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Ecosystem Functional Consequences Of Body Size Variation In An Apex Predator (Ambystoma jeffersonianum)

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ECOSYSTEM FUNCTIONAL CONSEQUENCES OF BODY SIZE VARIATION IN AN APEX PREDATOR (*AMBYSTOMA JEFFERSONIANUM*)

BY

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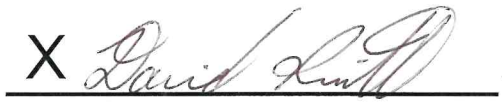
X 

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BY

DAVID S. SMITH

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ABSTRACT

Biodiversity is often emphasized at the species level where each species is assigned a mean functional trait value. However, populations within a species, and individuals within a population, often exhibit considerable intraspecific functional variation. Therefore, instead of focusing on species' mean trait values, we must incorporate intraspecific variation when considering species' ecological roles and conservation values. The primary objective of this study was to determine the effects of variation in body size (a functional trait in many aquatic taxa) in an apex predator on ecosystem functioning. We sought to characterize trophic cascades initiated by larval populations of *Ambystoma jeffersonianum* that varied in size structure based on diversity of maternal lines (i.e., sibship diversity) by quantifying the effect on larval salamanders, benthic macroinvertebrates, zooplankton, phytoplankton, and periphyton, as well as leaf-litter decomposition rates and release of soluble nutrients in cattle tank mesocosms. Although sibship diversity did not lead to populations of variable body size, it was positively related to larval density and survival to metamorphosis in *A. jeffersonianum*. Sibship diversity did not affect growth rates or dates of metamorphosis for *A. jeffersonianum*, nor did it have any significant effects on invertebrate communities and ecosystem function. This research emphasizes the importance of considering the effect of sibship diversity on predator density for intraspecific variation and the subsequent long-term effects on ecosystem function.

TABLE OF CONTENTS

CHAPTER PAGE

I. Introduction 1
II. Methods 9
Experimental Design 9
Mesocosm sampling 15
Larval densities 15
Macroinvertebrate density and rate of leaf litter decomposition 16
Zooplankton density 17
Algal productivity 18
Environmental conditions 19
Laboratory analysis 19
Larval densities 19
Macroinvertebrate densities and rates of leaf litter decomposition 20
Zooplankton density 21
Periphyton density 21
Nitrate and Phosphate 21
Statistical Analysis 22
III. Results 24
IV. Discussion 27
References 38
APPENDICES 45
Appendix A: Figures 46
Appendix B: Tables 54

LIST OF TABLES

TABLE PAGE

Table 1. Modified signed-likelihood ratio test (M-SLRT) for equality of size variation in *Ambystoma jeffersonianum*. 55
Table 2. ANOVA for hatchling size comparison of *Ambystoma jeffersonianum* between low, moderate, and high treatment levels immediately before being added to the mesocosms. 55
Table 3. ANOVA for the comparison of the survival to metamorphosis of larval *A. jeffersonianum* between treatment levels. 56
Table 4. MANOVA of the salamander subset of data comparing abundance of A. *jeffersonianum,* growth rate of A. *jeffersonianum,* and date of metamorphosis of A. *jeffersonianum* among low, moderate, and high sibship diversity treatment levels on May 30th, 2018. 56
Table 5. MANOVA of the invertebrate subset of data to compare macroinvertebrate and zooplankton densities among low, moderate, and high sibship diversity treatment levels on May 30th, 2018. 56
Table 6. MANOVA of the environmental subset of data to compare chlorophyll *a* and periphyton abundances, rate of leaf litter decomposition, phosphate, and dissolved oxygen among low, moderate, and high sibship diversity treatment levels on May 30th, 2018. 57
Table 7. Correlations among variables collected on May 30th, 2018 where response variables are (A) *A. jeffersonianum* abundance, (B) growth rate of *A. jeffersonianum*, (C) date of metamorphosis of *A. jeffersonianum*, (D) rate of leaf litter decomposition, (E) macroinvertebrate density, (F) zooplankton density, (G) periphyton abundance, (H) dissolved oxygen, (I) chlorophyll *a* abundance, and (J) phosphate. 58

LIST OF FIGURES

FIGURE PAGE

Figure 1. Intraspecific variation in community structure. 2
Figure 2. Mean functional traits underestimate the actual intraspecific trait variation present. 3
Figure 3. Food wed based on invertebrates recorded in this study and the known community structure of breeding ponds used by *A. jeffersonianum*. 5
Figure 4. As the number of egg masses represented in a population increases, assuming each egg mass is from locations outside the dispersal distance of egg mass parents, then sibship diversity will also increase. 7
Figure 5. Hypothesized effects of low, moderate, and high body size variation treatment levels on larval salamander density, macroinvertebrate density, zooplankton density, algal density, and the rate of leaf litter decomposition. 8
Figure 6. General locations of *A. jeffersonianum* egg mass collection sites. 12
Figure 7. Mesocosm array at the Taylor Fork Ecological Area at Eastern Kentucky University. 14
Figure 8. Mean (± 1 SE) for low, moderate, and high sibship diversity treatment levels where (A) is the coefficient of variation and (B) is the total length of hatchlings for each group of 24 immediately before they were initially added to the mesocosms. 47
Figure 9. Mean (± 1 SD) survival to metamorphosis of *A. jeffersonianum* among low, moderate, and high sibship diversity treatment levels. 48
Figure 10. Daily sum of metamorphosed *A. jeffersonianum* between low, moderate, and high sibship diversity treatment levels starting when the first metamorph was collected on May 28th until the last was collected on July 2nd. 49
Figure 11. Mean of (A) the larval abundance of *A. jeffersonianum* (± 1 SD), (B) larval growth rate of *A. jeffersonianum* (± 1 SE), and (C) the date of metamorphosis of *A. jeffersonianum* (± 1 SE) 50

Figure 12. Mean (± 1 SD) of (A) macroinvertebrate and (B) zooplankton densities among low, moderate, and high sibship diversity treatment levels for the invertebrate subset of data. 51
Figure 13. ANOSIM results comparing community dissimilarity in the macroinvertebrate taxonomic groups for the low, moderate, and high sibship diversity treatment levels. 52
Figure 14. Mean (± 1 SD) of (A) chlorophyll *a*, (B) periphyton abundance, (C) rate of leaf litter decomposition, (D) dissolved phosphate, and (E) dissolved oxygen between low, moderate, and high sibship diversity treatment levels for the environmental subset of data. 53

I. Introduction

Biodiversity loss and the successive deterioration of ecological resilience have prompted extensive research on the ecosystem functional consequences of species losses (Cardinale *et al.* 2012). Strategies to mitigate losses in biodiversity and maintain appropriate ecosystem functions frequently emphasize conservation of diversity at the species level because increased species diversity promotes ecosystem functional stability and increased protection from, and resilience to, environmental disturbances (Srivastava & Vellend 2005; Ives & Carpenter 2007; Cardinale *et al.* 2012). However, because species often exhibit some level of functional redundancy (Rosenfeld 2002), diversity of ecological function is often prioritized over species diversity (Tilman *et al.* 1997; Gagic *et al.* 2015). This approach has led to the characterization of communities based on functional groups and assigning mean functional trait values for all individuals of each species within the community, but this does not account for functional trait variation among populations within a species, or among individuals within a population (i.e., intraspecific functional variation; Violle *et al.* 2012). The importance of identifying and quantifying intraspecific functional variation and its consequences for community ecology has increased recently, but more empirical studies utilizing this approach are necessary (Bolnick *et al.* 2003, 2011; Hughes *et al.* 2008).

Intraspecific variation tends to maximize structural complexity and plasticity within ecological communities by expanding tolerance to abiotic (e.g. temperature, soil, and geology) and biotic (e.g. competition and predation) conditions, thereby

increasing the actual "functional" biodiversity in a community relative to using single mean functional trait values for each species (Figure 1; Valladares *et al.* 2015). The sole use of mean trait values typically exaggerates interspecific functional differences by not accounting for intraspecific functional variation (Figure 2; Violle *et al.* 2012). Increasing sampling effort for variation in intraspecific functional traits leads to an improved understanding of the actual overlap among species in a community (Figure 2; Violle *et al.* 2012). Recent studies have indicated that the effects of intraspecific

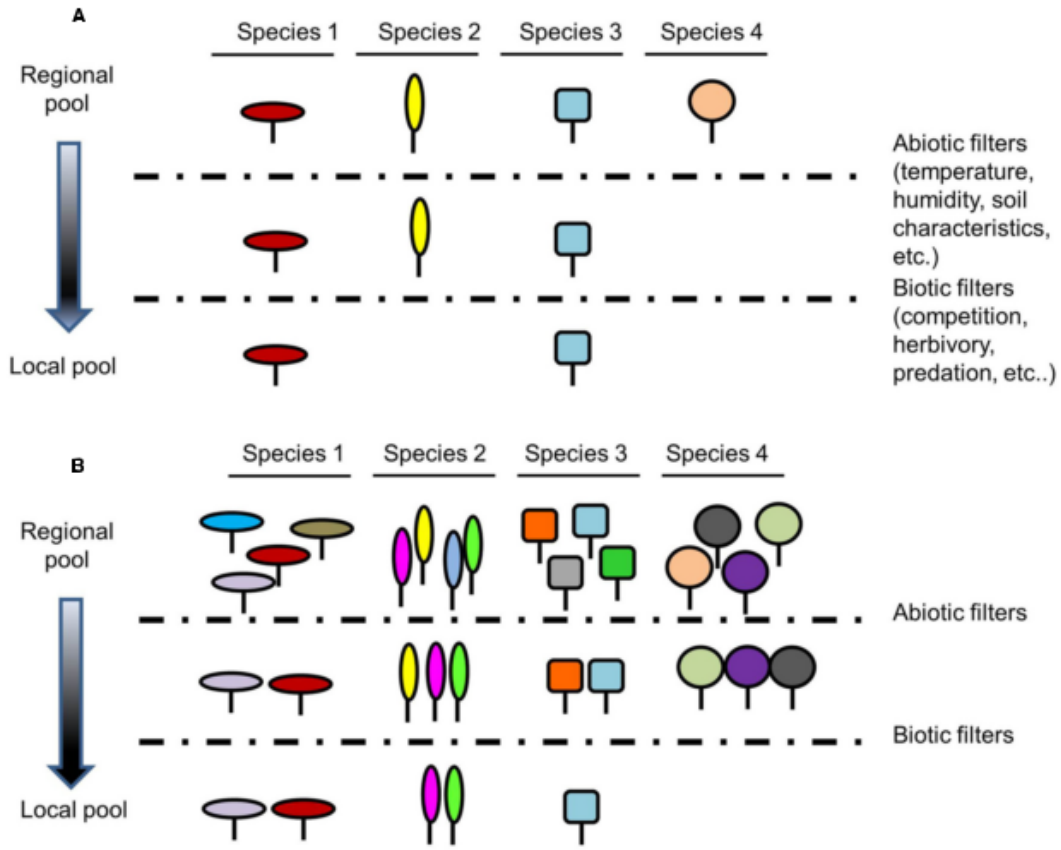

Figure 1. Intraspecific variation in community structure. (A) Excluding intraspecific variability underestimates the actual phenotypic plasticity and genetic variation, thereby misrepresenting a population's tolerance to abiotic and biotic filters (i.e. conditions), compared to (B) which includes intraspecific variability. Shapes signify species, colors signify trait values, and dashed lines signify filters. Source: Valladares, F., Bastias, C.C., Godoy, O., Granda, E. & Escudero, A. (2015) Species coexistence in a changing world. *Frontiers in Plant Science*, 6, 1–16.

