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Research

Extreme heat events and the vulnerability of endemic montane fishes to climate change

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Identifying how close species live to their physiological thermal maxima is essential to understand historical warm-edge elevational limits of montane faunas and forecast upslope shifts caused by future climate change. We used laboratory experiments to quantify the thermal tolerance and acclimation potential of four fishes (*Notropis leuciodus*, *N. rubrocreucus*, *Etheostoma rufilineatum*, *E. chlorobranchium*) that are endemic to the southern Appalachian Mountains (USA), exhibit different historical elevational limits, and represent the two most species-rich families in the region. All-subsets selection of linear regression models using AIC_c indicated that species, acclimation temperature, collection location and month, and the interaction between species and acclimation temperature were important predictors of thermal maxima (T_{max}), which ranged from 28.5 to 37.2°C. Next, we implemented water temperature models and stochastic weather generation to characterize the magnitude and frequency of extreme heat events ($T_{extreme}$) under historical and future climate scenarios across 25 379 stream reaches in the upper Tennessee River system. Lastly, we used environmental niche models to compare warming tolerances (acclimation-corrected T_{max} minus $T_{extreme}$) between historically occupied versus unoccupied reaches. Historical warming tolerances, ranging from +2.2 to +10.9°C, increased from low to high elevation and were positive for all species, suggesting that T_{max} does not drive warm-edge (low elevation) range limits. Future warming tolerances were lower (−1.2 to +9.3°C) but remained positive for all species under the direst warming scenario except for a small proportion of reaches historically occupied by *E. rufilineatum*, indicating that T_{max} and acclimation potentials of southern Appalachian minnows and darters are adequate to survive future heat waves. We caution concluding that these species are invulnerable to 21st century warming because sublethal thermal physiology remains poorly understood. Integrating physiological sensitivity and warming exposure demonstrates a general and fine-grained approach to assess climate change vulnerability for freshwater organisms across physiographically diverse riverscapes.

Keywords: Appalachian Mountains, freshwater fishes, range shift, stochastic weather generation, thermal tolerance, warming tolerance



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Introduction

Climate change poses a significant threat to biodiversity (IPCC 2014). In montane regions, upslope shifts in suitable thermal conditions have already forced species' distributions to higher elevations in the 20th century (Chen et al. 2011a, Tingley et al. 2012, Comte and Grenouillet 2013) and similar upslope movements are forecasted for the future (Loarie et al. 2009, Isaak et al. 2016). Other studies documented downslope shifts in species distributions, associated with changes in precipitation, land use and other elevation-correlated gradients (Comte and Grenouillet 2013, Radinger et al. 2016). These shifts will alter biodiversity patterns, with eroded richness at low elevation, mountaintop extinctions at high elevations, and changes in species' cooccurrences and subsequent interactions at all elevations (Feeley and Silman 2010, Sheldon et al. 2011, Freeman et al. 2018).

The magnitude of upslope shifts depends on the intrinsic physiological thermal sensitivity of the species and extrinsically on the species' exposure to increased temperatures where they presently occur. Sensitivity is affected by physiological tolerance (e.g. maximum thermal tolerance, hereafter T_{max}), short-term acclimation (i.e. acclimation response ratio, hereafter ARR), genetic adaptive potential and thermoregulatory behavior (Bürger and Lynch 1995, Kearney and Porter 2009, Sunday et al. 2011). Meta-analyses for freshwater fishes documented interspecific variation in T_{max} and ARR (Comte and Olden 2017a), indicating that some taxa are more sensitive to warming than others. Alternatively, exposure to warming varies spatially and depends on different rates of regional warming across the globe (Garcia et al. 2014, Wang and Dillon 2014). In freshwaters, exposure to warming depends not just on projected air temperature change but also hydrologic and landscape characteristics that mediate the air–water temperature relationship, e.g. riparian shading and groundwater influence (Caissie 2006).

The difference between future environmental temperature and T_{max} (i.e. warming tolerance) will ultimately determine whether warm-edge limits of populations will be forced upslope (Deutsch et al. 2008). A recent assessment of warming tolerances estimated a 3–19°C buffer for temperate freshwater fishes (Comte and Olden 2017b). However, these warming tolerances derived from mean temperature of the warmest month may be conservative because they do not take into account daily temperature extremes (hereafter $T_{extreme}$), which may drive warming tolerances during $T_{extreme}$ events to zero or less (hereafter lethal heat events). Incorporating such extreme weather events into future distributional projections of individual species is essential for vulnerability assessments of faunas (Jentsch and Kreyling 2007). For example, including extreme temperature and drought events as predictors in environmental niche models improved predictive performance of those models developed for mammals (Morán-Ordóñez et al. 2018) and birds (Bateman et al. 2016). The degree to which $T_{extreme}$ events in the future will affect freshwater taxa is not well-studied, but certainly will

depend on air–water temperature sensitivity, species-specific sensitivities and the warming tolerances afforded within historically-occupied habitats.

The southern Appalachian Mountains are a hotspot of freshwater fish biodiversity and a region with strong elevational temperature gradients (Pelayo-Villamil et al. 2015). Species exhibit a variety of elevational limits and breadths, and therefore associate with different thermal regimes (Etnier and Starnes 1993, Keck et al. 2014). However, thermal physiology of most species remains unknown, precluding understanding of historical elevational limits and preventing accurate forecasts of upslope shifts under 21st century climate change. To bridge these knowledge gaps, we combined new experimental data on the physiological sensitivity of endemic species with spatially explicit projections of $T_{extreme}$ events under historical and future climates to: 1) elucidate the role of thermal physiology and $T_{extreme}$ events in governing historical species elevational range limits; and 2) examine potential shifts in warm-edge elevation limits using high resolution (i.e. reach-scale) spatial maps of warming tolerance across 25 379 stream reaches in the upper Tennessee River system. We implemented our analyses in a species comparative framework, focusing on endemic species from the two most species- and endemic-rich families in the region: minnows (family Cyprinidae) and darters (subfamily Etheostomatinae). The Tennessee shiner *Notropis leuciodus* and saffron shiner *N. rubriroceus* occupy moderate-elevation and high-elevation streams, respectively, while the redline darter *Etheostoma rufilineatum* and greenfin darter *E. chlorobranchium* occupy low-elevation and high-elevation streams, respectively (Etnier and Starnes 1993).

Material and methods

Conceptual workflow

We implemented a four-step workflow to estimate warming tolerances associated with $T_{extreme}$ events across a physiographically diverse riverscape (Fig. 1). Specifically, we used physiology experiments to quantify T_{max} and ARR of our focal species (step A); multi-model ensembles to characterize landscape–air–water temperature relationships (step B); stochastic weather generation to project $T_{extreme}$ events under historical and future climate scenarios (step C); and environmental niche models (hereafter ENMs) to compare warming tolerances at historically occupied versus unoccupied reaches (step D).

