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INTERACTIONS BETWEEN EASTERN NEWTS AND WOOD FROGS IN AN ANTHROPOGENICALLY ALTERED WETLAND SYSTEM IN EASTERN KENTUCKY

By

Chelsea S. Kross

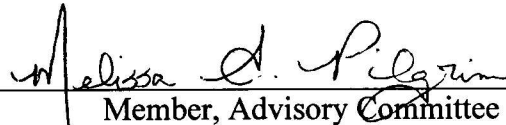Thesis Approved:

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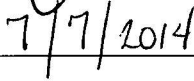
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INTERACTIONS BETWEEN EASTERN NEWTS AND WOOD FROGS IN AN
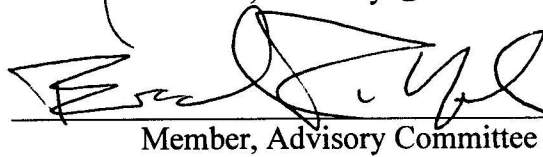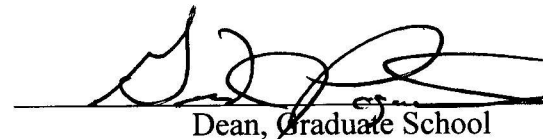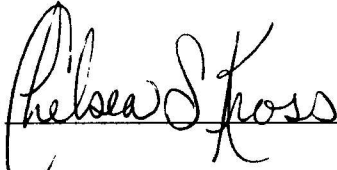ANTHROPOGENICALLY ALTERED WETLAND SYSTEM IN EASTERN
KENTUCKY

By

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Bachelor of Science
University of South Carolina Upstate
Spartanburg, South Carolina
2011

Submitted to the Faculty of the Graduate School of
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for the degree of
MASTER OF SCIENCE
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This thesis is dedicated to my parents, Jan and Jerry Kross. It is through their steady

encouragement and support that I was able pursue a graduate education and become a

better person. Thanks Mom and Dad! Additionally, I dedicate this thesis to Dr. Melissa

Pilgrim. Without her appropriate wording and confidence in my ability, I would never

have finished my B.S. in Biology and never been able to take advantage of the

opportunity to conduct my thesis research.

ABSTRACT

A key characteristic of the ridge-top ecosystem in eastern Kentucky is the presence of ephemeral wetlands. Ephemeral wetlands of the ridge-top ecosystem support an amphibian community assemblage characterized by species with short larval periods (e.g., Wood Frogs [*Lithobates sylvaticus*] and Marbled Salamanders [*Ambystoma opacum*]). Over the last 25 years, hundreds of wetlands have been constructed within the ridge-top ecosystem as permanent water sources for wildlife (i.e., game species). Consequently, the modified ridge-top ecosystem contains hundreds of constructed wetlands interspersed among natural, ephemeral wetlands. The altered ecosystem has been colonized by several amphibian species with larval periods that require a long hydroperiod (e.g., Eastern Newts [*Notophthalmus viridescens*], American Bullfrogs [*L. catesbeianus*], and Green Frogs [*L. clamitans*]). The new members of the amphibian community assemblage are top predators known to consume various amphibian life stages. Some ephemeral wetland species will breed in constructed wetlands; thus, the recent species additions could have negative impacts on the amphibian species historically associated with the ridge-top ecosystem. My study objectives were to: (1) determine if species of the ephemeral wetland and constructed wetland amphibian communities interact, and (2) evaluate the direction of species interactions (i.e., positive or negative impacts) within the communities. I selected Eastern Newts and Wood Frogs as representatives of the constructed and natural amphibian communities, respectively. I sampled six constructed and six ephemeral wetlands throughout 2013 and 2014. I counted Wood Frog egg masses and estimated survival bi-weekly throughout the breeding season (February–May) at each wetland in each year. I sampled Eastern Newt

populations once per month in May, July, September, and November 2013 and January–May 2014. I measured (i.e., SVL, Mass, and sex) newt captures to determine body condition. Data were analyzed using Wilcoxon Mann-Whitney U-tests and stepwise regression models. Eastern Newts and Wood Frogs interact within constructed wetlands. Wood Frog reproductive success was negatively impacted when eggs were laid in constructed wetlands: eggs were consumed $(15\% - 70\%)$ a higher number of Wood Frog larvae were found in natural wetlands than in constructed wetlands (W = 186.00, p = 0.039). Eastern Newts benefited from Wood Frog presence: newts in constructed wetlands with Wood Frog eggs had higher body condition (W = 402,474.00, p < 0.001). The creation of permanent constructed wetlands has allowed for colonization by amphibians that require long hydroperiods and are top predators. Wetland construction techniques need to be altered to have a more ephemeral hydroperiod, which can limit predation pressure and allow for use by ephemeral breeders. Understanding the impacts of how amphibian species interact as habitat loss and modification increase will continue to be critical for amphibian conservation.

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INTRODUCTION

Amphibian species distributions in freshwater habitats (e.g., wetlands) are largely a result of two factors, hydroperiod and predator-prey interactions (Wellborn et al. 1996). Hydroperiod limits species distributions because some species require a permanent water source (e.g., *Lithobates catesbeianus* [American Bullfrog]; Wang and Li 2009), while others require a more ephemeral water source (e.g., *Scaphiopus holbrookii* [Eastern Spadefoot]; Hansen 1958). However, some species are able to inhabit both permanent and ephemeral wetlands (e.g., *Ambystoma maculatum* [Spotted Salamander]; Rubbo and Kiesecker 2005, Denton and Richter 2013). Permanent water allows for predatory insects, anurans, and salamanders to persist throughout the year and may represent a hostile environment for organisms that usually inhabit ephemeral wetlands (Wellborn et al. 1996). Thus, predator-prey interactions can limit a species' ability to inhabit a permanent water source due to greater predator abundance (Wellborn et al. 1996, Azevedo-Ramos et al. 1999, Lardner 2000).

The presence of predators has important top-down effects on amphibian community structure (Morin 1986, Walls and Williams 2001). High predator densities can impact the reproductive success of amphibians breeding at permanent wetlands because of low embryonic and larval survival (Walls and Williams 2001). Thus, permanent wetlands might act as ecological sinks or traps for species that typically breed in ephemeral wetlands. An ecological trap refers to low-quality habitat used for reproduction, feeding, or cover in which survival and reproductive success are low,

elevating the risk of extinction. Ecological traps can be sinks because they provide little or no recruitment into the population (e.g. Cortwright and Nelson 1990, Vasconcelos and Calhoun 2006). These habitats are selected because individuals are misled by cues that suggest the habitat is suitable, which can lead to population decline and local extinction events (Schlaepfer et al. 2002, Kristan 2003, Battin 2004). As natural habitat continues to be modified and destroyed, more ecological traps are likely to be formed (Battin 2004). Understanding how ecological traps function in natural systems and what makes them attractive for use is important for amphibian conservation and management practices.

The ridge-top ecosystem of Daniel Boone National Forest (DBNF) in eastern Kentucky provides an example of an anthropogenically altered landscape that has resulted in community-level changes in the amphibian species assemblage (Drayer 2011, Denton and Richter 2013). A key characteristic of the ridge-top ecosystem in eastern Kentucky is the presence of ephemeral wetlands. Ephemeral wetlands of the ridge-top ecosystem support an amphibian community assemblage characterized by species with short larval periods (e.g., Wood Frogs [*Lithobates sylvaticus*] and Marbled Salamanders [*Ambystoma opacum*]; Denton and Richter 2013). However, during the last 25 years, over 400 wetlands have been constructed on ridge tops of the DBNF for the purpose of game and wildlife management (Drayer 2011, Denton and Richter 2013). Most of these ridge-top constructed wetlands serve as permanent water sources where permanent water was once absent (Brown and Richter 2012).

Permanent water allowed for amphibians that require a longer hydroperiod to colonize the constructed wetlands and ridge-top ecosystem. Thus, the constructed wetlands have a different assemblage of amphibians than the natural wetlands (Drayer

2011, Denton and Richter 2013). Many of the constructed wetlands have amphibians present that require larval overwintering (e.g. American Bullfrog) or have a fully aquatic adult stage (e.g. *Notophthamlmus viridescens* [Eastern Newt]), are top predators of amphibians (Morin 1986, Boone et al. 2004), and are potential reservoirs of disease (Richter et al. 2013). In contrast, the natural wetlands contain amphibians that metamorphose quickly and do not require a long hydroperiod (e.g. *L. sylvaticus*) and predators are limited primarily to insects in lower abundance than in constructed wetlands (Drayer 2011; Denton and Richter 2013).

