Final Project Report

Climate- and land-cover-induced shifts in the distribution and abundance of invasive fish and their impacts on native fish communities in the Tennessee and Cumberland River Basins

1. ADMINISTRATIVE

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Cooperative Agreement Number: G20AC00461-02
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2. PUBLIC SUMMARY

The Southeastern USA is extremely rich in aquatic biodiversity, which includes native fish species such as brook trout, largemouth bass, and smallmouth bass that are important for recreational fisheries and to local economies. This region is also home to a large number of native species that may be less well-known to the public but nevertheless are of great conservation concern due to their small range and population sizes. In particular, the Tennessee and Cumberland river basins (TCRB) are among the most biodiverse and important in the US. However, the climate of the Southeast is changing rapidly. Further, many areas are undergoing increased urbanization. These changes contribute to warming of streams, changes in streamflow, and decline in stream water quality and habitat, which in turn may facilitate the introduction and spread of non-native (i.e., invasive) fish species. Here, we collaborated with natural resource agency managers, land-trusts, nonprofits to (1) identify non-native fish that expanded their distributions in the TCRB; (2) identify non-native fish whose abundances have increased; (3) examine the effect of climate and land-cover changes on fish invasions; (4) investigate impacts of non-native fish on native fish; and (5) identify locations that may become suitable for invasive fish under future climates. We generated new scientific knowledge that has improved our understanding of invasive fish species, and will inform current and future fish monitoring and management efforts in the Southeast.

3. PROJECT SUMMARY

Climate change and land-cover change are major stressors to biota in aquatic ecosystems. The Southeastern USA is an aquatic biodiversity hotspot among temperate regions globally. Aquatic invasive species are increasing in the Southeast. Because they have the potential to negatively impact the freshwater ecosystems of this region and the unique native biota that these ecosystems support, state and federal agencies are focused on limiting the spread of existing non-native species, mitigating their impacts on ecosystems and the native species therein, and preventing the introduction and spread of new invasive species. However, the management of invasive species by agencies are compromised by the lack of information and data on how climate and land-cover changes affect the dynamics of species invasions, e.g., the rate at which non-native species increase its distribution across space, the rate at which they increase their local abundances, and which streams become suitable for these species in the future. In this project, we leverage on the field data and knowledge accrued by partners and collaborators in state and federal agencies, land-trusts, and nonprofits over the past ~40 years, along with publicly available fish occurrence data from GBIF and museum collections, to fill the knowledge gaps in how climate and land-use change has impacted the invasion dynamics of non-native species in the past ~30 years and how these changes are projected to affect invasion dynamics in the next ~30 years in the Tennessee and Cumberland River basins (TCRB). Specifically, our project (1) identified non-native fish that have occupied an increased number of streams in the TCRB; (2) identified non-native fish whose abundances have increased; (3) examined the ecological mechanisms by which factors associated with climate and land-cover changes might affect invasion trajectories; (4) investigated impacts of non-native fish on native fish; and (5) identified locations that may become suitable for invasive fish under future climates in 2035-2065.

Our analyses found that the four species of Asian carp (silver carp *Hypophthalmichthys molitrix*; grass carp *Ctenopharyngodon idella*; bighead carp *Hypophthalmichthys nobilis*; and black carp *Mylopharyngodon piceus*) are among the non-native species that showed the greatest rate of increase in the number of stream catchments occupied about the past ~30 years. Species that are native to the US but not to the TCRB such as striped bass (*Morone saxatilis*) and eastern mosquitofish (*Gambusia holbrooki*) showed sustained increase as well. Alabama bass, *Micropterus henshalli*, was not recorded in the TCRB before 2010, but was recorded in 10 unique stream reaches in our dataset by the end of 2024. In terms of local abundances relative to other species within individual sites, silver carp *H. molitrix*, blueback herring *Alosa aestivalis*, yellowfin shiner *Notropis lutipinnis*, and margined madtom *Noturus insignis*, showed evidence (ranging from weak to very strong) of increasing abundances.

So what climate and land-cover change-related ecological mechanism(s) may have driven changes in total abundances, as well as richness of non-native fish, relative to the total abundances and richness of native fish? We found evidence that decreasing month-to-month variation in stream flow through time (i.e., flow becoming less seasonal) was associated with an increase in the relative total abundance and richness of non-native fish across sampling sites. In addition, streams that drain an upstream watershed with lower percent tree cover were associated with a more positive trend in non-native abundance and richness. Lastly, we mapped habitat suitability for 37 non-native species under current (1980-2010) and future (2035-2065) climate and land-cover scenarios. In general, we found warmwater- and mainstem-adapted species that are currently invading in the lower reaches of the TCRB such as silver carp (*Hypophthalmichthys molitrix*; under RCP 8.5) and bighead carp (*H. nobilis*, under RCP 4.5 and 8.5) are likely to see an increase in climatically suitable habitat in the future (2035-2065), particularly along the mainstem and large tributaries of the Tennessee and Cumberland Rivers, upstream from their current distribution. By contrast, non-native species in the Upper Tennessee and French Broad-Holston regions that are currently occupying cooler streams in the Blue Ridge ecoregion, such as rainbow trout (*Onchorynchus mykiss*)and yellowfin shiner (*Notropis lutipinnis*) are projected to see declines in climatically suitable habitat as streams warm and/or precipitation patterns change.

Our project was the first to examine invasion dynamics (i.e,, changes in the spatial distribution and local abundances) of non-native fish species and project future locations that may become suitable for the spread of these species in the biologically important TCRB. By identifying non-native species that are increasing in occupied watersheds and/or abundance, our results can inform monitoring and eradication efforts conducted by agencies. By identifying the ecological mechanisms underlying increasing non-native abundances and richness, agencies and researchers can design programs that target (reduce) the effect of important variables such as maintaining high tree cover in important watersheds. Last, agencies and land-trusts can use habitat suitability maps to monitor the locations that non-native fish are likely to spread to and occupy under future climates. All these represent new scientific knowledge that has improved our understanding of invasive fish species, and will inform current and future fish monitoring and management efforts in the Southeast.

4. PURPOSE AND OBJECTIVES

The Southeastern USA is a highly dynamic region that has experienced rapid climate and land-cover change over the past half-century (Vose et al. 2017; Troia et al. 2019; Costanza et al. 2020). Both climate and land-cover change are expected to continue into the future (Terrando et al. 2014; Vose et al. 2017). Annual mean temperature is projected to increase by 1.9-2.4°C by mid-century. We would also expect more frequent and severe precipitation events, along with drier summers (Vose et al. 2017). Urban land-cover is expected to increase by 139% to 2060 (Terrando et al. 2014).

Climate and land-cover change is an important and pressing issue in the Southeast because of the unique and rich biodiversity it supports. In particular, the Southeast is globally important for freshwater biodiversity conservation because it is home to a diverse freshwater fauna, many species of which are highly range-restricted and found nowhere else. For fish, the Southeast contains nearly 80% of all fish species found in the US and Canada, including many found only in one or a few watersheds (Elkins et al. 2019; NatureServe 2010). Among the most important river basins in this region are the Tennessee and Cumberland River basins (hereafter, TCRB), which span 7 US states and support high total, range-restricted, and imperiled fish species richness (Elkins et al. 2019; Collen et al. 2014).

As climate and land-cover change continues into the future, an increase in air temperature, changing precipitation patterns, and land-cover change will likely interact to increase stream temperature, alter streamflow patterns, and stream habitat quality. Recent studies have examined how these changes may impact species communities and the distributions of native fish (Troia et al. 2019; Troia & Giam 2019; Comte et al. 2022). Less well-studied is the effect of climate and land-cover change on another important stressor in freshwater ecosystems: invasive species. Invasive fish may outcompete native fish, predate upon them, and/or alter their stream habitat, thus negatively impacting native fish species (Rahel & Olden 2008; Chick et al. 2020). We hypothesize that stream temperature warming, changes in flow magnitude and flow regime, and changes in land-cover may facilitate fish invasions.

As invasions of non-native aquatic species accelerate in the Southeast (Mangiante et al. 2018), state and federal agencies are working to limit the spread of existing non-native species, mitigate their impacts, and prevent future invasions (e.g., Tennessee Wildlife Resources Agency, undated; US Fish & Wildlife Service, 2015). A region-wide study that examines the effect of climate change on invasive fish and their impacts on native fish communities in the TCRB can provide important information to state and federal agencies about the ecological mechanisms that facilitate fish species invasions; however, up to now, no such study has been conducted. Here, we sought to fill in this crucial knowledge gap by collaborating with partners and collaborators from state and federal agencies, land-trusts, and nonprofits to answer the following questions: (1) Which invasive fish species have expanded their distributions over the past 15-30 years? (2) Which species have increased their abundances in the past 15−30 years? (3) How does climate- and land-cover-associated changes in stream temperature and flow dynamics impact invasions? (4) How does invasive fish species impact native fish communities? (5) Which streams are likely to be colonized by which invasive species in the next 30 years?

In our research, we have met our original objectives and goals by answering the questions listed above (see sections 6-8). In particular, we identified non-native fish species that have expanded their distributions over the last ~30 years, focusing on species that have accelerated in terms of their distribution increase over the last 15 years (e.g., the four Asian carp species). We also used a meta-analytic approach to synthesize relative abundance information on each non-native species at every site in its introduced range to identify species that have experienced the greatest increase in their relative abundance across sites that they occupy (e.g., silver carp, blueback herring, yellowfin shiner, and margined madtom). We also synthesized community sampling data from different state and federal agencies to identify the most important climate- and land-cover change related variables that were associated with the increase in the relative richness and total abundance of non-native species across sites.

At the project kickoff meeting, in our discussion with agency partners and collaborators, we leveraged their field knowledge and experience to identify which non-native fish species we should be focusing on in our research. We also asked them which non-native species are of particular interest or are management priorities for them. We also asked them about what information about these species would help inform their management of these species. Based on their answers (see section 5.1 Co-production approach), we fine-tuned some of our questions and discussions based on the species they identified, i.e., silver carp

(*Hypophthalmichthys molitrix*) and other Asian carp species, yellowfin shiner (*Notropis lutipinnis*), mosquitofish (*Gambusia* spp.), and redbreast sunfish (*Lepomis auritis*).

5. ORGANIZATION AND APPROACH

5.1. Co-production approach

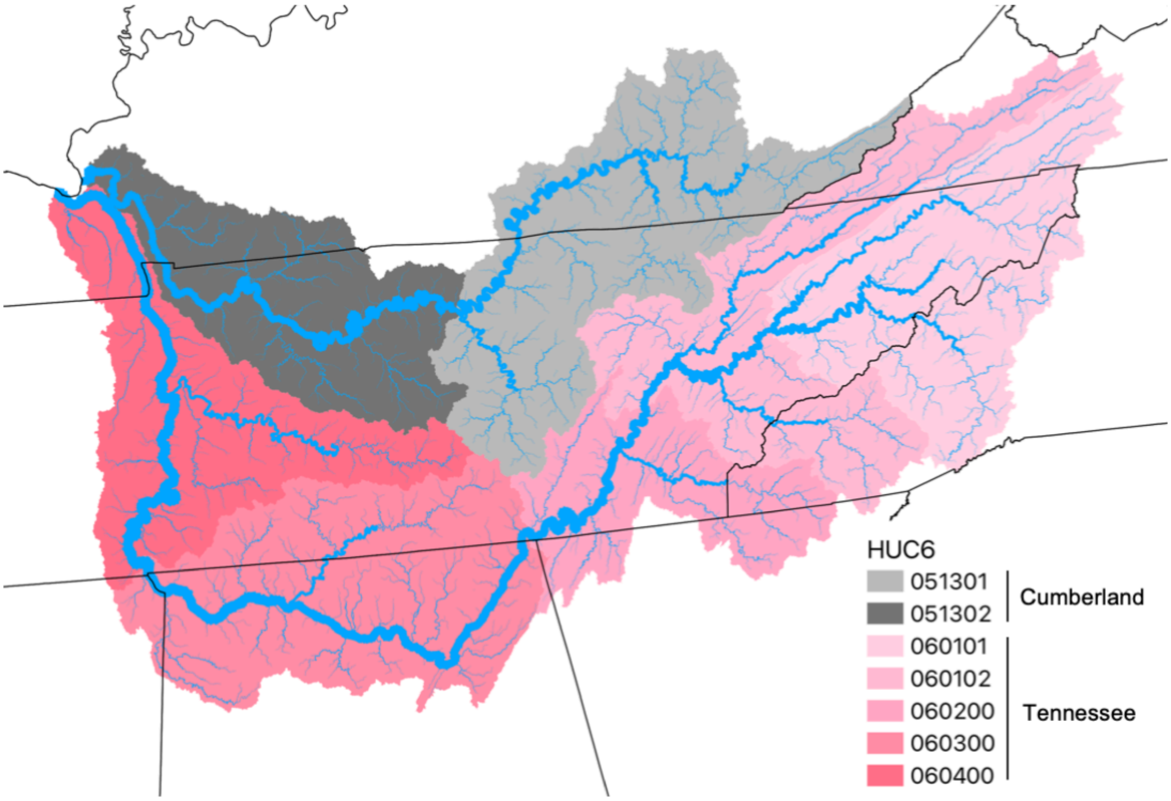
We designed and implemented this project using a co-production approach that incorporates the fish ecology and management expertise, field experience and knowledge, and data collected by collaborators and partners (comprising natural resource managers, fisheries biologists, conservation biologists, research hydrologists) from federal corporations and agencies (TVA, USGS), state natural resources/fisheries agencies (Tennessee Wildlife Resources Agency, NC Department of Environmental Quality, KY Department for Environmental Protection, KY Department of Fish and Wildlife Resources, Alabama Department of Environmental Management, Geological Survey of Alabama, Georgia Department of Natural Resources, Virginia Department of Wildlife Resources), land-trusts and nonprofits (Mainspring Conservation Trust and Conservation Fisheries). We worked with collaborators and partners to obtain fish and streamflow data at the start of the project and to complete the necessary data cleaning and processing. The project team organized a project kickoff meeting over Zoom to present preliminary data and to solicit feedback on additional research questions that might be of management interest to the respective agencies. Specifically, we received feedback to examine (1) the abundance and/or range changes of redbreast sunfish (*Lepomis auritis*) in the TCRB; (2) the status and invasive dynamics of yellowfin shiners (*Notropis lutipinnis*); (3) risk areas for silver carp (*Hypophthalmichthys molitrix*) invasion based on their dispersal capability; (4) the impact of mosquitofish (*Gambusia* sp.) on Barrens topminnow (*Fundulus julisia*); and (5) identify small-ranged native fish that occur in streams that are likely to be invaded in the future. Not all of these questions could be answered satisfactorily with the data that we have compiled; nevertheless, they provided a valuable perspective of which questions are most important for state agencies and managers, which informed our analyses and the discussions of our results.

5.2 The Tennessee and Cumberland River Basins (TCRB)

Our research focuses on the Tennessee River basin [Watershed Boundary Dataset (WBD) 2-digit hydrological unit code (HUC2) 05] and the Cumberland River basin (4-digit hydrological unit code (HUC4) 0513) (Fig. 1). The Tennessee River basin spans 7 states (Virginia, North Carolina, Tennessee, Georgia, Alabama, Mississippi, and Kentucky) and drains a total of 105,950 km^2 of land area. The headwaters of the Tennessee River basin originate in the mountains of the Appalachian-Blue Ridge forest ecoregion in southwestern Virginia, eastern Tennessee, and western North Carolina. Headwaters and tributaries of the French Broad and Holston rivers [6-digit hydrological unit code (HUC6) 060101] drain the mountains of these three states, and these two rivers eventually meet in Knoxville, TN, making the formal start of the Tennessee River. The Clinch, Emory, and Little Tennessee systems (which comprise HUC 060102) flow into the Tennessee River mainstem at various points downstream of the confluence of the French Broad and Holston. These two HUC6 units, 060101 French Broad-Holston and 060102 Upper Tennessee comprise the HUC4 0601 Upper Tennessee, which marks the Tennessee River basin above Watts Bar Dam. Continuing downstream of Watts Bar Dam, the Tennessee River receives flow from the Hiwassee (and upstream of it, the Ocoee that is located in Tennessee, North Carolina, and Georgia) and Sequatchie drainages along with several other smaller watersheds. Together this section of the Tennessee River and the basins draining into it makes up HUC4 0602 Middle Tennessee-Hiwassee. From there, the Tennessee River flows into Alabama and back up north into Tennessee to the Pickwick Dam in Hardin county, TN. This section of the Tennessee River and its upstream catchments is HUC4 0603, and important river systems include the Elk, which covers both Tennessee and Alabama, and the Bear, which is in Mississippi and Alabama. Downstream of Pickwick Dam is the Lower Tennessee (HUC4 0604), which comprises river systems such as the Duck and the Buffalo. Finally, the Tennessee River flows northward into Kentucky where it drains into the Ohio River at Paducah, KY.

Like the Tennessee River, the Cumberland River is a tributary of the Ohio River. It spans Kentucky and Tennessee, and drains a total land area of 46,390 km^2. The Cumberland River comprises two main sections, the Upper Cumberland (HUC6 051301) and the Lower Cumberland (HUC6 051302). The headwaters and tributaries of the Upper Cumberland originate in southwestern Kentucky in the Cumberland Plateau. Important river systems in the Upper Cumberland include South Fork Cumberland, Obey, and Caney Fork. Downstream of the confluence between Caney Fork and the Cumberland River mainstem marks the start of the Lower Cumberland, which comprises river systems such as the Harpeth and Stones. Finally, the Cumberland River flows into the Ohio River at Smithland, KY, just a little northeast of the confluence between the Tennessee River and the Ohio River.

