

Filial Cannibalism Leads to Chronic Nest Failure of Eastern Hellbender Salamanders (*Cryptobranchus alleganiensis*)

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ABSTRACT: In species that provide parental care, parents will sometimes cannibalize their own young (i.e., filial cannibalism). Here, we quantified the frequency of whole-clutch filial cannibalism in a species of giant salamander (eastern hellbender; *Cryptobranchus alleganiensis*) that has experienced precipitous population declines with unknown causes. We used underwater artificial nesting shelters deployed across a gradient of upstream forest cover to assess the fates of 182 nests at 10 sites over 8 years. We found strong evidence that nest failure rates increased at sites with low riparian forest cover in the upstream catchment. At several sites, reproductive failure was 100%, mainly due to cannibalism by the caring male. The high incidence of filial cannibalism at degraded sites was not explained by evolutionary hypotheses for filial cannibalism based on poor adult body condition or low reproductive value of small clutches. Instead, larger clutches at degraded sites were most vulnerable to cannibalism. We hypothesize that high frequencies of filial cannibalism of large clutches in areas with low forest cover could be related to changes in water chemistry or siltation that influence parental physiology or that reduce the viability of eggs. Importantly, our results identify chronic nest failure as a possible mechanism contributing to population declines and observed geriatric age structure in this imperiled species.

Keywords: riparian forest, Cryptobranchidae, parental care, amphibian conservation.

Introduction

Filial cannibalism—when a parent consumes its own offspring—has been observed in a wide variety of species, yet its adaptiveness remains uncertain in many cases. This peculiar behavior has been documented in many fishes (e.g., Rohwer 1978; Sargent 1992; Lindström and Sargent 1997; Manica 2002, 2004; Neff 2003a, 2003b; Klug and Bonsall 2007; Klug 2009), arthropods (e.g., Thomas and Manica 2003; Miller and Zink 2012), and rodents (e.g., Elwood 1992; Lonstein and De Vries 2000), with more isolated examples in primates (Culot et al. 2011), birds (Stanback and Koenig 1992; Gilbert et al. 2005), a squamate (Huang 2008), and amphibians (Okada et al. 2015; Takahashi et al. 2017; Unger and Williams 2018). Filial cannibalism is most common in species that have evolved parental care, particularly paternal care, where males guard, clean nests or eggs, or engage in other behaviors to increase offspring survival (Elgar and Crespi 1992; Sargent 1992; Manica 2002; Klug and Bonsall 2007). Cannibalism of offspring has been suggested to be advantageous to the caring parent when the costs of caring behaviors to parental self-maintenance or future reproductive success outweigh the fitness benefits expected from continuing current care (Klug and Bonsall 2007; Klug et al. 2012). Partial-clutch cannibalism in this scenario is intuitive, especially when reducing clutch/brood density increases per capita survival of young (e.g., Klug et al. 2006). Cannibalism of the whole clutch is an extreme version of this behavior that is more difficult to explain because it negates an entire reproductive event. Nevertheless, the occurrence of whole-clutch filial cannibalism in such a broad range of animals suggests that it may represent an adaptive form of brood termination that optimizes the parent's lifetime reproductive success (Sargent 1992; Manica 2004; Klug and Bonsall 2007).

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A wide variety of hypotheses have been proposed to explain the adaptive function of whole-clutch filial cannibalism, but the vast majority of studies and theory focus on the value of the brood as an easily accessible source of energy or nutrition. This energy-based hypothesis posits that consumption of the entire brood (whole-clutch cannibalism) occurs as a mechanism to shift resources from current to future reproduction (Manica 2002; Klug and Bonsall 2007). Thus, evolutionary theory predicts that whole-clutch filial cannibalism should be more prevalent in long-lived iteroparous species for which care is costly because the value of potential future reproduction can outweigh the expected fitness benefits from current reproduction, particularly for young adults, which may have smaller clutches, lower-quality nest sites, and a greater number of future reproductive opportunities than older adults (Sargent and Gross 1985; Manica 2002). In other words, the fitness benefits of caring for a clutch over an extended period may outweigh the costs of care only when clutch value exceeds a certain threshold.

Under this framework, the trade-off between costs and benefits of parental care behaviors and whole-clutch cannibalism could change with environmental conditions. For example, filial cannibalism of the entire clutch could be advantageous in environments where resources are scarce for parents and negatively impact the energetic status (e.g., body condition) of breeding individuals (Manica 2002; Klug and Bonsall 2007). Despite the intuitive appeal of this hypothesis, empirical support is lacking or contradictory, or alternative explanations, such as uncertain paternity, are more likely (e.g., Neff 2003a, 2003b; Payne et al. 2004; Klug and St. Mary 2005; Chin-Baarstad et al. 2009; Bose et al. 2014; Deal and Wong 2016; Vallon et al. 2016a, 2016b). If environmental degradation increases the propensity for individuals to forego care and cannibalize their entire clutch/brood, there could exist a tipping point at which cannibalism occurs at such high frequencies that it results in population declines due to low recruitment. This should be particularly true in long-lived and/or highly specialized species that have limited capacity to evolve in response to rapidly changing environmental conditions. In light of the exceptionally high rate of biodiversity loss associated with habitat degradation in the Anthropocene (Dudgeon 2019; Turvey and Cress 2019), empirical tests of this hypothesis are needed.

In this study, we examined the frequency of whole-clutch filial cannibalism in the eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*), a species of giant salamander in great need of conservation (USFWS 2018). Hellbenders and other cryptobranchid salamanders are known to engage in both partial and whole-clutch filial cannibalism, but the frequency of these behaviors in the wild and the factors that influence them are unknown, in large part because of the difficulty of studying these se-

cretive animals and their nest sites. Using innovative field techniques that employed underwater artificial shelters to observe nesting and document cannibalism, we simultaneously tested the common hypotheses that adult body size, adult body condition, and clutch size are important predictors of whole-clutch filial cannibalism. Moreover, we conducted our study across a gradient of upstream forest cover that influences instream habitat quality (Jachowski and Hopkins 2018) to determine whether environmental conditions were related to these predictors and/or the frequency of whole-clutch filial cannibalism. Our study provides new insight into the poorly understood reproductive biology of this imperiled species as well as novel information about the contribution of filial cannibalism to recently observed population declines.

