

1 Abbreviated Title: Stream Salamanders and Conductivity

2 Occupancy and Abundance of Stream Salamanders along a Specific Conductance Gradient

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25 **Abstract:** In the Central Appalachians (USA), mountaintop removal mining with valley fills
26 often leads to streams with elevated specific conductivity. Thus, the ionic composition of
27 freshwaters in this region is hypothesized as a driver of the distribution and abundance of
28 freshwater taxa, including stream salamanders. We examined the association between specific
29 conductivity and stream salamander populations by conducting salamander counts in 30
30 southeastern Kentucky streams across a continuous gradient of specific conductivity that ranged
31 from 30 to 1966 $\mu\text{S}/\text{cm}$. We counted 2,319 salamanders across 5 species and, using a hierarchical
32 Bayesian version of the N-mixture model, we found a negative association between specific
33 conductance and salamander occurrence; this finding was consistent across adults and larva of
34 the five species examined. Furthermore, we found that most salamander groups showing reduced
35 abundances given occupancy at greater specific conductivity levels. For example, estimated
36 mean abundance given occupancy of larval Southern Two-lined Salamanders (*Eurycea*
37 *cirrigera*) was 67.69 (95% CI 48.31–98.25) at 250 $\mu\text{S}/\text{cm}$ and 2.30 (95% CI 1.46–3.93) 2000
38 $\mu\text{S}/\text{cm}$. Although physical attributes, such as forest cover with stream catchments, also varied
39 along the specific conductance gradient, the consistent negative association across all species and
40 life stages support the hypothesis that salamander distributions and abundances are negatively
41 associated with elevated specific conductivity of streams in southeastern Kentucky. Restoration
42 of streams impacted by mountaintop removal mining should focus on restoring the ionic
43 compositions that naturally occur in this region.

44

45 **Keywords:** Amphibians, Appalachian, pollution, mining, salinization, water quality

46

47 **Introduction**

48 Human-accelerated weathering of rocks and soils is shifting the ionic composition of
49 freshwaters to saline systems (Kaushal et al. 2018). Land-cover change, particularly mining and
50 other resource extraction activities, is a major contributor to freshwater salinization (Cañedo-
51 Argüelles et al. 2013). In the central Appalachian Mountains (USA), mountaintop removal
52 mining is the primary driver of land-cover change (Bernhardt and Palmer 2011, Wickham et al.
53 2013). This type of surface mining involves the removal of large amounts of rock (i.e.,
54 mountaintops) to access coal seams; unconsolidated rocky material or overburden is often
55 discarded from the mine site into adjacent valleys forming a valley fill (Bernhardt and Palmer
56 2011). Valley filling results in the partial burial of low order streams and surface waters that
57 emerge from the fill area have elevated levels of major ions including Na^+ , Ca^{2+} , Mg^{2+} , K^+ , Cl^- ,
58 SO_4^{2-} , CO_3^{2-} and HCO_3^{2-} (Palmer et al. 2010, Griffith et al. 2012). Thus, freshwater systems
59 influenced by mountaintop removal mining frequently have specific conductance, a standard
60 measure of salinization, 30 times greater than unaltered streams (Lindberg et al. 2011, Price et al.
61 2016, Voss and Bernhardt 2017).

62 The Central Appalachians harbor one of most biologically diverse freshwater
63 assemblages in North America. Yet, thousands of km of streams have been impacted by valley
64 fills (Bernhardt and Palmer 2011, Bernhardt et al. 2012). Numerous studies have reported severe
65 declines in the abundances and species richness of freshwater taxa in streams affected by
66 mountaintop removal mining and valley filling (MTR-VF) (Pond et al. 2008, Cormier 2013, Hitt
67 and Chambers 2014, Muncy et al. 2014, Hitt et al. 2016). For example, Hitt and Chambers
68 (2014) found fish abundance, biomass, and diversity were 80%, 50%, and 49% lower in streams
69 influenced by MTR-VF compared to reference streams, respectively. The pathway linking MTR-

70 VF to biotic response is complex (Palmer et al. 2010, Wickham et al. 2013, Price et al. 2018);
71 however, elevated specific conductivity is regularly cited as a driver of the biotic patterns (i.e.,
72 Pond et al. 2008, Hitt and Chambers 2014). Elevated specific conductivity reduces or extirpates
73 local populations via osmoregulatory stress and direct mortality (McCulloch et al. 1993, Hassell
74 et al. 2006), increased immigration rates (i.e., downstream drift; Wood and Dykes 2002) or
75 changes in resource subsidies (Hitt and Chambers 2014). In the Central Appalachians, a specific
76 conductivity threshold of 300 $\mu\text{S}/\text{cm}$ is deemed protective of aquatic biota (US EPA 2011).

77 Stream salamanders comprise the majority of the vertebrate biomass in low-order stream
78 ecosystems in the Appalachian region (Hairston 1987, Petranka and Murray 2001). Stream
79 salamanders can regulate both freshwater and terrestrial macroinvertebrate communities, which
80 in turn influence ecosystem processes such as detritus processing and nutrient cycling (Burton
81 and Likens 1975, Keitzer and Goforth 2013a, b, Milanovich et al. 2015). Stream salamanders
82 allocate nearly 60% of prey consumed into growth and reproduction (Petranka and Murray 2001,
83 Johnson and Wallace 2005), and their diverse foraging behaviors aid in nutrient cycling in both
84 aquatic and terrestrial systems (Davic and Welsh 2004). Recent studies have shown that stream
85 salamander species diversity, abundance, and occupancy is reduced in MTR-VF streams
86 compared to reference locations (Wood and Williams 2013, Muncy et al. 2014, Price et al. 2016,
87 Price et al. 2018). Accordingly, elevated specific conductivity has been hypothesized as one of
88 the major factors driving salamander population declines.