Fig. 1. Map of the Tennessee and Cumberland River basins (TCRB). HUC6 units are color-coded: pink hues indicate HUC6 units within the Tennessee River basin and grey hues indicate HUC6 units within the Cumberland River basin. Grey lines are state lines and blue lines are major streams and rivers within the TCRB. Thicker lines represent stream reaches that drain a greater upstream catchment area (i.e, more “downstream” reaches).



5.3. Fish data

We compiled two main types of fish data in the TCRB: (i) fish *community monitoring* data (hereafter, *community data*) and (ii) fish *occurrence data*. *Community* data are collected by fish community monitoring programs in which the objective is to obtain a representative sample of the fish community at a given location on a given day. The sampling protocol and method/gear may differ across programs and among sites within a program. Sampling protocols include IBI sampling, CPUE sampling and n-pass electrofishing samples. Electrofishing was the main sampling method although there is variation in the gear used based on what gear is most appropriate for the stream, i.e., backpack for smaller wadeable streams, tow-barge for larger wadeable streams, and/or boat electrofisher for large, non wadeable streams. Each community sample comprises abundance counts of all species sampled in a unique sampling occasion (at a unique day and location based on site identifier or geographic coordinates provided in the dataset).

Occurrence data indicate the existence of a given species in a given location and time as evidenced by museum specimens (GBIF and University of Tennessee Etnier Ichthyological Collection [UTEIC]), material citations (e.g., citations of materials or specimens in taxonomic papers describing a new species), human observation (research-grade records provided by iNaturalist via Global Biodiversity Information Facility [GBIF]), and GBIF records that were classified as "Occurrence" (which includes general occurrence information included in the literature). Last, we included data from monitoring programs that were associated with targeted sampling (e.g., sampling programs targeting game fish by TWRA) in our *occurrence* dataset because they are likely not representative of the fish community at that location. To be conservative, data from monitoring programs that did not explicitly link a set of species (the presumed community) sampled to a given sampling occasion was included as *occurrence* and *not community* data.

For all data, we checked and corrected typographical errors in locality and geographical coordinates. We also standardized the taxonomy across the different datasets. We followed Integrated Taxonomic Information System (ITIS; www.itis.gov) taxonomy for most species except for newly described species e.g., *Percina freemanorum*, *Aphredoderus gibbosus*, *Forbesichthys papilliferus*, where we followed Catalog of Fishes. We excluded (i) fish records that are not identified to the species-level; (ii) hybrids; and (iii) marine species (as defined by FishBase; www.fishbase.org). Below we summarize the fish community (Table 1) and fish occurrence (Table 2) datasets we have compiled for our analyses.

Table 1. Fish *community* datasets. Year range denotes the first and last year of sampling. N_{record}: total number of records (each record is the abundance of a given species found in a unique sampling occasion). N_{sample}: total number of sampling occasions. N_{COMID}: number of stream reaches (each stream reach is a unique COMID in the National Hydrography Dataset Plus (NHDPlus) v21 National Seamless Geodatabase) sampled by each program.

No.	**Source**	**Year range**	N_{record}	N_{sample}	N_{COMID}
1	TVA Fish IBI Monitoring	1998-2022	91874	3855	1131
2	TWRA Fish Monitoring	1985-2018	16287	1411	1006
3	Mainspring Conservation Trust	1990-2022	12519	856	130
4	KYDEP Fish Monitoring	1978-2016	9684	726	204
5	NCDEQ Fish IBI Monitoring	1993-2022	5311	394	189
6	USGS National Water Quality Assessment	1996-2019	4197	182	131
7	EPA National Rivers and Streams Assessment	2008-2019	2974	143	86
8	ADEM/GSA Fish Monitoring	2013-2022	1144	46	36
9	GADNR Fish Monitoring	2002-2017	710	33	12
	All datasets combined	1978-2022	144700	7646	2647

Table 2. Fish *occurrence* datasets. Year range denotes the first and last year of a recorded occurrence in each dataset. N_{record} is the total number of records (each record is a given species observed to be occurring at a given location and time). N_{comid} represents the number of stream reaches (each stream reach is a unique COMID in the NHDPlus v21 National Seamless Geodatabase) associated with records in each dataset.

No.	**Source**	**Year range**	N_{record}	N_{COMID}
1	GBIF	1789-2025	95814	7683
2	KDFWR Fish Database	1925-2021	53597	1769
3	VADGIF Fish Database	1885-2017	40778	1007
4	Conservation Fisheries Inc. (CFI) Database	1986-2018	28518	717
5	UTKEIC Specimen Collection	1926-2023	17522	2720
6	TWRA Fish Monitoring	2000-2019	13643	942
7	USGS Nonindigenous Aquatic Species (NAS) Database	1875-2023	3452	1414
	All datasets combined	1789-2025	253324	11868

5.4. Stream network geospatial data

Our analyses are conducted at the stream reach (and the associated *catchment*) spatial grain as defined by the NHDPlus v21 National Seamless Geodatabase. We overlaid fish community and occurrence data with the catchment layer of the NHDPlus v21 National Seamless Geodatabase to identify the stream reach (COMID) from which each fish community and fish species occurrence was taken. We only considered COMIDs that are associated with a NHDPlus v21 flowline in our analyses, thus we excluded sinks (catchments that are not flow-connected to other catchments) from our analyses. Environmental data (see 5.5. Land-cover data, 5.7 Other environmental data; 5.8. Stream temperature data, 5.9. Streamflow data) were all generated at this same COMID spatial grain to match fish data.

We used functions from the R packages 'nhdplusTools' (Blodgett and Johnson, 2023) and 'hydroloom' (Blodgett, 2023) to identify the upstream catchments that contribute to each catchment. For a given focal catchment, the set of all upstream catchments together with the focal catchment constitutes its full upstream watershed (*sensu* Hill et al., 2016 and the StreamCat dataset; hereafter, the *watershed* scale). We use upstream catchment data to accumulate streamflow for each catchment (see 5.9. Streamflow data) as well as to calculate percent land-cover at the watershed scale (see 5.5. Land-cover data). We also obtain other environmental data at the watershed scale (see 5.7. Other environmental data), which we will use as environmental predictors to model stream temperature at each stream reach as the temperature at a given stream reach is affected by energy transfer and hydrological processes occurring in the entire watershed that contributes to a stream reach (Leach et al. 2013).

5.5. Land-cover data

We used different sets of land-cover data for different analyses to maximize consistency with the temporal range of the fish datasets used in each analysis as well as to ensure that input datasets used in each analysis is internally consistent. For example, the USGS LCMAP data (USGS, 2022) was used in the fish community analysis (Analysis 4) because its temporal coverage (1985-2021) coincided with the fish community data that we have compiled. Likewise, the Sohl et al. (2014) land-cover dataset was used to calibrate the historical and future streamflow projections used in species distribution modelling analysis (Analysis 6); therefore we used the Sohl dataset rather than the USGS LCMAP data for that analysis.

5.5.1. USGS LCMAP data - recent community change analysis (5.14. Analysis 4)

We used land-cover data from the USGS Land Change Monitoring, Assessment, and Projection (LCMAP) Collection v1.3, which includes eight main cover classifications over CONUS: Tree Cover, Developed, Grass/Shrub, Cropland, Water, Wetland, Snow/Ice, and Barren (USGS, 2022). For each stream reach (COMID) in the TCRB (and surrounding basins, *See Stream Temperature Dat*a for details), we calculated the percentage of the associated catchment occupied by each land-cover type for each year during the historical period from 1985 to 2021. Since LCMAP data for 2022 has not yet been released, we employed linear regression models to assess temporal trends over the last 10 years (2011–2021) at each COMID to extrapolate the land cover distributions for 2022. Using catchment-scale land-cover percentages and catchment area, we calculated watershed land-cover percentages for each COMID using functions from the ‘nhdplusTools’ (Blodgett and Johnson, 2023) and ‘hydroloom’ (Blodgett, 2023) packages in R (R Core Team, 2024).

5.5.2. Sohl land-cover data - species distribution modelling analysis (5.16. Analysis 6)

We used annual historical (1890-2010) and future projections (2035-2065) for land cover classes from Sohl et al. (2014). For historical data, we used a combination of observed (1980-2005) and forecasted (2006-2010) land-cover from the AB scenario to maintain consistency with the calibration of streamflow Lafontaine & Riley (2003). For each COMID, we averaged the percent coverage of each land-cover class for each period: historical and future.

5.6. Climatic data

As in the case of land-cover data described above, we used different sets of climatic data to maximize consistency with the temporal range of the fish datasets used in each analysis and to ensure that input datasets used in each analysis are internally consistent.

5.6.1. gridMet climatic data - recent community change analysis (5.14. Analysis 4)

gridMET provides daily precipitation, as well as minimum and maximum temperature, at ~4 km resolution from 1979 to the present for CONUS (Abatzoglou, 2013). We retrieved daily time series of these climatic variables from 154 monitored reaches across the Southeastern USA. from May 2017 to December 2020, corresponding to each logger monitoring stream water temperature. These data served as the primary input for building a water temperature model to analyze fish community-level changes.

5.6.2. Maurer et al. (2002) climatic data - species distribution modelling analysis (5.16. Analysis 6)

Maurer et al. (2002) provide daily and monthly downscaled climate projections at a ⅛-degree resolution for CONUS. While the initial publication covers data from 1950 to 2000, subsequent expansions include historical observed data up to 2010 and future projections extending up to 150 years for multiple scenarios (available at: https://gdo-dcp.ucllnl.org/downscaled_cmip_projections/dcpInterface.html).

For the SDMs analysis, we extracted daily minimum and maximum temperatures, as well as precipitation, for the period 1980–2010 as the historical baseline and for the future scenario (2035–2065) using the Bias-Corrected Constructed Analogs V2 (BCCAv2) dataset from the Coupled Model Intercomparison Project Phase 5 (CMIP5). The future scenarios included two Representative Concentration Pathways (RCPs; 4.5 and 8.5) from 12 General Circulation Models (GCMs) [also used for future flow scenarios modeled by Lafontaine & Riley (2023)], resulting in a total of 24 climate future scenarios. For each scenario, these data were aggregated to monthly means and spatially averaged at the COMID level for stream reaches across CONUS where fish occurrences were recorded. The resulting averaged reach values were then used to compute 19 bioclimatic variables for both historical and future scenarios.

5.7. Other environmental data

In addition to the land-cover and climatic data described above, we used a variety of data that describe the physical environment and geomorphology of the stream reach and its associated catchment and watershed. For example, we used mean % clay, mean water table depth, mean bedrock depth, base flow index, elevation, watershed area data from StreamCat (Hill et al., 2016). We also calculated a metric of northness (the extent to which the catchment is facing north as a measure of solar radiation intensity) from Amatulli et al. (2018). We also included the degree of regulation (DOR) metric, which quantifies the degree to which upstream dams affect the flow at each focal stream reach. These data were used as predictor variables for stream temperature modeling (see 5.8. *Stream temperature data*).

5.8. Stream temperature data

We used the *ranger* package in R to calibrate random forest models to predict maximum daily water temperature across the TCRB under historical conditions. We modeled the daily maximum temperature obtained from 154 monitored reaches (i.e., COMID) from May 2017 to December 2020, spanning nine sub-basins at the HUC8 level (167,714 temperature measurements) using stream temperature logger data from Troia et al. (2019) and USGS NWIS within the TCRB and adjacent drainages. We employed a set of 18 reach and upstream catchment scale predictors, including climatic, land cover, soil and hydrographic predictors annual land cover (Table 3). To select the best climatic proxies for maximum water temperature, we evaluated their lagged correlations with maximum air temperature and mean precipitation over average conditions for 1-7 days. The highest average correlations across the 154 stations with maximum air temperature and precipitation are found with average conditions from the past 4 (average r = 0.9498) and 7 days (average r = -0.06439), respectively.

Table 3. Climatic, land cover, and hydrographic variables considered for stream temperature modeling. We tested for potential collinearity issues among this initial set of variables to select a final set of 14 variables ($|r| < 0.7$) for inclusion as model predictors. Dataset source citations: [1]Abatzoglou (2013); [2]USGS (2022); [3]Hill et al. (2016); [4]NHDPlusv2 National Seamless Geodatabase (https://www.epa.gov/waterdata/nhdplus-national-data)

Variables	Scale	Type	Source	Included
4-day average of the daily maximum air temperature (°C)	Catchment	Climate	gridMET[1]	Yes
7-day average of daily precipitation (mm)	Catchment	Climate	gridMET[1]	Yes
Day of the year (DOY)	-	Climate	-	Yes
Tree cover (%)	Catchment, Watershed	Land-cover	LCMAP CONUS v1.3[2]	Yes
Developed (%)	Catchment, Watershed	Land-cover	LCMAP CONUS v1.3[2]	Yes
Cropland (%)	Catchment, Watershed	Land-cover	LCMAP CONUS v1.3[2]	No
Mean % Clay	Watershed	Hydrographic	StreamCat[3] (CLAYWS)	No
Mean Water Table Depth	Watershed	Hydrographic	StreamCat[3] (WTDEPWS)	Yes
Mean Bedrock Depth	Watershed	Hydrographic	StreamCat[3] (RCKDEPWS)	Yes
Base Flow Index	Watershed	Hydrographic	StreamCat[3] (BFIWS)	Yes
Elevation	Catchment	Hydrographic	StreamCat[3] (ElevCat)	Yes
Total area (km^2) (Watershed area)	Watershed	Hydrographic	NHDPlusv2[4]	Yes
Northness	Catchment	Hydrographic	Amatulli et al. (2018)	Yes
Degree of dam regulation (DOR)	Watershed	Hydrographic	Spinti et al. (2023)	Yes

To identify the best combination of hyperparameters in random forest fitting (node size, mtry, and sample fraction), we evaluated 125 candidate models with contrasting and different combinations of these parameters using all available data for training (node size: 2, 4, 6, 8, 10, 14, mtry: 2, 4, 6, 8, 10 and 14, and sample fraction: 0.2,0.4, 0.6, 0.8 and 1). For each model, we set the number of trees to 150 (10 times the number of predictors) and allowed resampling with replacement. We measured the goodness of fit of the candidate models by assessing the gain in RMSE, MAE, and R^2 on the training dataset (i.e., out-of-bag prediction).

Subsequently, we assessed the predictive performance of the top 10% of candidate models (n = 12) identified through hyperparameter tuning using a k-fold spatial cross-validation approach. Each top candidate model was trained nine times, with observations from the stations in each HUC8 omitted one at a time for testing. We quantified predictive performance by calculating RMSE, MAE, and R^2 using predictions from the testing datasets. The best-performing model in the spatial cross-validation (RMSE = 2.032°C, MAE = 1.494°C, R^2 = 0.906) (Figures 2 and 3) was selected to generate daily water temperature predictions under historical climatic conditions (1985–2022) in the TCRB. River basins. Daily values were then aggregated at the monthly level for historical conditions between 1985-2022.

5.9. Streamflow data

5.9.1. 1985-2021 PRMS flow data - recent community change analysis (5.14. Analysis 4)

Historical streamflow data for 1980–2021 were obtained from simulations using the Precipitation Runoff Modeling System (PRMS) v5.1.0, coupled with the USGS National Hydrologic Model Infrastructure (NHMI). PRMS was calibrated independently for the TCRB using climate forcings from gridMET (1985–2022) and dynamic land cover data from LCMAP v1.3. We estimated surface imperviousness annually (1985–2022) at each COMID using the National Land Cover Dataset (NLCD) Surface Imperviousness Product (2001–2021, available biennially) as input for a linear mixed model, which relates developed land cover percentage to imperviousness.

PRMS simulations yielded daily outflow values for relatively large-sized Hydrological Response Units (HRUs), from which we calculated the average monthly outflow for each HRU. We then downscaled monthly streamflow at the HRU spatial grain to the local catchment (COMID) spatial grain based on spatial intersections between HRU and COMID, proportionally disaggregating HRU outflows according to the relative areas of each intersection. Since a COMID may intersect multiple HRUs, we summed outflow values from each intersection to obtain the average monthly outflow of a given COMID. To obtain the total amount of streamflow at a given COMID, we summed outflow values from all upstream catchments that flow into that COMID (i.e., the watershed) using functions from the ‘nhdplusTools’ (Blodgett and Johnson, 2023) and ‘hydroloom’ (Blodgett, 2023) packages in R (R Core Team, 2024). This flow data was generated for the Tennessee River basin because it was the focal region of the community change analysis (see 5.14. Analysis 4).

5.9.2. Historical and future PRMS flow data - species distribution modelling analysis (5.16. Analysis 6)

For SDMs analysis, we used PRMS v5.1.0 simulations for historic (1980–2010) and future (2035-2065), calibrated by LaFontaine & Riley (2023) with climate forcings from Maurer et al. (2002) and land cover data from Sohl et al. (2014) for the conterminous USA. The streamflow downscaling process followed the same approach as above, from individual HRU outflows to the streamflow at individual stream reaches (COMID) via the steps described above in 5.8.1.: spatial intersections of HRU with COMID, proportional disaggregation of outflow at the HRU spatial grain to the COMID spatial grain, and summing outflow values from all upstream catchments that flow into each focal COMID.Finally, average monthly streamflow was converted into an initial set of 25 hydrological indicators for all future and historical scenarios, following the framework proposed by Olden & Poff (2003) to capture different dimensions of the hydrological regime (magnitude, frequency, duration, timing) across streams in the U.S. We followed the guidelines and code provided by Morden et al. (2023) to adapt 115 hydrological indicators from daily flow regimes to monthly flow regimes. Hydrological indicators from the rate-of-change dimension not because they are not transferable to monthly metrics (Morden et al. 2023).

