Habitat patchiness drives spatial structure in morphological trait variation and co-variation in spotted salamanders (*Ambystoma maculatum*)

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Author contributions

DRD and ETG designed the study and executed field sampling and trait measurements, with supervisorial support from AID and JAC. ETG completed the morphometrics and principle components analyses. DRD performed the rest of the statistical analyses. All authors contributed to the writing and revision of the manuscript.

Abstract

The influence of intraspecific trait variation in species interactions makes trait-based approaches critical to understanding eco-evolutionary processes. Given that species occupy habitats that are patchily distributed in space, advancement in trait-based ecology hinges on understanding how trait variation is spatially structured across the landscape. We sampled larval spotted salamanders (*Ambystoma maculatum*) across spatially discrete ponds to quantify spatial structure in morphology. Spatial structure explained 7-35% of total observed variation in the length and shape of salamander larvae, depending on the body segment measured (i.e., head, body, tail). Salamander tail morphology was more variable and exhibited more spatial structure than head or body morphology. Salamander mass was also highly variable, and was strongly correlated with total length. Analysis of allometry revealed that the slopes of mass-length relationships were similar across space, but the intercepts differed spatially. Preliminary evidence hints that newly constructed ponds are drivers of spatial differences in allometric intercepts. Pond construction may therefore bolster diversity in trait co-variation, and in so doing instil more adaptive potential of salamanders under current and future environmental change.

Key Words: Intraspecific trait variation, eco-morphology, allometry, phenotypic plasticity, allometry, amphibian, ecology, morphometrics, conservation

Introduction

Individual morphological traits underpin many eco-evolutionary processes. The range of morphology exhibited by individuals of a species, a form of intraspecific trait variation (Bolnick et al., 2011), shapes the niche breadth of populations, which in turn affects their resiliency to environmental disturbances and biological invasions (Tack, Horns & Laine, 2014). Models of population and community dynamics that incorporate intraspecific trait variation have therefore become central to ecology (Bolnick et al., 2011; Moran, Hartig & Bell, 2016). A key outstanding challenge for this 'trait-based' paradigm is to understand trait variation in a spatially explicit context (Violle et al., 2012; Moran, Hartig & Bell, 2016).

Species occur in landscapes comprised of spatially discrete habitat patches. Patchy habitat structure creates networks of spatially isolated groups of individuals, potentially structuring how traits of those individuals are distributed in space. For example, environmental or genetic variability across habitat patches may drive group-level differences in trait expression, especially when movement between patches is limited. Group-level trait differences produce spatially structured trait variation across the landscape. Theory predicts that spatial structure in trait variation fundamentally alters population and community dynamics (Moran, Hartig & Bell, 2016; Banitz, 2019). By partitioning trait variation among multiple habitat patches, spatial structure potentially allows for a broader range of adaptive responses to environmental disturbances (Moran, Hartig & Bell, 2016) and antagonistic interactions (Tack, Horns & Laine, 2014). Alternatively, spatial structure in trait variation may heighten extinction risk by geographically isolating trait diversity into specific habitat patches. Thus, distinguishing how traits are distributed across space, both within and among habitat patches, should aid with predicting community dynamics under environmental change.

Characterizing spatial structure of trait variation in natural populations is not straightforward. Within-patch sampling must be comprehensive enough to capture the range of traits expressed by individuals, and the number of habitat patches sampled must be extensive enough to capture the spatial structure. Additionally, trait measurements should be rapidly collected. Otherwise, temporal trait variation arising from seasonal life histories and ontogeny may confound spatial effects. The studies that have overcome these logistical challenges have focused primarily on plants (Des Roches et al., 2018). Empirical data on the spatial structure of trait variation in animals remains highly limited, particularly for vertebrates.

Even fewer studies have assessed spatial structure in trait co-variation (Evangelista et al., 2019). Co-variation describes relationships between multiple individual traits. Measures of trait co-variation are useful for describing species growth patterns (Hirst, Glazier & Atkinson, 2014), their adaptive constraints (Voje et al., 2014), and complex morphological characteristics such as body shape (Laughlin & Messier, 2015). Practically, they provide a tool for filling data gaps in ecological models (Madin et al., 2016). Allometric scaling of mass with body length is a basic form of trait co-variation that is widely used for this purpose (Madin et al., 2016). Morphometrics integrates co-variation between body length, depth, and sometimes width, to characterize body shapes of individuals. Body shape, being a strong proxy of performance and fitness, is arguably a better predictor of species adaptive responses to environmental change than linear body measurements (Laughlin & Messier, 2015). Understanding the eco-evolutionary processes in patchy landscapes therefore requires consideration of spatial structure in trait co-variation as well.

In this study, we assessed the spatial structure in trait variation and co-variation in a patchy metapopulation of spotted salamanders (*Ambystoma maculatum*). We sought to quantify the extent to which habitat patchiness drove spatial structure in

salamander mass and length, as well as allometric and morphometric relationships among multiple traits. We sampled larval spotted salamanders among a network of spatially discrete wetlands (i.e. ponds) and measured body length and mass of 519 individuals. We used these data to quantify individual variation in mass and length within and among ponds. We then examined the spatial structure in variation and co-variation of those traits by: a) quantifying the contribution of pond-level differences in mass and length to total observed variation in these traits; b) testing whether mass-length allometry differed among ponds; and c) quantifying the contribution of pond-level differences in salamander shape to total observed variation in shape.