89 To explore the hypothesis that elevated specific conductivity is associated with
90 reductions in salamander populations, we examined occupancy and abundance of species and
91 life-stages along a continuous gradient of specific conductivity that ranged from 30 to 1966
92 $\mu\text{S}/\text{cm}$. We used a hierarchical Bayesian modeling approach to provide estimates of occupancy,

93 abundance given occupancy, and detection probabilities; thus, incorporating species-level
94 attributes into the same modeling framework. In addition, we explored ion concentrations and
95 other physical attributes along the specific conductivity gradient.

96

97 **Methods**

98 *Study Sites*

99 We sampled salamanders at 30 first-order streams in the Cumberland Plateau in Breathitt,
100 Knott, and Letcher counties in southeastern Kentucky, USA (Figure 1). Stream sites were
101 selected across a continuous gradient of SC values ranging from 30 to 1966 $\mu\text{S}/\text{cm}$ (Table 1).
102 Low specific conductivity streams (30–70 $\mu\text{S}/\text{cm}$) were located in the main block of Robinson
103 Forest (RF) and Lilley Cornett Woods (LCW); see Martin and Shepherd (1973), Martin (1975)
104 and Phillippi and Boebinger (1986) for description of vegetative communities. Streams with
105 moderate specific conductivities (101–687 $\mu\text{S}/\text{cm}$) were in the main block of RF and adjacent to
106 the Laurel Fork Surface Mine (LFSM), and the second-growth forests adjacent to LCW; these
107 streams had elevated specific conductivity values due to previous timber harvest and surface
108 mining in a portion of their catchments (R. Watts and C. Osborne, *pers comm*). Streams with
109 very high specific conductivity (737–1966 $\mu\text{S}/\text{cm}$) were located within the LFSM, a surface mine
110 that was active from the late 1990s to early 2000s and released from bond in November 2007
111 after reclamation was determined satisfactory. Average forest cover within the stream
112 catchments declined steadily as specific conductivity increased (Table 1); however, riparian
113 zones and adjacent terrestrial areas were forested and riparian vegetation community
114 composition was similar across the specific conductivity gradient as the stream reaches sampled

115 were below the toe of the valley fill and the riparian area below the fill was relatively
116 undisturbed by the mining process.

117 High specific conductivity stream reaches were in the headwater streams below a valley
118 fill. Reference and moderate specific conductivity stream reaches were selected to contain stream
119 widths, depths, and current velocities similar to those found in the high specific conductivity
120 reaches. All stream reaches contained a pool, run, and riffle section to provide likely habitat to
121 increase detections of all possible salamander species and life stages.

122

123 *Salamander Sampling*

124 At each stream, we delineated 10-m reaches to sample for stream salamanders. Ten-meter
125 reaches were selected to compare stream salamander capture data to previous studies in the
126 eastern USA (e.g., Grant et al. 2009, Muncy et al. 2014). We counted salamanders in each 10 m
127 reach four times (approximately every 22 days) from April to July 2017. We conducted searches
128 during daylight hours (800–1700 h) and in baseflow conditions. Salamanders were captured
129 using systematic dipnetting and bank searches (Price et al. 2011). Dipnetting consisted of one
130 person, moving from downstream to upstream, searching for salamanders around and under
131 submerged rocks, logs, and other cover within the 10 m reach. One person then conducted bank
132 searches, which included searching under rocks, logs, leaf litter and other material within 1 m of
133 the wetted width of the stream. Stream searches were limited to 0.5 hours and bank searches to
134 0.25 hours (Price et al. 2011). We temporarily removed the salamanders from the stream reaches
135 during sampling. Visually-detected and identified salamanders that evaded capture were
136 recorded and were not likely to be recounted as sampling always continued upstream. After
137 sampling, we counted the number of individuals and recorded the species and life stage (larval or

138 adult).

139

140 *Habitat and Water Sampling*

141 Prior to conducting salamander counts, we collected 50 mL water samples that were later
142 analyzed at the Forestry Hydrology Lab (University of Kentucky, Department of Forestry and
143 Natural Resources) for concentrations of calcium (Ca^{+2}), magnesium (Mg^{+2}), potassium (K^{+}),
144 sodium (Na^{+}), sulfate (SO_4^{-2}), total organic carbon (TOC), pH, and specific conductivity (SC).
145 All sampling, preservation, and analytic protocols were followed per Greenberg et al. (1992).