Methods

Study Species

The eastern hellbender is among the most unique and imperiled amphibians in North America. It is one of two subspecies of *Cryptobranchus alleganiensis* that inhabit streams of the central and eastern United States (Nickerson and Mays 1973; Taber et al. 1975). These fully aquatic salamanders are long-lived (≥ 25 years) and are among the largest extant amphibians in the world (up to 74 cm total length and 2.2 kg; Nickerson and Mays 1973; Taber et al. 1975; Petranks 1998). Both hellbender subspecies have experienced population declines throughout their ranges over the past few decades (Wheeler et al. 2003; Foster et al. 2009; Burgmeier et al. 2011; Graham et al. 2011; Freake and DePerno 2017; Jachowski and Hopkins 2018). As a result, the Ozark subspecies (*C. alleganiensis bishopi*) and the evolutionarily distinct lineage of the eastern hellbender (*C. alleganiensis alleganiensis*) in Missouri are both federally protected as endangered species (USFWS 2011, 2021). The causes of hellbender declines are poorly understood, but loss of forest cover and associated changes to instream habitat, such as siltation and changes in water chemistry (e.g., increased dissolved ions leading to increased conductivity/salinity), are among the most common correlates implicated in declines (USFWS 2021). Importantly, declining populations also exhibit demographic shifts toward a geriatric population age structure, with very little evidence of young age classes in small populations (Wheeler et al. 2003; Burgmeier et al. 2011; Jachowski and Hopkins 2018). These observations suggest impaired reproduction and/or low survival of young age classes. However, reproduction of hellbenders has been difficult to study because they nest under inaccessible large boulders in streams (Nickerson and Mays 1973), and young age classes are notoriously difficult to detect (Unger et al. 2021).

Although some basic aspects of hellbender reproductive biology have been described, recent methodological advancements show promise for rapidly filling a diversity of important knowledge gaps related to hellbender reproductive physiology and ecology. Hellbenders generally take 5–8 years to reach sexual maturity (Peterson 1988). In the late summer and early fall, adult males establish nests under large submerged boulders, externally fertilize eggs, and then engage in solitary parental care of the eggs and larvae until the following spring (Bishop 1941; Nickerson and Mays 1973; Peterson 1988). Frequency of nesting by individuals, rates of nest success, causes of nest failure, paternal care behaviors, and the basic mating system of hellbenders are among the many aspects of hellbender reproductive biology that remain poorly understood. These fundamental knowledge gaps persist because hellbender nests cannot be easily or safely accessed under natural boulders. Over the last ~10 years, however, the use of artificial underwater shelters (Jachowski et al. 2020) has shown promise as a tool for studying hellbender reproductive biology and whether reproductive mechanisms contribute to population declines and shifts in population demographic structure.

Underwater Artificial Shelters and Study Design

We constructed artificial shelters by hand using a wire frame covered in thick concrete, and each shelter consisted of a single tunnel entrance leading to a rear chamber for nesting. We fitted shelters with a removable lid that allowed direct access to the chamber for nest monitoring. Two generations of shelter design were used in this study, the newer of which is heavier and has a more secure lid than the original, making it more stable in streams (Jachowski et al. 2020; Button et al. 2020b). Hellbenders are equally likely to occupy and nest in shelters of both designs (Button et al. 2020a). Additional details regarding our shelter design, hellbender use, and utility of artificial shelters for research are discussed in Jachowski et al. (2020) and Button et al. (2020a, 2020b).

We deployed underwater shelters from 2013 to 2020 in 10 reaches stratified across three rivers in the upper Tennessee River Basin in southwestern Virginia (fig. 1; table 1). River names are not included to prevent illegal collection and harassment of hellbenders and are hereafter referred to as rivers 1, 2, and 3. Two reaches were established in river 1, three reaches in river 2, and five reaches in river 3. Reaches were spatially arranged within streams such that movement of hellbenders between reaches is improbable and was never detected since the onset of mark-recapture in 2007. Reaches varied in length (206–376 channel meters) and in wetted area at baseflow (3,090–5,880 m²). We gradually established reaches as

long-term study sites from 2013 to 2018 by installing 30 artificial shelters in each reach, spaced an average of 10 linear meters apart (range $\approx$ 4–30 m) based on appropriate available habitat (e.g., depth, substrate characteristics). Several additional shelters were added to some reaches in 2019–2020, yielding a final range of 30–35 shelters per reach. Because we gradually established stream reaches over 8 years, the total number of opportunities for hellbenders to nest in our shelters varied among reaches (table 1).

Our previous work established that upstream catchment-wide riparian forest cover is a strong predictor of in-stream habitat quality (e.g., water quality), local hellbender abundance, and population demographic structure (Jachowski and Hopkins 2018). Therefore, we stratified stream reaches containing arrays of artificial shelters across a gradient of surrounding land use. We quantified upstream catchment-wide riparian forest cover (percent forest) from the USGS National Hydrography and 2019 National Land Cover datasets using methods similar to Jachowski and Hopkins (2018). Upstream catchment-wide riparian forest cover ranged from ~54%–68% among reaches (table 1), which provided enough variation in conditions to encapsulate both stable and declining populations. Population density estimates and size class distributions were previously established at six of the 10 reaches using multiyear mark-recapture surveys (reaches 2C and 3A–3E; Jachowski and Hopkins 2018; Button et al. 2020a; table 1). This subset of reaches illustrated that population density decreased and shifted toward a geriatric age class structure at reaches with upstream forest cover below ~63% (Jachowski and Hopkins 2018; Button et al. 2020a). As might be expected, the percentage of shelters used for nesting by hellbenders also decreases with reduced hellbender population density and loss of upstream forest cover (Button et al. 2020a; table 1).

Nest Monitoring

We monitored artificial shelters for nesting over an 8-year period (2013–2020). Beginning in mid- to late August each year, shelters were checked every 2–5 days for nest establishment. Once nesting activity was evident within a reach, we increased the frequency of shelter checks to every 2–3 days. We checked shelters for nesting by partially opening the lid enough for a skin diver to view the interior chamber using a dive light. Once a nest was detected, the date of detection was recorded (August 22 to September 20), which was typically within 48 h of oviposition (based on our high frequency of shelter checks).

We processed each nest and its attending adult male up to four times over the course of the parental care period, which in our study streams extends from oviposition until late April of the following year. The four nest-monitoring