5.10. Assigning native vs. non-native status to fish species

For each fish record, we assigned whether the species is native or non-native to the HUC6 (6-digit hydrological unit code) within which the locality of the fish record is embedded. There are seven HUC6 units in the TCRB: two in the Cumberland basin (051301 Upper Cumberland and 051302 Lower Cumberland) and five in the Tennessee basin (060101 French Broad-Holston; 060102 Upper Tennessee; 060200 Middle Tennessee-Hiwassee; 060300 Middle Tennessee-Elk; 060400 Lower Tennessee). We made native vs. non-native assignments based on information from a wide variety of literature sources including data from USGS Nonindigenous Aquatic Species (NAS) website (https://nas.er.usgs.gov/), fish atlases (e.g., Etnier and Starnes, 1993; Tracy et al. 2020), the Tennessee Aquarium Conservation Institute (TNACI) Freshwater Information Network (https://tnacifin.com/), NatureServe Explorer (https://explorer.natureserve.org/), species descriptions and taxonomic revisions, and expert assessment by Xingli Giam and Benjamin Keck (University of Tennessee, Knoxville). There were a small number of records in which there was no strong evidence or consensus if the species was actually native or non-native to the HUC6 they were sampled. For example, Etnier & Starnes (1993, on pg. 200) described the distribution and status of the golden shiner, *Notemigonus crysoleucas*, as "*Probably rare or absent from east and middle Tennessee prior to reservoir construction, but now established in larger waters of these areas as a result of bait bucket introductions or expansion of once-scattered populations.*" Another example is the warmouth, *Lepomis gulosus*, that was classified by USGS NAS as native to 060300 Middle Tennessee-Elk and 060400 Lower Tennessee but non-native in other HUC6 units in the TCRB. Tracy et al. (2020) in their NC Fish Atlas stated that *L. gulosus* was non-native to watersheds within 060101 French Broad-Holston and 060102 Upper Tennessee. However, Etnier & Starnes (1993) did not mention that the warmouth as being non-native to any specific watersheds in Tennessee (Etnier & Starnes, 1993; pg. 414) and classified it as a species that is native to Tennessee (Etnier & Starnes, 1993; Table 2, pg 24). For these two species and six others (*Dorosoma cepedianum*, *Lepomis marginatus*; *Morone mississippiensis*, *Pimephales promelas*, *Semotilus thoreauianus*, and *Gambusia affinis*) where there was uncertainty as to whether they were native or non-native at a given locality, we classified them as questionable. We also encountered some records of fish species that were not known to be native at the HUC8 they were found. However, there was also no corroborating evidence in the literature (e.g., USGS NAS or fish atlases) or from our field knowledge (Benjamin Keck and Xingli Giam) that such a non-native record was likely. We interpreted these records as a misidentification or a previously used name for a native species.

Last, based on the HUC6 designations, we assigned whether a fish record pertains to a species that is native vs. non-native (or questionable) at the basin level (HUC2 06 Tennessee; and HUC4 0513 Cumberland). We classified a species as native to the Tennessee or Cumberland basin if it was native to at least one HUC6 within that basin. For example, a species will be classified as non-native or questionable to the Tennessee basin if it was not recorded as native to any of the HUC6 units within the basin.

For the analyses in this report, we pooled the questionable records as native. However, this is an ongoing database, and we would revisit and revise the statuses of fish records as new evidence emerges from the literature or from field observations.

5.11. Analysis 1 - Trends in non-native fish

Using fish records with known collection dates (n = 386,828) from both *community* and *occurrence* datasets, we calculated the number of unique occurrences of non-native fish species recorded in the years up to 1979, and in each subsequent five-year period up to 2024 (i.e., 1979 and before; 1980-1984, 1985-1989…2015-2019, 2020-2024). Each unique occurrence is defined by one or more records of a given species sampled at a given stream reach (COMID) and date. The aggregation to the stream reach scale (rather than using raw program site identifiers or geographic coordinates) is conservative as it guards against counting the same fish record multiple times if that record is present in multiple databases (e.g., in the event of a fish collection divided into multiple lots and deposited in different museums; different monitoring programs collaborating on field sampling but including the community sample in their respective databases; and small changes in geographic coordinates during data entry and processing that prevents easy detection of such duplicated records). We compared these five-yearly unique occurrences of non-native species with the number of unique COMID sampling occasions graphically to assess if changes in non-native occurrences might be driven at least in part by changes in the frequency of sampling or if they reflect real trends.

5.12. Analysis 2 - Recent range changes of non-native species

We used fish records with known collection dates (n = 386,828) from both community and occurrence datasets to investigate range changes of non-native species over the last ~40 years. Our analysis focused on 38 species with non-native (i.e., introduced) occurrences in ≥10 stream reaches (COMIDs) (Table 4). In this analysis, non-native status was defined at the HUC6 level (see 5.9. *Assigning native vs. non-native status to fish species*). Consistent with Analysis 1, a unique occurrence is defined as a unique record of a species on a unique COMID on a given day to guard against duplicate records of the same individual(s) sampled that is archived in different datasets. In total, there were 335,661 unique occurrences.

Table 4. Species with non-native occurrences in at least 10 unique stream reaches (COMIDs). Non-native (introduced) status was defined at the HUC6 level. nCOMID: the number of unique stream reaches (COMIDs) within HUC6 units in which a given species was non-native. nCOMID-Yr: the number of unique COMID-year occurrences (e.g., a species recorded in one COMID in years 1989, 2006, and 2020 will have 3 unique COMID-year occurrences). minYear and maxYear means the earliest and most recent year of record, respectively. As an example, redbreast sunfish, *Lepomis auritis*, had known occurrences in 2070 stream reaches in our dataset, and 4728 COMID-year occurrences.

Species	**nCOMID**	**nCOMID-Yr**	**minYear**	**maxYear**
Lepomis auritus	2070	4728	1934	2024
Oncorhynchus mykiss	1429	3685	1888	2024
Salmo trutta	891	2620	1939	2024
Cyprinus carpio	774	1406	1936	2024
Perca flavescens	299	595	1955	2024
Dorosoma petenense	211	274	1948	2024
Hypophthalmichthys molitrix	175	260	1995	2024
Morone saxatilis	132	180	1962	2024
Nocomis leptocephalus	125	182	1941	2024
Ctenopharyngodon idella	121	179	1978	2024
Menidia audens	117	176	1990	2022
Carassius auratus	111	131	1939	2024
Micropterus coosae	107	142	1940	2024
Noturus insignis	95	221	1949	2024
Lepomis gibbosus	88	108	1940	2023
Notropis texanus	83	212	1953	2022
Notropis lutipinnis	75	405	1967	2024
Ameiurus platycephalus	73	133	1977	2023
Hypophthalmichthys nobilis	61	82	1995	2023

Alosa chrysochloris	47	49	1974	2024
Chrosomus oreas	45	63	1941	2024
Gambusia holbrooki	43	48	1965	2024
Ameiurus brunneus	38	121	1990	2023
Salvelinus fontinalis	37	84	1938	2020
Alosa pseudoharengus	30	30	1984	2019
Hypentelium etowanum	25	26	1949	2021
Cyprinella venusta	21	30	1991	2022
Salvelinus namaycush	17	20	1977	2023
Alosa aestivalis	16	20	1992	2022
Luxilus coccogenis	16	19	1973	2021
Ameiurus catus	15	18	1977	2008
Esox niger	15	25	1991	2024
Strongylura marina	14	16	1990	2017
Etheostoma fusiforme	13	17	1947	2021
Mylopharyngodon piceus	12	13	2017	2022
Esox lucius	11	12	1939	1994
Moxostoma poecilurum	11	12	1968	2020
Micropterus henshalli	10	10	2015	2024

For each of these 38 species (Table 4), we summed the number of unique stream reaches (COMID) it occupied in four time periods (before and up to 1979; 1980-1994; 1995-2009; 2010-2024) within its introduced range. We also calculated the cumulative number of unique stream reaches (COMID) that they occupied within its introduced range up to 1979, 1994, 2009, and 2024. The number of unique reaches represent a minimum bound of the number of stream reaches occupied by the species in a given time period whereas the cumulative number of unique reaches represent a higher estimate (but not maximal bound) of the number of reaches occupied by the species. The number of unique reaches represent known observations of the species during each time period; there would be other localities (some previously sampled while others never sampled before) that were not sampled during a given time period but may nevertheless be occupied by the species. The cumulative number of unique reaches represent a higher estimate of the magnitude of potential range expansion of an introduced species because it assumes any stream reach occupied by the species in a previous time period will remain occupied in future time periods. Yet, this is not a maximal bound due to the same reason as discussed before, i.e., that there will be established populations that have not yet been detected due to a lack of sampling in those reaches.

5.13. Analysis 3 - Recent abundance trends of non-native species

We used the community dataset to investigate recent trends in the relative abundance of each non-native species across its introduced range over the last few decades. We wanted to identify the species that are increasing across sites within their introduced ranges and those that are decreasing, as well as the strength of evidence associated with the trend. We employed a random effects meta-regression approach to combine relative abundance trends in each non-native species (across every occupied site in their non-native HUC6s) while taking into account the nonindependence among multiple non-native populations within a given reach to quantify the mean trend of each species. We used an evidence-based framework (Muff et al. 2022) to interpret the strength of evidence of each increasing or decreasing trend based on the P-value of the hypothesis test of whether the trend of each species is different from zero. Based on this framework, $P \leq 0.001$ can be interpreted as very strong evidence for a trend; $0.001 < P \leq 0.01$ as strong evidence; $0.01 < P \leq 0.05$ as moderate evidence; $0.05 < P \leq 0.1$ as weak evidence, and $P > 0.1$ as no evidence (Muff et al. 2022).

The relative abundance trend of a given non-native species at a given stream site is quantifying by performing a linear regression of the relative abundance (calculated as the log-response ratio of the abundance of the non-native species to the abundance of all other species within the same community) against year of sampling. Therefore, the trend of a non-native species at a given site—change in log[(abundance of the non-native species + 1) / (abundance of all other species + 1)] per year—is represented by the coefficient (slope) of the year term. A positive coefficient represents an increasing trend over time whereas a negative coefficient represents a decreasing trend. We used the year coefficient as the effect size in our random effects meta-regression and the squared standard error of the coefficient as its sampling variance (Becker & Wu, 2007; Babcock et al. 2017) . We entered species identity as a predictor in the meta-regression to estimate individual species-level trends while incorporating nonindependence among populations within individual sites. Random effects (intercepts) were individual populations (unique species-by-site combinations) and unique sites. We included only sampling occasions from March-August and sites (i.e. communities) that were sampled during this period in ≥ 4 unique years, over a span of ≥ 10 unique years, and with the last year of sampling in 2010 or after (464 sites). Of these 464 sites, 410 sites had at least one non-native species. A relative abundance trend was therefore estimated for each non-native species present in each of these sites. We used the rma.mv() function in the metafor R package (Vietchbauer, 2010) to fit the random effects meta-regression model.

5.14. Analysis 4 - Climate and land-use change effects on non-native fish abundance

We focused this analysis on 403 community sampling sites in the Tennessee River basin with at least one non-native species. We focused on the Tennessee River basin for this analysis because there were only 7 community sampling sites in the Cumberland River. Like in Analysis 3, these sites were sampled from March-August in ≥ 4 unique years, over a span of ≥ 10 unique years, and with the last year of sampling in 2010 or after. In each of these 403 sites, we calculated temporal trends in the relative richness and abundance of non-native species. Non-native status of species were defined based on HUC2 and HUC6 definitions consistent with all analyses in this report. For a given location, a given species was classified as whether it was non-native to the HUC2 (entire Tennessee River basin; e.g., species like silver carp, *H. molitrix*, which is non-native to the entire US, or rainbow trout, *O. mykiss*, which is non-native to the Tennessee River basin) of that location or to the HUC6 of that location (e.g., a species such as chain pickerel, *Esox niger*, which is native to the Lower Tennessee and Middle-Tennessee Elk but would be classified as a HUC6 non-native in sites within the French Broad-Holston).

We fitted linear regression models to quantify the trend of non-native fish over time in terms of their total species richness and total abundance pooled across all species, relative to the total species richness and total abundance of native fish species, relatively. Similar to Analysis 3, in each sampling occasion at each site, we calculated the log-response ratio of non-native to native species richness, as well as the log-response ratio of non-native to native total abundance adding a value of 1 to each quantity as continuity correction. At each site, we regressed each log-response ratio against year and recorded the coefficient (slope) of the year term as the mean change in log[(non-native species richness + 1) / (native species richness + 1)] or log[(total abundance of all non-native species + 1) / (total abundance of all native species + 1)] per year, i.e., the trend of non-native fish in a given in terms of the species richness and total abundance. We also recorded the squared standard error of the year coefficient as its sampling variance (Becker & Wu, 2007; Babcock et al. 2017) of the trend. There was one site in which there was no change in non-native richness through time, therefore the fitted slope of 0 had a perfect fit (standard error of zero). For the meta-analysis described below, we replaced the standard error of zero with the smallest non-zero standard error in the dataset.

We used the rma.mv() function in metafor R package (Vietchbauer, 2010) to fit random effects meta-regression models to identify climate-change and land-cover change related variables, along with other environmental and biotic variables, that may potentially drive variation in non-native fish trends across the 403 sites. We summarize fixed effects predictor variables in Table 5. All predictors were centered and scaled (mean=0, sd=1). We included a random intercept for HUC6, which accounts for nonindependence among sites; we hypothesized that sites within a given HUC6 may show more similar responses because of similarities in the overall species pool as well as more similar physiographic conditions. We used AICc (small-sample Akaike Information Criterion) to identify the most parsimonious model for statistical inference. Similar to Analysis 3, we used an evidence-based framework to interpret effects. Based on this framework, $P \leq 0.001$ can be interpreted as very strong evidence for a trend; $0.001 < P \leq 0.01$ as strong evidence; $0.01 < P \leq 0.05$ as moderate evidence; $0.05 < P \leq 0.1$ as weak evidence, and $P > 0.1$ as no evidence (Muff et al. 2022).

Table 5. Fixed effects predictor variables used to predict relative non-native richness and relative non-native abundance trends in meta-regression models. This set of variables is a smaller subset of the original variables considered after removing highly collinear (|r| > 0.7) variables. Dataset source citations: [1]Lafontaine et al. (2019) and section 5.9.1 of this report ; [2]Stream temperature modeling, section 5.8. of this report; [3]USGS (2022); [4]We modified the DOR metric proposed by Spinti et al. (2023) to produce local DOR (LDOR), which is calculated as local storage divided by annual flow at a stream reach using the data provided by Spinti et al. (2023); [4]Upstream watershed area is calculated by totalling area of focal catchment and all upstream catchments that drain into it (see section 5.4); [5]Mean native species richness is calculated from the dataset analyzed here.

Variables	**Description**	**Hypothesis**	**Source**
MeanFlow_Trend	Annual trend in mean monthly flow across the span of sampling years	Change in mean flow may benefit non-native species; directionality uncertain	PRMS[1] (this study)
CVFlow_Trend	Annual trend in the coefficient of variation (CV) of monthly flow across the span of sampling years	Decrease in flow seasonality would benefit non-native species	PRMS[1] (this study)
MeanTemp_Trend	Annual trend in mean monthly March-August water temperature across the span of sampling years	Increase in water temperature would benefit non-native species	This study[2]
CVFlow_Mean	Mean of the CV of monthly flow across the span of sampling years	More seasonal streams have less positive non-native trends	PRMS[1] (this study)
MeanTemp_Mean	Mean of mean monthly March-August water temperature across the span of sampling years	Warmer streams might be more invasible and have more positive non-native trends	This study[2]
Tree_Trend	Annual trend in upstream watershed tree cover across the span of sampling years	Streams with reduction in upstream tree cover favor non-natives	LCMAP[3]
Tree_Mean	Mean upstream watershed tree cover across the span of sampling years	Streams with less upstream tree cover have poorer water quality, and favor non-natives	LCMAP[3]
LDOR	A metric of local flow regulation modified from Spinti et al. (2023), calculated as local water storage (Norm_stor) divided by annual flow at a stream reach (QC_MA) in the NABD dataset provided by Spinti et al. (2023)	Streams that are more regulated (store more water relative to flow) are more lentic and favor non-natives	Spinti et al. (2023) (this study)[4]
logFF	A metric of recreational freshwater fishing demand at the HUC12 level,	Streams with higher recreational fishing demand	Davis & Darling

	log-transformed	are more likely to experience introductions due to bait buckets, accessibility, etc.	(2017)
logWSArea	Total upstream watershed area, log-transformed	Non-native trends may differ across the longitudinal gradient; directionality uncertain	This study[5]
logNativeRich	Mean native fish species richness across sampling occasions, log-transformed	Native species-rich streams might be less invasible due to increased biotic resistance	This study[6]

5.15. Analysis 5 - Impact of non-native fish on native fish species

Based on our discussions with agency partners and collaborators in the project kickoff meeting (see *section 5.1. Co-production approach*), we focus on investigating the potential impacts of silver carp (*Hypophthalmichthys molitrix*), yellowfin shiner (*Notropis lutipinnis*), and mosquitofish (*Gambusia affinis and G. holbrooki*) on native species. Note that *G. affinis* is classified as questionable in its native-vs-non-native status in our study; however, we pooled this species with the unambiguously non-native *G. holbrooki* because they appear to have the same ecological niche and because our partner is interested in the impact of mosquitofish in general (*section 5.1. Co-production approach*).

