

1 **Spatial variation in water loss predicts terrestrial**
2 **salamander distribution and population dynamics**

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19 Running head: Water loss predicts amphibian distribution

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23 analysed data, and wrote the manuscript. RDS contributed funding as well as
24 experimental and editorial advice.

25 **Abstract**

26 Many patterns observed in ecology, such as species richness, life history variation,
27 habitat use, and distribution have physiological underpinnings For many ectothermic
28 organisms temperature relations shape these patterns, but for terrestrial amphibians,
29 water balance may supersede temperature as the most critical physiologically-limiting
30 factor. Many amphibian species have little resistance to water loss, which restricts
31 them to moist microhabitats and may significantly affect foraging, dispersal, and
32 courtship. Using plaster models as surrogates for terrestrial plethodontid salamanders,
33 we measured water loss under ecologically-relevant field conditions to estimate the
34 duration of surface activity time across the landscape. Surface activity time was
35 significantly affected by topography, solar exposure, canopy cover, maximum air
36 temperature, and time since rain. Spatially, surface activity times were highest in
37 ravine habitats and lowest on ridges. Surface activity time was a significant predictor
38 of salamander abundance, as well as a predictor of successful recruitment; the
39 probability of a juvenile salamander occupying an area with high surface activity time
40 was two times greater than an area with limited predicted surface activity. Our results
41 suggest that survival, recruitment, or both are demographic processes that are affected
42 by water loss and the ability of salamanders to be surface active. Results from our
43 study extend our understanding of plethodontid salamander ecology, emphasize the
44 limitations imposed by their unique physiology, and highlight the importance of water
45 loss to spatial population dynamics. These findings are timely to understanding the
46 effects that fluctuating temperature and moisture conditions predicted for future
47 climates will have on plethodontid salamanders.

48 **Key-words:**

49 abundance, ecophysiology, foraging time, plaster models, *Plethodon albagula*

50 **Introduction**

51 An organism's physiology is dynamically related to its environment; physiology
52 dictates the habitats that are occupied and behaviour within these habitats, while the
53 environment can affect physiological performance and subsequently, ecological
54 performance (Huey 1991). In concert with the environment, physiology can affect an
55 organism's performance at the local scale (Brewster et al. 2013), life history at a
56 regional scale (Kearney 2012), and dictate limits on distribution (Buckley et al. 2010;
57 Gifford and Kozak 2012; Kearney and Porter 2009). Further, potential responses to
58 habitat or climate change can be modelled mechanistically by incorporating
59 physiology (Kearney et al. 2008; Keith et al. 2008; Sinervo et al. 2010). The role of
60 physiology is especially evident in ectothermic organisms, with the preponderance of
61 emphasis being placed on thermal aspects of behaviour, physiology, and life history
62 evolution (Angilletta 2009; Angilletta et al. 2004).

63 Although not independent of temperature and metabolic processes, water
64 balance is another critical physiological characteristic that weighs heavily on the
65 behaviour, distribution, and ecology of terrestrial taxa, especially amphibians (Tracy
66 et al. 2010; Wells 2007). The skin of most amphibians provides little to no resistance
67 to water loss (Spight 1968; Spotila and Berman 1976), even when the atmosphere is
68 near saturation (Adolph 1932). All terrestrial amphibians must manage their hydric
69 relations, but it is particularly critical for woodland salamanders of the genus
70 *Plethodon*. These salamanders are unique among terrestrial vertebrates in that they are
71 lungless and respire cutaneously by diffusion (Whitford and Hutchison 1967). As a
72 consequence, plethodontid skin must remain moist and permeable to facilitate gas
73 exchange, but these requirements impose physiological and ecological limitations.
74 Because of its permeability, the skin of plethodontid salamanders loses water at a rate

75 that is nearly identical to a free water surface of equivalent surface area (Peterman et
76 al. 2013; Spotila and Berman 1976). Uninhibited water loss impinges upon
77 salamander activity, potentially limiting foraging, dispersal, and reproductive efforts.
78 Terrestrial plethodontid salamanders spend the majority of their life under ground or
79 sheltered by cover objects such as rocks and logs on the ground surface (Petranka
80 1998). Surface activity and foraging of salamanders is greatest under moist conditions
81 (Grover 1998; Keen 1979; Keen 1984), and the duration of time spent foraging is
82 directly tied to water balance (Feder and Londos 1984). To minimize water loss,
83 salamanders are predominantly nocturnal, and are generally associated with cool,
84 moist microhabitats (Heatwole 1962; Peterman and Semlitsch 2013; Spotila 1972).

85 From a physiological perspective, four measurements are needed to predict the
86 duration of salamander surface activity: salamander mass (used to calculate surface
87 area; Whitford and Hutchison 1967), air temperature, relative humidity, and wind
88 speed (Feder 1983). These factors can be used to predict that surface activity will be
89 greatest for large salamanders when humidity is high, temperatures are cool, and there
90 is no wind. Ecologically, this means that adults may have an advantage over juveniles
91 in being able to sustain prolonged surface activity due to their lower surface area to
92 volume ratio, and microclimate variation produced by landscape features such as
93 topography may profoundly affect surface activity times by modulating temperature,
94 wind, and humidity. Limited surface activity may limit foraging time, and
95 consequently affect individual growth and reproduction. Dispersal may also be
96 curtailed, reducing gene flow among local populations.

97 Despite the intuitive effects that hydric constraints impose on terrestrial
98 plethodontid salamander activity time, habitat use, and population dynamics, direct
99 tests of these processes have been limited. Within a controlled laboratory setting,

100 Feder and Londos (1984) found that a stream salamander (*Desmognathus*
101 *ochrophaeus*, Cope) would abandon foraging in dry air twice as quickly as in moist
102 air (3.8% vs. 7.5% loss of body mass, respectively). Grover (1998) experimentally
103 demonstrated that surface activity of salamanders, especially juveniles, increased with
104 increased soil moisture. Peterman and Semlitsch (2013) found that terrestrial
105 salamander abundance was greatest in dense-canopy ravines with low solar exposure
106 and high moisture, and found evidence of differential reproductive success related to
107 these landscape features. Effects on population dynamics have indirectly been
108 observed through variation in egg production. Grover and Wilbur (2002) found that
109 salamanders in high moisture conditions produced more eggs, and both Milanovich et
110 al. (2006) and Maiorana (1977) found annual fecundity to increase with precipitation.
111 These findings suggest that wetter conditions may accommodate increased surface
112 activity and foraging, increasing the energy available to be allocated to reproduction.

