Reducing the Gap Between Science and Public Opinion

WILD TROUT SYMPOSIUM

Proceedings of the Wild Trout XIII Symposium

Holiday Inn, West Yellowstone, Montana
September 27 - 30. 2022

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Proceedings of the Wild Trout XIII Symposium

West Yellowstone, MT
September 27-30, 2022

Symposium Chair: Kevin Meyer
Proceedings Editor: Jim Gregory
Proceedings Designer: Kristi Stevenson

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A Reminder . . , WTXIV will be held in 2025
Stay in contact through:
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and through our Facebook site!

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Program Schedule

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12:00	Lunch
1:30	Contributed talks session 2
3:10	Break
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8:00	Fisheries Film Fest

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2:40	Free time, on your own
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6:30	Continental breakfast
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9:40	Break
10:00	Contributed talks session 8
11:20	Closing remarks
11:40	Adjourn
12:00	Post-symposium debrief and WTXIV planning

Event Sponsors

The Wild Trout Symposium gratefully appreciates the support provided by these agencies and individuals. These contributions help preserve, protect, and perpetuate wild trout around the world for the generations to come. For more information concerning sponsorship opportunities, visit us at www.wildtroutsymposium.com.

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Idaho Fish and Game

The mission of the IDFG is that all wildlife, including wild animals, wild birds, and fish, within the state of Idaho, is hereby declared to be the property of the state of Idaho. It shall be preserved, protected, perpetuated, and managed. It shall only be captured or taken at such times or places, under such conditions, or by such means, or in such manner, as will preserve, protect, and perpetuate such wildlife, and provide for the citizens of the state and, as by law permitted to others, continued supplies of such wildlife for hunting, fishing, and trapping.

Trout Unlimited

Founded in Michigan in 1959, Trout Unlimited today is a national non-profit organization with 300,000 members and supporters dedicated to conserving, protecting and restoring North America's coldwater fisheries and their watersheds. Our staff and volunteers work from coast to coast to protect, reconnect, restore and sustain trout and salmon habitat on behalf of today's anglers and coming generations of sportsmen and women who value the connection between healthy, intact habitat and angling opportunity.

Event Sponsors

The Southern Division of the American Fisheries Society

The Southern Division of the American Fisheries Society (SDAFS) was established in 1952 and it includes AFS members from the southern states who meet regularly to discuss research findings and management applications pertaining to the region. Current membership of SDAFS is composed of AFS members in good standing residing in, or having an interest in the fishery resources and related technical information of Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Maryland, Mississippi, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, Virginia, West Virginia, the District of Columbia, the Commonwealth of Puerto Rico, the Virgin Islands, and other Caribbean Islands.

Urbani Fisheries LLC

Urbani Fisheries LLC is a family owned and operated design-build firm consisting of biologists, geohydrologists, ecologists, and fly fishing enthusiasts that utilize a holistic, nature based approach to enhance cold water fisheries. As second generation River Restoration specialists, we've been at the forefront of active freshwater restoration for over 40 years. Our goal is to enhance, restore, and create biologically sound aquatic habitats from large rivers to small ponds.

Washington Fish and Wildlife

The Washington Department of Fish and Wildlife is dedicated to preserving, protecting, and perpetuating the state's fish, wildlife, and ecosystems while providing sustainable fish and wildlife recreational and commercial opportunities. Headquartered in Olympia, the department maintains six regional offices and manages dozens of wildlife areas and hundreds of water access areas around the state, offering fishing, hunting, wildlife viewing, and other recreational opportunities for the residents of Washington.

Supporting Sponsors

American Fisheries Society

The American Fisheries Society (established 1870 in New York City), is the "world's oldest and largest organization dedicated to strengthening the fisheries profession, advancing fisheries science, and conserving fisheries resources." It is a member-driven 501(c)(3) nonprofit organization governed by an executive director, a governing board, and officers who are guided by the AFS's organizational documents, a constitution, and a set of rules. The AFS's stated mission is "to improve the conservation and sustainability of fishery resources and aquatic ecosystems by advancing fisheries and aquatic science and promoting the development of fisheries professionals." AFS publishes five peer-reviewed fish journals, books, and the magazine Fisheries, organizes seminars and workshops that promote scientific research and fisheries management, and encourages fisheries education through 58 university-based student subunits. AFS has 48 chapters comprising four geographic regions in North America — North Central, Northeastern, Southern, and Western — and includes two "bi-national" chapters (the Atlantic International and Washington-British Columbia chapters) and a Mexico chapter.

Colorado Trout Unlimited

Founded in 1969, Colorado TU is the state's leading non-profit, non-partisan organization providing a voice for Colorado's River. As Colorado's financially self-sustaining, grassroots arm of the national organization Trout Unlimited, Colorado TU is independently governed by a 44-member volunteer board including the presidents of our 24 local chapters across Colorado. Colorado TU leverages the power of its over 12,000 members who contribute approximately 44,000 volunteer hours annually to restoration, education and other local conservation projects, equivalent to the power of 22 full-time employees.

Floy Tag

FLOY TAG Inc. is the leader in the manufacture of visual marking devices for fish & wildlife. Since 1957 FLOY TAG has been assisting marine biologists in obtaining valid data on a wide variety of fish and crustaceans. The company has produced more than 100,000,000 tags since that time. FLOY serves government agencies, international associations, university fisheries schools, and individual consultants worldwide.

Oregon Fish and Wildlife

Protecting and enhancing Oregon's fish and wildlife, and the habitats they use, for the use and enjoyment of present and future generations is at the heart of what the Oregon Department of Fish and Wildlife does. Oregon's year-round fishing spans the state from native redband trout in eastern deserts to rockfish and halibut in ocean waters – with lots of salmon, steelhead, trout and warmwater fishing in between.

Pennsylvania Trout Unlimited

The Pennsylvania Council of Trout Unlimited serves as a unified voice for the 49 local chapters representing more than 13,000 members of Trout Unlimited statewide.

Western Native Trout Initiative

The Western Native Trout Initiative (WNTI), established in 2006, is a public-private Fish Habitat Partnership that works collaboratively across 12 western states to conserve, protect, restore, and recover 21 native trout and char species. Covering over 1.75 million square miles of public and privately managed lands, WNTI and its partners combine science-based assessments with expert and local knowledge to establish joint priorities for native trout conservation at a landscape scale.

Wright Water Engineers

Wright Water Engineers, Inc. (WWE) is a full-service water resource, environmental, and civil engineering firm in Denver, Colorado (established 1961) with offices in Glenwood Springs, Colorado (established 1974) and Durango, Colorado (established 1990). WWE has a staff of approximately 45 people who include senior level engineers, hydrologists, scientists, biologists, chemists, geologists, and hydrogeologists. WWE professionals are Professional Engineers (P.E.s), Certified Professionals in Erosion and Sediment Control (CPESCs), Certified Floodplain Managers (CFMs), Ph.D.s, and Leadership in Energy and Environmental Design (LEED) Accredited Professionals. Most WWE employees have worked for the firm 15 years or longer and many are recognized experts in their respective disciplines. WWE staff members regularly testify as experts, publish, teach continuing engineering education, and are otherwise highly involved with professional activities.

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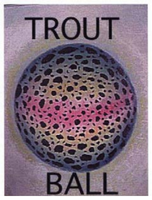
Since 1964, Smith-Root has proudly partnered with fisheries scientists to develop solutions for the fisheries conservation community.

Special Thanks

Performance Fly Rod

Dave Lewis, a lover of nature and visual arts, as well as stellar custom rod maker, spent many an hour capturing the beauty of fish and fishing across the country. The wild trout community lost Dave to cancer in 2008, after a long full life of appreciating the spirituality of wild trout country. His photos grace the WT Symposium web pages and we thank him for the opportunity to experience these extraordinary images and through them, him.



Trout Ball by Greg Keeler

Greg Keeler generously crafted the song "Born to be Wild" for the WTIX Symposium in 2007, in West Yellowstone, Montana. Professor Keeler teaches English by day at Montana State University, Bozeman and entertains the rest of us with wonderful, irreverent, original songs concerning all things fishing. Take a moment to visit his website for captivating art and prose. Thank you, Dr. Keeler, for sharing your gifts with the Wild Trout Symposium.

Advanced Telemetry Systems, Inc.

Advanced Telemetry Systems, Inc. is an innovative, science and engineering-based radio telemetry provider, dedicated to supporting biologists worldwide. Our commitment to our customer's success has helped us build a reputation as the leader in fisheries and wildlife research. We've partnered with preeminent researchers to design the most reliable radio tracking systems ever deployed. The experienced professionals at ATS possess a thorough understanding of the challenges you'll face in the field, and we're ready to provide you complete solutions - and valuable customer support - for your study's radio tracking equipment needs. The Wild Trout Symposium gratefully acknowledges ATS and their support of the Aldo Starker Leopold Medal. Dick Reichle and his wife, Laura, stepped forward to make the casting of these bronze medals possible. Thank you.

Wild Trout XIII Awards

Awards Committee:
Andy Dolloff and Bob Gresswell

Awards Selection

The Awards Committee is chosen from the membership of the Organizing Committee. Several months prior to the symposium, the committee posts requests for nominations along with applications and instructions on the symposium website. The following awards were made at Wild Trout XIII in 2022.

The applicant pool is solicited from university faculties and representatives of AFS student chapters, Trout Unlimited members and chapters, and a broad cross-section of scientists and managers.

Awards have been created over the years by the organizing committees of the Wild Trout Symposium in honor of individuals who have made outstanding contributions toward the advancement and stewardship of wild trout. Notifications of these awards occur in the summer prior to the meeting and recipients are encouraged to attend the symposium to personally receive their award during the Awards Banquet. The following awards were made at Wild Trout XIII in 2022.

Aldo Starker Leopold Wild Trout Award

The Wild Trout Symposium Organizing Committee established the Aldo Starker Leopold Wild Trout Medal in 1984 as a continuing memorial to this distinguished naturalist, teacher, author, and an important participant in these symposia, who was the son of Aldo Leopold. Typically, two Wild Trout medals are conferred, one to a professional and one to a non-professional individual who in the eyes of peers have made long-time and significant contributions to the enhancement, protection, and preservation of wild trout. In 2022, two medals were awarded to wild trout professionals; no nominations were received in the non-professional category.

Aldo Starker Leopold Wild Trout Award – Professional

The Aldo Starker Leopold Wild Trout Award in the Professional category is presented to an individual for outstanding contributions to the protection and conservation of wild trout resources.

A qualified nominee is or has been significantly involved in any or all of the following areas of wild trout management or conservation:

- Educational activities (mentoring, etc.)
- Raising awareness of wild trout issues and conservation
- Support for agency conservation activities(e.g. fund raising, volunteering time, etc.)

Congratulations Barry Nehring

Barry Nehring (Research Biologist, Colorado Parks and Wildlife retired) has dedicated a lifetime to the conservation and management of wild trout. While best known for his vast body of scientific contributions to the problem of Whirling Disease in Colorado and the intermountain west, Barry's research has been pivotal in efforts to improve wild trout management more broadly including seminal research in the areas of minimum in-stream flows, gas super-saturation below impoundments, special length limit regulations for wild trout fisheries, habitat suitability modeling, and electrofishing injury. As much as he is recognized as a leader, Barry is a consummate team player. From the beginning of his career, Barry used his intellect, knowledge, enthusiasm, and powers of persuasion to build a vast network of collaborators that has included biologists, domestic and international academic researchers, agency scientists, private landowners, and non-governmental organizations. Through the unofficial but very real "Nehring farm system" Barry mentored over 100 career professionals, many of whom fill vital roles in management, research, law enforcement, and administration not only within the ranks of his state agency but across the nation. His relevance to trout management in Colorado is enduring; today's management biologists depend not only on his foundational research publications but also on sampling methods and monitoring stations he established in the early 1980s. In this, Barry has achieved what is perhaps the ultimate career goal: to

Funded in 2000 by a grant provided by Richard and Laura Reichle and Advanced Telemetry Systems Inc.

leave a mark – both physically and virtually - that persists long after you hang up your waders.

WALKING AND WORKING IN THE PRESENCE OF GIANTS

R. Barry Nehring

When my dear friend and colleague Luciano Chiaramonte called and let me know that I was to be honored at this Wild Trout Symposium as a recipient of the **Aldo Starker Leopold Wild Trout Medal** I all but fell out of the chair I was sitting in. The phrase "Standing on the Shoulders of Giants" popped into my thought process and cycled through my brain for more than a week. While I am much too short of stature to have ever been able to climb up and stand on anyone's shoulders, I have known for decades that I have been blessed to be **Walking and Working in the Presence of Giants.**

The only other Wild Trout Symposium I ever attended was Wild Trout III in September 1984, just eight summers into my career as a fisheries researcher with the Colorado Division of Wildlife (CDOW). At that meeting, two things happened that I still remember to this day. **First**, at that meeting the **Aldo Starker Leopold Award** came into existence. **Second**, the closing keynote address titled ***The Worth of a Wild Trout,*** was given by Dan Abrams, angler, author and Minister of the First Baptist Church in Jackson, Wyoming. That address for me and many, many others present was **never** to be forgotten, nary a dry eye in the crowd of some 200+ attendees.

Dr. Robert Behnke was the first professional to receive the **Aldo Starker Leopold Award.** Bob Behnke was the first of numerous **"Giants"** in the **field of freshwater fisheries biology** that I have had the honor and pleasure of walking and working with over the past half-century. As junior in fisheries science at Colorado State University in the summer of 1970, I spent several weeks working with and traveling around Colorado in an old International Travel-All collecting Cutthroat Trout for Dr. Bob's fish collections. You could ask "Doc" a question and then just sit back and listen attentively for the next hour at least.

Dr. Behnke served as chairman of my graduate committee. Upon finishing my MSc degree in Fishery Biology, I landed a job as "Fisheries Advisor" to the Iran Department of Environmental Conservation in early 1973. In 1974 Dr. Behnke came to Iran and we worked together for the better part of a year. When I returned to Colorado in late 1977, Dr. Behnke was advisor of two Iranian graduate students sent to Colorado State University to major in Fisheries Biology and Range Science. Dr. Bob and I became dear friends and colleagues that lasted right up to the time of his death, spending many hours together each year talking about the research efforts that I was involved in.

In the early 1980s Dr. Kurt Fausch became the second professional **Giant** to grace my life. I could go on for hours about what this Giant of a man has meant to me. Cold water streams and the aquatic life that they support became a life-long passion we have mutually shared. Suffice it to say that together we have come to **DEEPLY** understand ***The Worth of a Wild Trout***.

Beginning in the fall of 1993, a truly unique and life-changing Latin term entered my vocabulary. For the next decade of my life, I never once held a flyrod or flicked a dry fly to a rising wild trout, as Myxobolus cerebralis, a microscopic parasite known to cause whirling disease in some species of trout and salmon dominated my research studies until my retirement in 2011, and still occupies some of my time right up to the present. By the mid-1990s, many more **"Giants"** came into my life. Droctors Ron Hedrick, Mansour El-Matbouli, Jerri Bartholomew, John Wood, and Karl Johnson, mentors all, came alongside and were critically important. They helped me discover and better understand the myriad and complex pathways that this pernicious parasite and whirling disease could have as it worked its way through aquatic ecosystems and ravaged wild trout populations across much of western North America.

Throughout my career there were four Colorado Division of Wildlife Directors that I considered **Giants** in the area of administration; early on men like the late Jack Grieb and John Mumma, and towards the end of my 36-year career, Russell George and Tom Remington. Three amazing aquatic research chiefs, Tom Powell, Mark Jones and George Schisler were **outstanding** supervisors that **ALWAYS** provided funding, support and encouragement to a sometimes overly enthusiastic if not outright at times "pain in the ass" researcher and always gave me the freedom to "go the extra mile."

And finally, from the beginning to the end of my career I was ***unbelievably blessed*** to always be in the presence of young **"Giants"**. These dedicated, extremely committed and incredibly hard- working men and women who may have started out as aquatic research technicians working with me, but continued on to college graduation, became graduate students, and went on to become **Giants** in their own right. **Rich Van Gytenbeek, Dirk Miller, Scot Shuler, Greg Espegren, Karen Tarman, Kevin Thompson, Matt Stinson, Bill Atkinson, Kevin Rogers, and Luciano Chiaramonte** just to name a few. Several of you are here in the room today.

Any success that I have had in my career is due in a **VERY LARGE WAY** to all of these **Giants** who came alongside of me at just the right time, and continued on as colleagues and dear friends. And in doing so, you RAISED ME UP, helping me to fly higher and accomplish far more than I ever thought possible. Thank you for gracing and enriching my life immensely. I am deeply honored to receive the **Aldo Starker Leopold Wild Trout Medal**.

Congratulations Bob Gresswell

Over the last nearly 50 years, Bob Gresswell a.k.a. "Cowboy Bob" (Research Scientist, US Geological Survey retired) has actively and significantly contributed to the understanding and management of native and wild trout. His research has spanned many ecosystems from coastal mountain watersheds to streams, rivers, and lakes in the intermountain west. Bob has used his considerable talent and passion for research to explore the habits, distribution, and relationships of many native species, including bull trout, Arctic graying, and a variety of cutthroat trout subspecies, providing invaluable information on non-native, wild brown, brook, rainbow, and lake trout populations along the way. His work has been especially useful to managers compelled to address the wicked problems of the day including the development of tactics to suppress non-native lake trout to restore the iconic Yellowstone cutthroat trout and leadership of efforts to understand watershed-scale impacts of fire on fish, fisheries, and habitats. Although Bob's scientific contributions are considerable, it may well be as an educator and mentor that Bob will be best remembered. Multiple generations of students, scientists and managers are reaping the benefits of his knowledge, experience, and generosity. He has been actively involved with the Wild Trout Symposium since its inception in 1974, contributing as coordinator, editor, and presenter.

Acceptance Message

Robert E. Gresswell, Department of Ecology, Montana State University, Bozeman, MT 59715, (406) 570-1486, bgresswell@gmail.com

It is a great honor to have been selected as a recipient of the A. Starker Leopold Award at Wild Trout XIII. Although I'm not sure that I deserve this award, I am humbled to receive it. In fact, to have my name mentioned in the same sentence as Starker Leopold is truly a compliment. I am indebted to numerous dedicated and creative colleagues and students with whom I have collaborated over the years; to my mind, they deserve to share this award.

As a young biologist working in Yellowstone National Park, I heard Starker speak at both Wild Trout I and Wild Trout II and was fortunate enough to meet him at a small gathering at a backcountry cabin during one of his visits to the park. My impression from these experiences is that Starker was one of those rare individuals who never lost sight of the relationships among the physical and biological components of the natural world; the whole is more than the sum of the parts. He championed the conservation of fish, wildlife, and the ecosystems that sustain them. I am inspired by his legacy and appreciate the recognition that comes with this award.

Marty Seldon Graduate Student Scholarship Awards

Marty loved wild trout and the places they lived. He continually challenged the organizing committees for each symposium to find the best research and management biologist internationally to come and present the results of their work. Marty also believed that students held the keys to the future and needed to play significant roles in the symposium. As the Organizing Committee developed plans for Wild Trout IX, they discussed ways to recognize Marty's 30+ years of involvement and many contributions to the Symposium. The result was the Marty Seldon Student Scholarship Award. Two awards are to be presented at each symposium.

The award is intended to support outstanding students who conduct research on coldwater fisheries ecology and management. The award for 2022 is for $600, to assist recipients with travel or other costs associated with attending the Wild Trout Symposium.

WTXIII Marty Seldon Graduate Student Scholarship

Congratulations Ridge Sliger

Ridge is a PhD student in the Wildlife Ecology Program at Clemson University. As a master's student, Riddge conducted research on wild Southern Appalachian Brook Trout, creating and publishing his results in the Journal of Zoology and Environmental Biology of Fishes. Ridge is a member of the National American Fisheries Society and a Member of the University of Georgia and Clemson University AFS subunits. He regularly collaborates with multiple nonprofit conservation organizations including TU to help with citizen training on topics including barrier identification and measurement. Ridge aspires to a university position where he can continue pursuing novel research on fish passage while engaging the next generation of fisheries scientists.

Congratulations Jeff Baldock

Jeff is a PhD student at the University of Wyoming where he is evaluating the role of spring-fed streams on Yellowstone cutthroat trout in the upper Snake River basin, WY. He has presented original research at the CO-WY Chapter and Western Division of AFS, and venues of National importance. He is a contributing member of the Jackson Hole Chapter of Trout Unlimited and assists with the TU Adopt-a-Trout program, where he leads hands-on activities to teach middle school students about cutthroat trout life history and conservation. Jeff's career goal is to do research for a state or federal agency to inform on-the-ground conservation and management practices.

Ron Remmick Undergraduate Student Scholarship Award

The Ron Remmick Undergraduate Student Scholarship Award was established at WT IX to memorialize Ron Remmick's 25-year career with Wyoming Game and Fish. A recognized authority on Colorado River and Bonneville cutthroat trout management, Ron was at the cutting edge of native fish conservation and restoration, and was a leader in public education. His knowledge was prodigious, his approach innovative, and his enthusiasm contagious. The Ron Remmick award recognizes one outstanding undergraduate student who has demonstrated an interest in the conservation and restoration of native trout – and encourages them to attend the Wild Trout Symposium.

The award includes a $600 stipend to assist with student travel or other costs incurred in attendance of the symposium. The Ron Remmick Scholarship is open to undergraduate students in Fisheries Management or related fields.

Congratulations to Logan Thompson

Logan is a sophomore at Brigham Young University-Idaho, where he is focusing his studies on Fisheries Management. He is a member of the BYUI subunit of AFS and has seasonal field experience as a fish tech for ID Fish & Game, for whom he helped electrofish small streams and large rivers and learned about the various tools and techniques of fishery managers. His near-term career goal is to finish his BS, get into graduate school, get a 'dream' job (!), and keep angling.

Plenary Session

The 2020 Vision: The fly-fishing community needs science to guide the future

Kirk Deeter

Editor-in-Chief, Trout Unlimited
Editor, Angling Trade Media

Fly-fishing has reached a crossroads.

The pandemic brought an influx of new anglers (as many as 1 million who at least tried fly-fishing). At face value, that is a good thing. Outdoor recreation is healthy! Fishing is good for families. Additionally, more people engaged in the outdoors equates to more potential for future stewards of the environment, as well as caretakers of the traditions and community that make fishing so valuable to many. From an economic standpoint, more participation amounts to a larger consumer base to buy products, more licenses sold, and generally more "commerce."

But with more participation comes more pressure on the fish. "How many" and "how big" have defined "success" in fishing for generations. But if everyone endeavors to catch as many fish as possible—even and especially under the premise of "catch-and-release," that is going to have a detrimental effect on fish populations in some rivers. Every day, anglers and guides are already seeing the effect and would value more scientific understanding of how catch-and-release pressure affects fisheries.

The methods used in "fly-fishing" have changed dramatically within the last 30 years. Now, the weighted nymph rig affords a person with no angling skill at all to experience the thrill of catching a trout. Again, at face value, that can be a good thing, because success sparks interest and engagement. But if a method increases the catch rate for many anglers, the pressure on the resource increases. We (anglers) are, seeing some rivers that are not only crowded but are also a shell of their former selves when it comes to production. We need to understand that anglers and angler pressure, as well as modern angling methods, are conservation considerations, in and of themselves.

All of this is also complicated by climate change. Our seasons are shifting. We see, firsthand, obvious changes, like higher water temperatures, lower flows, and impacts from floods and fires. We have seen all of this accelerate within the last 20 years.

Most anglers will support river closures, "hoot owl" and similar restrictions, because we ultimately see the value in sustaining fisheries. But we are also interested in what else science can teach us. For example, do hoot owl restrictions really work? Rather than an arbitrary time deadline, does it make more sense to restrict fishing gear or fishing methods? Perhaps fishing during warmer months could be restricted to dry fly-fishing only? Perhaps mandatory tippet strength or mandatory barbless hook restrictions could be enacted.

Science is the key to sustainability. Anglers understand this and will embrace what science tells us.

The good news is that anglers have learned and adapted in the past, and we will continue to do so in the future. A generation ago, we did not use rubberized mesh landing nets. Anglers have become much more conscious of harmful invasive species and what can be done to prevent their spread… and much more.

In an ideal scenario, the level of angler engagement would be maintained, but the angler mindset could be shifted—with the help of science—away from the "how many" benchmark toward an appreciation of the total angling experience.

Native and wild fish appreciation play into this. Most seasoned and dedicated anglers respect a hierarchy—native fish first, wild fish second, and anything after those are only okay insofar as they do not interfere with the first two. Most smart anglers would never advocate introducing a non-native species over a native species. That said, wild Brown Trout comprise a substantial fly-fishing opportunity, so demonizing wild Brown Trout is offensive to some anglers.

As anglers, we NEED science to guide us. We WANT science to help us. But the questions we wonder about might be a little different now than they have been in the past. Should we look at limiting "catches" and not just "keeps." The catch-and-release angler who beats up 50 fish in a day is likely causing more harm to the resource than the angler who keeps two fish to eat. We would like science to tell us if that is true. How so? How much?

What can we do to make fishing more sustainable? What should we recognize and appreciate amongst the fish themselves? How can we work together to inspire anglers to savor every experience?

As an editor who has spent the last 30 years covering fly-fishing throughout the world, those are topics where science and the angling community can more meaningfully intersect and cooperate.


Welcome to the
WILD TROUT
SYMPOSIUM XIII
"Reducing the Gap
Between Science
and Public Opinion"
EXIT


EXIT
Welcome to the
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SYMPOSIUM XIII
"Reducing the Gap
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Facts, Ideas, and the Post-Truth State of Play

Jesse Trushenski

Chief Science Officer, Riverence Holdings LLC

In pondering the plenary topic, I experienced mixed feelings. In part, I felt a bit beleaguered, because it was a reminder of the time and place we live and work in today as fisheries professionals. We are living in a post-truth or post-fact era, in which people's opinions are given as much credibility and weight as the facts.

But as I said, I experienced mixed feelings. Because intermixed with feelings of mild existential dread was the undeniable, unextinguishable feeling of hope. Hope that we might, through addressing the gap between scientific knowledge and public opinion, find ways together to bridge the gap and help the public remember that science is not some Poindexter's opinion or political talisman—it is a tool. It is the shovel that helps us dig deeper, it is the broom that sweeps away confusion, and it is the lens that lets us see the world more clearly. And, like any of these other tools, it is something we can all wield, so long as we allow ourselves to be shown which is the business end.

But before we get into the hopeful dessert of this presentation, I am afraid we need to eat some vegetables and talk about the post-truth world. A couple of years ago, Kurt Andersen wrote a fascinating, if somewhat disenchanting book called Fantasyland, in which he explores the growing disconnect between modern life and objective reality. "Post-truth" and "post-fact" are newer terms, but they both trace back to "truthiness," a word coined by Stephen Colbert in 2005. Truthiness is, "the belief in what you feel to be true rather than what the facts will support." Most of the discussion about truthiness and life in the post-fact era is centered around politics, but the 'do-it-yourself' approach to reality affects much more than just our civic discourse.

The internet has made it possible to consume information according to our own ideological leanings and to reaffirm what we already think. In research, we call this confirmation bias—only seeking out papers that align with our own observations. We are all guilty of it to a certain extent, but social media has made it that much easier to indulge the tendency to avoid anything that conflicts with our worldview.

The world is awash in 'fake news' that threatens the social licenses of fishing, fisheries management actions, hatcheries, and fish farms—even fishing itself. In turn, that threatens all of the work we do to conserve and restore fisheries, to provide food security and recreational opportunities, and to bring imperiled species back from the brink of extinction.

Today I am going to show you some of the ways that science is losing the battle for public hearts and minds, ways in which misinformation is sometimes spread, and point to a few techniques we might employ to become better communicators and consumers of fisheries information.

Let's start by examining a fishy 'fact' that has invaded the public consciousness: the idea that fish have a 3-second memory. I do not know the origins of this myth, but everyone seems to just know it. Ask the person standing in line next to you at the grocery store, ask school children, they all seem to know—or think they know—this fact. It is an idea that has really broken through and reached the public at large. If you think about it—even for just a few seconds—you can see how this cannot possibly be true, not really. How could fish learn and avoid the signs of a predator? How would fish ever become feed trained? How could they do much of anything if they really only had a 3-second memory? Fish might not have a memory like you or me, but it cannot be true that they do not have some type of memory that extends beyond the time that it takes me to complete this sentence. Despite the fact that this idea is demonstrably false, it is persistent and pervasive.

Whether people know the truth regarding fish memory or continue to believe that fish have three-second memories is not all that important. But I would like to pivot to another example about misinformation that is directly relevant to those of us in this room and that needs champions of objective reality to ensure the public does not get the wrong idea and run with it.

I am speaking of misinformation related to fish and fishing and efforts like the editorial that mischaracterized efforts to create and maintain fishing opportunities and trivialized the importance of fishing in the broader conservation landscape. This piece appeared in the New York Times (NYT) in 2015. The author made a number of spurious assertions about fish hatcheries and claimed that the stocking of nonnative trout was a major, if not the most important, cause of the decline of native trout species in the USA. After raising issues related to fish feeds, hatchery effluents, ecological and genetic interactions between so-called nonnative hatchery trout and native, wild trout, the author concluded that "If we continue to ignore the impact of hatchery fish on aquatic ecosystems, we will soon regret what has been lost." The author also stated that he had reluctantly given up fishing—presumably as a sort of protest against continued stocking of hatchery fish.

The American Fisheries Society (AFS) reached out to the NYT immediately after this article was published and offered a rebuttal to set the record straight. Unfortunately, the NYT essentially shrugged its shoulders and declined to publish the response—they do not allow rebuttals on pieces like this. So AFS opted to publish its response on its website and in Fisheries magazine. While this is good, there is no doubt that the AFS rebuttal was less-read than the original op-ed.

Reflecting on this experience, I sometimes wonder if it would have been better to leave well enough alone. I think the NYT piece was written not so much as an impassioned plea for a change in fisheries management, but with the intent to gin up attention to support book sales (the author of the op-ed also has a book for sale on the topic of hatcheries and the impacts of trout fishing). I hesitate to bring up this article again now—I do not want to give it any more oxygen, and I do not think it merits any more consideration in the public square. But I think it highlights some of the misconceptions about fish and fishing and some of the tactics we need to recognize as part of some types of media or communication strategies.

In retrospect, I think this op-ed—maybe most op-eds—are written as a sort of propaganda. The distinction is that opinion is just one's point of view—which can be right or wrong—whereas propaganda is typically of a biased or misleading nature. As the saying goes, everyone is entitled to their own opinion. But where I think the line is crossed, is where science and opinion are intermingled and presented as unassailable fact, as in the case in the NYT op-ed "The Cost of Trout Fishing" discussed earlier. And while you may be entitled to your opinions, you are not entitled to your own facts. So I think it is useful to examine this piece in light of what is known about propaganda and the tools that propagandists use to convey their messages.

Renee Hobbs is a scholar specializing in media literacy, and she identified four tools or strategies commonly implemented in propaganda pieces. The first tool is to activate strong emotions—to get people worked up about something. It does not necessarily have to be negative emotions. But as they say, "good news doesn't sell papers," and a lot of propagandists work to stir up negative emotions.

In the NYT op-ed piece, we see language that is intended to create concern or disgust: "…hatcheries are major polluters…much of the fish excrement, uneaten food, and dead and decaying fish leach nutrients into wastewater that is often then dumped untreated into the closest stream or river…." Everyone is sort of repulsed by the ideas of death and decay, so the piece is activating a natural aversive response. We also see the label "polluters" which does two things—again, nobody likes pollution, so it activates concern—but it also personifies the hatcheries and makes them the bad guy. We are not talking about how hatchery operations can result in elevated nutrient levels in discharge effluents or the associated permitting and oversight of water discharge. We are not talking about any of the realities with wastewater compliance in a neutral way—we are talking about the polluters that are harming our streams and rivers.

We also see the activation of another particularly powerful emotion: guilt. American society has been described as a guilt culture--much of our behavior is governed by wanting to avoid a guilty conscience. We hate to feel guilt and love to be redeemed from it—and this article gives us both. The NYT article states that, "…we are devastating populations of marine species simply to support a freshwater hobby." The article tells us that we are guilty of devastating populations of forage fish to feed hatchery fish, but also offers us the opportunity to be redeemed, if we would just give up this frivolous hobby called fishing.

Related to activating strong emotions is the second tool or strategy: appeal to hopes, fears, and dreams. In the op-ed article, we see the author tapping into feelings or beliefs that their target audience already has. It states, "…hatcheries are breeding fish that are poorly adapted to life in the wild. Even worse, these fish can pass on their undesirable traits to wild populations of native fish." Historically, genetic management was not a part of hatchery operations—not like it is today—and many people continue to incorrectly believe that hatcheries are factories of genetic pollution. So statements like that—that do not reflect modern operations and mix and match the concepts of outbreeding depression and hybridization—are not really correct anymore but are nonetheless likely to strike a chord.

The article also taps into the loss of faith in institutions, commenting that "Anglers and concerned citizens need to care about rivers and all the native species they contain, especially if organizations like AFS don't seem to care." Propagandists commonly adhere to the idea that the best defense is a good offense, and attacking opponents is a common feature of propaganda pieces. In this case, the author did not come out swinging against AFS—they saved their attacks for a response to the AFS rebuttal. But he did accuse AFS of 'blurring the lines' and a few other things, which we will discuss more in a moment.

Finally, propagandists understand that perhaps their most important tool is to keep things simple. In the NYT article, the author claimed that hatchery "…wastewater can also contain medicines…and disinfectants…." Ok, technically that may be true. But it is hardly the full story. There is no mention of the discharge restrictions that are in place or the strict limits on how therapeutants are used in hatcheries, or that pretty much any small neighborhood is likely to be discharging as much, if not substantially more, medications and disinfectants than a fish hatchery does. But most people are not going to know that this complexity even exists or try to unravel it—they are going to be satisfied by the simple message and accept it as the whole story.

Used together, these tools can create a very potent message that is difficult to counter in the public consciousness.

Another concept that is becoming more widely appreciated in the media and public communications is the concept developed by Jennifer Freyd, which is to: Deny, Attack and Reverse Victim and Offender (DARVO). This is a strategy that some employ when they have been criticized for their behavior or accused of crimes. It is basically 'flipping the script' and making it about anyone other than the person who was originally responsible for the situation at hand.

You can see shades of the DARVO approach in the response that ultimately came from the author of "The Cost of Trout Fishing," a few of which I will highlight.

"AFS…took umbrage with my piece 'The Cost of Trout Fishing' in the NYT last April. They call my piece inaccurate and complain about fundamental misunderstandings in fisheries management."

"I can assure the readers that **all my facts were meticulously referenced and were carefully fact-checked** by the editorial staff at the NYT…I thought it would be useful to **do a little factual checking on the claims AFS made** in their editorial."

There is the denial. Then come a series of attacks, as indirect or direct accusations of misconduct on the part of AFS.

"The AFS editorial seems to **intentionally blur the facts** through a change in terminology and misdirection with the stocking numbers they use. At the heart of the issue is a **somewhat disingenuous tactic** and alteration in wording AFS uses to switch the discussion from my focus on native versus nonnative species in the NYT piece, to an argument of hatchery versus wild trout in their response editorial."

Then come the attempts to reverse the victim and the offender.

"I am disappointed that a scientific organization like A**FS seemed to care more about the economic bottom line than the aquatic ecosystems** many of their members study."

"If money is really the bottom line behind hatchery production, perhaps AFS should argue for construction of more hydro**power dams or manufacturing plants with a cut going to state agencies** to more effectively generate income along these river systems…"

"I was not surprised when the commercial marine fisheries lobbying groups complained about the negative press reduction fisheries received in the NYT op-ed."

"The AFS decided to weigh in on my discussion of stocking of native versus nonnative trout and the impact they had on the environment with what seems to be a **fairly politically-motivated stance**…in doing so, **AFS revealed an industry bias that surprised me greatly**."

The message to the reader is that the author was simply championing native trout and aquatic resources—something that AFS should be doing if they were not so biased. But first, the author was attacked by the fishing industry, then—even worse—by AFS, taking a politically motivated stance and intentionally choosing data to support their positions and being just vague enough to be making statements that are technically true but belie the more troubling reality.

Do you see the reversal? The author is accusing AFS of many of the same things they did themselves in their op-ed, but casting themselves in the role of victim—first maligned by industry and now those that are supposed to be on the side of the fish. The author positioned himself as something of a David speaking out against the Goliath of the powers that be. Nevermind that this David was able to harness the power of the NYT, a giant if there ever was one.

This is part of the reality of the post-truth world. Many people now live in a do-it-yourself reality where they do not need facts or objective science to take a position and convert others to their way of thinking. All they need is an idea and a working knowledge of the tools of propaganda, DARVO, and other strategies to get their message across. It is something that fisheries professionals should recognize as part of the state of play for science communication. But I think it is also something that all of us would do well to recognize as media consumers. Most of us in this room are probably pretty savvy consumers of fisheries information—we likely view whatever we come across through a reasonably critical lens and can identify when we are being sold a bill of goods. But are we that savvy when it comes to other types of information? Probably not. We cannot know everything and so we cannot expect to know the facts or the data behind every news story. But we can learn to recognize these tools of propaganda and at least recognize when there may be more to the story than meets the eye.

So what is the message to fisheries professionals? How can we work to bridge the gap between science and public opinion? Well, I am certainly not suggesting that we try to become propagandists, but I think we need to try to become a little more effective science communicators if we are going to stand a chance of pushing back against those who are less scrupulous about what and how they communicate.

I am going to share some ideas I have been talking about for the last couple of years about making ideas infectious. Certain ideas are infectious—even the oldies like us talk about things going viral. We use the vernacular of diseases and epidemics to describe ideas that, seemingly all at once, take over public discourse or pop culture. But what makes an idea infectious?

These are questions that were explored by Malcolm Gladwell in his book, The Tipping Point. What defines the point at which a disease or idea tips and becomes an epidemic? In fish health, we always use the Venn diagram (Figure 1) to conceptualize the occurrence of disease. It shows the relationship between the host (or the fish), the pathogen (the virus, bacterium, parasite, what-have-you), and the environmental conditions that both are experiencing.

Only when all three of these things come together in the right way—or, depending on your perspective, the wrong way—can infectious disease occur. For example, you might have a host living in poor environmental conditions that weaken its immune system, making it vulnerable, but if the pathogen is not there—no disease. Even if the host and the pathogen are there, but the environmental conditions are not right—for example, it is the wrong temperature—you still will not get an infection. This model illustrates the importance of all three elements coming together for a disease event to occur.

In The Tipping Point, Gladwell also described three elements that he believes contribute to the virulence of an idea (Figure 1) —how rapidly it invades the collective consciousness and what effects it can have. He described these as the Law of the Few, the Stickiness Factor, and the Power of Context. Over the next few minutes, we will explore each of these elements in a bit more detail, think about how they are related to the fish disease model, and, hopefully, see how both of these models can help us better craft our messages of fisheries information.

The Law of the Few is essentially the idea that the right messenger is central to making an idea infectious. Gladwell wrote, “The success of any kind of social epidemic is heavily dependent on the involvement of people with a particular and rare set of social gifts.”

These archetypes make ideal messengers because they possess those rare social gifts. Connectors, for example,

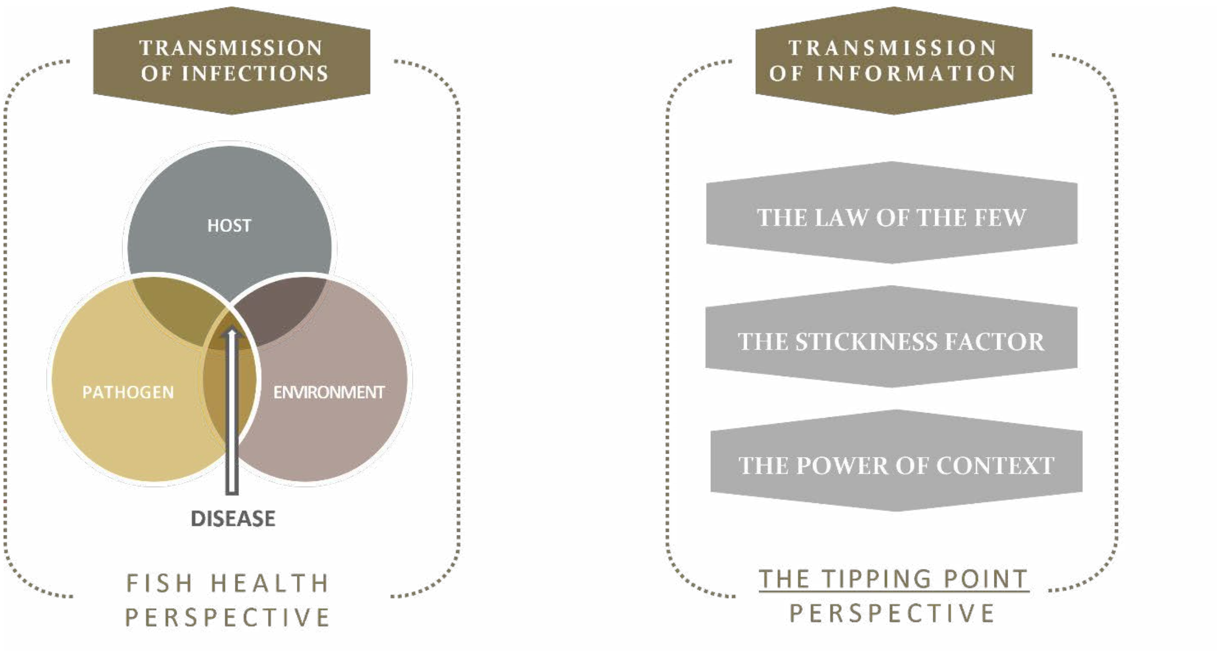


Figure 1. Malcom Gladwell diagrams of the relationship between the host, pathogen, and environment to produce disease and the elements that he believes contribute to the virulence of an idea.

are people who know people. Most of you have probably heard of the Six Degrees of Kevin Bacon game, in which it is possible to connect the actor to virtually any other actor through the movies they have been in, in six steps or less. There is a reason it is called Six Degrees of Kevin Bacon and not Six Degrees of Adam Sandler. Adam Sandler has not been in as many movies as Kevin Bacon, and he tends to act primarily in comedies and often with the same actors. Kevin Bacon has been in comedies, dramas, horror flicks, romances—all sorts of genres—and as a result, has acted with many more people.

Connectors are people who can distribute a message to a lot of people and a lot of different types of people through their social or professional networks.

Mavens, on the other hand, are people who know things. They are trusted experts in their chosen field or areas of interest and have a desire to help others learn more about it. The example I have used here is Cesar Millan, the Dog Whisperer. Nobody knows more about making dogs behave than he does, and he is widely recognized for sharing his knowledge with others. He is a maven and that makes him a trusted source of information—if Cesar Millan tells you something about dog training, you are likely to believe it and act upon his recommendations.

Salesmen are people who persuade people to ‘buy in’ to something, whether it is a car or an idea. Prior to his death, Peter Jennings was considered one of the most influential news anchors, not because he knew more or had a bigger network than the other anchors, but because he could sell. Through his words and especially nonverbal cues—body language and microexpressions—he could shape how viewers perceived the news. They bought what he was selling, without even knowing they were being pitched.

