

ABSTRACTS



**Southern Division American Fisheries Society
Annual Meeting**

April 6-9, 2021



**Hosted by the Virginia Chapter
of the American Fisheries Society**

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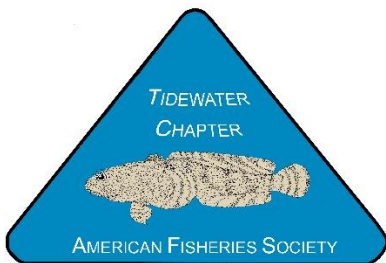


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**Southern Division American Fisheries Society
Virtual Annual Meeting – April 6-9, 2021**

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Thursday, April 8th

*Symposium: **Black Bass Biodiversity, Conservation, and Management***

Andrew Taylor, Brandon Peoples, and Martin Hamel, organizers

Taxonomy & Phylogenetics

[1.1] Phylogeny and time scale of diversification in the fossil-rich sunfishes and black basses (Teleostei: Percomorpha: Centrarchidae)

T. J. Near* and D. M. Kim

*Presenting

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Species of the North American freshwater fish lineage Centrarchidae are apex predators in their habitats and are among the world's most popular sport fishes. Centrarchids boast a rich fossil record that extends from the latest Eocene to the Pleistocene. To investigate the phylogeny and timing of diversification of Centrarchidae, we deploy both a dataset of DNA sequences of 16 nuclear genes sampled from nearly all of the recognized and undescribed species. We also utilize previously published morphological datasets to assess the phylogenetic placement of one of the oldest known centrarchid fossils, †*Plioplarchus whitei*. A multispecies coalescent species tree analysis provides insight on relationships that evaded resolution in earlier studies, such as the relationships of *Acantharchus pomotis*, the resolution of a clade consisting of species previously synonymized under the Spotted Bass, *Micropterus punctulatus*, and a clade of recently described species previously considered populations of the Redeye Bass, *Micropterus coosae*. This new molecular phylogeny and the inclusion of †*P. whitei* and other centrarchid fossils in the tip-dated fossilized birth-death analysis results in a new hypothesis of the timing of diversification in Centrarchidae that contextualizes the ages of centrarchid fossils to the timing of speciation among the extant species. In addition to providing new temporal perspectives on the diversification of freshwater fishes in North America, this study may close of the chapter of centrarchid phylogeny inferred using Sanger sequenced genes, as the use of genomic-scale datasets becomes mainstream in the phylogenetics of fishes.

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[1.2] Phylogenomics of Black Basses (*Micropterus*)

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The Black Basses (*Micropterus*) are iconic freshwater fishes of North America and well known for the biogeographical, ecological, and global recreational importance. The understanding of *Micropterus* phylogeny and species diversity have dramatically increased with the application of molecular data. However, there is a need for genetic markers with greater phylogenetic informativeness and a more comprehensive geographic sampling to better address the systematics and species delimitation hypotheses in *Micropterus*. The present study deploys a double-digest restriction-site associated DNA (ddRAD) genomic dataset based on complete sampling of all currently recognized and undescribed species of *Micropterus* with broad geographic sampling. Phylogenies from our analyses resolve the spotted basses (*M. punctulatus*, *M. henshallii*, *M. treculii*, and the undescribed Choctaw Bass) as a clade, support the delimitation of species in the Redeye Bass complex (*M. coosae*, *M. warriorensis*, *M.*

cahabae, *M. chattahoochae*, *M. tallapoosae*, and the undescribed Altamaha and Bartram's Basses), and delimit genetically and geographically distinct lineages within the Smallmouth Bass (*M. dolomieu*). Analysis of the ddRAD data reveal a high degree of genetic divergence between Largemouth Bass (*M. salmoides*) and Florida Bass (*M. floridanus*), which will serve as a basis for our species delimitation study of the two species. The phylogenomic analyses identify "intergrade" zones among species of *Micropterus* and patterns of ancient introgression among lineages, some of which are previously unrecognized.

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[1.3] Species delimitations among cryptic black basses

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Resource management relies upon accurate identification of management units. In particular, wildlife management agencies cannot effectively manage and recover cryptic undescribed species due to numerous political and regulatory hurdles. Recent publications have proposed recognition of several cryptic taxa of black basses, which include popular recreational species. Defining these species limits is of scientific importance and will facilitate responsible management of these species. Stream dwelling fishes all face challenges from landscape and hydrologic alteration; black bass populations are additionally challenged through hybridization and introgression with introduced congeners, which affects species persistence and hampers management success. Managers in SC and GA have launched a multi-collaborator effort to document the extent of non-admixed populations and provide formal descriptions of two cryptic taxa of black basses, the undescribed Altamaha Bass and Bartram's Bass. We have conducted a preliminary analysis of nuclear 3RAD and mitochondrial sequence data to explore the utility of these tools for both the delineation of these cryptic taxa and the discrimination of hybrid and pure individuals of 10 species of black basses. Encouragingly, individuals of Altamaha Bass and Bartram's Bass previously scored as pure using a nuclear gene, ITS2, and a mitochondrial gene, ND2, were recovered in reciprocally monophyletic clades based on 11,995 loci. We will use these and other tools to ensure the type series for both species descriptions are based on a minimum of 50 non-admixed individuals each, with a target date of Fall 2022 for completion. Collaborative studies documenting the occurrence and persistence of hybrids in black bass populations will continue through early 2023.

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Southeastern Endemics Conservation and Management

[1.4] Conservation and Taxonomic Status of the Endemic Savannah River Black Bass Species

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*Presenting

¹South Carolina Department of Natural Resources, ²Clemson University, ³US Forest Service

The Savannah River, an Atlantic Slope drainage of South Carolina and Georgia, harbors an endemic bass historically lumped together with the Redeye Bass (*Micropterus coosae*) of the Mobile Basin. Researchers in the 21st century have recognized the distinctiveness of this fish as a new species and proposed the name Bartram's Bass, although the formal description is still forthcoming. Rampant introgressive hybridization over the last few decades with non-native *Micropterus*, particularly Alabama Bass *M. henshalli* which were introduced without authorization to Savannah mainstem impoundments in the late 20th century, has placed the endemic bass in jeopardy of extinction even before formal description. The endemic bass genome has been largely extirpated in those impoundments where introgression has had the most time to operate. Conservation focus has now moved to fluvial habitats of the tributaries, where widespread hybridization has been documented but genetically pure endemic populations have nonetheless been identified. Management options are informed by programs elsewhere, such as the Guadalupe Bass of Texas, and include the immediate needs of documenting invasion rates by non-natives and their hybrids with telemetry, role of stream barriers in invasion dynamics, and habitat quality as an influence on hybridization. Aquaculture of endemic bass in the state hatchery system is currently being explored as a future option for various conservation stocking scenarios. These scenarios must be mindful of genetic structure in the system even while acknowledging that genetic diversity throughout the range is low. This conservation challenge is emerging as among the greatest in freshwaters of South Carolina.

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[1.5] Conservation ecology of Bartram's Bass: a Savannah River endemic

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Bartram's Bass *Micropterus sp. cf. cataractae* is an undescribed species of Shoal Bass endemic to the Savannah River basin of South Carolina and Georgia. Bartram's Bass is threatened by

habitat alteration and hybridization with invasive Alabama bass (*M. henshalli*) and other co-occurring congeners that may be better adapted for lentic or otherwise altered riverine habitats. Herein, I summarize several recent studies on the ecology and distribution of Bartram's Bass and outline work that is in its early stages. We have found widespread hybridization of Bartram's Bass in most Savannah River tributaries, and occurrence of hybrids is associated with increased land cover disturbance at both watershed and local scales. Important knowledge gaps include understanding how movement of black bass serves to transport nonnative alleles across the river network, as well as identifying fine-scale longitudinal patterns of hybridization and potential barriers that create upstream refugia. Characterizing genetic structure and estimating effective population size of Bartram's Bass across the basin will be critical for future reintroduction efforts.

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[1.6] Genetic diversity and population structure of Bartram's Redeye Bass in the tributaries of the Savannah Basin

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Bartram's Redeye Bass (*Micropterus sp. cf. M. cataractae*) is endemic to the Savannah Basin. It is one of three priority species included in National Fish and Wildlife Foundations' (NFWF) Native Black Bass Initiative (NBBI) and has been listed as a species of highest concern in South Carolina Department of Natural Resources' Comprehensive Wildlife Action Plan, due primarily to effects of habitat degradation and hybridization with the introduced Alabama Bass (*M. henshalli*). Previous research has documented that Bartram's Redeye Bass populations are diminishing due to introgression with Alabama Bass in Savannah River impoundments, but the extent of this process in tributary streams is unknown. There is also a need to evaluate the genetic diversity and population structure of Bartram's Redeye Bass throughout the basin. Samples were collected in 2017 and 2018 from adult individuals and eggs and larvae from nests throughout Savannah River tributaries in South Carolina and Georgia. We used a standard suite of microsatellite markers to compare the unknown field-collected samples against a reference set of 'pure' black bass species that potentially co-exist in the Savannah Basin. We identified hybrid and 'pure' Bartram's specimen and then analyzed the population structure and genetic diversity of the 'pure' specimens. Genetic results indicated that approximately 75% of the fish collected were predominantly Bartram's Redeye Bass and nearly 90% of these could be considered 'pure'. Data provided evidence of genetic population structure within the Savannah Basin and genetic diversity observed was low in the species of special concern across the study area.

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[1.7] Cryptic Phenotypes of Alabama Bass (*Micropterus henshalli*) X Redeye Bass (*M. coosae*) Complex Hybrids in the Mobile River Basin Require Genetic Identification

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Hybridization among the black basses (*Micropterus* sp.) occurs due to weak postzygotic reproductive barriers and anthropogenic factors such as habitat destruction and translocation. Introduction of previously allopatric species can result in hybrid swarms and in some cases, extirpation. The loss of a species to introgressive hybridization is a conservation concern in the more endemic black basses. This underscores the need for accurate identification of hybrids that can present with cryptic phenotypes. To that end, we collected 1,723 fish from the Mobile River Basin to compare phenotypic identification in the field based on morphology with genotype determined from SNP analysis using diagnostic SNP markers for the black basses. Only 4% of the fish collected were identified as hybrids in the field, while genotype analysis classified 22% of fish as hybrids. The majority of those misidentified in the field were hybrids between Alabama Bass (*M. henshalli*) and Redeye Bass (*M. coosae*) or Alabama Bass, Redeye Bass, and another black bass species. Results indicate that phenotypic identification among these cryptic hybrids is only 11% accurate. This underscores the need for genetic tools to augment classic field surveys in the black basses. The results also reveal abnormally high rates of natural hybridization (38%) in the Black Warrior River system relative to the Tallapoosa River system (8%) among sympatric Redeye Bass and Alabama Bass. Further studies are needed to better understand if this hybridization is a remnant of incomplete lineage sorting or due to anthropogenic factors that contribute to weak postzygotic isolating mechanisms in these co-evolved black basses.

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[1.8] Chattahoochee Bass distribution and genetics in the upper Chattahoochee River basin

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Chattahoochee Bass (*Micropterus chattahoocheae*) is a black bass species endemic to the Chattahoochee River basin in Georgia and Alabama. First described in 2013, Chattahoochee Bass were formerly considered conspecific with the Redeye Bass, *M. coosae*. The Georgia DNR Stream Survey Team began a project in 2018 primarily to document where extant populations of Chattahoochee Bass exist and to assess their genetic integrity. Our objectives were to delineate the current distribution of Chattahoochee Bass; assess the genetic integrity of Chattahoochee Bass populations; and to characterize the watershed and reach level habitat impacts on Chattahoochee Bass distribution. We sampled 154 sites from 2018 to 2020 and pure Chattahoochee Bass were found at 42 (27%) sites. Molecular surveys revealed that the genetic integrity of Chattahoochee Bass populations is threatened by introgression with the non-native Alabama Bass, *M. henshalli*. In addition, they are susceptible to disruptive anthropogenic land use and sedimentation. While genetically intact populations seem to persist in places above barriers in minimally disturbed watersheds, abundances have largely declined and have become fragmented, and some populations appear to have been extirpated.

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[1.9] Fishing for Understanding in the Black Bass Fishery of the Upper Chattahoochee River

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The Chattahoochee and Chestatee Rivers upstream of Lake Lanier (Georgia) are home to three known native black bass species (Largemouth Bass *Micropterus salmoides*, Shoal Bass *Micropterus cataractae*, and Chattahoochee Bass *Micropterus chattahoochee*) and one non-native congener: the Alabama Spotted Bass *Micropterus henshalli*. As fourth-order systems characterized by steep gradients, shoal complexes, and limited boat access, traditional fish survey methods are impractical and cost prohibitive. Accordingly, the status of these sympatric black bass populations is unknown, and a fundamental understanding of the fishery is necessary for sustainable management. Introgressive hybridization with Alabama Bass is also suspected to threaten endemic populations of Shoal and Chattahoochee Bass. Since 2017, a Southeastern Aquatic Resource Partnership-organized group of state, academic, and private sector stakeholders has collaborated to collect data supportive of objectives to assess 1) species compositions of black bass throughout the rivers, 2) habitat utilization, movement, and growth, and 3) genetic composition of black bass. Project volunteers angled for bass on guided kayak trips, collecting length, weight, photo, tagging, and genetic data. Preliminary results are

discussed including species-specific catch rates of angled bass, growth and movement summaries from recaptured fish, and genetic compositions. These efforts have been effective in promoting community awareness of Georgia's black bass diversity, improving watershed protection, and conserving native bass species. Several key watershed restoration projects have also been implemented concurrently, emphasizing the benefit of protecting and conserving habitat for native bass species. Future project direction is discussed, including opportunities for collaboration to maximize the utility of the data.

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[1.10] Conservation and Management of Endemic Black Basses in Georgia

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Black bass are the most sought-after sport fishes in the country and many state fisheries agencies, including Georgia, consider black bass management a high priority. Georgia's world-renowned black bass fisheries are made up of at least twelve species of black bass and produced the world record Largemouth bass, making Georgia the black bass capitol of the world. There has never been a more exciting time to study and promote black bass fisheries, but with this excitement brings the need to understand, conserve and manage these diverse and sometimes fragile natural resources. Georgia DNR has been actively increasing black bass management across the state over the past decade and has incorporated molecular tools to gather data on our black bass populations. Since 2013, GADNR has been working with Auburn's Aquatic Genetics and Genomics laboratory, using SNP's, to analyze genetic material from all our black bass populations to better understand the distribution of our endemic black bass genes. Our main objectives are to resolve the current levels of introgression among populations of Largemouth bass, Florida bass, and their interspecific hybrids from reservoirs, lakes and rivers across the state and to describe the hybridization impacts on our native black bass species. Our goal is to define and understand the genetic diversity partitioned across the state to not only preserve this abundant genetic diversity statewide across our diverse black bass fisheries but to also provide supportive conservation materials if populations are diminished. Determining these genetic population characteristics using the latest and most sensitive technology will inform future management efforts for black bass to protect the genetic heritage of our diverse populations and ensure the highest quality black bass fisheries for our anglers.

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[1.11] Suwannee Bass Movement and Life-History in the Withlacoochee River, Georgia

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The Suwannee bass is an obligate riverine species that is endemic to the SE United States and occupies one of the most limited ranges of all black basses, including three river systems in Georgia and six rivers in Florida. Most information regarding Suwannee bass is described from clear, spring-fed, calcareous streams in Florida, with scarce research available on Suwannee bass populations in Georgia. Prior research has indicated that presence of submerged aquatic vegetation could play a key role in nesting site selection, but Suwannee bass also occur in tannic, 'blackwater' streams with little-to-no aquatic vegetation. Basic information on life history of this fish - including movement and habitat selection - throughout the range is lacking and is essential for conservation measures. The objective of this study is to: (1) quantify sex-specific movement patterns, (2) identify home range, and (3) determine habitat associations. Telemetered Suwannee Bass (n=28) were tracked from February to September 2020. Distance, frequency, and trajectory of movement was highly variable among individuals. Male and female Suwannee bass were similar in mean total distance moved (7.28 vs. 6.71 km); however, the maximum distance moved was higher for males (28.5 vs. 18.9 km). Spawning occurred in late March in areas of flow refugia provided by limestone rock. Most Suwannee Bass locations were associated with large-diameter woody debris, deep pools downstream of shoals, and along steep limestone banks. These data are important for filling in critical knowledge gaps in Suwannee bass life history, providing fisheries managers necessary data for species conservation and management.

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Guadalupe Bass Conservation & Management

[1.12] Three Decades of Concerted Efforts to Conserve the State Fish of Texas (1991–2021): Reflecting on Strategies, Outcomes, Lessons Learned, and Future Directions

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Guadalupe Bass *Micropterus treculii* are endemic to rivers of central Texas, where populations are threatened with local extirpation from habitat degradation, flow alteration, and hybridization with non-native Smallmouth Bass *Micropterus dolomieu*. These threats are enormously challenging to address, but since 1991, cooperators have restored or conserved Guadalupe Bass populations in 14 rivers. This was accomplished through conservation stocking of over 2.4 million genetically pure Guadalupe Bass, implementation of nearly 50 habitat restoration or preservation projects, and watershed-scale management of riparian invasive plants in eight watersheds. Restoration of Guadalupe Bass populations is underway in another six rivers, while status assessments are planned for another eight rivers during 2021. Texas Parks and Wildlife Department currently manages 23 public river access areas that offer angling opportunities for Guadalupe Bass, which have served as focal points for engagement of fly fishing clubs, local conservation non-profits, and communities in efforts to restore and preserve Guadalupe Bass and their habitats. These efforts have been guided by a range-wide conservation plan established in 1991 and updated in 2017. With the initiative celebrating its 30th anniversary, this presentation will reflect on strategies, outcomes, lessons-learned, and the importance of staying focused on strategic goals while remaining flexible, adaptive, and responsive to opportunities.

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[1.13] Restoration, Conservation, and Recreation on Private Lands: Case Studies in Improving Habitat and Access in the Range of Guadalupe Bass, the State Fish of Texas

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In tandem with the effort to restore and conserve genetically pure populations of Guadalupe Bass (*Micropterus treculii*) within the central Texas rivers of their endemic home range, the Texas Parks and Wildlife Department (TPWD) has implemented a watershed approach to managing natural resources in an effort to improve habitat conditions at a landscape scale for the benefit of this keystone species. In addition to its role as a natural resource agency, TPWD is also tasked with providing the public with hunting, fishing, and outdoor recreation opportunities. To that end, TPWD has established 23 public river access areas that offer angling opportunities for Guadalupe Bass. With approximately 97% of Texas lands held in private

ownership, the Department has been compelled to develop novel approaches in achieving this dual mission of conservation and recreation. This presentation highlights restoration case studies and collaborative efforts to improve habitat and angler access on private lands in the home range of Guadalupe Bass, including: investment in private lands management; development of conservation demonstration and education sites; invasive species management; and recreation access leases.

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[1.14] Longitudinal Gradients in Guadalupe and Spotted Bass Allele Distributions: Implications for the Guadalupe Bass Restoration Initiative

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Guadalupe and Spotted Bass have historically been thought to be geographically separated in Texas with Guadalupe Bass primarily in streams of the Edwards Plateau and Spotted Bass in lower gradient streams of the Coastal Plains. The detection of Spotted Bass alleles among Guadalupe Bass populations has raised the question as to whether the alleles are naturally occurring or anthropogenically introduced and how their presence should be considered in regard to collection of Guadalupe Bass broodstock for restorative stocking purposes. We examined the occurrence and frequency of Guadalupe Bass and Spotted Bass alleles in individuals from sites in the Guadalupe and Medina River basins from headwater tributaries in the Edwards Plateau to their downstream reaches off of the plateau. Individuals with only Guadalupe Bass alleles were present only above Canyon Lake in the Guadalupe River and in the North Prong of the Medina River. Those with only Spotted Bass alleles were present only in the lower reaches of each river. Individuals with both Guadalupe and Spotted Bass alleles were present from the headwaters to the lower reaches. The observed gradients in allele frequencies and historical absence of stocking that could have translocated Spotted Bass alleles upstream or Guadalupe Bass alleles downstream suggests a natural hybridization zone or incomplete lineage sorting. As such, special consideration to ensure that hatchery propagated Guadalupe Bass reflect the populations into which they will be stocked should be taken.

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Smallmouth Bass Lineage Conservation & Management

[1.15] Population Genetic Investigations of Neosho Smallmouth Bass

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The Smallmouth Bass (*Micropterus dolomieu*) is one of the most popular freshwater sportfishes in North America. Previous studies of morphology and allozyme variation have supported the distinctiveness of two Smallmouth Bass forms from the Interior Highlands: the Neosho subspecies (*M. d. velox*) of the Ozark Mountains, and an undescribed genetic lineage from the Ouachita Mountains. Our recent work has focused on updating the conservation status of the Neosho subspecies using population genetic approaches. Using microsatellite markers, we quantified introgression with non-native strains of Smallmouth Bass. A case study of the Illinois River and Lake Tenkiller system uncovered geospatial patterns of invasion and introgression between Neosho and non-native strains of Smallmouth Bass that appeared to vary with both proximity to impoundment and stream size. We recently developed a low-density, 192 loci SNP panel to allow for further elucidation of hybridization and population-level genetic structuring in both the Neosho subspecies and Ouachita lineage. We anticipate that the application of this conservation-oriented SNP panel will allow for increased ecological understanding of the unique Smallmouth Bass forms endemic to the Interior Highlands.