Step A: physiology experiments

We conducted laboratory experiments to measure T_{max} and its ARR, which is the slope of the linear relationship between T_{max} and acclimation temperature (hereafter laboratory T_{acclim}). We estimated T_{max} using the dynamic critical thermal maximum method, whereby test individuals are exposed to a steady increase in temperature until a physiological or behavioral endpoint is reached (Lutterschmidt and Hutchison

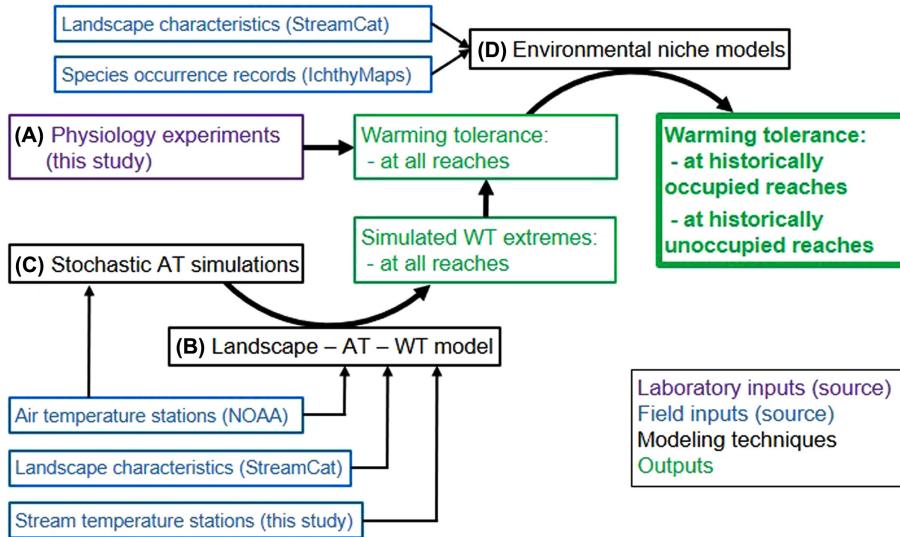


Figure 1. Conceptual workflow illustrating (A) laboratory experimentation, (B) stochastic weather generation, (C) landscape–air–water temperature modeling and (D) environmental niche modeling are integrated to compare warming tolerance of a species. This workflow was performed for the four study species in the upper Tennessee River system.

1997). This method yields an ecologically-relevant measure of susceptibility to heat stress because our physiological endpoint of choice – loss of righting ability – corresponds to an individual's inability to escape lethal thermal environments.

We collected test individuals from two locations for each species in the upper Tennessee River system using backpack electrofishing and seining (Supplementary material Appendix 1 Table A1). We selected the two collection locations to maximize the difference in summertime temperature experienced by individuals from the two populations. We collected individuals during two time periods (12–22 June 2017 and 1–22 August 2017) to assess differences in T_{\max} between early and late summer. All test fish were housed for 21–40 d in 114-liter aquariums at the indoor Univ. of Tennessee Animal Facility with a daily photoperiod of 14 h light and 10 h dark, which approximates the natural photoperiod in the upper Tennessee River system during summer. Fish were fed ad libitum rations of frozen brine shrimp and frozen blood worms once per day, with food withheld during the 24 h preceding tolerance assays. We separated individuals from the early summer collection into three groups and acclimated at 14, 18 or 22°C, while those collected in late summer were acclimated at 18, 22 or 26°C. These laboratory T_{acclim} levels approximate the elevational temperature gradient in the upper Tennessee River system during the early and late summer collection periods (Supplementary material Appendix 1 Table A1).

We performed T_{\max} assays in a five-gallon bucket filled full with water from acclimation aquariums and heated at a rate of $0.3^{\circ}\text{C min}^{-1}$ (Beitinger and Bennett 2000) using a 500-W aquarium heater. We performed assays on two individuals per trial, with each individual held in separate perforated 1-liter plastic containers submerged in the bucket. Each container was aerated to maintain oxygen saturation throughout the

duration of the trial. We used air lift pumps to circulate water from the bucket to each container. Temperatures were monitored using two digital thermometers per container. We used three behavioral cues to characterize loss of righting ability: first loss of righting ability, sustained loss of righting ability and cessation of muscular and opercular movements (Lutterschmidt and Hutchison 1997). All test fish were euthanized immediately following the final endpoint.

Endpoints derived from the three behavioral cues were highly correlated ($r=0.79\text{--}0.90$), therefore, statistical analyses are only presented for sustained loss of righting ability. We used all-subsets model selection based on small-sample Akaike information criterion (AIC_c) (Burnham and Anderson 2002) to identify the best linear regression model relating T_{\max} to species identity, collection month, collection location, laboratory T_{acclim} and two-way interactions.

Step B: landscape – air temperature – water temperature models

We modeled and mapped daily water temperature (hereafter WT) at all 25 379 National Hydrography Dataset flowlines (hereafter reaches; McKay et al. 2012) in the upper Tennessee River system using daily air temperature (hereafter AT) and landscape characteristics (Supplementary material Appendix 2 Fig. A1). We used a multi-model ensemble approach (Araújo and New 2007) to account for uncertainty in the complex relationships among WT, AT and landscape characteristics (DeWeber and Wagner 2014). Algorithms included linear and non-linear regression-based techniques (generalized linear models and generalized additive models, respectively) and a machine learning technique (random forests). We trained models using daily maximum WTs and daily maximum ATs from 153 stream temperature monitoring stations in the southern Appalachian Mountains

(Supplementary material Appendix 2 Fig. A2). Stations were nested within nine different fifth or sixth order watersheds representing the physiographic and anthropogenic landscape variation of the region. We established 149 of the 153 stations on 12–29 stream reaches per watershed and distributed these stations approximately proportional to Strahler order frequencies within each watershed. We installed one data logger (HOBO Water Temperature Pro v2 Data Logger; Onset) at each station between 3 May and 15 June 2017 following a standard field protocol (USEPA 2014) and programmed data loggers to record WT on 15-min intervals. We supplemented our 149 stations with four additional time series of equal temporal resolution and extent from the United States Geological Survey (gage numbers 03497300, 03539778, 03539600, 03538830), which we downloaded from the National Water Information System. We acquired daily maximum ATs for the calibration period (May–November 2017) from 451 NOAA weather stations in the southeastern United States (Supplementary material Appendix 2 Fig. A3) and used universal kriging to spatially interpolate these AT data to all 25 379 reaches in the upper Tennessee River system, including those 153 reaches on which we established WT monitoring stations. Universal kriging was implemented with elevation as a covariate in R using the automap library (Hiemstra et al. 2009). We acquired GIS-derived predictor variables describing landscape and hydrographic characteristics for the 25 379 reaches from the StreamCat dataset (Hill et al. 2016). We checked variable pairs for correlations (Pearson's r) and retained only uncorrelated variables ($|r| \leq 0.7$) known to influence stream temperature (DeWeber and Wagner 2014, Supplementary material Appendix 2 Table A2). Next, we used each of the three statistical algorithms to fit models, using several parameterizations for each statistical algorithm (Supplementary material Appendix 2 Table A3). We used this same procedure to model daily mean WT.

