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Supplementary material

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Future climate change drives lethal heat events for endemic fishes in montane biodiversity hotspot

- **Appendix 1.** Collection locations and sample sizes for laboratory T_{max} experiments.
- **Appendix 2.** Multi-model environmental niche modeling.
- **Appendix 3.** Multi-model projections of air-water temperature relationships and extreme heat events.
- Appendix 4. Supplementary results tables and figures.

Appendix 1. Collection locations and sample sizes for laboratory T_{max} experiments.

Table A1. Environmental characteristics of eight collection sites and sample sizes of T_{max} assays for four species (historical elevation affinity in parentheses).

	Elevation	Strahler _	Water tempe	erature (°C) ^A	Number of individuals		
Species	(masl)	order	June	August	June	August	
N. leuciodus (low)	261	5	21.3	n/a	17	0	
	549	5	17.9	18.4	14	18	
N. rubricroceus (high)	484	3	18.9	19.1	15	18	
	813	3	16.3	16.9	16	17	
E. rufilineatum (low)	310	6	24.6	25.3	16	16	
	469	4	19.8	21.5	16	17	
E. chlorobranchium (high)	513	5	22.7	24.4	17	18	
	549	5	16.9	18	17	18	

^A Measured at midday on day of collection.

Appendix 2. Multi-model projections of air-water temperature relationships and extreme heat events.

Table A2. Ten AT, landscape, and hydrographic variables used to model and map daily maximum WTs (*i.e.*, extreme heat events, T_{extreme}) in the upper Tennessee River system.

Predictor variable (units)	Min.	Median	Max
Maximum daily AT (5-day lag; °C)	14.6	26.7	32.5
Upstream drainage area (km²)	0.0009	94.8	9259.6
Mean sand content (% of dry mass) A	0	31.1	52.7
Mean soil organic matter content (% of dry mass) A	0	0.69	2.65
Mean soil permeability (cm/hour) A	0	7.99	31.23
Mean depth to bedrock (cm) A	0	114.4	152.4
Mean seasonal water table depth (cm) ^A	0	175.7	182.9
Mean clay content (% of dry mass) A	0	26.1	59.2
Mean composite topographic index ^A	154.2	292.4	935.1
Mean reach elevation (meters a.s.l.)	193.3	598.2	1747.7

^A Mean for upstream drainage area.

Table A3. Parameterizations for each of three statistical algorithms used for landscape-AT-WT models. Asterisks indicate the parameterization used in final model.

Generalized linear modeling (GLM):

- R library: base.
- Gaussian family.
- Log₁₀ transformed catchment area to normalize the right-skewed distribution.
- No interaction terms between predictor variables.
- No variable selection or reduction procedure.
- Compared models with differing response shapes: linear versus polynomial*

Generalized additive modeling (GAM):

- R library: gam.
- Gaussian family.
- Log₁₀ transformed catchment area.
- Smoother is a cubic-spline.
- Compared models with differing degrees of smoothing: 2 versus 4* versus 6 versus 10.

Random forests (RF):

- R library: randomForest.
- Maximum number of trees was set to 1000.
- Compared models with differing numbers of predictor variables randomly sampled as candidates at each split: 2 variables* versus 4 variables versus 6 variables.

Table A4. Four general circulation models (GCMs) used to project extreme heat events for the future (2071 to 2100) time period.

GCM	Institution
ACCESS1-0	Commonwealth Scientific and Industrial Research Organization and Bureau of Meteorology, Australia
CCSM4	National Center for Atmospheric Research, USA
CNRM-CM5	Centre National de Recherches Meteorologiques/Centre Europeen de Recherche et Formation Avancees en Calcul Scientifique, France
INM-CM4	Institute for Numerical Mathematics, Russia

Figure A1. Diagram of landscape—air temperature—water temperature modeling (Step B) and

stochastic weather generation (Step C).

