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Diet Composition: A Proximate Mechanism Explaining Stream Salamander Declines in Surface Waters with Elevated Specific Conductivity

Original Research Article

Diet composition: A proximate mechanism explaining stream salamander declines in surface waters with elevated specific conductivity

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A B S T R A C T

Changes in land use, such as mountaintop removal mining with valley fills (MTR-VF), often results in headwater streams with elevated specific conductivity (SC). Stream salamanders appear to be particularly sensitive to elevated SC, as previous studies have shown occupancy and abundance decline consistently among all species and life stages as SC increases. Yet, the proximate mechanism responsible for the population declines in streams with elevated SC have eluded researchers. We sampled salamander assemblages across a continuous SC gradient (30–1966 μS/cm) in southeastern Kentucky and examined the diet of larval and adult salamanders to determine if the ratio of aquatic to terrestrial prey (autochthony), total prey volume, aquatic prey importance (I_x), and body condition are influenced by SC. Further, we asked if threshold points for each diet component were present along a gradient of SC. Larval salamanders experienced a 12–fold decline in autochthony at 153 μS/cm, a 4.2–fold decline in total prey volume at 100 μS/cm, a 2.2-fold decline in aquatic I_x at 135 μS/cm, and a rapid decline in body condition as SC increased. Adult salamanders experienced a 3–fold decline in autochthony at 382 μS/cm, no change in prey volumes, a 2-fold decline in aquatic I_x at 163 μS/cm, and a decline in body condition as SC increased. Our results indicate that SC indirectly affects stream salamander populations by changing the composition of diet, which suggests that food availability is a proximate mechanism that leads to reduced population occupancy, abundance, and persistence in streams with elevated SC.

1. Introduction

In the central Appalachians of the United States, land-use disturbances have resulted in an environmental gradient of water chemistry; specifically, surface waters that vary in specific conductance (SC) in relation to land-use (Hartman et al., 2005; Fritz et al., 2010; Bernhardt and Palmer, 2011; Lindberg et al., 2011; Griffith et al., 2012). Specific conductance values are often 30 times greater in streams impacted by mountaintop removal coal mining and valley filling (MTR-VF) than streams within undisturbed, forested catchments (Lindberg et al., 2011; Price et al., 2016; Voss and Bernhardt, 2017), whereas streams in agricultural or urban watersheds often have SC values between these two extremes (Pond et al., 2008; Sweeten and Ford, 2016). Numerous studies have shown that

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elevated SC is negatively correlated with abundance and diversity of aquatic organisms (Pond et al., 2008; Cormier, 2013; Hitt and Chambers, 2014; Boehme et al., 2016; Hitt et al., 2016). While these correlative patterns have aided in a general understanding of the association between SC and abundance and diversity of aquatic organisms, the proximate mechanisms that may explain this pattern have rarely been investigated (Clements and Kotalik, 2016).

Elevated SC has shown to be negatively correlated with stream salamander occupancy and abundances in Central Appalachia (Muncy et al., 2014; Price et al., 2016). In a recent study, Hutton et al. (2020) examined the association between SC and stream salamander occupancy rates and abundances by conducting salamander counts in streams along a continuous gradient of SC that ranged from 30 to 1966 μS/cm. Hutton et al. (2020) found a negative association between SC and adult and larval salamander occupancy rates, and most salamander species and life stages showed reduced abundances given occupancy at greater SC levels. Appalachian streams with elevated SC from MTR-VF often have reduced aquatic macroinvertebrate abundance, biomass, and diversity (Chambers and Messinger, 2001; Kennedy et al., 2003; Hartman et al., 2005; Pond et al., 2008; Fritz et al., 2010; Pond, 2010, 2012; Merriam et al., 2011; Cormier et al., 2013; Boehme et al., 2016; Voss and Bernhardt, 2017). Aquatic macroinvertebrates are important prey for stream inhabiting amphibians (Martof and Scott, 1957; Greene et al., 2008), and a reduction in prey populations has been shown to decrease growth and body condition in larval salamanders (Johnson and Wallace, 2005). Thus, the reduction in aquatic macroinvertebrate abundance and diversity in streams with elevated SC may be a proximate mechanism explaining the consistent declines in the occupancy and abundance of salamanders along the SC gradient.

Stream food webs are connected with their riparian zones through cross-ecosystem movements of energy and nutrients. The use and impact of terrestrial subsidies on aquatic consumers is determined, in part, by in situ biomass of aquatic prey (Nakano et al., 1999; Sabo and Power, 2002; Baxter et al., 2007). Thus, stressors associated with elevated SC that reduce aquatic secondary production could increase the reliance of stream consumers on terrestrial resource subsidies (Carlisle and Clements, 2003; Kraus et al., 2016). Previous studies have shown that reductions in aquatic macroinvertebrate prey biomass may directly cause stream fish to shift foraging behavior towards terrestrial subsidies (Allan, 1982; Nakano and Murakami, 2001; Wipfli and Baxter, 2010; Wilson et al., 2014). Kraus et al. (2016) reported trout in streams affected by mining consumed a greater proportion and mass of terrestrial prey than fish in reference streams; thus, trout alter foraging behaviors to consume the more available, terrestrial prey. However, no studies have quantitatively tested for potential shifts in salamander diet due to elevated SC. Further, the estimation of dietary shifts, particularly threshold value point changes, along an environmental gradient can be critical for conservation efforts by estimating the specific level of influence required to significantly impact a population (Groffman et al., 2006).

