COMPARING THE EFFECTIVENESS OF DIURNAL ROCK-LIFTING AND NOCTURNAL DIVE-LIGHTING SURVEYS FOR EASTERN HELLBENDERS

A Thesis
by
FREDDY JUNIOR ORTEGA

Submitted to the School of Graduate Studies
at Appalachian State University
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

August 2020
Department of Biology

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July 2020

APPROVED BY:

____
Michael M. Gangloff, Ph.D.
Chairperson, Thesis Committee

____
Lynn M. Siefferman, Ph.D.
Member, Thesis Committee

____
John M. Davenport, Ph.D.
Member, Thesis Committee

____
Zack E. Murrell, Ph.D.
Chairperson, Department of Biology

____
Mike McKenzie, Ph.D.
Dean, Cratis D. Williams School of Graduate Studies

Abstract

COMPARING THE EFFECTIVENESS OF DIURNAL ROCK-LIFTING AND NOCTURNAL DIVE-LIGHTING SURVEYS FOR EASTERN HELLBENDERS

Freddy Junior Ortega
B.S., Appalachian State University
M.S., Appalachian State University

Chairperson: Michael M. Gangloff

Studies designed to better understand perceived hellbender population declines typically use diurnal rock-lifting surveys to detect individuals. However, these methods are invasive as they may alter sheltering or breeding habitat or result in injuries to hellbenders and surveyors. Further, diurnal surveys omit bedrock and large boulders that cannot be lifted. Between the months of June and August, 2019, I compared the number of detections and catch per unit effort (CPUE) of nocturnal snorkel surveys, followed by traditional diurnal rock-lifting surveys across 11 sites within the New, Watauga and Nolichucky river drainages in Western North Carolina. An additional late August - late September pass was conducted to reveal any breeding period effect on nocturnal detection rates. Wilcoxon signed-rank revealed that number of animals detected did not vary with method (diurnal to nocturnal summer: (Z = 37, df = 10, P = 0.08); nocturnal summer to nocturnal breeding: (Z = 9, df = 7, P = 0.68). Detections increased in 63% of sites during nocturnal surveys in both summer and breeding nocturnal surveys when compared to diurnal rock-lifting surveys. Paired t-tests comparison of hellbender catches across three survey treatments revealed that CPUE was statistically higher in the nocturnal summer treatment (t = 2.69, df = 9, P = 0.025); this difference was not observed between nocturnal-summer and nocturnal-breeding surveys (t = - 0.95, df = 7, P = 0.37). During nocturnal snorkel surveys, CPUE increased in 82% and 88% of sites for early summer and late summer treatments with 26% and 13% of detections being individuals sheltering in bedrock crevices during early summer and late summer nocturnal surveys respectively. Contrastingly, during early summer diurnal surveys, all detections were from beneath boulder substrate. By targeting the period of highest presumed activity in these cryptic salamanders, I was able to obtain more representative enumeration estimates of populations size likely because detection probabilities were equal or higher at most sites. These results suggest that both methods are similarly effective at detecting hellbenders. However, nocturnal surveys have the advantage of minimizing microhabitat impacts and are more efficient in terms of search effort. Additionally, non-invasive sampling can also be used to conduct surveys during the breeding season when nesting animals would presumably be more sensitive to disturbance associated with rock-lifting.

Key Words.—amphibians; hellbender; nocturnal; survey methods; rock-lifting; monitoring

Short Title. —Comparing diurnal and nocturnal surveys for Eastern Hellbenders

Acknowledgements

First, I'd like to thank Michael M. Gangloff and Lynn Siefferman for their support and encouragement throughout the entire project since its inception in 2018. Without their expertise, patience, and approachability, this project would not have been possible. I would also like to thank John Davenport for his support and very insightful comprehensive exam questions. A special thanks to Ashley Yaun whose friendship and knowledge made her a critical reference during the early days of scouting, site selection, and logistical planning for this project. A big thanks to my entire field crew: Michael Thompson, Samuel Fritz, Amber Olson, Vincent Santini, Hans Lohmeyer, Brandon Williams, David Burton, William McMahan, Chantelle Rondel, Tori Fowler, Danielle Everly, Chase Lorentz, all of the wonderful undergraduate capstone students (there are too many to list), and hellbender enthusiasts. I would also like to thank Ed Corey of the NC Division of Parks and Recreation and his field crew for their valuable assistance in surveying within the New River State Park. Lori Williams of the North Carolina Wildlife Resources Commission for being a valuable reference in best practices regarding permitting, site selection, and encouragement. Finally, I would like to thank Manley Worth Pugh for his guidance in the years prior to my graduate studies which provided me a foundation for the challenges that were soon to come. I feel very fortunate to have been surrounded by such a dedicated and bright group of individuals, whose passion for wildlife will surely lead to exciting and valuable discoveries in the future.

Dedication

This work is dedicated to my parents Maripili and Fredy Ortega whose love and support has allowed me to build a life in which I am able to pursue my dreams, this thesis is a clear example of that.

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Foreword

The research detailed in this thesis will be submitted to the peer-reviewed journal *Herpetological Conservation and Biology*. This thesis has been prepared according to the style guideline of that journal.

Introduction

Global assessment of amphibians has shown that amphibian declines and threats are occurring at alarming rates and many declines remain largely misunderstood (Pechmann et al. 1991; Stuart et al. 2004). Habitat loss and overutilization have been tied to 52% of global declines, however the remainder fall under a wide array of potential threats including the fungal disease chytridiomycosis (Berger et al. 1998; Lips et al. 2003), Ranavirus (Kik et al. 2011; Souza et al. 2012), anthropogenic habitat disturbance (Unger et al. 2017), overexploitation (Nickerson and Briggler 2007). Given the broad range of potential threats to amphibian populations, it has become increasingly important to develop methods to effectively and efficiently monitor populations over an extended period of time in order to distinguish natural population fluctuations from legitimate changes in abundances (Pechmann et al. 1991). Also, it is important to assess the effectiveness of survey methods for innate limitations mediated by behavior and the habitat use of target species (Pechmann et al. 1991; Hyde and Simons 2001) and possible negative effects including the spread of pathogens and trauma to individuals during capture (Browne et al. 2011; Franklin 2016).

The Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) is a large, cryptic aquatic salamander that can reach a total length of ~74 cm (Nickerson and Mays 1973). Hellbenders occur in cool-water, swift-flowing, and well-oxygenated streams with ample lithic substrate heterogeneity (Nickerson and Mays 1973; Fobes 1995; Pugh 2013). Eastern Hellbenders are widely-dispersed across the Ohio and Missouri river basins, however, they are believed to be declining across this range, likely due to anthropogenic habitat modification and land-use change (Wheeler et al. 2003; Quinn et al. 2013; Pugh et al. 2016; Pitt et al. 2017). The Ozark Hellbender (*C. alleganiensis bishop*i), an endemic subspecies, is listed as endangered under the U.S. Endangered Species Act (USFWS 2011) and USFWS has been petitioned to list the Eastern Hellbender multiple times during the last two decades (USFWS 2001; USFWS 2010; USFWS 2019).

