The remaining behaviors we observed (Fig. 3), collectively made up <1% of total video deployment time but included: approach by non-resident conspecifics (0.86%), bite-hold by resident males (0.87%), rocking behavior (0.54%), skin consumption (0.18%), territorial behavior (0.16%), and open mouth gape (0.11%) (Fig. 2). We observed nose-to-nose (non-resident conspecific directly in contact facing resident male; Fig. 2) for 0.06% of the time, while attack/chase behavior (resident male actively chasing a conspecific by leaving his shelter) was observed for 0.04% of the time. Finally, our least frequently observed behavior, biting (quickly) was observed 0.01% of the time.

Across the 21 resident males monitored, we observed 82 conspecific interactions. Non-residents involved in the interactions included mostly males (n = 51 interactions), followed by females (n = 14 interactions), and individuals of unknown sex (n = 17 interactions). The number of interactions per deployment varied from 0 (no interactions or other individual Eastern Hellbenders observed) to 13, with a mean of 2.3 and median of 1 interaction(s) per deployment. We observed non-resident conspecifics approaching in 50.00% (18 of 36) of deployments with 22 out of 36 deployments having evidence of 1 to 10 interactions by non-resident conspecifics. In 4 of 36 deployments, we observed non-resident conspecifics quickly swimming into the resident shelter entrance, but not approaching based on our definition of this behavior (Table 1). As these observations included non-resident conspecifics quickly entering an entrance, we did not classify these quick entrances, but instead tabulated these observations as non-resident conspecific individual in shelter.

<table>
<thead>
<tr>
<th>Date</th>
<th>Number of cameras deployed</th>
<th>Total hours recorded</th>
</tr>
</thead>
<tbody>
<tr>
<td>9/1/2018</td>
<td>2</td>
<td>7.3</td>
</tr>
<tr>
<td>9/5/2018</td>
<td>1</td>
<td>1.8</td>
</tr>
<tr>
<td>9/7/2018</td>
<td>3</td>
<td>14.5</td>
</tr>
<tr>
<td>9/8/2018</td>
<td>5</td>
<td>24.5</td>
</tr>
<tr>
<td>9/9/2018</td>
<td>5</td>
<td>15.6</td>
</tr>
<tr>
<td>8/31/2019</td>
<td>2</td>
<td>5.5</td>
</tr>
<tr>
<td>9/1/2019</td>
<td>6</td>
<td>24.7</td>
</tr>
<tr>
<td>9/6/2019</td>
<td>3</td>
<td>13.8</td>
</tr>
<tr>
<td>9/7/2019</td>
<td>4</td>
<td>14.3</td>
</tr>
<tr>
<td>9/8/2019</td>
<td>2</td>
<td>7.3</td>
</tr>
<tr>
<td>9/9/2019</td>
<td>3</td>
<td>12.9</td>
</tr>
</tbody>
</table>

Table 2. Summary of daily video data analyzed across tributaries of the French Broad River in North Carolina during 2018–2019 including date of camera deployment, total number of cameras deployed on individual resident male Cryptobranchus a. alleganiensis (Eastern Hellbender) shelters, and total hours of video assessed in this study.
We observed resident male defensive posturing behavior in over three-quarters of deployments (28 of 36 or 77.80%), most of which occurred in response to presence of other conspecific non-resident males. We observed biting and bite-holds (Fig. 2) in 42.00% of deployments (15 of 36), including 27 separate bites, with duration of bite-holds varying from 5 to 2989 seconds (mean = 326.5 seconds,