In this study, we used an environmental gradient approach to examine the relationship between SC and stream salamander diet composition. Specifically, we asked how do diet components, i.e., aquatic and terrestrial prey volume, aquatic/terrestrial (A/T) ratios (autochthony), prey diversity, prey importance values, and salamander body condition change along the SC gradient? Further, we ask if threshold points for each diet component are present that can provide potential specific SC values of importance to conservation, protection and enforcement, and restoration efforts? We hypothesized that as SC increases, overall aquatic prey volumes, A/T ratios, diversity, and importance will decrease, thus leading to a reduction in salamander body condition.

2. Methods

2.1. Study sites

Our study sites were 30 first-order streams in the Cumberland Plateau in Breathitt, Knott, and Letcher Counties in southeastern Kentucky, USA. Study sites were selected across a continuous gradient of SC values ranging from 30 to 1966 μS/cm (Hutton et al., 2020). Low conductivity streams (30–70 μS/cm) were primarily located in the main block of Robinson Forest (RF) and Lilley Cornett Woods (LCW); see Martin and Shepherd (1973), Martin (1975), and Phillippi and Boebinger (1986) for description of vegetative communities. Streams with moderate SC (101–687 μS/cm) were in the main block of RF adjacent to the Laurel Fork Surface Mine (LFSM) and the second-growth forests adjacent to LCW; these streams had elevated SC values due to previous timber harvest and surface mining in a small portion of their catchments (R. Watts and C. Osborne, *pers comm*). Streams with high SC (737–1966 μS/cm) were located within the LFSM, a surface mine that was active from the late 1990s to early 2000s and released from bond in November 2007 after reclamation was determined satisfactory. See Hutton et al. (2020) for additional details about the study sites.

At each stream, we delineated 10-m reaches to sample for stream salamanders. High SC stream reaches were located in the headwater streams below a valley fill. Reference and moderate SC stream reaches were selected to contain stream widths, depths, and current velocities similar to those found in the high SC reaches. All stream reaches contained a pool, run, and riffle section to provide likely habitat to increase detections of all possible species and life stages (Petranka, 1998). At LFSM, mining and tree removal occurred upstream from the sampling reaches and therefore the riparian canopy cover above the reaches was intact.

2.2. Salamander surveys and diet

We sampled each stream site four times (approximately every 22 days) from April to July 2017. Searches were conducted during daylight hours (0800–1700 h) and in baseflow conditions. Salamanders were captured using systematic dipnetting and bank searches (Price et al., 2011). Dipnetting consisted of one person, moving from downstream to upstream, searching for salamanders around and under submerged rocks, logs, and other cover within the 10-m reach. One person then conducted bank searches, which included searching under rocks, logs, leaf litter and other material within 1-m of the wetted width of the stream. Stream searches were limited to 0.5 h and bank searches to 0.25 h (Price et al., 2011). After sampling, we recorded the species and life stage (larval or adult, i.e.,

post-metamorphosis) of each individual. All captured salamanders were measured for snout-vent length (SVL: from the tip of the snout to the posterior angle of the vent), total length (TL: from tip of the snout to the tail's terminus) to the nearest 0.01 mm with a digital caliper, and mass (except larvae ≤ 30 mm TL) to the nearest 0.1 g with a digital scale. We calculated body condition (mass/TL) on all salamanders ≤ 30 mm TL; salamanders missing tails or parts of their tails were excluded.

After counting and collecting morphometric data, we haphazardly selected a subset of the individuals at each site to examine diet composition (Table S1). The selected salamanders were anesthetized in the field, using a solution of 1 g Maximum Strength Orajel®/1 liter of distilled water (Cecala et al., 2007). Once the salamanders failed to right themselves after being flipped over, their stomach contents were obtained using a non-lethal gastric lavage method (Fraser, 1976; Hutton et al., 2018, 2019). Specifically, salamanders were placed on their dorsum on a folded paper towel and an approximately 6.0 cm long piece of tubing was inserted into the esophagus until there was resistance, then distilled water was pumped into the tubing (Hutton et al., 2019). Specifically, we used Nipro® 3 mL syringes with 22-gauge needles and 0.9 and 1.3 mm OD PTFE tubing (Zeus Inc., catalog number AWG24). The salamanders were then placed in a recovery container of stream water until they could right themselves and responded to tapping. Salamanders were returned to their exact location of capture within 1.5 h. No anesthetization or lavage-based mortality occurred (Hutton et al., 2021).

Stomach contents were identified to family, genus, and species, if possible, using a dissecting microscope along with appropriate keys and guides (Peckarsky, 1990; Merritt and Cummins, 1996; Fisher and Cover, 2007; Bradley, 2012; Evans, 2014). Additionally, presumed habitat of origin (aquatic or terrestrial) and invertebrate life stage (larval or adult) were recorded, if applicable. For Shannon diversity calculations, different sized prey, or prey with unique characteristics in a single order, family, or genus were considered to be separate morphospecies. The individual prey items were then grouped into larger sections based on order/class, life stage, and presumed habitat of origin (Hutton et al., 2018). Samples were then placed into individually labeled vials containing 70% ethanol. Samples are stored in the Branson Museum collection at Eastern Kentucky University, Richmond, Kentucky.

2.3. Diet analysis

To examine how SC potentially affects salamanders, we calculated diet component values beginning with individual prey item volume. We measured the length and width of each prey item to the nearest 0.01 mm using a digital caliper and estimated prey volume as a prolate spheroid using the equation (Dunham, 1983):

Prey Volume $(v_x) = (4\pi/3)$ (length/2) (width/2)^2.