Methods

Study species

Spotted salamanders are broadly distributed throughout the Northeastern and Midwestern United States. These salamanders are semi-terrestrial pond breeders, annually migrating from terrestrial hibernacula to reproduce in fishless ponds, which in our study areas occurs between March–April. After hatching from eggs, aquatic larvae develop and metamorphose in 6–10 weeks. Larvae feed on invertebrates and anuran tadpoles, and are themselves prey for adult salamanders and larger aquatic invertebrates such as odonate larvae and beetles (Urban, 2010).

Spotted salamander populations provide useful systems to study morphological variation in a spatial context because: i) they occupy and move among spatially discrete ponds that comprise a functional metapopulation with patchy habitat structure (Patrick, Calhoun & Hunter, 2008); and ii) larval stages exhibit substantial morphological plasticity in response to heterogeneity in biotic and abiotic conditions (Scott, 1990; Urban, 2010), which enables a range of trait expressions across individuals and habitat patches.

Field sampling and husbandry

We sampled multiple ponds in east-central Missouri, distributed across three reserves – Tyson Research Center (800 ha), Forest 44 State Park (400 ha) and Shaw nature reserve (700 ha) (Fig. 1). We focused on six ponds in which pilot surveys confirmed salamander larvae were present. Three ponds (Mincke Pond, Arthur Christ Pond, Beth's Pond) were constructed in 2008 for research purposes and had similar sizes and dimensions (Burgett 2015). As part of a separate experiment, Rotenone was applied to Beth's pond in 2008. While this initially reduced microbial biodiversity (Woods et al., 2016) the micro-organismal community structure had returned to pre-treatment conditions well before sampling for this study (Woods et al., 2016). The other three focal ponds (Salamander Pond, Forest 44 Pond, Shaw Pond) were older and variable in size (Table S1). Salamander Pond was created in 1965. Forest 44 pond and Shaw pond were excavated between 1990 and 1996 (data extracted from Google Earth Historical Imagery). All ponds contained multiple predators of larval spotted salamanders: including dragonfly (*Anax* sp.) and damselfly (*Lestes* sp.) nymphs, diving beetles (*Dytiscidae* sp.), hyrdrophilid beetles (*Tropisternus* sp), and adult newts (*Notophthalmus viridescens*) (Tables S1 and S4; Biro unpublished). All ponds were located within forested habitats typical of temperate deciduous ecosystems found in the Midwestern U.S. A major highway bisected the study area, separating Forest 44 and Shaw ponds from the other four ponds.

We intensively sampled one pond per week (30 June–08 August, 2016) by dip-netting around the perimeter. We dipnetted until 10 minutes passed without a capture to maximize coverage of morphological variation within ponds. We kept all larvae that did not show overt signs of injury or illness (e.g. damaged tails or legs, tumorous growth) and immediately transported them to the National Great Rivers Research and Education Center (NGRREC) – less than 1 hr drive - where they were housed for seven days

147 before being returned to their original ponds. At NGRREC, we housed larvae individually
148 in circular plastic arenas (28 cm diameter) filled with 500 mL dechlorinated water
149 (approximately 2.5 cm depth). Larvae were maintained at 18 °C with a 14:10 h light:dark
150 cycle, consistent with ambient conditions at the surveyed ponds. Salamanders were fed
151 a single gray tree frog (*Hyla chrysoscelis-versicolor*) tadpole on the fifth day of the
152 housing period. Observing that not all salamanders ate the tree frogs fed to them the
153 prior day, we tested whether feeding influenced measures of mass for a random subset
154 of 237 individuals for which feeding data were available (see Supplementary Material for
155 detailed methods).
156
157 *Trait measurements*
158 On the sixth day after capture we measured the length and mass of salamanders,
159 distinguishing between head length, body length, and tail length. We photographed
160 lateral and dorsal images of larvae placed into clear tanks that minimized movement (Fig.
161 S1). We blot-dried individuals on paper towels before weighing. We measured the length
162 of salamander heads, bodies, and tails from images using ImageJ (Fig. S1) (Rasband,
163 1997). We classified larval development stage from our images according to Harrison,
164 (1969).
165 To obtain measurements of the shape of larvae we digitized landmarks on lateral
166 images using the software *tpsDig2* (Rohlf, 2006). Following Van Buskirk & Schmidt,
167 (2000) we tagged twenty landmarks that outlined larval shape (Fig. S1). Landmarks 1–3
168 described the shape of the head of the larvae, landmarks 4–11 described body shape,
169 and landmarks 9–20 outlined tail shape. Landmarks were rotated, scaled by size, and
170 aligned to a coordinated system using the Procrustes least-squares superimposition
171 available in the *geomorph* package for R statistical software (Adams and Otárola-Castillo
172 2013). We conducted four principal component analyses to explore the scaled two-