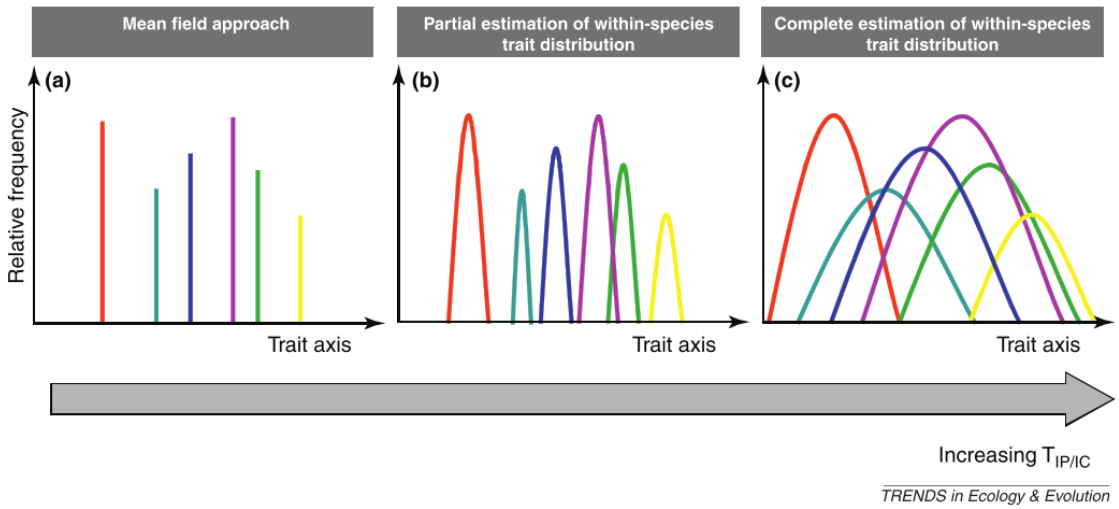

Figure 2. Mean functional traits underestimate the actual intraspecific trait variation present. Increasing the analysis of variation in intraspecific functional traits leads to increased overlap between species. (A) Mean trait values for a given species. (B, C) Include analysis of intraspecific trait variation. Different colors indicate different species. Source: Violle, C., Enquist, B.J., McGill, B.J., Jiang, L., Albert, C.H., Hulshof, C., Jung, V. & Messier, J. (2012) The return of the variance: intraspecific variability in community ecology. *Trends in Ecology and Evolution*, 27, 244–252.

variation in functional traits on community structure and ecosystem function may be as important or more important than the effects of interspecific variation (Palkovacs & Post 2009; Messier, McGill & Lechowicz 2010; Violle *et al.* 2012; Des Roches *et al.* 2018), but empirical studies on intraspecific functional variation are still limited (Palkovacs & Post 2009; Govaert, Pantel & De Meester 2016; Des Roches *et al.* 2018).

Typically studies of intraspecific variation have focused on body size (Woodward *et al.* 2005a; Doyle & Whiteman 2008; Carlson & Langkilde 2017). Body size variation is paramount to delineating trophic positions, direction and strength of species interactions, prey selection, competition, and mortality (Woodward, Speirs & Hildrew 2005b; Taborsky, Heino & Dieckman 2012; Trebilco *et al.* 2013), such that

intraspecific body size variation is a key evolutionary characteristic of populations and communities. In particular, predator body size variation governs trophic interactions among and within species due to scaling between body size and dietary niche breadth; as predator body size variation increases, niche overlap among individuals decreases (Woodward & Hildrew 2002), thereby reducing intraspecific competition. These trophic impacts of population size structure are especially notable in aquatic systems (Werner & Gilliam 1984), where ontogenetic niche shifts in top predators affect cannibalism rates (Rudolf & Armstrong 2008), which in turn directly or indirectly affect lower trophic levels (i.e., trophic cascade; Rudolf 2007; Rudolf & Armstrong 2008; Miller & Rudolf 2011). In gape-limited top predators, increased intraspecific body size variation leads to cannibalism (Miller & Rudolf 2011), decreasing top predator abundance/density and subsequently reducing threat-based behavioral changes that control lower trophic levels (Rudolf 2007; Miller & Rudolf 2011). The degree of phenotypic variation of top predators can alter the direction and intensity of top-down trophic cascades (Post *et al.* 2008; Ripple & Beschta 2012), thereby affecting processes controlling energy and nutrient cycling (i.e., ecosystem functions; Cardinale *et al.* 2012).

Ecosystem functions include how energy and biomass are stored and transferred, as well as the sustainability of their fluctuations over time (Pacala & Kinzig 2002). Examples of ecosystem functions include primary production, nutrient cycling, and decomposition (Cardinale *et al.* 2012). Further descriptions of ecosystem function may include resistance to invasive species, disease prevalence, and reproductive

productivity (Srivastava & Vellend 2005). Based on relationships between trophic cascades and ecosystem function, intraspecific functional variation, specifically in top predators, could cause stabilizing or destabilizing effects on other trophic levels (i.e., alter trophic cascades; Bolnick *et al.* 2011).

Body size variation in larval ambystomatid salamanders has important effects on trophic cascades in fishless pond ecosystems (Figure 3) by altering the presence, direction, and/or intensity of competition, predation, and cannibalism (Urban 2007; Wissinger *et al.* 2010), and thus these communities represent tractable systems for investigating the functional consequences of intraspecific body size variation. Relatively larger larvae exhibit broader dietary niche breadths and are more likely to

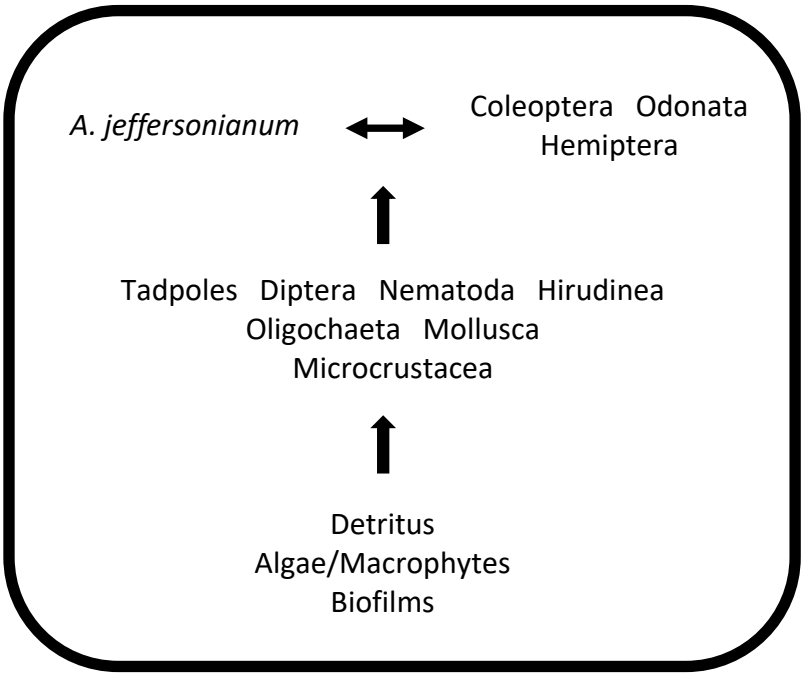

Figure 3. Food wed based on invertebrates recorded in this study and the known community structure of breeding ponds used by *A. jeffersonianum*. Double ended arrow shows reciprocal predation.

reach size refuge from, and prey on, macroinvertebrate predators which compete for similar resources (e.g., zooplankton basal prey; Urban 2007). In addition, body size determines the ability of ambystomatid larvae to engage in cannibalism and predate heterospecific larval salamanders and tadpoles (Walls & Williams 2001), which is expected to have subsequent effects on ecosystem function.

This study tested the effects of body size variation in *A. jeffersonianum* on water chemistry, nutrient cycling, algae, leaf-litter decomposition, basal prey density, macroinvertebrate density, and cannibalism frequency in cattle tank mesocosms. *Ambystoma jeffersonianum* is often an aggressive apex predator in fishless and ephemeral ponds where it is known to cannibalize conspecifics and alter the survival and behavior of smaller larval amphibians (Smith & Petranka 1987; Brodman 1999, 2004; Brodman & Jaskula 2002). Body size in ambystomatid salamanders is at least partially genetically determined by maternal identity, such that ovum and larval body size scales with maternal body size (Kaplan 1980) and population body size variation scales with sibship diversity (Mott *et al.* 2019). Based on these relationships, diversity of maternal lines (i.e., sibship diversity) was manipulated in an effort to create larval populations of varying size structure. Levels of low, moderate, and high sibship diversity were produced using larvae from different ratios of egg masses from three separate collection localities, thereby allowing the diversity of mothers of egg masses to act as surrogates for levels of low, moderate, and high body size variation in larval populations (Figure 4).

Figure 4. As the number of egg masses represented in a population increases, assuming each egg mass is from locations outside the dispersal distance of egg mass parents, then sibship diversity will also increase. As sibship diversity increases the body size variation is also suspected to increase.

I hypothesized that larval salamander populations exhibiting moderate levels of intraspecific body size variation would generate the strongest top-down trophic cascade (i.e., strongest top-down control) because moderate body size variation enables broader niche breadths than in populations with reduced size structure, but with less cannibalism than populations with increased size structure. Because prey choice in low size-variation populations results in narrow dietary niche breadths (Polis 1984; Scharf, Juanes & Rountree 2000), I speculated that reduced rates of cannibalism associated with reduced size structure would result in higher larval densities with relatively intense intraspecific competition (Figure 5). However, larvae exhibiting reduced size structure only consume specific size ranges of prey, leading to projected decreases in densities of prey inside their niche breadth and increases in densities of prey outside their niche breadth. These effects would reduce the overall invertebrate taxonomic diversity but increase the overall invertebrate density such that invertebrate primary consumers decrease algal productivity (Figure 5). Increases in overall invertebrate densities would likely include macroinvertebrate shredders, leading to higher rates of leaf litter decomposition (Figure 5). Larval salamander

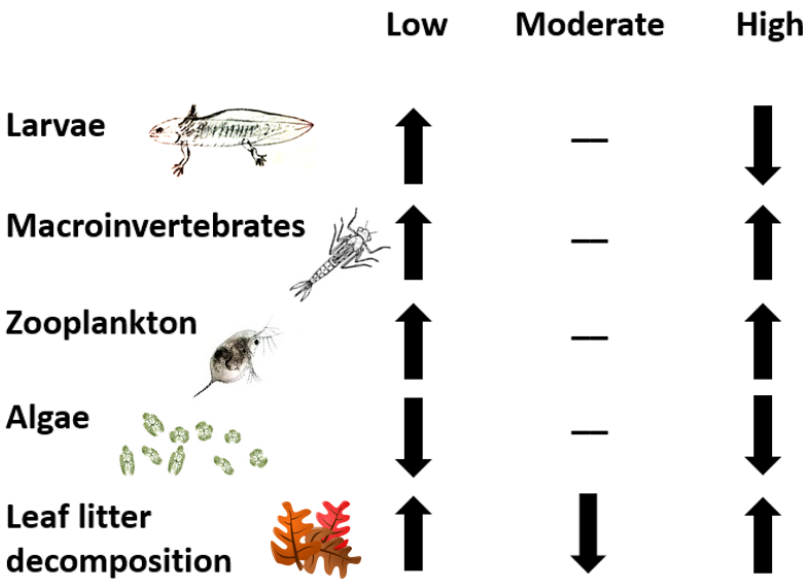

Figure 5. Hypothesized effects of low, moderate, and high body size variation treatment levels on larval salamander density, macroinvertebrate density, zooplankton density, algal density, and the rate of leaf litter decomposition. Arrows indicate whether a given factor is increasing or decreasing relative to the other treatment levels and dashes signify an intermediate response compared to the other treatment levels.

populations exhibiting higher intraspecific body size variation should exhibit the broadest dietary niche breadths (Woodward *et al.* 2005b), but with concomitant increases in rates of cannibalism. Self-thinning via cannibalism would reduce density-dependent predation on invertebrate prey, thereby increasing rates of detritus processing and decreasing algal production similar to the larval populations exhibiting reduced size structure (Figure 5). Because moderate intraspecific size variation enables broader niche breadths compared to the low variation populations and less cannibalism compared to high variation populations, I predicted intermediate levels of intraspecific body size variation would reduce invertebrate prey densities and associated rates of detritus processing and algal productivity compared to the other groups (Figure 5).

