

## Loss of catchment-wide riparian forest cover is associated with reduced recruitment in a long-lived amphibian



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### ABSTRACT

Land use alteration is recognized as a threat for many aquatic species, but demographic drivers of land use associated declines are poorly studied. We examined hellbender, *Cryptobranchus alleganiensis*, demography in six stream reaches stratified across a land use gradient to understand how land use might influence a long-lived species. We used robust-design surveys (2014–2015) to estimate abundance and demographic structure, and all captures recorded between 2007 and 2015 to estimate demographic rates. Catchment-wide riparian (CWR) forest predicted demography better than catchment or local riparian forest. Across space, sub-adult/adult abundance declined and demographic structure became increasingly skewed towards older adults as CWR forest declined. Demographic rates indicated sub-adults/adults were being lost from each reach at a similar rate and most populations remained stable over the period for which data were available (1–8 years per reach). Our findings suggest recruitment (via births, juvenile survival and/or immigration) of young age classes facilitated stability of high-density populations when CWR forest was relatively high. When CWR forest was lower, survivorship and longevity of old adults facilitated persistence of low-density populations for multiple years while recruitment of young age classes suffered. Fine sediment was not correlated with land use but water temperature, conductivity and pH declined as CWR forest increased, highlighting water quality as a possible mechanism linking forest cover to hellbender demography. Our findings suggest maintaining forest in upstream riparian areas is critical for conserving downstream biota, and emphasize the difficulty of detecting declines in long-lived species when environmental alterations act specifically on recruitment of young age classes.

### 1. Introduction

Identifying factors that influence the abundance and distribution of species is fundamental to effective conservation planning. Freshwater fauna rank among the most threatened on Earth, largely as a result of rapid environmental changes caused by humans (Dudgeon et al., 2006). Habitat degradation as a result of surrounding land use alteration is recognized as one of the greatest threats to freshwater biodiversity at a global scale (Sala et al., 2000). While numerous species are known to respond negatively to increases in human land use intensity (Dudgeon et al., 2006), few studies have attempted to investigate the demographic mechanisms responsible for declines associated with land use (Österling and Högberg, 2014). That is, for most species, we know little about whether land use associated declines are driven primarily by insufficient recruitment (births, juvenile survival, and/or immigration) and/or excessive losses (deaths and/or emigration).

Building a more mechanistic understanding of how environmental alterations influence the abundance and persistence of species can

benefit conservation planning in multiple ways. First, demographic studies can highlight the relative benefit of conserving adult age classes versus improving survivorship of immature age classes when goals are to bolster wild populations. For example, research has highlighted the importance of maintaining high adult survivorship and/or immigration rates to ensure persistence of some turtles (Congdon et al., 1994) and pond-breeding amphibians that experience high variance in annual reproductive success as a result of environmental stochasticity (Taylor et al., 2006). Second, understanding how demographic rates respond to environmental alteration can highlight important factors to consider during monitoring. Monitoring efforts are often designed to provide early warning of declines that threaten population persistence. While focusing monitoring efforts on any age class might be appropriate for species with short generation times (Carginan and Villiard, 2002), biologically relevant shifts in the abundance of young age classes may take several years before culminating in a measurable change in adult abundance for slower paced life history strategists. Identifying the sensitivity of specific demographic rates to environmental change can

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thus facilitate earlier detection of population declines and development of more effective conservation strategies.

In the current study, we investigated associations between land use, in-stream habitat alteration and demography of a fully aquatic, long-lived, stream dwelling salamander of increasing conservation concern (USFWS, 2011a, 2011b). The hellbender, *Cryptobranchius alleganiensis*, is a large (up to 74 cm), benthic amphibian with a slow-paced life history strategy (Peterson et al., 1988; Taber et al., 1975). Their high degree of habitat specialization and apparent sensitivity to anthropogenic disturbance make hellbenders a likely umbrella species for stream ecosystems throughout their range. Hellbenders have experienced enigmatic, range-wide, precipitous declines since the 1970s and 1980s (Burgmeier et al., 2011; Foster et al., 2009; Graham et al., 2011; Wheeler et al., 2003). Other extant members of Family Cryptobranchidae include the Japanese giant salamander, *Andrias japonicus*, and Chinese giant salamander, *A. davidianus*, which share similar habitat requirements and life history traits with hellbenders and are also threatened with extinction (Ota, 2000; Wang et al., 2004). Cryptobranchid declines are widely suspected to be the result of habitat loss as a result of land use alteration and an associated increase in fine sediment loads (i.e., sand, gravel and clay that fill interstitial space; Sutherland et al., 2002) and decrease in water quality (Brigglér et al., 2007; Okada et al., 2008; Wang et al., 2004). However, efforts to explicitly quantify the effects of land use on Cryptobranchid demography are lacking.

The overarching goal of our study was to better understand the demographic mechanisms through which land use might influence hellbenders. Our specific objectives were to 1) investigate associations between land use and demography (abundance, demographic structure and demographic rates) of local hellbender populations and between land use and in-stream abiotic conditions; and 2) quantify the relative strength of associations between land use within three spatial extents (catchment, catchment-wide riparian area, local-riparian area) and demographic responses. Our second objective was included largely because of variation in the spatial extent of land found to be most closely associated with demography of stream biota (see Willson and Dorcas, 2003). We predicted that local hellbender abundance would decline, demographic structure would become skewed towards older individuals (similar to patterns described by Okada et al., 2008; Wheeler et al., 2003), and that apparent survival ( $\Phi$ ) and population growth rates ( $\lambda$ ) would decline as forest cover declined in surrounding areas. We predicted that water quality would decline, fine sediment (particles < 4 mm) would increase, and the proportion of boulders with cavities would decline as forest cover declined in surrounding areas, based on well established relationships between landscapes and in-stream habitat quality (Allan, 2004). We predicted that abiotic and biotic responses would be more closely associated with the extent of land we defined as the catchment-wide riparian area (collective riparian area throughout the upstream catchment) than with land use in the local riparian area or entire catchment, based on recent work highlighting broad scale measures of riparian condition as the most reliable predictor of in-stream ecological response (Stanfield and Kilgour, 2013).

## 2. Material and methods

### 2.1. Species background

The hellbender is a fully aquatic salamander, typically associated with cool, highly oxygenated and swift flowing stream habitat. Adults (290–740 mm total length) rely heavily on rocky crevices beneath large boulders and bedrock for shelter and nest sites (Nickerson and Mays, 1973). Sub-adults and adults show extremely high site fidelity to stream reaches and specific cavities within them (Bodinof et al., 2012a). Hellbenders exhibit longevity of 25+ years (Taber et al., 1975) and delayed maturity (5–8 year; Peterson et al., 1988). Reproductive and

larval ecology is poorly understood. Spawning occurs annually during a brief (~14 d) period in early autumn, when members of both sexes congregate in and around potential nest cavities that are aggressively defended by a single male (Smith, 1907). Fertilization is external and males that are successful in attracting mates provide parental care for clutches of 100–1000+ eggs (Topping and Ingersol, 1981). Larvae measure 50–120 mm in length and use habitat ranging from deep gravel beds (Nickerson et al., 2003) to interstitial space among cobble and boulder substrate (Hecht-Kardasz, 2011). Following metamorphosis (~18 months, characterized by external gill resorption) hellbenders rely primarily on cutaneous respiration (Guimond and Hutchison, 1973) and have rarely been observed out of water (Coe et al., 2016). Little is known regarding hellbender tolerance to water quality parameters.

### 2.2. Study sites

Our study took place in a portion of the upper Tennessee River basin in southwest Virginia that encompasses portions of both the Blue Ridge and Ridge and Valley physiographic provinces. We studied hellbenders in six stream reaches (hereafter, R1-R6) staggered among three streams ( $n = 1-4$  reaches per stream). The catchment upstream of R5 fell predominantly in the Ridge and Valley (98%) while the majority of catchments upstream of other reaches fell predominantly within the Blue Ridge (range = 66–100%). We selected stream reaches where we knew hellbenders occurred that were also stratified across a relatively wide gradient of catchment land use (Table 1) and reflected the approximate range of land use conditions to which hellbenders are exposed in Virginia (Bodinof Jachowski et al., 2016). Average wetted width of each reach ranged from 13 to 18 m. We defined length (range = 93–129 m) of each reach such that the extent of wetted stream channel was equal (~1680 m<sup>2</sup>) among reaches, thus facilitating comparison of hellbender abundance estimates. Due to the sensitive status of our focal species and threats of illegal collection we do not refer to waterbodies by name.