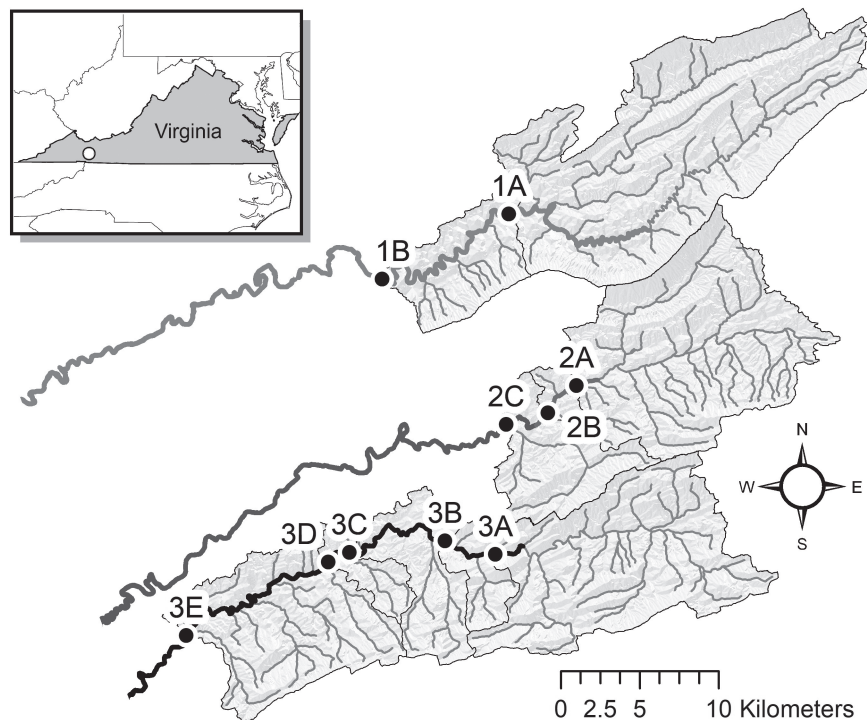


Figure 1: Map of study area in southwest Virginia. Ten stream reaches were stratified along a gradient of forest cover (54%–68%) in three rivers (rivers 1–3). Streams flow to the southwest; thus, reaches are labeled A (most upstream) to E (most downstream). The entire upstream catchment from the lowermost reach is depicted for each river along with tributaries within each catchment.

Table 1: Characteristics of stream reaches in southwestern Virginia used to study hellbender reproduction

Reach ID	First year monitored	% forest	No. nesting opportunities	Cumulative no. nests	Cumulative % nesting	No. larvae produced	No. larvae per nest	No. larvae per opportunity
1A	2018	60.0	90	4	4.44	42	10.50	.47
1B	2018	59.5	90	5	5.56	38	7.60	.42
2A	2018	55.4	90	3	3.33	93	31.00	1.03
2B	2018	54.1	81	2	2.47	0	0	0
2C	2014	55.2	237	19	8.02	0	0	0
3A	2016	67.8	161	26	16.15	450 ^a	18.00 ^a	2.81 ^a
3B	2015	67.9	210	32	15.24	928	29.00	4.42
3C	2013	66.5	225	28	12.44	1150	41.07	5.11
3D	2013	64.3	249	50	20.08	2101	42.02	8.44
3E	2014	61.9	210	13	6.19	17	1.31	.08

Note: Reaches were stratified across three rivers (rivers 1–3, upstream to downstream reaches A–E) and a gradient of upstream catchment-wide riparian forest cover (percent forest), calculated from the USGS National Hydrography and 2019 National Land Cover datasets. Shelters were gradually deployed over the course of the 8-year study at similar densities among reaches and were monitored each reproductive season thereafter (range = 3–8 reproductive seasons). Because of this unevenly distributed sampling effort, we report both raw values and values corrected for the number of nesting opportunities. The number of nesting opportunities is the sum of the number of shelters available at each reach across all years. Cumulative nests were summed within each reach across all years, and cumulative percent nesting was the cumulative number of nests expressed as a function of the number of nesting opportunities. The minimum total number of larvae emerging from nests summed over the course of the study (number of larvae produced) is expressed as a function of the number of nests produced at each reach as well as the per number of nesting opportunities at each reach.

^a Larval counts exclude one successful nest that produced larvae, but logistical constraints prevented us from estimating a minimum number of larvae present.

intervals coincided with key events relevant to early development and nest fate: day 0 (within 48 h of oviposition), about day 30 (approximately early October: midembryonic development), about day 60 (approximately early November: egg hatching), and about day 210 (approximately late March/early April: larvae preparing to emerge from nest). To process a nest, we blocked the entrance of the shelter and obstructed the stream current around the shelter using a large piece of plywood inserted vertically into the stream and perpendicular to flow. This technique created an eddy around the shelter and allowed us to safely open the shelter lid without its contents washing out. We removed the attending male hellbender from the nest at each nest-monitoring interval. We tagged (passive integrated transponder tags; Biomark, Boise, ID) unmarked males at oviposition and scanned all males each time they were captured to confirm their identity. Males underwent standard morphometric processing (mass, total length, and snout-to-vent length) and health assessments (documentation of injuries and abnormalities, external parasite assessments, blood sampling, tissue swabs for assessment of fungal infections) described in detail in our previous studies (e.g., Hopkins and DuRant 2011; DuRant et al. 2015; Hopkins et al. 2016, 2020; Jachowski and Hopkins 2018). Even though males from all sites were subjected to the same handling procedures, we sought to further confirm that repeated handling of males would not affect nest fates. In 2016, we handled only males ($N = 21$ nests) at oviposition to ensure that repeated handling of males did not differentially influence our estimates of nest fates across forest covers. This out-group validated our handling procedures: at sites with low forest cover, 100% of nests failed and 67% were cannibalized (compared with 85% and 44% in the full dataset, respectively; see table 2). At sites with high forest cover, 44% of nests failed and 16% were cannibalized (compared with 51% and 14% in the full dataset, respectively; see table 2).

At the first two nest-monitoring intervals (days 0 and 30), we removed the entire clutch of eggs and placed it in a large, sterilized plastic bin with ~2 cm of stream water. We placed a piece of plexiglass over the eggs and gently compressed the clutch, resulting in a layer one to three eggs deep spread across the bottom of the plastic bin. We then photographed the clutch and returned it to the nest. Photographs were later used to digitally quantify clutch size by counting eggs using ImageJ software (National Institutes of Health, Bethesda, MD). At the third nest-monitoring interval (day 60, November), we photographed and videoed the interior of each shelter chamber to provide a record of eggs hatching and the status of the clutch; nonviable eggs and dead embryos are easily distinguished from healthy embryos (e.g., eggs with dead embryos are cloudy and swollen). At the final nest-monitoring interval (March/April), we illuminated the nest with dive lights while photographing and videoing (360°) the interior of the shelter while moving debris that obstructed imaging. Photographs and videos were reviewed to provide minimum estimates of the number of gilled larvae present in the nest near the time of emergence. During the first 2 years of the study (2013–2014) and for a subset of nests (six of nine nests) processed in 2015, we generated actual counts of emerging larvae in the spring by removing the entire shelter from the stream and flushing it with stream water into a large plastic bin. This technique likely produces more precise larval counts than our photo- and video-generated estimates because larvae can possibly be missed in digital images if they are hidden beneath debris, but removal counts are highly destructive to the interior nest habitat, dangerous for the larvae, and logistically impractical with large numbers of nests. Therefore, in all subsequent years we relied on video and photographic records to provide minimum estimates of larvae in each nest prior to emergence. Thus, our estimates for the last five-plus years of the study likely underestimate the actual number

