2023

Using Mesocosms to Model Optimal Levels of Body Size Variation in Larval Spotted Salamanders (Ambystoma maculatum)

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Recommended Citation

Kratzer, Ian, "Using Mesocosms to Model Optimal Levels of Body Size Variation in Larval Spotted Salamanders (Ambystoma maculatum)" (2023). *Online Theses and Dissertations*. 797.
https://encompass.eku.edu/etd/797

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USING MESOCOSMS TO MODEL OPTIMAL LEVELS OF BODY SIZE VARIATION IN LARVAL SPOTTED SALAMANDERS (AMBYSTOMA MACULATUM)

BY

IAN KRATZER

Submitted to the Faculty of the Graduate School of
Eastern Kentucky University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

2023

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Cy Mott, for his support throughout all phases of this research project. I would also like to thank Drs. Jennifer Koslow and Stephen Richter for serving on my thesis committee. My fellow graduate students at EKU were all extremely helpful in all phases of this project, both theoretical and practical. I would especially like to thank Alex Funk, Sandra Elliot, and Brady Parlato for their assistance. My graduate education and research would not have been possible without financial support of the Graduate School, College of Science, and Department of Biological Sciences at Eastern Kentucky University (EKU). Access to the field station at the Taylor Fork Ecological Area was provided by the EKU Division of Natural Areas and was crucial for performing my thesis research. I greatly appreciate the Institutional Animal Care and Use Committee (IACUC) at EKU for approving experimental protocols, and the Kentucky Department of Fish and Wildlife Resources for providing the Educational Wildlife Collecting Permit (#SC2211149). This research was made possible by a Student Research Grant from the EKU Division of Sponsored Programs.

ABSTRACT

Body size is a critical aspect of an organism’s biological identity mediated by various biotic and abiotic factors. Body size historically been approached through “single optima” approaches, but body size variation modifies both inter- and intraspecific interactions and impacts competition-driven fitness outcomes within populations. Understanding optimal levels of body size variation will help illuminate how variation influences competitive outcomes and reproductive fitness. Populations may be structured through resource partitioning or competitive hierarchies, yet these structures predict contradictory size variation optima. This study sought to determine optimal levels of intraspecific body size variation in populations of larval salamanders to evaluate these mechanisms. Larval *Ambystoma maculatum* were raised in mesocosm populations along a continuum of body size variation at both high and low densities. Rates of larval growth, survival, size at metamorphosis, and length of the larval period were used to characterize optimum levels of body size variation. High density populations exhibited 38% lower survival than low density mesocosms, and metamorphs from high density populations were 11% smaller. Population size structures experienced shifts throughout the larval period, generally becoming less variable, though populations with higher initial levels of size variation exhibited shorter larval periods. Increased fitness through shorter larval periods in highly variable populations demonstrates fitness optima occur at high levels of intraspecific variation in body size, which is indicative of resource partitioning. However, the fitness benefits of this partitioning appear to be concentrated among larger individuals.

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I. Introduction

Body size mediates most of an animal's ecological interactions; thus, this trait is paramount when evaluating such emergent properties as metabolic rates (Peters 1989), population density and distribution (Damuth 1981), ecosystem function (Woodward et al. 2005), and evolutionary dynamics (LaBarbera 1989, Luhring and DeLong 2020). Body size is in turn mediated by resource availability (Pyke 1978, Kindlmann and Dixon 1992), conspecific density/competition (Marquet et al. 1990), and predation (Burley et al. 2006, Barriga et al. 2012), among other factors. Theoretical modeling within these contexts has produced general predictions of optimal body size for organisms ranging from zooplankton to large mammals (Ziółko and Kozłowski 1983, Brown et al. 1993), as well as taxon-specific predictions including, but not limited to, mammals (Ziółko and Kozłowski 1983, Demment and Van Soest 1985), birds (Blackburn and Gaston 1994), reptiles (Naganuma and Roughgarden 1990, Boback and Guyer 2003), amphibians (Werner 1986, Hom 1988), fish (Chaparro-Pedraza and Roos 2020), and insects (Pyke 1978, Kindlmann and Dixon 1992). Theoretical models of body size evolution indicate that optimal body sizes maximize rates of development (Werner 1986, Chaparro-Pedraza and Roos 2020), foraging (Pyke 1978, Demment and Van Soest 1985), predator avoidance (Ludwig and Rowe 1990), and ultimately, reproductive success (Hom 1988, Brown et al. 1993), adding to our understanding of the adaptive significance of ontogenetic and evolutionary shifts in body size. However, despite demonstrating the ecological and evolutionary implications of body size, trait-based theoretical approaches primarily tend to predict single body size optima within species (Werner 1986, Brown et al. 1993) while discounting the substantial implications of intraspecific body size variation for population, community, and ecosystem processes (Bolnick et al. 2011, Violle et al. 2012, Des Roches et al. 2018).

Recent studies on the evolutionary and ecological importance of intraspecific trait variation have documented its effects on individual specialization (Woodward et al. 2005), intensity of interspecific interactions (Miller and Rudolf 2011, Violle et al. 2012, Carlson and Langkilde 2017) and resulting community structure (Post et al. 2008), ecosystem trait diversity (Cianciaruso et al. 2009, Albert et al. 2012), ecosystem function (Raffard et al. 2018, Des Roches et al. 2018), and speciation rates (Bolnick et al. 2011). Intraspecific variation in body size is positively correlated with niche breadth, leading to competitive release among size classes in size-structured populations (Roughgarden 1972, Bolnick et al. 2007). Intraspecific competitive release through niche partitioning is widespread among animals, having been observed in insects (Polis 1984), amphibians (Székely et al. 2020), reptiles (Schoener and Gorman 1968, Bolnick et al. 2007), fish (Bolnick et al. 2007), birds (Traba et al. 2015), and mammals (Jones et al. 2020). Although niche partitioning due to intraspecific body size variation can reduce competition, increased size variation can also increase rates of cannibalism (Cushing 1992, Maret and Collins 1994, Woodward and Hildrew 2002) and non-lethal injury (Wissinger et al. 2010, Mott and Steffen 2014), while altering refuge use and decreasing activity in smaller conspecifics (Buskirk 1992, Rudolf 2006). Ecological effects of intraspecific trait variation in populations can then be compounded in communities composed of multiple species of variable size to produce novel patterns of niche overlap and functional diversity (Violle et al. 2012).

Conspecifics have historically been modeled as exhibiting identical ecological functions within communities, but incorporating intraspecific trait variation increases the complexity of community interactions, stabilizing communities and leading to novel ecosystem-level effects (Cianciaruso et al. 2009, Violle et al. 2012, Des Roches et al. 2018). In populations or communities exhibiting increased trait variation, interactions may occur between discrete phenotypic classes within species rather than solely between and/or among species, leading to increased quantities of relatively lower intensity ecological interactions. In size-structured predator populations, cannibalism and variation in individual trophic position among size classes interact to decrease consumptive pressure on basal resources as foraging interactions increase in breadth and decrease in intensity through size variation (Woodward and Hildrew 2002, Woodward et al. 2005, Miller and Rudolf 2011). In prey populations, relative predation risk is mediated by predator:prey body size ratios, and therefore body size variation decreases proportions of individuals available as prey to gape-limited predators (Miller and Rudolf 2011, Carlson and Langkilde 2017). As intraspecific interactions become more numerous, yet less intense, communities are stabilized due to general weakening of individual trophic interactions, with resulting effects on ecosystem processes including rates of respiration, primary production, and decomposition (Hughes et al. 2008, Rudolf and Rasmussen 2013a, 2013b). Recent studies of intraspecific body size variation have dramatically increased our understanding of its wide-ranging population, community, and ecosystem effects; however, optimality approaches to characterize the fitness consequences of the emergent trait of intraspecific body size variation (as opposed to single mean body size optima) are necessary to represent the ecological complexity of size-structured populations.

Experimental investigations of intraspecific size variation that identify peaks of maximized fitness (size variation optima – SVO) will offer insight into competitive interactions in populations, which are hypothesized to exhibit one of two opposing structures (fig. 1). The competitive hierarchy hypothesis (CHH) predicts resource requirements change little with increasing individual size, but with larger individuals consistently functioning as superior competitors (Wilson 1975, Persson 1985, Smith 1990). This hypothesis therefore predicts one body size optima (i.e., "largest") which offers competitive advantages, with sub-optimal body sizes occupying lower positions on competitive gradients. If the SVO is "low variation" because large individuals consistently outcompete smaller ones and monopolize fitness, then intraspecific competitive relationships are hierarchically structured. Alternatively, the resource partitioning hypothesis (RPH) predicts resource use changes dramatically with body size, allowing competitive release between size classes due to niche partitioning (Roughgarden 1972, Werner and Gilliam 1984, Polis 1984). Under these conditions, the SVO is "high variation" because smaller individuals avoid larger conspecifics and utilize different resources, which is indicative of populations structured through resource partitioning. The specific mechanism of competitive organization for populations will be illuminated by population SVO, and evaluating competing hypothesized mechanisms adds additional incentive to explore the field of optimal size variation as an emergent property of biological populations.

Larval amphibians present a tractable system with which to examine intraspecific size variation optima, as larval size variation affects competition and cannibalism (Urban 2007, Wissinger et al. 2010), which mediate fitness via effects on time to and size at metamorphosis (Wilbur 1972, Wildy et al. 1998) that produce carryover effects on survival and reproduction in metamorphs, juveniles, and adults (Semlitsch et al. 1988, Earl and Whiteman 2015). Previous studies have identified single amphibian body size optima for larval and adult life stages (Werner 1986, Olalla-Tárraga and Rodríguez 2007, Cvetković et al. 2009) and documented effects of intraspecific size variation on ecological interactions and associated larval development (Smith 1990, Maret and Collins 1994, Brunkow and Collins 1996). However, no studies to our knowledge have investigated optimality in intraspecific body size variation and its relationships to resource use.