Conservation biologists and resource managers have conducted an array of studies designed to determine presence-absence, quantify abundance and demography or examine genetic connectivity to understand the mechanisms driving hellbender population declines (Briggler et al. 2007; Unger et al. 2013; Franklin 2016; Pugh et al. 2016; Wineland et al. 2019). Because activity rates are highly variable among amphibian taxa, it is important to understand fundamental aspects of an organism's behavior when designing abundance or occupancy-based surveys (Pechmann et al. 1991; Humphries and Pauley 2000; Buderman and Liebgold 2012; Spear et al. 2015; Murphy et al. 2016)**.** Variability in behavioral traits influence population estimates, and survey methodologies that account for these differences can be used to improve detection rates. In Red-backed Salamanders, scotoperiod and its effect on diel activity patterns, has been found to influence detection rates (Buderman and Liebgold 2012). Hellbenders are considered to be nocturnally-active salamanders, however surface-active individuals have been observed during the middle of the day (Noeske and Nickerson 1979). Although these trends have not been well-studied, a range of observations appear to suggest that diel activity varies on a seasonal basis (Nickerson and Mays 1973; Nickerson and Tohulka 1986; Humphries 2007; Takahashi et al. 2018; Michael Gangloff pers. obs.; Worth Pugh pers. obs.). Japanese Giant Salamanders (*Andrias japonicus*) exhibit strong breeding/nest site defense with bouts of intense intraspecific combat and cannibalism documented among breeding males (Kawamichi and Ueda 1998). Nocturnal surveys are also more efficient at capturing terrestrial salamanders (family Plethodontidae) compared with both artificial cover boards and leaf litter searches (Hyde and

Simons, 2001). Nocturnal searches for Red-backed Salamanders (*Plethodon cinereus*) consistently had higher initial encounter probabilities compared to daytime searches (Buderman and Liebgold 2012). It is possible that salamanders occupying cover objects during the day are exhibiting territorial behavior whereas surface counts represent all foraging individuals (i.e., counts include both territorial and non-territorial animals) (Jaeger 1979; Mathis 1990; Mathis 1991; Jaeger et al. 1995). Thus, targeting the period of peak hellbender activity may provide a more accurate estimate of population sizes as nocturnal searches are less likely to be biased by substrate composition and territorial behaviors.

Traditional hellbender studies have used diurnal snorkel/rock-lifting surveys to detect animals in water with up to a 1 m depth (Nickerson and Krysko 2003; Browne et al. 2011). However, despite widespread use of this technique, researchers have recently raised concerns about rock-lifting surveys (Browne et al. 2011; Santas et al. 2013; Franklin 2016). Main concerns revolve around rock-lifting altering hellbender habitats and causing injury to animals if rocks are dropped (Browne et al. 2011; Santas et al. 2013; Franklin 2016). Additionally, lifting large boulders is both effort intensive and hazardous to investigators and hellbenders commonly escape capture when large clouds of sediment become suspended in the moments following a rock-lifting event (Browne et al. 2011; Franklin 2016). In previous reports the maximum activity of hellbenders was reported to occur ~2 hours after sunset (Noeske and Nickerson 1979). Blais (1996) investigated nighttime activity of hellbenders in south-central New York but reported very low amounts of nocturnal activity, seemingly contradicting similar studies in the Allegheny River (e.g., Swanson 1948).

Seasonal changes in hellbender behavior including surface activity, sheltering rates and feeding strategies at night remain unknown, likely because diurnal rock turning techniques do not overlap with periods of nocturnal, or breeding activity (Smith 1907; Green 1934; Bishop 1941; Nickerson and Mays 1973; Browne et al. 2011; Pugh et al. 2018). In West Virginia, Humphries and Pauley (2000) conducted repeated surveys for hellbenders within a 215 x 20 m by wading with spotlights. Between May and October 1998, they captured 59 individuals, with 12 detections of sheltering individuals, and observations of as many as 10 individuals sheltering and/or active on the streambed on some nights. Hellbender surface activity decreased and sheltering rates increased over the course of summer and into the breeding period, and this result is contrary to prior reports of increased surface activity during the breeding season (Smith 1907; Green 1934; Bishop 1941; Humphries and Pauley 2000). However, it remains unclear whether the hellbenders in this study were changing their sheltering behavior in response to seasonal life history cues (i.e. increased breeding season territoriality), or to the frequency (every 2 weeks) of surveys. Additionally, because individuals were not marked, detection probability remained unknown. Despite this study’s limitations, Humphries and Pauley (2000) did provide evidence that nocturnal surveys can yield hellbender detection rates that are comparable to those of daytime rock-lifting surveys (Pugh et al. 2016, Pugh et al. 2018).

To date, no prior studies have directly compared the effectiveness of diurnal and nocturnal hellbender surveys. Indeed, only a few studies have attempted to quantify seasonal changes in hellbender activity periods (Humphries and Pauley 2000; Humphries 2007). Nocturnal surveys have been shown to be broadly effective for detecting other cryptobranchid salamanders. Nocturnal spotlighting is the most common survey method employed to detect populations of the Japanese and Chinese (*Andrias davidianus*) Giant Salamanders and is often used independently or in conjunction with diurnal rock-lifting (Kawamichi and Ueda 1998; Wang et al. 2004; Okada et al. 2008; Browne et al. 2011; Takahashi et al. 2016). Here, I test the hypothesis that a survey method targeting the period of increased surface activity in hellbenders can provide estimates of hellbenders abundance that are equivalent to those obtained from diurnal rock-lifting surveys. I predict that nocturnal survey methods will provide similar estimates but the effectiveness of this approach may be influenced by habitat conditions. Additionally, by conducting nocturnal dive-lighting surveys across two seasons, I tested the hypothesis that season influences detection of hellbenders. Because hellbenders tend to begin exhibiting pre-breeding behaviors (e.g., increased levels of territoriality and male-male combat) beginning in August 15 and continuing through early autumn (Lori Williams pers. obs.; Humphries 2007; Spear et al. 2015). I predict that hellbender detections and CPUE will increase during late summer and early autumn surveys.

Materials and Methods

Hellbender surveys

I selected 11 study sites for surveys within the New, Watauga and Nolichucky river drainages in western North Carolina based on results of previous hellbender surveys (e.g., Pugh 2013; Franklin 2016; Pugh et al. 2018; Yaun 2019; Gangloff et al. unpubl. data). I conducted an initial round of mid-summer surveys between 14 June 2019 and 10 August 2019. First, I used nocturnal surveys aided by dive lights and then 1-6 days later, I surveyed the same reach using the traditional diurnal rock-lifting method. Finally, I conducted a second nocturnal pass at eight sites between 23 August and 29 September to reveal any breeding period effect on nocturnal detection rates. Due to logistical constraints (i.e. time, weather, crew availability etc.), nocturnal breeding period surveys did not follow the same order as those conducted during the summer and passes ranged from 32 to 84 days apart (Table. 1).

I used the spatially constrained transect-based survey method described by Pugh et al. (2018) to maintain a standardized protocol to compare data across space and time. For each site, I surveyed a 150-m stream reach divided into 15 cross-channel transects spaced 10-m apart. Snorkelers proceeded in an upstream direction and survey teams of two to five searchers (with the exception of one survey that involved 25 searchers) completely searched each transect. To minimize disruption of substrates and natural behavioral patterns, I first conducted nocturnal surveys and then returned to the site, typically within 1-2 days (site 6 was sampled five days apart) to conduct diurnal surveys. This minimized both habitat disturbance and the effects of spacing out surveys over the course of a summer field season (i.e., seasonal effects).

During both nocturnal and diurnal surveys, snorkelers moved in an upstream direction searching the streambed for active hellbenders and examining the spaces beneath boulders and bedrock for sheltering hellbenders (Nickerson and Krysko 2003). I quantified search time, number of searchers, and number of hellbender detections/captures separately for each transect. I calculated catch per unit effort (CPUE) to account for variation in effort (time and number of individuals searching) for each survey. During nocturnal surveys, snorkelers used Underwater Kinetics SL4 ELED (Model MK2 600 Lumen) dive lights® and 3AAA Vizion Z3 (210 Lumens) Herculite headlamps® to observe both individuals that were sheltering and those active on the streambed. I only captured or attempted to capture hellbenders that were active on the streambed. I marked shelter rocks and/or position of capture of all individuals by deploying a numbered glow marker. I constructed glow markers of bright orange duct tape with two 4.5 cm round steel washers at one end, and a 10.2 cm glow stick attached to the other end. I recorded the location of all captured hellbenders and hellbender shelter rocks using a Bad Elf GNSS Surveyor Bluetooth GPS unit® (Bad Elf, LLC).