113 By incorporating physiology with spatial and temporal climate variation,
114 mechanistic biophysical models are capable of accurately predicting the distribution
115 (Kearney and Porter 2009), biotic interactions (Buckley and Roughgarden 2005;
116 Gifford and Kozak 2012), and life history variation (Kearney 2012; Tracy et al. 2010)
117 of species. To encompass spatial heterogeneity, most of these studies cover broad
118 geographical or elevational ranges. However, environmental gradients can occur over
119 significantly smaller spatial scales in topographically complex landscapes (Bennie et
120 al. 2008; Chen et al. 1999). Further, fine-scale variation in microclimate can affect
121 species occurrence, population dynamics, and resilience to changing climatic
122 conditions, especially in species with low vagility (Antvogel and Bonn 2001;
123 Peterman and Semlitsch 2013; Scherrer and Körner 2011; Weiss et al. 1988).
124 Although the importance of fine-scale microclimatic variation is well-understood

125 (Huey 1991), most analyses of physiological processes have not been spatially
126 explicit.

127 The objective of our study was to explicitly test, for the first time, the
128 hypothesis that water balance is a limiting factor for terrestrial salamanders (Feder
129 1983). Specifically, that spatial variation in water loss and surface activity time affects
130 the distribution of salamanders as well as population dynamics across the landscape.
131 We model physiological landscapes describing fine-scale spatial variation in water
132 loss rates for a terrestrial plethodontid salamander, *Plethodon albagula* (western slimy
133 salamander), and then convert these rates to potential surface activity times. In
134 calculating rates of water loss surface activity time we seek to (1) determine the
135 landscape and environmental factors influencing spatial variation in water loss in a
136 topographically complex landscape, (2) determine whether salamander distribution on
137 the landscape can be predicted by the physiological limitations imposed by water loss
138 and activity time, and (3) assess the effects of surface activity time on spatial
139 population dynamics. We hypothesized that rates of water loss would be dependent
140 upon both topographical landscape features as well as climatic conditions.
141 Specifically, we predicted that topographic complexity would result in heterogeneous
142 water loss rates across the landscape and that ravine habitat with low solar exposure
143 would have the lowest rates of water loss. Temporally, we predicted that abiotic
144 factors such as time since rain, air temperature, and relative humidity would
145 significantly affect daily and seasonal patterns of water loss. Because Peterman and
146 Semlitsch (2013) found salamander abundance to be greatest in sheltered ravine
147 habitats and lowest on ridges, we hypothesized that spatial patterns of water loss
148 would corroborate these patterns with ravines exhibiting low rates of water loss and
149 ridges high rates of water loss. We also hypothesized that water loss, as an integrated

150 measure of the landscape and climate, would significantly predict the spatial
151 distribution of salamander abundance. Lastly, as a mechanism limiting population
152 growth, we hypothesized that evidence of successful reproduction would be greatest
153 in ravines with lower rates of water loss.

154

155 **Materials and methods**

156 STUDY SPECIES

157 *Plethodon albagula* (western slimy salamander, Grobman) are a large plethodontid
158 salamander of the *P. glutinosus* species complex that live in forested habitats
159 throughout the Ozark and Ouchita mountains of Missouri, Arkansas, eastern
160 Oklahoma, and northeastern Texas, USA (Highton 1989). Within these forested
161 habitats, salamander abundance is greatest in moist, forested ravines (Peterman and
162 Semlitsch 2013). Surface activity varies seasonally, with peak activity occurring in
163 spring and to a lesser extent during autumn (Milanovich et al. 2011); terrestrial
164 plethodontid salamanders generally seek subterranean refuge during hot, dry summer
165 conditions (Taub 1961).

166

167 PLASTER MODELS

168 We assayed water loss across the landscape using cylindrical plaster of Paris models
169 (hereafter “replicas”) as analogues for live salamanders. Plaster replicas were made
170 following methods described by Peterman et al. (2013), and had surface areas
171 equivalent to adult- and juvenile-sized salamanders that were 7.25 g and 2.25 g,
172 respectively. Previous research has shown these replicas lose water linearly and at
173 rates equivalent to similarly-sized salamanders (Peterman et al. 2013). Models were
174 cured in a drying oven for 24 h at 70°C, and then weighed to the nearest 0.01 g on a

175 portable digital balance (Durascale, My Weigh, Vancouver, BC). Prior to deployment,
176 all replicas were soaked in water for at least four hours; replicas were deployed within
177 one hour of sunset, and retrieved within one hour of sunrise.

178 Replicas were deployed at Daniel Boone Conservation Area (DBCA; Fig. 1)
179 along 250-m long transects, spaced at approximately 50-m intervals (n=18 transects;
180 108 locations). Locations of replica deployment were marked in the field using a
181 handheld GPS (Garmin 62sc, Olathe, Kansas, USA) with multiple locations being
182 taken until the estimated precision was ≤ 3 m. Replicas were deployed in both spring
183 (8 April–8 May 2012) and summer (15 August–28 August 2012). At each location,
184 adult- and juvenile-sized replicas were deployed under the leaf litter, and another pair
185 was deployed on top of the leaf litter. Because the focus of this study was the effects
186 of landscape and climate features on water loss, all replicas were housed within
187 cylindrical cages made of 3 mm hardware cloth to prevent replicas from coming in
188 direct contact with leaf litter or soil, which could have confounding effects on water
189 loss rates (Peterman et al. 2013). Each replica was weighed with the portable digital
190 balance upon deployment and retrieval.