The spotted salamander (*Ambystoma maculatum*) is an ideal species for examining intraspecific size variation optimality and its association with competitive structure, as populations of larval *A. maculatum* exhibit considerable size variation and are under intense competitive pressure from conspecifics (Voss 1993, Petranka 1998). *Ambystoma maculatum* breed mostly in ephemeral, fish-free ponds in mature deciduous forests (Petranka 1998). Breeding occurs from late January to mid-February, often during several mass breeding events (Welter and Carr 1939, Green 1956, Keen 1975), after which females deposit two to four egg masses of up to 250 eggs each (Bishop 1941, Shoop 1974). This pattern of explosive breeding leads to larval populations with densities as high as 258 larvae/m^2, though densities vary in response to predation and habitat quality (Cortwright 1988, Wilbur 1997, Anderson et al. 2017). Initial variation in hatchling larval size arises due to intraspecific variation in egg size, phenology of oviposition, location of egg masses within ponds, and developmental temperatures (Dushane and Huthcinson 1944, Worthington 1968, Voss 1993, Petranka 1998). Larvae are gape limited predators and prey diversity is positively correlated with larval growth. All larvae consume zooplankton, while relatively larger individuals incorporate wider varieties of macroinvertebrates, and even other larval amphibians, allowing for niche partitioning through increasing size variation (Branch and Altig 1981, Freda 1983, Nyman 1991, Petranka 1998). Though cannibalism is rare among larval *Ambystoma maculatum*, interference competition is common; larger individuals regularly lunge at and bite conspecifics, providing the former with potential competitive advantages (Walls and Semlitsch 1991, Mott and Sparling 2016). Intense competition can reduce larval survival rates to less than 10% (Petranka 1998), with larval size and growth rates negatively correlated with density (Smith 1990, Brunkow and Collins 1996, Wilbur 1997, Anderson et al. 2017). Under this scenario, reduction in competition may occur through intraspecific variation in larval size and related prey selection.

Based on the presence of both competitive gradients and niche partitioning associated with intraspecific body size variation in *Ambystoma maculatum* larval populations, competitive structures could follow either the CHH or the RPH, and determining SVO would clarify which hypothesis best explains larval competition. To identify SVO, I created populations of larval spotted salamanders (*Ambystoma maculatum*) along a gradient of intraspecific size variation using experimental mesocosms and monitored survival to, time to, and size at metamorphosis as indicators of fitness. Since competitive intensity affects size variation in aquatic larval amphibian populations (Peacor and Pfister 2006, Huss et al. 2007), I will examine the effects of size variation on these response variables at relatively high and low larval densities. These data will be used to identify SVO (the level of variation which optimizes population fitness) and characterize the shape and direction of the size variation vs fitness relationship for larval *Ambystoma maculatum*. The SVO and direction of the size variation vs fitness relationship will serve to illuminate which competitive structure (CHH or RPH) serves to structure populations of larval *Ambystoma maculatum*.

II. Materials and Methods

Mesocosm Design

This study used experimental mesocosms to assess fitness optimality across a gradient of intraspecific body size variation and at two levels of larval density in *A. maculatum*. Mesocosms were constructed at Eastern Kentucky University's (EKU) research station at Taylor Fork Ecological Area (TFEA). The mesocosm array consisted of 86 20-gallon stock tanks (MacCourt Plantainer, Denver Colorado) covered with 1-mm window screen to prevent colonization by non-target organisms. Mesocosms were filled with rainwater and contained oak (*Quercus spp*.) and American sycamore (*Platanus occidentalis*) leaf litter at various stages of decomposition from several previous field seasons. In preparation for the study, an additional 0.2 kg *Quercus spp.* and *Platanus occidentalis* litter were added to all mesocosms, and water was homogenized across mesocosms prior to leaf addition. Zooplankton and macroinvertebrates characteristic of natural ponds containing *Ambystoma maculatum* already inhabited mesocosms, so mesocosms provided microhabitat and food resources typical of breeding ponds used by *Ambystoma maculatum* (Wilbur 1997, Anderson and Whiteman 2015).

Gradients in intraspecific size variation were created across mesocosms by using combinations of lab-reared larvae and larvae collected from natural ponds. Eggs for lab-rearing were collected in mid-March from ponds in the Miller-Welch Central Wildlife Management Area (CWMA) near Richmond, Kentucky and hatched in two environmental chambers maintained at 12°C or 7°C on a 12L:12D photoperiod (Ling et al. 1986) in the EKU Vivarium facilities. Temperature differences between environmental chambers and variation in time of hatching among egg masses created variation in larval size, as larval growth rates are correlated with temperature (Voss 1993). In late April, unhatched egg masses were moved from environmental chambers to 10- or 20-gallon mesocosms at TFEA to hatch. Mesocosm size differences led to variation in water temperature and resource availability, which produced additional larval size variation. To supplement lab-reared populations with additional individuals, larval *Ambystoma maculatum* were collected from CWMA in late March and from the Daniel Boone National Forest near Morehead, Kentucky in late April. At these times, larval *Ambystoma maculatum* have hatched but not attained advanced development towards metamorphosis (Petranka 1998).

After hatching of eggs and collection of larvae from field sites, mesocosms were populated between May 16 - 25 with larval salamanders at one of two larval densities for four gradient levels of intraspecific body size variation. Before introduction into mesocosms, larvae were measured and assigned to small, medium, and large relative size classes. Mesocosms populations were then systematically established from these size classes to yield coefficient of body size coefficient of variation (BCV) values in low (0.09 – 0.18), medium low (0.18-0.28), medium high (0.25-0.33), and high (0.33-0.53) ranges. This systematic assignment of individuals created mesocosm populations with varying levels of body size variation while maintaining equivalent mean body size across mesocosms. Prior to introduction, all mesocosm populations were photographed in a white collecting tray with a 15-cm ruler for scale. These photographs were analyzed in ImageJ to measure the precise total length of all larvae. BCV and mean total length were then calculated for each mesocosm population.

The mesocosm array consisted of ten blocks of eight mesocosms, with each block containing low, medium low, medium high, and high BCV mesocosms at two levels of density (fig 2). Mesocosms in “low” and “high” density treatment levels received 8 and 16 larvae (105 and 210 larvae/m^3, respectively); these densities are within the range of natural densities for ambystomatid larvae observed in field studies (Cortwright 1988, Mott and Sparling 2016). One additional block was created to make the total distribution of BCV levels more uniform. This block did not conform to the other block layouts and contained only three high density and three low density mesocosms.

Sampling

Mesocosms were sampled twice (between June 13 - 23 and between July 5 - 13) to measure larval density and growth rates while also tracking progress towards metamorphosis. At each sampling event, all mesocosms were dip netted five times. The first sweep covered the center of the water column without disturbing the substrate, with four subsequent sweeps passing around the mesocosm edge while agitating the substrate. Larvae were placed in a white collecting tray with a ruler and photographed for analysis in ImageJ to measure density and total length of larvae. Image analyses were used to quantify growth rates along the continuum of BCV and between densities, as well as to identify potential departures from the experimentally established initial BCV levels during larval ontogeny. During sampling, individuals were monitored for signs of metamorphosis (reabsorption of gills and/or tailfin). Once larvae approached metamorphosis in late May, sections of polyethylene foam were added to mesocosms as floating platforms onto which metamorphs could emerge. Mesocosms were thereafter sampled daily for emerging metamorphs. Any metamorphs observed either on the platform or floating at the water surface were removed from tanks, anesthetized, and euthanized using benzocaine, and preserved in 10% buffered formalin. Preserved larvae were photographed for subsequent measurement. Dates of metamorphosis were recorded to determine the total length of the larval period. Beginning on July 22, when emergence had slowed, mesocosms were sampled for emerging metamorphs every other day. Sampling for metamorphs continued daily until no new metamorphs were observed for five consecutive days.

Fitness Metrics

Larval counts from larval sampling periods and length measurements of both larvae and metamorphs were used to calculate five fitness metrics for each mesocosm population: adjusted change in larval density, larval growth, average time to metamorphosis, average size at metamorphosis, and percent survival to metamorphosis. Adjusted density was calculated as the change in larval density between the first and second sampling periods divided by the initial mesocosm population size (8 or 16 individuals). Growth rate was calculated by dividing average length (determined through ImageJ measurements) of larvae at the first larval sampling period minus the average length of larvae at mesocosm population establishment by the number of days between establishment and larval sampling (29 days). Time to metamorphosis was calculated as the average number of days between mesocosm population establishment and emergence dates for individual metamorphs. Size at metamorphosis was calculated by averaging metamorph lengths from ImageJ analysis. Percent survival to metamorphosis was calculated by dividing the number of metamorphs by the total number of larvae in the initial mesocosm population (8 or 16).

Data Analysis

Changes in intraspecific body size variation and the effects of intraspecific body size variation and larval density in *A. maculatum* on indicators of population fitness were assessed using analysis of variance (ANOVA) and multivariate analysis of covariance (MANCOVA) in R (Version 3.6.3). One-way ANOVA was used to indicate potentially significant changes in BCV between mesocosm establishment and at the first sampling period. A subsequent factorial ANOVA was used to characterize the effects of initial BCV, density, and/or their interaction, on final change in BCV. MANCOVA was used to characterize the effects of intraspecific body size variation, larval density, or their interaction, on our combined fitness parameters (adjusted change in density, growth rate, average size at metamorphosis, average time to metamorphosis, and percent survival to metamorphosis). In MANCOVA analysis, density level was the categorical variable, and BCV was the continuous covariate. Size variation is a continuous variable because, though mesocosms were populated with four variation "levels", there was considerable variability BCV present at each level, creating a continuum of body size variation among all mesocosms.

There was a need to remove mesocosms from analyses that either produced no metamorphs or had no larvae present at the first sampling period. These null value mesocosms unnaturally skewed data by yielding negative size change values and null time to metamorphosis and size at metamorphosis values. Because of this, separate MANCOVAs were run for response variables from the two life stages: larval (change in density, growth rate) and metamorph (percent survival, average size at metamorphosis, average time to metamorphosis). This division maximized the sample size of the

MANCOVA for larval metrics, as there were many mesocosms which produced no metamorphs but did have larvae present at the first sampling period.

III. Results

Of the 1,032 larval *A. maculatum* added to experimental mesocosms, 116 survived to metamorphosis, resulting in a larval survival rate of 11.2%. However, subsequent MANCOVA reflects survivorship percentages excluding mesocosms that produced no metamorphs so as not to confound other fitness metrics by producing inaccurate zero values for size at metamorphosis and time to metamorphosis. In mesocosms with larvae present at the first larval sampling period, population BCV decreased by 36% from establishment ($\bar{x} = 0.266$) to the first larval sampling period ($\bar{x} = 0.17$), a period of 28 days (ANOVA $F_{1,148} = 36.47$, $P < 0.001$ (Figure 3). The coefficient of variation in population BCV (i.e., the variation of BCV values among mesocosms) among all experimental mesocosms also decreased by 37% from establishment (0.27) to first larval sampling (0.17), with BCV values becoming more homogenous among mesocosms between the establishment and the first larval sampling period (Figure 4). The change in population BCV was not consistent across all mesocosms, as those with higher initial BCV values experienced larger rates of BCV decrease (ANOVA $F_{1,76} = 61.34$, $P < 0.001$) (Figure 5). Additionally, BCV change was negatively correlated with survival at both high ($r = -0.25$) and low ($r = -0.09$) densities (Figure 6).