Step C: stochastic AT simulations

We used stochastic weather generation to downscale monthly AT minima and maxima for historical (1951–1980) and future (2071–2100) time periods (Supplementary material Appendix 1 Fig. A1). This finer resolution of temporal temperature variability emulates the T_{extreme} events likely to cause physiological stress and affect population persistence (Jentsch and Kreyling 2007). Stochastic weather generation quantifies the statistical properties of a daily temperature time series during a calibration period and then generates an analogous daily time series with identical statistical properties for a projection period. First, we fit vectorized autoregressive models to a 10-yr calibration period (Cordano and Eccel 2016). Input data for the calibration period included daily and monthly minimum and maximum ATs for the years 2007–2016, which we acquired from the aforementioned 451 NOAA weather stations. We used universal kriging to spatially interpolate these AT data to all 25 379 reaches in the upper Tennessee River system. Second, we used Gaussianized principal components

analysis to simulate 100 annual time series of daily maximum AT for projection periods representing historical and future climates (Cordano and Eccel 2016). We clarify that each year of the 100 yr is calibrated on a climate scenario (e.g. averaged over the 2071–2100 time period), rather than the gradual change in climate over a 100-yr time period (e.g. 2001–2100). Input data for each projection period included monthly minimum and maximum ATs from a GCM representing the historical climate, four GCMs representing the Representative Concentration Pathway (hereafter RCP) 4.5 emissions scenario for the future period, and four GCMs scenarios representing the RCP 8.5 emissions scenario for the future period (Supplementary material Appendix 2 Table A4), which we spatially interpolated using the ClimateNA program (Wang et al. 2016). We implemented calibration and projection of the stochastic weather generator with the RMAWGEN library in R.

We used the simulated daily maximum ATs from step C and landscape-AT-WT-model from step B to project a 100-yr time series of daily maximum WTs to each of the 25 379 reaches. This same procedure was used to project daily mean WTs. Lastly, we summarized the frequency of T_{extreme} events using the return interval concept from hydrologic science (Gordon et al. 2004). For the 100 annual WT time series, the maximum daily maximum WT for each year (hereafter T_{extreme}) was used to compute the return interval associated with a given magnitude of a T_{extreme} event using the equation:

$$\text{RI} = \frac{N + 1}{m}$$

where RI is the return interval in years, N is the number of years (i.e. 100) and m is the rank of the maximum daily maximum WT for a year.

Step D: environmental niche models

We used ENMs to map historical occurrence probabilities of each species to the 25 379 reaches in the upper Tennessee River system. We extracted occurrence records for each species from the IchthyMaps dataset (Frimpong et al. 2016). We acquired GIS-derived predictor variables describing landscape and hydrographic characteristics for the 25 379 reaches from the StreamCat dataset (Hill et al. 2016). We checked variable pairs for correlations (Pearson's r) and retained only uncorrelated variables ($|r| \leq 0.7$) known to constrain distributions of stream fishes (Huang and Frimpong 2015). In total, ten uncorrelated landscape and hydrographic variables were used as predictors in our ENMs (Supplementary material Appendix 3 Table A5). We used a multi-model ensemble approach to account for uncertainty in reach occurrence probabilities associated with different model classes (i.e. statistical algorithms) and initial conditions (i.e. subsets of presences and absences) (Araújo and New 2007, Supplementary material Appendix 3 Fig. A4) and explored several parameterizations (Supplementary material Appendix 3 Table A6).

Warming tolerance

We combined the laboratory-measured T_{\max} with simulated T_{extreme} to estimate warming tolerances for each species across the 25 379 reaches in the upper Tennessee River system. Because T_{\max} depends on recent thermal acclimation, we estimated T_{\max} based on acclimation to environmental temperatures experienced during the 30 d prior to the T_{extreme} event of a given year. We identified the T_{extreme} for each of the 100 annual WT time series and computed the mean of the daily mean WT over the 30 preceding days (hereafter field T_{acclim}). We used this field T_{acclim} value to predict the T_{\max} of each species using the species-specific ARR. The difference between this acclimation-corrected T_{\max} and T_{extreme} for that year was computed as the warming tolerance. This procedure was implemented for each of 12 historical ensemble runs (three WT modeling algorithms, four ARRs) and 96 future ensemble runs (two emissions scenarios, four GCMs, three WT modeling algorithms, four ARRs). The four ARRs of each species were acquired from the slope parameters of simple linear regression models fit separately for each experimental treatment (i.e. high- versus low-elevation locations, early versus late summer collections).

To examine whether lethal heat events may have contributed to historical range limits, we compared warming tolerances in historically occupied versus unoccupied reaches. We classified historical occupancy using ENM projections from Step D, assuming conservatively that reaches with modeled suitability > 0.9 were occupied and reaches with modeled suitability < 0.1 were unoccupied. We also assessed variation (i.e. uncertainty) in warming tolerance among future ensemble runs with a linear regression model, which included species, emissions scenario, GCM, WT modeling algorithm, collection location ARR and collection month ARR as covariates. We used all-subsets AIC_c model selection to identify the best linear regression model (Burnham and Anderson 2002). Relative variable importance was evaluated by comparing the F-statistics of likelihood ratio tests after excluding each variable from the best model (Giam and Olden 2018).

Data deposition

Data available from the Dryad Digital Repository: <<https://doi.org/10.5061/dryad.55s9h5r>> (Troia and Giam 2019).