Figure 2. Some of the behaviors of Cryptobranchus a. alleganiensis (Eastern Hellbender) that were documented in this study, including (A) male active guarding a shelter entrance against conspecific non-resident approach, (B) resident male performing a bite-hold on conspecific non-resident, (C) resident male defensive posturing, (D) gravid female entering shelter, (E) resident male performing open mouth gape, and (F) nose-to-nose behavior of resident male and female conspecific (for video footage of documented behaviors, see supplemental Files 1–6, available online at https://www.eaglehill.us/SENA.online/suppl-files/s19-4-S2643-Unger-s1, https://www.eaglehill.us/SENA.online/suppl-files/s19-4-S2643-Unger-s2, https://www.eaglehill.us/SENA.online/suppl-files/s19-4-S2643-Unger-s3, https://www.eaglehill.us/SENA.online/suppl-files/s19-4-S2643-Unger-s4, https://www.eaglehill.us/SENA.online/suppl-files/s19-4-S2643-Unger-s5, and https://www.eaglehill.us/SENA.online/suppl-files/s19-4-S2643-Unger-s6, and for BioOne subscribers, at https://dx.doi.org/10.1656/S2643.s1, https://dx.doi.org/10.1656/S2643.s1, https://dx.doi.org/10.1656/S2643.s2, https://dx.doi.org/10.1656/S2643.s3, https://dx.doi.org/10.1656/S2643.s4, https://dx.doi.org/10.1656/S2643.s5, and https://dx.doi.org/10.1656/S2643.s6).
median = 43 seconds). Prolonged biting behavior, or bite-holds, primarily involved non-resident male conspecifics. We observed 15 bite-holds that involved male conspecifics, 1 that involved an unknown sex conspecific, and no bite-holds that involved females. The time females spent in shelters (from entrance by a gravid female to emergence of the same female, presumably for breeding) during breeding behavior averaged 2243 seconds or 37.4 minutes (median = 2000 seconds or 33.3 minutes) and varied from 21 to 58.6 minutes.

In 83.33% (30 out of 36) of deployments, we documented residents performing at least 1 form of shelter entrance or internal shelter maintenance. Shelter maintenance typically consisted of the following behavior pattern: male leaves entrance and turns around, re-enters head first, and then excavates the nest entrance with a combination of their rear limbs and tail with side-to-side movements. Other examples of maintenance we noted included Eastern Hellbenders using their head to nudge sand and gravel out of an entrance or perform maintenance farther inside the shelter exemplified by large amounts of silt moving out of shelter rock, typically observed just after resident males returned to shelter rocks. We observed individual resident males opening their mouths (open mouth gape) in about half (19 of 36 or 52.80%) of deployments, illustrating the commonness of this behavior.

We observed resident males leaving their shelter rock at least once during 20 of 36 (55.00%) deployments. For these deployments in which resident males were leaving shelter rocks, we observed the number of away bouts, or absent from rock, averaged 3.3 (median = 2, min–max = 1–15). On average, the cumulative time that resident males spent away from shelter was 200.95 seconds (median = 168 seconds,
min–max = 22 to 758 seconds). For residents that left shelter during deployments, time spent away from the shelter varied from 0.38% to 14.40% of total camera deployment time.

We observed several species, other than conspecifics, in close proximity to shelters and apparently instigating defensive posturing by resident Eastern Hellbenders. Species that elicited defensive posturing included: *Lepomis* sp. (sunfish), *Oncorhynchus mykiss* (Walbaum) (Rainbow Trout), *Notropis* spp. (shiners), *Erimyzon oblongus* (Mitchill) (Creek Chubsucker), *Etheostoma* spp. (darters), *Semotilus atromaculatus* (Mitchill) (Creek Chub), and *Hypentelium nigricans* (Lesueur) (Northern Hogsucker). Lastly, in 1 deployment we observed a North American River Otter briefly exploring a shelter rock occupied by a resident male before moving upstream.

**Discussion**

Our deployment of portable action cameras provided novel documentation of diurnal Eastern Hellbender behavior and interspecific interactions at natural shelters during the brief (~14 d) Eastern Hellbender breeding season in the French Broad watershed in North Carolina. This study increases our understanding of breeding biology and shelter-guarding behavior in Eastern Hellbenders under natural conditions. Although we observed resident males leaving their shelters periodically, they spent most of the time actively guarding the single entrance to their shelter. At least 4 of the shelters where we deployed cameras were confirmed to be nests (Unger et al. 2020), and 2 of these nests were in crevices under large pieces of bedrock that was embedded in the stream bank, as has been observed for *Cryptobranchus alleganiensis bishopi* Grobman (Ozark Hellbender; Nickerson and Tohulka 1986). Our findings are consistent with previous studies that report visits by multiple conspecifics (male and female) to individually guarded Eastern Hellbender nests (Floyd and Unger 2016). For example, we documented visits to a single shelter by 3 males, 3 females, and 1 individual of unknown sex within ~38 minutes during 1 deployment. The ultimate reason for these visits remains unclear. Conspecific males may have been attempting to fertilize eggs (Unger and Williams 2015). Conspecific females may have been attempting to breed, evaluate potential mates, or potential nest sites. Alternatively, either sex may have been attempting to consume pre-existing eggs in the focal shelter (Smith 1907). During our study, the time females spent in shelters was consistent with time required for successful breeding in captivity by Ozark Hellbenders (Settle et al. 2018b) and *Andrias davidianus* (Blanchard) (Chinese Giant Salamander) (Lou et al. 2018). However, as our cameras were placed directly outside of guarded shelters to limit interference and minimize disturbance, we only confirmed breeding for a subset of shelters. Collectively, our findings highlight that resident male Eastern Hellbenders can experience a high rate of encounters with conspecifics during a relatively short time period, which emphasizes the need for additional research to investigate the drivers and potential implications of these interactions.