During daytime surveys, researchers searched for juvenile/larval hellbenders beneath cobble (64-256 mm), boulder (>256 mm) and bedrock substrates. I used log-peaveys to lift boulders coupled with placing dip-nets directly downstream of cobbles and boulders to capture larger hellbenders (Gordon et al., 2004). Dip-nets were placed directly downstream of cobbles and boulders to reduce escapement. For all captured hellbenders (night and day), I recorded the following morphological measurements: total length (TL), snout-vent length (SVL), tail width (TW), weight, and any abnormalities (i.e. missing limbs, scars etc.). Sex was not discernable as no captures were made during the breeding period when swollen cloacae could be observed (Nickerson and Mays, 1973). Additionally, I used a Marsh McBirney Flo-Mate model 2000® to measure flow at the upstream end of each occupied shelter object. At each occupied shelter object, I also recorded: water depth (highest point of rock), rock length (RL), and rock width (RW). After processing, I measured depth and mid-channel flow velocity at five equi-distant points across the stream channel using a Marsh McBirney Flo-Mate model 2000® and a meter stick. At each locality at which hellbenders were encountered, depth (top of rock or stream bed), flow (immediately upstream of location), and rock length was recorded. Finally, I returned hellbenders to their respective cover objects or their approximate point of capture in the study reach.

Statistical Analysis

I compared the numbers of detections between diurnal and nocturnal surveys during the summer, and between nocturnal-summer and nocturnal-breeding treatments using a Wilcoxon signed-rank test because the distribution of detections was not normal for these data. CPUE data were compared using a paired sample t-test however. I also excluded a site in New River State Park

(Site 7) because this site was sampled as part of a larger inventory effort. At this site, 25 searchers located only two hellbenders and generated an artificially-low CPUE of 0.04. I used IBM SPSS Statistical Software 26® to analyze data.

Results

Hellbender Detections

During my 2019 summer and breeding period surveys, I detected a total of 122 hellbenders during 19 nocturnal dive-lighting surveys and 11 diurnal rock-lifting surveys. Diurnal (summer) surveys yielded 32 hellbender detections, all of which were from captures of individuals sheltering below boulder substrate that could be lifted (with one exception). I detected 43 individuals using nocturnal-summer surveys. Of these, 29 (67.4%) were detected beneath boulders, and 11 (25.6%) were detected beneath bedrock. Only 3 (7%) hellbenders were observed to be exposed on the streambed and not sheltering beneath substrate. I detected 47 hellbenders using nocturnal-breeding season surveys. Of these, I found 37 (78.7%) beneath boulders, 6 (12.8%) beneath bedrock, and 4 (8.5%) active on the streambed (Fig. 1). I did not detect any individuals during diurnal surveys at two sites that were occupied during nocturnal surveys (Site 6 in the Watauga and Site 8 in the Nolichucky River Drainage, Table 1).

During summer surveys, detections increased in 7 of 11 sites during nocturnal surveys and CPUE increased in 8 out of 10 sites when compared to diurnal surveys (Fig. 3). During the nocturnal surveys in the breeding season, CPUE increased in 5 locations when compared to nocturnal summer surveys (Fig. 3). At two other sites, hellbender detections increased substantially during the breeding season: site 9 detections increased from 13 during the nocturnal-summer to 22 during the nocturnal-breeding surveys and Site 11 nocturnal detections increased from 3 to 7 (Fig. 2).

Comparisons Across Methods

I found marginally significant increases in hellbender detections using nocturnal summer surveys compared to diurnal-summer surveys (Z = 37, df = 10, P = 0.08) (Fig. 4). However, contrary to findings by Humphries and Pauley (2000), I found no significant differences in summer versus breeding season hellbender detection rates using nocturnal methods (Z = 9, df = 7, P = 0.68) (Fig. 4). CPUE comparisons revealed that more animals were detected per survey effort during nocturnal versus diurnal methods in the summer (t = 2.69, df = 9, P = 0.025) (Fig. 4). There was no significant difference in CPUE when comparing nocturnal-summer and nocturnal-breeding surveys which further suggests that activity increases in the breeding season (t = - 0.95, df = 7, P = 0.37) (Fig. 5). Despite not finding statistical differences, we surpassed our summer season number of 43 detected hellbenders during the breeding season by 5 individuals, even with the omission of three sites. These increases can be attributed to spikes in Site 11 going from 3 to 7, and a very successful survey at Site 9 in which water levels were extremely low and 22 hellbenders were detected (13 during the summer) (Fig. 2, Table 1).

Discussion

My data show that nocturnal surveys can detect hellbenders in comparable numbers to diurnal methods (Fig. 4). When effort (i.e., CPUE) was incorporated into the comparisons, I found that nocturnal dive-lighting methods were significantly more efficient in locating hellbenders than diurnal surveys (Fig. 5). A likely explanation for the increased efficiency of nocturnal surveys lies in the fact that considerable time is invested by field teams in lifting shelter rocks. Additionally, during nocturnal surveys I had higher detection of hellbenders that were sheltering under large, unliftable boulders and bedrock shelves. In a study aimed at characterizing optimal release sites for Ozark Hellbenders, resource selection models indicated that bedrock provided important microhabitat in areas where coarse substrate, the most positively associated microhabitat type in resource utility models, was patchy (Bodinof et al. 2012b). Bodinof et al. (2012b) also noted that estimates of resource utility were substantially higher in sites with less continuous bedrock (i.e., bedrock with more crevices). Given that diurnal rock-lifting methods are limited by substrate that can be lifted, it is possible for investigators to locate hellbender nests beneath bedrock (Nickerson and Tohulka 1986). Bedrock has proven to be a challenging microhabitat in which to access hellbenders in the past (Bodinof 2010). Peterson (1988) and Nickerson and Tohulka (1986) used crowbars and steel bars to fracture bedrock to expose individuals and/or nests with mixed success. Bodinof et al. (2012a) followed translocated hellbenders and found an unequal detectability because of the researchers' limited access to bedrock sheltering individuals.

During both the 2019 summer and breeding season, nocturnal surveys were more efficient than diurnal surveying for a variety of reasons. First, when lifting and searching large boulders, field teams of 3-5 searchers are required to safely and effectively lift large boulders as well as to help prevent escapement. Also, when an animal was located visually during nocturnal surveys, searchers were able to drop a marker, and almost immediately return to searching. These two reasons lead to uninterrupted sampling efforts in which much efficiency can be gained during the time of search (Fig. 3, Fig. 5) over diurnal rock lifting. Lastly, some substrates could not be lifted safely with log peaveys. During summer diurnal surveys the largest lithic substrate (omitting outliers) was 1570 mm (Fig. 6), while during both nocturnal surveys the size of the largest occupied shelter rock was 3110 mm (Fig. 6). Nickerson et al. (2002) predicted the utility of nocturnal surveying in accessing hellbenders that sheltered under crevices and rocks that are too large to turn; I was able to confirm this in my study.

Hellbender Behavior

Of the 90 hellbender detections observed during nocturnal-summer and nocturnal-breeding surveys, only 7 individuals were active on the surface with an overwhelming majority observed sheltering beneath lithic substrate. In hellbenders, aggressive intraspecific combat between males has been widely-observed along with cutaneous abrasion, loss of limbs, and death, most frequently during the breeding season (Pfingsten 1990; Wheeler et al. 2002; Miller & Miller 2005). Sheltering individuals were typically observed with only their heads protruding from beneath cover objects (Fig. 7). Minimal exposure by hellbenders reflects a territorial/nest defense behavior that has been observed both within and outside of the breeding period (Smith, 1907; Bishop, 1941; Hillis and Bellis 1971; Nickerson and Mays 1973). In populations of *P. cinereus,* territoriality in superior competitors is a way of guaranteeing exclusive access to certain resources, thereby increasing their fitness. Excluded individuals experienced lower fitness and were potentially failing to breed altogether (Mathis 1991). Similar exclusion behavior has been observed in hellbenders (Hillis and Bellis 1971) and male size can determine the outcome of bouts of intraspecific competition for breeding habitat in *A. japonicus* (Kawamichi and Ueda 1998). Cover object size was correlated with temperature and moisture and the role of cover objects as predation refuges and prior residence under an object mediated cover selection.