Presence of permanent constructed wetlands provides the potential for interactions between species of the natural and constructed assemblages (Brown and Richter 2012). An interaction between one predatory species from constructed wetlands, Eastern Newt, and a species from natural wetlands, Wood Frog, has been observed (S. Richter and A. Drayer unpubl. data). Wood Frog larvae were only detected in natural wetlands by Drayer (2011) and Denton and Richter (2013). However, Richter and Drayer (unpubl. data) conducted egg masses surveys and found Wood Frog eggs in constructed wetlands, and Eastern Newts were observed consuming them to the extent that no embryos appeared to survive to free-swimming larvae. Thus, based on published and anecdotal accounts at these constructed wetlands, it appeared that they function as ecological traps for Wood Frogs, in particular because of predation from newts. However, this hypothesis needed to be empirically tested and the effects of newts and constructed wetlands on Wood Frog reproductive success needed to be quantified.

The objectives of my research were to determine if species of the ephemeral and constructed assemblages interact and evaluate positive and negative impacts on these

species. Specifically, the following questions were addressed: (1) Do Wood Frogs reproduce successfully in constructed wetlands? (2) Do newts in constructed wetlands with Wood Frogs benefit from the additional food source in terms of abundance and body condition?

Site Selection and Description

 I studied species interactions at 12 wetlands within the Cumberland District of the Daniel Boone National Forest (DBNF; Figure 1[1]), which includes areas of Bath, Estill, Lee, Menifee, Morgan, Rowan, and Wolfe counties in eastern Kentucky. Six of the ridge-top wetlands were ephemeral and six were constructed permanent wetlands (Table 1). All known natural wetlands were selected. Constructed wetlands were selected based on the following criteria: (1) known presence and absence of Wood Frog eggs (A. Drayer and S. Richter unpubl. data), so that comparisons in newt body condition and abundance could be made, and (2) located within 1 km of a natural wetland. Known constructed wetlands that fit the criteria were then randomly selected. All wetland sites were fishless, hydrologically isolated, located on a ridge-top, and surrounded by deciduous forest. The area of each wetland was estimated using the area of an ellipse during each newt sampling period.

Focal Species

Eastern Newt (*Notophthalmus viridescens*)

 Eastern Newts are classified within the Salamandridae family and are widespread throughout the eastern United States (Hunsinger and Lannoo 2005). They breed in an aquatic environment and have a multi-phase life cycle, in which most individuals go through an egg, larval, and terrestrial eft stage prior to maturing into an aquatic adult.

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[1] All tables and figures are in the appendix.

Habitats used for breeding include permanent and semi-permanent wetlands and streams. The newt breeding season is from February to April (Hunsinger and Lannoo 2005, Regosin et al. 2005).

Oviposition lasts throughout the spring and summer months (Barbour 1971). On average, females lay 200–375 eggs over several days. Each egg is individually laid at the bottom of the wetland and wrapped in a piece of detritus. Eggs incubate for 20–35 d and hatch, producing larvae. Timing of metamorphosis varies across their range but generally occurs two to five months post-hatching (Hunsinger and Lannoo 2005). Most larvae develop into a terrestrial eft stage, but in some cases larvae will metamorphose directly into an adult (Takahashi et al. 2011). Adult newts inhabit permanent or semi-permanent wetlands, and will overwinter in the same wetland or migrate to terrestrial habitat; newts inhabiting a drying wetland will leave the wetland to avoid desiccation and heat stress (Grayson et al. 2011). Additionally, adults will abandon a wetland for forested habitat if daily water temperature is too high and will return when temperatures begin to cool (Hunsinger and Lannoo 2005).

Eastern Newts are a top-predator and carnivorous as larvae and adults. Their diet includes invertebrates, amphibian eggs, and amphibian larvae. They are opportunistic predators and will take advantage of seasonal food sources (Hunsinger and Lannoo 2005). Due to their predatory behavior, newts can influence the abundance of invertebrate and vertebrate populations and community structure (Kurzava and Morin 1994, Hunsinger and Lannoo 2005).

Wood Frog (*Lithobates sylvaticus*)

The Wood Frog is classified within the Ranidae family and is the most widespread amphibian species in North America. Wood frogs exhibit a biphasic life cycle that includes an egg, larval, juvenile, and adult stage (Redmer and Trauth 2005). Wood Frogs typically breed in wetlands that include fish-free vernal pools (Baldwin et al. 2006). Breeding usually occurs directly after the first warm rain during late winter or early spring and is explosive (i.e., only lasting a few days; Berven 1982a, 1982b).

Most oviposition is completed during March and up to 1,500 eggs can be laid by a single female (Redmer and Trauth 2005). The eggs incubate for a short time (e.g., 13–36 days; Berven 1982b). Total time from egg laying to metamorphosis is 65–98 d (Berven 2009). After metamorphosis, juveniles disperse to the terrestrial habitat, remaining there for 1–3 years until they mature into adults (Berven 2009).

Wood Frogs occupy multiple niches within the aquatic and terrestrial habitats. Larval Wood Frogs are opportunistic feeders, eating detritus and small invertebrates (Schiesari et al. 2009). Larvae are a source of food for larger amphibians and invertebrates within the wetland. Predators, including newts, have been known to impact pre-metamorphic survival of Wood Frog larvae (Berven 1990).

Newt Sampling

Newt sampling occurred during the months of May, July, September, and November of 2013 and January–May of 2014, for a total of nine sampling events. Each sampling event included the use of mesh minnow traps and dipnetting. During each sampling period minnow traps were distributed evenly along the outer edge of the wetland. Six minnow traps were set for every 100 m area. Wetland area was calculated

prior to setting traps during each sampling period and the number of traps set was adjusted based on the estimated area. Traps were visually inspected for tears and then placed deep enough that the water would cover the funnel opening. All traps were checked within a 24-hour period. All individuals within the traps were recorded. Following trap removal, dipnetting was used to supplement and maximize the number of newts caught at each wetland. A D-frame net was repeatedly jabbed into the substrate in 1 m arcs along the edge and shallow areas (i.e., less than 5 ft deep) of the wetland and repeated until no newts were caught within 20 dips. Due to time constraints, dipnetting did not occur if more than 75 newts were captured within traps at a wetland. This was the case for 4 of 9 sampling events at HEA and 1 of 9 sampling events at GLA. To limit the amount of disturbance to egg masses, dipnetting did not occur during the March 2014 sampling event.

All newts were then measured, marked, and released back into the wetland at the end of processing. Snout-vent length (SVL), tail length, and tail width were measured using a cloth tape measure and estimated to the closest millimeter. Mass was measured using a Pesola spring scale to 0.01 g. Additionally, each newt was identified as male or female and given an individual code using Visual Implant Elastomer (VIE; Northwest Marine Technology, Inc., Shaw Island, WA) for mark-recapture in four body locations (behind each fore-limb and in front of each hind-limb). Recaptures were remeasured and released, and codes were updated in cases of fading. Because newts were marked in four areas, I was less likely to miss identify a recapture if fading occurred.

Wood Frog Sampling

Egg mass surveys were completed every other week throughout the Wood Frog breeding season (February–March in 2013 and 2014) at each site. Egg mass abundance was estimated using visual counts. Prior to counting egg masses, I performed a visual inspection of the entire wetland to ensure all egg masses were included and their location was recorded. Counts were taken twice. In the event the first and second counts were different, a third count was taken and the three values were averaged. Predator presence in the wetland and predator-egg interactions were documented. The percent of egg predation and egg mortality due to abiotic factors (e.g. freezing and wetland drying) were estimated based upon visual inspection of the egg masses and comparison to previous observations. The percent of egg predation was determined by estimating the percentage of an egg mass where embryos were missing and only fragments of jelly remained. Mortality caused by abiotic factors was estimated by counting the number of white eggs within an egg mass (freezing) and the number of egg masses in an area of the wetland not containing water (drying).

Once eggs were hatched, mesh minnow traps were set at each wetland following the newt sampling protocol. Captures were identified to species and life stage. In the event traps could not be set due to low water level, I determined larval presence by dipping a D-frame net into the substrate in a 180 degree arc around the entire shoreline. Larvae captured were identified to species and life stage. Captures were released immediately after identification.