191

192 SPATIAL AND TEMPORAL COVARIATES

193 Spatial covariates used in this analysis were calculated in ArcGIS 9.3 (ESRI,
194 Redlands, CA, USA) and are described in detail in (Peterman and Semlitsch 2013).
195 Previously, these covariates were used to predict the spatial distribution of abundance
196 of *P. albagula* (see details below). In the current study we assessed the effects of
197 topographic position (TPI), topographic wetness index (TWI), potential relative
198 radiation (PRR), and distance from stream. These variables have a resolution of 3 m,
199 and were derived from 1/9 arc second National Elevation Dataset

200 (<http://seamless.usgs.gov/products/9arc.php>). Canopy cover was also estimated at
201 DBCA using the normalized difference vegetation index (NDVI), which was
202 calculated from cloud-free Landsat 7 satellite images of our study area taken on 15
203 June, 20 July, 9 August 2012 (<http://glovis.usgs.gov/>). A mean NDVI was calculated
204 by averaging these days together. The resolution of the NDVI layer was 30 m, so it
205 was resampled to a resolution of 3 m. Because the majority of our spring trials were
206 conducted prior to full leaf-out, NDVI was not included in the spring models. For this
207 analysis, we used time since rain, maximum overnight humidity, and maximum
208 temperature of the previous day as temporal climatological covariates. These data
209 were collected from the Big Spring weather station (<http://www.wunderground.com>),
210 which is located 8 km west of DBCA. For extrapolating our model to the entire
211 DBCA landscape, we determined averages for these measures in spring (1 April–31
212 May) and summer (1 June–31 August) from data collected 2005–2012.

213

214 STATISTICAL ANALYSES

215 For each replica we calculated the proportion of water lost per hour (*proportion loss*
216 $= [deployed\ mass - retrieved\ mass] / [deployed\ mass - dry\ mass] / time\ deployed$),
217 which became our dependent variable. For this analysis, we did not have competing *a*
218 *priori* hypotheses concerning the factors that would affect water loss, but rather, we
219 were interested in fitting the best model possible to explain the spatial and temporal
220 patterns of water loss in our plaster replicas. As such, we did not conduct extensive
221 model selection on parameters to include or exclude from each model, but instead fit a
222 small number of meaningful parameters to each model. Our modeling work flow
223 proceeded as follows. We first divided our data by replica size and season (size-
224 season) to create four independent data sets (juvenile-spring, juvenile-summer, adult-

225 spring, and adult-summer). We then assessed the correlation of each of our
226 independent variables with each other, as well as their correlation with the dependent
227 variable. If two variables had a Pearson's correlation $r \geq 0.70$, we excluded the variable
228 that had the lowest correlation with the dependent variable. Lastly, to limit complexity
229 we did not include interactions of independent variables, and excluded variables that
230 had $r < 0.10$ correlation with the dependent variable. To account for heterogeneous
231 variance in our data, we fit different variance structures to our data using 'nlme' in R
232 (Pinheiro et al. 2013; R Core Team 2013; Zuur et al. 2009). Model selection was
233 based on AIC (Akaike 1974). Using the model with the best-fit variance structure, we
234 then tested different random effects parameterizations to account for the nested nature
235 of our data (i.e. models within location, locations within transect, transects within
236 date). The percent variance explained by our top model for each size-season
237 combination was assessed using the marginal R^2 measure of Nakagawa and Schielzeth
238 (2013) and calculated with 'MuMIn' (Barton 2013). The marginal R^2 describes the
239 percent variation explained in the fixed effects model alone. The full list of variance
240 structures and random effects parameterizations tested in model selection can be
241 found in Appendix S1.

242 The fixed effects parameter estimates for the top size-season models were then
243 used to predict water loss rates across the DBCA landscape. Replica position (under
244 leaves or on the surface) was a factor in each model, so for each size-season
245 combination, we calculated a surface and a leaf water loss estimate. For the remainder
246 of this paper we consider salamander surface activity to be evenly divided between
247 these two states (i.e. 50% surface, 50% under leaves). Therefore, to calculate a single
248 size-season water loss rate, we averaged the model predictions from surface and leaf
249 models. Because the main objective in this study is to demonstrate water loss as a

250 limiting factor for terrestrial salamanders, we converted water loss rates to surface
251 activity times (SAT). There is no empirical data describing the threshold of water loss
252 when terrestrial plethodontid salamanders cease surface activity and seek refuge, and
253 only one study has experimentally assessed this in a stream-associated salamander
254 (Feder and Londos 1984). Previous studies have used 10% of body mass lost as the
255 point at which salamanders stop foraging (Feder 1983; Gifford and Kozak 2012). For
256 our study, we used 10% of total water lost as the threshold; SAT was calculated as the
257 time (hrs) to 10% water loss. It should be noted that the proportion of a salamander's
258 body mass comprised of water decreases as mass increases (Peterman et al. 2013):

259
$$\text{Proportion Water} = (-0.0168 * \text{live salamander wet mass (g)}) + 0.8747.$$

260 Ten percent mass loss for juvenile and adult salamanders of sizes equivalent to our
261 replicas would result in 11.9% and 13.3% loss of water, respectively.

262 One of our objectives in this study is to determine how predicted SAT relates
263 to the predicted spatial distribution of abundance. The methods and model used to
264 predict salamander abundance across the landscape are described in detail by
265 Peterman and Semlitsch (2013). Briefly, we surveyed 135 plots at DBCA that were
266 spaced ≥ 75 m apart seven times in the spring of 2011. We fit binomial mixture
267 models to our repeated count data using a Bayesian framework (Royle 2004). To
268 account for imperfect observation of salamanders in space and time, we modeled
269 salamander detection probability as a function of survey date, the number of days
270 since a soaking rain event (rain ≥ 5 mm), and temperature during each survey. After
271 correcting for imperfect detection, abundance was modeled as a function of NDVI,
272 TPI, TWI, and PRR. We then projected the fitted abundance model across the
273 landscape to spatially represent the distribution of salamanders at DBCA.

274 We conducted Pearson product-moment correlation tests between the
275 abundance estimates at the 135 survey plots from Peterman and Semlitsch (2013) and
276 the spatial SAT predictions made in this study to get a point estimate correlation. We
277 also assessed spatial patterns of correlation between SAT and abundance within
278 ArcGIS using a moving window correlation (Dilts 2010) with a window size of 51 m
279 (17 x 17 pixels). SAT is a physiological measure estimated from several of the same
280 landscape covariates included in the abundance model of Peterman and Semlitsch
281 (2013). To estimate the strength of SAT as a predictor of abundance, we re-ran the
282 binomial mixture model of Peterman and Semlitsch (2013) in this study, but modeled
283 abundance at each of the 135 survey plots solely as a function of SAT. Details of the
284 model parameterization and settings can be found in Appendix S2.