In addition to the encounters with conspecifics, several of the behaviors we observed, including time spent active guarding, are comparable to those from
quantitative behavioral observations from Settle et al. (2018a) and Settle et al. (2018b) in hellbenders. Previous studies on hellbender behavior utilized 4 cameras positioned directly above and recorded either primarily nocturnal video in a captive setting (artificial stream; Settle et al. 2018b) or nest-guarding activity from a single male nest in the field (Settle et al. 2018a). Our study contributes to the available knowledge of Eastern Hellbender male behaviors and activities during the breeding season in natural conditions of streams in the Blue Ridge ecoregion of North Carolina. Okada (2015) observed Japanese Giant Salamanders opening and closing their mouth for no obvious reasons (open mouth gape) and also eating their own shed skin (skin consumption), both of which were minor behaviors observed in our study. Consumption of shed skin (dermatophagy) may be a mechanism to reclaim nutrients and has been observed in other salamanders (Fontenot and Pojman 2016, Lamb 2019, Mitchell et al. 2006). Our ability to document a wide variety of behaviors over a relative short period of time emphasizes the utility and potential of underwater cameras to facilitate non-invasive behavioral studies of Eastern Hellbenders during the breeding season and advance our understanding of breeding and nesting ecology for this imperiled species.

Among the aggressive behaviors we observed in this study, several biting and bite-hold encounters by shelter residents were mostly directed toward conspecific non-resident males and widely variable in duration. We observed both single or short bites and longer bite-holds or “seizing” which has been observed in other groups of salamanders (Clay and Gifford 2016, Verrell and Donovan 1991), and was often followed by the non-resident conspecific twisting and thrashing in an effort to be released by the resident male. Residents were less likely to bite a female conspecific versus male conspecifics, suggesting that resident males were able to clearly discern between female and males and may not have perceived females to be as threatening as males. We documented resident Eastern Hellbenders performing defensive posturing behavior, often protruding their body out from the shelter just before leaving it, facing off a potential conspecific non-resident Eastern Hellbender or even a fish predator (e.g., sunfish, trout), and we are uncertain whether the intruder was perceived either through visual or chemosensory detection. We observed resident males performing defensive posturing in the presence of fish, whereby on multiple occasions an individual was actively guarding with most of his anterior body directly facing a potential intruder (Rainbow Trout or sunfish). These aggressive behaviors are likely mechanisms for residents to remain vigilant at shelters while active guarding.

We observed several non-aggressive behaviors that, to our knowledge, have rarely been observed in wild Eastern Hellbenders. Examples include nest entrance and shelter maintenance behavior by Eastern Hellbenders utilizing natural rock shelters. This behavior was performed with shelter substrate composed of either coarse sand or gravel. We hypothesize this behavior allows males to “maintain” the entrance size to exclude predators but still permit gravid females. Lou et al. (2018) reported sand pushing in simulated streams occurring across several days when male Chinese Giant Salamanders were modifying dens just prior to breeding.
Similar cleaning behavior observed in Japanese Giant Salamanders is thought to represent a form of preoviposition parental care (Terry et al. 2019) but has yet to be studied or reported for Eastern Hellbenders, to the best of our knowledge. We also noted maintenance by individuals that moved sand out of their entrance, as well as instances of large amounts of debris exiting the shelter when individuals were under the shelter. The energetic costs of maintenance and active guarding remain unclear, but additional studies on this subject may be warranted. However, the value of these activities may facilitate successful breeding and reproduction while also reducing the probability of conspecific cannibalism or predation of eggs being guarded by resident males. Moreover, shelters occupied by Eastern Hellbenders in streams which have elevated levels of fine sediment may require greater maintenance to successfully facilitate breeding and reproduction. The camera systems utilized here could be useful for comparing time budgets for nesting males across a gradient of habitat quality and as a first step towards understanding the implications of habitat modification on Eastern Hellbender behavior.