Other Study Organisms

The number of *A. jeffersonianum* (Jefferson Salamander) clutches was counted at each wetland using the same protocol as Wood Frog egg masses. Jefferson Salamander egg masses are deposited around the same time as Wood Frog clutches and serve as another source of food for newts. Ranid (i.e. American Bullfrog and Green Frog) larvae also consume Wood Frog eggs (Petranka and Kennedy 1999). Thus, the number of ranid larvae within minnow traps was used to determine ranid abundance.

Data Analyses

Wood Frog Reproductive Success

Egg mass survey and minnow trap data were used to compare Wood Frog reproductive success between natural wetlands and constructed wetlands with Wood Frogs. To determine the impact of newts on Wood Frog reproductive success a Wilcoxon Mann-Whitney U-test was used to compare catch-per-unit-effort (CPUE) of late-stage larval Wood Frogs between natural and constructed wetlands. The CPUE was used as an estimate of abundance. Only wetlands with Wood Frogs present were included. The percent egg mortality by predation and abiotic factors (i.e., freezing and wetland drying) was compared between wetland types.

Newt Body Condition

Body condition is related to the health of an organism (e.g., Legagneux et al. 2013, Maceda-Veiga et al. 2014). Many non-destructive Condition Indices (CI) have been developed to quantify body condition, but there is much debate on which CI is the most reliable indicator of body condition (Peig and Green 2010). For the purposes of this study, I used the Scaled Mass Index (SMI) proposed by Peig and Green (2009). I did not

sacrifice any newts for body fat analysis; I chose the SMI because the SMI better estimated the mass and length relationship when compared to dry weight measurements (Peig and Green 2009, 2010, Legagneux et al. 2013, Meaceda-Vega et al. 2014). The SMI accounts for changes in mass and length by standardizing mass at a fixed value based on a scaled relationship between length and mass (Peig and Green 2010). In addition, the SMI accounts for ontogenetic variation and sexual dimorphism in body size and is ideal to use when comparing multiple populations (Peig and Green 2010). I calculated SMI following the recommendations of Peig and Green (2009). Snout-vent length was the morphological metric (L_0) most correlated with body mass (M_i) on a log-log scale ($r = 0.44$, $p < 0.01$), and was used as the indicator of body size (L_i; Peig and Green 2009). Bivariate plots, one for each wetland type (i.e. Wood Frog absent and Wood Frog present), were created to determine which M and L data were most correlated. The correlation between M and L was highest for the absent group ($r = 0.545$); thus, I ran a standardized major axis (SMA) regression using ln-transformed M and L to determine the slope of the fitted line, or b_{sma} value. For L_o, the average SVL from the whole group (i.e. newts from both wetland types) was used (Table 2). Finally, SMI was calculated for each individual from both wetland types (n = 1263).

Once SMI was calculated, Generalized Linear Models (GLMs) were used to determine if the presence of Wood Frogs and habitat affect newt body condition. The global model included two fixed factors: sex and wetland type, and six covariates: Newt CPUE, ranid CPUE, Number of Wood Frog Clutches, Number of Jefferson Salamander Clutches, SVL, and an interactive effect between Number of Wood Frog Clutches and newt CPUE. I ran a correlation matrix prior to processing the GLM to address

multicollinearity assumptions. Correlations with an r ≥ 0.70 resulted in the elimination of one parameter per correlated pair by removing the variable that was correlated to multiple parameters or had the lowest correlation to body condition. Additionally, I performed log-transformations on the SMI, SVL, and wetland size data to reduce heteroscedasticity. Using a reverse stepwise approach, I determined the model that best explained newt body condition (SMI). I removed factors one at a time, with the highest p-value until all remaining factors had a p-value < 0.10. Only factors with a $p < 0.05$ were considered to be statistically significant.

Newt Abundance

Newt abundance data were calculated using captures from funnel traps. Dipnetting was not included because I did not dipnet during the month of March or at individual sampling events at GLA and HEA (for reasons described above). Funnel trap count data were converted to newt CPUE, which was standardized for effort by dividing the total number of individual newts captured by the total number of funnel traps set (Shono 2008; Denton and Richter 2013).

To address the effect of Wood Frog presence on newt abundance, newt CPUE was used as the response variable in a compound Poisson (Tweedie) distribution model with a log-link function (Shono 2008, Shulse et al. 2010, Denton and Richter 2013). Because count data become more continuous when converted to CPUE, the Tweedie distribution was chosen. The index parameter value (*p*) can be any value between 1 and 2, and varies depending on how continuous the data are. Models were run with parameter values within this range, and a parameter value of 1.1 was supported based on the lowest log likelihood value. Additionally, I performed log-transformations on the SMI, SVL,

and wetland size data to reduce heteroscedasticity and improve the overall fit of the model. The global model contained two fixed factors: sex and wetland type, and five covariates: ranid CPUE, Number of Jefferson Salamander Clutches, Number of Wood Frog Clutches, SVL, and SMI. For CPUE, I did not include funnel trap data from November and January due to low sample size across all wetlands. Using a reverse stepwise approach, I determined the model that best explained newt CPUE. I removed factors one at a time, with the highest p-value until all remaining factors had a p-value < 0.10. Only factors with a $p < 0.05$ were considered to be statistically significant.

All natural wetlands dried during the summer (977N/ELN/GLN: May 2013; BPN/JRN/HEN: July 2013). All constructed wetlands had permanent hydrology. Wood Frog eggs were laid at all natural wetlands and three constructed wetlands during the 2013 breeding season (February–March), and at all natural wetlands and four constructed wetlands during the 2014 breeding season (February–March; Table 3). Jefferson Salamander egg masses were observed and counted at all wetlands, except HEA (Table 4). Nine newt sampling events were completed at the constructed wetlands and seven were completed at natural wetlands throughout 2013 and 2014. A total of 14,286 amphibians were captured and identified from funnel traps (Table 5). I documented the presence of 11 amphibian species in my study system: ten species at constructed and seven species at natural wetlands. Ranid larvae were captured at all constructed wetlands and CPUE was calculated (Table 4). A total of 1,030 newts were captured in funnel traps (Table 5). With the addition of dipnet captures, 1,275 newts were marked at six constructed and two natural wetlands. Of the 1,275 marked newts, 1,263 were captured within constructed wetlands and 12 were captured in natural wetlands. I did not recapture any newts at natural wetlands, but 162 individuals were recaptured at constructed wetlands. Eighty-three percent of all newt captures were male. Most females were captured during the newt breeding season (Figure 2). Average wetland size was variable (Table 4), and in general, the number of individual newts and ranid larvae was the same within each wetland regardless of wetland size (Figure 3).

Wood Frog Reproductive Success

The number of Wood Frog egg clutches deposited at each wetland varied by wetland and year (Table 3). In constructed wetlands, Wood Frog egg mortality was caused by predation and freezing. In contrast, freezing and pond drying were the primary causes of egg mortality in natural wetlands. In 2013 and 2014, natural wetland 977N dried and all eggs died prior to hatching. Constructed wetland JRC dried down, and 50% of Wood Frog eggs died prior to hatching. The number of Wood Frog egg masses laid in constructed wetlands decreased between 2013 and 2014 (Figure 4). The number of free-swimming Wood Frog larvae captured at wetlands where eggs were laid was significantly higher in natural wetlands compared to constructed wetlands (Mann-Whitney U-test, W = 186.00, p = 0.039; Figure 5).

Newt Body Condition

Average *L*, *M*, and SMI values varied across all wetlands (Table 2). Newt SMI was highest in JRC and lowest in HEA (Table 1). Average SMI was significantly higher in wetlands with Wood Frogs than wetlands without Wood Frogs (Mann-Whitney U-test, W = 402,474.00, p < 0.001; Figure 6). However, newt SMI in constructed wetlands with Wood Frogs had a higher body condition after Wood Frog eggs were laid (March–May), but during the summer and winter months, newts in constructed wetlands without Wood Frogs had a higher body condition (Figure 7). Average SMI of newts within constructed wetlands with Wood Frogs mostly decreased from 2013 to 2014, while average SMI of newts within constructed wetlands without Wood Frogs remained relatively constant from 2013 to 2014 (Figure 8).

Prior to running the GLMs, ranid CPUE and Number of Jefferson Salamander Egg Masses were removed from the global model due to high correlation with newt CPUE (r > 0.70). The model that best explained newt SMI was sex, number of Wood Frog clutches, newt CPUE, and the interaction between Wood Frog clutches and newt CPUE. All parameters were significant (p < 0.001). Generally, newts in wetlands with lower newt abundance and more Wood Frog clutches had a higher SMI (Figure 9).