285 Peterman and Semlitsch (2013) also used multistate models to identify a
286 potential disconnect between reproductive effort (presence of gravid females) and
287 realized recruitment (presence of juveniles). We generalize that analysis for this study
288 to estimate the probability of juvenile and adult occurrence at each of the 135 plots
289 surveyed by Peterman and Semlitsch (2013). We constructed multistate models using
290 a conditional binomial parameterization in program PRESENCE v3.1 (MacKenzie et
291 al. 2009). Models were fit separately for adult and juvenile salamanders, with three
292 states being present in each model: (1) no salamanders present (site unoccupied); (2)
293 salamanders present, but focal size class absent; (3) focal size class present, where the
294 focal size class is either adult (snout-vent length [SVL] ≥ 55 mm; Milanovich et al.
295 2006) or juvenile (SVL < 55 mm), respectively. As in the abundance model described
296 above, we replaced the individual landscape covariates used by Peterman and
297 Semlitsch (2013) with our integrated SAT measure. From this model we estimated the
298 conditional probability of occurrence, which is the probability of a focal demographic

299 group occurring at a site, given that a site is suitable to be occupied. Extended details
300 of this analysis and model parameterization are in Peterman and Semlitsch (2013) and
301 Appendix S2.

302 Lastly, we determined the mean SVL of salamanders observed at each of the
303 135 survey plots, and used a linear model to assess the relationship between SVL and
304 SAT. Our objectives in re-analysing the data of Peterman and Semlitsch (2013) are to
305 determine if SAT, as an integrated multivariate parameter, predicts abundance and
306 occupancy of demographic groups, thereby providing a physiological mechanism for
307 the effects of environmental gradients.

308

309 **Results**

310 Correlations among independent variables revealed that TPI and distance from stream
311 were highly correlated ($r = 0.74$), but TPI had a greater correlation with rate of water
312 loss in the spring data sets, and distance to stream had a greater correlation in summer
313 data sets. We also found TWI and maximum overnight humidity to have low
314 correlation with water loss across all size-season combinations ($r \leq 0.07$), so these
315 variables were not included in the mixed effects models. To account for heterogeneity
316 within our data, an exponential variance structure was fit to both the juvenile and
317 adult spring data, a combined identity-exponential variance structure was fit to the
318 juvenile summer data, and an identity variance structure was fit to the adult summer
319 data (Table 1). Random-effects fit to each model had both slopes and intercepts
320 varying by covariates (Table 1). The average interval between rainfall events, as
321 determined from the seven years of climate data, is 1.5 days (± 1.98 SD) and 2.2 days
322 (± 2.85 SD) and the average daily maximum temperature is 22.5°C (± 6.22) and 31.2°C

323 (± 4.00 SD) for spring and summer seasons, respectively. These seven-year mean
324 estimates were used to make spatial predictions of water loss.

325 Our final mixed effects models explained the majority of the variance in our
326 data ($R^2_{\text{GLMM}(m)} = 82.90\% - 98.69\%$; Table 1). Notably, simple linear regression
327 models that do not properly account for heterogeneity in variance or the nestedness of
328 our sampling design described 67.15%–81.60% of variation in our data (Table 1).
329 Plaster replica position was a significant predictor of water loss rate for both replica
330 sizes in both seasons, with replicas on the surface losing 1.26%–2.64% more water
331 per hour than adjacent replicas placed under leaves (Table 1). In the spring, water loss
332 in juvenile replicas increased significantly with topographic position (TPI) meaning
333 that water loss was greatest in ridge-like habitat and least in ravine-like habitats. In
334 contrast, topographic position had no effect on adult replicas. Distance from stream
335 had a significant effect on both juvenile and adult replica water loss in the summer
336 with water loss rates increasing with distance from streams. Solar exposure (PRR) had
337 no effect in the spring, but significantly increased rates of water loss in the summer
338 (Table 1). The number of days since rainfall also significantly increased the rate of
339 water loss in all size-season replicas. As anticipated, water loss increased with
340 maximum temperature in the spring for both juvenile and adult replicas. Surprisingly,
341 temperature had no effect on adult replica water loss in the summer, and had a
342 negative effect on juvenile replica water loss (Table 1). Lastly, canopy cover, as
343 measured by NDVI, was found to have no effect on juvenile replica water loss, but
344 had a significant effect on adult replica water loss; as canopy cover increased, adult
345 replica water loss decreased.

346 Spatially, there is extensive congruence among each size-season SAT map
347 (Fig. S1), and correlations among these ranged from 0.62–0.95 (Table S1). The

348 highest SAT are concentrated within ravine habitats, which are separated by ridges
349 with lower SAT. Mean SAT on the landscape ranged from 1.94 hrs for juveniles in
350 the summer, to 9.90 hrs for adults in the spring (Table 2). Paired t-tests revealed that
351 juvenile SAT is significantly less than adult SAT in spring and summer, and that all
352 SAT are significantly less in the summer (all tests $P < 0.0001$). In general, the
353 estimated SAT is 3 times longer in spring than summer, and is about 1.5 times longer
354 for adults than juveniles, regardless of season (Table 2, Fig. S1). Correlations of
355 predicted salamander abundance with size-season SAT at the 135 survey plots were
356 also high ($r = 0.35\text{--}0.63$; Table 2). Adult summer SAT had the highest correlation with
357 predicted abundance ($r = 0.63$), largely because of the significance of canopy cover in
358 mitigating water loss (Table 1). Spatial similarities between predicted salamander
359 abundance and adult summer SAT are evident (Fig. 3a–b); the correlation between
360 abundance and SAT is generally highest in areas of low predicted abundance and low
361 SAT (Fig. 3c).