Results

Environmental niche modeling confirmed interspecific variation in ecoregional and elevational associations of the four study species (Fig. 2). *Notropis rubricroceus* and *E. chlorobranchium* were restricted to the highest elevation streams of the Blue Ridge ecoregion, with the occurrence probability of the former greatest in the northeastern portion of the

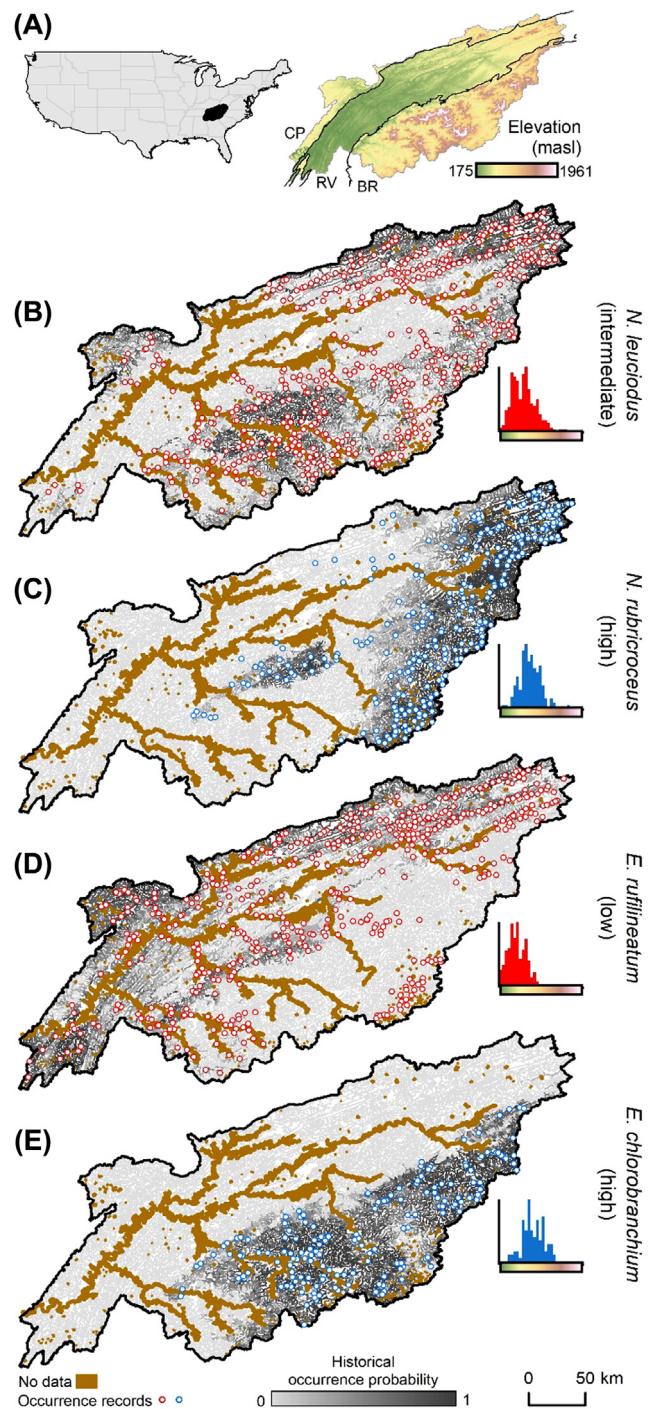


Figure 2. (A) Physiographic characteristics of the upper Tennessee River system in southeastern United States and (B-E) historical distributions of four endemic fishes. Historical distributions are shown as IchthyMaps point occurrences (colored circles) and ENM-based occurrence probability (grayscale). Black lines in (A) delineate Cumberland Plateau (CP), Ridge and Valley (RV) and Blue Ridge (BR) ecoregions. Inset histograms show frequency of IchthyMaps occurrences across elevations.

UTB and occurrence probability for the latter greatest in the southeastern portion. By contrast, occurrence probability for *E. rufilineatum* was highest in the Ridge and Valley ecoregion and to a lesser extent in Cumberland Plateau ecoregions of the central and northwestern UTB. Occurrence probability for *N. leuciodus* was intermediate to the other species, with the highest values distributed sparsely throughout the Blue Ridge ecoregion. Median elevation of occurrence records was 818 m above sea level (hereafter m a.s.l.) for *E. chlorobranchium*, followed by 736 m a.s.l. for *N. rubricroceus*, 669 m a.s.l. for *N. leuciodus* and 405 m a.s.l. for *E. rufilineatum*. Low-elevation limits highest for *N. rubricroceus* (447 m a.s.l.) and *E. chlorobranchium* (445 m a.s.l.) and lowest for *E. rufilineatum* (220 m a.s.l.) and *N. leuciodus* (296 m a.s.l.). ENMs performed better for the high-elevation species, *N. rubricroceus* ($AUC=0.81-0.83$) and *E. chlorobranchium* ($AUC=0.81-0.82$), than for the low- and intermediate-elevation species, *E. rufilineatum* ($AUC=0.75-0.79$) and *N. leuciodus* ($AUC=0.69-0.73$) (Supplementary material Appendix 4 Table A7).

T_{\max} measured on laboratory-acclimated fish ranged from 28.5 to 37.2°C. According to the best linear regression model, T_{\max} was associated with species identity, laboratory T_{acclim} , the interaction between species identity and laboratory T_{acclim} , collection location and collection month (Supplementary material Appendix 4 Table A8, A9). T_{\max} increased with laboratory T_{acclim} (Fig. 3, Supplementary material Appendix 4 Table A9). *Etheostoma rufilineatum* exhibited a lower ARR than the other three species, particularly in late summer, indicating a lower ability of this darter to acclimate to seasonal temperature fluctuations. Individuals collected from the lower elevation location and in late summer had lower T_{\max} .

The magnitude and frequency of T_{extreme} varied spatially (i.e. high- versus low-elevation reaches) more so than temporally (i.e. historical versus future climates) (Fig. 4, Supplementary material Appendix 4 Table A10, Fig. A5). We project T_{extreme} events to occur with greater magnitude and increased frequency from upstream to downstream and from high to low elevation. The magnitude of T_{extreme} events in reaches in the Blue Ridge ecoregion is 24.8°C (range: 19.0–33.8°C) on average compared with 26.4°C (21.6–31.3°C) for the Cumberland Plateau and 27.1°C (23.8–40.3°C) for the Ridge and Valley. Under future climate scenarios, our models estimate the magnitude of these T_{extreme} events to increase by 2.2–3.5°C in the Blue Ridge ecoregion, 2.9–4.3°C in the Cumberland Plateau ecoregion, and 1.6–3.0°C in the Ridge and Valley ecoregion.