II. Methods

Experimental Design

Experimental mesocosms were established at Eastern Kentucky University's Taylor Fork Ecological Area, Madison Co., KY (37.720051, -84.296051). Containers used for mesocosms were 1,136-L plastic cattle tanks (Rubbermaid structural foam stock tanks) filled with rain water to a depth of 45 cm. Single 1-cm holes were drilled into each tank ~ 7 cm to avoid overflowing; however, to prevent organisms from escaping, holes were stuffed with mesh (1 mm mesh size). One liter of water collected from a mesocosm with only rainwater and planktonic algae was added to each experimental mesocosm to ensure thorough phytoplankton colonization. Due to freezing temperatures from December 25th, 2017 to January 7th, 2018, water in mesocosms was thoroughly frozen. After the ice thawed, leaf litter was added by homogenizing an accumulation of wet leaves (consisting of mostly *Quercus* sp., *Acer* sp., *Platanus occidentalis*, and *Cornus* sp.) collected from yard waste, filling a 62.46-L plastic Hefty® tote (one tote for each tank), and then spreading these evenly across the water surface in each tank on January 12th. We added to each mesocosm 2 L of homogenized pond water containing zooplankton (1 L on January 12th and 1 L on March 5th) collected with an 80-µm Fieldmaster conical zooplankton net (Wildlife Supply Company, Yulee, FL) from a 0.65 ha pond (calculated using DaftLogic; 37.726593, -84.301888) or other nearby sources. Homogenized pond sediment samples (1,215 cm^3) containing predominantly non-biting midges (Chironomidae), worms (Oligochaeta), and leeches (Hirudinea) were collected from the same pond and added

to each mesocosm on January 30th. Equal numbers of bladder snails (Physidae, N = 6 / tank), crawling water beetles (Haliplidae, 6 / tank), narrow-winged damselflies (Coenagrionidae, N = 5 / tank), and scuds (Amphipoda, N = 8 / tank) were also added to each mesocosm. In addition, other aquatic insect taxa were allowed to colonize *ad libitum* until mesh lids were placed on the mesocosms, after which time lids were removed most days for at least an hour to allow continued colonization and emigration. These included dragonflies (Anisoptera), predaceous diving beetles (Dytiscidae), water scavenger beetles (Hydrophilidae), water striders (Gerridae), giant-water bugs (Belostomatidae), water scorpions (Nepidae), backswimmers (Notonectidae), and water boatmen (Corixidae). All macroinvertebrates added or naturally colonizing tanks were characteristic of those typically found in ponds with larval salamanders (Anderson & Whiteman 2015). On May 8th, mesocosms were covered with 1-mm mesh lids to provide artificial shade, prevent unwanted colonization and oviposition by Cope's Gray Tree Frog (*Hyla chrysoscelis*; Anderson & Whiteman 2015), and prevent escape of metamorphosing *A. jeffersonianum* and *H. chrysoscelis*. Although it was unnecessary due to sufficient rain accumulation in mesocosms, additional rainwater was collected in two 11,356-L holding tanks to maintain mesocosm water levels.

Based on previously reported relationships between sibship diversity and size variation in larval salamanders (Kaplan 1980; Mott *et al.* 2019), we attempted to vary size structure among treatment levels by varying the diversity of maternal lines (i.e., sibship diversity) in larval populations of *A. jeffersonianum*. Treatment levels utilized

different combinations of egg masses of *A. jeffersonianum* from widely separated geographic locations to produce three sibship diversity groups: a) “low”; b) “moderate”; and c) “high”, which was predicted to correspond with “low”, “moderate”, and “high” size structure in larval populations. For example, a population of larvae created using one egg mass (i.e., sibship) would exhibit “low” sibship diversity and intraspecific body size variation, whereas larval populations of larvae created using multiple egg masses collected from widely separated geographic locations would exhibit “high” sibship diversity leading to “high” intraspecific body size variation. Egg masses of *A. jeffersonianum* were collected from four wildlife management areas (WMA) across four Central Kentucky counties from March 3 - 4, 2018. These included the Miller-Welch Central Kentucky WMA in Madison Co., KY, the John Kleber WMA in Franklin Co., KY, the Kentucky River Gilbert Tract WMA in Owen Co., KY, and the Taylorsville Lake WMA in Spencer Co., KY (Figure 6). The sites were separated by distances much greater than the known adult dispersal distance of 625 m – 1,600 m (Bishop 1941; Downs 1989), thereby assuring no movement of individual females between or among sites. Based on studies from other ambystomatid salamanders (e.g. *A. talpoideum*), egg masses were selected from separate mothers (i.e., separate sites) in an attempt to increase the probability that larvae would exhibit varying sizes at hatching and subsequent growth rates, thereby producing different degrees of size structure in experimental populations (Kaplan 1980; Alcobendas, Buckley & Tejedo 2004; Moore, Landberg & Whiteman 2015; Mott *et al.* 2019). Egg masses were maintained in plastic trays (one tray per egg mass of ~ 24 larvae) with 0.5 L of

Figure 6. General locations of *A. jeffersonianum* egg mass collection sites. Bold labels correspond to county names and numbers represent multiple ponds per county. Created using QGIS mapping software (Version 3.4; QGIS Development Team 2019).

deionized water. The trays were placed in an environmental chamber at 11.4^{o} C with a 12L:12D photoperiod in the vivarium facilities at Eastern Kentucky University until they hatched and were sorted into treatment levels (12 - 14 days post-collection).

Hatch dates of each egg mass were recorded on the first day any larvae hatched; however, multiple days passed before all larvae from a given egg mass hatched. Egg mass hatch date ranged from March 6 - 16th, and for all but two mesocosm assignments, only larvae with the same hatch dates were mixed prior to addition to mesocosms. For the two exceptions, the maximum difference in egg mass hatch date was three days. To create low sibship diversity treatment levels, 24 hatchlings from the same egg mass were assigned to each mesocosm. For moderate treatment levels, mesocosms contained 24 larvae from two egg masses from two collection sites (12 larvae from each), and high treatment levels contained 24 larvae from three egg masses from three collection sites (eight larvae from each). Six replicate mesocosms were constructed for each treatment level (Figure 7), which were assigned randomly across the mesocosm array (using the RAND function in Microsoft Excel 2013) to account for variation in the initial community composition (Carlson & Langkilde 2017). Remaining hatchlings (from each egg mass used in the project) not assigned to mesocosms were anesthetized and euthanized by immersion in an aqueous solution of 250 mg L^{-1} of benzocaine and preserved in 70% ethanol to provide representative samples for future genetic analyses. Before being added to the tanks on March 16-17th, all larvae were photographed to measure total length (TL; the length from the anterior tip of the snout to the posterior tip of the tail) using ImageJ (Mott *et*

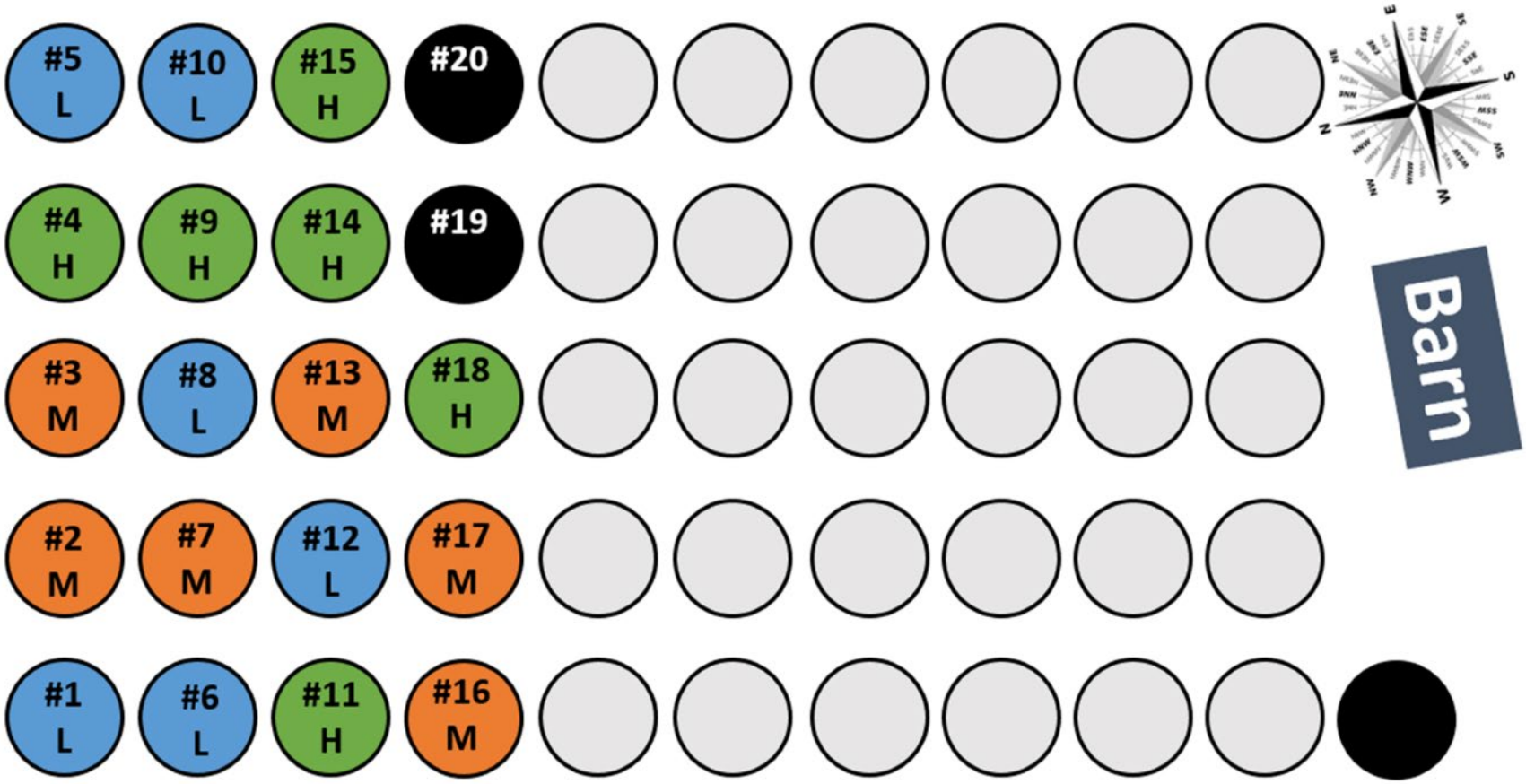

Figure 7. Mesocosm array at the Taylor Fork Ecological Area at Eastern Kentucky University. Circles represent mesocosms and those used for larval *Ambystoma jeffersonianum* are labeled #1 – 18. The low diversity treatment (L – blue) was assigned 24 larvae from 1 egg mass, the moderate treatment (M – orange) had 12 larvae each from 2 egg masses, and high treatment (H – green) had 8 larvae each from 3 egg masses. The assigned treatment was determined randomly for each mesocosm. The black mesocosms were spare cattle tanks and the gray were used in another project. Mesocosms #19-20 contained unused leaf litter, invertebrates, and salamander larvae. Figure is not to scale.

al. 2010; Rasband 2014). These, and all subsequent photographs for ImageJ analysis, were taken with a Nikon Coolpix P530 camera (Nikkor 42X wide optical zoom ED VR 4.3-180 mm 1:3-5.9). On May 25th, newly hatched *H. chrysoscelis* tadpoles were collected from two spare cattle tank mesocosms situated adjacent to the north side of the barn (Figure 7) containing only rain water and phytoplankton. On the same day, they were homogenized in 18.9 L buckets, divided into groups of 30, photographed, and added to mesocosms haphazardly as additional prey sources for larval *A. jeffersonianum* and as vertebrate primary consumers.

Mesocosm sampling

To ensure homogeneous starting densities, a preliminary sampling was conducted for zooplankton density (10 days after larvae were added to the mesocosms) and algal productivity (24 days after larvae were added). The first formal sampling event began on April 29th (43 days after larvae were added), the second on May 30th, and the final on July 4th, when nearly all salamanders had metamorphosed. Subsequent "post-salamander" sampling events were conducted beginning August 2nd and September 4th. Each sampling event typically spanned two to three days.