362 Because adult summer SAT had the highest correlation with abundance, we
363 explored in more detail its relations with abundance, salamander size distribution, and
364 probability of occurrence. We do note, however, that the other size-season models
365 also had significant correlations with predicted abundance ($r = 0.35\text{--}0.48$; Table 2).
366 The binomial mixture model fit with predicted adult summer SAT as the sole
367 independent variable in the abundance model fit the data well, and SAT had a
368 significant effect on abundance, with abundance increasing as predicted SAT
369 increased (Appendix S2; Fig. 3a). Further, we found that the mean SVL of
370 salamanders observed at 88 of the 135 surveyed plots ($n = 407$ unique salamanders
371 measured; Peterman and Semlitsch 2013) significantly increased as predicted SAT
372 decreased ($F_{1, 86} = 8.38$; $P = 0.005$; $R^2 = 0.089$; Fig. 3b), suggesting that, on average,

373 larger salamanders are found in areas with limited SAT. Similarly, we found that the
374 conditional probability of juvenile salamander occupancy at the 135 surveyed plots,
375 correcting for imperfect detection, significantly increased as predicted SAT increased
376 (Appendix S2, Fig. 3c). In contrast, the conditional probability of adult occupancy
377 was not significantly related to adult summer SAT (Fig. 3c), and there was little
378 variation in predicted adult occupancy probability across the range of predicted SAT
379 (adult conditional occupancy probability = 0.91–0.95). Predicted conditional
380 occupancy probabilities of juveniles at the same 135 sites ranged from (0.35–0.92;
381 Fig. 3c).

382

383 **Discussion**

384 Our study assessed patterns of water loss as a process that varies spatially and
385 temporally as a function of fine-scale environmental gradients and temporal climatic
386 conditions. We found that spatial estimates of SAT derived from rates of water loss
387 were significantly correlated with predicted salamander abundance and that SAT was
388 a significant predictor of abundance as well as population demographic
389 characteristics. Importantly, our SAT estimates were independently derived from
390 plaster replicas deployed under field conditions, and were in no way contingent upon
391 actual salamander distributions. Results from our study extend our understanding of
392 plethodontid ecology and emphasize the limitations imposed by their unique
393 physiology. Previous research has only logically conjectured the importance of hydric
394 relations and surface activity as mechanisms underlying local distribution and
395 population dynamics by extrapolating results from controlled laboratory experiments
396 or indirectly through field observations (Feder 1983; Spotila 1972). As an integrated
397 measure of the local environment and climate, SAT was a significant predictor of

398 abundance as well as population dynamics. Combined with our findings that SAT and
399 abundance are spatially correlated, we have compelling evidence that water loss is a
400 physiologically-limiting factor underlying the abundance-habitat and population
401 dynamic relationships described by Peterman and Semlitsch (2013).

402 Water balance can be particularly critical for smaller organisms, and we found
403 that juvenile-sized plaster replicas lost water at 1.5–3 times greater rate than adult-
404 sized replicas. Such differences significantly curtail surface activity, and could lead to
405 differential survival across the landscape. In support of this, we found that the mean
406 body size of salamanders was smaller in plots with lower rates of water loss and high
407 SAT (Fig. 3b). Further, we found that the probability of encountering a juvenile
408 salamander in areas of high SAT was significantly greater than areas of low SAT. In
409 contrast, we found that adults were more uniformly distributed across the landscape,
410 regardless of SAT (Fig. 3c). These patterns suggest that reproductive rates may be
411 greater in high SAT regions of the landscape, or that survival of juvenile salamanders
412 is higher in high SAT areas. Either or both of these processes would contribute to the
413 increased abundance of salamanders in high SAT regions (Fig. 3a). Differentiating
414 these processes as the mechanisms underlying the spatial variation in size distribution
415 will likely only be possible through long-term, detailed studies of local demographic
416 processes.

417 In corroboration with seasonal patterns of surface activity of salamanders in
418 the field (Milanovich et al. 2006), estimated SAT differed significantly among spring
419 and summer seasons (Table 2; Fig. S1). Although SAT was three times greater in the
420 spring, there is still pronounced spatial heterogeneity in SAT due to the influence of
421 topographic position in affecting water loss. The mixed effects models describing the
422 spatial patterns of water loss for adult- and juvenile-sized replicas in the spring were

423 nearly identical (Table 1). In the summer, juvenile replicas had no relationship with
424 canopy cover, while adult replicas lost significantly less water as canopy cover
425 increased. We speculate that the rate of water loss was so rapid in the high surface
426 area juvenile models that canopy cover did little to attenuate losses. Although
427 Peterman et al. (2013) found water loss rates of plaster replicas to be linear over an 8-
428 hr laboratory test with up to 35% water loss, we note the possibility that rates of water
429 loss could become non-linear as dehydration deficits approaches 100% (summer
430 dehydration deficit for juvenile replicas: mean=60.3%, max=98.5%; adult replicas:
431 mean= 39.4%, max=82.1%). Such non-linearity could contribute to the observed
432 differences in parameter estimates for adult and juvenile models.

433 If reproductive success differs across the landscape, then *P. albagula* may best
434 be described as existing as a spatially-structured population (Harrison 1991; Thomas
435 and Kunin 1999). Specifically, reproductive rates and success may be greatest within
436 forested ravines with high SAT, and be negligible or non-existent where SAT is low.
437 As such, the presence of salamanders in low SAT areas of the landscape would
438 predominantly depend upon salamanders dispersing from high SAT regions, implying
439 fine-scale source-sink dynamics (Pulliam 1988). Little is known concerning dispersal
440 in plethodontid salamanders, but as adults they are generally considered to be highly
441 philopatric with small home ranges (Kleeberger and Werner 1982; Ousterhout and
442 Liebgold 2010). *Plethodon cinereus* (Green), a smaller species of woodland
443 salamander, have been found to have significant genetic differentiation over small
444 spatial scales within continuously forested habitat (200 m; Cabe et al. 2007) and to
445 have male-biased dispersal (Liebgold et al. 2011). Marsh et al. (2004) also found the
446 majority of dispersing *P. cinereus* to be young adults. From a water loss perspective,
447 smaller individuals with higher surface areas will incur the greatest cost of dispersing,

448 so the finding of Marsh et al. (2004) that young adults are the dispersing size class
449 may indicate a trade-off between maximizing the benefits of dispersing (e.g.,
450 reduction of kin competition, metapopulation processes, inbreeding avoidance;
451 Hamilton and May 1977; Olivieri et al. 1995; Waser et al. 1986) while minimizing
452 costs by not dispersing as very small, desiccation-prone juveniles. Explicit testing of
453 how spatial variation in activity time affects population genetic structure may provide
454 greater insight into how physiology and behaviour shape population processes.

