ESTIMATES OF EASTERN HELLBENDER (*CRYPTOBRANCHUS ALLEGANIENSIS ALLEGANIENSIS*) OCCUPANCY AND DETECTION USING TWO SAMPLING METHODS

A Thesis
THOMAS WILLSON FRANKLIN

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

May 2016
Department of Biology

ESTIMATES OF EASTERN HELLBENDER (*CRYPTOBRANCHUS ALLEGANIENSIS ALLEGANIENSIS)* OCCUPANCY AND DETECTION USING TWO SAMPLING METHODS

A Thesis
by
THOMAS WILLSON FRANKLIN
May 2016

APPROVED BY:

Lynn M. Siefferman, Ph. D.
Chairperson, Thesis Committee

Michael M. Gangloff, Ph. D.
Member, Thesis Committee

Lori A. Williams
Member, Thesis Committee

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

Max C. Poole, Ph.D.
Dean, Cratis D. Williams School of Graduate Studies

Abstract

ESTIMATES OF EASTERN HELLBENDER (*CRYPTOBRANCHUS ALLEGANIENSIS ALLEGANIENSIS*) OCCUPANCY AND DETECTION USING TWO SAMPLING METHODS

Thomas Willson Franklin
B.S., Appalachian State University
M.S., Appalachian State University

Chairperson: Dr. Lynn Siefferman

Traditional survey methods for rare benthic organisms are expensive, time consuming, labor intensive and can be dangerous to the researchers as well as stressful to the target animals. Environmental DNA (eDNA) is a non-invasive survey method that is an increasingly popular alternative for detecting rare aquatic species. Although recent studies have demonstrated the efficacy of eDNA in detecting the presence of aquatic species, many studies do not incorporate detection estimates and the potential covariates affecting detection. Further, the factors affecting eDNA detection show great variability between study species and aquatic systems. Hellbenders (Cryptobranchus alleganiensis) are currently experiencing rapid, range-wide population declines and are considered at-risk by many state and federal management agencies. I collected eDNA via water samples at 25 sites, and, at the same locations, conducted exhaustive traditional hellbender surveys (i.e., snorkeling, rock-turning), and characterized instream habitat three times each per site in 2015.

Based upon repeated surveys, my occupancy model approach utilized both eDNA and traditional survey methods to estimate the occupancy rate and detection probability of hellbenders. Site- and survey-specific covariates were used to investigate the factors affecting occupancy and detection for both survey methods. Both survey methods yielded similar detection estimates (p = ~0.90), but eDNA surveys detected hellbenders at 20% more sites. Occupancy covariates were not significant in the best fit models, but hellbenders were more likely to occur at sites with increased substrate sizes. Detection estimates for traditional surveys were highest at sites with larger populations and individuals. Environmental DNA survey detection estimates were most affected by eDNA concentrations, hellbender abundance, animal size and the amount of sand at a site. Thus, I argue that eDNA concentrations can be used to estimate biomass and relative abundance for hellbenders in their natural environment. By integrating repeated eDNA surveys into occupancy and detection models, the covariates that predict occupancy and detection become more reliable. Moreover, this project expands upon the current knowledge of eDNA detection by demonstrating the importance of accounting for substrate composition in eDNA surveys as well as demonstrating the positive relationship between eDNA concentrations and population estimates in lotic systems.

Acknowledgments

Firstly, I would not be where I am or who I am today without the never-ending love, support, and encouragement from my family. To Susan, Ken and Brittany, thank you for all you have done to inspire me and show me what can be accomplished a strong work ethic, care for all, and some creativity. Secondly, I would not be the biologist I am today without the guidance and support from my graduate advisors, Drs. Lynn Siefferman and Mike Gangloff. I would be hard-pressed to find any other advisors that would be able to push me in the right direction all while accompanying my weirdness. Although I may be a tad more independent than traditional students, you both were always there when I needed it and I cannot begin to express my gratitude towards you two. I would also like to thank my committee member, Lori Williams, for her endless support, advice, and guidance throughout the entirety of my graduate career. Lori, you provide an excellent role model to look up to as an agency biologist. Further, I am grateful for the support I received from Dr. Stephen Spear throughout my research. Not only could the project have been completed without you, your input and feedback were critical to the success of this research. I also thank John Groves for his support and knowledge you lent for this project. Thank you to Dr. Grant Connette for the guidance you extended throughout the process of learning, understanding, and analyzing occupancy and detection modeling. You greatly contributed to the integrity of this project as well as helping me not lose my mind with your code revisions and feedback. This research received external funding from the North Carolina Wildlife Resources Commission, The

Orianne Society, North Carolina Zoological Society, Chopsticks for Salamanders, and the Cryptobrachid Interest Group. Additionally, the Appalachian State University Graduate Student Association Senate, the Cratis D. Williams Graduate School, and the Appalachian State University Office of Student Research all provided internal funding towards this project.

To all of the members from the Siefferman and Gangloff labs, thanks for coming together and all being weirdos with me. My field season could not have been completed with all of your help. From hip-hop hour to trips to the Boone Saloon, you guys kept me together. For those of you who I have not photoshopped, your doom is quickly approaching. And Perkins, it has been great to be able to look up to you every day. To Alex and Dan, thank you two for your never-ending support in the field, lab, and friendship. To Ryan, Jeph, and Bryce, your years of support have kept me grounded, enthused with good times, and striving for adventures. Last but certainly not least, thank you to Mowgli for getting me through all the ruff times.

Table of Contents

Foreword

The research detailed in this thesis will be submitted to the peer-reviewed journal *Conservation Biology*. The body of this thesis has been prepared according to the style and formatting requirements for publication in this journal.

Introduction

As freshwater biodiversity remains threatened, it is becoming increasingly important to employ the proper ecological approaches and survey methods to study declines (Dudgeon et al. 2006; Vie et al. 2009). Innovative detection methods such as environmental DNA (hereafter, eDNA) are revolutionizing the ability to detect rare and non-native organisms (reviewed by Rees et al. 2014). This non-invasive sampling method has shown varying success detecting aquatic taxa including: fishes (Jerde et al. 2011), amphibians (Ficetola et al. 2008), mammals (Thomsen et al. 2012), and invertebrates (Thomsen et al. 2012; Goldberg et al. 2013) in both marine and freshwater systems. Further, Thomsen et al. (2012) were able to detect terrestrial species in close distances to ponds by using high-throughput sequencing of DNA from pond water. In addition to being sensitive at detecting animals in low densities (Pilliod et al. 2013; Spear et al. 2015), the advantages of eDNA surveys lie in being cheaper, safer, and requiring less effort compared to traditional aquatic surveys (Biggs et al. 2015; Sigsgaard et al. 2015). While variation has been seen in eDNA detection across taxa and habitat (Ficetola et al. 2008; Thomsen et al. 2012), eDNA can be integrated with repeated surveys to provide a unique framework to develop estimates of detection and occupancy (Schmidt et al. 2013; Hunter et al. 2015).

Whether managing for imperiled species or non-native species, the reliability and accuracy of eDNA detecting rare animals has significant management implications (Dejean et al. 2012). Although many of the potential factors affecting eDNA detection have been

studied, much more research is needed to see how these factors and other affect detection with different taxa and habitats. Fluctuations in hydraulic, spatial, and temporal parameters have been seen to have varying roles influencing the detectability and quantification of target DNA (Thomsen et al. 2012; Pilliod et al. 2013; Jane et al. 2015). In lentic systems, eDNA has shown a high degree of temporal precision and successfully detected animals two weeks after the species was removed (Thomsen et al. 2012). However, in lotic systems precise estimates of biomass, density, distribution, and even DNA concentrations can be influenced by increased habitat complexity. When compared to pond samples, the detection rates of European weather loaches (*Misgurnus anguillicaudatus*) was reduced from 100% success in lentic systems to only 54% success when sampling from running water systems (Thomsen et al. 2012). Increased flow can results in lower eDNA counts and reduced detectability at varying distances from the source (Jane et al. 2015). Flow also likely affects the downstream detection distance on a species specific scale. Pilliod et al. (2014) lost detectability 50 m downstream of caged Idaho giant salamanders (*Dicamptodon aterrimus*), while Jane et al. (2015) were able to detect brook trout (*Salvelinus fontinalis*) up to 239.5 m downstream of their location regardless of flow.

To fully utilize eDNA as a monitoring tool, eDNA sampling would be able to make estimates of population status and size. Assuming individuals release eDNA in proportion to their biomass, many studies have attempted to infer population sizes from eDNA samples. Studies combining field survey estimates with PCR replicates have shown mixed results in correlating larger populations with higher proportions of positive PCR replicates (Ficetola et al. 2008; Goldberg et al. 2011). Greater success has been demonstrated in controlled environments by using quantitative PCR (qPCR) to estimate concentrations of target DNA in

a sample (Takahara et al. 2012; Pilliod et al. 2013). Relationships are less clear in field studies (Biggs et al. 2015; Spear et al. 2015). To use eDNA as a successful management tool, researchers need a better understanding of how these biological, chemical, and physical processes effect eDNA detectability.

Site occupancy has long been used to estimate the viability and distribution of populations, and but failing to account for imperfect detection can severely bias occupancy estimates (Moilanen 2002). For species that are rare, difficult to detect, and/or occur in small numbers, occupancy models can provide unbiased estimates of occupancy and detection that can direct conservation biologists and wildlife stakeholders to make more informed management decisions (MacKenzie et al. 2002; Mazerolle et al. 2007; Connette & Semlitsch 2013). The most notable benefit of occupancy modeling is its ability to minimize the effects of imperfect detection. Detection may be affected by factors like population size, life history, and habitat use (MacKenzie et al. 2002; Connette & Semlitsch 2013; Albanese et al. 2014). As a principle of occupancy modeling, each repeated visit reduces the probability of false negatives and adds confidence to the true occupancy state (Mackenzie 2002). False absences pose a large problem to conservation biologists; by not accounting for a present animal, biologists underestimate levels of true occupancy resulting in artificially small range size and population estimates (Moilanen 2002; Mackenzie 2002). By including repeated visits and detection covariates into eDNA sampling framework, the factors affecting eDNA detection can be properly investigated. Repeated eDNA surveys can come from 1) separate PCR assays, 2) separate temporal visits or 3) a combination of both (Schmidt et al. 2013; Hunter et al. 2015).

As previously mentioned, eDNA is a viable method for detecting amphibians and more specifically hellbender salamanders (*Cryptobranchus alleganiensis*) (Olson et al. 2012; Spear et al. 2015). Hellbenders are large, elusive, aquatic salamanders that inhabit cavities under large rocks in clean, well-oxygenated streams (Smith 1957; Hillis & Bellis 1971). This species is currently experiencing local population declines from habitat degradation (Wheeler et al. 2003, Foster et al. 2009; Burgmeier et al. 2011a; Unger et al. 2013). Because their skin plays a critical role in osmoregulation and respiration, aquatic amphibians are unusually sensitive to changes in stream physicochemical parameters, making many species indicators of high water quality (Feder & Burggren 1985; Duellman & Trueb 1986). Traditional survey methods for hellbenders consist of exhaustive snorkel surveys in which researchers turn over large rocks in streams to capture hellbenders underneath (Nickerson & Krysko 2003; Browne et al. 2011). During these surveys, hellbenders may be injured or killed. False negatives during traditional surveys are not unlikely because hellbenders can hide in areas that researchers are unable to fully search or they can escape from their cavity while the rock is being lifted. The micro-habitat under the rock is also disturbed during the lifting and moving of the rock (Burgmeier et al. 2011b). Further, researchers can be injured while reaching under large, heavy rocks. False negative detection during traditional surveys is not unlikely because hellbenders can hide in areas that researchers are unable to be effectively searched and because hellbenders can also escape from their cavity while the rock is being lifted.

The objectives of my study were to 1) compare estimates of detection and occupancy probabilities between traditional field methods and eDNA survey methods and 2) investigate the factors affecting detection and occupancy for both survey methods.

To address my first objective, I sampled 25 sites in northwestern North Carolina three times each conducting an eDNA survey and a traditional survey at every visit. A critical assumption of single-season occupancy models is that sites are closed to changes in occupancy during the sampling season. Due to the small home ranges of hellbenders, this assumption was satisfied by using a large traditional sampling site (150 m) which could contain at least one hellbender territory (Peterson & Wilkinson 1996). Further, I collected factors to use as covariates that could affect detection and/or occupancy estimates during habitat characterization surveys. In combination with extrapolated covariates from spatial land use land cover modeling, I used all of these covariates to address the second objective.

Methods

Study Design

My project was based on the single-season occupancy model framework described by MacKenzie et al. (2002). I sampled hellbenders at 25 sites across the New River and Watauga River drainages in North Carolina during May-August 2015 (Fig. 1). The sampling season occurred from May – August to avoid elevated concentrations found during the breeding season (Spear et al. 2015). Sites were selected based on historical and anecdotal reports of hellbender captures or sightings. All sites consisted of a 150-m reach with transects at 10-m intervals (n = 16). One critical assumption of single-season occupancy models is that sites are closed to changes in occupancy during the sampling season. Based on a hellbenders home range, I decided that a 150-m reach is a large enough to contain at least one hellbender territory if one were present while satisfying the assumption that the site is closed (Peterson & Wilkinson, 1996; Burgmeier et al. 2011a). Sites were visited three times each where an 1) eDNA survey, 2) a traditional hellbender survey, and 3) a habitat characterization survey were completed each visit.