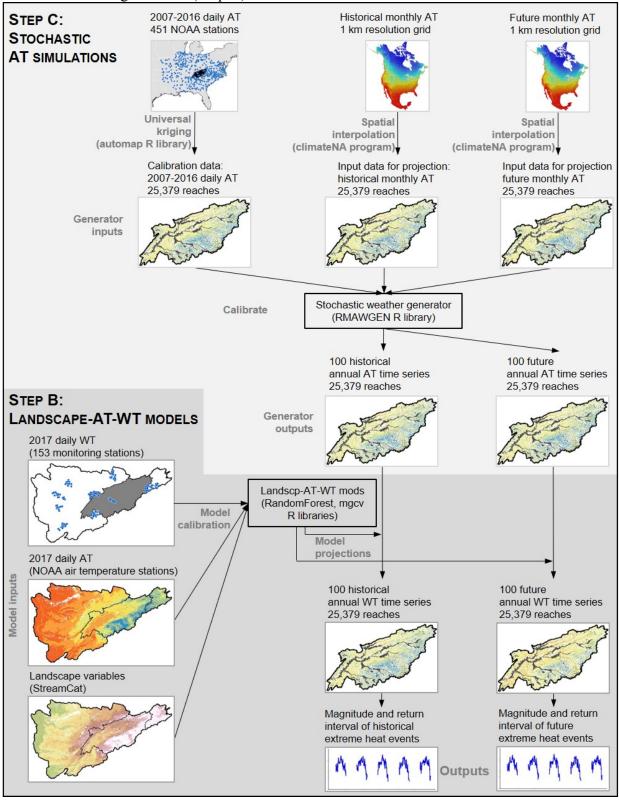


Figure A2. Locations of 153 WT monitoring stations in southern Appalachian Mountains.

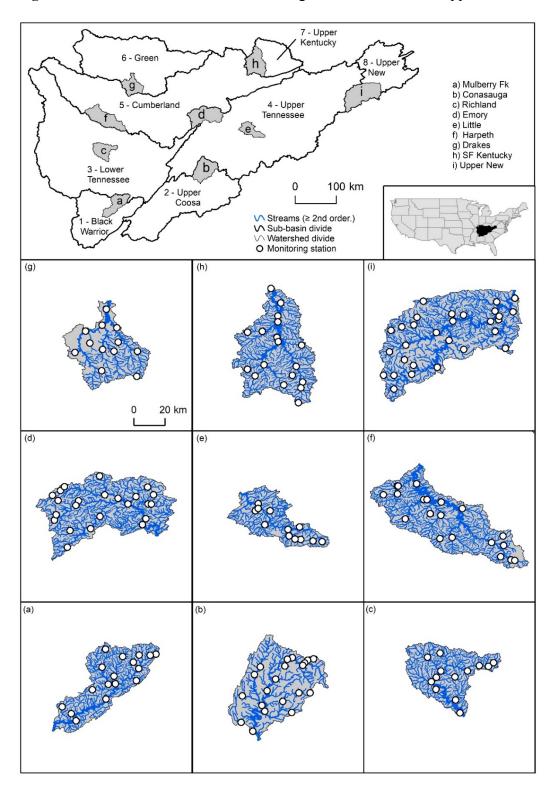
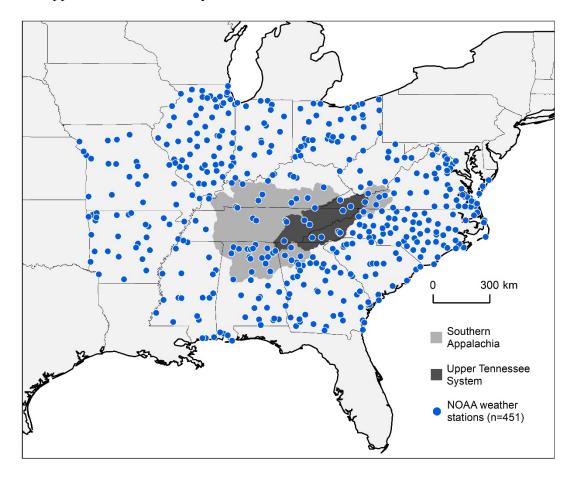


Figure A3. Locations of National Oceanic and Atmospheric Administration (NOAA) weather stations used to spatially interpolate daily ATs to WT monitoring stations and 25,379 reaches in the upper Tennessee River system.



Appendix 3. Multi-model environmental niche modeling.

We modeled species' occurrence probabilities using linear and non-linear regression based techniques (generalized linear modeling and generalized additive modeling, respectively) and a machine learning technique (random forests). Because these statistical algorithms require both presences and absences and the IchthyMaps dataset does not include true absence reaches for each species, we selected pseudoabsences at random from reaches in which one or more other non-game fish species (but not the focal species) was present in the IchthyMaps dataset to increase the likelihood of selecting true absences (Huang and Frimpong 2015). We generated ten pseudoabsence datasets where pseudoabsences were equal in number to occurrence records (i.e., 50% prevalence) (Figure A4). We implemented modeling using an internal and external split sample cross validation procedure (Guisan et al. 2017; Figure A4). We split each of the ten presence-pseudoabsence datasets into an 80% external training dataset and a 20% external testing dataset, while maintaining 50% prevalence for both training and testing datasets. We further split each external training dataset into a 75% internal training dataset and a 25% internal testing dataset. This internal splitting procedure also maintained 50% prevalence and was repeated ten times for each of the ten datasets, producing 100 datasets per species. Next, we used each of the three statistical algorithms to fit models to each internal training dataset and to predict occurrence probabilities for the paired internal testing dataset, resulting in 300 models per species. We explored several parameterizations for each statistical algorithm (Table A6). An optimal threshold occurrence probability was identified where sensitivity and specificity are equal, thus minimizing the frequencies of both false absences and false positives (Guisan et al. 2017). We used fitted models to predict probabilities of occurrence for the paired external testing datasets that were then converted to presence or absence according to each model run's optimal threshold. We assessed model performance using area under the curve (AUC) of the receiver operator characteristic for the internal validation and classification success, sensitivity, and specificity for the external validation. We projected the ensemble of models to all 25,379 reaches in the upper Tennessee River system for each species, and occurrence probability was evaluated as a committee average (Guisan et al. 2017). We implemented all ENM analyses in the R statistical environment (R Core Team 2017).