Mean acclimation-adjusted warming tolerances (i.e. $T_{\max} - T_{\text{extreme}}$) across species and historically occupied reaches was +7.4°C (range: +2.2 to +10.9°C) under historical climate, 6.3°C (+0.1 to +10.2) under the future low emissions scenario and +5.5°C (-1.2 to 9.3) under the future high emissions scenario, indicating that lethal heat events are unlikely throughout nearly all reaches of the upper Tennessee system under either historical or future climate scenarios (Fig. 5). Variation in warming tolerance across the 96 future ensemble

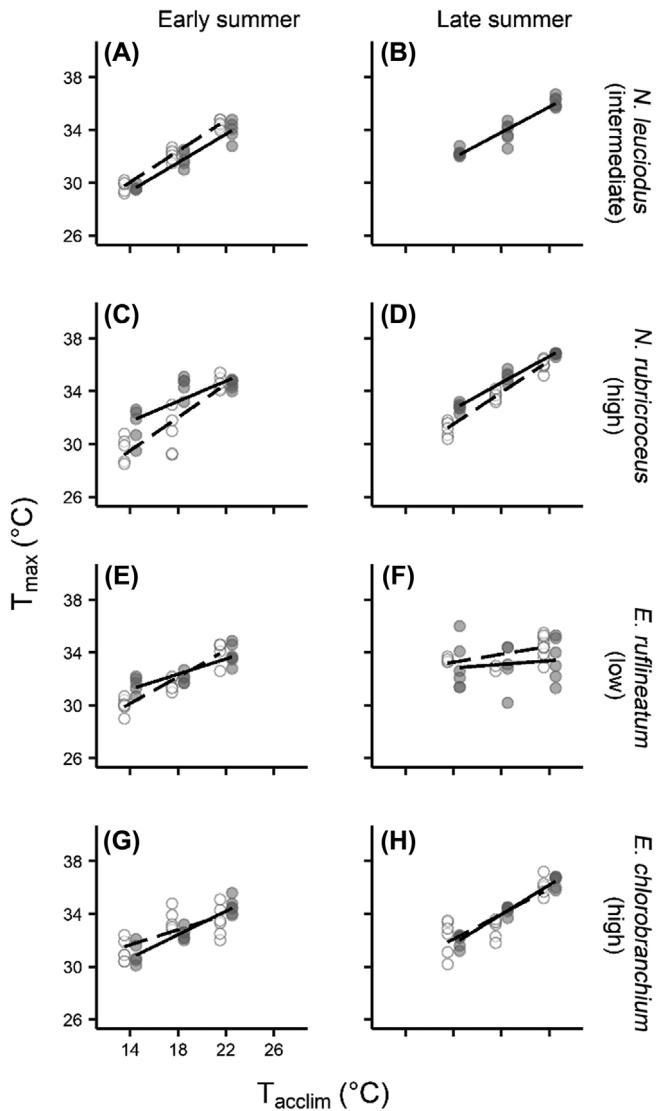


Figure 3. T_{\max} as a function of laboratory T_{acclim} for four species (putative elevation affinity in parentheses). Individuals were collected from high-elevation (filled circles and solid lines) and low-elevation (open circles, dashed lines) locations in the upper Tennessee River system in early (left panel) and late (right panel) summer.

runs was mostly associated with the WT modeling algorithm, followed by species identity, and GCM (Supplementary material Appendix 4 Table A11, A12). Median warming tolerances under historical climate was similar for historically high-suitability reaches versus low-suitability reaches of *N. leuciodus* (+7.2°C versus +7.2°C), *N. rubricroceus* (+7.9 versus +7.5°C) and *E. chlorobranchium* (+7.9 versus +7.1°C). For the low-elevation darter, *E. rufilineatum*, median warming tolerance was lower for high suitability reaches (+5.7°C) compared to low suitability reaches (+8.1°C) which suggests that habitat suitability and risk of experiencing a lethal heat event are negatively correlated. Warming tolerances

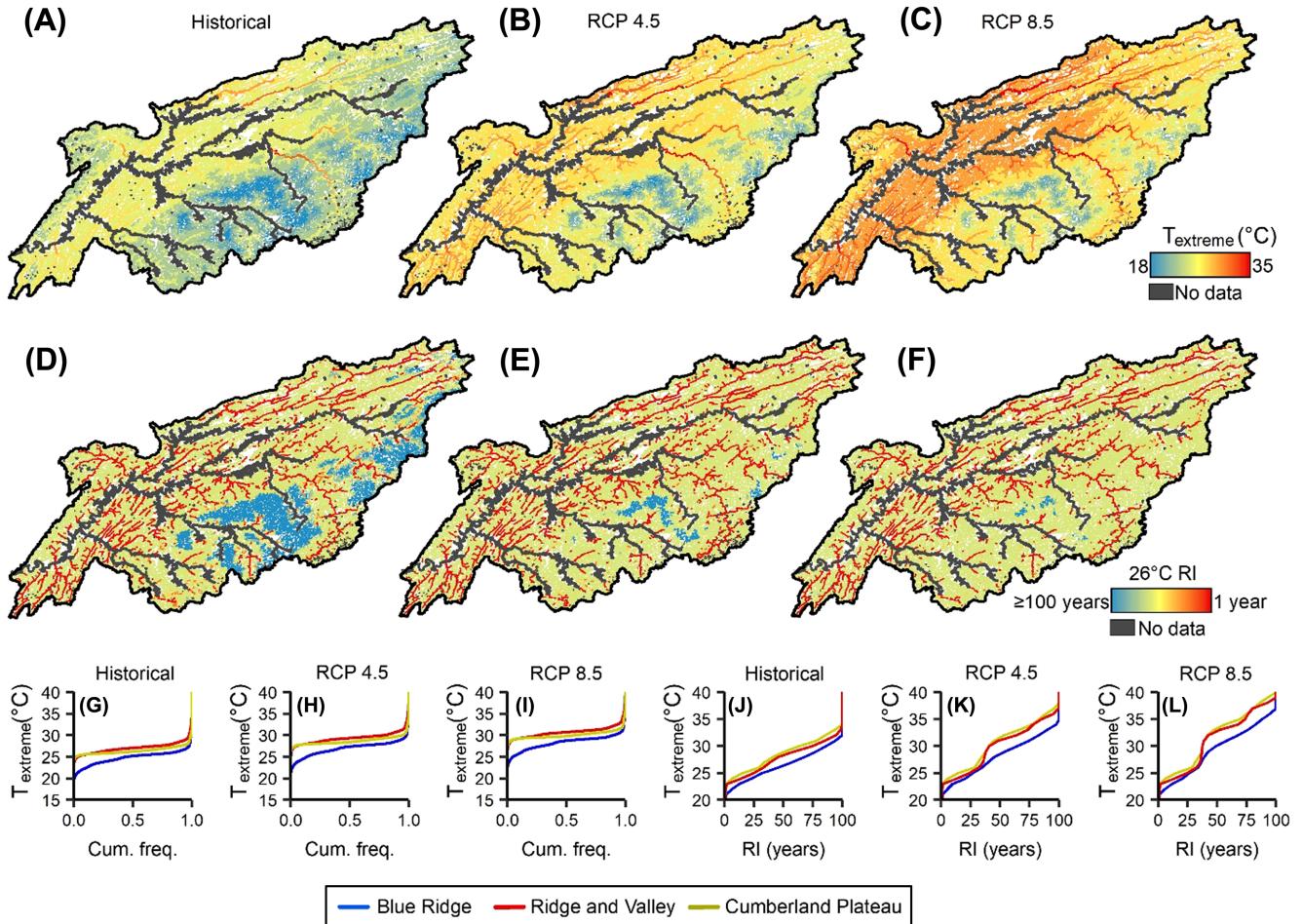


Figure 4. (A–C) Magnitude of T_{extreme} and (D–F) return interval of 26°C event across 25 379 reaches in the upper Tennessee River system under (A, D) historical climate and future climate based on (B, E) low and (C, F) high emissions scenarios. Plots show (G–I) cumulative frequencies and (J–L) return intervals of extreme WTs among three ecoregions.