### 2.3. Quantifying land use

We considered human land use within the local riparian area, the catchment-wide riparian area and throughout the upstream catchment as a whole (Fig. 1). We use 'riparian area' to refer to the 50 m terrestrial corridor along each side of a stream (i.e., 100 m wide in total). Local riparian areas extended 1000 m upstream of a sampling reach (Fig. 1A) while catchment-wide riparian areas included the collective extent of riparian area surrounding all upstream tributaries (Fig. 1B); where tributaries included streams delineated in the National Hydrography Dataset (NHD) Plus version 2.1 (1:100,000 scale; USGS and USEPA, 2012). Catchments included all land within the contributing area upstream of a sampling reach (Fig. 1C). We used ArcMap version 10.1 (Esri, Redlands, CA) to delineate catchments and quantify the percent of each land use type within each spatial extent. We classified land use as forest (deciduous, evergreen, mixed forest and shrub and brush), agriculture (crop and grassland), low-intensity development (< 50% impervious surface) or medium-high intensity development (50–100% impervious surface) based on the 2011 national land cover database (NLCD; USGS, 2014)). For simplicity, we used percent forest as a univariate proxy of land use in each extent (hereafter, LR, CWR or C forest for local riparian, catchment-wide riparian and catchment areas, respectively). Due to concerns that the NLCD might underestimate forest cover in small spatial extents (Nowak and Greenfield, 2010), we validated our estimates of CWR and LR forest cover by visually generating 100 random points within polygons representing each spatial extent, overlaying points onto high resolution (0.3 m) aerial imagery, and visually classifying locations of points as either forest or non-forest. On average, percent forest cover estimated with the NLCD differed from the percent of points located in forest by only  $-0.75\% \pm 2.17\%$  SE; thus we concluded that our NLCD estimates of forest cover were

**Table 1**  
Land use/land cover characteristics for six stream reaches (R1–R6) inhabited by *Cryptobranchius alleghaniensis* and number of mark-recapture sampling occasions used to estimate demographic rates, abundance and demographic structure in each reach. For = Forest, Ag = agriculture, LID = low intensity development, MHD = medium-high intensity development.

|    | Upstream Catchment (%) |    |     |     |     |    | Catchment-wide riparian area (%) |    |     |     |     |    | Local riparian area (%) |    |     |     |      |      | No. sampling occasions used to estimate demographic rates (abundance/demographic structure) |      |      |      |      |      |      |       |
|----|------------------------|----|-----|-----|-----|----|----------------------------------|----|-----|-----|-----|----|-------------------------|----|-----|-----|------|------|---|------|------|------|------|------|------|-------|
|    | For                    | Ag | LID | MHD | For | Ag | For                              | Ag | LID | MHD | For | Ag | For                     | Ag | LID | MHD | 2007 | 2008 | 2009  | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | Total |
|    |                        |    |     |     |     |    |                                  |    |     |     |     |    |                         |    |     |     |      |      |   |      |      |      |      |      |      |       |
| R1 | 92                     | 5  | 3   | <1  | 83  | 7  | 11                               | 11 | <1  | 83  | 3   | 14 | 0                       | 1  | 1   | 1   | 1    | 1    | 1   | 1    | 1    | 1    | 1    | 1    | 1    | 10    |
| R2 | 78                     | 18 | 3   | <1  | 69  | 22 | 9                                | 9  | <1  | 24  | 47  | 29 | 0                       | 0  | 0   | 0   | 0    | 0    | 0   | 0    | 0    | 0    | 0    | 0    | 0    | 7     |
| R3 | 74                     | 23 | 4   | <1  | 69  | 23 | 9                                | 9  | <1  | 45  | 46  | 9  | 0                       | 0  | 1   | 0   | 0    | 0    | 0   | 0    | 0    | 0    | 0    | 0    | 0    | 25    |
| R4 | 71                     | 25 | 4   | <1  | 66  | 25 | 9                                | 9  | <1  | 16  | 62  | 20 | 0                       | 0  | 0   | 0   | 0    | 0    | 0   | 0    | 0    | 0    | 0    | 0    | 0    | 23    |
| R5 | 67                     | 25 | 7   | 2   | 53  | 30 | 13                               | 13 | 3   | 4   | 2   | 64 | 30                      | 0  | 0   | 0   | 0    | 0    | 0   | 0    | 0    | 0    | 0    | 0    | 0    | 8     |
| R6 | 66                     | 30 | 3   | <1  | 62  | 30 | 8                                | 8  | <1  | 74  | 11  | 16 | 0                       | 0  | 0   | 0   | 0    | 0    | 0   | 0    | 0    | 0    | 0    | 0    | 0    | 11    |

reliable.

2.4. Field sampling

2.4.1. Hellbender sampling

We conducted robust design (Pollock, 1982) mark-recapture surveys during summers of 2014 and 2015 to quantify abundance and demographic structure in each stream reach (Table 1). Robust design surveys included two primary and two secondary occasions. We defined primary occasions by year and defined secondary occasions as passes within a year. The model we used to estimate abundance assumed populations in each reach were open between years but closed between sampling occasions within a year. To minimize potential for violating assumptions of closure we conducted secondary occasion surveys within each year as close together in time as possible. We conducted robust design surveys between 6 July and 27 Aug in 2014 and between 23 June and 5 Aug in 2015. We completed secondary surveys within each reach in ≤4 days, with the exception of secondary surveys that were interrupted by heavy rain and required 9 days (R1 in 2014) and 34 days (R5 in 2015) to complete.

During robust-design surveys we searched each reach exhaustively, where we visually scanned the entire wetted stream channel while snorkeling and searched beneath all rocks measuring ≥30 cm in diameter. We searched smaller objects if we noticed a crevice beneath them. When rocks were too large to lift or crevices were located beneath bedrock we used dive lights and tactile searches to locate and capture hellbenders. We marked individuals ≥ 130 mm in total body length with uniquely coded passive integrated transponder (PIT) tags (models HPT8 or HPT12; Biomark Inc., Boise, ID, USA). We implanted PIT tags subcutaneously along the lateral tail musculature and used surgical glue to close the epidermis prior to release. We recorded sex based on external morphology (cloacal swelling in males) and measured total length (mm) and mass (g) of each individual. We recorded capture locations using a hand held Garmin global positioning system (GPS; accuracy ± 3 m). We released individuals at the point of capture after making an effort to return all substrate to the condition in which it was found.

To estimate demographic rates, we pooled data collected during robust-design surveys in 2014–2015 with all available mark-recapture data available from each reach. Data collected outside of robust-design surveys included capture records recorded as early as 2007 during efforts to examine relative abundance, health and physiology of hellbenders in R1–R3 (DuRant et al., 2015; Hopkins and Durant, 2011; Hopkins et al., 2011; Hopkins et al., 2016) and during efforts to examine patterns of species occurrence and seasonal variation in physiology of hellbenders (R1-R6) between 2013 and 2015 (Bodinof Jachowski, 2016; Bodinof Jachowski et al., 2016). Methods used to process, mark and release individuals were consistent across all surveys. However, the frequency and intensity of surveys conducted outside of robust-design surveys varied depending on research goals. For simplicity, we pooled captures by calendar month such that the data could be structured in terms of monthly sampling occasions. As an exception, we considered robust design surveys as separate sampling occasions within a month due to the relatively large number of captures that occurred during each pass. The final number of sampling occasions per reach (range = 7–25) varied considerably, especially prior to 2014 (Table 1).

While estimates of demographic rates can be sensitive to sampling intensity and the timespan over which data are collected, we justified our analysis on multiple grounds. First, we pursued our analysis of demographic rates largely as a complement to our analyses of abundance and demographic structure, rather than a stand-alone study. That is, we suspected that demographic rates could help elucidate underlying demographic drivers of any variation that we detected in abundance or demographic structure. Second, though reaches were monitored for relatively short timespans (range = 1–8 years per reach) relative to hellbender longevity (25+ years; Taber et al., 1975), we viewed these

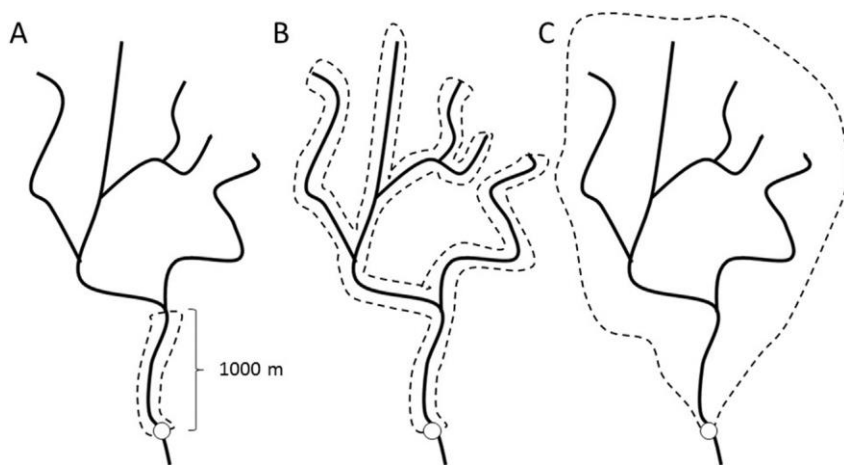


Fig. 1. Various spatial extents used to calculate land use statistics associated with stream reaches (each ~100 m long) where *Cryptobranchus alleganiensis* demography was studied. Extents were defined as the local riparian area (A), catchment-wide riparian area (B) and upstream catchment as a whole (C). Riparian areas include 50 m on each side of a stream (100 m wide total).

timespans as biologically relevant because we were investigating demographic rates in response to a hypothesized threat. More explicitly, we considered it plausible that adult survivorship and population stability might vary across a land use gradient over relatively short time spans, particularly given recent enigmatic and rapid population declines (Wheeler et al., 2003). Third, though sampling intensity varied across time, our modeling approach allowed us to account for observational error related to sampling by incorporating heterogeneity in capture probability among stream reaches and sampling occasions. Finally, despite its imperfections, our dataset ranks among the most robust in existence for hellbenders, thus our findings provide valuable baseline information for the species.

