

FINDING HELLBENDERS (*CRYPTOBRANCHUS ALLEGANIENSIS*): A COMPARISON OF DIURNAL ROCK-LIFTING AND NOCTURNAL DIVE-LIGHTING SURVEYS

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Abstract.—Studies designed to understand trends in Hellbender (*Cryptobranchus alleganiensis*) populations typically use diurnal rock-lifting surveys; however, these methods may alter sheltering or breeding habitat, potentially injure Hellbenders and surveyors, and may fail to detect animals sheltering under bedrock and large boulders. Here, we compare the number of Hellbender detections and detection efficiency between nocturnal dive-lighting surveys and diurnal rock-lifting surveys conducted at 11 sites in four streams in western North Carolina, USA. We conducted an additional nocturnal survey during the breeding season to examine breeding-period effects on detection rates. Hellbender detection efficiency (measured as catch-per-unit effort, CPUE) was higher during nocturnal dive-lighting than during diurnal searches. We did not observe an effect of breeding season on the number of Hellbender detections or CPUE. Overall, our nocturnal sampling protocol was less invasive and more efficient (i.e., required fewer person hours to detect Hellbenders) than diurnal surveys. Although nocturnal dive-lighting surveys provide limited demographic data, they may be more effective in deeper streams where larger boulders and bedrock comprise the majority of habitat. Moreover, they can help identify reaches to be targeted for more intensive surveys. Furthermore, nocturnal dive-lighting surveys are low-impact and can be used during the breeding season thereby extending temporally constrained field seasons. Although nocturnal Hellbender surveys will likely never completely replace diurnal surveys, they provide important supplemental data and improve our understanding of how the range and population status of these imperiled giant salamanders is changing.

Key Words.—amphibian; breeding period; conservation; detection efficiency; non-invasive

INTRODUCTION

Recent assessments suggest that amphibians are Earth's most threatened vertebrate class with a wide range of taxa experiencing declines in their range and abundance due to the cumulative effects of disease, habitat loss, and climate change (Stuart et al. 2004; Luedke et al. 2023). To understand the causes of and develop remedies for amphibian declines, it is important to track population fluctuations via long-term monitoring, and it is helpful to refine methods to monitor populations effectively and efficiently (Pechmann et al. 1991). Furthermore, because behaviors vary among amphibian taxa, fundamental aspects of the behavior of an organism must also be considered when designing abundance or occupancy-based surveys (Humphries and Pauley 2000; Buderman and Liebgold 2012; Murphy et al. 2016).

The Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*, hereafter Hellbenders) is a large, cryptic aquatic salamander that can reach a total length of about 74 cm (Nickerson and Mays 1973). Hellbenders occur in cool, well-oxygenated streams with low substrate embeddedness (Smith

1907; Hillis and Bellis 1971; Nickerson and Mays 1973; Fobes 1995). Hellbenders are widely dispersed across the Ohio and Missouri river basins in the U.S.; however, they are 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). Studies designed to understand the mechanisms driving Hellbender population declines reveal that water-quality, local habitat, and catchment land-use are frequent predictors of site occupancy. Many declining populations are spatially or genetically isolated and exhibit a lack of recruitment characterized by low rates of larval and subadult detections (Wheeler et al. 2003; Unger et al. 2013; Pugh et al. 2016; Wineland et al. 2018; da Silva Neto et al. 2023). Currently, the Ozark Hellbender (*C. alleganiensis bishopi*), an endemic subspecies, is listed Federally as Endangered under the U.S. Endangered Species Act (U.S. Fish and Wildlife Service [USFWS] 2011) and the USFWS recently proposed listing the Eastern Hellbender as Endangered range wide (USFWS 2010, 2019, 2024a, 2024b).

Hellbenders are typically nocturnal and peak surface activity rates are reported to occur about 2 h after sunset (Noeske and Nickerson 1979; Coatney 1982). Although these trends have not been well-studied, a range of observations suggests that diel activity varies seasonally (Nickerson and Mays 1973; Humphries 2007). Individuals are active on the streambed in the middle of the day during the late August to September breeding period and, to a far lesser extent, during the spring months (Smith 1907; Nickerson and Mays 1973; Humphries 2007). Hellbenders exhibit pre-breeding behaviors including increased levels of territoriality and male-male combat beginning in mid-August (Smith 1907; Nickerson and Mays 1973; Spear et al. 2015; Lori Williams, pers. obs.).