were generally higher in the Blue Ridge ecoregion and high-elevation (i.e. northeastern) portions of the Ridge and Valley ecoregion compared to larger streams in the low-elevation (i.e. southwestern) portions of the Ridge and Valley (Fig. 6). Mean return interval of lethal heat events (i.e. $T_{\text{max}} < T_{\text{extreme}}$) across species and historically occupied reaches was 86 yr (range: 40 to > 100 yr) under historical climate, 92 yr (37 to > 100) under the future low emissions scenario and 80 yr (37 to > 100) under the future high emissions scenario (Fig. 5).

Discussion

Historical elevation limits

Freshwater fishes – including the southern Appalachian species in the present study – exhibit a variety of elevational range limits, the physiological and ecological drivers of which often are understood poorly (Sexton et al. 2009, Bhatt et al. 2012). Our findings revealed positive warming tolerances and infrequent lethal heat events for all four species from

low to high elevations, indicating that T_{max} and acclimation potentials are adequate for these species to persist anywhere in the upper Tennessee River system under historical climate. This suggests that T_{extreme} events and T_{max} play little role in low-elevation (i.e. warm-edge) limits for these species. This notion is further substantiated by the lack of concordance between warming tolerances in reaches with historically high versus low habitat suitability. It is not uncommon that species do not ‘fill’ their fundamental thermal niche. For example, a global analysis revealed frequent underfilling of the equatorward (i.e. warm edge) geographic limit for a majority of terrestrial ectotherms – a pattern attributed to moisture availability, constraints of alternative thermal regime components, and biotic interactions (Sunday et al. 2012). There are several mechanisms specific to southern Appalachian fishes that may explain the variation in elevational range limits observable among these four species and among the broader fauna of southern Appalachian minnows and darters. First, laboratory T_{max} estimates may overestimate T_{max} in streams for several reasons. The warming rate of $0.3^{\circ}\text{C min}^{-1}$ used our laboratory assay exceeds that of diel warming rates in the field

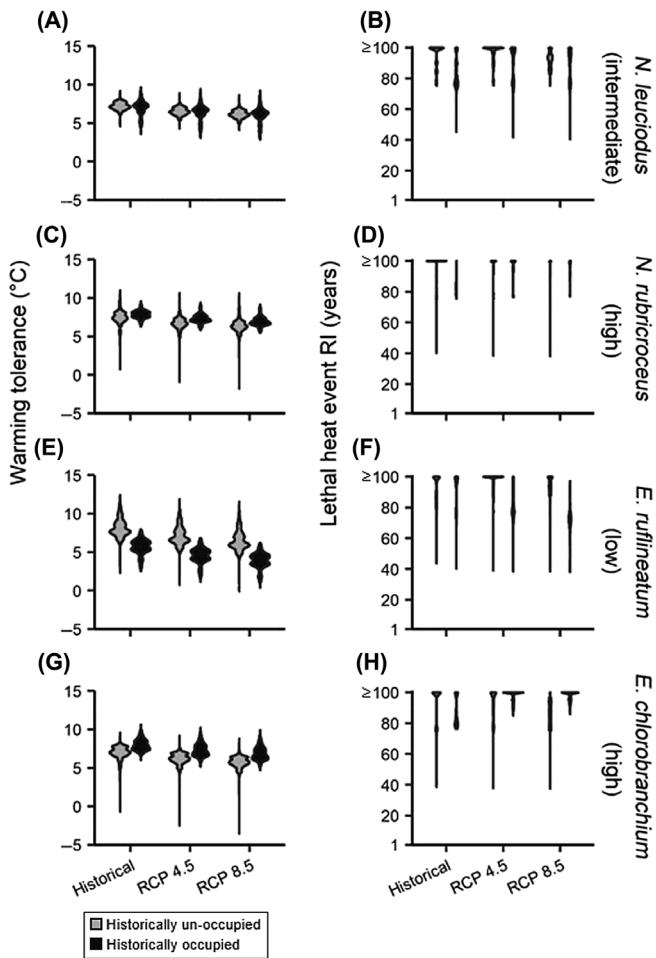


Figure 5. Acclimation-adjusted warming tolerances ($T_{\max} - T_{\text{extreme}}$; right column) and return intervals of lethal heat events (warming tolerance ≤ 0 ; left column) for four species (putative elevation affinity in parentheses) in the upper Tennessee River system under historical and future climate scenarios. Symbols are violin plots showing distribution of responses in reaches with low (< 0.1 ; light gray) versus high (> 0.9 ; dark gray) ENM-based occurrence probability.

(Maheu et al. 2016) and therefore may overestimate T_{\max} . Tolerance to physiological stressors – including temperature – decreases with increasing duration of exposure, meaning that exposure to temperatures several degrees Celsius lower than our T_{\max} estimates for several hours during the diel temperature crest could be lethal (Rezende et al. 2014). Lastly, laboratory-acclimated fish are released from potentially interacting stressors including chemical pollutants and hypoxia that would reduce realized T_{\max} in the field (Patra et al. 2007, Pörtner and Knust 2007). Indeed, such stressors are more prevalent in Ridge and Valley streams and would likely interact with the higher thermal stress of this low-elevation ecoregion (Keck et al. 2014).

Several non-temperature environmental factors correlated with elevation could drive interspecific differences in elevational limits. Geological differences among Blue Ridge,

Cumberland Plateau and Ridge and Valley ecoregions influence channel geomorphology, substrate and water chemistry (Etnier and Starnes 1993). Correlations between the composition of stream fish communities and in-stream geomorphology are known from the southern Appalachian Mountains (Angermeier and Winston 1999) and throughout the world (Lamouroux et al. 2002). These community–geomorphology interactions are mediated through diet, body morphology and reproductive traits (Lamouroux et al. 2002, Walters et al. 2003). Given the similarity of these traits between the high- and low-elevation congeners we studied (Keck et al. 2014), geomorphic factors seem unlikely to contribute substantially to the elevational range differences between congeners. Biotic interactions could also explain interspecific differences in elevational limits. Investigators have elucidated evidence of competitive exclusion among darters and minnows via observational (Winston 1995, Geheber and Geheber 2016) and experimental (Resetarits 1995, Taylor 1996) inquiries. The restriction of *E. chlorobranchium* and *N. rubricroceus* to high-elevation streams could reflect diffuse competition whereby warm-edge range limits are limited by functionally and taxonomically rich assemblages in low-elevation streams (Sunday et al. 2012). Exclusion via pairwise competition with ecologically similar species is also possible. Indeed, *E. chlorobranchium* occurs in parapatry with its sister taxon, *E. camurum*, which occupies low-elevation streams of the Ridge and Valley ecoregion (Etnier and Starnes 1993). Positive biotic interactions may also be important drivers of historical distributions, particularly for *N. rubricroceus* and *N. leuciodus* which reproduce in association with nest-building fishes (Peoples et al. 2015).