173 dimensional shape variation, again distinguishing head shape, body shape, and tail
174 shape. The first principal component (PC) score accounted for most of the variation in
175 head shape (37 %), body shape (33%), and tail shape (33%). We therefore used PC1 as
176 the shape metric in our analyses.
177
178 *Data Analysis*
179 We calculated the coefficient of variation – the ratio of the standard deviation to
180 the mean – as a standardized measure of individual morphological variation within
181 ponds. We assessed spatial structure of variation in salamander length and mass with
182 generalized linear mixed models (GLMM), using the *lme4* package in R (R Core Team,
183 2013). Specifically we calculated the intra-class correlation coefficient (ICC) from
184 GLMMs that included 'pond' as a random intercept term. The ICC, also called the
185 variance partitioning coefficient, is the proportion of total variation in response variables
186 that is attributable to group-level differences. In our analysis, the ICC indicated the
187 proportion of total observed variation in salamander length and mass that came from
188 pond-level differences in those traits. Trait variation would become increasingly spatially
189 structured as pond-level differences explained proportionally more of total trait variation,
190 that is, with higher ICC values. We ran separate GLMMs for mass and the length
191 measurements – head length, body length, tail length, and total length. All models used
192 a Gaussian error structure, as all morphological data were normally distributed (Fig. 3).
193 We omitted developmental stage from the models because all larvae were in the final
194 Harrison stages (45-46).
195 We then tested for spatial structure in mass-length allometry, a form of trait co-
196 variation. Specifically, we ran GLMMs to test whether the slopes and intercepts of mass-
197 length regressions differed across the six focal ponds, again distinguishing head length,
198 body length, and tail length. GLMMs included mass as the response, length as a fixed

199 effect, pond as a random intercept term, and length as a random slope term. We log-
200 transformed both length and mass and used a Gaussian error structure for the
201 normalized data in all models. To enable convergence of these more complex models,
202 we multiplied (log-transformed) mass by a factor of 10 to standardize the units with
203 length measurements. To test for differences in regression intercepts and slopes across
204 focal ponds, we used likelihood ratio tests comparing the fit of models that included both
205 terms with models omitting the random intercept or random slope term. We also
206 calculated the marginal and conditional R^2 of the models using the *MUMIn* package in R.
207 Marginal R^2 is a measure of the amount of variation in mass that was explained by the
208 fixed effect of length, whereas conditional R^2 considers variation explained by both fixed
209 and random effect terms (Johnson, 2014).
210 To further assess spatial structure in co-variation of morphological traits, we
211 determined the extent to which pond-level variation in salamander head, body, tail, and
212 overall (all segments combined) shape contributed to total observed variation in these
213 multidimensional morphological traits. Again, we calculated the ICC from GLMMs
214 including 'pond' as a random intercept term. PC1 scores were used as response
215 variables. The shape data were also normally distributed (Fig. 3), and so we used a
216 Gaussian error structure for all models.
217
218 **Results**
219 We obtained measurements of 519 spotted salamander larvae (Forest 44: N =
220 101, Shaw: N = 88, Salamander pond: N = 65, Arthur Christ: N = 116, Beth's: N = 30,
221 Mincke: N = 119). Salamander mass was not influenced by their feeding the previous
222 day in the subset of 227 individuals tested ($F_{(1\text{-}82)} = 3.43$, $p = 0.068$). Within all ponds,
223 salamander mass varied more among individuals than any of the length measurements
224 (Fig. 2). Morphological variation was consistently lower in Beth's pond (Fig. 2), though

this pattern was likely due to the lower sample size. Salamanders in Mincke pond generally varied more morphologically than salamanders from other ponds (Fig. 2). Otherwise, there was no indication that specific ponds harbored most of the morphological variation (Figs. 2 and 3).

Differences in average trait values across ponds accounted for 7–35% of the total observed variation in salamander mass and length, depending on the specific body section measured (Table 1). Specifically, pond-level differences accounted for proportionally more of the observed variation in salamander mass (35%), total length (35%), and tail length (27%) than in head (11%) and body length (17%) (Table 1).

Salamander mass co-varied most strongly with total length, which makes sense because we took single measures of mass that incorporated all body segments. Mass also co-varied strongly with body and tail lengths, but was associated less with head lengths, likely because heads are the smallest body segment of salamanders (Fig. S1). Accounting for pond-level differences in the intercepts of mass-length relationships improved explanations of variance in mass (Table 2), resulting in detectable differences in intercepts of mass-length relationships across ponds in terms of their intercepts (including pond as a random intercept term improved model fit; mass-head length: X^2_1 = 111.84, $p < 0.001$; mass-body length: X^2_1 = 141.09, $p < 0.001$; mass-tail length: X^2_2 = 39.089, $p < 0.001$; total length: X^2_1 = 81.136, $p < 0.001$; Fig. 4). For a given length, individuals from Salamander pond tended to be heavier than individuals from other ponds (Fig. 4) whereas individuals form Beth's pond were generally lighter in mass per unit length (Fig. 4). Pond-level differences in the slopes of mass-length relationships played a lesser role in explaining variation in salamander mass (Table 2). Only the slopes for mass-head length and mass-tail length relationships differed across ponds (mass-head length: X^2_2 = 8.68, $p = 0.013$; mass-tail length: X^2_2 = 11.25, $p = 0.004$; Fig. 4); the slopes of mass-body length and mass-total length relationships were consistent

251 across sampled ponds (mass-body length: X^2_2 4.24, p = 0.120; mass-total length: X^2_2 =
252 5.05, p = 0.080; Fig. 3). The slope exponents were always < 3 (Table S3), indicating
253 that larger salamander larvae generally had more elongate heads, bodies and tails than
254 smaller larvae.