Newt Abundance

The number of newts captured varied by time, wetland, and wetland type (Figure 10). More individual newts were captured in constructed wetlands without Wood Frogs than constructed wetlands with Wood Frogs (Table 2), but average newt CPUE did not differ between wetland types (Mann-Whitney U-test, W= 9, p = 0.700). Prior to running the GLMs, ranid CPUE and Number of Jefferson Salamander egg masses were removed from the global model due to high correlation with newt CPUE (r > 0.70). The model that best explained newt CPUE contained sex, wetland type, number of Wood Frog clutches, wetland size, and SMI. All parameters in the final model were significant (p < 0.02).

CHAPTER IV

DISCUSSION

I studied the use of permanent constructed wetlands as breeding habitat by Wood Frogs, an amphibian that typically breeds in ephemeral wetlands, over two breeding seasons. Previous research in the DBNF system did not quantify the use of constructed wetlands by Wood Frogs (Drayer 2011, Denton and Richter 2013). However, I found that Wood Frogs used constructed wetlands as breeding habitat and that presence of Eastern Newts had a negative impact on Wood Frog egg and larval survival. Additionally, my study determined that newts benefit from the presence of Wood Frog eggs and larvae via an increased body condition. Finally, I found strong support that constructed wetlands act as sink habitats for Wood Frogs in this anthropogenically altered wetland ecosystem.

Wood Frogs were negatively impacted by the interaction with newts. Eggs were predated upon prior to hatching, and no larvae were captured at the constructed wetlands where eggs were present. Wood Frogs are early breeders and eggs provide an easy food source for predators during late winter and early spring (e.g. Vasconcelos and Calhoun 2006). Wood Frog larvae are small and remain relatively immobile on top of the egg mass immediately after hatching; thus, they are especially susceptible to predators before becoming free-swimming larvae. If larvae survive post-hatching, they might be more active foragers because they are usually in low-predator wetland, which also increases their susceptibility to predators (Julian et al. 2006). Under natural conditions, Wood Frog larvae have a high detectability because they occur in high abundance (Drayer 2011, Denton and Richter 2013, this study). Thus, I feel confident that they were absent or in very low abundance in constructed wetlands because of predation. Wood Frogs are

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apparently being predated upon at both the egg and larval stage because not all eggs were consumed and no larvae were captured post-hatching. Multi-stage predation can have important consequences on population growth and species distributions (Rubbo et al. 2006).

The repeated failure to successfully reproduce can lead to local population decline and extinction (Semlitsch 2000), unless a source habitat is able to provide individuals for recolonization (Calhoun et al. in press). In the DBNF, populations of Wood Frogs at the natural wetlands appear to act as a source. Egg predation was not observed and hundreds to thousands of Wood Frog larvae were captured in all but one natural wetland. Wood Frog breeding and successful metamorphosis are positively related to an ephemeral hydroperiod (Green et al. 2013), and predation can greatly reduce reproductive success (Vasconcelos and Calhoun 2006). All of the natural wetlands in this study dried during the summer effectively excluding amphibians that require longer hydroperiods. In all but one natural wetland, Wood Frogs were reproductively successful, likely due to the absence of top predators.

Newts benefit from the presence of Wood Frogs; overall, average newt body condition was higher in wetlands that had Wood Frog eggs. Specifically, newt body condition increased in constructed wetlands with Wood Frogs directly after Wood Frogs bred, while newt body condition in wetlands without Wood Frogs remained lower and relatively constant or decreased during the same time period. The number of Wood Frog clutches available likely influenced the fluctuation of newt body condition observed at most constructed wetlands with Wood Frogs present. An increase in prey availability can lead to a higher body condition in predators (Pope and Matthews 2002; Brown and Shine

2007; Sztatecsny et al. 2013). My results suggest that in wetlands without Wood Frogs, there is more competition for potentially fewer resources, leading to lower body condition. The Wood Frogs were likely a nutritious prey item for newts at a time when few other resources were available, as expressed by the increased body condition of newts in wetlands with Wood Frogs compared to those without. During the fall and winter months, newts in wetlands without Wood Frogs had a higher body condition than newts in wetlands with Wood Frog. During this time few newts were captured (0.3% of total captures at Wood Frog Present and 4% of total captures at Wood Frog Absent); thus, newts that remained in the wetlands were likely able to consume more resources and increase in body condition.

Newt abundance varied widely over time, which was unexpected. In permanent wetlands, adult newts typically overwinter in the wetland (Sever 2006), but at all of my constructed wetlands, newt abundance dropped substantially during the fall and winter months (November and January). The majority of newt captures were male and most females were captured during the spring months (May 2013, March and April 2014). Females might be migrating to wetlands during the breeding season and migrating to terrestrial habitat following oviposition while males have a longer residency period to maximize mating opportunities (e.g. Grayson and McLeod 2009, Grayson et al. 2011). The newt populations inhabiting the constructed wetlands appear partially migratory; although unusual, partially migratory populations of newts have been documents before (e.g. Grayson and Wilbur 2009). In 2011, the winter was milder, and newts appeared to be more abundant earlier in the season and consumed all or nearly all Wood Frog eggs prior to hatching (Richter and Drayer, unpubl. data). The trend in newt abundance might

explain why low egg predation was observed over the course of my study. Few newts (12% of total captures) were present in the wetlands containing ovipositing Wood Frogs (February–March). Although newt abundance increased from March–April, Wood Frog eggs had already hatched at the peak of newt abundance. Relative to 2011, the lower egg mortality due to predation I observed was likely due to the timing of peak abundance and lower abundance of newts present during Wood Frog oviposition. Thus, predation was likely higher in during the Wood Frog larval stage when newts were in higher abundance.

Eggs were typically laid in lower abundance at constructed wetlands compared to eggs laid in natural wetlands. The difference in abundance between wetland types provides support for the idea that constructed wetlands are a secondary choice for breeding adults. Additionally, the higher number of Wood Frogs present in natural wetlands might influence individuals that breed later in the season to breed in constructed wetlands to reduce competition. This hypothesis requires further testing, but the potential for competition among conspecifics has led to female amphibians ovipositing at sites containing predators where they might not otherwise breed (e.g. Crump 1991; Matsushima and Kawata 2005).

An understanding of how different species interact in a human-altered habitat is key to the conservation of amphibians (Boone et al. 2004, Vasconcelos and Calhoun 2006). Anthropogenic alterations within DBNF have led to an increase in predator-prey interactions that might lead to local population declines of amphibians that use ephemeral habitats. My research demonstrates the negative impacts one species of one community assemblage can have on a species of a different assemblage. Although newts are native to the DBNF area historically, they likely remained in lowland basins where permanent

water was available for breeding habitat. The presence of constructed wetlands has likely

allowed newts and other predators to migrate and colonize wetlands near natural

wetlands, likely reducing the ability of Wood Frogs to use the constructed wetlands.

Ephemeral wetlands are important habitats that support specialized wetland breeders

(Drayer 2011, Denton and Richter 2013, Calhoun et al. in press).

Anthropogenic alteration to natural habitats is an important factor related to the

decline of amphibians (Vitousek 1994, McKinney 2002). Although wetland creation is an

important tool for the conservation of amphibians, my study demonstrates how

constructed wetlands with permanent hydroperiod can have a negative impact on

amphibian species. Improving construction techniques to discourage amphibians that do

not naturally occur within an ecosystem could reduce the possibility of local population

declines (Calhoun et al. in press). Understanding how such alterations impact species

interactions in freshwater habitats is important for the conservation of amphibians.

Conclusions

Natural wetlands are important source habitats, providing Wood Frogs with lower

predation risk and increasing larval recruitment into the local population. In contrast,

constructed wetlands are acting as population sinks for Wood Frogs. Larvae are unable to

reach metamorphosis and be recruited into the breeding population due to the high level

of predation by newts. Additionally, the presence of Wood Frogs in constructed wetlands

benefit newts by providing a supplemental food source, increasing overall newt body

condition. My results indicate that the two amphibian assemblages interact and that the

presence of constructed wetlands and the amphibian assemblage inhabiting them are

having a negative effect on at least one species of the natural amphibian assemblage. I

was able to demonstrate the impacts of interactions between species representing both assemblage types within the DBNF. However, other species from the communities need to be studied to determine how broadly my results apply within the system. I recommend that wetland creation techniques be modified to allow for annual pond drying, providing a habitat that reduces predator abundance and better supports ephemeral breeders, such as Wood Frogs.

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APPENDIX

Table 1. List of wetland names and abbreviations for constructed and natural wetland studied within the Daniel Boone National Forest, Kentucky.