For each of the three focal non-native species (1. silver carp; 2. yellowfin shiner; 3; mosquitofish comprising the non-native *G. holbrooki* and the questionable *G. affinis*), we identify all community sampling sites within the Tennessee River basin with records of that species. We then identify the 8-digit hydrological unit code (HUC8) drainage units that contain all these sampling sites, and select all sites (including those without the focal non-native species within the HUC8 units. On each sampling occasion at each site, we calculate the relative abundance of the focal non-native species as log[(abundance of the focal non-native species + 1) / (abundance of all other species + 1)]. We examined the association of this variable (which quantifies the degree of invasion of this species at a given site and time) with response variables that appropriately reflect the hypothesized impact of invasion. The response variable to examine the impact of silver carp was the number of native species because we hypothesized that silver carp invasion would likely result in a decline of native species because silver carp is known to alter and degrade stream habitats important for native species and that they also compete with native species for food resources. The response variable to examine the impact of yellowfin shiner was the richness of native *Notropis* spp. [log (richness+1) transformed because of zeroes] because we hypothesize that yellowfin shiner might potentially compete with native congenerics for food as they share similar ecological niches. Similarly, we hypothesized that *Gambusia* spp. would compete with *Fundulus* sp. because of their shared ecological niches. *Gambusia affinis* has been implicated in the decline of the federally endangered barrens topminnow *F. julisia*

(Ennen et al. 2021) and one of our partners requested for us to examine if this was the case using our dataset. However, *F. julisia* was not in any of our community sampling sites; therefore we used the richness of *Fundulus* spp. [log (richness+1) transformed because of zeroes] as the response variable to investigate impacts of *Gambusia* spp.

We used the glmmTMB() function in the glmmTMB R package (Brooks et al. 2017) to fit linear mixed-effects models (LMM) that predict the response (silver carp impact model: native species richness; yellowfin shiner impact model: native *Notropis* species richness; mosquitofish impact model: *Fundulus* relative abundance) as a function of the log-transformed relative abundance of the non-native species or species group (LRRAbun) in question. Each observation is a sampling occasion at each site. We included stream-reach-level environmental covariates: (1) mean of mean monthly flow across the span of sampling years (MeanFlow_Mean); (2) mean of the coefficient of variation of monthly flow across the span of sampling years (CVFlow_Mean); (3) mean of mean monthly March-August water temperature across the span of sampling years (MeanTemp_Mean); (4) mean upstream watershed tree cover across the span of sampling years (Tree_Mean); (5) local degree of regulation metric (LDOR; Table 5); and (6) log-transformed upstream watershed area (logWSArea) to control for site-level effects on native fish species. All predictor variables were centered and scaled to mean=0, sd=1. To account for the fact that we are analyzing repeated community samples (within each site), we allowed for the coefficient of the focal predictor variable LRRAbun to vary across sites. We also included random intercepts at the site level and at the HUC8 level whenever sites are located in multiple HUC8 units. Like in the previous analyses, we used an evidence-based framework to evaluate the strength of evidence of a given effect.

5.16. Analysis 6 - Projecting future range shifts using species distribution modeling

We modeled the ranges of 37 non-native species with at least 10 records in the TCRB at any time, as well as at least 25 records across the entire CONUS between 1980 and 2010. To more comprehensively characterize the environmental niche of each species species, we supplemented our dataset with additional occurrence records from GBIF for the entire CONUS. This broader dataset helps prevent niche truncation, which could otherwise impair future predictions.

We used four commonly employed machine learning techniques to fit SDMs CONUS-wide for each species: Random Forest (RF), Maximum Entropy (Maxent), Generalized Boosted Regression Models (GBM), and Extreme Gradient Boosting (XGBoost). SDMs were fitted to sing the 'biomod2' R package (Thuiller et al. 2024) with default settings to establish the relationships between CONUS-wide occurrences and pseudoabsences with historical environmental conditions (climate, streamflow, land cover, and other factors). To evaluate model predictive performance, we conducted both spatial cross-validation and random k-fold validation by partitioning occurrences and pseudoabsences into five independent spatial folds and five random folds, repeating this process five times for each modeled species. We then assessed the performance of each machine learning technique using the mean Area Under the

Curve (AUC) for both cross-validation (AUC_{cross}) and k-fold validation (AUC_{kfold}) across repetitions and folds. In total, we fitted 100 models per species for each validation type (cross-validation and k-fold validation), with 25 models per machine learning technique (five repetitions across five folds) to evaluate predictive performance.

For each non-native species, we retained only machine learning techniques that performed moderately well in spatial cross-validation (mean $AUC_{cross} \geq 0.6$). If no species performed moderately well with any technique, the species was dropped for subsequent analyses. We then fitted full SDMs for each technique using all available occurrences to project them onto the historical period (1980–2010) and the 24 future climatic scenarios (2035–2065) along the whole TCRB. These future scenarios aligned between climate and flow data combine two Representative Concentration Pathways (RCPs; 4.5 and 8.5) from 12 General Circulation Models (GCMs) [also used for future flow scenarios modeled by Lafontaine & Riley (2023)]. In all future scenarios, we applied the same land cover projection from the AB scenario in Sohl et al. (2014).

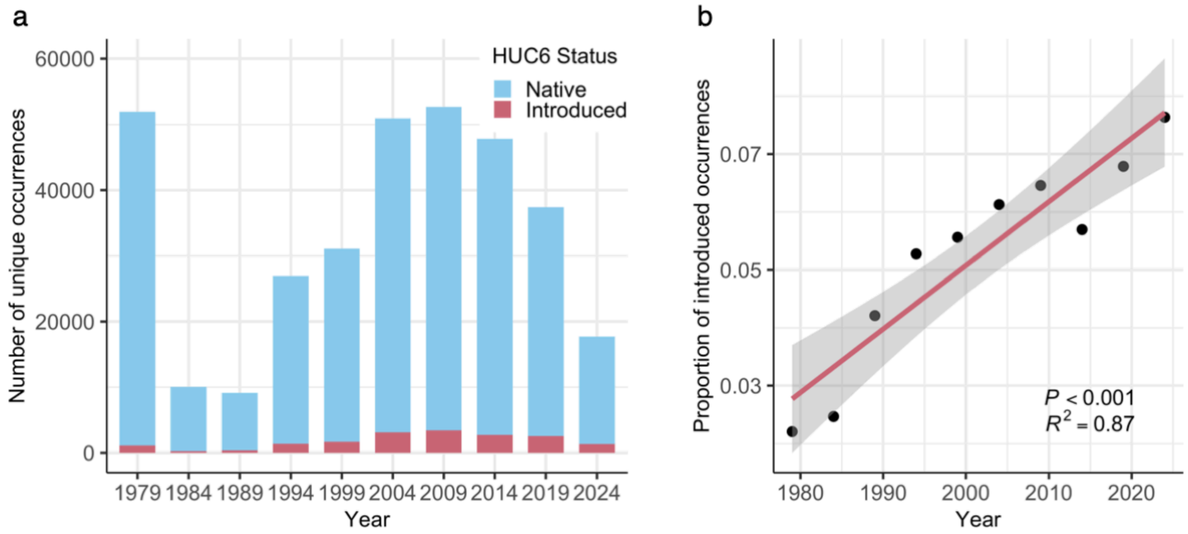
For each species, we generated an ensemble consensus prediction for both historical and future scenarios by weighting the average continuous suitability (ranging from 0 to 1) based on AUC values. For future predictions, we averaged projected suitabilities across the 12 GCMs scenarios for each RCP (4.5 & 8). We used the weighted average threshold across cross-validation repetitions that maximized the sum of specificity and sensitivity (maxSSS) (Liu et al. 2013, Liu et al. 2016) and then applied a weighted average across techniques to classify catchments as suitable (1) or unsuitable (0) in both historical and future scenarios.

6. PROJECT RESULTS

6.1. Analysis 1 - Trends in non-native fish

Up to 1979, there were 51958 total unique occurrences of freshwater fish within the TCRB across both community and occurrence datasets. 1146 (2.2%) of these occurrences were of fish that were non-native (i.e., introduced) to the HUC6 that their respective sites were located (Fig. 2a). In each subsequent five-year period until 2005-2009, there was an increase in the number of introduced species occurrences (from 248 occurrences in 1980-1984 to 3401 occurrences in 2005-2009). However this was also accompanied by an increase in the number of native species occurrences (Fig. 1a). Both introduced and native species occurrences declined in each successive five-year period from 2005-2009 (3401 introduced occurrences; 49272 native occurrences) to 2020-2024 (1353 introduced occurrences; 16366 native occurrences). Importantly, the proportion of introduced occurrences (out of all occurrences) showed a strong increase through time ($P < 0.001$; $R^2 = 0.87$) (Fig. 2b)

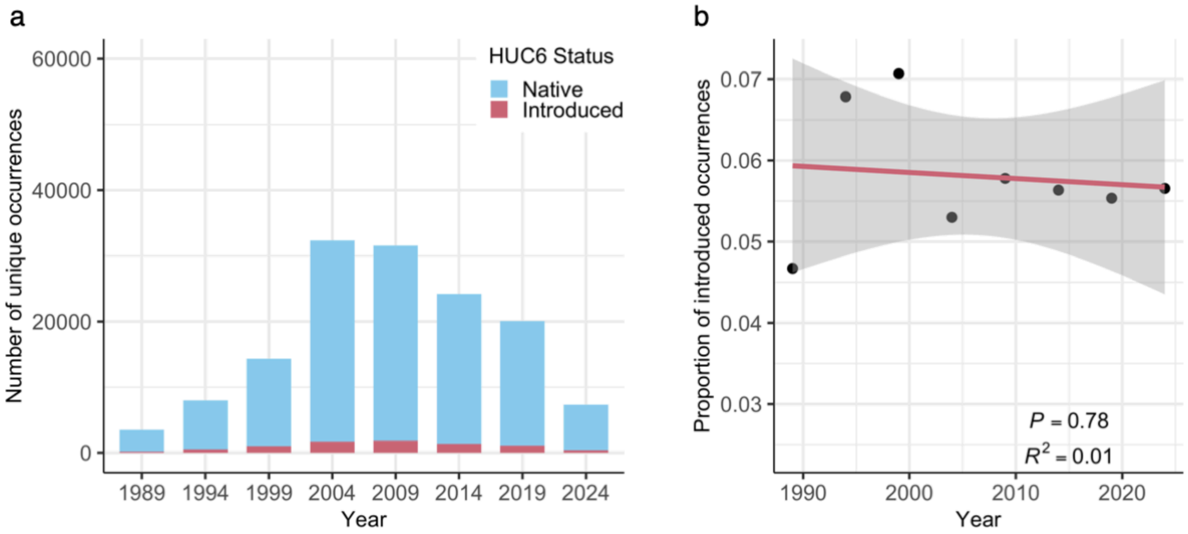
Fig. 2. Temporal trend in all unique occurrences of introduced fish species. (a) Number of unique occurrences of introduced fish species (red) compared with native fish species (blue) and (b) trend in the proportion of unique occurrences of introduced species up to the year 1979 (the first data point in each panel) and within subsequent five-year periods up to 2024. Introductions are defined at the HUC6 level.



We also analyzed the trend in unique occurrences from only the community dataset. The rationale is that while using data combined from both occurrence and community datasets can inform the trend in all known occurrences of introduced species, any increase in occurrences of any group of species might be due in part to biases in sampling and reporting. For example, we found a significant increase in the proportion of introduced occurrences through time (Fig. 1b). However, this increase may be a result of increased targeting of non-native species by sampling in localities they were already known to occur or were suspected to have spread to, and therefore an increased collection/reporting of introduced species

Our community dataset showed a similar increasing trend in both introduced and native species occurrences from 1989 and before to each subsequent five-year period to around 2000-2004 and 2005-2009, followed by a decreasing trend in both groups until 2020-2024 (Fig. 3a). However, in contrast with the trend for all occurrences, there was no temporal trend for the proportion of introduced occurrences (P = 0.78; Fig. 3b). In community samples recorded up to 1989, 5% of all occurrences were those of introduced species (163 out of 3491 total occurrences). In 1990-1994 and 1995-1999, the proportion of introduced occurrences peaked at 6.8% and 7.1% respectively, followed by proportions ranging from 5.3% to 5.8% in the next 5 five-year time periods. Despite the lack of an overall trend, the last 5 five-year time-periods had higher proportions of introduced occurrences than the first period (pre-1990).

Fig. 3. Temporal trend in unique occurrences of introduced fish species within the community dataset only. (a) Number of unique occurrences of introduced fish species (red) compared with native fish species (blue) and (b) trend in the proportion of unique occurrences of introduced species up to the year 1989 (the first data point in each panel) and within subsequent five-year periods up to 2024. Introductions are defined at the HUC6 level.



6.2. Analysis 2 - Recent range changes of non-native species

Using our community and occurrence datasets, we examined the change in the number of unique stream reaches occupied by 38 non-native species in the past 45 years. These 38 introduced species comprised 35 species that were not native to any HUC6 within the Tennessee or Cumberland basin and 3 species (*Alosa chrysochloris*, *Cyprinella venusta*, and *Esox niger*) that were native in some HUC6 units within the Tennessee or Cumberland basin but were introduced to others within the same basin. For the latter group, we only analyzed range changes within the introduced portion of their range.

For most introduced species, the number of unique stream reaches continued to increase from the pre-1980 period through the subsequent three 15-year time periods ending in 2024 (Fig. 4). In particular, the largest increase in the number of reaches occupied generally occurred between 1994 to 2009, which coincided with the period in which most fish records were taken (Figs. 1 and 2). However, our analysis revealed notable species that greatly expanded in their range (i.e., number of stream reaches occupied) in the most recent time period (2010-2024). The most extreme example is the silver carp, *Hypophthalmichthys molitrix*, which was not recorded pre-1980 and 1980-1994 and occurred in only 5 stream reaches in 1995-2009 before expanding its range of known occurrences to 175 stream reaches (Table 6). The bighead carp, *Hypophthalmichthys nobilis*, showed similar temporal dynamics. Grass carp, *Ctenopharyngodon idella*, had a longer history of invasion, but nevertheless, continued to show accelerating dynamics in the number of stream reaches occupied. Black carp, *Mylopharyngodon piceus*, was found in fewer stream reaches (12) in our dataset; however, occurrences in all of these reaches were observed in the most recent time period (2010-2024).

Fig. 4. The number of stream reaches (COMID) occupied by introduced fish species through time. The panels are arranged in descending sequence of the total number of reaches (COMIDs) occupied (see Table 3); *Lepomis auritis* has occupied the largest number of reaches (2070), followed by *Onchorhynchus* mykiss (1429) and *Salmo trutta* (891). The upper line with solid dot symbols represent the cumulative number of unique stream reaches occupied by a given species up to a given time period whereas the lower line with hollow dot symbols represent the number of unique stream reaches within each given time period.