255 Similar to length measures, the shape of salamander tails exhibited more spatial
256 structure than did the heads or bodies. Pond-level trait differences contributed 25% of
257 the total observed variation in tail shape, compared with 11%, 9%, and 7% of head, body,
258 and overall shape, respectively (Table 1). Further, there was little evidence that PC
259 scores were clustered by pond (Fig. 5), indicating a weak signal of spatial structure in
260 salamander shapes.

261

262 **Discussion**

263 There was a stronger signal of spatial structure in larval salamander tails
264 compared with their heads or bodies. Spatial structure in trait co-variation was also
265 evident in salamander tails. Scaling of mass with salamander tail lengths differed across
266 ponds both in terms of the intercepts and the slopes of the relationships, and pond-level
267 differences contributed more to total observed variation in tail shapes than for head or
268 body shapes. The spatial structure of pond habitats therefore seems to act particularly
269 strongly on salamander tails, perhaps because tails play an important role in swimming
270 performance. Being meso-predators, swimming performance for salamanders is critical
271 to both capturing prey and evading predators (Van Buskirk & Schmidt, 2000; Urban,
272 2010). Tails may therefore be more closely linked to fitness, hence under stronger
273 selection, than heads and bodies, at least in habitats where predation is a significant
274 threat. Multiple predators of larval salamanders were found in our focal ponds, and so
275 predation risk is likely to be a strong selective force in the salamander metapopulation
276 studied here. In a preliminary analysis of the data, we did not detect an influence of

predator density on tail length of salamanders (see Supplementary Material for detailed methods and results), but this analysis was based on our limited sample of ponds and warrants further investigation. Regardless of the factors driving salamander tail variation, our findings suggest that salamander responses to habitat alterations, biological invasions, and other pond-level disturbances may manifest as changes in tail morphology as opposed to changes in head and body morphology. If this prediction holds, the more common body size measure for amphibians, snout-to-vent length, which only takes head and body lengths into account, would be insufficient for predicting the eco-evolutionary responses of this species to landscape-scale environmental changes in aquatic habitats.

Even with respect to salamander tails, much of total morphological variation that we observed was unstructured (i.e. random). The substantial within-pond variation in salamander morphology suggests that local factors, such as microhabitat heterogeneity, also influence salamander morphology and mediate the extent to which habitat patchiness spatially structures morphological variation. Given that many ponds were spaced within the documented dispersal ranges of salamanders (Zamudio & Wieczorek, 2006; Patrick, Calhoun & Hunter, 2008), movement between ponds could also have mediated spatial structure in morphological variation by sustaining mixing of genotypes and phenotypes. At the metapopulation scale, local and spatial factors likely interact to shape varying degrees of spatial structure in morphological variation like that we observed in salamanders. This points out that spatial structure in morphological variation occurs along a continuum rather than in a binary, structured versus unstructured, fashion. The impact of trait variation on population and community dynamics likely depends the degree to which traits are spatially structured. This argument is supported by recent modelling (Banitz, 2019) and calls for empirical testing of how population and community dynamics change as trait variation becomes

increasingly spatially structured by habitat patchiness.

Spatial structure in the allometric relationship between salamander mass and total length arose specifically from pond-level differences in intercepts; slopes of the relationship were highly consistent. Spatial pond structure therefore appears to drive spatial differences in salamander mass relative to total body length, but does not appear to alter how mass scales with total body length. This spatially robust scaling of mass and total body length may explain why the two traits separately exhibited nearly identical degrees of spatial structure. More broadly, this pattern of allometric scaling, in which intercepts but not slopes of trait relationships differ, is consistent with allometric relationships documented across many other taxa (Voje et al., 2014), suggesting a general constraint to the plasticity and evolution of the slopes of trait relationships.

The inclusion of morphological diversity data in biodiversity conservation stems from the idea that different populations of the same species are not equal in terms of eco-evolutionary history. As such, exploring various approaches to the conservation of morphological diversity is important to developing strategies for reducing biodiversity losses under global change (Des Roches et al., 2018). The mix of within- and between-pond morphological variation in salamanders provides promise that pond construction can utilize local and spatial processes to bolster morphological diversity. Capitalizing on the presence of new constructed ponds in our study area, we made a preliminary comparison of salamander morphology and allometry between ‘new’ and ‘old ponds’ (see Supplementary Material for detailed methods and results). This analysis did not detect differences in mass or length of salamanders between ‘new’ and ‘old’ ponds, but pond age did influence mass-length relationships and body shapes (Supplementary Material Table S4). Habitat restoration through pond construction may then bolster diversity in trait co-variation, and in so doing may instill more adaptive potential under environmental change (Laughlin & Messier, 2015). Although there are a number of

studies for various taxa that quantify functional connectivity between habitat patches (and local populations) using genetic techniques, we encourage additional studies on morphological parameters and patterns to better understand the mechanisms that promote long-term population persistence in fragmented landscapes.