#### 2.4.2. In-stream habitat quality

We quantified abiotic characteristics of in-stream habitat for each reach during 2014–2015. We deployed HOBO® (Onset Corp, Bourne, MA, USA) model Pro v2-U22–001 temperature loggers in each reach. We set loggers to record water temperature (accuracy =  $\pm 0.2$  °C) at 1 h increments between Sep 2014 and Aug 2015. We visited each reach approximately monthly from Sep 2014–Aug 2015 to measure dissolved oxygen (mg/L), conductivity ( $\mu\text{S}/\text{cm}$ ), salinity, total dissolved solids (mg/L) and pH using a YSI 556 MPS portable water probe (Yellow Springs, OH, USA). We quantified substrate characteristics in May 2015 using a modified Wolman (1954) Pebble Count. In our modification we measured substrate at 100 points from 20 cross-sectional transects (five points per transect) stratified evenly across each reach. We recorded the Wentworth (1922) size class of substrates at each sampling point as either bedrock, boulder ( $\geq 256$  mm), cobble (64–256 mm), pebble (4–64 mm) or fine sediment ( $< 4$  mm). Because hellbenders are cavity dwellers, we estimated the proportion of boulders with cavities based on 100 randomly selected boulders per reach; where we sampled the nearest boulder to each pebble count location without replacement. We classified boulders as having a cavity when an observer was able to slide a 3 cm wide ruler beneath the boulder to a depth  $\geq 3$  cm; otherwise we classified boulders as having no cavity.

#### 2.5. Data analysis

Due to the low number of larvae detected (see Section 3) we excluded larvae from all analyses, thus restricting our inference to the pooled populations of individuals measuring  $\geq 131$  mm total length in each reach, hereafter referred to as the sub-adult/adult population.

##### 2.5.1. Abundance

To estimate local abundance of sub-adults/adults we fit a Huggins (1989, 1991) version of the closed robust design model to data collected during robust-design surveys in 2014–2015. We chose the

Huggins version of the model because it allowed us to model probability of capture ( $p$ ) as a function of individual covariates. Preliminary data suggested that  $p$  increased as hellbender total length increased. Prior to analysis we compiled capture histories for all PIT tagged sub-adults/adults captured during primary occasions. We coded capture histories as live encounters where on each occasion an individual was either encountered alive ('1') or not-encountered ('0'). We pooled data from all reaches and defined each reach as a separate group using dummy variable coding.

One caveat of the Huggins model is that, while it allowed us to account for variation in capture probability, it precluded our ability to model abundance ( $\hat{N}$ ) as a function of predictors (e.g., land use) directly. Rather, the Huggins model estimates  $\hat{N}$  separately for each group (i.e., reach) and each primary occasion and as a derived parameter. As a result, our four candidate models differed only in how  $p$  was modeled (Table A1). We considered various combinations of stream reach, year and total length as covariates of  $p$ . We included reach in every model to account for site specific effects that influenced detection (e.g., substrate, water depth, etc.). Our null detection model assumed  $p$  was constant within each reach. Our most saturated detection model ([Reach \* Year + Total length]) assumed  $p$  was influenced by total length of individuals and varied by both year and reach. The Huggins model facilitates estimation of apparent survival ( $\Phi$ ), defined as the probability that sub-adults/adults present in year one both survived and remained faithful to the reach between primary occasions. Because we estimated apparent survival using a more robust dataset (see Section 2.5.3), we only report  $p$  and  $\hat{N}$  from our robust design model. Some additional parameters available within the Huggins model (temporary immigration and emigration [ $\gamma'$  and  $\gamma''$ , respectively] and probability of a marked animal being recaptured in each primary occasion [ $c$ ]) were inestimable due to the fact that our design included only two primary and two secondary sampling occasions. Thus, we constrained  $\gamma' = \gamma'' = 0$ , and constrained  $p = c$  in all models. We fit models in program MARK (White and Burnham, 1999) and ranked them using Akaike's Information Criterion adjusted for small samples (AICc).

We used mean estimates of sub-adult/adult abundance from our top-ranked closed robust design model as a Poisson distributed response variable within a generalized linear mixed model framework to investigate associations between land use and sub-adult/adult hellbender abundance. We fit four candidate models representing our hypotheses regarding factors driving variation in abundance (Table 2). We used logarithmic forms of forest cover ( $\log(\text{Forest})$ ) to represent our hypothesis that abundance would increase as forest cover increased, up to some threshold. We included stream reach as a random effect in every model to account for the fact that we estimated abundance separately in 2014 and 2015. We fit models using the Laplace approximation and a log link in Program R (Team, 2013) and ranked models using AICc. We

Table 2

Ranking of candidate models used to investigate associations between land use and either abundance or demographic structure of sub-adult/adult *Cryptobranchus alleganiensis* populations in six stream reaches during 2014 and 2015. Forest<sub>c</sub> = catchment forest; Forest<sub>cwr</sub> = catchment-wide riparian forest; Forest<sub>lr</sub> = local riparian forest; R = random effect representing stream reach.

| Response                | Rank | Model structure                 | K <sup>a</sup> | AICc <sup>b</sup> | ΔAICc | w <sub>i</sub> <sup>c</sup> | Likelihood |
|-------------------------|------|---------------------------------|----------------|-------------------|-------|-----------------------------|------------|
| Abundance               | 1    | log(Forest <sub>cwr</sub> ) + R | 3              | 91.33             | 0.00  | 0.41                        | 1.00       |
|                         | 2    | R                               | 2              | 91.75             | 0.42  | 0.33                        | 0.81       |
|                         | 3    | log(Forest <sub>c</sub> ) + R   | 3              | 92.84             | 1.51  | 0.19                        | 0.47       |
|                         | 4    | log(Forest <sub>lr</sub> ) + R  | 3              | 94.93             | 3.60  | 0.07                        | 0.16       |
| Proportion sub-adults   | 1    | R                               | 3              | -17.77            | 0.00  | 0.73                        | 1.00       |
|                         | 2    | log(Forest <sub>cwr</sub> ) + R | 4              | -14.07            | 3.70  | 0.12                        | 0.16       |
|                         | 3    | log(Forest <sub>c</sub> ) + R   | 4              | -13.34            | 4.43  | 0.08                        | 0.11       |
|                         | 4    | log(Forest <sub>lr</sub> ) + R  | 4              | -13.17            | 4.60  | 0.07                        | 0.10       |
| Proportion young adults | 1    | log(Forest <sub>cwr</sub> ) + R | 4              | 1.34              | 0.00  | 0.76                        | 1.00       |
|                         | 2    | log(Forest <sub>c</sub> ) + R   | 4              | 4.41              | 3.07  | 0.16                        | 0.22       |
|                         | 3    | R                               | 3              | 6.17              | 4.83  | 0.07                        | 0.09       |
|                         | 4    | log(Forest <sub>lr</sub> ) + R  | 4              | 9.76              | 8.42  | 0.01                        | 0.01       |
| Proportion old adults   | 1    | log(Forest <sub>cwr</sub> ) + R | 4              | 4.92              | 0.00  | 0.58                        | 1.00       |
|                         | 2    | R                               | 3              | 6.87              | 1.95  | 0.22                        | 0.38       |
|                         | 3    | log(Forest <sub>c</sub> ) + R   | 4              | 7.39              | 2.46  | 0.17                        | 0.29       |
|                         | 4    | log(Forest <sub>lr</sub> ) + R  | 4              | 11.03             | 6.11  | 0.03                        | 0.05       |

<sup>a</sup> Number of estimated parameter in each model.

<sup>b</sup> Akaike information criterion adjusted for small samples.

<sup>c</sup> Akaike model weight.

only considered land use variables to be meaningful predictors of abundance when they occurred in models that outranked our null model. We report model derived estimates of any supported land use effects and their 95% confidence intervals unless otherwise noted.

### 2.5.2. Demographic structure

We used the sample of sub-adults/adults captured during robust design surveys to describe demographic structure in each reach, separately for 2014 and 2015. Each year we classified individuals as either sub-adults (131–289 mm), young adults (290–419 mm), or old adults ( $\geq 420$  mm) according to age-length relationships developed by Taber et al. (1975; Fig. 2). We based the lower size threshold for young adults on the total length when males in our system first exhibit secondary sex characteristics (290 mm) and based the lower size threshold for old adults on the approximate total length at which growth rate reaches an

asymptote ( $\sim 420$  mm; Taber et al., 1975). We calculated the proportion of our sample that fell into each age class where, for each reach and year combination, proportions summed to one.

We used generalized linear mixed models to investigate associations between demographic structure and land use. For each age class, we fit a set of four candidate models representing our hypotheses regarding factors driving variation in demographic structure (Table 2). To aid model convergence we replaced values of 0, for age classes that were not represented in a reach, with very small values (0.0001). We used logarithmic forms of forest cover ( $\log(\text{Forest})$ ) to represent our hypothesis that our response variable would increase or decrease as forest cover increased, up to some threshold. Stream reach was included as a random effect in every model to account for the fact that we estimated age class composition in both 2014 and 2015. We fit and ranked models according to methods described in Section 2.5.1.