MANCOVA of the effects of density and initial BCV of mesocosm populations on the larval fitness metrics of size change and percent density reduction showed a significant multivariate effect of density, but not initial BCV or the interaction between density and BCV ($F_{2,76} = 3.17$, $P = 0.047$) (Table 1). Subsequent univariate analyses revealed the multivariate effect was driven by density effects on growth over the first 28 days of the larval period. Average growth rates were significantly lower ($\bar{x} = 0.653$

mm/day) in high density mesocosms than in low density mesocosms (x̄ = 0.772 mm/day) (Figure 7).

MANCOVA of the effects of density and initial BCV of mesocosm populations on the metamorph fitness metrics of percent survival, size at metamorphosis, and length of the larval period revealed that both density and initial BCV had significant multivariate effects on these metrics (Density: $F_{3,54} = 5.07$, $P = 0.004$; BCV: $F_{3,54} = 5.11$, $P = 0.003$) (Table 2). Subsequent univariate analyses indicated significant effects of density on both percent survival and size at metamorphosis, with high density mesocosms having lower average survivorship (x̄ = 15.4%) than low density mesocosms (x̄ = 24.6%), and smaller metamorphs (x̄ = 4.75 cm) than low density mesocosms (x̄ = 5.34 cm) (Figures 8 - 9). The significant multivariate effect of initial BCV is explained by a significant univariate effect of initial BCV on time to metamorphosis. Initial BCV (a continuous variable) was negatively associated with length of the larval period, resulting in right (i.e., early) skewed emergence density distributions for higher BCV mesocosms, while mesocosms from lower BCV groups were more normally distributed in their emergence density distributions (Figures 10 - 12).

IV. Discussion

Results Summary

Initial body size coefficient of variation (BCV) for larval *Ambystoma maculatum* populations had no significant effect on changes to larval density, larval size change, size at metamorphosis, or percent survival to metamorphosis. However, there was a significant effect of initial BCV on time to metamorphosis, with more variable populations requiring less time to reach metamorphosis. Conversely, initial larval density significantly affected larval size change, size at metamorphosis, and percent survival to metamorphosis but did not impact change in larval density or time to metamorphosis. Larvae in high density populations experienced smaller size increases, were smaller at metamorphosis, and exhibited reduced survival to metamorphosis. BCV did not remain constant throughout the larval period, as populations generally experienced size variation channelization wherein body size variation was reduced more dramatically for populations with higher initial BCV.

Larval BCV

Identifying size variation optima (SVO) is necessary to determine which hypothesis (resource portioning or competitive hierarchy) best characterizes competitive outcomes in larval *Ambystoma maculatum* populations. However, most fitness metrics, such as survival to metamorphosis, growth rate, and size at metamorphosis were not affected by initial larval coefficients of variation of body size (BCV). The absence of discrete BCV values that maximize fitness makes it challenging to identify SVO and evaluate the efficacy of either the RPH or CHH, but temporal patterns in BCV indicate size variation channelization (a reduction in variation in population size structure) may explain the limited effects of initial BCV on fitness metrics. If initial differences in larval size variation created during establishment of experimental mesocosms decreased quickly during the beginning of the larval period, impacts of increased size variation on fitness outcomes hypothesized by either the RPH or CPH would be prevented from occurring. By homogenizing BCV across mesocosms, such variation channelization may have unified niche breadth across all larval populations, resulting in equivalent competitive and aggressive hierarchies. Recent studies of larval salamanders in natural ponds and experimental mesocosms show ontogenetic decreases in BCV (Mott unpublished data, Smith 2019, Anderson 2023). Beyond general ontogenetic decreases in BCV, the extents of ontogenetic decreases in BCV positively scaled with initial BCV, supporting predictions that extreme initial BCVs may be quickly channelized, and reduce their relevance for agonistic interactions and subsequent fitness outcomes.

Channelization of BCV in experimental mesocosms, if present, may have been driven by mortality-dependent and/or mortality-independent ecological processes. If driven by mortality-dependent processes, channelization would be mediated by mortality of small individuals due to predation or competition from larger individuals in high variation mesocosms (Wildy et al. 1998, Anderson and Whiteman 2015), leading to increases in average larval size but corresponding reductions in BCV. If driven by non-mortality processes, channelization would be mediated by relatively earlier metamorphosis of larger individuals (Wilbur and Collins 1973), leading to similar decreases in BCV but relative decreases in average larval size if sampling occurred after metamorphosis of larger individuals. Analysis of emergence density distributions across size structure classes offers some insight into how these mechanisms functioned in mesocosm populations. Mesocosm populations with the most variable size structures

(BCV 0.33-0.53) exhibit right-skewed (i.e., positively skewed) emergence density distributions, indicating most metamorphs emerged early in the study period and were presumably from the "large" initial size class. That this distribution is right-skewed, and not bimodal, indicates individuals from the "small" initial size class were unlikely to emerge, and therefore channelization of BCV during ontogeny represents a mortality-driven process associated with the loss of small individuals. Larval amphibians are highly aggressive interference competitors and may lunge at, bite, or otherwise harm and disturb conspecifics (Petranka 1998, Mott and Sparling 2009, Petrović et al. 2020, Fouilloux et al. 2022). These interactions collectively drive increased mortality among smaller individuals while simultaneously allowing larger individuals to grow more rapidly, survive at a higher rate, and achieve metamorphosis earlier as superior competitors (Wilbur and Collins 1973, Doyle et al. 2010). Based on my collective evidence, both mechanisms of variation channelization seem to be occurring simultaneously in experimental mesocosms, with smaller individuals suffering relatively increased mortality and larger individuals exhibiting relatively increased survival rates and shorter larval periods.

Previous studies of larval ambystomatid populations initially appear at odds with the phenomena of variation channelization, indicating that large ambystomatid larvae tend to suppress growth rates of smaller individuals through interference competition, leading to increasingly right-skewed size distributions with increasingly larger coefficients of variation for body size during ontogeny (Wilbur and Collins 1973, Wilbur 1997, Ziemba and Collins 1999). However, such studies typically characterized size distributions soon after hatching, when populations exhibited limited size structure, and used repeated measurements of size structure that occurred relatively early in the larval period. (Wilbur and Collins 1973, Wilbur 1997, Ziemba and Collins 1999). These studies may therefore have missed changes in size structure occurring later in ontogeny. Informed by previous studies, interpretation of data from my mesocosm populations suggests larval salamander populations experience initial periods of competition-induced increases in BCV followed by periods of variation channelization via metamorphosis of large individuals or mortality of small individuals (Wilbur 1997, Anderson et al. 2023). Similar ontogenetic channelization of BCV was also recently observed in mesocosm populations of *Ambystoma opacum* (Anderson et al. 2023). To truly characterize patterns of intraspecific body size variation and the potential fitness implications of these shifts, it would be necessary to measure natural body size variation at hatching and then track ontogenetic changes in body size variation and fitness metrics for populations of ambystomatid salamander larvae. However, this approach could not be used for my study, as manipulation of population size structure required establishing populations several weeks after some larvae have hatched. Examining ontogenetic size structure shifts and their effects *in situ* may reveal that larval amphibian populations undergo variation channelization more rapidly than initial variation can affect fitness.

Although most fitness metrics for larval *Ambystoma maculatum* were not impacted by larval size variation because of variation channelization, differences in the length of the larval period across variable size structures may illuminate competitive and fitness consequences of high degrees of intraspecific variation. Timing of metamorph emergence is an important fitness metric. Delays in metamorphosis can expose larvae to stressors unique to aquatic environments, including elevated competition, predation, and the threat of desiccation following pond drying (Werner 1986, Petranka 1998). Date of metamorphosis has also been linked to fitness parameters for terrestrial juveniles and adults, including size and age at first reproduction (Semlitsch et al. 1988). Earlier reproduction at larger sizes serves to maximize the number of reproductive events and clutch size, thus increasing fitness (Voss 1993). Despite mesocosm populations with higher initial BCV exhibiting shorter larval periods, I observed equivalent larval growth rates and sizes at metamorphosis among all mesocosms regardless of initial size structure, which aligns with previous observations that larval growth rates appear unaffected by differences in population size structure (Smith 1990, Brunkow and Collins 1996).

While growth rates were unaffected by population size structure, ontogenetic characteristics of larval amphibians highlight the distinction between “growth” and “development”, as previous studies have demonstrated decoupled rates of growth and development in response to various stressors. Developmental suppression is distinct from reduced growth rates in that the former manifests as slowed progression through the stages of larval development, rather than reduced size increases or increased mortality rates. Development towards sexual maturity (e.g., Gosner and Harrison stages; Harrison 1924, Gosner 1960) progresses separately from growth for many amphibian taxa (Ryan and Semlitsch 1998, Gomez-Mestre et al. 2010, Bonett et al. 2022). This “decoupling” of growth and development occurs in tadpoles in response to stressors including accelerated pond drying, which increases the threat of desiccation, as well as increasing density and thus competitive pressure (Gomez-Mestre et al. 2013). Additionally, paedomorphic salamanders provide an extreme example of decoupling of sexual and somatic development in salamanders (Ryan and Semlitsch 1998, Bonett et al. 2022). Given this precedent, and observations that mesocosm populations with low variability exhibited reduced developmental rates without showing reduced growth rates, it can be inferred that developmental suppression may be responsible for longer larval periods in mesocosm populations with less variable size structures. If larvae in low variation populations responded to increased competition from equivalently sized conspecifics by allocating resources to growth rather than development, developmental suppression would be undetected based on the fitness parameters I monitored (i.e., larval growth, time to and size at metamorphosis). Studies of intraspecific interactions in low-variation populations indicate changes in size structure may lead to physiological and behavioral changes in response to elevated competition from equivalently-sized conspecifics that drive suppression of developmental rates independent of growth rates in ambystomatid larvae (Maret and Collins 1994, Brunkow and Collins 1998).

Variation channelization or developmental suppression may each function individually to shape population fitness metrics, but larval amphibian populations and communities are frequently shaped by multiple interactive factors (Kerby et al. 2011, Relyea 2012, Ortiz-Santaliestra et al. 2012, Johnson et al. 2013, Hanlon and Parris 2014). The predominant mechanisms mediating fitness may change during ontogeny in larval amphibian populations. If early ontogeny is characterized by periods of increasing size structure due to increased growth by a subset of individuals (Wilbur 1972), such increased variation may reduce developmental suppression compared to low-variation populations through decreased competition and increased niche breadth (Whiteman et al. 2012, Shanbhag et al. 2016). Following initial increases in variation,

variation channelization late in ontogeny may occur as the largest larvae undergo metamorphosis. In contrast, populations not characterized by initial increases in variation will continue to experience developmental suppression due to limited size structure, resulting in later dates of metamorphosis. Data from my mesocosm populations present evidence for the simultaneous action of variation channelization and developmental suppression in size-structured larval salamander populations. Knowledge of the mechanisms creating and maintaining size structure expands our understanding of the ecology of larval amphibian populations and presents opportunities for further study. Studies that are more robust in their temporal scale, incorporate studies of behavioral interactions between size classes, and focus on tracking success of individual larvae related to their position in the population size structure (*sensu* Maret and Collins 1994), would all expand our understanding of size-structured populations and the relative importance of variation channelization and developmental suppression within these populations.

