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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22   Author Contributions: WEP conceived and designed the experiments, collected and

23   analysed data, and wrote the manuscript. RDS contributed funding as well as

24   experimental and editorial advice.

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Abstract

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

Key-words:

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Introduction

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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,

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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

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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

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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.

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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).

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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

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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.

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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.

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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

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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   *Proportion Water = (-0.0168*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 ≥5mm), 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.

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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] $\geq$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

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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

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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

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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 ($r=0.35–0.63$; 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 ($r=0.35–0.48$; 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,

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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

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

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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

15

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,

16

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

18

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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704

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.

| | Spring water loss models | | | | | | Summer water loss models | | | | | |
| | Juvenile | | | Adult | | | Juvenile | | | Adult | | |
Fixed effects parameters	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P
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[b]
$R^2_{GLMM(m)}$	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 / 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

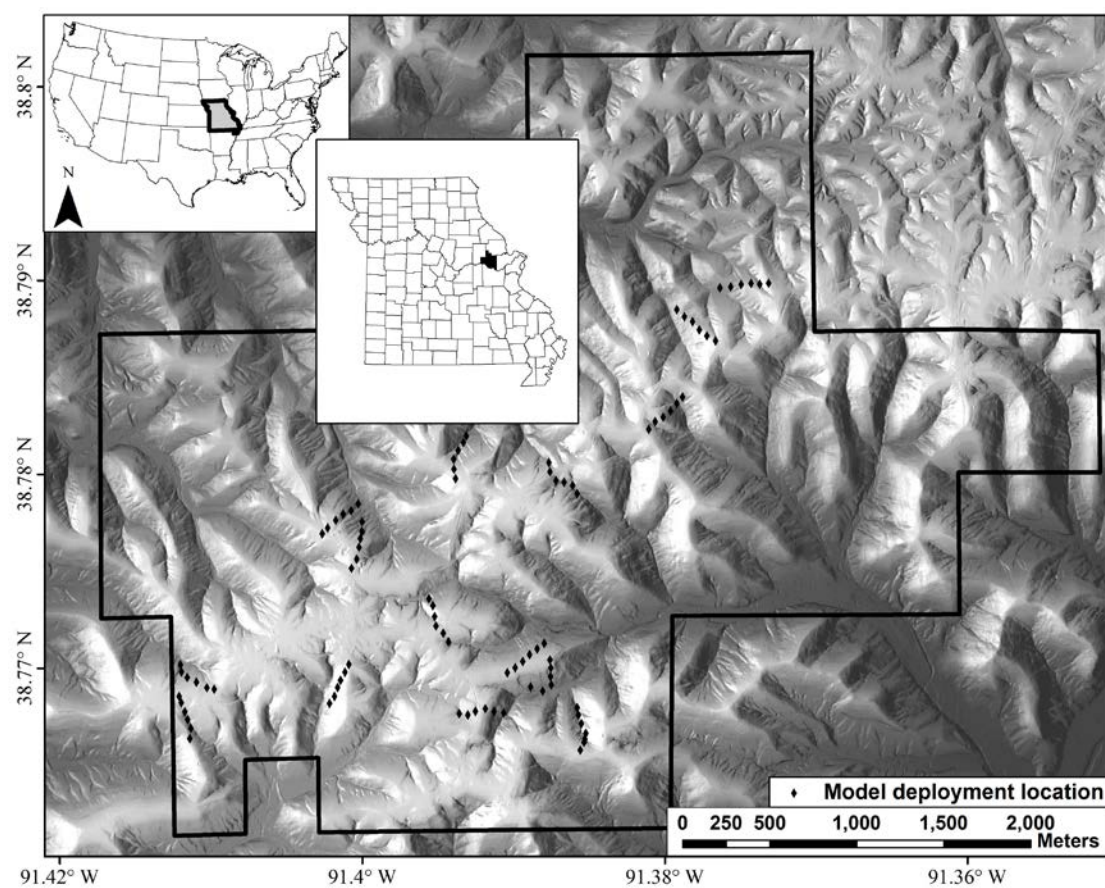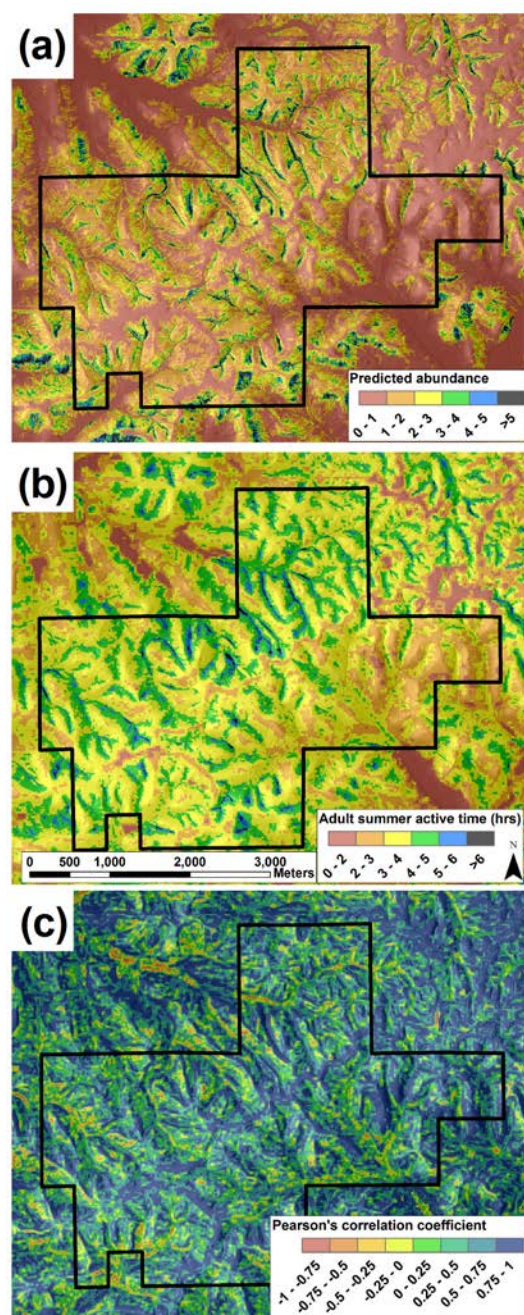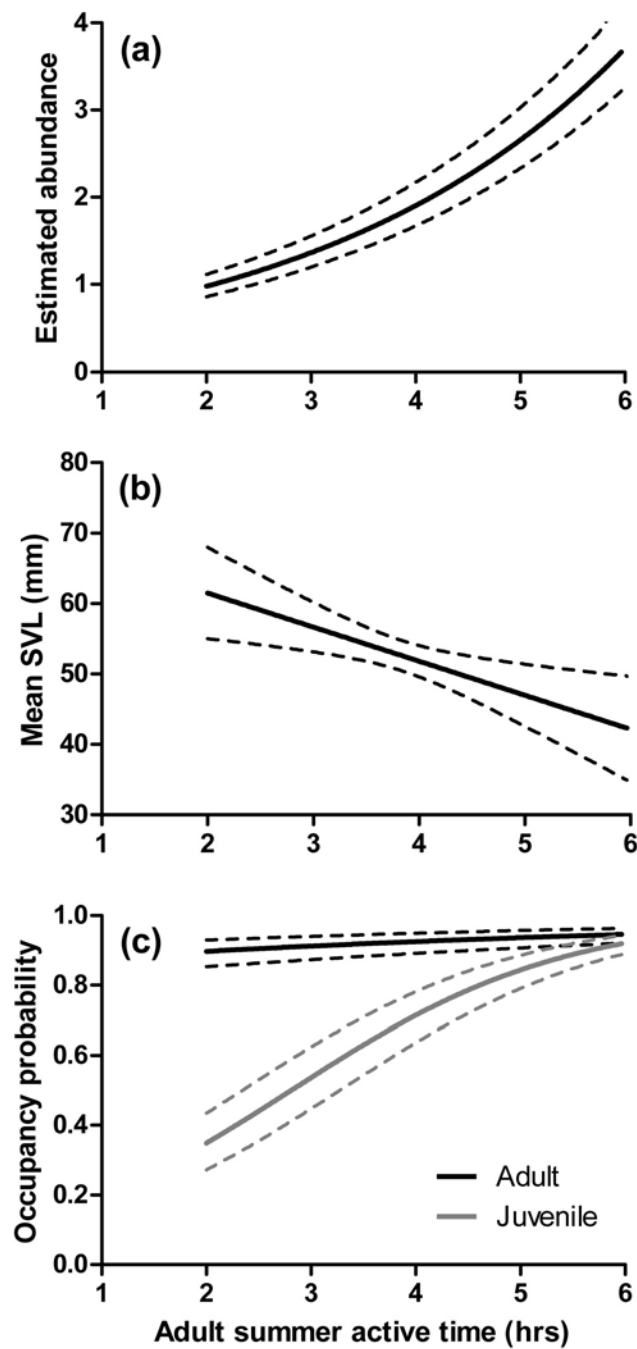746 estimated from adult- and juvenile-sized models in spring and summer.

747

748   Figure 1

749

750   Figure 2

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751

752   Figure 3

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