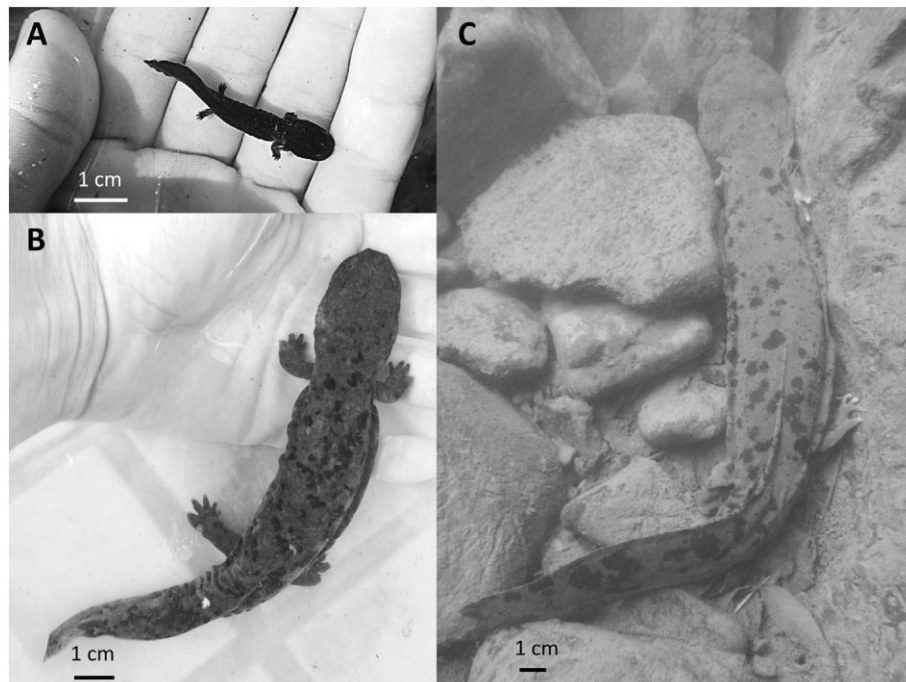


Fig. 2. Photographs of larval (A), sub-adult (B) and adult (C) *Cryptobranchus alleganiensis* encountered during mark-recapture surveys used to estimate abundance and demographic rates.

### 2.5.3. Demographic rates

We used Pradel (1996) models to estimate average monthly apparent survival ( $\Phi$ ) and the realized average monthly per-capita population growth rate ( $\lambda$ ) of sub-adult/adult population in each reach. Because our analysis was retrospective, apparent survival ( $\Phi$ ) refers to the joint probability that individuals both survived and remained faithful to a site between time  $t$  and time  $t+1$ . The complement to apparent survival ( $1-\Phi$ ) represents the rate at which sub-adults/adults were lost from each reach due to either mortality and/or movement. Lambda ( $\lambda$ ) is an estimate of the per-capita population growth rate between sampling occasions and essentially reflects the change in density of individuals within a reach between occasions. Values of  $\lambda < 1$  indicate a declining population,  $\lambda = 1$  indicates a stable population and  $\lambda > 1$  indicates population growth. Other important characteristics of Pradel models include the fact that  $\Phi$  and  $\lambda$  are specific to the age class that is the focus of sampling; which in our study was sub-adult/adult population. Pradel models also assume that populations are open between sampling occasions, the study area remains constant over time, and that the act of sampling does not influence survival or fidelity to the reach. We feel confident that our study generally meets these assumptions given that we used GPS coordinates of capture locations to filter records for inclusion in our analysis and because capture records from robust design surveys (Section 2.4.1) suggest hellbenders showed high site fidelity to the reach in days immediately following a capture event. For example, the majority (71% in 2014; 73% in 2015) of individuals captured during the first pass of secondary occasion surveys (Section 2.4.1) were captured in the same reach on the second pass. Anecdotally, many individuals were found in the same location on both passes.

Prior to analysis we constructed capture histories for all PIT tagged individuals  $\geq 131$  mm total length. We coded capture histories as live encounters and defined time intervals between sampling occasions in terms of months. Due to the variation in timespan over which each reach was monitored (Table 1) we conducted a separate analysis for each reach. In each analysis, we fit a virtually identical set of candidate models to our data (Table A2). We allowed  $p$  to vary among sampling occasions in every model to account for variation in sampling intensity over time and included total length at first capture as a covariate of  $p$  to represent our hypothesis that capture probability was higher for larger individuals. We considered total length at first capture as a covariate of  $\Phi$  to represent our hypothesis that survival or site fidelity increased as size/age increased. The suite of candidate models for R5 and R6 was slightly reduced because individuals encountered there were all relatively large which precluded our ability to model  $\Phi$  as a function of total length. Models failed to converge when we modeled  $\Phi$  or  $\lambda$  as fully time dependent, thus we constrained  $\Phi$  and  $\lambda$  in each reach to be constant across months. As a result, demographic rates we estimated represent average monthly rates over the time frame a given population was monitored.

We fit Pradel models in program MARK (White and Burnham, 1999) and ranked them using Akaike's Information Criterion adjusted for small samples (AICc). While we wanted to account for variation in  $p$  among sampling occasions, we viewed it largely as a nuisance variable and thus only report model averaged estimates of  $\Phi$  and  $\lambda$  and their 95% confidence intervals. When the upper 90% of model weight was distributed across multiple models we obtained model averaged (Burnham and Anderson, 2002) estimates of  $\Phi$  and  $\lambda$ . To facilitate comparison among sites, we report estimates of  $\Phi$  for individuals measuring 400 mm total length, which represented the smallest size present in all reaches.

### 2.5.4. In-stream characteristics

We used simple linear regression to examine associations between forest cover in each spatial extent (C, CWR and LR) and each dependent abiotic variable. Because physiographic provinces are distinguished by

unique geology and topography which can often influence abiotic stream conditions, we also evaluated associations between physiography (percent of catchment in the Blue Ridge) and each abiotic response variable. We used average values of conductivity ( $\mu\text{S}/\text{cm}^3$ ), dissolved oxygen (mg/L) and pH from each reach as water quality response variables (Table A2). Our temperature logger in R2 was lost during a high flow event in spring 2015, thus our analysis of water temperature involved data from only five reaches. We calculated mean weekly mean, mean weekly minimum, and mean weekly maximum temperature using two steps. In step one we calculated mean weekly temperature values (mean, min and max) during each sequential seven-day period in our dataset. In step two we calculated a grand mean value based on mean weekly values (Table A2). We used the percent of each reach characterized by bedrock, boulder, cobble, pebble and fine sediments and the proportion of boulders with cavities as substrate response variables (Table A2). We fit simple linear regression models in program R (Team, 2013) and recognized statistical significance at the  $\alpha = 0.05$  level.

## 3. Results

We recorded 411 capture events ( $n = 203$  unique individuals) during robust design surveys in 2014–2015, which we used to estimate abundance and demographic structure. We pooled these data with 257 additional capture events recorded outside of robust-design surveys to estimate demographic rates. The pooled dataset of 668 capture events included records for 274 unique hellbenders ( $n = 60$  [R1], 27 [R2], 119 [R3], 49 [R4], 11 [R5] and 8 [R6]). The majority of individuals (268 of 274 or 98%) were sub-adults/adults (i.e.,  $> 131$  mm total length). As a result, we excluded larvae from all our analyses thereby restricting our inference to sub-adult/adult populations in each reach. Among sub-adults/adults that we encountered (49 U, 111F, 108 M), 107 were captured only once, 74 were captured twice, 33 were captured three times and 54 individuals were captured four to 16 times.

### 3.1. Abundance

A single model received the majority of support explaining differences in detection probability ( $p$ ) and abundance ( $N$ ) among reaches ( $w_i = 0.99$ ; Table A1). As we predicted,  $p$  varied among reaches and increased with total body length in every reach. The probability of capturing an average sized adult (ca. 400 mm) during a single survey was relatively high 0.78 (range = 0.59 [0.45–0.71 95% CI] to 0.93 [0.82–0.97 95% CI] per reach). In contrast, the probability of capturing a recently matured adult (ca. 300 mm) averaged only 0.63 (range = 0.35 [0.21–0.51 95% CI] to 0.83 [0.62–0.93] per reach) and the probability of capturing relatively old individuals ( $\geq 500$  mm) averaged around 0.88 (range = 0.76 [0.6–0.97 95% CI] – 0.97 [0.89–0.99 95% CI] per reach).