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Conflicts of interest

We declare not conflicts of interest

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417 **Tables and Figures**

body segment	pond-level variance	SD	residual variance	SD	ICC (%)	95% CI - lower	95% CI - upper
mass							
	0.013	0.112	0.023	0.152	35.3	8.62	58.71
length							
head	0.15	0.39	1.27	1.13	10.8	0.73	26.11
body	0.92	0.96	4.36	2.09	17.4	8.72	53.62
tail	4.27	2.07	11.29	3.36	27.5	4.08	52.07
combined	18.23	4.27	32.91	5.74	35.6	6.25	57.80
shape							
	pond-level variance	**SD**	**residual variance**	**SD**	**ICC**	**95% CI - lower**	**95% CI - upper**
head	0.0004	0.019	0.003	0.053	11.0	0.7	26.5
body	0.0002	0.016	0.002	0.049	9.4	0.3	23.6
tail	0.001	0.026	0.002	0.049	22.8	1.2	35.0
combined	0.000	0.009	0.001	0.033	7.60	0.2	17.7

418

419 **Table 1. Proportion of mass, length, and shape variation attributable to pond-level**
420 **differences.** Displayed are the variance components of generalized linear mixed models
421 used for our analyses of spatial structure in salamander mass, length, and shape. The
422 intra-class correlation coefficient (ICC), or the variance partitioning component, is the
423 proportion of pond-level variation explaining total observed trait variation (pond-level
424 variance + residual variance). Higher ICC values denote higher degree of spatial
425 structure in salamander trait variation. CI = confidence intervals of the ICC derived from
426 bootstrapping over 500 resampling events.

427

mass-length co-variation								
body segment	**intercept variance**	**SD**	**slope variance**	**SD**	**residual variance**	**SD**	**marginal R^2**	**conditional R^2**
head	0.11	0.33	0.09	0.29	0.02	0.13	0.26	0.49
body	0.04	0.20	0.02	0.13	0.01	0.09	0.65	0.77
tail	0.08	0.28	0.04	0.19	0.01	0.08	0.73	0.79
combined	0.07	0.26	0.00	0.07	0.00	0.07	0.81	0.87

Table 2. Variation in intercepts and slopes of mass-length relationships. Displayed are the variance components of generalized linear mixed models used for our analyses of spatial structure in the scaling of salamander mass with different length measurements. Models included length (log-transformed) as a fixed effect and a random slope term, and pond as a random intercept term. Marginal R^2 denotes the amount of variation in mass explained by the length measurement alone, whereas conditional R^2 considers variation explained by the random intercept and slope terms.

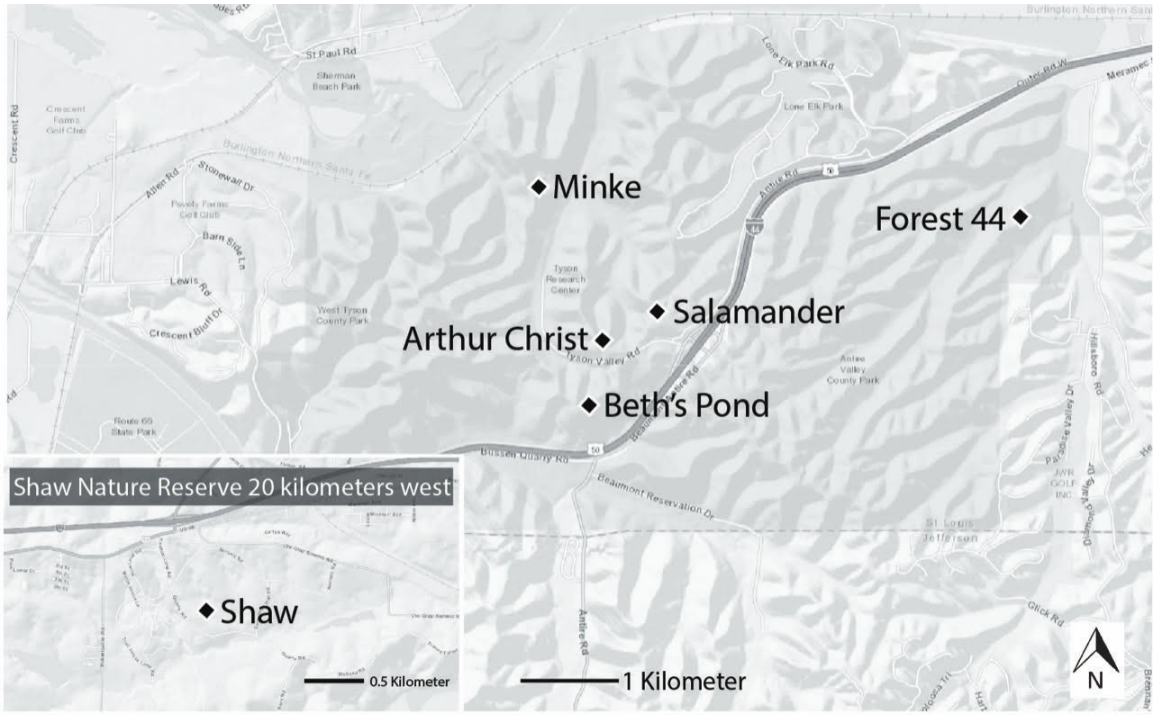

Figure 1. Map of study area. The six focal ponds were located in Eastern Missouri (U.S.A.) across three conservation areas. Mincke Pond, Arthur Christ Pond, and Beth's Pond were located in Tyson Research Center. Shaw pond was located in the Shaw Nature Reserve. Forest 44 pond was located in Forest 44 Conservation Area. All ponds occurred in Oak-Hickory forests typical of the region.