Table 2: Summary of nest fates at all sites in all years as well as sites with high and low catchment-wide riparian forest cover

	All sites ($n = 180$)	Forest cover	
		<63% ($n = 46$)	>63% ($n = 134$)
Success	41 (17–65)	15	49
Failure	59 (35–83)	85	51
Whole-clutch cannibalism	22 (1–43)	44	14
Abandonment	3 (0–8)	4	2
Predation	2 (0–10)	4	2
High flows	5 (0–20)	2	7
Unknown	27 (13–41)	31	26

Note: Data are percentages. Nest failures are broken down by specific causes when known. Forest cover was handled as a continuous variable in all statistical models but is presented here categorically for visual purposes only. Numbers in parentheses are 1 standard deviation away from the mean. Two small aberrant nests (see “Methods”) are not included.

of larvae emerging from successful nests. However, failed nest numbers are not affected by our estimation technique because once a nest failed we were able to dismantle the interior of the nest to confirm that no larvae were present. Of the nests that failed over the course of the entire study, 83% did so by November (day 60, hatching).

We categorized the fate of nests based on our cumulative observations at the four nest-monitoring intervals. In brief, we categorized nests as successful if they contained at least one larva in the spring and the attending male was present during at least three of the four monitoring intervals. Nests were considered to have failed if they no longer contained eggs or larvae. We attributed failure of nests to the following causes: abandonment, predation, destruction due to high-river-flow events, filial cannibalism of the whole clutch, and unknown causes (table 2). Abandoned nests were characterized by the male leaving the clutch permanently unattended. Predated nests were characterized by complete loss of the clutch and the male displaying fresh, severe wounds (e.g., deep cuts, complete limb loss) that were not present at nest establishment. The identity of predators was never confirmed but was possibly other hellbenders because the wounds documented after nest predation events were sometimes consistent with wounds in males leading up to the breeding season, when hellbender combat over nest sites is common. Although rare, high flows associated with large storms occasionally dislodged shelters, dislodged shelter lids, or filled the nest cavity with sediment, causing nest failure. Whole-clutch cannibalism was often confirmed at day 30 or 60 because the male regurgitated eggs during capture, and typically at least two of the following characteristics were present: extreme bloating and an obviously distended abdomen (which is easily differentiated from feeding on crayfish by simple palpation), significant weight gain ($>10\%$ since the prior monitoring interval, compared with the only 1.5% weight gain typical of males not engaging in whole-clutch cannibalism), and the clutch was torn apart with eggs missing. However, males did not always regurgitate eggs during capture. Therefore, we classified individuals as whole-clutch cannibals if the nest and the male exhibited two of the three characteristics listed above in a monitoring interval leading up to the nest's complete failure. Our method of categorizing cannibals is likely conservative, given that some failures of unknown causes were possibly cannibalistic events that we could not detect because of the timing of our sampling strategy. Nests that could not be categorized as abandonment, predation, destruction, or filial cannibalism based on our conservative criteria were classified as failing for unknown causes. For example, on several occasions we found males at day 30 or 60 with no clutch, with no injuries, without multiple lines of evidence for cannibalism, and without experiencing high-flow events.

Data Analysis

Prior to statistical analyses, we removed two nests from our dataset that had fewer than 20 eggs and failed immediately with no attending male; these two nests were most likely aberrant partial clutches representing either a failed breeding attempt or remnant eggs deposited after a female's nearby breeding attempt. We also removed six nests from models because of missing data for body size and/or body condition of the attending male as well as a further six nests that lacked clutch size information.

Forest Cover and Hellbender Traits. Because male characteristics (size/age and body condition) and clutch size are commonly hypothesized drivers of cannibalism, we first sought to investigate how these biological traits may have varied across the gradient in upstream forest cover. Furthermore, our past work showed that population density and age structure are correlated with upstream catchment-wide riparian forest cover (Jachowski and Hopkins 2018), further necessitating an exploration of the relationship between upstream forest cover and these traits. Therefore, we used linear mixed effects models to investigate the relationships between our continuous variables of interest: (1) forest cover and body size (total length [TL]) and (2) forest cover and body condition (scaled mass index [SMI]; Peig and Green 2009). SMI is a way of quantifying the body condition of an individual by scaling the individual's mass and TL relative to a reference measure of body size. In our populations, we used the median TL for adult males (41.0 cm) for this reference measure. We used SMI as our measure of body condition because it has been shown to be a more accurate indicator of relative energy reserves than more commonly used indices of condition (e.g., residuals from an ordinary least squares regression) for a wide range of taxa, including amphibians (Peig and Green 2009). Based on a representative set of 1,303 capture events of adult males in our populations, the median SMI is 415 (range = 272–605, with a normal distribution), with 80% of males falling within a “normal” range of 357–485. The 10% of individuals above this range typically have ample stomach contents (e.g., multiple crayfish from recent foraging) and/or extraordinary fat stores in their tails. The rare 10% below the normal range are lean and sometimes show signs of injury or illness.

TL and SMI of attending males at nest establishment was used in all models. Both linear models included random intercepts for each site, year, and individual, to account for nonindependence of observations. Standardized parameters were obtained by centering and scaling body size measurements and body condition. To understand whether the environmental and biological variables we measured explained variation in clutch size, we used a

generalized linear mixed effects model with TL, SMI, and forest cover as fixed effects. We modeled the discrete response variable (clutch size) with a negative binomial distribution and a log link function. This model also included site, year, and individual as random effects. Confidence intervals (CIs) and P values for all three models were computed using the Wald approximation (Wald 1947; Makowski et al. 2020).

Factors Associated with Nest Fate and Larval Emergence. We next sought to understand the effect of environmental factors (forest cover) and biological factors (clutch size and male characteristics) on nest fates. We used a Bayesian multinomial logistic regression with three outcomes: success, failure due to whole-clutch cannibalism, and failure due to other reasons. We first fitted models with single predictors of nest fate, including percent upstream catchment-wide riparian forest cover, initial clutch size, and male TL and SMI at nest establishment. We then considered candidate models with pairwise interactions between each of these biological traits and forest cover. Individual male identity was included as a random effect (random intercept) in all models considered. We were unable to include site and year as random effects because of overparameterization issues (see below for a discussion of model diagnostics). We view this approach as conservative and the most likely to lead to robust inferences, as it minimizes overfitting and also minimizes the chance of spurious inferences that could arise as a result of underlying covariance between predictor variables, which could be an issue in more complex models (Vehtari and Ojnenen 2012; Gelman and Shalizi 2013).