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[1.16] Phylogeographic divergence, local adaptation, and admixture history in populations of Smallmouth Bass (*Micropterus dolomieu*) in the Central Interior Highlands

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Genomic technologies allow us to investigate eco-evolutionary patterns in rare, non-model taxa at risk of local extinction. The Neosho Smallmouth Bass (*Micropterus dolomieu velox*) is range-restricted to the Arkansas River Basin (ARB). To promote angling, the more widely-distributed Northern Smallmouth Bass (*M. d. dolomieu*) has been used to supplement natural populations. Hybridization in the Neosho range may erode genetic diversity and undermine locally adapted genome complexes, decreasing fitness. To understand these impacts, we must resolve patterns of Smallmouth diversity, incorporating markers under selection. We sought to assess: 1) population divergence, 2) local adaptation, and 3) the extent and origins of hybridization between Smallmouth populations. We genotyped 127,429 SNPs using ddRADseq methods for 64 Neosho Smallmouth, 24 Northern Smallmouth, and 4 Spotted Bass (outgroup) distributed across the CIH. After rigorous filtering, we retained 50,828 SNPs for analysis. We found strong differentiation between the subspecies, identifying four distinct lineages. In the Northern range, one lineage encompassed Skiatook Lake and two Missouri River tributaries; another encompassed the White River drainage. In the Neosho range, one contained the upper and middle ARB; another the lower ARB. We discovered 29 SNPs driving diversifying selection between the middle and lower ARB populations. We detected extensive admixture in all but two Neosho populations. Populations in the upper ARB were admixed with alleles from the White River drainage, while populations in the middle ARB were admixed with Northern hatchery strain alleles. Our results demonstrate that these subspecies are independent lineages that have hybridized, possibly due to human introductions.

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[1.17] Development of Growth Standards for Lotic Smallmouth Bass Populations

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Comparisons of local population growth rates to species averages are valuable for natural resource managers because growth rates provide information on abiotic and biotic factors that regulate growth in a fishery. Thus, species-specific standard growth models pave the way for fisheries scientists to efficiently compare, evaluate the status, and determine the potential of a fishery for meeting goals and objectives. Growth standards have been published for numerous freshwater fishes; however, growth standards commonly neglect differences among populations of the same species that reside in different habitat types (e.g., lotic vs lentic habitats). Furthermore, studies may utilize data sets based on age estimates from various aging structures, even those with known biases, when creating standard growth models. Therefore, the purpose of this study was to develop a standard growth model for lotic dwelling Smallmouth Bass based on over 11,000 Smallmouth Bass across their native range in the United States. Due to differences in parameters estimates between standard growth equations generated with otolith and scale-aged fish, separate standard growth models were created for each structure. In

addition, standard growth models were used to calculate growth standards including: predicted age-specific standard length, percentile distributions of age-specific standard length, and estimates of the time required to reach specified size classes for each aging structure. Thus, standard growth models and growth standards are presented for the two most common aging structures, which will facilitate comparisons of lotic Smallmouth Bass populations by providing equitable growth equations regardless of aging structure used by researchers.

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*Symposium: Systematics, Ecology, and Conservation of Southeastern U.S.
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Systematics

**[2.1] Anchored Hybrid Enrichment Resolves the Phylogeny of *Lacunicambarus*
(Decapoda: Cambaridae)**

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North American crayfish taxonomy is in the midst of a revolution, with an increasing number of researchers working to disentangle species complexes and describing species. This revolution is timely as crayfish are facing numerous threats including habitat loss, invasive species and climate change. Accurate taxonomy is an important precursor to conservation but has historically proven difficult to elucidate for some crayfish species. Next-generation sequencing (NGS) methods that can rapidly sequence large portions of genomes have opened up new possibilities for taxonomic research. One promising class of NGS methods is hybridization-based target enrichment, which uses short DNA sequences called probes to bind with specific targets within the genome that are then sequenced. Amongst NGS methods, anchored hybrid enrichment (AHE) is distinct because its probes target conserved regions of the genome flanked by less-conserved regions, making it informative across broad and shallow taxonomic scales. Here, we use AHE to resolve the phylogeny of the burrowing crayfish genus *Lacunicambarus*. Previous efforts using morphology and mitochondrial DNA have failed to answer important questions about species identities and relationships in this genus. Our AHE *Lacunicambarus* phylogeny is the most accurate and complete to date, and we use it to justify the resurrection of *L. nebrascensis* and recognition of *L. erythrodactylus*. Based on this phylogeny, we also present evidence for the existence of two undescribed species, *L. aff. thomai* and *L. aff. acanthura*. Our

study demonstrates the power of AHE to resolve challenging taxonomic problems, and we encourage others to adopt similar techniques to effectively increase understanding and conservation of North American crayfish biodiversity.

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[2.2] Unraveling the Distribution and Genetics of *Cambarus* Species C in Virginia: Preliminary Results and Focuses Moving Forward

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The provisional name, *Cambarus* sp. C, refers to a widespread crayfish species complex that inhabits Atlantic slope drainages from central South Carolina north to northeastern Pennsylvania. Despite known substantial morphological and genetic variation within this complex, it has historically remained a taxonomic Pandora's Box, with few exceptions, all in North Carolina (e.g., *C. hobbsorum*, *C. hystricosus*, *C. johni*). In 2019, Williams et al. redescribed *Cambarus acuminatus* - a name that had previously been used interchangeably with *C. sp. C* - and restricted its distribution to the Saluda River drainage in northwestern South Carolina, from which it was described by Walter Faxon in 1884. This action was significant as it effectively 'freed up' the entirety of the *C. sp. C* complex for taxonomic investigation, necessary for effective conservation and management. In the same year, Perkins et al. described *Cambarus franklini*, splitting it from the complex, from the upper Catawba River in western North Carolina. These two publications represented the beginning of a large, highly collaborative, integrative effort to split apart the *C. sp. C* complex throughout its range. To date, a total of 1,283 sites have been sampled range-wide. Here, we provide results from preliminary analyses of our multi-locus molecular genetic dataset comprising nearly 1,750 individuals. We focus on patterns observed across 280 sites sampled in Virginia; phylogenetic reconstruction indicates a minimum of six species in the State within the *C. sp. C* complex. Several of these putative taxa are highly restricted in distribution, and therefore are of immediate conservation concern.

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Ecology

[2.3] Translocation and Monitoring of the Meadow River Mudbug (*Cambarus pauleyi*), a Primary Burrowing Species of Crayfish: Efforts, Results, and Implications

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The Meadow River Mudbug (*Cambarus pauleyi*) is a primary burrowing crayfish endemic to West Virginia's Meadow River drainage in southeast West Virginia. It was described in 2015. Due to its narrow range, it was suggested that it be listed as Endangered under the American Fisheries Society criteria. Conservation listing efforts are a rigorous and time-consuming process, and although the species has not yet been awarded formal protection, a recent project crossing its habitat recognized its potential future listing status and agreed to treat the animal as if it was protected. The West Virginia Division of Natural Resources (WVDNR) issued a request for habitat surveys along the project's footprint through the crayfish's range and two large colonies were identified. WVDNR requested relocation of the colonies, and in 2018 and 2020 salvage and relocation efforts for the crayfish were conducted using baited lines, net jabbing, nocturnal searches, and burrow excavation. In 2018, 186 *C. pauleyi* including 75 juveniles, 48 females, 14 Form II males, and 49 Form I males were collected and relocated. In 2020, an additional 115 including 59 juveniles, 35 females, 14 Form II males, and 7 Form I males of *C. pauleyi* were collected for relocation. Herein we discuss the efforts utilized during the survey as well as our results. We also discuss the implications the success of the project has, not only for other species of imperiled burrowing crayfish, but for imperiled species of fauna that utilize the burrows of these crayfish as their primary habitat.

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[2.4] Exploring the linkages between physiology and thermal tolerance in crayfish and mussels

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Understanding the physiological mechanisms behind thermal tolerance is of increasing importance in the face of ongoing climate change. In aquatic ectotherms, metabolic rates initially increase with increasing temperature but eventually decline with further temperature increases – a change called metabolic depression. The implications of metabolic depression are not well understood. Metabolic depression has been interpreted as an indicator of severe thermal stress and imminent mortality, but, conversely, has also been described as a mechanism to reduce thermal stress and delay mortality. We used respirometry to compare relationships between temperature, metabolic depression, and mortality among crayfish (mobile) and unionid mussel (sessile) species. We hypothesized 1) metabolic depression indicates severe thermal stress and imminent mortality, and 2) mobile taxa exhibit a lower temperature of metabolic depression (MD_t) and increased thermal sensitivity relative to sessile taxa. Preliminary results generally support these hypotheses. Mussels had a higher MD_t and thermal tolerance relative to crayfish. Mortality of most taxa consistently occurred $\sim 4^\circ\text{C}$ past the onset of metabolic depression – suggesting MDT is generally an indicator of severe thermal stress and imminent death. An important exception was *L. straminea* which had a lower MD_t (38°C) than other mussel species, but were still alive at 50°C – suggesting that metabolic depression may be a coping mechanism for thermal stress in some taxa. Additional crayfish and mussel species are being tested to determine the generality of these patterns and how they may explain differences and similarities in thermal tolerance between two highly threatened freshwater taxa.

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[2.5] Advancements in the Understanding of *Cambarus monongalensis* Life History and Ecology

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Primary burrowing crayfish life history and ecology is largely due to their cryptic habitat preferences. *Cambarus monongalensis*, arguably the most charismatic of all crayfishes, lacks basic life history and habitat data because of the aforementioned obstacles. This study seeks to determine the life history and ecological preferences of populations of *C. monongalensis* in the northern panhandle of West Virginia and Southwestern Pennsylvania with notes on its natural history. Life history data will include form change and reproductive patterns including juvenile size of departure from the maternal burrow. Habitat data were collected using external burrow morphology and burrow locations within a standardized area collected seasonally to determine the preferences and cyclical patterns of crayfishes. With these data, the conservation of this species, and others that follow a similar life history strategy, is possible.

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[2.6] Trophic niches of terrestrial and lotic crayfishes

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Understanding the trophic ecology of a species gives insight into food webs, functional roles of a species, and the flow of energy throughout the system. This benefits our understanding of both species-specific natural histories, community-level interactions, as well as provides invaluable information for conservation planning. Crayfishes can have significant impacts on their environment and have been reported to be both ecosystem engineers and potential keystone species due to their ability to modify microbenthic and macrophyte community compositions. However, the trophic ecology of crayfishes is greatly lacking, with very little species-specific data. We are assessing the trophic position of sympatric crayfishes (four lotic, three lentic, and two terrestrial crayfish species) within the Sumter National Forest through stable isotope analyses. To obtain data on the trophic position, we collected tissue from the chela of crayfishes and representative food groups. Our work highlights the similarities and differences between the trophic positions of sympatric crayfishes and ecologically different crayfishes (e.g., terrestrial vs. lotic).

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[2.7] Modeling the Distribution of a Rare, Endemic Crayfish

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Understanding distribution and habitat needs is crucial to properly managing and conserving rare and endemic species. Species distribution models are a popular and effective tool for studying what drives the distribution of those species. In our study we are using the modeling program Maxent to analyze the distribution of the Boston Mountain Crayfish (*Cambarus causeyi*), an understudied burrowing crayfish species endemic to the Boston Mountains of

Arkansas. *Cambarus causeyi* is a Species of Greatest Conservation Need in Arkansas due to its historically small range, and it is purported in recent literature to have a shrinking range. This study's intent is to model what drives *C. causeyi*'s distribution. We used a combination of historical presence-only data and geographic data layers to create a model predicting its range. Habitat variables in our model include elevation, Euclidean distance from a stream, available soil water storage, precipitation, solar radiation, and slope. A jackknife analysis of model results showed that precipitation was the most important driver of *C. causeyi* distribution, with Euclidean distance from a stream as the second most important predictor variable. Precipitation contributed 61.9 percent of the training gain in the model, and Euclidean distance from a stream contributed 18.8 percent of the training gain. Additionally, we hope to present the results of our traditional, fine-scale sampling and field validation of this model. Our results will provide much needed information on the distribution and habitat preferences of *C. causeyi* to resource managers hoping to conserve this rare species.

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[2.8] Contrasting patterns of population structure in sympatric southeastern US crayfishes with distinct burrowing traits

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Trait-based frameworks have been used to predict spatial patterns of genetic diversity and population connectivity of aquatic organisms based on their dispersal ability. However, these frameworks often over-estimate population connectivity in crayfishes because they assume that all crayfishes are equally capable of terrestrial dispersal. Prior work in Alabama has suggested strong population structure based on stream network connectivity in stream-dwelling species as well as irrespective of network connectivity in primary burrowing species. We hypothesize that crayfish burrowing propensity reflects the dispersal model of population structure, as burrowing behavior is likely linked to habitat requirements and overland movements. We compared the population structure of *Cambarus striatus*, a secondary burrower and inhabitant of intermittent streams, with *Faxonius validus*, a tertiary burrower and inhabitant of permanent streams, in north Alabama. Mitochondrial cytochrome oxidase subunit I (COI) sequences were utilized to examine the population structure of these two sympatric species from 15 locations within 4 drainages in the Bankhead National Forest. For *F. validus*, most genetic variation (82%) was among drainages, and genetic and stream distances were correlated, suggesting reduced overland movement reflecting a stream hierarchy dispersal model. In contrast, most genetic variation (54.5%) for *C. striatus* was within populations, and genetic and stream/geographic distances were not correlated, suggesting considerable stream and overland movement reflecting a panmictic dispersal model. Population structure was stronger in *F. validus* than *C. striatus*, which is consistent with observations and hypotheses. These results suggest that burrowing traits of crayfishes may be a useful heuristic for predicting population structure.

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Management

[2.9] Preliminary comparison of three trap types for recreational crayfish harvest

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Arkansas has diverse fauna of 61 crayfish taxa, several of which are of conservation concern. The potential of overexploitation as a threat is unknown, yet harvest is mostly unregulated in Arkansas and most of the southeastern United States. The Arkansas Game & Fish Commission was asked to consider allowing larger mesh size and throat diameter traps, such as those sold by Crayster LLC, to harvest crayfish for human consumption. It was suggested that the larger openings in these traps may reduce capture of smaller species that are of conservation concern. The Commission's philosophy is that utilization of this resource should be allowed if it does not adversely affect the resource. To begin to understand the effectiveness of these traps, six each of the Snake River Trap and Lake Trap sold by Crayster LLC were fished along with six standard minnow traps currently allowed under Commission code. Traps were baited with cut fish and fished overnight in stream and reservoir habitats between October 2019 and October 2020. Crayfish sex, carapace length, and body length were recorded by trap. Three hundred five trap-nights of sampling captured 533 crayfish of 10 species. Lake Traps captured a mean of 0.55 crayfish/night, which was significantly lower than the other trap types. The Snake River Traps were most effective, capturing 3.74 crayfish/night, which was marginally more than the 2.37 captured in minnow traps. Lake Traps captured significantly larger crayfish than minnow traps, while Snake River Traps were intermediate between the other trap types and not significantly different than either. Overall, the size distribution of catches in minnow traps appears to be skewed toward smaller crayfish relative to the other trap types. Preliminary results do not suggest that the currently prohibited traps tested pose a greater threat than minnow traps to crayfish of high conservation concern.

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[2.10] Utilizing Opportunistic Data in Order to Aid in the Recovery of a Federally Threatened Crayfish Species: A Case Study on the First Biological Opinion Issued for the Big Sandy Crayfish (*Cambarus callainus*)

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Recovery of a listed species is a lofty goal, often involving years if not decades of research, effort, and capital before results can be shown. Some species never reach this goal, while others, with just the right touches of effort, diligence, and luck can take drastic steps toward recovery in a short period of time. During the past few years, we believe we have helped with a project in the latter category. In May of 2016, the Big Sandy crayfish (BSC) (*Cambarus callainus*) was listed under the Endangered Species Act. In 2018, potential replacement of a railroad bridge over the Tug Fork in Matewan, WV triggered surveys for the crayfish. Two individuals were collected, leading to formal consultation with USFWS and an eventual issuance of a Biological Opinion (BO) for the project. Following guidelines set forth in the BO, 50 BSC were collected and held in offsite housing until completion of the project. Additionally, 42 other BSC were fitted with elastomer tags. In addition to Elastomer tags, twelve of the largest BSC were fitted with radio telemetry tags and monitored until completion of the project. In addition to this, some female crayfish that were held in offsite housing dropped viable eggs, resulting in the rearing of juvenile BSC. In this talk we outline the efforts and results of our study, opportunistic data gathered, how this will aid in recovery of the Big Sandy crayfish, and how these lessons can be applied to other species in the future.

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[2.11] Integrating functional biology with the conservation and management of crayfish

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Integrative organismal research aims to test ecological and evolutionary hypotheses by studying animals in both natural and laboratory environments. By contrast, conservation and management officials strive to protect and conserve biodiversity by conducting species surveys, natural history studies, and imperilment analyses. Until recently, these two research fields have worked independently, despite a substantial opportunity for these disparate fields to collaborate and provide information relevant to the goals of both groups. Here, I describe how research on crayfish conservation can benefit from collaborations with functional biologists, and vice-versa. Specifically, because crayfish are model organisms for studies of behavioral ecology and they are a group of conservation concern, uniting these two fields can produce outcomes that are beneficial to both fields. Within this talk, I will describe several case studies in non-crayfish animals that have successfully bridged this intersection. Ultimately, I propose lucrative areas of

future studies that can provide a system of mutually informative research to both organismal biologists and conservations/management officials.

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[2.12] Evaluation of Carbon Dioxide and Water Flow as Control Techniques for Invasive Red Swamp Crayfish

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Procambarus clarkii (Red Swamp Crayfish) is a highly invasive crayfish species that is difficult to eradicate from infested waterbodies. We conducted pond and laboratory experiments to test whether carbon dioxide (CO₂) mixed into water could be used to push crayfish towards refuges with low CO₂ for collection and removal. Pond experiments consisted of a control (no CO₂) and a treatment (CO₂ diffusion) pond. Refuge areas in one end of each pond were demarcated with a ¼"-mesh netting arranged as a large funnel trap. We found no significant difference in the number of crayfish trapped in pond refuges between control and treatment ponds; however, in treatment ponds we observed crayfish moving towards pond edges and climbing aquatic plants to the water surface. We subsequently conducted raceway experiments to test whether CO₂ and/or water inflow could push/pull crayfish towards a refuge or induce crayfish to climb to the water surface. All raceways contained climbing substrate as well as a refuge area. In experiment I, control and treatment raceways received freshwater inflows; the treatment raceway received CO₂. In experiment II, no raceways received CO₂; the treatment raceway received a freshwater inflow. Carbon dioxide caused significantly more crayfish to climb to the surface, but not to move towards the refuge. Freshwater inflows caused significantly more crayfish to move into the refuge but not to climb to the surface. These findings are currently being used to modify crayfish traps using a combination of freshwater inflow and CO₂ to enhance crayfish removal.

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[2.13] The conservation concern of *Faxonius sp.* (Decapoda: Cambaridae) from the Lower Tennessee-Beech subbasin

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Crayfishes are among the most imperiled freshwater taxa in North America with nearly 50% of US species at some level of conservation concern. The southern Appalachian Mountains of the US are home to the greatest crayfish diversity in the world. In the fall of 2019, the West Liberty University Crayfish Conservation Laboratory conducted surveys in the Lower Tennessee-Beech subbasin. Within the Indian Creek watershed, *Faxonius sp.* was found which we believe is an evolutionary significant unit (ESU) based on genetic and morphological data. Data is currently being compiled and analyzed with NatureServe's Conservation Rank Calculator to determine the degree of imperilment of *Faxonius sp.* However, field surveys of the Indian Creek watershed and surrounding areas indicate *Faxonius sp.* has a narrow range and is not locally abundant. We believe that habitat competition from *F. compressus* and *F. durrelli* has forced *Faxonius sp.* into headwaters of the Indian Creek watershed. Thus, *Faxonius sp.* is a punitive taxon in need of immediate conservation attention.

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[2.14] Evaluation of fish predators as biological control agents to manage and eradicate Red Swamp Crayfish (*Procambarus clarkii*) invasions in Michigan

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Red Swamp Crayfish (*Procambarus clarkii*) is a prolific invasive species and is demonstrated to have negative effects on recipient ecosystems around the world. In 2017 established populations of *P. clarkii* were located in several waterbodies throughout southeast Michigan. In an effort to manage the infestation, we evaluated several options for control and eradication, including supporting elevated abundances of predatory fishes for biological control. However, there is little evidence to evaluate what fish species are effective biological control agents for red swamp crayfish. This study used a laboratory consumption experiment to evaluate the potential efficacy of three candidate fish species (Bluegill, Green Sunfish hybrids, and Largemouth Bass) for biological control. Results indicate that Largemouth Bass are capable of consuming red swamp crayfish up to 50mm carapace length, whereas Bluegill and Green Sunfish consumed fewer than 50% of crayfish at sizes above 20mm. For crayfish < 14mm, Bluegill have higher consumption than Green Sunfish, but significantly lower probabilities of consumption at sizes > 14mm. In comparison, Largemouth Bass possessed a higher consumption rate than either Bluegill or Green Sunfish across all crayfish sizes. Results suggest that while Largemouth Bass are more likely to be most effective on larger individuals, Bluegill and Green Sunfish within the size we tested (155-200mm) are likely to be effective agents on juveniles. These findings will help inform management and stocking decisions of waters invaded by *P. clarkii*.