Importance values (I_x), ranging from 0 to 3, were then calculated and used to compare the overall importance of a particular prey group or origin group (aquatic or terrestrial) to the overall diet (Anderson and Mathis, 1999). To calculate I_x for the prey groups, we used the equation:

$I_x = [(n_x/N) + (v_x/V) + (f_x/F)]/3$

where n_x, v_x, and f_x represent the number of a particular prey type, the volume of the prey type, and frequency or the number of stomachs containing that prey group, respectively, and N, V, and F represent their sums across all prey types (Hantak et al., 2016). We additionally calculated the dietary niche breadth, representing the variety of prey types consumed and their origins, estimated by calculating Shannon diversity indexes. Lastly, we calculated the relative occurrence (RO), or the percentage of each prey group's occurrence relative to all of the prey items, using the equation:

RO = (P*100)/T

where P is total number of occurrences for that prey type and T is the total number of prey items recovered (Loveridge and Macdonald, 2003). Empty stomachs were not included in the analyses.

We used the package "segmented" in the statistical program R (Version 3.4.3) to estimate larval and adult salamander SC thresholds for autochthony (the ratio of aquatic to terrestrial prey: A/T), total prey volumes per salamander, total aquatic and terrestrial prey volumes per salamander, Shannon aquatic prey diversity, and aquatic prey importance (I_x). Additionally, we created simple linear regression models (lm) using the R package "ggplot2" to examine the larval and adult average number of prey items, the adult total prey volume, total aquatic and terrestrial prey volumes, Shannon terrestrial prey diversity, terrestrial importance (I_x), and larval and adult salamander body condition in relation to SC because they failed to converge "segmented" threshold estimates. Specific conductance thresholds for the I_x and RO of the most important aquatic prey groups were also calculated for larval salamanders. The Shapiro-Wilk test was used to assess the normality of the data residuals. To attain the assumptions, the data were either log, square-root, or cube-root transformed, and estimates were then back-transformed to the scale of the data.

3. Results

We detected 9 salamander species during our active searches. However, we only considered 5 species (*Desmognathus fuscus* (DF), *D. monticola* (DM), *Eurycea cirrigera* (EC), *Gyrinophilus porphyriticus* (GP), and *Pseudotriton ruber* (PR)) in our analysis, as these species are primarily associated with streams. Due to low numbers of adult GP (n = 1) and PR (n = 2) captures, adult diets were not included. At our high SC sites, few individuals were found and lavaged, therefore, to allow for statistical dietary comparisons across the SC gradient, we combined the diet data of all species for each life stage (Table S1). Thus, for analyses, the larval (L) group consisted of diet data from DF(l), DM(l), EC(l), GP(l), and PR(l) and the adult (A) group consisted of data from DF, DM, and EC.

We stomach flushed 988 salamanders (424 A and 564 L). Fifty-four of the stomachs were empty (12 A and 42 L) and were all from sites with SC > 100 μS/cm. In larval salamanders, we identified 1130 aquatic prey items to 150 morphospecies (15 prey groups from 40 families/orders; Table S2) and 700 terrestrial prey items to 122 morphospecies (17 prey groups from 41 families/orders; Table S3). In the adult salamanders, we identified 318 aquatic prey items to 114 morphospecies (16 prey groups from 17 families/orders; Table S4) and 1356 terrestrial prey items to 378 morphospecies (24 prey groups from 59 families/orders; Table S5).

In the larval salamanders, autochthony (A/T) declined faster and more pronounced than in adults. Larval autochthony decreased rapidly along the gradient, specifically there was a 12-fold reduction (12:1–1:1) at a threshold of 153 μS/cm (95% CI: 64–243 μS/cm; Fig. 1a). However, adult salamanders were found to eat proportionately more terrestrial prey as SC increased. Adult autochthony decreased 3-fold (3:4–1:4) at a threshold of 382 μS/cm (95% CI: 12–752 μS/cm; Fig. 1b).

Larval salamanders consumed less prey volumetrically as SC increased, compared to adults. In the larvae, the total prey volume decreased approximately 4.2-fold (25–6 mm^3) at a threshold of 100 μS/cm (95% CI: 42–157 μS/cm; Fig. 1c). Further, the aquatic prey volume decreased approximately 2.6-fold (13–5 mm^3) at 99 μS/cm (95% CI: 34–164 μS/cm; Fig. 2a), and the terrestrial prey volume decreased 12-fold (12–1 mm^3) at 36 μS/cm (95% CI: 25–47 μS/cm; Fig. 2c). In adult salamanders, however, we found no statistical differences in the total, aquatic, or terrestrial prey volumes along the SC gradient ($P = 0.347$, 0.756, and 0.149, respectively; Figs. 1d, 2b, 2d, respectively).