Traditional Field Surveys

While snorkeling in an upstream direction, the field team searched cobble and small rocks by hand and lifted mid-size to large rocks using log peaveys to expose potential hellbenders for capture (Nickerson & Krysko 2003; Browne et al. 2011). Hellbenders were captured with dip nets and/or by hand, then transferred the animals to mesh bags submerged in the stream or

water-filled buckets prior to processing. After each transect, the search time was number of animals captured was recorded to calculate catch per unit effort (CPUE, hellbenders per person hour). For the first round of surveys, the full 150 m was sampled in attempt to capture every hellbender in the reach to use as a relative abundance estimate. For survey rounds two and three, surveys were conducted until a hellbender was caught or when the full 150 m reach had been searched.

For all captured hellbenders, morphology measurements (total length, snout-vent length, tail width, weight), sex (if possible), age class (larval, juvenile, or adult, see Nickerson & Mays, 1973 for age classes), and any abnormalities (ie. missing limbs, scars, etc.) were recorded. In addition, each animal was scanned for the presence of a Passive Integrated Transponder (PIT) tag to identify recaptured individuals. If a PIT tag was not detected, a new tag was implanted in the subcutaneous tissue at the dorsum of the base of the tail and PIT tag numbers was be recorded in a PIT tag reader (BioMark Inc, Boise, ID, USA). Once processing was complete, animals were released to the specific area where it was captured.

Habitat Characterization

After the completion of the traditional snorkel survey, detailed habitat parameters were recorded at each of the 16 transects in the 150-m reach. At each transect, the team collected substrate measurements using a modified Wolman Pebble Count (25 particles per transect), five mid-channel flow and depth measurements per transect using a Marsh-McBirney Flo-Mate model 2000 electronic flow-meter and a meter stick, and the total wetted width of the stream (Wolman, 1954). Substrate composition was classified as measurable stream particles (length 2 – 2000 mm), boulders (>2 m length), bedrock, silt, sand, organic matter, or woody

debris (modified from Wentworth Scale; Wentworth, 1922). Additionally, the following measurements were recorded at the downstream-most transect at each survey using a YSI Pro Series Multi-Meter (Yellow Springs Instruments, Yellow Springs, OH): water temperature, pH, specific conductivity, dissolved oxygen and NO_3^- levels. This three-step survey process (eDNA water sample, traditional snorkel survey, habitat characterization) was completed every time the site was visited.

Environmental DNA

Field Collection

I followed the collection protocol described in Spear et al. (2015). Specifically, at each site, I collected and filtered two, 1 L water samples using a Nalgene Bottle at the downstream most transect of the site. Each sample was collected using sterile gloves upstream of the collecting personnel. Additionally, collections were made prior to any survey personnel entering the water. Water was vacuum pumped through a 0.45 µm cellulose nitrate filter. After filtering, the filter paper was removed with forceps treated with DNA Away (Molecular Bioproducts) to ensure no contamination between samples. Filters were stored in centrifuge tubes containing 95% ethanol. All Nalgene© Bottles were autoclaved between sampling events.

Laboratory Methods

I followed the protocols described in Spear et al. (2015) for the handling, storing, and extraction of DNA from each filter. DNA was extracted from each filter using DNeasy® Blood and Tissue kits (Qiagen). The primers and quantitative PCR (qPCR) protocols described in Spear et al. (2015) on an Applied Biosystems® StepOne Plus system (Life Technologies™) were used to amplify a 104 bp region of mitochondrial cytochrome b and estimate the amount of DNA in each filter. Three qPCR replicates were run per filter for total

of six DNA estimates per survey. Samples were considered samples ambiguous that only amplified DNA for one of three qPCR replicates. Three new qPCR replicates were run on the already extracted DNA in order to ensure amplification repeatability.

Raw DNA concentrations estimates from qPCR replicates were transformed by multiplying these values by the concentration of the DNA extract used for the standard to produce an estimate of actual DNA amount in ng. This was then extrapolated to represent the amount of DNA on the entire filter assuming constant concentration across filter and extraction. To investigate how eDNA concentrations related to other detection parameters, I used Spearman's and Pearson correlations.

Land Use and Land Cover Analysis

Land-use and land-cover (LULC) for the New River and Watauga River Drainages were analyzed at both the catchment and riparian scales using ArcGIS 10.3 and the ArcHydro Toolset (ESRI, Redlands, CA) to delineate drainages for each sampling site following a slightly modified protocol from Merwade (2012). I delineated the drainages using Digital Elevation Models (DEM) from the United States Geological Survey (USGS) National Elevation Dataset. Using the 1992 and 2011 National Land Cover Dataset (30 m resolution), I quantified the catchment land use for each site locality by clipping the raster to the delineated site watersheds. On a finer spatial scale, the riparian LULC analysis consisted of a 100-m buffer of the upstream catchment. Land use classes pre-defined by the USGS were modified so that deciduous, evergreen, and mixed forest were combined into forest cover, herbaceous, hay/pasture, and cultivated crops were combined into agriculture, developed spaces (open, low, medium, and high intensities) were combined into disturbed, and open water, barren land, shrub, and wetlands were combined into miscellaneous. Land use classes

were also calculated for the percent change between 1992 and 2011. All categories were combined into % forest cover change, % forest cover, % forest cover (no evergreen), % agricultural, % disturbed, and % miscellaneous for statistical analysis. Pearson and Spearman's correlations were used to investigate potential relationships between land use classes and instream habitat factors. Paired *t*-tests and Wilcoxon signed rank tests were used to analyze differences between 2011 and 1992 land use.

Occupancy and Detection Modeling

Prior to analysis, all traditional hellbender survey data were converted from count to presence-absence data. For eDNA samples, if at least two out of three qPCR replicates per filter amplified hellbender DNA, the survey was considered present. Site covariates used in the models consisted of LULC outputs (1992, 2011) and substrate composition (% sand, % bedrock, median substrate, etc.). Survey specific covariates included number of surveyors as well as measurements of instream habitat, water chemistry, and hellbenders (length, mass, etc.) I derived site discharge (m^3/sec) from measurements of flow velocity, depth, and width. Additionally, all continuous variables used as covariates were standardized before analysis to reduce parameter estimation biases.

Using package "unmarked" (Fiske & Chandler 2011) in program R (R Core Team 2016), I used presence-absence data from to create separate models investigating detection probabilities and site occupancy estimates for each sampling method. The first batch of analyses assumed that both detection and occupancy probabilities were constant [$\psi(.)p(.)$] to assure the robust detection probabilities needed for occupancy modeling. Further, I explored the importance of both site and survey specific covariates by modeling the parameters for each sampling method because the constant model did not best represent the data for each

sampling method. I initially explored the effects of each covariate separately for eDNA and traditional survey models [ψ(Cov)p(.), ψ(.)*p*(Cov)]. Moreover, I incorporated multiple sample- and survey-specific covariates into each survey method's models to help best explain occupancy and detection estimates [ψ(Covs)*p*(Covs)]. I used Akaike's information criterion (AIC) (Burnham & Anderson 2002) to identify the best supported models for each sampling method and model covariates were significant if the 95% confidence intervals exclude zero.

Results

Traditional Survey Results

Using traditional survey methods, 89 hellbenders were detected at 18 of the 25 independent sites in 49 of the 75 surveys. Detections were relatively consistent between survey rounds one, two, and three (18 (36.7%), 14 (28.6%), and 17 (34.7%), respectively). While keeping ψ and p constant [$\psi(\bullet)$ $p(\bullet)$], I obtained precise estimates of both occupancy (ψ = 0.72, SE = 0.089) and detection (p = 0.90, SE = 0.04). Hellbender occupancy was most strongly positively associated with catchment size, average discharge, average depth, average width, and median substrate.

Hellbender detection probability was positively associated with hellbender length, hellbender mass, and relative abundance. The positive additive relationships between hellbender length and relative abundance [$\psi(\bullet)$ p(Average Hellbender Length + Relative Abundance)] and hellbender mass and relative abundance [$\psi(\bullet)$ p(Hellbender Mass + Relative Abundance)] received continuous support in the models (Table 3, Fig. 2). When combined with detection covariates, covariates affecting occupancy became nonsignificant. Therefore, constant occupancy [$\psi(\bullet)$] received the highest model support in with the additive covariates mentioned above (Table 3). The three best fit models are all significantly different models than the null model [$\psi(\bullet)$ $p(\bullet)$, $p < 0.0001$]. The best fitting model for traditional surveys did not differ strongly from the next two highest models (Δ AIC = 1.64, 3.16) likely

due to the hellbender length and hellbender mass being significantly related measures of size ($p < 0.001$). Additionally, the total hellbender mass and average biomass estimates positively affected the detection estimates while divers, time, and replicate number showed no effect on detection probability.

eDNA Survey Results

eDNA detected hellbenders were detected at 23 of the 25 localities in 62 of 75 surveys. In survey round one, two, and three, 22 (35.5%), 19 (30.6%), and 21 (33.9%) detections occurred, respectively, again showing a relatively consistent detection method among survey rounds. Similarly to the traditional survey results, eDNA methods also produced high estimates of both occupancy ($\psi = 0.92$, SE = 0.054) and detection ($p = 0.90$, SE = 0.037) with ψ and p held constant [$\psi(\bullet)$ $p(\bullet)$]. Although occupancy covariates did not receive strong model support when added to detected models, average substrate size, depth, width, and subsequently discharge were positively associated with the probability of hellbender occupancy. Best fit models accounting for occupancy and detection using eDNA survey methods were strongest supported by constant occupancy levels [$\psi(\bullet)$] due to the high proportion of occupied sites (Table 3).

Overall, detection estimates with a base model accounting for concentration of DNA in a sample [$\psi(\bullet)$ p(eDNA Conc. +)] yielded the strongest supported models (Table 3, Fig. 2). Best fit models included an additive function between DNA concentration and 1) the percent of sand [$\psi(\bullet)$ p(eDNA Conc. + Sand)], 2) the median hellbender length [$\psi(\bullet)$ p(eDNA Conc. + Median Hellbender Length)], and 3) the hellbender mass per site [$\psi(\bullet)$ p(eDNA Conc. + Hellbender Mass)] (Table 3, Fig. 2). Using the DNA concentration as a detection covariate, I found that 0.0025292 ng of DNA was ensured 95% probability of detection

hellbender DNA is present (Fig. 3). All models were significantly different than the null model (Table 3). Neither, survey round, flow, nor water quality covariates significantly affected the ability to detect hellbenders using eDNA survey methods.

eDNA and Biomass

Environmental DNA concentrations were significantly related to survey measurements of discharge ($r_s(74) = 0.428$, $p < 0.001$). Site estimates of relative abundance ($r_s(24) = 0.457$, $p = 0.022$) and biomass ($r_s(24) = 0.432$, $p = 0.031$) show a significantly positive relationship with eDNA concentrations (Fig. 4). Further, site discharge levels were positively correlated with biomass ($r_s(24) = 0.628$, $p = 0.001$) and relative abundance ($r_s(24) = 0.597$, $p = 0.002$).

Land Use Land Cover

The percent forest cover significantly decreased from 1992 (M = 85.15, SD = 7.74) to 2011 (M = 74.68, SD = 10.52; (t (24) = -4.348, $p < 0.001$; z (24) = -4.372, $p < 0.001$) while the percent of disturbed land significantly increased from 1992 (M = 1.81, SD = 2.30) to 2011 (M = 9.16, SD = 6.56; z (24) = -4.238, $p < 0.001$). Because each land use class is a ratio of the site catchment, forest cover and discharge were inversely related (1992: $r_s(24) = -.542$, $p = 0.005$; 2011: $r_s(24) = -.654$, $p < 0.001$). The percent of forest cover in 2011 was inversely related to silt and nitrate levels across the sites ($r(24) = -0.496$, $p = 0.012$; $r_s(24) = -0.579$, $p = 0.002$). Further, as more forest was removed from 1992 to 2011, sites increased in silt, conductivity, and nitrate levels ($r(24) = 0.417$, $p = 0.038$; $r(24) = 0.396$, $p = 0.050$; ; $r_s(24) = 0.498$, $p = 0.011$). Disturbed land in 2011 demonstrated a positive relationship with conductance ($r_s(24) = 0.553$, $p = 0.004$) and nitrate levels ($r_s(24) = 0.517$, $p = 0.008$). Agricultural land from 1992 to 2011 did not significantly increase, but sites with increased

percentage of agricultural land in 2011 had significantly more silt and nitrate levels ($r(24) = -0.496$, $p = 0.012$; $r_s(24) = -0.421$, $p = 0.036$).