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[2.15] Evaluation of five trap designs for eradication of invasive red swamp crayfish (*Procambarus clarkii* Girard) in Southern Michigan: Catch per unit effort, size, and sex biases

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Trapping and removing crayfish is one of the most common forms of eradication and control, however gear-specific biases can limit effectiveness of such methods. We evaluated 5 trapping gears (Gee Minnow Traps [GMT], Pyramidal traps [PYR], Artificial Refuge Traps (ART), Additional Partition Refuge Traps (APART), and juvenile traps (JUV)) for their effectiveness in a red swamp crayfish eradication effort among 4 Southern Michigan ponds between May and October 2019. Our objectives were 1) determine which gear(s) produce the highest CPUE; 2) assess gears for body size and sex biases; 3) ascertain the degree of seasonal dependency of gear-specific catches and biases. We found that GMT and PYR traps substantially outperformed the ART, APART, and JUV traps with respect to CPUE. However, catches of artificial refuge traps trended positively over the season. Size biases were moderately prevalent, with GMT and PYR traps consistently recording individuals > 30mm carapace length. ART and APART traps caught relatively smaller individuals, but trended seasonally towards larger individuals. We observed no sex biases or between form 1 and form 2 males) among the gears. Our findings supported recommendations of employing multiple gears for crayfish eradication, but novelly demonstrated that trapping protocols can remove more crayfish (on a CPUE basis) and capture body size trends by accounting for timing. Further, the lack of sex bias suggests strategies to target females may not be effective with these commonly used gears. Our results have been incorporated into Michigan's adaptive response strategy to inform future red swamp crayfish eradication attempts.

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[3.1] Rapid genetic sex identification in Brook Trout

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Identifying sex in salmonoids using non-lethal methods is valuable for controlled breeding in aquaculture and understanding behavior and dispersal for conservation. Although salmonoids are male heterogametic, genetic sex identification has been problematic because a specific sex determination locus has been elusive. We implemented a blind analysis to identify sex in Brook Trout *Salvelinus fontinalis* using genetic primers (sdY) developed for Brown Trout *Salmo trutta* along with cytochrome oxidase internal control (CO1-BT1). Fin clips were taken from 24 Brook Trout and analyzed via PCR and gel electrophoresis, while fish were simultaneously sacrificed and dissected to identify sex via gonads. The genetic and phenotypic analysis matched in 96% of the Brook Trout sampled, suggesting that this primer combination is successful in genetic sex determination. The single mismatch was probably due to a mistake in dissection because the fish were not reproductively mature, making the identification of the gonads problematic. The ability to sex Brook Trout genetically will allow scientists to observe the difference in male and female Brook Trout behavior in natural stream ecosystems without using lethal methods.

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[3.2] Host selectivity and local environmental conditions determine cutaneous microbiome structure of North Texas stream fishes at the onset of drought

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Fishes native to drought-prone regions are adapted for population persistence during drought or rapid recolonization after cessation of drought. However, anthropogenic stressors are exacerbating the intensity and duration of drought events and will likely push species to the extremes of their tolerances. Microbial communities living in or on an organism (host microbiome) are affected by environmental conditions and have consequences for host behavior and health. Thus, host microbiome interactions may play an important role in

mediating fish population responses to drought. Relatively little research to date has addressed the factors that affect the composition of the fish host microbiome. We determined cutaneous microbiome structure for eight fish species across three upstream and downstream sites in an intermittent stream in North Texas and analyzed the relative importance of host phylogeny and local environmental factors affecting the cutaneous microbiome at the onset of drought. DNA was extracted from fish cutaneous and environmental microbial samples and library prepped for next generation sequencing targeting the V4 region of 16S bacterial ribosomal RNA. We found differences between environmental and cutaneous microbiomes, demonstrating host-microbiome selectivity. That said, there was a difference in microbiome composition of hosts between upstream and downstream sites with overlap among host species within sites, but little overlap within host species across sites, suggesting that local habitat conditions are important in structuring the cutaneous microbiome. Data collected during drought conditions will enable us to further assess factors affecting the host microbiome as assemblages are restricted to refugia pools and exposed to increasing stressors.

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[3.3] A genetic evaluation of dam impacts on Sauger (*Sander canadensis*) movement patterns in the Arkansas River

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Dams have led to significant changes in freshwater ecosystems including altered flow dynamics, nutrient cycling, sedimentation, water quality and overall ecosystem function. Dams act as a barrier to movement for migratory species. Decreased habitat connectivity can result in genetic isolation and lower species diversity in fish communities. Sauger (*Sander canadensis*) is a migratory freshwater species native to the Arkansas River. Little is known about population genetics of Sauger in the Arkansas River and what influence the McClellan-Kerr Arkansas River Navigation System (MKARNS) may have on fish passage. Sauger fin clips were collected in the winters of 2019, 2020, and 2021 below five consecutive dams in MKARNS. Samples were taken from one pool in the upper reach of the system in Oklahoma and in the lower reach near Pine Bluff, Arkansas. Fin clips were also provided by the Kansas Department of Fish and Wildlife from two reservoirs to serve as reference of a distinct population. Once DNA samples are processed and sequenced, genetic differentiation (F_{ST}), allelic richness (A_R), heterozygosity, and rate of hybridization will be evaluated. My hypothesis is that fish from the upper and lower reaches of the river will have greater differences in genetic structure when compared to the consecutive pools. It is expected that there will still be distinctions between the adjacent sites due to restricted movement among pools, but these may be more subtle. This study will identify

areas if Sauger populations have become isolated and face the threats of decreased genetic diversity and fitness.

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[3.4] Movement and Home Range of the Sickle Darter (*Percina williamsi*) in the upper Emory River Watershed

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The Sickle Darter *Percina williamsi* is a species of fish endemic to the upper Tennessee River basin in eastern Tennessee, southwestern Virginia, and western North Carolina. It has been listed as threatened by the states of Tennessee and Virginia and is being petitioned for federal listing under the Endangered Species Act. Little is known about the movement of this species. We wanted to assess the movement and home range of this species. A total of 8 Sickle Darters from Rock Creek and the Emory River were implanted with an 8-mm PIT tag and released back in the respective creek. Each individual pit-tagged was at least 55 mm in length. The mean (\pm SD) length and weight of all fish PIT was 70.1 \pm 3.4 mm and 3.08 \pm 1.4 g. Movement for each individual was tracked every two weeks for 6 months, environmental variables were also monitored throughout the study. Each time a fish was detected, GPS coordinates and microhabitat data were collected. Mean total effort for all the tracking events was 70 \pm 39.4 min, mean catch-per-effort was 9.3 \pm 6.6 (min/fish), and mean (\pm SE) detection was 69.5 \pm 12 %. Mean (\pm SD) distanced moved of all individuals throughout the study was 7.1 \pm 4.5 m. Early analyses suggest that Sickle Darter movement is related to water temperature (C). Home range for each individual varied very little in size (m²) and overlap existed with some individuals. The results from this study will be beneficial for informing future conservation decisions regarding this species.

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[3.5] Influence of salinization on freshwater crustaceans (Decapoda: Amphipoda, Astacidea Brachyura, Caridea, and Isopoda)

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Crustaceans are important contributors to freshwater diversity and resulting ecosystem services. However, crustaceans are among the most imperiled freshwater taxa and it is unclear the extent to which they will be affected by the global rise in secondary freshwater salinization. We reviewed the literature concerning individual, population, and assemblage responses to salts and conductivity of amphipods, crayfishes, crabs, shrimp, and isopods. A total of 55 studies with 226 unique response variables were reviewed. Most studies were conducted in Europe, North America, or Asia. Study questions were mostly related to basic biology or ecology, aquaculture, or a mix of anthropogenic threats; however, a few studies focused on specific threats such as road salts. Studies investigated the response of shrimp ($n = 108$), amphipods ($n = 60$), crayfishes ($n = 39$), crabs ($n = 10$), and isopods ($n = 9$) to salinization. Most response variables focused on individual toxicity, survival, growth, or population size. However, most crayfish experiments focused on osmoregulation and development, whereas the crab experiments focused on biomolecular (e.g., glucose levels) and body fluid changes (e.g., hemolymph ion concentrations). In general, increasing concentrations of salt were more toxic, resulting in decreased survival, body size, and population sizes across taxa. Data were lacking for South American and African species, freshwater crabs and isopods, less common species, and how these effects manifest at the ecosystem level. Despite these deficiencies, our results begin to fill the knowledge gaps concerning secondary freshwater salinization and show that it could alter freshwater crustacean populations.

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[3.6] Phenotypic Plasticity in Size and Growth of Invasive Mayan Cichlids from South Florida

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Mayan cichlids are a tropical species native to Central America that have become invasive in south Florida. Our primary goal was to compare age, size structure, and growth between Mayan cichlids located north and south of the Everglades ecosystem. In July-Nov 2017, 43 Mayan cichlids were collected from the L-28 Interceptor and Hillsboro canals (pooled to represent the “northern population”) and 49 cichlids were collected from the C-111 canal (southern population). Each fish was processed in the laboratory, including otolith extraction for age estimation. Mayan cichlids from the northern population [mean age = 5.0 ± 0.2 (SE) years; max = 10 years] were significantly older than cichlids from the southern population (3.5 ± 0.2 years; max = 7 years; $K-S = 1.89$, $P < 0.01$). Mayan cichlids from the northern population also grew to larger sizes (mean TL = 240 ± 5 mm) than cichlids from the southern population (170 ± 4 mm; $K-S = 3.87$, $P < 0.01$), and ANCOVA revealed that northern cichlids grew at a higher rate than their southern counterparts ($P < 0.01$). The northern population also showed significant sexual dimorphism in size, with the males being longer than females ($K-S = 1.47$, $P = 0.03$). While holding for the effect of sex on growth, we determined that females from the northern population grew to significantly larger sizes at ages 3, 4, and 5 than females from the southern population ($P < 0.001$). Our findings suggest that these genetically similar Mayan Cichlids show great phenotypic plasticity in growth and size across their nonnative range, and that targeted removal of large, reproductive males could potentially assist in controlling the growth and expansion of invasive cichlids.

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[3.7] Capture-mark-recapture assessment of two populations of Tennessee heelsplitter (*Lasmigona holstonia*) in southwestern Virginia and southeastern Tennessee

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The Tennessee heelsplitter (*Lasmigona holstonia*) is listed as a state endangered species in Virginia and is a candidate for listing under the U.S. Endangered Species Act. Due to its preference for atypical habitat in small headwater streams, *L. holstonia* often has been overlooked during long-term monitoring surveys. In order to estimate population sizes for *L. holstonia*, we conducted a long-term capture-mark-recapture assessment at two sites (South Fork Clinch River, Tazewell, VA and Cloud Branch, Benton, TN), which likely represent some of the largest remaining populations of this species. Mussels were first captured and marked in summer 2019 in June and July, and both sites were re-visited five times between August 2019 and July 2020. At the South Fork Clinch River site (area = 200 m²), 60 mussels were initially marked and an additional 117 mussels were collected during later visits. At Cloud Branch (area = 100 m²), 43 mussels were initially marked and an additional 42 mussels were subsequently collected. Recapture rates ranged from 21–48% at the South Fork Clinch River site and 3–56%

at Cloud Branch. In general, more mussels were collected during the fall at both sites, likely due to mussels surfacing for reproduction. We estimate a population of 192 mussels at the South Fork Clinch River site and 87 mussels at the Cloud Branch site, indicating that we likely encountered most mussels at each site. During the final visit to both sites, evidence of high flows and sediment scouring were observed. This likely caused mussels to wash downstream and contributed to our low recovery rate at Cloud Branch. Results from this study will provide critical information for monitoring *L. holstonia* populations and for possible listing of this species.

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[3.8] The effect of climate change on southeastern U.S. headwater systems

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Headwater ecosystems, stream orders of three or less, are an essential resource for downstream ecological interactions, refugia for aquatic fauna, and water sources for rural and small town communities, yet these ecosystems have the least amount of regulatory protection. We examined the distributional changes of madtom catfishes, benthic fishes endemic to headwater ecosystems of the southeastern U.S., using hydro-climatic informed species distribution models (SDMs) as a proxy to predict and better understand the threat headwater systems are combating under current climate model policies. SDMs included 20 variables, combining factors that were biologically significant to the life history of these species. We compared model outputs for present day environmental conditions for two madtom species, Mountain Madtom (*Noturus eleutherus*) and the Tadpole Madtom (*Noturus gyrinus*), with two global climate models, IPSL and CNRM, from WoldrClim under the socioeconomic scenario of active global climate change (ssp126). The resulting distributional shifts of these species will provide an indication of what environmental features have the most significant effect on what is considered suitable habitat and provide a better understanding of how climate change and anthropogenic activities could exacerbate negative conditions without targeted conservation efforts in predicted stressed regions. Future work will include four madtom species to compare predictions across the southeastern U.S. for highland and lowland geographic regions, and the inclusion of genetic interactions in SDMs to assess for local adaptation. This study will provide intuitive context for potential risks of southeastern U.S. headwater ecosystems and the effects it could have on the associated community.

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[3.9] Investigating ecological links between floodplain forests and aquatic communities

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Forested floodplains are a complex mosaic of periodically flooded aquatic habitats with variable levels of connectivity. While there is a clear link between riparian forests and freshwater organisms, floodplain forests are seldom investigated due to difficulties in sampling structurally complex and periodically inundated habitat. Therefore, we aimed to determine how bottomland hardwood forests influence fish taxonomic and functional diversity. To accomplish this goal, we: (1) assessed species taxonomic diversity (i.e., species richness and composition) and functional diversity (i.e., standard length and body shape), and (2) quantified habitat complexity. We hypothesized that fish taxonomic and functional diversity are driven by forest complexity. Overall, a total of 51 fish species (1,487 individuals) were captured. Ordination analyses per hydrological period revealed consistently different assemblages in floodplain forest sites compared to river channel sites, yet periodic connectivity facilitated longitudinal movement of fishes across the floodplain during the annual flood. Floodplain forests also contained a higher taxonomic diversity and functional richness than the river channel. Regression models showed that fish standard length was negatively affected by increased water surface temperature in the river channel. However, water surface temperature had no effect on fish standard length in the floodplain forest. Interestingly, the water surface temperature in floodplain forest sites was cooler than in river channel sites, even in the warmer months of the year, which suggests that floodplain forests act as a thermal refugia for fish. By linking floodplain forests to greater fish taxonomic and functional diversity, this research further emphasizes the importance of floodplain forests to inland fisheries conservation.

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[3.10] Effects of voltage threshold on the response of Flathead Catfish to low-frequency electrofishing fields

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Low frequency electrofishing (LFE; 15 pps) results in an atypical response from Flathead Catfish, whereby they surface as far as 50m from the electrodes. Little is known about why Flathead Catfish respond the way they do in a field setting. Our goal was to quantify the electrical settings that elicit this “surfacing response” (i.e. allowing them to be netted in the field)

to help scientists better understand what electrofishing power settings would be most effective in the field. Flathead Catfish (n=64) collected from Oklahoma reservoirs were shocked using a Smith Root L24 Backpack Electrofisher in a 3-m long rectangular tank with metal plates at either end serving as cathode and anode. All fish were tested at each of 4 electrofishing treatments (in random order) to determine the minimum voltage threshold for the surfacing response. All trials were conducted with 15 Hz pulsed DC current and 15% duty cycle. The randomly assigned treatments were: testing for 2 minutes at a fixed voltage in low (50-100V), medium (100-150V), or high (150-200V) voltage ranges, or testing over a range from 50-200V, with voltage increasing 50 volts every 30 seconds. Fish exhibited the surfacing response in 9.8% trials. The increasing treatment of 50-200v was the most successful treatment. It took ≥ 40 seconds before any Flathead Catfish surfaced in any treatment. Other responses were recorded including tucking pectoral spines against body, muscle twitching, and erratic swimming, but many fish showed no reaction movement indicating individual variation exists in Flathead Catfish responses to LFE.

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[3.11] Effects of temperature on gear bias of low-frequency electrofishing for Flathead Catfish *Pylodictis olivaris* in reservoirs

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Flathead Catfish are popular among anglers. Unfortunately, information about sampling Flathead Catfish is limited, making it difficult for management agencies to get the population data they need to properly manage this species. Research on the best method for sampling Flathead Catfish is needed. Low-frequency electrofishing (LFE; < 30 pulses per second [pps], commonly 15 pps) is the most commonly used method for sampling Flathead Catfish. Although the accuracy and precision of this gear is unknown, many think it may be biased against fish >600mm. To quantify the accuracy and precision of LFE for Flathead Catfish at different temperatures, we created known populations by tagged Flathead Catfish in Lake Carl Blackwell (n=1,073), Lake McMurtry (n=795), and Boomer Lake (n=130) with numbered modified Carlin Dangler tags. Preliminary results from sampling these marked populations suggest that Flathead Catfish catch rates begin to decrease when the surface temperature is <21° C, and Flathead Catfish are generally captured in proportion to their actual abundance despite LFE being thought to be size bias against larger fish. We plan to continue our mark-recapture study throughout the next spring to compare the catch rates over a range of temperatures.

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[3.12] Let's eat! A look at the diet of the invasive Flathead Catfish (*Pylodictis olivaris*) in the Susquehanna River Basin, PA

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Introductions of nonnative fishes often alter food web structure and trophic interactions in aquatic ecosystems. In such cases, it is important to understand such alterations in order to effectively manage native and naturalized fisheries of recreational and conservation importance. The Flathead Catfish is a large bodied piscivore that can disrupt river systems through direct predation effects. Flathead Catfish have been introduced throughout the eastern United States, including the Susquehanna River Basin in central Pennsylvania. To date, there have been no diet studies performed on this invader within this system, and as a result their impact on prey species remains unknown. This project aims to (1) quantify the diet composition of the Flathead Catfish within the Susquehanna River Basin using molecular methods, and (2) identify prey species that are preferentially consumed by Flathead Catfish. A total of 155 fish were collected during 2020, with 63 fish having prey items in their stomachs. Molecular analysis of the diets is currently ongoing with a subset (n=18) of the analyzed samples identifying primarily benthic fish and crayfish species. Visual analysis of all stomachs showed 25 contained only fish, 24 contained only crayfish, 6 contained both fish and crayfish, and 7 were unidentifiable through visual analysis alone. Analyses will investigate differences in diet by location throughout the basin and by Flathead Catfish length class. The prey items identified in Flathead Catfish diets will allow characterization of risks to species of interest and allow managers to account for these risks when developing policy.

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[3.13] Comparison of first and second pectoral fin ray for use in aging Shortnose Sturgeon (*Acipenser brevirostrum*) in Georgia

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The endangered Shortnose Sturgeon (*Acipenser brevirostrum*) inhabits coastal rivers of eastern North America. Careful assessment of the extant population is vitally important for determining recovery status and making management decisions for species conservation. Currently, the abundance of Shortnose Sturgeon in Georgia is assessed by using mark-recapture models that take fish age into account; an improved understanding of population age structure, particularly among juveniles, will help refine those models. Currently, fish are aged using the first bony pectoral fin ray but shifting policy may require the use of the second ray instead. The objective of this study was to determine if fish ages could similarly be determined using the second pectoral fin ray instead. In order to determine whether similar age results can be achieved, during the summer of 2020 we obtained samples of both rays from 271 Shortnose Sturgeon in the Altamaha and Savannah rivers; fish varied in size from 300-1000 mm (TL). First and second fin rays for each fish were sectioned into ~1 mm thick sections, attached to slides, and then digitally photographed using a dissecting microscope with attached camera. Each image (stripped of identifying information) was aged by 3 independent readers; if readers could not reach a consensus on the age of a fin ray, it was removed from analysis. The data from this study will demonstrate whether aging results using the second fin ray are comparable to the first fin ray and help calibrate comparisons in fish ages based on the two structures.

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[3.14] Pathogen Lesions and Parasite Invasions in American eels

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American eel (*Anguilla rostrata*) is commercially, recreationally, and ecologically important to the Chesapeake Bay. Eel aquaculture, comprising of holding live animals in rudimentary facilities, experiences up to 20% disease-associated mortality from an infectious disease characterized by severe skin lesions. This condition, known as the 'red disease', may also be contributing to the declining wild eel stocks in addition to other eel parasites. There isn't much scientific knowledge about the etiology, epidemiology, and impacts of red disease on the fishery. However, anecdotal evidence suggests a bacterial pathogen, and spatial and temporal variation in the distribution of this disease in the wild. Extensive studies have been carried out for similar diseases in European and Japanese eels but research on pathogenic lesions of American eels is limited. Using standard bacteriological and molecular methods, we primarily isolated and identified species of *Vibrio* and *Aeromonas* from the external lesions and internal organs of diseased fish from aquaculture. We also observed clinical signs of the red disease in fish caught from wild. There is a need to better understand the red disease due to its potentially severe impacts on the eel fishery, and emerging aquaculture industry. Our future work is focused on elucidating the environmental correlates of

this disease, and experimentally quantifying the impacts of environmental stressors on disease expression. These results will be instrumental in effective disease mitigation and management of eel losses, both in aquaculture and wild.