Gladwell argued that having one or more of these types of people as your messenger makes the idea more likely to be infectious. The Law of the Few tells us that communication is not just about reaching large numbers of people, but reaching a few key types of people that can carry your message from there.

The second element Gladwell described was what he called the Stickiness Factor. The idea here is that the nature of a message—both the message itself and the way in which it is presented—can determine whether it is memorable or forgettable. One of the most important attributes of a message is its simplicity. This picture (Figure 2) illustrates a light switch, or at least a Rube Goldberg machine version of one. This switch has many interesting but unnecessary components. If you want the light to be turned on, you could simply walk over and pull the chain rather than hitting the bowling ball, which strikes the pin, which pulls the string, which opens the birdcage, and so on.

People have short attention spans and limited ability to absorb complex ideas quickly. The myth that goldfish have a 3-second memory is short, simple, and easy to remember—the truth is much more complicated and involves various types of memory, ways of measuring memory spans, experimental conditions, statistical methods, interpretation of results, etc. The truth is more like a Rube Goldberg machine, whereas the myth is a simple light switch. The simple, easy-to-remember message is the one that will stick in people’s minds.

Complex ideas aren’t very likely to be infectious—they simply are not as virulent, sticky, or seemingly right as simple, truthy ideas. Simplicity is one of the tools of propaganda, too. But the idea here is not to tell a simple half-truth—it is to find a way to simplify our messages and make the whole truth more readily understood.

Simplicity certainly matters, but here is an example that brings us to Gladwell’s third element, the Power of Context. Sesame Street and Blue’s Clues are two well-known, highly popular children’s shows. Both have the same message and content—they are attempting to make learning infectious through messages about the fundamentals of reading, mathematics, etc. Kids are entertained by both shows, but in terms of reaching their common goal, Blue’s Clues has proven much more effective in getting kids to retain the information and achieve learning outcomes. Why is that?

Blue’s Clues’ message is stickier because it was presented more effectively to the intended audience, and it understood the context within which it was working. What makes children love Blue’s Clues—and learn better from it—is precisely what makes adults *hate* it. Blue’s Clues is highly repetitive, has only a few characters, and includes long, punctuated pauses in the storyline that are excruciating to adults. Sesame Street has multiple storylines, puns and ‘inside jokes’ that only adults would appreciate, celebrity guest stars that kids are not likely to recognize, etc., all of which can be confusing to children. The content of the message is the same, but Blue’s Clues ideas are stickier because they are better tailored to the intended audience.

The Power of Context tells us that even the most infectious idea will not go viral if it falls on deaf ears. People have to be receptive to a message for it to be understood, so you need to meet your audience where they are at, recognize that different audiences will respond to different parts of your message, and tailor your message accordingly. If we can craft our messages to tap into the Power of Context, the Stickiness Factor, and the Law of the Few, we can

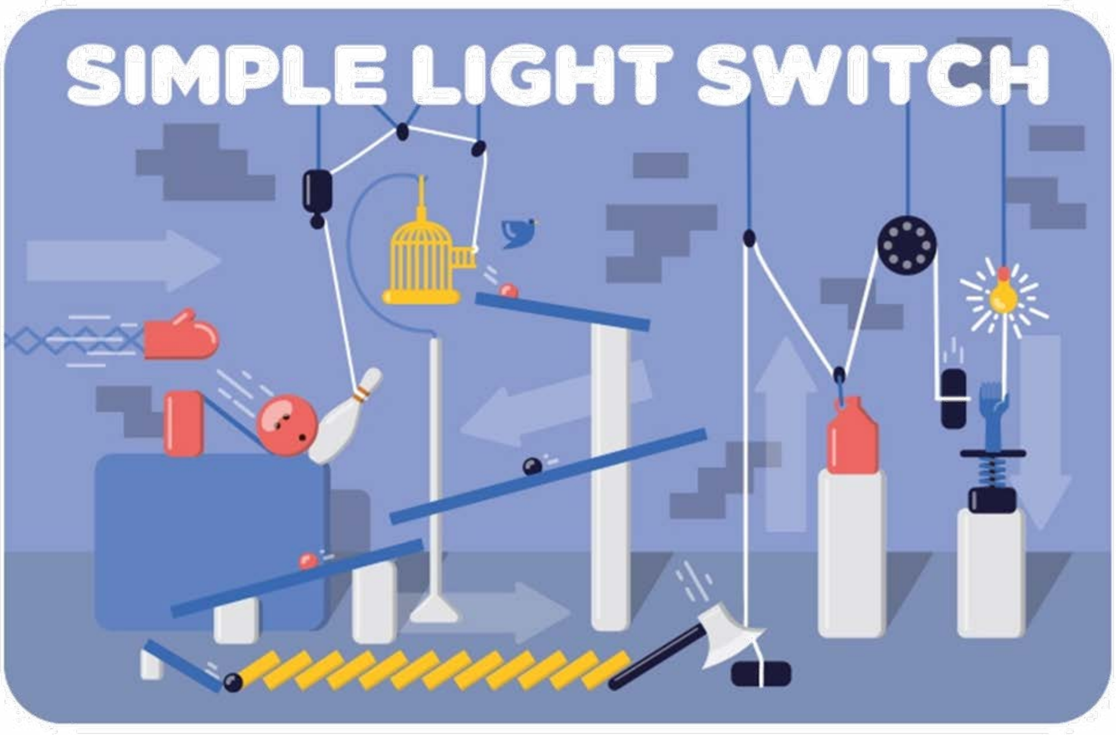


Figure 2. Rube Goldberg machine version of a light switch.

communicate fisheries information much more effectively.

I have personally found Gladwell's model of infectious ideas helpful in trying to distill and convey my messages about fisheries and aquaculture. But I have recently been introduced to another framework that might be even easier to adopt, and it has to do with anecdotes. Unlike longer narratives, anecdotes are short, memorable vignettes that quickly engage listeners and get to the point without indulging in unessential details. An anecdote has four parts:

- The setting: Time and place to which speakers transport their listeners
- The characters: One or two individuals who animate the setting and make the situation come to life
- The plot: The incident that takes place with the proper context
- The moral: The lesson learned or the solution to the identified problem

Together, these four parts form a "picture frame"—a mental snapshot that captures the essence of the anecdote and makes it real and meaningful to listeners.

The proponents of this style of science communication argue that the moral is the most important part of the anecdote; after all, this is the take-home, "so what?" message you are trying to convey. However, I would argue that the plot is where many would-be narrators are likely to stumble and that it is equally, if not more important to get this part right. Note that this part of the anecdote framework is called "the plot" and not "the story." A quip from famed author Edward Morgan Forster illustrates the difference between the two:

"'The king died and then the queen died' is a story. But 'the king died and then the queen died of grief ' is a plot." The basic facts are the same in both cases, but knowing the cause of the queen's demise provides a much richer context and stirs an emotional reaction to both deaths. It is not enough for an anecdote to relay facts; it must convey meaning. For too long, scientists have labored fruitlessly under the "deficit model," believing that if only people understood the scientific method better and knew the facts about, say climate change, public views of science and the need to reduce carbon emissions would change. 'If they just saw one more figure or data point, then they would understand!' Of course, this is laughably (if lamentably) false. This is the difference between story and plot: if you speak to someone's heart or appeal to their gut, they do not need to know the facts to believe; but if you fail to connect with them at a visceral level, all the facts in the world will not convince them.

As scientists, we have been talking about two dead monarchs, when we should have been telling listeners about the grief-stricken queen's broken heart. As fisheries professionals, we should have been talking about how fish, fishing, management, culture and conservation help to maintain our way of life and conserve resources and biological and cultural legacies for current and future generations.

As an example, I thought I might share the anecdote I have been using to tell my own story about fish culture. When people ask me about my career and why I help raise fish, I tell them the setting, characters, plot, and moral as outlined below (Figure 3).

That is why I get up in the morning and do what I do. I think many of your stories would illustrate a similarly compelling motivation. We work in fisheries not because of fame or money, but because without people like us, fisheries will dwindle, rare species will blink out of existence, and ecosystems will falter. Without our work, there would be less fish, less clean water, less financial stability, less resilience to change—less of everything we consciously or unconsciously rely on aquatic ecosystems to provide, and less conservation-minded people to care about any of it.

We do our work for many compelling reasons, and it is up to us to share our stories, our anecdotes, in a way that is simple, sticky, and speaks to people's hearts as much as their minds.

Waves of misinformation wash over the public consciousness, but fisheries professionals are not powerless against the rising tides of do-it-yourself, post-truth reality. We have mourned the old ways of sharing our science long enough; it is time to move on and find more productive ways to cope.

In his final thoughts on the way forward in a post-factual fantasyland, Kurt Anderson offered a few recommendations.

"What is to be done? We must call out the dangerously untrue and unreal, and fight the good fight in the public sphere. You do not need to get into an argument with a stranger, but do not give acquaintances and friends and family members free passes."

When you hear someone say something blatantly false, speak up. Point them in the direction of the truth, and take a few minutes to walk that path with them.

There are no better standard-bearers for fish and fisheries than the people in this room, and ours is a story worth telling. "Progress is not inevitable," Anderson tells us (in

The time and place to which speakers transport their listeners

One or two individuals who make the situation come to life

The incident that takes place with the proper context

The lesson learned or the solution to the identified problem

*The human population is **8 billion** strong and growing*

*Every day, hundreds of millions of people go to bed **hungry***

***Seafood** provides food security where it's needed most, but the ocean isn't a **bottomless** resource*

*If we try to feed the next billion people with wild-caught fish, the **oceans** will soon be **empty***

We can't catch more fish, but we can raise them

*Through **aquaculture**, we can have our fish and eat them, too*

Figure 3. Setting, character, plot, and moral used to explain why I raise fish.

conservation or communication), “but it is not impossible either.”

Finally, I would encourage everyone here to reflect on Simon Sinek’s idea of the Golden Circle. Whether you are a fisheries scientist or a salesman for a widget company, there are three aspects to what you do: there is the what, the how, and the why. For myself, my what is that I lead Research and Development groups for aquaculture companies in the U.S. and Norway. My how is that I work with farmers to understand their current and future problems, I conduct independent science with several teams of other scientists, and we use our findings to try to develop practical solutions that farmers can use in the real world. Unless you are really interested in fish farming or applied science, you probably do not care about any of that. But you might care about my why. I do what I do because I believe in the promise of aquaculture—I believe that hatcheries and farms can allow us to continue to have wild fish on the ends of our lines and wholesome seafood on our plates, now and in the future.

People do not care what you do, they care why you do it. I encourage all of us to remember that and to start with why. And when it comes time to talk about the how and the what, remember the importance of keeping it simple and tailoring your message to meet the audience where they are. Not in a craven manner that taps into their fears and casts others as the villains of the story, but out of a genuine interest in ensuring the scientific method and its fruits are made accessible and understandable to the public.

Reducing the Gap Between Science and Public Opinion: A State Agency Perspective

Dirk Miller

Wild Trout 2022

There is occasionally a gap between the best available scientific information and what some anglers want. Knowing if the vocal anglers represent the broader public is often difficult. Fishery managers spend most of their time evaluating fish populations and aquatic ecosystems. Less effort is expended evaluating angler interests. Managers should attempt to understand public desires and incorporate them with biological information when making management decisions.

Fisheries managers are expected to use scientific management to conserve populations for future generations while providing diverse, high-quality fishing opportunities. Maximizing sport fishing and conserving populations for future generations in every water is impossible. Striking a balance is a challenge that fisheries managers face. The North American Model of Wildlife Conservation suggests that wildlife is allocated according to the democratic rule of law and that every person has an equal opportunity to participate. As such, resource managers must communicate with resource users to explain when their desires cannot be met and why it is not possible. Sometimes there are different desires for the same fishery, and the conflicting objectives can not be met. Other times it is simply not biologically or economically feasible.

There is a great deal of variety in fishing opportunities and even more diversity of opinion and motivations among anglers. It is challenging to determine what the "average angler" wants. Modes of interaction have changed, people do not attend meetings, and the number of information outlets has increased dramatically. Some anglers and advocacy groups work to influence agency decisions. Fisheries managers' opinions can be biased by relatively few people with strong opinions and by their own personal interests.

Many fisheries management decisions are not controversial. Some decisions have diverse alternative solutions, and the public does not clearly support one approach. Anglers expect that fisheries managers will listen to their input. While the public trust in government has declined, public confidence in fish and wildlife agencies has remained high. Recent impacts of COVID-19 and national politics have decreased trust in science, and the increasingly uncivil political discourse may trickle into resource management.

Public trust provides the foundation for state wildlife agencies. Resource managers can build and maintain trust by developing evidence-based recommendations and showing the public that they are working for the public. Managers should embrace scientific uncertainty and learn from, and admit, mistakes. It is important to be transparent and share findings in an understandable way.

Human dimensions science is well developed, and many tools are available to fisheries managers. However, most agencies do not have staff that are trained in this area. Fish biologists spend relatively little time trying to understand what anglers want. Fish biologists like to collect fish data and have a lot of cool gear to do so; consequently, it is easier and more fun to do fish stuff than to try to influence or understand human behavior.

Fisheries managers would benefit from spending more time with people. Managers should invest time analyzing the needs and expectations of all affected stakeholders. It is a challenge to find ways to connect with more stakeholders. Managers should work with social scientists as they design and conduct analyses of stakeholder interests. They should also share social science data when they have it.

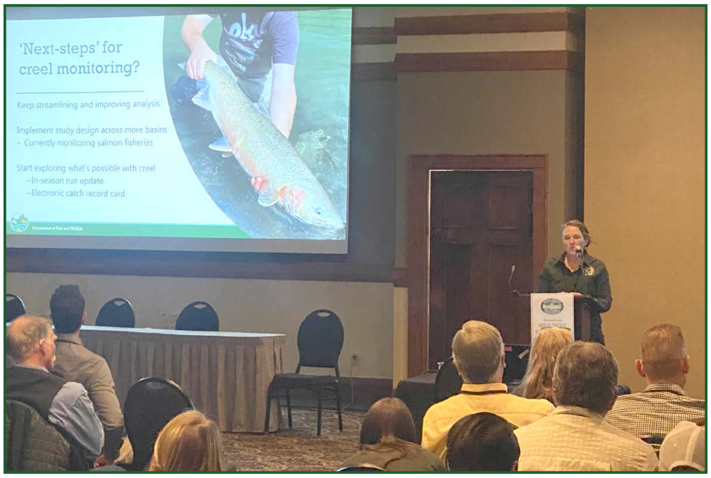
Making science-based management decisions is important, and fisheries managers should be deliberate about having the right data. As such, managers should collect social science data along with fisheries data. To close the gap between fishery science and public opinion, resource managers need to understand both.


The gap between fishery science and public opinion
EXIT


What does the public want?
Modes of interaction have changed, people don't come to meetings
Information outlets have increased
A few TV fishing shows and magazines
Many more of those plus YouTube, social media apps, online forums, etc.
Social media is popular, may also suffer from squeaky wheel bias
NGOs that advocate for resource issues and specific interests have increased
EXIT
Welcome to the
WILD TROUT
SYMPOSIUM XIII
"Reducing the Gap Between Science and Public Opinion"


WILD TROUT SYMPOSIUM
WILD TROUT SYMPOSIUM

Session 1
Socioeconomics and Public Engagment



Assessment of the Wild Brown Trout Fishery in the Deerfield River Tailwater, Massachusetts, USA

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Abstract - The Deerfield River tailwater in northwest Massachusetts has long been considered a high-quality trout fishery, especially for trophy Brown Trout *Salmo trutta*. However, until recently the Massachusetts Division of Fisheries and Wildlife (MassWildlife) has not had the capability to thoroughly assess the fishery or its management. Therefore, there has been ongoing debate amongst anglers, guides, and other parties regarding whether this fishery consists wholly of stocked trout or if natural reproduction is able to maintain a viable wild trout fishery. To assess key metrics (e.g., population size) and determine the contribution of wild Brown Trout to this fishery, we used a simple marking scheme for hatchery Brown Trout and a subsequent mark-recapture survey focusing on 13 km of the Deerfield River tailwater. Since 2018, hatchery Brown Trout were marked with an adipose fin clip prior to being stocked. Concurrently, we performed electrofishing surveys each year (2019-2022) to collect and tag Brown Trout with external T-bar tags. Survey results indicated that the fishery supports a higher than statewide average, but lower than expected (given available habitat), abundance of Brown Trout (320 Brown Trout/km). Reproduction is occurring but recruitment is low, with relatively few young-of-the-year present. Furthermore, it appears that hatchery Brown Trout are not a substantial proportion of the population (~21%) making this primarily a wild trout fishery. Our results to date have led to discussions regarding shifts in management strategies (e.g., cessation of stocking, extending catch-and-release regulations). Additional monitoring is scheduled for the next several years to assess changes in the Brown Trout population following potential management changes and following a shift in flow regime at Fife Brook Dam, which will increase minimum flows from October to April. Public engagement, partnerships, and ongoing communication with anglers, guides, and conservation organizations have been key for securing the necessary equipment and supplies to perform this work. Working closely with these groups has shaped the direction of the project in ways that are meaningful from both a management perspective, as well for those that use and care for this resource.

Introduction

In a state full of wild Brook Trout *Salvelinus fontinalis* streams, there seems to be an inordinate interest in the few river systems where introduced Brown Trout *Salmo trutta* thrive. The Deerfield River in northwestern Massachusetts is one of those places. The Deerfield River also happens to be the largest river trout fishery in the state and it provides a unique opportunity for anglers to target Brown Trout and Rainbow Trout *Onchorhyncus mykiss* in a setting more reminiscent of the western USA than of southern New England. In addition, being a tailwater, the river can be fished and floated year-round, which is a relative rarity in the region. Because of this, the Deerfield River has become a very popular fishery that supports numerous guides and anglers, some traveling from as far away as Boston and New York City, as well as thriving local Trout Unlimited chapters with whom the Massachusetts Division of Fisheries and Wildlfie (MassWildlife) has developed a close working partnership. Indeed, without the support and engagement of Trout Unlimited and the local angling community, this project would not have been possible.

Although the Deerfield River tailwater has long been one of the most popular trout fisheries in the state, because of its size, depth, and swift currents, MassWildlife biologists have had difficulty surveying the river effectively with the equipment available (e.g., backpack electrofishers). Thus, until recently, managers have had limited fisheries data to guide management of the fishery. This relative lack of information has resulted in some confusion and untenable expectations among anglers, guides, and other interested parties regarding various aspects of the fishery. MassWildlife stocks hatchery Brown Trout and Rainbow Trout in the Deerfield River, and there is ongoing debate regarding whether the Brown Trout that anglers are encountering are predominantly of wild or hatchery origin. Prior to this study, MassWildlife has been unable to adequately answer the wild-stocked debate or other basic fisheries questions such as population abundance. This has led to tension regarding the reasoning behind the strategies employed to manage the Deerfield River tailwater trout fishery.

This project was developed partly in response to the ongoing debate regarding the origin of the Brown Trout inhabiting this section of the river. Answering this question not only appeases the curiosity of fishing constituents but also provides MassWildlife the necessary information to make decisions about stocking practices in the future. Additionally, this project provided the opportunity to obtain abundance data on the Brown Trout population inhabiting the Deerfield River tailwater. Having this basic fisheries data improves our ability to communicate with fishing constituents about what is going on with the fishery and also allows more informed decisions regarding angling regulations in the future. This is of great interest to those who fish and guide on the Deerfield River. Finally, the timing of these investigations was fortuitous because the minimum baseflow from Fife Brook Dam, which is the source of this tailwater fishery, is scheduled to increase from the current minimum of 3.5 cms up to 7 cms from October through April starting in 2023. This project has provided an opportunity for the collection of baseline data to assess any shifts in Brown Trout population metrics following changes to stocking practices and angling regulations . Therefore, the objectives of this project were 1) to determine the proportion of Brown Trout in the Deerfield River tailwater comprised of wild versus hatchery origin fish, and 2) obtain abundance data on the Brown Trout population prior to changes in upstream dam operations, stocking practices, and angling regulations.

StudyArea

Our study area was approximately 13 km of the Deerfield River tailwater in northwestern Massachusetts from Fife Brook Dam downstream to the Route 2 bridge in the town of Charlemont (Fig. 1).

Figure 1. Map of Deerfield River watershed in northwest Massachusetts. The 13 km Deerfield River tailwater study area is highlighted in red.

This 13 km is considered to be the extent of coldwater influence from Fife Brook Dam releases and therefore the extent of a year-round coldwater fishery. We used raft electrofishing to survey approximately 11 km of the 13 km study reach. We did not sample 0.5 km of the study reach directly downstream of the outlet of Fife Brook Dam nor the 1.5 km of the study reach in the vicinity of Zoar Gap rapids due to safety concerns.

Fife Brook Dam, along with the associated Bear Swamp pump-storage system, is a hydroelectric facility on the Deerfield River in Monroe, Massachusetts. Minimum permitted flow releases from Fife Brook Dam to the Deerfield River downstream is 3.5 cms and power plant maximum capacity is 40 cms. Flow releases above the minimum 3.5 cms during part of the year (October 1 – April 30) are irregular and based on both electricity demand as well as the need to pass excess water released from upstream impoundments. From May 1 – September 30, in addition to any flow releases for power generation, there are 106 scheduled releases for whitewater recreation. These whitewater releases are required to be >20 cms but are often closer to 23-25 cms. There is no specified ramp-up period for either whitewater or peaking power flows. Therefore, the transition from baseflow to peak flow is almost instantaneous.

Methods

Beginning in May 2018, MassWildlife biologists began marking, with an adipose fin clip, the 1000 hatchery Brown Trout stocked into the Deerfield River tailwater each spring to clearly identify them as hatchery-origin rather than wild fish. These fish were stocked shortly (<1 week) after marking and allowed to distribute themselves prior to electrofishing surveys.

In May 2019, we began our multiple mark-recapture surveys using raft electrofishing equipment (12-foot raft outfitted with Smith-Root 2.5 GPP) to collect Brown Trout in the study area. Brown Trout > age-0 were tagged with an individually numbered external T-bar tag inserted into the muscle tissue near the dorsal fin. Mark-recapture surveys were done monthly May-September each year from May 2019 through September 2022. We used a Jolly-Seber multiple mark-recapture methodology to estimate the population size of Brown Trout > age-0 in the study area (Jolly 1965, Seber 1965) during each survey period. We also made a simple length-frequency histogram to visually assess survival across size/age classes of Brown Trout to aid in our overall assessment of the fishery.

We began annual monitoring of young-of-the-year Brown Trout abundance during the summer of 2020 and continued through 2022. We used single-pass backpack electrofishing to collect, count, and measure young-of-the-year Brown Trout from bankside habitat at four 100-m reaches in the upper half of the study area where most Brown Trout spawning was thought to occur.

Results and Discussion

To date, population estimates from our mark-recapture survey results indicate that the Deerfield River tailwater supports between 2470-4345 juvenile and adult Brown Trout (> age-0) (Figure 2), or approximately 320 Brown Trout/river km, on average across the four years of the project. The Brown Trout population estimate in the Deerfield River tailwater was greater than the mean abundance of Brown Trout in other Brown Trout fisheries elsewhere in Massachusetts (MassWildlife unpublished data). Wild Brown Trout abundance across the 140 other Massachusetts Brown Trout streams averaged 168 Brown Trout/km. However, the Deerfield River is also the largest coldwater river in the state and therefore may be expected to have Brown Trout abundances higher than other rivers; moreover, as a tailwater fishery, its coldwater habitat is more consistent throughout the summer. None of the other wild Brown Trout fisheries in Massachusetts are tailwaters and most suffer from high summer water temperatures in many cases forcing trout into isolated coldwater refugia and limiting the extent of coldwater habitat available (MassWildlife unpublished data). In addition, other wild Brown Trout fisheries in Massachusetts are generally only a fraction of the size of the Deerfield River. The Deerfield River averages 45 m in width while the mean channel width of other Massachusetts Brown Trout streams is only 8 m. Therefore, simple abundance measures may be misleading with average Brown Trout density in the Deerfield River tailwater at 55 Brown Trout/ha and average Brown Trout density in other streams in Massachusetts at 70 Brown Trout/ha. Additionally, population abundance estimates for other Brown Trout stream fisheries are based simply on single-pass backpack electrofishing and so are likely underestimating the actual population size.

Hatchery Brown Trout make up an average of just over 20% of the population present in the Deerfield River tailwater according to our survey results, although it varies somewhat from year to year (Figure 2). Some of the individuals that we considered to be wild Brown Trout in the first year or two of our study may have been holdover, unmarked, hatchery fish that had been stocked in the years

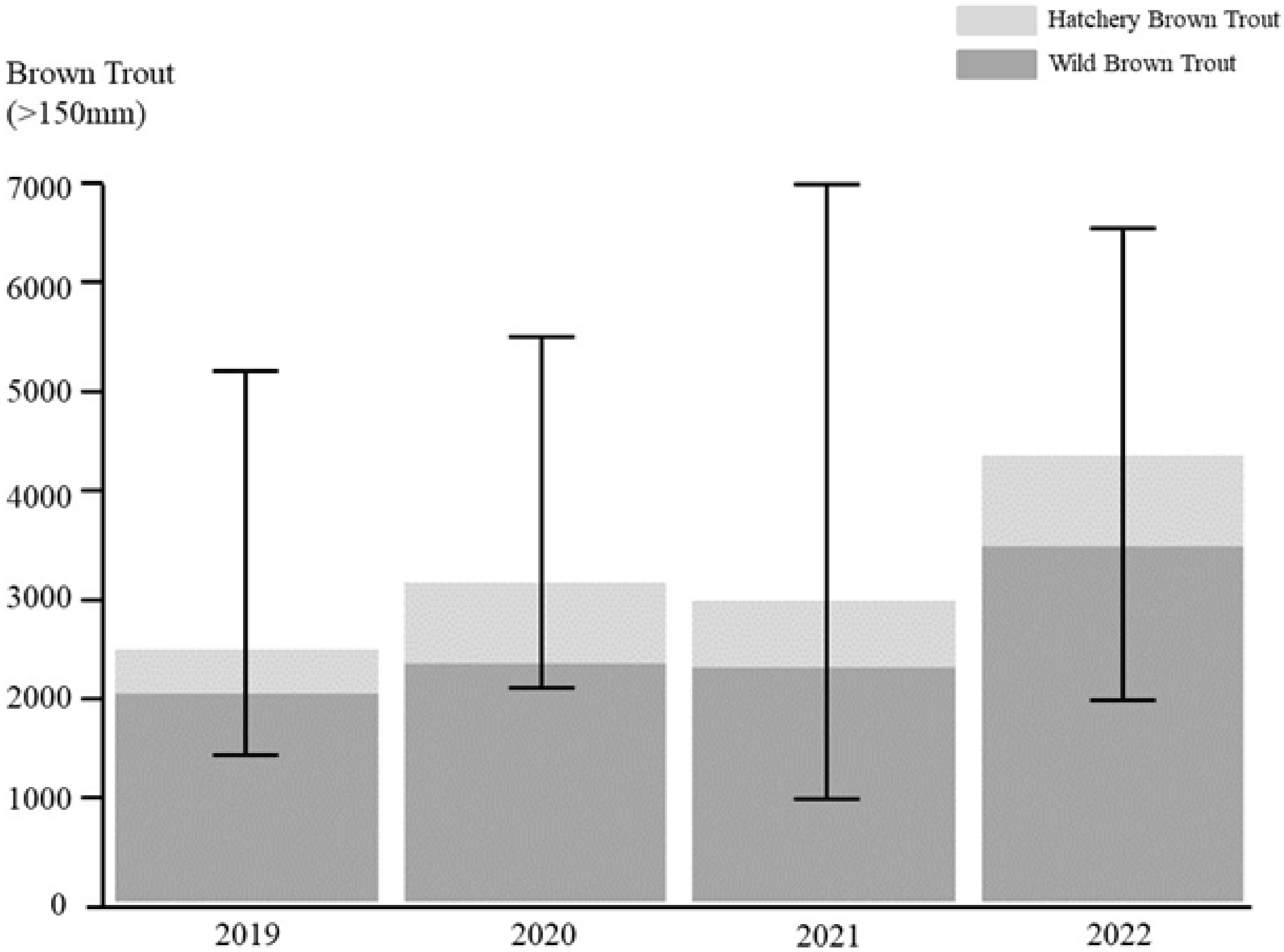

Figure 2. Population estimates and 95% confidence intervals for Brown Trout in the Deerfield River tailwater based on Jolly-Seber multiple mark-recapture methodology 2019-2022. Proportion of wild versus hatchery-origin Brown Trout that comprise the total population is indicated.

prior to the onset of this work. However, this seems unlikely given the low survival of stocked trout that MassWildlife has observed in the Deerfield River as well as in other Massachusetts trout streams (MassWildlife unpublished data). Other than a few individual instances, we have not found clear evidence of hatchery Brown Trout holding over for subsequent years during our work in the Deerfield River (i.e., no recaptures of tagged hatchery Brown Trout in subsequent years and no hatchery Brown Trout collected were substantially larger than their size at stocking, which would be expected if they were holdovers from the previous year). Furthermore, recent work on the Swift River tailwater in central Massachusetts, a comparatively more favorable environment for trout, showed that nearly 70% of stocked Brown Trout are unaccounted for within eight months post-stocking. This is likely due to natural mortality or harvest (emigration was assumed to be negligible in the Swift River because it is bound on both ends by dams and there are no tributaries; MassWildlife unpublished data). In general, hatchery Brown Trout stocked at catchable size tend to have higher mortality rates than wild fish in the same size class (Weiss and Schmutz 1999; Wills 2006; Flowers et al. 2019).

Because of the size of the river, habitat available, and water temperature, we would have expected the abundance of wild Brown Trout in the Deerfield River tailwater to be higher than our results showed. Once Brown Trout make it past their first year, mortality appears to be similar for most age classes over the course of their lifespan according to a visual interpretation of the length-frequency histogram (Figure 3).

Therefore, recruitment seems to be a factor in limiting the population of adult Brown Trout. Young-of-the-year Brown Trout, while present, tended to be scarce in the Deerfield River tailwater for most of the study. During the first two years of dedicated young-of-the-year sampling, we only found nine (2020) and 12 (2021) age-0 Brown Trout. The reason for this could include daily hydropeaking and lower-than-ideal minimum flows, which can lead to dewatered redds (personal observations by MassWildlife and Deerfield River Trout Unlimited). The dam operator has agreed to raise minimum baseflows from October through April at Fife Brook Dam from 3.5 cms to 7 cms as an initial attempt to remedy this issue. However, redd dewatering may not be the only factor limiting the recruitment of Brown Trout in the tailwater. Hydropeaking has numerous, often negative, effects on trout in regulated river systems. Low Brown Trout population abundance overall and, in particular, the low abundance of age-0 Brown Trout may be a consequence of the hydropeaking operation of Fife Brook Dam. Hydropeaking can be particularly detrimental to the early life stages of Brown Trout, limiting their survival and recruitment (Dibble et al. 2015). For example, rapid reduction in flows following a hydropeaking event can lead to stranding and mortality of younger age classes of trout (Saltveit et al. 2001). Lower growth rates of juvenile Brown Trout have also been shown in hydropeaking systems (Flodmark et al. 2006), which can

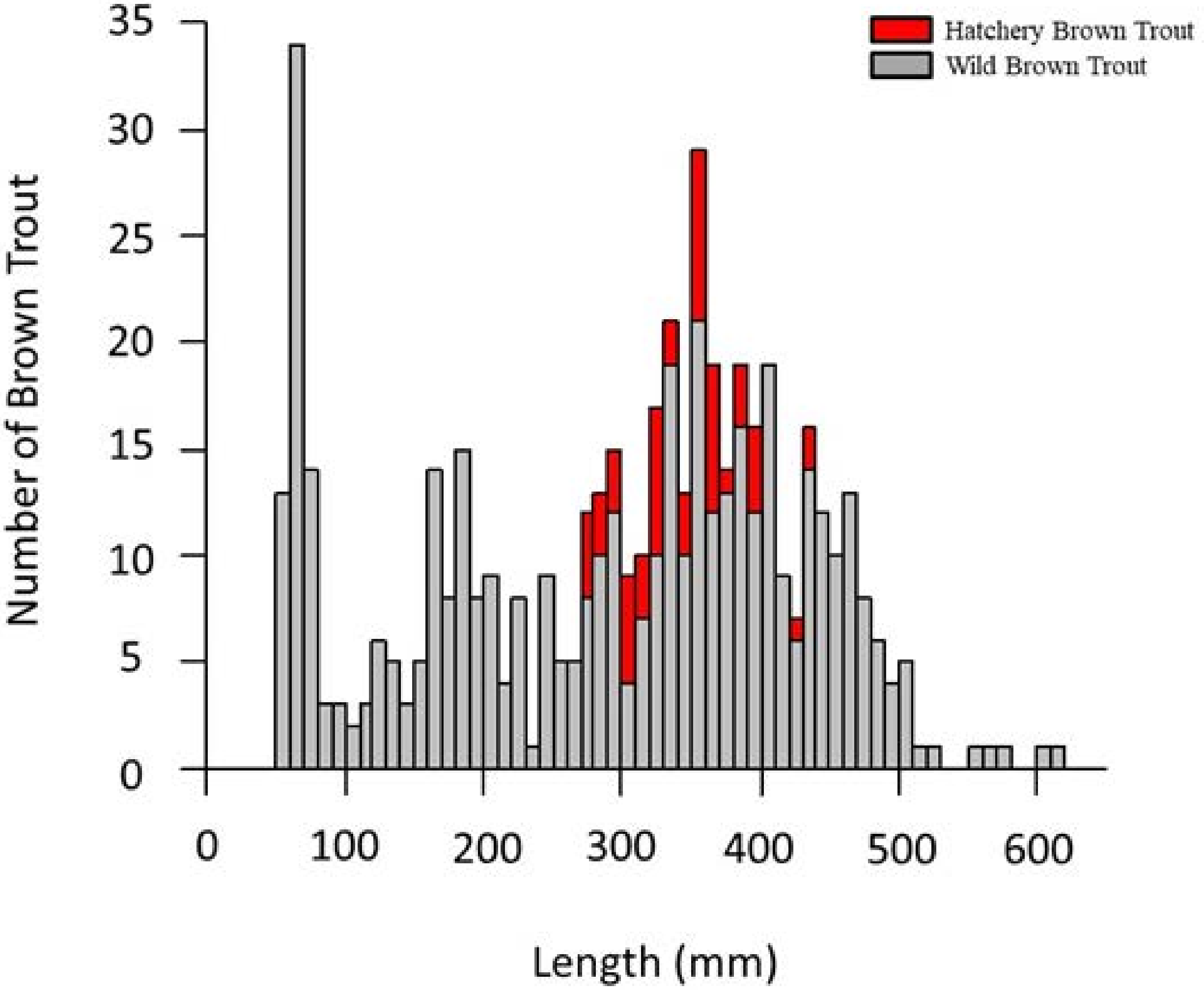

Figure 3. Length-frequency histogram for Brown Trout in the Deerfield River tailwater 2019-2022. All Brown Trout measured were included here. Hatchery-origin Brown Trout are indicated in red.

lead to lower overwinter survival (Borgstrom and Museth 2005). In the Deerfield River tailwater, flow fluctuations are likely to be broadly detrimental to the survival of eggs and fry as trout commence spawning in areas during water releases which then become dewatered during the subsequent drawdown phase (Hayes et al. 2019).

In comparison, the Deerfield River upstream of Fife Brook Dam (the "Bypass Reach") is also a tailwater but with far fewer scheduled peak flow days (32 versus 106 for Fife Brook Dam) from May-September. In the Bypass Reach, most of the flow from hydropower generation is routed through a bypass canal adjacent to the river channel before being released into Fife Brook Reservoir downstream. Therefore, the river upstream of Fife Brook Dam does not experience the same amount of flow variation. Fish survey data, although collected more than 20 years ago, indicate that abundance of age-0 Brown Trout, recruitment, and overall trout abundance were once greater in this upper section than in the more heavily impacted section of the Deerfield River below Fife Brook Dam. More investigation and updated surveys in the Bypass Reach are needed for this comparison to be meaningful.

We plan to extend the current monitoring for the next several years to observe changes in the Brown Trout population following a shift in flow regime with higher minimum baseflows. We expect that recruitment will be higher partly because eggs deposited in redds that were created during higher flows will not be as susceptible to dewatering. In addition, there is likely to be more suitable habitat available for newly hatched Brown Trout with the higher baseflows inundating the shallow nearshore habitat and side channels. Initial evidence supporting this inference comes from our most recent young-of-the-year surveys (2022) where we found a nearly threefold increase in age-0 Brown Trout across the same study reaches we have been surveying the past several years. Streamflow in the Deerfield River tailwater, during the 2021-2022 spawning and incubation periods, dropped below 8.5 cms only briefly four times, all during a a single week in late January and early February 2022. In contrast, streamflow fell below 5.5 cms multiple times throughout the fall spawning period in both 2019 and 2020, potentially resulting in redds being dewatered and trout eggs being exposed to desiccation and freezing temperatures.

Because of the relatively low apparent survival of stocked Brown Trout past the year of stocking, and thus a presumably low return to anglers, there have been discussions about curtailing stocking of Brown Trout in the Deerfield River tailwater. This would make the Deerfield River tailwater a wild and self-sustaining Brown Trout fishery, a condition supported by local guides, anglers, and wild trout advocates. Though considered to be minor, partly because of the relatively low number of Brown Trout stocked into this section of river, there may be additional benefits to wild fish as competition with hatchery fish is reduced and the potential genetic introgression of hatchery Brown Trout with wild Brown Trout is eliminated.

Our results have prompted discussions regarding extending the current catch-and-release area boundaries to include the entire section of year-round trout habitat – i.e., the extent of our study area. As mentioned previously Brown Trout population density in the Deerfield River tailwater is lower than would be expected given the space, habitat, and water temperature. Currently the tailwater is broken into two catch-and-release only sections that cover 4 km of the 13 km study area. Extending the catch-and-release regulations beyond their current boundaries may be a way to limit angling mortality. Even though overall mortality of Brown Trout > age-0 appears to be low, an encompassing catch-and-release regulation may lead to a potential, if somewhat minor, increase in Brown Trout abundance. It is unlikely that the regulation alone would make up for the entire loss in abundance once hatchery Brown Trout are no longer stocked in this part of the river. However, a catch-and-release regulation, together with the future change in flow regime from Fife Brook Dam, should provide the opportunity for the Brown Trout population to increase in abundance.

Summary

This project has allowed MassWildlife to answer some important questions pertinent to the effective management of the Brown Trout fishery in the Deerfield River tailwater. Namely, that 1) this fishery is primarily a wild Brown Trout fishery with relatively minimal contribution from stocked Brown Trout and 2) that Brown Trout abundance is lower than expected, likely because of low recruitment. This project has also brought about crucial discussions on updating management strategies such as no longer stocking Brown Trout in the river and potentially extending the catch-and-release regulations to cover the entire tailwater. Furthermore, with a strong baseline dataset from this initial project, MassWildlife is well set to be able to assess the potential changes to the wild Brown Trout fishery in the Deerfield River tailwater following shifts in management of the fishery as well as anticipated changes to the flow regime coming from Fife Brook Dam. Therefore, results from this study have important management implications for this specific fishery.

Acknowledgements

This project was made possible almost entirely through a close partnership with the Deerfield River Watershed Chapter and the Greater Boston Chapter of Trout Unlimited. These organizations donated the equipment and funds to outfit an electrofishing raft which used to complete this project. Having strong public engagement was critical for this project. We could not have answered these important management questions without interest from concerned constituents who continually advocated for a closer look into the Brown Trout fishery in the Deerfield River tailwater. These groups were also willing and had the capacity to relatively quickly provide the equipment and funds to outfit an electrofishing raft. The success of this partnership has led to additional opportunities for public engagement in the management of our wild trout fisheries across Massachusetts.

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Investigating Angler Use and Harvest Using Creel Surveys and Fish Tagging

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Abstract - Anglers began voicing their concerns regarding perceived overharvest in 2018, after Rainbow Trout *Oncorhynchus mykiss* (RBT) abundance declined from historical levels in the Big Lost River downstream of Mackay Reservoir (tailwater). We conducted a roving creel survey to investigate angler use, catch rates, harvest, and angler satisfaction in the Big Lost River tailwater from 2021 to 2022. We also tagged 238 RBT in the tailwater in 2021 with t-bar anchor tags and used tag return data to estimate angler use and harvest. Data collected from creel surveys indicates that catch rates for RBT were 1.1 fish/h for all tackle combined. The majority (57%) of anglers interviewed said that they would rather catch big fish rather than more fish when fishing the tailwater, and 80% of anglers reported that the size of fish they caught met or exceeded their expectations. Based on the number of RBT reported as harvested during the creel survey, we estimated that about 12% of RBT caught were harvested. Based on the number of tags that were reported, we estimated that 5% of all tagged fish were caught and released, and no tagged fish were harvested over the duration of the study. Angler satisfaction appears to be met in the Big Lost River tailwater despite abundances that are below historical levels.

Introduction

The Big Lost River watershed is located in central Idaho, originating in Copper Basin and eventually flowing southward to the sinks on the Idaho National Engineering Laboratory (INL) site (Figure 1). Because of the scenic quality of the area, its numerous recreational opportunities, and its proximity to the resort area of Sun Valley, the Big Lost watershed receives a considerable amount of recreational use. Fishing is one of the most popular recreational activities in the area (Corsi 1989).

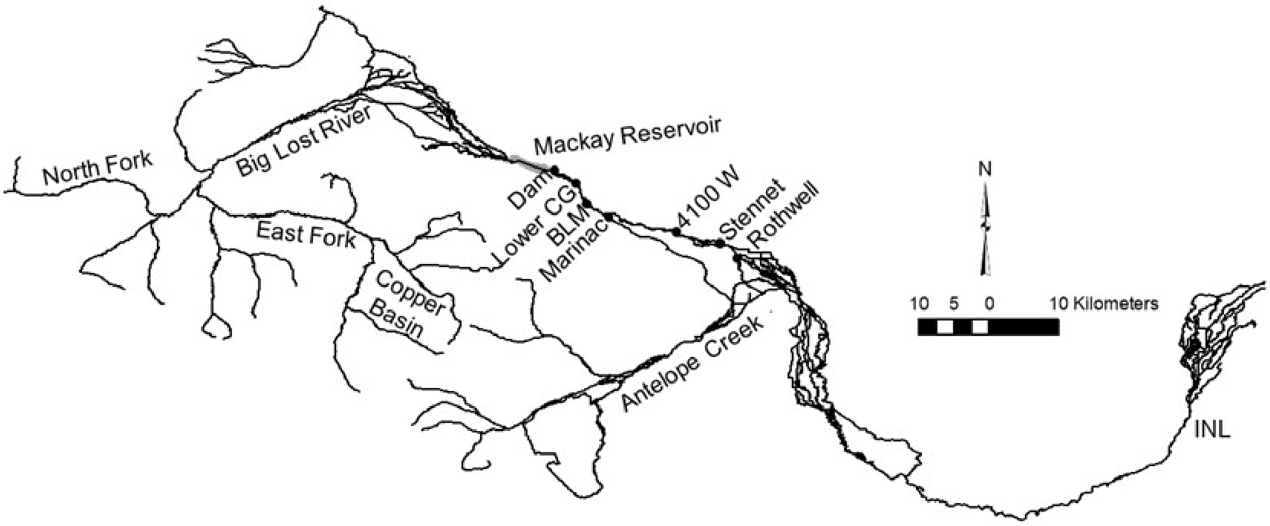

Figure 1. The Big Lost River watershed from the headwaters to the sinks at INL. The seven public access sites in the tailwater reach are labeled and identified by black circles.