146 Several other environmental attributes were measured at each site. Specifically, we
147 recorded water temperature ($^{\circ}\text{C}$), the number of cover objects (logs ≥ 8 cm diameter, rocks ≥ 15
148 cm diameter), the number of trees within 2 m of the stream channel within the 10 m transect that
149 were $<$ or $>$ 2 m tall, and percentage of detritus in the stream substrate of each transect (per Pond
150 et al. 2008). We calculated the catchment area and percent catchment in forest cover for each
151 stream site using a geographic information system (ArcGIS 10.1 ESRI) and Watershed tool in
152 ArcToolBox. To calculate catchment area, a post-mining 10 ft. digital elevation model (DEM)
153 data was used as the base layer for catchment delineation (Muncy et al. 2014). Forest cover was
154 obtained via United States Geological Survey 2013 7.5-min image map for Noble, KY
155 quadrangle; both mature and second growth forest classes were considered as forest cover in the
156 analysis of each stream catchment. Prior to regression analyses on the environmental and water
157 parameters, Shapiro-Wilk normality tests were conducted. To improve normality, non-normally
158 distributed attributes (i.e. p-value > 0.05) were either log, square root, or cube root according to
159 skew direction.

160

161 ***Occupancy and Abundance Analysis***

162 Salamander count data were separated by species and life stage for occupancy and
163 abundance analyses. We detected 9 salamander species during our active searches. However, we
164 only considered 5 species (i.e., Northern Dusky Salamander (*Desmognathus fuscus*), Seal
165 Salamander (*D. monticola*), Spring Salamander (*Gyrinophilus porphyriticus*), Red Salamander
166 (*Pseudotriton ruber*), and Southern Two-lined Salamander (*Eurycea cirrigera*) in our analysis,
167 as these species are primarily associated with streams and were captured in sufficient numbers to
168 allow statistical analyses. We then separated the salamanders into 8 groups: adult *D. fuscus*, *D.*
169 *monticola*, and *E. cirrigera* and larval *D. fuscus*, *D. monticola*, *E. cirrigera*, *G. porphyriticus*,
170 and *P. ruber*. Due to low numbers (i.e., 2) of adult *G. porphyriticus* and *P. ruber* captures, adults
171 were combined with the larvae.

172 We used a hierarchical Bayesian modeling approach to estimate species-specific and life
173 stage-specific responses to specific conductivity. This method fits an N-mixture model to each
174 species with a prior that relates the different parameters across species; where O_{ij} denotes
175 whether species i is present at site j , N_{ij} denotes the abundance of species i at site j , and n_{ijk}
176 denotes the number of individuals counted on visit k (Dorazio et al. 2013). The model assumes:

177

178 1. $O_{ij} | \psi_{ij} \sim \text{Bernoulli}(\psi_{ij})$

179

180 2. $N_{ij} | O_{ij} > 0, \lambda_i \sim \text{ZTPoisson}(\lambda_i)$

181

182 3. $n_{ijk} | N_{ij}, p_i \sim \text{Binomial}(N_{ij}, p_i)$

183

184 where ψ_i , λ_i , and p_i represent the occupancy probability, mean abundance per occupied site, and
185 individual detection probability for species i , respectively. The distribution of N_{ij} is assumed to
186 be a zero-truncated Poisson if $O_{ij} = 1$ and N_{ij} is fixed to be 0 if $O_{ij} = 0$ (i.e., an occupied site must
187 have at least one individual present and an unoccupied site must have no individuals present).
188 We further modelled the occupancy and abundance parameters dependent on the mean observed
189 specific conductivity at site j , denoted by x_j :

190

191 1. $\text{logit}(\psi_{ij}) = \beta_{0i} + \beta_{1i}\text{Conductivity}_j$

192

193 2. $\log(\lambda_{ij}) = \gamma_{0i} + \gamma_{1i}\text{Conductivity}_j$.

194

195 The detection probability was modelled on the logistic scale as:

196

197 $\text{logit}(p_{ijk}) = \delta_{0i} + \delta_{1i}\text{Days Since Last Rain}_{jk} + \delta_{2i}\text{Day of Year}_{jk}$.

198

199 This allowed the detection probability to vary by species and also allowed for species specific
200 effects of the number of days since last rain and the day of year. The regression parameters were
201 then assigned hierarchical priors such that:

202

203 $\beta_{0i} \sim \text{Normal}(\mu_{\beta 0}, \tau^2_{\beta 0})$

204

205 $\beta_{1i} \sim \text{Normal}(\mu_{\beta 1}, \tau^2_{\beta 1})$

206

207 and similar for γ_{0i} , γ_{1i} , and δ_{0i} , δ_{1i} , and δ_{2i} . These priors relate the parameters across the species,
208 but the strength of the relationship was determined by the data.

209 We fit the models with Markov chain Monte Carlo sampling implemented via JAGS
210 (Plummer 2003). Specifically, we ran the sampler with three chains started at diffuse initial
211 values. Each chain was run for 5,000 iterations burn-in and 100,000 sampling iterations.
212 Convergence was assessed with the Brooks-Gelman-Rubin diagnostics (Gelman and Rubin
213 1992). We found no evidence for lack of convergence. Specifically, point estimates of the
214 potential scale reduction factors were below 1.01 for all of the species specific parameters and
215 below 1.04 for all hyperparameters. We approximated posterior summary statistics including the
216 mean, standard deviation (SD) and credible intervals (95%) for each of the model parameters as
217 well as derived quantities including occupancy probability and abundance for each species or life
218 stage.