Larval densities

During each sampling event, densities of larval *A. jeffersonianum* were estimated using three 25 x 46-cm Promar Collapsible Minnow Traps (1.6 mm mesh; Cabela's Inc., Sidney, NE) per mesocosm over a 20-hour period (Doyle & Whiteman 2008). Each trap had a 7.6 x 25.4 cm section of foam pool noodle inside to enable buccal pumping by older larvae. Larvae from minnow traps were examined for injuries

from competition and attempted cannibalism, photographed for subsequent measurement, and immediately returned to tanks. Additional larvae were collected opportunistically using dip-nets and included in the injury examination and photographs for determining larval size variation, but not incorporated into larval density estimates. As larvae underwent metamorphosis (determined by conspicuous floating at the water's surface paired with gill reabsorption) individuals were collected from mesocosms, anesthetized and euthanized by immersion in an aqueous solution of 250 mg L^{-1} of benzocaine, and preserved in 70% ethanol. During the second sampling event, relative abundances of *H. chrysoscelis* tadpoles were also estimated using the same minnow traps as those used for *A. jeffersonianum*, but were not included in the final analysis due to low sample size. Tadpole metamorphs were preserved in the same way as the salamanders. Experimental methods followed Eastern Kentucky University's animal care guidelines (IACUC Protocol #: 09-2017, 10-2017), and egg mass collection was permitted by the Kentucky Department of Fish and Wildlife Resources (Permit # SC1811119).

Macroinvertebrate density and rate of leaf litter decomposition

During each sampling event, sediment samples were collected using an 18.5 x 15.0 cm benthic dredge (WILDCO® Fieldmaster® Mighty Grab II Dredge; Luo *et al.* 2015). Dredge contents were preserved in Whirl-Paks with 70% ethanol and Rose Bengal stain. Each mesocosm was divided into four equal-sized quadrants separated by an imaginary cross, such that a different quadrant would be used during each sampling event.

Leaf litter decomposition was examined using mesh leaf packs (Boulton & Boon 1991; Robinson, Gessner & Ward 1998). Leaf packs were constructed of 18 x 24 cm polypropylene produce packs (5 mm mesh size; Miller Supply Inc., Rancho Santa Margarita, CA) and filled with 5.0 g of leaf litter (consisting of mostly *Quercus* sp., *Acer* sp., *Platanus occidentalis*, and *Cornus* sp. collected from yard waste) that had been dried at 80-90^{o} C for 24 hours. Prior to adding leaf packs to experimental mesocosms, ten leaf packs were submerged in spare mesocosm #20 (Figure 7), placed in a Whirl-Pak, and returned immediately to the lab to be dried and weighed to account for handling error. Eleven leaf packs were then placed along the southeastern edge of each experimental mesocosm on March 27th and individually anchored with a small gravel rock. For each sampling event, one leaf pack was removed from each tank and preserved in a Whirl-Pak with 70% ethanol and Rose Bengal stain.

Zooplankton density

During each sampling event, zooplankton were sampled using one haul of a 80-µm Fieldmaster conical zooplankton net (Wildlife Supply Company, Yulee, FL). The net was submerged on the bottom edge of the mesocosm and allowed to sit for at least 30 seconds (to limit zooplankton disruption prior to sampling). The net was then pulled up at a ~45^{o} angle so that it emerged in the center of the mesocosm at the water's surface. Samples were poured into a 100 mL specimen cup and preserved by adding an equivalent volume of 70% ethanol with Rose Bengal stain.

Algal productivity

During each sampling event, periphyton growth was analyzed using 48 x 48 x 6-mm glazed ceramic mosaic tiles (American Olean©) following Karouna & Fuller (1992). Eleven tiles were strung along the inside of each mesocosm with mason line secured to the lips of mesocosms, such that tiles were suspended 15 cm above the leaf litter. During each sampling event, one tile from each mesocosm was removed, with periphyton collected by scraping the tile's glazed surface with a 38-mm single edge razor blade. Periphyton was transferred to a 100-mL specimen cup containing ~10 mL of 2% glutaraldehyde.

Chlorophyll *a* (a measure of algal productivity) was measured during each sampling event using a AquaFluor® handheld fluorometer (Turner Designs, San Jose, CA) and turbidimeter (P/N: 8000-010), calibrated with an adjustable solid secondary standard (red; P/N 8000-952) to analyze relative fluorescent units (RFU; Krohn *et al.* 2011; Marino, Srivastava & Farjalla 2013). For each sampling event, the fluorometer was blanked using a water sample filtered through No.3 Whatman filter paper from one of the mesocosms to remove algal cells and other contaminants. Water samples were collected by first dividing each tank into four equal sized quadrants separated by an imaginary cross. Using a 100-mL syringe, four 20-mL subsamples were collected sequentially (one from each quadrant) at a depth of 4 cm, thereby creating an 80-mL homogenized sample in the syringe. Approximately 3 mL of sample was transferred to a cuvette for fluorometric analysis, and remaining sample was used for nitrate and phosphate analyses (see below).

Environmental conditions

The remaining water sample from fluorometer readings was divided between two 10-mL vials for nitrate and phosphate analyses in the field, transferred to an ice cooler, and ultimately refrigerated at -20°C in an Isotemp® freezer (Fisher Scientific, Waltham, MA). Dissolved oxygen and temperature in mesocosms were measured using an Oakton® DO 6+ Dissolved Oxygen/Temp meter (Model: WD-35643-10) at a depth of 5 cm. Because the DO 6+ probe was new, and thus newly calibrated, the temperature sensor's factory calibration was trusted to accurately record temperature. Percent saturation calibration was used to measure the percent saturated dissolved oxygen, and the milligrams per liter dissolved oxygen. Barometric pressure settings were adjusted accordingly to ensure correct calibration, and calibration and sample reading procedures followed the Oakton® DO 6+ Manual. During the final "post-salamander" sampling event, some DO readings exceeded their max (200.0% and 20.00 mg/L), likely due to high mid-day temperatures; therefore, maximum estimates were recorded for those samples. pH readings were taken using the Oakton® pH 6+ (Model: WD-35613-24), and calibration and sample reading procedures were outlined in the Oakton® pH 5+, pH 6+, Ion 6+ instruction manual.

Laboratory analysis

Larval densities

Although hatchling measurement for *A. jeffersonianum* utilized total length (TL), snout-to-vent length (SVL; length from the anterior tip of the snout to the opening of the cloaca) was recorded for subsequent measurements due to tail damage

on some of the salamanders that would artificially reduce TL measurements. For the May 30th sampling event, SVL was measured from photographs using ImageJ (Mott *et al.* 2010; Rasband 2014). Preserved metamorphs were blotted with a paper towel, weighed using a 60-g scale (Fisher Scientific; accuracy = 0.0001 g), photographed, and SVL was measured using ImageJ. SVL and body size variation analyses for the May 30th sampling event were based on measurements of preserved metamorphs from May 28-30th and salamanders captured (using minnow traps and dip-nets) on May 31st. This was necessary because salamanders began metamorphosing on May 28th and the actual salamander sampling for the "May 30th sampling event" did not occur until May 31st.

Macroinvertebrate densities and rates of leaf litter decomposition

Macroinvertebrates were identified to order or family and enumerated under 32x dissection microscopy. To calculate macroinvertebrate densities in mesocosms from dredge samples, the area sampled by the dredge was extrapolated to square meters. Macroinvertebrates from leaf packs were picked and identified following procedures for benthic samples to provide another measure of relative macroinvertebrate abundance. Leaf packs picked of macroinvertebrates were dried at 66° C for 5 days (time determined to completely dry samples) and weighed (Boulton & Boon 1991). A Thermo Scientific drying oven and Isotemp® drying ovens (Fisher Scientific) were used for drying the leaves. Dried leaf mass was measured using a 500-g scale (Fisher Scientific; accuracy = 0.1 g), and leaf litter decomposition rates were

expressed as milligrams per day lost (mass loss = mass at addition – mass at removal – mean handling error).

Zooplankton density

Zooplankton samples were drawn down to 20 mL, and one 1-mL subsample per sample was transferred to a Sedgewick-Rafter counting cell. Zooplankton were then enumerated and identified to Order under 32x dissection microscopy (Smith 2001). Zooplankton density was calculated as total zooplankters per liter by extrapolating counts from 1-mL subsamples to 20-mL samples, and dividing by the volume of the water sampled formed when retrieving zooplankton net hauls.

Periphyton density

Periphyton samples were transferred to aluminum weigh boats and dried in a Thermo Scientific drying oven for 48 hours at 85^{o} C to calculate biomass for each tile using a 60-g Fisher Scientific scale (accuracy = 0.0001 g). This mass was then extrapolated to square meters based on the area of the ceramic tile.

Nitrate and Phosphate

Nitrate and phosphate samples were thawed and filtered through 80-µm mesh to remove particulates. Nitrate and phosphate concentrations were then recorded using nitrate and phosphate absorbance modules (P/N: 7200-074 and 7200-070, respectively) in Turner Designs Laboratory Fluorometer (P/N: 7200-000). Analytical protocols followed Turner Designs nitrate procedure using the LaMotte test kit and the phosphate procedure for the Trilogy™ Laboratory Fluorometer (Forms: S-0094 and S-0077, respectively).

Statistical Analysis

To test the assumption that hatchling body size variation was initially equivalent among treatment levels, the coefficient of variation (CV) of hatchling total lengths was compared using a modified signed-likelihood ratio test for equality of CVs' (M-SLRT; Krishnamoorthy & Meesook 2014) from the R package "cvequality" (Version 0.1.3; Marwick & Krishnamoorthy 2019). This package also used to compare body size variation among treatment levels for the May 30th sampling event. However, due to low sample sizes in low sibship diversity replicates, we compared CV using: a) summary statistics for individual mesocosms; and b) pooled, raw measurements of like replicates for each treatment level.

Three separate multivariate analyses of variance (MANOVAs) were used on subsets of related data (i.e., response variables dealing with salamanders, invertebrates, and ecosystem functions) to assess treatment effects while increasing power that would otherwise be lost by conducting a single MANOVA using all response variables (Scheiner 1993; Chalcraft & Resetarits Jr. 2003). The first MANOVA assessed the effects of sibship diversity treatment levels on the abundance, growth rates, and dates of metamorphosis for *A. jeffersonianum* larvae and metamorphs, respectively. A second MANOVA assessed the effects of sibship diversity treatment levels on macroinvertebrate and zooplankton densities; and a third MANOVA assessed treatment effects on chlorophyll *a* concentration, periphyton mass, rates of leaf litter decomposition, phosphate, nitrate, and dissolved oxygen. If any MANOVA indicated a significant overall treatment effect, subsequent univariate analyses of variance

(ANOVAs) were conducted to determine which individual response variables were affected by treatment. Separate from the MANOVAs, an initial ANOVA was conducted to test for differences in hatchling TL by treatment, and after all metamorphic *A. jeffersonianum* were collected, an ANOVA was conducted on rates of survival to metamorphosis by treatment. If significant differences were detected in following ANOVA, pairwise comparison of means was conducted using a Tukey's honest significant difference (HSD) test. Because of the high variability in the data within treatment levels, relationship between response variables was analyzed using a Pearson pairwise correlation test. An analysis of similarity (ANOSIM) of Bray-Curtis similarity measures was used to test differences in macroinvertebrate taxonomic diversity among treatment levels (Marchant, Wells & Newall 2000; Clarke, Somerfield & Chapman 2006; Rudolf & Rasmussen 2013). All data analyses were conducted using the R statistical software environment (Version 3.4.1; R Core Team 2017).