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[3.15] Towards Standardized Fisheries Management Plans

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Organizing and planning are among the most demanding tasks fisheries professionals routinely face. This process can be overwhelming and explicit links between fishery objectives (OBJs), monitoring metrics (METs), and management actions (ACTs) can be uncertain or not explicitly specified. However, when OBJs, METs, and ACTs are made explicit in fisheries management plans (FMPs) tend to be similar throughout an agency and reflect agency-wide goals. Thus, an opportunity to streamline and regiment FMPs is made apparent. We propose a framework that links OBJs and ACTs to standardized METs shared among fisheries and revises the pool of OBJs, METs, and ACTs and their linkages through an iterative process between the agency and a technical support team. Working with the Mississippi Fisheries Bureau, we have begun synthesizing common BOJs and ACTs from existing FMPs and making links to METs (phase I). We developed a prototype web application that provides users with a retrospective summary of system- and species-specific METs and corresponding agency target ranges to identify where OBJs may not have been reached and ACTs may be triggered. The tool guides users from retrospective summaries to selection of OBJs and then to selection of ACTs specific to OBJs. User selections are summarized in a downloadable table that makes explicit the linkages between OBJs, METs, and ACTs. The alpha version of the application is set to be deployed fall 2021. In phase II, the accumulation of FMPs can be used to identify gaps in OBJs and ACTs, and better understand how ACTs influence one or more METs and fulfill OBJs. The framework provides fisheries professionals an efficient and effective way organize and plan ACTs in the context of OBJs and paves the way for improved future decision making.

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[3.16] Seasonal diet composition of hybrid Striped Bass and Largemouth Bass in DeGray Lake, Arkansas: Preliminary results

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Hybrid Striped Bass *Morone saxatilis* x *M. chrysops* are stocked in reservoirs across the eastern and midwestern United States to provide anglers additional fishing opportunities and control baitfish populations. Despite being stocked for over 50 years, information on basic life history, such as diet composition, is relatively scarce. We compared hybrid Striped Bass diets on a seasonal basis in relation to those of Largemouth Bass *Micropterus salmoides* in DeGray Lake, Arkansas. Adult hybrid Striped Bass and Largemouth Bass were collected via gillnetting and boat-mounted electrofishing throughout 2019 and 2020. Stomach contents were removed using gastric lavage. Diet composition was analyzed using percent by weight. Three hundred and thirty Largemouth Bass and 159 hybrid Striped Bass have been collected. The importance of fish prey—particularly shads *Dorosoma* spp.—in hybrid Striped Bass diets varied seasonally, but fishes were the most common diet item throughout all seasons for both species. Seasonal diet overlap, measured by Schoener's index of overlap, ranged from 9.4% in the fall to 61.5% in the summer. These results indicate that diet overlap may or may not be concerning, depending on season. While not directly studied, high mean relative weights for both species across most seasons suggests competition for food resources may be minimal. Interspecies competition cannot be ruled out without knowledge of forage fish availability in the lake. Further clarification of prey consumption patterns, particularly using DNA barcoding techniques, will better inform future management decisions by elucidating the extent of diet overlap between these two popular sport fish.

Keywords: fisheries management, diet overlap, reservoirs, sport fish

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[3.17] Navigating the paradoxes of invasivorism: a techno-economic analysis for a conceived alternative Asian carp supply chain

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Invasivorism, the enlistment of human consumption to suppress invasive species populations, is gaining global appeal as an intuitive mechanism of invasive species management. Among such appeal is the potential for private entities and free markets to drive suppression efforts,

alleviating burdens on natural resource agency budgets. Yet, despite this and human's historical tendencies to deplete abundant resources, invasivorism remains an unproven method that has spurred much contention within the bio-invasion community. Central to the contention are the paradoxes that arise when an invasivore campaign is implemented and extirpation has not yet been abandoned, as is the case for the U.S Asian carp invasion. Indeed, commercial fisheries may slow range expansion and mitigate eco-economic impacts, buying time for novel control mechanisms of improved efficacy (e.g. gene drives, pheromones, engineered pathogens). But if opened to free market forces, ensuing dependents of the commercial fisheries may perversely oppose the future deployment of said mechanisms. Here, we explore shortcomings of the current Asian carp supply-chain framework, identifying critical barriers to overstep if likely outcomes are to better align with management goals. Drawing influence from novel supply chains emerging across commercial marine fisheries, we present an alternative supply chain. This, we hypothesize, could mitigate current barriers, providing managers a resilient, long-term and fiscally independent iteration of invasivorism that could be dismantled should extirpation prove more feasible. A techno-economic analysis is reported to test deliberations of the proposed framework, with particular focus on an endpoint for and return on government investments.

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[4.1] Population genetics of Brook Trout (*Salvelinus fontinalis*) in the southern Appalachian Mountains

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Broad-scale genetic assemblages of the Brook Trout remain poorly elucidated in its native range in eastern North America. We characterized variation at 12 microsatellite DNA loci in 22,020 Brook Trout among 836 populations from Georgia to Quebec to the western Great Lakes region. Within-population diversity was typically lower in the southern Appalachians than in the mid-Atlantic and northeastern regions. Effective population sizes in the south were often very small, with many estimates less than 30. Population genetics of Brook Trout in the southern Appalachians are more complex than the conventional “northern” versus “southern” dichotomy would suggest. Bayesian clustering and discriminant analysis of principal components showed two major assemblages of populations mainly differentiated at the Eastern Continental Divide. Contemporary population genetic variation showed the signatures of geographic expansion of Brook Trout from Mississippian, mid-Atlantic and Acadian glacial refuges, as well as differentiation among drainages within these broader clades. Genetic variation was pronounced among drainages (57.4% of variation among HUC10 or larger units), but was considerable even at fine spatial scales (13% of variation among collections within HUC12 drainage units). Remarkably, 87.2% of individuals were correctly assigned to their collection of origin. While some impacts were apparent at the population level, stocking did not overwhelm broad-scale patterns of population genetic structure. Although our results reveal deep genetic structure in Brook Trout over broad spatial extents, fine-scale population structuring is prevalent across the southern Appalachians. Additional work is necessary to provide managers with genetic information on a scale relevant to specific management activities.

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[4.2] Comparing the use of multiple molecular techniques for identification of prey items in invasive Flathead Catfish (*Pylodictis olivaris*) diets

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Flathead Catfish are a piscivorous invasive fish species introduced throughout the United States. It is important to understand how Flathead Catfish introductions alter ecosystem function and disrupt community structure through predation. Flathead Catfish are commonly collected with empty stomachs or degraded prey items in stomach contents making it difficult to identify prey using meristic or morphometric techniques. Molecular identification of individual prey items using DNA barcoding and Sanger sequencing is a more suitable approach for unidentifiable items but could be complicated when the diet is comprised of an inseparable slurry of prey. We investigated the use of universal fish DNA barcoding primers and both Sanger sequencing and next generation sequencing in the identification of prey items from a subset of Flathead Catfish diet samples (n=5). Sanger sequencing of individual prey items produced consistent species level identification. Sample preparation for next generation sequencing was labor intensive and it was often difficult to get desired DNA amplification from diet slurries. Next generation sequencing has been completed and samples are currently being analyzed through a bioinformatics pipeline for species identification. Next generation sequencing may be most advantageous to use in samples where contents are inseparable, whereas Sanger sequencing may be an easier and more direct approach for samples with separable prey items. Prey identification from fish species with degraded diet samples likely requires different methods to accommodate varying sample quality and quantity.

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[4.3] Linking male reproductive success to nesting effort within and among nests in a co-breeding stream fish, bluehead chub

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Nest construction is common in fishes and this is an energetically costly activity for males. In some fishes, males share the same nest with their co-breeders, and they may also construct multiple nests in a single spawning season. However, little is understood about how allocation of nesting effort within and among nests affects their reproductive success. We characterized reproductive effort of male bluehead chub (*Nocomis leptcephalus*) using individual tracking techniques (PIT antennas) deployed around nests and linked effort within and among nests to reproductive success in a small stream in South Carolina, USA. We observed 34 males on 18 monitored nests during the spawning season in 2017. The inference resulting from our Bayesian

hierarchical model showed that larger males spent more time constructing and maintaining a given nest, and consequently were more reproductively successful than smaller males. Combined with aggressive behavior displayed by larger males toward smaller males, this finding suggested that reproductive effort, including agonistic interactions within nests, was a determinant of reproductive success. At the same time, more males constructed larger nests, which led to higher reproductive success of members that constructed those nests. The number of nests that males constructed, a measure of effort across nests, did not predict reproductive success well, indicating that reproductive success varied among nests due to nest size. Our study showed that male reproductive success was determined by both aggressive and cooperative behaviors in this co-breeding species.

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[4.4] Diet analysis in Blue Catfish illustrates advantages of high-throughput sequencing compared to morphological methods

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Blue Catfish *Ictalurus furcatus* are an invasive, yet economically important, species in the Chesapeake Bay. In order to provide in-depth analysis of predation by Blue Catfish, we identified prey items using high-throughput DNA sequencing (HTS) of entire gastrointestinal tracts from 134 samples using two genetic markers, mitochondrial cytochrome c oxidase I (COI) and the nuclear 18S ribosomal RNA gene. We compared our HTS results to a more traditional “hybrid” approach that coupled morphological identification with DNA barcoding. The hybrid study was conducted on additional Blue Catfish samples (n=617 stomachs) collected from the same location and season in the previous year. Taxonomic representation with HTS vastly surpassed that achieved with the hybrid methodology in Blue Catfish. Significantly, our HTS study identified several instances of at-risk and invasive species consumption not identified using the hybrid method, supporting the hypothesis that previous studies using morphological methods may greatly underestimate consumption of critical species.

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[4.5] Evaluation of Striped Bass Stocking at Badin Lake Using Parentage Based Genetic Tagging

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Stocking has been used since the 1970's to establish Striped Bass fisheries in reservoirs across the United States. While Striped Bass stocking has been evaluated previously using multiple metrics, no studies to date have looked at the contributions of specific stocking sites to the overall population in a reservoir. Parentage based tagging, which utilizes genetic information collected from broodstock, was used in this study to stock genetically unique cohorts of Striped Bass at three different stocking sites at Badin Lake, North Carolina from 2013—2016. Genetically unique cohorts of Striped Bass were also stocked in three reservoirs upstream of Badin Lake during this time as well to measure any immigration of Striped Bass from these reservoirs. Gillnetting and electrofishing were used to collect Striped Bass beginning in Fall 2014 and ending in Fall 2017. Percent contribution of Striped Bass from these stocking sites in our samples varied over time for all cohorts except for the 2016 cohort where only one sample collected these fish. However, pooled results from each cohort over the entire study suggested that no differences occurred in the percent contribution of Striped Bass from the stockings in 2013 and 2014 but differences were apparent in the contributions from the 2015 and 2016 stockings. Immigration of striped bass from upstream sources was detected in every sample and the percent contribution varied widely over the study. The results of this study indicate stocking site selection may be an important factor in successful reservoir Striped Bass fisheries and that immigration from upstream sources should be considered when management decisions are made.

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[4.6] Population structure of Atlantic blackfin tuna *Thunnus atlanticus* as inferred from microsatellite markers

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The blackfin tuna, *Thunnus atlanticus* is a tropical tuna commonly found in waters of the Southeastern United States and Gulf of Mexico as well as the Caribbean Sea. Blackfin tuna are increasingly targeted by recreational fisheries in the US and by commercial fisheries in the Caribbean region and South America. Information on stock structure is essential in order to develop sustainable management plans for this important species. In this work, a panel of homologous microsatellite markers and multiplex assays were developed and used to perform a first assessment of genetic stock structure of blackfin tuna in its West Atlantic range. Thirteen polymorphic microsatellites were available to survey genetic variation among populations. A total of 470 blackfin tunas from 9 geographic populations were assayed. Four of the localities were sampled in two different years. Both temporal (among years) and spatial (among localities) components of molecular variance did not differ significantly from zero. The overall divergence among locality-samples was low ($F_{ST} = 0.0004$) and no clear pattern of cryptic structure was detected. A weak isolation by distance pattern was however evidenced ($r = 0.3$, $P = 0.040$). Further study incorporating high-density genome scans is warranted and in progress.

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[5.1] Freshwater Fishes of North America

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Over 1,200 native freshwater fish species occur on the North American continent, comprising the largest temperate, freshwater fish fauna on Earth. The importance of freshwater fishes in North American ecosystem function, their value economically and as providers of ecological services, and the growing need to conserve this fauna cannot be over emphasized. In the last 30 years, major scientific advances have been made for these fishes across multiple disciplines (e.g., systematics, genetics, physiology, behavior, ecology, and conservation). These advances are marked by increased specialization and fragmentation of knowledge about the diverse North American fish fauna. Our book is the first, fully illustrated multi-volume work synthesizing the

diversity, natural history, ecology, and biology of 52 fish families with coverage including Canada, Alaska and the coterminous United States, and Mexico (south to Isthmus of Tehuantepec). Non-taxonomic chapters provide syntheses on evolution and ecology of fish communities, mating behavior, effects of introduced fishes, and conservation. Our goal is a near-comprehensive synthesis of information on freshwater fishes in North America. We have about 80 authors engaged in the project. Volume 1 (chapters 1-13) was published in 2014 and Volume 2 (chapters 22-33) in 2020, both by Johns Hopkins University Press. Volume 3 is in preparation.

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[5.2] Satellites, Sondes, and Shallow Water Fishes: A Habitat Restoration Evaluation

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Water level stabilization and excessive growth of invasive aquatic plants have contributed to an accelerated rate of lake succession in East Lake Tohopekaliga (ELT; Osceola County, FL). A lake drawdown was started on October 1, 2019 and the habitat restoration was conducted in spring/summer 2020 by controlling/removing monocultures of invasive emergent vegetation via herbicide treatment and prescribed burning as well as the mechanical removal of woody vegetation, tussocks, and associated organic material from the littoral zone. Our objective is to evaluate the impacts to shallow water ($\leq 0.61\text{m}$) fish communities, diel dissolved oxygen regimes (DO) and habitat structure/composition across these habitat restoration actions (i.e., pre- vs post-treatment). Mini-fyke nets and dissolved oxygen sondes were used to respectively sample the fish community and DO in September 2016, 2018, and 2019 (i.e., pre-treatment). Quantitative and qualitative assessments of organic sediment depths and aquatic vegetation were respectively completed at each site during all years of pre-treatment sampling. Whole-lake submersed and emergent vegetation maps for ELT were made for the pre-treatment condition via EcoSound and EcoSat surveys (BioBase, Minneapolis, MN), respectively. Assessments of fish communities, DO, and habitat structure/composition will be conducted each September for three years post-treatment. Spatial and temporal analyses evaluating the effects of the restoration on the limnological properties of ELT will be conducted upon completion of post-treatment sampling in 2022. Understanding how a range of restoration actions influences the ecology of littoral habitats in lakes will provide managers with a framework to guide future restorative efforts.

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Student posters - Finfishes and general ecology

[5.3] Structure and scale in spatially synchronous southeastern US trout populations

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Brook trout (*Salvelinus fontinalis*), rainbow trout (*Oncorhynchus mykiss*), and brown trout (*Salmo trutta*) are important cultural and economic resources in the southeastern USA. As projected climatic changes increase water temperatures and alter flow regimes, these coldwater species will face major challenges, particularly in their southern native range. However, not all populations will be similarly affected by climate change, and quantifying synchrony in temporal population trends across the region is needed for broad-scale conservation planning. We tested whether geographically close populations exhibit similar population trends by compiling and comparing trout electrofishing data at 326 sites in 8 states collected between 1982 and 2014. Preliminary results show signs of region-wide trout population synchrony, but its strength and spatial scale differ by life stage (i.e., young-of-the-year versus older). We are currently expanding and updating the trout data set and incorporating stream temperature data to understand linkages between abiotic conditions and trout population size. This effort will inform regional interagency cooperation to monitor and conserve trout resources by accounting for the spatial scales of synchrony.

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[5.4] A typology of anglers in Tennessee: Catch orientation and species preference for stocking public lakes.

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Angler populations are often heterogenous, implying that they seek a variety of fishing experiences. Understanding the typology of anglers that value different experiences and comparing management preference helps fishery managers meet angler expectations and enhance satisfaction. This study segmented a sample of the Tennessee angler population based on the importance placed on catch aspects of fishing and compared segments in terms of their unique characteristics, fishing behavior, and species preference for stocking of public lakes. For this study, we conducted a mixed mode (email and mail) survey of Tennessee fishing license holders and collected data on fishing participation and satisfaction, fishing tournament, awareness and use of agency lakes and demographic. A hierarchical cluster analysis of survey responses from 2,222 respondents (29% response rate) identified four distinct segments that varied in terms of their catching aspects: *Trophy anglers* (30%), *Native Fish Consumers* (45%), *Subsistence Consumers* (17%), and *Non-consumptive Anglers* (8%). Statistically significant differences were observed across the identified segments in terms of demographic characteristics, fishing behavior, and preference for fish species. Among the seven different fish taxa considered, all the four angler-segments preferred crappie and Largemouth Bass. Findings from this study offer important insights to fishery managers on the underlying variation angler populations, which can help inform decisions related to selecting fish taxa for stocking of public lakes.

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[5.5] Effects of intracoelomically injected Microcystin-LR on channel catfish (*Ictalurus punctatus*)

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Cyanobacterial blooms are becoming increasingly common in natural water bodies and aquaculture systems. These blooms can release several toxins that are health risks for many species including humans, domesticated animals, wildlife, and fish. The most commonly studied environmentally stable toxin produced by cyanobacteria is the hepatotoxin, Microcystin (MC-LR). Channel catfish (*Ictalurus punctatus*) ponds often have blooms that express MC-LR, but losses due to the toxin are rare. However, we believe that the toxin may be a predisposing factor of losses in catfish to summer pathogens due to the effects that MC-LR has on the liver. In this preliminary study, we investigated the effects of a known dosage of MC-LR on the channel catfish liver. Treatment fish were intracoelomically injected with a 500 ng/g bw dose and were sampled along with control fish over a 6-day period. The MC-LR treated fish were not visibly affected but completely stopped eating, when sampled all treated fish through day 4 had

no ingesta and had full gall bladders, most control fish demonstrated ingest a in the gut and lighter colored typical gall bladders. Serum AST and ALT levels were significantly elevated from 6 hours through 96 hours post-exposure indicating hepatotoxicity. Alkaline phosphatase and bilirubin levels were not substantially affected. Histology confirmed substantial hepatic injury among the treated fish. Our study demonstrated exposure to doses of MC-LR below levels that cause visible disease signs, can compromise the function of the liver and digestive system and these are critical organs of the innate immune defenses.

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[5.6] Habitat associations of Blotchside Logperch (*Percina burtoni*) and the availability of habitat for a reintroduced population in Abrams Creek, Tennessee

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Great Smoky Mountains National Park is seeking to reintroduce Blotchside Logperch (*Percina burtoni*) into Abrams Creek, where they, along with thirty other species, were extirpated in 1957. This extirpation was the result of an ill-fated effort to eliminate competition with stocked Rainbow Trout. Nine of the thirty-one extirpated species have been determined to have potential for reintroduction; however, efforts to reestablish those nine species have varied in success. The National Park Service is applying lessons learned from past reintroduction attempts and has deemed an a priori habitat assessment necessary for Blotchside Logperch. This study seeks to identify the habitat associations of Blotchside Logperch in nearby Little River, and determine if those habitats are still present in Abrams Creek after sixty years and inundation of the lower reaches by the Chillhowee Reservoir. Fifty-nine Blotchside Logperch (thirty-two adults and twenty-seven juveniles) were observed across thirty-one random sites in Little River during the 2020 field season. These data are being analyzed using a three-tiered hierarchical approach (microhabitat scale, site scale, and landscape scale) with results forthcoming. The results of these analyses will be used to direct our efforts during the 2021 field season to assess the habitat suitability of Abrams Creek for Blotchside Logperch.

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[5.7] Spatial Assessment of Sickle Darter (*Percina williamsi*) Diet and Microhabitat Utilization in the Upper Tennessee River Basin.

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The Sickie Darter (*Percina williamsi*) is a species of fish that is endemic to the upper Tennessee River drainage in eastern Tennessee, western North Carolina, and southwestern Virginia. Because of its narrow range and presumed decline in occupied sites over the last half century, it has been listed as threatened by the states of Tennessee and Virginia and is being petitioned for federal listing under the Endangered Species Act. This species has been relatively understudied until recently. Little is known about the diet, and microhabitat utilization of the Sickie Darter. We wanted to assess spatial variation in the diet and microhabitat utilization of this species. We sampled Sickie Darters across their native range, Emory River, Little River, and Middle Fork Holston River sub-basins. During sampling, microhabitat (n=51) and diet (n=18) data were collected from a subset of individuals observed. There was little variation in microhabitat utilization among the three sub-basins. The substrate sand was the dominant substrate utilized by this species in all three-subbasins. Sickie Darter diet varied among the three sub-basins, diet was mostly comprised of Ephemeropterans (mayflies; 53%) and Dipterans (true flies; 34%) across all three sub-basins. The results from this study will be beneficial in determining management and conservation measures for this species.