A long history of fish stocking has occurred throughout the Big Lost River watershed to provide more opportunities for anglers, since Mountain Whitefish *Prosopium williamsoni* are the only native salmonid in the basin. Fish stocking occurs annually in the watershed upstream of Mackay Dam, but stocking downstream of the dam ceased in 2006. Brook Trout *Salvelinus fontinalis*, kokanee *Oncorhynchus nerka*, and Yellowstone Cutthroat Trout *Oncorhynchus clarkii bouvieri* have been stocked throughout the Big Lost watershed and are found in Mackay Reservoir and in the tailwater. Abundance estimates conducted in 2018 indicated that Rainbow Trout *Oncorhynchus mykiss* (RBT) abundance has been declining throughout the watershed since the 1990s (Heckel et al. 2021). As a result, anglers began voicing their concerns of low catch rates in 2018 and they believed overharvest was limiting the RBT population abundance. In addition, an assessment of angler use and exploitation has not occurred in the watershed downstream of Mackay Dam since 2007 (Garren et al. 2009). Our objectives for this study were to 1) investigate angler use, harvest, angling effort, catch rates, and compare those results to a creel survey conducted in 2007 (Garren et al. 2009); 2) investigate angler satisfaction using a questionnaire during the creel survey; and 3) investigate angler use and harvest using fish tagging. The creel survey and fish tagging were conducted in the tailwater portion of the Big Lost River (Figure 1).

Methods

Fish tagging

In 2021, we randomly selected and tagged 238 RBT, which were collected during electrofishing surveys conducted in two 1-km sections of the tailwater. This number of fish was 20% of the 2018 abundance estimates in those two sections of the tailwater (Heckel et al. 2021). Fish were tagged with non-reward, T-bar anchor tags inserted at the base of the dorsal fin according to standard methods (Dell 1968). Tags were printed with a unique identification number, phone number, and website address where anglers could report the tag using the “Tag You’re It” statewide tag reporting system (Meyer and Schill 2014). We used data from the reported tags to estimate exploitation, caught-and-released fish, and total angler use.

We estimated the angler reporting rate (λ) using the average reporting rate of non-reward tags in the current study relative to the high-reward tags of RBT as estimated by Meyer et al. (2012):

$$\lambda = \frac{\mathrm{R}r \div \mathrm{R}t}{\mathrm{N}r \div \mathrm{N}t}’$$

where Rr and Rt are the numbers of non-reward tags released and reported, respectively; and Nr and Nt are the numbers of high-reward tags released and reported. We assumed a $200 reward tag reporting rate of 100% (Meyer et al. 2012). In the current study, we used statewide averages to estimate tag loss and tagging mortality of RBT (Meyer and Schill 2014). We estimated angler exploitation (u') using the equation:

$$u’ = \frac{u}{\lambda\,(1 - Tag_l)(1 - Tag_m)}’$$

where u is the number of non-reward tagged fish that were reported as harvested divided by the total number of non-reward tagged fish stocked, Tag_l is the first year tag loss rate (i.e., 0.088), and Tag_m is the tagging mortality rate (i.e., 0.01). We also estimated total angler use by modifying u to include fish reported as caught-and-released.

Creel Survey

Following the methods of previous creel studies (Corsi 1989; Garren et al. 2009), we randomly selected two weekdays and two weekend days in every two-week period throughout the creel survey duration (i.e., from May 2021 through April 2022). We considered holidays that fell on a weekday as a weekend day. We delineated the creel survey day to occur from one hour after sunrise until sunset; based on sunrise and sunset times for Idaho Falls. Start times for creel shifts were randomly generated and occurred during one of two shifts: morning (i.e., A.M.) or afternoon (i.e., P.M.). The roving creel survey was designed to collect counts of cars at each of seven public access sites from Mackay Dam downstream to the Rothwell sportsman’s access (approximately 35 river km). Creel shifts were six hours long and two roves were conducted during each shift. Creel technicians would begin the shift at either the most upstream public access (i.e., Mackay Dam) or most downstream public access (i.e., Rothwell sportsman’s access). Technicians would start counts at the randomly assigned access and work in the direction of the opposite access site, stopping to count cars at each public access site. This process was considered a rove. Between roves, technicians would return to access sites where anglers were present and conduct interviews. We recorded access sites where anglers were interviewed, if they were on a guided trip, in a group, how many anglers were in their group, how many anglers were in their car, number of hours they fished, whether their trip was completed, and what time the interview occurred. We also asked anglers which state they were from, what fishing gear type they were using, what species of fish they caught, the number of fish they caught, the number of fish that they harvested, what species they were targeting, and the length(s) of fish they caught. We then asked anglers to rate how they felt about the number of fish they caught on a scale of 1 to 3 (1 being fewer than expected, 2 being met my expectations, and 3 being more than expected). Next, we asked anglers how they felt about the size of fish they caught on a scale of 1 to 3 (1 being smaller than expected, 2 being met my expectations, and 3 being larger than expected). We also asked them if they would rather catch "big fish" or "more fish" on trips to fish the Big Lost River tailwater. Lastly, we asked anglers to rate their fishing experience that day on a scale of 1 to 5 (1=poor, 2=fair, 3=average, 4=good, and 5=excellent).

In this study, a model-based estimator was used to estimate angling effort and catch instead of a traditional design-based estimator. The model-based estimator provides more accurate estimates of effort and catch because it leverages information about the temporal dependence among the parameters of interest as opposed to treating them independently, which is common with design-based estimators.

More specifically, a state-space model was used to estimate effort and catch. The state-space model included process models for effort and catch rate that described the true but unknown states of effort and catch, and observation models that were conditional on the respective process models.

The process model for effort was defined as:

$$log(N_t) = log(N_{t-1}) + r_{e,t},$$

where N_t is the true, but unknown, number of angler vehicles present at day t, and $r_{e,t}$ is the rate of change in angler vehicles from the previous day which was defined as:

$$r_{e,t} \sim normal(\mu_{r,e}, \sigma^2_{r,e}),$$

where $\mu_{r,e}$ and $\sigma^2_{r,e}$ are the mean and variance of the distribution of the rate of change in effort.

The observation model for effort was defined as:

$$Y_{e,t} \sim Poisson(N_t \times \pi_t \times 1/a),$$

where $Y_{e,t}$ is the sum of cars counted on day t, π is the sampling probability on day t, which was defined as the number of counts divided by the total hours in the fishing day, and a is the average number of anglers per vehicle and was estimated as:

$$Y_a \sim Poisson(a),$$

where Y_a is the angler per car data collected during angler interviews.

The process model for catch rate was similar to the process model for effort and was defined as:

$$log(R_{i,t}) = log(R_{i,t-1}) + r_{i,c,t},$$

where $R_{i,t}$ is the true, but unknown, catch rate on day t for species and disposition i (e.g., Rainbow Trout released), and $r_{i,c,t}$ is the rate of change in catch rate from the previous day, which was defined as:

$$r_{i,c,t} \sim normal(\mu_{i,r,c}, \sigma^2_{i,r,c}),$$

where $\mu_{i,r,c}$ and $\sigma^2_{i,r,c}$ are the mean and variance of the distribution of the rate of change in catch rate.

The observation model for catch rate was defined as:

$$F_{i,t} \sim Poisson(R_{i,t} \times H_t),$$

where $F_{i,t}$ is the number of fish of species and disposition (i) caught and reported to creel clerks during angler interviews on day t and H_t is the number of angler hours reported to creel clerks during angler interviews.

Daily catch of species and disposition i, $C_{i,t}$ was estimated as:

$$C_{i,t} = N_t \times R_{i,t}.$$

Daily estimates of effort and catch were summed to estimate effort and catch for the fishing season.

The model was fit using Bayesian methods. Markov Chain Monte Carlo algorithms were used to estimate posterior distributions for all model parameters. Analyses were performed using the JAGS program (Plummer 2003) implemented in R using the R2jags package (Su and Yajima 2012; R Development Core Team 2015). Posterior distributions were generated using three chains of 20,000 iterations that were thinned by six with a burn-in of 10,000. Parameters were checked for convergence based on the Gelman-Rubin statistic (i.e., $\hat{R}$ less than 1.05; Brooks and Gelman 1998). Estimates of all parameters were summarized as the median of the posterior distributions. Prior distributions used for all model parameters along with the JAGS model code are given in Appendix A.

We calculated the percent of harvest for all salmonid species combined for the duration of the creel survey (i.e., one year) by dividing the total number of fish harvested for all salmonid species by the total number of fish caught for all salmonid species, then multiplied that quotient by 100 and rounded to the nearest whole number. We further calculated the percent of harvest by species for the duration of the creel survey (i.e., one year) by dividing the total number of fish harvested for the respective species (e.g., Rainbow Trout, Brook Trout) by the total number of fish caught for the respective species, then multiplied that quotient by 100 and rounded to the nearest whole number. We made the same calculations for creel survey data from 2007 to compare the two surveys.

RESULTS

Fish tagging

We tagged 238 RBT with non-reward, T-bar anchor tags in the tailwater reach. Anglers reported catching six of those fish, and all of these fish were reported to have been released. Therefore, we were unable to estimate exploitation of fish based on tag returns. We calculated the adjusted reporting rate (Meyer et al. 2012) at 11% and total angler use at 5%, which accounts for tag loss and tagging mortality at the rates reported by Meyer et al. (2012).

Creel Survey

We interviewed anglers from 23 different states that fished the Big Lost River tailwater from May 2021 through April 2022. The number of anglers per group varied, but we collected data from 324 interviews. The average number of anglers per vehicle was 2.42. We estimated 33,325 h (97.5% credible interval, ± 6,167; Table 1; Figure 2) of angling effort for the 12-month creel survey period. Furthermore, we estimated catch rates in the tailwater were 1.1 fish/h for all tackle combined (Figure 3). Fly anglers reported catching 1.23 fish/h, lure anglers reported catching 0.61 fish/h, and bait anglers reported catching 0.60 fish/h. Bait anglers reported the highest rate of harvest (76%), followed by lure anglers at 20%, and fly anglers at 3%. We calculated the total percent of harvest for the duration of the creel survey at 11% for all species combined (12% in 2007), with 12% for Rainbow Trout (15% in 2007) and 2% for Brook Trout (1% in 2007). Anglers did not report harvesting kokanee during the current study, but they reported 23% of kokanee harvested in 2007. We estimated that the catch-and-release rate for the duration of the creel survey was 1.0 fish/h (Figure 4), and the harvest rate was 0.1 fish/h. Of all anglers interviewed, 52% were fly anglers, 26% were bait anglers, and 22% of anglers

Table 1. Summary of creel survey data from May 2021 through April 2022, and 2007 (Garren et al. 2009) for comparison, in the Big Lost River tailwater.

	2021	2007
Number of interviews	324	375
Hours of angling effort	33,325	30,340
Number of fish caught	38,085	50,482
Catch rate (fish/h)	1.1	1.7
Harvest rate (fish/h)	0.1	0.2
Release rate (fish/h)	1.0	1.5
Mountain Whitefish caught	415	558
Rainbow Trout caught	34,721	38,492
Brook Trout caught	2,514	8,567
Kokanee caught	435	823
Rainbow Trout harvested	4,064	5,681
Brook Trout harvested	41	125
Kokanee harvested	0	190

reported using lures. Anglers reported harvesting fish at four of the seven access sites, with the most harvest occurring at the Mackay Dam access at 87%, followed by the Lower Campground access at 8%, 4% at the Rothwell access, and 1% at the 4100 W access (Figure 1). Angler interviews showed that 57% of those interviewed would rather catch big fish instead of more fish when fishing the Big Lost River tailwater, and 43% of interviewed anglers would rather catch more fish. In addition, 65% of anglers interviewed rated their day of fishing as good or excellent, 19% responded average, and 16% responded that their day of fishing was poor or fair. Eighty percent of anglers interviewed said that the size of fish that they caught met or exceeded their expectations, whereas 20% reported that it did not meet their expectations. Of all the anglers that reported catching fish, 68% reported that the number of fish they caught met or exceeded their expectations, and 32% reported that they caught fewer fish than expected.

The design-based estimator was used on days that were surveyed and indicated that over 300 h of angling effort occurred seven times during the 12-month study (i.e., May 2021 through April 2022; Figure 2). The design-based estimator was used to inform the model-based estimated daily angling effort, which showed the trends of daily angling effort and indicated that there was little to no angling effort during the winter months (e.g., days 200 to 300). We also calculated that "observed" catch rates from days that were surveyed exceeded 2 fish/h eight times (Figure 3). The daily estimates of Rainbow Trout caught and released exceeded 300 fish six times during the 12-month study (Figure 4).

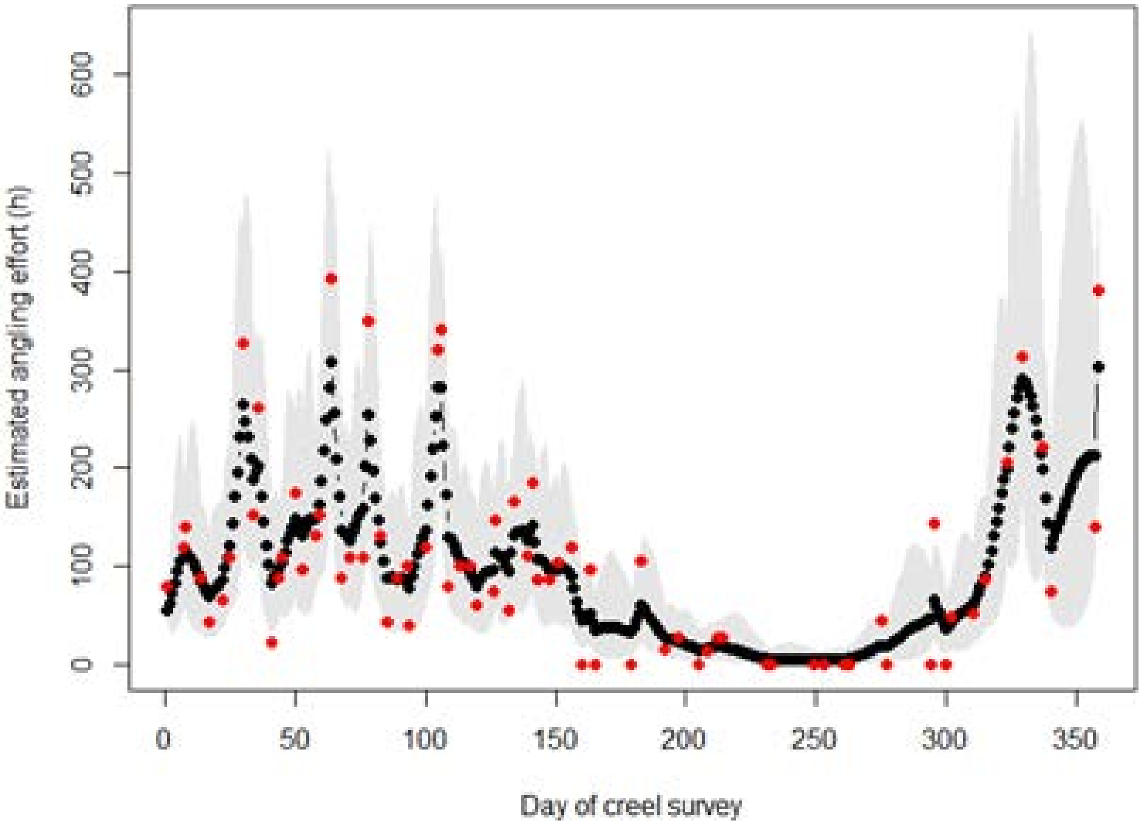

Figure 2. Model-based estimate of daily angling effort (black points and lines) and daily 'observed' estimates of angling effort (red points). Day 0 represents the first creel survey day (May 1, 2021) and the end of the study (April 22, 2022) is beyond day 350.

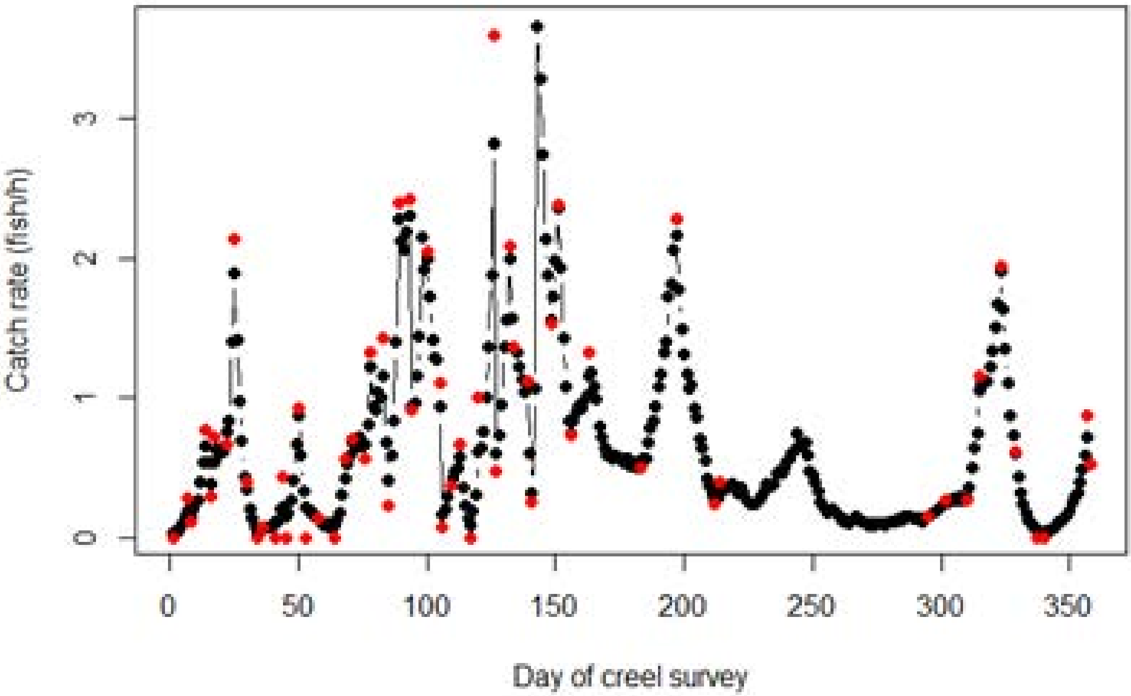

Figure 3. Model-based estimate of daily catch rate of Rainbow Trout released by anglers (black points and lines) and daily 'observed' estimates of daily catch rate of Rainbow Trout released by anglers based on angler interviews (red points). Day 0 represents the first creel survey day (May 1, 2021) and the end of the study (April 22, 2022) is beyond day 350.

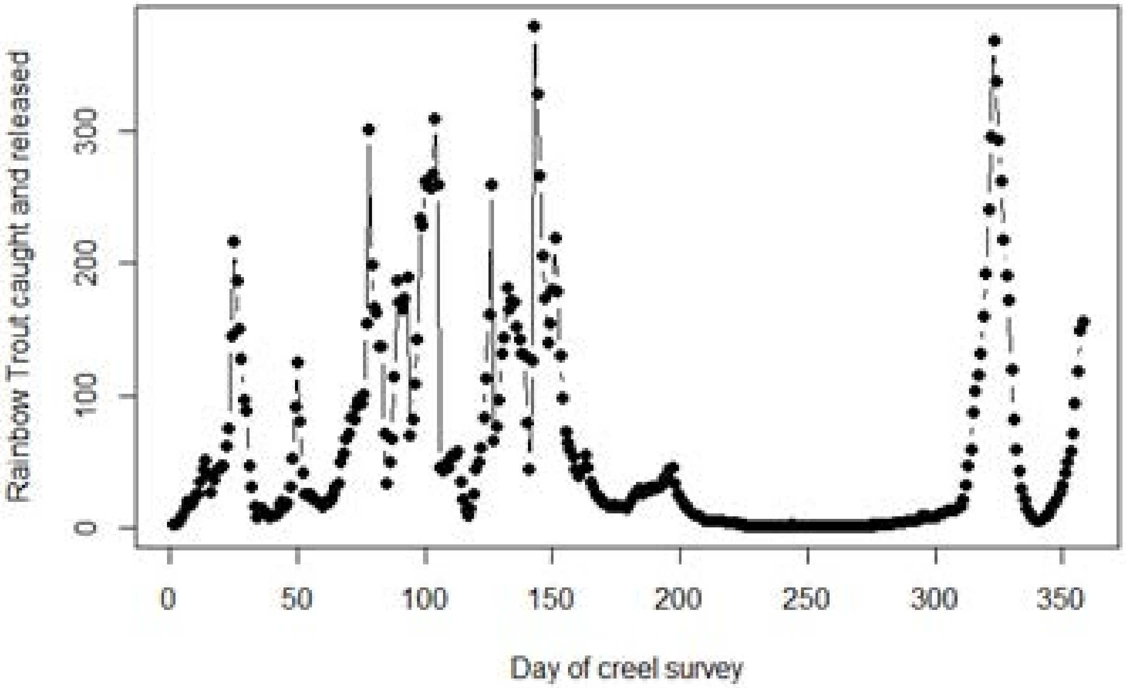

Figure 4. Model-based estimates of the daily number of Rainbow Trout caught-and-released on the Big Lost River. Day 0 represents the first creel survey day (May 1, 2021) and the end of the study (April 22, 2022) is beyond day 350.

Discussion

Using the compensatory mortality hypothesis (Allen et al. 1998), we believe that the level of angler harvest (i.e., 11%) does not appear to have a population level effect based on total annual mortality (i.e., 65%) that was estimated for the Big Lost River tailwater wild RBT population in 2021 (Kennedy et al. in press). Environmental factors and entrainment in canals (Kennedy 2009) are likely contributing to a greater level of mortality on the RBT population in the Big Lost River tailwater than harvest. Entrainment into irrigation canals and river dewatering have also been suggested to contribute to unstable populations and low abundance of Mountain Whitefish in the Big Lost River (Kennedy 2009), which could also be occurring for the Big Lost River RBT population.

Based on our creel survey data and angler responses to the questionnaire, anglers appear to be satisfied. The majority of anglers expressed satisfaction with the size of fish that they caught, the number of fish they caught, and rated their day of fishing as good or excellent. Despite anglers reporting low catch rates and declines in abundance due to perceived overharvest, our creel survey and tagging data indicate a different reality. Overall, average catch rates for the duration of the creel survey were similar to the 2007 creel survey catch rate estimate. Additionally, anglers reported catch rates greater than 2 fish/h during multiple two-week periods of the creel survey. The harvest rate per hour and the total amount of harvest have declined since 2007, additionally, the tailwater remains closed to harvest from December until Memorial Day weekend. Furthermore, angler effort declines to nearly zero from December through March. Taking these factors into consideration, there is a period of time when the fish population gets a partial rest from angling pressure and a complete rest from harvest.

The majority of harvest occurred at the Mackay Dam access. Despite hatchery fish not being planted in the tailwater, we believe hatchery RBT contributed substantially to the harvest in that area for several reasons. Hatchery RBT were planted in Mackay Reservoir and likely moved downstream through the dam into the tailwater as has been observed for kokanee, a lacustrine species present in Mackay Reservoir. Additionally, one study has shown that hatchery RBT typically move downstream after being out-planted (High and Meyer 2009). Most of the harvest occurred in the area of large pools immediately downstream from Mackay Dam (i.e., close to source of the hatchery fish). Additionally, creel survey personnel observed fin erosion, which is typical of fish held in concrete raceways, on some of the fish they observed to have been harvested. Lastly, no angler reported a tagged, wild RBT as being harvested. Research is ongoing to determine the contribution of hatchery fish to fish harvest in the tailwater. Fin clips collected from harvested RBT during creel surveys are being tested to determine whether harvested fish were diploid or triploid, as only triploid RBT are stocked in Mackay Reservoir.

The level of angling effort remains consistent in the Big Lost River tailwater with slightly more angling effort in 2021-2022 than in 2007, but less harvest reported in 2021-2022 than in 2007. Therefore, fewer fish are being harvested now than in the past. In addition, based on the number of anglers from other states and an increase in angling effort since 2007, the Big Lost River tailwater is a popular fishery. The Big Lost River tailwater fishery also contains fish that, on average, exceed angler's expectations for fish size and quantity and has opportunities for anglers to access sections of the river at seven public access sites.

Acknowledgements

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Appendix A

JAGS code (including prior distributions) for the state-space model.

```
model {

  #Effort model

 logN[1]~dpois(log(100))

 for(t in 2:T){
  logN[t]<-logN[t-1]+r[t]
  r[t]~dnorm(mean.r, tau.proc)
 }

 for(t in 1:T){
  E[t]~dpois(N[t]*pi[t]*(1/Apc))
 }

 for(t in 1:T){
 N[t]<-exp(logN[t])
 }

 mean.r~dnorm(0, 0.0001)
 sigma.proc~dunif(0, 5)
 sigma2.proc<-pow(sigma.proc, 2)
 tau.proc<-pow(sigma.proc, -2)



 for(i in 1:Ncar){
  A[i]~dpois(Apc)
 }

 Apc~dunif(0, 5)


  #Catch rate model
  for(i in 1:I){
   logR[i,1]~dunif(log(0.001), log(0.1))
  }

  for(i in 1:I){
   for(t in 2:T){
     logR[i,t]<-logR[i,t-1]+ir[i,t]
   }
  }
```

```
 for(i in 1:I){
   for(t in 1:T){
   ir[i,t]~dnorm(mean.ir[i], tau.procir[i])
  }
 }

 for(i in 1:I){
  for(t in 1:T){
   F[t,i]~dpois(R[i,t]*H[t])
  }
 }

 for(i in 1:I){
  for(t in 1:T){
   R[i,t]<-exp(logR[i,t])
  }
 }

for(i in 1:I){
  mean.ir[i]~dnorm(0, 0.001)
  sigma.procir[i]~dunif(0, 3)
  sigma2.procir[i]<-pow(sigma.procir[i], 2)
  tau.procir[i]<-pow(sigma.procir[i], -2)
}
```

```
 #derived parameters
#total effort
 totN<-sum(N)

#daily catch
for(i in 1:I){
  for(t in 1:T){
  dailycatch[i,t]<-R[i,t]*N[t]
  }
}

#season catch
for(i in 1:I){
seasoncatch[i]<-sum(dailycatch[i,]) } }#end model
```

Returning Rotenone to the Restoration Tool Box in Banff National Park, Canada: Reflections on Public Engagement and Communications Tools to Address Public Interest in a National Park Context

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Abstract - Rotenone has not been commonly used in the province of Alberta or the Canadian mountain national parks in recent decades. In 2018, Banff National Park completed the first of a series of eight planned rotenone applications to restore threatened Westslope Cutthroat Trout *Oncoryhnchus clarkii* lewisi after over 40 years of non-use. We were not sure what to expect in terms of public concern or interest. We have now completed six of the eight applications and can reflect on our progress. We will outline some of the public communication activities completed before, during, and after the rotenone treatments that we believe contributed to low public concern. Some activities such as typically interested stakeholder meetings and conventional media including radio, television and newspaper were utilized, but we have also used some less common methods including active interpretive stations during application, use of drone footage to create video suitable for social media content, satellite-linked stream side school events and guided interpretive hikes focussed on species at risk and our restoration process.

Introduction

Westslope Cutthroat Trout *Oncorhynchus clarkii lewisi* are a threatened species in Canada with Bow Lake, in Banff National Park, the northern limit of their distribution. From 2011 – 2017 we attempted manual removal of Eastern Brook Trout *Salvelinus fontinalis* from Hidden Lake and associated stream segments to support restoration for threatened Westslope Cutthroat Trout. Hidden Lake is in the heart of Banff National Park near the hamlet of Lake Louise. It previously contained Westslope Cutthroat Trout, but they were displaced by Eastern Brook Trout 50 years ago. Although we were aware of rotenone as a tool, and had referenced it as an option in our original environmental assessment from 2011, we were persuaded to start with manual removal because of the recent success of the removal of fish from the Devon Lakes (Pacas and Taylor 2015) in our adjacent work unit, also in Banff National Park. Rotenone had not been used in Banff National Park in over 40 years. By 2016 we knew we were failing at manual removal and needed to investigate other options. Unsuccessful manual removal methods at Hidden Lake dominated our restoration efforts and kept us from addressing other emerging issues. We contacted colleagues from Montana to gather direct experience with rotenone. We sought additional funds, hired additional staff, completed a detailed impact assessment, increased our training and experience, and prepared for our first rotenone application at a suite of five locations planned to start in 2018.

There were some expected and unexpected problems including internal disagreements about the use of rotenone, which contributed to a one-year delay in our special conservation funding, and the need for completion of a systematic review of fish removal methods (Rytwinski et al. 2018). We were also unable to engage in formal public meetings, formal consultation, or proactive media engagement due to conflicts from other higher-priority projects with legal consultation requirements. Finally, changes to legislation meant we were no longer required to circulate the impact assessment for this type of project, which we would have routinely done under previous legislation and policy. This meant that many people, including our highly engaged local and regional media and most active stakeholders, would only hear about our project and the use of rotenone for the first time when the legal area closures went into place in the days leading up to the first rotenone application. Some park visitors would discover that they would not be permitted to hike to a fairly popular location when arriving at the trailhead. We were concerned that this could stop our project from advancing, so we revised our pre-treatment communication planning to rely less on conventional public participation activities which were not available to us.

We have now completed six (seventh is planned for late August 2022) of the eight planned rotenone treatments in Canada's highest-profile national park and we have generated very limited public concern. We are now actively introducing threatened Westslope Cutthroat Trout into the first basin in which we used rotenone and are contemplating other candidate locations for future work. We believe our communication activities have increased public knowledge and possibly support for the need for trout restoration as part of our national park mandate. Below we will outline some of the communication tools that we used before, during, and after our rotenone treatments.

METHODS

Pretreatment

We consulted with as many people involved in rotenone projects as possible. These conversations were invaluable to help us understand issues and concerns on similar projects and gather input to address these issues and concerns. This influenced our planning and the basis of an extensive question-and-answer document and detailed communication plan. As we were unable to host formal public participation meetings and had no requirement to post or circulate our impact assessment, we relied on some existing regional Weststlope Cutthroat Trout stakeholder meetings to communicate our intent. This allowed communication to our larger region but did not inform our specific local groups and residents. We then scheduled one-on-one meetings or phone calls with members of all local or regional groups or individuals believed to be interested or concerned. One stakeholder suggested the addition of interpretive signs next to the legally required placards, which we did (Figure 1). Within our agency, we hosted lunchtime meetings, webinars, and attended all Banff National Park spring start-up meetings to discuss the project and gather feedback and reactions. As expected, the posting of the legal closure generated immediate questions from the media. In anticipation of this, a detailed media package, including high-quality images, was made available and we had a spokesperson availabel for all reactive interview requests.

During Treatment

The Hidden Lake area is a several-hour walk from the nearest road along a popular backcountry backpacking circuit, to which we had been instructed to maintain access. The hiking trail paralleled the treatment stream for approximately 1 km through the temporary area closure. To accommodate maintaining access along this trail during the area closure and treatment days, we established five communication nodes – one on each side of the treatment area and one at each of the three stream crossings within the treatment area. Each station had a placard and interpretive sign but also a uniformed interpreter or communication officer to speak with people about the project. The interpreter informed people about where they could take water and what they might see as they crossed the stream coming from the lake (e.g., pink or green water, dead fish, and people in the distance wearing Tyvek suits and masks). They also had a list of alternate day hikes, angling lakes, or summits available to visitors encountering our closure. Over five days, hundreds of visitors passed by and interacted with our staff during the first treatment. We also included a senior biologist from Trout Unlimited Canada in the fish salvage and mobilization day, so members of this group would have direct access to knowledge of the project intent and elements, including mitigations.

Figure 1. Area closure, pesticide warning and interpretive habitat restoration work in progress sign.

Post Treatment

After treatment, we reopened the area as quickly as possible while meeting the legal requirements. This meant that the public resumed hiking, climbing, and backpacking into the treatment lake while we were still doing daily fish clean-up and completing deactivation. We retained one interpreter on-site to handle questions. Many visitors also approached the uniformed biologists while we were working, with curious but informed questions – it was clear that they had read our interpretive signage.

During the treatments we had dedicated communication officers gathering official imagery and overseeing drone image collection. These images were provided to the media as part of the follow-up interviews we offered. We have now developed a series of small videos explaining these projects and methods. These short 30-second and 1-3 minute videos will be launched in August 2022 and are intended for social media, conferences, and our website.

Parks Canada interpreters have developed a theatre program, delivered at the Lake Louise campground, and trailhead roving kits, that the interpreters can set up anywhere including special events. These materials explain how removal of Eastern Brook Trout benefits native Westslope Cutthroat Trout trout and what actions Parks Canada is taking, including the use of rotenone, to increase populations of Westslope Cutthroat Trout.

We have also developed a high-quality interpretive conservation hike into the Hidden Lake restoration area where a certified interpretive hiking guide takes small groups of visitors and explains the restoration project and provides the latest updates. Last year park visitors on the hike were able to join us while we released the first of the hatched Westslope Cutthroat Trout, which generated many positive comments. This year permanent interpretive signage has been installed throughout the restoration area (Figure 2).

In spring 2022, we partnered with Exploring By The Seat of Your Pants to deliver, via satellite link, a fully interactive program from a remote location at the edge of a small stream with Westslope Cutthroat Trout, to 90 classrooms across North America. We showed the students live fish and explained our work. The hour-long program was fully interactive with the classroom participants but was also recorded and is available for viewing at any time.

We continue to provide annual updates at stakeholder meetings, technical meetings, staff updates meetings, and media follow-up events. We also routinely provide site tours for senior managers and media.

Figure 2. Interpretative signage has been developed and installed in the Hidden Lake area.

RESULTS

Since our first rotenone treatment in 2018, we have completed six treatments (seven by late August 2022) at four lakes and associated streams. No rotenone has escaped deactivation or resulted in unintended consequences. Post project monitoring using eDNA, benthic invertebrates, and zooplankton shows our work has accomplished all early biological goals. Westslope Cutthroat Trout reintroduction commenced in 2021 and will continue.

Public concern has been extremely low consisting of a few emails to the biologist questioning some aspects of the science, some questions from Parks Canada staff, and a few comments on social media. The restoration location in 2021 generated a few more comments as it was a more popular angling location that contained non-native Yellowstone Cutthroat Trout Oncorhynchus clarkii bouvieri, but still, the comments were on the low end of the public concern continuum (Figure 3), with less than five anglers voicing concern.

We have had no delays once project implementation began, and have been able to attract additional funding. Banff National Park has an international reputation and is carefully covered by local and regional media – the stories about our work that have been aired by the press and have gone national or international have been positive and have generated additional media opportunities in print, radio, and magazine. We have been contacted by other groups to support their rotenone projects in Canada and, more recently, in Argentina.

We are aware of other rotenone projects that have been affected by public concerns, so we do not assume that we will always have low public concern for our work.

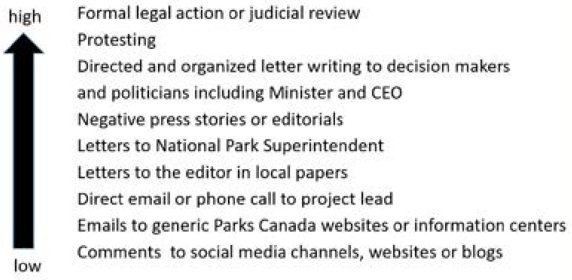

Figure 3. Typical ways public concern is expressed to Parks Canada along a continuum of concern from low to high.

Dissussion

Area closures of desirable locations in one of Canada's most beloved national parks during the peak of summer visitation and the use of chemicals to restore fish in those same areas have the potential to generate high public interest and complaints. People use Banff National Park in different and sometimes surprising ways. Resident and non-resident anglers, climbers, horseback riders, all get their information in different places. A comprehensive communication plan with diverse tools and a well-informed staff is needed to address the many audiences and users that we have in Canada's busiest national park. We believe the items listed below are some of the communication elements that have allowed this work to continue and build momentum. Dedicated communication staff funded by our project included a full-time communication officer and an interpreter. These two staff members are funded by our project but report to their respective sections, which are not within resource management. Embedding them into other sections makes accessing additional interpreters, communication officers, and media relations staff seamless and allows us to receive input from additional staff with varying expertise.

- Early meetings with rotenone users to get advice and plan our communication approaches were invaluable. They correctly identified prospective issues which allowed advanced preparations to be implemented.
- One-on-one meetings with key people. These meetings are less efficient than larger public or stakeholder meetings but we believe they were very effective when a larger public meeting were not an option. This is also an important and respectful way to treat people and allow them to share.
- The use of on-site interpreters and communication officers to explain the purpose of the work, distribute fact sheets, answer questions, and redirect the public to other locations worked very well to dissipate any concerns. Staff at these stations had zero negative comments or interactions.
- Internal audiences can not be overlooked and downplayed and often have excellent suggestions. Although we all work for the same conservation organization, not everyone will agree with what has been proposed and the plan to get it done.
- Respond quickly to emails and letters and incorporate unique questions into a regularly updated question-and-answer document.

Other agencies have expressed interest in using rotenone but are concerned about generating controversy. We hope that our experience can be used to show that we were able to add this important restoration tool back into our toolbox with little public concern. We know not every agency or department has the same mandate or budget for public communication, but we hope that our investments in this area, and some of the tools we have developed, will be directly or indirectly helpful to other practitioners. We think proactive communication is also important to attract attention to this project, this allows our work to continue to attract funds, as our agency addresses the financial challenges of managing multiple species at risk.

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People involved in rotenone projects in La Maurice National Park of Canada and from Montana Fish Wildlife and Parks were very helpful by sharing the details of their experience. We are grateful for their time. The manuscript does not necessarily constitute an endorsement by Parks Canada.

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Creel Monitoring of Washington Coast Recreational Winter Steelhead Fisheries

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Abstract - Sport fisheries targeting anadromous *Oncorhynchus mykiss* (steelhead) in streams along the coast of Washington State provide an important recreational opportunity for anglers and revenue for local communities. Following the decline of wild steelhead abundance over the past decade, fisheries managers have relied on catch-and-release regulations to maximize fishing opportunities while limiting impacts on wild steelhead. To quantify the impact (e.g., release mortality) of these fisheries on wild populations, we conducted on-site creel surveys in select fisheries. Specifically, the Washington State Department of Fish and Wildlife utilized a roving creel survey design to conduct angler counts and interviews to sample fishing effort and catch rates, respectively. Creel survey data were analyzed using a newly developed Bayesian state-space model to generate estimates of catch, effort, and catch-per-unit-effort with associated uncertainty. When generated weekly, these estimates provide a high-resolution tool for fisheries managers to monitor impacts and identify the need for in-season adjustments to fisheries regulations. Additionally, creel surveys create an opportunity for fisheries managers to interact with the public while providing valuable data to sustainably manage fisheries.

Introduction

Winter steelhead *Oncorhynchus mykiss* fisheries provide economic and cultural significance to communities along the coast of Washington State. Providing recreational fishing opportunities for steelhead has become increasingly difficult for Washington State Department of Fish and Wildlife (WDFW) managers due to declines in the abundance of wild steelhead over the past three decades. To protect populations and maximize recreational angling opportunities, managers have implemented catch-and-release (C&R) regulations for wild steelhead. While C&R regulations should dramatically reduce fishing-related impacts relative to harvest, steelhead released from a C&R fishery are still susceptible to post-release mortality due to stress and injuries. Therefore, the first step in understanding the impact a C&R fishery is having on a population is to estimate catch, which, for our purposes, is the total number of fish encountered (i.e., brought to hand) and released.

For over a half-century, angler surveys (also known as creel surveys) have been used to gather data from anglers to estimate catch (Robson 1960; Malvestuto 1978; Pollock 1994; Jones and Pollock 2012). There are many types of angler surveys, but most are generally categorized as either an on-site or off-site survey. An off-site catch record card system to monitor recreational fisheries has been utilized by WDFW for over sixty years and today this system generates estimates of catch for harvested steelhead along with salmon, sturgeon, halibut, and crab for all fisheries in Washington State. However, under its current construct, the catch record card system has two main limitations. First, the program only collects data on harvested fish, meaning catch of released fish cannot be quantified. Second, the catch record card system is still paper based, meaning estimates of catch are delayed one to two years after harvest occurs. On-site creel surveys provide an alternative approach to estimate recreational catch of non-retained fish where C&R regulations are mandated, like the coast of Washington State, where all retention of wild steelhead was halted in 2016.

On-site creel surveys allow real-time data to be collected on fishing pressure and catch rates of all species encountered, which together provide the information needed to estimate catch. Over the past few years, WDFW developed a standardized study design approach for conducting on-site creel surveys that are rooted in established methods (Pollock 1994). The overall goal of this approach is to generate unbiased estimates of catch that are consistent and comparable with other fisheries. The specific creel survey study design is known as a roving creel survey and is most appropriate for fisheries where anglers can access a waterbody from many points over a relatively large area. Along with the standardized creel survey study design, WDFW has also developed an array of supporting products, which include a creel survey schedule generator, electronic data collection, a statewide creel survey database, and script-based analyses.

Currently, WDFW staff are refining methods to streamline analysis and reporting to ensure catch estimates are generated in a timely manner to better inform in-season management decisions. Using current forecasting methods, fish managers can establish goals of allowable catch of wild steelhead in a season. These goals are likely to change each year due to variability in returning runs. By utilizing the stream-lined creel survey process during the fishing season, estimates of catch can be produced weekly or even daily, allowing managers to track catch compared to the pre-season goal. If the number of wild steelhead caught during the season is higher or lower than the goal, managers can use this information to help decide if regulation changes are appropriate to further protect steelhead or provide extended opportunity for anglers. During the 2022 winter steelhead season, stream-lined creel survey methods were partially implemented in two tributaries (Willapa and Naselle Rivers) located within Willapa Bay in southwest Washington. Creel surveys had not been conducted in this area since the 1970s and therefore it provided the opportunity to implement standardized creel survey methods in a new system. While the final analysis is not yet complete, the pilot creel survey in Willapa Bay provided valuable experience for WDFW managers to successfully implement creel survey programs in new systems along the coast. Additionally, creel monitoring depends upon the interaction between management agencies and the public and in this way increases the public's awareness and understanding of management objectives.

Methods

A roving creel survey is used by WDFW to monitor recreational fisheries. This approach is well suited for fisheries with many diffuse access points. Unlike an on-site access creel survey design, where anglers are enumerated and interviewed at select sites, a roving creel survey design requires surveyors to move throughout a fishing area to collect spatially and temporally representative effort and catch rates data (Malvestudo 1978; Pollock and Jones 2012; Hahn 2000). Data collected during a roving creel survey come from three main survey components – index effort counts, census (also known as tie-in) effort counts, and angler interviews – that are implemented in accordance with the creel survey schedule.