Lastly, sub-lethal thermal physiology associated with chronic (days to weeks) exposure to sub-optimal temperatures could mechanistically underlie interspecific differences in elevational limits. Fish bioenergetics models have putatively documented interspecific differences, particularly among salmonids, in the temperature dependence of food consumption and assimilation, metabolic rates and subsequent energy budgets, survival and growth (Deslauriers et al. 2017). These differences in sub-lethal thermal physiology constrain elevational range limits of salmonids in southern Appalachia (Petty et al. 2014) and throughout montane regions of the world (Budy et al. 2013). Detailed data on these sub-lethal thermal physiological traits are comparatively limited for minnows and darters (Deslauriers et al. 2017). However, Hasnain et al. (2013) documented 7.2°C and 5.9°C interspecific ranges in optimal growth temperatures for a subset of North American cyprinids and percids, respectively. These insights suggest that sub-lethal thermal physiology may constrain elevational limits of southern Appalachian minnows and darters. Temperature dependent rates of consumption depend on competition for prey and abiotic conditions influencing prey abundance and quality (Petty et al. 2014). Consequently, elevational limits likely depend on interactive effects of gradients of temperature, physical habitat and strength of biotic interactions along elevation gradients (Case et al. 2005).

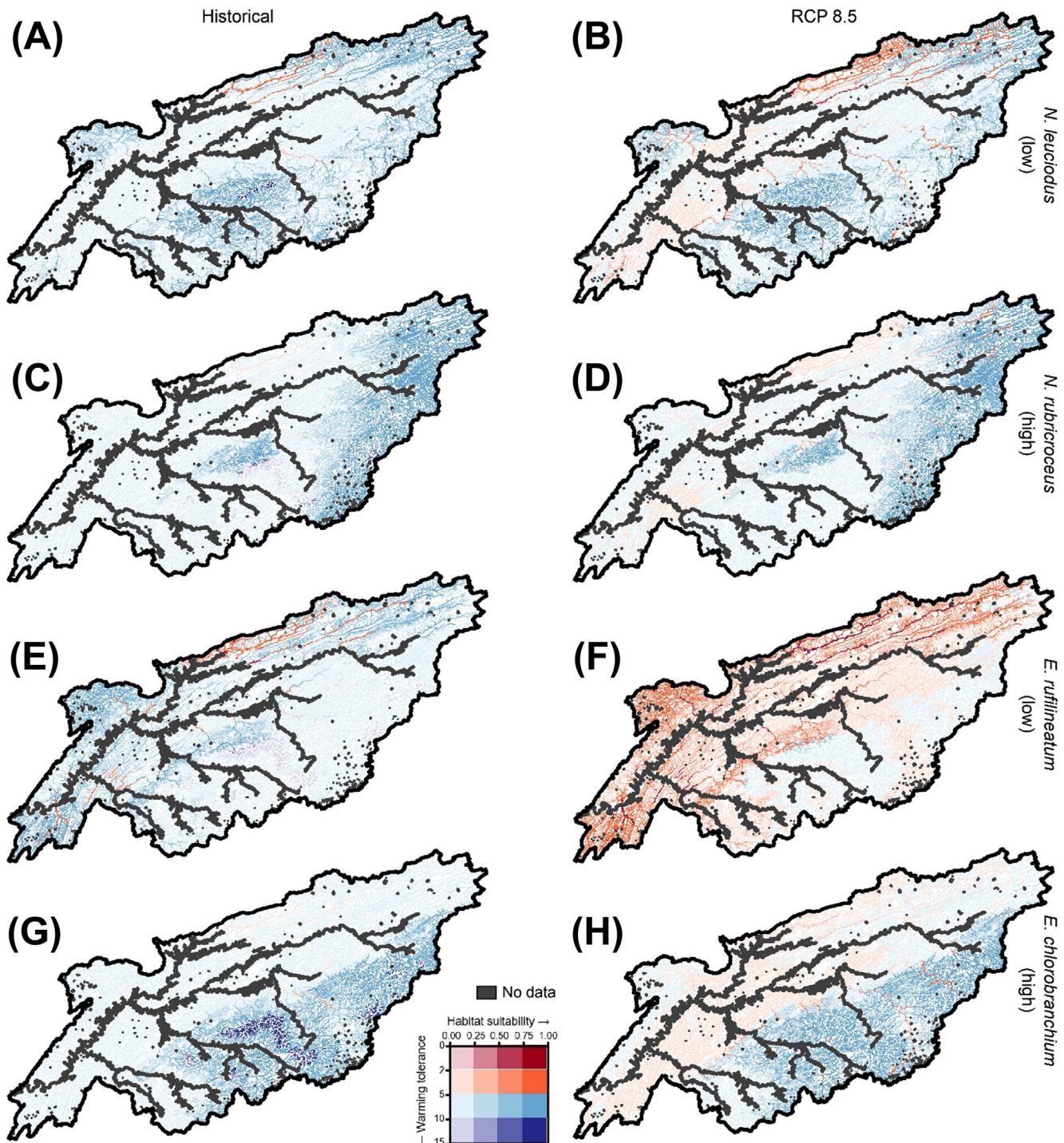


Figure 6. Acclimation-adjusted warming tolerances ($T_{\text{max}} - T_{\text{extreme}}$) for four species (putative elevation affinity in parentheses) mapped to 25379 reaches in the upper Tennessee River system under historical (left column) and future (right column) climate scenarios. Bivariate choropleth coloration shows low (red) to high (blue) warming tolerance and low (light) to high (dark) ENM-based occurrence probability.

Although our weather generation approach provides a realistic means to estimate T_{extreme} event frequency and magnitude and account for short term acclimation to these events, complete integration of laboratory-based physiology in a natural setting remains challenging (Kearney and Porter 2009). While

the findings of our current study cannot explain why southern Appalachian fishes differ in elevation range limits, we can rule out the role of insufficient T_{max} (and its ARR) and T_{extreme} events. Future research efforts should be directed toward the remaining potential drivers and their interactions outlined above.