Discussion

Site occupancy rates have long been used as a useful method of investigating species status and ranges (Moilanen 1999). As the development and success of eDNA as a conservation biology tool continues to progress, the ability to accurately take into account the factors affecting detection has been a long standing problem. Without accounting for imperfect detection and potential covariates affecting detection, management practices are compromised. I have successfully developed a framework for estimating detection and occupancy probabilities by conducting temporally repeated surveys of eDNA while measuring both site- and survey-specific covariates. My data suggest that eDNA in an occupancy framework is a much more suitable tool for gaining more precise estimates of occupancy and detection compared to traditional survey methods. I had no sites where a hellbender was captured in a traditional survey, but the eDNA survey failed to detect it at least once (i.e., false negative). I found that, even between the two survey methods, there were 20% fewer occupied sites at the same 25 sampling locations. Although there is the possibility that the eDNA detected at the site washed downstream from animals upstream of the 150 m searched site, the ability to detect outside of the 150 m site adds to the power of this tool. Further, the effort and time that would be needed to traditionally search the full potential of the eDNA's search area would be unrealistic for repeated surveys while taking into account seasonal, monetary, and personnel constraints.

I confirmed hellbender presence at 92% of sites using eDNA and 72% of sites using traditional methods; however hellbender presence/absence was not variable enough to accurately estimate occupancy site covariates when also accounting for detection covariates. Although traditional survey models had more variation between occupied and unoccupied sites, no occupancy covariates were significant in the best fit models. Both survey methods yielded occupancy-specific models with covariates that corroborate published data on hellbender habitat selection (Nickerson and Mays 1973; Petranka 1998; Rossell et al. 2013). Predicted occupancy of hellbenders was greatly increased as the median substrate size increased (Table S9). Larger substrate sizes provide more possible shelter rocks and available habitat to boast hellbender populations (Nickerson and Mays 1973; Rossell et al. 2013). Further, hellbenders were more likely to occur at sites with larger catchments and consequently increased depth, width, and discharge (Table S9).

Land use changes including urbanization and agriculture have altered many stream ecosystems (Flynn et al. 2009; reviewed by Barret & Price 2014). For example, when deforestation occurs in a watershed, aquatic sediment loads and nutrient inputs increase which may dramatically affect native aquatic taxa (Price et al. 2006; Helms et al. 2009; Barrett & Price 2014). Aside from drainage size, catchment-scale covariates such as land-use parameters did not play a significant role in the combined models due to the high site occupancy levels. However, land use parameters were important in the occupancy-specific models. My data show that more intact watersheds, either with higher forest cover in 2011 or less forest cover removed from 1992 – 2011, are experiencing lower levels of silt, conductivity, and NO_3^-. I suggest these results are consistent with a lag affect from recent land use change in the region where the effects are occurring over a gradual period of time

rather than rapidly. This occurs in sites with less intact watersheds and impaired instream habitats. Pugh et al. (2015) found that hellbender occurrence in this region may be more accurately predicted by local habitat parameters than catchment-scale parameters. Although hellbenders are believed to also be sensitive to water quality (Nickerson & Mays 1973), hellbenders cannot occupy a site where the suitable shelter habitat does not exist. Although water quality remains impaired at more deforested sites, the older hellbenders from these sites to not appear to be driven out yet. The decreased water quality may not have reached a threshold to affect hellbenders physiologically or behaviorally. After examining 13 major taxonomic groups including anurans and caudates, Kerby et al. (2010) suggest that amphibians may not be the "canary in the coal mine" as commonly suggested. Specifically, amphibians do not appear as sensitive to environmental contaminants such as heavy metals, pesticides, or phenols as expected. While this is a more broad taxonomic generalization, other research suggests that hellbenders are not as sensitive to instream water chemistry as previously thought. It seems unlikely that animals will move to other reaches because hellbenders are territorial.. Similarly, the silt levels in these high-gradient mountain streams may have not become high enough to completely eliminate suitable habitat for larger individuals. Increased silt levels may disproportionately affect larval and juvenile habitat by filling interstitial spaces in cobble and gravel in run and riffles commonly associated with smaller individuals. Moreover, land use mediated changes in water quality may be putting larvae and juveniles at a further disadvantage due to increased sensitivity to silt and water quality parameters (S.D. Unger, pers. comm.). Even though these results demonstrate that larger hellbenders are more easily detected, only two of the 59 individuals captured in this study represented non-adult age classes. The lack of larvae and juveniles, in combination

with a patchy distribution shown in previous surveys (Pugh et al. 2015; Franklin, unpublished data), suggest some populations may be experiencing extinction debt in this region (Jackson & Sax 2010). It may be that many of these disconnected, older populations are unlikely to persist in the face of declining water quality and land use practices. By using presence/absence data in occupancy models, I cannot investigate viability of populations and age classes. My findings support local parameters are currently driving hellbender occurrence in this region while the potential negative effects of land use on hellbenders may be expected to become more important and clear in the future.

As a principle of occupancy models and imperfect detection, there is generally a positive relationship between abundance of target individuals and the probability of detection. Indeed, detectability was highest for traditional surveys at sites with larger hellbender size (mass or length) and relative abundance (Table 3, Fig. 2). Aside from relative abundance, the larger an animal is, the easier it should be to detect it. This has been shown in numerous aquatic taxa including freshwater mussels (Meador et al. 2011) and fish (Bozec et al. 2012). Further, hellbender territoriality may have added to the high detection estimates seen in traditional surveys. On many occasions, individuals were recaptured under the same rock as previous survey rounds. Alternatively, hellbenders were not detected in many surveys where all possible habitat was searched including rocks individuals were previously captured under. Hellbenders likely go undetected in traditional surveys because they may be occupying unsearchable habitat or because individuals escape before they are captured. The detection estimates were not affected by search effort, the number of surveyors, or the survey replicate. All field personnel were experienced with hellbender surveys and less than 10% of

animals encountered were not captured. These factors likely aided in the consistently high detection estimates seen in my traditional field surveys.

Sites with higher eDNA concentrations and more sand had the highest probability of detection during eDNA surveys (Table 3, Fig. 2). Increased concentrations of eDNA positively affected my ability to detect hellbenders. I found that 0.0025292 ng of eDNA is needed for 95% confidence in detection (Fig. 4). At the occupied field sites, eDNA concentrations were positively associated with discharge, biomass, and relative abundance (Fig. 4), suggesting that eDNA concentrations follow the expected trend in which the larger the individual or population size, the higher the detection probability because there is more eDNA is present in the stream. The repeated visits to the sites add confidence in the ability to estimate biomass and abundance with eDNA concentrations in natural environments.

Similar to traditional surveys, detection probabilities also increased with hellbender length and mass using eDNA (Table 3, Fig. 2). Previously Takahara et al. (2012) assumed biomass to be proportional to the amount of eDNA released in common carp (*Cyprinus carpio*), but hellbenders may not fit this assumption as soundly. Biomass, and consequently surface area, is likely one of the most important factors affecting the amount of eDNA introduced into a system by an individual (Kylmus et al. 2015). Klymus et al. (2015) showed that invasive carp species (*Hypophthalmichthys nobilis* and *Hypophthalmichthys molitrix*) produced eDNA proportional to their biomass in lab experiments. A similar trend should be expected in aquatic salamanders where larger individuals introduce more eDNA into the stream from increased surface area for eDNA to shed from compared to a smaller individual. When investigating eDNA and population sizes, the relationship becomes much more complex. Large numbers of smaller individuals may shed more DNA than a single, large

individual with the same total mass because the smaller individuals comprise more total surface area. Hellbender age may add more complexity to relationships between skin sloughing and body size. As hellbenders increase in size and age, they appear to develop more dorsolateral skin folds resulting in added surface area. While larvae add surface area with external gills, juveniles and larvae typical have tighter skin conceivably resulting in comparatively less surface area. Overall, the relationship between animals and the amount of eDNA is likely complicated, but my results suggest that hellbender size positively affects eDNA detection probabilities due to higher eDNA concentrations.

In addition to the intuitive relationship between eDNA concentrations and eDNA detection, the percent of sand at a site greatly increased the eDNA detection estimates (Table 3, Fig. 2). Studies have shown DNA fragments to form sand-DNA bridges after binding to available cations such as Mg^{2+}, Ca^{+}, and Na^{+} in aquatic systems (Aardema et al. 1983; Lorenz & Wackernagel 1987). Without this bridging between DNA and a cation, the DNA would not absorb into the sand due to electrostatic repulsion. The bridged molecule lessens the charge of the eDNA fragment, thus eliminating the electrostatic repulsion found between DNA and sand. The bound cations and eDNA fragments are then absorbed to the sediment. DNA bound to sand has been shown to be more resistant to enzymatic degradation (DNase I) than DNA free in the water column (Lorenz et al. 1981; Aardema et al. 1983; reviewed in Nielsen et al. 2006). Deere et al. (1996) studied the persistence of DNA in lake water and sediment. They found DNA was detectable for three weeks longer in sandy sediment compared to water samples. In turn, sand in lotic systems may act as a reservoir for eDNA from upstream sources. If sandy areas are accumulating eDNA and aiding in positive detections, the inferred occupied area may need to be expanded based on substrate

classification. It may be that once the eDNA is protected by binding to sand, it experiences reduced degradation rates and can be transported downstream to either interstitial space or to accumulate with other sand particles. One advantage of sand bound eDNA in lotic compared to lentic systems is that continuous and varying flow velocities may allow for the redistribution of eDNA-sand bound particles back into the water column. This may help explain the increased detectability of hellbenders using eDNA compared to traditional surveys.

My sites are primarily swift, headwater streams, and this may increase detectability of eDNA while the opposite effect may occur in slower moving systems if eDNA is more likely to adhere to the sediments. Therefore, water samples from slow flowing habitats may reduce detection probabilities compared to sediment samples in faster flowing habitats. By integrating measurements of sand into the eDNA detection framework, we may increase the variable distance of detection from the eDNA source. If sandy areas are accumulating eDNA and increasing detection rates, the inferred occupied area may need to be expanded based on substrate classification. The relationship between flow, sand particles and eDNA detection deserves more attention from future research. For hellbenders in particular, detection may increase with increased sand, but a threshold may be present where by a certain amount of sand is needed at a site to have increased detection but exceptionally sandy locations are typically not suitable hellbender habitat.

Previous studies focusing on lotic species have yielded varying results when attempting to associate eDNA to population metrics while accounting for various environmental and life history factors. My data suggest eDNA sampling should be repeated at least three times per a site if there are no existing detection estimates for the target species.

If detection rates are high as seen in this study, only two surveys per site would be needed for 95% detection confidence. For species that are much more mobile and include a larger territory/habitat compared to hellbenders, the detectable range of eDNA in a stream will be increasingly important to discern. Hellbenders are an excellent species for eDNA studies due to their defined fall breeding season and relatively small individual range from their shelter rock. Additionally, in both hellbenders (Spear et al. 2015) and fishes (Furlan et al. 2015), eDNA detection increases during the breeding season likely because more eDNA is shed into the system from reproductive activities. If the goal of a study is to establish the occupancy of a species across sites, sampling effort may be best utilized by sampling during the breeding season. Contrarily, the influx of eDNA from gametes may inhibit the ability to accurately make population estimates and certain detection covariates. If multiple sampling rounds were conducted throughout the proposed nonbreeding season to develop a baseline eDNA concentration for a site, more intensive sampling closer to the breeding season could lead to more accurate temporal estimates of when the breeding season is beginning on a site scale. This could be imperative for protecting sensitive species at site- or regional-scales compared to range-wide generalizations. This may be even more important at sites where breeding populations appear to declining. To draw conclusions between eDNA concentrations and biomass, relative abundance, and other detection covariates of interest, studies should be designed such that sampling is conducted well outside of the breeding season. More accurate estimates of occupancy lead to more informed and better management decisions. By using eDNA at same sites as traditional survey methods, I show further support for eDNA by detecting hellbenders at 20% more sites. For both eDNA and traditional survey models, I found 95% confidence in site absence after two rounds of surveys due to high detection rates.

Nonetheless, because hellbender are elusive and field crews vary in skill, three traditional surveys are recommended. Compared to traditional field surveys, the benefits of an eDNA approach include: reduced costs and time and greater ability to standardize methodology. This also lends to the integration of eDNA as a citizen science tool (Biggs et al. 2015). The number of repeated visits needed to accurately detect is likely to vary among study species using eDNA. For smaller species, more survey replicates may be necessary due to smaller amounts of eDNA shed per individual. If traditional surveys are conducted, researchers should be aware of the potential skew in age classes found due to size affecting detection.

The best fit models, along with high correlations between eDNA concentrations and size measurements, show that eDNA is a suitable tool to predict biomass estimates in a natural environment. The repeated samples integrated into this study design increases the confidence in this relationship. While being able to estimate biomass from eDNA concentrations adds to the power of eDNA as a conservation tool, it cannot replace the valuable information that traditional surveys yield, including information about age, body size distribution, individual health and behavior- all of which can contribute to management decisions about species re-introduction, propagation, or population viability.

In conclusion, I integrated eDNA survey methods into an occupancy model framework and showed that eDNA detection of hellbenders in most affected by the amount of eDNA in the sample and the substrate composition. eDNA surveys were slightly more sensitive than traditional survey methods and eDNA surveys increased occupancy estimates by 20% compared to traditional survey estimates. When using eDNA to monitor biodiversity or for the conservation of species, studies should consider the probability of detection as well as the substrate in which the eDNA is travel over. However, more research is needed towards

degradation rates sediment bound eDNA in lentic systems and in varying water quality parameters. Future studies on cell settling and the degradation of eDNA bound to sediment would also greatly improve our ability to designing projects based on whether taking water samples or sediment samples is more appropriate for the question in mind.