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[5.8] Climate Change and Multiple Stressors in Marine Environments: A Meta-Analysis and Modeling Study of Interactions Between Ocean Acidification and Changing Seasonality

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Anthropogenic activity has caused an increase in atmospheric CO₂, shifting the equilibrium of marine carbonate chemistry and decreasing oceanic pH. Ocean acidification (OA) has the potential to negatively impact the development of early life stages of marine organisms by reducing calcification rates and increasing mortality. Warming temperatures associated with climate change have also influenced the timing of life history events of marine organisms, such as spawning and migration, that rely on environmental triggers to initiate these events. As climate change impacts both marine pH and phenology, there is a potential for interactions between the two stressors. Little research has been done on the potential interactions between impacts of OA and shifting phenology on marine organisms, raising the question as to how early life stages can adapt to differences in pH due to seasonality and ocean acidification. A meta-

analysis was performed to shed light on the overlap and gaps between literature on these two stressors. Preliminary analysis of 170 studies of OA's effect on marine life shows that four phyla, Echinodermata, Arthropoda, Mollusca, and Chordata, are represented equally while Cnidarians were only represented in five papers. Chordata was the most studied phyla in the reviewed literature on phenology. Further analysis will explore the representation of habitats, experimental methods, and life history events and stages in published literature on OA and phenology – both independently and with a focus on overlapping species. The modeling portion of this project will focus on 70 papers representing 22 species for which literature on potential impacts of both stressors were studied. Each species will be assessed consideration as a potential case study to examine whether OA and phenology changes may have interacting impacts on organisms. Preliminary modeling has been initiated using Atlantic cod. Another species, market squid), was identified as a candidate for modeling.

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[5.9] A novel method to assess substrate compaction in lotic systems

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Many benthic aquatic species including fish, crayfish, mussels, and macroinvertebrates are associated with specific substrate conditions and require them to carry out vital life-history events. Compaction can influence bed stability in lotic systems, the ability of organisms to create burrows, and amount of interstitial space. Existing methods used to assess substrate compaction typically rely on qualitative measures and are susceptible to multiple biases. We propose a new method for measurement of compaction in lotic systems. Our objectives were to use a soil penetrometer to 1) develop and refine methods for quantifying compaction in a controlled environment and 2) determine efficacy of use in a field setting and relation to Neosho Madtom (*Noturus placidus*) presence and abundance. Using multiple size classes of homogenous, natural substrate in a lab setting, we performed twenty repetitions in each size class at three predetermined pressure readings by pushing the penetrometer into the substrate and recording depth at target pressure. Mean compaction reading (depth) and substrate midpoint diameter were negatively correlated across substrate sizes and median depth at psi-150 was significantly different among most paired substrate types. Although we found no relationship between Neosho Madtoms and substrate compaction during field tests, we gained insight from data collected in a natural compared to a controlled setting. Preliminary analysis suggested that the penetrometer method has potential for linking compaction to organism response in streams with predominantly rocky substrate.

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[5.10] Detecting sublethal effects of harmful algal bloom cyanotoxins in animal cells

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Algal and cyanobacterial blooms pose risks to human and animal health and ecosystem sustainability. Some blooms produce toxins that can lead to illness or mortality to animal species upon exposure; such blooms are called harmful algal blooms (HABs). Natural global freshwater and marine phenomena, HABs are composed of a complex mix of cyanobacterial species that can produce specific cyanotoxins. In freshwater, the most common is microcystin-LR (MC-LR), which can cause liver cancer. The cyanotoxins each cause alterations at the cell and molecular level along pathways, considered adverse outcome pathways (AOPs). Biomarker changes along an AOP can be quantified after exposing a gradient of MC-LR concentrations to mouse (*Mus musculus*) liver cells. Thus, predictions on mammalian health outcomes can be projected and connections can be made about relevant environmental threshold concentrations. Flow cytometric bioassays with hepatocytes measure metabolic function, apoptosis, mitochondrial function, and genotoxicity, and livers from mallard (*Anas platyrhynchos*) hatchlings previously exposed to MC-LR via oral ingestion have been studied. Data showed an average hepatocyte metabolic dysfunction of 15.2% in MC-LR-exposed birds, as opposed to 9.4% for control birds. Additionally, sperm quality parameters of MC-LR-exposed male channel catfish (*Ictalurus punctatus*) will be measured, as testis is a secondary target organ for this cyanotoxin. This presentation will provide data and will illustrate a literature-derived AOP from MC-LR as well as from anatoxin a, saxitoxin, and cylindrospermopsin.

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[5.11] Imperilment of the Tennessee – Tombigbee Waterway due to the presence of bigheaded carps

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The Tennessee – Tombigbee Waterway was the largest earth moving project in history in order to create a water-based route from Mobile Bay in Alabama to the Tennessee River in Mississippi. This system is now in danger of being invaded by Silver Carp (*Hypophthalmichthys molitrix*) and Bighead Carp (*H. nobilis*) collectively referred to as the bigheaded carps. These

fishes are potentially moving from the Tennessee River through the Yellow Creek Embayment of Pickwick Lake, into Bay Springs Lake which is the northernmost reservoir in the Tennessee – Tombigbee Waterway. This invasion could potentially be detrimental to the various recreational and commercial opportunities this waterway provides. The objectives of this study were to 1) document the capture of any bigheaded carps that have moved into Yellow Creek or Bay Springs Lake, and 2) document the CPE to show relative abundance of fish coming into this system. So far, the capture rates of bigheaded carps have been extremely low within the headwaters of the Tennessee – Tombigbee Waterway. The implications of capturing such low numbers within this system means that the invasion of the Tennessee – Tombigbee waterway is just in its early stages. Bay Springs may potentially act as a natural barrier to these fishes due to its oligotrophic nature and thus, it may not be able to provide enough of a food source for these fishes.

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[5.12] A Comparison of Population Dynamics of Invasive Bigheaded Carp from Established Populations

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During the 1970s, the initial introduction of two invasive fish, Bighead Carp, *Hypophthalmichthys nobilis*, and Silver Carp, *H. molitrix*, (known collectively as bigheaded carp) into the U.S. occurred in Arkansas. These two species now reside in much of the Mississippi River Basin. How the dynamics of the Arkansas population differ from other established populations from across the U.S. is not known. As such, we documented trends in Asian Carp population dynamics in five Arkansas water bodies, Arkansas River, Cache River, St. Francis River, White River, and Lower Mississippi River, and compared them to established population from across the U.S. Overall, 550 silver carp and 22 bighead carp were collected with boat electrofishing, and population structure, mortality, growth and recruitment were compared to populations within the Upper Mississippi River System, Missouri River System, Ohio River System, and Lower Mississippi River System. The mean (SD) total length (mm) of Arkansas Silver and Bighead Carp were 799.55 (84.13) and 915.45 (83.92), respectfully. The mean (SD) ages (years) were 5.07 (2.08) and 5.00 (4.35). Total annual mortality (A) is estimated at 39.1% for Silver Carp and 28.9% for Bighead Carp. Based on length-weight regressions, Arkansas Silver Carp are larger at stock size than just 19% (4/21) other populations but larger at preferred size than 71.4% (15/21) of other populations.

The von Bertalanffy growth equation for Arkansas Silver Carp indicates a slower growth rate but higher maximum sizes than other populations. Recruitment appears to be stable for both Silver Carp (RVI = 0.60) and Bighead Carp (RVI = 0.40), which differs from sporadic recruitment in other populations.

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[5.13] The trophic ecology of an invasive species in a novel ecosystem: Green Sunfish (*Lepomis cyanellus*) in an urban stream

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Urbanization is occurring rapidly in the southeastern United States. Urbanization poses significant threats to small stream ecosystems through ecological degradation and increased proliferation of invasive species. Stream restorations aim to remediate ecological degradation, but the effectiveness of these efforts is not well understood. Green Sunfish (*Lepomis cyanellus*) is a highly tolerant and common invader that is known to suppress native fishes, but its trophic ecology is currently understudied. We assessed the role of Green Sunfish in the food web of Rocky Branch, a restored urban stream on North Carolina State University's campus. We sampled the fishes of Rocky Branch to determine species composition, size structure, and abundance. Green Sunfish diet samples were collected across two seasons for comparison against the benthic macroinvertebrate assemblage to determine if Green Sunfish were employing a selective feeding strategy. Chironomidae was the most common taxa in both the benthic macroinvertebrate assemblage and Green Sunfish diets during the summer. Green Sunfish exhibited selective predation on four macroinvertebrate families in the summer and two families in the winter. Principal components analysis suggest that Chironomidae are disproportionately important in the diets of small fish (<70mm) in the summer. Green Sunfish comprised ~99% of all individuals collected during our sampling efforts. Our findings suggest that Green Sunfish are able to thrive in degraded urban streams as the sole top predator by employing a flexible generalist feeding strategy overall, with some seasonal prey selectivity.

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[5.14] Impervious Surfaces and Stream Health Factors: A Study Using Machine Learning Techniques and Multivariate Statistical Analysis

L. R. Tao* and K.M. Morrow

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Impervious surface area is projected to triple within the next three decades as a direct consequence of proliferating urbanization. Impervious surfaces, man-made architectural features that prevent absorption of water, e.g. buildings and roads, play a profound role in affecting surface runoff and physio-chemical properties of stream systems. Thus, quantifying and studying impervious surfaces is crucial to understanding the breadth of anthropogenic influence. Current methods of quantifying impervious surfaces require complex procedures, expensive software, and experienced personnel. As an alternative, we designed a novel machine learning approach that utilizes Google Maps and a K-Nearest-Neighbors (KNN) supervised algorithm to quantify the percentage of impervious surfaces (PoIS) surrounding 21 urban stream sites in Fairfax County, VA. Non-metric Multidimensional Scaling (nMDS) was conducted using the *vegan* package in R to analyze the relationship between PoIS and 10 water quality parameters on the Bray (Sorenson) distance matrix. Permutational Multivariate Analysis of Variance (PERMANOVA) was used to detect the strength of dissimilarities among stream sites. Our research demonstrates that impervious surfaces are negatively correlated with the ecological health of Fairfax County streams based on a novel machine learning algorithm used to quantify PoIS. This new tool may serve as a useful mechanism for better understanding and preserving our urban stream environment, serving as a foundation for cost-effective water-resource and fisheries management.

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Student posters - Crayfishes

[5.15] Water Quality Monitoring for the Central Appalachian Coalfields Crayfishes

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Primary economic drivers over the years in the central Appalachian coalfields include coal mining, timbering, fossil fuel extraction, and all-terrain vehicle tourism. These industries cause impacts such as sedimentation, elimination of headwater streams (resulting from valley fills), destruction of riparian vegetation, and increased physiochemical pollution in montane streams. As a result of this significant habitat destruction, two species of endemic crayfishes, *Cambarus callainus* and *Cambarus veteranus*, were both federally listed in April 2016. This study will sample 12 selected sites – four degraded streams, four medium-impacted streams, and four reference condition streams – within the coalfields region of Kentucky, Virginia, and West Virginia. Sampling will occur for one year, alternating monthly between two sets of six sites. General crayfish sampling will occur at each site to document the crayfish community present. Water quality monitoring will be split into four categories: long-term, point-source, photometer, and water samples analyzed for heavy metal content. Understanding the chemical composition

of the target species' habitat will enable us to properly and efficiently aid *C. callainus* and *C. veteranus* conservation efforts.

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[5.16] Trophic Partitioning of *Cambarus carinirostris*, *Cambarus robustus*, and *Faxonius obscurus* in Kings Creek, WV by Stable Isotope Analysis

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The competitive exclusion principle states that no two species can share the same niche in nature. Partitioning among coexisting species occurs through utilization of various food sources or differences in microhabitat preference. The purpose of this study is to assess potential trophic partitioning between *Cambarus carinirostris*, *Cambarus robustus*, and *Faxonius obscurus*, three species of crayfish present in Kings Creek, Weirton, WV. Analysis and comparison of individual carbon-13 and nitrogen-15 isotope signatures were used to establish organic matter transfer pathways and trophic structures in the ecosystem. Representative trophic groups were sampled, spanning from macro-predators (e.g., smallmouth bass) to primary producers (e.g., macrophytes and algae). All samples were analyzed for carbon-13 and nitrogen-15 by the Stable Isotope Ecology Laboratory at the University of Georgia. Interspecific partitioning within the community was identified. Smallmouth bass were top predators and crayfish were the second highest group. Between the three species of crayfish, interspecific partitioning was suggested. The lowest and highest trophic levels were occupied by *C. carinirostris* and *F. obscurus*, respectively. In addition, intraspecific partitioning within each crayfish species suggested that juveniles occupy a lower trophic level than adults, potentially signifying ontogenetic dietary shifts. All analyzed data represent the summer season. Winter results are pending and once available, the two seasons will be compared for any differences in trophic structure.

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[5.17] A Behavioral Study of *Lacunicambarus thomai* in a Field Setting

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Crayfish respond rapidly to environmental changes, this responsiveness to environmental changes makes them good detectors for degradation in the environment. Contaminants in the environment may influence crayfish by making them absent in what would be prime habitat. It may also affect the population densities of areas by decreasing the number of individuals in area that superficially look like prime habitat. This study was conducted on *Lacunicambarus thomai* in a field setting to gather information on the life history of a primary burrowing species of crayfish. Some pre-existing information on the behavior and parts of the life history of *L. thomai* were incomplete and, in some cases, incorrect. The data showed that what was perceived to be a fully nocturnal species or spends most of its time underground may not necessarily be true. Findings of this study conflicted on multiple instances with the previously believed notions. This project provides a better understanding of the life history of a primary burrowing crayfish species: the life history of primary burrowing crayfish has been less than stream dwelling species. This could lead to more studies being conducted on other species to obtain a better understanding of this elusive type of crayfish. The results of this study could be used to get a baseline of the basic life history of these animals as well as other types of burrowing crayfish species.

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[5.18] Evaluation of Pet Trade Regulations for Management of Invasive Crayfish in Midwestern States

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The aquatic pet trade is increasing in popularity in Europe and North America, leading to widespread transport and introductions of non-native species, including crayfishes, into natural waters. Over 30 crayfish species have invaded North American waters, often changing aquatic ecosystems and impacting native species. The live bait industry has historically been one of the most important pathways for non-native crayfish introductions in the United States; however, increasing numbers of recent crayfish introductions have been linked to the pet trade, and this pathway also requires proper attention from natural resource managers. The Missouri Department of Conservation (MDC) responded to this problem in 2014, implementing regulations restricting the sale of all crayfish species except the Virile Crayfish (*Faxonius virilis*) by Missouri bait and pet stores. However, MDC has never monitored regulation compliance by pet stores. We designed a survey of all 125 Missouri pet stores in 2019 to determine their level of compliance. Missouri Department of Conservation Agents visited all stores and gathered evidence of crayfish sales by inspecting displayed livestock and interviewing owners/employees. Simultaneously, we conducted a comparative survey of 60 pet stores in Ohio, a state where minimal regulations exist to limit the sale of crayfish in pet stores. Crayfish were sold (illegally) at 11 of 125 (8%) Missouri stores, and included 4 non-native species. Thirty-

one of 49 (63%) Ohio stores sold crayfish, including 3 non-natives. Results suggest that the Missouri regulation has been effective at managing and possibly reducing pet store sales of potentially invasive crayfish.

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[5.19] The Presence and Impacts of Pollutants On Central Appalachian Crayfishes

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Cambarus veteranus and *Cambarus callainus* are federally endangered and threatened crayfish, that live in the coalfields region of central Appalachia. The dominant industry and land use in this region is coal mining. Currently, the most common form of coal mining in this region is surface mining, also known as mountaintop removal mining. There is a lack of data on the specific impacts of pollutants from mining on crayfish and their congeners. Chemical pollutants along with increased sedimentation from industrial sources are known to cause adverse effects in crustaceans and can even be lethal. It is crucial to better understand how these pollutants are affecting crayfishes inhabiting coalfield streams. This study will assess the bioaccumulation of the metals arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), and selenium (Se) in non-imperiled cambarid crayfish that are being used as surrogate species for *C. veteranus* and *C. callainus* by analyzing the hepatopancreas and gill tissues. Physical maladies, such as deformed dactyl and gill melanization, will also be quantified. This data, as well as species abundance at each site, will be analyzed to better understand the effects of mining on crayfish

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[5.20] Genetic Haplotype Mapping of the Allegheny Crayfish (*Faxonius obscurus*)

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Non-indigenous species are a significant threat to freshwater biodiversity. In the Potomac Basin there are several known invasive crayfish species including *F. virilis* (virile crayfish) and *F. rusticus* (rusty crayfish). It is imperative to discover whether *F. obscurus* is in fact an invasive crayfish that needs a management plan or simply another native species that needs protection. *Faxonius obscurus* is a wide-ranging crayfish species that is native to the northeastern United States. Populations found in the Atlantic Slope Drainage basins are considered invasive. Using CO1 and 16S primers, we will be creating a range-wide haplotype map to investigate these invasions.

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[5.21] Human Dimensions of Crayfish Conservation in Appalachian Coalfields

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Cambarus veteranus and *Cambarus callainus* are two closely related crayfish federally protected under the Endangered Species Act. While government protection provides vital funding for research and management initiatives, it is rare for the average citizen to be involved or impacted by this process. Less popular animal groups, such as invertebrates, lack public conservation support contributed by apathetic perceptions. The objective of this project is to foster appreciation for *C. veteranus* and *C. callainus* through public engagement. The Project Crawdad pilot is an education module designed for 3rd grade students. Five lesson plans, aligned with state standards, will allow students to explore local aquatic ecosystems, interact with native species, and develop experiments of their own design. Pre and post tests accompanied with each lesson will be facilitated to gauge student development. As community understanding and familiarity increases, potential pre-existing negative associations are likely to be supplanted by appreciation and sense of personal responsibility. This environmental education study aims to increase conservation literacy and stewardship of *C. veteranus* and *C. callainus* and the ecosystems they depend on.

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[5.22] Specific microhabitat utilization and resource partitioning of the federally protected species of crayfish (Decapoda: Cambaridae) *Cambarus callainus* and the sympatric Cambarid species *Cambarus hatfieldi*

K. Scott

Following the description of *Cambarus callainus*, a range-wide survey was completed to determine the current distribution and conservation status for the species, resulting in federal protection enforced by the United States Fish and Wildlife Service. While significant efforts evaluating physiochemical and physical habitat associations for *C. callainus* have been completed, a more specific analysis of microhabitat utilization, habitat preference, resource partitioning, and movement patterns remains incomplete. We aim to resolve these unknowns for *C. callainus* as well as the sympatric species *Cambarus hatfieldi* by observing potential species interactions regarding habitat preference and individual movement patterns, assessed via radiotelemetry. Ten adult *C. callainus* individuals at each of two selected sites will be fitted with transmitters, as well as ten adult *C. hatfieldi* individuals from a single collection site. Tracking will occur hourly over the course of ten days of varying lunar luminosity during the summer and repeated the following year. Qualitative Habitat Evaluation Index forms and hydrology data will be documented for observed microhabitat. Efforts will also include attempting to locate young of the year and determine movement patterns within the stream, as that has not yet been observed and would be beneficial to designing and implementing appropriate conservation protocols.

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[5.23] Big Sandy Crayfish (*Cambarus callainus*) Habitat Affinity in Proximity to Bridges and Assessed via Radio Telemetry

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Cambarus callainus (Big Sandy Crayfish) is a federally threatened species endemic to the coalfields of Kentucky, Virginia, and West Virginia. Existing datasets involving this species reflect broad/seasonal natural history trends, but conservation managers are still in need of a fine-scale, high resolution understanding of daily microhabitat utilization and movement patterns. The WVDOT has identified multiple locations within *C. callainus* range where in-stream bridge infrastructure requires complete reconstruction in a timely manner. Construction projects occurring within waterways supporting *C. callainus* could represent a threat to the crayfish community, but the extent of such effects is currently unknown. To identify potential disruptions to *C. callainus* populations we aim to determine the extent of site fidelity, peak and minimum activity periods, and preferred microhabitat utilization assessed by radiotracking *C. callainus* during and after active construction. Collected data will be interpreted to determine further conservation and management practices necessary for protection of *C. callainus*. Findings will allow organizations such as the WVDOT to move forward with necessary infrastructure projects while mitigating further anthropogenic impact to this vulnerable species.