Sub-adult/adult abundance spanned an order of magnitude (range = 4–48 individuals in 2014; range = 6–51 individuals in 2015; Fig. 3A) along our land use gradient. We observed some evidence to suggest that temporal proximity of a primary occasion to the onset of breeding may have influenced abundance in each year. For example,  $\bar{N}$  was similar between years for the three reaches (R3, R5 & R6) that were sampled at approximately the same time each year (mid-late July); while  $\bar{N}$  varied by 30–40% between years in reaches (R1, R2 & R4) that were sampled at different times each year (Fig. 3A). In all cases  $\bar{N}$  was higher when a given reach was sampled closer in time to the onset of breeding. Regardless of survey timing, we found that abundance increased as forest cover increased and was best explained by CWR forest, as predicted (Table 2; Fig. 3A). Our top-ranked model indicated that sub-adult/adult abundance nearly doubled with every 10% increase in catchment-wide riparian forest cover over the range of land use values we examined. However, uncertainty regarding land use effects also increased as forest cover increased, suggesting local site characteristics

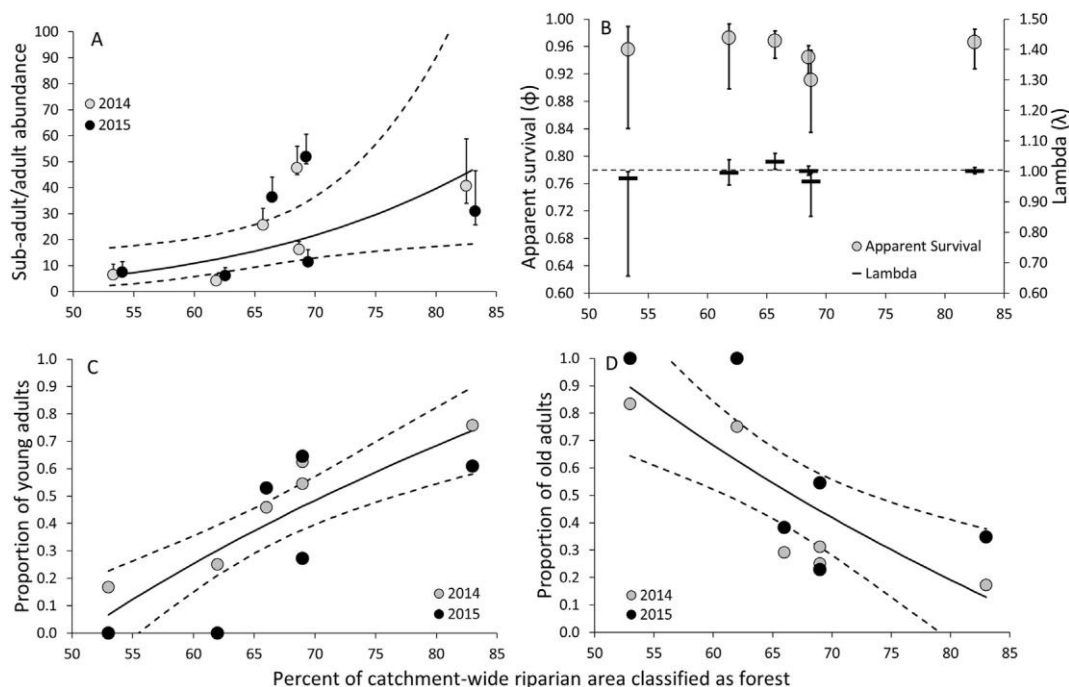


Fig. 3. Associations between catchment-wide riparian forest cover and abundance (A), demographic rates (B), and demographic structure (C–D) of sub-adult/adult *Cryptobranchus alleganiensis* populations within an approximate 100 m length of stream (wetted extent = 1668 m<sup>2</sup>). Points represent mean estimates and error bars represent 95% confidence intervals. The horizontal line (B) is placed at  $\lambda = 1$  for visual reference. Solid lines (A, C–D) represent mean estimated land use effects based on top-ranked generalized linear mixed models and dashed lines represent 95% confidence intervals based on uncertainty of fixed effects.

(e.g., substrate composition, biotic interactions, etc.) likely functioned as major determinants of sub-adult/adult hellbender abundance above some minimal threshold of CWR forest cover (Fig. 3A).

### 3.2. Demographic structure

We observed strong support for our hypothesis that land use was an important driver of hellbender demographic structure. Most variation in structure was driven by the proportion of young vs. old adults inhabiting a reach. Sub-adults made up a relatively small percentage of sampled individuals from any reach in both 2014 (range = 0–25% of individuals) and 2015 (range = 0–18% of individuals) and were only ever detected in the most heavily forested reaches (R1–R4). However, model rankings failed to support our hypothesis that sub-adult representation increased as forest cover increased (Table 2); suggesting that relative abundance of sub-adults was driven by factors other than land use or low detectability of sub-adults precluded our ability to detect any land use effects that may have been present.

Young adults were the most abundant or second-most abundant age class present in every reach (range = 16–75% of individuals in 2014 and 0–64% of individuals in 2015). Model rankings indicated that CWR forest ( $w_i = 0.76$ ) was around five times more likely to explain the proportion of a population composed of young adults than C forest ( $w_i = 0.16$ ; Table 2) and suggested that the proportion of a population composed of relatively young adults increased as CWR forest increased in a highly predictable fashion (Fig. 3C).

Old adults were the only age class detected in every reach in both years (range = 17–83% of individuals in 2014 and 22–100% of individuals in 2015) and made up the majority of individuals sampled from the two reaches subject to the lowest levels of forest cover (Fig. 3D). The proportion of a population composed of old adults increased as CWR forest decreased (Table 2; Fig. 3D).

### 3.3. Demographic rates

Given variability in the time span used to estimate demographic

rates for each of our populations (Table 1), we make comparisons among demographic rates with caution. In contrast to our predictions, we observed little to no evidence that stability of sub-adult/adult abundance was associated with land use over the range of time spans we considered. We observed moderate evidence indicating that sub-adult/adult populations declined ( $\lambda < 1$ ) over time in two stream reaches (R2:  $\lambda = 0.97$  [0.85–1.00 95% CI]; R5:  $\lambda = 0.98$  [0.66–1.00 95% CI]), including one (R2) of the more heavily forested stream reaches (Fig. 3B). Mean realized monthly  $\lambda$  for the remaining four sub-adult/adult populations ranged from 1.00–1.03 (Fig. 3B) and suggested that even the lowest abundance sub-adult/adult population with the most skewed size class distribution (R6) remained stable over the time frame for which data were available.

Also in contrast to our predictions, mean estimates of  $\Phi$  were extremely similar, suggesting that averaged sized adults were being lost at a similar rate along our land use gradient (Fig. 3B). Due to the relatively small size of each study reach (Section 2.2) and the fact that mortality and emigration were confounded as a result of our study design, we urge extreme caution against interpreting estimates of  $\Phi$  reported here as true survival. Mean apparent survival was noticeably lower in R2 ( $\Phi = 0.93$ ) than in other reaches (range of  $\Phi = 0.96$ –0.97), suggesting that declines ( $\lambda < 1$ ) indicated there were at least partially attributable to a relatively high rate of adult losses through either mortality or emigration.

### 3.4. In-stream characteristics

For simplicity, we restrict our reporting of results regarding in-stream characteristics to statistically significant correlations. Among the 12 abiotic parameters that we measured (Table A3), five (mean weekly maximum and mean weekly mean temperature, conductivity, pH and percent of substrate categorized as pebble) were significantly ( $p < 0.05$ ) associated with land use in at least one of the spatial extents that we considered (Table 3). Notably, none were significantly associated with LR forest. Conductivity was the only abiotic parameter that was significantly associated with both land use and physiography.

Table 3

Statistics for simple linear models used to examine associations between abiotic characteristics of stream reaches occupied by *Cryptobranchus alleganiensis* and landscape characteristics. Forest<sub>C</sub> = catchment forest; Forest<sub>CWR</sub> = catchment-wide riparian forest; Forest<sub>LR</sub> = local riparian forest; MW = Mean Weekly; DO = Dissolved oxygen.

| Response variable         | Forest <sub>C</sub> |                |                    | Forest <sub>CWR</sub> |                |                    | Forest <sub>LR</sub> |                |      | Physiography     |                |                   |
|---------------------------|---------------------|----------------|--------------------|-----------------------|----------------|--------------------|----------------------|----------------|------|------------------|----------------|-------------------|
|                           | F <sub>1,4</sub>    | R <sup>2</sup> | p                  | F <sub>1,4</sub>      | R <sup>2</sup> | p                  | F <sub>1,4</sub>     | R <sup>2</sup> | p    | F <sub>1,4</sub> | R <sup>2</sup> | p                 |
| MW Mean Temp <sup>a</sup> | 44.47               | 0.94           | <0.01 <sup>b</sup> | 5.71                  | 0.66           | 0.09               | 0.52                 | 0.15           | 0.52 | 0.75             | 0.20           | 0.45              |
| MW Min Temp <sup>a</sup>  | 7.72                | 0.72           | 0.06               | 2.07                  | 0.41           | 0.24               | 0.01                 | 0.00           | 0.91 | 0.23             | 0.07           | 0.67              |
| MW Max Temp <sup>a</sup>  | 91.65               | 0.97           | <0.01 <sup>b</sup> | 13.23                 | 0.82           | <0.05 <sup>b</sup> | 2.36                 | 0.44           | 0.22 | 1.70             | 0.36           | 0.28              |
| Conductivity              | 33.89               | 0.89           | <0.01 <sup>b</sup> | 675.90                | 0.99           | <0.01 <sup>b</sup> | 5.02                 | 0.55           | 0.08 | 8.65             | 0.68           | 0.04 <sup>b</sup> |
| DO                        | 0.01                | 0.00           | 0.90               | 0.04                  | 0.01           | 0.85               | 0.16                 | 0.04           | 0.70 | <0.00            | <0.00          | 0.98              |
| pH                        | 31.27               | 0.88           | <0.01 <sup>b</sup> | 12.72                 | 0.76           | <0.05 <sup>b</sup> | 6.32                 | 0.61           | 0.06 | 1.52             | 0.28           | 0.28              |
| % Bedrock                 | 0.42                | 0.09           | 0.55               | 0.11                  | 0.03           | 0.77               | 0.43                 | 0.10           | 0.55 | 0.30             | 0.07           | 0.61              |
| % Boulder (≥256 mm)       | 2.88                | 0.42           | 0.16               | 1.30                  | 0.24           | 0.32               | 1.93                 | 0.33           | 0.24 | 0.06             | 0.01           | 0.82              |
| % Cobble (64–256 mm)      | 0.41                | 0.01           | 0.85               | 0.02                  | 0.00           | 0.90               | 0.35                 | 0.08           | 0.59 | 0.00             | 0.00           | 0.97              |
| % Pebble (4–64 mm)        | 11.91               | 0.75           | 0.03 <sup>b</sup>  | 5.29                  | 0.57           | 0.08               | 1.62                 | 0.29           | 0.27 | 1.92             | 0.32           | 0.24              |
| % Fine sediment (<4 mm)   | 0.24                | 0.06           | 0.65               | 0.36                  | 0.08           | 0.58               | 2.94                 | 0.42           | 0.16 | 0.07             | 0.02           | 0.81              |
| % Boulders with cavities  | 0.11                | 0.02           | 0.75               | 0.11                  | 0.02           | 0.75               | 0.93                 | 0.18           | 0.38 | 0.66             | 0.14           | 0.46              |