.

Literature Cited

Aardema BW, Lorenz MG, Krumbein WE. 1983. Protection of sediment-adsorbed transforming DNA against enzymatic inactivation. Applied and Environmental Microbiology **46**:417–420.

Albanese B, Litts T, Camp M, Weiler DA. 2014. Using occupancy and species distribution models to assess the conservation status and habitat use of the goldline darter (*Percina aurolineata*) in Georgia, USA. Ecology of Freshwater Fish **23**:347–359.

Bailey LL, Simons TR, Pollock KH. 2004. Estimating site occupancy and species detection probability parameters for terrestrial salamanders. Ecological Applications **14**:692–702.

Barrett K, Price SJ. 2014. Urbanization and stream salamanders: A review, conservation options, and research needs. Freshwater Science **33**:927–940.

Biggs J et al. 2015. Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt (*Triturus cristatus*). Biological Conservation **183**:19–28. Elsevier Ltd.

Bodinof CM, Briggler JT, Junge RE, Beringer J, Wanner MD, Schuette CD, Ettling J, Millspaugh JJ. 2012. Habitat attributes associated with short-term settlement of Ozark hellbender (*Cryptobranchus alleganiensis bishopi*) salamanders following translocation to the wild. Freshwater Biology **57**:178–192.

Bozec YM, Kulbicki M, Laloë F, Mou-Tham G, Gascuel D. 2011. Factors affecting the detection distances of reef fish: Implications for visual counts. Marine Biology **158**:969–981.

Browne RK, Li H, Mcginnity D, Okada S, Zhenghuan W, Bodinof CM, Irwin KJ, Mcmillan a MY, Briggler JT. 2011. Survey techniques for giant salamanders and other aquatic Caudata. Amphibian and Reptile Conservation **5**:1–16.

Burgmeier NG, Unger SD, Sutton TM, Williams RN. 2011a. Population status of the eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*) in Indiana. Journal of Herpetology **45**:195–201.

Burgmeier NG, Sutton TM, Williams RN. 2011b. Spatial Ecology of the Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) in Indiana. Herpetologica **67**:135–145.

Burnham, KP, Anderson, DR. 2002. Model selection and mutitimodel inference: a practical information-theoretic approach. 2nd edition. Springer-Verlag, New York.

Connette GM, Semlitsch RD. 2013. Life History as a Predictor of Salamander Recovery Rate from Timber Harvest in Southern Appalachian Forests, U.S.A. Conservation Biology **27**:1399–1409.

Deere D, Porter J, Pickup RW, Edwards C. 1996. Survival of cells and DNA of Aeromonas salmonicida released into aquatic microcosms. Journal of Applied Bacteriology **81**:309–18.

Dejean T, Valentini A, Miquel C, Taberlet P, Bellemain E, Miaud C. 2012. Improved detection of an alien invasive species through environmental DNA barcoding: the example of the American bullfrog *Lithobates catesbeianus*. Journal of Applied Ecology **49**:953–959.

Dudgeon D et al. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological Reviews of the Cambridge Philosophical Society **81**:163–82.

Duellman WE, Trueb L (1985) Biology of Amphibians. McGraw-Hill, New York.

Feder ME, Burggren WW. 1985. Cutaneous gas exchange in vertebrates: design, patterns, control and implications. Biological reviews of the Cambridge Philosophical Society **60**:1–45.

Ficetola GF, Miaud C, Pompanon F, Taberlet P. 2008. Species detection using environmental DNA from water samples. Biology Letters **4**:423–425.

Fiske IJ, Chandler RB. 2011. Unmarked: An R package for fitting hierarchical models of wildlife occurrence and abundance. Journal of Statistical Software **43**:1–23.

Flynn DFB, Gogol-Prokurat M, Nogeire T, Molinari N, Richers BT, Lin BB, Simpson N, Mayfield MM, DeClerck F. 2009. Loss of functional diversity under land use intensification across multiple taxa. Ecology Letters **12**:22–33.

Foster RL, Mcmillan AM, Roblee KJ. 2009. Population status of hellbender salamanders (*Cryptobranchus alleganiensis*) in the Allegheny river drainage of New York state. Journal of Herpetology **43**:579–588.

Furlan EM, Gleeson D, Hardy CM, Duncan RP. 2015. A framework for estimating the sensitivity of eDNA surveys. Molecular Ecology Resources **16**:641–654.

Gillooly JF, Allen AP, West GB, Brown JH. 2005. The rate of DNA evolution: effects of body size and temperature on the molecular clock. Proceedings of the National Academy of Sciences of the United States of America **102**:140–5.

Goldberg CS, Pilliod DS, Arkle RS, Waits LP. 2011. Molecular detection of vertebrates in stream water: a demonstration using Rocky Mountain tailed frogs and Idaho giant salamanders. PloS one **6**:e22746.

Goldberg CS, Sepulveda A, Ray A, Baumgardt J, Waits LP. 2013. Environmental DNA as a new method for early detection of New Zealand mudsnails (*Potamopyrgus antipodarum*). Freshwater Science **32**:792–800.

Gu W, Swihart RK. 2004. Absent or undetected? Effects of non-detection of species occurrence on wildlife–habitat models. Biological Conservation **116**:195–203.

Helms BS, Schoonover JE, Feminella JW. 2009. Assessing influences of hydrology, physicochemistry, and habitat on stream fish assemblages across a changing landscape. Journal of the American Water Resources Association **45**:157–169.

Hillis RE, Bellis ED. 1971. Some aspects of the ecology of the hellbender,*Cryptobranchus alleganiensis alleganiensis*, in a Pennsylvania stream. Journal of Herpetology **5**:121–126.

Hunter ME, Oyler-McCance SJ, Dorazio RM, Fike JA, Smith BJ, Hunter CT, Reed RN, Hart KM. 2015. Environmental DNA (eDNA) sampling improves occurrence and detection estimates of invasive burmese pythons. Plos One **10**:e0121655.

Jackson ST, Sax DF. 2010. Balancing biodiversity in a changing environment: extinction debt, immigration credit and species turnover. Trends in Ecology & Evolution **25**:153–160. Elsevier Ltd.

Jane SF, Wilcox TM, Mckelvey KS, Young MK, Schwartz MK, Lowe WH, Letcher BH, Whiteley AR. 2015. Distance, flow and PCR inhibition: EDNA dynamics in two headwater streams. Molecular Ecology Resources **15**:216–227.

Jane SF, Wilcox TM, McKelvey KS, Young MK, Schwartz MK, Lowe WH, Letcher BH, Whiteley AR. 2015. Distance, flow and PCR inhibition: eDNA dynamics in two headwater streams. Molecular Ecology Resources **15**:216–27.

Jerde CL, Mahon AR, Chadderton WL, Lodge DM. 2011. "Sight-unseen" detection of rare aquatic species using environmental DNA. Conservation Letters **4**:150–157.

Kerby JL, Richards-Hrdlicka KL, Storfer A, Skelly DK. 2010. An examination of amphibian sensitivity to environmental contaminants: Are amphibians poor canaries? Ecology Letters **13**:60–67.

Kleiber M. 1932. Body size and metabolism. Hilgardia: A Journal of Agricultural Science **6**:315–353.

Klymus KE, Richter CA, Chapman DC, Paukert C. 2015. Quantification of eDNA shedding rates from invasive bighead carp *Hypophthalmichthys nobilis* and silver carp *Hypophthalmichthys molitrix*. Biological Conservation **183**:77–84.

Lorenz MG, Aardema BW, Krumbein WE. 1981. Interaction of marine sediment with dna and dna availability to nucleases. Marine Biology **64**:225–230.

Lorenz MG, Wackernagel W. 1987. Adsorption of DNA to sand and variable degradation rates of adsorbed DNA. Applied and Environmental Microbiology **53**:2948–2952.

Mackenzie DI. 2005. What are the issues with presence – absence data for wildlife managers? Special Section : The value and utility of presence – absence data to wildlife monitoring and research. Journal of Wildlife Management **69**:849–860.

MacKenzie DI, Nichols JD, Lachman GB, Droege S, Royle JA., Langtimm CA. 2002. Estimating site occupancy rates when detection probabilities are less than one. Ecology **83**:2248–2255.

Mackenzie DI, Royle JA. 2005. Designing occupancy studies: General advice and allocating survey effort. Journal of Applied Ecology **42**:1105–1114.

Mazerolle MJ, Bailey LL, Kendall WL, Royle JA, Converse SJ, Nichols JD. 2007. Making great leaps forward: accounting for detectability in herpetological field studies. Journal of Herpetology **41**:672–689.

Meador JR, Peterson JT, Wisniewski JM. 2011. An evaluation of the factors influencing freshwater mussel capture probability, survival, and temporary emigration in a large lowland river. Journal of the North American Benthological Society **30**:507–521.

Merwade V. 2012. Watershed and stream network delineation using ArcHydro tools. School of Civil Engineering. Purdue University:1–22.

Moilanen A. 1999. Patch occupancy models of metapopulation dynamics: Efficient parameter estimation using implicit statistical inference. Ecology **80**:1031–1043.

Moilanen A. 2002. Implications of empirical data quality to metapopulation model parameter estimation and application. Oikos **96**:516–530.

Nickerson MA, Mays CE. 1973. The Hellbenders: North American "Giant Salamanders." Milwaukee Public Museum, Milwaukee.

Nickerson MA, Krysko KL. 2003. Surveying for hellbender salamanders, *Cryptobranchus alleganiensis* (Daudin): A review and critique. Applied Herpetology **1**:37–44.

Nickerson MA, Krysko KL, Owen RD. 2003. Habitat differences affecting age class distributions of the hellbender salamander, *Cryptobranchus alleganiensis*. Southeastern Naturalist **2**:619–629.

Nielsen KM, Calamai L, Pietramellara G. 2006. Stabilization of Extracellular DNA and Proteins by Transient Binding to Various Soil Components. Pages 141–157 Soil Biology.

Olson Z, Briggler J, Williams R. 2012. An eDNA approach to detect eastern hellbenders (*Cryptobranchus a. alleganiensis*) using samples of water. Wildlife Research **39**:629–636.

Peterson CL, Wilkinson RF. 1996. Home Range Size of the Hellbender (*Cryptobranchus alleganiensis*) in Missouri. Herpetological Review **27**:126–127.

Petranka JW. 1998. Salamanders of the United States and Canada. Smithsonian Institutional Press, Washington DC.

Pilliod DS, Goldberg CS, Arkle RS, Waits LP. 2014. Factors influencing detection of eDNA from a stream-dwelling amphibian. Molecular Ecology Resources **14**:109–116.

Pilliod DS, Goldberg CS, Arkle RS, Waits LP, Richardson J. 2013. Estimating occupancy and abundance of stream amphibians using environmental DNA from filtered water samples. Canadian Journal of Fisheries and Aquatic Sciences **70**:1123–1130.

Price SJ, Dorcas ME, Gallant AL, Klaver RW, Willson JD. 2006. Three decades of urbanization: Estimating the impact of land-cover change on stream salamander populations. Biological Conservation **133**:436–441.

Pugh MW, Hutchins M, Madritch M, Siefferman L, Gangloff MM. 2015. Land-use and local physical and chemical habitat parameters predict site occupancy by hellbender salamanders. Hydrobiologia. **770:** 105-116.

R Core Team. 2016. R: A language and environment for statistical computing. R Core Team, Vienna, Austria.

Rees HC, Maddison BC, Middleditch DJ, Patmore JRM, Gough KC. 2014. Review: The detection of aquatic animal species using environmental DNA - a review of eDNA as a survey tool in ecology. Journal of Applied Ecology **51**:1450–1459.

Rossell CR, McNeal P, Gillette DP, Williams LA, Patch SC, Krebs AG. 2013. Attributes of shelters selected by eastern hellbenders (*Cryptobranchus a. alleganiensis*) in the French Broad River Basin of North Carolina. Journal of Herpetology **47**:66–70.

Schmidt BR, Kéry M, Ursenbacher S, Hyman OJ, Collins JP. 2013. Site occupancy models in the analysis of environmental DNA presence/absence surveys: A case study of an emerging amphibian pathogen. Methods in Ecology and Evolution **4**:646–653.

Sigsgaard EE, Carl H, Mller PR, Thomsen PF. 2015. Monitoring the near-extinct European weather loach in Denmark based on environmental DNA from water samples. Biological Conservation **183**:46–52.

Smith PW, Minton SA. 1957. A Distributional Summary of the Herpetofauna of Indiana and Illinois. American Midland Naturalist **58**:341–351.

Spear SF, Groves JD, Williams LA, Waits LP. 2015. Using environmental DNA methods to improve detectability in a hellbender (*Cryptobranchus alleganiensis*) monitoring program. Biological Conservation **183**:38–45.

Takahara T, Minamoto T, Yamanaka H, Doi H, Kawabata Z. 2012. Estimation of fish biomass using environmental DNA. PloS one **7**:e35868.

Thomsen PF, Kielgast J, Iversen LL, Wiuf C, Rasmussen M, Gilbert MTP, Orlando L, Willerslev E. 2012. Monitoring endangered freshwater biodiversity using environmental DNA. Molecular Ecology **21**:2565–73.