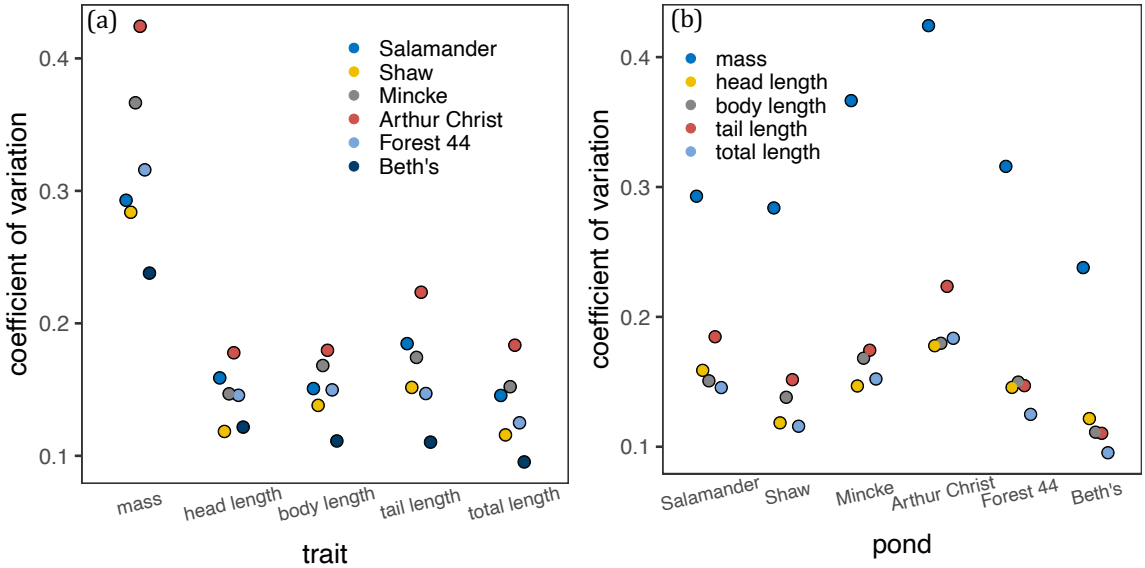

Fig. 2. Within-pond variation in salamander morphology. The coefficient of variation, or the extent of variance in relation to mean trait values, is shown for **(a)** mass and length measures and **(b)** the six ponds where we sampled salamanders, ordered from left to right in the chronological order in which they were sampled. Colors distinguish pond of capture in (a) and the focal trait in (b). Note that the same data are reported in (a) and (b).