Creel survey Schedule

Roving creel surveys are implemented using a multi-stage stratified sample design. First, the length of the fishery is delineated, and individual dates are assigned as either weekdays or weekends. Individual survey dates are randomly selected each week based on their day type and the total number of surveys per week. Second, each survey day is divided into shifts (e.g., AM/PM) and a randomized survey start time is selected typically based on legal fishing hours (e.g., 1-hr before sunrise and 1-hr after sunset). Third, index effort counts times are selected in a randomized manner with systematic review to ensure counts can be logistically deployed in the field. Lastly, census effort count dates and times are selected on a sub-set of survey dates.

Index Effort Counts

Angler effort is estimated by enumerating cars and trailers at designated angler access points throughout the fishing area. The access points surveyed provide an index of effort. Cars and trailers provide indirect representations of the number of anglers present within an area and are counted more efficiently than individual anglers along streams. Surveyors drive pre-determined routes at least twice per shift providing a 'snapshot' of the effort occurring in that location on that specific day and time. Often it is logistically infeasible to enumerate fishing effort across the entire spatial extent of the fishery. Therefore, index effort counts are typically conducted in sub-sections to provide an index of fishing effort. Index effort count sections consist of standardized areas such as parking lots, boat ramps, pull-outs, and/or specific reaches of a waterbody that are easily accessible. Fishing effort enumerated within index reaches can be expanded to the entire fishing area by conducting census effort counts (see below).

Census (aka Tie-in) Effort Counts

Data collected at index effort count sites do not encompass the complete angler effort within a fishery. Anglers outside surveyed index reaches may not be encountered, leading to under-estimation of effort. In areas where mixed-use of access occurs, counts of non-anglers may lead to an over-estimation of effort. Tie-in counts relate the effort enumerated at index effort counts sites to census effort occurring within the entire fishery area, providing a correction factor to generate an unbiased estimate of angling effort (Bentley and Buehrens *in prep*). Like index effort counts, tie-in surveys provide a 'snapshot' of the fishery in time and space. The tie-in survey occurs over the entire length of stream where anglers are actively participating in the fishery and must overlap a concurrent index effort count. Census effort counts utilize helicopters, motorized boats, non-motorized boats, or foot surveyors to cover the stream channel and enumerate anglers. Individual anglers are grouped into sub-categories based on their fishing location (e.g., boat vs. shore) and watercraft type.

Angler Interviews

Angler interviews are conducted by surveyors each sample day to, at minimum, gather catch rate data, which provides the complementary information to effort count data necessary for estimating total catch. The standard list of questions includes fishing time, gear, method (boat vs. shore), number of anglers in the party, number of cars brought to the area, species retained, species released, and guided vs. non-guided trips (Bentley and Buehrens *in prep*). Surveyors attempt to conduct as many interviews as possible, taking care to collect a representative sample of the fishery by spreading interviews across user-groups and areas. Data is stratified across angler types based on how anglers access the fishery (boat vs. shore) to account for variation in catch-per-unit-effort (CPUE). Interviews from complete and incomplete angling trips are both used. Additionally, interviews provide the opportunity to collect basic demographic information from anglers. For example, anglers are currently asked for their zip code, which allows managers to track movement of users across different fisheries and the number of anglers entering from outside the local area.

Data Management

Electronic data capture is used by creel surveyors to record all information. Each surveyor is issued an Apple iPad loaded with a standard data collection form created by WDFW staff in iForms. At the end of each creel survey shift, surveyors review and submit data by syncing their device with a PostgreSQL database hosted on an Amazon Web-based Server, which serves as the central repository for WDFW's creel survey data. Managers access and review data through a front-end application for WDFW employees (Fish Apps), as well as a public-facing website (https://data.wa.gov/) that is updated daily.

Analysis

Two methods are used to produce estimates of angler effort, CPUE, and total catch. Both methods use scripted analyses in Program R which directly query data from the cloud database (Buehrens and Bentley 2020; Booher and Auerbach 2022). The "point estimate" method uses deterministic equations to calculate angler effort, CPUE, and associated variance (Hahn 2000; Pollock 1994; Jones and Pollock 2012). The method is computationally simple and produces estimates quickly, but the uncertainty around the estimates do not fully account for error propagation, and thus must be interpreted with caution. Data is also analyzed using a Bayesian state-space (BSS) model. This model allows for serial autocorrelation of data in space and time across angler-types and survey sections. The estimates produced are statistically unbiased and accurately quantify uncertainty. Both methods provide results with estimates of uncertainty but serve separate purposes. The processing time for the 'point-estimate' is quick and generates estimates from datasets with low records of catch. The BSS model provides estimates with more precision and reduces assumptions by auto-correlating data. However, processing time takes several hours, and the model does not converge well with low-catch (i.e., zero-inflated) data sets. These analyses result in an estimate of catch or encounters in the case of catch-and-release fisheries. To understand the impact on the number of steelhead, a catch-and-release mortality is applied to the number of encounters. Numerous studies exploring variation in hooking mortality rates through angling techniques, fish handling, and environmental conditions are available (Muoneke and Childress 1994; Whitney 2019). For WDFW a ten percent mortality rate for all C&R fisheries is generally applied.

Willapa Bay Pilot Study Methods

Creel surveys were implemented in the Willapa River and Naselle River from January 1st through February 28th during the 2022 winter steelhead season. Both rivers are tributaries to Willapa Bay, which is the second largest estuary on the Pacific coast of the United States and located in southwest Washington State. Hatcheries are located on both systems and operate segregated winter steelhead programs. During the 2022 season, four staff conducted effort counts and interviews to estimate catch of wild steelhead. Two weekdays and two weekend days were sampled during the month of January. Three weekdays and two weekend days were sampled during the month of February due to increased daylight. Sample days during the week were randomly selected out of the five available days. Census effort counts were conducted using single-person pontoon rafts due to stream size.

Willapa Bay Study Results

Over the course of the season, a total of 104 index effort counts, three census effort counts, and 285 interviews were conducted in the Willapa River. In the Naselle River a total of 99 index effort counts, two census effort counts, and 166 interviews were conducted. From the interviews conducted, 38 and 51 wild steelhead were recorded caught and released in the Willapa and Naselle River respectively. For the Naselle River, the total angler hours estimated for bank anglers was 2,198, with a variance of 14,633, and was 295 with a variance of 3,178 for boat anglers. For the Willapa River, the total angler hours estimated for bank anglers was 8,437, with a variance of 140,603, and was 191 with a variance of 10,845 for boat anglers. The expansion ratio calculated from census effort surveys for the Willapa River was 1.48 for bank anglers. A default ratio of 1.00 was used in the Willapa River due to a lack of boat anglers in the fishery. The expansion ratios calculated from the census effort surveys for the Naselle River were 0.61 and 1.25 for bank and boat anglers respectively. Once expanded by effort, the estimated catch of wild steelhead for the Willapa River was 317 and was 222 for the Naselle River. The data collected is preliminary and could be subject to change with further review. Analysis was completed using the point-estimator. Estimates using the BSS model have not yet been completed.

Discussion

Fishery managers along the coast of Washington continually operate within narrow margins when prosecuting winter steelhead fisheries, due to population declines and fluctuating returns across years. Creel surveys provide managers with the ability to monitor catch of species of concern (such as wild steelhead) during the fishing season, compare catch with goals based on pre-season forecasts, and use that information to change fishing regulations when necessary. Unbiased catch estimates with quantified uncertainty are produced using unique analysis techniques, such as the BSS model, from data collected using a statistically sound study design. Staff at WDFW continue to stream-line workflow with electronic data collection, cloud database systems, and R scripts for data analysis to ensure estimates are produced in a timely manner.

With the availability of established sampling and analysis techniques, creel monitoring can be applied to fisheries as needs arise. The pilot study in Willapa Bay provided a valuable learning experience for WDFW fishery managers as we implemented a new creel survey in small, tributary systems where angling was predominately conducted from the bank and few boats were used to access the fishery. Conducting census counts using single-person pontoon boats in fisheries with low angler numbers led to uncertainty with the census effort ratios and contributed to the high variance in estimated effort. This emphasized the importance of conducting census effort counts as often as weekly over the course of the season and during times when high numbers are anglers are present in the fishery.

As long-term datasets become available in areas with consistent creel monitoring, new management tools may be developed. Standardized methods and analyses reduce variability and allow for comparisons of effort, CPUE, and catch between years and stream systems. Comparisons between years could help managers determine if steelhead runs are returning above or below forecast, while comparisons between streams could indicate if changes to fishery regulations are having the desired impact. Exploration of these possibilities comes from continued collection of quality creel survey data from the public. Creel surveys provide a unique opportunity for anglers to collaborate with fish managers and help fulfill management needs through the interview process. Face-to-face interactions with the public and WDFW staff provide the opportunity for establishing positive relationships and encouraging anglers to continue providing vital and accurate data.

Acknowledgements

Thanks to the many WDFW biologist and staff who helped develop and implement creel survey methods across western Washington over many years. Special thanks to all the staff and technicians on the front-line collecting data and implementing creel surveys.

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Angler Perspectives on Wild, Native, and Genetically Pure Trout

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Abstract - Across many western U.S. states, conservation and restoration objectives for fisheries now include goals targeting genetic purity. Although categories such as *wild fish* or *native fish* have been used for decades as priority designations in a number of states, the increasing reach of genetic analyses in recent years has led to additional considerations for fisheries managers and conservation. Reclassified by new molecular findings, fish may now be held to account for genealogies that go well beyond recent histories of dispersal, *in situ* reproduction, or watershed boundaries. As the science of fish genetics has changed, however, it remains uncertain if angler knowledge or preferences have experienced similar shifts to recognize or value genetic purity and nativeness. This paper examines results from angler surveys to evaluate and understand this prospective gap between fisheries science and angler perspectives. Our findings indicate that many anglers struggle to differentiate between categories of wild or native, and that values surrounding the experience of fishing likely outweigh concerns about the genetic composition of the fish.

Introduction

Colorado anglers often have a Greenback Cutthroat Trout *Oncorhynchus clarkii stomias* story. Many start with a rugged drive or a hike into a headwater stream, continue through a day of dodging willows and wading through pools and riffles, then culminate in hooking a lovely parr-splotched trout and exalting in at last finding and catching the elusive Colorado state fish. These stories typically carry fond memories and resonate with some of the most cherished qualities highlighted by fly fishing enthusiasts – time spent outdoors amid mountains and streams, pursuing wild trout in their native waters, maybe sharing the day with a companion or two – but they stumble upon one common flaw: almost without exception, the fish at the center of these stories are not actually Greenback Cutthroat Trout. The fish these anglers caught and celebrated as Greenback Cutthroat Trout were, genetic tests now tell us, most likely a variant of Colorado River Cutthroat Trout *Oncorhynchus clarkii pleuriticus*, mis-identified and in some cases mistakenly restored and protected by federal law for more than four decades in Colorado's high country (Metcalf et al. 2007; Rogers 2010, 2012; Metcalf et al. 2012; but see Behnke 1979; 1992).[1] In many cases, these fish were also caught well outside the South Platte River watershed where Greenback Cutthroat Trout likely ought to be considered native.

Complete understanding of the historical range and distribution of the Colorado state fish is still a work in progress (Trotter et al. 2015; Bestgen et al. 2019), but the event that first catalyzed the upheaval in Colorado fisheries conservation, taxonomy, and identification was a series of genetic analyses that disrupted long-standing assumptions about Cutthroat Trout *Oncorhynchus clarkii* lineages and their location (Metcalf et al. 2007; 2012). While the significance of these genetic tests continues to reverberate among fisheries scientists and managers, the impact on the fishing public is less clear. This gap in understanding angler perspectives is important for at least two reasons: 1) fisheries management and hatchery production commonly center around perceptions of what the fishing public desires; 2) state fisheries programs often depend upon angler license fees for their operations. The emerging genetic insights

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[1] This is not to suggest that the Colorado River Cutthroat Trout did not warrant conservation attention, but rather to highlight that the fish protected and restored by the U.S. Endangered Species Act(s) as Greenback Cutthroat trout since 1968 were not, genetically, the fish categorized during that time as *Oncorhynchus clarkii stomias*.

also often have life or death implications for various trout populations across the region, with fish meeting a certain standard for genetic composition qualifying as "conservation populations," while those falling beneath this threshold may be managed as recreational fisheries or targeted for elimination (Havlick and Biermann 2021). Additionally, a growing array of restoration and conservation-related policies, ranging from water rights to Wild and Scenic River protections are mapping onto designations of genetically-pure populations of native fish (Biermann and Havlick 2022).

Our research seeks to address this gap in understanding angler perspectives. We report on preliminary data collected by surveying members of Colorado-based fishing organizations and the staff and clientele of fly fishing shops, and by conducting creel surveys of anglers accessed along popular fishing sites in Colorado. In this report, we briefly analyze three main categories of questions asked in the two surveys: 1) respondents' views on wild, native, and genetically pure trout; 2) respondents' attitudes toward stocking; and 3) respondents' knowledge of native fish species or sub-species in Colorado.

Methods

In order to address these three areas of focus we developed two survey instruments. The first was designed for members of advocacy organizations or trout enthusiasts: participants in Colorado Springs chapters of Trout Unlimited and Pikes Peak Flyfishers, as well as staff and patrons of fly shops located within a 100 km radius of Colorado Springs (hereafter, the "dry survey"). The second was used for creel surveys of anglers along popular stretches of the South Platte, Frying Pan, and Roaring Fork rivers and Spinney Mountain Reservoir in Colorado (hereafter, "creel survey"). The two surveys included distinct but overlapping questions designed to access respondents' views about wild, native, and genetically pure trout; which fish are considered native; and views about fish stocking and values surrounding the fishing experience. Portions of the creel survey were modeled after components of previous Colorado Parks and Wildlife surveys to collect relevant information about angler success, species caught, and hours fished (for complete surveys, refer to Appendix 1).

The dry survey was distributed in-person at Colorado Springs chapter meetings of two trout fishing-focused organizations in May and June 2022. Surveys were handed out in hard copy with pens and clipboards, and respondents completed the forms and returned them by the end of the meetings. We also visited all local fly fishing shops within a 100 km radius of Colorado Springs and asked willing staff and clientele to complete surveys and informally discuss their views on fisheries management in Colorado. We are in the process of converting this dry survey to an online format and distributing it to additional chapters and organizations across Colorado.

For the creel surveys, research teams worked in groups of 2-4 individuals (and solo in three instances) by driving to popular fishing locations where they then approached anglers on foot either along the rivers/reservoirs or in parking areas or adjacent campgrounds/picnic grounds. Creel surveys are continuing through August 2022, but at the time of this writing have been conducted on the following dates: June 19, July 1-3, July 8-10, July 17, July 19, and July 22. Willing participants were then read the questions from the survey and researchers marked the responses on the survey forms. Creel surveys have been conducted at the following locations: Eleven Mile Canyon Recreation Area (South Platte River); Deckers, Colorado, and Cheeseman River trail (South Platte River); Spinney Mountain Reservoir and "Dream Stream" (South Platte River); Frying Pan River; and Roaring Fork River. Additional surveys are scheduled to occur in August 2022 at these same locations and the Arkansas River between Cañon City and Leadville, Colorado.

For the two types of survey, we created a separate Excel spreadsheet and entered data fields numerically for questions that accommodated quantitative inputs, and as text for open-ended responses. This latter field collected responses on what species survey takers considered native to Colorado or the water body in which they were fishing. For this initial analysis, we ran simple descriptive statistics (mean, median) for the numeric responses and applied a content analysis of open-ended responses to identify recurring themes and terms.

Results

In both of our surveys, responses revealed a hierarchy of values for wild, native, and genetically pure trout in descending order of importance. Respondents to the dry survey considered catching a wild trout and a native trout both to be important (wild trout mean=4.1; native trout mean=3.9), while catching a genetically pure fish was only marginally important (mean=3.2) (Table 1).

Responses from the creel survey showed similar patterns, but slightly different values: catching a wild trout was only moderately important (mean=3.3), catching a native trout was neutral (mean=3.0), and catching a genetically pure fish was considered moderately unimportant (mean=2.3) (Table 1).

When asked if “trout should be stocked in Colorado’s rivers and streams,” a majority of respondents to the dry survey said “yes” (69 percent), 8 percent said “no,” and 22 percent indicated they were “not sure” or did not respond (Table 2).

The two surveys also asked about respondents’ knowledge of native fish. These open-ended questions varied slightly between the two surveys, inquiring more broadly in the dry survey about any species of trout (or sub-species) that respondents considered native to Colorado, and in the creel surveys asking more locally which fish respondents considered native “here,” in the body of water they had just fished. We record simple tallies of these responses in Table 3.

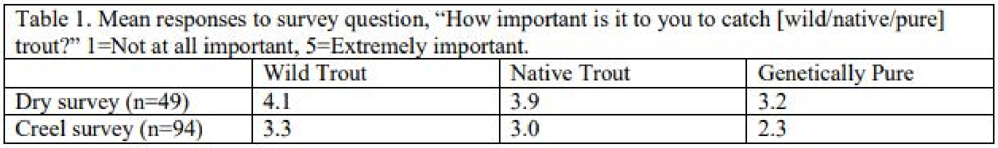

Table 1. Mean responses to survey question, “How important is it to you to catch [wild/native/pure] trout?” 1=Not at all important, 5=Extremely important.

	Wild Trout	Native Trout	Genetically Pure
Dry survey (n=49)	4.1	3.9	3.2
Creel survey (n=94)	3.3	3.0	2.3

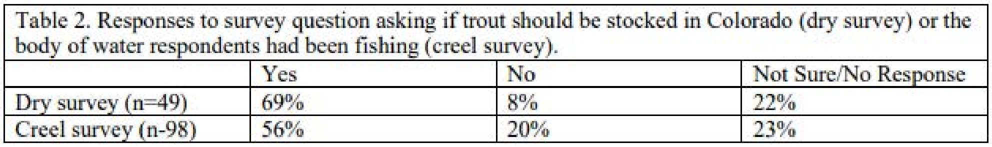

Table 2. Responses to survey question asking if trout should be stocked in Colorado (dry survey) or the body of water respondents had been fishing (creel survey).

	Yes	No	Not Sure/No Response
Dry survey (n=49)	69%	8%	22%
Creel survey (n-98)	56%	20%	23%

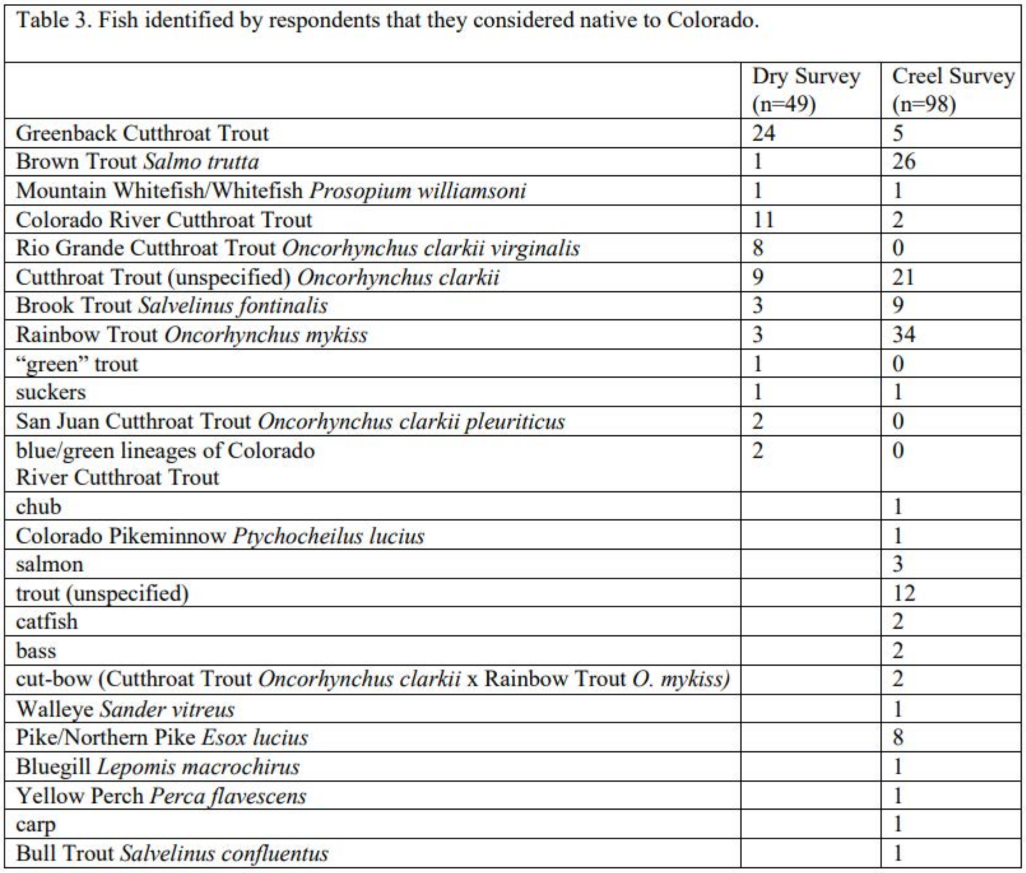

Table 3. Fish identified by respondents that they considered native to Colorado.

	Dry Survey (n=49)	Creel Survey (n=98)
Greenback Cutthroat Trout	24	5
Brown Trout *Salmo trutta*	1	26
Mountain Whitefish/Whitefish *Prosopium williamsoni*	1	1
Colorado River Cutthroat Trout	11	2
Rio Grande Cutthroat Trout *Oncorhynchus clarkii virginalis*	8	0
Cutthroat Trout (unspecified) *Oncorhynchus clarkii*	9	21
Brook Trout *Salvelinus fontinalis*	3	9
Rainbow Trout *Oncorhynchus mykiss*	3	34
“green” trout	1	0
suckers	1	1
San Juan Cutthroat Trout *Oncorhynchus clarkii pleuriticus*	2	0
blue/green lineages of Colorado River Cutthroat Trout	2	0
chub		1
Colorado Pikeminnow *Ptychocheilus lucius*		1
salmon		3
trout (unspecified)		12
catfish		2
bass		2
cut-bow (Cutthroat Trout *Oncorhynchus clarkii* x Rainbow Trout *O. mykiss)*		2
Walleye *Sander vitreus*		1
Pike/Northern Pike *Esox lucius*		8
Bluegill *Lepomis macrochirus*		1
Yellow Perch *Perca flavescens*		1
carp		1
Bull Trout *Salvelinus confluentus*		1

Discussion

Our findings suggest that a majority of anglers place a value on catching wild trout, and that at least for members of the trout-related organizations we surveyed, catching native trout is also considered desirable. Anglers we queried via the dry survey expressed only moderate interest in the genetic composition of Colorado trout, and those responding to the creel survey indicated this was not important to them. A majority of respondents to both surveys expressed support for continued fish stocking in Colorado, although nearly one-fourth of respondents indicated they were unsure or qualified their responses by location, fishing type (catch and release vs. put and take), or species.

Open-ended responses on the two surveys reveal a wide range of understanding about which fish are native to Colorado or to particular water bodies. Respondents to the dry survey demonstrated considerable accuracy and depth of knowledge about native trout, with nearly half of all respondents correctly identifying the Greenback Cutthroat Trout, nearly one-quarter of respondents naming the Colorado River Cutthroat Trout, and only a handful mistakenly naming Brown Trout, Brook Trout, or Rainbow Trout as natives. Creel survey respondents expressed relatively less awareness of Greenback Cutthroat Trout, Colorado River Cutthroat Trout, or other specific trout native to Colorado, and listed Rainbow Trout, Brown Trout, Cutthroat Trout (unspecified) and trout (unspecified) as the most common responses.

Very briefly, we offer several observations about the data we have collected so far. First, if we are to take angler preferences seriously in influencing fisheries management policies, it may be important to be cautious about promoting programs that foreground genetic purity. Management and conservation programs have highlighted wild and native trout fisheries for decades in many states, while the identification of and management for genetic purity is a relatively new consideration. It is possible that increasing familiarity with scientific arguments promoting conservation genetics will shape angler opinions in the future, but in conversations we had alongside our survey work, a number of anglers expressed opposition toward programs that eradicated wild (but non-native or hybridized) trout in order to establish pure-strain conservation populations.

Our survey findings suggest that while anglers value opportunities to catch wild and native trout, angler understanding of native fisheries is rather variable. Our two surveys framed the questions about native fish slightly differently – with the dry survey asking about native trout in Colorado and the creel survey asking about fish native to the particular water body – but the wide-ranging responses to the creel survey suggest a lack of local ecological knowledge among many anglers. This, in turn, likely makes it difficult for anglers to accurately evaluate if they are catching native fish or not.

In addition, our findings suggest that accurate knowledge of native fish does not necessarily correspond with placing a high importance on catching native fish. Among anglers who rated catching native fish as important or extremely important, only 8 of 41 (19.5%) correctly identified Cutthroat Trout as a native species, while 18 of 41 respondents (43.9%) incorrectly identified Rainbow Trout as native, and 15 of 41 (36.6%) incorrectly identified Brown Trout as native. Surprisingly, anglers who rated catching native fish as not at all important or not very important were more likely to correctly identify Cutthroat Trout as a native species (12 of 39 respondents, 30.8%) and less likely to incorrectly identify Rainbow Trout (12 of 29 respondents, 30.7%) or Brown Trout (8 of 39 respondents, 20.5%) as native. This suggests that efforts to educate anglers on native species may not necessarily translate directly into support for native trout conservation or restoration programs.

Responses regarding fish stocking also suggest some potential conflicts of understanding or values. Both the dry and creel surveys found relatively strong interest in catching wild fish, which the surveys defined as fish "reproducing in the wild/not hatchery-raised." This would seem to contradict these same respondents' strong support for continued fish stocking, which can either undermine or directly conflict with anglers encountering fish reproducing in the wild or those that are not raised in hatcheries.

Acknowledgements

We appreciate the cooperation of employees of Colorado Parks and Wildlife; members of local chapters of Trout Unlimited and Pikes Peak Flyfishers; fly shop owners, clientele, and staff; and anglers in Colorado who contributed to data presented in this paper. We also thank University of Colorado Colorado Springs student researchers Meghan Cazier, Melissa Fisher, Gabriel Garcia, Rece Lehberger, Sydney Nelson, Saloni Patel, and Hayden Strait for their work on this project. This research is supported by the National Science Foundation grant #SES-1922157.

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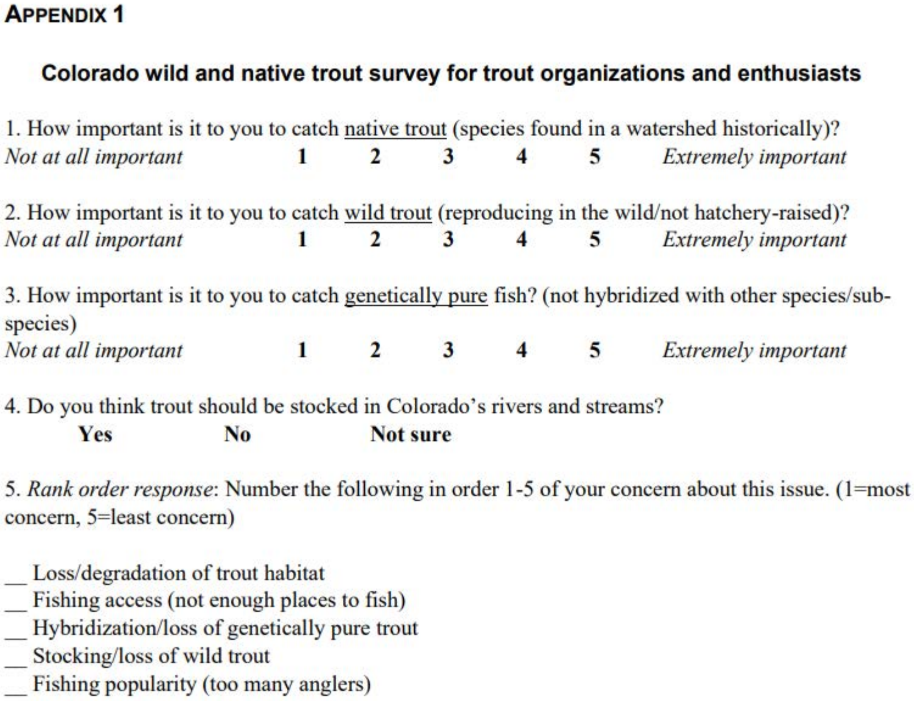
APPENDIX 1



APPENDIX 1

Colorado wild and native trout survey for trout organizations and enthusiasts

1. How important is it to you to catch native trout (species found in a watershed historically)?
Not at all important **1 2 3 4 5** *Extremely important*

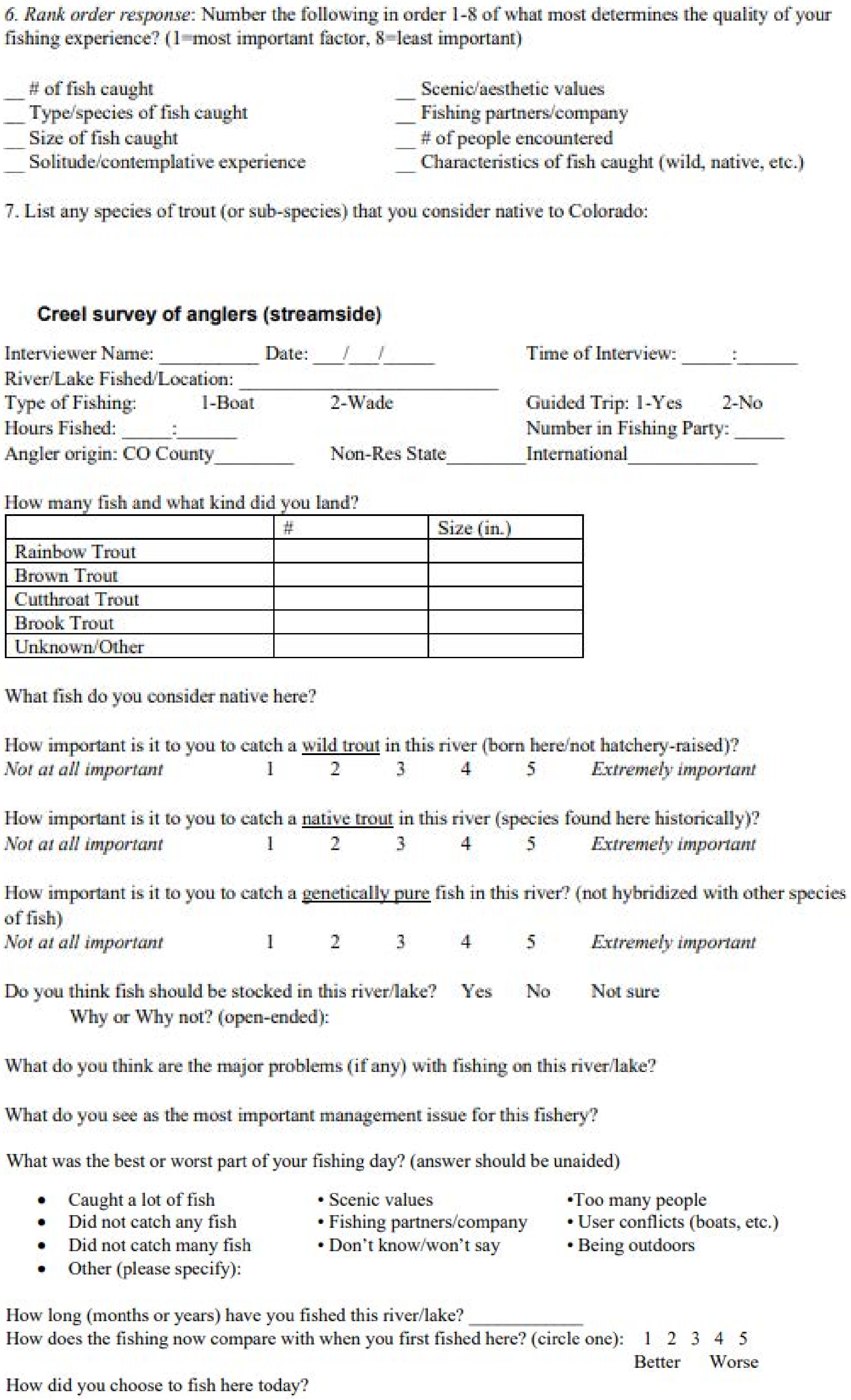
2. How important is it to you to catch wild trout (reproducing in the wild/not hatchery-raised)?
Not at all important **1 2 3 4 5** *Extremely important*

3. How important is it to you to catch genetically pure fish? (not hybridized with other species/sub-species)
Not at all important **1 2 3 4 5** *Extremely important*

4. Do you think trout should be stocked in Colorado's rivers and streams?
Yes **No** **Not sure**

5. *Rank order response*: Number the following in order 1-5 of your concern about this issue. (1=most concern, 5=least concern)

__ Loss/degradation of trout habitat
__ Fishing access (not enough places to fish)
__ Hybridization/loss of genetically pure trout
__ Stocking/loss of wild trout
__ Fishing popularity (too many anglers)



6. *Rank order response*: Number the following in order 1-8 of what most determines the quality of your fishing experience? (1=most important factor, 8=least important)

__ # of fish caught
__ Type/species of fish caught
__ Size of fish caught
__ Solitude/contemplative experience
__ Scenic/aesthetic values
__ Fishing partners/company
__ # of people encountered
__ Characteristics of fish caught (wild, native, etc.)

7. List any species of trout (or sub-species) that you consider native to Colorado:

Creel survey of anglers (streamside)

Interviewer Name: _________ Date: ___/___/_____ Time of Interview: _____:______
River/Lake Fished/Location: ________________________
Type of Fishing: 1-Boat 2-Wade Guided Trip: 1-Yes 2-No
Hours Fished: _____:______ Number in Fishing Party: _____
Angler origin: CO County________ Non-Res State________International_____________

How many fish and what kind did you land?

	#	Size (in.)
Rainbow Trout		
Brown Trout		
Cutthroat Trout		
Brook Trout		
Unknown/Other		

What fish do you consider native here?

How important is it to you to catch a wild trout in this river (born here/not hatchery-raised)?
Not at all important 1 2 3 4 5 *Extremely important*

How important is it to you to catch a native trout in this river (species found here historically)?
Not at all important 1 2 3 4 5 *Extremely important*

How important is it to you to catch a genetically pure fish in this river? (not hybridized with other species of fish)
Not at all important 1 2 3 4 5 *Extremely important*

Do you think fish should be stocked in this river/lake? Yes No Not sure
Why or Why not? (open-ended):

What do you think are the major problems (if any) with fishing on this river/lake?

What do you see as the most important management issue for this fishery?

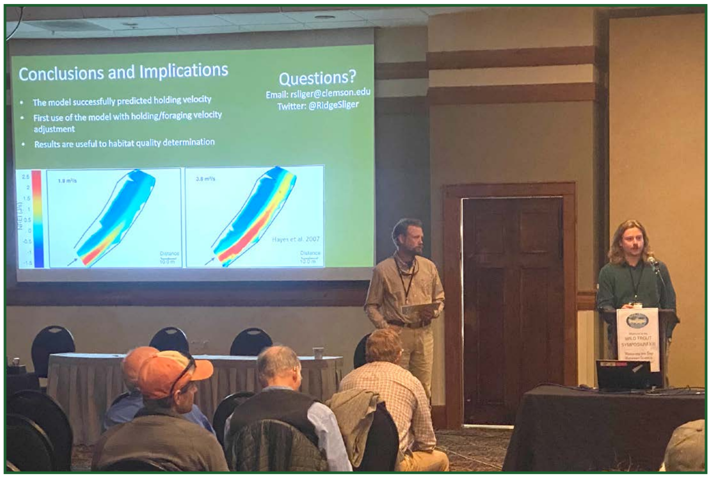
What was the best or worst part of your fishing day? (answer should be unaided)

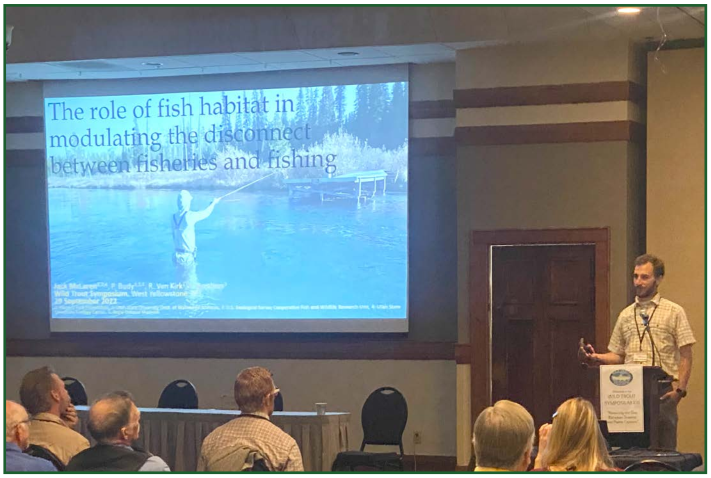
- Caught a lot of fish
- Did not catch any fish
- Did not catch many fish
- Other (please specify):
- Scenic values
- Fishing partners/company
- Don't know/won't say
- Too many people
- User conflicts (boats, etc.)
- Being outdoors

How long (months or years) have you fished this river/lake? ___________
How does the fishing now compare with when you first fished here? (circle one): 1 2 3 4 5
Better Worse
How did you choose to fish here today?

Session 2

Cold, Clean, Complex, and Connected: Understanding and Restoring Wild Trout Habitat to Prepare for a Changing World I





A Test of a Generalized, Optimal Habitat Selection Model for Drift-Feeding Fishes: Brook Charr

R. Sliger, and G. D. Grossman

Journal of Zoology 315:313-318

Synopsis

Efficient preservation of trout habitat requires a thorough understanding of habitat requirements. Quality habitat must allow suitable levels of fitness for population sustainability. In a changing world, understanding the mechanisms by which habitat affects fitness is critical. Optimal Foraging Theory, which assumes organisms forage in ways that maximize their fitness, is a useful framework for determining necessary habitat characteristics. But measuring lifetime fitness and correlating it with habitat use is rarely feasible. Therefore, the use of more tractable models that can quantify the effects of habitat use on fitness proxies, such as net-energy-intake (NEI), are particularly useful for identifying characteristics of quality habitat. Grossman et al. (2002) developed such a model, in which they used a simple relationship between water velocity and prey capture success to infer at which velocities drift-feeding fishes should forage to maximize their NEI. This model has successfully predicted the microhabitat selection of three salmonids and four leuciscids (Grossman et al. 2002; Donofrio et al. 2018; Bozeman and Grossman 2019a,b), demonstrating the importance of water velocity to NEI through its effects on prey capture success. However, there have been some contexts in which this model has failed to predict habitat use, demonstrating the need to further understand its generalizability. In this work, we applied a modified form of the Grossman et al. (2002) model to wild southern Brook Charr Salvelinus fontinalis (hereafter Brook Trout). We used feeding trials in an artificial stream flume to parameterize the relationship between water velocity and prey capture success of 21 individuals from Lynn Camp Prong, Great Smoky Mountains National Park. This parameterization allowed us to predict the water velocity at which Brook Trout should maximize NEI. Additionally, we quantified the linear relationship between the experimental water velocity in which fish captured prey (treatment velocity), and the water velocity at which they held before moving to capture prey (holding velocity). We used this relationship to translate the optimal foraging velocity prediction to an optimal holding velocity prediction. Using the Grossman et al. (2002) model parameterization and the holding velocity adjustment, we generated an optimal holding velocity prediction of 18.5 cm/s. We used snorkeling observations to measure the holding velocities of 26 naturally foraging fish in Lynn Camp Prong. The 95% confidence interval of trout in Lynn Camp Prong was 13.5—20.5 cm/s. Because this confidence interval encompassed our optimal holding velocity prediction, the modified Grossman et al. (2002) model successfully predicted wild southern Brook Trout microhabitat use, and these trout appear to forage in ways that maximize their NEI. The results from our study can be useful to managers conserving or restoring suitable southern Brook Trout habitat and can allow inference concerning the effects of flow regimes on habitat quality. This information will be particularly useful as flow regimes are altered by climate change and land and water use practices.

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The Role of Fish Habitat in Modulating the Disconnect Between Fisheries and Fishing

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Abstract - Fisheries management often aims to improve the recreational fishing experience by improving fish abundance, but fish abundance does not always align with the fishing experience. In one section of the Henry's Fork of the Snake River, Idaho, a suboptimal fishing experience led to studies of in-stream salmonid habitat quality and quantity to identify strategies to increase the availability of salmonids for anglers. Previous research has found fish in Island Park Reservoir migrate into the Henry's Fork to spawn and these migrations are temporally divorced from most recreational use. We hypothesized that improving productivity and food availability could encourage more fish to remain in the Henry's Fork, where they are available to anglers. We modeled current bioenergetic conditions and found potential fish growth is lower in Island Park Reservoir than in the Henry's Fork. Thus, improving productivity and food availability in the Henry's Fork is unlikely to alter current unfavorable out-migration patterns. However, we also found that increasing submerged macrophyte coverage could affect fish habitat and fish habitat preferences, thereby allowing fish to aggregate in areas consistent with angler expectations (e.g., macrophyte-rich glides). Creating habitats consistent with angler expectations may better connect fish abundance with the fishing experience

Introduction

Fisheries management often aims to improve the fishing experience by improving fish abundance, size, and population structure, but these metrics do not always align with the recreational fishing experience. Often, angler catch is not linearly related to fish abundance; recreational fisheries are usually "hyperstable" with unchanging catch rates despite changes in fish abundance (Dassow et al. 2020; Solomon et al. 2020). In the case of fisheries with poor catch rates, hyperstability could lead to frustration wherein fisheries managers successfully improve overall fish abundance with little corresponding return to creel. For example, the Henry's Fork of the Snake River, Idaho ("Henry's Fork") upstream of Island Park Reservoir is a recreational angling destination for iteroparous adfluvial Rainbow Trout (*Oncorhynchus mykiss*; Flinders et al. 2016). However, adfluvial spawning migrations are temporally divorced from peak recreational use (Van Kirk et al. 2019), resulting in a disconnect between fish abundance and recreational fishing experience (Laatsch et al. 2017).

One potential solution to this disconnect is improving habitat to encourage fish to remain in waters where they are likely to be captured (Beard and Carline 1991; Magee et al. 1996; Penaluna et al. 2021) while simultaneously providing anglers with more locations fitting angler expectations (Dassow et al. 2020). In the Henrys Fork, improving habitat starts with increasing submerged macrophyte coverage. Higher submerged macrophyte coverage increases food availability and reduces water velocities, thereby reducing swimming costs and resulting in greater growth opportunities, measured as "Net Rate of Energy Intake" (NREI) in joules per second (McLaren et al. 2022 *in review*). Net Rate of Energy Intake is a measure of the difference between the metabolic and swimming costs of a fish and the potential invertebrate food available in the drift. Higher NREI is thought to indicate higher-quality fish habitat; microhabitats with higher NREI tend to hold fish (Fausch 1984) and reaches with higher NREI should theoretically have higher carrying capacity (Wall et al. 2016; Jensen 2017). If we assume habitats with greater NREI will hold iteroparous out-migrating fish such as Rainbow Trout longer, especially if NREI is greater than what is available in Park Reservoir downstream (Fausch 1984; Armstrong et al. 2021; Penaluna et al. 2021), then we hypothesize current NREI values in the summertime are lower in the Henrys Fork than in Island Park Reservoir, and we hypothesize increased growth of submerged macrophytes will increase NREI to the point of exceeding what is available in Island Park Reservoir and is therefore a viable strategy for closing the temporal gap between peak migration and peak angler use.