219

220 **Results**

221 *Salamander Occupancy and Abundance*

222 Overall, we had 2,319 salamander counts across the study: 657 adults and 1,662 larvae.
223 Specifically, we counted 280 *D. fuscus* adults, 284 *D. monticola* adults, 89 *E. cirrigera* adults,
224 191 *D. fuscus* larva, 205 *D. monticola* larva, 1015 *E. cirrigera* larva, 183 *G. porphyriticus* and 72
225 *P. ruber*. We found a general decline in occupancy probabilities of all salamander groups as
226 specific conductivity increased (Figure 2). For example, estimated occupancy probability of *D.*
227 *fuscus* adults at 250 $\mu\text{S}/\text{cm}$ was 0.95 (95% CI 0.89, 0.98) and decreased to 0.23 (95% CI = 0.05,
228 0.52) at 2000 $\mu\text{S}/\text{cm}$. Furthermore, posterior means and 95% credible intervals for the parameters
229 modelling the effect of specific conductivity on occupancy (β_{1j}) were negative for all salamander

230 groups (Figure 3; Table 3). Thus, when all salamanders were considered together, the mean
231 response to specific conductivity was negative ($\mu_{\beta 1} = -0.24$, 95% CI = -0.33, -0.15) indicating
232 that salamanders, as a group, occur less frequently at elevated specific conductivity levels
233 (Figure 3; Table 4). Furthermore, the response was similar across all species and life stages as
234 indicated by the posterior variance parameters (Table 5).

235 We also found a decline in mean salamander abundance given occupancy as specific
236 conductivity increased (Figure 4). Five groups exhibited decreases in abundance at elevated
237 conductivity levels. For example, the estimated abundance of larval *E. cirrigera* decreased from
238 63.38 (44.92-92.33) individuals per 10 m at 250 $\mu\text{S}/\text{cm}$ to 0.38 (0.04-1.04) individuals per 10 m
239 at 2000 $\mu\text{S}/\text{cm}$. However, we found no significant effect of specific conductivity on the
240 abundances of larval *D. fuscus*, *P. ruber* or adult *E. cirrigera* as 95% CI of posterior means
241 overlapped with zero (Figure 4, Table 3). Nonetheless, when all salamander species were
242 considered together, the mean response to specific conductivity was negative ($\mu_{\gamma 1} = -0.09$, 95%
243 CI = -0.16, -0.002) suggesting that salamanders, as a group, are less abundant given occupancy at
244 elevated specific conductivity levels (Figure 3, Table 4). Additionally, the association between
245 specific conductivity and salamanders was similar across all species and life stages as indicated
246 by the small posterior variance parameters (Table 5).

247 The effects of sampling covariates (i.e., days since last rain and day of year) on detection
248 probabilities varied among species and life stages (Figure 5). When we considered all
249 salamander groups together, we found that as days since last rain increased, detection probability
250 increased ($\delta_{1i} = 0.09$ 95% CI = 0.03, 0.14). Adult and larval *D. fuscus*, adult *E. cirrigera* and *G.*
251 *porphyriticus* had higher detection probabilities during early sampling events, whereas larval *D.*

252 *monticola*, larval *E. cirrigera* and *P. ruber* had higher detection probabilities in later samples
253 (Figure 4; Tables 3).

254

255 ***Physical Attributes***

256 Our analysis of the association of physical attributes across the specific conductivity
257 gradient showed no difference in the catchment size, number of trees > or < 2 m tall within 2 m
258 of the stream, number of logs, water temperature, pH, or TOC (Table 2). However, the
259 concentrations of the dissolved ions SO_4^{-2} , Ca^{+2} , Mg^{+2} , K^+ , and Na^+ increased as specific
260 conductivity increased (Table 2). For example, at reaches 40–100 $\mu\text{S}/\text{cm}$, SO_4 was on average
261 122.79 (± 158.22 SD) and 786.64 (± 72.89 SD) at sites 1750–2000 $\mu\text{S}/\text{cm}$. The number of rocks
262 in the 10-m reaches declined steadily as specific conductivity increased (Table 2); sites 40–100
263 $\mu\text{S}/\text{cm}$ had approximately 4.5 rocks/m, whereas, sites 1750–2000 $\mu\text{S}/\text{cm}$ had approximately 2
264 rocks/m. Additionally, the percentage of detritus in the stream substrate increased as specific
265 conductivity increased (Table 2); detritus substrate composition at sites 40–100 $\mu\text{S}/\text{cm}$ was
266 approximately 14%, whereas, at sites 1750–2000 $\mu\text{S}/\text{cm}$, detritus composition was
267 approximately 30%. Average forest cover within the stream catchments declined steadily as
268 specific conductivity increased (Table 2); sites 40–100 $\mu\text{S}/\text{cm}$ had approximately 95% forest
269 cover, whereas, sites 1750–2000 $\mu\text{S}/\text{cm}$ had approximately 40% forest cover.