Traditionally, Hellbenders are studied using diurnal snorkel and rock-lifting surveys to detect animals in water at depths < 1.0 m (Nickerson and Krysko 2003; Browne et al. 2011). Despite widespread use of this technique, researchers have raised concerns about the unintended consequences of rock-lifting surveys (Browne et al. 2011; Santas et al. 2013; Franklin 2016). Rock-lifting can alter habitats and cause injury to animals if rocks are dropped (Browne et al. 2011; Santas et al. 2013; Franklin 2016). Additionally, lifting large boulders is effort intensive, hazardous to investigators and disruptive to sheltering or brooding Hellbenders. Furthermore, Hellbenders may escape capture when sediment becomes suspended in the moments following a rock-lifting effort (Franklin 2016). Finally, Hellbenders frequently occupy shelters beneath rocks that are too large to be lifted by investigators or bedrock ledges, thus limiting search efficacy although dive-lighting during the day can sometimes be used to detect animals beneath very large substrates (Burgmeier et al. 2011).

Seasonal changes in Hellbender behavior, activity, sheltering rates, and feeding strategies at night remain unknown, likely because diurnal rock-lifting surveys do not, by design, overlap temporally with periods of nocturnal or breeding activity in North Carolina, USA (Smith 1907; Green 1935; Nickerson and Mays 1973; Browne et al. 2011; Pugh et al. 2018). In West Virginia, USA, Humphries and Pauley (2000) conducted repeated nocturnal surveys for Hellbenders by using passive nocturnal spotlighting searches with researchers walking through shallow stream reaches and using flashlights to locate Hellbenders. Between May and October 1998, they observed up to 10 individuals per night in a 65.6 m² area either sheltering beneath rocks or active on the streambed. This study

provided evidence that passive nocturnal surveys can yield Hellbender detection rates comparable to those of daytime rock-lifting surveys observed in Pugh et al. (2016, 2018) and with limited effort and level of disturbance. Furthermore, nocturnal spotlighting is the most common survey method employed to detect populations of the Japanese Giant Salamander (*Andrias japonicus*) and Chinese Giant Salamander (*A. davidianus*), and it 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).

Due to a lack of previous effort and considerable evidence that nocturnal surveys could be used to expand the temporal scope of Hellbender surveys we compared the effectiveness of diurnal rock-lifting and nocturnal dive-lighting surveys. In addition to expanding the duration of Hellbender surveys, nocturnal dive-light surveys could allow investigators to monitor sensitive populations with minimal habitat disturbance and to conduct surveys in deeper water or other habitats where traditional rock-turning is difficult. Further, only a few prior studies have quantified seasonal changes in Hellbender behavior, and dive-lighting provides a method for assessing abundance and behavior that is minimally disruptive to focal animals (Humphries and Pauley 2000; Humphries 2007). We used nocturnal surveys prior to and during the breeding season to examine how seasonality influences Hellbender detection rates. We expected that Hellbender detections and catch per unit effort (CPUE) would be greater in surveys conducted during the breeding season compared to those conducted during the summer months.

MATERIALS AND METHODS

Hellbender surveys.—We selected 11 study sites in four streams within the New, Watauga, and Nolichucky river drainages in western North Carolina, USA. All sites were known to be occupied based on results of prior Hellbender surveys (Pugh 2013; Franklin 2016; Pugh et al. 2018; Yaun 2019). We 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, we surveyed the entirety of 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 (except for one survey that involved 25 searchers and was removed from CPUE comparisons)

completely searched each transect. We conducted an initial round of mid-summer surveys between 14 June and 10 August 2019.

To minimize disruption of substrates or displacement of Hellbenders, we used nocturnal dive-lighting surveys first and then we surveyed the same reach using the traditional diurnal rock-lifting method (described below) typically within 1–2 d (site 6 was sampled 5 d apart due to limited crew availability). Finally, we conducted a second pass using nocturnal dive-lighting at eight sites between 23 August and 29 September 2019 to evaluate potential breeding season effects on nocturnal detection rates. Due to frigid water temperatures and fewer crew available, it became difficult to continue nocturnal surveys, thus we surveyed only eight sites during the breeding season. All comparisons between nocturnal non-breeding and breeding season surveys involved the eight sites shared between both seasons only.