Methods

Study site

Island Park Reservoir and the Henrys Fork are within the Columbia River drainage in eastern Idaho (Figure 1). Island Park Reservoir is a 167 million m^3, 34 km^2, earthen-dam, hypolimnetic-release reservoir with a full-pool surface elevation of 1921 m above mean sea level, a maximum depth of 23 m, and a mean depth of 5.25 m. The fishery is composed of migratory sportfishes such as Rainbow Trout and kokanee salmon (Van Kirk and Benjamin 2000; Van Kirk and Gamblin 2000; High et al. 2015).

The Henrys Fork is low-gradient, wide (50-100 m) and shallow (x = 0.52 m, o = 0.23) and fed by large first-order springs with a mean annual discharge of about 9 $m^3 \cdot s^{-1}$. The Henrys Fork has a low-to-high longitudinal gradient of submerged macrophyte coverage and fishery quality with distance downstream from headwater springs (Henry 2010; High et al. 2015; Flinders et al. 2016). We thereby categorized our sampling locations into three types of reaches: first-order "Headwaters" reaches near springs and without upstream human or tributary influence, third-order "Middle" reaches downstream of the first tributaries and human infrastructure, and fourth-order "Tailwater" reaches downstream of multiple major tributaries and Island Park Reservoir (Figure 1).

Data Collection

To evaluate our hypothesis that increasing NREI in the Henrys Fork upstream of Island Park Reservoir would encourage more fish to stay in the stream longer after the spawning migration, we compared NREI values between the Henrys Fork and Island Park Reservoir during spring and summer. Potential NREI values were calculated for fish observed in two reaches upstream of Island Park Reservoir and for a hypothetical fish living at the deepest point of Island Park Reservoir near the dam (Figure 1). To calculate NREI, we used the software program BioenergeticsHSC (Naman et al. 2020) for fish in the Henrys Fork and the Wisconsin bioenergetics model (Deslauriers et al. 2017) for fish in Island Park Reservoir. Inputs for BioenergeticsHSC were invertebrate drift density ($g \cdot m^{-3}$), fish size (g), fish position in the water column (%), water velocity ($m \cdot s^{-1}$), channel roughness (% of depth), water depth (m), water temperature (˚C), and turbidity (NTU). For Fish Bioenergetics 4, our inputs were temperature (˚C), fish size (g), proportion of maximum consumption ("Bioenergetic efficiency; BioEff"; Budy et al. 2013), and temporal extent (d). Both models also used pre-determined bioenergetics and metabolic parameters for Rainbow Trout (Railsback and Rose 1999) and a default value for prey energy density (5200 J·g-1).

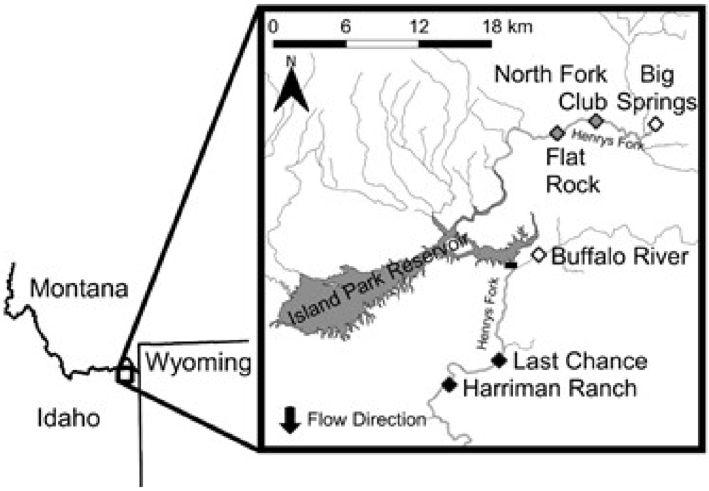

Figure 1. Map of the Henrys Fork watershed, including all sampling locations, with regional context within the U.S.A states of Montana, Wyoming, and Idaho.

We measured invertebrate drift density (mg·m-3) using three 250 µm mesh drift nets at each location with short soak times (15-30 minutes) to account for biofouling (Baxter et al. 2017). We measured fish size and position in the water column with snorkeling surveys (Thurow 1994). If submerged macrophyte growth compromised visibility, we used visual wading surveys with polarized sunglasses, which have been shown to be effective at enabling counting and observing fish in relatively shallow, clear streams (Hankin and Reeves 1988). Wading surveys consisted of 3-5 surveyors with polarized sunglasses walking from the downstream end of the sampling reach to the upstream end in a zig-zag pattern. Two separate surveys were conducted in May and July, 2021. We estimated fish size using categories, with large being > 300 mm, medium between 150 and 300 mm, and small being <150. We converted to grams using the relative weight equation reported in Flinders et al. (2016), with large fish set at 450 mm (735 g), medium at 225 mm (130 g), and small at 100 mm (11 g). We measured water velocity, channel roughness, and water depth at each fish focal point. Consistent with the BioenergeticsHSC model, we defined channel roughness as a percentage of depth of the larger value between substrate size and macrophyte growth height. We measured water temperature and turbidity by using data from the nearest Henry's Fork Foundation multiparameter sonde (Henry's Fork Foundation 2022).

In Island Park Reservoir, we recorded vertical profiles of water temperature with a multiparameter sonde at the deepest point in the reservoir. Assuming Rainbow Trout would seek out optimal water temperatures for growth, we used the warmest temperature available below the threshold for Rrainbow tTrout consumption (22.5 ˚C; Railsback and Rose 1999) and at a depth with a dissolved oxygen above 6 $mg \cdot l^{-1}$. Without observations of individual fish, we assumed fish sizes were the same in Island Park Reservoir as in the Henrys Fork upstream, and therefore ran three sets of models, one each for 735 g, 130 g, and 11 g fish. For each fish size, we used three BioEff values, 0.25, 0.5, and 0.75 to span the realistic limits of potential consumption in Island Park Reservoir (Railsback and Rose 1999; Dieterman et al. 2004; van Poorten and Walters 2014). To check our assumption, we ran two additional models wherein small, medium, and large fish approximated age-2, age-3, and age-4 Rainbow Trout in the Henrys Fork (Angradi and Contor 1989). With known beginning and end fish weights, and assuming trout growth occurs during the May 1 to September 1 time period, we calculated BioEff values and NREI for Small and Medium fish in Island Park Reservoir. In review, we had a total of eleven models: three models for each fish size and three BioEff values for each fish size, plus two more "fitted" models where the BioEff value was fitted to inferred fish weight change over time.

To determine whether reach-scale macrophyte coverage could affect overall NREI values, we used a random-point physical habitat survey across all reaches in the watershed (Figure 1). We used a geographic information systems program to select 30 random points in each reach, for a total of 60 points per location. We used the same methods and equipment to collect physical habitat data at each of the 30 points: submerged macrophyte cover, height, water depth, velocity, and substrate. We set fish species to Rainbow tTrout, and calculated NREI for each fish size (small, medium, and large) at three relative depths (at 20%, 60%, and 80% of depth), resulting in nine NREI values per sample point.

Data Analysis

We ran a BioenergeticsHSC model for each individual fish observed in each snorkel survey , thereby producing mean and variance estimates of NREI experienced by each fish size in the Henrys Fork in both the spring and summer time periods, for a total of six mean/variance estimates. For eleven models of fish bioenergetics in Island Park Reservoir, model outputs included total consumption in joules for each day between May 1 and September 1. We assumed fish consumption occurred during approximately 12 hours of daylight, and so we divided daily total consumption by 43200 seconds to obtain NREI in joules per second. We extracted NREI estimates for each model from the days corresponding to snorkel surveys in the Henrys Fork to ensure comparability of results. We compared the estimated mean and variance of NREI from the Henrys Fork to the outcomes of each model from Island Park Reservoir for each fish size and season using an ANOVA and Tukey's Honest Significant Difference Test, for a total of 18 comparisons: three models with different BioEff values per fish size category between two seasons. For reach-scale comparisons of submerged macrophyte growth and NREI, we logit-transformed submerged macrophyte growth and $\log_e$-transformed NREI and performed a Pearson's correlation. All analyses and graphical production were completed in R (R core team 2019) with packages reshape 2 (Wickham 2007), lattice (Sarkar 2008), latticeExtra (Sarkar and Andrews 2022), and RColorBrewer (Neuwirth 2014).

Modeled NREI in Island Park Reservoir varied based on season, fish size, and BioEff value (Figure 2). NREI and resulting growth is higher for larger fish and BioEff value (Figure 2). BioEff values have the greatest relative effect on NREI (Figure 2). Outputs from models fitted to known weights between small- and medium-sized fish and medium- and large-sized fish indicate BioEff values are likely between 0.5 and 0.25 given estimated prey energy density and temperatures (Figure 2). Thus, we feel confident that a combination of bioenergetics models with BioEff values between 0.25 and 0.75 give a realistic range of possible growth rates and resulting NREI values.

Net Rate of Energy Intake for Rainbow Ttrout observed in the Henrys Fork varied based on fish size and season, as invertebrate drift, water temperature, turbidity, and metabolic parameters changed over time. Greater invertebrate drift, lower turbidity, and higher water temperatures increased observed NREI in the Henrys Fork by an approximate factor of 10 from spring to summer ($p < 0.001$). Across seasons, medium-sized fish experienced significantly higher NREI than small ($p = 0.002$) or large fish ($p < 0.001$), with large and small fish experiencing the same NREI ($p = 0.93$). These differences are due to differences in metabolic parameters and habitat selection among fish sizes (McLaren et al. 2022 in review). Observed NREIs in the Henrys Fork were significantly greater than the highest modeled NREI in Island Park Reservoir in all season-size cases ($p<0.001$) except for large fish in the springtime, where maximum modeled NREI in Island Park Reservoir exceeded observed NREI in the Henrys Fork ($p = 0.004$; Figure 3).

At the reach scale, average macrophyte coverage is broadly related to average NREI (Pearson's $r = 0.21$), though summertime insect hatches greatly increase NREI independent of macrophyte coverage and create a source of uncertainty (Figure 4).

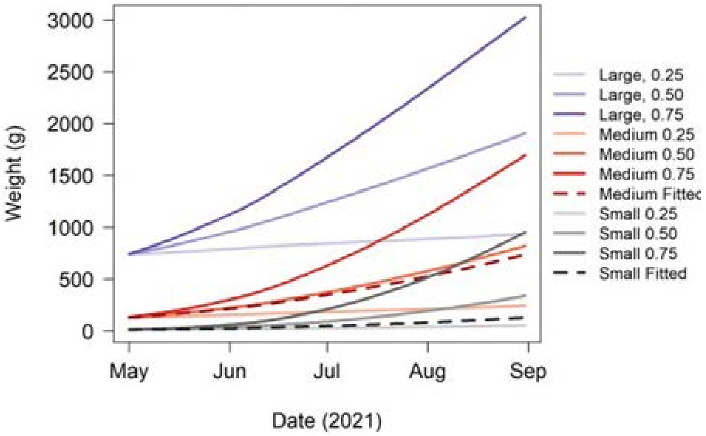

Figure 2. Modeled fish weights (g) for Island Park Reservoir during 2021. Solid lines represent models where the proportion of maximum consumption ("BioEff value") was manipulated from 0.25 to 0.75. Dashed lines represent models where beginning and end weights were known and we allowed the BioEff value to vary to fit. Lines are grouped by starting fish weight; purple for Large (>300 mm) fish, red for Medium (150-300 mm) fish, and gray for Small (<150 mm) fish.

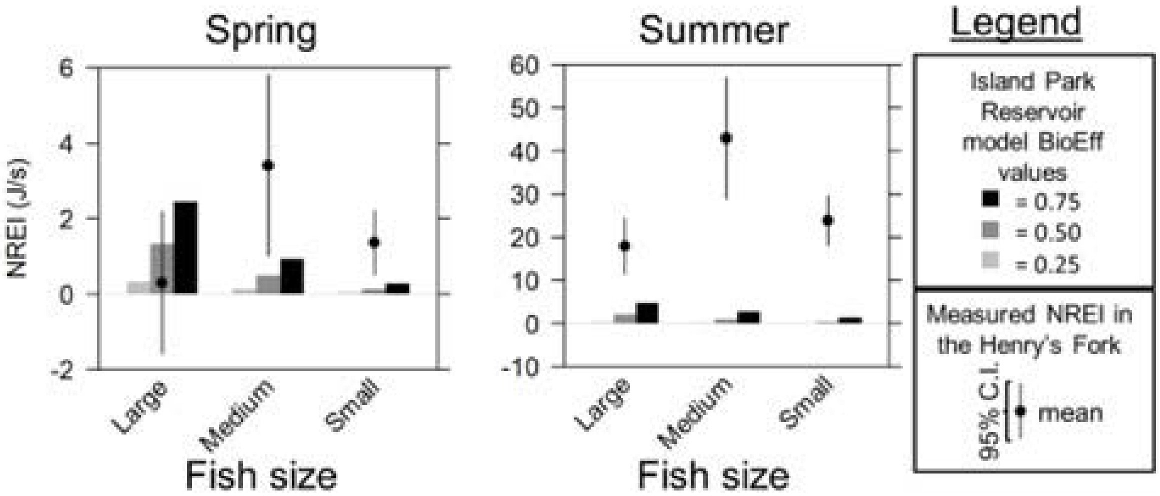

Figure 3. Modeled Net Rate of Energy Intake (NREI, J-s^{-1}) for Island Park Reservoir fish and fish observed in the Henrys Fork across the spring and summer seasons and across three fish size categories.

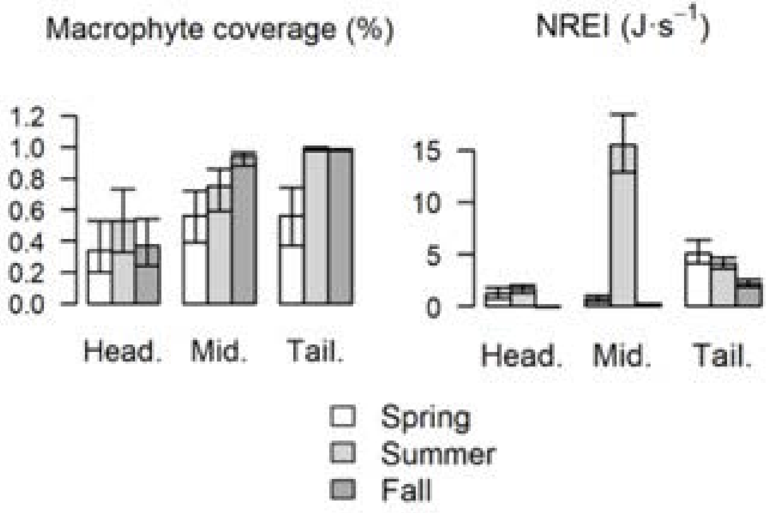

Figure 4. Submerged macrophyte coverage (%) and NREI ($J\text{-}s^{-1}$) across three sampling locations in the Henrys Fork, showing the spatiotemporal gradient in macrophyte coverage and corresponding differences in NREI.

Discussion

We found that post-spawn adult fish experience better energetic conditions within the Henrys Fork than in Island Park Reservoir. We hypothesized iteroparous post-spawn adult fish would remain available to anglers in the Henrys Fork for as long as NREI remains higher than what is available in the reservoir (Fausch 1984; Armstrong et al. 2021; Penaluna et al. 2021), but this is not the case. Previous research indicates excellent Rainbow Trout growth and relative weight in the Henrys Fork (Flinders et al. 2016), with zooplankton studies in Island Park Reservoir indicating a low likelihood of inter- and intra-specific competition between fishes (Flinders et al. 2016). Previous research also concluded fish were not selecting habitats in the Henrys Fork based on food availability (McLaren et al. 2022 *in review*) but based on water depth, presumably to avoid predation (Penaluna et al. 2016, 2021). Island Park Reservoir lacks fish predators and has good availability of deep water. Thus, despite higher NREI values, fish have little incentive to remain in the Henrys Fork post-spawn despite higher NREI values , and projects to increase NREI are unlikely to increase fish availability for anglers.

There is uncertainty in our models, but since even the most liberal NREI estimates in Island Park Reservoir are lower than NREI values in the Henrys Fork in all but one case, we are confident in our conclusion that fish are more likely to experience favorable growth conditions in the Henrys Fork as compared to Island Park Reservoir. Because of some required assumptions, we modeled a wide range of potential conditions. Our potential growth rates bracketed growth rates found in previous studies (Angradi and Contor 1989; Flinders et al. 2016). In addition, our BioEff values based on models using inferred fish weight endpoints were reasonable (Railsback and Rose 1999; Dieterman et al. 2004; Budy et al 2013; v an Poorten and Walters 2014). Realistic BioEff and resultant NREI values for Island Park Reservoir may be even lower, given our conservative assumption that all growth occurs between May 1 and September 1. One critical source of uncertainty arose from invertebrate drift sampling methods. Invertebrate drift sampling only occurred for 15-30 minutes due to biofouling and was thereby susceptible to high variability due to short-duration insect hatches. Future work should better constrain variability in invertebrate drift measures and account for hatch timing and duration.

Submerged macrophyte growth was positively associated with NREI, but the absolute magnitude of the relationship is small. However, submerged macrophytes may affect the Henrys Fork, its fish, and its users in other unique ways. Increasing submerged macrophyte coverage creates more areal habitat, allowing fish to use more of the riverscape (McLaren et al. 2022 *in review*). Local and regional anglers in the Henrys Fork preferentially target medium-depth glides with heavy macrophyte growth to target fish feeding on emerging macroinvertebrates (Harrop 2010; Lawson 2012). Since macroinvertebrates favored by anglers are abundant in the Henrys Fork upstream of Island Park Reservoir (Van Kirk, *personal communication*), and good fishing experiences on the Henrys Fork are tied to fish eating macroinvertebrates from the surface (Laatsch et al. 2017), it appears that creating macrophyte-heavy glide habitats may increase the number of areas that meet angler expectations. Providing more locations that meet angler expectations has the potential to improve fishing even without evidence linking fish habitat improvement to total fish numbers (Dassow et al. 2020).

Fisheries managers traditionally increase fish numbers through stocking, regulations, and fish habitat improvement. However, the success of these management actions can have less to do with increasing fish numbers and more to do with affecting perceived fish availability to the angler (Dassow et al. 2020; Solomon et al. 2020). To increase fish availability for the angler, agencies may consider stocking non-triploid fish species, such as native Yellowstone Cutthroat Trout *Oncorhynchus clarkii bouvieri* that complete their spawning migration in high-use months, or by increasing macrophyte growth to create angler-friendly habitats. Future study in the Henrys Fork should focus on including predation in models of fish occupancy, such as in models by Railsback et al. (2021), in order to determine if creating refugia from predators would affect out-migration.

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Utah's North Slope Uinta Mountain Native Cutthroat Trout Renovation Project 2019-2026

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Colorado River Cutthroat Trout *Oncorhynchus clarkii pleuriticus* currently exist in fragmented habitats with current occupancy estimated at 11% of their native historic range (Hirsch et al. 2013). Their decline is attributed to loss of habitat, interspecific competition with introduced nonnative trout species (i.e., Brook Trout *Salvelinus fontinalis*, Brown Trout *Salmo trutt*a, Rainbow Trout *O. mykiss,* and nonnative sub-species of Cutthroat Trout *O. clarkii spp*.) and loss of genetic purity from hybridization with Rainbow Trout and other subspecies of Cutthroat Trout (Binns 1977; Behnke and Zarn 1976; Behnke 1992; Young 1995; Lentsch and Converse 1997; CRCT Coordination Team 2006).

Colorado River Cutthroat Trout (CRCT) were petitioned for listing as a threatened species under the Endangered Species Act in 2000 (Greenwald 2000); however, in April 2004, the U.S. Fish and Wildlife Service ruled that Endangered Species Act listing for CRCT protection was not warranted at that time (USFWS 2004). Efforts by state and federal agencies to restore CRCT and the existence of signed conservation strategies (Lentsch and Converse 1997; CRCT Task Force 1999; CRCT Coordination Team 2006) for the species were cited as providing the basis, in part, for not listing CRCT.

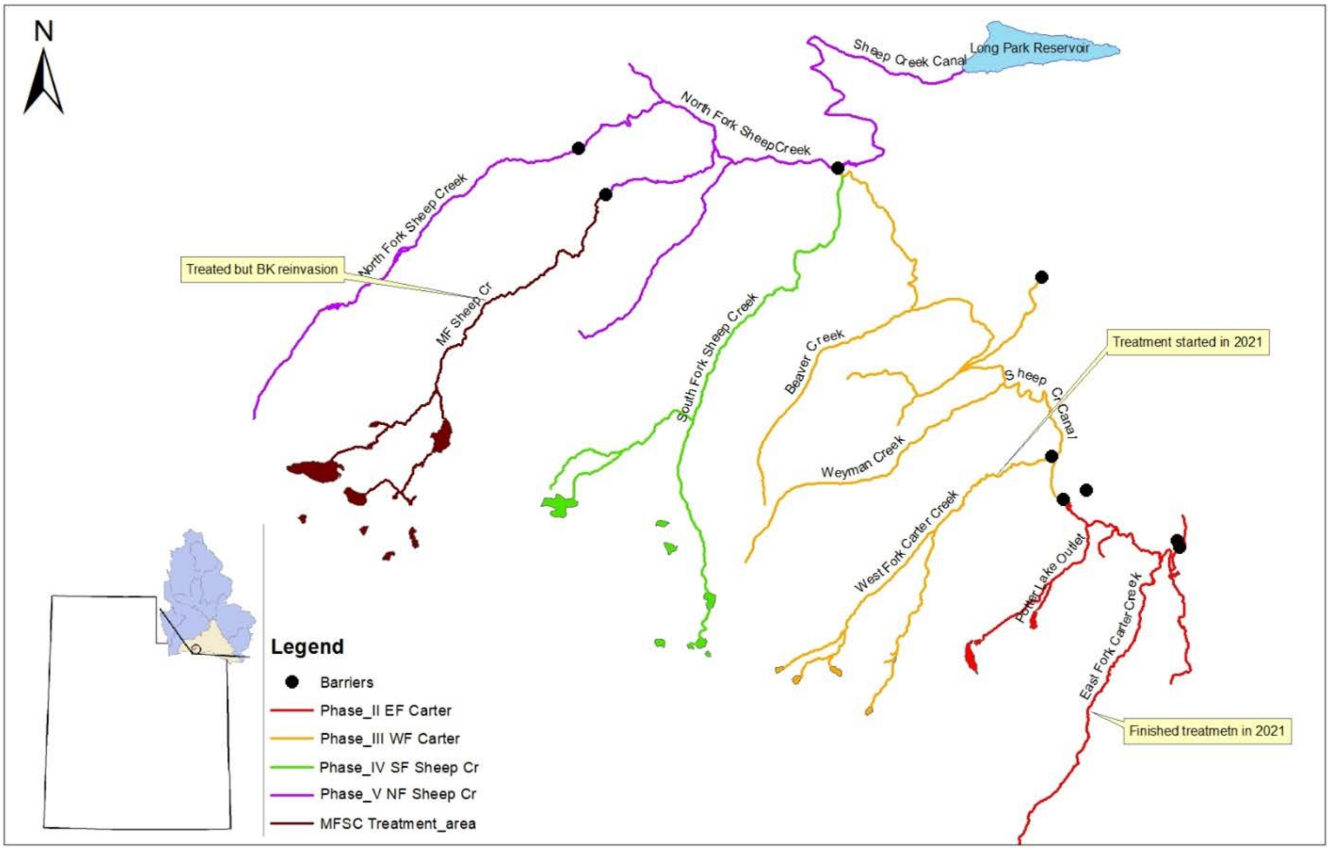
The State of Utah, in partnership with other organizations and agencies, has taken an aggressive approach to CRCT restoration. End goals of this approach include an agency-point-of-completion where program goals will shift away from active restoration projects in favor of monitoring and habitat conservation. In order to reach that goal, we are actively pursuing large, achievable, meaningful restoration projects. These projects are considered to have the best chance for repatriating native trout populations that can survive small and large stochastic events such as wildfires, floods, diseases, lack of recruitment, or invasions by nonnative fish species. One of these large restoration projects is a nonnative fish removal project located on the north slope of the Uinta Mountains and encompasses the Sheep Creek and Carter Creek drainages in northeastern Utah (Figure 1). If successful in removing nonnative trout, this project will result in the restoration of nearly 100 stream miles for native CRCT. There are also 32 lakes in the drainage that are managed as fisheries. Following the treatment project, 20 of these lakes will be stocked with CRCT resulting in 271 surface acres of occupied habitat.

Tributaries in the Carter Creek and Sheep Creek drainages are artificially connected by the Sheep Creek canal that delivers water to Long Park Reservoir for irrigation use in Manila, Utah. Water from all these tributaries are collected in the canal during the summer months and the tributaries are dry immediately downstream from the canal. During the fall, winter, and spring seasons, most of the water is allowed to pass below the canal in the natural stream channel. Nine barriers in the project area are essential for project completion and to prevent reinvasion of nonnative trout. Three of the barriers are natural and did not require construction, and the remaining six barriers were constructed specifically for the project.

To make the project manageable, we divided the project area into five separate phases of treatment (Figure 1). Rotenone treatment started with phase I on the Middle Fork Sheep Creek with three treatments from 2012 to 2014. Unfortunately, Brook Trout were detected in the treatment area in 2016 due to what we believe was barrier failure. We believe this was the case because Brook Trout below the barrier were marked with an adipose clip and in the year following marking, we captured marked fish above the barrier.

After additional planning and subsequent barrier construction, we continued with the next phase of the treatment in 2019 on the East Fork Carter Creek drainage. For this tributary, we completed two full treatments in 2019 and 2020 and a partial treatment in 2021. Following these treatments, we believed this phase of the treatment was complete and CRCT stocking occurred in parts of the drainage.

In 2021 we continued with the next phase of the treatment on the West Fork Carter Creek and tributaries. At the time of this writing, we were planning a second treatment in 2022 and depending on the results of this treatment, we will decide if a third treatment is necessary. After the West Fork phase is complete we plan to treat the South Fork Sheep Creek in 2023 or 2024, and then finish the project with treatment of



the North Fork Sheep Creek and a retreatment of the Middle Fork Sheep Creek beginning in 2025 or 2026.

The treatment area is a popular destination for anglers, particularly some of the lakes. Within the two drainages, there are 32 lakes managed as fisheries by the Utah Division of Wildlife. Before the treatment, there were 20 lakes managed for Brook Trout, 6 lakes managed for Cutthroat Trout, 5 lakes managed for Arctic Grayling (*Thymallus arcticus*) and one lake containing Rainbow Trout (Spirit Lake). Daggett Lake, which was managed for Arctic Grayling, also has a remnant population of Rainbow Trout and has been invaded by Brook Trout. When the Utah Division of Wildlife started CRCT restoration efforts in the mid 1990s, Rainbow Trout stocking in Daggett Lake was discontinued and Arctic Grayling were stocked instead. Of the 20 lakes containing Brook Trout, 15 were stocked cyclically (e.g., every three to five years) and the remaining 5 were maintained through natural reproduction. All of the lakes managed for Cutthroat Trout or Arctic Grayling were stocked cyclically. Cutthroat Trout stocking in these lakes was also discontinued in the mid 1990s until a brood of genetically pure fish was developed. While efforts to develop the brood were taking place, the lakes were stocked with either Arctic Grayling or Tiger Trout (*Salmo trutta x Salvelinus fontinalis*). It took many years to develop the broodstock for the North Slope of the Uinta Mountains, and the first CRCT from the brood were not stocked until 2018.

Post treatment, one of our goals was to maintain species diversity for anglers while reducing the risk of reinvasion by Brook Trout. Post treatment, only four lakes are managed for Brook Trout, and they are lakes that are not connected to the tributaries. Additionally, all Brook Trout stocked by the Utah Division of Wildlife are triploid and cannot reproduce. There are 15 lakes that will be stocked with CRCT post treatment and another five lakes that will be stocked with a combination of CRCT and Tiger Trout. Of these 20 lakes, natural reproduction by CRCT could occur in 11 of them. If natural reproduction does occur, these populations will be classified as CRCT conservation populations. Post treatment, there will still be five lakes stocked with Arctic Grayling and another three that will be stocked with Tiger Trout. In summary, in the post-treatment fishery, CRCT will be the featured species instead of Brook Trout, and anglers will still be provided with the species diversity they desire when fishing Uinta Mountain lakes.

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Effects of Streamflow on Trends in Native Salmonid Abundance Indices in Southwest Idaho

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Abstract - Idaho Department of Fish and Game (IDFG) fisheries staff conducts snorkel surveys to index populations of salmon, trout and other fishes across the state. Previous studies have found annual mean stream discharge three years prior to the survey had a strong correlation to observed fish density of Bull Trout *Salvelinus confluentus* and Brook Trout *Salvelinus fontinalis*. In IDFG's Southwest Region, snorkel surveys are conducted on a number of streams, including the North Fork Boise River (NFBR) and Middle Fork Boise River (MFBR). While the NFBR and MFBR have similar drainage areas, elevations, and aspect, the two streams have different fisheries regulations and recreational access. During 2017-2019, IDFG Southwest Region fisheries staff conducted snorkel surveys at 15 sites per year on the NFBR. During 2020 – 2022, IDFG Southwest Region fisheries staff conducted snorkel surveys at 13 sites per year on the MFBR. Native gamefish species in both systems are predominantly wild Rainbow/Redband Trout *Oncorhynchus mykiss* and Mountain Whitefish *Prosopium williamsoni*. Based on snorkel survey data, wild Redband/Rainbow Trout densities are strongly correlated to average stream discharge three years prior. However, Mountain Whitefish densities are less strongly correlated to average stream discharge three years prior.

Introduction

Snorkel surveys have been widely utilized by numerous agencies across space and time to quantify relative abundance indices for a variety of species, including native salmonids both resident and anadromous. It is generally accepted that snorkel surveys provide a relatively unbiased observation method (Heggenes et al. 1990). As with all sample techniques, there are pros and cons of utilizing snorkel surveys to estimate trends in relative abundance of fishes. As mentioned, snorkel surveys provide a relatively unbiased observation method and allow observers to make behavioral observations (Helfman 1983), including spawning, feeding, and habitat selection. Once data is collected, snorkel observations can be correlated to other environmental variables, including temperature, flow, and depth (Thurow 1994; Copeland and Meyer 2011). Observers rarely physically capture fish; thus, individual fish length is estimated visually (Grunder and Corsi 1988). Additionally, there may be detection biases with snorkel data, such as duplicate or missed observations (Griffith et al. 1984; Heggenes et al. 1990), species or size selectivity (Hillman et al. 1992; Helfman 1993), behavioral selectivity, and habitat influences (Rodgers et al. 1992). Finally, snorkel surveys are often utilized in systems where water chemistry is not suitable for other methods of capture (i.e., water conductivity limiting the efficacy of electrofishing).

Similar to many of the streams and rivers in the Idaho Batholith, both the Middle Fork Boise River (MFBR) and the North Fork Boise River (NFBR) are relatively unproductive rivers with low levels of dissolved solids and nutrients, and low conductivity. Historically, the drainages within the Idaho Batholith received marine-derived nutrients from the carcasses of returning anadromous fishes. However, anadromous returns to the Boise River basin were extirpated after the construction of numerous dams in the system starting as early as the completion of the Boise River Diversion Dam in 1909. Geology in the basin consists of granitic rocks and sand that result in shallow soil that is prone to high rates of erosion. Erosion is further amplified following wildfires, and large portions of the basin have been affected by wildfire. Due to the low productivity and resulting low fish densities, coupled with low dissolved solids, low conductivity, and high visibility, snorkel surveys are the most effective sampling tool currently available for Idaho Department of Fish and Game (IDFG) regional fisheries staff to implement on streams such as the NFBR and MFBR.

Both the MFBR and NFBR have been sampled intermittently by IDFG staff over the past 40 years. Fifteen NFBR sites have been surveyed using snorkeling techniques since the late 1980s, with the most recent prior surveys being conducted in 2004. Thirteen MFBR sites have been surveyed using snorkeling techniques since the late 1980s, with the most recent prior surveys being conducted in 2000. Copeland and Meyer (2011) showed stream flow three and four years previous to sampling was the most important bioclimatic condition influencing Brook Trout *Salvelinus fontinalis* and Bull Trout *Salvelinus confluentus* densities in Idaho rivers. Following record-setting snowfall and subsequent runoff throughout the greater Boise River basin during water year 2017, we sampled the NFBR for three consecutive years, followed by the MFBR for three consecutive years, to compare fish densities to determine if salmonid populations responded to changes in annual flow conditions.

Methods

The NFBR originates on the west side of the Sawtooth Mountain Range and flows in a southwesterly direction for approximately 80 km before joining the MFBR. Ridgeline elevations at the head of the drainage are around 2,500 m, while the elevation at the confluence with the MFBR is approximately 1,060 m. The NFBR loses approximately 960 m in elevation over the 75 km from where it becomes a third-order stream to its mouth, dropping an average of 12.8 m per kilometer over that distance. Recreation along the NFBR is variable due to topography and access. The lower 15 km are in a steep, narrow, non-trailed canyon section. This section is moderately popular among floaters in the spring, but experiences little angling effort most of the year. The middle section (river kilometers 15 - 45) is roaded, with numerous camping areas and one developed campground. This section of river receives the highest amount of recreation and angling effort. The upper 35 km are also remote, consisting of a trailed canyon section immediately above the roaded section. The uppermost portion of the basin is accessible via a primitive and long forest road or by flying into a remote airstrip at the U.S. Forrest Service's Graham Guard Station. As a result, the upper portion of the basin is also only moderately used for recreation. The NFBR falls under general regulations for IDFG's southwest region; trout limit is six, with no length or gear restrictions.

The MFBR originates on the west side of the Sawtooth Mountain Range and flows in a southwesterly direction for approximately 84 km before the NFBR confluence. Ridgeline elevations at the head of the drainage are approximately 2,800 m, while the elevation at the confluence with the NFBR is approximately 1,060 m. The MFBR loses approximately 1000 m in elevation over the 84 km from where it becomes a third-order stream to its mouth, dropping an average of 11.9 m per kilometer over that distance. United States Forest Service Road #268 runs parallel to the MFBR from the NFBR confluence to Atlanta, Idaho. As such, recreation along the MFBR is much more consistent compared to the neighboring NFBR. Above the confluence with the NFBR, the MFBR is a special regulations water; trout limit is two, none under 35.6 cm, no bait is allowed, and barbless hooks are required. Nearby waters, such as the NFBR, do not have special regulations. Native game fish in both the NFBR and MFBR consist of wild Rainbow/Redband Trout *Oncorhynchus mykiss* (WRBT), Mountain Whitefish *Prosopium williamsoni* (MWF) and Bull Trout. Additionally, both rivers are stocked annually with catchable-sized triploid hatchery Rainbow Trout *Oncorhynchus mykiss*.

During the summers of 2017 to 2019, 15 historic trend sites of various lengths were surveyed using entire-width snorkeling on the NFBR. During the summers of 2020 to 2022, 13 historic trend sites of various lengths were surveyed using entire-width snorkeling on the MFBR (Figure 1). For both rivers, we identified sites using historical accounts from previous sampling that included written descriptions, drawings, photos, and GPS coordinates. This allowed for reasonably precise relocation of sites. Sites were sampled with three snorkelers completing an entire-width snorkel survey. Methods for conducting fish abundance surveys by snorkeling followed the methods outlined by Apperson et al. (2015). Snorkelers moved upstream or downstream (depending on the site characteristics), counted all fish within their respective lanes, and estimated fish total lengths to the nearest 2.5 cm. Species, counts, and visually estimated lengths were recorded on PVC wrist cuffs by each snorkeler during the survey, then transcribed to a datasheet immediately after completing each survey. Following completion of each snorkel survey, staff measured and recorded site length, as well as quartile widths using a handheld laser rangefinder (Leupold RX-1000).

We compared differences in average observed length and average observed fish densities between the two streams for both WRBT and MWF using a two-tailed t-test ($\alpha = 0.05$).

Trends in relative abundance were compared by calculating species-specific density estimates for each site and comparing amongst years and river sections. Density was calculated as the count of each gamefish species divided by site area (site length multiplied by average width). Density was then standardized to fish per 100 m2 to account for differences in area. Average observed density for each basin was calculated by averaging standardized site densities within that basin for each year. Average observed density and cumulative average annual streamflow were evaluated with a linear regression model. Cumulative average annual streamflow was calculated for the three years preceding each of the surveys. Annual streamflow data was gathered from the Twin Springs, Idaho gauge (USGS #13185000). While there is no gauge station on either stream, we used the Twin Springs gauge as an indicator of basin-wide trends in streamflow as it is the nearest streamflow gauge and is located below the confluence of the NFBR and the MFBR.

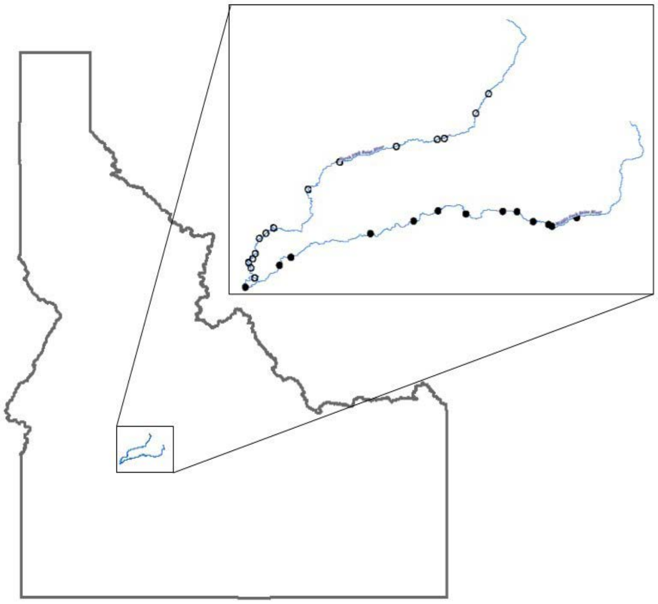

Figure 1: Snorkel survey sites in both the NFBR (white) and MFBR (black) completed by IDFG Region 3 fisheries staff between 2017 – 2022.

Results

During the sample period, average observed densities and estimated fish total lengths varied widely across years, species and rivers (MFBR and NFBR). Average observed density varied across river, sample year and species; typically, the MFBR had higher average observed fish densities across WRBT and MWF than the NFBR (Table 1). Average estimated fish total length varied by species and across years; typically, the MFBR had higher average estimated total lengths for both WRBT and MWF (Table 1).

During the survey period, cumulative total number of WRBT observed was similar in the two streams (NFBR n = 713, MFBR n = 706), however cumulative total number of MWF observed was higher in the MFBR (n = 768) than the NFBR (n = 443). There was a significant difference in average observed fish total length between the two streams for both WRBT ($p < 0.0001$) and MWF ($p < 0.0001$). We did not detect a statistically significant difference in average observed density of WRBT between the two streams ($p = 0.14$) but did detect a statistically significant difference in average observed density of MWF between the two streams ($p = 0.02$).

Trends in linear regression correlations for WRBT and MWF varied across the two streams during the sample period. In the NFBR, average observed density was positively correlated with increased streamflow for both WRBT ($R^2 = 0.99$) and MWF ($R^2 = 0.22$). In the MFBR, the opposite patterns were observed; both WRBT ($R^2 = 0.84$) and MWF ($R^2 = 0.97$) densities were negatively correlated with increased streamflow (Figure 2).

Discussion

Overall, average observed densities were correlated with cumulative average annual streamflow for WRBT and MWF. However, opposite trends in the relationship were observed; average observed density was positively correlated for both WRBT and MWF in the NFBR, yet average observed density was negatively correlated for both WRBT and MWF in the MFBR. While these results are curious, we acknowledge the relatively short time series of this study, and thus limited sample size.

Similar to other Idaho Batholith streams, snorkeling remains one of the most effective means of estimating fish densities in the MFBR and NFBR, partially due to extremely low conductivity. As mentioned previously, snorkel estimates can be biased by variation in observers, visibility, and flow. Snorkel surveys in the MFBR and NFBR were sampled at similar times annually during periods of low flow and

Table 1: Average observed density ± SE (fish/ 100 m²) average total length ± SE (mm) by river, year and species and cumulative average streamflow (three years prior; m³/s) for snorkel surveys completed by IDFG Region 3 fisheries staff between 2017 – 2022.

		Total fish observed (n)		Average observed density ± SE (fish/ 100 m²)		Average total length ± SE (mm)		Cumulative average annual streamflow (m³/s)
River	Year	WRBT	MWF	WRBT	MWF	WRBT	MWF	
NFBR	2017	143	98	0.94 (± 0.28)	0.79 (± 0.13)	200.9 (± 6.93)	305.2 (± 7.74)	32.7
	2018	282	212	1.93 (± 0.33)	1.36 (± 0.28)	210.4 (± 5.59)	300.4 (± 7.81)	43.8
	2019	288	133	1.99 (± 0.41)	0.86 (± 0.23)	204.2 (± 4.11)	305.2 (± 7.09)	45.8
MFBR	2020	132	169	1.24 (± 0.45)	1.29 (± 0.43)	203.2 (± 8.17)	328.7 (± 5.69)	47.0
	2021	324	296	2.99 (± 0.75)	2.26 (± 0.64)	156.9 (± 4.35)	311.7 (± 5.60)	32.9
	2022	250	303	2.61 (± 0.72)	2.81 (± 1.22)	188.9 (±4.64)	346.8 (± 3.75)	29.2

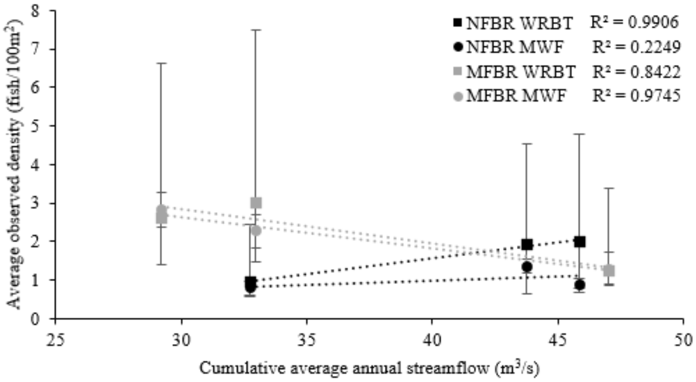

Figure 2: Linear regression of average observed density (fish/ 100 m²) and cumulative average streamflow (m³/s) for WRBT (squares) and MWF (circles) in both the NFBR (black) and MFBR (grey) species for snorkel surveys completed by IDFG Region 3 fisheries staff between 2017 – 2022.

favorable weather conditions. Additionally, sites can change across years due to spring runoff or other landscape-level disturbances. This is especially true when sites occur at the mouths of tributaries, as do many of the sites on the MFBR and NFBR. Both the MFBR and the NFBR have experienced landscape-level disturbances and habitat alterations (i.e., Little Queens fire in 2013 and Trinity Ridge fire in 2012). Unfortunately, no snorkel surveys were conducted during this period, and as such establishing correlations directly following these disturbances is difficult.