III. Results

Coefficient of variation (CV) for hatchling total length (TL) among *A. jeffersonianum* prior to addition to mesocosms was not significantly different among treatment levels [1](Modified signed-likelihood ratio test statistic = 0.952, p = 0.621; Figure 8, Appendix A; Table 1). Significant differences were detected in the absolute TL of hatchling larvae (ANOVA $F_{2,15} = 4.589$, p = 0.028; Table 2), with hatchlings assigned to low sibship diversity mesocosms being 5.8% larger than hatchlings assigned to high diversity mesocosms (Tukey 95% CI, p = 0.022; Figure 8). To account for differences in initial sizes of hatchlings at the time of introduction to mesocosms, we chose to report subsequent measurements of larval size as rates of growth (mm/day) as opposed to absolute measurements of body size. Hatchling TL in the moderate diversity treatment level did not differ significantly from those in low (Tukey 95% CI, p = 0.228, Figure 8) or high (Tukey 95% CI, p = 0.421, Figure 8) diversity treatment levels. Before adjusting for outliers, the final CV (based on snout-to-vent lengths [SVL]) was significantly different between all individual mesocosms, even within treatment levels (Modified signed-likelihood ratio test statistic = 52.511, p < 0.001, Table 1), as well as among treatment levels (Modified signed-likelihood ratio test statistic = 6.410, p = 0.041, Table 1). After removing single outliers (determined using "boxplot.stats" function in the R statistical software environment) from mesocosms #3 and #4 there was still a significant difference in final CV between individual mesocosms (Modified signed-likelihood ratio test statistic = 24.554, p = 0.039, Table 1), but after removing the single outliers from

[1] All tables are presented in appendix B at end of thesis.

mesocosm #3, #4, and #17 there was no significant difference in final CV between treatment levels (Modified signed-likelihood ratio test statistic = 4.456, p = 0.108, Table 1).

Of the 432 hatchlings used in the mesocosms, a total of 224 survived to metamorphosis, resulting in an estimated overall survival rate of 51.8%. Numbers of larvae surviving to metamorphosis differed significantly by treatment level (ANOVA $F_{2,15} = 5.209$, p = 0.019; Table 3). High diversity mesocosms exhibited significantly higher survival to metamorphosis (72.9%) than the low diversity level (27.8%; Tukey 95% CI, p = 0.015; Figure 9, Appendix A). Survival in moderate diversity mesocosms (54.9%) was not significantly different from either low diversity (Tukey 95% CI, p = 0.166, Figure 9) or high diversity mesocosms (Tukey 95% CI, p = 0.426, Figure 9).

Metamorphosis of *A. jeffersonianum* was first recorded on May 28th (Figure 10, Appendix A). On May 30-31st, 45.9% of the total metamorphs were collected (Figure 10). An average of six metamorphs (3%) were collected daily from June 1st through June 19th (Figure 10). After this, only seven more metamorphosed until the last metamorph was recorded on July 2nd (Figure 10). MANOVA indicated a significant difference by sibship diversity in larval abundance in minnow traps, growth rate (based on SVL), or average date of metamorphosis in *A. jeffersonianum* ($F_{2,12} = 6.215$, p = 0.032; Figure 11, Appendix A; Table 4). Subsequent one-way ANOVAs revealed a significant difference in larval abundance in minnow traps ($F_{2,15} = 6.318$, p = 0.010; Table 4, Figure 11) but not growth rate ($F_{2,12} = 1.314$, p = 0.305; Table 4, Figure 11) or date of metamorphosis ($F_{2,12} = 0.981$, p = 0.403; Table 4, Figure 11). High sibship

diversity mesocosms exhibited significantly higher larval abundance than the low diversity level (Tukey 95% CI, p = 0.008, Figure 11). Abundance in moderate sibship diversity mesocosms was not significantly different from either low sibship diversity (Tukey 95% CI, p = 0.253, Figure 11) or high sibship diversity mesocosms (Tukey 95% CI, p = 0.175, Figure 11).

There were no significant influences of sibship diversity on macroinvertebrate or zooplankton densities (MANOVA $F_{2,15}$ = 1.205, p = 0.329; Figure 12, Appendix A; Table 5), and ANOSIM indicated that sibship diversity did not influence macroinvertebrate taxonomic diversity (*R* = 0.032, p = 0.264; Figure 13, Appendix A). Sibship diversity did not significantly affect chlorophyll *a* or periphyton abundances, rate of leaf litter decomposition, dissolved oxygen, or phosphate (MANOVA $F_{2,15}$ = 2.056, p = 0.072; Figure 14, Appendix A; Table 6). Nitrate concentrations in the mesocosms were relatively non-existent, so it was not included in the MANOVA. Based on correlation analyses at the mesocosm level, aspects of larval *A. jeffersonianum* were associated with response variables associated with primary production. There was a positive correlation between the abundance of larval *A. jeffersonianum* in minnow traps and dissolved oxygen (R = 0.54, p = 0.02; Table 7) and chlorophyll *a* (R = 0.68, p < 0.01; Table 7). The growth rate of *A. jeffersonianum* was negatively correlated with chlorophyll *a* (R = -0.56, p = 0.03; Table 7) and positively correlated with phosphate (R = 0.55, p = 0.04; Table 7). Dates of metamorphosis for *A. jeffersonianum* were positively correlated with dissolved oxygen (R = 0.75, p < 0.01; Table 7) and chlorophyll *a* (R = 0.79, p < 0.01; Table 7).

IV. Discussion

Although sibship diversity did not lead to populations of variable body size, it was positively related to larval density and survival to metamorphosis in *A. jeffersonianum*. Sibship diversity of egg masses used in stocking mesocosms did not affect growth rates or dates of metamorphosis for *A. jeffersonianum*, nor did it have any significant effects on invertebrate communities and ecosystem function. The lack of differences in body size variation among sibship diversity treatment levels challenges the viability of manipulating size variation by kinship shown in other studies (Mott et al. 2019).

Important considerations for our observations of sibship diversity not leading to populations of variable size structure include the overall effects of larval salamander density and the viability of manipulating intraspecific body size variation by controlling sibship diversity in *A. jeffersonianum*. Larval body size in multiple ambystomatid salamanders has been linked to maternal body size (Kaplan 1980). Most recently, larval *A. talpoideum* exhibited 30% higher body size variation in populations of mixed sibship diversity, compared to populations composed exclusively of sibships (Mott *et al.* 2019). However, in this study it was difficult to analyze body size variation in low sibship diversity replicates due to low sample sizes from the May 30th sampling event (N = 15 across all low diversity mesocosms). Instead of comparing CV for each mesocosm between low, moderate, and high diversity treatment levels, we compared CV among: a) individual mesocosms; and b) pooled, raw measurements of replicate mesocosms for each treatment level. Considerable variability in CV among individual mesocosms

within treatment levels, and among treatment levels, largely resulted from a small number of outliers, and intraspecific body size variation did not scale with sibship diversity. Therefore, altering levels of sibship diversity was not a viable method for manipulating body size variation in this experimental design. Carlson & Langkilde (2017) generated body size variation in *Lithobates sylvaticus* by keeping tadpoles in holding tanks at high densities to produce size variation. They then mixed 100 tadpoles from different size classes to create high and low variation populations for each mesocosm. Based on their results, lower risk methods of manipulating body size variation similar to their procedure should be considered in the future. While this would be difficult to do this while maintaining sibship, due to the small clutch size for *A. jeffersonianum*, it may be possible for ambystomatids with higher clutch sizes (e.g. *A. maculatum* and *A. opacum*).

Another possible explanation for why body size variation did not scale with sibship diversity is due to the relatively low initial density of hatchlings per mesocosm (24 / m^2). Because the low sibship diversity treatment consisted of hatchlings from one egg mass, population sizes were limited by the smallest numbers of embryos in the egg masses collected. Egg masses in central Kentucky average 23 embryos per mass (Smith 1983), and thus this number approximated larval population sizes used in the low sibship diversity treatment level and therefore all other treatment levels. This initial density was roughly half the size of other mesocosm studies with *A. jeffersonianum* (Brodman 2004; Chambers *et al.* 2011) and at the lower end of the range of natural densities (Cortwright 1987). Because intraspecific body size variation is positively

correlated with both cannibalism and density (Brodman 2004), I hypothesize that increasing initial densities would increase the ability to create treatment levels with significantly different degrees of body size variation. A possible solution to this would be using more egg masses relative to each treatment level. This would allow increasing the initial density while still maintaining lower numbers of sibships in the low diversity treatment level and would also allow for greatly increasing the sibship diversity in the high treatment level. However, a disadvantage to this would be the much higher sampling effort and expense due to the necessity of collecting eggs from many more ponds to ensure eggs are from different females. Another solution is to use the same or smaller initial densities of larvae but with smaller mesocosms to increase effective densities.

Despite the absence of an association between sibship diversity and intraspecific body size variation, there were nevertheless differences in larval salamander densities and survival to metamorphosis between treatment levels. Reduced larval salamander densities in the low sibship diversity treatment level with larvae from the same egg mass is possibly due to sibship competition and/or cannibalism (i.e., “negative” kin selection). Kin selection is inclusive fitness resulting from preservation of alleles either directly through offspring or indirectly through relatives (Hamilton 1964; Pfennig 1997). In some cases, *A. tigrinum* larvae will cannibalize kin indiscriminately (Pfennig, Sherman & Collins 1994), and *A. opacum* are known to prefer cannibalizing kin (i.e. "negative" kin selection; Walls & Blaustein 1995). If this occurred in the low treatment level, it may be an example of altruism

where it is genetically best for smaller sibships to sacrifice themselves to increase the fitness of sibship cannibals (Pfennig 1997).

The hypothesis that increased sibship diversity would create higher competition and higher rates of cannibalism was not supported based on patterns of larval abundance and survival to metamorphosis. I believe this was most likely a result of the relatively low salamander starting density (24 hatchlings / m^2) compared to the relatively high numbers of estimated prey items, which ranged from 144 – 4,721 total macroinvertebrates / m^2, 108 – 4,685 Chironomids / m^2, and 25,076 – 266,312 total zooplankters / m^3 on the May 30th sampling event. In the spare mesocosm (#19; Figure 7) ~ 200 extra hatchlings were added along with extra leaf litter and invertebrates not used in the experiment. Although observations from this spare mesocosm were singular and qualitative, my personal observations demonstrated that a much higher starting density lead to more extreme body size variation and evidence of competition (missing tails, gills, and limbs) when compared to the experimental mesocosms.

The difference in the initial total length of hatchlings between the low and high sibship diversity treatment levels was not expected to explain the differences in salamander density or survival. Measurements of the hatchlings immediately before they were added to the mesocosms revealed that those used in the low diversity treatment level were 0.8 mm larger on average than those used in the high diversity treatment level. This likely resulted from the hatching date being an average of four days earlier in the low diversity replicates. Because increased larval size is generally associated with faster growth rate and higher survival when compared to smaller

larval size (Kaplan 1980; Travis 1983; Semlitsch & Gibbons 1990; Dziminski & Alford 2005; Räsänen, Laurila & Merilä 2005; Ficetola & De Bernardi 2009), the initial size differences did not seem to factor into the final salamander densities and survival to metamorphosis.

This study used diversity of maternal lines to manipulate sibship diversity, but it did not consider paternal effects, which can be important to determining larval survival in some amphibians (Howard 1978; Woodward 1987). However, because ambystomatid salamanders have aggregate (i.e., explosive) breeding, females are known to collect sperm from more than one male, leading to multiple and unequal paternity in individual clutches (Arnold 1976; Myers & Zamudio 2004). Without genetic analysis or controlled breeding, paternal diversity in a single clutch is unknown, thereby complicating our understanding of the paternal effects on offspring and possibly creating increased variation in the sibship diversity treatment levels. Further consideration should be made for the effects of multiple paternity in larval ambystomatid growth and survival.

There were no major trophic cascades mediated by sibship diversity of larval salamander apex predators, as evidenced by no significant influences of sibship diversity on macroinvertebrate or zooplankton densities, measures of primary productivity or leaf litter decomposition, or aspects of water chemistry. I believe this was most likely a result of the low initial salamander densities and the high invertebrate densities observed across mesocosms. High macroinvertebrate densities may have acted as a mitigating factor to the predatory impact of larval salamanders

(Strong 1992; Holomuzki, Collins & Brunkow 1994). If invertebrate densities are relatively high compared to salamander densities, then it is less likely the salamanders would affect invertebrates. We may have recorded stronger trophic cascades using initial salamander densities of 50 – 100 hatchlings/m^2; however, there is also some evidence that salamander predator density and average prey density are unrelated (Van Buskirk & Smith 1991). There was also no significant difference in the macroinvertebrate community composition, but, on average, 90.4% (SD = ± 13.6) of the total macroinvertebrate community sampled in each mesocosm using the Mighty Grab sampler was chironomids, which likely overshadowed other macroinvertebrate taxa in mesocosms. The macroinvertebrate analysis was strictly based on benthic macroinvertebrates at the expense of other macroinvertebrate taxa observed on the water surface, mesocosm sides, and in the water column, including Odonata, Gerridae, Belostomatidae, Nepidae, Notonectidae, Corixidae, Physidae, Planorbidae, and Hirundinea. Experimental mesocosms were also only a few months old and only contained macroinvertebrate taxa that had been added or colonized immediately prior to experimentation; therefore, they were likely missing taxonomic diversity that results from colonization over extended periods. Large macroinvertebrate predators tended to colonize after zooplankters (i.e., basal prey) was added in late March, and therefore most macroinvertebrate predator populations were only first established a few weeks after adding salamander hatchlings and were not expected to have significant predatory effects on larval salamanders. Although efforts were made to assess abundances of the additional macroinvertebrate taxa, future research should

include a concerted effort to quantify these groups due to their importance to ecosystem function as predators, grazers, and collector-gatherers. While these taxa were not included in the analysis, based on multiple visual encounter surveys, they seemed to colonize the mesocosms randomly and were not expected to increase variation between treatment levels.