455 Our findings suggest that water relations temporally and seasonally shape
456 activity times, locally dictate habitat use, and regionally delineate distributions.
457 Nonetheless, water loss is not a physiological process working in isolation. Metabolic
458 rates of ectotherms are temperature dependent, increasing with environmental
459 temperature. Because evaporative water loss also increases with temperature (Spotila
460 1972; Tracy et al. 2010), plethodontid salamanders are doubly challenged under hot,
461 dry conditions. As metabolic demands increase with temperature there is a greater
462 need for energy intake, but surface activity will likely be curtailed at higher
463 temperatures due to increased rates of water loss. The relationship of energy
464 expenditure and intake, as a function of temperature and foraging time (limited by
465 water loss), was incorporated into a mechanistic energy budget model and used to
466 accurately predict the elevational distribution of a montane woodland salamander
467 (Gifford and Kozak 2012). Although temperature variation exists across our
468 landscape and correlates with predicted abundance (Peterman and Semlitsch 2013),
469 the independent (or interactive) role that spatial variation in temperature has on
470 salamander metabolic rate, and subsequently on abundance and population dynamics,
471 is unclear. Mechanistic modelling approaches, as used by Gifford and Kozak (2012),
472 may be able to provide insight into these questions.

473 Although we observed significant spatial correlation between SAT and
474 predicted salamander abundance, correlations were not perfect. Included in the
475 original abundance model of Peterman and Semlitsch (2013) were topographic
476 wetness and an interaction between topographic wetness and solar exposure. These
477 terms were not included in our mixed effects models to limit model complexity and
478 because there was minimal correlation with measured rates of water loss. Exclusion of
479 these factors could explain some of the SAT-abundance discrepancies, although our
480 mixed effects models were able to explain the majority of the variation in our data,
481 leaving little unexplained variance to be accounted for by other factors.

482 Plaster replicas effectively mimicked water loss rates of living salamanders
483 (Peterman et al. 2013), but we nonetheless made several simplifying assumptions.
484 First, evaporative water loss in wet-skinned amphibians is determined by the moisture
485 content of the air and the difference in the water vapour density at the surface of the
486 animal (Spotila et al. 1992), but atmospheric moisture can vary over small spatial
487 scales and as a function of topography and vegetation (Campbell and Norman 1998).
488 While we attempted to account for humidity variation by using synoptic
489 meteorological measurements, relative humidity did not correlate with water loss and
490 was omitted from our mixed effect models. Fine-scale estimation of variation of
491 relative humidity is likely necessary to more accurately estimate evaporative water
492 loss in salamanders, but we note that TPI and distance from stream in our study likely
493 correlate strongly with fine-scale humidity variation (Holden and Jolly 2011). Second,
494 under wind-free conditions, a boundary layer will form around a stationary object
495 (Tracy 1976), which reduces the rate of evaporative water loss. Our estimates of water
496 loss from plaster models are therefore likely conservative as foraging or dispersal
497 movements of surface active salamanders would disrupt the boundary layer and

498 increase rates of water loss. Third, a critical aspect of terrestrial salamander water
499 balance is their ability to rehydrate by absorbing water across their skin (Spotila
500 1972), but we sought to avoid contact of our replicas with the leaf litter and soil to
501 minimize the potentially confounding effects of these factors on evaporative water
502 loss.

503 Our study is the first to estimate spatially-explicit rates of water loss for a
504 terrestrial amphibian under relevant ecological field conditions. Previous research has
505 carefully detailed the physiological relationships of amphibians with their
506 environment (reviewed by Feder 1983; Shoemaker et al. 1992; Spotila et al. 1992;
507 Wells 2007), but only superficial attempts have been made to relate physiology with
508 patterns observed in nature (Spotila 1972). While water loss is unlikely to be the only
509 factor limiting terrestrial salamander activity and spatial distributions, our results
510 provide strong support that it is critical. Future work in this system should explore
511 how temperature, metabolic rate, and spatial energy budgets (Gifford and Kozak
512 2012) relate to patterns of abundance and population processes. Additionally, spatial
513 genetic processes of terrestrial salamanders are largely unknown, but understanding
514 how fine-scale environmental gradients relate to population and landscape genetics
515 may provide critical insight into how physiology affects local population dynamics
516 and dispersal. Lastly, our findings that abundance and spatial demographic patterns
517 can be predicted by SAT have implications for the future persistence of terrestrial
518 salamanders. Across seasons, we found that maximum temperature and time since
519 rain were critical predictors of water loss. Current climate change scenarios are
520 forecasting more extreme temperatures and increased variability in the interval and
521 amount of rainfall (Field et al. 2012), and changes in these climatological parameters
522 may profoundly affect terrestrial salamanders (Milanovich et al. 2010; Walls 2009).

523 By incorporating water loss and surface activity time into biophysical or dynamic
524 population models, it may be possible to gain a better understanding of the effects that
525 changing environmental and climatological conditions have on plethodontid
526 salamanders.

527

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537

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- 705

706 **Figures**

707 Figure 1. Locations of plaster replica deployment at Daniel Boone Conservation Area,
708 Missouri, USA. Replicas were deployed at six locations separated by 50 m along each
709 transect, and there were 18 transects (n = 108 locations).

710

711 Figure 2. Maps of (a) predicted salamander abundance, (b) summer surface activity
712 time (hrs) estimated from adult-sized plaster replicas, and (c) spatial Pearson's *r*
713 correlation values. There is generally very high, positive correlation between
714 estimated abundance and surface activity time; as activity time increases, so does the
715 predicted abundance of salamanders.

716

717 Figure 3. Relationship of estimated adult summer active time with (a) abundance; (b)
718 mean SVL of salamanders; and (c) conditional probability of occurrence of adult and
719 juvenile size classes. Dashed lines around the estimates represent 95% prediction
720 intervals. Increased surface activity time resulted in more salamanders being present,
721 and juvenile salamanders were more likely to be found in areas with higher surface
722 activity times.