Wetland Pair Name	Constructed Abbreviation	Natural Abbreviation
977	977C	977N
Big Perry	BPA2	BPN
Elk Lick	ELA	ELN
Gas Line	GLA	GLN
High Energy	HEA	HEN
Jones Ridge	JRC	JRN

Table 2. Summary of newt captures and body condition information used to calculate Scaled Mass Index (SMI) at each individual wetland and wetland type (Wood Frog Absent/Present).

Population	*n*	*L*	*M*	SMI	Wood Frog +/-
977C 2013	28	4.49 ± 0.06	3.54 ± 0.14	3.76 ± 0.10	-
977C 2014	59	4.78 ± 0.04	3.79 ± 0.08	3.67 ± 0.06	+
BPA2	65	4.51 ± 0.04	3.26 ± 0.07	3.45 ± 0.06	+
ELA	171	4.59 ± 0.03	3.33 ± 0.05	3.44 ± 0.04	-
GLA	242	4.67 ± 0.02	3.76 ± 0.05	3.79 ± 0.05	+
HEA	539	4.77 ± 0.02	3.23 ± 0.03	3.14 ± 0.02	-
JRC	159	4.64 ± 0.02	3.89 ± 0.06	3.96 ± 0.05	+
WF ABSENT	738	4.72 ± 0.01	3.27 ± 0.03	3.23 ± 0.02	
WF PRESENT	525	4.65 ± 0.01	3.74 ± 0.03	3.79 ± 0.03	

Table 3. Summary of Wood Frog egg mass survey and larval captures at all wetlands.

Wetland	Wetland Type	# Clutches	% Predation	% Frozen	# Larvae Captured (May)
			2013		
977N	Natural	37	0	0	0
977C	Construct	0	-	-	-
BPN	Natural	54	0	0	0
BPA2	Construct	68	70	10	0
ELN	Natural	125	0	68	0
ELA	Construct	0	-	-	-
GLN	Natural	143	0	20	907
GLA	Construct	89	15	50	0
HEN	Natural	76	0	40	28
HEA	Construct	0	-	-	-
JRN	Natural	46	0	50	82
JRC	Construct	69	30	25	0

Table 3 continued.

	2014			
# Clutches	% Predation	% Frozen	# Larvae Captured (April)	# Larvae Captured (May)
5	0	<1	0	0
24	42	<1	0	0
170	0	<1	1083	784
40	15	10	0	0
111	0	<1	2048	P
0	-	-	-	-
171	0	<1	2673	44
42	28	<1	0	0
121	0	0	1731	1071
0	-	-	-	-
33	0	0	176	P
47	7	<1	0	0

Table 4. Summary of other environmental factors used in newt body condition and abundance regression analyses.

Wetland	# Jefferson clutches ($\overline{x}$)	Newt CPUE	Ranid CPUE	Wetland Size ($\overline{x}$)
977C	212 ± 56	0.88	1.8	170.62 ± 6.45
BPA2	140.5 ± 3.5	0.73	2.2	145.83 ± 7.80
ELA	17.5 ± 17.5	3.694	1.33	88.70 ± 5.11
GLA	202.5 ± 89.5	1.73	2.3	237.54 ± 10.90
HEA	0 ± 0	5.13	1.0	243.99 ± 13.01
JRC	7.5 ± 2.5	4.81	1.69	40.50 ± 6.75

Table 5. Cumulative list of all amphibian species identified and captured within funnel traps at all twelve wetlands surveyed from 2013 and 2014.

Organism	Life Stage (A/L)	Count (*n*)
Ambystoma jeffersonium	A	367
A. maculatum	A	12
A. jeffersonium/maculatum	L	876
A. opacum	L	109
Anaxyrus spp.	L	85
Hyla chrysoscelis	L	35
Lithobates catesbeianus	A	17
L. clamitans	A	42
L. catesbeianus/clamitans	L	886
L. palustrus	A	1
L. sylvaticus	A	156
	L	10627
Notophthalmus viridescens	A	1030
	L	20
Pseudacris crucifer	A	1
Pseudacris spp.	L	22

Note: Life stage is represented by A (Adult) and L (Larvae).
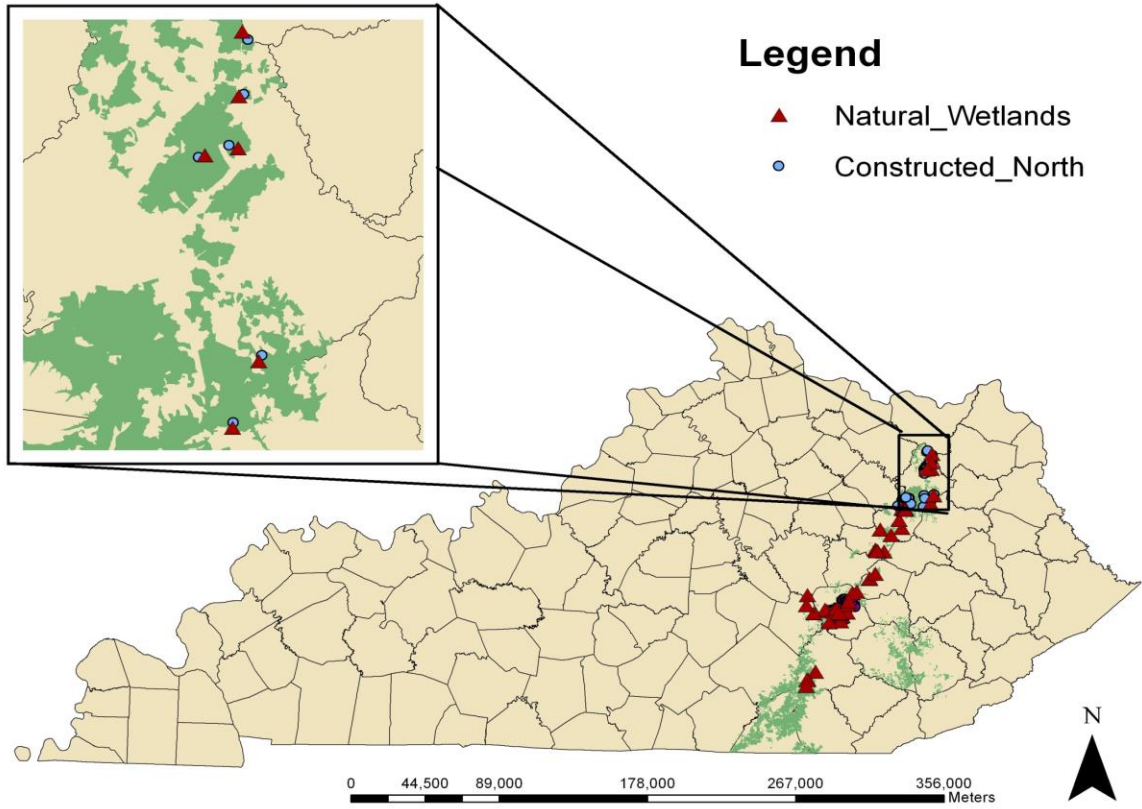
Figure 1. Map of Daniel Boone National Forest (DBNF).