270

271 **Discussion**

272 Stream salamander populations are known to be reduced in streams impacted by MTR-
273 VF (i.e., Muncy et al. 2014, Wood and Williams 2014, Price et al. 2016); yet, few studies have
274 examined their response as it relates to specific stressors associated with MTR-VF, such as

275 elevated specific conductance. We found that salamander occupancy decreased as specific
276 conductivity increased across all species and life-stages. Furthermore, abundance given
277 occupancy of most species and life-stages decreased as specific conductivity increased. Our
278 results support previous research on salamanders and specific conductance. Miller et al. (2007)
279 found that larval *E. cirrigera* abundance was negatively related to specific conductance in urban
280 streams, and Schorr et al. (2013) found that the occurrence of four salamander species in the
281 Cumberland Plateau were negatively correlated with elevated specific conductance. In addition,
282 our findings compliment previous studies conducted in streams impacted by MTR-VF mining
283 which found declines in occurrence, abundance or species richness of fish and
284 macroinvertebrates as specific conductivity increased along a continuous gradient (Cormier et al.
285 2013, Hitt and Chambers 2014, Hitt et al. 2016).

286 Specific conductivity may have both direct and indirect effects on stream salamanders.
287 Amphibians, like most freshwater organisms, osmoregulate to maintain greater internal salinity
288 than the external environment; that is, they are hyperosmotic to the external environment
289 (Schoffeniels and Gilles 1970, Shoemaker and Nagy 1977, Evans 2008). When the salinity of the
290 external environment becomes greater than the internal salinity, they expel excess ions to
291 maintain homeostasis. This can be energetically costly and result in adverse effects ranging from
292 increased stress to mortality (Konnick 1977, McCulloch et al. 1993, Ferrari et al. 2004, Evans
293 2008, Cañedo-Argüelles et al. 2013). Chambers (2011) found that increased conductivity led to
294 elevated corticosterone levels and altered activity and feeding behaviors in larval Jefferson
295 Salamanders (*Ambystoma jeffersonianum*). In addition, studies in saline environments,
296 specifically those contaminated with road salt (NaCl), have shown significant reductions in
297 embryonic and larval survival of Spotted Salamanders (*A. maculatum*) and Wood Frogs

298 (*Lithobates sylvaticus*) (Sanzo and Hecnar 2006, Karraker et al. 2008). Furthermore, elevated
299 specific conductivity (>250 $\mu\text{S}/\text{cm}$) has been linked to malformations in developing amphibian
300 larvae (Sanzo and Hecnar 2006, Karraker 2007). Collectively, these studies suggest that elevated
301 specific conductance results in osmoregulatory stress and reduced survivorship in amphibians.
302 However, previous studies on the response of amphibians to elevated conductivity have largely
303 focused on pond-breeding amphibians and road salts (NaCl). As such, our research fills an
304 important gap by examining the responses of stream amphibians to elevated conductivity. Future
305 research is needed on amphibian responses to both component ions typical of streams draining
306 MTR-VF landscapes as well as combined toxicity of solutes.

307 Elevated specific conductivity is also known to reduce the abundance, biomass, and
308 diversity of aquatic macroinvertebrates (Kennedy et al. 2003; Hartman et al. 2005, Pond et al.
309 2008, Pond 2010, 2012, Merriam et al. 2011, Cormier et al. 2013). Dietary studies have reported
310 high diversities and occurrences (68–82%) of aquatic macroinvertebrates in the stomach contents
311 of larval stream salamanders, primarily comprised of larvae from the orders Ephemeroptera,
312 Plecoptera, Trichoptera, and Diptera (Martof and Scott 1957, Caldwell and Houtcooper 1973,
313 Davic 1991, Brophy and Pauley 1997, Cecala et al. 2007, Hutton et al. 2018); these orders are
314 particularly sensitive to elevated specific conductivity (Pond et al. 2008, Cormier et al. 2013).
315 Thus, a reduction of prey populations may be a possible explanation for the decreased occupancy
316 and abundance of stream salamanders along the specific conductivity gradient. This pathway is
317 not unprecedented as Johnson and Wallace (2005) indicated a reduction in prey populations may
318 decrease growth and body condition in larval salamanders. In addition, Kraus et al. (2016)
319 reported trout alter foraging behaviors and consume sub-optimal terrestrial prey in streams
320 impacted by mining activity. Furthermore, declines in abundance of some fish species in streams

321 with elevated conductivity appear to be linked to decreased availability of aquatic
322 macroinvertebrate prey (Hitt and Chambers 2014). Although we did not measure the
323 macroinvertebrate community, our data suggest that density of certain macroinvertebrates may
324 be reduced. We found an association between increased specific conductivity and increased
325 detritus (Table 2), which previous studies have linked to changes in macroinvertebrate shredder
326 diversity (Fritz et al. 2010). Future research should focus on the diet of stream salamanders and
327 how diet changes in relation to specific conductance.

328 Our findings on physical attributes of study sites suggests that specific conductivity may
329 not be the only factor associated with patterns of occupancy and abundance in Central
330 Appalachian streams. First, our study sites ranged from 1250 ft to 884 ft above sea level.
331 Elevation influences local climate and stream water temperature; these factors have been
332 associated with occupancy and abundance of some stream-associated salamanders (Grant et al.
333 2005, Gould et al. 2017, Cecala et al. 2018). In addition, we found that forest cover within the
334 stream catchments declined as specific conductivity increased along our survey gradient. Forest
335 loss from logging, agriculture, or urban development can lead to population decline in some
336 stream salamander species (e.g., Barrett et al. 2010, Price et al. 2011, Grant et al. 2016).
337 Consequences of forest loss for stream salamanders include changes to base flow conditions and
338 warmer and drier conditions in forests adjacent to streams (Wood and Williams 2003b), which
339 reduces the quality of terrestrial microhabitats and may inhibit population processes (i.e.,
340 dispersal; Price et al. 2018). Reductions in forest cover near streams also impacts in-stream
341 habitat conditions, such as substrate composition (Lowe et al. 2004, Barrett et al. 2010). Indeed,
342 we found a negative association between the number of rocks within our sampling reach and
343 specific conductivity. Fewer rocks within streams with elevated conductivity is likely due to the