Due to relatively easy access, we speculate the MFBR likely receives higher recreational use compared to the

NFBR. Additionally, the MFBR and NFBR are managed under different fisheries regulations and have different levels of anthropogenic impacts (i.e., roads, hydropower impoundments, campgrounds, etc.). We did not observe a statistically significant difference in average observed WRBT densities between the two streams but observed statistically significant differences in MWF average observed density, as well as average observed total length for both species. Despite these differences, streamflow likely remains the most highly variable factor affecting the river and subsequent fish populations. Relationships between different flow metrics and fish populations are not unique in fisheries management literature. Generally, extreme streamflow events exhibit some regulatory impacts on fish populations (Blum et al. 2018), whether that is associated with dry season streamflow limiting growth (Harvey et al. 2006) or high flow events limiting recruitment (Sweka and Wagner 2022).

Snorkel surveys have been conducted infrequently on the NFBR, MFBR and other Idaho Batholith streams. In the last four decades, only six surveys have been conducted on the MFBR (1988, 1993, 2000, 2020, 2021, and 2022) and seven surveys have been conducted on the NFBR (1987, 1995, 1999, 2002, 2017, 2018, and 2019). Previous work in southwest Idaho (Cassinelli et al. 2017; Peterson et al. 2018; D'Amico et al. 2019) concluded that due to large annual fluctuations in average observed densities, infrequent (once per decade) snorkel sampling of Idaho Batholith rivers (i.e., NFBR, MFBR, and South Fork Payette River) may not be sufficient for trend monitoring in unregulated streams with dynamic flow regimes. Instead, short-term (< five years) intensive (annual) snorkel surveys were proposed to relate stream discharge patterns and trends in relative abundance in Idaho batholith streams. Based on the preliminary findings from this work, we encourage continued snorkel surveys to better inform trends in relative abundance of fishes and their correlation with environmental variables.

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Homing Behavior and Spawning Frequency of Yellowstone Cutthroat Trout in Three Tributaries to the South Fork of the Snake River, Idaho

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Abstract - Homing behavior for migratory fluvial Yellowstone Cutthroat Trout *Oncorhynchus clarkii bouvieri* (YCT) has been documented in tributaries of the South Fork Snake River (SFSR), Idaho. Tributaries of the SFSR have a moderate to high amount of genetic diversity, and the various life-history strategies of fluvial YCT in the SFSR may contribute to this diversity. We captured YCT with various electrofishing gears in both the SFSR and in tributaries to the SFSR, and in traps at weirs associated with tributaries. We tagged captured YCT with 12 mm full duplex PIT tags. We interrogated 1,052 individual PIT-tagged YCT that returned multiple times to the tributaries during the spawning run over a five-year period. We documented that most fluvial YCT in the SFSR spawn annually and that both male and female YCT have similar spawning frequencies. Ninety-nine percent of recaptured YCT expressed homing behavior. The operation of tributary weirs and traps during spawning runs does not appear to be adversely impacting YCT migratory patterns. The strong homing behavior by SFSR YCT is beneficial for the achievement of Idaho Department of Fish and Game's (IDFG) primary management objective for the SFSR, which is to maintain the genetic integrity and population viability of native YCT.

Introduction

In a broad sense, homing is defined as an adult fish returning to its natal area to spawn (Quinn et al. 1991). Salmonid families with differing life-history patterns have been documented displaying patterns of homing (Quinn 1997). Additionally, there is evidence that strong homing patterns in salmonids are a result of local adaptation (Quinn et al. 1991). Among those salmonids that display patterns of homing, are Yellowstone Cutthroat Trout *Oncorhynchus clarkii bouvieri* (YCT). Yellowstone Cutthroat Trout are native to the upper Snake River and Yellowstone River basins (Behnke 1992). Yellowstone Cutthroat Trout populations exhibit various life history patterns, including adfluvial and migratory fluvial forms (Behnke 1992; Gresswell et al. 1994). Adfluvial YCT in Yellowstone Lake have been documented to exhibit homing behavior (Varley and Gresswell 1988). Homing behavior for migratory fluvial YCT has also been documented in Burns Creek, a tributary to the South Fork Snake River (SFSR) (Jeppson 1971; Moore and Schill 1984), but homing rates have rarely been estimated for fluvial YCT.

The SFSR contains a robust population of YCT, and a migratory fluvial component of this population has been documented spawning in its tributaries (Jeppson 1971; Moore and Schill 1984; Thurow et al. 1988). However, homing rates have not been quantified for multiple tributaries of the SFSR, nor has homing in these tributaries been quantified over multiple years. Additionally, tributaries of the SFSR have a moderate to high amount of genetic diversity in terms of allelic richness and heterozygosity (Cegelski et al. 2006) and the various life-history strategies of fluvial YCT (e.g., homing behavior) in the SFSR may contribute to this genetic diversity. Conserving native YCT genetic integrity and population viability is a primary objective for the Idaho Department of Fish and Game (IDFG) (IDFG 2013). The biggest threat to YCT in the SFSR is introgression with non-native Rainbow Trout *Oncorhynchus mykiss* (RBT) (High et al. 2013). Rainbow Trout spawn timing and spawning locations have some overlap with YCT in the SFSR (Henderson et al. 2000). While hybridization occurs, low levels of introgression have been documented for the YCT population in the SFSR (Gunnell et al. 2008).

In 2004, the IDFG altered its management plan for the SFSR to address increasing RBT and decreasing YCT abundance trends (Schoby et al. 2013). This resulted in the entire SFSR being opened to fishing year round with no harvest of YCT and unlimited harvest of RBT (Schrader and Fredericks 2006). Additionally, the IDFG began working to protect against hybridization and introgression in four major spawning tributaries of the SFSR. This was done by operating weirs and traps on these streams, hand-sorting fish throughout the spawning runs, passing YCT upstream, and removing RBT and hybrids (High et al. 2013). Host (2003) demonstrated that with proper training, fisheries personnel were able to phenotypically differentiate between YCT and RBT and hybrids with 99% accuracy. Thus, in addition to managing against introgression, the weirs also offer an opportunity to study YCT life-history strategies. Our objective was to use data from these weirs and PIT antennas to quantify homing and straying rates of fluvial YCT in SFSR tributaries. Understanding patterns of homing and straying of YCT in spawning tributaries may help identify mechanisms for maintaining genetic diversity in the highly connected system (Van Doormik and Berejikian 2014) and inform future conservation strategies for the species in each tributary and within the SFSR.

Study Area

This study took place in eastern Idaho on four tributaries to the SFSR downstream from Palisades Dam including: Burns Creek, Palisades Creek, Pine Creek, and Rainey Creek (Figure 1). Weirs with traps are present on all four of these spawning tributaries and were operational during almost all spawning runs from 2009 – 2014. Exceptions were the trap on Rainey Creek, which was not operated in 2013 due to poor weir function (High et al. 2013), and the Palisades Creek trap, which was only operational during a portion of the 2011 spawning season (High et al. 2011). Additionally, PIT antenna arrays are located in all of these tributaries except Rainey Creek. These permanent arrays were used to remotely detect PIT-tagged fish moving into or out of the tributaries to spawn.

Methods

Tagging and Recapture

Beginning in 2008, we used boat-mounted electrofishing gear to capture YCT during SFSR population surveys. We continued this practice from 2009 – 2014, while also using backpack electrofishing in tributaries, and at traps associated with weirs in tributaries to capture YCT. Following capture, we anesthetized YCT using MS-222 and tagged them with 12 mm full duplex PIT tags. Tags were placed in the peritoneal cavity of fish following the technique described in Columbia Basin Fish and Wildlife Authotity (1999). We recorded total length, the date, and the capture location for all fish tagged with PIT tags. We also removed the adipose fin as a secondary mark. Additionally, at tributary weirs and whenever possible, we identified and recorded gender according to egg or milt expression and head morphology. These same parameters (i.e., total length, sex, capture location, and date) were also recorded when we recaptured fish that had previously been tagged with PIT tags. We recaptured PIT-tagged fish throughout the year during fisheries surveys in the SFSR drainage, but recapture events at spawning tributary weirs were the focus of this study.

Each spring during spawning runs, we trapped trout ascending study tributaries, differentiated YCT from RBT and hybrids (hereafter RBT and hybrids will collectively be referred to as RBT) based on phenotype, and passed YCT upstream of the weir. During this sorting, we scanned any adipose-clipped YCT for a PIT tag. We evaluated homing and spawning frequency using these recapture data. Fixed location PIT antenna arrays on Burns, Palisades, and Pine creeks also provided recapture information including PIT tag identification, location, and date. All recaptured YCT were placed into one of two categories: homing or straying. We defined homing as individual spawners returning to the same tributary (e.g., Burns Creek 2010 and Burns Creek 2012) during spawning season (mid-April through early-July), in two or more years. Conversely, we defined straying as individual spawners entering different tributaries (e.g., Burns Creek 2011 and Palisades Creek 2012) during spawning season over two or more years.

Analysis

We assessed homing using Program R and analyzed ‘subsequent’ capture data over a period of six consecutive years from 2009 to 2014. Only a single recapture of YCT occurred at Rainey Creek, where fluvial YCT escapement is low. This one record was removed from analyses to reduce bias. In the homing analysis, the response variable was fidelity (i.e., homing) and the predictor variables included tributary, year, gender, and their interactions. Summarized data in Program R showed that the number of strays was low, did not include any stray YCT for two of the years, and did not include any female YCT in Burns Creek. Thus, we could not construct meaningful models with interactions that included year or interactions between tributary and sex. We then constructed all possible logistic regression models (full and reduced after removing interactions as described earlier) using a general linear model and calculated Akaike information criteria corrected for small sample sizes (AICc) to select the best model. We then used the coefficients of the best model to interpret model results to assess direction and degree of influence for each of the variables.

We used logistic regression to assess spawning frequency with the observed probability of spawning each year as the response variable and year and gender as predictor variables. By using the observed probability of spawning each year as the observed variable, more weight was given to fish with greater number of years they were recaptured during spawning runs. We used the general linear model function in Program R to perform the logistic regression analysis. First, however, we fit the full model using a dispersion parameter to test for overdispersion. With overdispersion not evident, we fit all available models using the predictor variables and then used AICc scores to choose the best model. Once the best model was selected, we used coefficients to interpret model results and calculated the probabilities for fish in each of the tributaries during the spawning season.

Difference Between Proportions

We evaluated the difference between proportions among all interrogated fish (±95% confidence interval [CI]) as the proportion of straying males minus the proportion of straying females (Schaeffer et al. 2006).

We calculated the lower limit using the following equation:

$$(p_1 - p_2) - c_{\propto/2}\sqrt{\frac{p_1q_1}{n} + \frac{p_2q_2}{n}}$$

and the upper limit using the following equation:

$$(p_1 - p_2) + c_{\propto/2}\sqrt{\frac{p_1q_1}{n} + \frac{p_2q_2}{n}}$$

where p_1 is the proportion of straying males, p_2 is the proportion of straying females, $q_1 = 1 - p_1$, $q_2 = 1 - p_2$, $c_{\infty/2}$ is 1.96, and n is the sample size.

Results

We observed homing patterns of fluvial YCT that spawn in tributaries of the SFSR. Over a 6-year period, we interrogated 1,052 individual PIT-tagged fish that were repeat spawners. Ninety-nine percent of these recaptured fish were observed in the same spawning tributary in which they were initially observed. Of the thirteen YCT that strayed among tributaries, eight strayed from Palisades Creek (two female and six male), three strayed from Pine Creek (one female and two male), and two males strayed from Burns Creek. Logistic regression analysis and AICc scores indicated tributary was the most important predictor, occurring in the top four models, and the best model for predicting spawning stream fidelity included all three predictor variables (Table 1).

With Burns Creek, 2009, and female YCT set as the reference group, the exponentiated coefficients of the log (odds) indicated straying was not significantly different between Burns Creek and Pine Creek, but YCT had 112% higher odds of straying from Palisades Creek than Burns Creek. Also, the odds of straying was 350% higher for male than female YCT. While male YCT strayed at a greater proportion than female YCT, the differences between

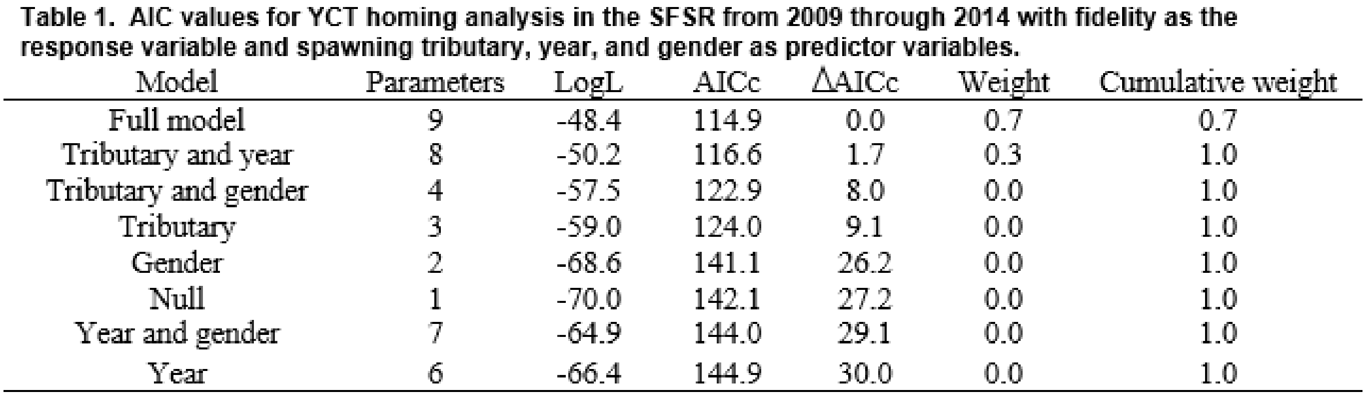

Table 1. AIC values for YCT homing analysis in the SFSR from 2009 through 2014 with fidelity as the response variable and spawning tributary, year, and gender as predictor variables.

Model	Parameters	LogL	AICc	ΔAICc	Weight	Cumulative weight
Full model	9	-48.4	114.9	0.0	0.7	0.7
Tributary and year	8	-50.2	116.6	1.7	0.3	1.0
Tributary and gender	4	-57.5	122.9	8.0	0.0	1.0
Tributary	3	-59.0	124.0	9.1	0.0	1.0
Gender	2	-68.6	141.1	26.2	0.0	1.0
Null	1	-70.0	142.1	27.2	0.0	1.0
Year and gender	7	-64.9	144.0	29.1	0.0	1.0
Year	6	-66.4	144.9	30.0	0.0	1.0

proportions were not statistically significant as the bounds overlapped zero (0.0 CI; ± 0.015).

Fluvial YCT in the SFSR that spawn in tributaries, spawn frequently. The spawning frequency of YCT was 1.2 years for fish spawning in Burns Creek, 1.4 years for fish in Pine Creek, and 1.5 years for fish spawning in Palisades Creek. We included records from the same fish used in the homing analysis to investigate if tributary or gender affected spawning frequency. Tributary was the most important predictor variable, and we chose the model with tributary as the only predictor variable as the best model (Table 2).

Table 2. AIC values for YCT spawning frequency analysis in the SFSR from 2009 through 2014 with the observed probability of spawning as the response variable and spawning tributary and gender as predictor variables.

Model	Parameters	LogL	AICc	∆AICc	Weight	Cumulative weight
Tributary	3	-954.7	1915.3	0.0	0.5	0.5
Tributary and gender	4	-954.2	1916.4	1.1	0.3	0.8
Full	6	-952.4	1916.9	1.5	0.2	1.0
Gender	2	-972.8	1949.5	34.2	0.0	1.0
Null	1	-974.5	1951.0	35.7	0.0	1.0

Discussion

Previous research has demonstrated that adfluvial (McCleave 1967) and fluvial YCT (Jeppson 1971; Moore and Schill 1984; Schoby et al. 2014) display patterns of tributary homing during spawning season. Results from our analysis demonstrate strong patterns of tributary homing (99%). We attempted to identify factors that affect homing such as tributary, gender, and year. While straying rates were extremely low, our models indicated the source tributary was the most important predictor of YCT straying. In our study, YCT from Palisades Creek were more likely to stray than fish from the other tributaries. To the best of our knowledge, findings of differential straying rates among tributaries have not been documented in resident or anadromous trout. Secondary variables that influenced straying include gender as well as annual variability. Straying among salmonids has been well documented (Lindsey 1959; McCleave 1967; Quinn et. al 1991; Fraser et. al 2004). Male-based dispersal among salmonids has also been documented. Fraser et. al (2004) observed that male Brook Trout *Salvelinus fontinalis* in an adfluvial population in Mistassini Lake, Québec, Canada demonstrated a higher rate of straying than did females among three of four tributaries. In our study, we observed male YCT having a higher propensity than female YCT to stray during spawning runs, but the difference was not statistically significant.

Fluvial YCT in SFSR tributaries had an average spawning frequency of 1.2 – 1.5 years, indicating that a majority of fish were annual spawners and some were alternate year spawners. Other studies have demonstrated similar patterns. In both Young and Hungry creeks in Montana, repeat spawning occurs on an annual basis by fluvial Westslope Cutthroat Trout *Oncorhynchus clarkii lewisi* (Liknes and Graham 1988). However, in other drainages in Montana, alternate-year spawning tends to predominate in repeat spawning behavior of Westslope Cutthroat Trout (Liknes and Graham 1988).

The most important predictor affecting spawning frequency in our models was tributary. This may be the result of one of the limitations of our study. We relied on recapture events of PIT-tagged YCT at each of the spawning tributaries in order to compile data for our analyses. In order for the analysis of spawning frequency to be truly comparable among tributaries, we would need capture efficiencies to be equal at all of the streams. This was not the case. For example, High et al. (2013) found that trapping efficiencies at Burns Creek (98%) are generally higher than at Pine Creek (89%) or Palisades Creek (96%). This was especially true in 2011, when damage caused by high flows at Palisades Creek terminated trapping efforts early in the spawning season. Because spawning frequencies were higher in streams where we generally had higher trapping efficiencies, we could assume that trapping efficiencies were potentially the cause of the observed difference in spawning frequencies between tributaries. Even so, spawning frequencies we estimated using our models, yielded results that we interpreted as evidence that fluvial YCT in the SFSR tributaries are predominately annual spawners with some fish spawning in alternate years. Interestingly, we observed SFSR YCT of both genders spawning at the same frequency. There are a number of studies that have documented repeat spawning frequency of various resident salmonid species: adfluvial RBT (Lindsey 1959), fluvial YCT (Moore and Schill 1984), and fluvial Westslope Cutthroat Trout (Schmetterling 2001). However, to the best of our knowledge, the difference, or the lack of difference, in spawning frequency between male versus female trout has not been documented.

The YCT population in the SFSR and its tributaries is robust and has been identified as a conservation priority for the IDFG (IDFG 2007). In 2004, the IDFG altered its management plan for the SFSR to address the increasing RBT and decreasing YCT abundance trends (Schoby et al. 2013). The entire SFSR was opened to fishing year round with no harvest of YCT and unlimited harvest of RBT (Schrader and Fredericks 2006). Another change in management efforts in 2004 placed an emphasis on maintaining multiple tributaries (i.e., Burns, Palisades, Pine, and Rainey creeks) as spawning strongholds, where YCT can spawn without the threat of introgression by RBT. Because the SFSR is one of the strongholds for YCT (Thurow et al. 1988; Van Kirk and Benjamin 2001; Meyer et al. 2006) protecting the genetic integrity of YCT in the SFSR is critical to ensuring the conservation of the species. Available information suggests the genetic integrity of YCT in the SFSR remains intact (Cegelski et al. 2006; Loxterman and Keeley 2012). The strong homing behavior of fluvial YCT shows that spawning tributary may be a factor contributing to the continued genetic integrity of the population (Van Doornik and Berejikian 2014). The operation of tributary weirs and traps and maintaining spawning refugia for YCT, free of RBT introgression risks, is an important part of YCT conservation in the SFSR (Van Kirk et al. 2010), and weir/trap operations do not appear to be negatively affecting YCT migration and spawning patterns in the SFSR. High homing rates of migratory fluvial YCT in the SFSR result in spawners consistently returning to spawn in areas where RBT are actively excluded. This is beneficial for attaining IDFG's primary management objective for the SFSR, which is maintaining the genetic integrity and population viability of YCT.

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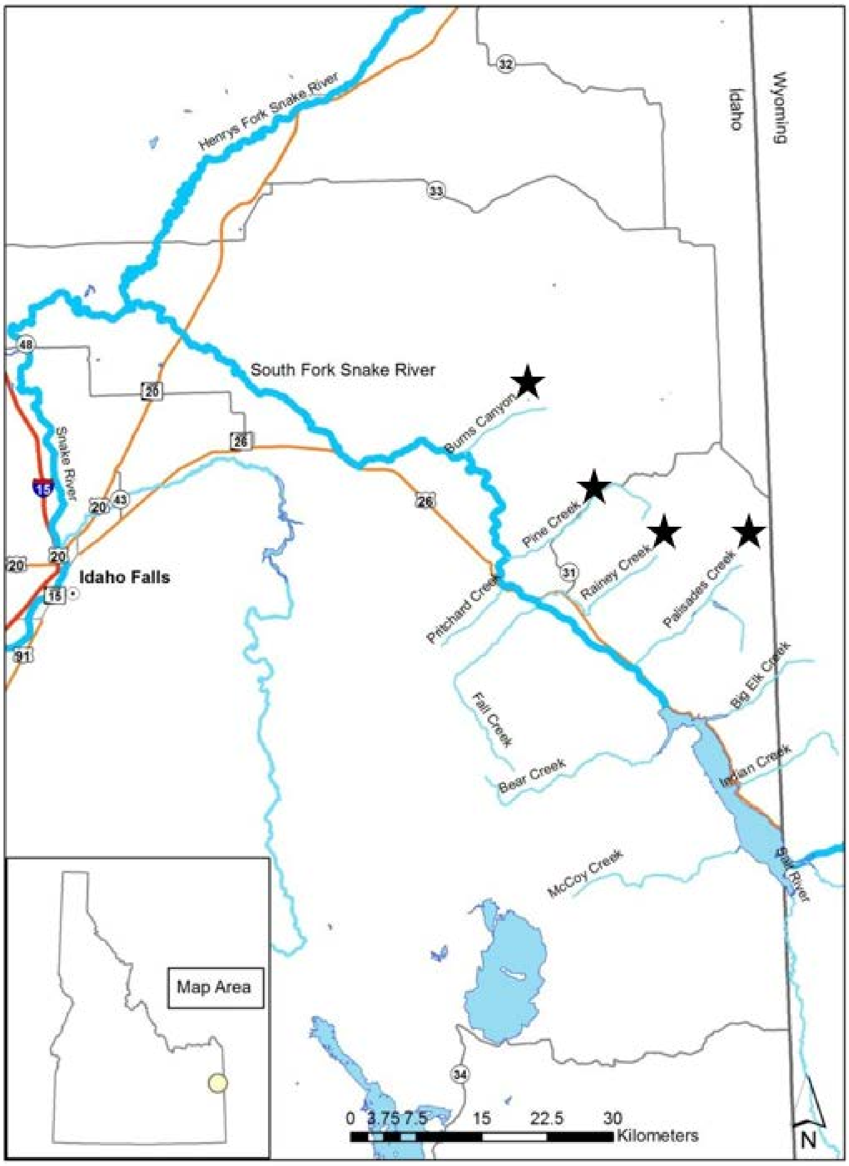

Figure 1. The SFSR in eastern Idaho. Black stars indicate study tributaries.

Session 3

Cold, Clean, Complex, and Connected: Understanding and Restoring Wild Trout Habitat to Prepare for a Changing World II

Scaling Up Restoration: Recovery of Native and Wild Trout Populations and Other Downstream Responses Due to Abandoned Mine Drainage Remediation in a Large River System

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Abstract - Impaired by abandoned mine drainage (AMD), the West Branch Susquehanna River was once devoid of life, and recovery was considered impossible. Through multiple partnerships, numerous remediation projects have been completed over the last 30 years. The objective of this study was to document current water quality and biological conditions and identify changes in response to the efforts to restore the watershed. Pollution-sensitive fish species have increased over time and Brook Trout *Salvelinus fontinalis* were documented in the main stem of the river for the first time in 2019. In addition, sections of tributaries and the main stem of the river have been documented to support natural trout reproduction (~320 kilometers) with several supporting Class A trout fisheries (~90 kilometers) since 2009. Nearly 42 kilometers of the main stem now support natural trout reproduction, demonstrating the improved conditions of the watershed. Some tributaries sampled only contained young-of-the-year trout, indicating that adult trout use the main stem of the river. Increased pH and alkalinity and decreased metal concentrations, conductivity, and acidity have occurred since 2009 due to AMD treatment. Macroinvertebrate communities have also shifted towards reference conditions. The presence of a wild trout fishery is attributed to the cumulative improvements in water quality from active and passive AMD treatment systems and abandoned mine land (AML) reclamation. Treatment of AMD in the West Branch watershed has increased the amount of available habitat, coldwater refugia, and spawning areas; increasing resiliency of trout populations to current and future stressors such as climate change. The results of this project demonstrate the cumulative impact of numerous, strategic restoration projects in headwater areas and tributaries of large rivers.

Introduction

The West Branch Susquehanna River basin drains approximately 18,000 km2 of mainly (83%) forested land in northcentral Pennsylvania and contains over 18,000 stream kilometers of tributaries. The watershed contains numerous strongholds of Brook Trout *Salvelinus fontinalis* (Fesenmyer et al. 2017). However, the area's true economic and ecological potential continues to be negatively impacted by historical coal extraction. Coal mining between the late 1700s and 1970s occurred with little to no regulation and resulted in over 1,900 kilometers of water polluted by AMD and more than 160 km2 of unreclaimed and scarred mine lands. A 1972 report for the West Branch Susquehanna River stated that "conditions in the study area are such that no more than 30 miles [48 km] could possibly be restored for fishing and recreational use" (Commonwealth of Pennsylvania 1972). Rummel and Wolfe (2019) provide a review of the historical impacts of AMD and restoration efforts within the watershed. The acidic water and toxic metals in AMD can negatively influence the growth rate, behavior, and metabolic processes of fish. Additionally, AMD can cause a reduction in the abundance and diversity of aquatic insect populations and the metal precipitates can armor the stream substrate, thereby reducing habitat availability and diminishing the food supply for other aquatic organisms.

In 2009, Trout Unlimited developed the West Branch Susquehanna Recovery Benchmark Project to document and quantify the results from dozens of AMD remediation projects and millions of dollars that have been invested in mine cleanup across the watershed (Trout Unlimited

2011). In partnership with the Pennsylvania Department of Environmental Protection (DEP), Pennsylvania Fish & Boat Commission (PFBC), Susquehanna River Basin Commission, and others. This study noted substantial improvements in water chemistry compared to 2009. The improvements were attributed to AMD treatment and AML restoration, improved mining practices and regulation, and natural attenuation (Trout Unlimited 2011). Since the completion of the initial study, AMD remediation efforts have continued throughout the West Branch Susquehanna River watershed, including the construction of new passive and active treatment systems and AML reclamation. In 2017, Trout Unlimited began the West Branch Susquehanna Recovery Benchmark Project II to replicate and expand the 2009 project. The objective of this study was to document current water quality and biological conditions and identify changes through time in response to the continued efforts to restore the West Branch Susquehanna River watershed to its full ecological potential.

Methods

A total of 110 sites were sampled (Figure 1). Data were collected from 2017-2019, a majority of sites were replicates of the 2009 project ("replicate sites") for comparisons. Thirty reference sites were located within the West Branch Susquehanna River watershed, had no listed impairments (including AMD impairment) on the Pennsylvania 303(d) list and were designated as Class A (>30 kg/ha Brook Trout biomass) trout waters. Identification of treatment system locations was completed in ArcGIS using publicly available data and satellite imagery. Additional treatment systems and reclamation projects may exist within the study area. A watershed was determined to have AMD treatment if AML reclamation, passive treatment, or active treatment was present upstream of the sampling point. The five groups used in the analysis were: [1] reference sample sites, [2] active treatment upstream regardless of other treatment types, [3] passive treatment upstream regardless of other treatment types (excluding sites with active treatment), [4] only land reclamation present upstream in the watershed, and [5] sites with no known treatment.

A total of 108 and 110 sites were sampled for water quality in May and July 2017, respectively. Streamflow was measured at the time of water quality sampling using a Swoffer 3000 meter. U.S. Geological Survey (USGS) stream gauge data were used where available. In the case of larger tributaries or mainstem river sample locations, 3 to 6 samples from across the sample site were composited. A 500 mL raw water sample, as well as unfiltered and 0.45 micron filtered 250 mL samples fixed with 15 to 20 drops of nitric acid were collected from each site. Samples were analyzed at a DEP accredited laboratory. Pollutant and alkalinity loadings (kg/day) were calculated at sites with streamflow measurements.

Benthic macroinvertebrate sampling was completed according to DEP's Instream Comprehensive Evaluation protocols (Chalfant 2015). Macroinvertebrates were identified by taxonomists, certified by the North American Benthological Society, to genus or the lowest taxonomic level possible. Samples were evaluated according to metrics comprising the DEP's Index of Biological Integrity (Chalfant 2015). Biological metrics were standardized and used to determine if the stream met the Aquatic Life Use threshold. Macroinvertebrate community composition was examined using non-metric multidimensional scaling (NMDS) plots using the five groups of AMD treatment. Vector arrows of water quality parameters were added to the NMDS plots and a metal index was used to incorporate all metals analyzed at the lab (Goher et al. 2014) in order to see how sites separate based on water quality.

PFBC staff sampled fish at five historic sample sites and one additional site in October 2019. Data collection protocols followed those of past surveys (Detar and Kristine 2009) using backpack and mini-boom boat electrofishing gear. Shannon diversity and Simpson's diversity were calculated for fish collected during 1998, 2009, and 2019. Coldwater fisheries data from historical PFBC data collection efforts since 2009 were also included to evaluate changes in trout presence and classification of those streams by the PFBC. Trout biomass data from PFBC was also used to compare replicate sites to reference sites.

Kruskal-Wallis statistical tests with their associated multiple comparison tests, as well as Mann-Whitney and Mann-Whitney pairwise tests were used for comparisons among years and treatment groups. Data were assessed prior to analysis to determine if they met assumptions for parametric tests. Non-parametric tests were used when assumptions were violated and transformation failed. Permutational analysis of variance (PERMANOVA) was used to identify differences between the different group clusters in the NMDS (Anderson 2001).

Results

Water Quality

Long-term data from USGS gaging stations in the main stem of the West Branch Susquehanna River documented

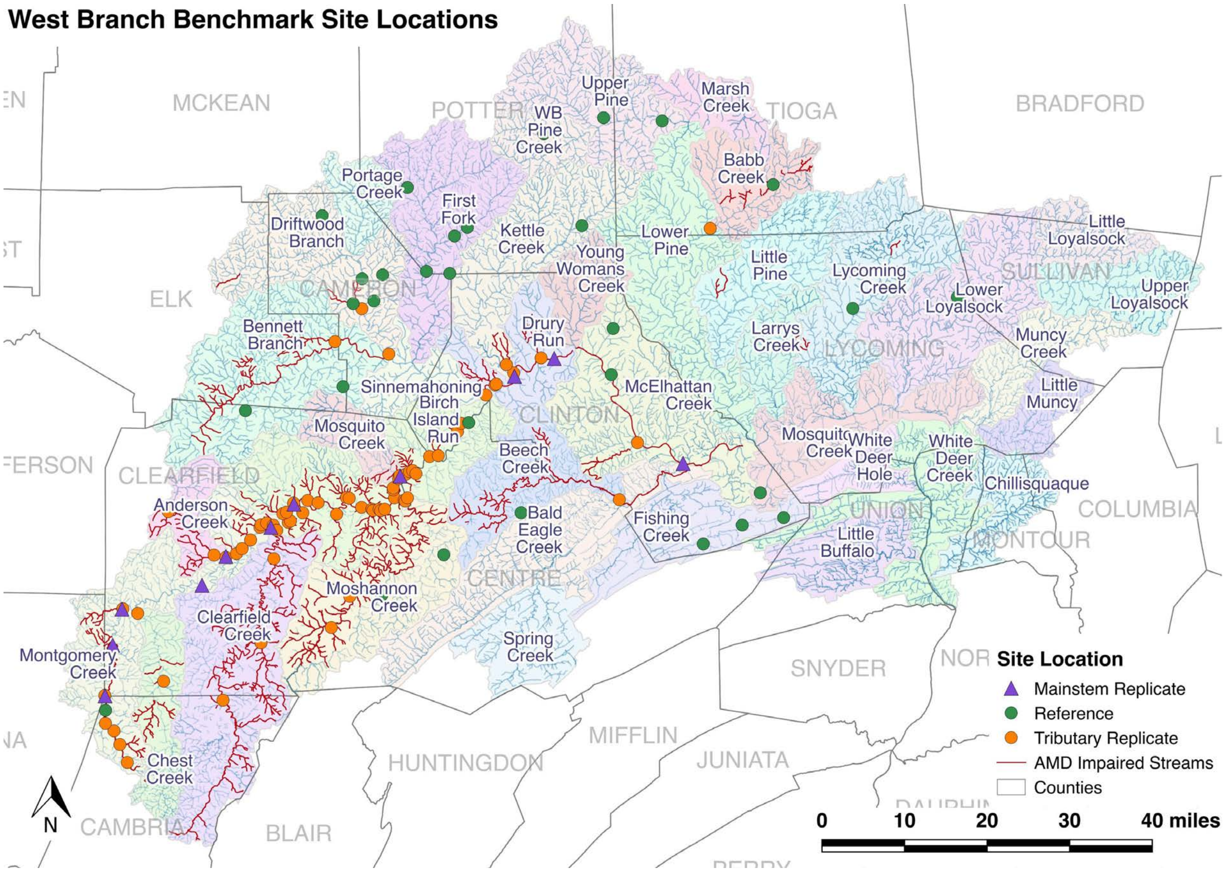

Figure 1. Sample site locations for the West Branch Susquehanna Recovery Benchmark II project.

increasing pH, stabilizing sulfate concentrations, and decreasing acidity concentrations over time. In the current study, water quality at replicate sites improved from 2009 to 2017. Mann-Whitney pairwise comparisons of replicate sites between 2009 and 2017 showed statistically significant increases for pH ($V = 3159.5$, $P < 0.001$), alkalinity ($V = 1454$, $P < 0.001$), total iron ($V = 7174$, $P < 0.001$), and dissolved iron ($V = 4049$, $P < 0.001$); while conductivity ($V = 8623$, $P < 0.001$), acidity ($V = 8738$, $P < 0.001$), total manganese ($V = 9428$, $P < 0.001$), total aluminum ($V = 6477$, $P = 0.03$), sulfate ($V = 8125.5$, $P < 0.001$), total dissolved solids ($V = 8191.5$, $P < 0.001$), dissolved manganese ($V = 9234$, $P < 0.001$), dissolved nickel ($V = 6638.5$, $P < 0.001$), total nickel ($V = 6559.5$, $P < 0.001$), and total zinc ($V = 6476$, $P = 0.003$) significantly decreased between the sample years.

Active and passive treatment groups were combined for the water quality Kruskal-Wallis comparisons because there were only five samples within the active treatment group. Sites with active and/or passive treatment had significantly higher pH ($Z = 3.73$, $P = 0.001$) and alkalinity ($Z = 3.90$, $P < 0.001$), and lower acidity ($Z = -3.81$, $P < 0.001$) and metal concentrations (Iron: $Z = 3.51$, $P = 0.002$; Aluminum: $Z = 3.35$, $P = 0.003$; Manganese: $Z = 5.03$, $P < 0.001$) than impaired sites without any AMD treatment. Sites with land reclamation projects had significantly higher pH ($Z = 2.82$, $P = 0.02$), alkalinity ($Z = 2.89$, $P = 0.015$), and lower acidity ($Z = -2.75$, $P = 0.023$) than sites without treatment. There were no significant differences in metal concentrations between sites with land reclamation and those without AMD treatment. Reference sites had significantly lower conductivity, total iron, total manganese, total aluminum, sulfates, total dissolved solids, dissolved iron, dissolved manganese, dissolved aluminum, dissolved nickel, and dissolved zinc compared to all other treatment groups. Reference site pH was significantly higher than impaired sites that had no AMD treatment ($Z = -4.08$, $P < 0.001$).

Benthic Macroinvertebrates

Mann-Whitney tests found significant increases in the Index of Biological Integrity score ($W = 120.5$, $P < 0.001$), total taxa richness (W = 10.5, P <0.001), EPT taxa richness (W = 43.5, P <0.001), Beck's index (W = 91, P <0.001), and Shannon diversity (W = 209, P <0.001) from 2009 to 2017-18 macroinvertebrate surveys among the 59 replicate sites that were sampled in both 2009 and 2017-18. Analysis of the data using NMDS and PERMANOVA of the 2017-18 data indicated that reference sites were a significantly different cluster than the other four treatment groups and the passive treatment group was significantly different than untreated sites (Figure 2). Any results from PERMANOVA analysis may confound centroid location versus dispersion (Warton et al. 2012). However, in Figure 2 the reference sites occupy a visibly different space and a much tighter cluster than untreated (F_1 = 7.66, P = 0.01)), land reclamation (F_1 = 9.16, P = 0.01), passive treatment (F_1 = 7.85, P = 0.01), and active treatment (F_1 = 4.03, P = 0.01) groups. Dispersion and centroid location of the points are both likely to be significantly different. For passive treatment versus no treatment (F_1 = 2.26, P = 0.04), it is more difficult to determine if the groups are occupying different centroid locations; this comparison likely differs in dispersion. Water quality parameters were fitted to Figure 2 using an environmental fit with the length of the vector arrow indicating the strength and direction of the trend. Most untreated sites had the highest values of metals (mg/L), acidity (mg/L), and sulfate (mg/L) (Figure 2). There was a significantly higher percentage of scrapers at reference sites compared to both 2009 (Z = 8.23, P <0.001) and 2017-18 replicate sites (Z = 6.44, P <0.001). There was also a significantly higher percentage of shredders (Z = -3.80, P <0.001) and scrapers (Z = -2.46, P = 0.014) in the 2017 replicates compared to 2009 replicates.

Fish Communities

Twenty-four total fish species were captured in 1998, 29 in 2009, and 31 in 2019. Kruskal-Wallis comparisons of percent intolerant individuals between 1998 (1.89 ± 2.95) and 2019 (21.8 ± 26.75) showed a significant increase (H = -2.39, P = 0.049). Comparisons of abundance were not completed due to variance in streamflow conditions between sample years and associated sampling efficiency variation. No significant differences were found for Shannon or Simpson diversity across sampling years, but a general increasing trend could be seen when comparing Shannon diversity between 1998 to 2009.

Data collected on both the main stem of the river and its tributaries were compiled to evaluate the coldwater fishery in

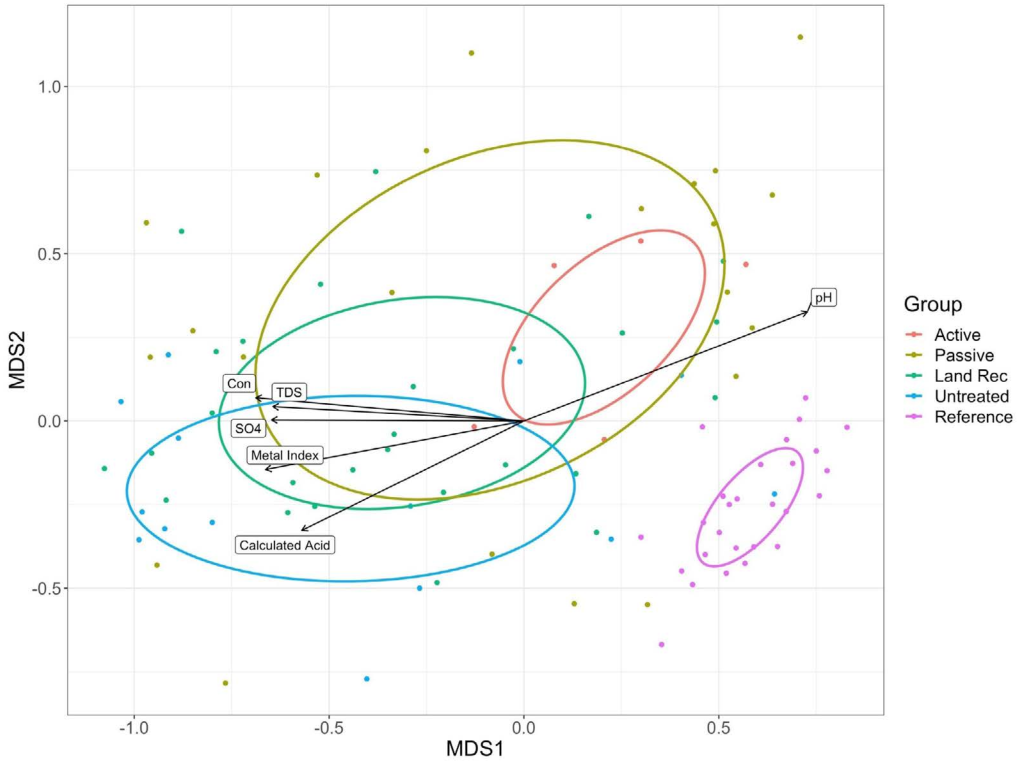

Figure 2. Macroinvertebrate community and abundance data NMDS of treatment groups and reference sites showing relation to water quality parameters in 2017.

relation to historic AMD issues in the watershed. Since 2009, approximately 1,010 kilometers of Class A trout streams and approximately 4,500 kilometers of streams supporting natural trout reproduction have been added throughout the entire West Branch Susquehanna River watershed. The majority of these additions were made through the PFBC's Unassessed Waters Initiative, which aims to document trout presence in streams previously lacking fishery surveys. Of the sites sampled as part of this project, 12 sections of streams and the mainstem of the river have been added as supporting natural reproduction (~320 stream kilometers) with several supporting Class A trout fisheries (~90 stream kilometers) since 2009. The majority of these sections remain listed as AMD impaired by the DEP.

Trout biomass data from 2010-2018 was compiled for the replicate and reference sites in the current study for comparisons. Data were non-normal and data transformation failed, so a non-parametric Mann-Whitney test was used for group comparisons for each trout biomass (combined Brook Trout and Brown Trout *Salmo trutta*, Brook Trout only, and Brown Trout only). Some sites had multiple samples taken across multiple years. Biomass in replicate tributaries was significantly lower than in reference sites (Figure 3) for combined species (W = 1279.5, P < 0.001) and Brook Trout (W = 1343, P < 0.001), but not for Brown Trout (W = 652.5, P = 0.298). Among reference tributary samples, one had no trout, 20 had only Brook Trout, and 7 were a mix of Brook and Brown Trout. And among replicate tributary samples, 17 had no trout, 14 had only Brook Trout, 9 had only Brown Trout, and 12 were a mix of Brook and Brown Trout.