Note: The box shows location of the twelve wetlands (six natural and six constructed) used to determine interactions between Eastern Newts and Wood Frogs.
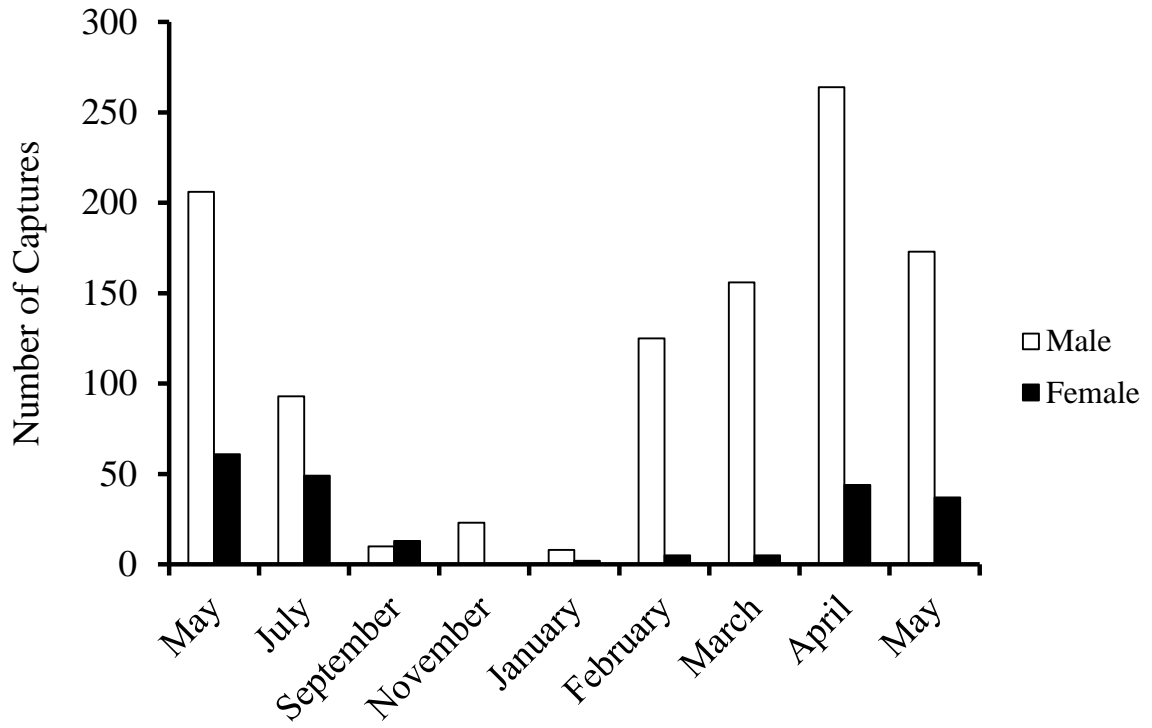
Figure 2. Comparison of the total number of male and female newts captured from May 2013 to May 2014.
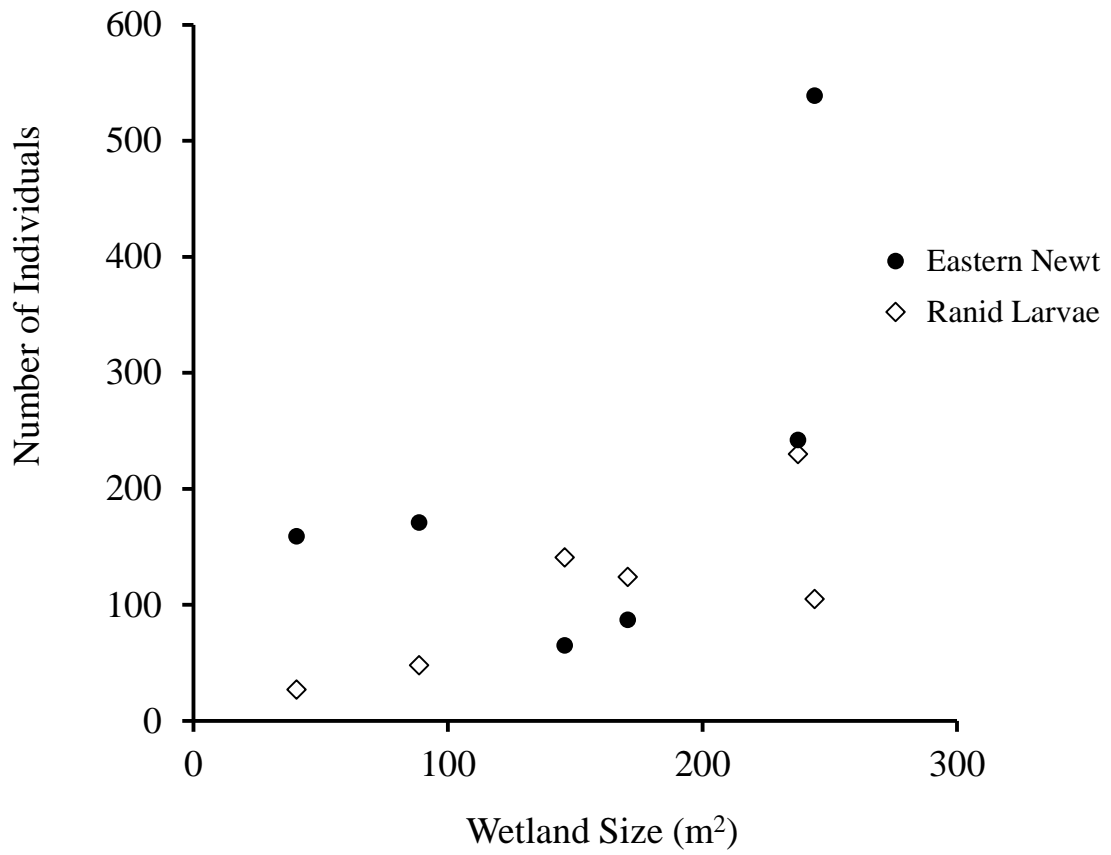
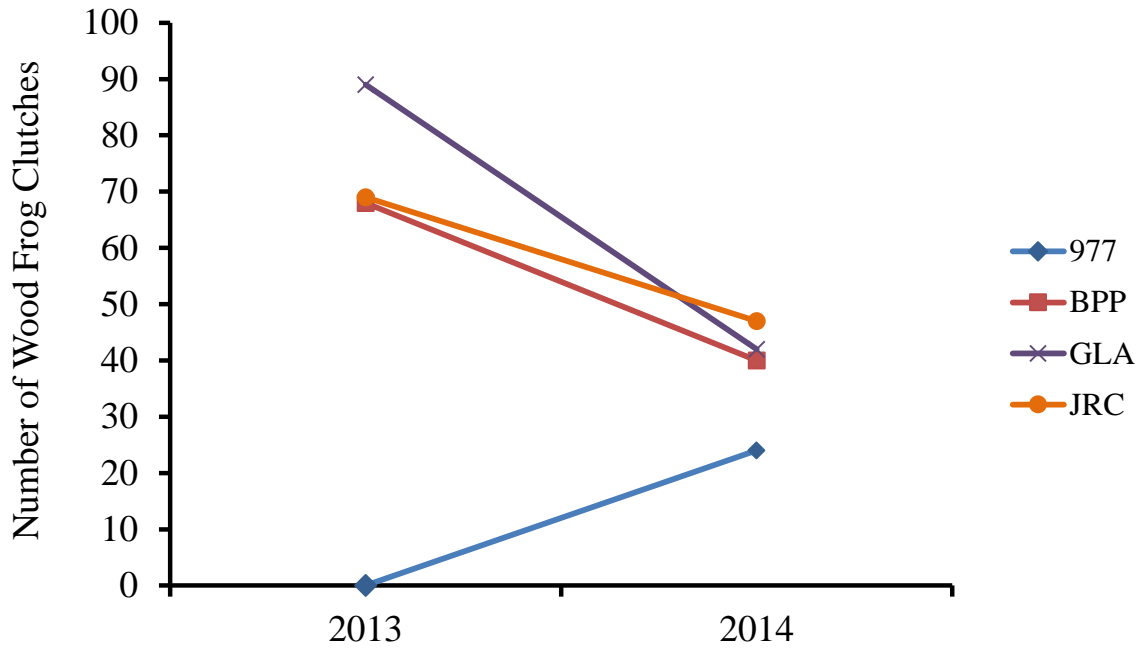Figure 3. Number of Ranid (Bullfrog and Green Frog) larvae and Eastern Newt individuals captured compared to wetland size (m^2).

Figure 4. Change in the number of Wood Frog clutches laid at constructed wetlands from 2013–2014.
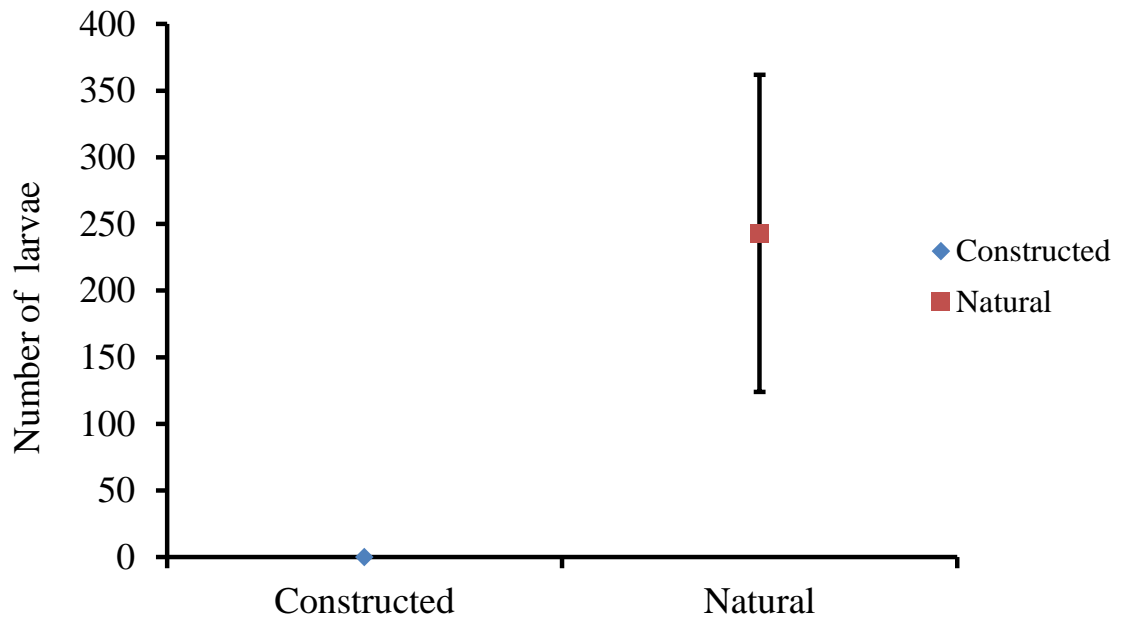
Figure 5. Average number of late-stage Wood Frog larvae captured from constructed and natural wetlands within the Daniel Boone National Forest.