During the nocturnal dive-lighting surveys and diurnal surveys, snorkelers moved upstream, searching the streambed for active Hellbenders and cover objects (Browne et al. 2011). We quantified search time, number of searchers, and number of Hellbender detections/captures separately for each transect and calculated CPUE as the number of Hellbenders/(# searchers * time searched in hours) to account for variation in effort (time and number of individuals searching) for each survey. At each occupied shelter object, we recorded rock length (longest aspect of the rock) and rock width (perpendicular to the length at its widest point). Sex was not discernable, as no captures were made during the breeding period when swollen cloacae could be observed (Nickerson and Mays 1973). Finally, we returned Hellbenders to their respective cover objects or their approximate point of capture.

During nocturnal dive-lighting surveys, snorkelers used SL4 ELED Model MK2 600 Lumen dive lights® and Z3 (210 Lumens) Herculite headlamps® (Underwater Kinetics, San Diego, California, USA) to observe Hellbenders that were sheltering or active on the streambed. We only attempted to capture Hellbenders that were active on the streambed during the non-breeding period (May through mid-August). We marked shelter rocks and position of capture of all individuals by deploying a numbered glow marker, which we constructed with bright orange duct tape and two 4.5 cm round steel washers at one end and attached a 10.2 cm (4 in) white light sticks (Lumistick, Stafford, Texas, USA). During daytime surveys, researchers searched for juvenile/larval

Hellbenders beneath cobble (64–256 mm), boulder (> 256 mm), and bedrock substrates. To capture larger Hellbenders, the team lifted boulders with log-peaveys, reached under rocks for Hellbenders, and placed dip-nets directly downstream of cobbles and boulders to reduce escape (Gordon et al. 2004; Browne et al. 2011). We used dive lights to examine cervixes beneath shelter rocks but we did not use cameras to examine cervixes during diurnal searches.

Statistical analysis.—We compared the numbers of detections between diurnal rock-lifting and nocturnal dive-lighting surveys during the summer, and between summer and breeding nocturnal dive-lighting surveys. Because Shapiro-Wilk Normality tests indicated that distribution of the paired differences was not normal, we used a non-parametric Wilcoxon Signed-rank Test to examine differences in abundance data. We then compared normally distributed CPUE data using a parametric Paired Sample *t*-test. We excluded CPUE data from Site 7 (New River State Park site) because it was sampled as part of a larger inventory effort with 25 searchers and thus generated an artificially low CPUE. We used SPSS Statistical Software 26® (IBM Corporation, Chicago, Illinois, USA) and considered results significant when *P*-values were ≤ 0.05.

RESULTS

Hellbender detections.—We detected 122 Hellbenders during 19 nocturnal dive-lighting surveys (11 non-breeding and eight breeding) and 11 diurnal rock-lifting surveys (all non-breeding; Fig. 1). We found 32 Hellbenders during the diurnal (summer) rock-lifting surveys, all were captured while sheltering underneath boulder substrate (Fig. 2) as large as 2.25 m. Nocturnal dive-lighting surveys conducted during the summer and breeding period were more effective at locating Hellbenders under a wider range of substrate size classes compared to diurnal rock-lifting surveys (Fig. 3). Moreover, 92% of Hellbenders detected during nocturnal surveys were located by illuminating crevices in boulder and bedrock substrates. Of the 43 individuals detected during summer-nocturnal surveys, 29 (67.4% of detections) were beneath particles ranging from 0.42 m to 3.1 m, and 11 (25.6%) were beneath bedrock. We observed only three Hellbenders (7% of detections) walking on the streambed during summer nocturnal surveys. Of the 47 Hellbenders we detected during nocturnal breeding-season surveys, 37 (78.7%) were beneath boulders as large as 3.1 m, six (12.8%) were