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Friday, April 9th

*Symposium continued: **Black Bass Biodiversity, Conservation, and Management***
Andrew Taylor, Brandon Peoples, and Martin Hamel, organizers

Largemouth Bass Conservation & Management

[6.1] Diagnostic SNPs for determining genetic integrity and hybridization among Delta, Largemouth Bass, and Florida Bass

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The Mobile-Tensaw Delta is home to an estuarine population of Largemouth Bass (*Micropterus salmoides*) with larger relative condition and smaller adult size compared to inland populations. To determine the evolutionary relationships between these Delta bass populations, Largemouth Bass (*M. salmoides*), and Florida Bass (*M. floridanus* or *M. s. floridanus*), putatively pure and intergrade populations of all three groups were sampled across the eastern U.S. Phylogenetic analyses of the ND2 mitochondrial gene and 8,582 nuclear SNPs derived from genotype-by-sequencing determined that Delta bass populations stem from a recently diverged lineage of Largemouth Bass. A panel of 73 diagnostic SNPs was developed for the three lineages, evaluated for accuracy, and then used to screen 906 samples from 50 sites for genetic integrity and hybridization. Populations with at least 20% mean Delta bass ancestry were found across Alabama, eastern Georgia, coastal Louisiana, and Tennessee. Furthermore, Delta bass ancestry was shown to contribute significantly to the previously described intergrade zone between Largemouth Bass and Florida Bass, suggesting a more complex pattern of secondary contact and introgression among these diverged *Micropterus* lineages.

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[6.2] Stocking Spatial Planning: Examining Trade-offs in Largemouth Bass Recreational Fisheries Systems

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Recreational fisheries are typified by two common management objectives—conserving wild fish populations and providing angler satisfaction. Trade-offs between these objectives commonly exist in the short-run. Stock enhancement has been historically proposed to circumvent these trade-offs, but recent research suggests this is unlikely at the water-body scale. Here, using Largemouth Bass as a case study, we consider if spatially explicit stocking might work better and increase both conservation and socioeconomic objectives at a landscape scale. To address this, we employ a socioecological spatially-explicit simulation model representing a heterogeneous landscape, as well as key fish population dynamics, angler behaviors, and management decisions. We find that at a landscape scale, win-win approaches to conservation-socioeconomic trade-offs are possible with stock enhancement, but only under specific conditions of wild recruitment, angler movement, and stocking strategy. Embedding this model in a management strategy evaluation showed that positive outcomes also required specific criteria for deciding which waters were stocked. Additionally, detecting these outcome improvements would be challenging with variable wild fish recruitment. All results were particularly sensitive to societal valuations of wild versus hatchery fish. This work promotes increased incorporation of spatial planning into the management of recreational fisheries where multiple social or conservation objectives are likely to occur.

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[6.3] Evaluating Mechanical Removal Rates for Rehabilitating Over-Crowded Largemouth Bass Populations in Alabama Small Impoundments

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Largemouth bass populations in small impoundments exhibit poor growth and body condition under strong density dependence. The effectiveness of bass harvest at reducing these conditions and restore overcrowded populations back to desirable population size structures is unclear. We evaluated mechanical removal rates for rehabilitating over-crowded bass population in Alabama small impoundments. Via boat electrofishing, we removed 0-80% of bass populations under 356 mm from eleven Auburn University Fisheries Research Unit ponds with a mean surface acreage of 4 acres (1.1-13.2) in 2019 and 2020. Upon initial population assessment in 2019, bass populations less than 356 mm were composed of bass age 1-7 with a mean relative weight of 89 (82-98), mean annual growth increments were 158 mm (119-232) for age-1 and 74 mm (50-103) for age-2, and mean proportional size distribution (PSD-Q) was 22 (2-57). Following removals in 2019 and 2020, we evaluated compensation in bass recruitment and growth as well as estimated changes in bass energy allocation as a function of age and size across mechanical removal rates. Removal effort necessary to achieve a mechanical

removal rate of 0.5 ranged from 4-9 complete shoreline circuits as bass catchability declined with removal effort.

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[6.4] Changes in Largemouth Bass *Micropterus salmoides* population characteristics in the early stages of an Alabama Bass *Micropterus henshalli* invasion of Lake Wylie, North Carolina/South Carolina

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It is challenging for invasion ecologists and resource managers to determine if nonnative freshwater fishes are definitively impacting ecosystems in their introduced range. It is also difficult to predict if similar impacts of the same nonnative species will occur in comparable bodies of water. Alabama Bass *Micropterus henshalli* had documented impacts in Lake Norman, North Carolina and have recently been detected in Lake Wylie, North Carolina/South Carolina, which is an impoundment of the Catawba River located downstream of Lake Norman. Concerns about the impacts seen at Lake Norman spurred an analysis of historical and contemporary electrofishing data to determine if any trends existed in the Largemouth Bass *Micropterus salmoides* population in Lake Wylie that resembled the early stages of the Alabama Bass invasion of Lake Norman. Largemouth Bass abundance and condition declined while Alabama Bass abundance increased in the time since Alabama Bass were first detected in Lake Wylie. While still early in the invasion process, these trends are similar to those seen in Lake Norman and might presage a long-term decline in Largemouth Bass abundance for the next 10+ years in Lake Wylie.

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Bookend Talks & Discussion

[6.5] Can bioenergetics modeling refine spatially-explicit assessments of climate change vulnerability?

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*Presenting

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Rising water temperature under climate change is changing the physiology, population dynamics, and geographic distribution of freshwater taxa. We propose a novel application of individual-based bioenergetics modeling (BeM) to assess the physiological impacts of warming on freshwater fishes across broad spatial extents. We test this approach using the Guadalupe bass (*Micropterus treculii*), a species of conservation and recreational significance that is endemic to central Texas, USA. We projected historical-to-future changes (middle 20th century to end 21st century) in daily bioenergetics of individual fish across 8,119 stream reaches and compared this output to changes in reach occupancy derived from traditional species distribution models (SDMs). SDMs project a 4.6 to 44.1% decrease in reach occupancy, depending on model parameterizations and climate change scenarios. Persistence is projected in the central Edwards Plateau region, whereas extirpations are projected for the warmer southeastern region. BeM projected a median 103.6% and 192.0% increase in somatic growth of age-1 GB across historically-occupied reaches under moderate and severe climate change scenarios, respectively. Higher end-of-year body size under future climate was caused by longer growing seasons for moderate (mean increase of 36 days) and severe (increase of 71 days) scenarios. Projected growth was geographically discordant with SDM-based habitat suitability, suggesting that SDMs do not accurately reflect fundamental thermal niche dimensions. Our assessment suggests that Guadalupe bass may benefit from warming via increased capacity to consume prey from November through March, although realized consumption gains will depend on seasonal changes in prey availability and other biotic and abiotic factors.

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[6.6] Georgia's Newly Revamped Angler Awards

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The Wildlife Resources Division's core mission is to conserve, enhance and promote Georgia's fish and wildlife resources and outdoor heritage through science-driven research, management, regulation and education. Staff are investigating ways to increase fishing participation through R3 (recruitment, retention and reactivation) efforts. One implemented strategy was to expand and promote DNR sponsored programs like the existing Angler Award Program and the new Georgia Bass Slam. DNR created and rolled out the Bass Slam in 2017 and revamped the Angler Award Program in 2019. New angler award categories included a youth category, trophy bass category, and Public Fishing Area (PFA) lake records. Additionally, the program offered

more incentivized prizes and staff created a list of locations where anglers can use certified scales. Since the introduction and expansion of programs, angler participation has steadily increased. Georgia Bass Slam participants increased from 15 in 2017 to a high of 41 in 2020. The average total angler awards turned in more than quadrupled from a 2001-2018 average of 41 per year to 190 per year for 2019-2020. Much of this increase in participation can be attributed to youth participation (40% of all angler awards turned in for 2020). The top 5 species submitted for angler awards are Shoal Bass (7.9%), Flathead Catfish (7.7%), Largemouth Bass (7.2%), Black Crappie (6.6%) & Striped Bass (5.9%). Anglers have expressed gratitude for the certified scale list, the PFA records and the trophy bass awards.

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[6.7] The Native Black Bass Initiative: 10 years of Success

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Founded in 2010, the Native Black Bass Initiative (NBBI) Business Plan, began as an auspicious undertaking with the National Fish and Wildlife Foundation to promote conservation of native fluvial bass species and warmwater streams in the Southeast. Guiding science into action, this plan created a regional platform for funding and execution to increase our understanding of key life-history traits and deliver measurable actions to forward the protection of three focal species, the Guadalupe Bass, Bartrams Red-eye Bass, and Shoal Bass. From partner engagement, restoration, research and reintroductions, the NBBI has facilitated over \$4 million dollars for black bass conservation work across the Southeast for these keystone species and their associated habitats.

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Symposium: American Eel Research and Conservation in the Southeast: A Look at Progress and Next Steps

Robby Maxwell, Sean Kinney, Stephen Curtis, and Kevin Mayes, organizers

Gulf of Mexico

[7.1] Conservation and biology of American Eel in the Gulf of Mexico

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The American Eel *Anguilla rostrata* is a highly migratory catadromous species with multiple life-history stages and an extremely large distribution. The species has been intensively studied in Atlantic Coast watersheds. However, limited information is available for a vast area including Gulf of Mexico (GoM) watersheds, Central American, and portions of South America. The goal of this presentation is to review recent studies and conservation efforts from GoM basins. Significant evidence indicates eels have declined from multiple factors, and five long-term data sets from the GoM support the decline. Although eel densities are thought to be low in the GoM, moderate abundance of eels below large dams has been observed. Eel ladders are needed in the Gulf of Mexico region to index fish recruitment. However, state resource agencies are usually unable to commit to the required long-term maintenance costs of eel ladders at U.S. Army Corps of Engineers owned dams.

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[7.2] Preliminary Assessment of American Eel Demographics at the Toledo Bend Hydropower Project, Sabine River, Gulf of Mexico

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A range-wide decline in the distribution and abundance of the American Eel *Anguilla rostrata* has prompted efforts to facilitate passage at migration barriers to restore eel to historical freshwater habitat. In 2014, the Federal Energy Regulatory Commission issued a new license to the Sabine River Authorities for the continued operation of the Toledo Bend Hydropower Project on the Sabine River, Texas and Louisiana. Included in this license is a condition to pass American Eel upstream of the project dam. Upstream passage operations and electrofishing surveys were initiated at the project in 2017. A total of eight eels were captured in ramp traps sited at the project in 2017 and 2018. Boat electrofishing catch rates ranged from 0 – 48 eels per hr in 2017 and 0 – 11 eels per hr in 2018. Three hundred and thirty four eels were captured

during a backpack electrofishing event in 2019, during which the catch rate was 95 eels per hr. The length of eels captured at the project ranged from 140 – 700 mm, with the majority of eels <350 mm. This presentation will review the results of the first year and a half of upstream passage operations at the project, including total catch, relative abundance, size structure, and habitat use patterns of eel captured. The presentation will also outline unforeseen obstacles and operational changes proposed to increase the effectiveness of the upstream passage program.

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[7.3] Status and Survey of American Eel (*Anguilla rostrata*) in Alabama

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In recent years, more attention and focus has been directed towards American eel (*Anguilla rostrata*) due to increased global pressure on stocks in the United States. Although several petitions have been filed to list American eel (AME) under the Endangered Species Act, status reviews in 2007 and 2015 found listing AME was unwarranted. However, Gulf of Mexico AME populations from Texas to Florida are understudied compared to other regions in the U.S. To date, AME has not been studied or surveyed in the waters of Alabama. Therefore, we began compiling and summarizing historical and contemporary AME collection records to begin to understand AME distribution in Alabama. In addition, we launched a campaign to enlist the assistance of anglers and citizen scientists to provide catch records throughout Alabama. We also initiated a fisheries-independent targeted sampling project (as time permits) using eel traps to gather specimens for basic life-history information. These data will provide current distribution, abundance, habitat preferences, presence/absence of parasites, diet, genetics, age and growth, and sex ratios of AME populations in Alabama. We present initial results that will assist with directing future research, conservation, and management efforts of AME in Alabama.

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[7.4] Distribution and detection of American eels *Anguilla rostrata* in Mississippi

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The American eel, *Anguilla rostrata*, is listed as Endangered by IUCN. It is a catadromous species whose migration is an important indicator of stream connectivity. American eels are difficult to capture especially in non-wadeable streams and therefore distributional status may be underestimated. Understanding the current distribution and capture methods is important to evaluate population distribution. The objectives of this project was to evaluate American eel distribution in interior Mississippi Rivers and evaluate passive capture methods for non-wadeable streams. Historic detections of American eels were evaluated from distributional databases. American eels have been detected throughout in the state with the highest number of current and historical detections in rivers draining to the Gulf of Mexico. Detections occurred in 259 out of 22256 sampling efforts withing Mississippi. The Noxubee River is a non-wadeable river in the Mobile basin that is representative of interior Mississippi Rivers. American eel was not commonly detected in the Noxubee River, with detections occurring in 2 out of the 363 samples conducted between 1880 and 2018. The last American eel detection in the Noxubee River was in 1983. Few detections in the Noxubee River despite over 300 sampling efforts suggest that American eels are rare and possibly declining or difficult to capture using conventional stream fish sampling gears. We evaluated several passive capture gears were in the Noxubee River at an American ell historical locality. Net traps, wire box traps, tube traps, and modified limb lines all with varying baits were evaluated for American eel catch. A single American eel was captured in a net trap. This preliminary result indicates that historical locality remains occupied by American eels and suggests that capture and detection is difficult.

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[7.5] American Eel in Texas: Ongoing and Future Research

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American Eel *Anguilla rostrata* have a unique and complex life history that is fairly well-studied on the eastern coast of the United States, but few studies have been done on Gulf of Mexico drainages. To inform conservation and management decisions, efforts to better understand the population structure, seasonal dynamics, and life history of American Eel are underway. The primary objectives of our efforts are to assess the current and historical distribution and

abundance, habitat use, movement patterns, parasite occurrence, diet and population structure (genetics, age, sex, etc.) of American Eel across all life stages in Gulf of Mexico drainages of Texas. Over 100 specimens were collected from ten river basins and several coastal tributaries; most were considered yellow eels and one putative silver eel was found on a Mustang Island beach. Total lengths ranged from 153-1059 mm. *Anguillicoloides crassus*, an exotic parasitic swimbladder nematode, was found in ten American Eel (range 1-9 nematodes) from three basins. Tissue from these specimens are also being analyzed for gut content, otolith microchemistry, stable isotopes, genetics, and heavy metal concentrations. Eel mops and fyke nets were deployed at 153 sites along the Texas Coast from 2018 to 2020 to sample for glass and elver eel. While no American Eel were collected, sampling gear collected other elopomorphs that undergo similar life history stages as American Eel.

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[7.6] American Eel (*Anguilla rostrata*) in Louisiana: Ages, growth rates, diet, and emerging patterns

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Recently, there has been increasing interest in American Eel (*Anguilla rostrata*) populations in Gulf of Mexico drainages due to pressure on the species from markets in Europe and Asia, impacts from passage barriers, critical habitat loss, effects of *Anguillacolooides crassus* infections, and the lack of available life history information. Very little is known about Louisiana populations, so in 2017 LDWF began work on a two-year State Wildlife Grant to collect baseline data on American Eels found in state waters, primarily utilizing bycatch from standardized sport fish sampling efforts in the Department. Data collected from specimens includes length, weight, sex, age, gonad development, stomach contents, and presence of *A. crassus*. Of the 420 eels collected, otoliths were collected from 314 specimens. Ages ranged from zero up to 16 years old, with a mean age of five years. Eels aged at nine years and below made up 95.6% of samples. Sizes ranged from 106 to 940 mm total length. Notably reduced growth rates have been observed at one site, and various stomach contents have been detected with crayfish being the most abundant prey item across the state. *A. crassus* has been detected in 104 specimens. Results will be used to inform future methods and focus of eel stock research in Louisiana, and contribute to a growing body of knowledge regarding eel conservation and management in Louisiana and the Gulf South.

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[7.7] Migration Dynamics of American Eels *Anguilla rostrata* in Ouachita-Black-Red-Atchafalaya River Navigation System, Arkansas and Louisiana

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The American Eel is a migratory, facultative catadromous species that spawns in the Sargasso Sea. We used ultrasonic telemetry to determine migratory chronology, outmigration success, and the impacts of multiple navigation dams on migrations in the Ouachita-Black-Red-Atchafalaya River navigation system, Arkansas and Louisiana. A total of 83 American Eels (597-836 mm TL) were surgically implanted with acoustic tags from 2017 to 2020 at three rivers within the Ouachita River basin. A total of 17 VR2 stations was used to detect migrating eels. Receivers were deployed in the Atchafalaya River at Morgan City and the Wax Lake Outlet, and these stations were established to confirm outmigration from fresh water to the Gulf of Mexico. Migratory chronology was highly variable among years, migrations were triggered by high flow events, and long-range movements occurred from July to February. The percentage of tagged eels that migrated varied among years between 16% and 44%. The probability of outmigration was 18% at 700 mm, 63% at 750 mm, and 93% by 800 mm TL. For 2020-2021, 94% of migrants have been detected as they reached the Gulf of Mexico. Therefore, out-migration survival through the navigation system appears high despite the abundance of large apex predators, numerous tropical hurricane disturbances, and multiple navigation dams. Migration of eels through the Gulf of Mexico or along the Florida Coast has not been documented by iTag or FACT network collaborators. This case exemplifies the utility of maintaining a passive acoustic telemetry array and the importance of open communication between multi-agency researchers as a cost-effective means for obtaining valuable biological information on species of interest over large spatial scales.

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Atlantic coast

[7.8] American Eel Updates from the Atlantic States Marine Fisheries Commission

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American eels in territorial seas and inland waters along the Atlantic coast from Maine to Florida are managed by the Atlantic States Marine Fisheries Commission. The last stock assessment for American eel was updated in 2017 and a new stock assessment for the species is currently being developed and will be available in 2022. The stock assessments use landings data and fishery-independent survey data to look at trends regionally and coastwide. Past stock assessments concluded that the American eel stock is depleted due to significant downward trends in multiple surveys, but the assessment still lacks a model that would allow for reference points, abundance estimates, or other outputs that would better inform the current management. This talk will present current trends for American eel, ongoing monitoring programs, and the results of an ageing workshop for the Atlantic states.

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[7.9] American Eel Recruitment, Demographics and Disease in Virginia

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Recent investigations of American Eel (*Anguilla rostrata*) in the Virginia portion of Chesapeake Bay have focused on glass eel recruitment and yellow eel relative abundance, demographics, and disease status due to *Anguillicoloides crassus*. Glass eel recruitment has been monitored since 2001 in the Potomac, Rappahannock, York, and James river estuaries and the timing of arrival of glass eels at the monitoring sites is consistent with the distance of each site from the mouth of Chesapeake Bay. Relative abundance of glass eels varies by multiple orders of magnitude among sites and years. Yellow eel abundance has been monitored through a standardized trawl survey and relative abundance has declined since the late 1980s and remains below the historic average. Age composition of yellow eels taken from these same systems from trawl and electrofishing surveys from 2013 to 2015 range from age-0 to age-15 with most eels around three or four years old. Adult nematode prevalence in yellow eels averaged 46.2% and the annual survival rate of disease-positive eels was lower than that of disease-negative eels. Management of American Eel can be improved through further study of eel population dynamics throughout its range in support of developing biological reference points.

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[7.10] Comparative Analysis on a Decade of American Eel Passage at Roanoke Rapids, NC and at Three Additional East Coast Hydropower Projects

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Dams impede the upstream migration of juvenile American eel, *Anguilla rostrata*, limiting their access to freshwater habitat and contributing to population declines across their range. The Federal Energy Regulatory Commission issued a new license in 2005 to Dominion Energy for operation of the Roanoke Rapids Hydroelectric Project including a condition to pass American eel upstream. Since 2009, project operators have passed eels upstream by trapping them below the dam using standard eelways and releasing the eels at various locations further upstream. We analyzed the captures at the Roanoke Rapids Dam and examined the relationships between captures and select environmental variables using Generalized Estimating Equations (GEE). In order to contextualize the results, we similarly analyzed three additional east coast rivers with a comparable decade of data and sample techniques; Conowingo River, MD, Holyoke River, MA, and St. Lawrence River, NY. The largest number of eels and longest passage season was observed at Roanoke Rapids Dam, on the southernmost river. Over two million eels were passed over the last decade, however, only 12% of that total occurred in the last five years. The number of eels captured varied annually and between projects. The GEE

analysis supported models that included terms for river discharge, water temperature, and lunar illumination at all four dams, indicating environmental conditions are interdependent. American eel were found to be highly opportunistic in their upstream movements, with peak movement events associated with high water discharge coming from the dams.

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[7.11] Long-term Monitoring Project Developed for Spring Runs in Florida's St. Johns River Suggest These Ecosystems May Be Important Habitats for American Eels

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Florida's commercial American Eel fishery is almost entirely based in the St. Johns River Basin, yet little is known about these populations. Routine biological data are collected annually in some systems in the basin through a statewide long-term monitoring (LTM) program which began in 2006. Electrofishing for LTM sampling has primarily focused on collecting community data in lentic systems and mainstem reaches of the river, but not in the adjacent spring-fed tributaries. Thus, the relative importance of these tributaries remains unknown. In 2019, development of a new monitoring program began which included eight large freshwater spring runs which flow into the river. Depending on habitat, three types of electrofishing boats were used for fish collection. Randomly selected sites were sampled in spring/summer 2019 and winter 2020 in each spring run. American Eel numbers ranged broadly within these systems, but overall fish communities did not differ significantly between seasons. From as few as three individuals up to 100 were collected in a single sampling season in individual spring runs. Eels ranged in size from 102 – 677 mm in total length. Heavily degraded systems generally produced the fewest numbers of eels but length of run, water quality parameters, and aquatic vegetation differences may have influenced population numbers or capture efficiency.