Density-Dependence

Effects of size variation on aquatic populations are often modified by density-dependence (Schindler et al. 1997, Benard and Maher 2011, Bolnick et al. 2011), and associated with shifts in morphology, physiology, and behavior with far reaching implications for many fitness metrics, including survival, growth, and timing of metamorphosis (Petranka 1989, Wildy et al. 2001, Doyle and Whiteman 2008). Morphological and behavioral responses to density include shifts in foraging behavior, habitat use, and pronounced life history changes such as paedomorphosis and trophic polyphensism (Jaskula and Brodman 2000, Ortiz-Santaliestra et al. 2012, Anderson and

Whiteman 2015, McLean et al. 2016). Decreased survival at high larval densities has been widely observed in larval amphibian populations in a wide variety of experiments (Wilbur 1972, 1997, Petranka 1989, Boone and Semlitsch 2001), including my experimental mesocosm populations. Increased mortality at higher densities is often accompanied by decreased growth rates that prolong the time to metamorphosis and produce smaller metamorphs (Semlitsch 1987, Petranka 1989, Wildy et al. 2001). In my mesocosm populations, growth rates were reduced, and metamorphs were smaller, in high-density populations. Reduced size at metamorphosis from high-density populations has important fitness implications, as larger juvenile pond-breeding salamanders are more resistant to desiccation, survive to reproduction at higher rates, reproduce earlier, and are larger during initial reproductive events (Smith 1987, Semlitsch et al. 1988, Messerman and Leal 2021). A major factor that may contribute to reduced average growth rates in high-density populations is repressed growth of smaller individuals by larger individuals, which primarily results from intraspecific aggression and interference competition (Wilbur 1976, 1980). Decreased growth under high intra- and interspecific densities serves to extend larval periods by extending times required to reach size thresholds for metamorphosis (Scott 1990), and can increase larval mortality by exacerbating predation risk or leaving larvae vulnerable to pond drying (Stenhouse et al. 1983). Intense density-dependent mortality in larval stages can reduce metamorph recruitment in *Ambystoma maculatum* populations to 1%, and recruitment rarely exceeds 15% (Shoop 1974, Ireland 1989, Petranka 1998). Similarly, my experimental mesocosm populations exhibited rates of larval survival of 11.2%. High larval mortality due to many density-dependent factors indicates that *Ambystoma maculatum* populations are density-regulated in larval stages, wherein larvae experience saturation densities inversely proportional to larval mortality and rates of metamorphosis (Wilbur 1972, 1980, Van Buskirk and Smith 1991). Conversely, in terrestrial adult habitats, survival is relatively constant among individuals, as resources are non-saturated (Wilbur 1980).

Despite myriad effects of increased density on larval fitness, including reduced larval growth rates, increased density did not significantly lengthen time to metamorphosis in my experimental mesocosms, despite this change being noted in other experiments (Wildy et al. 2001). However, theoretical models of larval amphibian ecology point towards a potential explanation. Emergence from aquatic environments has been modeled as a trade-off between the resource/risk ratios of aquatic and terrestrial habitats (Werner 1986, Chaparro-Pedraza and Roos 2020). For individuals in low-density treatments, it may be advantageous to remain in aquatic environments after surpassing metamorphosis size thresholds due to abundant aquatic resources and reduced competition (Wilbur and Collins 1973, Werner 1986). Conversely, individuals in high-density treatments may emerge as soon as size thresholds for metamorphosis are reached to escape highly competitive aquatic environments (Werner 1986, Chaparro-Pedraza and Roos 2020). Additional research may help characterize the cues which mediate the metamorphosis size threshold in response to these tradeoffs. Despite some inconsistencies, results from mesocosm populations indicate pronounced actions of density-dependent mechanisms in regulating larval mesocosm populations of *Ambystoma maculatum*.

Competitive Structure of Ambystomatid Larval Populations

Significant effects of both size variation and density on larval fitness metrics indicate broad influences of density-dependent regulatory mechanisms and size structure in dictating fitness outcomes, and further examination of trends in average metamorph timing and patterns of metamorph emergence among size structure classes serve to illuminate the nature of these effects, and thus which hypothesis (RPH or CHH) best characterizes the competitive structure of *Ambystoma maculatum* larval populations. The resource partitioning hypothesis (RPH) predicts populations with highly variable size structures experience competitive release through increased population niche breadth, leading to increased average fitness. Under the RPH, resource use scales with body size, and small and large individuals are released from competition due to divergent patterns of resource use (Roughgarden 1972, Werner and Gilliam 1984, Polis 1984), leading to predicted size variation optima (SVO) at maximum levels of body size variation. Under the CHH, large and small individuals compete for similar resources and average fitness is reduced through increases in interference competition, as well as lethal and nonlethal aggressive encounters (Wilson 1975, Persson 1985, Smith 1990). The CHH predicts SVO at minimal levels of body size variation, as such size structure will maximize competitive symmetry. Previous research on the competitive ecology of pond breeding salamander species, in combination with SVO identified in experimental mesocosms, may offer support for a particular competitive hypothesis.

Evidence exists for partitioning between co-occurring amphibian species (Toft 1985, Fasola 1993, Crawford and Peterman 2013, Cudmore and Bury 2014), and although more limited, for intraspecific habitat partitioning in a variety of larval amphibians (Alford and Crump 1982, Denoël 2005, Mott and Steffen 2014). Studies addressing the fitness implications of partitioning in larval ambystomatid populations have yielded inconclusive results that have led researchers to infer limited fitness consequences of size structure in these populations (Smith 1990, Brunkow and Collins 1996, Anderson et al. 2023). However, these studies did not track metamorphs throughout ontogeny, and thus would not have detected potential fitness consequences linked to altered developmental rates and lengths of larval periods based on body size variation. In my study, negative relationships between larval BCV and average larval period length indicate high-variation populations experience improved fitness via shorter larval periods, demonstrating a high SVO for larval populations of *Ambystoma maculatum*. This increased fitness may be due to competitive release minimizing developmental suppression in high-variation populations and would offer support for the resource partitioning hypothesis. Attempts to compare these results to the literature revealed only one study which specifically tracked developmental rates in populations of larval ambystomatid salamanders with varying size structures. Developmental rates increased in populations with low size variability, the inverse of trends I observed in mesocosm populations (Brunkow and Collins 1996). However, this experiment tracked only “focal” larvae from a medium size class, making this study less apt for comparison to examinations of average development rates from populations. Though trends in average larval period length across mesocosm populations seem to support the RPH, examination of differences in patterns of metamorph emergence across broad size structure categories indicates some potential complications.

Examination of emergence patterns across BCV ranges indicate the largest individuals benefit disproportionately from competitive release in populations with increased size structure. Emergence histograms of high-variation mesocosms show metamorphosis is highly concentrated relatively early in the larval period, supporting previous observations that larger, or earlier-hatching, individuals are more likely to survive to metamorphosis (Smith and Petranka 1987, Ryan and Plague 2004). This emergence phenomenon indicates that, while competitive release is beneficial for larger larvae and allows them to reach metamorphosis sooner, the benefits of competitive release do not extend to individuals in smaller size classes, because otherwise survival would have been relatively higher in high-variation populations. When fitness metrics are averaged across populations, it appears that fitness benefits conferred on larger individuals in high-variation populations is sufficient to outweigh the fitness consequences to smaller individuals, resulting in overall higher fitness for high-variation populations as expressed by shorter average larval periods. The apparent competitive superiority of larger individuals in high-density populations indicates that, although average fitness is maximized under highly variable size structures, competitive hierarchies are still exacerbated by such variation. The action of these two hypothesized mechanisms (RPH and CHH) in concert is unexpected and offers insight into the competitive structure of ambystomatid larval populations.

Competition-mediated fitness outcomes in mesocosm populations of *Ambystoma maculatum* larvae reveal an important nuance of the RPH/CHH question. Research across many taxa has shown that larger individuals generally have competitive advantages in both inter- and intraspecific contexts (Persson 1985, Buskirk 1992, Liu et al. 2021). This advantage may manifest through interference competition, exploitation competition, reduced predation risk, heightened cannibalistic ability, or increased reproductive success (Honěk 1993, Byström and García-Berthou 1999, Ziemba and Collins 1999, Rausch et al. 2014, Fouilloux et al. 2022). Often, the competitive advantage of larger size manifests in the form of competitive gradients, which have been observed in mammals (Sharpe et al. 2016), birds (Moreno-Opo et al. 2020), reptiles (Hollis et al. 2004), fish (Pettersson et al. 1996), and invertebrates (Savolainen and Vepsäläinen 1988, Balfour et al. 2003, Liu et al. 2021). These trends manifested in my mesocosm populations, with larger individuals experiencing relatively higher survival rates. Under these conditions, populations of uniformly large individuals may experience the highest average fitness (following the CHH hypothesis of a low SVO and high mean body size). However, even in competitive hierarchies, increased variation caused by the presence of smaller individuals may lead to competitive release. Smaller individuals often forage less efficiently (Ayers and Shine 1997, Persson et al. 1998), utilize habitat differently (Branch and Altig 1981, Santoyo-Brito et al. 2021), and are less threatening aggressors (Mitchem et al. 2019). When smaller individuals are present, competitive release may occur for members of larger size classes, allowing for elevated average fitness when compared to uniformly “large-sized” population. In my experimental mesocosms, highly variable mesocosms did show evidence of competitive release through shorter average larval periods, and the benefits of competitive release appeared concentrated in individuals of larger size classes. When considering any highly competitively structured population, it is important to consider that fitness benefits of competitive release may not be uniformly distributed throughout a population, and instead may be another “resource” to be distributed unevenly across a competitive gradient, with superior competitors experiencing more release.

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APPENDICES

Appendix A: Tables

Appendix A: Tables

Table 1. MANCOVA and subsequent univariate ANCOVAs for the effects of *Ambystoma maculatum* larval density and body size coefficient of variation (BCV) on larval fitness metrics of average size change (over the first 28 days of the larval period) and percent density reduction (over the first 28 days of the larval period). MANCOVA results are reported on the same line where the independent variable is shown, whereas one-way ANCOVA results are reported on the same line as the corresponding dependent variables. Bolded items indicate significant treatment effects, and "n,d" represents the numerator and denominator degrees of freedom.