Nocturnal spot-lighting observations of *A. japonicus* at nesting sites showed relatively sedentary behaviors during much of the year (Kawamichi and Ueda 1998). However, during the spawning period (late summer and early fall), large males referred to as ‘den masters’ were observed with their heads protruding from the entrances of desirable nest cavities, presumably in an effort to exclude other males. Males approaching the nest were frequently attacked and forced to fight the (often larger) male or flee (a behavior most commonly observed among smaller males). Den masters frequently left the nest to patrol the area and attack any males in the vicinity. These conflicts sometimes resulted in severe injury or mortality of individuals involved in territorial disputes. The aggressive intraspecific competition observed among *A. japonicus* in this study was largely constrained to the spawning season. During much of the remainder of my study, individuals had no reaction even when incidental physical contact between individuals sheltering in the same crevices occurred. Future behavioral studies could build on my observations and potentially address many questions regarding hellbender life history and behavior including: (1) How long does the spring period of surface activity extend? (2) When do breeding-associated aggressive behaviors begin? (3) How frequent are agonistic interactions among competing males during the breeding season and perhaps most interestingly from a conservation perspective (4) How important might these agonistic encounters be to density-dependent regulation of population density. I observed hellbender mortalities coincident with the breeding season at one of my study sites during a nocturnal-breeding survey, and in another western North Carolina stream where freshwater mussels were being surveyed.

During nocturnal surveys, 92% of detected individuals were observed with their heads protruding from beneath cover (in a few exceptions, another portion of their bodies were visible from shelter openings by searchers). Nocturnal observations suggest that hellbenders are able to remain undetected by prey items such as crayfishes (Smith 1907; Netting 1929; Green 1935; Bishop 1941; Swanson 1948; Nickerson and Mays 1973; Peterson et al. 1989) and fishes (Swanson 1948, Yaun 2019) and/or predators by only exposing a small portion of their body i.e. their head. During the nocturnal-summer survey at Site 5, one hellbender was observed attempting, but failing, to eat a passing fish with only its head exposed from the shelter rock; this is a previously undocumented sit-and-wait feeding strategy. I was unable to distinguish whether individuals were males defending nest rocks (Smith 1907; Hillis and Bellis 1971), males or females attempting to ambush their prey, or both. Sheltering individuals rarely reacted to the presence of observers during nocturnal surveys with responses restricted to retreating slightly into shelters but were never observed fleeing. Humphries and Pauley (2000) observed individuals sheltering and feeding in a similar manner, however, their observations were made from above the water's surface. Interestingly, during two early-summer nocturnal surveys (May 1998), all individuals detected were encountered fully exposed on the stream bottom.

In only a single case (Site 2), was I able to locate a hellbender sheltering beneath a boulder with its head protruding during a diurnal survey. Otherwise, all individuals were located after lifting boulders during diurnal surveys. In several instances, boulders where a hellbender was observed to be sheltering previously during nocturnal surveys were not found to be occupied during subsequent diurnal surveys, suggesting that individuals may not use the same shelters from day to day, at least during the summer. A nocturnal radio-telemetric study by Coatney (1982) revealed that monophasic activity in Ozark Hellbenders occurred in the first two hours of darkness in which a mean elliptical home range for males and females was 90.01 m^2), although this was in August. At Site 1, two individuals located in the second transect were not detected during rock-lifting surveys just one day after the initial observation. However, during nocturnal-breeding surveys, two hellbenders were located in the same transect with their heads protruding from beneath the same shelter rocks. Interestingly, this was also the only time two individuals were observed sheltering together beneath the same rock; limited reports exist for this behavior (Smith 1907; Hillis and Bellis 1971; Nickerson and Mays 1973). During nocturnal-summer surveys in the Watauga River (Site 6), a single individual that was spotted at 22:34 through a narrow opening between two boulders. Only its leg was visible. After completing the survey (23:41), I returned to the marked rock and the hellbender's head was now protruding from the opening. Although nocturnal activity does not always translate to exposure on the streambed, these observations may indicate that hellbenders are considerably more active nocturnally at the sites sampled during my study.

Nocturnal surveys may also improve the effectiveness and accuracy of occupancy-model based surveys for hellbenders. My results suggest that nocturnal visual surveys could, despite their logistical challenges, provide a more efficient, low-impact method for assessing the current distribution and change in status of hellbenders. One potential drawback with nocturnal surveys is a lack of population demographic information (i.e. sex and a distinction between juvenile and adult age class) for sheltering hellbenders. Horchler (2010) was able to capture sheltering hellbenders using a crushed crayfish lure placed near the crevice of a shelter rock; I have not tested this myself however. Nocturnal surveys could be used to help managers target reaches for more time-intensive diurnal rock-lifting surveys to obtain demographic parameters (Keitzer et al. 2013; Pugh et al. 2016). Because hellbenders did not react strongly to the presence of nocturnal observers in my study (possibly in an effort to reduce detection by a presumed predators- otters, water snakes, large turtles, native and non-native fishes, it may be possible to use images from a waterproof camera with a size standard in the image to estimate body size of sheltering animals

(Nickerson and Mays 1973; Kniest et al. 2010; Bendik et al. 2013; Browne et al. 2014; Nickerson et al. 2017).

My study reveals that nocturnal searches using a timed, spatially-segregated approach supported by powerful dive-lights provide an efficient, non-invasive, albeit logistically challenging method for detecting hellbender populations. Future studies designed to assess changes in hellbender detectability and occupancy, especially those with limited resources, or studies in streams with small or declining populations may want to consider employing nocturnal surveys. Unfortunately, occupancy and detection models using pseudo-occupancy were not possible due to limitations in the quantity of occupied transects in my data; this prevented our models from converging. Finally, if hellbenders are listed range-wide as endangered species, nocturnal surveys may prove to be an effective way of assessing distribution and population sizes without risking substantial ‘take’ (defined by USFWS as ‘killing, removing, harassing or altering the habitat’) of a federally-protected species during monitoring (ESA 1973).

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Tables and Figures

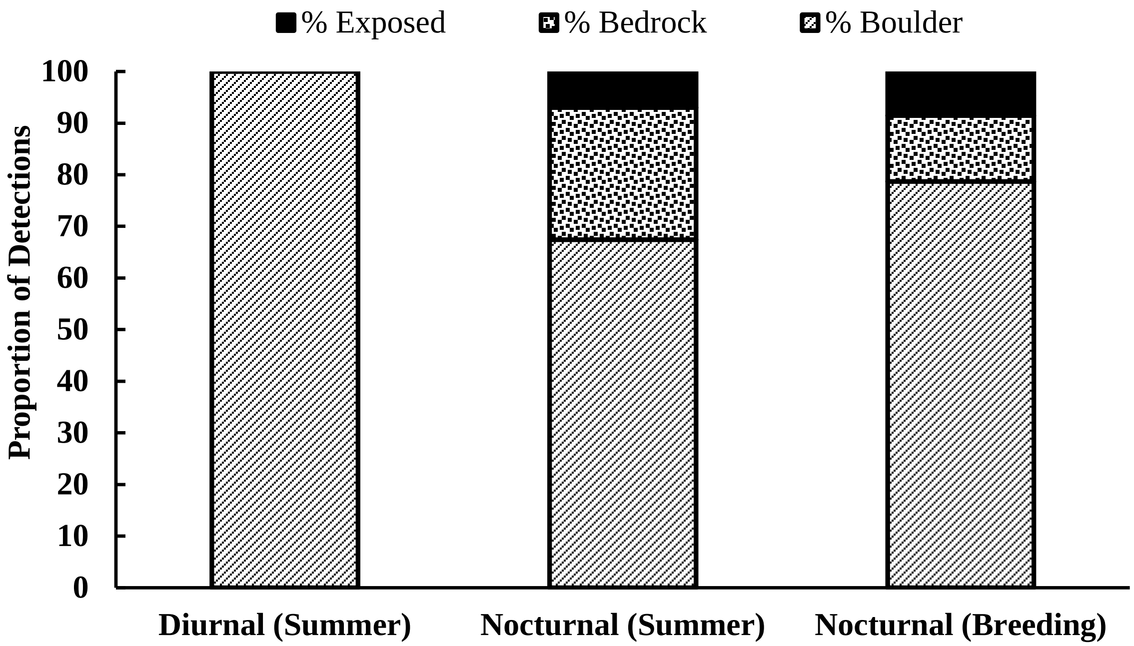

Figure 1. Proportion of all hellbenders that were detected as either exposed on the streambed, or sheltering beneath bedrock or boulder substrates during diurnal and nocturnal surveys in summer (June-early August), and breeding season (late August-September) periods in 2019.