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[7.12] Non-Lethal Detection of the Invasive Swimbladder Parasite *Anguillicola crassus* in American Eels (*Anguilla rostrata*)

Danielle Lavoie & Ken Oliveira

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Anguillicola crassus is an invasive species of nematode that infects the swimbladders of anguillid eel species. This parasite is native to southeast Asia but was introduced to European and American eels through the transportation of eels for aquaculture. Heavy repeated infections cause thickening and scarring of an eel's swimbladder which can lead to changes in swimbladder gas composition. Infected eels must exert more energy to overcome these changes and effectively control their buoyancy. Silver eels can migrate thousands of kilometers to spawn in the Sargasso Sea, and any increase in energetic needs during the migration may be detrimental to breeding success. Currently, the only way to confirm that an individual eel is infected with *A. crassus* is to dissect and examine the swimbladder. This study uses a portable digital X-ray to determine the presence of *A. crassus* in individual American eels. Silver phase eels were anesthetized and X-rayed from multiple angles. Eels were then dissected to compare the contents of the swimbladder to the X-rays. Work is ongoing to analyze X-ray images and determine if the percent of infected area of the swimbladder is related to infection load. Current results show no false positives and an accuracy of greater than 70%. This digital X-ray method is a quick and non-lethal process that could be incorporated into existing monitoring programs to determine the presence of *A. crassus* in American eels without the need to sacrifice individual eels.

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Symposium: Fishin' in the City: Strategies and Techniques to Connect Urban Anglers to Diverse Fishing Opportunities

Cynthia Fox Holt, Raphael Brock, and Tom Lang, organizers

[8.1] It's Just Fishing ... or Is It? The Strategic Service of Urban and Community Fisheries

T. Lang

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Anglers are a driving force in fisheries conservation. Their financial contributions, political support, contributions of time to habitat restoration and other projects, and one-on-one angler recruitment efforts are and have been astronomical. By definition, urban and community fisheries are geographically located amongst high human population levels. They therefore inherently provide opportunities to engage many people about nature and fisheries conservation through angling. Many of these people would have otherwise never known about the work of fish and wildlife agencies. Urban fishing programs have been occurring for more than 50 years. Overtime, urban fisheries objectives, techniques, understanding and support have changed. Urban fisheries have evolved from a way to combat social unrest, to special programs with publicity as a primary benefit, to a legitimate strategic portion of fisheries management and R3 (recruit, retain, reactivate) efforts. As natural resources agencies began to realize the diversity of the angling public and the subsequent discovery of "churn" and segmentation of license buyers, programs have further evolved and diversified efforts. Regulations, management,

habitat, stocking, human dimensions, marketing, public outreach and education, funding, and partnerships are each mixed into the stew of these fisheries. They are legitimate fisheries, with intensive efforts required for success, but they also produce significant rewards. Urban and community fisheries management efforts are and will continue to be a key strategic component of agency efforts to engage current and future generations in a meaningful and positive way.

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[8.2] An overview of Kentucky's urban fishing program: challenges, opportunities, and strategies going forward

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The Kentucky Department of Fish and Wildlife Resources created the Fishing in Neighborhoods (FINs) program in 2006, partnering with local municipalities in most cases to provide anglers with quality fishing opportunities close to home. The FINs program currently includes 44 lakes statewide in cities of all sizes. Lakes are regularly stocked with "keeper-sized" catfish, rainbow trout, and sunfish throughout the year to maintain high catch rates. The FINs program is marketed heavily as an urban angling opportunity for new anglers, families, or those limited on time. Additional opportunities for urban angling in Kentucky exist, especially for more experienced anglers; however, these opportunities are not as formalized as the FINs program and messaging/marketing has been inconsistent in the past. We will review challenges to creating diverse urban angling opportunities through the perspective of a fisheries manager, noting successes, failures, and strategies moving forward.

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[8.3] Building upon success – Virginia's FishLocalVA Program

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Virginia's population has increased by more than 1.5 million since 2000, with most growth occurring in the urban and suburban areas of the state. The Virginia Department of Wildlife

Resources (DWR) manages freshwater fisheries in these developed areas by adding habitat, providing access, monitoring fish assemblages, and stocking warm and cold water fish species. Quality fishing opportunities for bass, panfish, catfish and trout have been established and maintained for several decades at urban impoundments across the state. These fisheries have proven to be quite popular with anglers in addition to adding value to outdoor recreation in our urban metropolitan areas. To better raise awareness about these fisheries and to further connect people in urban and suburban areas with fishing opportunities, Virginia launched the FishLocalVA initiative as a promotional campaign. This initiative aims to raise awareness of fishing opportunities through workshops, social media, press releases, and internet content. Our efforts are informed by human dimensions research and are prioritized as part of DWR's efforts to recruit, retain, and reactivate. The success of FishLocalVA will be measured by tracking views, page statistics, creel surveys, and license sales. Potential barriers include the availability of funding and difficulties in measuring angler awareness; DWR is planning to alleviate this by leveraging partnerships, utilizing long-term strategic planning, and expanding the human dimensions program. Preliminary results are promising and we intend to modify the program based on incoming information to best suit the needs of existing and potential anglers in urbanized areas.

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[8.4] Family and Community Fishing Program Family Fun Day Events: A Recruitment, Retention, and Reactivation (R-3) approach to the outdoors in Arkansas

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Surveys have shown that people don't fish because of a number of things, but some of the top are that it takes too long, locations are too far away, and they don't catch fish. In 1998, the Family and Community Fishing Program (FCFP) was formed. FCFP tears down those barriers by offering convenient, close to home locations and stocking them with catchable fish that make for a high success level. In an effort to increase awareness and participation in angling opportunities at FCFP locations, Family Fun Day (FFD) events were implemented as a new way of reaching a diverse population of potential outdoor enthusiasts in cities such as Pine Bluff, Texarkana, West Memphis, and Springdale. FFD events consisted of community partners and various AGFC divisions working together to provide the ultimate outdoor experience that would recruit, retain, and reactivate people into the outdoors. The main fish species promoted during the events were catchable channel catfish. An array of outdoor activities including archery, air rifles, canoeing, wild game cooking and tasting stations, focused on how to cook and clean your catch were marketed to cities in both English and Spanish. A chance to win an outdoor related activity prize package such as guided hunting and fishing trips were offered to families who registered online for a FFD event. Onsite free health and wellness screenings were also offered during FFD events. The use of AGFC's media outlets (website, newsletter, magazine, TV, radio and social media accounts) allowed for more public awareness of FFD events. This

presentation will focus on FFD events as a method to potentially increase fishing license sales and public interest in fishing and in the outdoors.

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Symposium: Managing Cold Water Designated Uses with Traditional Concepts of Natural Resource Management

Jonathan Leiman, Anna Kasko, Guido Yactayo, and Jeff White, organizers

[9.1] Managing Cold Water Designated Uses with Traditional Concepts of Natural Resource Management

Jonathan Leiman*, Anna Kasko, Guido Yactayo, Jeff White

*Presenting

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The Clean Water Act mandates that waters be characterized based on use designation, e.g., “fishable”. The ultimate intent of a cold water temperature Total Maximum Daily Load (TMDL) is to support the designated use of a wild trout fishery. Therefore, a temperature TMDL is essentially a baseline habitat model for trout fisheries. The Maryland Department of the Environment’s (MDE) Integrated Water Planning Program (IWPP) assists local jurisdictions in Maryland with implementing TMDLs. IWPP is using resource-based management in the form of geospatial analysis to drive the implementation of temperature TMDLs. The methodology parses out resources that are interconnected with the cold water designated use, and risks posed to it by linking datasets that are conventionally disjointed across multiple State programs and agencies. This framework then provides a risk assessment, and clearinghouse for data related to cold water resources for local resource managers to direct investments in the improvement of trout habitat. Concepts that aggregate information such as “mitigation potential”, are key to this framework. Furthermore, jurisdictional reporting requirements may become more useful using this methodology, because they can be linked to the resource of concern. Phase 1 Municipal Separate Storm Sewer System (MS4) permits in Maryland require that an implementation plan be developed for TMDL wasteload allocations. However, the impervious area metric is the underlying regulatory standard by which these permitted jurisdictions are assessed. As a result, the more biological components that can be connected to the TMDL model, the more impactful the permit required implementation plans can become.

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[9.2] Development of Maryland Coldwater Resources Mapping Tool

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Watersheds that support coldwater aquatic communities represent some of the most threatened ecosystems in the mid-Atlantic region. A key focus of resource agencies is identifying these stream systems and providing them the highest level of protection. The implementation of a more efficient and refined environmental review process was the impetus behind the design and development of the Maryland Coldwater Resources Mapping Tool. The Maryland Department of Natural Resources Freshwater Fisheries Program worked to compile and map all available coldwater taxa records and stream temperature data in an online GIS application. This information is projected at both the HUC12 watershed and sample point scale. Having all relevant data layers centralized in one mapping function allows for the thorough review of proposed projects on the landscape with potential thermal impacts. The Maryland Coldwater Resources Mapping Tool is currently being used by state and federal agencies, local governments, and NGOs to screen environmental review plans, target areas for additional protection/conservation, and identify locations for restoration activities.

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[9.3] Evaluating the Thermal Impact of Small Ponds in Maryland Trout Watersheds

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Surface water discharges from small farm ponds and impoundments can have adverse thermal impacts on downstream coldwater aquatic communities. These impacts can be acutely exacerbated during hot summer months when runoff events compound surface water releases with already thermally stressed conditions. The Maryland Department of Natural Resources Freshwater Fisheries Program has launched a project to locate small ponds in sensitive watersheds and attempt to quantify the impact they have on downstream water temperatures and trout resources. Over 4000 small ponds were identified in coldwater watersheds across Maryland. On average, HUC12 watersheds in the Piedmont had twice as many small ponds as HUC12 watersheds on the Appalachian Plateau. The thermal regimes of many Piedmont trout streams are already approaching the upper critical limit for trout survival. Regression analysis indicated a positive relationship with the number of ponds per HUC12 watershed and increased stream temperature regime. The direct and cumulative thermal effects of small ponds have

caused observed decreases in trout densities and inhibited the movement of trout. Extirpation of trout from many coldwater watersheds is likely due to the presence of small ponds in thermally sensitive watersheds. Identifying the location of these small ponds has allowed Maryland DNR to start prioritizing their removal and prevent additional ponds to help mitigate the thermal impacts to existing coldwater resources.

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[9.4] Groundwater dynamics define Brook Trout thermal habitat and restoration opportunities in Canaan Valley National Wildlife Refuge

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Coldwater fisheries management requires an understanding of groundwater-surface water interactions that influence stream temperature and thermal resiliency to climate change. Here we investigate the role of groundwater on thermal habitat and restoration opportunities for native Brook Trout in the Canaan Valley National Wildlife Refuge in West Virginia. We estimated the relative importance of groundwater on fish thermal habitat based on stream temperature correspondence to air temperature in summer months of 2018 and 2019 (38 and 33 sites, respectively). Results revealed several discrete zones of high groundwater influence which kept stream temperatures below physiological stress thresholds for Brook Trout. Although mainstem river sites exceeded thermal thresholds for trout, we discuss the potential for tributary confluences and beaver dam management to create thermal refugia for native trout in the mainstem river. Our study highlights the importance for spatially complex groundwater dynamics for coldwater fisheries conservation and restoration planning.

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Chas Gowan and Amanda Rosenberger, organizers

[10.1] Assessing Hydrilla Spread and Subsequent Impacts on Sportfish Communities in Lake Sinclair, Georgia

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Hydrilla is an invasive aquatic macrophyte that has negatively impacted freshwater ecosystems across the globe. It is a detriment to anthropogenic use of waterways for navigation, commercial fishing, irrigation, and hydroelectric power generation. Additionally, hydrilla has been shown to negatively impact aquatic food web dynamics when it surpasses a certain density.

Understanding how fish communities in a system are affected by hydrilla is critical so that officials can make educated decisions when managing these species. Equally important is having clear and effective methods for assessing hydrilla abundance in a system. Therefore, the objectives of this study were to 1.) determine the efficacy of satellite imagery for identifying hydrilla spread across a large reservoir, and 2.) compare pre- and post-hydrilla assessments of Largemouth Bass relative abundance, age structure, size structure, and body condition. A normalized difference vegetation index combined with PLANET satellite imagery was effective at distinguishing hydrilla cover, providing an efficient and effective means at quantifying hydrilla colonization through time. Comparative assessments of Largemouth Bass pre- and post-hydrilla resulted in few differences in dynamic rate functions. However, body condition increased, with mean relative weight being highest the year after hydrilla became established in the lake. These results equip lake managers with a more efficient and cost-effective method for accurately identifying hydrilla and provide additional insight to further our understanding on the relationship between sportfish and hydrilla in large reservoirs.

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[10.2] Bigheaded carps influence on a Clupeid species within the Tennessee River

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The Tennessee River basin and its cascade of reservoirs are home to some of the most diverse assemblages of fishes in the world. This unique system is being threatened by the ongoing invasion of Silver Carp (*Hypophthalmichthys molitrix*) and Bighead Carp (*H. nobilis*), collectively referred to as the bigheaded carps. Bigheaded carps directly compete for food resources with native Clupeid species such as the Gizzard Shad (*Dorosoma cepedianum*), and this potential

interaction could have devastating ecological and even economic consequences. Large abundances of Gizzard Shad are crucial to the Tennessee River fisheries due to their role as a forage base for carnivorous species of this system. Gizzard Shad also serve an important ecosystem function as indicator species for the condition of water quality within this system. We analyzed an extensive dataset of annual gillnetting and electrofishing data between 1990 and 2017 to assess how Gizzard Shad abundances might have changed in the Tennessee River reservoirs since the invasion of bigheaded carps began. We used a BACI design to test the changes in overall Gizzard Shad abundance before and after the arrival of bigheaded carps using an ANOVA and an ANCOVA. A Change-Point Regression was applied to test for potential pivotal changes in Gizzard Shad abundances before and after the arrival of bigheaded carps. We report shifts in abundances since the invasion of bigheaded carps, but more research is needed to connect these changes to bigheaded carps directly.

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[10.3] Impervious Surfaces on Stream Health and Biodiversity: A Study on Virginian Stream Health Using Machine Learning

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Increased population density and urbanization around the Chesapeake Bay watershed over the past 70 years has resulted in a drastic increase in impervious surfaces and is worsening the water quality of streams and tributaries flowing into the Bay. Promoting environmental stewardship with simple methods for quantifying impervious surfaces can mitigate the effects of urban development. Many softwares calculate surface types (e.g. ArcGIS); however, none are easy-to-use and readily accessible. *Tar Print* was created and published to the App Store to generate accurate impervious surface percentages without requiring surface classification to be done by the user. *Tar Print* uses a boosted tree machine learning model, which was trained with 2,250 data points labeled by surface type and has an 86% validation rate and a 98% testing rate. This study used biological stream health data that was gathered through the Virginia Save Our Streams (VASOS) program in the spring and summer of 2018 and 2019. VASOS assessed water quality at 139 unique stream sites and computed a multi-metric score for each location based on populations of indicator macroinvertebrate species, including stoneflies, worms, and mayflies. *Tar Print* quantified the impervious surface percentage (ISP) within a three-mile square around each testing site. The generated ISP values were stratified into three categories: (1) streams with 0%-5% ISP, (2) 5%-20% ISP, and (3) >20%ISP, which were classified as good, fair, and poor respectively. Results from running a one-way ANOVA with Tukey post-hoc pairwise comparisons demonstrated a significant difference in multi-metric stream health scores

among and between the three ISP health categories. These results suggest that as ISP increases, stream health is negatively impacted with regards to biodiversity.

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[10.4] Exploring Synchrony in Black Bass Angler Activity and Management Actions Across Tennessee Reservoirs

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Management agencies and other groups allocate large proportions of funding, time, and effort towards increasing angler usage and fishing quality. Despite the high cost, the results of these investments often remain unquantified by post hoc studies leaving managers to question results of their efforts and agency investments. The diversity of Tennessee reservoirs, and inherent fisheries regulatory agency infrastructure, leads to questions about lake-specific, regional, and statewide returns for investments and agency actions. In this study, we will utilize a Bayesian model choice procedure bolstered by a hierarchical modeling framework to detect landscape scale patterns in *Micropterus* spp. angler effort across a spatiotemporal gradient of large Tennessee reservoirs, and to identify reservoirs whose patterns are asynchronous with the statewide trends. We will then model these results against environmental and management action covariates to attempt to identify the primary drivers of changes in angler effort. Of particular interest is an assessment of how *Micropterus* spp. stocking could potentially increase angler effort. Effort data will be obtained from historical Tennessee Wildlife Resource Agency creel reports from 2000 to 2018. This study will help elucidate managers as to the spatiotemporal conditions in which management actions are likely to have a strong effect, as well as identifying circumstances where their efforts are not having the desired effect and could be modified or directed elsewhere to maximize efficiency.

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[10.5] Movement Patterns of Striped Bass (*Morone saxatilis*) in the Robert S. Kerr River-Reservoir Complex

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Striped Bass (*Morone saxatilis*) is an anadromous sportfish, but a few land-locked populations have been documented that are self-sustaining, including fish in the Arkansas River, Oklahoma. Gaining insight into the biotic and abiotic factors underpinning landlocked Striped Bass populations is essential for developing meaningful conservation and management actions. It is not known what portion of fish from the Arkansas River population use tailwater habitats seasonally and whether these fish represent resident populations or are transient. The objective of this study is to examine the segment-scale movement of Striped Bass in the Arkansas River, Oklahoma. Striped Bass were tagged with dual acoustic/radio telemetry tags ($n = 52$; but 10 tags were deemed expulsions or mortalities and three fish were never relocated). A total of 222 tag detections were obtained by manual tracking. Initially, we tracked fish in the lower Illinois ($n = 131$ relocations), Canadian ($n = 42$ relocations), and Arkansas ($n = 19$ relocations) rivers from Webbers Falls Lock and Dam to Robert S. Kerr Reservoir. Beginning November 2020, Robert S. Kerr Reservoir ($n = 30$ relocations) was also tracked. Five fish moved between the lower Illinois and Canadian river tailwater habitats. Ten fish were relocated in both the Arkansas River and tailwaters. Preliminary results suggest Striped Bass use tailwaters seasonally. As water temperatures drop, Striped Bass began to move out of tributaries and into the Arkansas River and Robert S. Kerr Reservoir. Future tagging and tracking will allow us to formulate spatially explicit capture-recapture models.

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[10.6] Projected Changes of the Distribution of Nassau Grouper Spawning Habitat and Its Management Implications

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Nassau Grouper (*Epinephelus striatus*) is an endangered, iconic Caribbean reef fish whose spawning and larval success may be threatened by climate change. These fish spawn within aggregations on coral reefs from December to April. Climate change threatens to reduce spawning habitat and population connectivity via thermal stress and changing currents. Previous research projects a reduction of up to 80% of spawning habitat utilizing one earth system model, but additional research is needed to assess the robustness of this

projection and understand how fisheries may be affected. This research aims to identify how climate change will impact Nassau Grouper by assessing changes in spawning habitat under climate change conditions utilizing multiple models, as well as how these changes will impact current management strategies such as MPAs and seasonal fishing bans. By utilizing a suite of climate models, climate conditions can be simulated over different time periods. These simulations can then be coupled with a non-parametric probabilistic ecological niche (NPPEN) model to assess changes in habitat suitability for spawning aggregations and how these changes will alter management effectiveness. It is expected that under future climate conditions, the probability of spawning at current-day aggregation sites will decline throughout the range of Nassau Grouper, also altering the effectiveness of management strategies. This work will be significant in that it will provide key insight into how to reduce the declines of a charismatic endangered reef fish as well as how to improve ineffective conservation strategies.

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[10.7] Population Estimates of Juvenile Atlantic Sturgeon (*Acipenser oxyrinchus oxyrinchus*) in the Edisto River, SC

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Atlantic Sturgeon (*Acipenser oxyrinchus oxyrinchus*) are an anadromous species of fish native to the eastern coast of the United States and Canada. Atlantic Sturgeon were once widely abundant, serving as a primary food source in coastal states. Decades of overfishing and habitat degradation have led to population decline, placing Atlantic Sturgeon on the federally endangered species list. Although populations of adult Atlantic Sturgeon are closely studied within South Carolina rivers, data on juvenile population abundance is lacking. Determining the abundance of juvenile Atlantic Sturgeon can show the health of the current population and act as a baseline in predicting the status of future sturgeon populations. Juvenile Atlantic Sturgeon were captured using mark-recapture sampling over a 25-year period in the Edisto River, SC. Using model POPAN in program MARK and RMark, monthly populations and yearly super populations were estimated. Yearly population estimates ranged from 263 to 2566 juveniles. Ongoing analysis of the data will look at the relationship between water quality, river discharge, and population size.