Note: Error bars indicate ± 1SE.
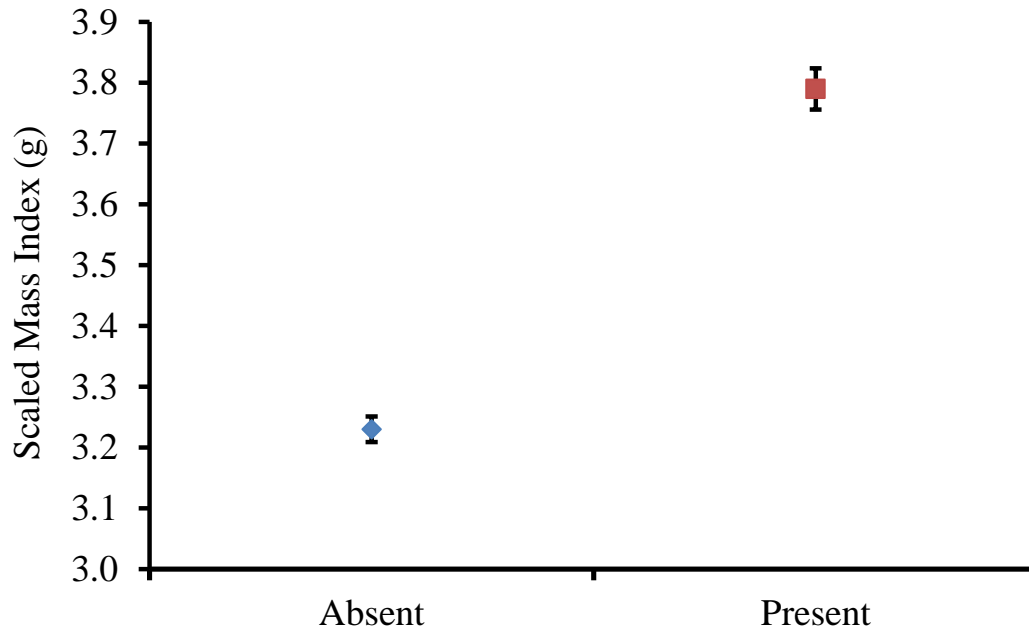
Figure 6. Average body condition (scaled mass index) of Eastern Newts in response to the presence of Wood Frogs in constructed wetlands.
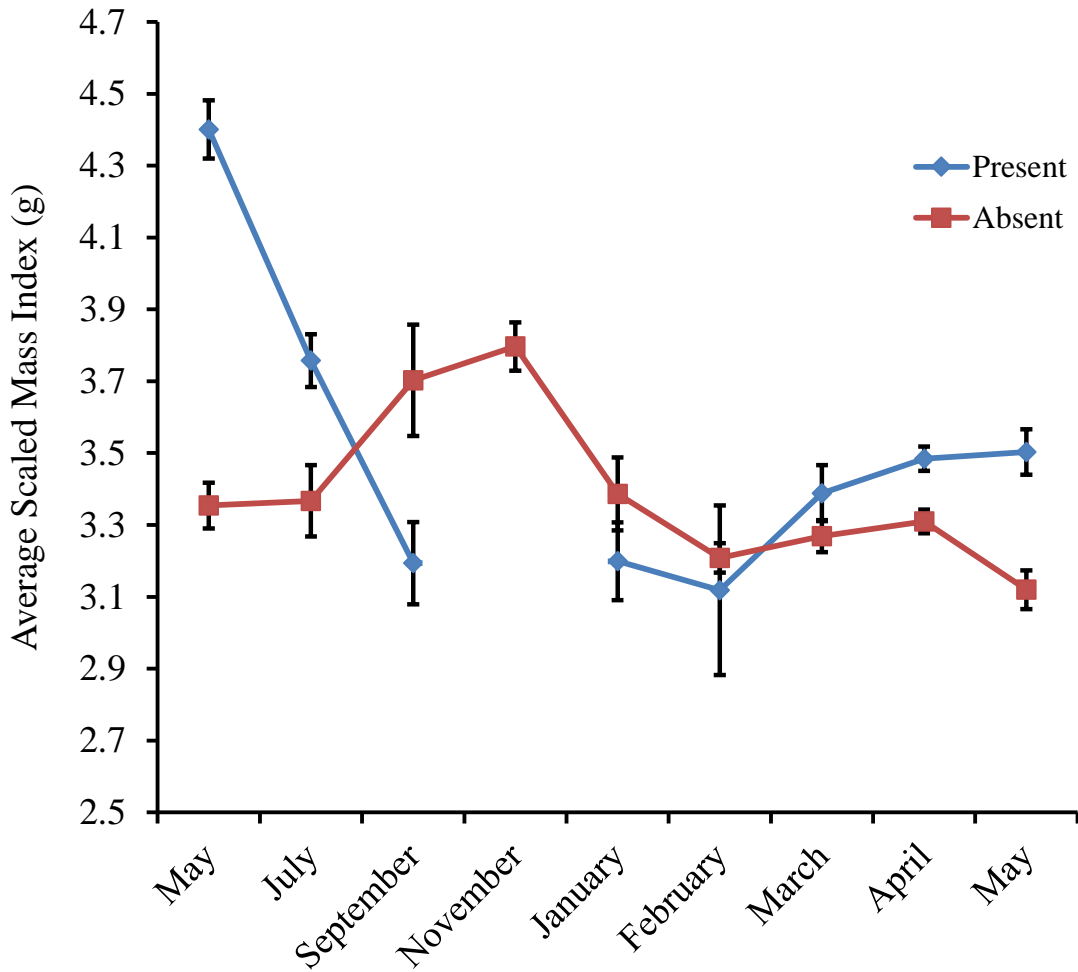
Note: Error bars represent ± 1SE.

Figure 7. Change in average Eastern Newt body condition (Scaled Mass Index) from May 2013 to May 2014.

Notes: Present represents newt SMI averages from constructed wetlands with Wood Frogs.
Absent represents newt SMI averages from constructed wetlands without Wood Frogs.
No newts were captured at Wood Frog Present wetlands in November 2013.
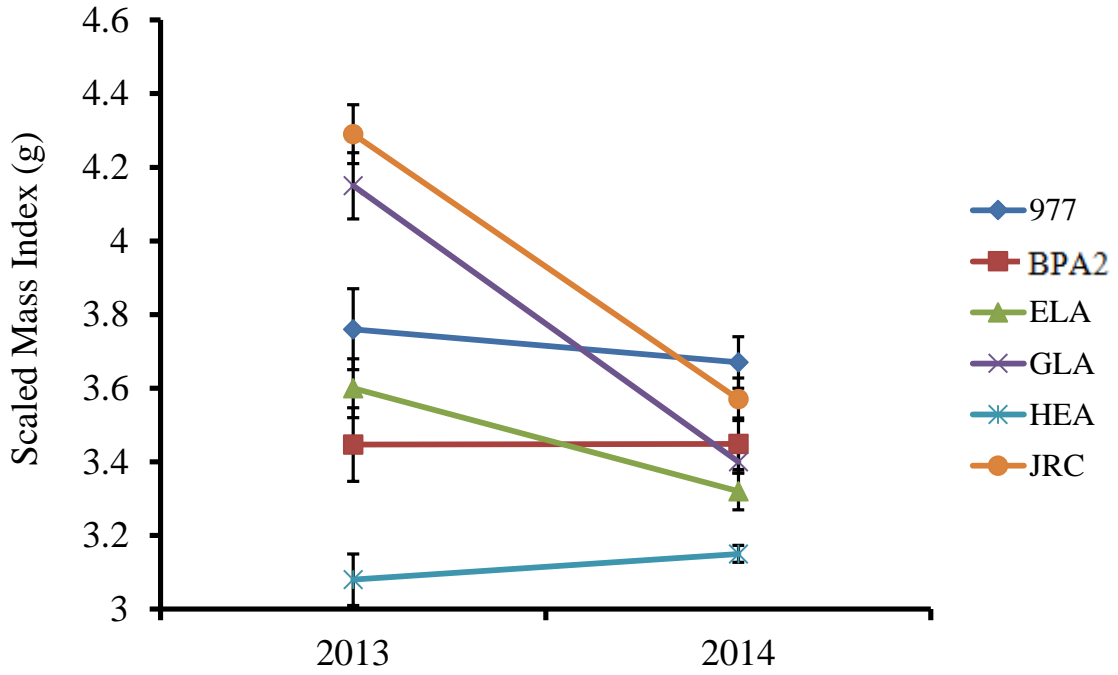Error bars represent ± 1SE.