344 higher proportion of fine sediment in streams impacted by MTR-VF, which results poorer habitat
345 for salamanders and can lead to reduced abundances (Lowe et al. 2004). Studies on other aquatic
346 taxa have also noted the difficulty of teasing apart the effects of specific conductivity from other
347 physical attributes on freshwater taxa (Hitt and Chambers 2004, Pond et al. 2008).

348 Yet, we argue that elevated specific conductivity is an important driver of salamander
349 occupancy and abundances in our study area. Stream salamander species and stages tend to
350 exhibit differential responses to physical attributes, such as elevation, forest cover and
351 sedimentation due to variations in larval period, larval body size and terrestrial habitat use by
352 adults (Price et al. 2011, Gould et al. 2017, Cecala et al. 2018). For example, Cecala et al.
353 (2018) found Blue Ridge Two-lined Salamander (*Eurycea wilderae*) larvae did not exhibit
354 predictable occupancy patterns in response to forest cover, whereas occupancy of Black-bellied
355 Salamanders (*Desmognathus quadramaculatus*) were strongly predicted by forest cover within
356 stream catchments. Similarly, previous studies have shown species often respond differently to
357 alterations of in-stream habitat, with some species (i.e., *Eurycea* sp) being tolerant of
358 sedimentation (Keitzer and Goforth, 2012). Thus, we would expect to see variation in responses
359 if forest cover, sedimentation or other factors were determining patterns of salamander
360 occupancy and abundance in our study area. Instead, we found similar responses across species
361 and life-stages to specific conductivity which suggests that specific conductance may be a
362 strongly associated with patterns of occupancy and abundance at our study sites.

363 If specific conductivity is a mechanism driving patterns of salamander occupancy and
364 abundance in Central Appalachian streams, restoration activities may need to emphasize
365 repairing water chemistry. Current restoration activities focus on restoring native forests on
366 MTR-VF sites (Angel et al. 2005). While reforestation will clearly benefit some salamander

367 species and ameliorate some water quality issues, failure to address elevated ionic composition
368 may prevent recovery of stream salamander populations in streams impacted by MTR-VF.
369 Elevated specific conductance is known to persist for decades in streams draining MTR-VF sites
370 (Merricks et al. 2007, Pond et al. 2008), and thus, treatment methods such as desalinization or
371 stream creation may be necessary to restore habitat. However, to further tease out the influence
372 of elevated specific conductivity on salamander populations, physiological and dietary studies
373 should be conducted along the specific gradient to identify the mechanisms behind the patterns
374 of salamander occupancy and abundance observed in our study area.

375

376 **Author Contributions**

377 JMH, SJP, SCR and CDB framed ideas and designed the study. JMH and SJP conducted the
378 field work; JMH, SJP and SJB analyzed the data. All authors contributed to the interpretation of
379 results and writing the manuscript.

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613 **Table 1.** Site names, average specific conductivity, elevation, county, catchment size, and
 614 percent forest cover for 30 study stream sites in southeastern Kentucky (USA).

Site	SC ($\mu\text{S}/\text{cm} \pm \text{SE}$)	Elevation		Catchment Size	Forest Cover
		(ft)	County	(ha)	%
Miller	31.5 (± 3)	1241	Breathitt	7.98	98.75
Falling Rock B	36.2 (± 6)	1074	Breathitt	17.47	100.00
Little Millseat A	38.4 (± 4)	1087	Breathitt	14.99	100.00
Field Branch A	39.6 (± 12)	1145	Breathitt	17.28	100.00
Falling Rock A	39.7 (± 7)	1053	Breathitt	12.06	100.00
Boardinghouse	40.8 (± 4)	958	Breathitt	31.13	99.04
Bucklick	43.8 (± 3)	914	Breathitt	15.64	100.00
Tome	47.7 (± 8)	982	Breathitt	30.08	100.00
Cole's Fork A	51.6 (± 10)	1050	Knott	87.27	100.00
Big Everidge	69.6 (± 5)	1114	Letcher	55.3	100.00
Bear Branch #3	100.5 (± 8)	957	Breathitt	5.42	69.13
Mart Branch	108.1 (± 16)	886	Breathitt	67.02	98.71
Pole Branch	130.3 (± 9)	1109	Letcher	90.61	97.42
Rich Hollow #2	286.8 (± 45)	1242	Breathitt	8.78	70.11
Island Branch	417.8 (± 49)	1245	Letcher	143.84	95.35
Rich Hollow #3	418.3 (± 86)	1144	Breathitt	12.42	100.00
Whitaker Branch	442 (± 92)	1222	Letcher	28.14	74.15
White Oak Left	480.3 (± 117)	1140	Breathitt	10.81	49.58
Rich Hollow #1	501.8 (± 226)	1250	Breathitt	8.61	15.31