Thomsen PF, Willerslev E. 2015. Environmental DNA – An emerging tool in conservation for monitoring past and present biodiversity. Biological Conservation **183**:4–18.

Unger SD, Sutton TM, Williams RN. 2013. Projected population persistence of eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*) using a stage-structured life-history model and population viability analysis. Journal for Nature Conservation **21**:423–432.

Vie J-C, Hilton-Taylor C, Stuart SN. 2009. Wildlife in a changing world: An analysis of the 2008 IUCN Red List of Threatened Species. IUCN, Gland, Switzerland.

Wentworth CK. 1922. A scale of grade and class terms for clastic sediments. The Journal of Geology **30**:377–392.

Wheeler BA, Prosen E, Mathis A, Wilkinson RF. 2003. Population declines of a long-lived salamander : a 20 + year study of hellbenders, *Cryptobranchus alleganiensis*. Biological Conservation **109**:151–156.

Wolman MG. 1954. A method of sampling coarse river-bed material. American Geophysical Union Transactions **35**:951–956.

Tables and Figures

Table 1. Abbreviated list of site covariates and definitions used to estimate occupancy probabilities for traditional and eDNA survey methods.

Covariate	**Definition**	***Unit***
Average Depth	Depth measured across site and averaged between surveys	*cm*
Average Flow	Flow velocity measured across site and averaged between surveys	*m/s*
Average Stream Width	Wetted width of site averaged between surveys	*m*
Catchment Size	Area of upstream catchment from sampling location	m^2
Average Discharge	Calculated from site depth, width, and flow then averaged between surveys	m^3/s
Forest Cover Change	Percent change in forested upstream catchment from 1992-2011	*%*
Mean Substrate	Mean substrate size in site	*mm*
Median Substrate	Median substrate size in site	*mm*
Sand	Percent sand in site	*%*

Table 2. Abbreviated list of covariates and definitions used to estimate detection probabilities.

Covariate	Definition	*Unit*
Average Hellbender Length	Average length of all individual hellbenders per site	*cm*
Biomass	Average mass of all individual hellbenders multiplied by relative abundance per site	*g/150m^2*
Depth	Average depth of site per survey	*cm*
Discharge	Average discharge of site per survey	*m^3/s*
Divers (T)	Number of divers per survey	*people*
DNA Concentration (E)	Average of corrected DNA from qPCR	*ng*
Flow	Average flow velocity of site per survey	*m/s*
Hellbender Mass	Average mass of all individual hellbenders per site	*g*
Median Hellbender Length	Median length of all individual hellbenders per site	*cm*
Median Substrate Size	Median substrate size per site	*mm*
Relative Abundance	Number of individual hellbenders captured per site	*hellbenders*
Sand	Percent sand in site	*%*
Stream Width	Average wetted width of site per survey	*m*
Survey Number	Survey round number (1-3)	*survey*
Time (T)	Time spent searching for hellbender per survey	*min*

Total Hellbender Mass	Total mass of all individual hellbenders per site	*g*

* Covariates with an (T) represent a covariate only used in traditional survey models.

Covariates with an (E) represent a covariate only used in eDNA survey models

Table 3. Summary of AICc table for three best fit models for both traditional and eDNA survey methods.

Model Name	Model Covariates	K	AICc	Δ AICc	ω_i
eDNA Survey Method					
	$\psi(\bullet)$ p(DNA Conc. + Sand)	4	10	0	0.98
	$\psi(\bullet)$ p(DNA Conc. + Median Hellbender Length)	4	18.42	8.41	0.01
	$\psi(\bullet)$ p(DNA Conc. + Hellbender Mass)	4	19.15	9.15	0.01
	$\psi(\bullet)$ $p(\bullet)$	2	63.73	53.73	0
Traditional Survey Method					
	$\psi(\bullet)$ p(Average Hellbender Length + Relative Abundance)	4	39.87	0	0.61
	$\psi(\bullet)$ p(Hellbender Mass + Relative Abundance)	4	41.51	1.64	0.27
	ψ(Average Discharge^{X}) p(Hellbender Mass + Relative Abundance)	5	43.03	3.16	0.13
	$\psi(\bullet)$ $p(\bullet)$	2	67.48	27.61	0

* Nonsignificant model covariates are denoted with (^{X}).The • symbol in certain models indicates that no covariates were fitted to occupancy. *K* represents the number of parameters in a model. Δ AICc represents the difference in AICc value between each model and the best model in the set. ω_i gives the Akaike weight for each model.

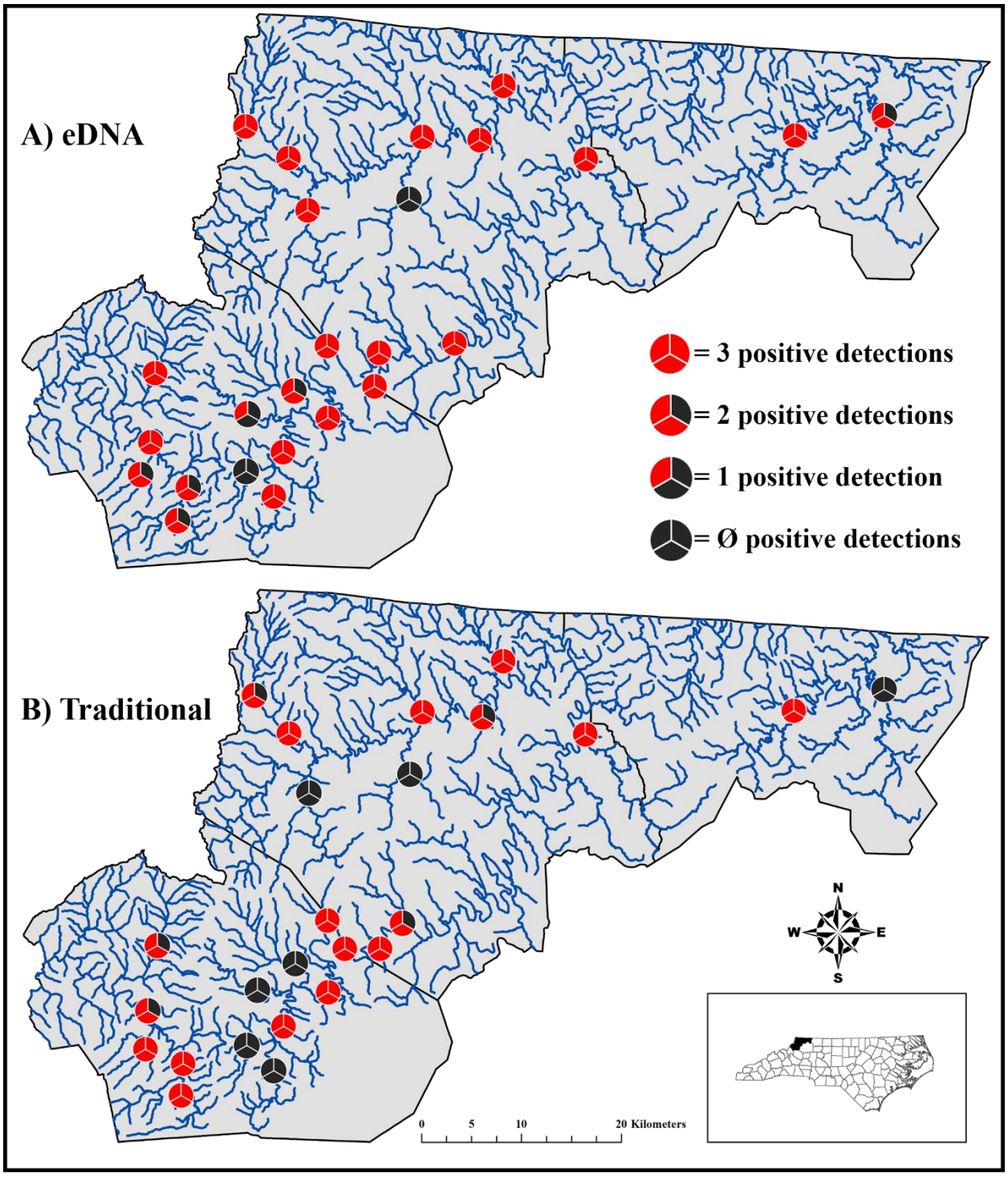

Figure 1. A map of study streams and sampling locations in northwestern North Carolina for A) eDNA surveys and B) traditional surveys. The circles represent each sampling location (n=25). A red section of a circle represents a positive detection for a

survey round. A black section of a circle represents a negative survey round. Together, the black and red sections make up the three repeated visits for each site.

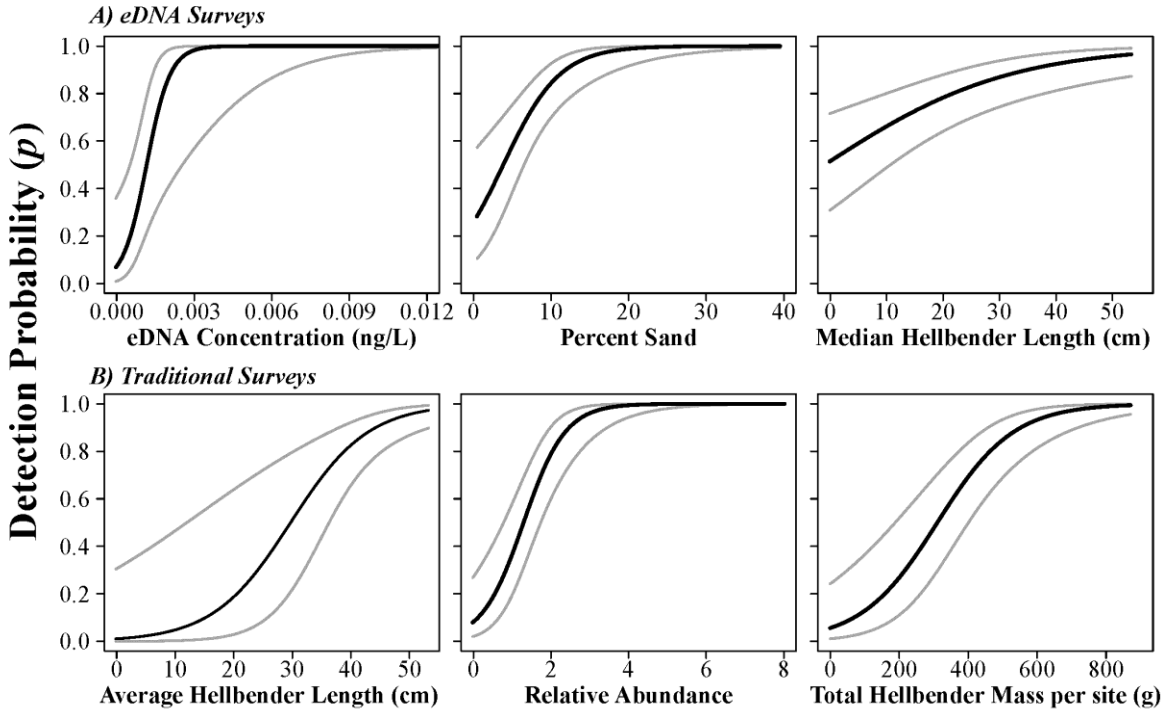

Figure 2. The predicted relationships between hellbender detection and significant covariates in best fitting models for A) eDNA Surveys and B) Traditional Surveys. 95% CI depicted by grey lines.

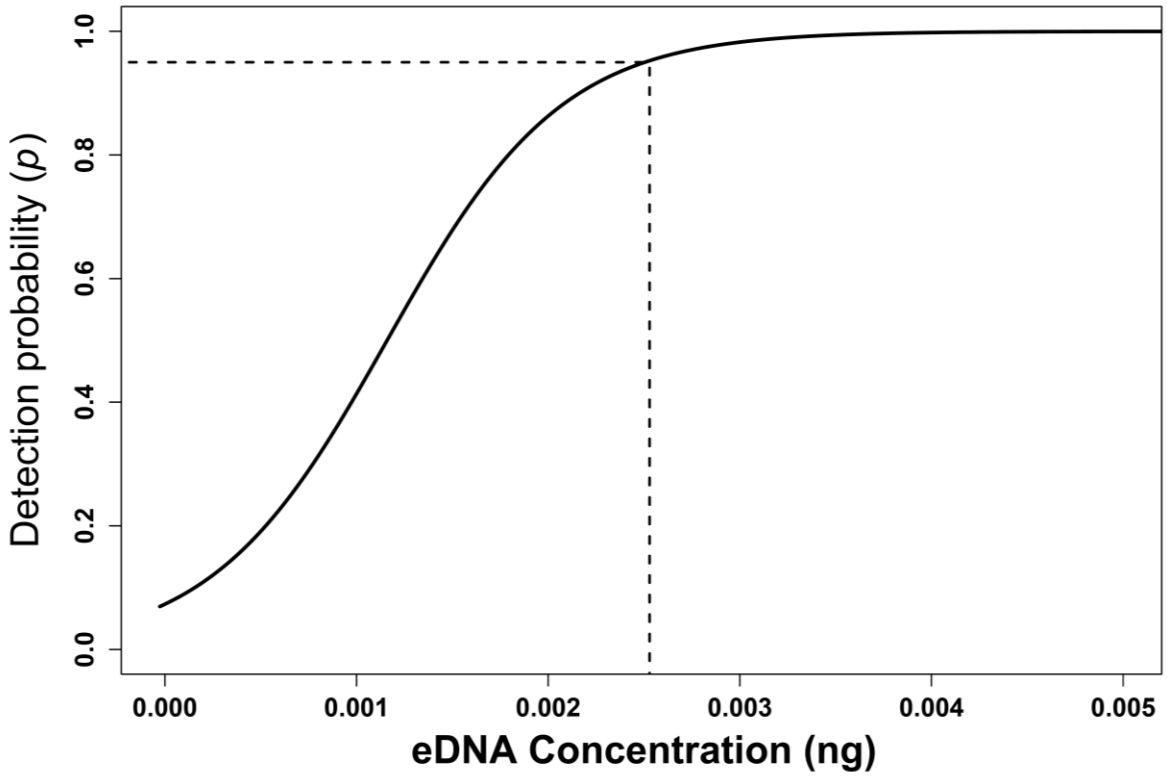

Figure 3. The predicted relationship between the amount of DNA (ng) needed to ensure 95% probability of detection. The dashed lines represents the 95% detection estimate and the corresponding DNA value (0.0025292 ng).