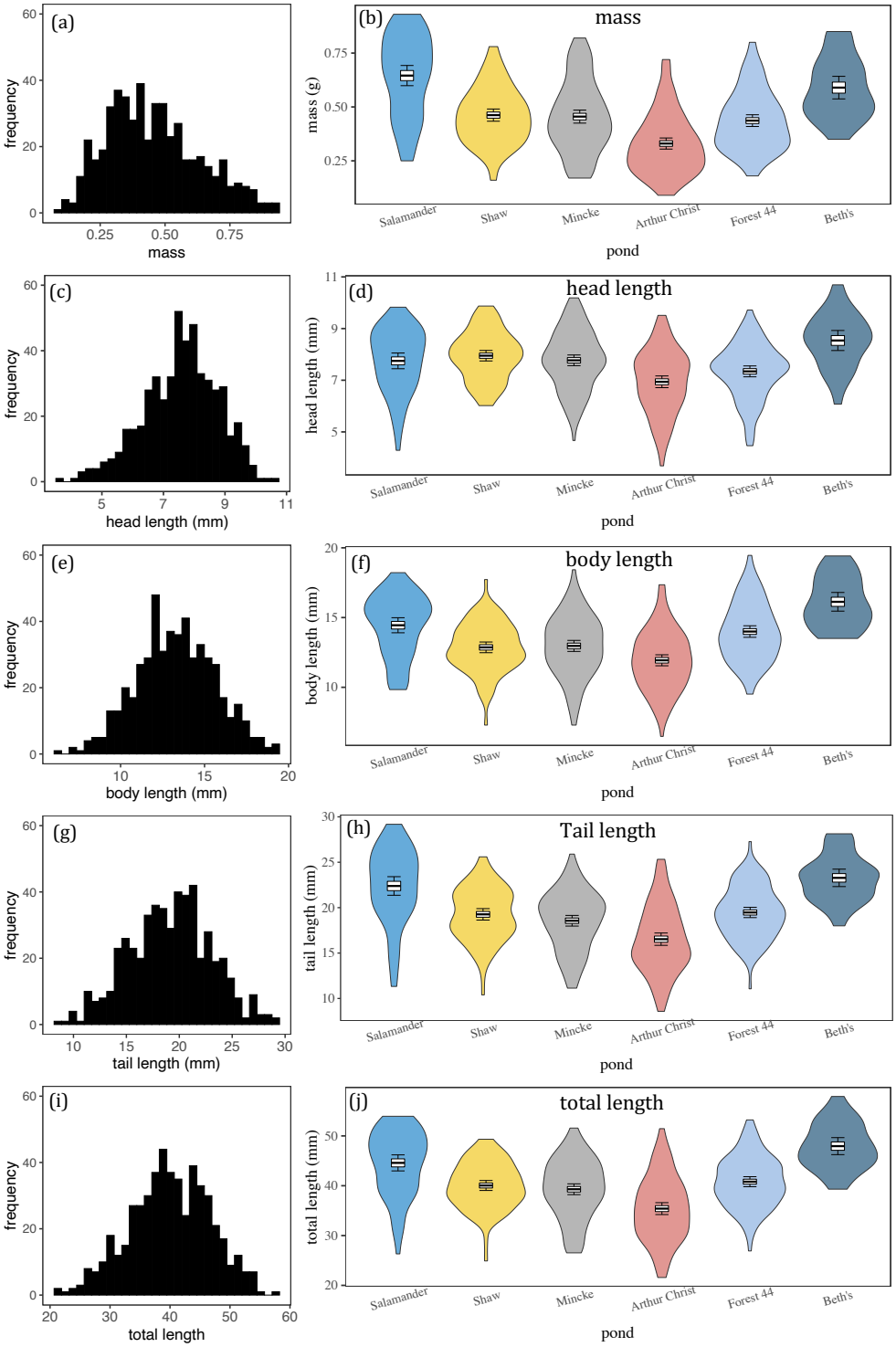
(a)
frequency
mass
(b)
mass
mass (g)
Salamander
Shaw
Mincke
Arthur Christ
Forest 44
Beth's
pond
(c)
frequency
head length (mm)
(d)
head length
head length (mm)
pond
(e)
frequency
body length (mm)
(f)
body length
body length (mm)
pond
(g)
frequency
tail length (mm)
(h)
Tail length
tail length (mm)
pond
(i)
frequency
total length
(j)
total length
total length (mm)
pond

Fig. 3. Salamander mass and length variation within and between ponds. Panels on left **(a,c,e,g,i)** are frequency distributions of mass and length measurements across all focal ponds. Violin plots on the right-side panels **(b,d,f,h,j)** distinguish individual-level and pond-level morphological variation to show how the traits were spatially structured. Box plots within the violin plots denote the mean, standard error, and 95% confidence intervals of trait measures. Ponds in the right-side panels are ordered from left to right in the chonological order in which they were sampled.