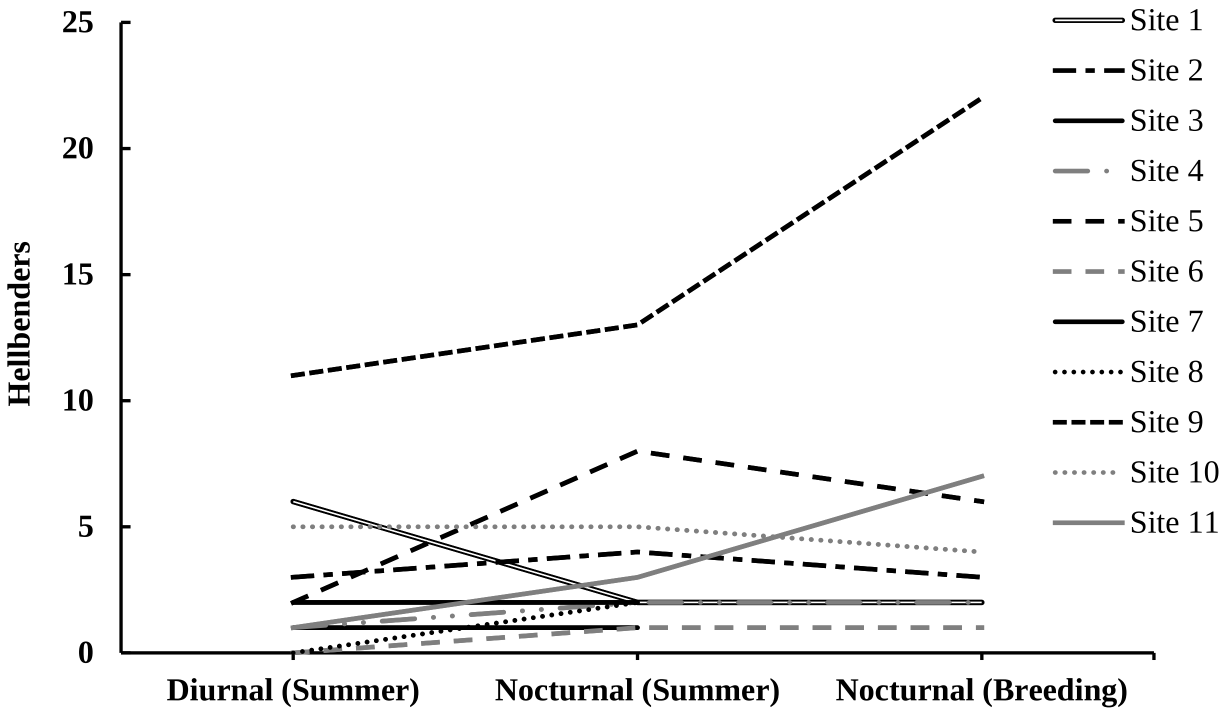

Figure 2. Number of hellbenders detected during each survey during the 2019 survey season organized by field site, survey method, and season.

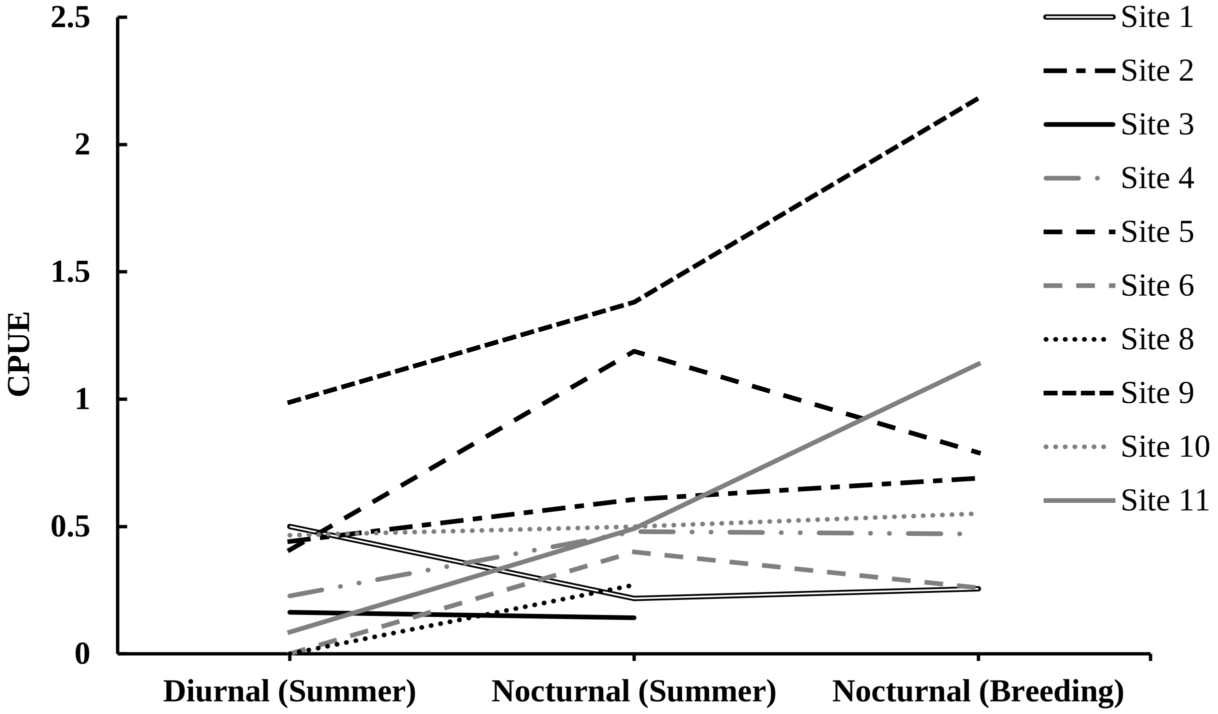

Figure 3. CPUE for surveys conducted during the 2019 survey season organized by field site, survey method, and season.