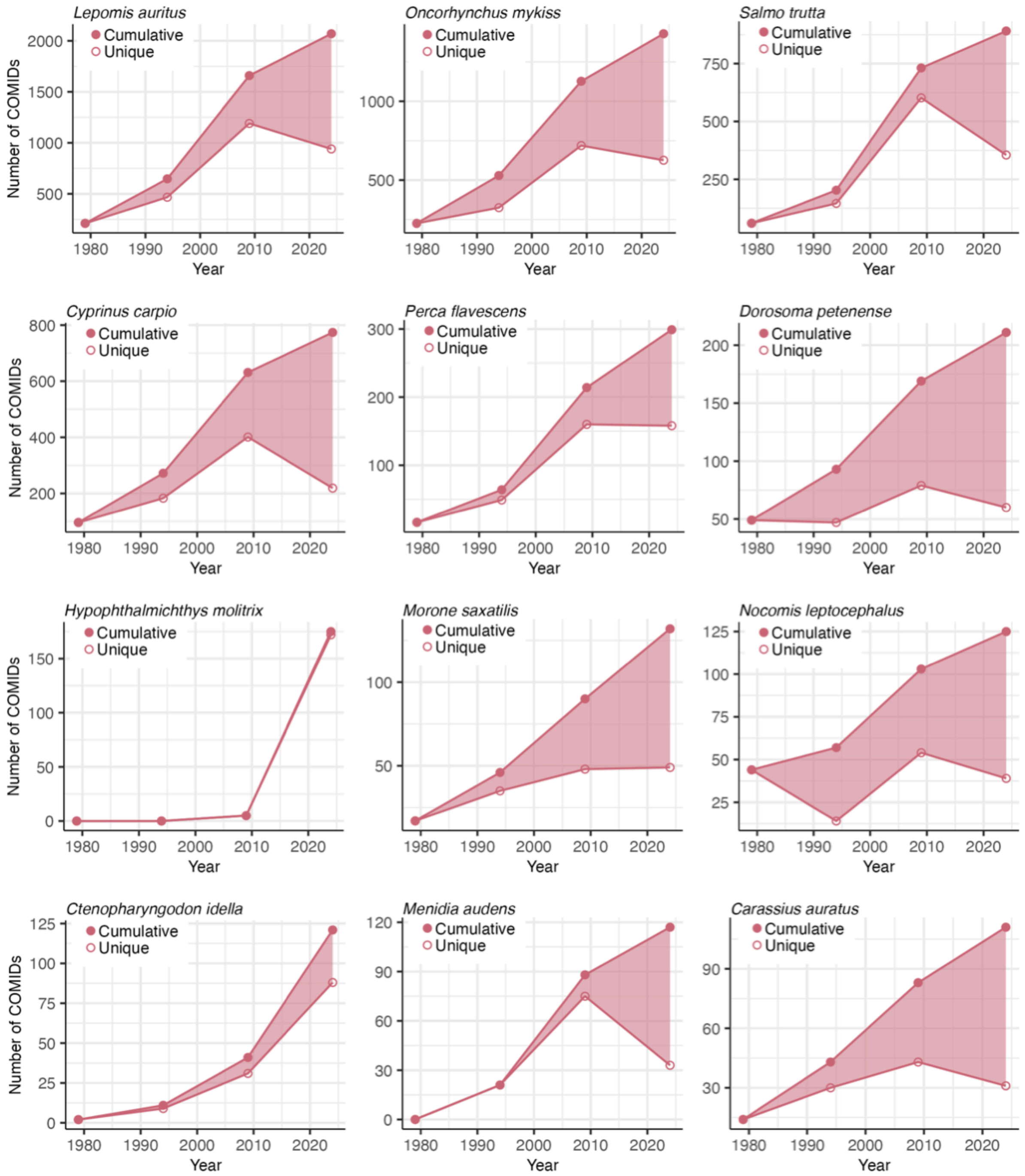


Fig. 4. continued

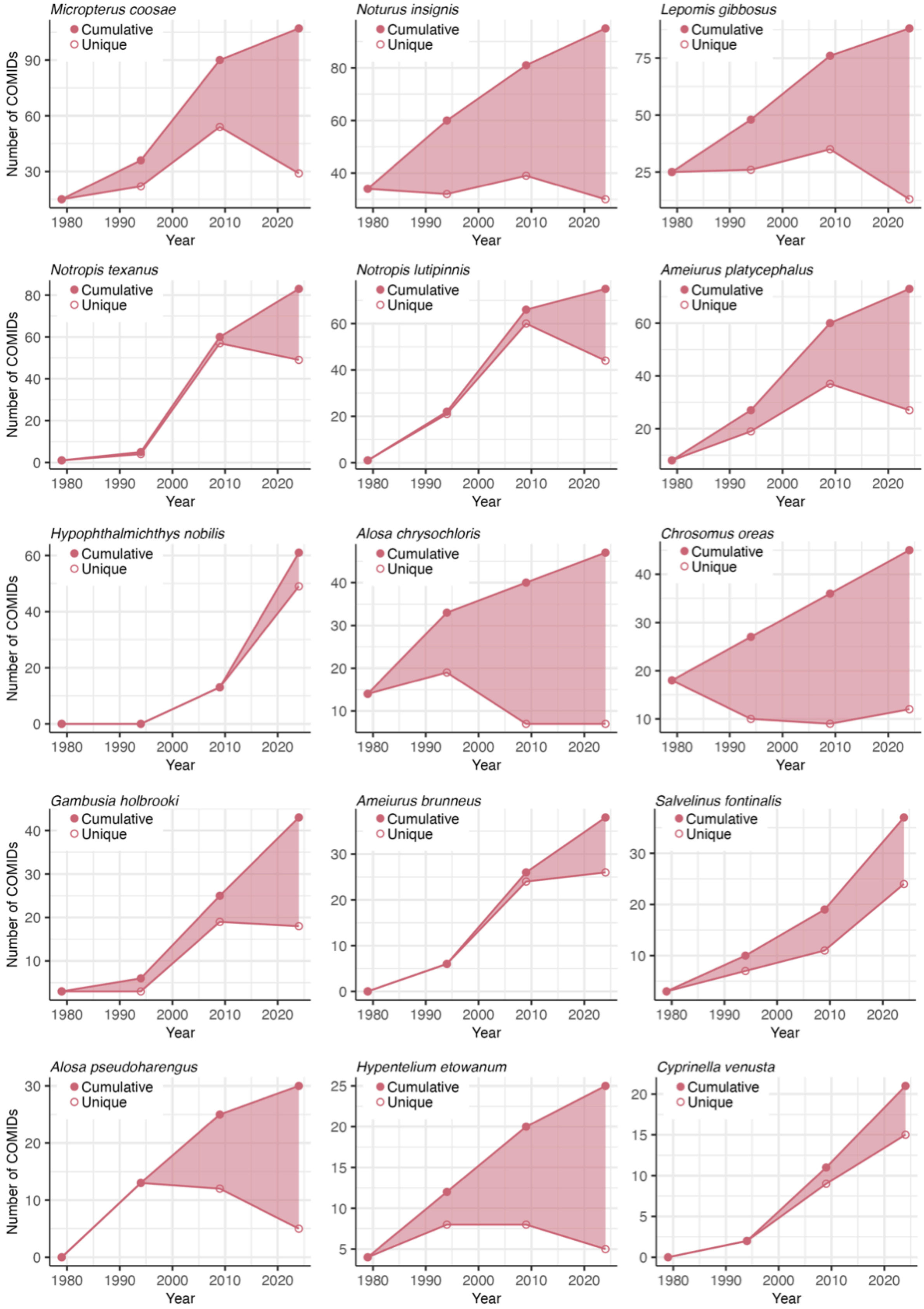
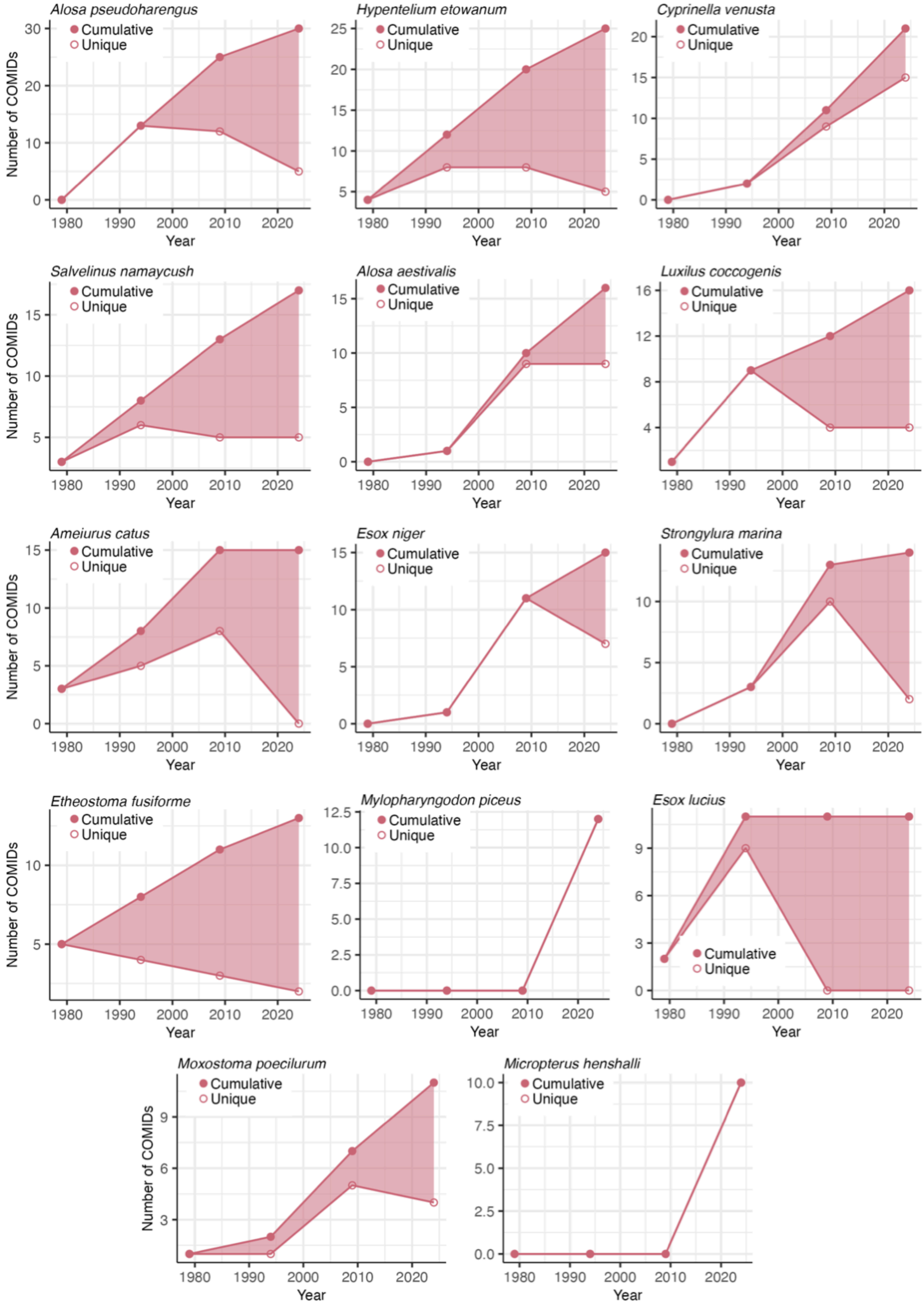


Fig. 4. Continued (last panel)



Alosa pseudoharengus

Hypentelium etowanum

Cyprinella venusta

Salvelinus namaycush

Alosa aestivalis

Luxilus coccogenis

Ameiurus catus

Esox niger

Strongylura marina

Etheostoma fusiforme

Mylopharyngodon piceus

Esox lucius

Moxostoma poecilurum

Micropterus henshalli

Table 6. The cumulative number of unique stream reaches (COMID) occupied by the four species of non-native Asian up to the years 1979, 1994, 2009, and 2024.

Species	**1979**	**1994**	**2009**	**2024**
Hypophthalmichthys molitrix	0	0	5	175
Ctenopharyngodon idella	2	11	41	121
Hypophthalmichthys nobilis	0	0	13	61
Mylopharyngodon piceus	0	0	0	12

Accelerating or continued increase in the number of occupied reaches was not limited to exotic species like the four carp species discussed above. Species native to the US, but not to the TCRB (HUC2 non-natives), e.g., striped bass (*Morone saxatilis*) and eastern mosquitofish (*Gambusia holbrooki*) showed sustained increase as well. Alabama bass, *Micropterus henshalli*, was not recorded in the TCRB before 2010-2024, but expanded its distribution to 10 stream reaches in our dataset by the end of 2024. Last, we also noted that brook trout (*Salvelinus fontinalis*; introduced in the Upper Cumberland HUC6 051301 and the Middle Tennessee-Elk HUC5 060300)—an example of a species native to some HUC6 in the TCRB but not others (HUC6 non-natives)—shows accelerating increase in its non-native range.

In terms of the increase in unique stream reaches in which a given species was recorded from 1995 to 2024, *L. auritis*, *O. mykiss*, and *S. trutta* showed the largest increase in reaches occupied (1423, 900, 688, respectively; Table 7). In terms of relative (i.e., percent) increase, *H. molitrix*, *H. nobilis*, *M. piceus*, *Micropterus henshalli*, each increased from 0 to 175, 61, 12, and 10 stream reaches occupied; among species with pre-1995 records, the cumulative number of streams occupied by *Notropis texanus*, *Alosa aestivalis*, *Esox niger*, *C. idella*, *Cyprinella venusta*, *Gambusia holbrooki* and *Ameirus brunneus* by 2024 increased by more than 5-fold in a 30-year timespan from 1995 to 2024 (Table 7).

We were interested to examine whether the increase in the number of occupied reaches within the last time period (2010-2024) was due to increased sampling in reaches that have not been sampled before, which may reflect new discoveries of existing introduced species, or whether it actually reflects new populations of non-native species. We therefore chose stream reaches that had known species occurrences (therefore, sampled) before 2010 and from 2010-2024, and calculated the change in cumulative number of reaches between these two time periods. Because we only analyzed sites that were sampled in both time periods, an increase in cumulative number of reaches between 2010 to 2024 for a given species would indicate that in this time period, the range of that species expanded to stream reaches that did not have known occurrences of that species previously (before 2010), reflecting actual spread.

34 of the 38 non-native species had new occurrences in previously sampled stream reaches in their introduced range in 2010-2024 (Table 8), representing an actual expansion of the known distributions of these species rather than new discoveries of existing populations. The number of reaches occupied by each non-native carp species increased > 2 fold; *S. fontinalis*

increased by 144%; *G. holbrooki* and *S. namaycush* increased by 75%; *M. saxatilis* increased by 56% (Table 8).

Table 7. Change in the cumulative number of unique stream reaches (COMID) occupied from 1994 to 2024. 1994 and 2024 represents the cumulative number of COMIDs in which a species was recorded by the end of 1994 and 2024 respectively. Increase is the number of new stream reaches gained between 1995-2024. FoldIncrease is the relative change in cumulative COMIDs between 1995-2024 [($nCOMID_{2024}$-$nCOMID_{1994}$)/$nCOMID_{1994}$]. The table is sorted in descending order from the greatest to the smallest FoldIncrease.

Species	**$nCOMID_{1994}$**	**$nCOMID_{2024}$**	**Increase**	**FoldIncrease**
Hypophthalmichthys molitrix	0	175	175	Inf
Hypophthalmichthys nobilis	0	61	61	Inf
Micropterus henshalli	0	10	10	Inf
Mylopharyngodon piceus	0	12	12	Inf
Notropis texanus	5	83	78	15.60
Alosa aestivalis	1	16	15	15.00
Esox niger	1	15	14	14.00
Ctenopharyngodon idella	11	121	110	10.00
Cyprinella venusta	2	21	19	9.50
Gambusia holbrooki	6	43	37	6.17
Ameiurus brunneus	6	38	32	5.33
Menidia audens	21	117	96	4.57
Moxostoma poecilurum	2	11	9	4.50
Perca flavescens	64	299	235	3.67
Strongylura marina	3	14	11	3.67
Salmo trutta	203	891	688	3.39
Salvelinus fontinalis	10	37	27	2.70
Notropis lutipinnis	22	75	53	2.41
Lepomis auritus	647	2070	1423	2.20
Micropterus coosae	36	107	71	1.97
Morone saxatilis	46	132	86	1.87
Cyprinus carpio	272	774	502	1.85
Ameiurus platycephalus	27	73	46	1.70
Oncorhynchus mykiss	529	1429	900	1.70
Carassius auratus	43	111	68	1.58
Alosa pseudoharengus	13	30	17	1.31

Dorosoma petenense	93	211	118	1.27
Nocomis leptocephalus	57	125	68	1.19
Salvelinus namaycush	8	17	9	1.13
Hypentelium etowanum	12	25	13	1.08
Ameiurus catus	8	15	7	0.88
Lepomis gibbosus	48	88	40	0.83
Luxilus coccogenis	9	16	7	0.78
Chrosomus oreas	27	45	18	0.67
Etheostoma fusiforme	8	13	5	0.63
Noturus insignis	60	95	35	0.58
Alosa chrysochloris	33	47	14	0.42
Esox lucius	11	11	0	0.00

Table 8. Change in the cumulative number of unique stream reaches (COMID) occupied from 2010 to 2024 among reaches that were sampled in both pre-2010 and 2010-2024. 2010 and 2024 represents the cumulative number of COMIDs in which a species was recorded by the end of 2010 and 2024 respectively. Increase is the number of new stream reaches gained between 2010-2024. FoldIncrease is the relative change in cumulative COMIDs between 2010-2024 [(nCOMID$_{2024}$-nCOMID$_{2010}$)/nCOMID$_{2010}$]. The table is sorted in descending order from the greatest to the smallest FoldIncrease.

Species	**nCOMID$_{1994}$**	**nCOMID$_{2024}$**	**Increase**	**FoldIncrease**
Mylopharyngodon piceus	0	8	8	Inf
Micropterus henshalli	0	2	2	Inf
Hypentelium etowanum	0	1	1	Inf
Hypophthalmichthys molitrix	1	99	98	98.00
Hypophthalmichthys nobilis	9	37	28	3.11
Ctenopharyngodon idella	18	61	43	2.39
Salvelinus fontinalis	9	22	13	1.44
Gambusia holbrooki	8	14	6	0.75
Salvelinus namaycush	4	7	3	0.75
Morone saxatilis	39	61	22	0.56
Ameiurus brunneus	11	17	6	0.55
Perca flavescens	90	139	49	0.54
Cyprinella venusta	7	10	3	0.43
Chrosomus oreas	12	17	5	0.42
Menidia audens	49	69	20	0.41

Carassius auratus	35	49	14	0.40
Oncorhynchus mykiss	579	760	181	0.31
Notropis texanus	32	42	10	0.31
Cyprinus carpio	293	383	90	0.31
Nocomis leptocephalus	43	56	13	0.30
Micropterus coosae	40	52	12	0.30
Lepomis auritus	826	1066	240	0.29
Salmo trutta	388	497	109	0.28
Dorosoma petenense	54	69	15	0.28
Lepomis gibbosus	33	42	9	0.27
Ameiurus platycephalus	30	38	8	0.27
Luxilus coccogenis	8	10	2	0.25
Notropis lutipinnis	37	45	8	0.22
Etheostoma fusiforme	5	6	1	0.20
Noturus insignis	43	51	8	0.19
Strongylura marina	7	8	1	0.14
Esox niger	8	9	1	0.13
Alosa pseudoharengus	14	15	1	0.07
Alosa chrysochloris	22	23	1	0.05
Alosa aestivalis	5	5	0	0.00
Ameiurus catus	5	5	0	0.00
Esox lucius	1	1	0	0.00
Moxostoma poecilurum	3	3	0	0.00

6.3. Analysis 3 - Recent abundance trends of non-native species

We analyzed the relative abundance trends in the non-native ranges (HUC6) of 30 species that were present in community sampling sites that had ≥ 4 unique sampling years over a time span of ≥ 10 unique years, and with the last year of sampling in 2010 or after (*n* = 410 sites; Fig. 5). There were a total of 1071 non-native populations (i.e., 1071 species-by-site combinations). The first sampling year in this dataset is in 1982 and the last sampling year is 2022. Across sites, the median number of unique years of sampling is 5 [interquartile range (IQR: 4-7)], the median first year of sampling is 2000 (IQR: 1999-2002), the median last year of sampling is 2019 (IQR: 2016-2022), and the median timespan of sampling is 18 years (IQR: 16-21).

There was broad heterogeneity in relative abundance trends across species (Fig. 6).There was very strong evidence for increasing trend in the non-native populations of yellowfin shiner

(*Notropis lutipinnis*; *P* = 0.00004), strong evidence for the increasing trend in blueback herring (Alosa aestivalis; *P* = 0.006) and silver carp (*Hypophthalmichthys molitrix*; *P* = 0.006), and weak evidence for the increasing trend in margined madtom (*Noturus insignis*; *P* = 0.09). Conversely, we identified very strong evidence for the decreasing trend in common carp (*Cyprinus carpio*; *P* = 0.000001) and moderate evidence for decreasing trends in rainbow trout (*Oncorhynchus mykiss*; *P* = 0.000001) and mountain redbelly dace (*Chrosomus oreas*; *P* = 0.000001). Of the other 23 species with no (inadequate) evidence of a trend due to the high uncertainty in the mean effect size (90% CI overlapping with zero), mean effect size was positive for 19 species while it was negative for the other 4 species.