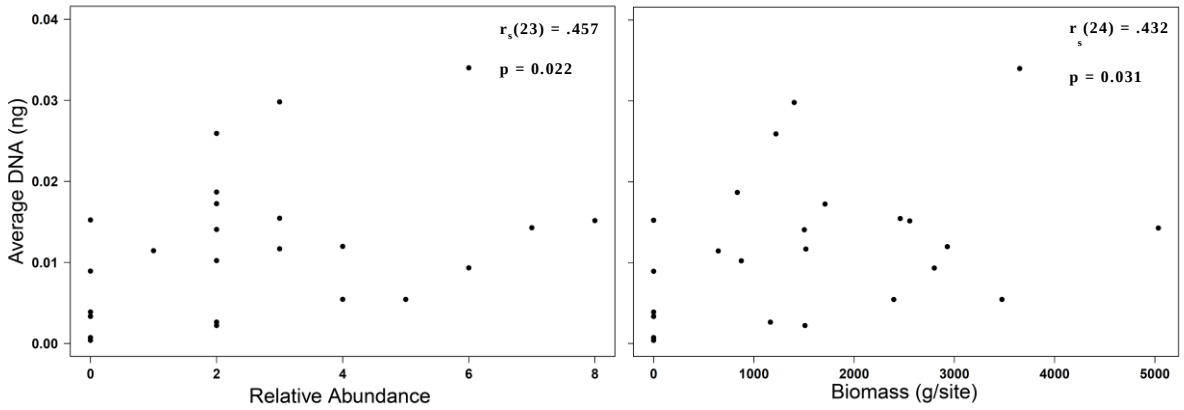

Figure 4. The positive relationships between DNA concentrations and relative abundance and biomass measurements.

Supporting Information

Table S1. The full list of site covariates and the corresponding definitions and units.

Covariate	Definition	*Unit*
Agriculture 1992	Percent agricultural land in the upstream catchment from 1992 LULC	*%*
Agriculture 2011	Percent agricultural land in the upstream catchment from 2011 LULC	*%*
Average Depth	Depth measured across 150m site and averaged between surveys	*cm*
Average Flow	Flow velocity measured across 150m site and averaged between surveys	*m/s*
Average Stream Width	Wetted width of 150m site averaged between surveys	*m*
Bedrock	Percent bedrock in 150m site	*%*
Boulder	Percent boulder in 150m site	*%*
Catchment Size	Area of upstream catchment from sampling location	m^2
Conductivity	Conductivity averaged between surveys	*µS/cm*
Discharge	Calculated from site depth, width, and flow then averaged between surveys	m^3/s
Disturbed 1992	Percent disturbed land in the upstream catchment from 1992 LULC	*%*
Disturbed 2011	Percent disturbed land in the upstream catchment from 2011 LULC	*%*
DO	Dissolved oxygen averaged between surveys	*mg/L*
Evergreen Forest Cover 1992	Percent evergreen forest cover in the upstream catchment from 1992 LULC	*%*
Evergreen Forest Cover 2011	Percent evergreen forest cover in the upstream catchment from 2011 LULC	*%*
Evergreen Forest Cover Change	Percent change in evergreen forest cover in the upstream catchment from 1992-2011	*%*
Forest Cover 1992	Percent forest cover in the upstream catchment from 1992 LULC	*%*
Forest Cover 1992 (No Evergreen)	Percent non-evergreen forest cover in the upstream catchment from 1992 LULC	*%*
Forest Cover 2011	Percent forest cover in the upstream catchment from 1992 LULC	*%*

Forest Cover 2011 (No Evergreen)	Percent non-evergreen forest cover in the upstream catchment from 2011 LULC	*%*
Forest Cover Change	Percent change in forested upstream catchment from 1992-2011	*%*
Forest Cover Change (No Evergreen)	Percent non-evergreen forest change in forested upstream catchment from 1992-2011	*%*
Mean Substrate	Mean substrate size in 150m site	*mm*
Median Substrate	Median substrate size in 150m site	*mm*
Miscellaneous Land 1992	Percent Miscellaneous land in the upstream catchment from 1992 LULC	*%*
Miscellaneous Land 2011	Percent Miscellaneous land in the upstream catchment from 2011 LULC	*%*
Nitrate	NO3- averaged between surveys	*mg/L*
Organic	Percent organic material in 150m site	*%*
pH	pH averaged between three surveys	*pH*
Sand	Percent sand in 150m site	*%*
Silt	Percent silt in 150m site	*%*
Specific Conductivity	Specific conductivity averaged between surveys	*µS/cm*
Temperature	Instream temperature averaged between surveys	*C*
Wood	Percent wood in 150m site	*%*

Table S2. The full list of survey-specific covariates and the corresponding definitions and units.

Covariate	Definition	*Unit*
Average Hellbender Length	Average length of all individual hellbenders per site	*cm*
Average Hellbender Mass	Average mass of all individual hellbenders per site	*g*
Bedrock	Percent bedrock per site	*%*
Biomass (average)	Average mass of all individual hellbenders multiplied by relative abundance per site	$g/150m^2$
Biomass (median)	Median mass of all individual hellbenders multiplied by relative abundance per site	$g/150m^2$
Boulder	Percent boulder per site	*%*
Depth	Average depth of site per survey	*cm*
Discharge	Average discharge of site per survey	m^3/s
Divers (T)	Number of divers per survey	*people*
DNA Concentration (E)	Average of corrected DNA concentration from qPCR	*ng/L*
Flow	Average flow velocity of site per survey	*m/s*
Mean Substrate	Mean substrate size per site	*mm*
Median Hellbender Length	Median length of all individual hellbenders per site	*cm*
Median Hellbender Mass	Median mass of all individual hellbenders per site	*g*
Median Substrate	Median substrate size per site	*mm*
Organic	Percent organic material per site	*%*
Relative Abundance	Number of individual hellbenders captured per site	*hellbenders*
Sand	Percent sand of site	*%*
Silt	Percent silt of site	*%*
Stream Width	Average wetted width of site per survey	*m*
Survey Number	Survey round number (1-3)	*survey*
Time (T)	Time spent searching for hellbender per survey	*min*
Total Hellbender Mass	Total mass of all individual hellbenders per site	*g*
Wood	Percent wood per site	*%*

* Covariates with an (T) represent a covariate only used in traditional survey models. Covariates with an (E) represent a covariate only used in eDNA survey models.

Table S3. Instream substrate measurements collected for each site.

Site	Sand (%)	Wood (%)	Bedrock (%)	Organic (%)	Silt (%)	Boulder (%)	Mean Substrate Size (cm)	Median Substrate Size (cm)
1	0.24	0.02	0.02	0.06	0.10	0.00	164.25	120.00
2	0.20	0.04	0.18	0.01	0.03	0.04	141.53	110.00
3	0.19	0.10	0.03	0.03	0.02	0.00	115.28	51.00
4	0.01	0.01	0.04	0.01	0.00	0.00	305.32	181.00
5	0.13	0.06	0.05	0.03	0.13	0.00	95.21	50.00
6	0.02	0.02	0.01	0.01	0.05	0.00	101.87	70.00
7	0.17	0.01	0.30	0.01	0.17	0.00	159.22	110.00
8	0.40	0.01	0.02	0.01	0.10	0.00	174.99	120.00
9	0.15	0.01	0.04	0.02	0.06	0.00	156.28	93.50
10	0.15	0.02	0.08	0.02	0.06	0.01	152.85	102.50
11	0.09	0.02	0.13	0.03	0.05	0.00	153.71	100.00
12	0.19	0.04	0.05	0.03	0.07	0.02	250.43	140.00
13	0.07	0.01	0.20	0.00	0.02	0.00	139.19	85.00
14	0.17	0.01	0.18	0.02	0.07	0.03	137.80	50.00
15	0.18	0.04	0.18	0.12	0.02	0.01	155.77	90.00
16	0.12	0.00	0.25	0.02	0.03	0.01	293.84	174.00
17	0.14	0.01	0.18	0.01	0.00	0.00	120.97	76.00
18	0.12	0.02	0.31	0.10	0.03	0.00	163.31	80.00
19	0.09	0.08	0.00	0.10	0.04	0.00	338.75	230.00
20	0.17	0.07	0.24	0.05	0.21	0.01	134.33	70.00
21	0.19	0.03	0.09	0.09	0.07	0.00	176.06	95.00
22	0.06	0.01	0.16	0.03	0.01	0.00	134.60	90.00
23	0.04	0.01	0.28	0.01	0.01	0.11	176.39	145.00
24	0.08	0.00	0.04	0.00	0.02	0.00	126.72	103.00

25	0.02	0.03	0.55	0.01	0.00	0.00	238.18	102.00
Total	**0.14 (0.02)**	**0.03 (0.01)**	**0.14 (0.03)**	**0.03 (0.01)**	**0.05 (0.01)**	**0.01 (0)**	**172.27 (12.73)**	**105.52 (8.51)**

***** "Total" represents the mean and the parentheses denote the standard error of the mean.

Table S4. Instream habitat measurements of water quality and stream size.

Site	Conductivity (µS/cm)	Depth (cm)	Discharge (m^3/s)	DO (mg/L)	NO3 (mg/L)	pH	SPC (µS/cm)	Temperature (C)	Velocity (m/s)	Width (m)
1	52.37	39.86	25.67	86.57	0.28	7.30	58.00	19.77	0.27	12.41
2	42.30	30.59	19.41	87.00	0.20	7.30	45.53	21.33	0.32	10.16
3	32.45	24.41	11.45	89.53	0.30	7.09	37.73	16.17	0.34	7.37
4	24.55	30.13	16.40	87.17	0.19	7.09	26.77	17.13	0.19	14.11
5	37.37	40.74	20.38	89.43	0.37	6.89	40.23	21.30	0.26	13.45
6	67.37	19.57	8.46	85.10	0.51	7.66	73.00	20.87	0.33	6.77
7	85.97	26.12	9.89	86.53	0.46	7.69	98.97	18.07	0.21	9.30
8	38.50	42.18	29.09	87.80	0.38	7.22	43.07	19.33	0.32	11.59
9	51.27	30.57	9.28	86.27	0.30	7.12	58.20	18.67	0.25	7.86
10	58.27	42.11	25.71	85.63	0.45	7.41	65.73	19.17	0.30	11.58
11	48.90	20.99	6.65	81.57	0.22	7.27	54.93	19.23	0.26	7.40
12	38.73	31.87	26.75	85.07	0.35	7.04	42.70	20.20	0.29	14.57
13	48.07	24.35	15.24	84.47	0.16	7.32	55.70	17.83	0.36	8.27
14	88.35	40.89	15.41	86.53	0.38	7.30	100.23	17.20	0.19	12.52
15	64.40	43.15	136.72	88.30	0.70	7.64	67.57	22.53	0.46	35.30
16	101.33	40.78	61.62	94.53	1.29	8.04	106.63	22.27	0.28	28.70
17	98.93	43.85	86.29	97.50	0.99	7.97	103.73	22.47	0.39	25.74
18	94.87	42.12	109.40	90.27	1.26	8.06	98.33	22.97	0.40	31.99
19	125.30	38.73	20.42	86.40	1.51	7.37	141.20	18.90	0.19	17.27
20	117.03	74.96	43.41	80.10	1.09	7.46	134.37	18.00	0.14	20.56
21	116.60	52.66	61.24	94.43	2.02	7.95	111.57	21.07	0.34	15.69
22	39.47	25.05	18.33	84.37	0.17	7.20	45.17	18.20	0.33	10.84
23	43.80	45.60	21.93	89.07	0.22	7.15	50.03	18.33	0.20	16.01
24	63.33	35.90	30.80	90.47	0.25	7.55	68.80	20.77	0.24	18.52

25	34.47	17.70	5.22	84.40	0.12	6.88	38.17	19.97	0.24	7.04
Total	**64.56 (6.01)**	**36.2 (2.46)**	**33.41 (6.67)**	**87.54 (0.78)**	**0.57 (0.1)**	**7.4 (0.07)**	**70.65 (6.4)**	**19.67 (0.37)**	**0.28 (0.02)**	**15 (1.59)**

***** "Total" represents the mean and the parentheses denote the standard error of the mean.

Table S5. Land Use Land Cover (LULC) measurements from 1992 USGS data for each site.