References

Guisan, A. W. *et al.* 2017. Habitat suitability and distribution models: with applications in R. Cambridge: Cambridge University Press.

Huang, J. and Frimpong, E. A. 2015. Using historical atlas data to develop high-resolution distribution models of freshwater fishes. – PLOS One 10: e0129995.

Table A5. Ten landscape and hydrographic variables used in ENMs to model and map occurrence probabilities of four species in the upper Tennessee River system.

Predictor variable (units)	Min.	Median	Max
Upstream drainage area (km²)	0.0009	94.8	9259.6
Mean sand content (% of dry mass) A	0	31.1	52.7
Mean soil organic matter content (% of dry mass) A	0	0.69	2.65
Mean soil permeability (cm/hour) A	0	7.99	31.23
Mean depth to bedrock (cm) A	0	114.4	152.4
Mean seasonal water table depth (cm) A	0	175.7	182.9
Mean clay content (% of dry mass) A	0	26.1	59.2
Mean composite topographic index ^A	154.2	292.4	935.1
Reach slope (meters/kilometer)	0.00001	0.0420175	3.02
Mean reach elevation (meters a.s.l.)	193.3	598.2	1747.7

^A Mean for upstream drainage area.

Table A6. Parameterizations for each of three statistical algorithms used for ENMs. Asterisks indicate the parameterization used in final ENMs.

Generalized linear modeling (GLM):

- R library: base.
- Binomial family with logit link function.
- Log₁₀ transformed catchment area to normalize the right-skewed distribution.
- No interaction terms between predictor variables.
- No variable selection or reduction procedure.
- Compared models with differing response shapes: linear versus polynomial*

Generalized additive modeling (GAM):

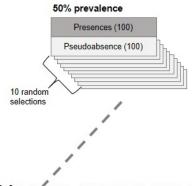
- R library: gam.
- Binomial family with logit link function.
- Log₁₀ transformed catchment area.
- Smoother is a cubic-spline.
- Compared models with differing degrees of smoothing: 2 versus 4* versus 6 versus 10.

Random forests (RF):

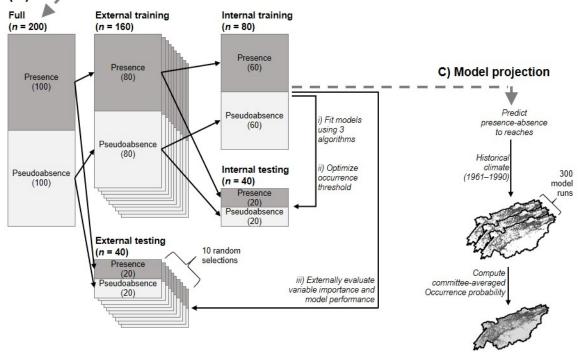
- R library: randomForest.
- Maximum number of trees was set to 1000.
- Compared models with differing numbers of predictor variables randomly sampled as candidates at each split: 2 variables* versus 4 variables versus 6 variables.

Figure A4. Schematic depiction of ENM procedure.

(A) PSEUDOABSENCE SELECTION



(B) INTERNAL-EXTERNAL SPLIT SAMPLE CROSS-VALIDATION



Appendix 4. Supplementary results tables and figures.

Table A7. Performance and output of ENMs across three algorithms and four species.

	Historical Number of		ENM performance				ENM output				
Species	elevation affinity	IchthyMaps records	Algorithm	AUC	Acc.	Sens.	Spec.	Occup.	Elev ₅	Elev ₅₀	Elev ₉₅
Notropis leuciodus	moderate	653	GLM	0.69	0.62	0.62	0.62	0.32	279	664	1,240
			GAM	0.72	0.66	0.66	0.66	0.31	332	675	1,163
			RF	0.73	0.66	0.64	0.68	0.31	333	653	1,176
N. rubricroceus	high	290	GLM	0.83	0.75	0.77	0.72	0.23	447	736	1,146
	_		GAM	0.83	0.75	0.78	0.72	0.23	448	734	1,131
			RF	0.81	0.74	0.76	0.72	0.26	443	732	1,146
Etheostoma rufilineatum	low	878	GLM	0.75	0.67	0.67	0.68	0.31	219	340	648
·			GAM	0.80	0.71	0.70	0.72	0.26	218	428	719
			RF	0.79	0.70	0.69	0.71	0.29	225	448	782
E. chlorobranchium	high	160	GLM	0.82	0.74	0.73	0.74	0.25	443	823	1,300
	_		GAM	0.82	0.74	0.72	0.76	0.24	459	825	1,272
			RF	0.81	0.74	0.73	0.75	0.24	443	823	1,252