<sup>a</sup> Data were only available for five of six stream reaches, thus F-statistic was calculated for 1 and 3 degrees of freedom (F<sub>1,3</sub>).

<sup>b</sup> Indicates a statistically significant association ( $\alpha = 0.05$ ).

Therefore, we suspect both factors may have played an important role in determining conductivity, though fit statistics indicated that land use explained a greater proportion of the variation in conductivity than did physiography (Table 3).

Water quality generally improved as C and CWR forest cover increased. Mean weekly mean water temperature declined as C forest increased ( $R^2 = 0.94$ ,  $F_{1,3} = 44.47$ ,  $p \leq 0.01$ ) but was not significantly associated with CWR forest ( $R^2 = 0.66$ ,  $F_{1,3} = 5.71$ ,  $p = 0.09$ ). In contrast, mean weekly maximum water temperature declined as both C forest ( $R^2 = 0.97$ ,  $F_{1,3} = 91.65$ ,  $p \leq 0.01$ ) and CWR forest increased ( $R^2 = 0.82$ ,  $F_{1,3} = 13.23$ ,  $p = 0.03$ ). Conductivity declined in a highly linear fashion as both C and CWR forest cover increased but was most closely associated with CWR forest ( $R^2 = 0.99$ ,  $F_{1,4} = 675.90$ ,  $p < 0.001$ ; Fig. 4B). We observed a negative association between pH and both C ( $R^2 = 0.88$ ,  $F_{1,4} = 31.27$ ,  $p \leq 0.01$ ) and CWR forest ( $R^2 = 0.75$ ,  $F_{1,4} = 11.86$ ,  $p = 0.02$ ; Fig. 4C). However, the pH trend appeared to be largely driven by slightly acidic conditions in R1, which we attribute to the fact that the catchment upstream of R1 included spruce-fir ecosystems which are characterized by acidic soils

(Stephenson and Adams, 1984).

In contrast to our predictions, we detected only mild support of an association between land use and substrate characteristics, and no significant correlation between land use and fine sediment (Tables 3, A3) or between land use and the proportion of boulders with cavities (Tables 3, A3). The only statistically significant correlation that we detected between substrate parameters and land use was that between pebble and C forest ( $R^2 = 0.72$ ,  $F_{1,4} = 11.91$ ,  $p = 0.03$ ; Fig. 4D). Pebbles were about three-times more common (30% pebble) when only 65% of a catchment was forested compared to when 90% was forested (10% pebble). We observed some evidence of a similar trend between pebble and CWR forest, but the correlation was not statistically significant ( $p = 0.08$ ; Tables 3, A3).

#### 4. Discussion

Our study provides novel insight into the spatial extent of land that influences ecological conditions in streams and the demographic mechanisms by which land use might influence abundance and persistence

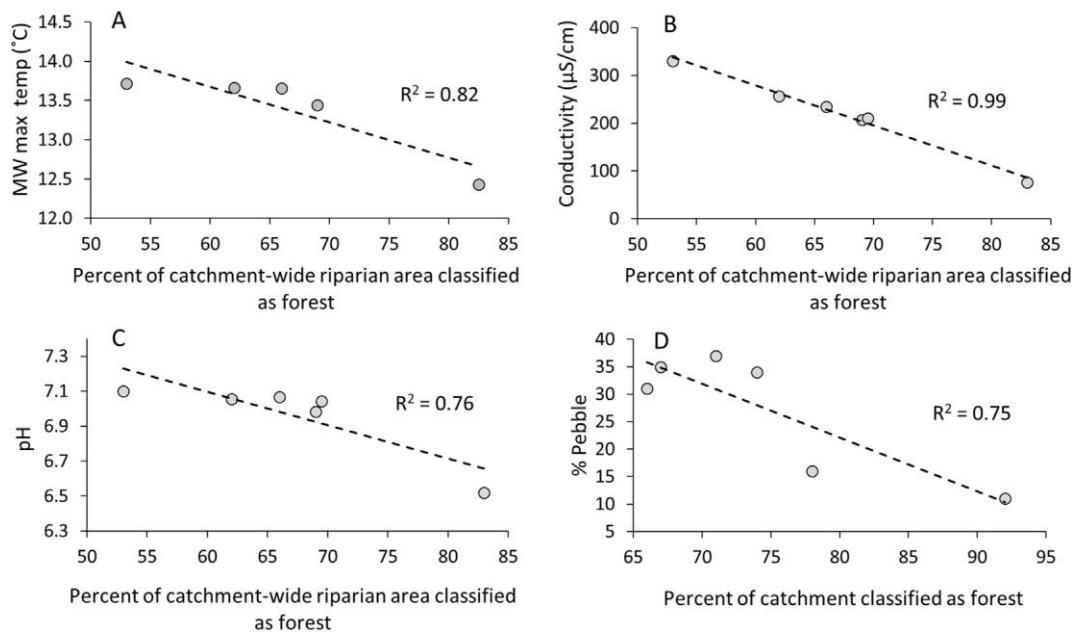


Fig. 4. Associations between land use and abiotic characteristics in six stream reaches where *Cryptobranchus alleganiensis* demography was studied. Points represent observed values and dashed lines represent trends based on simple linear regression. All correlations were statistically significant ( $p < 0.05$ ). Note that temperature data (A) were only available for five of six reaches.



of long-lived stream dwelling species. We found that catchment-wide riparian (CWR) forest cover predicted hellbender demography better than land use in the catchment or local riparian area, emphasizing the importance of upstream riparian areas as critical determinants of downstream ecology. Across space, local sub-adult/adult hellbender abundance declined and demographic structure became increasingly skewed towards older adults as CWR forest declined. While neither apparent survival or lambda estimates were associated with land use, they helped identify some notable patterns when viewed alongside spatial trends in abundance and demographic structure. For example, demographic rates highlighted the potential for both low- and high-density hellbender populations to persist at relatively stable densities for multiple years. The stability of high-density populations in areas subject to relatively high CWR forest appeared to be at least partially facilitated by recruitment (i.e., addition of individuals via reproduction, survival of juveniles and/or immigration) of relatively young age classes, as evidenced by demographic structure. In contrast, the stability of low-density populations subject to relatively low CWR forest appeared to be driven entirely by high survivorship and longevity of older adults, with relatively young individuals being rare or absent. Collectively, our findings provide compelling evidence that loss of CWR forest, or some correlate, has the potential to influence local hellbender abundance by acting specifically on recruitment of young individuals. However, given hellbender longevity, a significant lag time (i.e., multiple years) may separate the onset of mechanisms responsible for land-use associated declines and a detectable difference in sub-adult/adult abundance.

While our findings suggest variation in recruitment was the most likely explanation for variation in abundance across our land use gradient, more work is needed to elucidate the specific mechanisms responsible for poor recruitment in lesser forested areas. Insufficient recruitment of young age classes could have been the result of low immigration rates, reduced reproductive success, reduced survival of larvae and smaller sub-adults or perhaps some combination of these factors. Immigration via dispersal is poorly understood for hellbenders, and most stream-dwelling species, but is recognized as an important determinant of local population persistence for many amphibians (Marsh and Trenham, 2001). Barriers to dispersal in stream networks can include physical obstructions such as waterfalls, dams and road crossings (Warren and Pardew, 1998) as well as gaps in suitable habitat (Cecala et al., 2014). Barriers not only inhibit movement, but may also decrease the likelihood of an individual surviving an attempt to disperse, and life stages may vary considerably in their resilience to traversing barriers (Bodinof et al., 2012a–2012b). Reduced reproductive success and decreased survival of young age classes have both been hypothesized to be drivers of enigmatic hellbender declines noted throughout the species' range (Wheeler et al., 2003; Briggler et al., 2007; Burgmeier et al., 2011). However, the lack of effective methods to monitor hellbender nest success and to detect (i.e., as in the current study) and monitor larval survivorship has historically precluded collection of empirical data to test these hypotheses. Recently, however, Bodinof Jachowski (2016) presented compelling evidence regarding the use of artificial nest boxes as a novel tool to investigate hellbender nest and larval ecology. Thus, our ability to test hypotheses about the effects of land use on young life stages of hellbenders may no longer be limited. We encourage additional research to better understand the sensitivity of local population persistence to reproductive success versus survival of smaller sub-adults and dispersal, and how each of these processes might be influenced by land use.