Site	Agriculture 1992	Disturbed 1992	Evergreen Forest Cover 1992	Forest Cover 1992	Forest Cover 1992 (No Evergreen)	Miscellaneous Land 1992
1	9.92	0.13	8.63	89.46	80.84	0.47
2	8.14	0.00	9.04	91.19	82.15	0.67
3	9.30	0.00	9.75	90.31	80.57	0.39
4	29.23	1.21	31.24	68.55	37.32	0.65
5	20.26	1.07	7.23	78.25	71.02	0.39
6	22.08	0.01	19.91	77.83	57.92	0.07
7	8.00	0.06	11.74	91.88	80.14	0.04
8	13.93	0.03	8.47	86.01	77.54	0.02
9	6.55	0.21	7.18	93.05	85.88	0.13
10	14.33	0.07	4.39	85.41	81.02	0.13
11	11.73	5.80	19.37	81.03	61.66	0.88
12	16.87	4.09	10.76	78.30	67.54	0.56
13	16.33	3.48	11.23	79.49	68.26	0.50
14	9.22	0.53	8.91	89.91	81.00	0.31
15	16.39	3.26	11.98	79.66	67.68	0.49
16	4.90	0.02	29.95	93.73	63.78	0.37
17	6.22	0.45	6.21	92.82	86.62	0.48
18	22.35	2.78	14.70	73.56	58.86	1.25
19	14.10	8.82	15.31	75.77	60.46	0.94
20	15.17	4.80	10.46	79.16	68.70	0.65
21	3.16	0.00	7.81	96.68	88.87	0.11
22	4.79	1.67	21.90	92.75	70.85	0.36
23	16.13	3.60	10.51	79.54	69.03	0.52
24	6.22	2.91	18.02	89.92	71.90	0.67

25	4.43	0.37	12.09	94.39	82.30	0.05
Total	**12.39 (1.33)**	**1.81 (0.46)**	**13.07 (1.38)**	**85.15 (1.55)**	**72.08 (2.33)**	**0.44 (0.06)**

* All LULC classes are percentages of the total catchment area. “Total” represents the mean and the parentheses denote the standard error of the mean.

Table S6. Land Use Land Cover (LULC) measurements from 2011 USGS data for each site.

Site	Agriculture 2011	Disturbed 2011	Evergreen Forest Cover 2011	Forest Cover 2011	Forest Cover 2011 (No Evergreen)	Miscellaneous Land 2011
1	13.48	4.04	3.56	80.19	78.45	2.29
2	13.17	2.76	8.96	80.35	78.46	3.72
3	12.58	2.35	3.35	81.43	79.65	3.64
4	4.04	8.66	1.75	85.60	82.48	1.70
5	35.06	11.02	0.59	51.69	35.94	2.24
6	8.96	2.15	1.89	86.52	85.94	2.37
7	18.85	8.31	1.78	71.30	70.80	1.54
8	30.37	5.74	1.04	59.44	50.47	4.46
9	8.43	4.78	2.72	85.88	84.75	0.91
10	17.87	4.26	2.82	75.53	72.18	2.34
11	9.45	4.88	3.19	82.62	82.06	3.05
12	30.40	9.00	0.23	56.61	53.05	3.99
13	16.59	4.23	0.50	76.84	76.61	2.33
14	9.93	23.70	0.56	65.05	60.18	1.31
15	12.25	3.88	2.18	81.60	79.85	2.27
16	14.95	15.17	15.76	67.75	65.03	2.13
17	15.31	13.37	1.74	69.06	66.24	2.27
18	15.80	12.78	2.56	68.92	65.73	2.51
19	9.82	26.18	3.58	62.66	59.08	1.35
20	13.44	16.51	2.67	68.07	65.88	1.98
21	14.82	13.69	1.13	69.31	66.75	2.18
22	5.27	1.19	2.29	91.22	90.17	2.32
23	4.90	11.15	4.87	82.61	80.15	1.34
24	6.30	13.37	2.46	79.02	76.73	1.30

25	5.12	5.71	3.11	87.77	85.10	1.39
Total	**13.89 (1.6)**	**9.16 (1.31)**	**3.01 (0.64)**	**74.68 (2.1)**	**71.67 (2.58)**	**2.28 (0.18)**

*****All LULC classes are percentages of the total catchment area. "Total" represents the mean and the parentheses denote the standard error of the mean.

Table S7. Changes in forest cover from 1992 to 2011 in which a negative value represents forest was removed from 1992 to 2011.

Site	Catchment Size	Forest Cover Change	Forest Cover Change (No Evergreen)	Evergreen Forest Cover Change
1	161649	-9.27	-2.38	-5.06
2	68655	-10.84	-3.69	-0.07
3	39171	-8.88	-0.91	-6.40
4	27031	17.05	45.17	-29.49
5	89167	-26.56	-35.09	-6.64
6	36389	8.69	28.01	-18.02
7	51978	-20.58	-9.34	-9.96
8	106015	-26.58	-27.07	-7.43
9	30383	-7.17	-1.12	-4.45
10	127033	-9.88	-8.84	-1.57
11	26214	1.59	20.40	-16.19
12	88853	-21.69	-14.49	-10.53
13	57424	-2.65	8.35	-10.73
14	34193	-24.85	-20.82	-8.34
15	619957	1.93	12.17	-9.80
16	252428	-25.98	1.25	-14.19
17	299844	-23.77	-20.38	-4.46
18	322449	-4.65	6.87	-12.14
19	89404	-13.11	-1.38	-11.73
20	212010	-11.10	-2.82	-7.79
21	289348	-27.37	-22.12	-6.68
22	65810	-1.54	19.32	-19.61
23	64216	3.07	11.11	-5.64
24	106574	-10.89	4.84	-15.56

25	16490	-6.62	2.80	-8.97
Total	**131307.4 (27510.23)**	**-10.47 (2.41)**	**-0.41 (3.59)**	**-10.06 (1.26)**

* "Total" represents the mean and the parentheses denote the standard error of the mean.

Table S8. Site estimates for hellbender abundance, mass, and length based upon captures during round one of traditional surveys.

Site	Average Mass (g)	Average Total Length (cm)	Biomass (Average)	Biomass (Median)	Median Mass (g)	Median Total Length (cm)	Relative Abundance	Total mass (g)
1	610	47	1220	1220	610	47	2	1210
2	583	45	1165	1165	583	45	2	1896
3	438	41	875	875	438	41	2	875
4	468	46	1403	1433	478	45	3	1870
5	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
7	418	39	835	835	418	39	2	835
8	820	46	2460	1365	455	40	3	2460
9	755	51	1510	1580	790	50	2	2265
10	733	51	2930	2990	748	53	4	2930
11	0	0	0	0	0	0	0	0
12	719	47	5035	5075	725	47	7	5035
13	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0
15	645	49	645	645	645	49	1	645
16	609	48	3652	3921	654	50	6	3652
17	467	43	2800	2685	448	45	6	2800
18	479	42	2396	2538	508	46	5	2875
19	869	50	3475	3660	915	52	4	3475
20	506	43	1519	1320	440	42	3	2025
21	752	50	1503	1480	740	52	2	2255
22	0	0	0	0	0	0	0	0

23	319	35	2555	2600	325	39	8	3194
24	855	53	1710	1710	855	53	2	1710
25	0	0	0	0	0	0	0	0
Total	**441.69 (62.68)**	**32.97 (4.27)**	**1507.5 (274.34)**	**1483.84 (279.35)**	**430.84 (61.86)**	**33.33 (4.32)**	**2.56 (0.47)**	**1680.26 (285.19)**

* For the last row, “Total” represents the mean and the parentheses denote the standard error of the mean.

Table S9. The AICc table for the 30 best fit traditional occupancy-only models.

Model Name	K	AIC	Δ AIC	ω_i
p_._psi_Depth_avg_site_Median_Substrate_avg_site_	4	55.93	0	0.12
p_._psi_Catchment_Size_site_Median_Substrate_avg_site_	4	56.23	0.3	0.10
p_._psi_Avg_Discharge_site_Median_Substrate_avg_site_	4	56.33	0.39	0.10
p_._psi_Width_avg_site_Median_Substrate_avg_site_	4	57.83	1.9	0.05
p_._psi_Catchment_Size_site_Mean_Substrate_avg_site_	4	58.41	2.48	0.03
p_._psi_Avg_Discharge_site_Mean_Substrate_avg_site_	4	58.63	2.69	0.03
p_._psi_Avg_Discharge_site_DO_avg_site_	4	59.14	3.2	0.02
p_._psi_Catchment_Size_site_DO_avg_site_	4	59.16	3.23	0.02
p_._psi_Avg_Discharge_site_	3	59.24	3.3	0.02
p_._psi_Median_Substrate_avg_site_DO_avg_site_	4	59.35	3.42	0.02
p_._psi_Catchment_Size_site_	3	60.23	4.3	0.01
p_._psi_Depth_avg_site_Mean_Substrate_avg_site_	4	60.34	4.41	0.01
p_._psi_Avg_Discharge_site_MeanSubBoulder_	4	60.62	4.69	0.01
p_._psi_Width_avg_site_DO_avg_site_	4	60.66	4.73	0.01
p_._psi_Width_avg_site_	3	60.71	4.78	0.01
p_._psi_Depth_avg_site_DO_avg_site_	4	60.72	4.79	0.01
p_._psi_Avg_Discharge_site_pH_avg_site_	4	60.94	5.01	0.01
p_._psi_Median_Substrate_avg_site_pH_avg_site_	4	61.04	5.11	0.01
p_._psi_Avg_Discharge_site_Depth_avg_site_	4	61.07	5.14	0.01
p_._psi_Avg_Discharge_site_Width_avg_site_	4	61.15	5.22	0.01
p_._psi_Depth_avg_site_	3	61.15	5.22	0.01
p_._psi_Avg_Discharge_site_organic_site_	4	61.21	5.28	0.01
p_._psi_Avg_Discharge_site_Catchment_Size_site_	4	61.22	5.29	0.01
p_._psi_Catchment_Size_site_Width_avg_site_	4	61.25	5.32	0.01
p_._psi_Width_avg_site_MeanSubBoulder_	4	61.26	5.32	0.01
p_._psi_Width_avg_site_Mean_Substrate_avg_site_	4	61.31	5.38	0.01
p_._psi_Catchment_Size_site_Depth_avg_site_	4	61.43	5.49	0.01
p_._psi_Catchment_Size_site_MeanSubBoulder_	4	61.75	5.81	0.01
p_._psi_Width_avg_site_Depth_avg_site_	4	62	6.07	0.01
p_._psi_Depth_avg_site_pH_avg_site_	4	62.06	6.13	0.01
p_._psi_._	2	66.94	11	0.00

Table S9. The AICc table for the 30 best fit eDNA occupancy-only models.

Model Name	K	AIC	Δ AIC	ω_i
p_._psi_MeanSubBoulder	3	51.34	0	0.03
p_._psi_Depth_avg_site	3	51.35	0.0053	0.03
p_._psi_Width_avg_site	3	51.39	0.0531	0.03
p_._psi_Depth_avg_site_MedSubBoulder	4	53.3	1.9605	0.01
p_._psi_Depth_avg_site_organic_site	4	53.3	1.961	0.01
p_._psi_MeanSubBoulder_Depth_avg_site	4	53.3	1.961	0.01
p_._psi_sand_site_Misc_1992_site	4	53.3	1.9614	0.01
p_._psi_MedSubBoulder_Disturbed_2011_site	4	53.3	1.9614	0.01
p_._psi_Width_avg_site_sand_site	4	53.3	1.9616	0.01
p_._psi_Width_avg_site_MedSubBoulder	4	53.3	1.9622	0.01
p_._psi_MeanSubBoulder_Disturbed_2011_site	4	53.3	1.9626	0.01
p_._psi_MeanSubBoulder_Width_avg_site	4	53.3	1.963	0.01
p_._psi_Avg_Discharge_site_MedSubBoulder	4	53.31	1.965	0.01
p_._psi_Misc_1992_site_FinePart	4	53.31	1.9655	0.01
p_._psi_Misc_1992_site_Forest_2011_site	4	53.31	1.967	0.01
p_._psi_Depth_avg_site_Misc_1992_site	4	53.31	1.9674	0.01
p_._psi_Depth_avg_site_FinePart	4	53.31	1.9674	0.01
p_._psi_Depth_avg_site_sand_site	4	53.31	1.9678	0.01
p_._psi_MeanSubBoulder_Avg_Discharge_site	4	53.31	1.9686	0.01
p_._psi_Misc_1992_site_Forest__No_EG__2011_site	4	53.31	1.9689	0.01
p_._psi_Depth_avg_site_Disturbed_1992_site	4	53.31	1.9689	0.01
p_._psi_MedSubBoulder_Misc_1992_site	4	53.31	1.9691	0.01
p_._psi_MeanSubBoulder_Forest__No_EG__2011_site	4	53.31	1.9691	0.01
p_._psi_MeanSubBoulder_Misc_1992_site	4	53.31	1.9697	0.01
p_._psi_MeanSubBoulder_sand_site	4	53.31	1.97	0.01
p_._psi_MeanSubBoulder_Catchment_Size_site	4	53.31	1.97	0.01
p_._psi_MeanSubBoulder_Forest_2011_site	4	53.31	1.9702	0.01
p_._psi_MeanSubBoulder_Disturbed_1992_site	4	53.31	1.9704	0.01
p_._psi_sand_site_Disturbed_1992_site	4	53.31	1.9711	0.01
p_._psi_FinePart_Disturbed_1992_site	4	53.31	1.9729	0.01

Table S10. The AICc table for the 30 best fit traditional detection-only models.