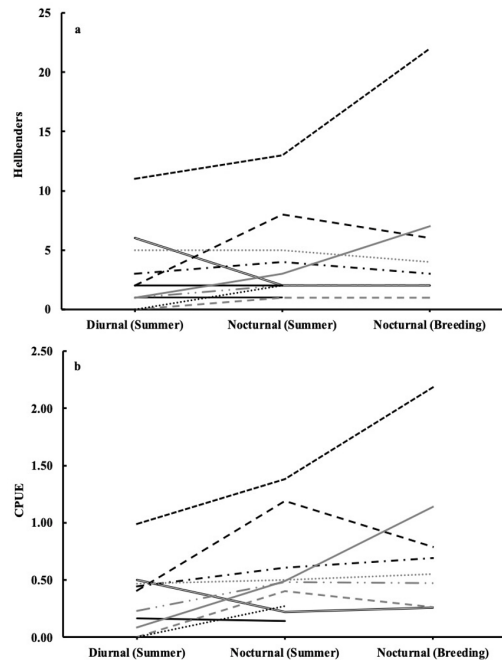


FIGURE 1. (a) Number of Hellbenders (*Cryptobranchus alleganiensis*) detected and (b) Hellbender CPUE (animals per person hour, during each survey during the 2019 survey season in the New, Watauga, and Nolichucky river drainages in western North Carolina, USA. Lines represent individual field sites during each survey method and season during which surveys occurred.

beneath bedrock, and four (8.5%) were active on the streambed (Fig. 2). For all nocturnal dive-lighting surveys, if not fully exposed walking the streambed, individuals were observed with their heads protruding from a rock or bedrock crevice (Fig. 4).

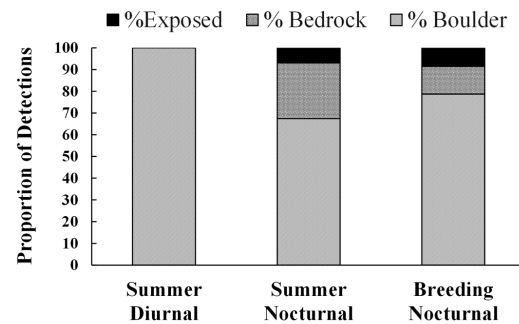


FIGURE 2. Proportion of Hellbenders (*Cryptobranchus alleganiensis*) found exposed on the streambed, or either sheltering beneath bedrock or boulder substrates during diurnal and nocturnal dive-lighting surveys in summer (June-early August) and the breeding season (late August-September) in 2019 in the New, Watauga, and Nolichucky river drainages in western North Carolina, USA.

Comparisons across methods.—During the summer diurnal rock-lifting surveys, we detected a mean of $2.9 \pm$ [standard error] 1.00 Hellbenders per hour and a mean of 3.9 ± 1.10 Hellbenders per hour during nocturnal dive-lighting surveys. During the breeding season, we detected a mean of 5.9 ± 2.4 Hellbenders per hour. We found no significant increases in Hellbender detections between nocturnal summer surveys compared to diurnal-summer surveys ($W = 37$, $df = 10$, $P = 0.080$). We also found no significant differences in summer versus breeding season Hellbender detections using nocturnal dive-lighting surveys ($W = 9$, $df = 7$, $P = 0.684$). During