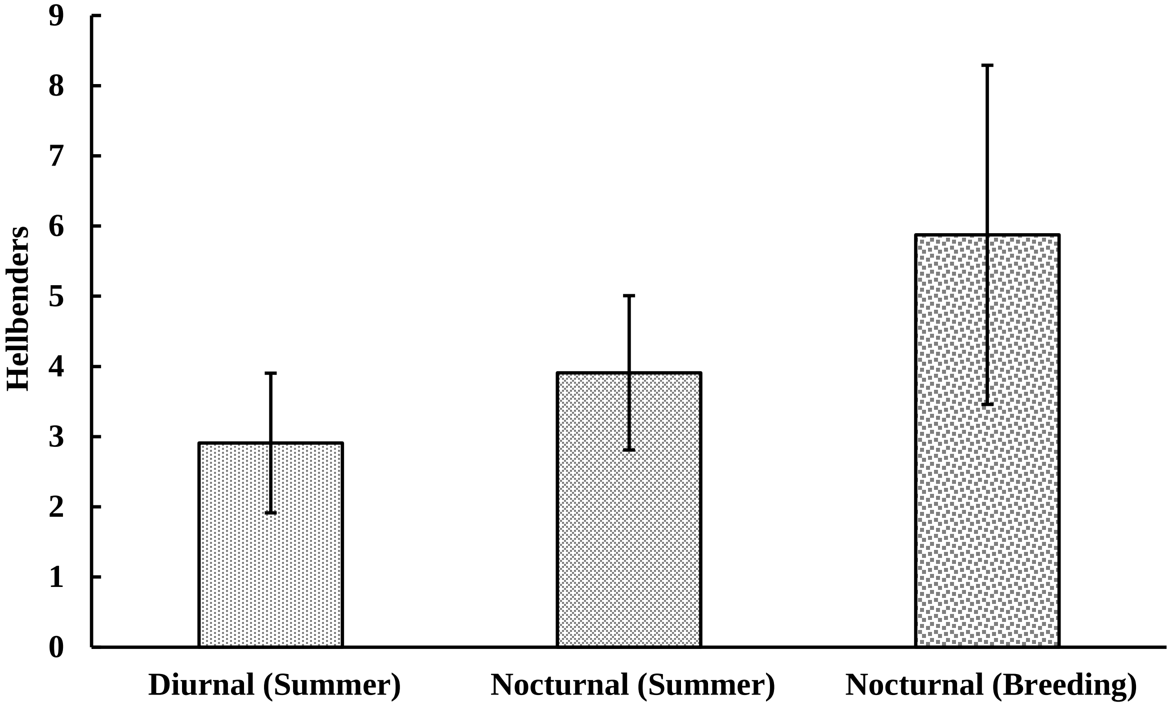

Figure 4. Comparison of hellbender detections across three survey methods in western North Carolina during 2019; error bars represent standard error. Number of animals detected did not vary with method (diurnal to nocturnal-summer: (Z = 37, df = 10, p = 0.08); nocturnal-summer to nocturnal breeding: (Z = 9, df = 7, p = 0.68).

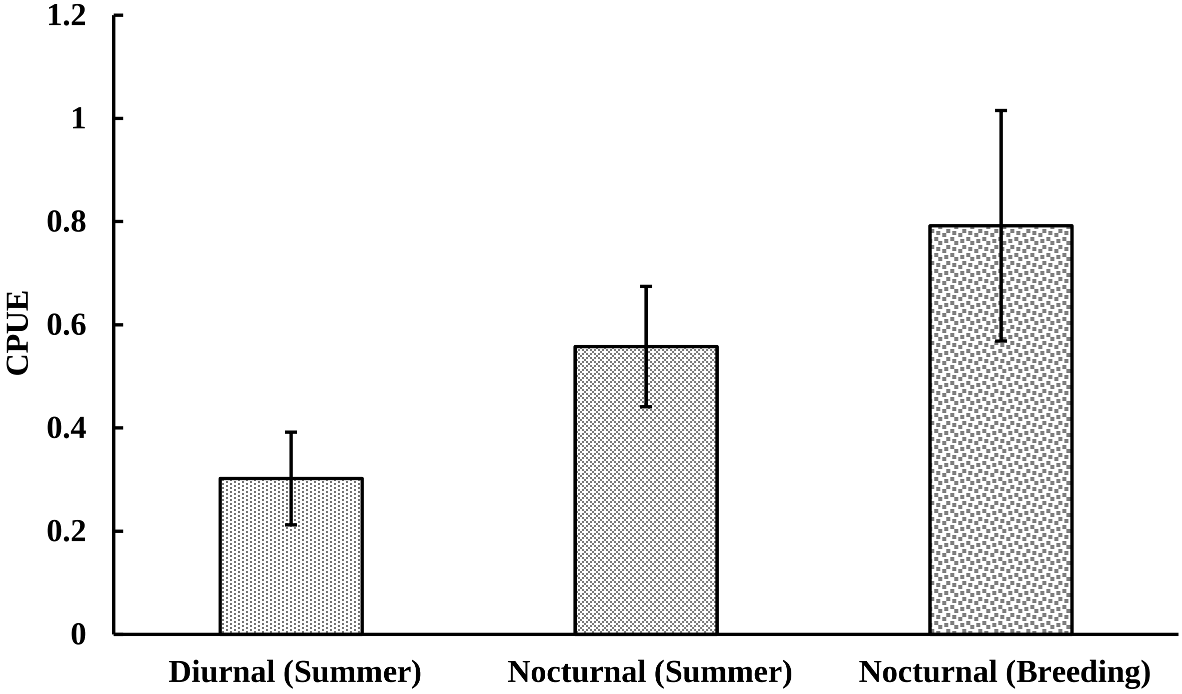

Figure 5. Comparison of hellbender CPUE across three survey methods; error bars represent standard error. CPUE did vary with method (diurnal to nocturnal-summer: (paired t = -2.69, df = 9, p = 0.025); nocturnal-summer to nocturnal breeding (paired t = - 0.95, df = 7, p = 0.37).

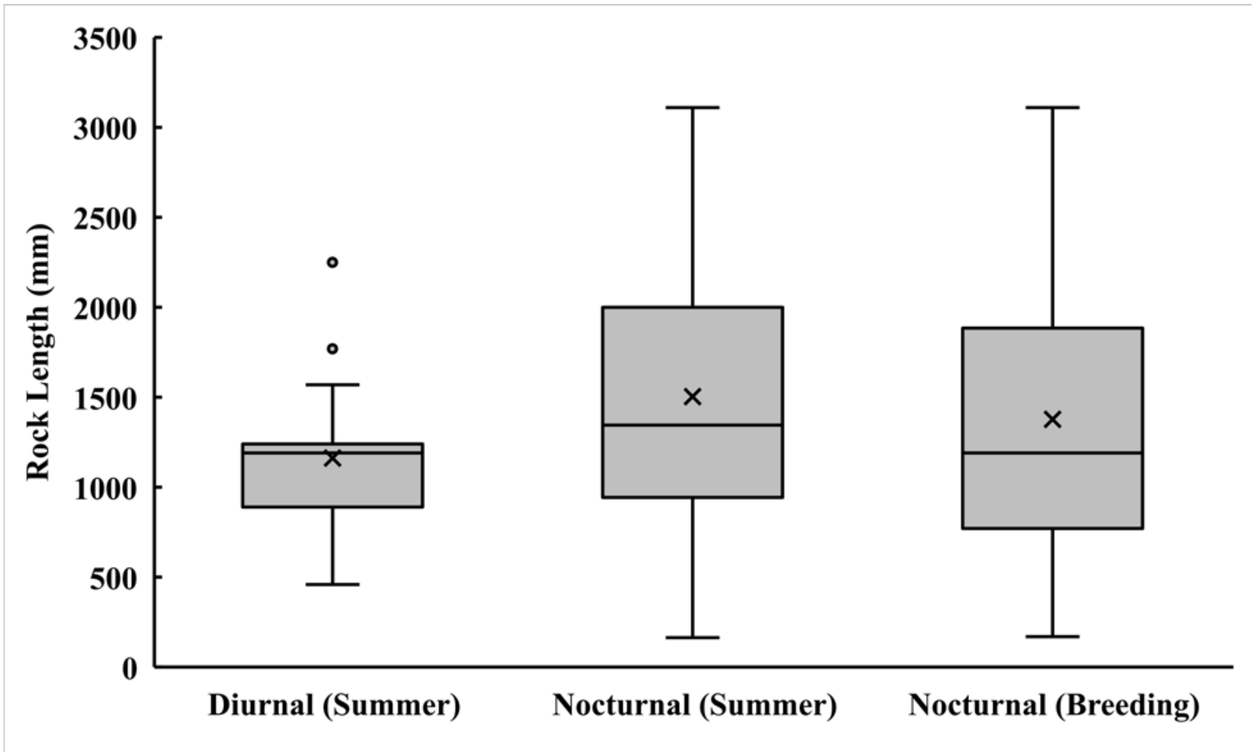

Figure 6. Boxplot of rock lengths (mm) under which hellbenders were located during 2019 hellbender surveys.