The concurrence between declining water quality and increasingly skewed population structure of our focal species suggests that altered water quality may have functioned as a more proximate mechanism linking land use to recruitment of relatively young age classes in our study system. Among abiotic variables that were correlated with land use, conductivity was most closely associated with CWR forest cover and thus hellbender demographic structure (Fig. 4B). Conductivity

refers to the concentration of impurities, namely salts and other inorganic ions, in water and can vary as a result of underlying geology (i.e., physiography), weather patterns and addition of pollutants like those introduced via urban and agricultural runoff (Allan and Castillo, 2007; Gibbs, 1970). Though physiography and CWR forest were somewhat confounded,  $R^2$  values (Table 3), suggest that CWR land use was likely a critical determinant of water quality in our system. While hellbender tolerance to most water quality parameters has not been examined directly, multiple studies have recently reported negative associations between hellbender occurrence and conductivity (Bodinof Jachowski et al., 2016; Pitt et al., 2017; Pugh et al., 2016). Our findings are strikingly consistent with recent work by Pitt et al. (2017) who reported complete absence of hellbenders from areas in Pennsylvania where conductivity exceeded 278  $\mu\text{S}/\text{cm}$ , which is similar to conductivity levels in areas where recruitment was most severely impaired in the current study (Fig. 4B). A multitude of pathways exist by which conductivity might have influenced hellbender recruitment. While freshwater species have evolved various traits to cope with some fluctuation in conductivity, extreme fluctuations can result in mortality (Evans, 1980) and juvenile life stages are often more vulnerable than adults (James et al., 2003). Elevated conductivity can also interrupt spawning cues, inhibit sperm development and motility and inhibit embryonic development in some aquatic species (Alavi and Cosson, 2006; Bonislawski et al., 2015; James et al., 2003). Notably, recent captive breeding success for Ozark hellbenders was at least partially attributed to reduction of conductivity in captive enclosures (Ettling et al., 2013); suggesting conductivity levels may be particularly important determinants of hellbender reproductive success. While maximum water temperature was also correlated with CWR forest, the range of water temperatures that we observed (Table 3A) fell within the range of temperatures preferred by adult hellbenders within a lab setting (11–21 °C; Hutchison and Hill, 1976). However, water temperature can be an important determinant of metabolism, growth, reproduction and behavior of many cold-water species (Ficke et al., 2007) and temperature preferences of young hellbenders have not been studied. We encourage additional research to understand how conductivity and water temperatures might influence reproductive success and survival of young hellbenders and other freshwater species.

We detected only moderate evidence that substrate conditions varied across our land use gradient and no evidence that fine sediment loads co-varied with sub-adult/adult abundance or recruitment of young hellbender age classes. The lack of correlation between land use and fine sediment in our study directly contrasts with our predictions and well established relationships between land use and substrate (e.g., Allan, 2004). For example, development of land for residential, urban and agricultural use is a well-known source of pollution in the form of fine sediment which can pose major challenges to aquatic species (Sutherland et al., 2002). Fine sediments increase turbidity and decrease availability of interstitial spaces (Wood and Armitage, 1997) which translates to habitat loss for many benthic species. Hellbenders have an extended larval period (18+ months) and require at least five years to reach sexual maturity, during which they are suspected to depend heavily on relatively small interstitial spaces among pebbles and cobble (Nickerson et al., 2003). As a result, fine sediment loading that fills space among pebble and cobble is a widely suspected driver of impaired recruitment and enigmatic hellbender declines throughout the species' range (Foster et al., 2009; Graham et al., 2011; Wheeler et al., 2003). Suspicions that fine sediment may drive hellbender declines have been supported by recent evidence that fine sediment loads are negatively associated with hellbender occurrence (Keitzer et al., 2013; Pugh et al., 2016; Quinn et al., 2013). Thus, we were surprised by the lack of correlation between land use and fine sediment in the current study. The small number of study sites examined, the range of land use we considered, our methodology, the small extent of our sampling reaches (~100 m), or timing of surveys (i.e., substrate can be dynamic within a reach) could have precluded our ability to detect biologically

relevant difference in fine sediment if they existed (Table A3); thus we encourage others to interpret our findings regarding fine sediment with some caution.

## 5. Conservation implications

Our findings highlight the importance of upstream riparian areas as critical determinants of downstream ecological responses. We found that catchment-wide riparian condition was a better predictor of most ecological responses than catchment and local riparian condition. Our findings contrast somewhat with recent suggestions that conservation of entire catchments, as opposed to riparian buffers, is necessary to protect amphibians in headwater streams of North Carolina USA (Willson and Dorcas, 2003). The disparity may be related to differences in lifespan and habitat requirements of hellbenders versus relatively small aquatic salamanders, or the fact that our focal sites were higher order streams than those examined in the North Carolina study. The disparity might also be related to the difference in resolution of land use data used in each study, or the fact that our study area largely fell within the Blue Ridge while Willson and Dorcas (2003) studied Piedmont streams. Regardless, our findings corroborate a growing body of evidence that forest cover in close proximity to water is often a major determinant of ecological integrity throughout broader stream networks (Sheldon et al., 2012), though local riparian conditions are often overridden by processes occurring upstream (Stanfield and Kilgour, 2013). These findings collectively emphasize the need to consider broad spatial extents in conservation planning for stream-associated species and indicate that protection and restoration of riparian buffers in the immediate vicinity of occupied habitats may offer little benefit to stream biota unless upstream riparian areas are largely intact (Lorenz and Feld, 2013).

The response of our focal species to loss of forest cover may be a proxy of processes affecting many other long-lived species native to the broader Tennessee River basin. The Tennessee-Cumberland freshwater ecoregion is home to some 200 species of fish (67 endemic species), 125 species of freshwater mussel, 65 species of crayfish and is a global hotspot for salamander diversity (Abell et al., 2000). Hellbenders exhibit a slow-paced life history strategy characterized by longevity of 25+ years, delayed maturity and high egg/larval mortality. Similar traits are exhibited by many amphibians (Morrison and Hero, 2003), turtles (Congdon et al., 1994), mussels (Strayer et al., 2004) and fishes (Winemiller, 2005). Life history strategies are ultimately shaped by ecological pressures and reflect optimal strategies for a given environment (Stearns, 1992). When dominant selection forces are sufficiently altered, some strategies are favored over others leading to shifts in community assemblages (Mims and Olden, 2012). Because life histories reflect strategies, suites of life history traits often function as useful predictors of how a species, and ultimately communities, will respond to change. Importantly, life history is just one of several factors, including behavior, physiology and ecology, likely to influence the vulnerability of species to loss of CWR forest cover. However, the power of life history traits to predict how freshwater species respond to some broad scale environmental alterations suggests that many long-lived fishes, mussels and salamanders that inhabit streams throughout the Tennessee River Basin may also face an increased risk of extirpation as a result of declining CWR forest. Additional work is needed to identify key traits that might function as reliable indicators of species vulnerability to land use alteration.

Our findings highlight the need to consider demographic mechanisms of decline that might be at play when designing conservation and monitoring strategies for species with considerable longevity. Persistence of long-lived species is generally much more sensitive to adult survivorship than to acute changes in reproductive success (Congdon et al., 1994). Resultantly, conservation strategies for long-

lived species often focus on reducing adult mortality (Heppell, 1998) or augmenting adult density through translocation (Bodinof et al., 2012b; Cope and Waller, 1995; Haag and Williams, 2014). While maintenance of adult populations is critical for any long-lived organism, strategies focused solely on adult age classes may contribute little towards species recovery when recruitment is chronically suppressed. Our study demonstrates that certain combinations of longevity and high adult survivorship can facilitate the persistence of mature adults at stable densities for multiple years while recruitment suffers (also see Okada et al., 2008). While these remnant adult populations are highly susceptible to eventual extirpation, species biology can facilitate considerable time lag between the onset of declines and local extinction. This lag time likely explains why Bodinof Jachowski et al. (2016) failed to detect a significant effect of current land use on occurrence of hellbenders; and suggests occupancy (i.e., presence-absence) monitoring may be particularly ill-suited for detecting declines in long-lived species suffering recruitment deficits. Even when monitoring strategies for long-lived species focus on tracking abundance, survey efforts that are biased towards adult age classes might be prone to yielding overly optimistic estimates of population status. Considering abundance and demographic structure alongside demographic rates allowed us to distinguish between stable populations that were small and non-recruiting and stable populations that were moderate to high density and clearly recruiting. Thus, we suspect changes in demographic structure may function as the most reliable and earliest indicator of decline for many long-lived species, such as the hellbender. We recommend focusing on multiple age classes when designing monitoring and conservation strategies for long-lived species and considering multiple demographic parameters when assessing population status.