Model Name	K	AIC	Δ AIC	ω_i
p_._psi_Depth_avg_site_Median_Substrate_avg_site_	4	55.93	0	0.12
p_._psi_Catchment_Size_site_Median_Substrate_avg_site_	4	56.23	0.3	0.10
p_._psi_Avg_Discharge_site_Median_Substrate_avg_site_	4	56.33	0.39	0.10
p_._psi_Width_avg_site_Median_Substrate_avg_site_	4	57.83	1.9	0.05
p_._psi_Catchment_Size_site_Mean_Substrate_avg_site_	4	58.41	2.48	0.03
p_._psi_Depth_avg_site_XEvergreen2011_site_	4	58.59	2.65	0.03
p_._psi_Avg_Discharge_site_Mean_Substrate_avg_site_	4	58.63	2.69	0.03
p_._psi_Avg_Discharge_site_DO_avg_site_	4	59.14	3.2	0.02
p_._psi_Catchment_Size_site_DO_avg_site_	4	59.16	3.23	0.02
p_._psi_Avg_Discharge_site_	3	59.24	3.3	0.02
p_._psi_Median_Substrate_avg_site_DO_avg_site_	4	59.35	3.42	0.02
p_._psi_Catchment_Size_site_	3	60.23	4.3	0.01
p_._psi_Depth_avg_site_Mean_Substrate_avg_site_	4	60.34	4.41	0.01
p_._psi_Avg_Discharge_site_MeanSubBoulder_	4	60.62	4.69	0.01
p_._psi_Width_avg_site_DO_avg_site_	4	60.66	4.73	0.01
p_._psi_Width_avg_site_	3	60.71	4.78	0.01
p_._psi_Depth_avg_site_DO_avg_site_	4	60.72	4.79	0.01
p_._psi_Avg_Discharge_site_pH_avg_site_	4	60.94	5.01	0.01
p_._psi_Median_Substrate_avg_site_pH_avg_site_	4	61.04	5.11	0.01
p_._psi_Avg_Discharge_site_Depth_avg_site_	4	61.07	5.14	0.01
p_._psi_Avg_Discharge_site_Width_avg_site_	4	61.15	5.22	0.01
p_._psi_Depth_avg_site_	3	61.15	5.22	0.01
p_._psi_Avg_Discharge_site_organic_site_	4	61.21	5.28	0.01
p_._psi_Avg_Discharge_site_Catchment_Size_site_	4	61.22	5.29	0.01
p_._psi_Catchment_Size_site_Width_avg_site_	4	61.25	5.32	0.01
p_._psi_Width_avg_site_MeanSubBoulder_	4	61.26	5.32	0.01
p_._psi_Width_avg_site_Mean_Substrate_avg_site_	4	61.31	5.38	0.01
p_._psi_Catchment_Size_site_Depth_avg_site_	4	61.43	5.49	0.01
p_._psi_Catchment_Size_site_MeanSubBoulder_	4	61.75	5.81	0.01
p_._psi_Width_avg_site_Depth_avg_site_	4	62	6.07	0.01

Table S11. The AICc table for the 30 best fit eDNA detection-only models.

Model Name	K	AIC	Δ AIC	ω_i
p_Average_eDNA_sand_site_psi_._	4	8.95	0.95	0.37
p_Average_eDNA_Median_Total_Length_per_site_psi_._	4	16.42	8.41	0.01
p_Average_eDNA_Avg_mass_per_site_psi_._	4	17.15	9.15	0.01
p_Average_eDNA_Total_mass_per_site_psi_._	4	19.17	11.17	0.00
p_Average_eDNA_MeanSubBoulder_psi_._	4	19.62	11.62	0.00
p_Average_eDNA_Biomass_per_site__Average__site_psi_._	4	20.58	12.58	0.00
p_Average_eDNA_Discharge_psi_._	4	20.61	12.6	0.00
p_Average_eDNA_Mean_Depth_psi_._	4	21.53	13.53	0.00
p_Average_eDNA_psi_._	3	22.44	14.44	0.00
p_Average_eDNA_Mean_Width_psi_._	4	23.46	15.45	0.00
p_sand_site_Mean_Width_psi_._	4	41.61	33.61	0.00
p_Discharge_MeanSubBoulder_psi_._	4	42.93	34.93	0.00
p_Mean_Width_MeanSubBoulder_psi_._	4	45	36.99	0.00
p_Discharge_Avg_mass_per_site_psi_._	4	47.07	39.06	0.00
p_Discharge_Median_mass_per_site_psi_._	4	47.27	39.26	0.00
p_Discharge_Average_Total_Length_per_site_psi_._	4	47.42	39.42	0.00
p_Mean_Depth_MeanSubBoulder_psi_._	4	47.48	39.48	0.00
p_Discharge_Median_Total_Length_per_site_psi_._	4	47.62	39.61	0.00
p_sand_site_Median_Total_Length_per_site_psi_._	4	47.66	39.66	0.00
p_sand_site_Biomass_per_site__Average__site_psi_._	4	47.74	39.74	0.00
p_sand_site_Average_Total_Length_per_site_psi_._	4	47.78	39.78	0.00
p_sand_site_Biomass_per_site__Median__site_psi_._	4	47.84	39.84	0.00
p_sand_site_Mean_Depth_psi_._	4	48.04	40.04	0.00
p_sand_site_Median_Substrate_avg_site_psi_._	4	48.1	40.1	0.00
p_Discharge_Sal_psi_._	4	48.21	40.21	0.00
p_sand_site_Avg_mass_per_site_psi_._	4	48.44	40.44	0.00
p_sand_site_Median_mass_per_site_psi_._	4	48.59	40.59	0.00
p_sand_site_Total_mass_per_site_psi_._	4	48.76	40.75	0.00
p_Median_Total_Length_per_site_MeanSubBoulder_psi_._	4	49.79	41.79	0.00
p_Discharge_psi_._	3	50.3	42.29	0.00
p_._psi_._	2	63.19	55.18	0.00

Table S12. The AICc table for the 30 best fit traditional single species, single season occupancy models.

Model Name	K	AIC	Δ AIC	ω_i
p_Average_Total_Length_Relative_Abundance_psi_._	4	37.87	0	0.16
p_Avg_mass_Relative_Abundance_psi_._	4	39.51	1.64	0.07
p_Avg_mass_Relative_Abundance_psi_Avg_Discharge_	5	39.87	2	0.06
p_Median_Total_Length_Biomass_Median_psi_._	4	40.32	2.45	0.05
p_Average_Total_Length_psi_._	3	40.53	2.65	0.04
p_Median_Total_Length_psi_._	3	40.73	2.86	0.04
p_Relative_Abundance_Median_mass_psi_._	4	41.06	3.19	0.03
p_Average_Total_Length_psi_Width_Median_Substrate_	5	41.44	3.57	0.03
p_Average_Total_Length_psi_Depth_Median_Substrate_	5	41.44	3.57	0.03
p_Average_Total_Length_Median_Substrate_psi_Depth_Median_Substrate_	5	41.51	3.64	0.03
p_Average_Total_Length_Median_Substrate_psi_Width_Median_Substrate_	5	41.51	3.64	0.03
p_Average_Total_Length_AvgDepth_psi_Catchment_Size_Median_Substrate_	5	41.57	3.7	0.03
p_Average_Total_Length_AvgDepth_psi_Avg_Discharge_Median_Substrate_	5	41.57	3.7	0.03
p_Average_Total_Length_psi_Avg_Discharge_	6	41.87	4	0.02
p_Average_Total_Length_psi_Median_Substrate_	6	41.87	4	0.02
p_Average_Total_Length_psi_Avg_Discharge_Median_Substrate_	6	41.87	4	0.02
p_Average_Total_Length_psi_Catchment_Size_Median_Substrate_	6	41.87	4	0.02
p_Median_Total_Length_Median_mass_psi_._	4	41.9	4.03	0.02
p_Average_Total_Length_Avg_mass_psi_._	4	42.28	4.41	0.02
p_Average_Total_Length_Relative_Abundance_psi_Median_Substrate_	4	42.52	4.65	0.02
p_Average_Total_Length_Relative_Abundance_psi_Avg_Discharge_	4	42.53	4.65	0.02
p_Median_Total_Length_Avg_mass_psi_._	4	42.71	4.84	0.01
p_Total_mass_psi_._	3	42.72	4.85	0.01
p_Average_Total_Length_Relative_Abundance_psi_Depth_Median_Substrate_	6	43.44	5.57	0.01
p_Average_Total_Length_Relative_Abundance_psi_Width_Median_Substrate_	6	43.44	5.57	0.01
p_Average_Total_Length_Relative_Abundance_psi_Catchment_Size_Median_Substrate_	6	43.44	5.57	0.01

p_Average_Total_Length_Relative_Abundance_psi_Avg_Discharge_Median_Substrate_	6	43.44	5.57	0.01
p_Avg_mass_Relative_Abundance_psi_Width_Median_Substrate_	6	43.51	5.64	0.01
p_Avg_mass_Relative_Abundance_psi_Depth_Median_Substrate_	6	43.51	5.64	0.01
p_Avg_mass_Relative_Abundance_psi_Catchment_Size_Median_Substrate_	6	43.51	5.64	0.01
p_._psi_._	2	66.94	29.07	0.001

Table S13. The AICc table for the 30 best fit eDNA single species, single season occupancy models.

Model Name	K	AIC	Δ AIC	ω_i
p_Average_eDNA_sand_site_psi_._	4	8.95	0.95	0.37
p_Average_eDNA_Median_Total_Length_per_site_psi_._	4	16.42	8.41	0.01
p_Average_eDNA_Avg_mass_per_site_psi_._	4	17.15	9.15	0.01
p_Average_eDNA_Total_mass_per_site_psi_._	4	19.17	11.17	0.00
p_Average_eDNA_MeanSubBoulder_psi_._	4	19.62	11.62	0.00
p_Average_eDNA_Biomass_per_site__Average__site_psi_._	4	20.58	12.58	0.00
p_Average_eDNA_Discharge_psi_._	4	20.61	12.6	0.00
p_Average_eDNA_psi_._	3	22.44	14.44	0.00
p_Average_eDNA_Mean_Width_psi_._	4	23.46	15.45	0.00
p_Average_eDNA_NO3_mg_L_psi_._	4	24.2	16.2	0.00
p_Discharge_sand_site_psi_._	4	41.15	33.14	0.00
p_sand_site_Mean_Width_psi_._	4	41.61	33.61	0.00
p_Discharge_MeanSubBoulder_psi_._	4	42.93	34.93	0.00
p_Mean_Width_MeanSubBoulder_psi_._	4	45	36.99	0.00
p_Mean_Width_FinePart_psi_._	4	45.92	37.92	0.00
p_Discharge_Avg_mass_per_site_psi_._	4	47.07	39.06	0.00
p_Discharge_Median_mass_per_site_psi_._	4	47.27	39.26	0.00
p_Discharge_Average_Total_Length_per_site_psi_._	4	47.42	39.42	0.00
p_Mean_Depth_MeanSubBoulder_psi_._	4	47.48	39.48	0.00
p_Discharge_Median_Total_Length_per_site_psi_._	4	47.62	39.61	0.00
p_sand_site_Median_Total_Length_per_site_psi_._	4	47.66	39.66	0.00
p_sand_site_Biomass_per_site__Average__site_psi_._	4	47.74	39.74	0.00
p_sand_site_Average_Total_Length_per_site_psi_._	4	47.78	39.78	0.00
p_sand_site_Biomass_per_site__Median__site_psi_._	4	47.84	39.84	0.00
p_sand_site_Mean_Depth_psi_._	4	48.04	40.04	0.00
p_sand_site_Median_Substrate_avg_site_psi_._	4	48.1	40.1	0.00
p_sand_site_Avg_mass_per_site_psi_._	4	48.44	40.44	0.00
p_sand_site_Median_mass_per_site_psi_._	4	48.59	40.59	0.00
p_sand_site_Total_mass_per_site_psi_._	4	48.76	40.75	0.00
p_._psi_._	2	63.19	55.18	0.00

Vita

Thomas W. Franklin was born in Santa Rosa, California to Susan and Ken Franklin in October 1991. Thomas has been enthused by nature and streams since he was a toddler. He received his Bachelor of Science degree from Appalachian State University in May 2014 where he was a Biology Department Honors Student and was awarded the Meritorious Senior Award. Thomas was also inducted into the Cratis Williams Society of Outstanding Graduates of the Graduate School at Appalachian in April 2016.