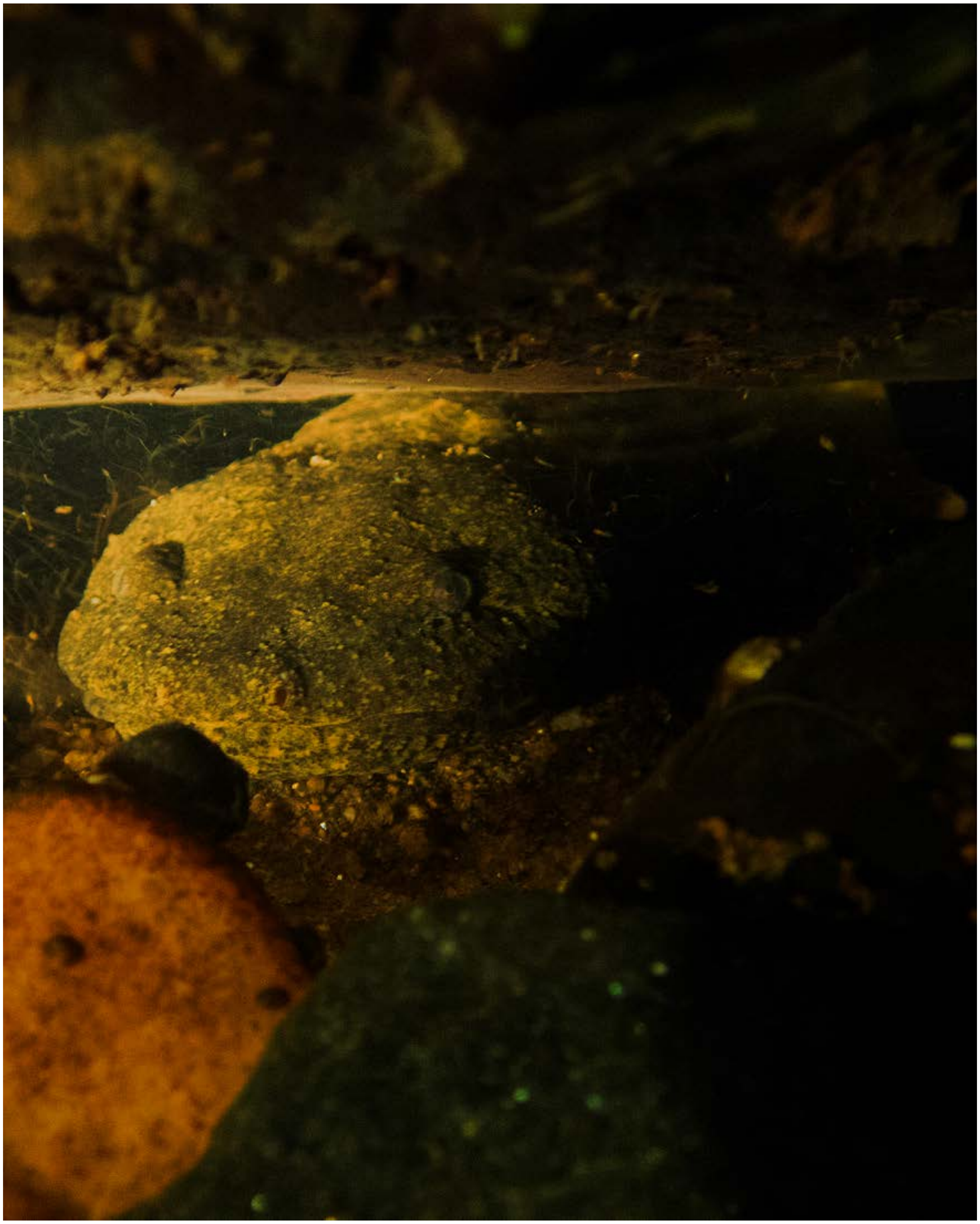

Figure 7. Photo of a hellbender with its head exposed from under its shelter rock. 91% of hellbenders were located by illuminating crevices in boulder and bedrock 5substrate during summer and fall 2019 nocturnal surveys. Photo was taken at Site 11 in the South Fork of the New River.

Table 1. Sites surveyed for Eastern Hellbenders during Diurnal-summer (D-S), Nocturnal-summer (N-S), and Nocturnal-breeding (N-B) surveys in the New, Nolichucky and Watauga drainages during summer of 2019.

	Site	Date	Drainage	Detections	Time Searched (min)	Searchers	CPUE
D-S	1	6/15	New	6	180	4	0.5
	2	6/27	Nolichucky	3	102	4	0.4
	3	6/28	Nolichucky	1	92	4	0.16
	4	6/31	New	1	88	3	0.23
	5	7/8	Watauga	2	147	2	0.41
	6	7/15	Watauga	0	119	3	0.00
	7	7/17	New	2	108	25	*0.04
	8	7/22	Nolichucky	0	179	4	0.00
	9	7/26	Watauga	11	167	4	0.99
	10	8/7	New	5	161	4	0.47
	11	8/10	New	1	235	3	0.09
N-S	1	6/14	New	2	138	4	0.22
	2	6/25	Nolichucky	4	99	4	0.61
	3	6/26	Nolichucky	1	106	4	0.14
	4	6/30	New	2	125	2	0.48
	5	7/7	Watauga	8	101	4	1.19
	6	7/10	Watauga	1	75	2	0.40
	7	7/16	New	2	87	3	0.46
	8	7/20	Nolichucky	2	111	4	0.27
	9	7/24	Watauga	13	113	5	1.38
	10	8/6	New	5	150	4	0.50
	11	8/8	New	3	122	3	0.49
N-B	1	8/23	New	2	94	5	0.26
	2	8/26	Nolichucky	3	87	3	0.69
	4	8/30	New	2	85	3	0.47
	5	9/29	Watauga	6	114	4	0.79
	6	9/1	Watauga	1	77	3	0.26
	9	9/15	Watauga	22	121	5	2.18
	10	9/7	New	4	109	4	0.55
	11	9/22	New	7	123	3	1.14

* CPUE was omitted from analysis.

Table 2. Morphometric and microhabitat data for each individual that was captured during surveys conducted in the summer and fall of 2019; HB # (order in which hellbender was detected), TL (total length), SVL (snout-vent length), WT (weight), TW (tail width), RL (rock length), RW (rock width), DNR (did not recover). Depth was taken at the top of shelter rock or at approximate location in which individual was first observed, flow was taken directly upstream of location detected.

	Site #	Transect #	HB #	TL (cm)	SVL (cm)	WT (g)	TW (mm)	Depth (cm)	Flow (m/s)	RL (mm)	RW (mm)
N-S	1	2	1	-	-	-	-	44	0	3100	1820
	1	2	2	-	-	-	-	44	0	3100	1820
	2	3	1	-	-	-	-	98	0.91	2000	1500
	2	3	2	-	-	-	-	119	0.85	2250	1650
	2	8	3	-	-	-	-	57	57	1600	1100
	2	15	4	-	-	-	-	36	36	850	450
	3	6	1	-	-	-	-	126	1.28	BR	BR
	4	5	1	-	-	-	-	32	0.47	1250	1400
	4	15	2	40	25	370	30.8	73	0.46	E	E
	5	3	1	0.65	40	-	4.7	41	0.11	42	30
	5	7	2	-	-	-	-	0	0.35	1300	1250
	5	7	3	-	-	-	-	30	0.46	1390	1000
	5	7	4	-	-	-	-	0	0.04	1290	860
	5	8	5	-	-	-	-	0	0.03	1350	820
	5	10	6	-	-	-	-	14	0.6	910	480
	5	13	7	41	25	420	31	20	0.51	E	E
	5	14	8	-	-	-	-	1	0.41	650	520
	6	2	1	-	-	-	-	0	0.09	950	590
	7	6	1	-	-	-	-	12	0.41	2000	1900
	7	8	2	-	-	-	-	16	0.27	BR	BR
	8	3	1	39	25	350	34	78	0.38	E	E
	8	5	2	-	-	-	-	20	0.24	1600	1100
	9	2	1	-	-	-	-	35	0.29	1290	1280
	9	2	2	-	-	-	-	0	0.44	2700	900
	9	3	3	-	-	-	-	0	0.54	BR	BR
	9	3	4	-	-	-	-	0	0.05	2080	1190
	9	3	5	-	-	-	-	61	0.17	560	1150
	9	4	6	-	-	-	-	27	0.43	1340	630