Fig. 5. Sampling sites containing non-native species analyzed in the relative abundance trend random effects meta-regression analysis. The number of non-native species (# NN species) at a site is represented by the size of points (larger points = more non-native species).

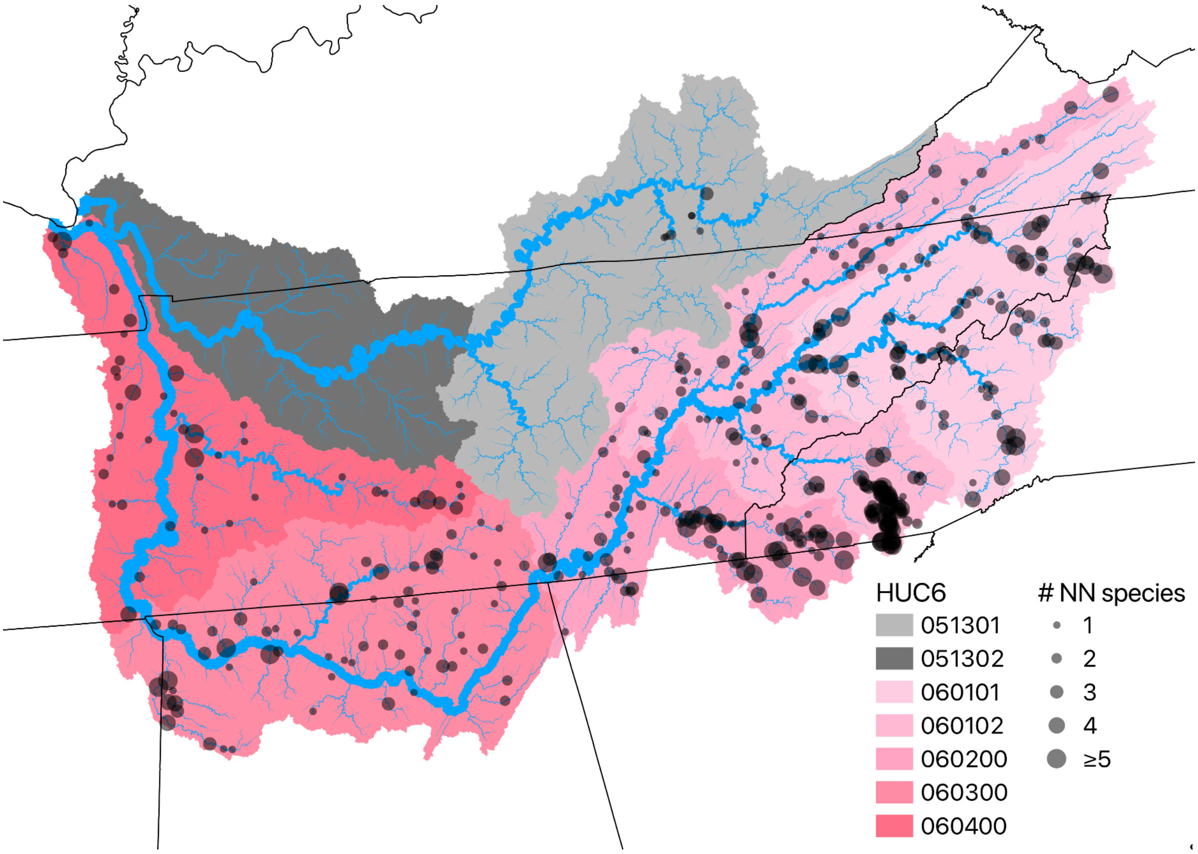
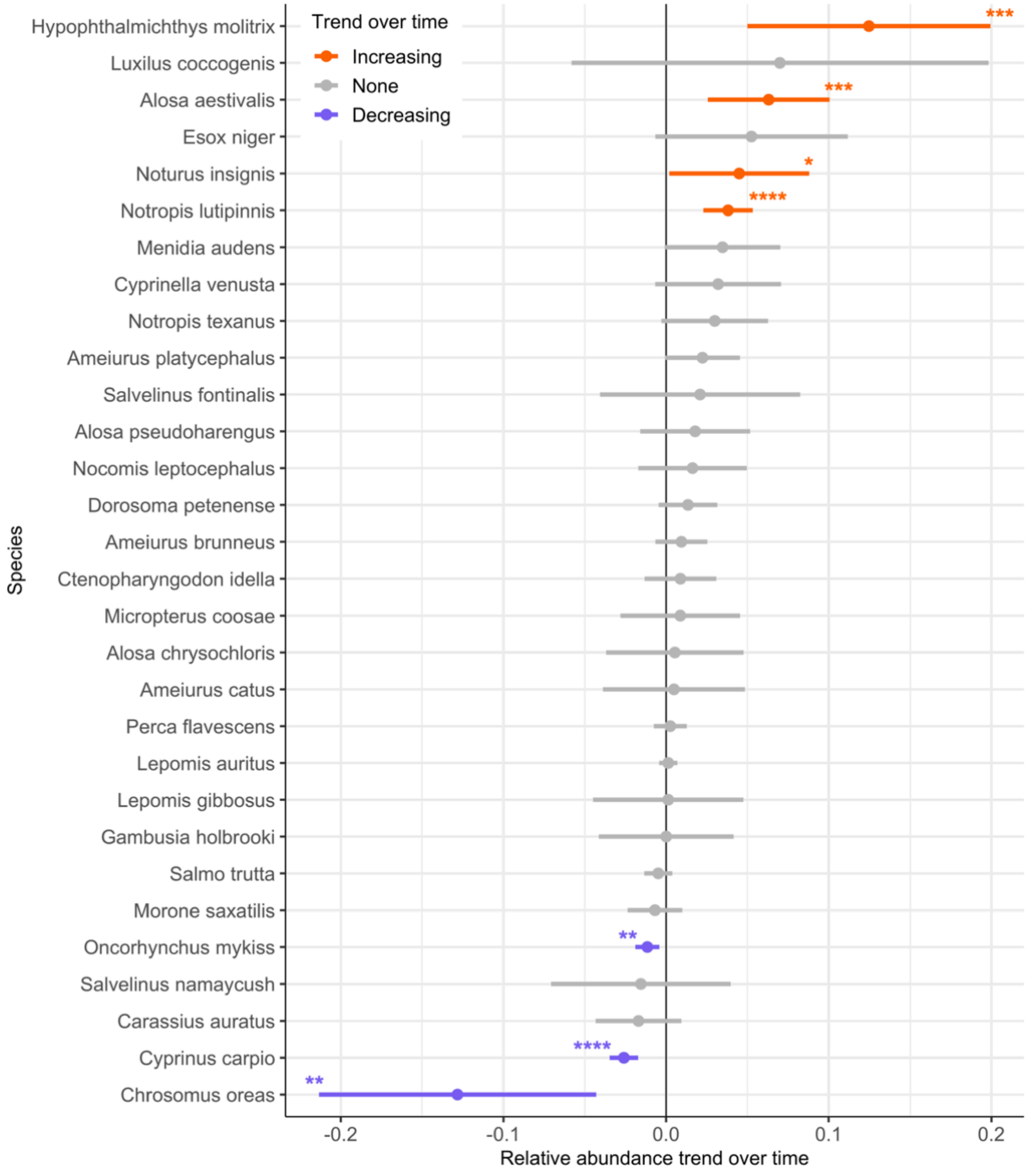


Fig. 6. Meta-analysis of the temporal trend in relative abundance of non-native species across sites. The effect size is the change in log[(abundance of focal non-native species + 1) / (abundance of all other species + 1)] per year at a given site. The mean effect size is denoted by the solid point while the error bars represent 90% confidence intervals. Orange points and bars represent species with evidence of an increasing relative abundance trend through time; blue dots and bars represent species with evidence of decreasing trend. Asterisks represent the strength of evidence: **** $P \leq 0.001$, very strong evidence; *** $0.001 < P \leq 0.01$, strong evidence; ** $0.01 < P \leq 0.05$, moderate evidence; * $0.05 < P \leq 0.1$, weak evidence.



6.4. Analysis 4 - Climate and land-use change effects on non-native fish abundance

We present the results for non-native fish classified at the HUC6 unit level. The top AICc meta-regression model that predicts the trend in the relative richness of non-native species included the fixed effect predictors: CVFlow_Trend, logFF, and Tree_Mean (Table 9). There is strong evidence that sites located in stream reaches with decreased variability in flow tend to have an increasing trend of relative richness of non-native species (*b* = -0.0036; *P* = 0.0047). There is also strong evidence that sites located in stream reaches draining an upstream watershed with lower tree cover tend to have an increasing trend of relative richness of non-native species (*b* = -0.0036; *P* = 0.0042). The mean effect of recreation fishing usage on relative non-native richness trends were positive; however, there is quite a bit of uncertainty around the mean. We therefore conclude that there is no or little (Muff et al. 2022) evidence of this effect. The rest of the predictor variables listed in Table 5 were not in the top model, therefore we conclude that there is no evidence that the relative non-native richness trend was associated with any of those excluded variables.

Table 9. Top AICc meta-regression model predicting the trend in the relative richness of non-native species. *b*: mean coefficient estimate for the predictor variable; *s.e.*: standard error of the mean coefficient; *P*: *P*-value of the effect.

Predictor variables	***b***	***s.e.***	***P***	**90% CI**
CVFlow_Trend	-0.0036	0.0013	0.0047	-0.0057, -0.0015
logFF	0.0014	0.0012	0.2273	-0.00052, 0.00339
Tree_Mean	-0.0030	0.0010	0.0042	-0.0047, -0.0013

In terms of the trend in the relative abundance of non-native species, the top AICc meta-regression model provided very strong evidence that sites on streams that drain upstream watersheds with lower tree cover tend to have an increasing trend of non-native relative abundance (*b* = -0.015; *P* = 0.00003) (Table 10). Unexpectedly, there was also moderate evidence that streams with upstream watersheds with an increasing tree cover trend tend to have increasing non-native relative abundance (*b* =0.0091; *P* = 0.012). There was also moderate evidence for the negative association between upstream watershed area and non-native relative abundance trend (*b* =-0.0085; *P* = 0.043). Finally, there was weak evidence for negative associations between trend in flow variability (CVFlow_Trend) and trend in flow magnitude (MeanFlow_Trend) versus the trend in non-native relative abundance ($0.05 < P \leq 0.1$). Streams with a decreasing trend in flow variability and a decreasing trend in flow magnitude were more likely to increase in non-native relative abundance across years. Interestingly, there was weak evidence for the positive association between the mean number of native species across sampling occasions and the trend in non-native relative abundance (*b* = 0.0070; *P* = 0.077).

Table 10. Top AICc meta-regression model predicting the trend in the relative abundance of non-native species. *b*: mean coefficient estimate for the predictor variable; *s.e.*: standard error of the mean coefficient; *P*: *P*-value of the effect.

Predictor variables	***b***	***s.e.***	***P***	**90% CI**
CVFlow_Mean	-0.0053	0.0037	0.1492	-0.0030, 0.0055
CVFlow_Trend	-0.0062	0.0033	0.0621	-0.0113, 0.0007
MeanFlow_Trend	-0.0057	0.0033	0.0835	-0.0112, -0.0003
Tree_Mean	-0.0151	0.0036	0.00003	-0.0211, -0.0091
Tree_Trend	0.0091	0.0036	0.0123	0.0031, 0.0151
logWSArea	-0.0085	0.0042	0.0428	-0.0155, -0.0016
logNativeSpp	0.0070	0.0040	0.0772	0.0005, 0.0135

Our results of the analysis on non-native richness and abundance trends based on a HUC2 non-native classification were largely consistent with the results above. For relative richness trends, there was very strong evidence that streams that are becoming less flow variable have more positive non-native relative richness trends; and moderate evidence that watersheds with greater tree cover tend to guard against the increase of non-native relative richness. For relative abundance trends, there was very strong evidence that watersheds with greater tree cover guard against an increase in non-native relative abundance and strong evidence that smaller streams (those that drain a smaller upstream watershed area) increased in non-native relative abundance. There was moderate evidence that streams that were becoming less variable and streams that saw an increase in tree cover over time experienced a greater increase in non-native relative abundance. Last there was weak evidence for the association between mean native species richness and the trend in non-native relative abundance.

6.5. Analysis 5 - Impact of non-native fish on native fish species

Among the 33 sites (n = 154 sampling occasions) within the HUC8s that silver carp, *H. molitrix*, occupied, there was very strong evidence for the negative association between number of native fish species recorded in each sampling occasion and the relative abundance of silver carp ($b = -0.152$; $P = 3.6 \times 10^{-9}$) (Table 11). This is after controlling for site-level variables and accounting for the repeated measures structure of the data. Of the site-level variables, watershed area was very strongly and positively related to the number of native fish species ($b = 0.343$; $P = 2.2 \times 10^{-9}$).

Table 11. Linear mixed-effects model (LMM) in predicting log(native richness) across sites within the HUC8s that silver carp occupied. *b*: mean coefficient estimate for the predictor variable; *s.e.*: standard error of the mean coefficient; *P*: *P*-value of the effect. LDOR (local degree of regulation metric) was not included because all sites had a zero value).

Predictor variables	***b***	***s.e.***	***P***
LRRAbun	-0.152	0.026	3.62×10^{-9}
MeanFlow_Mean	-0.024	0.050	0.631
CVFlow_Mean	0.027	0.024	0.268

MeanTemp_Mean	-0.028	0.041	0.488
Tree_Mean	0.033	0.024	0.171
logWSArea	0.343	0.057	2.22×10^{-9}

Yellowfin shiner, *N. lutipinnis*, was only present in one HUC8 unit 06010202 in our community sampling dataset. Various native *Notropis* spp. were also present in this HUC8. Among the 60 sites (n = 599 sampling occasions) within HUC8 06010202, yellowfin shiner relative abundance was not associated with the species richness of native *Notropis* spp after controlling for site-level variables and accounting for the repeated measures structure of the data (Table 12). Of the site-level variables, mean temperature from March to August, upstream watershed tree cover, and watershed area was positively related to the number of native *Notropis* species (Table 12).

Table 12. Linear mixed-effects model (LMM) in predicting log(native Notropis richness+1) across sites within the HUC8s that yellowfin shiner occupied. *b*: mean coefficient estimate for the predictor variable; *s.e.*: standard error of the mean coefficient; *P*: *P*-value of the effect.

Predictor variables	***b***	***s.e.***	***P***
LRRAbun	-0.024	0.026	0.357
MeanFlow_Mean	-0.028	0.050	0.569
CVFlow_Mean	-0.027	0.032	0.395
MeanTemp_Mean	0.163	0.048	0.0007
Tree_Mean	0.127	0.040	0.0016
LDOR	-0.039	0.029	0.180
logWSArea	0.146	0.067	0.028

We limited our analysis of the impact of mosquitofish (*Gambusia* spp.) on topminnow species (*Fundulus* spp.) to the HUC8s within which both groups of species were present. We therefore analyzed a total of 291 sites (n = 1686 sampling occasions). We found no evidence that the relative abundance of *Gambusia* spp. was related to the relative richness of *Fundulus* spp. *Fundulus* richness, instead, was driven by moderate effects of mean flow magnitude, and strong effects of mean March-August temperature and watershed tree cover (Table 13).

Table 13. Linear mixed-effects model (LMM) in predicting log(native *Fundulus* richness+1) across sites within the HUC8s that both *Gambusia* and *Fundulus* occupied. *b*: mean coefficient estimate for the predictor variable; *s.e.*: standard error of the mean coefficient; *P*: *P*-value of the effect. LDOR was not included because all sites had zero values.

Predictor variables	***b***	***s.e.***	***P***
LRRAbun	0.0063	0.009	0.479

MeanFlow_Mean	-0.0737	0.029	0.012
CVFlow_Mean	0.0051	0.020	0.797
MeanTemp_Mean	0.0994	0.028	0.00032
Tree_Mean	0.0626	0.017	0.00031
logWSArea	0.0426	0.027	0.109

6.6. Analysis 6 - Projecting future range shifts using species distribution modeling

The 37 modeled species performed moderately well on average across machine learning techniques (mean AUC_{cross} = 0.7, SD AUC_{cross} = 0.05; mean AUC_{kfold} = 0.87, SD AUC_{kfold} = 0.04). Each species had at least one model that had at least acceptable performance (AUC_{cross} >= 0.6) that was consequently used for predicting suitability across TCRB and for analysis of changes in habitat suitability Table 14). The SDM approach used here highlights variability in species-specific responses to future climate scenarios, as evidenced by changes in catchment suitability. Four species (10.1% of those modeled) did not exhibit changes in the total number of suitable reaches under RCP 4.5 or 8.5, balancing gains and losses in future suitable reaches. This group includes species with a predicted historically low number of suitable catchments, such as northern pike (*Esox lucius*), chain pickerel (*Esox niger*), grass carp (*Ctenopharyngodon idella*), and pumpkinseed (*Lepomis gibbosus*) (Fig. 7).