Future shifts in elevation limits

There is growing concern that rising temperatures are pushing warm-edge limits of species ranges upslope, with concomitant impacts on montane biodiversity (Colwell et al. 2008, Sheldon et al. 2011). Our assessment of warming tolerances suggests that neither high- nor low-elevation species will experience lethal heat events across their geographic range under even the direst scenarios of 21st century warming. One exception is a small proportion of reaches occupied by the low-elevation darter, *E. rufilineatum*. Despite these optimistic findings, we strongly caution the assertion that southern Appalachian fishes are invulnerable to climate change for several reasons. First, sub-lethal thermal physiology could increase or decrease individual energy budgets depending on seasonal changes in growing-degree days and prey availability (Ries and Perry 1995). These processes could push warm-edge limits upslope if, for example, high temperatures and metabolic demand at low elevations cause future energy deficits (Petty et al. 2014). Alternatively, warming at cold-edge limits could enhance growth and recruitment, allowing upslope range expansions (Lawrence et al. 2015). Second, changes in precipitation patterns and runoff could alter elevational ranges directly via changes to flow regimes and subsequent habitat size and heterogeneity within streams (Xenopoulos et al. 2005). Altered flow regimes may also affect fishes indirectly by altering air–water temperature relationships and subsequent thermal habitat conditions (Merriam et al. 2017). The paucity of data on the temperature dependence of key physiological processes and ecological interactions precludes a complete understanding of climate change vulnerability of southern Appalachian minnows and darters, and indeed, freshwater fishes worldwide.

Ecological forecasting is challenging because of uncertainty in forecasting future exposure to climate change and inexact understanding of species' sensitivities to thermal regimes and interacting stressors. A key step in estimating the frequency and magnitude of T_{extreme} events in streams is accurately characterizing air–water temperature relationships, which involve a multitude of heat transfer pathways that change along environmental gradients and will differ between historical and future climates (Luce et al. 2014). Indeed, our inability to consistently model water temperatures from air temperature and landscape features using different statistical algorithms contributed the most uncertainty to our projections of warming tolerance. Increasing daily temperature variation is also likely to increase in the future (Wang and Dillon 2014). This statistical property is not explicitly incorporated into our stochastic air temperature simulations, meaning that our estimation of T_{extreme} events could be conservative. Nevertheless, our multi-model ensemble forecasts revealed substantial variation in the magnitude and frequency of T_{extreme} events in the upper Tennessee River system under future climate scenarios. It is likely that this range of projections encompasses the true nature of T_{extreme} event frequencies and magnitudes, but more multi-year paired air–water temperature time-series are

necessary to reduce uncertainty in forecasts. Another consideration is that our projections of T_{extreme} events do not explicitly incorporate the role of streamflow in mediating air–water temperature relationships which, in addition to temperature, is forecasted to change in southern Appalachia (Anandhi and Bentley 2018) and elsewhere in the world (Santiago et al. 2017). For example, Merriam et al. (2017) identified low flows in combination with high air temperatures contributing to thermal stress of Brook Trout in central Appalachian streams. Another important consideration is the spatial resolution of exposure forecasts. We estimated T_{extreme} events at the spatial resolution of the reach while WTs may vary at the sub-reach scale (e.g. groundwater intrusions), offering fine scale thermal refugia (Kanno et al. 2014). Lastly, shifting spatial gradients of warming exposure may be mitigated by temporal shifts in the phenology of life history events (Soclar et al. 2017). Although exposure to T_{extreme} events cannot be avoided by non-migratory fishes, phenological shifts in reproduction can mitigate sub-lethal thermal physiology including the temperature dependence of larval hatch success, survival and growth (Tao et al. 2018).

Our physiology-based approach to assessing organismal sensitivity to climate change offers advantages over correlative ENM (i.e. climate envelope) approaches in terms of characterizing fundamental niche dimensions (Kearney and Porter 2009, Briscoe et al. 2016). Correlative ENMs that fit historical occupancies to temperature gradients would suggest alternative mechanisms driving low-elevation range limits under historical and future projections. In this case, ENMs would not adequately characterize the fundamental (i.e. physiological) upper thermal limit of *E. rufilineatum* because this species occurs in the warmest streams throughout its geographic range which is restricted to the Tennessee River system. This would make future projections of these ENMs uncertain because a warmer future would require ENM extrapolation to a novel environment. In an alternative case, ENMs fitting historical occupancies of high-elevation species (e.g. *N. rubricroceus* and *E. chlorobranchium*) to temperature gradients would erroneously underestimate the upper thermal limit. Projections of these ENMs to future climate scenarios would likely overestimate the upslope shift of the low-elevation limit. Still, several limitations regarding sensitivity persist. On one hand, laboratory-based thermal tolerance may overestimate the future thermal tolerances in the field if other stressors such as hypoxia and chemical pollution become more pervasive (e.g. with increased human landscape disturbance) in the future. On the other hand, although our approach accounts for short term thermal acclimation, it does not account for evolutionary change. Given the relatively short generation time of these species (i.e. one to two years), there is more opportunity for minnows and darters to undergo evolutionary adaptation than longer-lived organisms. Thus, our intragenerational laboratory estimates of T_{max} may underestimate future T_{max} realized following 50–100 generations of thermal adaptation (Bürger and Lynch 1995).

Broader implications

Previous studies have assessed historical geographic range limits (Sunday et al. 2011) and climate change vulnerability (Deutsch et al. 2008, Sunday et al. 2012, Comte and Olden 2017b) using thermal physiology (e.g. warming tolerances) for multiple taxonomic groups in terrestrial, freshwater and marine habitats. These studies have largely assessed latitudinal patterns in exposure and physiological sensitivity at global extents and at necessarily coarse spatial and temporal resolutions. Alternatively, studies on elevation limits have mostly used climate envelope approaches (Chen et al. 2011b, Sheldon et al. 2011), making it difficult to assess the role of physiological sensitivity (Feeley and Silman 2010). Our study builds on this literature in several key ways. First, we use physiology to evaluate fundamental range limits along elevation gradients. Second, we use stochastic weather generation which provides realistic estimation of exposure to T_{extreme} events. This allowed us to estimate return intervals of threshold temperatures (i.e. lethal heat events) which, when considered in the context of metapopulation dynamics, provides an intuitive way of assessing population persistence at range edges by linking individual-level physiology apparent in the short term (i.e. hours to days) to population dynamics and persistence operating in the long term (i.e. years to decades) (Case et al. 2005). Third, stochastic weather generation produces a daily time series, which provides the opportunity to explicitly incorporate acclimation potential.

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Supplementary material (available online as Appendix ecog-04576 at <www.ecography.org/appendix/ecog-04576>). Appendix 1–4.