We compared this suite of candidate models using leave-one-out cross validation (LOOCV) and the leave-one-out information criterion (LOOIC; Vehtari et al. 2017). This method assesses the predictive ability of each of our suite of models by iteratively leaving out each data point and calculating each model's ability to predict that point. We chose this method because it is a commonly accepted way to capture both the within-sample and the out-of-sample predictive ability of each model and it was computationally feasible for this analysis (Gelman et al. 2014; Vehtari et al. 2017). Similar to other information criteria, LOOIC includes a penalty that accounts for the effective number of parameters (Gelman et al. 2014). Model selection was then performed by comparing LOOIC values, as well as the standard error of the difference between candidate models, to help determine whether models were substantially different (Vehtari et al. 2017). We computed LOOIC statistics and LOOIC weights for each of our fitted models using the *loo* package (Vehtari et al. 2021).

For all nests with eggs, the number of larvae emerging from artificial shelters was modeled in a Bayesian nonlin-

ear modeling framework with clutch size as a predictor and a negative binomial error distribution to account for overdispersion. We modeled the relationship between initial clutch size and number of larvae emerging as a logistic function such that

$$y = \frac{a}{1 + (a - 1)e^{-bx/100}},$$

where x represents initial clutch size, y is the number of emergent larvae, and a and b are estimated parameters that determine the curve's asymptote and inflection point, respectively. Our choice of a nonlinear model was driven by visual inspection of the relationship between clutch size and emergent larvae and lower residual variance compared with linear parameterizations (results not shown). While the nonlinear structure matched the biological expectation that density-dependent survival of offspring occurs within nests, it precluded the inclusion of either random effects or environmental covariates, such as those used in the nest fate model.

Details of Model Fitting Methods. We used R version 4.0.3 (R Core Team 2021) for all statistical analyses. The relationships between forest cover and characteristics of attending males and clutch sizes were modeled with the *lme4* and *report* packages (Bates et al. 2015; Makowski et al. 2020). Bayesian modeling of nest fates and count data of larvae emerging from nests was performed using the *brm()* function in the *brms* package (Bürkner 2017). We obtained highest density intervals and posterior predictive draws using the *bayestestR* and *tidybayes* packages (Makowski et al. 2019; Kay 2021). Plots were constructed using the *ggplot* and *see* packages (Wickham 2016; Lüdtke et al. 2021).

For both the nest fate model and the larval emergence model, we performed Markov chain Monte Carlo simulations using a Gibbs's sampler, running 11,000 iterations of three chains and discarding the first 1,000 draws as burn-in. Every tenth estimate was drawn from each chain to produce posteriors composed of 1,000 samples. We visually inspected trace plots to assess convergence and estimated potential scale reduction factors and effective sample sizes for each parameter. We used vague priors for all parameters and set bounds based on biologically reasonable limits to prevent impossible outcomes (e.g., negative numbers of emergent larvae). All fixed effects in the multinomial regressions were assigned normal priors with a mean of 0 and a standard deviation of 5, and all random effects were assigned half-Cauchy priors. For the emergent larvae model, parameters a and b were both assigned normal priors with means of 1 and standard deviations of 5. All priors were considered to be uninformative, and model results were not sensitive to the prior specifications in either case.

Results

During this 8-year study, we monitored 182 hellbender nests established in our artificial shelters at sites spread across a gradient of upstream forest cover (table 1). Nests were guarded by 101 unique males over the course of the study. We were unable to identify the guarding male in six instances because of nest failure within days of detection (e.g., immediate nest abandonment). Nests were established in 108 unique artificial shelters, 45 of which were nested in more than once during the course of the study (range = 2–6 nests per shelter). Some males nested in multiple years in shelters: 57 males nested once, 25 males nested twice, 11 males nested three times, four males nested four times, and four males nested five times. Of the 44 males that nested multiple times in our shelters, 28 (64%) nested more than once in the same artificial shelter (range = 2–4 by the same male in the same shelter).

Characteristics of Males and Their Nests

Characteristics of males varied within and among reaches. The size of males at nest initiation was highly variable (TL = 33–56 cm; range in mass = 212–1,020 g). Most males with nests had a relatively high body condition (mean SMI = 411; range = 299–517). We rarely encountered a nesting male that was visibly thin; only 5.9% of males captured at nest initiation were below the population's historical 10th percentile SMI (i.e., SMI = 357). We found no clear relationship between forest cover and body size ($\beta = -0.28$, 95% CI = -0.57 to 0.01 , $P = .06$; fig. 2) or forest cover and body condition ($\beta = -0.07$, 95% CI = -0.31 to 0.16 , $P = .55$; fig. 2), although there was a statistically insignificant tendency for males to be longer at sites with low forest cover.

Clutch sizes were highly variable among nests (mean = 304, range = 51–1,139; excluding the two aberrant nesting attempts mentioned above), but this variation was not explained by factors examined in our study. We did not detect relationships between clutch size and male size (TL; $\beta = 0.05$, 95% CI = -0.04 to 0.15 , $P = .26$), male body condition (SMI; $\beta = -0.05$, 95% CI = -0.13 to 0.04 , $P = .26$), or forest cover ($\beta = -0.04$, 95% CI = -0.15 to 0.07 , $P = .45$). Although the full model with random effects had substantial explanatory power (conditional $R^2 = 0.28$), only 3% of the variability in clutch size was explained by our three fixed effects.

Summary of Patterns of Nest Fate and Larval Emergence at Different Sites

At the four sites (stream reaches) with >63% of catchment-wide forest cover ("high"-forest-cover sites, with healthy