Ind. & Dep. Variables	**n,d**	**F**	**P**
Density	2,76	3.17	**0.047**
Growth Rate		4.61	**0.035**
Percent Density Reduction		1.81	0.182
Body Size Coefficient of Variation (BCV)	2,76	.052	0.599

Table 2. MANCOVA and subsequent univariate ANCOVAs for the effects of *Ambystoma maculatum* larval density and body size coefficient of variation (BCV) on metamorph fitness metrics of time to metamorphosis, size at metamorphosis, and percent survival to metamorphosis. MANCOVA results are reported on the same line where the independent variable is shown, whereas one-way ANCOVA results are reported on the same line as the corresponding dependent variables. Bolded items indicate significant treatment effects, and "n,d" represents the numerator and denominator degrees of freedom.

Ind. & Dep. Variables	**n,d**	**F**	**P**
Density	3,54	5.07	**0.004**
Time to Metamorphosis		0.35	0.556
Size at Metamorphosis		6.07	**0.017**
Percent Survival		7.83	**0.007**
Body Size Coefficient of Variation (BCV)	3,54	5.11	**0.003**
Time to Metamorphosis		12.77	**<0.001**
Size at Metamorphosis		1.11	0.296
Percent Survival		1.29	0.261

Appendix B: Figures

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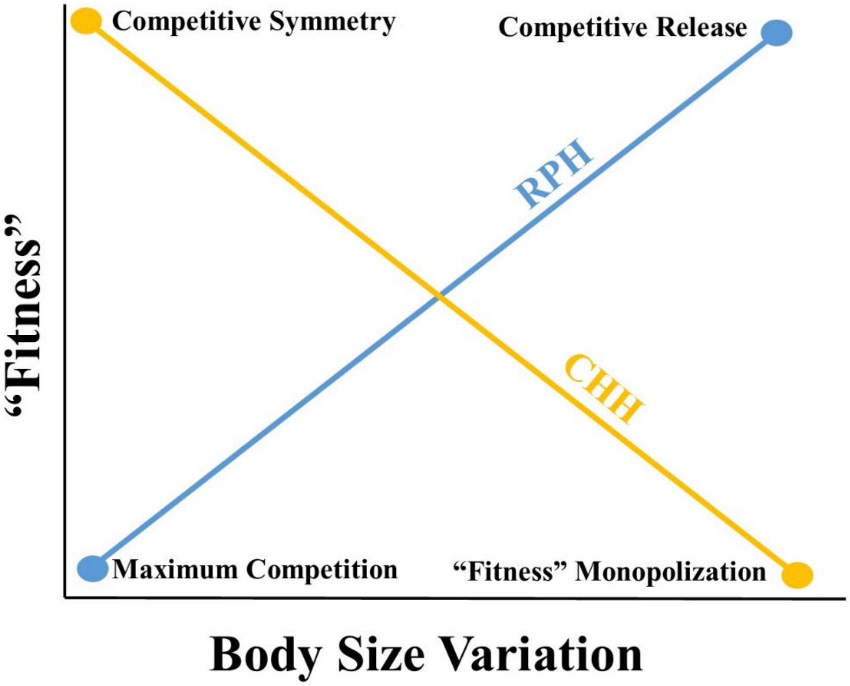
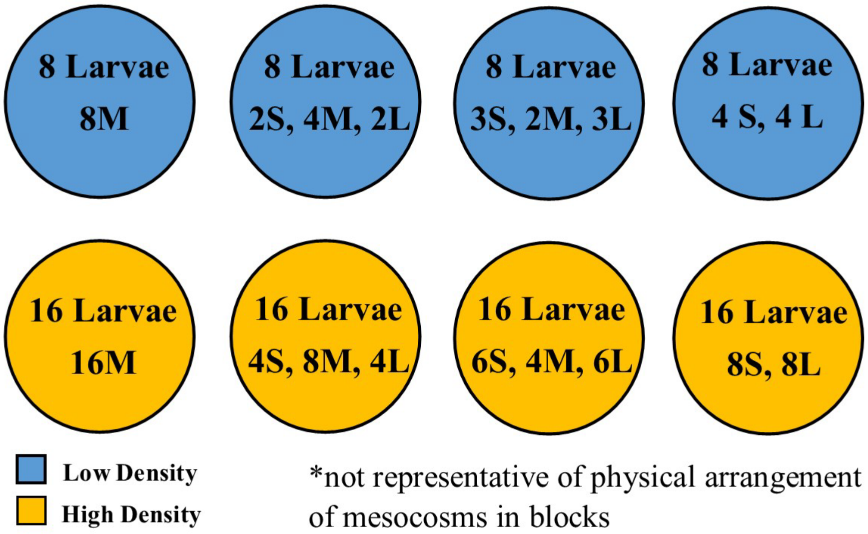


Figure 1. Proposed relationships between population "fitness" and intraspecific body size variation according to both the competitive hierarchy hypothesis (yellow) and the resource partitioning hypothesis (blue).



*not representative of physical arrangement of mesocosms in blocks

Figure 2. One block from the mesocosm array. Circles represent individual mesocosms, with larval numbers corresponding to density treatment and size class numbers indicating the number of small (S), medium (M), and large (L) size class larvae in each mesocosm. Positioning of mesocosms within each block at the field site was randomly assigned. CV values generated in mesocosm populations fell within range observed within natural ambystomatid communities (Mott, unpublished data)

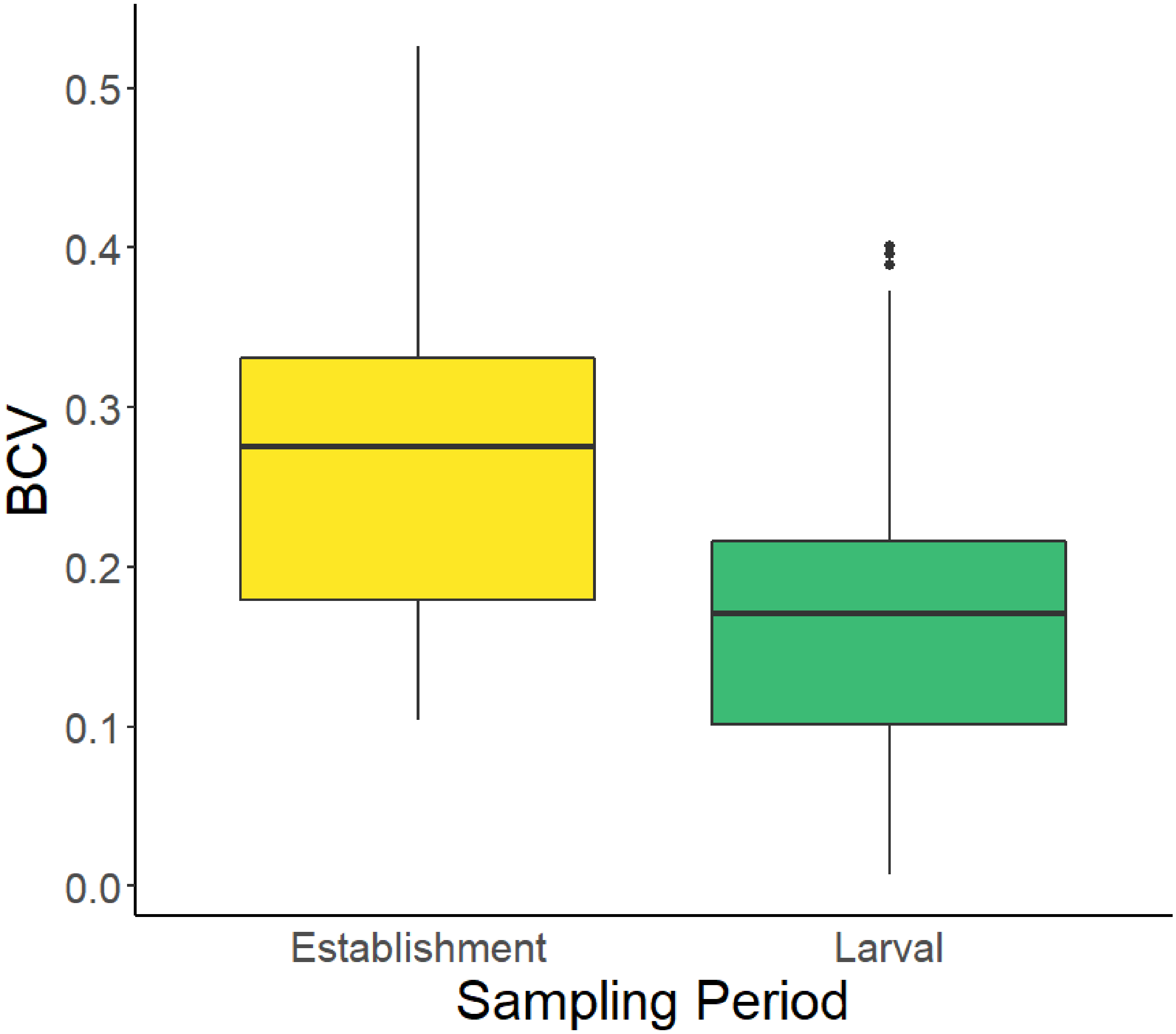


Figure 3. Changes in body size coefficient of variation (BCV) in larval *Ambystoma maculatum* populuations between establishment of the larval mesocosm populations and a sampling period 28 days into the larval period.