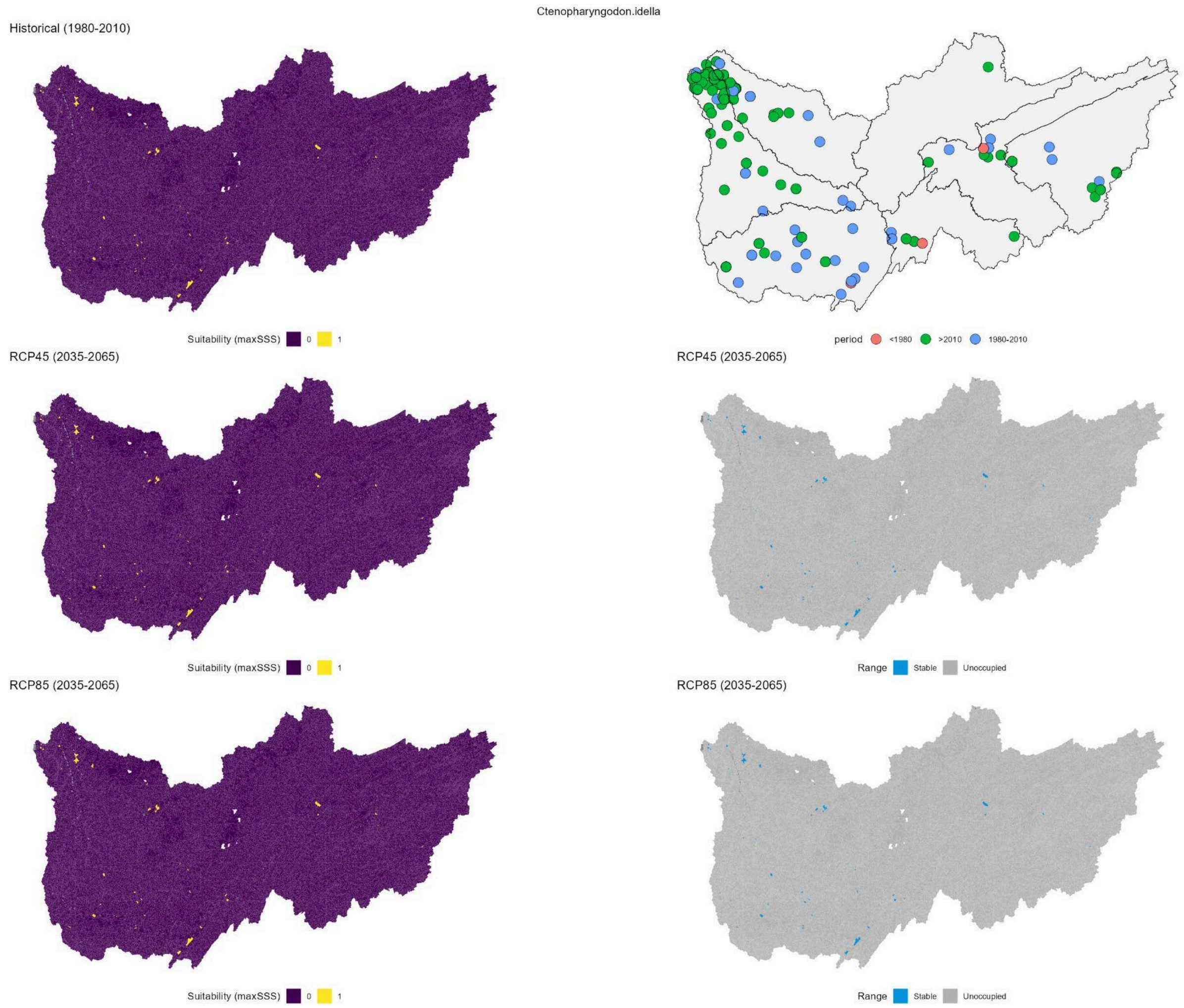
Table 14. Predicted overall changes in suitable reaches along the TCRB for the 37 non-native species modeled using SDMs. The change in the total number of suitable reaches for future scenarios (RCP 4.5 & RCP 8.5) is calculated by comparing it with the total number of catchments predicted to be suitable under historical conditions (1980-2010) and future conditions (2035-2065).

Species	**Historical**	**RCP 4.5**	**Change RCP 4.5 (%)**	**RCP 8.5**	**Change RCP 8.5 (%)**
Alosa aestivalis	4562	7791	70.78	6273	37.51
Alosa chrysochloris	4400	3571	-18.84	3462	-21.32
Alosa pseudoharengus	1183	3	-99.75	0	-100
Ameiurus brunneus	142	239	68.31	51	-64.08
Ameiurus catus	888	0	-100	0	-100

Ameiurus platycephalus	1171	0	-100	0	-100
Carassius auratus	1182	0	-100	0	-100
Chrosomus oreas	3597	10	-99.72	0	-100
Ctenopharyngodon idella	57	57	0	57	0
Cyprinella venusta	74	731	887.84	976	1218.92
Cyprinus carpio	2758	48	-98.26	35	-98.73
Dorosoma petenense	2373	6085	156.43	6976	193.97
Esox lucius	0	0	NA	0	NA
Esox niger	281	281	0	281	0
Etheostoma fusiforme	312	14347	4498.4	16353	5141.35
Gambusia holbrooki	33	74943	227000	75712	229330.3
Hypentelium etowanum	1947	1618	-16.9	1432	-26.45
Hypophthalmichthys molitrix	1036	770	-25.68	1401	35.23
Hypophthalmichthys nobilis	1029	6492	530.9	9672	839.94
Lepomis auritus	16568	62	-99.63	1	-99.99
Lepomis gibbosus	77	77	0	77	0
Luxilus coccogenis	12860	3891	-69.74	2220	-82.74
Menidia audens	2381	8111	240.66	9104	282.36
Micropterus coosae	2240	845	-62.28	556	-75.18

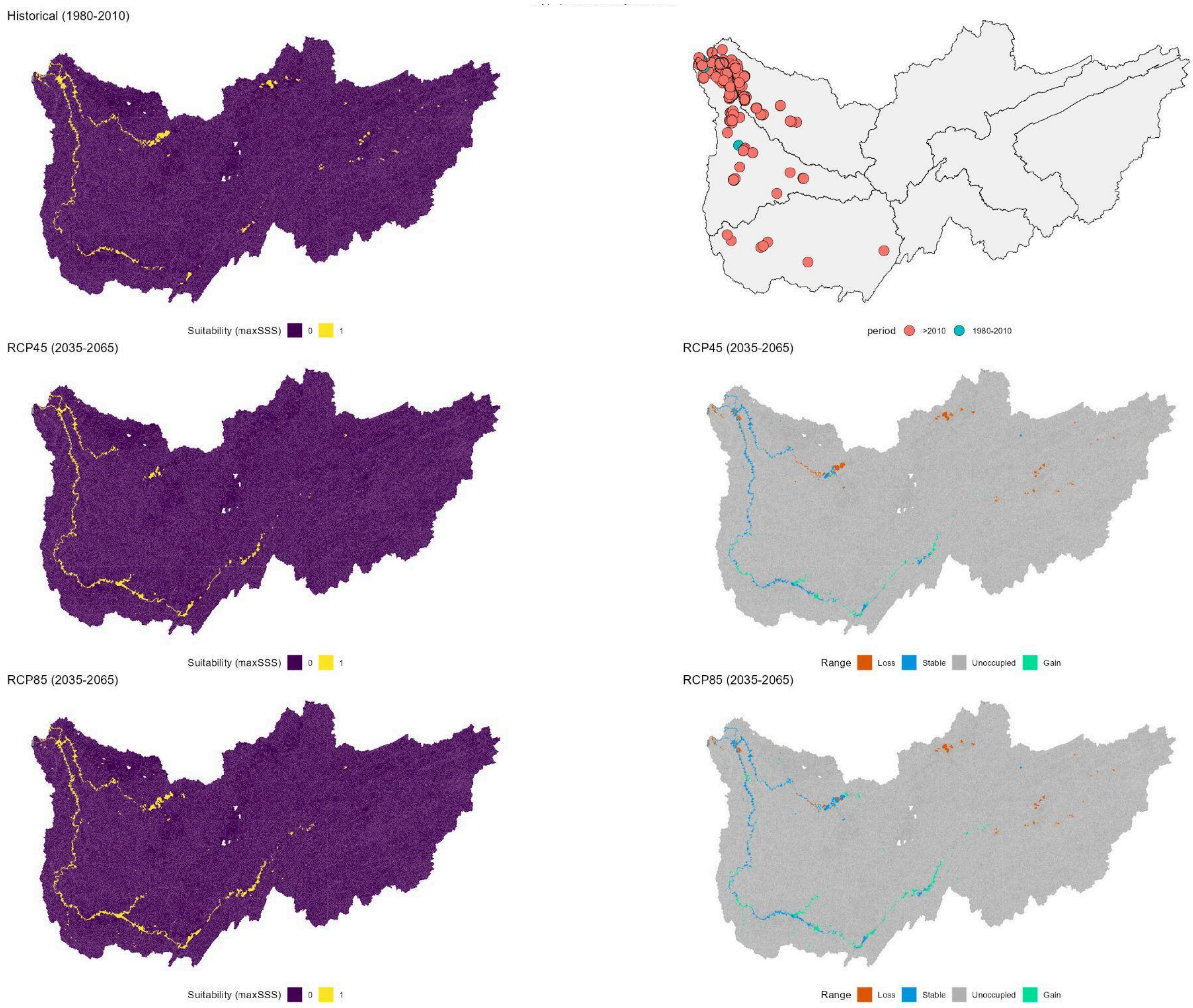
Micropterus henshalli	2687	5687	111.65	5511	105.1
Morone saxatilis	5235	1334	-74.52	1381	-73.62
Moxostoma poecilurum	443	125	-71.78	48	-89.16
Nocomis leptocephalus	914	0	-100	0	-100
Notropis lutipinnis	2863	0	-100	0	-100
Notropis texanus	372	1311	252.42	2314	522.04
Noturus insignis	3465	17	-99.51	0	-100
Oncorhynchus mykiss	15435	7735	-49.89	5438	-64.77
Perca flavescens	22	0	-100	0	-100
Salmo trutta	5752	908	-84.21	492	-91.45
Salvelinus fontinalis	1799	344	-80.88	98	-94.55
Salvelinus namaycush	99	256	158.59	433	337.37
Strongylura marina	414	1053	154.35	1853	347.58

Fig. 7. Suitable catchments of stream reaches (COMID) for the grass carp (*Ctenopharyngodon idella*) under different climatic scenarios in the TCRB. The left panel shows historical (1980-2010) and future suitable catchments (2035-2065; RCP 4.5 & RCP 8.5). The right panel indicates current known occurrences in the TCRB (top), and the range changes in suitable catchments for RCP 4.5 (middle) and RCP 8.5 (bottom).



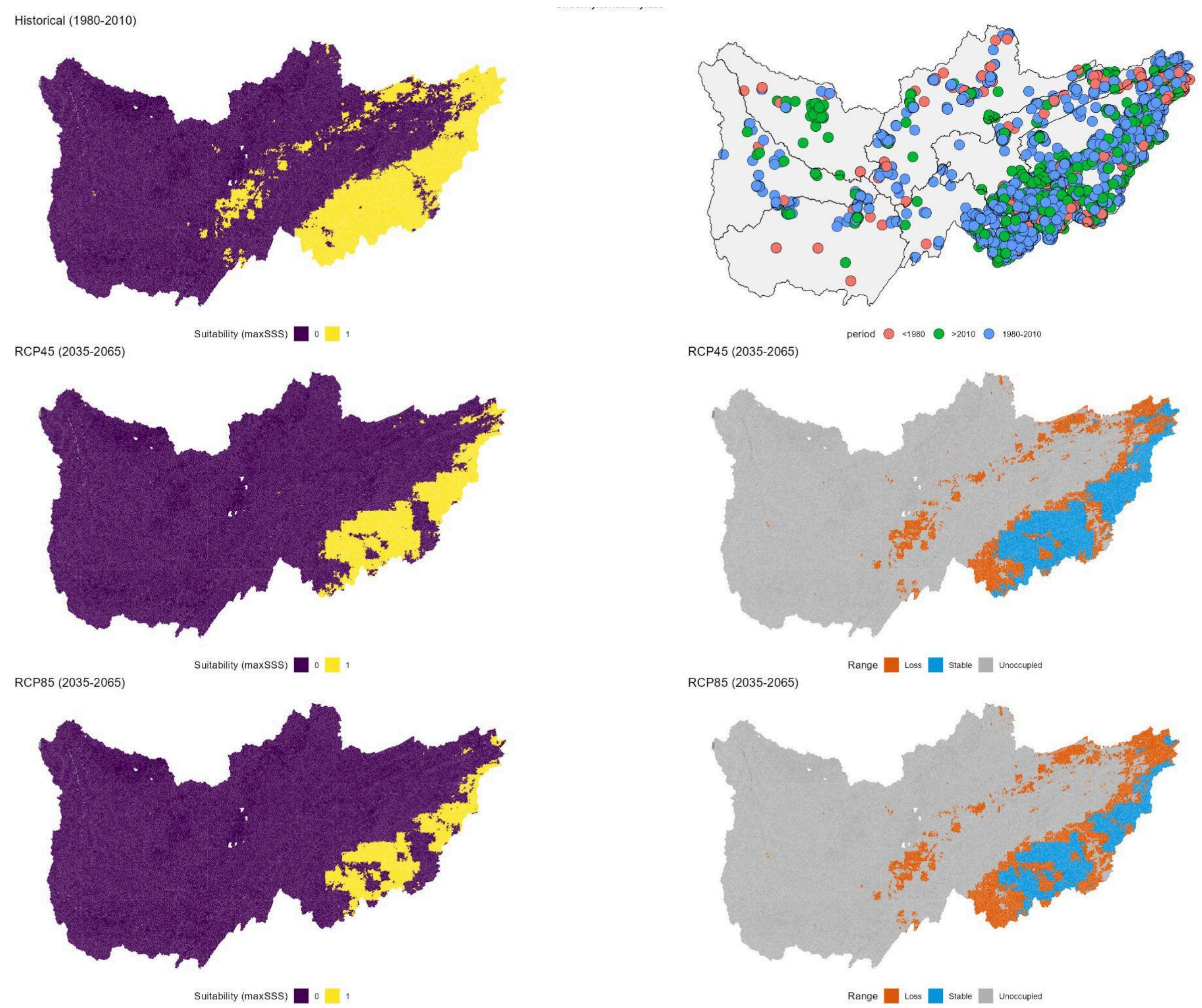
Twelve species (32.4% of those modeled) are predicted to increase their total number of suitable reaches in the future. The average increase of suitable reaches for most species is +648% (SD=1300) for RCP 4.5 and +823.76% (SD=1476) for RCP 8.5. These figures exclude the eastern mosquitofish (G. holbrooki), for which our SDMs projected a very large increase of suitable reaches from 33 to >70,000 (which was not surprising given that the questionable *G. affinis* is broadly distributed across the TCRB, and that G. holbrooki is thought to have a similar niche to *G. affinis*). Representative species in this increasing group include those that historically colonized downstream mainstems of the TCRB, such as the silver carp (*Hypophthalmichthys molitrix*) and Mississippi silverside (*Menidia audens*), where the expansion of suitable areas occurs further upstream in the mainstem and tributaries, with increases of up to 35.23% and 282.36% under RCP 8.5, respectively (Fig. 8).

Fig. 8. Suitable catchments of stream reaches (COMID) for the Silver carp (*Hypophthalmichthys molitrix*) under different climatic scenarios in the TCRB. The left panel shows historical (1980-2010) and future suitable catchments (2035-2065; RCP 4.5 & RCP 8.5). The right panel indicates current known occurrences in the TCRB (top), and the range changes in suitable catchments for RCP 4.5 (middle) and RCP 8.5 (bottom). This example illustrates the increase in the number of suitable catchments under future conditions.



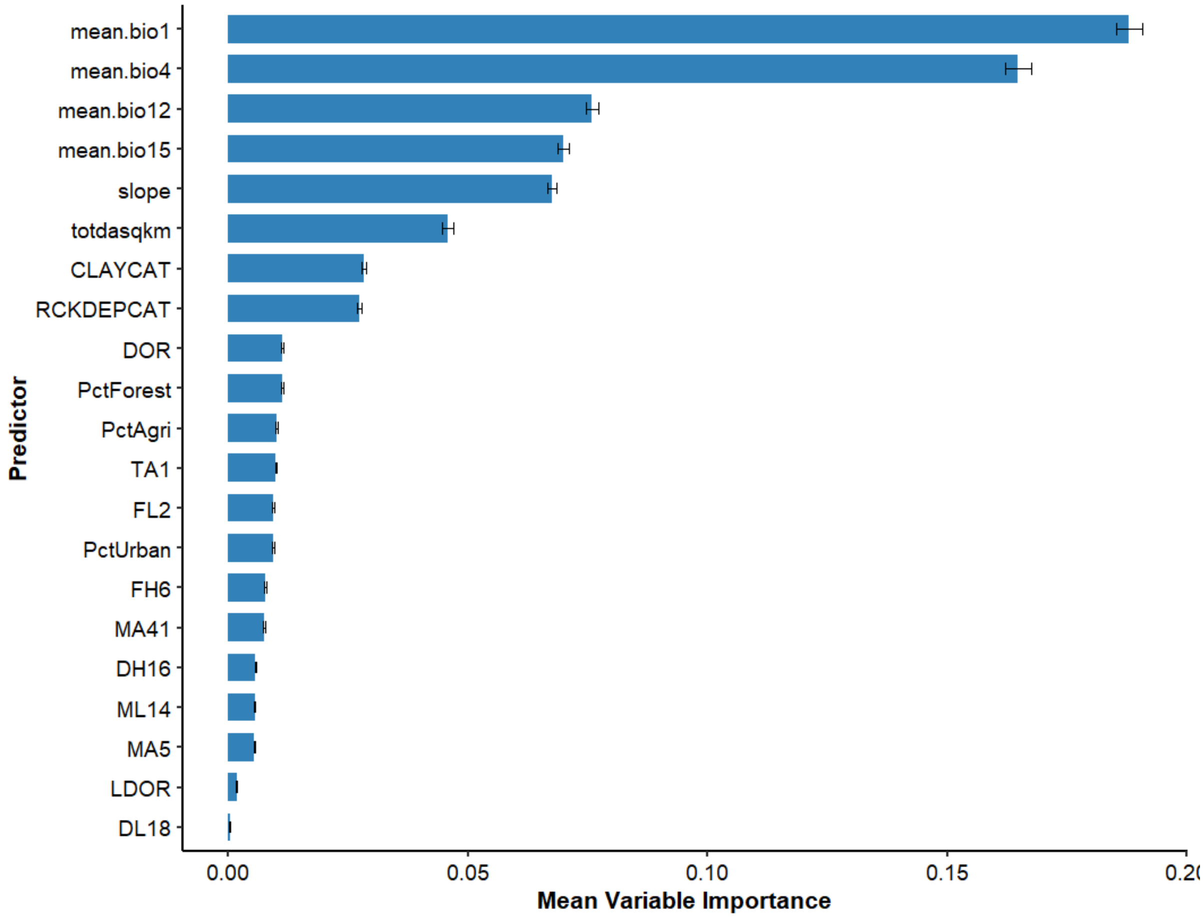
An unexpected majority of 22 species (59% of those modeled) are predicted to experience a decline in the total number of suitable reaches in the future. These species will lose an average of 78.6% (SD=28.6) for the RCP 4.5 and -84.8% (SD=23.6) These species mainly include coldwater species that currently occur in the upper elevation reaches of the TCRB, such as rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*). Their most significant losses in suitable areas occur in the downstream portions of their historically suitable ranges, with declines of up to 64% for rainbow trout and 91% of historical suitable reaches for brown trout under RCP 8.5 (Fig. 9).