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[10.8] Two-dimensional photogrammetry as a tool for morphological analysis: an example with shark teeth

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For many mobile, marine species, collecting functional trait data is a nontrivial, costly endeavor. Natural history museum collections can be an instrumental resource for morphological analysis of these species. Digitizing collections has increased the accessibility of material and the potential utility of invaluable collections, but there is a lack of standardized protocol for the best practices of capturing specimen data. Two-dimensional photogrammetry is an increasingly appealing approach, as it requires minimal training, can accommodate a wide range of specimen sizes, and is time- and cost-effective. We applied two-dimensional photogrammetry to the digitization of shark teeth to exemplify a comprehensive methodology for morphological analysis. We captured tooth crown shape for 57 species and compared them via elliptical Fourier and principal components analyses. We described this process's key components—locating specimens, choosing material, photographing, post-processing, and analysis— and their associated challenges. Through collating our experience into a set of recommendations, we aim to streamline this process for future photogrammetric studies and to define the instances where this technique is applicable.

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[10.9] Collapse of an Historic Oyster Population: Impacts of Habitat Loss on Estuarine Populations in Apalachicola Bay, FL

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Reef structures created by eastern oysters (*Crassostrea virginica*) provide important habitat to many estuarine species, utilized for foraging, reproduction, and refuge from predation. In 2012, the historically productive oyster population in Apalachicola Bay, FL collapsed suddenly. While the causes of the collapse have undergone much scrutiny, little has been done to understand the effects of the loss of the oyster population, and thereby the reef habitat, on other members of the estuarine community. Here we utilize fisheries-independent monitoring data collected by the Florida Fish and Wildlife Conservation Commission in Apalachicola Bay from 1998-2018 to create a standardized time series of catch per unit effort for each of twelve estuarine species that rely or are frequently encountered on oyster reefs. These time series represent the standardized relative abundance of each species through time and can be used to identify changes in the individual populations. We examine each time series using change-point analysis methods to detect significant changes in fish population abundance mean and variance with respect to the oyster population status. Change-points identified concurrently with or shortly after the oyster population collapse may indicate an important relationship between oyster reef habitat and the species of interest. This study may provide further evidence that oyster reefs constitute essential habitat for several estuarine species, and mismanagement of oyster populations may have wide-reaching implications for community dynamics and ecosystem function.

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[10.10] Effects of physical and oceanographic characteristics on reef fish community structure at GRNMS both inside and outside of an MPA

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Understanding the physical and oceanographic differences across reef habitats can help scientists to evaluate how effective MPAs are at preserving and protecting the fish community. However, the traditional methods used to assess fish communities on temperate reefs are limited. In this study, Gray's Reef National Marine Sanctuary (GRNMS) was surveyed using echosounder technology to assess the physical and environmental controls that drive reef fish community structure. Here we focus on four echosounder transects, two within the boundaries of the MPA and two outside of it. Oceanographic information was collected along each echosounder transect simultaneously using a CTD profiler. Preliminary results from the echosounder data suggest that there are differences in the structure of fish communities driven by physical characteristics, such as the presence of relief and biofouling

community density along the seafloor. Stratification, which varies with time, also plays a role in these community differences. Gathering and assessing more data on the physical and oceanographic characteristics at each GRNMS location will help researchers better understand the fish community structures and the driving factors behind these communities, both inside and out of the MPA.

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[10.11] Olfactory discrimination in juvenile bonefish (*Albula goreensis*) in response to food, predator, and habitat cues

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Taxis and orientation within the environment via sensory signals is essential for survival throughout ontogeny. Bonefish *Albula* spp., fishes that are important to coastal recreational fishing throughout the Caribbean, are at their most vulnerable to predation as juveniles, and are physically limited in their ability to emigrate from sub-optimal environmental conditions at scales beyond their realized habitat. Therefore, the early onset ability to orient and move within suitable nursery habitat is critical to survival. Little is known about how juvenile bonefish use sensory systems like olfaction to find food, avoid predation, and locate suitable habitat. Understanding the role of olfactory discrimination, an organism's ability to differentiate scents and make decisions based upon those scents, is imperative to assessing how coastal anthropogenic activities could result in a population bottleneck and reduced recruitment, as current bonefish stocks in South Florida are experiencing. We used a Y-maze decision experiment to determine if juvenile bonefish, *Albula goreensis*, use olfaction for locating food (commercial feed), avoiding predation (predator discharge), and finding suitable habitat (near-shore reef water). Olfactory cues of food and near-shore reef water significantly influenced juvenile bonefish movement, while the predator scent elicited a random response. These results suggest that olfactory cues and chemotaxis play an important role in juvenile bonefish movement and behavior, a process that may be susceptible to disruption through anthropogenic activities. (Funding provided by the Florida Institute of Technology).

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[10.12] Temporal and spatial dynamics of sympatric Redbreast Sunfish and Green Sunfish populations in an increasingly urbanized watershed

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We studied the dynamics of sympatric Green Sunfish (*Lepomis cyanellus*) and Redbreast Sunfish (*Lepomis auritus*) in an urban tributary of the Yellow River in Lawrenceville, GA. Specifically, we investigated changes in recruitment and abundance of both species in an increasingly developed watershed. Standardized backpack electrofishing was conducted to sample and measure sunfish (mm TL) at four fixed transects, with two transects established upstream and two downstream (near the confluence with Yellow River) to account for spatial variation in sunfish abundance. Total shock time (sampling effort in min.) was recorded to calculate CPUE for each species. Eighty-one total sampling surveys were conducted from 2016 to 2020, with 39 surveys performed at upstream transects and 42 downstream. A major deforestation event in spring 2018 adjacent to our upstream transects coincided with significant changes in recruitment and abundance of each species, although annual hydrologic and temperature variation also played a role. Over time, recruitment and abundance of redbreast sunfish declined, whereas recruitment and abundance for green sunfish markedly increased (species*year interactions; abundance: $F = 7.59$, $P < 0.001$, recruitment: $F = 2.61$, $P = 0.03$). We accounted for a highly significant spatial effect in both models (higher recruitment and abundance at upstream transects; $P < 0.001$). Annual variation in the hydrologic regime also provided evidence that green sunfish thrive in highly disturbed aquatic habitats with increased recruitment in years with higher variation in waterflow and in years with highest maximum flows. Our study highlights the importance of considering temporal and spatial effects of urbanization on stream fish populations in relation to increased urban land use. Additional analyses of size structure shifts and comparisons of age, growth, and mortality between species are currently underway.

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Contributed papers – Professionals

Eric Hallerman, organizer

[11.1] Land-use and habitat characteristics associated with the presence of the Sandhills Chub (*Semotilus lumbee*)

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The Sandhills Chub (*Semotilus lumbee*) is a leuciscid only found in the Sandhills ecoregion of North and South Carolina, and prefers small, clear, headwater streams with sand or gravel substrate and little aquatic vegetation. Because of its limited geographic distribution, the Sandhills Chub is a species of conservation concern, and as a result, has been listed as imperiled by the American Fisheries Society and as a Species of Special Concern by the South Carolina Department of Natural Resources (SCDNR). It has been extirpated from several locations in South Carolina and is at risk to disappear from others due to habitat alteration as a result of impoundment of streams, residential and industrial development, and agriculture. This study aims to identify the limiting habitat factors for Sandhills Chub populations by comparing habitat, land-use, and biological characteristics of locations where Sandhills Chub are present with locations where they have been extirpated or are absent. Logistic regression will be used to identify which habitat, land-use, and biological features are associated with the presence and absence of Sandhills Chub. Results from this study will help guide management decisions for the future conservation of Sandhills Chub.

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[11.2] Population Demographics of Small Impoundment Bluegill Fisheries in Northern West Virginia

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Bluegill are often one of the most abundant sportfishes in public waters and are popular with many anglers. However, in West Virginia, little management of this species occurs, and no specific regulations exist. We assessed Bluegill populations within five small impoundments in northern West Virginia with varying management regimes. We compared estimated population demographics across impoundments to aid future management efforts. From 2016–2018, 3,382 Bluegills were collected utilizing boat electrofishing, of which 561 individuals were used for age and growth analysis. Bluegill population characteristics (CPUE, age, growth, mortality) varied across each small impoundment. Bluegill in impoundments with high densities of Gizzard Shad and Common Carp grew slower and failed to reach preferred length (200 mm). Bluegill in impoundments with high Largemouth Bass densities had faster growth and reached preferred length in 3 – 5 years. Total annual mortality was higher (65 – 80%) in impoundments with good angler access and high fishing pressure compared to total annual mortality in other impoundments (42 – 53%). Bluegill as old as age-9 were collected, but proportion of older individuals varied across impoundments. Old-age Bluegill were less common in impoundments

with the highest mortality rates. Results suggest that Bluegill in impoundments with high mortality rates and fast growth have the potential to reach large sizes and may benefit from reduced harvest of larger, older individuals. These results will be beneficial for future management efforts geared toward enhancing Bluegill fisheries.

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[11.3] Investigating Drivers of Seasonal Shifts in Fish Abundance in the Homosassa River System

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In the southeastern U.S., some subtropical marine fishes use thermal refugia during winter at the northern limits of their range. The Florida Fish and Wildlife Conservation Commission (FWC) recently completed the Springs Coast Fish Community Assessment Project which found marine species abundance (dominated by Common Snook and Grey Snapper) increased in several spring-fed rivers during winter, consistent with use of the warm springs as thermal refugia. The Homosassa River, located centrally among several spring-fed rivers in north-central Florida, was chosen to investigate: 1) seasonal water quality parameters (temperature, salinity) and their influence on the timing of marine species immigration, 2) the timing of the winter influx and subsequent interactions between marine (Common Snook, Grey Snapper) and freshwater fishes (Largemouth Bass, Redear Sunfish), and 3) fish movement and habitat associations by marine and freshwater fish species in the mainstem and backwater habitats of the river system. The Homosassa project uses acoustic telemetry, electrofishing, mark-recapture, habitat assessment and abiotic measurements to investigate species interactions, distribution and movement in the Homosassa River system. The Homosassa project used an ecosystem-based approach to better understand and protect the species that depend on this unique freshwater environment. Data provided in this study can assist resource managers with enhancing aquatic habitat for resident freshwater fish species, while providing important refugia for migratory marine species. Additional work, using the methods outlined in this study, can be adapted to benefit other coastal spring-fed rivers similar in ecological nature.

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[11.4] The extent of seasonally suitable habitats may limit forage fish production in a temperate estuary

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The sustained production of forage is critical to advancing ecosystem-based management, yet factors that affect local abundances and habitat conditions necessary to support forage production remain largely unexplored. We quantified suitable habitat in the Chesapeake Bay and its tidal tributaries for four key forage fishes: bay anchovy, juvenile spot, juvenile weakfish, and juvenile spotted hake. Observations from monthly surveys from 2000 – 2016 were coupled with hindcasts from a spatial-interpolation model of dissolved oxygen (DO) and a 3-D hydrodynamic model of the Chesapeake Bay. Boosted regression trees were used to identify influential habitat covariates from among 18 covariates of salinity, temperature, DO, depth, and current speed that were considered for each species. Habitat suitability models were constructed from the species-specific influential covariates, and habitat suitability indices were used to estimate seasonal and annual extents of suitable habitats. Seasonal variations in suitable habitat extents were more pronounced than annual variations. Areas near shorelines served as suitable habitats for juvenile spot and juvenile weakfish, indicating the importance of these shallow areas for production. The relative abundances of juvenile weakfish and juvenile spotted hake varied independently of the spatial extent of suitable habitats, indicating that habitat was not limiting for these species in Chesapeake Bay. In contrast, the relative baywide abundances of juvenile spot and bay anchovy were significantly related to the extent of suitable habitats in summer and winter, respectively; as such, estimates of the minimum habitat area required to produce a desired abundance or biomass of these forage fishes may be used to establish quantitative habitat targets or spatial thresholds that may serve as reference points for management. In an ecosystem-based approach, areas that persistently provide suitable conditions for forage species, such as shoreline and tributary habitats, may be targeted for protection or restoration, thereby ensuring sufficient production of forage for predators.

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[11.5] A meta-analysis of environmental flow standards and thresholds in Texas rivers

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The protection of natural flow regimes promotes the sustenance of riverine ecosystems and their services to society. The Texas Parks and Wildlife Department, in collaboration with other agencies and stakeholders, have developed strategies for restoring and protecting natural hydrologic regimes. These strategies have included research programs aimed at identifying environmental flow targets, hydrologic alteration, and flow deficits. Among these concerted efforts is the development of a statewide Environmental Flow Information Toolkit (EFIT), which can be used to evaluate environmental flow targets and flow deficits to design strategies for environmental flow implementation. To inform the EFIT, literature examining ecological responses to hydrologic alteration was compiled for the entire state of Texas and surrounding regions, such as the Great Plains Landscape Conservation Cooperative. Over 1,500 sources were identified and, of these, roughly 260 contained information relevant to fish species of greatest conservation need in Texas. Through a meta-analysis, information on hydrologic variation and ecological responses were extracted from literature with particular emphasis on documenting scientifically based hydrologic thresholds, i.e., tipping points beyond which river ecosystems experience ecological degradation, such as loss of fish species, habitat, or important services. These thresholds were compared to previous hydrologic thresholds established by Texas Instream Flow Program studies and thresholds recommended by expert science teams during the Texas environmental flow standards process. Additionally, the literature review yielded a gap-analysis of fish SGCN and river systems that would benefit from more instream flow studies.

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[11.6] Validity of daily and annual age estimation and back-calculation methods for juveniles of a subtropical-tropical species, the Atlantic tarpon (*Megalops atlanticus*)

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Understanding early life history processes for fishes is critical because they can affect other life history characteristics. Additionally, habitats used by young fishes are often the target of conservation and restoration efforts and growth has been proposed as a metric for evaluating the quality of habitats. Accurate estimates of growth rely on the validation of aging and back-calculation methods, which in turn allow for informed fisheries management decisions. We used oxytetracycline to chemically mark juvenile tarpon (*Megalops atlanticus*) for an annual increment

validation study, a controlled back-calculation validation study, and a controlled daily increment periodicity study. Oxytetracycline marks were realized on all the otoliths of recaptured tarpon ($n = 22$), prior to a newly formed annulus, validating true age and that one annulus is deposited yearly. However, annuli in scales were more easily identified by readers, thus leading to more accurate and precise estimates of age from scales (100% accuracy of age estimates for age-1 fish) compared to otoliths (88% accuracy). Marginal increment analyses indicated that annuli are deposited during spring (March-April) on juvenile tarpon scales and otoliths. Back-calculated lengths from otoliths were significantly different compared to measured lengths, and bias of length estimates differed between months. Daily increment estimates from otoliths were relatively accurate during September but not during October, leading to mean error of 21 d (37% error) across the 56-d study period. Results from this study highlight the importance of validating age estimates for juvenile fishes, particularly for subtropical and tropical species that have protracted spawning seasons.

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[11.7] Documentation of Extreme Catch-and-Release Exploitation and Abundance in a Southern Riverine Muskellunge Population from a 4-Year Tagging Study

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Muskellunge *Esox masquinongy* fisheries have increased in abundance and popularity in numerous southern and mid-Atlantic rivers. However, the paucity of information for these fisheries has limited biologists' ability to effectively manage these resources. We simultaneously utilized a four-year fishery dependent tag-return and a fishery independent capture-recapture study to estimate angler exploitation and abundance of the James River Muskellunge fishery in Virginia. From 2016 through 2019, we tagged 756 individual Muskellunge with passive integrated transponder (PIT) and dart tags in the James River, Virginia during winter electrofishing surveys and conducted an angler tag-return study to estimate rates of catch-and-release exploitation and abundance. Estimates of catch-and-release exploitation, based on a novel use of the Brownie dead-recovery model, indicated differences in catch-and-release exploitation between the upper ($u_j = 0.59$) and lower ($u_j = 1.04$) reaches of our study area. These estimates of catch-and-release exploitation are high, warranting further investigation into the effects of this level of angling on the James River Muskellunge population. Densities of adult (≥ 762 mm) Muskellunge in the upriver (0.80/ha) and downriver (0.50/ha) reaches of the upper James River were also high, but comparable to densities observed in lakes and at least two other riverine systems. However, when we evaluated the site-specific density for a 1.6 km reach

below Scott's Mill Dam in the lower portion of our study area, the population densities were extreme (4.69/ha).

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[11.8] NCIFD: An R Package for the Inland Fisheries Division of the North Carolina Wildlife Resources Commission

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The Inland Fisheries Division (IFD) of the North Carolina Wildlife Resources Commission has several online databases that field staff use to store and retrieve data; however, many of our databases produce query results that are messy and difficult to analyze. Unfortunately, our agency IT resources are currently focused elsewhere, so we are exploring how we can use R to make our data more useful and accessible. The surging popularity of R is partly due to its package system which makes it relatively easy to share data and functions. Therefore, we developed an internal R package 'NCIFD' to store and distribute data that is otherwise only available from our online databases. We discovered that R packages are excellent data containers for the following reasons: First, R scripts automate the clean-up of messy data while saving the data in the package. Second, additional R scripts perform automated tests to verify the integrity of the data. Third, R packages can store metadata which is retrieved with R's built-in help() function. Fourth, data stored in an R package typically requires 90% less hard drive space than equivalent spreadsheets. Finally, R scripts can also export the package datasets in CSV format for traditional spreadsheet users. In addition to storing data, NCIFD also stores homemade functions that the staff develop to address their specific data analysis needs. Thus, NCIFD provides an internal outlet for us to publish and share our code accomplishments.

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[11.9] Statewide Evaluation of Oklahoma's Lotic Blue and Flathead Catfish Populations

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Surveys of anglers identified Blue Catfish *Ictalurus furcatus* and Flathead Catfish *Pylodictis olivaris* as two of the most targeted species in Oklahoma's rivers. Despite their popularity, management of lotic catfish populations has received very little attention in Oklahoma. Consequently, lotic catfish population dynamics data are almost non-existent. To document contemporary Blue Catfish and Flathead Catfish population dynamics and establish a baseline for monitoring of trends, lotic catfish surveys were initiated in 2016 by the Oklahoma Department of Wildlife Conservation's Stream Program. Since then, seven rivers throughout Oklahoma have been sampled using low-frequency electrofishing. The data collected provides insight into relative abundance, size/age structure, growth rates, mortality rates, age at maturity, sex ratios, and fecundity. Initial results suggest there may be intraspecific differences in population characteristics among rivers, but inferences in some rivers are hindered by sample size, especially for Flathead Catfish. To compare growth rates among rivers and/or regions, a statewide standard growth equation was estimated for each species to calculate relative growth indices. Mortality caps were assessed to determine the maximum mortality rates that can sustain various size structure objectives. To guide future survey efforts, we used current survey data to estimate the precision of relative abundance estimates and determine the number of surveys required to attain a relative standard error of 25%. Future surveys will continue to add new rivers to the dataset and augment initial sample sizes. Ultimately, data will be used to set management objectives and evaluate various catfish harvest regulations under an adaptive management framework.

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[11.10] Investigation of Fish Species Susceptibility to Antimycin a for Control Efforts with Invasive Asian Carp

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Invasive fish are often detrimental to aquatic ecosystems, and past eradication efforts in the U.S. and Canada have used a variety of piscicides, including the discontinued formulation Fintrol. The goal of this research is to contribute data toward a potential U.S. Environmental Protection Agency re-registration of Antimycin A (Ant-A), the active component of Fintrol. Ant-A binds to the Qi subunit of cytochrome B within complex III of the inner mitochondrial membrane, resulting in cell death due to a halt in adenosine triphosphate (ATP) production, thus inhibiting

respiration. Past studies which exclusively tested organismal level response show variation in lethality among species. Differences in species susceptibility may be determined by gaining insight on variation of the binding site of Ant-A. In line with the Asian Carp Action Plan, the primary objective of this research is to measure effects on mitochondrial function *in vitro* after exposure to analytical Ant-A concentrations. Blood from Bighead Carp (*Hypophthalmichthys nobilis*), Silver Carp (*Hypophthalmichthys molitrix*), Grass Carp (*Ctenopharyngodon idella*), Black Carp (*Mylopharyngodon piceus*), Common Carp (*Cyprinus carpio*), Bluegill Sunfish (*Lepomis macrochirus*), Fathead Minnows (*Pimephales promelas*), Walleye (*Sander vitreus*), Yellow Perch (*Perca flavescens*), and Channel Catfish (*Ictalurus punctatus*) will be incubated with a range of Ant-A concentrations for eighteen hours, then mitochondrial function will be measured. Additionally, the species' target protein amino acid sequences of the Qi subunit from an international database will be compared as evidence of sequence similarities and differences that may further aid in understanding mitochondrial and species sensitivities.

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[11.11] Implementing Integrated Pest Management Strategies on Small Impoundments

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Determining possible treatment techniques for the control of invasive and undesired species of aquatic vegetation and algae in small impoundments can be a struggle for resource managers. Small impoundments can become overrun with nuisance aquatic vegetation and harmful algal blooms that inhibit users from participating in recreational activities, and often pose unique challenges when trying to manage and influence fisheries. Implementing an Integrated Pest Management (IPM) allows for a comprehensive approach using a variety of methods to control algae and aquatic vegetation in a small impoundment. Common management strategies are grouped into three categories: biological, chemical, and mechanical. Each management strategy plays a key role in successful pest management and knowing when to implement them, along with an understanding of their advantages and disadvantages associated with their efficacy and public perception, is critical to the success and health of a pond and fishery.

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