My hypothesized trophic cascade was such that zooplankton density would be higher in low- and high-body size variation populations of salamanders, thereby decreasing primary productivity. This pattern was expected to result from reduced niche breadth with low salamander body size variation, and low larval survival due to cannibalism with high body size variation. Although there was no significant difference in zooplankton densities and measures of primary productivity among treatment levels, general trends were observed wherein average zooplankton densities decreased and average chlorophyll *a* (measure of phytoplankton primary productivity) increased with higher sibship diversity. There was also a significant positive correlation between chlorophyll *a* and salamander density. In addition, *Hyla chrysoscelis* tadpoles were added to mesocosms a few days before the May 30th sampling event and, based on a visual encounter survey, they were less abundant in mescosms with higher salamander density (not included in the analysis due to low sample size). Though not evidenced in the MANOVA, these factors in concert suggest increased salamander density may have directly increased phytoplankton abundance via increased ammonia excretion or indirectly by decreasing herbivore density or foraging rate.

Ecosystem stability among the sibship diversity treatment levels appeared to be relatively high, as evidenced by the lack of major ecosystem functional consequences in associate with differences in densities of apex predators (Cardinale *et al.* 2012). In this project, high invertebrate densities seemed to have a mitigating effect on larval salamander predation and therefore the anticipated trophic cascades (Strong 1992; Holomuzki *et al.* 1994). In natural systems, high tadpole densities, variable rates of tadpole hatching and metamorphosis, and competition and predation from salamander congeners and large macroinvertebrate predators could also have mitigating effects (Brodman & Jaskula 2002; Brodman 2004). While this study does not indicate significant top-down regulation, it does not consider potential bottom-up regulation (Carpenter & Kitchell 1988; Power 1990; Hunter & Price 1992). For example, increased primary productivity could promote higher invertebrate densities, buffering the effects of top-down control by salamanders (Holomuzki *et al.* 1994). With this in mind, decreases in predator density may facilitate more bottom-up effects, whereas increases in predator density could lead to less bottom-up effects, thus complicating ecosystem functional consequences of intraspecific functional variation (McQueen *et al.* 1989). In lentic systems, mixtures of both top-down trophic cascades and seasonal bottom-up effects from nutrient input and mixing are fairly common (Carpenter & Kitchell 1988). A lack of a strong, top-down trophic cascade could also be an artifact of this project's relatively short-term analysis in newly established mesocosms. Invertebrate communities were not fully established, and leaf litter was less than one year old. Although release of soluble nutrients and leaf litter decomposition begin

almost immediately upon introduction to the water (Benfield, Fritz & Tiegs 2017), mesocosms lacked leaves from a variety of decomposition stages and therefore a pool of initially available dissolved organic material.

Despite the lack of size structure between treatment levels at the conclusion of the experiment, the differences in salamander survival to metamorphosis, abundance, and possible minor trophic cascades may result from intraspecific genetic diversity effects between larvae from different egg masses (i.e. mothers). Examples of this in ambystomatid salamanders include genetic adaptations specific to characteristics of source ponds (e.g. hydroperiod, presence of interspecific predation, and invertebrate community composition, etc.) or populations and parentage (e.g. cannibalism tendencies, kin selection, aggression, avoidance behavior, diet preference, size and date of metamorphosis, etc.). Some populations may be adapted to particular hydroperiods (Rowe & Dunson 1995; Denton & Richter 2013; Drayer & Richter 2016), avoidance of fish predators (Davenport *et al.* 2017), competition with intraguild predators (Brodman & Jaskula 2002; Mott & Maret 2011), or kin selection (Pfennig *et al.* 1994; Mott *et al.* 2019). These factors illustrate the many intraspecific genetic adaptations that could affect populations of larval ambystomatid salamanders.

In this study larvae from the moderate and high sibship diversity treatment levels were mixed from different ponds across different geographic locations, possibly creating populations with a variety of genetic adaptations and therefore increased intraspecific niche variation and individual specialization (*sensu* Bolnick *et al.* 2002, 2003). Such diversity would have allowed these populations to exhibit niche

partitioning, avoid competition, have a higher survival, and increase their long-term stability and adaptability (Bolnick *et al.* 2003). This would compare to larvae from low sibship diversity treatment levels are likely genetically similar and have low individual specialization, increasing their competition, and decreasing their survival. However, because the eggs were collected from a limited geographic range and the larvae were mixed in relatively small numbers further testing should be performed to determine the actual genetic variation.

It can be difficult to identify the factors causing intraspecific functional variation, as evidenced by the absence of body size variation between sibship diversity treatment levels in this study. However, previous studies of intraspecific functional variation tend to focus on populations with highly exaggerated morphological variation, such as fish foraging morphology (Scharf *et al.* 2000; Bush & Adams 2007; Bonaldo & Bellwood 2008; Post *et al.* 2008; Palkovacs & Post 2009; Bassar *et al.* 2010), ontological variation in salamanders (Urban 2007; Wissinger *et al.* 2010), or salamander polyphenism or paedomorphosis (Lannoo & Bachmann 1984; Ziemba & Collins 1999; Doyle & Whiteman 2008; Whiteman *et al.* 2012; Mott *et al.* 2019). In comparison, this study sought to explore the effects of body size variation in a species with less drastic levels intraspecific variation that is likely more reflective of most species.

To confirm the effect of sibship diversity in *A. jeffersonianum* on larval survival, abundance, and body size variation, I recommend repeating this project on a finer scale in a laboratory with aquaria microcosms and higher initial larval densities. This

would allow for increased replicates, monitoring, and precise measures of larval density at any given time. This approach in conjunction with an analysis of genetic variation would help pinpoint the cause of decreased survival to metamorphosis in the low diversity treatment level (e.g. cannibalism, competition, etc.). Further analysis should also consider the effects of sibship diversity on more abundant ambystomatids such as spotted salamanders (*A. maculatum*) and marbled salamanders (*A. opacum*), as their survival to metamorphosis may be affected by sibship diversity proportionally to those in *A. jeffersonianum*.

These results highlight the importance of sibship diversity to the survival of ambystomatid salamanders and the potential significance for apex predator conservation in general (Estes *et al.* 2011). When designing studies involving manipulation of intraspecific phenotypic variation, careful consideration should be given to initial species density and the viability of the method of manipulating body size. Future research is necessary to better understand the ecosystem effects of intraspecific functional variation, due to evidence indicating its effects on community structure and ecosystem function may be as important or more important as those of interspecific functional variation (Palkovacs & Post 2009; Messier *et al.* 2010; Violle *et al.* 2012; Des Roches *et al.* 2018). The increasing shift to trait-based ecology from species-based ecology for measures of ecosystem function (Messier *et al.* 2010; Violle *et al.* 2012; Gagic *et al.* 2015; Laughlin 2018) reveals the need to better understand intraspecific functional traits when preserving biodiversity and determining conservation value.

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APPENDICES

Appendix A: Figures

Appendix A: Figures

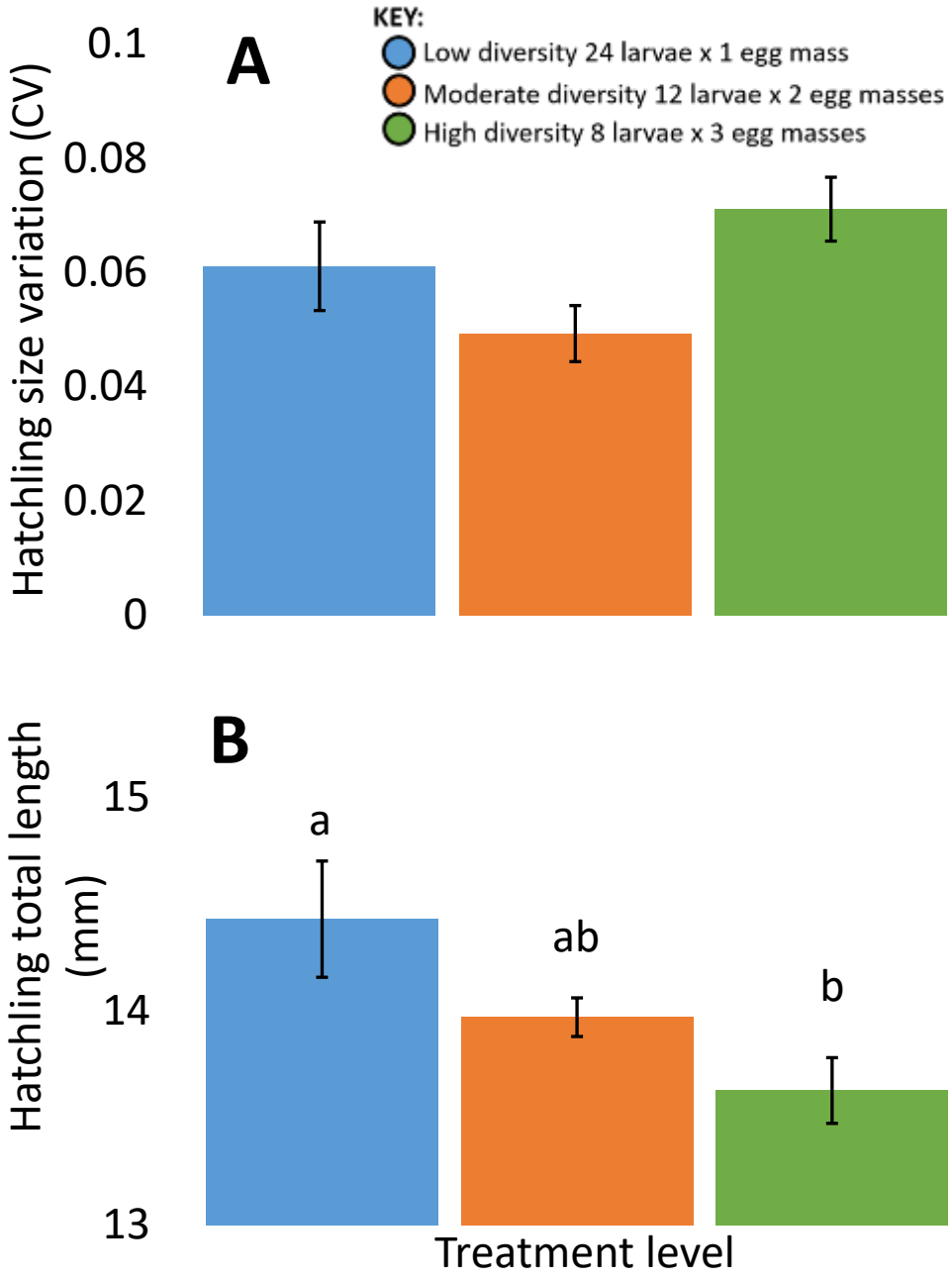

Figure 8. Mean (± 1 SE) for low, moderate, and high sibship diversity treatment levels where (A) is the coefficient of variation and (B) is the total length of hatchlings for each group of 24 immediately before they were initially added to the mesocosms. Letters above bar graphs indicate significant differences based on Tukey HSD following a one-way ANOVA ($\alpha = 0.05$). "CV" = coefficient of variation.