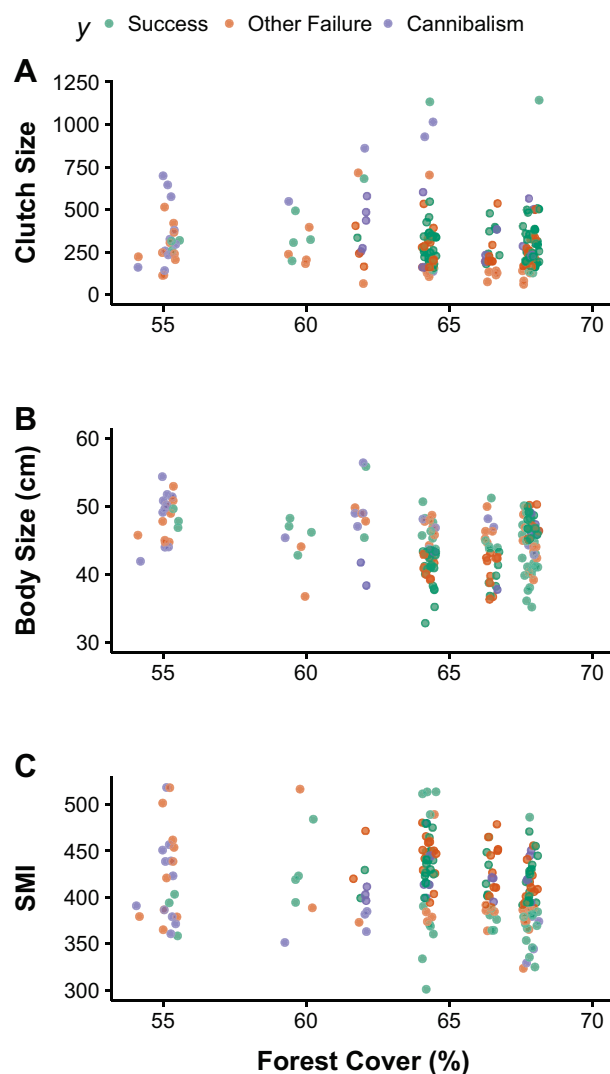


Figure 2: Morphometrics of male hellbenders and their clutch sizes. Shown is the relationship between forest cover and initial clutch size (A; number of eggs), the body size of attending males (B; total length in centimeters), and body condition (C; scaled mass index [SMI]) at nest establishment. There was no discernible relationship between forest cover and these three biological traits (see text for details). Points display the raw data, are color coded by nest fate, and are offset slightly on the x-axis to enhance clarity of overlapping individual points.

hellbender populations; Button et al. 2020a), approximately half of the attending males successfully reared young to emergence, in contrast to only 15% of males at the six reaches in watersheds with "low" forest cover (<63%; table 2). Of the causes of nest failure that we were able to document, whole-clutch filial cannibalism was the most common across all stream reaches. However, whole-clutch filial cannibalism was three times more prevalent at low-forest-cover reaches compared with reaches with higher

forest cover (table 2). Other sources of nest failure, including predation, abandonment, and major storms, appeared relatively constant (cumulatively 10%–12%; table 2) across the gradient of upstream forest cover in our study. Unknown sources of nest failure accounted for roughly a quarter of total nest failures in reaches with both low and high forest cover (failure rates from unknown causes were 31% and 26%, respectively; table 2).

Biological and Environmental Factors Contributing to Nest Fate and Larval Emergence

In our analyses with forest cover as a continuous covariate, model ranking indicated that nest fate was strongly influenced by both upstream forest cover and clutch size but was not related to characteristics of the attending male hellbender (body size or condition). Specifically, our top-ranked model indicated strong evidence for an interaction between upstream forest cover and clutch size (table 3), such that nest success was positively correlated with clutch size at nest establishment in reaches with high forest cover but showed no relationship with clutch size at reaches with low forest cover (fig. 3). Failure specifically due to cannibalism at low-forest-cover sites was strongly associated with clutch size, with the largest clutches being much more likely to be cannibalized than the smallest clutches (fig. 3a). In contrast, at high-forest-cover sites, the probability of failure due to cannibalism remained low across all clutch sizes (fig. 3b). Nest failure due to reasons other than cannibalism disproportionately affected smaller clutches regardless of forest cover (fig. 3). We found no support for models that included body size and body condition at nest establishment as predictors of nest fate and no support for any of the other interactive models we considered (table 3). Posterior distributions for all parameter estimates are provided in figure S1.

Of all the eggs laid during the course of the study, 9% survived to emergence the following spring. When considering only successful nests, egg survival was 22% on

average, but there was substantial variation among successful nests (fig. 4). The number of larvae surviving to emergence from artificial shelters was positively correlated with clutch size at nest establishment, but the relationship reached an asymptote at ~150 emerging larvae (95% credible interval = 107–200; fig. 4). Counts of the number of emerging larvae were highly overdispersed; the largest clutches, nests containing more than 1,000 eggs, could completely fail, yielding no larvae, or could succeed with half of the initial clutch surviving to emergence (95% prediction interval = 3–500; fig. 4). Posterior distributions for estimated parameters are provided in figure S2.

Bayesian Model Diagnostics

For both Bayesian models, all potential scale reduction factors were below 1.1 and all effective sample sizes were greater than 1,000, indicating adequate model convergence. For the multinomial model comparison, Pareto k diagnostics for all of the LOOCV calculations were below 0.5.

Discussion

In this study, we provide clear evidence that whole-clutch filial cannibalism may be a normal part of the life history of eastern hellbender salamanders but that the behavior may reach unsustainable frequencies in degraded habitats and may thus be a previously overlooked mechanism of population declines. The probability of nest failure due to filial cannibalism was low (14%) in stream reaches with relatively high upstream riparian forest cover and unaffected by clutch size in such reaches. In contrast, nearly half (44%) of all nests at reaches with substantial loss of upstream riparian forest cover (<63% intact) failed because of whole-clutch cannibalism, with larger clutches most likely to be cannibalized in these degraded reaches. Neither body size nor body condition of males were important predictors of cannibalism. Other sources of nest

Table 3: Comparison of Bayesian multinomial models of nest fate

Model	P_{LOO}	LOOIC	LOO _{SE}	ΔLOOIC	$\Delta\text{LOOIC}_{\text{SE}}$	w_i
Fate ~ clutch size × forest cover	41	339.7	15.8	.0	...	.79
Fate ~ forest cover	41.8	343.0	12.7	3.3	4.5	.15
Fate ~ clutch size	40.4	346.3	13.5	6.6	3.7	.03
Fate ~ body size × forest cover	48.1	347.6	13.8	7.9	5.1	.01
Fate ~ body condition × forest cover	50.9	348.1	14.4	8.4	4.9	.01
Fate ~ body size	40.1	349.0	11.9	9.3	6.1	.01
Fate ~ 1	45.1	351.9	10.8	12.2	5.5	.00
Fate ~ body condition	46.9	354.2	11.6	14.5	5.6	.00

Note: Models were ranked using leave-one-out cross validation (LOOIC) and LOOIC weights (w_i). Standard errors for both LOOIC and ΔLOOIC are reported, and P_{LOO} is the effective number of parameters.