Aquatic Shannon prey diversity and importance (I_x) were also found to disproportionately characterize the diets of larval salamanders. As SC increased, larval Shannon aquatic prey diversity declined approximately 1.3-fold (4.1–3.1) at a threshold of 119 μS/cm (95% CI: 45–194 μS/cm), whereas terrestrial prey diversity was positively associated with SC ($P < 0.05$; Fig. 3a). In adult salamanders, we found the Shannon aquatic prey diversity to decline approximately 1.4-fold (2.5–1.8) at a threshold of 682 μS/cm (95% CI: 151–1213 μS/cm), whereas the terrestrial prey diversity did not change as SC increased ($P = 0.538$; Fig. 3b). In larval salamanders, aquatic prey I_x declined approximately 2.2-fold (1.3–0.6) at a threshold of 135 μS/cm (95% CI: 98–172 μS/cm), whereas terrestrial prey I_x increased rapidly as SC increased ($P < 0.05$; Fig. 4a). In adults, aquatic prey I_x declined 2-fold (0.6–0.3) at a threshold of
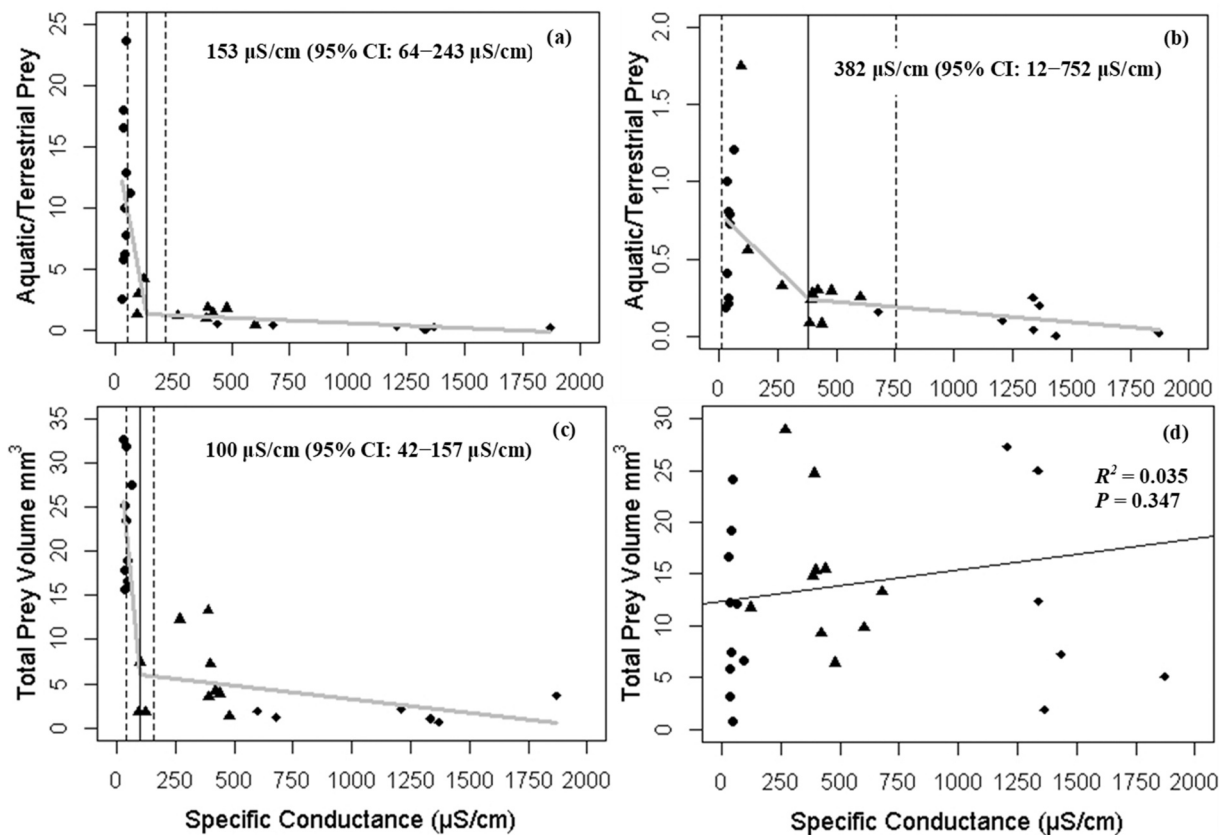