Fig. 9. Suitable catchments for the rainbow trout (*Oncorhynchus mykiss*) under different climatic scenarios in the TCRB. TThe left panel shows historical (1980-2010) and future suitable catchments (2035-2065; RCP 4.5 & RCP 8.5). The right panel indicates current known occurrences in the TCRB (top), and the range changes in suitable catchments for RCP 4.5 (middle) and RCP 8.5 (bottom). This example shows decreased suitability of catchments in the Blue Ridge ecoregion of the upper Tennessee River basin in 2035-2065.



The SDM approach used here highlights variability in species-specific responses to future climate scenarios, as evidenced by changes in catchment suitability. However, it also provides insights into generalized patterns of range maintenance for non-native species. For instance, bioclimatic variables are the most important predictors of the observed ranges of non-native species, along with slope. Other hydrographic variables, as well as land cover and DOR, follow bioclimatic variables in importance. Interestingly, hydrological indices rank among the predictors with the lowest overall importance (Fig. 10).

Fig. 10. Predictor importance across the 37 non-native species with modeled SDMs. Blue bars indicate the mean variable importance, while error bars indicate standard deviations across species. Bioclimatic data consist of mean annual temperature (mean.bio1), temperature seasonality (mean.bio4), annual precipitation (mean.bio12), and precipitation seasonality (mean.bio15). Topographic information is represented by slope. Hydrological data including total upstream drainage area (totdasqkm), degree of river regulation (DOR) and local degree of regulation (LDOR). Soil data encompass soil clay content (CLAYCAT) and depth to bedrock (RCKDEPCAT). Land cover data include the percentage of forest cover (PctForest), agricultural land (PctAgri), and urban area (PctUrban). Hydrological indices include FL2, FH6, MA41, DH16, ML14, MA5, DL18 and TAL1 [see Morder et al. (2023) for definitions].



Lastly, we also produced an R script which can incorporate information on the dispersal ability of a given non-native species to identify stream reaches (COMID) that are both projected to be suitable for the species by our SDMS as well as reachable by the species based on its dispersal ability. Our analyses on silver carp (*H. molitrix*) found that based on its dispersal ability of 22.6 km/year (Pretchtel et al. 2018; based on the mean 3-year range), 99.9% of the stream reaches projected to be suitable in 2035-2065 under RCP 4.5 are also reachable by the species, whereas 99.6% of suitable reaches in 2035-2065 under RCP 8.5 are estimated to be reachable.

7. ANALYSIS AND FINDINGS

This project generated new information and data on the status of non-native fish species in the Tennessee and Cumberland River basins (TCRB). The results of our analyses and the data generated by this project fills a crucial knowledge gap in our understanding of the impacts of recent climate and land-cover change on fish invasions in the TCRB. They can also help inform the management of existing invasive species as well as to prevent novel invasions.

Based on our discussion and feedback with our federal and state agency partners, we focused our project on answering 5 main questions that would be useful to advance the science as well as practice of managing the spread and mitigating the impact of invasive fish in the TCRB. These questions are:

1. Which invasive fish species have expanded their distributions over the past 15-30 years?
2. Which species have increased their abundances in the past 15−30 years?
3. How does climate- and land-cover-associated changes in stream temperature and flow dynamics impact invasions?
4. How does invasive fish species impact native fish communities?
5. Which streams are likely to be colonized by which invasive species in the next 30 years?

For clarity we will summarize our analysis of our results in these sections below.

1. Which invasive fish species have expanded their distributions over the past 15-30 years?

We used fish records from both community and occurrence datasets to answer this question. The number of stream reaches occupied by non-native fish species has continued to increase over the past two 15-year periods (1995-2009 and 2010-2024). It is difficult to ascertain whether this expansion in distribution is a result of novel sampling in sites that have never been previously sampled, and thus represents merely a detection of a previously established population or if it represents a true expansion in distribution. To try to tease these two processes apart, we reanalyze the data from streams that have been sampled in the most recent 15-year period (2010-2014) as well as years prior to that (pre-2010). Because we only included sites that were sampled in both time periods in this new analysis, an increase in the cumulative number of stream reaches occupied from pre-2010 to 2010-2024 time periods will present actual range expansions of non-native fish species. In this analysis we found that in the period 2010-2024, 34 of our focal 38 non-native species (non-native fish that were recorded in at least 10 unique stream reaches) occupied new stream reaches. Among species with the largest percentage increase in the number of streams occupied from pre-2010 to 2010-2024 and with at least five new stream reaches were the four species of Asian carp (*Hypophthalmichthys molitrix*, *H. nobilis*, *Ctenopharyngodon idella*, and *Mylopharyngodon piceus*), brook trout (*Salvelinus fontinalis*) outside of its native range in the Blue Ridge ecoregion, eastern mosquitofish (*Gambusia holbrooki*), striped bass (*Morone saxatilis*).

2. Which species have increased their abundances in the past 15−30 years?

We analyzed the relative abundance trends of 30 non-native species present in sites sampled in our community dataset that have been sampled by the same monitoring agency or organization at around the same time of the year (March to August) that had ≥ 4 unique sampling years over a time span of ≥ 10 unique years, and with the last year of sampling in 2010 or after. The focal region for this analysis is the TCRB. There was wide heterogeneity in the relative abundance trends among the 30 species. There was very strong evidence for yellowfin shiner (*Notropis lutipinnis*) increasing in abundance relative to other species in the community. There was also strong evidence for silver carp (*H. molitrix*) and blueback herring (*Alosa aestivalis*) increasing in relative abundance, as well as weak evidence for the increase in margined madtom (*Noturus insignis*). In addition to these four species, 19 other species have a mean relative abundance trend that is positive, but the variation across sites was high enough that there is inadequate evidence to include that it represents an actual positive trend.

It was exciting that we found very strong evidence for a positive abundance trend in yellowfin shiners. In the co-production process, one of our agency partners remarked that based on their field experience, they think that yellowfin shiner has been increasing and they wanted the project team to confirm if it was true with the data, and if so, what would their impact be on native species. We can confirm with quantitative evidence based on data from many different sources and monitoring programs that our partner's anecdotal observations are correct. Conversely, our partners were also concerned about the abundance trend of redbreast sunfish (*Lepomis auritis*) and wanted us to investigate this. Our results showed that redbreast sunfish are increasing in their relative abundance on average across sites, but there was relatively large uncertainty around the mean trend, therefore the data did not show evidence that there was an overall positive trend.

3. *How does climate- and land-cover-associated changes in stream temperature and flow dynamics impact invasions?*

We focused our community-level analysis in the Tennessee River basin because the large majority of our community sampling sites were in this basin. Here, we identified climate and land-cover change associated drivers of the trends in the relative richness and abundance of non-native species across different stream reaches. We found that streams that were experiencing a decreasing trend in flow variability through time and streams that had lower tree cover in their upstream watershed were more likely to experience an increase in the richness of non-native species relative to native species through time. In terms of trends in the relative abundance of non-native fish, we detected a similar effect of a decreasing trend in flow variability and lower watershed-scale tree cover being associated with an increasing trend in non-native fish. We also found reduction in flow magnitudes to be associated with an increasing trend in non-native relative fish abundance. Interestingly, for the abundance trend, there was a moderate association between an increasing trend in tree cover with an increasing trend in non-native fish. This result is unexpected and warrants a closer look at the data to examine whether this effect was being driven by one or a few sites other factors may have resulted in an increase in non-native fish abundance.

4. How does invasive fish species impact native fish communities?

Based on our discussions with project partners from federal and state agencies, we focused on investigating the impacts of silver carp (*H. molitrix*), yellowfin shiner (*N. lutipinnis*), and mosquitofish (*Gambusia* spp.) on native fish. We found very strong evidence for the negative association between silver carp and native fish richness, after controlling for other environmental factors that may drive native richness. This suggests that silver carp likely has a negative impact on native fish species. Previous research has shown that silver carp competes with native species for food resources and reduces stream habitat quality for native fish species. Further research has to be performed to ascertain the directionality of this effect as it is also possible that streams with fewer native species may be more invasible and/or easily established by silver carp. In the case of yellowfin shiner and mosquitofish, we tested for the association between the relative abundance of these species with the richness of putative native competitors (yellowfin shiner: native *Notropis* spp.; mosquitofish: *Fundulus* spp.), We found no evidence for both of these associations, indicating that there is little impact of yellowfin shiner and mosquitofish on their respective native competitors in terms of reducing the species richness of their competing native species groups. However, richness declines are only one of the many possible impacts. The impact of these non-native species on the abundance of individual native competitors might be a more relevant measure of impact. However, we were not able to compare the relative abundance of these non-native species to the relative abundance of their native competitors due to statistical artefacts that would be introduced in such an analysis. Future work can focus on testing whether these non-native species were excluding certain native species from stream reaches using co-occurrence (Giam & Olden 2016) or similar analyses.

5. Which streams are likely to be colonized by which invasive species in the next 30 years?

To answer this question, we conducted species distribution modeling (SDM) of non-native species. We produced maps of habitat suitability under current (1980-2010) climatic and land-cover conditions as well as under future (2035-2065) projected climate and land-cover scenarios. Warmwater- and mainstem-adapted species that are currently invading in the lower reaches of the TCRB such as silver carp (*Hypophthalmichthys molitrix*; particularly under RCP 8.5) and bighead carp (*H. nobilis*, under RCP 4.5 and 8.5) are likely to increase their distribution up the mainstem and large tributaries of the Tennessee and Cumberland Rivers, upstream from their current distribution. By contrast, non-native species in the Upper Tennessee and French Broad-Holston regions that are currently occupying cooler streams in the Blue Ridge ecoregion, such as rainbow trout (*Onchorynchus mykiss*) and yellowfin shiner (*Notropis lutipinnis*) are projected to see declines in climatically suitable habitat as streams warm and/or precipitation patterns change.

8. CONCLUSIONS AND RECOMMENDATIONS

Our study indicates that climate change and land-cover change will continue to affect stream ecosystems both directly and through their effects on non-native fish. We found that non-native fish have continued to invade new stream reaches in the TCRB over the past 15-30 years, expanding their distributions. Many fish have also increased their abundances relative to other species in their community. State and federal agencies should focus on species that have expanded their ranges and increased their local abundances most rapidly in recent years. These species include the four species of Asian carp, which has increased both its range and local abundance greatly in the last 15 years. Other species that deserve attention include weed shiner (*Notropis texanus*), blueback herring (*Alosa aestivalis*), striped bass (*Morone saxatilis*), eastern mosquitofish (*Gambusia holbrooki*), and yellowfin shiner (*Notropis lutipinnis*).

Based on our analyses on community-level trends in relative non-native richness and abundance, it is clear that maintaining a high level of tree cover in the watershed guards against increases in richness and abundance of non-native relative to native species. To protect against invasive species and their potential impacts of native, policymakers should aim to maintain a high tree cover in the upstream watersheds. We also found that climate change may increase non-native species richness and abundance in streams that become less variable in their month-to-month flow. Therefore, managers and practitioners should focus on monitoring the populations of existing non-native species and preventing new introductions into streams with flow regimes that are becoming less variable and seasonal.

Based on our impact analysis, increasing silver carp abundance was linked to lower native fish species richness. We understand that silver carp, along with the three other Asian carp species, are current priorities for control by state and federal agencies within the TCRB. Our research provides evidence on the negative impact of silver carp, thus providing further support for controlling and reducing its population to the maximum extent possible.

Lastly, we provided current and future habitat suitability maps for non-native fish species. State and federal agencies can use the maps to determine which locations to sample and monitor for expanding populations of non-native fish based on current known records and suitable habitat areas that are proximal to current populations.

Our work is ongoing. This project provided our team with a unique opportunity to work with partners and collaborators from different agencies in learning about the most pressing knowledge gaps and questions related to invasive fish management. Importantly, this collaboration also resulted in the compilation of a large fish occurrence and community sampling database in the Southeast. This compiled dataset is currently being used in a NSF-funded project to incorporate bioenergetics in determining fish distributional limits. We have also used this dataset to organize a K-12 teacher professional development workshop at the Tennessee Science Teachers Association annual meeting in 2023.

We will continue to build this dataset to further understand how climate change, land-cover change, and fish invasions interact to affect native species. We have started preliminary work on understanding how these stressors interact (additively or synergistically, etc.) to affect range and abundance shifts of native species. Using the data we have compiled, we also have plans to build species distribution models to understand how distributions of fish species of greatest conservation concern (SGCN) would change under future climate and land-cover change.

9. MANAGEMENT APPLICATIONS AND PRODUCTS

We generated new scientific knowledge and data products that can be applied and used in the management of invasive fish species. Our list of non-native species and their rates of range expansion and abundance increase can inform monitoring and eradication efforts conducted by agencies. By identifying drivers of non-native richness across fish communities, agencies and researchers can design programs that target (reduce) the effect of important variables such as maintaining high tree cover in important watersheds. Last, we generated habitat suitability maps that agencies can use to monitor and target enforcement in locations where non-native fish are likely to spread to and occupy under future climates. All these represent new scientific knowledge that has improved our understanding of invasive fish species, and will inform current and future fish monitoring and management efforts in the Southeast.

The products we deliver in our project are as follows:

1. PRMS streamflow data for the Tennessee River basin disaggregated at the COMID grain
2. Modelled stream temperature data for the Tennessee and Cumberland River basins at the COMID grain
3. Fish modeling results (habitat suitability maps for present, 1980-2010, and the future, 2035-2065 under different climate scenarios)
4. Example R code for species distribution modelling include identifying suitable stream reaches (COMIDs) that are reachable by a species

10. OUTREACH AND COMMUNICATION

We organized a project kickoff meeting in July 2021 where we presented our preliminary research questions, and preliminary summaries of the data and some analyses to our project partners and collaborators from Tennessee Valley Authority, Alabama Department of Environmental Management, USGS, Conservation Fisheries Inc. and Mainspring Conservation Trust as well as academic institutions University of Alabama and University of Tennessee, Knoxville. From this meeting, we were able to further fine-tune our research questions to meet the informational needs of our stakeholders.

We also worked with project cooperator, Jacob LaFontaine, and his hydrological modelling team at the USGS South Atlantic Water Science center in a 3-hour online workshop where we worked towards adapting the PRMS model to produce streamflow outputs tailored toward our project needs.

Members of the project team also introduced the project and described the research questions and methodology, and data compilation efforts undertaken in this project in several seminars given at professional meetings as well as academic settings. These seminars include:

1. Giam X. 2021. Conserving fish biodiversity under climate change in the Tennessee River Basin. Tennessee River Basin Network (TRBN) Annual Meeting 2021. Online.

2. Giam X. 2021. Projecting freshwater fish responses to climate change. Departmental Seminar Series, Warnell School of Forestry and Natural Resources, University of Georgia.

We also presented preliminary results of our analyses in this project as well as related analyses using the data compiled in the course of this project at ecology conferences:

3. Giam X. et al. 2023. Multidecadal effects of climate and land-use change on stream fish communities in the southeastern US. Ecological Society of America Annual Meeting 2023, Portland, OR. Contributed talk.

4. Giam X., Herrera-R. G., Keck B. 2024. Distributional range shifts of fish species in the Tennessee and Cumberland river basins. Ecological Society of America Annual Meeting 2024, Long Beach, CA. Poster.

Data compiled in the course of this project has also been used for the educational outreach and professional development of K-12 teachers. We used fish community data compiled in this project along with other datasets (e.g., lake ice and temperature) to demonstrate example lesson plans to integrating data in teaching climate science, freshwater ecology and ecosystem ecology:

5. Aydeniz M., Giam X. 2023. Engaging Students in Scientific Inquiry and Computational Thinking through Local Fish Investigations: A Hands-On Inquiry Lesson. Tennessee Science Teachers Association Annual Meeting 2023. 3-hr in person workshop.

Last, we most recently presented results from a previous version of our analyses of this project in a SE CASC Science Seminar in October 2024.

6. Giam X. 2024. Climate and land-use change impacts on the distribution and abundance of non-native fish in the Tennessee and Cumberland River Basins with Xingli Giam. SE CASC Science Seminar Series. October 23 2024.

We are currently preparing multiple manuscripts for submission to several peer-reviewed journals.

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