AUC = area under the curve; Sens. = sensitivity; Spec. = Specificity; Acc. = prediction accuracy

Occup. = proportion of reaches occupied; $Elev_5 = 5$ th percentile of elevation at occupied reaches; $Elev_{50} = 50$ th percentile; $Elev_{95} = 95$ th percentile

Table A8. Top five linear regression models relating laboratory T_{max} to species identity, laboratory T_{acclim}, collection location, and collection month. All-subsets selection was based on Akaike Information Criterion corrected for small sample size (AIC_c). Top five models are sorted from best to worst (*i.e.*, low to high AIC_c).

Model formula	AICc	Weight
Tmax ~ species + Tacclim + location + month + species × Tacclim	714.0	0.645
Tmax ~ species + Tacclim + location + species × Tacclim	715.2	0.352
Tmax ~ species + Tacclim + species × Tacclim	726.1	0.001
Tmax ~ species + Tacclim + month + species × Tacclim	726.2	0.001
Tmax ∼ species + Tacclim + location + month	743.1	0.000

Table A9. Coefficients for the best fitting linear regression model from Table A8 predicting laboratory T_{max} .

Parameter	Estimate	SE	<i>t</i> -value	P-value
Intercept	24.818	0.688	36.070	< 0.001
Species - E. rufilineatum	2.226	0.881	2.527	0.012
Species - N. leuciodus	-2.428	0.990	-2.453	0.015
Species - N. rubricroceus	-1.659	0.906	-1.830	0.069
Tacclim	0.431	0.032	13.306	< 0.001
Month - June	0.267	0.148	1.813	0.071
Location - low-elevation	-0.479	0.127	-3.773	0.000
Species - E. rufilineatum × Tacclim	-0.139	0.043	-3.268	0.001
Species - E. leuciodus × Tacclim	0.101	0.049	2.051	0.041
Species - E. rubricroceus × Tacclim	0.087	0.044	1.969	0.050

Table A10. Performance and output of statistical models linking daily WT to daily AT and landscape predictors.

		Model performance				
Response	Algorithm	RMSE	Percent bias	NSC		
Daily maximum WT (i.e., Textreme)	GLM	1.70	-0.007	0.77		
	GAM	1.59	-0.006	0.80		
	RF	0.85	-0.003	0.95		
Daily median WT (i.e., field T _{acclim})	GLM	1.50	-0.006	0.82		
	GAM	1.34	-0.005	0.86		
	RF	0.68	-0.003	0.96		

RMSE = root mean squared error; NSC = Nash-Sutcliffe coefficient

Table A11. Top five linear regression models relating future (2071 to 2100) warming tolerance in historically occupied reaches to species identity, RCP scenario, general circulation model, WT modeling algorithm, collection location ARR, and collection month ARR. All subsets selection was based on Akaike Information Criterion corrected for small sample size (AIC_c). Top five models are sorted from best to worst (*i.e.*, low to high AIC_c).

Model formula	AICc	Weight
WmTol ~ species + scenario + gcm + algorithm + location + month	2,336,878	1.0
WmTol ~ species + scenario + gcm + algorithm + month	2,349,991	0.0
WmTol ~ species + scenario + gcm + algorithm + location	2,351,603	0.0
WmTol ∼ species + gcm + algorithm + location + month	2,356,986	0.0
WmTol ~ species + scenario + gcm + algorithm	2,364,451	0.0

Table A12. Analysis of variance table for the best fitting linear regression model from Table A11 relating future (2071 to 2100) warming tolerances in historically occupied reaches to species identity, RCP scenario, general circulation model, WT modeling algorithm, collection location ARR, and collection month ARR.

Covariate	d.f.	S.S.	M.S.	F-value	P
Species identity	3	672,474	224,158	145,307	< 0.001
Emissions scenario	1	31,462	31,462	20,395	< 0.001
GCM	3	159,511	53,170	34,467	< 0.001
WT modeling algorithm	2	2,882,931	1,441,466	934,408	< 0.001
ARR collection location	1	20,418	20,418	13,236	< 0.001
ARR collection month	1	22,953	22,953	14,879	< 0.001
Residuals	714324	1,101,953	2		

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