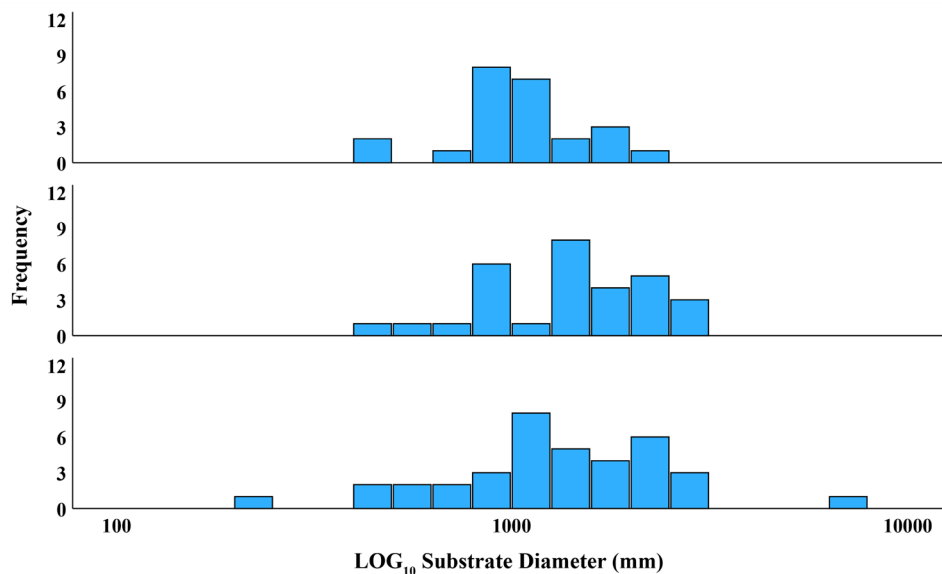


FIGURE 3. Histogram of the distribution of Hellbender (*Cryptobranchus alleganiensis*) shelter rock lengths (mm) occupied by animals during 2019 (A) diurnal, (B) summer nocturnal, and (C) breeding season nocturnal surveys in the New, Watauga, and Nolichucky river drainages in western North Carolina, USA. Substrate diameter (mm) has been log-transformed using base 10.



FIGURE 4. A Hellbender (*Cryptobranchus alleganiensis*) sheltering beneath rock in the South Fork of the New River, North Carolina, USA. The photograph was cropped and lightened as needed to enhance contrast. (Photographed by Freddy J. Ortega).

the summer, the mean CPUE for diurnal rock-lifting surveys was 0.3 Hellbenders per hour $\pm$ 0.10, while the mean CPUE for nocturnal dive-lighting surveys was 0.6 Hellbenders per hour $\pm$ 0.13. During the breeding season, the mean CPUE for nocturnal dive-lighting surveys was 0.8 Hellbenders per hour $\pm$ 0.22. We found that CPUE was greater in summer during nocturnal dive-lighting surveys compared to diurnal rock-lifting surveys ($t = 2.69$, $df = 9$, $P = 0.025$), but CPUE was not different between nocturnal-summer and nocturnal-breeding dive-light surveys ($t = -0.95$, $df = 7$, $P = 0.372$).

DISCUSSION

Nocturnal dive-lighting surveys may provide, despite their logistical challenges, a lower-impact method than diurnal rock-lifting surveys when the primary research objectives include assessing catch-per-unit effort or species occupancy across a watershed. Our results suggest that nocturnal dive-lighting yields Hellbender count data that are similar and sometimes higher than those obtained from diurnal rock-lifting surveys while requiring less time and smaller crew sizes. Nocturnal dive-lighting methods do not allow for the collection of

tissue samples, pathogen samples, or morphometric/demographic information but they are less disruptive to Hellbenders, can be used in deeper-water habitats, and can survey large areas for relatively little cost.

Another attractive aspect of nocturnal surveys is that they produce abundance estimates that are relatively consistent across seasons. Although Humphries and Pauley (2000) found that nocturnal Hellbender detections began to decline in July and continued to decrease into the breeding period, we found that nocturnal Hellbender detections and detection rates (CPUE) were not significantly different among surveys occurring during breeding and non-breeding periods. These data suggest that abundance estimates obtained from nocturnal dive lighting surveys conducted outside the traditional survey period are comparable with those conducted at other times of year.

Because nocturnal dive-lighting searches are non-invasive and unlikely to alter microhabitat conditions within shelters and disturb sheltering or egg-guarding Hellbenders than are cameras or rock turning, they may be particularly useful during the breeding season. Many U.S. states now restrict the dates during which rock-turning surveys may be conducted to better safeguard nest-guarding males and reduce nest predation. In North Carolina, rock-turning surveys generally cease on 15 August and this limitation was a major driver of our study. Because streams are generally workable through mid-October in western North Carolina, non-invasive surveys have the potential to greatly expand the number of sites that can be sampled in a field season.

During diurnal rock-lifting searches, we located all but one Hellbender after a cover object was lifted, whereas most individuals located during the nocturnal dive-lighting surveys were observed with their heads protruding from beneath cover objects. Numerous diurnal and nocturnal behavioral observations also suggest that Hellbenders can remain undetected by prey items such as crayfishes (Cambaridae; Smith 1907; Green 1935; Swanson 1948; Nickerson and Mays 1973; Peterson et al. 1989), small fishes (Swanson 1948; Yaun 2019), and potential predators by only exposing a small portion of their heads. Hellbenders that exposed only their heads from beneath cover objects may also reflect a territorial/nest defense behavior that has been observed in males both within and outside of the breeding period (Smith 1907; Hillis and Bellis 1971; Nickerson and Mays 1973). Although we were unable to distinguish whether individuals were defending nest rocks

(Smith 1907; Hillis and Bellis 1971), attempting to ambush prey, or simply preparing to leave the shelter to search for food, our data suggest that the likelihood of detecting Hellbenders sheltering at the openings of cervixes increases after sunset.