Fig. 1. Specific conductivity (SC) threshold estimates (with 95% confidence intervals) for autochthony (A/T) in (a) larval salamanders (cube root: $Y = -0.094x + 15.07$; $R^2 = 0.587$, $P < 0.05$; segmented regression line back-transformed to the scale of the data), (b) adult salamanders (cube root: $Y = -0.001x + 0.799$; $R^2 = 0.559$; $P < 0.05$; segmented regression line back-transformed to the scale of the data), and total prey volume in (c) larvae (log: $Y = -0.305x + 35.35$; $R^2 = 0.759$; $P < 0.05$, segmented regression line back-transformed to the scale of the data), and (d) adults (log: $Y = 0.0031x + 12.30$; regression line back-transformed to the scale of the data) along a continuous SC gradient in SE Kentucky. Symbols represent SC influence where circles are low SC (reference) streams, triangles are moderate SC streams, and diamonds are high SC (MTR-VF) streams. Solid vertical lines indicate the threshold estimate and dashed vertical lines represent the 95% confidence intervals.
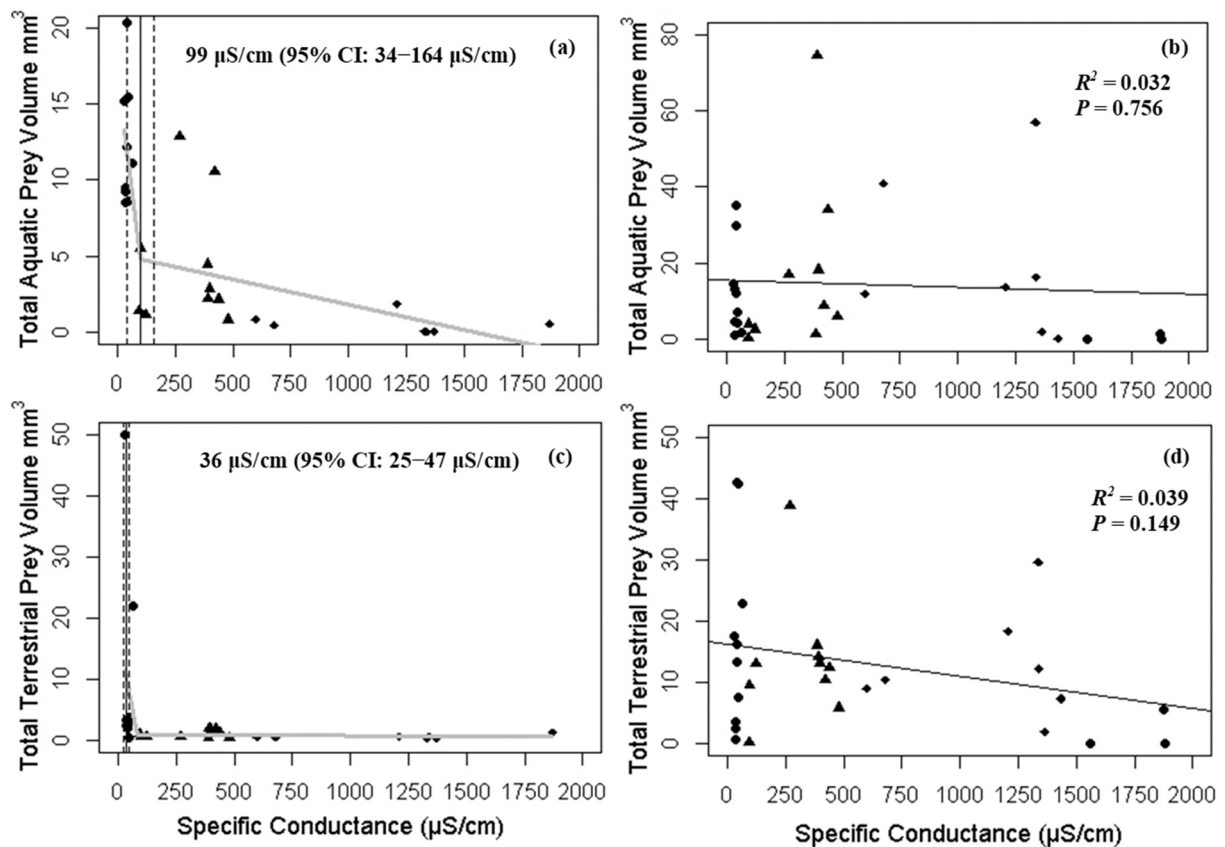
Fig. 2. Specific conductivity (SC) threshold estimates (with 95% confidence intervals) for (a) larval salamander aquatic prey volume (log: $Y = -0.134x + 17.55$; $R^2 = 0.634$, $P < 0.05$; segmented regression line back-transformed to the scale of the data), (b) adult salamander aquatic prey volume (log: $Y = -0.0017x + 15.306$; regression line back-transformed to the scale of the data), (c) larval salamander terrestrial prey volume (log: $Y = -10.25x + 367.16$; $R^2 = 0.832$, $P < 0.05$; segmented regression line back-transformed to the scale of the data), and (d) adult salamander terrestrial prey volume (log: $Y = -0.0052x + 16.117$; regression line back-transformed to the scale of the data) along a continuous SC gradient in southeastern Kentucky. Symbols represent SC influence where circles are low SC (reference) streams, triangles are moderate SC streams, and diamonds are high SC (MTR-VF) streams. Solid vertical lines indicate the threshold estimate and dashed vertical lines represent the 95% confidence intervals.

163 μS/cm (95% CI: 66–260 μS/cm), whereas terrestrial prey I_x increased as SC increased ($P < 0.05$; Fig. 4b). Specifically, in larval salamanders, the I_x of five major aquatic prey groups: Plecoptera larva (stoneflies), Caudata larva (salamanders), Ephemeroptera larva (mayflies), Cyclopoida (copepods), and Diptera larva (flies) decreased as much as 5-fold at thresholds from 43 to 128 μS/cm (Table 1).

In adults, both the terrestrial Shannon diversity and terrestrial I_x were already greater than the aquatic Shannon and I_x estimates at the lowest SC streams; thus, there was a greater original (i.e., at low SC streams) prominence of terrestrial prey in adult salamander diets than in larvae. In both larval and adult salamanders there was no change in the average number of prey items consumed as SC increased ($P = 0.117$ and 0.994, respectively). Lastly, larval and adult body condition decreased as SC increased ($P < 0.05$); however, the decline was more rapid in larval salamanders (Fig. 5a-b).