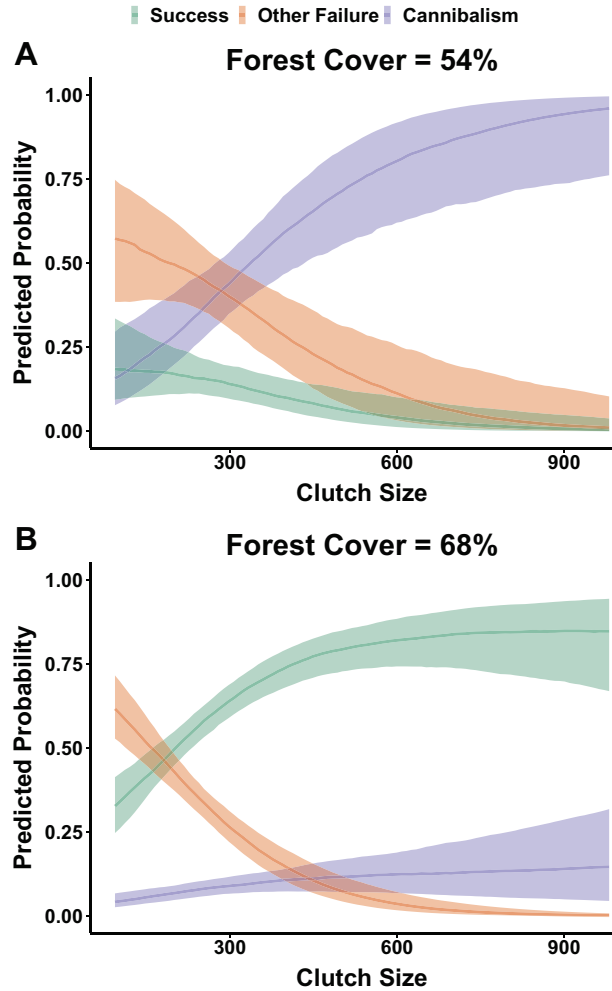


Figure 3: Influence of forest cover and clutch size on nest fate. Shown is the predicted probability of nest fate (success, failure due to whole-clutch cannibalism, failure due to other reasons) given an interactive effect between clutch size and catchment-wide riparian forest cover. *A* shows the predicted probabilities of nest fate in relation to initial clutch size at the lowest forest cover (54%) in our study, and *B* shows the predicted probabilities of nest fate in relation to initial clutch size at the highest forest cover (68%) in our study.

failure, such as abandonment, predation, high-flow events, and unknown causes, occurred at similar frequencies across reaches.

Our results did not support one of the most common hypotheses explaining the occurrence of filial cannibalism. The energy-based hypothesis predicts that guarding male hellbenders may consume their eggs to meet energy deficits, prioritizing self-maintenance over their immediate reproductive opportunity (Klug and Bonsall 2007; Klug et al. 2012). Such a decision may be adaptive in long-lived animals like hellbenders that invest consider-

able time into parental care (~8 months) during each reproductive season. Thus, under this energy-based prediction the frequency of filial cannibalism should be highest in individuals in relatively low body condition. In contrast to this prediction, we rarely found lean males with nests, and we found no evidence to indicate that reduced body condition was related to the decision to cannibalize young. Energy-based explanations also failed to explain the pronounced differences in cannibalism rates among stream reaches with low and high forest cover upstream; on average, males were in similar body condition across all reaches represented in this study (fig. 2).

In long-lived iteroparous species, theory also predicts that filial cannibalism may be more advantageous in smaller/younger adults that have greater expected future reproductive opportunities than older individuals (Klug and Bonsall 2007; Klug et al. 2012). We found no support for this prediction either; the probability of whole-clutch filial cannibalism was not related to body length, regardless of upstream forest cover conditions (fig. 3). We did find that males were ~10% longer at sites with the lowest forest cover compared with higher forest cover (fig. 2), which was consistent with prior findings from mark-recapture studies in our study system (Jachowski and Hopkins 2018). Although TL of hellbenders can be used as a rough proxy for their age (Taber et al. 1975), larger body size in areas of reduced forest cover also may be partly related to reduced population density and therefore less intraspecific competition for resources (Jachowski and Hopkins 2018). Thus, body size also failed to explain the high rates of cannibalism at our most degraded sites because males were slightly larger at these sites, which is the opposite of theoretically expected relationships between body size and cannibalism.

The reproductive value of small clutches is lower than that of larger clutches of eggs (Stearns 1992), and thus the probability of whole-clutch filial cannibalism should be greater for smaller clutches (Sargent and Gross 1985; Manica 2002). We found strong evidence that clutch size was an important predictor of whole-clutch filial cannibalism, but this relationship was highly dependent on the interaction of clutch size with forest cover (table 3; fig. 3). In reaches with comparatively high forest cover, the frequency of cannibalism was low and fairly consistent across a wide range of clutch sizes. In these same stream reaches, the probability of nests succeeding increased steeply with clutch size, which is consistent with theoretical predictions based on higher reproductive value of larger clutches. In stark contrast, the probability of cannibalizing eggs actually increased steeply with clutch size in reaches with lower forest cover. From a life history perspective, this pattern should be highly maladaptive and suggests a possible pathological basis of filial cannibalism

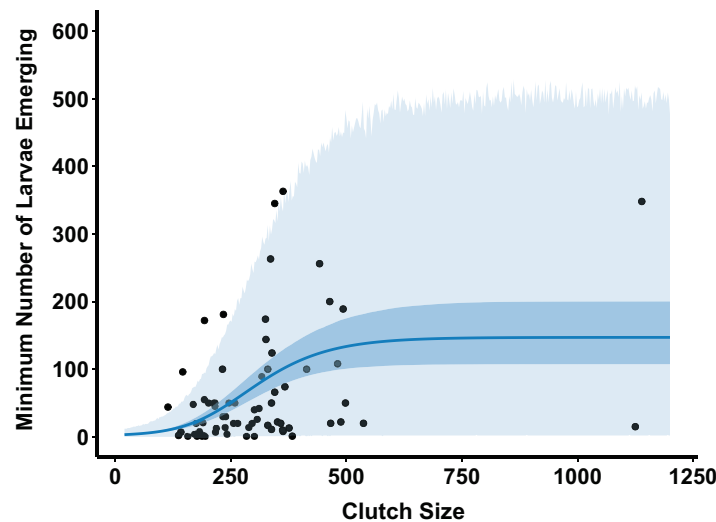


Figure 4: Emergent larvae as a function of clutch size. Shown is the predicted minimum number of larvae emerging from nests given initial clutch size. Counts were modeled in a Bayesian nonlinear modeling framework assuming a logistic relationship and a negative binomial error distribution. The dark shaded area represents 95% credible intervals for the mean response, and the light shaded area represents 95% prediction intervals based on the overdispersion estimated from the negative binomial formulation.

in degraded reaches. It seems plausible that the oxygen demands of large clutches can exceed that available within a nest cavity, triggering the male to adopt self-maintenance behaviors, abandon care, and switch to a cannibalistic strategy. Indeed, in some fish species that provide paternal care, low oxygen is known to promote tail fanning and construction of large nest entrances that improve oxygenation, as well as partial filial cannibalism to presumably improve the developmental conditions for remaining eggs (Payne et al. 2002, 2004; Lissåker et al. 2003; Klug et al. 2006; Olsson et al. 2016). Unlike some of these fishes, hellbender clutches can be enormous and thus possibly deplete the oxygen available for the attending father. In our system, clutch volumes range up to 5,300 cm³, which is equivalent to 79% of the nest chamber's total volume. Thus, we hypothesize that the sheer physical size of the clutch within the nest cavity coupled with its oxygen demands can exceed the space and dissolved oxygen available for the male within the nest cavity. Given that clutches were of similar sizes across all stream reaches (fig. 2), this hypothetical negative effect of clutch size on oxygen availability may be apparent and cause high rates of whole-clutch cannibalism only in degraded reaches because reductions in upstream forest cover are associated with greater siltation and changes to water chemistry (e.g., Wood and Armitage 1997; Sutherland et al. 2002; Sheldon et al. 2012; Jachowski and Hopkins 2018). Hellbenders may be particularly sensitive to even modest reductions in dissolved oxygen because of their large body size and reliance on cutaneous respiration.