Our study highlights the value of considering processes occurring at multiple spatial scales in order to improve our understanding of how terrestrial landscapes influence aquatic species. Though we used a relatively simple approach to investigate the extent of the terrestrial environment most closely associated with in-stream responses, our approach was useful for helping to refine our understanding of the spatial extent of land that should be the target of restoration and conservation in order to enhance habitat for our focal species. Until recently, surprisingly few studies have considered land use in multiple spatial extents when attempting to explain drivers of ecological patterns (Sheldon et al., 2012; Stanfield and Kilgour, 2013; Willson and Dorcas, 2003). Considering land use in multiple spatial extents enhanced our ability to detect associations between forest cover and ecological parameters that may have otherwise been missed. Software advances and the growing availability of high resolution spatial data should continue to improve our ability to consider more realistic and complex relationships between terrestrial landscapes and aquatic habitats. We encourage future studies aimed at understanding the response of stream species to land use alteration to consider multiple spatial extents whenever possible.

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Appendix A

Table A1

Ranking of Huggins closed robust design models used to generate derived estimates of sub-adult/adult *Cryptobranchus alleganiensis* abundance in six stream reaches stratified across a land use gradient.

| Rank     | Model structure                           | K <sup>a</sup> | AICc <sup>b</sup> | ΔAICc | w <sub>i</sub> <sup>c</sup> | Likelihood |
|----------|---|----------------|-------------------|-------|-----------------------------|------------|
| Step one |   |                |                   |       |                             |            |
| 1        | Φ (Reach) p (Reach + Total length)        | 12             | 625.26            | 0.00  | 0.99                        | 1.00       |
| 2        | Φ (Reach) p (Reach * Year + Total length) | 17             | 634.77            | 9.51  | 0.01                        | 0.01       |
| 3        | Φ (Reach) p (Reach)                       | 11             | 642.48            | 17.22 | 0.00                        | 0.00       |
| 4        | Φ (Reach) p (Reach * Year)                | 16             | 651.86            | 26.60 | 0.00                        | 0.00       |

<sup>a</sup> Number of estimated parameters in each model.

<sup>b</sup> Akaike information criterion adjusted for small samples.

<sup>c</sup> Akaike model weight.

Table A2

Ranking of candidate models used to estimate monthly apparent survival (Φ) of sub-adult/adult *Cryptobranchus alleganiensis* and the per-capita population growth rate (λ) of sub-adult/adult populations in six stream reaches (R1-R6).

|    | Model  | K <sup>a</sup> | AICc <sup>b</sup> | ΔAICc | w <sub>i</sub> <sup>c</sup> | Likelihood |
|----|--|----------------|-------------------|-------|-----------------------------|------------|
| R1 | Φ(.) p (Survey) λ(.)                           | 11             | 418.43            | 0.00  | 0.54                        | 1.00       |
|    | Φ(.) p (Survey + Total length) λ(.)            | 12             | 420.40            | 1.97  | 0.20                        | 0.37       |
|    | Φ(Total length) p (Survey) λ(.)                | 12             | 420.77            | 2.34  | 0.17                        | 0.31       |
|    | Φ(Total length) p (Survey + Total length) λ(.) | 13             | 421.93            | 3.51  | 0.09                        | 0.17       |
| R2 | Φ(Total length) p (Survey) λ(.)                | 10             | 165.50            | 0.00  | 0.36                        | 1.00       |
|    | Φ(.) p (Survey) λ(.)                           | 9              | 165.99            | 0.50  | 0.28                        | 0.78       |
|    | Φ(.) p (Survey + Total length) λ(.)            | 10             | 166.71            | 1.22  | 0.20                        | 0.54       |
|    | Φ(Total length) p (Survey + Total length) λ(.) | 11             | 167.25            | 1.75  | 0.15                        | 0.42       |
| R3 | Φ(.) p (Survey + Total length) λ(.)            | 28             | 1420.23           | 0.00  | 0.57                        | 1.00       |
|    | Φ(Total length) p (Survey + Total length) λ(.) | 29             | 1420.82           | 0.59  | 0.43                        | 0.75       |
|    | Φ(.) p (Survey) λ(.)                           | 27             | 1437.32           | 17.09 | 0.00                        | 0.00       |
|    | Φ(Total length) p (Survey) λ(.)                | 28             | 1439.77           | 19.54 | 0.00                        | 0.00       |
| R4 | Φ(.) p (Survey + Total length) λ(.)            | 26             | 581.56            | 0.00  | 0.73                        | 1.00       |
|    | Φ(Total length) p (Survey + Total length) λ(.) | 27             | 583.58            | 2.02  | 0.27                        | 0.36       |
|    | Φ(.) p (Survey) λ(.)                           | 25             | 609.16            | 27.61 | 0.00                        | 0.00       |
|    | Φ(Total length) p (Survey) λ(.)                | 26             | 609.23            | 27.68 | 0.00                        | 0.00       |
| R5 | Φ(.) p (Survey) λ(.)                           | 9              | 114.89            | 0.00  | 0.60                        | 1.00       |
|    | Φ(.) p (Survey + Total length) λ(.)            | 10             | 116.00            | 1.11  | 0.35                        | 0.57       |
|    | Φ(Total length) p (Survey + Total length) λ(.) | 10             | 120.89            | 6.01  | 0.03                        | 0.05       |
|    | Φ(Total length) p (Survey) λ(.)                | 11             | 121.50            | 6.61  | 0.02                        | 0.04       |
| R6 | Φ(.) p (Survey) λ(.)                           | 13             | 148.79            | 0.00  | 0.52                        | 1.00       |
|    | Φ(.) p (Survey + Total length) λ(.)            | 14             | 150.40            | 1.61  | 0.23                        | 0.45       |
|    | Φ(Total length) p (Survey) λ(.)                | 14             | 151.52            | 2.73  | 0.13                        | 0.26       |
|    | Φ(Total length) p (Survey + Total length) λ(.) | 15             | 151.67            | 2.88  | 0.12                        | 0.24       |

<sup>a</sup> Number of estimated parameters in each model.

<sup>b</sup> Akaike information criterion adjusted for small samples.

<sup>c</sup> Akaike model weight.

Table A3

Abiotic characteristics of six stream reaches (R1-R6) in southwest Virginia where *Cryptobranchus alleganiensis* demographics were studied. Forest<sub>c</sub> = % catchment forest, Forest<sub>cWR</sub> = % catchment-wide riparian forest; Forest<sub>LR</sub> = % local riparian forest.

|                        | R1  | R2  | R3  | R4  | R5  | R6  |
|------------------------|-----|-----|-----|-----|-----|-----|
| Forest <sub>c</sub>    | 92  | 78  | 74  | 71  | 67  | 66  |
| Forest <sub>cWR</sub>  | 83  | 69  | 69  | 66  | 53  | 62  |
| Forest <sub>LR</sub>   | 83  | 24  | 45  | 16  | 4   | 74  |
| Elevation (m)          | 652 | 691 | 642 | 635 | 645 | 595 |
| Catchment physiography |     |     |     |     |     |     |
| % Blue Ridge           | 100 | 93  | 83  | 79  | 2   | 66  |
| % Ridge and Valley     | 0   | 7   | 17  | 21  | 98  | 34  |
| Water quality          |     |     |     |     |     |     |

|                            |                       |                      |                       |                       |                       |                       |
|----------------------------|-----------------------|----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| MW mean temp (°C)          | 11.33<br>[0.05–20.88] | –                    | 12.08<br>[0.42–19.90] | 12.25<br>[0.36–20.34] | 12.20<br>[1.44–19.86] | 12.56<br>[0.16–20.97] |
| MW min temp (°C)           | 10.31<br>[0.04–19.52] | –                    | 10.87<br>[0.02–18.41] | 10.97<br>[0.03–18.73] | 10.87<br>[0.47–18.07] | 11.57<br>[0.00–19.86] |
| MW max temp (°C)           | 12.42<br>[0.06–22.38] | –                    | 13.44<br>[1.26–21.56] | 13.65<br>[0.99–22.21] | 13.71<br>[2.73–22.01] | 13.66<br>[0.54–22.74] |
| Conductivity (µS/cm)       | 75<br>[55–133]        | 207<br>[110–337]     | 210<br>[121–350]      | 234<br>[140–632]      | 330<br>[216–559]      | 257<br>[171–423]      |
| Dissolved oxygen (mg/L)    | 11.23<br>[8.02–14.81] | 10.95<br>[8.71–14.8] | 11.21<br>[8.84–14.31] | 11.57<br>[8.88–14.16] | 11.19<br>[8.51–15.3]  | 10.92<br>[8.47–15.27] |
| pH                         | 6.52<br>[5.86–7.14]   | 6.98<br>[5.84–7.74]  | 7.04<br>[6.57–7.37]   | 7.06<br>[6.19–7.58]   | 7.09<br>[6.36–7.61]   | 7.05<br>[6.66–7.72]   |
| Substrate (%)              |                       |                      |                       |                       |                       |                       |
| Bedrock                    | 10                    | 45                   | 5                     | 3                     | 9                     | 0                     |
| Boulder (≥256 mm)          | 17                    | 6                    | < 1                   | 7                     | 8                     | 7                     |
| Cobble (64–256 mm)         | 34                    | 21                   | 52                    | 31                    | 32                    | 35                    |
| Pebble (4–63 mm)           | 11                    | 16                   | 34                    | 37                    | 35                    | 31                    |
| Fine sediments (< 4 mm)    | 28                    | 12                   | 9                     | 22                    | 16                    | 27                    |
| Boulders with cavities (%) | 63                    | 69                   | 54                    | 59                    | 68                    | 55                    |

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