4. Discussion

Elevated SC from MTR-VF has been shown to be negatively correlated with stream salamander occupancy and abundances in Central Appalachia (Muncy et al., 2014; Price et al., 2016, 2018; Hutton et al., 2020). Appalachian streams with elevated SC also have reduced aquatic macroinvertebrate abundance, biomass, and diversity (Chambers and Messinger, 2001; Kennedy et al., 2003; Hartman et al., 2005; Pond et al., 2008; Fritz et al., 2010; Pond, 2010, 2012; Merriam et al., 2011; Cormier et al., 2013; Boehme et al., 2016; Voss and Bernhardt, 2017). Because stream salamanders rely heavily on aquatic prey, we hypothesized that as SC increases, reductions in adult and larval overall A/T ratios, aquatic prey volumes, aquatic Shannon diversities, and aquatic prey importance would lead to declines in salamander body condition. Our results show that as SC increased, the A/T ratio, aquatic volume, aquatic Shannon diversity, and aquatic prey importance declined precipitously; however, aquatic larval salamanders had stronger responses to SC than adults. Particularly, our diet parameter threshold estimates were consistently at lower SC values in larval salamander compared to adults. Therefore, our results suggest SC appears to directly affect the diet composition of larval and adult salamanders and serves as a
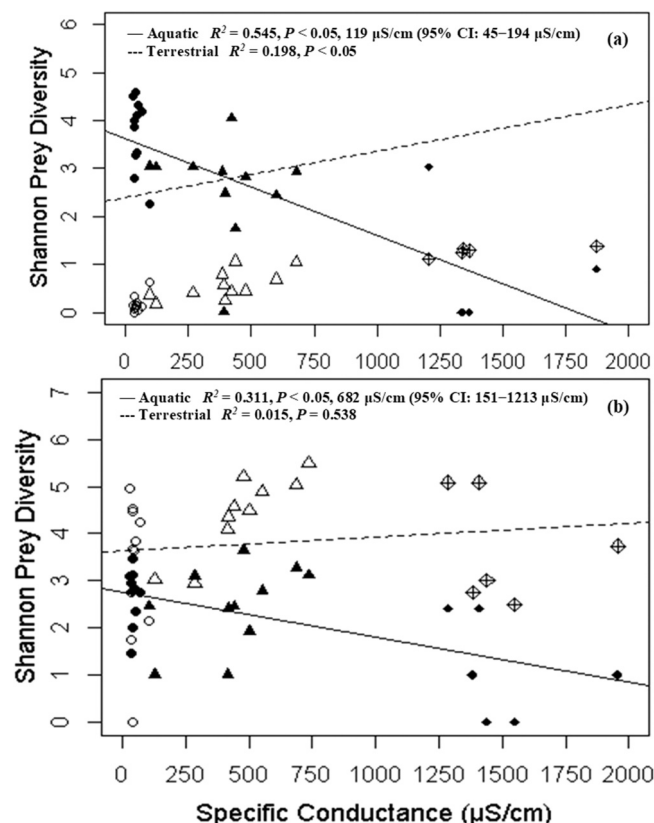
Fig. 3. Larval salamander (a) Shannon aquatic prey diversity (no transformation: $Y = -0.002x + 3.6192$) and Shannon terrestrial prey diversity (no transformation: $Y = 0.001x + 2.3827$), and adult salamander (b) Shannon aquatic prey diversity (no transformation: $Y = -0.001x + 2.751$) and Shannon terrestrial prey diversity (no transformation: $Y = 0.0003x + 3.649$) along a continuous SC gradient in southeastern Kentucky. The solid regression line represents the aquatic prey and the dashed regression line represents the terrestrial prey. Symbols represent SC influence where circles are low SC (reference) streams, triangles are moderate SC streams, and diamonds are high SC (MTR-VF) streams, closed symbols and represent aquatic prey and open symbols represent terrestrial prey. Specific conductivity thresholds were also calculated for the Shannon aquatic prey diversities.

proximate mechanism likely responsible for the patterns of reduced abundance, occupancy and species diversity along a SC gradient.

Few studies have estimated thresholds across a continuous SC gradient on aquatic organisms. Cormier et al. (2013) estimated that 163 aquatic macroinvertebrate genera are eliminated from Appalachian streams at a threshold of 295 µS/cm. Further, Pond et al. (2008) and Cormier et al. (2013) reported Ephemeroptera (mayflies), Plecoptera (stoneflies), and Diptera: Chironomidae (midge flies) diversity and abundance to be significantly lower in MTR-VF streams, which are important prey for many aquatic taxa (Wallace and Webster, 1996). Kraus et al. (2016) examined the effects of mining on trout along a metal gradient and reported a 9.4-fold and a 5-fold decrease in autochthony (A/T) and terrestrial prey dry mass, respectively. We found a 12-fold reduction in autochthony in larvae at a threshold of 153 µS/cm and observed a 3-fold reduction at 382 µS/cm in adults. Although our results correspond to prey found in salamander stomachs, overall autochthony suggests rapid declines in aquatic macroinvertebrate diversity and abundance similarly to as reported by Cormier et al. (2013). Although autochthony data provide important details alone, they fail to provide enough support to elucidate systematic patterns that can be better explored with finer scale information, such as prey volume.