Bear Branch #1	552.5 (± 91)	943	Breathitt	3.33	90.82
Bear Branch #2	686.8 (± 99)	884	Breathitt	4.37	68.29
Turkey	736.8 (± 123)	961	Breathitt	6.89	78.21
Bee Branch Near	1286.5 (± 176)	1059	Breathitt	37.17	35.59
White Oak Right	1382.3 (± 100)	1107	Breathitt	32.03	44.19
Bee Branch Far	1409.5 (± 168)	916	Breathitt	22.47	29.60
White Oak	1439.5 (± 175)	1113	Breathitt	24.5	40.59
Stillrock	1549.5 (± 272)	1129	Breathitt	12.69	46.31
Big Hollow	1609 (± 113)	1040	Breathitt	18.74	23.41
Wharton	1954.8 (± 196)	1088	Breathitt	61.53	21.66
Hickory Log	1965.5 (± 193)	995	Breathitt	13.88	45.16

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628 **Table 2.** Regression coefficients (R^2) and associated P-values for stream site attributes along a
629 continuous gradient of specific conductance in southeastern Kentucky. Data were either log,
630 square root, or cube root transformed to improve normality. Transformed attribute estimates
631 were calculated by back-transformation.

Site Attribute	Slope Estimate	95% CI	P-Value	R^2	Transformation
Catchment Size (ha)	1.000	(-0.999, 1.007)	0.739	0.004	log
# Trees > 2 m	0.00032	(-0.0015, 0.0021)	0.772	0.005	none needed
# Trees < 2 m	1.0002	(0.9998, 1.0007)	0.163	0.076	log
# Logs	2.99E-8	(-3.20E-8, 2.76E-7)	0.448	0.021	square-root
Water Temp	2.31E-4	(-0.0007, 0.0011)	0.555	0.016	none needed
pH	1.68E-5	(-0.0003, 0.0004)	0.919	0.0004	none needed
TOC	-0.00159	(-0.0079, 0.0047)	0.610	0.009	none needed
% Detritus	1.0007	(1.000, 1.001)	< 0.001	0.381	log
SO ₄	3.53E-8	(1.12E-8, 8.06E-8)	< 0.001	0.628	cube-root
Ca	1.56E-4	(7.62E-5, 2.62E-4)	< 0.001	0.782	square-root
Mg	1.0012	(1.0008, 1.0016)	< 0.001	0.698	log
K	1.001	(1.0008, 1.0012)	< 0.001	0.779	log
Na	1.31E-7	(6.59E-8, 2.30E-7)	< 0.001	0.817	log
# Rocks	-23.9	(-36.0, -11.8)	< 0.001	0.369	none needed

% Forest Cover	-17.510	(-22.484, 12.537)	< 0.001	0.595	log
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653 **Table 3.** Mean and 95% credible intervals for occupancy (β_0, β_1), abundance (γ_0, γ_1) and detection ($\delta_0, \delta_1, \delta_2$) parameters across salamander
654 groups in relation to specific conductivity. Specifically, β_{1i} and γ_{1i} are the effect of conductivity on salamander occupancy and
655 abundance, respectively. Detection parameters included number of days since last rain (δ_1) and day of year (δ_2).
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Parameters	<i>Desmognathus fuscus</i>		<i>Desmognathus monticola</i>	
	Adult	Larva	Adult	Larva
β_0	3.56 (2.56, 4.79)	3.61 (2.60, 4.90)	3.62 (2.61, 4.93)	3.35(2.29, 4.44)
β_1	-0.24 (-0.35, -0.15)	-0.23 (-0.33, -0.13)	-0.23 (-0.33, -0.13)	-0.23 (-0.33, -0.12)
γ_0	2.32 (1.97, 2.71)	3.01 (2.34, 4.04)	2.50 (2.16, 2.90)	2.70 (2.25, 3.27)
γ_1	-0.04 (-0.08, 0.00)	-0.02 (-0.05, 0.02)	-0.09 (-0.13, -0.05)	-0.10 (-0.16, -0.05)
δ_0	-0.43 (-1.09, 0.18)	-1.91 (-3.07, -1.07)	-0.97 (-1.54, -0.45)	-2.50 (-3.14, -1.96)
δ_1	0.12 (0.06, 0.20)	0.12 (0.06, 0.20)	0.09 (0.02, 0.16)	0.11 (0.04, 0.18)
δ_2	-0.14 (-0.20, -0.08)	-0.14 (-0.20, -0.09)	-0.01 (-0.06, -0.04)	0.17 (0.11, 0.24)
	<i>Eurycea cirrigera</i>		<i>Gyrinophilus porphyriticus</i>	<i>Pseudotriton ruber</i>
	Adult	Larva	Adults and Larva	Adults and Larva