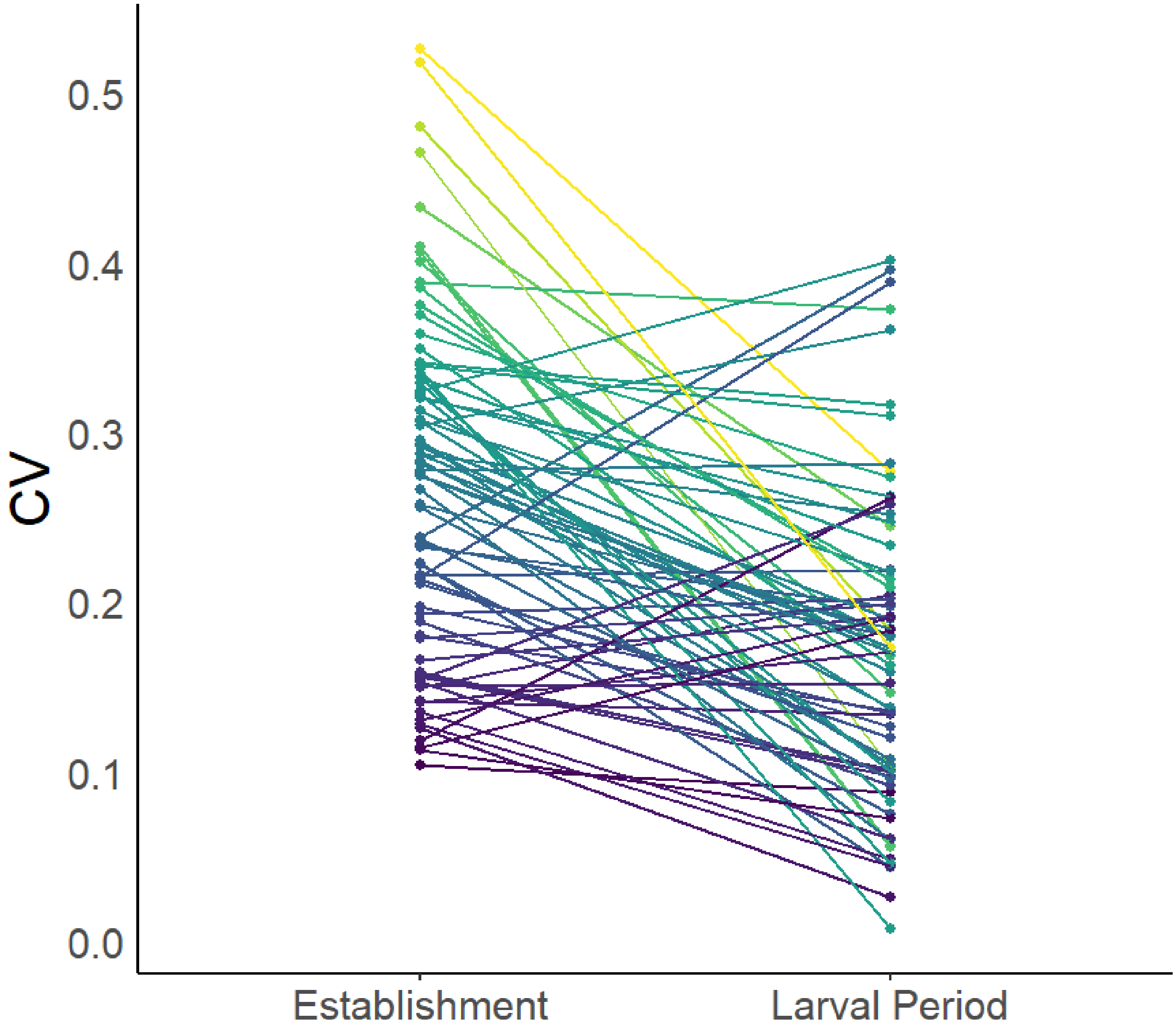


Figure 4. Changes in body size coefficient of variation (BCV) for individual *Ambystoma maculatum* mesocosm populations between establishment and larval period sampling 28 days post-establishment. Each connected set of dots represents one mesocosm.

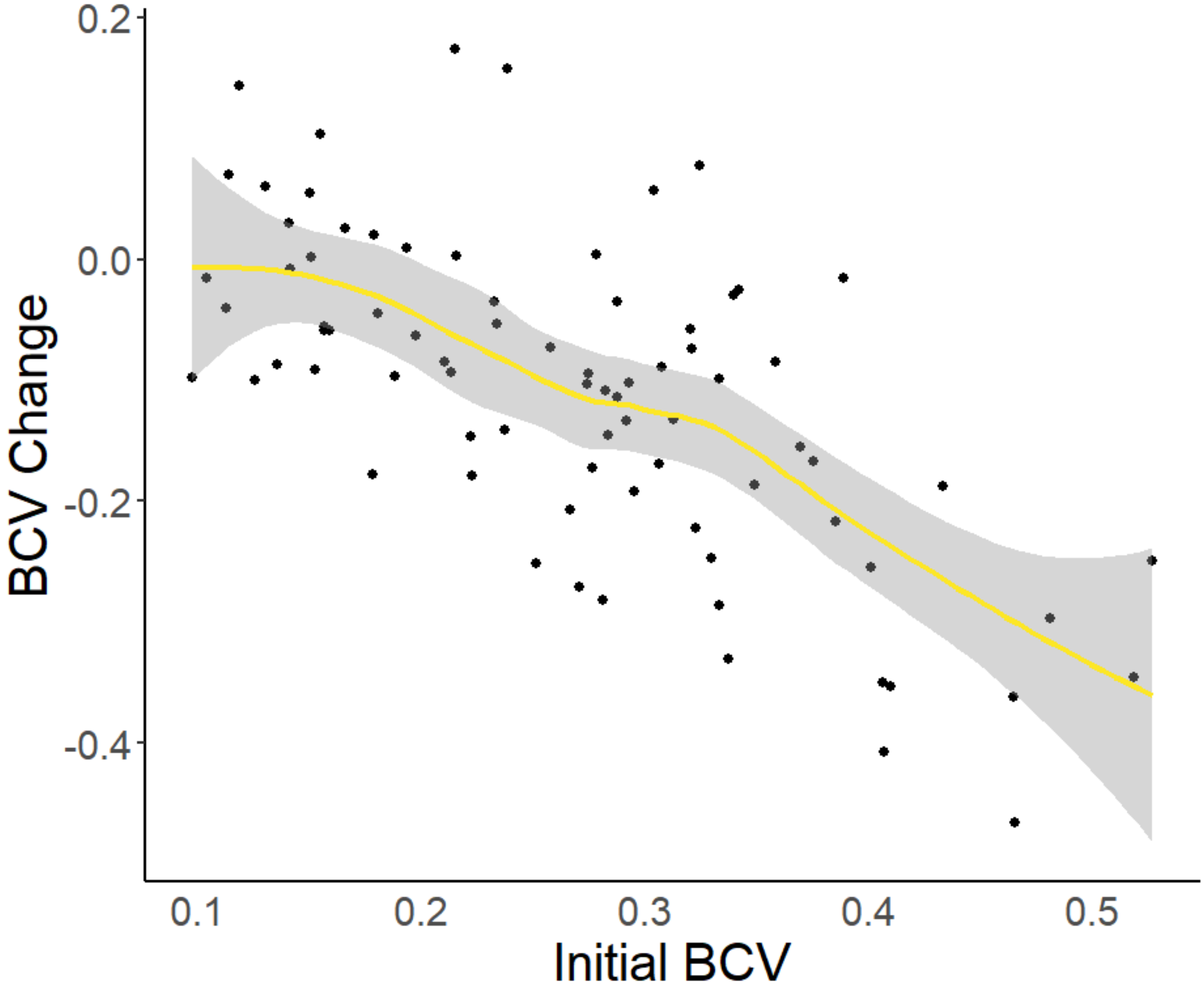


Figure 5. Relationship between initial body size coefficient of variation (BCV) and change in BCV at 28 days post-establishment for mesocosm populations of *A. maculatum*. Line of best fit generated using local regression fitting.

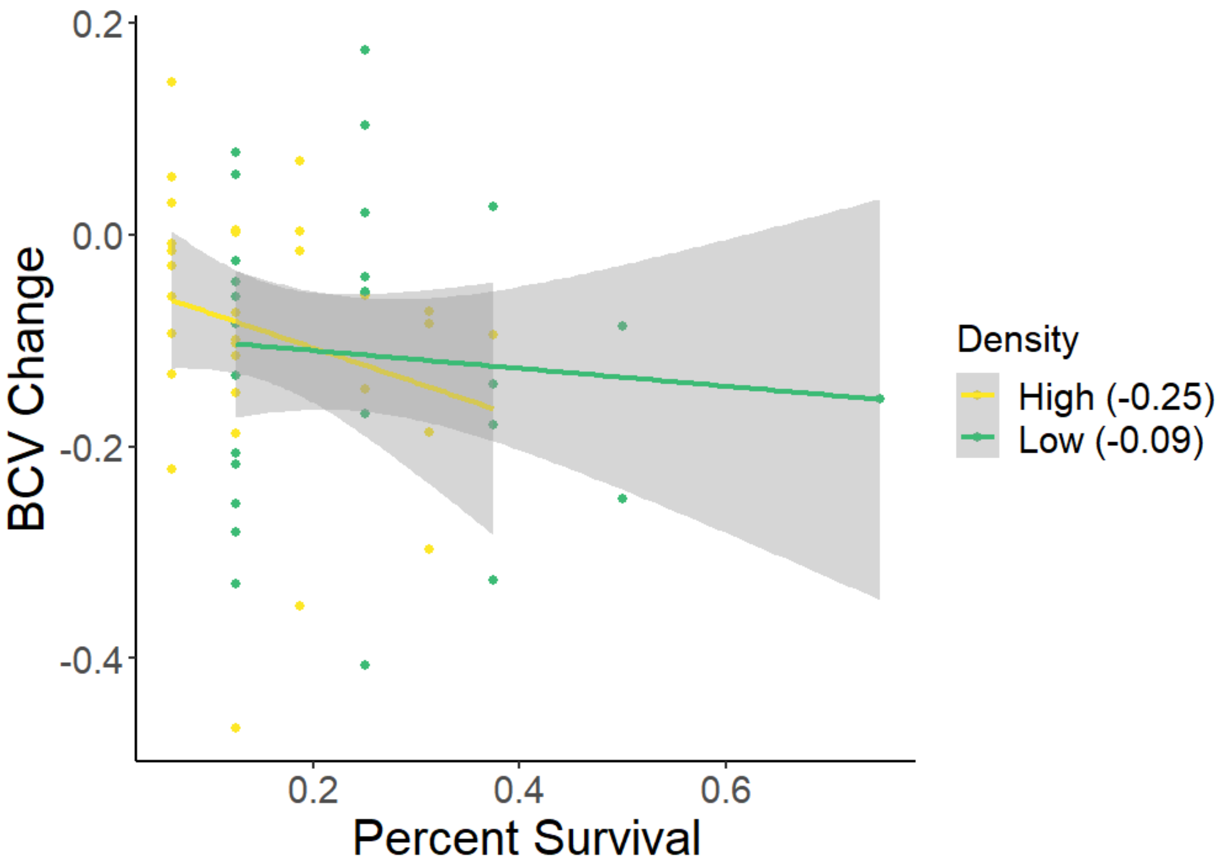


Figure 6. Correlation between body size coefficient of variation (BCV) change over the first 28 days of the larval period and percent survival to metamorphosis for mesocosm populations of *Ambystoma maculatum*.