Discussion

Water quality in the main stem of the West Branch Susquehanna River has changed dramatically over the past 60 years. Throughout the 1970s and 1980s, predominantly or intermittently acidic conditions and high metal concentrations existed; currently neutral pH, low metal concentrations, and net alkaline conditions exist along the entire mainstem of the river. The most notable improvements in the river's water quality occurred from 1980-2009 due to substantial AMD remediation (Trout Unlimited 2011). The results of this project demonstrate that an overall trend towards recovery has continued at the watershed scale. However, at the sample site/stream reach level, results are highly variable, with AMD related parameters increasing at some sites while other sites have substantially improved since 2009. Grouping sample

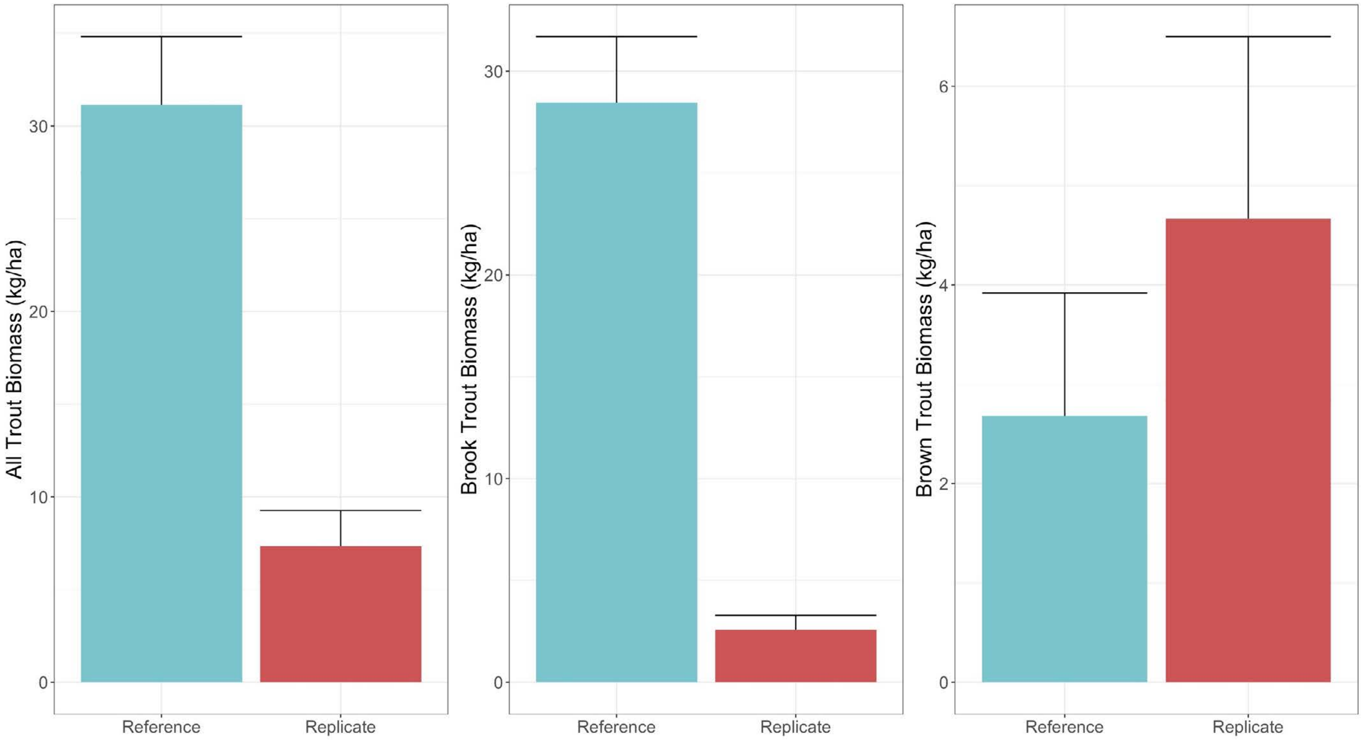

Figure 3. Mean biomass across all years and sites for replicate and reference sites sampled for trout biomass from 2010-2018. Error bars represent standard error of the mean.

sites into treatment categories reveal that sites with some form of treatment have significantly improved water quality compared to sites lacking AMD treatment. Sites with only land reclamation present upstream of the sample point did show some improvements in water quality over untreated sites, but not at the levels of passive or active treatment. This indicates that land reclamation alone may not be removing metals effectively from the stream, which could impair aquatic life recovery and prevent these streams from being restored (DEP 1998). Since land reclamation projects encompass a wide variety of techniques, further evaluation is needed to determine the effectiveness of site-specific treatments. Sites with active and/or passive treatment showed significantly lower metal concentrations than sites with land reclamation alone, indicating that these treatment techniques may be more efficient at treating AMD impaired water (DEP 1998).

Macroinvertebrate community results showed significant increases in most metrics between 2009 and 2017-18. The Hilsenhoff biotic index and percent sensitive individuals were the only metrics that were not significantly improved in 2017-18. Both of these indices use pollution tolerance values that are primarily based on nutrient pollution (Bode et al. 1996), which may explain the lack of change in these metrics. The reference sites were significantly different than the other four treatment groups; in addition, sites with passive treatment were significantly different than sites without AMD treatment, separating along vectors of pH and metal concentrations. Macroinvertebrate communities for some sites with passive treatment were more closely related to the reference condition, while others were more closely related to sites without AMD treatment. These results indicate the varying degrees of treatment success and may be useful to determine which sites may not be effectively treating water quality. The functional feeding group composition of benthic macroinvertebrate samples revealed that replicate sites had fewer shredders and scrapers than reference sites. This supports previous findings that decomposition rates in AMD impacted streams is impaired (Hogsden and Harding 2012).

The fishery in the mainstem of the river has also improved since 2009. The increase in fish species diversity and the percent pollution intolerant species may be attributed to the improving water quality. However, similar to the water quality results, improvements in the fish community were moderate compared to 1999-2009. The designation of nearly 42 kilometers of the main stem of the river, from its headwaters downstream to the confluence of Cush Creek, as supporting natural trout reproduction is a testament to the improved conditions in this region of the watershed. The presence of a wild trout fishery is attributed to the cumulative improvements in water quality from AMD mitigation efforts in this region. In addition to the main stem of the West Branch Susquehanna River, many tributaries within the watershed have been designated as supporting natural trout reproduction since 2009, but not all additions can be attributed to improvements in water quality. In 2009, PFBC launched its Unassessed Waters Initiative (PFBC 2013) to document trout distribution in streams that did not previously have survey data. Many of the waters in the West Branch Susquehanna River have been surveyed by PFBC and its partners through this initiative. Therefore, without historical fishery data for many of these sites, it is impossible to determine if a trout fishery was always present at these sites and was documented through the Unassessed Waters Initiative, or if conditions have improved and trout have recolonized these areas. It is also possible that some streams that have been listed as AMD impaired by DEP were incorrectly classified as not every stream reach in the watershed was sampled to determine impairment.

The results of this study can attribute trout populations in tributaries that were sampled as part of this project to improvements in water quality from AMD restoration. Eight of the replicate sites in this study have been designated as supporting wild trout populations since 2009. The presence of trout populations in these historically AMD impaired waters is encouraging; however, biomass comparisons demonstrate that Brook Trout biomass is lower in replicate sites than in reference sites. This may be an indication that water quality or other environmental factors (e.g., water temperature, detrimental land uses, etc.) may suppress biomass in replicate sites through decreased reproduction, limited habitat availability, population isolation, or other mechanisms. Further study is required to determine the causes and underlying mechanisms limiting trout biomass in these streams.

Overall, the results of this project demonstrate that a trend towards recovery has continued at the watershed scale, however, most sites remain distant from a “fully recovered” state. In order to continue the trajectory of recovery within the watershed, water treatment, AML reclamation, and protection of the current water quality/biological improvements will be required.

Acknowledgments

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A Comparative Review of Two Species of "Sea Trout" from Opposite Sides of the Globe: Biology, Ocean Ecology and Management of Anadromous Cutthroat and Brown Trout

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Abstract - Anadromous Trout and char, commonly referred to as sea trout, are important economically, ecologically and culturally, yet are poorly understood across the globe, making management of these species challenging. The lack of scientific attention is due, in large part, to their reduced commercial value relative to larger-bodied and more numerous salmonids, but also their complicated life history. Nevertheless, the scientific community has made significant strides in the last two decade, particularly around filling data gaps associated with anadromous Brown Trout *Salmo trutta* and Coastal Cutthroat Trout *Oncorhynchus clarkii clarkii*. Comparative analyses can shed light on recent advances in one area of study or species that may have applicability to another, accelerating broader understanding. We reviewed the available literature to compare the biology, ocean ecology and management practices associated with these two species that inhabit protected marine waters on opposite sides of the globe. We highlight similarities between species as well as key differences that provide important insight for lesser understood anadromous trout and help to prioritize future research projects.

Introduction

Anadromous Brown Trout *Salmo trutta* and anadromous Coastal Cutthroat Trout *Oncorhynchus clarkii clarkii* have received comparatively little attention by scientists and managers in relation to larger bodied andromous salmonids that are the focus of commercial and recreational fisheries management (i.e., Atlantic Salmon, *Salmo salar*, and Pacific salmon, *Oncorhynchus spp*.). Consequently, management and conservation plans associated with these trout are less developed and often lack extensive and reliable monitoring data as foundation. This is concerning given their high recreational value, and their unique role in the ecology of nearshore ecosystems (Knutsen et al. 2001; Quinn 2018).

The anadromous form of Brown Trout and Coastal Cutthroat Trout evolved allopatrically, in the northeastern sides of their respective oceans, yet these species inhabit a similar realized ecological/trophic/behavioral niche in their respective ecosystems. While research is limited on anadromous trout globally, recent work on anadromous Brown Trout has accelerated understanding and provided insight for the less-studied Coastal Cutthroat Trout (Figure 1). By applying a comparative approach to the behavior and ecology of these species, the function and adaptive value of behaviors can be more clearly determined and the recent advances by scientists across the globe can stimulate communication and growth of current research programs and prioritize data gaps for these ecologically similar species.

Origin/Phylogeny

The genera *Oncorhynchus* and *Salmo* diverged from a common ancestor but the time period when this occurred, and the most recent common ancestor is not known. However, a fossil discovered in 1964 assigned to *O. rastrosus*, the saber-toothed salmon, was estimated to originate from the late Miocene to Pleistocene period (Sepkoski 1992). This fossil provides evidence that the *Salmo* genus diverged from *Oncorhynchus* well before the Pliocene and as early as the Miocene (20 MYA). While cutthroat and Brown Trout are direct descendants of unique ancestors, *O. rastrosus* and *S. salar*, respectively (McKay et al. 1996; Bernatchez

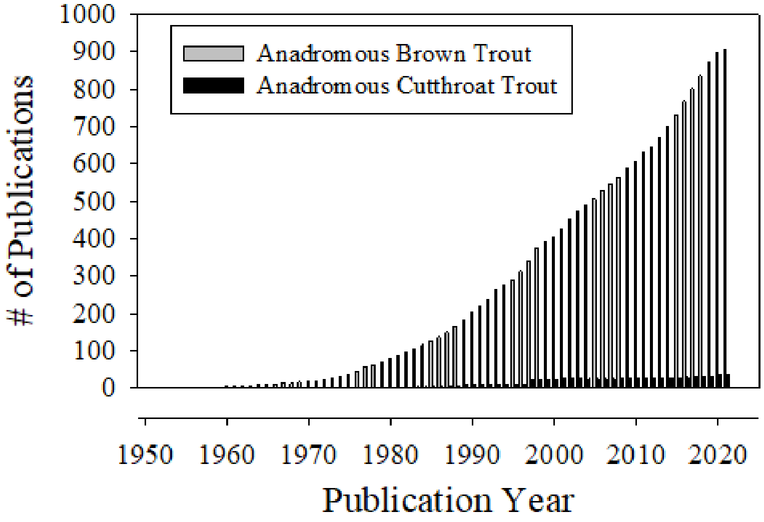

Figure 1. Cumulative number of peer reviewed papers and reports published on anadromous Brown Trout (grey bars) and Coastal Cutthroat Trout (black bars) between 1950 and 2021. Source: Web of science search terms included "anadromous" or "sea run and cutthroat" and "sea trout" or "anadromous and Brown Trout" or "seatrout".

et al. 2004; Trotter et al. 2018), their origin is the product of a similar set of circumstances (glaciation, dispersal limitations, geographic barriers and other climactic factors) occurring in the Pleistocene era. Specifically, Brown Trout diverged from Atlantic Salmon into several isolated geographic lineages (Bernatchez 2001, Crete-Lafreniere et al. 2012) that remained separate from each other for roughly 2 million years, and all retained the ability to produce the anadromous life form (Sloat et al. 2014). In this way, the anadromous form is not phylogenetically distinct from freshwater forms (Ferguson et al. 2019). Within these lineages, some authorities recognize multiple subspecies (Page 2008; Whiteley et al. 2019; Schöffmann 2021) and this is an area of rapidly developing science (Tougard 2022) but for the purpose of this paper these putative subspecies will be discussed generally as sea trout in the species *trutta*, given that they all have access to the marine environment and commonly express anadromy.

Outside the native range of Brown Trout, Coastal Cutthroat Trout evolved in the northeastern Pacific for at least 10 million years (Smith and Stearley 2018). They occur on both sides of the Rocky Mountains (a major faunal divide for North American fishes) including the headwaters of the Rio Grande, Colorado, and Missouri rivers, and interior basins that do not flow into marine waters. Numerous subspecies of Cutthroat Trout *Oncorhynchus clarkii* have been described; however, Coastal Cutthroat Trout represent the oldest existing lineage (Behnke 1992), and the only anadromous one, and therefore are the focus of this review. Following the divergence of the coastal lineage, 24 other subspecies developed, 3 of which are now extinct (Trotter et al. 2018). In contrast to the long history of Brown Trout and Coastal Cutthroat Trout, the marine habitat they inhabit today is relatively young, and thus the fish must have colonized and been extirpated repeatedly between glacial retreats and advances.

Distribution

The distribution of Coastal Cutthroat Trout is from northern California to south-central Alaska (Trotter 2008). Brown Trout is even more widely distributed, with a native range that historically occupied much of Europe and a large area in Asia (Snoj et al. 2021) (Figure 2). As a result of stocking by Europeans, beginning in the mid to late 1800's (MacCrimmon and Marshall 1968; MacCrimmon et al. 1970; Klemetsen et al. 2003), naturalized populations of Brown Trout exist on every continent except Antarctica, and they are often anadromous.

Brown Trout often remain within the fjord into which the home river empties. However, anadromous Brown Trout inhabit geographic areas without large, fjord-like inlets in both their native ranges such as the coastlines of North Africa (Lobón-Cerviá et al. 2019) and France ((Nevoux et al. 2019) as well as non-native areas such as Japan (Honda et al. 2012), South America (O'Neal and Stanford 2011; Minett et al. 2021), Newfoundland (Westley et al. 2011), and the Kerguelen Islands in the southern Indian ocean (Lecomte et al. 2013). Similarly, spawning populations of anadromous cutthroat are present in numerous small independent tributaries of the Pacific Ocean in Oregon and California that lack protected fjord-like environments (Johnson et al. 1999b). Here, anadromous cutthroat have been observed along open coastlines and beyond 30 km offshore (Loch and Miller 1988; Pearcy et al. 1990). Together, this information highlights the broad spatial distribution of anadromous cutthroat and Brown Trout within their respective ranges.

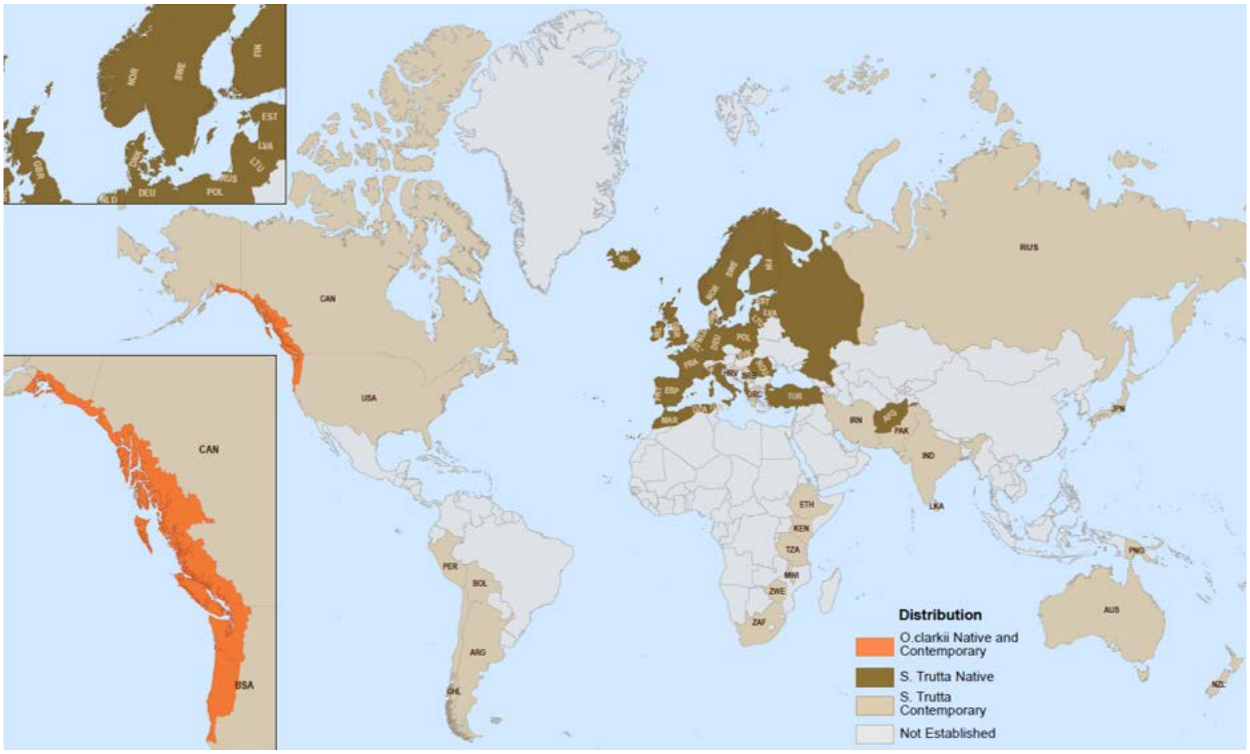

Figure 2. Map showing native and contemporary range of Brown Trout and Coastal Cutthroat Trout, Revised from MacCrimmon and Marshall (1986); Pearcy et al. (2018) and Mulfield et al. (2019).

Physical Characteristics

Anadromous Brown Trout and cutthroat share important physical similarities, and differ from many non-anadromous trout species. Both have numerous irregularly shaped spots, as opposed to the round spots that are common among fish in the *Oncorhynchus* family (Figure 3). The maxillary of anadromous Brown Trout and cutthroat extends beyond the eye, creating a relatively wide gape that supports an ontogenetic shift to a piscivorous diet at a small body size relative to other salmonids (Brodeur et al. 1987; Knutsen et al. 2001). In the marine environment, Brown Trout and Coastal Cutthroat Trout have a silvery coloration similar to other marine fish of similar size (Denton and Nicol 1966) but with heavier spotting and countershading highlighting the importance of camouflage in the nearshore marine environment. Upon their return to freshwater as adults, yellowish or brown coloration is common. These changes in color are used as characters for biologists and anglers to identify recent transitions from one environment to the other.

Both Brown Trout and Coastal Cutthroat Trout display great variation in color patterns among populations and subspecies, but there are nevertheless some consistent differences between them. Notably, Coastal Cutthroat Trout

Figure 3. Illustration of anadromous Brown Trout (above) and Coastal Cutthroat Trout (below). By Joseph Tomelleri.

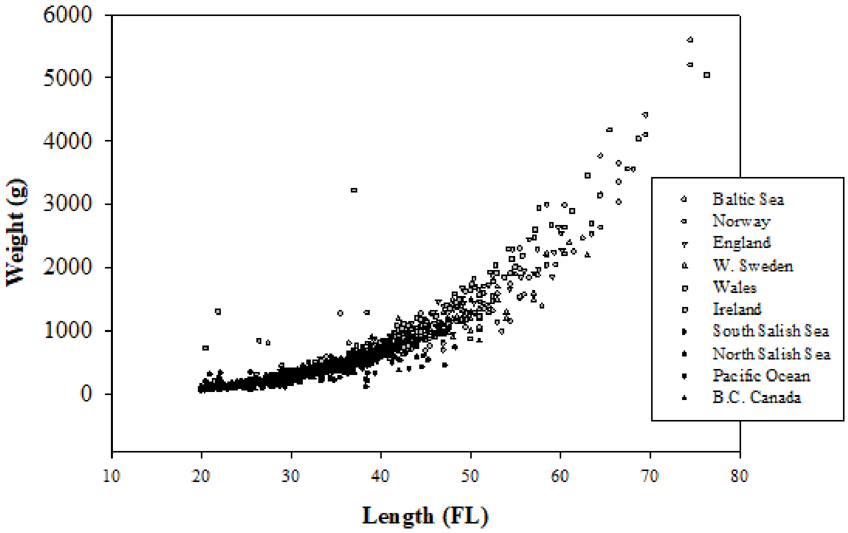

Figure 4. Fork length vs. mass of Coastal Cutthroat Trout (black) and Brown Trout (grey). Different shapes represent different data sets from Norway (J. Davidsen Pers. Comm.), United Kingdom (B. Brown, Pers. comm.), Baltic Sea (Sciences 2022), South Puget Sound (WDFW, Pers. comm.), North Puget Sound (T. Quinn, Pers. Comm.), Vancouver Island, B.C. (B. O'Connor Pers. Comm.) and Pacific Ocean (Pearcy et al. 2018).

have red slashes under the jaw (especially prominent when they are in fresh water) that are the source of their common name, and spotting on both the dorsal and ventral lobes of the caudal fin. In contrast, Brown Trout often lack spots on the tail or carry light spotting on the upper lobe. When Brown Trout are in freshwater, red spotting is common with a white or blue "halo" around spots, but these are less common or absent in the anadromous form (Klemetsen et al. 2003).

Adult anadromous Brown Trout and cutthroat exhibit similar body condition as is seen when comparing weight at length between the two species when sampled in the marine environment (Figure 4). At sea, both typically range in size from 20 to 56 cm (Jonsson and Jonsson 2007; Quinn 2018) but larger specimens are common in some areas although the average size vary considerably across season (L'Abée-Lund 1991; Figure 5; Table 1), particularly for Brown Trout which can reach lengths of greater than 80 cm.

Feeding

As visual, opportunistic predators, Brown Trout and cutthroat feeding habits are diverse, and studies of their diet provide insight into habitat utilization and prey availability. Diet analysis from anadromous Brown Trout and cutthroat suggests the prey consumed vary with the trout's size, age, and season, but are typically dominated by prey associated with the marine littoral zone, reflecting movements largely restricted to the nearshore environment.

Feeding studies are much more common for anadromous Brown Trout than cutthroat but both rely heavily on stomach content analysis. For anadromous Brown Trout, broad scale patterns of feeding have been well described with a diet dominated by nearshore invertebrates and fish. Piscivory is consistently reported as important with increasing contribution of fish in the diet with increasing trout size, especially in the summer and fall (May – October) (Lyse et al. 1998; Knutsen et al. 2001; Sánchez-Hernández 2020). For instance, in coastal waters of west Scotland, Pemberton (1976) observed increased feeding on small fish among Brown Trout > 21 cm, especially in summer when clupeids such as Atlantic herring *Clupea harengus* and sand eels Ammodytidae were abundant. For anadromous cutthoroat, diet is dominated by nearshore biota including small, schooling, planktivorous fishes, juvenile salmonids, and invertebrates (Brodeur et al. 1987; Loch and Miller 1988; Pearcy et al. 2018). In the southernmost reaches of South Puget Sound, where Chum Salmon *Oncorhynchus keta* are the most abundant anadromous fish species (Losee et al. 2019), Jauquet (2005) demonstrated the importance of fry, flesh and eggs of Chum Salmon to the diet of Coastal Cutthroat Trout in marine waters. Eggs are likely much less available to Brown Trout, however differences in diet by season and geographical area of both Brown Trout and Coastal Cutthroat Trout highlight the opportunistic nature of anadromous trout.

Life History and Migratory Patterns

Brown Trout and Coastal Cutthroat Trout exhibit four basic life history pathways: lacustrine (lake dwelling), fluvial (riverine), adfluvial (river and lake), and anadromous (marine) (Trotter 1989; Lobón-Cerviá and Sanz 2017; Arostegui et al. 2019; Jonsson et al. 2019). The anadromous forms are the focus of this report; however, it is worth noting that individuals entering marine water can express multiple life history patterns during their life span, either before or after their initial freshwater emigration (Saiget et al. 2007; Thorstad 2016). Additionally, offspring from anadromous parents may remain in freshwater their entire life (Rohtla et al. 2017; Claiborne et al. 2020) and vice versa. For instance, approximately 12% of sea trout sampled in the Baltic Sea near Estonia were produced by non-anadromous mothers, based on otolith microchemistry analysis (Rohtla et al. 2020).

Cutthroat and Brown Trout may rear for 1 to 6 years prior to ocean entry in spring but 2 is typical (Trotter 1989; Johnson et al. 1999b), as indicated from scale analysis. However, other, less common, rearing and migration patterns may exist, including autumn downstream migration (Birnie-Gauvin and Aarestrup 2019), and dispersal of juvenile Brown Trout from natal waters and into neighboring streams via the marine environment, reported by Taal et al. (2018).

While a comprehensive understanding of movement patterns for anadromous Brown Trout and cutthroat is lacking, general behaviors have been described (Figure 5). For instance, they occupy similar ecological niches in their respective marine habitats and exhibit repeatability in migratory behaviors at the juvenile (Bohlin et al. 1996; Goetz et al. 2013; Zydlewski et al. 2014) and adult (Losee et al. 2017; Halttunen et al. 2018; Birnie-Gauvin et al. 2021) life stages. Specifically, they rarely migrate more than 80 km from land (Moore et al. 2010; Thorstad 2016; Quinn 2018; Schöffmann 2021) as mentioned previously, making them different from their congeners. Similarly, Klemetsen et al. (2003) compared the movement patterns of Brown Trout and Atlantic Salmon and highlighted significantly reduced migratory distance of Brown Trout.

Abundance, Threats, and Management

Trait	*S. trutta*	*O. clarkii clarkii*	References
Dominant food	Fish, zooplankton, benthic invert.	Fish, zooplankton, benthic invert., salmon eggs	(Brodeur et al. 1987, Jauquet 2005, Rikardsen et al. 2006, Harris and Milner 2008)
Mass at maturity (g)	300-4000	174-1,111	(Saiget et al. 2007, Haque 2008, Harris and Milner 2008, Pearcy et al. 2018)
Length at maturity (mm)	199-800	222-575	(Peoples et al. 1988, Saiget et al. 2007, Harris and Milner 2008, Pearcy et al. 2018)
Maximum growth rate (mm/day)	1.1-1.8	0.6-2.6	(Berg and Jonsson 1990, Pearcy et al. 1990, Degerman et al. 2012, Losee et al. 2018)
Sexual dimorphism	Yes	Yes*	(Gruchy and Vladykov 1968, Jonsson and Jonsson 2015)
Marine survival	0.01% to 14.9%	2 to 20%*	(Michael 1989, Pearcy et al. 1990, Jonsson and Jonsson 2009b)
Typical distance from shore (km)	<80	<80	(Berg and Berg 1987, Berg 1989, Pearcy et al. 1990, Kallio-Nyberg et al. 2002, Thorstad 2016, Losee et al. 2017b)
Maximum recorded migration distance	800	600	(Kallio-Nyberg et al. 2002, Pearcy et al. 2018)
Typical depth (m)	<6	<5*	(Haque 2008, Eldøy et al. 2017, Sturlaugsson 2017)
Max depth (m)	70	Unknown	(Sturlaugsson 2017)
Redd length (m)	1.65	1.12	(Nika et al. 2011, Losee et al. 2016)
Redd depth (m)	0.08 to 0.35	0.06-0.37	(Jones and Report 1975, Ottaway 1981, Nika et al. 2011, Losee et al. 2016)
Water velocity at redd ($m\ s^{-1}$)	0.3 to 0.91	0.34-0.96	(Ottaway 1981, Nika et al. 2011, Losee et al. 2016)
Sediment size (cm)	0.2-8.6	0.2-7.6	(Ottaway 1981, L'Abée-Lund 1991, Losee et al. 2016)
Estuary spawning	Yes	Unknown	(Limburg et al. 2001, Gabrielsen et al. 2021b)
Sex ratio in marine water (% female)	58-79	Unknown	(Nevoux et al. 2019)
Fecundity (eggs/female)	291-8273	220-4420	(Jones and Report 1975, Johnston and Mercer 1976, Solomon 1997)
Age at marine entry	0 to 6	0 to 6	(Loch and Miller 1988, Peoples et al. 1988, Trotter 1989, Johnson et al. 1999, Sturlaugsson 2017)
Maximum age	11 to 12	7 to 10	(Jones and Report 1975, Peoples et al. 1988, Behnke 2002, Sturlaugsson 2017)
Maximum size (FL, mm)	758	756*	(L'Abée-Lund 1991, Behnke 2002, Sciences 2022) Ron Ptolemy, Ministry of Justice, British Columbia pers. Comm.
Scale count on lateral line	118-130	120-180	(Linnaeus 1758, Page and Burr 1991, Behnke 1992)
Gill rakers	13-18	15-21	(Linnaeus 1758, Behnke 1992)

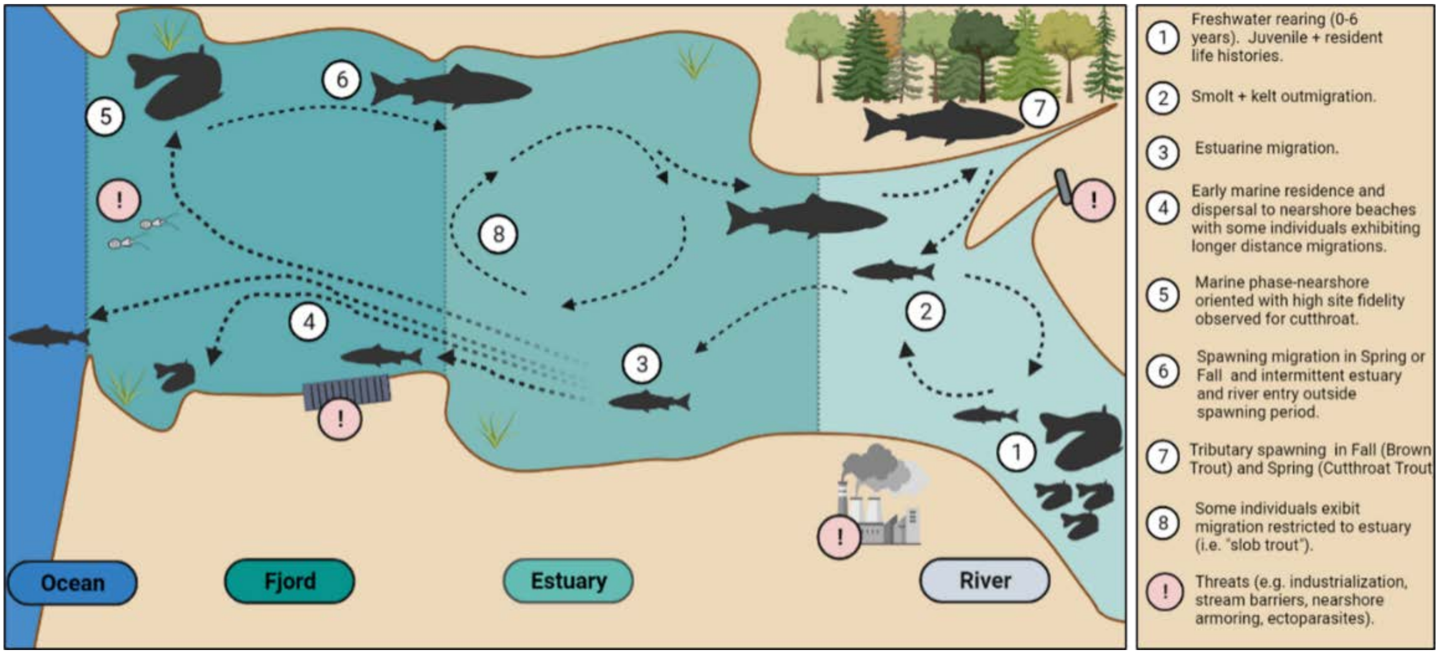

Figure 5 Generalized life cycle, examples of common patterns and some of the major threats of anadromous Brown Trout and anadromous Coastal Cutthroat Trout; adapted from Nevoux et al. (2019).

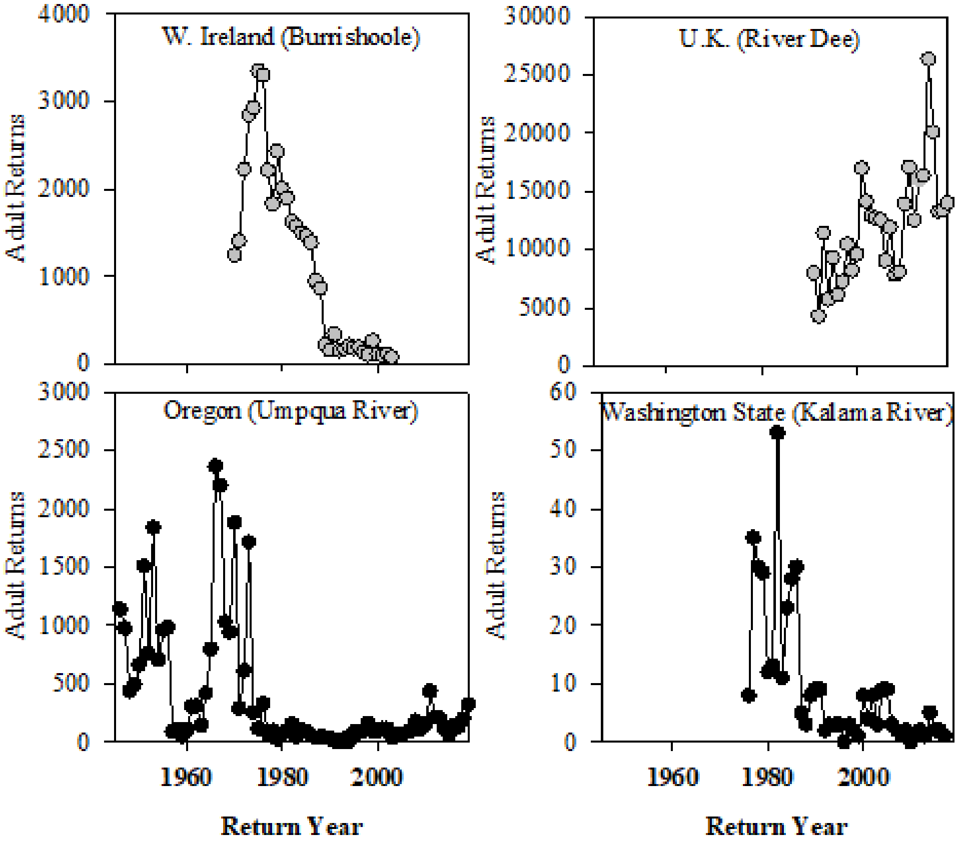

Figure 6. Trends in abundance for anadromous Brown Trout (upper tiles in grey) and Coastal Cutthroat Trout (lower tiles in black) from trapping locations in Europe and the United States.

Despite the limited number of comprehensive assessments of population size for anadromous trout, declines in abundance have been documented across the respective ranges of cutthroat (Giger 1972; Jones and Report 1975; Hooton 1997; Johnson et al. 1999a; Pearcy et al. 2018) and Brown Trout (ICES 2013, Pedersen et al. 2017) and represent the norm across their native distribution. The consensus among managers and biologists is that fewer anadromous cutthroat and Brown Trout are present relative to historic levels of abundance, but trends may have stabilized at current levels following declines in the 80's and 90's (Figure 6). Similarly, several populations of anadromous Coastal Cutthroat Trout declined in the 1980's and 1990s but information on abundance is much more limited compared to Brown Trout. Catch of anadromous trout from traps in tributaries of Oregon and Washington states demonstrate a significant decline followed by a period of stability at lower abundances in recent years (Figure 6). Together, the trends observed for anadromous Brown Trout and Coastal Cutthroat Trout highlight the importance of long-term time series to avoid the masking of past declines (i.e., shifting baseline; see Pauly 1995) and the importance of the late 1980's and 1990's in altering baseline abundance levels for many populations of anadromous fish.

Causes cited for the declines observed for both Brown Trout and Coastal Cutthroat Trout are consistent with other anadromous salmonids and include habitat degradation, hydropower operation, hatchery production and harvest. Additionally, a rapidly changing climate represents one of the greatest threats for species across the globe and is expected to exacerbate many of the threats and challenges highlighted above. Altering harvest regulations for commercial fisheries has shown promise in recovering wild trout populations in some areas and some assessment of this work from areas of the Baltic Sea has been published (Jutila et al. 2006). Furthermore, formal evaluations of management strategies are uncommon, in part due to the lack of well-defined management objectives (ICES 2020). This is concerning, given the mixed stock nature (Degerman et al. 2012; Losee et al. 2017) and nearshore orientation of sea trout in the marine environment which makes them vulnerable to capture and overexploitation (Blyth and Rönnbäck 2022).

Data Gaps

The most conspicuous data gap for anadromous cutthroat and Brown Trout is the lack of good abundance estimates across a range of rivers, and thus the lack of good information on possible trends in abundance. With a greater understanding of stock abundance and status, more refined management approaches to support the recovery of anadromous trout in some places and improved fishing opportunity more broadly could be put in place. If traditional tools like redd counts, riverscape and genetics, could be tailored and used for smaller salmonids with diverse life histories, there is great promise for these two species and the management around them. Additionally, new tools being developed for other focal species will be available to apply to anadromous trout in the near future including acoustic telemetry, SONAR (Gaudet 1990), seismic monitoring (Dietze et al. 2020) and drones (Groves et al. 2016).

Along with anadromous Brown Trout and Coastal Cutthroat Trout, numerous other closely related species exhibit similar behavior but suffer from the same lack of information needed to support sustainable fisheries management. Nearshore distributions of different species of char have been documented, including brook trout, *Salvelinus fontinalis* referred to as "coasters" and "salters" (Morinville and Rasmussen 2006), bull trout, *S. confluentus* (Hayes et al. 2011; Goetz et al. 2021), Dolly Varden, *S. malma* (Bond et al. 2013), grayling *Thymallus thymallus* and Arctic charr, *S. alpinus* (Mulder et al. 2020), though several of these species may also occupy offshore waters. Each of these facultatively anadromous species shows some similarities with Brown Trout and Coastal Cutthroat Trout as described here, hence future research may highlight important aspects of the behavior and biology of any one species that can help understand and refine management approaches to others.

Conclusion

The work presented here aims to advance understanding of anadromous cutthroat and Brown Trout, aid in the progression of science relating to anadromous coastal salmonids across the globe and identify data gaps for these chronically understudied fishes. Research is limited on anadromous trout globally, however it is clear that these smaller bodied salmonids, with their use of many small, short coastal streams for spawning, restricted migrations at sea and occupancy of primarily nearshore marine habitats necessitates a unique approach to management and conservation. Brown Trout and Coastal Cutthroat Trout that reside in close proximity to marine waters rely on diverse life history strategies spanning the freshwater, estuary and marine environments adding to their resilience in the face of a changing climate and increasing human population growth. With declining abundance across much of the range of these fish, managers should focus on improving connection between diverse habitat types, conservative fishing regulations that protect resident and anadromous forms and development of comprehensive management plans that seek to rebuild populations to historic abundance and diversity and protect fish populations that are performing well.

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The Influence of Beaver Dams on Coldwater Habitat and Trout in Wisconsin Streams

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Abstract - American beaver *Castor canadensis* play a complex role in the dynamics of low-gradient streams of the Upper Midwest, especially where dams alter important habitat for trout. For this reason, the control of beaver to maintain free-flowing conditions in select coldwater streams has been a core part of the Wisconsin Department of Natural Resources' (WDNR) management of Brook Trout *Salvelinus fontinalis* and Brown Trout *Salmo trutta*. However, beaver control on trout streams is often misunderstood, with deeply divided opinions among both the public and managers, leaving many unanswered questions concerning the science behind the control program. Calls for research to address management needs were included in recent WDNR management plans for both beaver and trout. In 2018, the WDNR initiated a study to measure the effects of beaver dams on coldwater stream habitat and trout populations in different ecological regions and beaver management zones across Wisconsin. A key approach to this study is the experimental manipulation of beaver management. Beaver control was removed from 24 free-flowing streams to allow for recolonization and dam building, and beaver and beaver dams were removed from 1 stream to restore free-flowing conditions. Here I will discuss how beaver dams physically reconstruct habitat for trout in streams across different gradients in the Lake Michigan, Lake Superior, and Mississippi River basins in Wisconsin; the transience versus permanence of beaver dams in Wisconsin streams; how beaver dams affect short-term trout population and fish community dynamics; and evidence for the warming of stream temperatures by beaver dams.

Introduction

Brook Trout Salvelinus fontinalis and American beaver Castor canadensis have a storied history in Wisconsin and other Upper Midwest states in the western Great Lakes region of the United States (Willging 2017; Johnson-Bice et al. 2018). Both species are native to the region, but changes in land use through the 19th and 20th centuries have altered the population dynamics of these species and how they interact (WDNR 2015; WDNR 2019). During this period, populations of both beaver and trout were alternately decimated and recovered. Beavers are generally considered to have negative effects on trout in Wisconsin streams, and this is reflected in management actions that maintain free-flowing conditions in select coldwater trout streams by controlling beaver.

Trout streams in Upper Midwest states are generally of low gradient compared to streams in the Intermountain West and Appalachian Mountain streams in the East. As such, beaver dams in Wisconsin tend to backup streams and flood large areas. New beaver dams may create deep pools, supporting the growth of larger trout, and open the riparian canopy, offering easy access to angling. However, in low-gradient meandering streams, beaver ponds extending far outside the original stream channel may be largely shallow (Knudsen 1962). As sediment settles behind beaver dams, initially deep pools fill to become shallow, saucer-shaped ponds, and initially improved trout fisheries may be lost. Coarse spawning substrates may become buried in silt and detritus, and dams may become "mortared," making it difficult for larger trout to traverse for access to seasonally important habitat. Such hardened dams may also achieve a level of "permanence" because flooding in low gradient watersheds may generally be insufficient to dislodge dams.

The scientific literature is somewhat equivocal about the effects of beaver dam construction or removal on coldwater streams and trout populations therein. Wisconsin's beaver control efforts to maintain free-flowing conditions on trout streams are largely based on research on the Pemonee River and seven of its tributaries in northeastern Wisconsin from 1982 to 2000 (Avery 2002). Avery's 18-year study showed that the removal of 546 beaver dams by 1986 and the maintenance of free-flowing conditions through 2000 resulted in decreases in stream temperatures and increases in Brook Trout abundance and size structure. Although the data suggest the removal of beaver dams improved the trout fishery in the Pemonee River system, questions have been raised on the lack of control streams in the study and the extent to which the study results can be generalized to coldwater streams elsewhere in Wisconsin. For example, McRae and Edwards (1994) found no consistent relationship between the size or number of beaver dams and their effect on downstream water temperatures in four other northeastern Wisconsin streams, and according to Pollock et al. (2003), detrimental population-level effects of beaver dams on trout have not been demonstrated in the scientific literature.