β_0	3.40 (2.35, 4.56)	3.44(2.45, 4.61)	3.65 (2.62, 5.11)	3.50 (2.36, 4.91)
β_1	-0.26 (-0.40, -0.17)	-0.27 (-0.40, -0.17)	-0.22 (-0.33, -0.10)	-0.22 (-0.32, -0.08)
γ_0	2.49 (1.51, 3.78)	4.71 (4.39, 5.10)	2.73 (2.15, 3.54)	2.53 (1.56, 3.80)
γ_1	-0.03 (-0.10, 0.03)	-0.21 (-0.25, -0.17)	-0.16 (-0.23, -0.09)	-0.04 (-0.11, 0.01)
δ_0	-1.34 (-2.90, 0.10)	-3.05 (-3.46, -2.70)	-1.32 (-2.27, -0.55)	-3.45 (-4.84, -2.34)
δ_1	0.07 (-0.04, 0.15)	0.10 (0.06, 0.14)	0.03 (-0.09, 0.12)	0.08 (-0.02, 0.16)
δ_2	-0.30 (-0.42, -0.20)	0.21 (0.18, 0.24)	-0.06 (-0.12, -0.01)	0.13 (0.04, 0.23)

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666 **Table 4.** Posterior summaries for hierarchical mean parameters for occupancy ($\mu_{\beta 0}$, $\mu_{\beta 1}$),
 667 abundance ($\mu_{\gamma 0}$, $\mu_{\gamma 1}$) and detection ($\mu_{\delta 0}$, $\mu_{\delta 1}$, $\mu_{\delta 2}$). The means can be interpreted as representing the
 668 average values of their respective parameters across all groups of salamanders in the study.

Parameter	Mean Estimate (95% Credible Interval)
$\mu_{\beta 0}$	3.47 (2.61, 4.47)
$\mu_{\beta 1}$	-0.24 (-0.33, -0.15)
$\mu_{\gamma 0}$	2.75 (1.90, 3.52)
$\mu_{\gamma 1}$	-0.09 (-0.16, -0.02)
$\mu_{\delta 0}$	-1.75 (-2.65, -0.78)
$\mu_{\delta 1}$	0.09 (0.03, 0.14)
$\mu_{\delta 2}$	-0.03 (-0.19, 0.14)

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690 **Table 5.** Posterior summaries for hierarchical variance parameters for occupancy ($\sigma_{\beta 0}$, $\sigma_{\beta 1}$),
 691 abundance ($\sigma_{\gamma 0}$, $\sigma_{\gamma 1}$) and detection ($\sigma_{\delta 0}$, $\sigma_{\delta 1}$, $\sigma_{\delta 2}$). The variance parameters represent the variation in
 692 the species and life stages. Parameters with smaller variance parameters are more similar across
 693 the groups while those with larger variance parameters are less similar.

Parameter	Mean Estimate (95% Credible Interval)
$\sigma_{\beta 0}$	0.39 (0.01, 1.29)
$\sigma_{\beta 1}$	0.04 (0.00, 0.15)
$\sigma_{\gamma 0}$	1.02 (0.56, 1.89)
$\sigma_{\gamma 1}$	0.09 (0.05, 0.18)
$\sigma_{\delta 0}$	1.33 (0.70, 2.39)
$\sigma_{\delta 1}$	0.05 (0.00, 0.14)
$\sigma_{\delta 2}$	0.22 (0.12, 0.42)

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FIGURE CAPTIONS

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707 Figure 1. Study area and sampling locations for 30 stream reaches in Breathitt, Knott, and
708 Letcher counties, Kentucky, USA. Symbols represent specific conductivity influence where
709 circles are low specific conductance (reference) streams, triangles are streams with moderate
710 specific conductance values, and diamonds are high specific conductivity (MTR-VF) streams.

711

712 Figure 2. Mean estimated occupancy probabilities (with 95% credible intervals) for salamanders
713 detected at stream reaches along a continuous SC gradient in southeastern Kentucky. Groups are
714 denoted as *Desmognathus fuscus* (DF), *D. monticola* (DM), *Eurycea cirrigera* (EC),
715 *Gyrinophilus porphyriticus* (GP), and *Pseudotriton ruber* (PR) and adults are represented by (A)
716 and larvae are represented by (L).

717

718 Figure 3. Salamander group estimates of (a) occupancy probability and (b) mean abundance
719 across a continuous SC gradient in southeastern Kentucky. The points represent posterior means,
720 the wide bands central 50% CI, and the thin bands 95% central CI. Groups are denoted as
721 *Desmognathus fuscus* (DF), *D. monticola* (DM), *Eurycea cirrigera* (EC), *Gyrinophilus*
722 *porphyriticus* (GP), and *Pseudotriton ruber* (PR) and adults are represented by (A) and larvae are
723 represented by (L).

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725 Figure 4. Estimated mean abundances (with 95% credible intervals) for salamanders detected at
726 stream reaches along a continuous SC gradient in southeastern Kentucky. Groups are denoted as
727 *Desmognathus fuscus* (DF), *D. monticola* (DM), *Eurycea cirrigera* (EC), *Gyrinophilus*

728 *porphyriticus* (GP), and *Pseudotriton ruber* (PR) and adults are represented by (A) and larvae are
729 represented by (L).

730

731 Figure 5. Model estimated detection parameters and 95% credible intervals (95% CI) for each
732 salamander group observed at stream reaches across a continuous SC gradient in southeastern
733 Kentucky, the points represent posterior means, the wide bands central 50% CI, and the thin
734 bands 95% central CI. Groups are denoted as *Desmognathus fuscus* (DF), *D. monticola* (DM),
735 *Eurycea cirrigera* (EC), *Gyrinophilus porphyriticus* (GP), and *Pseudotriton ruber* (PR) and
736 adults are represented by (A) and larvae are represented by (L).