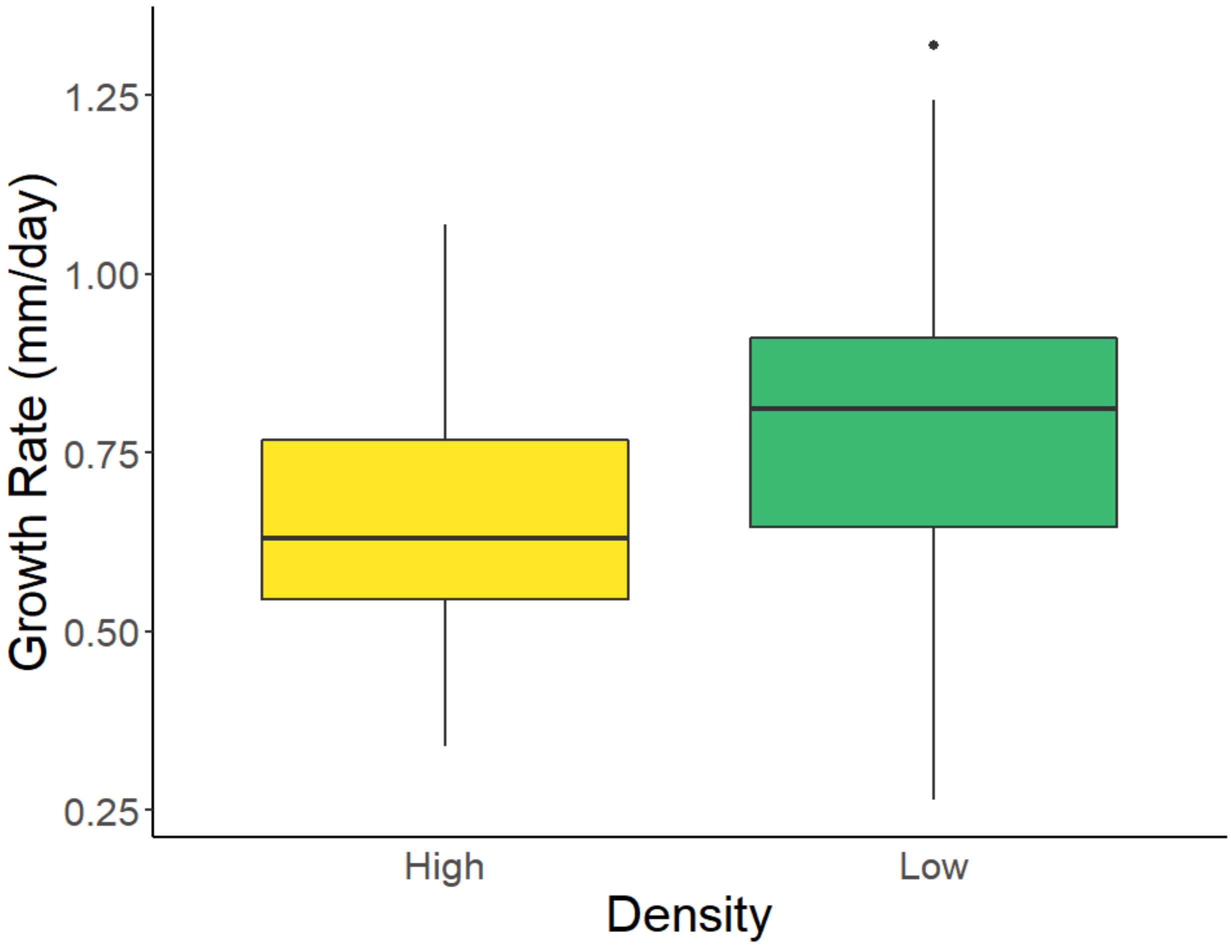


Figure 7. Average growth rate in a larval *Ambystoma maculatum* populuations between establishment of the larval mesocosm populations and the sampling period 28 days into the larval period for mesocosms with high (16) and low (8) larval densities.

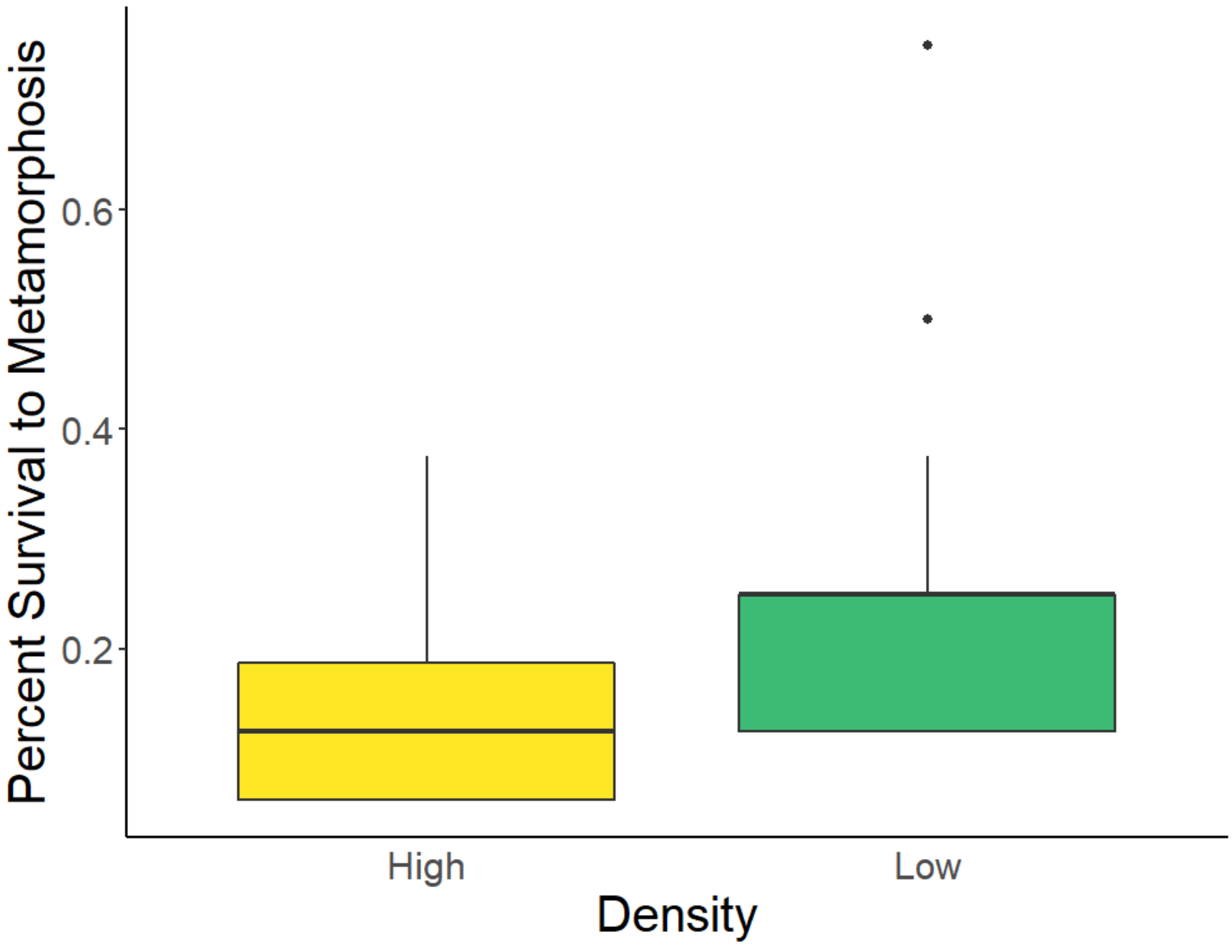


Figure 8. Average percent survival to metamorphosis for mesocosm populations of *Ambystoma maculatum* with high (16) and low (8) larval densities.

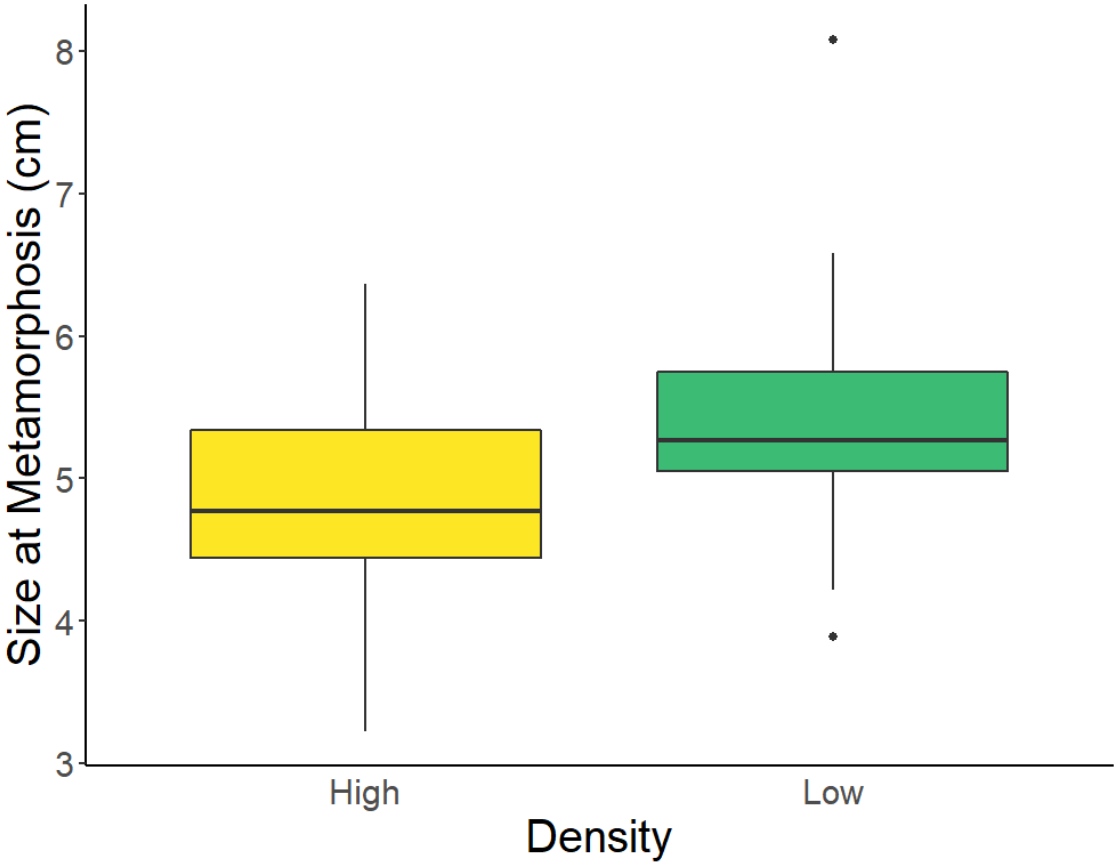


Figure 9. Average size at metamorphosis for mesocosm populations of *Ambystoma maculatum* with high (16) and low (8) larval densities.