There are several alternative mechanisms that could explain high rates of whole-clutch filial cannibalism in reaches with reduced forest cover upstream. For example, selective consumption of nonviable or slow-developing eggs is known to occur in other systems (Klug and Lindström 2008), including the closely related Japanese giant salamander (Okada et al. 2015; Takahashi et al. 2017). It is possible that females in degraded reaches produce eggs of low quality, that males inadequately fertilize them, and/or that water chemistry in degraded reaches is not conducive to embryonic development. Each of these possibilities could trigger cannibalism of clutches that have excessive numbers of eggs unlikely to develop properly. Therefore, one hypothesis for the increased frequency of cannibalism at larger clutch sizes in reaches with reduced forest cover upstream is that larger clutches may be more challenging for males to care for and thus be more prone to embryonic mortality in situations where water quality has deteriorated (e.g., higher siltation on eggs, lower dissolved oxygen in nest), triggering the male to consume them because they are dead. Alternatively, changes to water chemistry could alter male hormonal or neuroendocrine signals (e.g., endocrine disruption) necessary for the maintenance of parental care behaviors (Rodgers et al. 2013). For example, evidence from captive breeding of hellbenders suggests that their reproductive physiology may be sensitive to changes in the ionic composition of the water (Ettling et al. 2013), one of the strongest instream habitat correlates of forest cover in our study system (Jachowski and Hopkins 2018). Finally, uncertain paternity

is known to trigger nest abandonment or filial cannibalism in some fish (Neff and Gross 2001; Neff 2003a, 2003b; Alonzo and Heckman 2010). It is plausible that similar mechanisms influence male hellbenders that cannibalize eggs. However, the frequency of multiple paternity in hellbender nests is unknown. Clearly, future work that identifies the proximate cue or cues that promote filial cannibalism in hellbenders will increase our understanding of the natural history of the species and how the frequency of this intriguing behavior is being affected by rapid environmental change.

Our findings of impaired reproduction due to filial cannibalism in stream reaches with relatively low forest cover reveal important insights into potential mechanisms underlying precipitous population declines of hellbenders. One of the hallmark characteristics of their population declines at sites with low forest cover upstream is a shift toward a geriatric age class structure, with little to no evidence of young age classes in low-density populations (Jachowski and Hopkins 2018). These observations have led to the hypothesis that reproductive impairment and/or poor survival of early larval age classes are the cause of declines (Wheeler et al. 2003; Briggler et al. 2007; Burgmeier et al. 2011; Jachowski and Hopkins 2018). Importantly, we found that hellbenders are able to produce eggs and mate in reaches with low forest cover upstream, reducing the likelihood that problems with egg production are the limiting factor for hellbender populations. However, at our sites with low forest cover upstream, the vast majority of hellbender egg clutches are cannibalized or otherwise destroyed within the first 2 months of the parental care period. The net result is that larvae rarely emerge from hellbender nests in these degraded reaches. Moreover, on the rare occasions that nests at degraded sites succeeded in producing larvae, they produced far fewer larvae than at higher-quality sites; we observed more than 100 larvae emerging from nests in reaches with >63% forest cover on 13 occasions but never documented this in reaches with <63% forest cover. Collectively, our results support the hypothesis that reproductive impairment—including cannibalism—could be driving population declines. While our observations do not exclude the possibility that poor larval survival also contributes to population declines, they suggest that an increased focus on reproductive impairment and parental care is warranted.

Although the underlying mechanisms that trigger high rates of whole-clutch cannibalism and nest failure in degraded reaches remain unclear for hellbenders, our results have practical management implications for this species and freshwater streams more generally. First and foremost, our results highlight the unpredictable negative effects that the loss of riparian forest can have on freshwater biodiversity and contribute to a growing body of evidence that the loss of upstream riparian forest cover is associated

with hellbender population declines (USFWS 2021). Thus, protection of existing riparian forest, restoration of depleted riparian forest across entire catchments, and implementation of best land management practices is of paramount importance to hellbender conservation. The benefits of such management decisions are far-reaching, as they will also benefit other freshwater fauna, such as native mussels, macroinvertebrates, snails, and fishes. Second, augmentation of instream nesting habitat may not be an effective near-term management strategy for hellbenders. Placement of artificial nest boxes and large flat rocks in degraded habitats is becoming a common practice in several states, but its efficacy as a restoration tool has not been established. Although such practices are useful for collecting eggs for captive propagation and to provide needed shelter for adult hellbenders in situations where physical habitat is limited (e.g., due to siltation), our results indicate that this strategy is unlikely to address underlying causes of population declines because most hellbender nests fail in degraded reaches due to lack of adequate parental care. Last, because the vast majority of hellbender nest failures occur early in offspring development, our results point to the need for a management approach that combines upstream forest cover restoration with interventions that mitigate the effects of chronic nest failure. Pinpointing the proximate cues in streams that cause males to cannibalize their eggs will inform management and restoration strategies to promote paternal care and nest success in hellbenders.

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Statement of Authorship

W.A.H.: conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, writing—original draft, visualization, supervision, project administration, funding acquisition; B.F.C.: methodology,

validation, formal analysis, investigation, data curation, writing—review and editing, visualization; J.G.: methodology, formal analysis, investigation, data curation, writing—review and editing, visualization, supervision; G.C.B.: formal analysis, writing—original draft, visualization; C.M.B.J.: conceptualization, methodology, investigation, data curation, writing—review and editing, supervision; S.T.B.: investigation, formal analysis, writing—review and editing; J.J.H.: methodology, investigation, data curation, writing—review and editing; R.S.M.O.: investigation, writing—review and editing; H.K.K.: formal analysis, writing—review and editing.

Data and Code Availability

The data, statistical code, and examples of nest videos and clutch photos that support the findings of this study have been archived in the Dryad Digital Repository (<https://doi.org/10.5061/dryad.7m0cfxp21>; Hopkins et al. 2023).

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An adult male eastern hellbender salamander (*Cryptobranchus alleganiensis*) captured while actively cannibalizing his eggs, visible in his mouth. The male also regurgitated partially digested eggs upon capture. Photo credit: Brian Case.