Our study demonstrates the potential utility of underwater cameras to function as an effective method for monitoring some aspects of Eastern Hellbender biology and behavior during the breeding season, and perhaps during other times of the year. Affordable waterproof cameras with prolonged battery life have the potential to provide information on important ecological interactions involving hellbenders within streams, including food studies (Unger 2018), determining the presence of individuals within sites if deployed when individual movement and activity is likely, or assessing the presence of predators. Our observation of a North American River Otter swimming around one of our confirmed nest rocks indicate the potential for long battery life underwater cameras to be used to monitor potential predators, especially since North American River Otters are known to prey on adult Eastern Hellbenders (Hecht et al. 2014). Subsequently, baited underwater videos may also provide data on potential predators of Eastern Hellbenders such as otters and may offer a low-cost alternative to other surveys for aquatic mammals (Rolim et al. 2019). Lastly, underwater cameras can be used to monitor movement of individual Eastern Hellbenders between sites, and potentially compare diurnal and nocturnal activity patterns across the geographic range of the species.

Limitations of our field method using underwater cameras include limited nocturnal guarding or breeding observations within actual shelters as our action cameras lack infrared capabilities and were deployed at nest entrances. Therefore, we recommend future studies incorporate infrared (IR) video (possibly attaching waterproof IR lights to cameras; see Hellinger et al. 2020) to further characterize nocturnal behaviors across the species range, and encourage the development of IR camera systems capable of deployment within shelters. Nocturnal nesting behavior may provide additional valuable video as it has for studies of sea turtles using night-vision video recording (Lindborg et al. 2019), behavioral studies in lakes (Chidami et al. 2007), or research on emergence time of larval fish (Chaput et al. 2019). Many underwater camera technologies, including IR, used to detect spawning fish behavior are reliant on an external power source or connection to
video recording equipment or computers, so they have been used to a lesser extent (Holubova et al. 2020, Ruggirello et al. 2020), possibly due to their increased cost or lack of portability (Fukuba et al. 2015).

The use of multiple underwater cameras has recently been documented to provide similar detections for rare aquatic species and may be recommended as a sample method over conventional survey methods in aquatic systems with low turbidity (Castaneda et al. 2019). An additional caveat of our study was video deployments occurred primarily under ideal visibility and low-flow conditions. Researchers may have to first determine stream visibility before using non-invasive action cameras. We noted slight differences in ability to utilize cameras if large amounts of sediment were present following rain events, or if entrances were deep into unlit cavities of bedrock. Subsequently, other survey methodologies (e.g., environmental DNA) could be utilized to study potential spikes in reproductive behavior related to elevated number of animals moving or producing gametes during the breeding season (Milhau et al. 2019) if surveying in low visibility or streams with high sediment loads. Researchers may also consider artificial nest structures (Bodinof-Jachowski et al. 2020) combined with cameras to control visibility and obtain breeding behavior video.

Conclusions

Breeding behaviors documented with waterproof action cameras have the potential to function as a proxy for overall local population presence and health, and highlight the need for more research and camera surveys to be conducted in streams. We further encourage managers to concomitantly incorporate both waterproof-camera trapping and passive breeding surveys into their regular monitoring protocol, as we found passive surveys to yield numerous Eastern Hellbender detections at 1 location in a period spanning a few hours. Moreover, our extended video deployment of wild Eastern Hellbender shelters allowed us to monitor and confirm nests on a yearly basis. Our approach with diurnal, underwater camera traps has the potential to be utilized for population monitoring, as we noted morphological differences (e.g., coloration, scars, tail notches, etc.), which permits positive individual identification and allows for repeated observation of the same male over time. Moreover, underwater video footage obtained from these GoPro® action cameras can be used in outreach and to increase public awareness of cryptic aquatic species (Ulrich and Bonar 2020). Therefore, we recommend researchers consider this technique in combination with snorkel surveys when monitoring breeding populations of Eastern Hellbenders and other aquatic organisms (e.g., fish, mussels, crayfish, macroinvertebrates, and other salamanders) encountered in southeastern streams of the Blue Ridge ecoregion of North Carolina.

Acknowledgments

Surveys were conducted under NCWRC permit 19-ES00286 and followed Wingate University’s Research Review Board animal care and use guidelines. We thank Jelsie Kerr and
Zeb Hull for their help in obtaining video for this study and Lorie Stroup from the Pisgah National Forest for project support.

**Literature Cited**