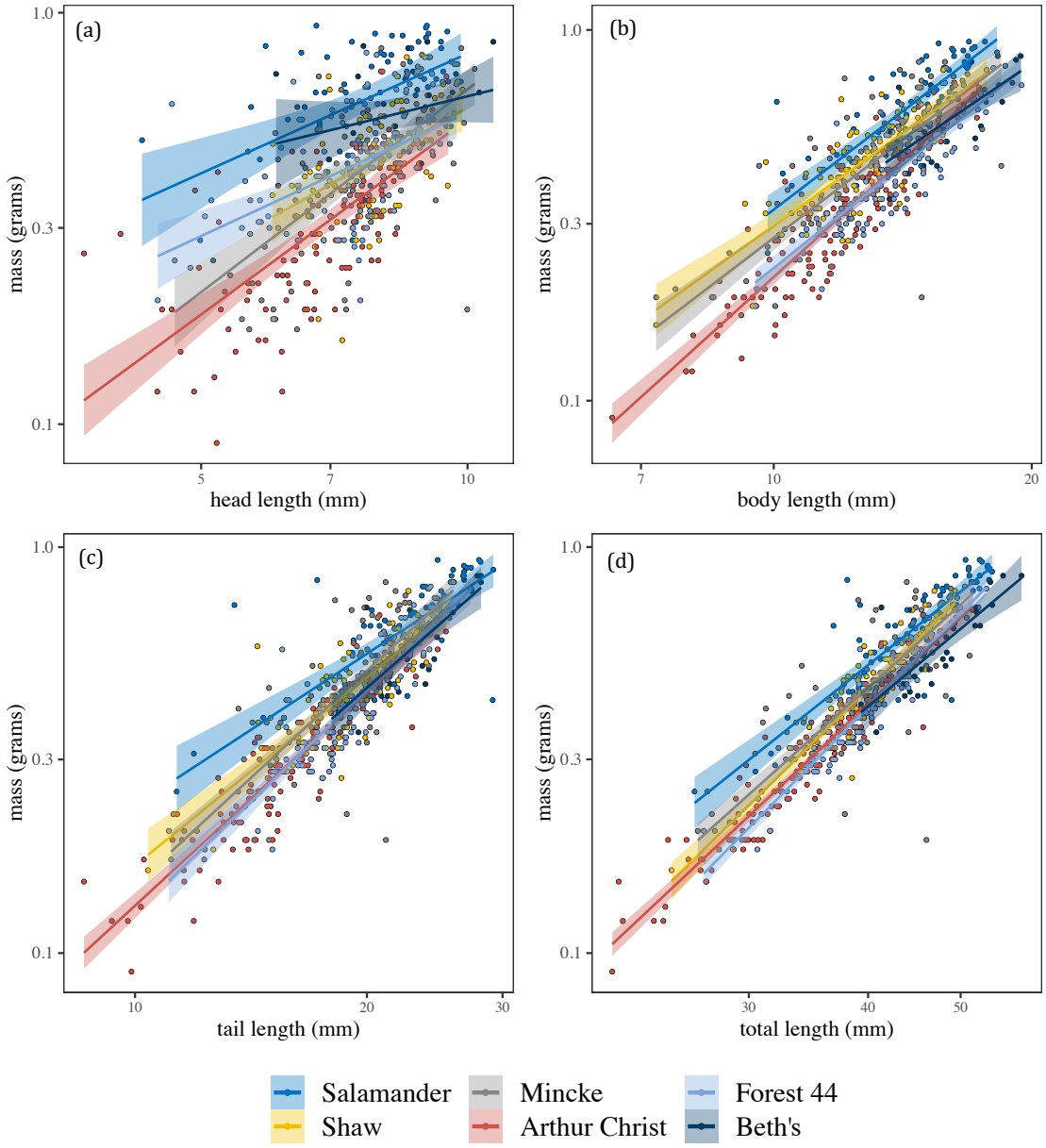

Fig. 4. Spatial structure in mass-length allometry in salamanders. Regression lines of salamander mass with (a) head length, (b) body length, (c) tail length, and (d) are shown for the six focal ponds, distinguished by line colors. Shaded areas show the 95% confidence intervals of the regression lines. Mass and length are plotted on a $\log_{10}$ scale in all cases.

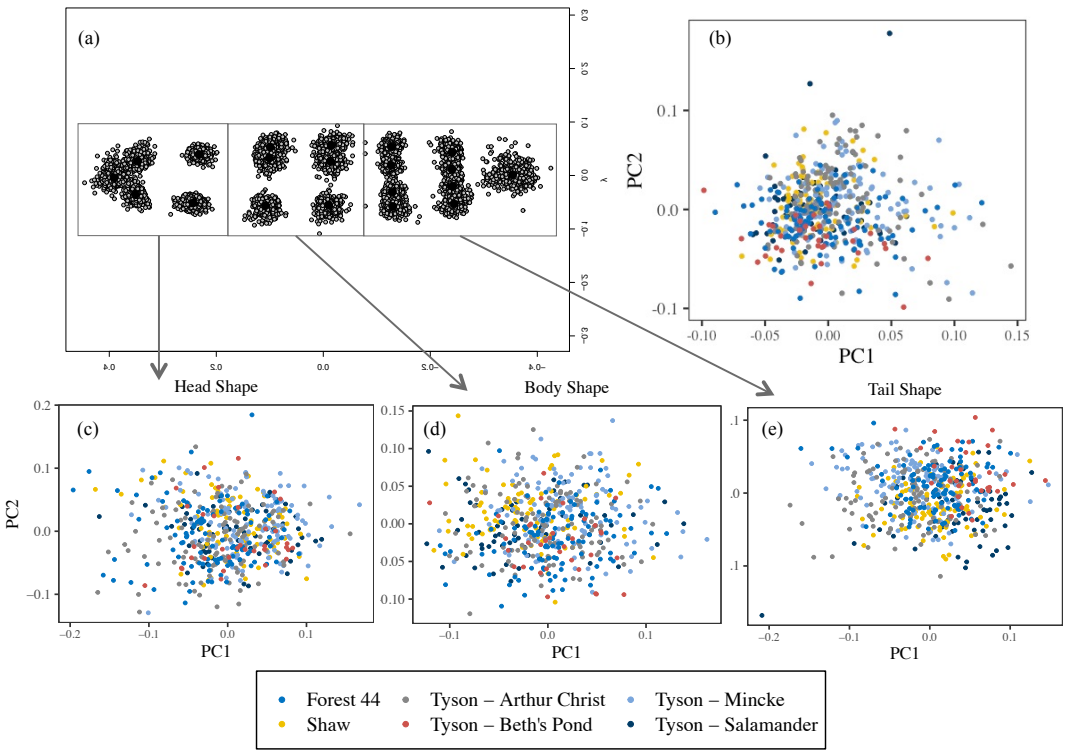

Fig. 5. Salamander shape variation within and among ponds. The shape values are based on sets of landmarks at different points along the lateral surface of salamander bodies (a). The overall (b), head (c), body (d), and tail (e) shape of salamanders collected from the six focal ponds. The shape values are based on sets of landmarks at different points along the lateral surface of salamander bodies. PC1 and PC2 values increase with elongation of shape and increasing length:height ratio.