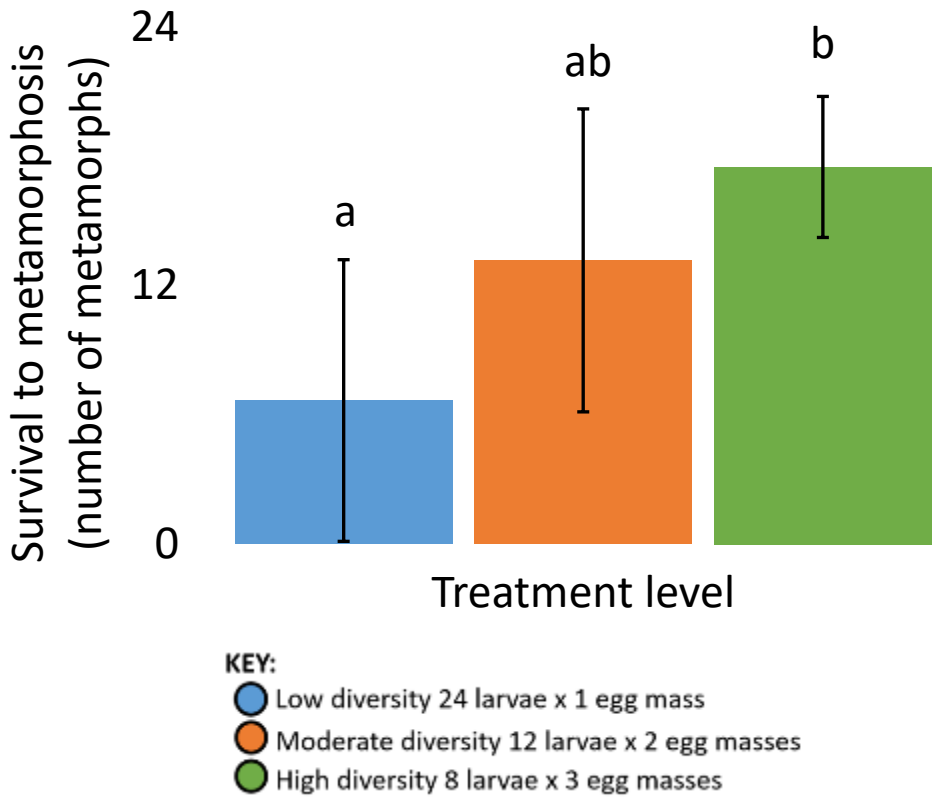

Figure 9. Mean (± 1 SD) survival to metamorphosis of *A. jeffersonianum* among low, moderate, and high sibship diversity treatment levels. Letters above bar graphs indicate significant differences based on Tukey HSD from a one-way ANOVA ($\alpha = 0.05$).

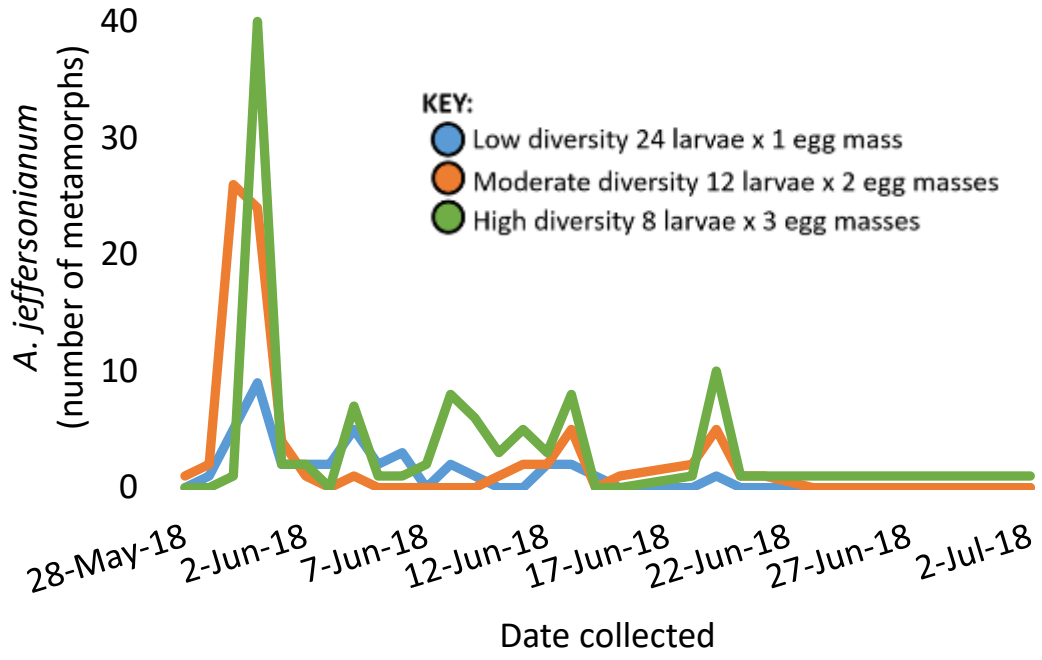

Figure 10. Daily sum of metamorphosed *A. jeffersonianum* between low, moderate, and high sibship diversity treatment levels starting when the first metamorph was collected on May 28th until the last was collected on July 2nd.

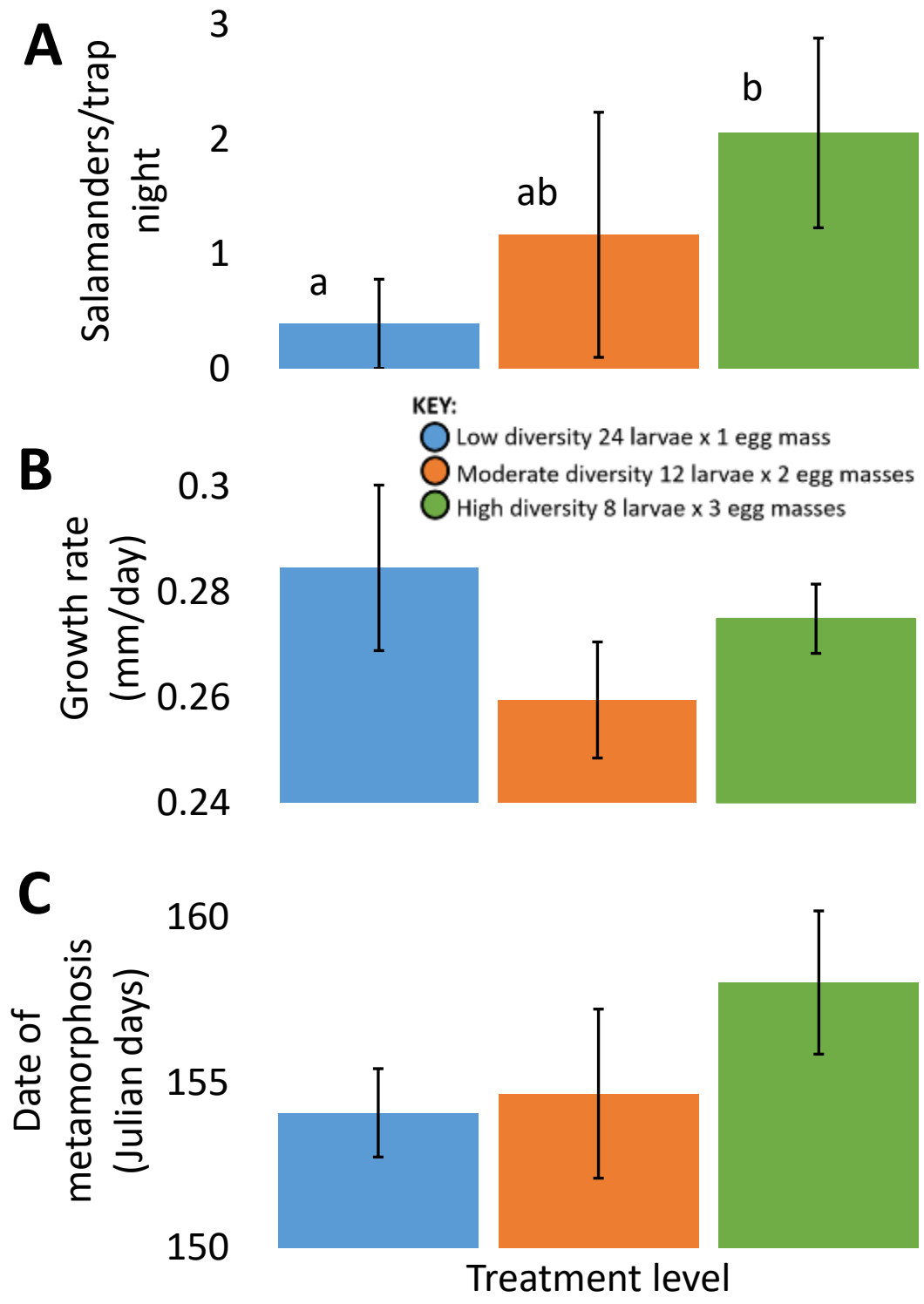

Figure 11. Mean of (A) the larval abundance of *A. jeffersonianum* (± 1 SD), (B) larval growth rate of *A. jeffersonianum* (± 1 SE), and (C) the date of metamorphosis of *A. jeffersonianum* (± 1 SE) among low, moderate, and high sibship diversity treatment levels. Letters above bar graphs indicate significant differences based on Tukey's HSD following a two-way ANOVA ($\alpha = 0.05$).

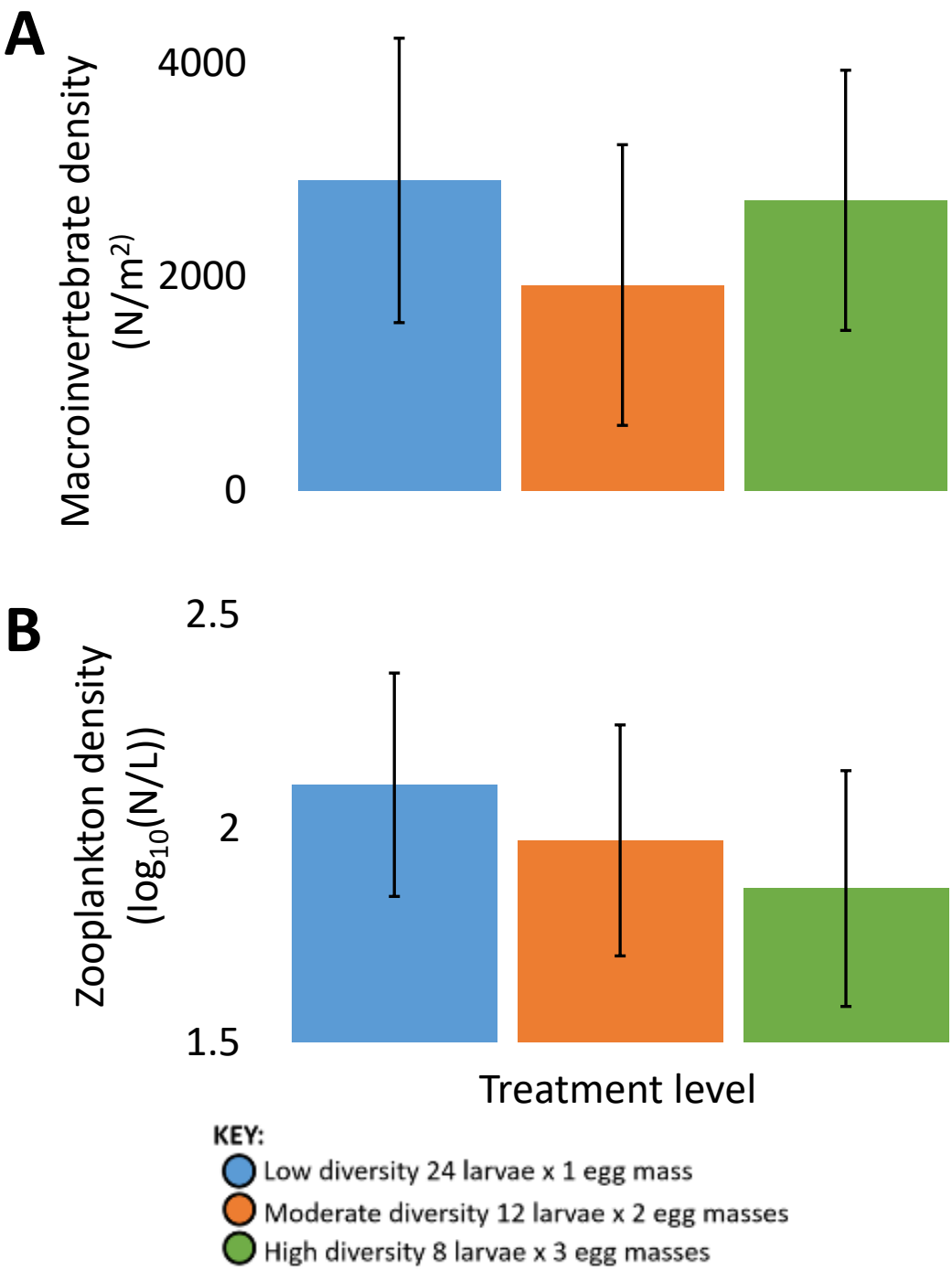

Figure 12. Mean (± 1 SD) of (A) macroinvertebrate and (B) zooplankton densities among low, moderate, and high sibship diversity treatment levels for the invertebrate subset of data. "N" = number of individuals.