723

724 Table 1. Parameter estimates, standard errors (SE), and parameter significance from mixed effects model analyses of water loss rate for adult-
 725 and juvenile-sized plaster of Paris replicas.

| Fixed effects parameters | Spring water loss models | | | | | | Summer water loss models | | | | | |
|-------------------------------|------------------------------------|----------|----------|------------------------------------|----------|----------|--|----------|----------|----------------------|----------|----------|
| | Juvenile | | | Adult | | | Juvenile | | | Adult | | |
| | Estimate | SE | <i>P</i> | Estimate | SE | <i>P</i> | Estimate | SE | <i>P</i> | Estimate | SE | <i>P</i> |
| Intercept | -3.968 | 0.931 | <0.0001 | -1.794 | 0.402 | <0.0001 | 16.075 | 6.874 | 0.022 | 6.360 | 4.903 | 0.201 |
| Position (surface) | 2.264 | 0.352 | <0.0001 | 1.155 | 0.184 | <0.0001 | 1.721 | 0.669 | 0.012 | 1.256 | 0.109 | <0.0001 |
| TPI ^a | 0.060 | 0.017 | 0.0006 | 0.004 | 0.007 | 0.546 | - | - | - | - | - | - |
| Dist from stream ^a | - | - | - | - | - | - | 0.007 | 0.002 | 0.005 | 0.007 | 0.002 | 0.006 |
| PRR | 0.000053 | 0.000060 | 0.378 | 0.000034 | 0.000027 | 0.206 | 0.000334 | 0.000110 | 0.003 | 0.000254 | 0.000107 | 0.022 |
| Max. Temp | 0.142 | 0.024 | 0.028 | 0.059 | 0.010 | 0.028 | -0.537 | 0.170 | 0.002 | 0.063 | 0.101 | 0.534 |
| Time since rain | 0.415 | 0.060 | 0.021 | 0.205 | 0.024 | 0.013 | 0.256 | 0.020 | <0.0001 | 0.167 | 0.013 | <0.0001 |
| NDVI ^b | - | - | - | - | - | - | 0.348 | 6.922 | 0.960 | -15.830 | 6.464 | 0.019 |
| $R^2_{GLMM(m)}$ | 89.79% | | | 93.68% | | | 98.69% | | | 82.90% | | |
| Linear model R^2 | 70.93% | | | 67.15% | | | 73.74% | | | 81.60% | | |
| Variance structure | Exponential (Max. Temp*position) | | | Exponential (Max. Temp*position) | | | Combined Identity(position) Exponential (Max. Temp*position) | | | Identity (position) | | |
| Random effects | ~1 + position date/transect/locale | | | ~1 + position date/transect/locale | | | ~1 + position date/transect/locale | | | ~1 + position locale | | |

726 ^a These parameters were correlated with each other; the parameter with the highest correlation with water loss rate was retained

727 ^b Spring models were deployed pre leaf-out, so canopy cover was not used as a predictor of water loss

728 Table 2. Summary of estimated surface activity times (SAT*) and the correlation
729 (Pearson's r) of SAT with predicted abundance at Daniel Boone Conservation Area
730 for juvenile- and adult-sized replicas in spring and summer seasons. Correlations are
731 between predicted abundance from Peterman and Semlitsch (2013) and SAT from this
732 study at 135 survey plots.

| Model | Surface active time (hrs) | | | Predicted abundance |
|-----------------|---------------------------|------|------------|---------------------|
| | Mean | SD | Range | Pearson's r^{**} |
| Juvenile spring | 6.25 | 0.62 | 5.00–9.48 | 0.48 |
| Adult spring | 9.9 | 0.49 | 8.96–12.57 | 0.45 |
| Juvenile summer | 1.94 | 0.15 | 1.59–2.65 | 0.35 |
| Adult summer | 3.67 | 0.73 | 1.15–8.97 | 0.63 |

733 * $SAT = 10 / \text{percent water lost}^{-hr}$

734 ** All correlations are significant at $P < 0.0001$

735 **Supporting Information**

736 The following Supporting Information is available for this article online.

737

738 Appendix S1. Detailed methods of how mixed effect models were made to estimate

739 rates of water loss.

740

741 Appendix S2. Details of field methods used to collect salamander abundance and size

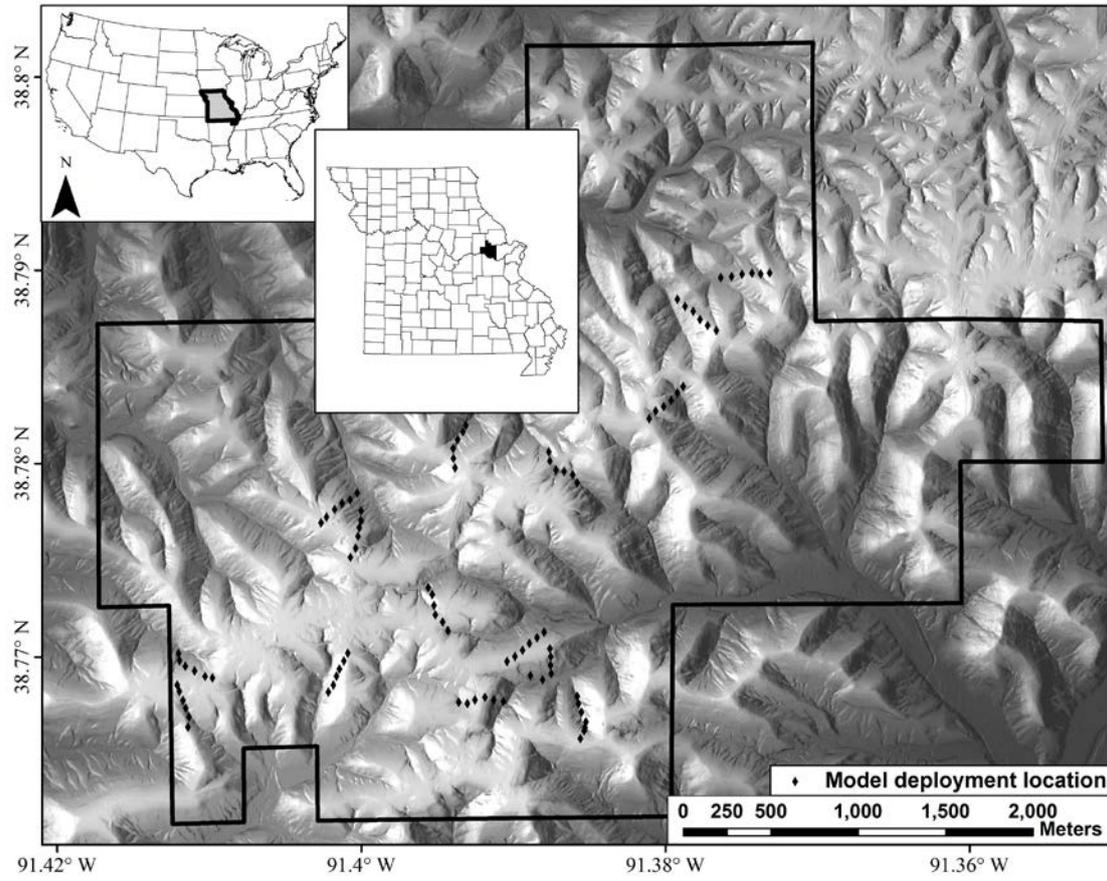
742 data, as well as detailed description of abundance and multistate modelling