We can think of two reasons to explain the increased Hellbender CPUE during summer nocturnal versus diurnal surveys. First, crews employing diurnal rock-lifting are limited by which shelter objects they can lift and how quickly they can lift them. During diurnal surveys, field teams lift and manually search beneath substrates ranging from cobbles (larval and subadult habitat) to large boulders. The pace of these searches is dictated by the rate at which the slowest searcher(s) are moving upstream so sites with abundant large boulders that require multiple team members to lift may be time-consuming. During visual searches, survey team members take advantage of nocturnal sheltering behaviors (Hellbenders were most frequently observed with just their exposed but beneath shelter objects) and detect animals without having assistance to move cover objects or disturb animals and potentially nests. When an animal was located during nocturnal surveys, searchers placed a marker on the shelter rock and returned to searching to allow for a more efficient and uninterrupted survey. Second, the diurnal approach is limited in that some substrates including large boulders and bedrock crevices were either unliftable or unsafe to lift with log peaveys, omitting portions of habitat from the search effort. During nocturnal surveys, the largest occupied lithic substrate (omitting outliers) was > 3 m compared to about 1.5 m during the diurnal surveys. Our data indicate that investigators who use the diurnal rock-lifting method alone were unable to access some occupied habitats during searches and that more resources including time and personnel are necessary to sample a population.

Despite the limitations of the diurnal rock-lifting method, both methodologies offer distinct avenues for obtaining abundance estimates, each with their own strengths and limitations. For example, both survey methods tend to overlook smaller Hellbenders sheltering beneath cobbles somewhat by necessity because larval and sub-adult Hellbenders tend to use smaller cobbles compared to adults and many surveys overlook these substrates because catch rates are very low (Hecht et al. 2019). Studies designed to obtain demographic data may wish to use a combination of the two methods compared in this study whereas studies attempting to understand the distribution of animals across a large area or those with logistical or

financial constraints may find that visual surveys are more effective.

In contrast, although nocturnal surveys are minimally invasive and can detect animals beneath a larger range of substrate sizes when compared to rock-turning, potential drawbacks include the difficulty of collecting population demographic information for sheltering Hellbenders, the inability to collect pathogen data and the potential to overlook larval and subadult animals. In addition to providing demographic and animal health data, diurnal rock-lifting also permits investigators to search during the typical workday hours, which may work better with less experienced survey crews, and makes navigation to and from sites easier. Also, nocturnal surveys can be logistically challenging as experienced personnel are required to navigate field sites with dangerous river conditions (e.g. deep or fast-moving waters), which are more hazardous at night.

Our study reveals that nocturnal dive-lighting, despite its logistical limitations, provides an efficient and less invasive manner of acquiring statistically equivalent abundance estimates to using the diurnal rock-lifting method alone. Further, our data suggest that nocturnal dive-lighting surveys may be a particularly useful approach during the breeding season where diurnal rock-lifting is likely to disturb nesting animals. Although a comparison of diurnal dive-lighting surveys to nocturnal dive-lighting surveys was beyond the scope of this study, a future study that compares nocturnal and diurnal dive-lighting could reveal to what degree method or time of day affects our ability to obtain abundance estimates. Although obtaining population estimates was beyond the scope of this study, we hope that these findings can inspire future investigators to apply these passive search methods to obtain population estimates via occupancy modeling approaches (MacKenzie et al. 2018). Future studies designed to assess changes in Hellbender detectability and occupancy may want to consider employing nocturnal dive-lighting if sites include larger streams with deep water habitat or streams dominated by bedrock or larger substrates. If demographic parameters are required, nocturnal dive-lighting surveys could be used to help managers passively identify higher density field sites for more time/crew-intensive diurnal rock-lifting surveys.

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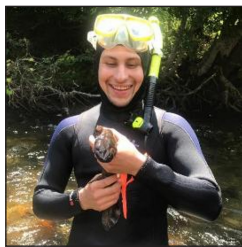
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