	9	5	7	-	-	-	-	0	0.11	BR	BR
	9	7	8	-	-	-	-	16	0	1820	1320
	9	8	9	-	-	-	-	22	0.14	2500	760
	9	8	10	-	-	-	-	43	0.75	BR	BR
	9	8	11	-	-	-	-	40	0.67	BR	BR
	9	8	12	-	-	-	-	20	0.13	BR	BR
	9	10	13	-	-	-	-	60	1.36	1440	480
	10	5	1	-	-	-	-	52	0.35	1270	900
	10	5	2	-	-	-	-	54	0.4	960	910
	10	6	3	-	-	-	-	90	0.6	BR	BR
	10	11	4	-	-	-	-	40	0.6	BR	BR
	10	13	5	-	-	-	-	0	0.64	1590	920
	11	2	1	-	-	-	-	45	0.05	920	750
	11	2	2	-	-	-	-	7	0.14	870	800
	11	3	3	-	-	-	-	104	0.08	BR	BR

	Site #	**Transect #**	**HB #**	**TL (cm)**	**SVL (cm)**	**WT (g)**	**TW (mm)**	**Depth (cm)**	**Flow (m/s)**	**RL (mm)**	**RW (mm)**
D-S	1	2	1	27	17	200	286	23	-	-	-
	1	4	2	DN	DN	DN	DNR	41	-	-	-
	1	9	3	53	36	1,0	37	45	-	-	-
	1	9	4	41	25	540	348	50.5	-	-	-
	1	9	5	52	32	1,0	47.2	70.1	-	-	-
	1	10	6	30	30	645	41.3	64	-	-	-
	2	3	1	44	29	570	45.3	113	1.03	1770	670
	2	3	2	-	-	-	-	119	0.85	2250	1650
	2	15	3	44	29	410	34.4	5	-	1230	800
	3	2	1	41.5	25	380	36	56	0.38	-	-
	4	9	1	51	32	810	45.2	65	0.4	1240	740
	5	1	1	47	29	500	33	0	0.21	1190	590
	5	8	2	-	-	-	-	0	0	1220	1000
	7	7	1	49	29.3	670	36.5	52	0.28	1780	840
	7	10	2	24	17	90	24.4	62	0.32	950	550
	9	1	1	47	28	520	38.7	-	-	1160	610
	9	4	2	-	-	-	-	-	-	490	540
	9	4	3	43	27	520	38.4	-	-	690	530
	9	5	4	45	25	400	32.2	-	-	460	330
	9	7	5	45	27	480	35.4	-	-	910	750
	9	9	6	38	32	330	35	-	-	880	640
	9	9	7	48	29	560	38.6	-	-	970	760
	9	9	8	53.5	31	720	43.7	-	-	1220	630

	9	11	9	43	26	430	38.8	-	-	1570	680
	9	-	10	34	25	480	37.5	-	-	-	-
	9	15	11	43	26	520	37	-	-	1200	640
	10	5	1	54	32	820	41.9	60	0.55	930	-
	10	8	2	-	-	-	-	81	0.4	900	-
	10	8	3	52.5	31.5	710	50.6	66	0.53	855	-
	10	13	4	42.5	27	490	34.5	47	0.55	1270	-
	10	14	5	46	28	570	34.8	30	0.83	1590	-
	11	8	1	50	31	800	39.8	490	0.17	890	590

	Site #	Transect #	HB #	TL (cm)	SVL (cm)	WT (g)	TW (mm)	Depth (cm)	Flow (m/s)	RL (mm)	RW (mm)
N-B	1	2	1	-	-	-	-	18	0.84	3110	1820
	1	2	2	-	-	-	-	18	0.84	3110	1820
	2	1	1	-	-	-	-	-	0.42	E	E
	2	2	2	-	-	-	-	-	0.32	2200	950
	2	3	3	-	-	-	-	-	0.07	2150	2100
	4	5	1	-	-	-	-	35	-	E	E
	4	7	2	-	-	-	-	15	-	1000	535
	6	3	1	-	-	-	-	18	0.37	1070	900
	10	6	1	-	-	-	-	5	0.24	1600	1170
	10	6	2	-	-	-	-	87	0.19	1190	880
	10	6	3	-	-	-	-	51	0.35	1000	770
	10	12	4	-	-	-	-	38	0.28	1320	650
	9	1	1	-	-	-	-	30	0.17	E	E
	9	2	2	-	-	-	-	0	0.01	2420	1580
	9	5	3	-	-	-	-	5	0.1	1500	1010
	9	5	4	-	-	-	-	19	0.02	1750	465
	9	5	5	-	-	-	-	19	0.02	2020	1390
	9	6	6	-	-	-	-	0	0.2	2085	1080
	9	6	7	-	-	-	-	0	0.09	2455	1330
	9	7	8	-	-	-	-	48	0.04	600	540
	9	7	9	-	-	-	-	30	0.13	1495	630
	9	7	10	-	-	-	-	43	0.2	1650	830
	9	7	11	-	-	-	-	40	0.06	600	420
	9	7	12	-	-	-	-	10	0.2	1135	1130
	9	7	13	-	-	-	-	18	0.04	930	635
	9	8	14	-	-	-	-	49	0.7	460	385
	9	9	15	-	-	-	-	39	0.17	E	E
	9	12	16	-	-	-	-	41	0.2	170	110
	9	12	17	-	-	-	-	50	1.05	BR	BR
	9	12	18	-	-	-	-	63	0.25	3075	2030
	9	13	19	-	-	-	-	58	0.13	490	690
	9	13	20	-	-	-	-	61	0.13	1070	560

9	15	21	-	-	-	-	84	0	1190	690
9	15	22	-	-	-	-	48	0	810	735
11	2	1	-	-	-	-	31	0.22	BR	BR
11	2	2	-	-	-	-	0	0.17	710	620
11	5	3	-	-	-	-	33	0.23	730	600
11	6	4	-	-	-	-	120	0.08	910	660
11	7	5	-	-	-	-	47	0.56	BR	BR
11	8	6	-	-	-	-	49	0.09	250	170
11	9	7	-	-	-	-	5	0.1	BR	BR
5	1	1	-	-	-	-	8	0.18	1320	1320
5	1	2	-	-	-	-	3	0	680	1600
5	4	3	-	-	-	-	14	0.18	BR	BR
5	4	4	-	-	-	-	6	0.28	1550	1430
5	6	5	-	-	-	-	0	0.09	BR	BR
5	12	6	-	-	-	-	0	0.57	1130	575

Vita

Freddy Junior Ortega was born in Foggy Bottom, Washington D.C. to Maripili Arce Ortega and Fredy Humberto Ortega in June of 1993. In 2013, he transferred to Appalachian State University to pursue a degree in Ecology, Evolution, and Environmental Studies and in 2016 he was awarded a bachelor of science. Freddy volunteered at the university Zoological collection transitioning into his role as a research technician in the Aquatic Conservation Research Lab, there he developed an interest in lotic systems and the herpetofauna of Western North Carolina. He began pursuing a Master's Degree in August of 2018 and was awarded a Master of Science degree in August of 2020.