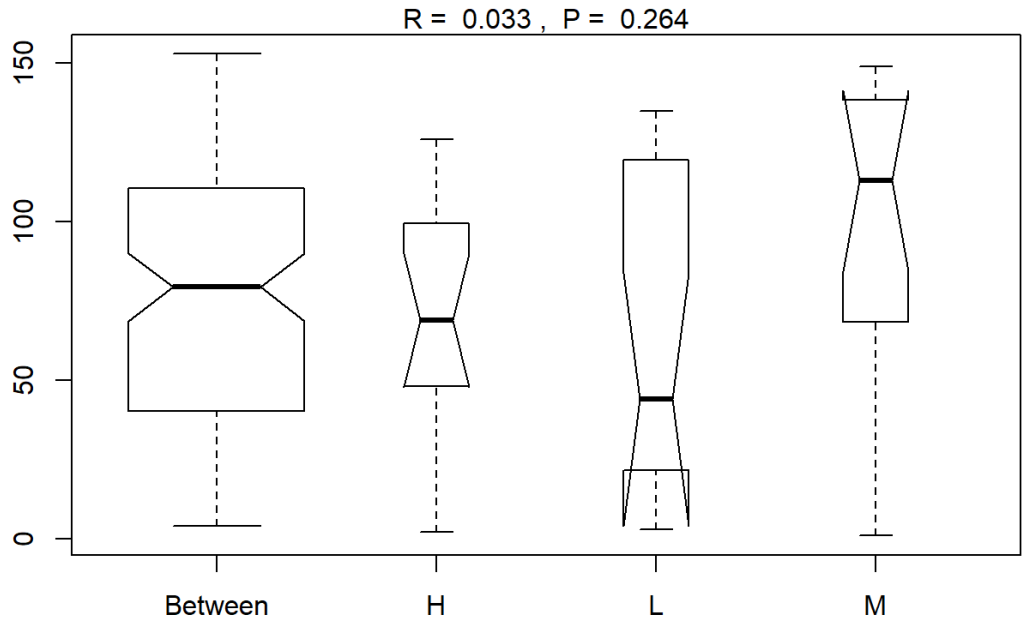

Figure 13. ANOSIM results comparing community dissimilarity in the macroinvertebrate taxonomic groups for the low, moderate, and high sibship diversity treatment levels. R = ANOSIM test statistic or the difference between mean ranks between groups and within groups; P = probability statistic (α = 0.05); L = low diversity 24 larvae x 1 egg mass, M = moderate diversity 12 larvae x 2 egg masses, H = high diversity 8 larvae x 3 egg masses.

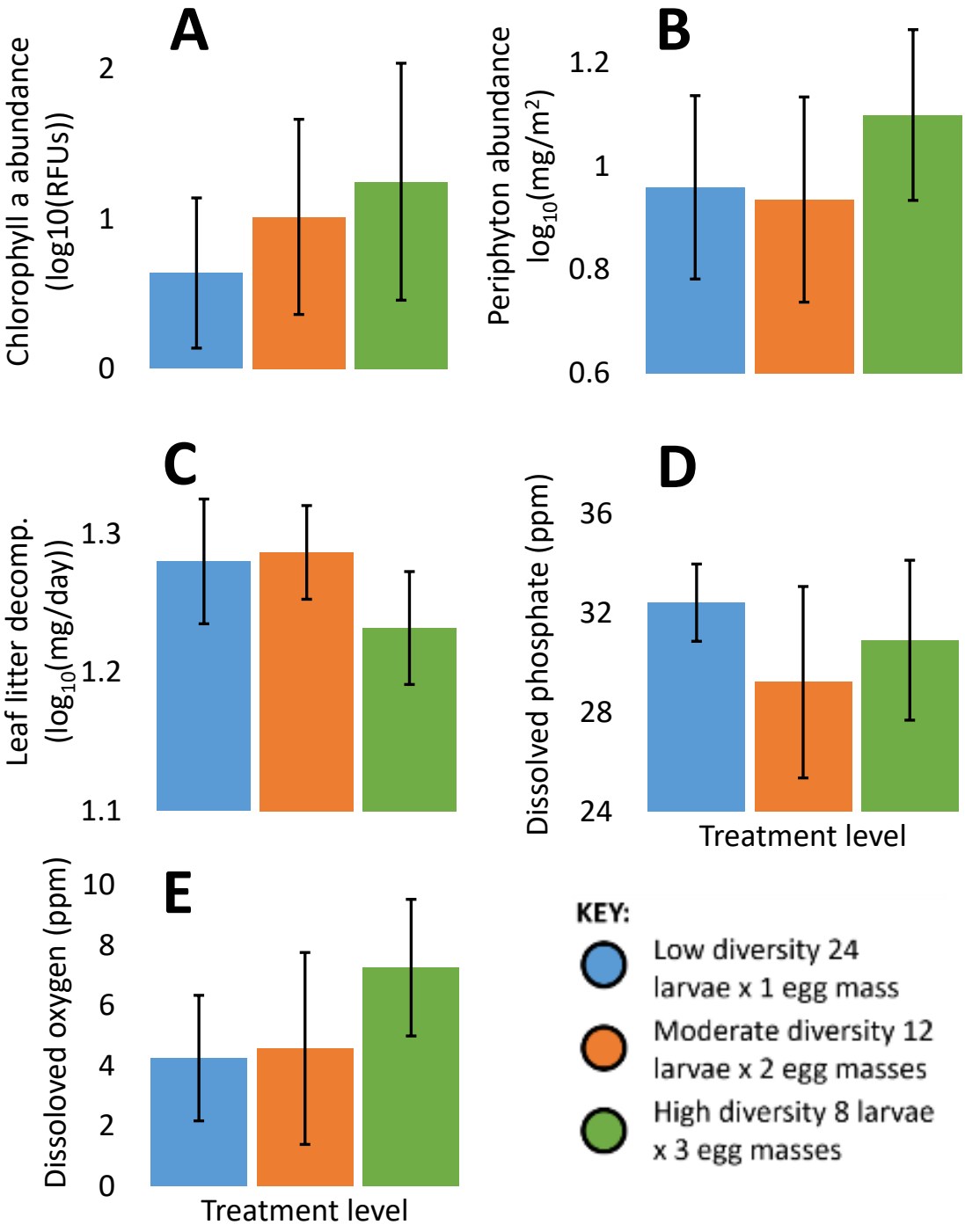

Figure 14. Mean (± 1 SD) of (A) chlorophyll *a*, (B) periphyton abundance, (C) rate of leaf litter decomposition, (D) dissolved phosphate, and (E) dissolved oxygen between low, moderate, and high sibship diversity treatment levels for the environmental subset of data. "RFUs" = relative fluorescent units.

Appendix B: Tables

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Table 1. Modified signed-likelihood ratio test (M-SLRT) for equality of size variation in *Ambystoma jeffersonianum*. (A) The coefficient of variation of hatchlings for each mesocosm between low (L), moderate (M), and high (H) sibship diversity treatment levels immediately before they were added to the mesocosms. (B) Comparison of size variation for *A. jeffersonianum* collected on May 30th, 2018 among mesocosms, (C) among mesocosms after removing the outlier from mesocosm #3 and #5, (D) among treatment levels, and (E) among treatment levels after removing the outlier from mesocosm #3.

Response	Sample size			M-SLRT test statistic	p
	L	M	H		
(A) Initial size variation	144	143	144	0.952	0.621
(B) Final size variation among mesocosms	14	55	56	2.511	0.001
(C) Final size variation among mesocosms after removing outliers	13	54	56	22.851	0.063
(D) Final size variation among treatment levels	15	53	57	6.41	0.041
(E) Final size variation among treatment levels after removing outlier	15	52	57	2.241	0.326

Table 2. ANOVA for hatchling size comparison of *Ambystoma jeffersonianum* between low, moderate, and high treatment levels immediately before being added to the mesocosms.

Response	df	*F*	p
Initial TL	2, 15	4.589	0.028

df = treatment, residuals; TL = total length

Table 3. ANOVA for the comparison of the survival to metamorphosis of larval *A. jeffersonianum* between treatment levels.

Response	df	*F*	p
Survival to metamorphosis	2, 15	5.209	0.019

df = treatment, residuals

Table 4. MANOVA of the salamander subset of data comparing abundance of A. *jeffersonianum,* growth rate of A. *jeffersonianum,* and date of metamorphosis of A. *jeffersonianum* among low, moderate, and high sibship diversity treatment levels on May 30th, 2018.

	df	*F*	p
MANOVA			
Subset			
Salamander	2, 12	2.881	0.032
ANOVAs			
Response			
Abundance	2, 15	6.318	0.010
Growth rate	2, 12	1.314	0.305
Date of metamorphosis	2, 12	0.981	0.403

df = treatment, residuals

Table 5. MANOVA of the invertebrate subset of data to compare macroinvertebrate and zooplankton densities among low, moderate, and high sibship diversity treatment levels on May 30th, 2018.

	df	*F*	p
MANOVA			
Subset			
Invertebrate	2, 15	1.205	0.329

df = treatment, residuals

Table 6. MANOVA of the environmental subset of data to compare chlorophyll *a* and periphyton abundances, rate of leaf litter decomposition, phosphate, and dissolved oxygen among low, moderate, and high sibship diversity treatment levels on May 30th, 2018.

	df	*F*	p
MANOVA			
Subset			
Environmental	2, 15	2.056	0.072

df = treatment, residuals

Table 7. Correlations among variables collected on May 30th, 2018 where response variables are (A) *A. jeffersonianum* abundance, (B) growth rate of *A. jeffersonianum*, (C) date of metamorphosis of *A. jeffersonianum*, (D) rate of leaf litter decomposition, (E) macroinvertebrate density, (F) zooplankton density, (G) periphyton abundance, (H) dissolved oxygen, (I) chlorophyll *a* abundance, and (J) phosphate.

Response variables	(A) Abundance	(B) GR	(C) Date of metamorph.	(D) Leaf decomp.	(E) Macroinvertebrates	(F) Zooplankton	(G) Periphyton	(H) DO	(I) Chlorophyll *a*	(J) P
(A) Abundance	1.00									
(B) GR	-0.38	1.00								
(C) Date of metamorph.	0.66*	-0.66**	1.00							
(D) Leaf decomp.	-0.12	0.00	-0.31	1.00						
(E) Macroinvertebrates	0.10	0.27	0.02	-0.07	1.00					
(F) Zooplankton	-0.06	0.36	-0.25	0.54*	-0.19	1.00				
(G) Periphyton	-0.02	0.18	-0.01	-0.55*	-0.34	-0.14	1.00			
(H) DO	0.54*	-0.51*	0.75**	-0.45	0.12	-0.49*	0.29	1.00		
(I) Chlorophyll *a*	0.68**	-0.56*	0.79**	-0.20	0.12	0.02	-0.24	0.52*	1.00	
(J) P	-0.46	0.55	-0.49	0.12	-0.18	0.42	0.11	-0.65**	-0.40	1.00

* $P < 0.05$, ** $P < 0.01$; "GR" = growth rate