Prey volume data can be used to provide more detailed dietary information for a system or study. Across the SC gradient, we observed precipitous declines in larval salamander total, aquatic, and terrestrial prey volumes that were not mirrored in the adult salamander diets. We estimated a larval total prey volume threshold with a 4.2-fold (25–6 mm^3) decline at 100 µS/cm, whereas no discernible differences were found in the adult volume parameters. Reductions in both the larval salamander's aquatic and terrestrial volumes suggests dietary changes associated with SC. However, the lack of changes in adult salamander volumes with increased SC may be explained by foraging natural histories. Fully aquatic larval salamanders are restricted to foraging in aquatic environments (Petranka, 1998), where they primarily prey on larvae from the orders Ephemeroptera (mayflies), Plecoptera (stoneflies), Trichoptera (caddisflies), and Diptera (flies; Martof and Scott, 1957; Caldwell and Houtcooper, 1973; Davic, 1991; Brophy and Pauley, 1997; Cecala et al., 2007; Keitzer and Goforth, 2013; Hutton et al., 2018). Further, many salamander species' larval natural histories include sit-and-wait aquatic suction predation; thus, they may refrain from actively foraging (Jaeger and Barnard, 1981; Anthony et al., 1992; Deban and Wake, 2000). Therefore, a reduction in aquatic prey can be immediately detrimental if they are unable to readily switch to a secondary foraging mode (Leff and Bachmann, 1988; McWilliams and Bachmann, 1989). Thus, in order to persist, the salamander
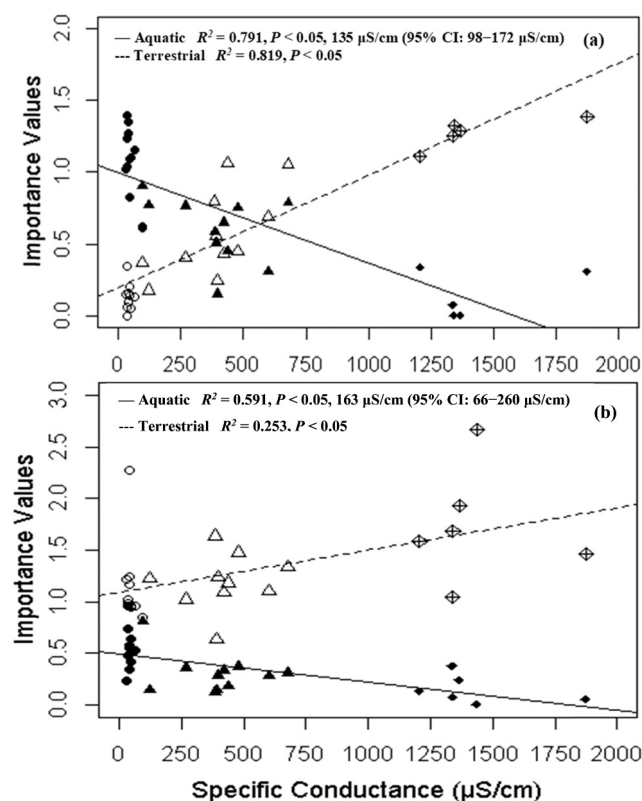
Fig. 4. Larval salamander (a) aquatic prey importance (no transformation: $Y = -0.0054x + 1.371$) and (a) terrestrial prey importance (no transformation: $Y = 0.0008x + 0.191$), and adult salamander (b) aquatic prey importance (no transformation: $Y = -0.002x + 0.649$) and terrestrial prey importance (no transformation: $Y = 0.0004x + 1.094$) along a continuous SC gradient in southeastern Kentucky. The solid regression line represents the aquatic prey and the dashed regression line represents the terrestrial prey. Symbols represent SC influence where circles are low SC (reference) streams, triangles are moderate SC streams, and diamonds are high SC (MTR-VF) streams, closed symbols and represent aquatic prey and open symbols represent terrestrial prey. Specific conductivity thresholds were also calculated for the Shannon aquatic prey diversities.

Table 1
Major aquatic prey importance (I_x) and relative occurrence (RO) threshold estimates with 95% confidence intervals and relative changes found in larval *Desmognathus fuscus*, *D. monticola*, *Eurycea cirrigera*, *Gyrinophilus porphyriticus*, and *Pseudotriton ruber* diets across a continuous SC gradient in southeastern Kentucky.

Order	I_x Threshold	95% CI	I_x Change	RO Threshold	95% CI	RO Change
Plecoptera larva	54 µS/cm	30–78 µS/cm	0.45–0.35	53 µS/cm	38–68 µS/cm	22–2%
Caudata larva	91 µS/cm	45–137 µS/cm	0.15–0.07	125 µS/cm	24–227 µS/cm	3–0.2%
Ephemeroptera larva	98 µS/cm	60–137 µS/cm	0.35–0.1	95 µS/cm	51–139 µS/cm	28–7%
Cyclopoida	128 µS/cm	58–139 µS/cm	0.1–0.02	145 µS/cm	49–148 µS/cm	13–2%
Diptera larva	43 µS/cm	26–62 µS/cm	0.3–0.1	125 µS/cm	24–227 µS/cm	35–15%

larvae must alter their foraging behavior or consume less optimal prey (Kraus et al., 2016).

At higher SC streams, most larval diets were primarily comprised of small terrestrial Acari (mites), collembolans (springtails), and adult and larval dipterans (flies). However, if terrestrial prey are not adequately available after the disappearance of aquatic prey, they may be too infrequent and volumetrically insignificant for the larval salamander's necessary metabolic processes. In our study, the volume of aquatic and terrestrial prey in larval diets declined 2.6-fold (13–5 mm³) and 12-fold (12–1 mm³) at 99 and 36 µS/cm, respectively. Therefore, in order to supplement the drastic decline in both aquatic prey and larger terrestrial prey, the larvae would need to forage much more frequently and in partially terrestrial conditions, thus increasing predation and desiccation risk. Contrarily, it is also possible that the only prey larval salamanders can consume in the absence of aquatic prey are terrestrial prey subsidies that fall or land in the stream (Duellman and Trueb, 1986; Deban and Wake, 2000), yet we are unaware of any in situ observations for either mode in larval plethodontids. However, in low-water flow captive settings, the larvae of the focal species have been observed predating upon terrestrial prey floating on the water's surface (JMH *pers obs*; T. Herman *pers comm*).