Figure 8. Change in average Eastern Newt body condition (scaled mass index) from 2013–2014.

Notes: Constructed wetlands with Wood Frogs were BPA2, GLA, and JRC.
Constructed wetlands without Wood Frogs were 977, ELA, HEA.
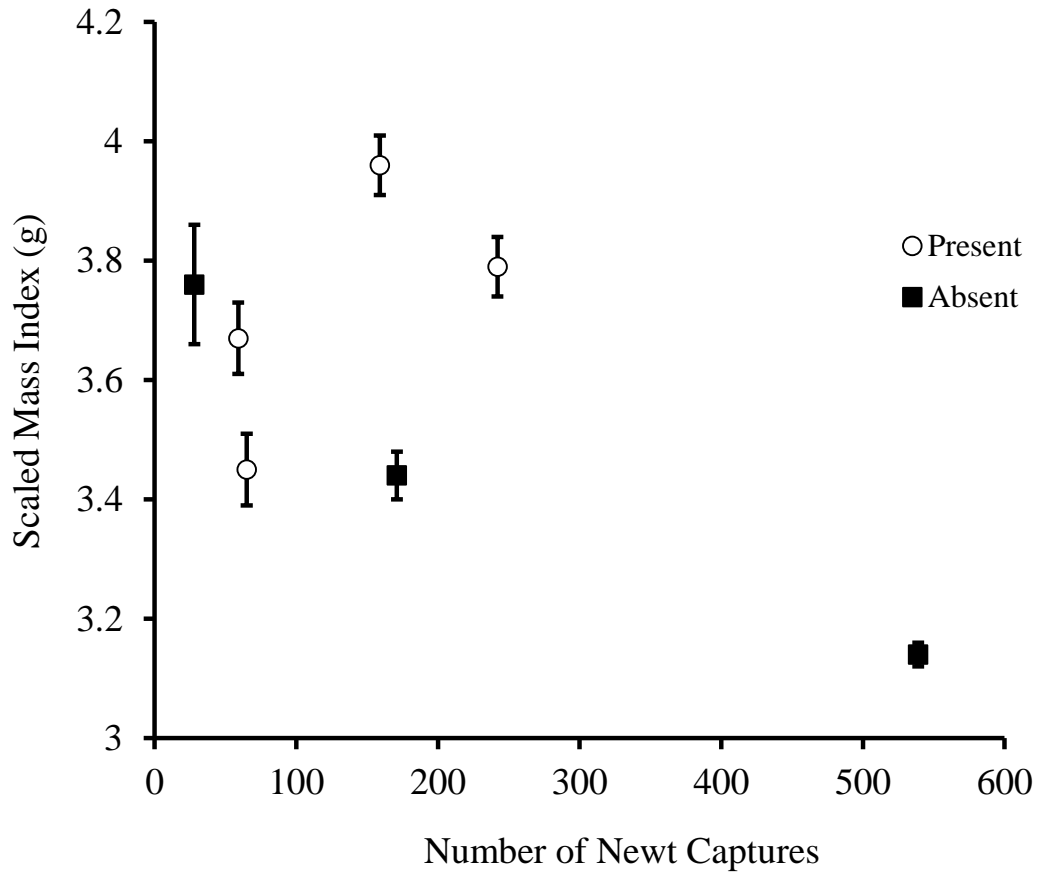Error bars represent ± 1SE.

Figure 9. The interactive effect of Wood Frog Presence (Present or Absent) and newt abundance on average body condition of newts (Scaled Mass Index).
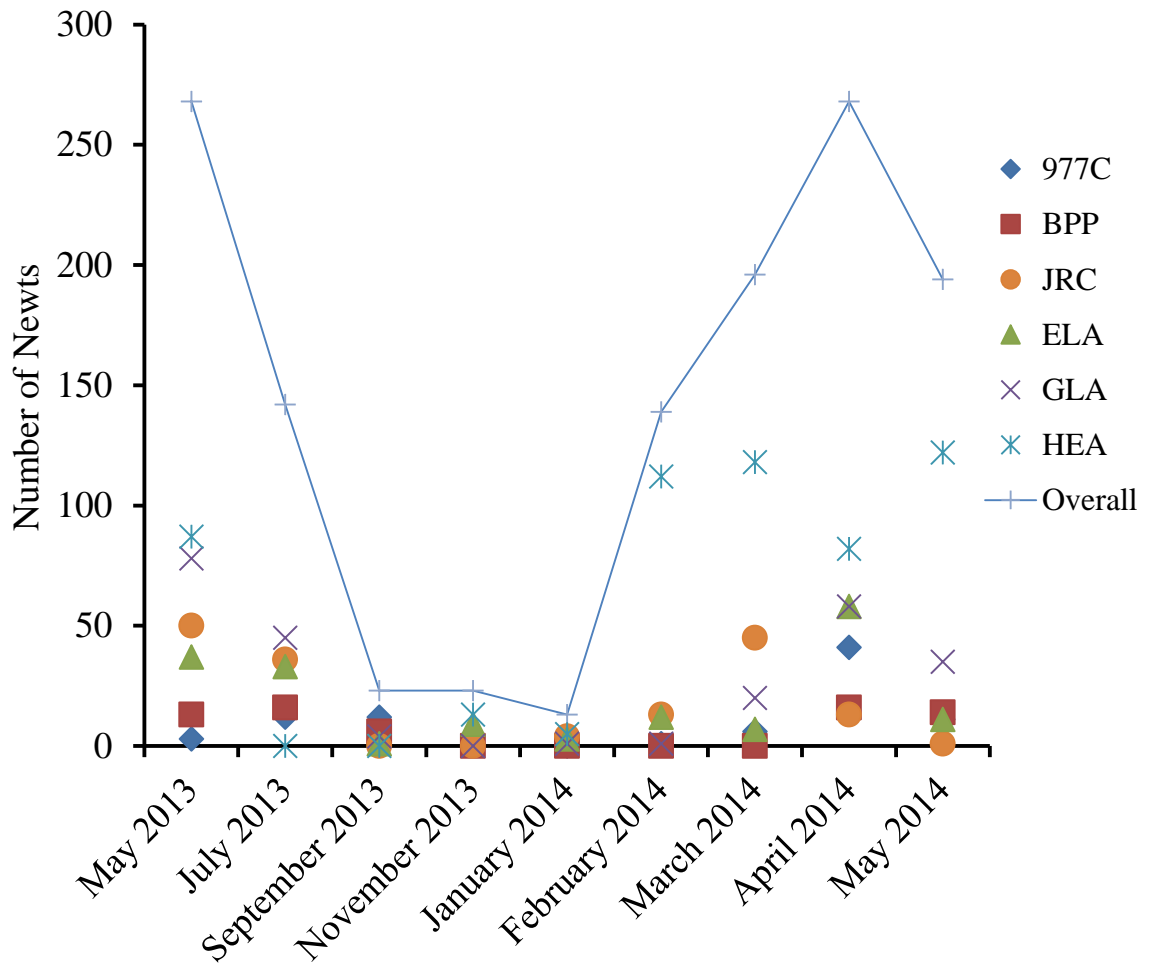
Notes: Error bars represent ± 1SE.

Figure 10. Total newt captured during each sampling event at each constructed wetland.

Note: The line shows the overall trend in changes of total newt abundance.