743 procedures.

744

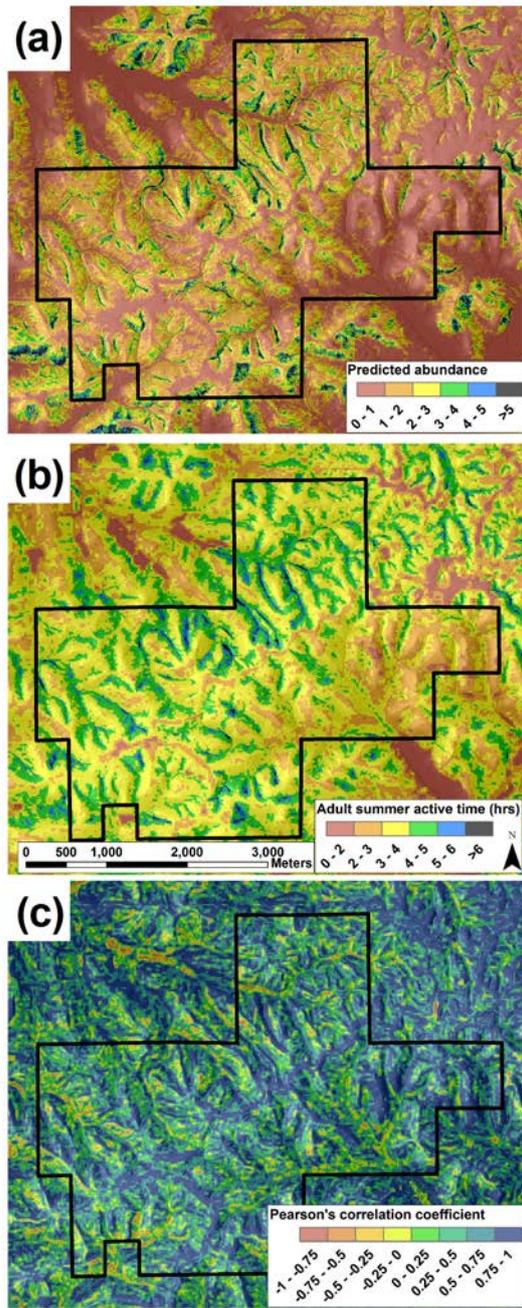
745 Table S1. Table of Pearson's r correlation coefficients for surface activity times

746 estimated from adult- and juvenile-sized models in spring and summer.



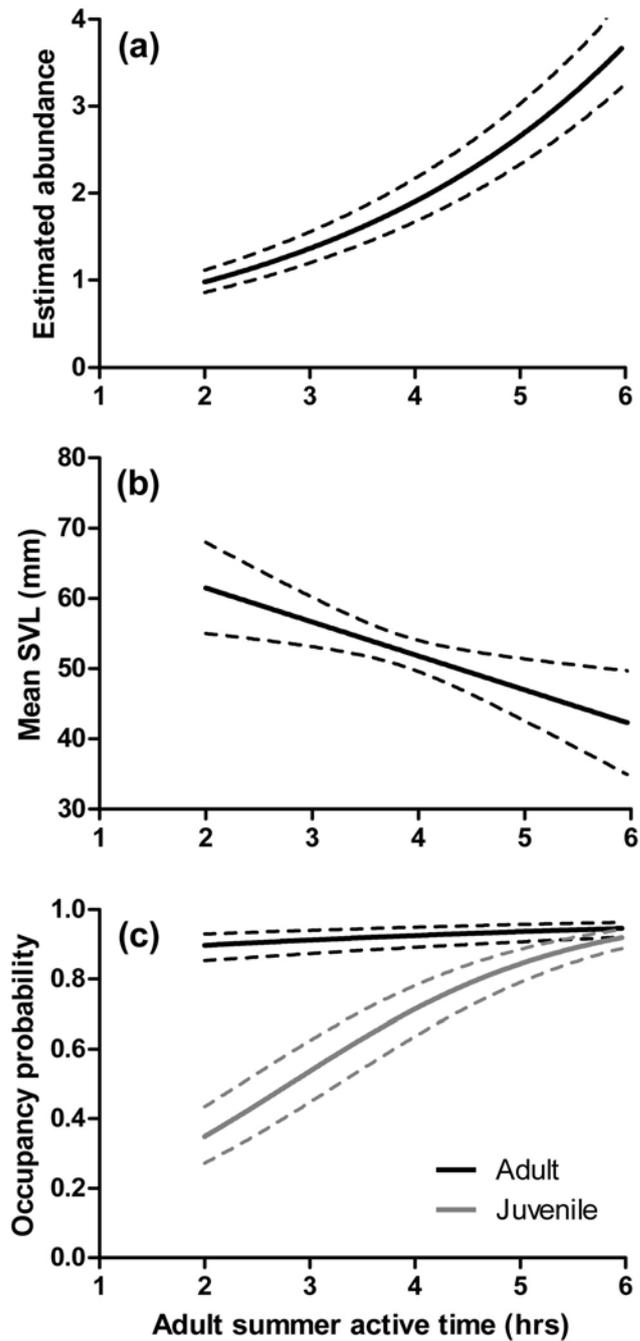
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748 Figure 1



749

750 Figure 2



751

752 Figure 3