Unlike the larval salamander diets in this study, adult diets at the low SC sites were comprised primarily of terrestrial prey. Numerous adult stream plethodontid dietary studies from high quality sites (i.e., low SC or undisturbed) have also reported terrestrial
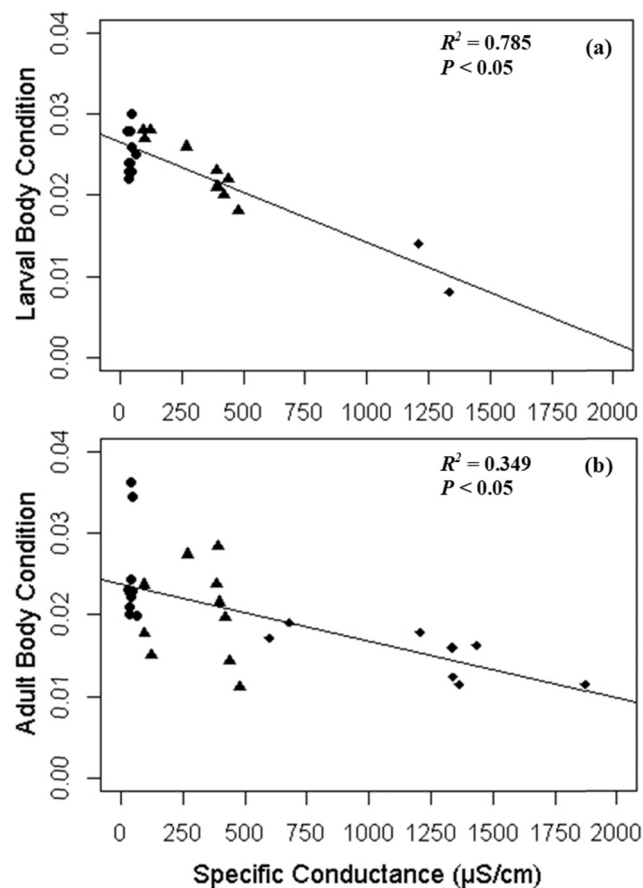
Fig. 5. Larval salamander (a) body condition (no transformation: $Y = -1.23\text{E-}5x + 0.0267$) and (b) adult salamander body condition (no transformation: $Y = -7.01\text{E-}6x + 0.0237$) along a continuous SC gradient in southeastern Kentucky. Symbols represent SC influence where circles are low SC (reference) streams, triangles are moderate SC streams, and diamonds are high SC (MTR/VF) streams.

prey to make up the majority of the diet (Sites, 1978; Davic, 1991; Shipman et al., 1999; Felix and Pauley, 2006; Hutton et al., 2018). Adult salamanders are also reported to consume a wider diversity and frequency (65–85%) of terrestrial prey in their diets than larvae, which is mainly comprised of adult and larval Diptera (flies) and Coleoptera (beetles), Collembola (springtails), and Hemiptera (true bugs; Sites, 1978; Davic, 1991; Shipman et al., 1999; Felix and Pauley, 2006; Hutton et al., 2018). Therefore, a reduction in aquatic prey due to moderate catchment disturbance is not expected to have a severe impact on adult stream salamanders' overall diets and volume. In this study, there were no statistical differences in the adult salamander's total, aquatic, or terrestrial prey volumes across the SC gradient. Furthermore, at the low SC streams, terrestrial prey were already considerably more diverse and important than aquatic prey. Thus, in low and high SC streams, terrestrial prey appear to be more readily available to adult salamanders because they are capable of leaving the stream margin to forage and obtain more numerous and larger prey, unlike fully aquatic larval salamanders.

Although we found no differences in the prey volumes of adult salamanders in this study over the gradient, there was a noticeable change in overall composition. Land use disturbances have also been shown to reduce terrestrial invertebrate biomass and abundance (Edwards and Huryn, 1996; Attwood et al., 2008). In our study, the adult terrestrial I_x only increased slightly, whereas the aquatic I_x declined much more rapidly over the SC gradient. The Shannon diversity of aquatic prey declined rapidly along the SC gradient, whereas the terrestrial prey diversity did not statistically change. At high SC sites, there was a considerably lower diversity of prey consumed. There was a decline in aquatic and terrestrial prey available to the adult salamanders. Nonetheless, there appears to be a decreasing trend in volume, which is supported by the decline in adult salamander body condition along the SC gradient. Overall, our diet results indicate severe reductions in aquatic and terrestrial prey in salamander stomach contents as well as salamander body condition, but the effects were more abrupt and pronounced in larvae than adults. Thus, at sites with reduced aquatic and terrestrial prey availability (i.e., high SC), we have seen declines in larval and adult salamander abundance, which likely leads to reduced population sizes (Hutton et al., 2020). Local extinction may result due to the inability of larvae to persist and be recruited into the population.

Previous salamander studies have also seen stronger effects of MTR-VF on larvae than adults (Wood and Williams, 2013; Muncy et al., 2014; Price et al., 2016). However, since these studies only examined salamander occupancy, abundance and richness, the proximate mechanism(s) driving these observations could not be tested or determined. Our results indicate that diet is potentially an

important factor driving the observed declines, as well as the differences in adult and larval salamander abundances (Hutton et al., 2020). Our results also illustrate the importance of sampling along ecological gradients to elucidate specific mechanisms and pathways that explain the observed system. From a management perspective, our results indicate riparian buffers around headwater streams that provide foraging habitat with an abundant and diverse invertebrate community may be necessary to sustain salamander populations in streams with high SC from MTR-VF and other land uses. Revegetation practices have been shown to increase the amount of terrestrial prey that enter the stream (Wipfli, 1997; Kawaguchi and Nakano, 2001; Saunders and Fausch, 2012; Wipfli and Baxter, 2010). An increase in terrestrial prey subsidies could potentially provide sufficient resources for the larvae to reach metamorphosis and to survive to reproductive age, thus aiding population persistence (Clements et al., 2010; Kraus et al., 2016). Since adult salamanders rely almost exclusively on terrestrial prey, a further increase in availability and diversity of terrestrial prey items is also expected to increase occupancy, abundance, and body condition. Therefore, in order to protect stream salamander populations in MTR-VF landscapes, we recommend riparian protection from mining, widening of buffer zones, and if possible, mitigating SC levels via buffering.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.gecco.2021.e01719.

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