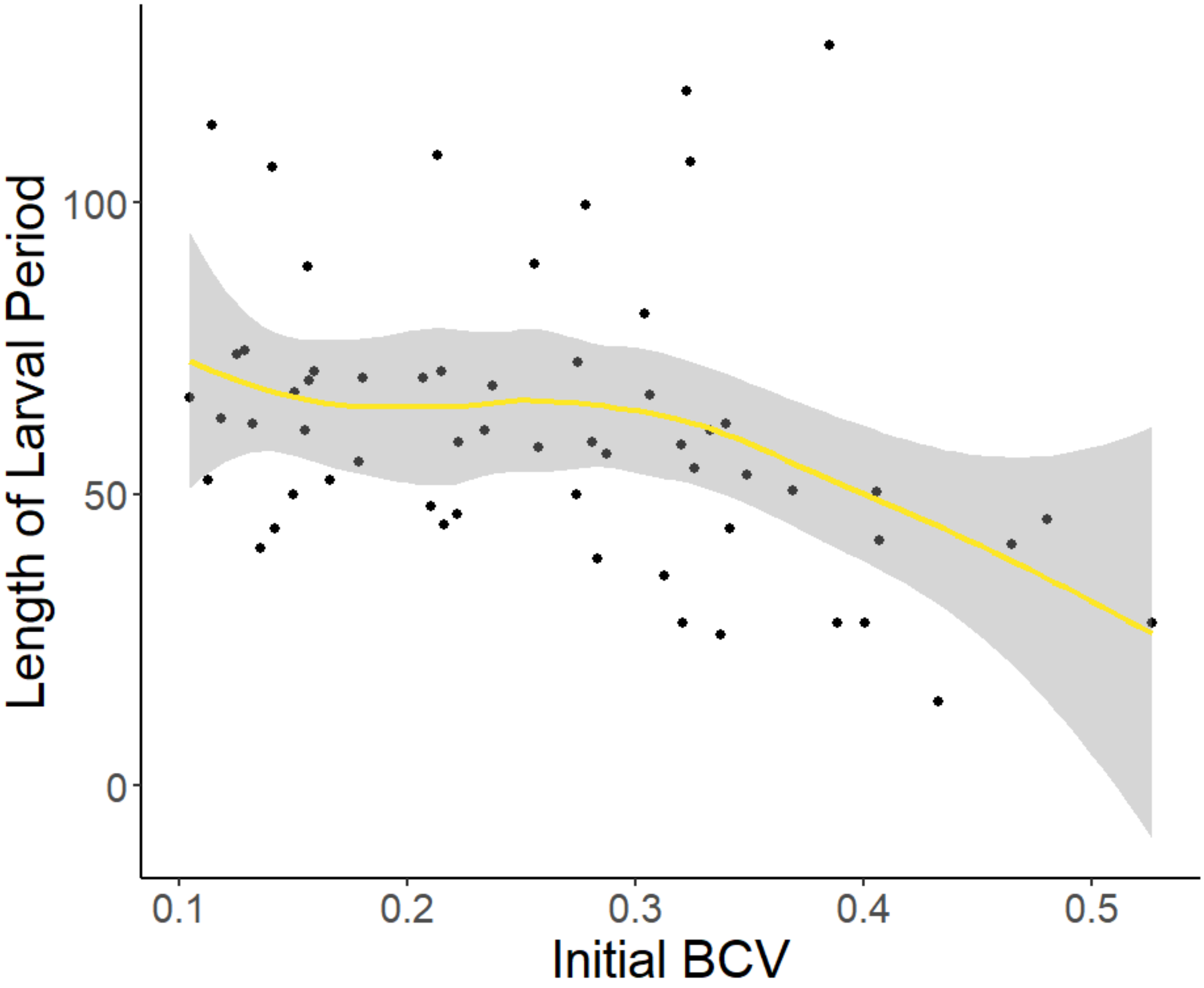


Figure 10. Relationship between initial body size coefficient of variation (BCV) and length of the larval period for mesocosm populations of *A. maculatum*. Line of best fit generated using local regression fitting.

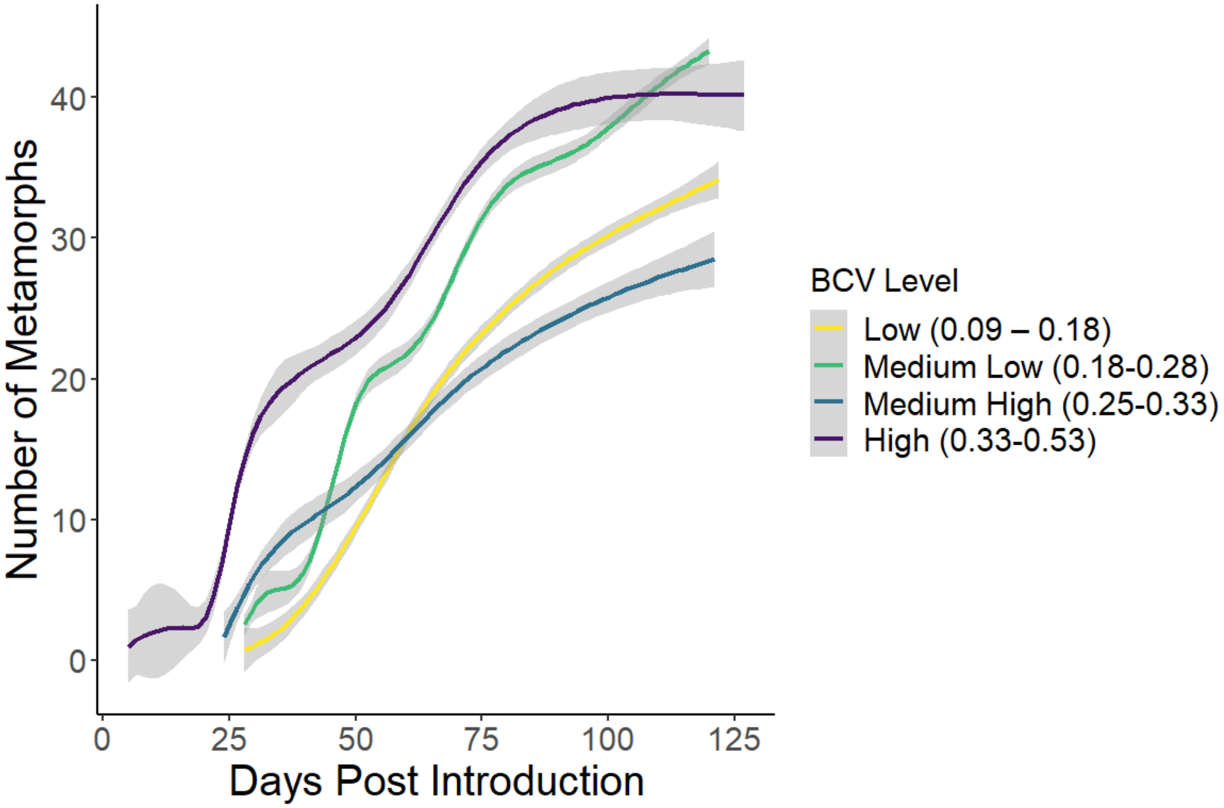


Figure 11. Accumulation of *Ambystoma maculatum* metamorphs from mesocosm populations at four different levels of body size coefficient of variation (BCV) over the course of the larval period.

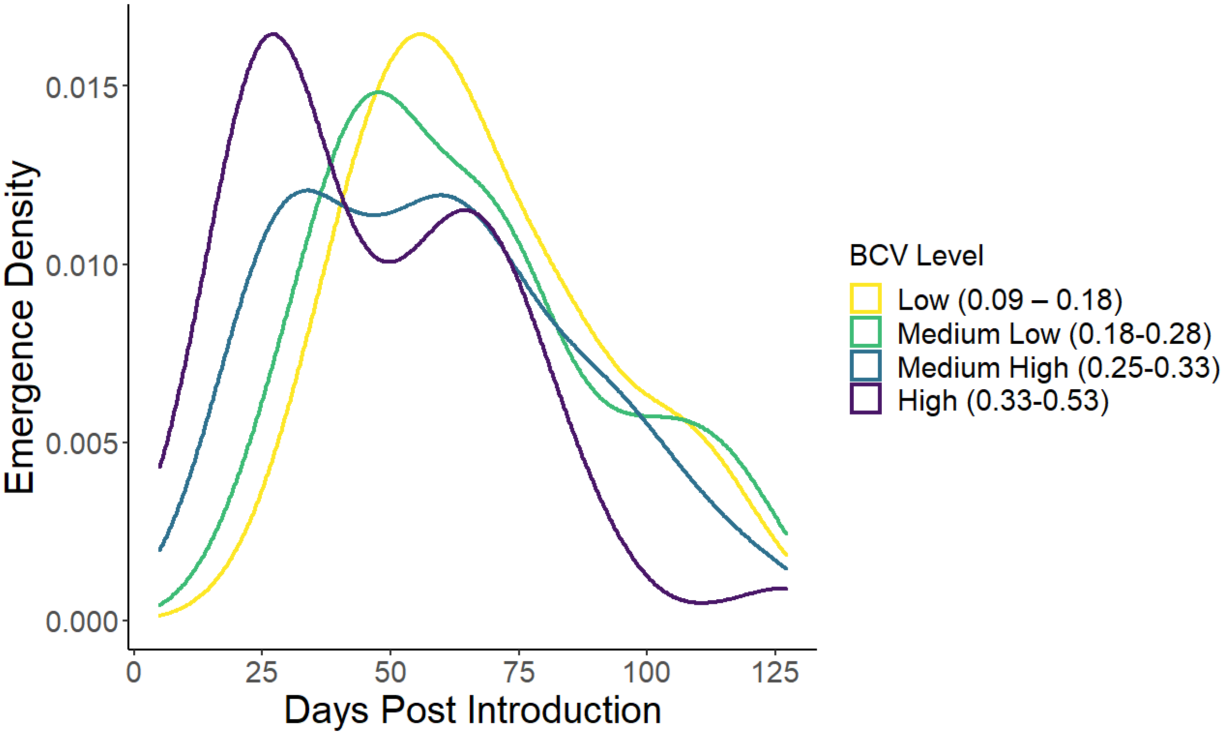


Figure 12. Emergence density of *Ambystoma maculatum* metamorphs from mesocosm populations at four different levels of body size coefficient of variation (BCV) over the course of the larval period.