Studies on the influence of beaver dams on Midwestern trout streams have been limited (Johnson-Bice et al. 2018). Research is critically needed to better understand the effects of beaver dam construction on coldwater stream habitat and existing trout populations and to better understand the effects of removing beaver dams to restore coldwater streams in different regions throughout Wisconsin (WDNR 2015, 2019). Here I describe an ongoing study to test for the effects of beaver dam construction and beaver dam removal on coldwater stream habitat and trout populations in different ecological regions and beaver management zones (BMZ) across Wisconsin. Specific study objectives include (1) quantifying the effects of beaver recolonization of free-flowing streams on stream habitat, trout populations, and fish community structure and (2) quantifying the effects of removing beavers and beaver dams, creating free-flowing conditions in streams currently colonized by beaver.

Methods

In autumn 2018, we began the process of identifying potential study streams in different regions and management zones of Wisconsin by (1) using a list of streams the Wisconsin Department of Natural Resources (WDNR) has contracted with Wildlife Services (U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services) to maintain under free-flowing conditions; (2) consulting with regional biologists; and (3) conducting field assessments of stream suitability for the study. We are studying how stream habitat, trout populations, and fish communities respond to beaver colonization of streams and to the removal of beaver and beaver structures to restore free-flowing conditions. Beaver control was removed from a sample of streams currently maintained under free-flowing conditions and beaver control was applied to a sample of streams heavily colonized with beaver, with each group compared to control streams in which beaver management was not changed.

We identified 19 potential study streams for beaver recolonization and 7 for beaver removal in 2018. Following pilot surveys in 2019, 8 recolonization and all 7 removal streams were dropped from consideration. In 2020, we evaluated 16 new streams of which 8 were added for recolonization and 2 were added for removal. In 2021, we added 5 more recolonization streams and 1 more removal stream. As of the start of 2022, we had 24 recolonization streams and 3 removal streams (Table 1 and Figure 1). Beaver recolonization began on two streams prior to the start of this study: stream #24 in 2016 and stream #26 in 2018. We removed beaver control from streams #1-6, 17, 18, 25, and 29 beginning in 2020; from streams #15, 20-23, 27, 28, and 30 in 2021; and from streams #7-9 and 13 in 2022 (Table 1). We removed beavers and beaver dams from stream #12 in 2022. Two removal streams (#10 and 14) have been dropped from the study in 2022, and three recolonization streams (#20, 29, and 30) will be dropped in 2023.

Study streams were selected to represent different ecological landscapes and salmonid fisheries across Wisconsin in areas where classified trout waters are managed to control beaver activity (Figure 2). Brook Trout predominate in northern Wisconsin streams (BMZ A and B), and Brown Trout are dominant in Driftless Area streams in southwestern Wisconsin (BMZ C) (Table 1 and Figure 1). Lake Superior tributaries also provide spawning and nursery habitat for lake-run salmonids including Coho Salmon *Oncorhynchus kisutch*, steelhead trout *Oncorhynchus mykiss*, and Brown Trout. Stream gradient is relatively higher in Driftless Area streams and Lake Superior tributaries and is relatively lower elsewhere in northern Wisconsin streams.

We used standardized stream habitat survey protocols to measure habitat metrics including riffle-pool-run sequences, channel structure, substrate composition, canopy and fish cover, and riparian land use (Simonson et al. 1993, 1994). HOBO U22-001 Water Temperature Pro v2 data loggers were used to monitor stream temperature and HOBO U20-001-01 30-foot depth water level data loggers were used to monitor baseflow and flood events (hourly, year-round) (Onset Computer Corporation, Bourne, MA). We measured stream flow during each fish or habitat survey, and we used a YSI ProQuatro Multiparameter Meter to measure water quality parameters including dissolved oxygen (YSI Incorporated, Yellow Springs, OH).

We mapped the occurrence and change over time of beaver dams using GPS coordinates and physical measurements of beaver dams. We characterized beaver dams by measuring dam length (bank to bank), dam width (upstream-downstream), and dam height and water level upstream and downstream at multiple points across each dam (Figure 3). We also noted whether water flowed under, through, or over beaver dams.

We used standard electrofishing survey protocols to sample trout and other fish species. For fish community analyses, we collected all species to calculate coldwater indices of biotic integrity (Lyons et al. 1996). For salmonid populations, we collected relative abundance and size structure data, and in some streams, we tagged trout with either Visible Implant Elastomer tags (Northwest Marine Technology Incorporated, Anacortes, WA) or passive integrated transponder tags (Oregon RFID, Portland, OR) to obtain capture-tag-recapture data for estimating apparent survival, recruitment, growth, production, and movement.

Results

As we are still in the early stages of an ongoing study, here I present preliminary results from a subset of study streams to illustrate the data we are collecting.

Beaver recolonization. As of 2022, beavers are present and have built dams on 12 of the 24 study streams designated for recolonization by removing beaver control. Three of these streams are in BMZ A, one is in BMZ B, and eight are in BMZ C (Table 1 and Figure 1). To date, there has been no beaver activity on any of the Lake Superior tributaries in our study area. We are pursuing the installation of a beaver dam analog on one of these tributaries should no beaver activity occur by 2023.

Substrate composition. Lepage Creek (Florence County, BMZ B) supports a wild Brook Trout fishery and has been maintained in free-flowing condition by controlling beaver. A new beaver dam (<1 year old) was found in 2019 and scheduled for removal. We surveyed the stream habitat upstream and downstream of the dam prior to its removal in September 2019 and post-removal in September 2020. Downstream of the beaver dam, the stream had a mean width of 2.8 m and its substrate comprised a mix of rubble/cobble (2%), gravel (25%), sand (47%), silt (15%), and detritus (11%) (Figure 4). The beaver dam was 70 m long and the mean width of the stream within 0 to 60 m upstream of the dam was 50 m. Upstream of the beaver dam, the substrate comprised a mix of gravel (1%; only present at the furthest upstream transect 180 m from the dam), sand (26%), silt (45%), and detritus (29%). One-year post dam removal, coarser substrates again became visible (6% gravel, 74% sand, 10% silt, and 11% detritus).

We similarly observed rapid deposition of fine substrates upstream of beaver dams in other parts of the state. West Branch Tainter Creek (Vernon County, BMZ C) is a relatively high gradient Driftless Area stream with a typically coarse substrate (14% boulder, 43% rubble/cobble, 19% gravel, 10% sand, 14% silt, and 1% detritus), but within a year of a beaver dam being built, fine substrates predominated in proximity to the dam (1% boulder, 16% rubble/cobble, 4% gravel, 7% sand, 71% silt, 1% clay, and 1% detritus) (Figure 5).

Stream temperature. Comparison of stream temperature at two long-term trout trend sites on Elk Creek (Richland and Vernon counties, BMZ C) showed that the July mean stream temperature was consistently about 2 °C cooler at the downstream site from 2011 through 2017 prior to the first beaver dams being built between these two sites in the latter half of 2017 (Figure 6). As the number of beaver dams increased, the July mean stream temperature at the two survey sites began to converge, were statistically similar by 2020, and became slightly warmer at the downstream site in 2021.

Trout size structure and relative abundance. We observed changes in trout size structure and relative abundance upstream of newly formed beaver dams that created deep pool habitat previously not present. For example, there was an increased abundance of larger trout ≥ age-2 and a significantly lower abundance of age-0 trout upstream of a beaver dam on Big Spring Branch (Iowa County, BMZ C) (Figure 7). In Driftless Area streams in southwest Wisconsin where Brown Trout predominate, trout survey data suggest Brook Trout respond favorably to pools formed by newly built beaver dams. Trout populations were comprised of 11% Brook Trout versus 89% Brown Trout upstream of a beaver dam in West Branch Tainter Creek (Vernon County, BMZ C), compared to 2% Brook Trout and 98% Brown Trout downstream of the beaver dam. In Big Spring Branch (Iowa County, BMZ C), Brook Trout constituted 3% of the trout population upstream of a beaver dam versus <1% downstream of the dam.

Fish community structure. Fish community structure varied widely among streams and ecological regions and was related to stream temperature. In colder streams, only salmonids and either Mottled Sculpin *Cottus bairdii* or Slimy Sculpin *C. cognatus* were typically present. These streams were widely distributed throughout the Driftless Area in southwest Wisconsin, along the shore of Lake Superior, and in scattered locations across northern Wisconsin. Many other streams, typically distributed across northern Wisconsin, were of marginal quality and, while trout were often present, many coolwater fish species (e.g., Northern Redbelly Dace *Phoxinus eos*, Central Mudminnow *Umbra limi*, Creek Chub *Semotilus atromaculatus*, White Sucker *Catostomus commersonii*) and warmwater fish species (e.g., Green Sunfish *Lepomis cyanellus,* Fantail Darter *Etheostoma flabellare*, Horny Head Chub *Nocomis biguttatus*) were also present, often in large numbers. In marginal-quality trout streams where non-salmonid fishes were present, they tended to increase in abundance following the construction of beaver dams, with some streams having as many as 17 coolwater and warmwater fish species present (e.g., Armstrong Creek in Lincoln County, BMZ B).

Discussion

The legacy of beaver control and stream habitat improvement as tools for managing trout in Wisconsin has challenged our ability to conduct this study. Since 1974, Wisconsin has funded a stream habitat development program using trout angler license fees to invest in improving stream habitat to support better trout fisheries (WDNR 2019). Some WDNR fisheries biologists have been reluctant to allow beaver to recolonize streams for study purposes, where doing so may threaten to undo efforts that have fixed streambank erosion or improved adult trout habitat availability by installing instream structures, all at significant expense. Many such streams are our best trout streams. Consequently, with few exceptions, most of our study streams are lesser quality trout streams.

Conversely, beaver control on trout streams has been so pervasive that we had difficulty finding trout streams heavily colonized by beaver where longstanding dams could be removed to recreate free-flowing conditions. Heavy rain events causing excessive flooding in high gradient streams, which can limit the longevity of beaver dams (e.g., Driftless Area streams in southwest Wisconsin), have also reduced the availability of trout streams heavily colonized by beavers for this study. One heavily colonized stream from which we removed beavers and an extensive network of beaver dams was Armstrong Creek (Lincoln County, BMZ B; Figure 8). The removal occurred from a 2-km headwater reach on county forest land in May 2022. Armstrong Creek is classified as a trout stream, but we were unable to find any trout in the stream. We will monitor Armstrong Creek over the next few years to determine if the restoration of free-flowing conditions leads to cooler summer temperatures more conducive to supporting trout.

One of the most pervasive contentions about beaver dams is that they warm stream temperatures in trout streams, but empirical data to support this assertion is often lacking (McRae and Edwards 1994; Avery 2002; Johnson-Bice et al. 2018). As with most ecological issues, the relationship between beaver dams and stream temperature is complicated by many confounding factors. Any effects of beaver dams on stream temperature will be dependent on site-specific stream attributes such as additions of cold water from springs and tributaries, which occurs, for example, between the two monitoring sites on Elk Creek (Richland and Vernon counties, BMZ C). The difference in stream temperature observed between two monitoring sites on Elk Creek was remarkably consistent for the seven years of data collected prior to the building of beaver dams between the two sites. Stream temperature varied from year to year in relation to variability in air temperature. Absent the beaver dams, the stream temperature at the downstream site should have been cooler in recent years had the 2 °C difference in July mean stream temperature remained consistent. We will analyze similar paired stream temperature data sets from control streams and other beaver recolonization streams to further explore the influence of beaver dams as a controlling influence on stream temperature.

Pools formed behind beaver dams in streams that remain thermally suitable for trout appear to quickly lead to an increased abundance of larger trout. This is somewhat expected and is a reason why some anglers prefer to fish streams with beaver dams (Petchenik 2014). However, the pool habitat and abundance of large trout may be transient (Willging 2017; Johnson-Bice et al. 2018). Brook Trout appear to benefit from newly created pool habitat, but in streams in which Brown Trout predominate, the effect appears relatively small. However, given that Brook Trout tend to be more susceptible than Brown Trout to being caught by anglers, the effect of beaver dams on improving Brook Trout abundance, small though it may be, may be noticeable to anglers (Mitro, personal observation).

During the to-date short-term length of this study, beaver dams, where they have occurred on study streams, have led to significant changes in the physical structure of streams. Beaver dams, and in some cases series of beaver dams, have created lengthy pools that have obscured formerly present riffles and runs. Fines like silt and detritus have quickly accumulated and covered coarser substrates like rubble/cobble and gravel. The long-term effects of these changes are still to be determined. Gravel substrates, for example, are critical to supporting natural reproduction of trout. The loss of spawning opportunities may lead to long-term decreases in recruitment and population growth, depending on the extent to which movement among habitat types is limited.

Beaver dams are likely to restrict the movement of some trout, but the extent to which this occurs and how it varies among salmonid species and streams in Wisconsin is unknown. Seasonal movement of trout appears to vary among regions, with movements among summer, spawning, and winter habitat appearing extensive in streams that experience near-freezing temperatures during winter and relatively warm temperatures, for trout streams, during summer (Lundberg and Mitro 2022). Movement of trout through dams may likely depend on dam structure and trout size. We have been tagging trout to quantitatively describe such movement, or lack thereof, in multiple study streams.

Though still in its early stages, this study has begun to fill the gap in our knowledge about how beavers influence habitat quality, trout population attributes, and fish community structure in Wisconsin's coldwater streams in different ecological regions of the state.

Acknowledgments

We thank WDNR Fisheries Research staff B. Breaker, N. Hoffman, E. Lundberg, M. Mataya, A. Nolan, K. Renik, S. Vanderbloemen, and D. Walchak and WDNR Wildlife Research staff N. Roberts and W. Dodge for their assistance in trout-beaver research across the state. Thanks also to the many WDNR biologists in the Bureau of Fisheries Management and to the United States Department of Agriculture Wildlife Services for their cooperation on this study. Funding was provided by the Sport Fish Restoration program and the Wildlife Restoration program.

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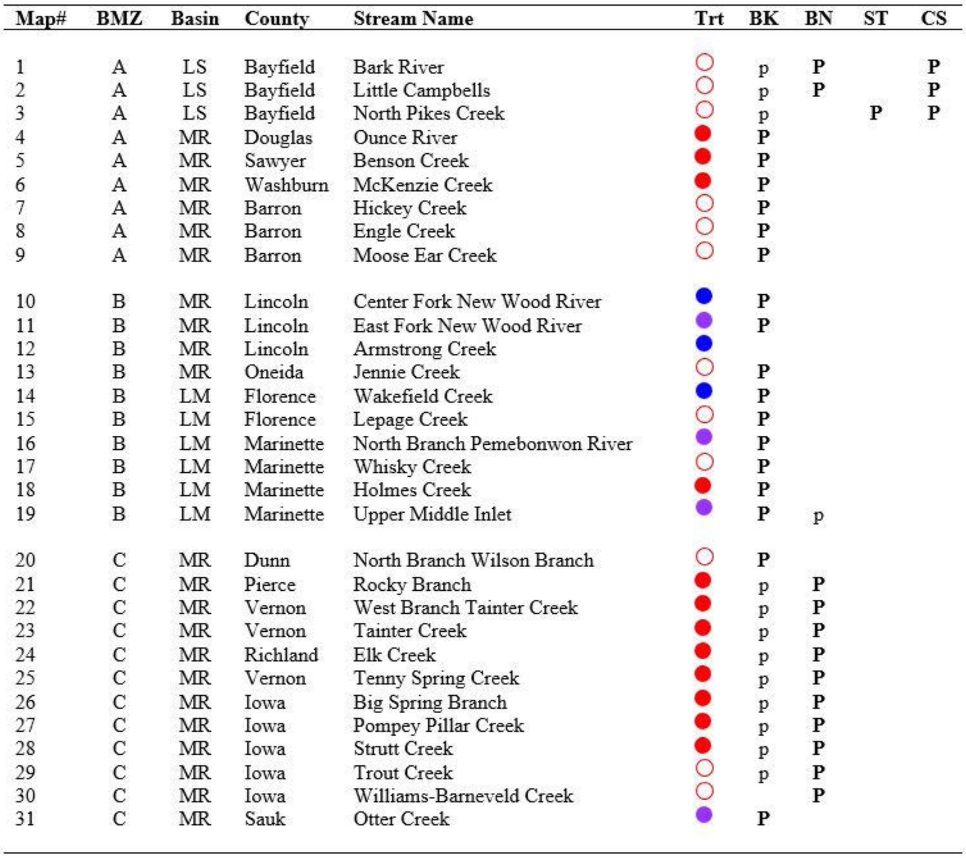
Table 1. Wisconsin study streams in beaver management zones (BMZ) A, B, and C, which are identified in the map in Figure 1. Basins include Lake Michigan (LM), Lake Superior (LS), and Mississippi River (MR). Treatments (Trt) include allowing beaver recolonization (red), removing beavers and beaver dams (blue), and no change (purple). Solid symbols indicate recolonization has begun or removal has been completed. Salmonid species present (p) in each stream may include Brook Trout (BK), Brown Trout (BN), steelhead trout (ST), and Coho Salmon (CS). Bold, uppercase P indicates the numerically dominant salmonids in each stream. Only a subset of control streams (i.e., no change in beaver management) are included in this table.



Map#	BMZ	Basin	County	Stream Name	Trt	BK	BN	ST	CS
1	A	LS	Bayfield	Bark River	⭕	p	**P**		**P**
2	A	LS	Bayfield	Little Campbells	⭕	p	**P**		**P**
3	A	LS	Bayfield	North Pikes Creek	⭕	p		**P**	**P**
4	A	MR	Douglas	Ounce River	🔴	**P**			
5	A	MR	Sawyer	Benson Creek	🔴	**P**			
6	A	MR	Washburn	McKenzie Creek	🔴	**P**			
7	A	MR	Barron	Hickey Creek	⭕	**P**			
8	A	MR	Barron	Engle Creek	⭕	**P**			
9	A	MR	Barron	Moose Ear Creek	⭕	**P**			
10	B	MR	Lincoln	Center Fork New Wood River	🔵	**P**			
11	B	MR	Lincoln	East Fork New Wood River	🟣	**P**			
12	B	MR	Lincoln	Armstrong Creek	🔵				
13	B	MR	Oneida	Jennie Creek	⭕	**P**			
14	B	LM	Florence	Wakefield Creek	🔵	**P**			
15	B	LM	Florence	Lepage Creek	⭕	**P**			
16	B	LM	Marinette	North Branch Pemebonwon River	🟣	**P**			
17	B	LM	Marinette	Whisky Creek	⭕	**P**			
18	B	LM	Marinette	Holmes Creek	🔴	**P**			
19	B	LM	Marinette	Upper Middle Inlet	🟣	**P**	p		
20	C	MR	Dunn	North Branch Wilson Branch	⭕	**P**			
21	C	MR	Pierce	Rocky Branch	🔴	p	**P**		
22	C	MR	Vernon	West Branch Tainter Creek	🔴	p	**P**		
23	C	MR	Vernon	Tainter Creek	🔴	p	**P**		
24	C	MR	Richland	Elk Creek	🔴	p	**P**		
25	C	MR	Vernon	Tenny Spring Creek	🔴	p	**P**		
26	C	MR	Iowa	Big Spring Branch	🔴	p	**P**		
27	C	MR	Iowa	Pompey Pillar Creek	🔴	p	**P**		
28	C	MR	Iowa	Strutt Creek	🔴	p	**P**		
29	C	MR	Iowa	Trout Creek	⭕	p	**P**		
30	C	MR	Iowa	Williams-Barneveld Creek	⭕		**P**		
31	C	MR	Sauk	Otter Creek	🟣	**P**			

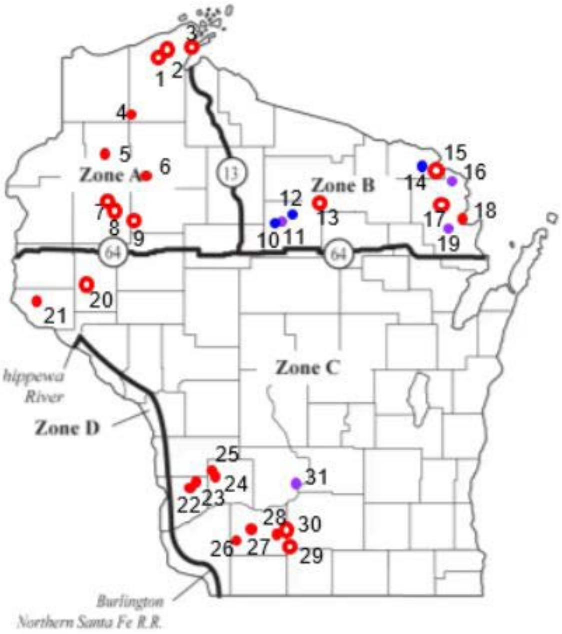


Figure 1. Location of study streams in beaver zones A, B, and C in Wisconsin. Treatments include allowing beaver recolonization (red), removal of beavers and beaver dams (blue), and no change (purple). Solid symbols indicate streams in which recolonization has begun or removal has been completed. Only a subset of control streams (i.e., no change in beaver management) are displayed on this map. Details for each numbered stream are in Table 1.

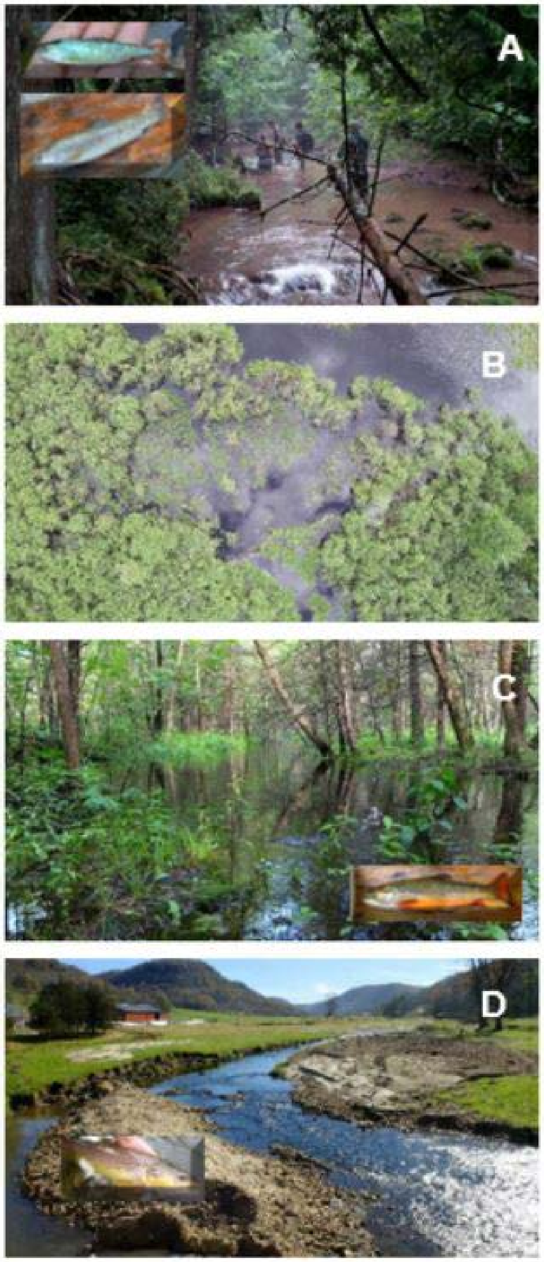


Figure 2. (A) Lake Superior tributaries in northern Wisconsin are relatively high gradient streams important to lake-run salmonids including Brown Trout, steelhead trout, and Coho Salmon, as well as stream-resident Brook Trout. Clay in the watershed causes turbid condition during flood events, and the clay can mortar or harden beaver dams. (B) Low gradient streams predominate in north central Wisconsin, where beaver dams can cause extensive flooding in forested areas. (C) Low gradient streams also predominate in northeastern Wisconsin and largely support Brook Trout. Waters tend to rise and fall slowly following precipitation events. (D) Driftless Area streams in southwest Wisconsin are higher gradient and are prone to flash flooding during heavy precipitation events, which can cause extensive streambank erosion. Brown Trout predominate in these streams.

Figure 3. Beaver dams were described by measuring dam length (bank to bank), dam width (upstream-downstream), and dam height and water level upstream and downstream at multiple points across each dam.

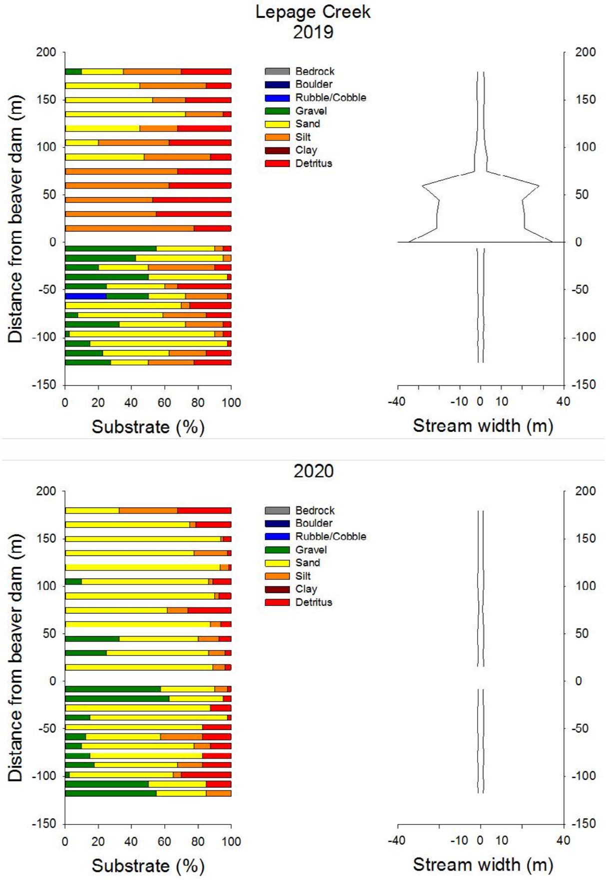


Figure 4. Substrate composition and stream width at multiple transects on Lepage Creek upstream and downstream of a beaver dam (<2 years old), which was removed post-survey in 2019.

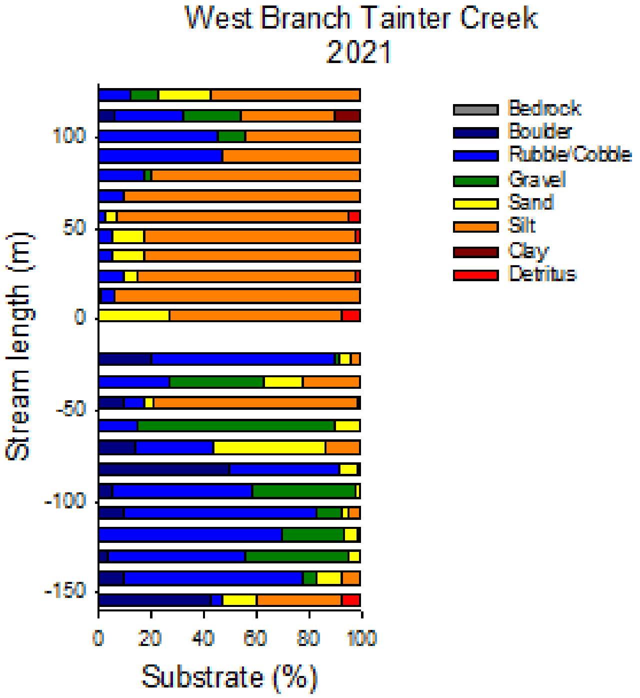


Figure 5. Substrate composition at multiple transects on West Branch Tainter Creek upstream and downstream of a new beaver dam (<1 year old).

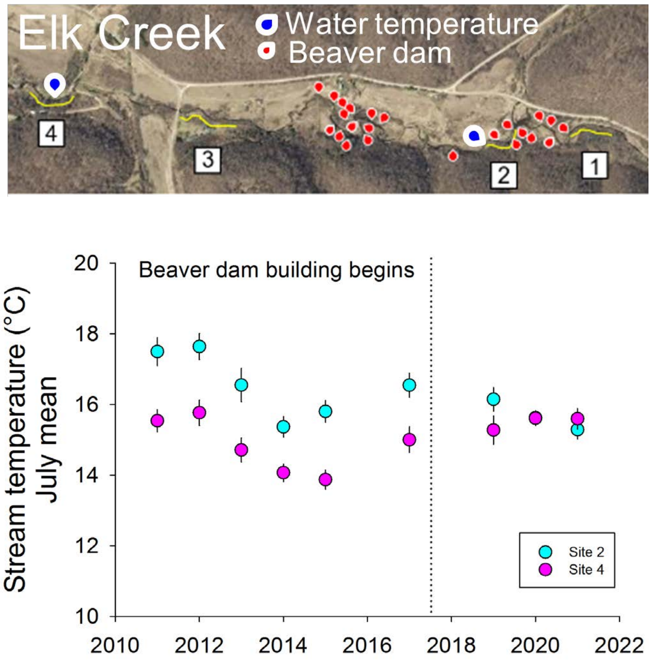


Figure 6. July mean stream temperature (°C) upstream (site 2) and downstream (site 4) of 14 beaver dams built in Elk Creek in 2017-2021.

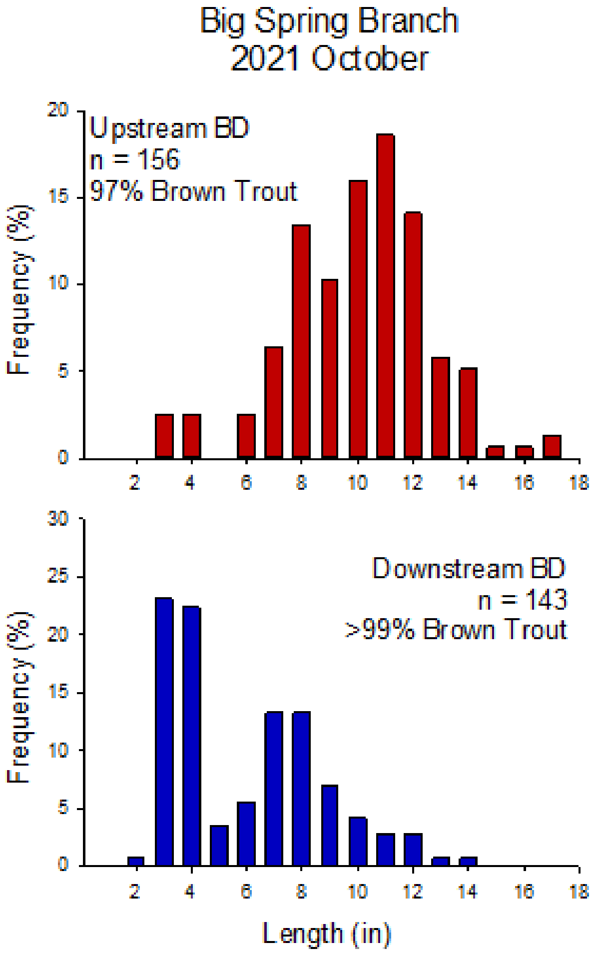


Figure 7. Length-frequency of Brown Trout and Brook Trout upstream (red) and downstream (blue) of a beaver dam on Big Spring Branch in October 2021.

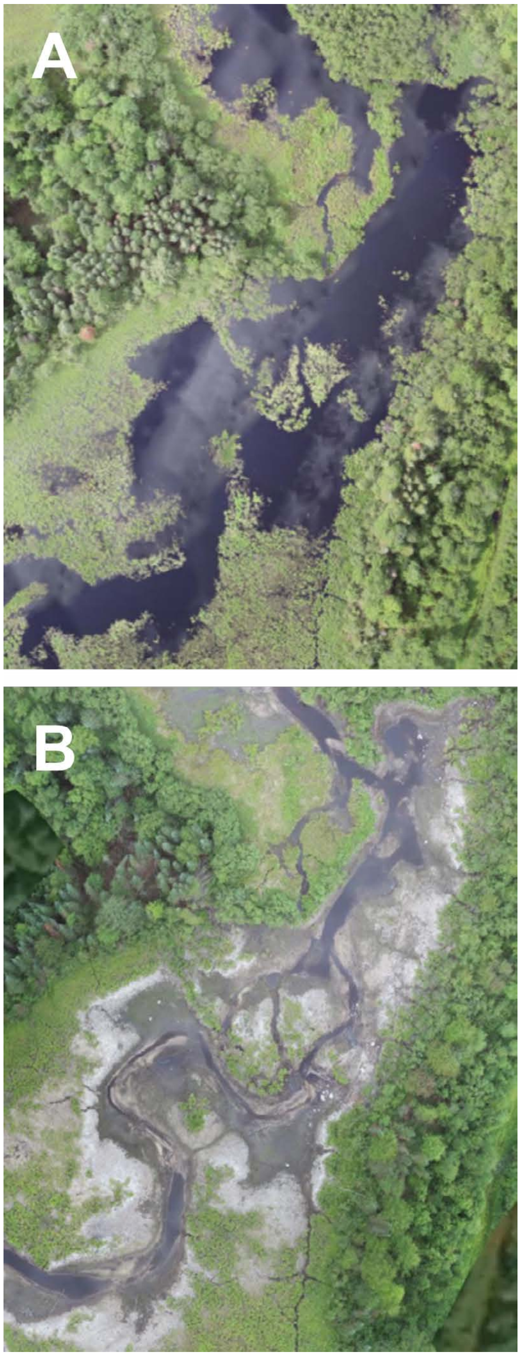


Figure 8. Armstrong Creek (Lincoln County, BMZ B) before (June 2021) and after (July 2022) (June 2021; A) and after (July 2022; B) removal of beavers and beaver dams.

Canyon Creek Culvert Fish Passage Retrofit

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Abstract - The Canyon Creek Culvert Fish Passage Retrofit (Project) was designed to improve upon a failed 1970s fish passage culvert to better provide for future upstream fish migration along Canyon Creek. The Project evaluated a range of fish passage techniques and configurations via two-dimensional hydraulic modeling to determine the best technique or combination of techniques to provide fish passage during low flows when Brown Trout *Salmo trutta* are attempting to access headwater spawning areas. The goal of this Project was also to develop a design that could be implemented in similar locations without completely re-constructing culverts. The existing Canyon Creek culvert is a long, over wide, and steep double cell box culvert that has minimal flow depths (less than three inches) and high velocities (greater than eight feet/second [ft/s]) during the fall spawning season for Brown Trout. During high flows, velocities in the culvert are also high with limited boundary roughness to allow efficient movement of spring spawning Rainbow Trout *Oncorhynchus mykiss*. To reconnect the Colorado River's cold-water fish with the productive spawning and juvenile rearing habitat of Canyon Creek, our design reflected a combination of fish passage techniques and retrofitting the previous fish passage design to provide a new, resilient approach using natural channel design principles and innovative modeling. Our team researched, modeled, and combined proven techniques to devise a unique solution to promote fish passage during low flows. The Project was constructed in the late fall of 2021 and will be monitored during fall of 2022 Brown Trout spawning migration.

Introduction

Reconnecting fragmented cold-water fisheries throughout the United States is a goal of Trout Unlimited and Wright Water Engineers, Inc. (WWE). The Canyon Creek culvert, located beneath Interstate 70 approximately seven miles west of Glenwood Springs, Colorado (Figure 1), is a 220 foot long, 20-foot wide, double-cell box culvert at approximately 3% slope. This is considered a very long, wide, and steep culvert. Its dimensions resulted in shallow (less than 4 inches), supercritical flow and created a hydraulic barrier that limited fish's ability to migrate to the productive habitat upstream (Figure 2). As a result, the high-quality habitat of Canyon Creek was largely inaccessible to many of the Colorado River's cold-water species.

During the construction of the culvert in the 1970s, 12 wooden baffles were installed that, at the time, represented innovative techniques for fish passage and were intended to concentrate flows to provide adequate water depth for fish movement upstream. Over time, the baffles caused culvert floor scouring and many of the structures rotted or washed away. In 2020, only three baffles remained.

The primary goal of this Project was to improve fish passage through the existing culvert. In support of the Project goal, the following objectives were also identified:

1. Evaluate various fish passage techniques for optimal configuration and placement within the Canyon Creek culvert.
2. Make the Canyon Creek drainage accessible to spawning cold-water fish migrating from the Colorado River.
3. Conduct necessary maintenance on the culvert's previous fish passage design.
4. Increase knowledge of fish passage design.
5. Increase wild fish population size in Canyon Creek and the Colorado River.
6. Improve the local economy via increased angler success rates.
7. Increase community awareness and collaboration.
8. Evaluate and monitor trout migration through the culvert before and after implementation.

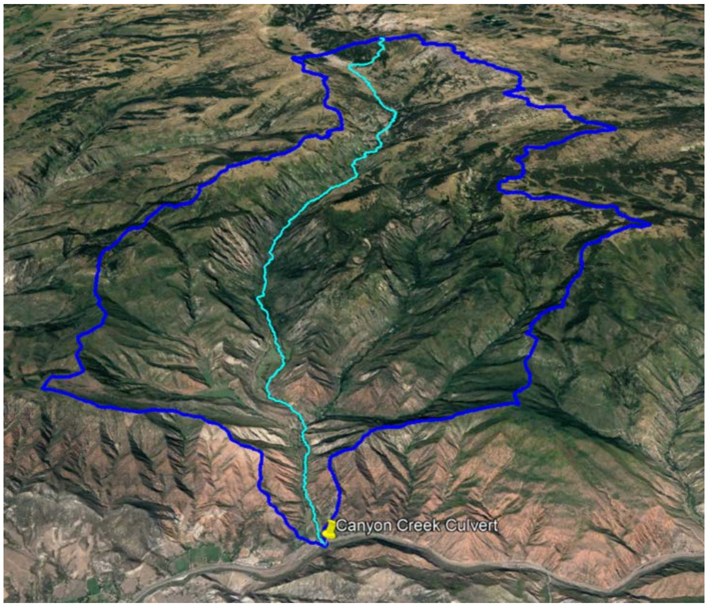

Figure 1. Canyon Creek drainage area and Canyon Creek culvert location (Photo Credit Google Earth).

Figure 2. Brown Trout Salmo trutta at the entrance of the Canyon Creek culvert that are unable to migrate upstream through the culvert.

Methods

Wright Water Engineers, along with Trout Unlimited, evaluated various fish passage techniques, based on experience and interviews with other fish passage professionals, and hydraulic modeling to determine the best configuration of fish passage elements. Wright Water Engineers researched various fish passage techniques and lessons learned from other fish passage projects to help prioritize the preferred alternative. The preferred alternative needed to provide for a combination of greater flow depths with reduced velocities without affecting the conveyance capacity of the culvert. The design also needed to be feasible to construct in a culvert without extensive interruption of flows.

Prior to Project implementation, in the fall of 2020, Colorado Parks and Wildlife and WWE conducted an electrofishing survey of Canyon Creek approximately one mile upstream of the culvert to assess the pre-project condition. The pre-project condition data will be compared to post-project electrofishing data that will be collected during the fall spawning migration of Brown Trout to evaluate Project success.

Wright Water Engineers utilized the U.S. Army Corps of Engineers HEC-RAS program Version 6.2 (U.S. Army Corps of Engineers, 2022) to develop two-dimensional hydraulic modeling of existing conditions and alternatives. Wright Water Engineers used Autodesk Civil 3D Computer Aided Drafting program to develop detailed digital elevation models of existing conditions and alternatives (Figure 3). The digital elevation models were highly detailed to represent the proposed ‘roughness features’ as opposed to only utilizing variations in Manning’s roughness. Wright Water Engineers evaluated and compared the flow depths and velocities of the existing conditions and alternatives to determine the most beneficial configuration of fish passage techniques. For the evaluation, WWE used a design flow of 17 cubic feet per second (cfs), which is the November mean monthly flow statistic from the U.S. Geological Survey StreamStats (2009) application.

Wright Water Engineers used research conducted by Colorado Parks and Wildlife and presented in “Fish Passage at River Structures” (Colorado Parks and Wildlife 2022) for fish passage criteria for Salmonidae (i.e., trout) to evaluate the alternatives. Based on Colorado Parks and Wildlife research, Salmonidae or trout have prolonged speeds from 2.3 feet per second and 4.0 feet per second and burst speeds of 4.5 feet per second to 7.5 feet per second.

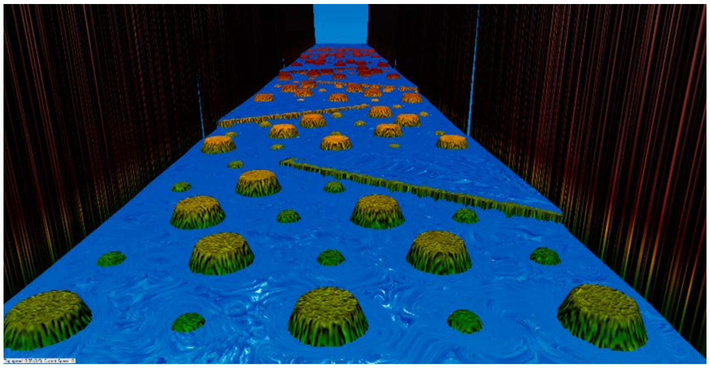

Figure 3. Perspective view of digital elevation model used in hydraulic modeling.

The culvert is a 226-linear-foot, double-cell reinforced concrete box culvert, with each cell being 10 feet wide and 9 feet tall with an approximate longitudinal slope of three percent (Figure 4). It is presumed that during the initial construction of the culvert, 12 wooden baffles were installed, which were approximately 8 inches tall, 8 inches wide, and 8 feet long, aligned along the river left side of the river left culvert cell. It, therefore, provided approximately two feet between the edge of the baffle and the culvert cell wall for fish passage. In 2019, only three of the original 12 wooden baffles remained in place. describe such movement, or lack thereof, in multiple study streams.

Though still in its early stages, this study has begun to fill the gap in our knowledge about how beavers influence habitat quality, trout population attributes, and fish community structure in Wisconsin's coldwater streams in different ecological regions of the state.

Figure 4. Condition of the Canyon Creek culvert in 2019 looking downstream. The left cell has some remaining wooden baffles originally installed in the 1970s.

Wright Water Engineers conceptually evaluated numerous potential designs that consisted of various fish passage techniques in solo and some in combination (Figure 5). Approximately 13 different alternatives were further developed in computer-aided drafting programs to provide a graphical representation of the various alternatives. The alternatives were developed from the premise of utilizing baffles and/or hemispheres to provide roughness elements to increase the water depth and reduce water velocities. The hemispheres were designed to mimic boulders in a natural stream system. During the initial evaluation process, the idea to combine baffles and hemispheres was not developed. As the alternative analysis continued, the use of a combination of baffles and hemispheres was introduced to create additional bedform diversity and optimal hydraulics.

The baffles provide many benefits for fish passage. The baffles would not only help to concentrate low flows but would also provide an area of backwater upstream of the baffles. The baffles were also profiled to provide better hydraulics and sediment transport by conveying flows away from the culvert walls and towards the center of the culvert. This would also help to reduce the potential for scour along the culvert walls. The size of the baffles was limited so as to not reduce the conveyance capacity of the culvert compared to existing conditions. Additional inner-channel sinuosity was provided by manipulating some of the baffle configurations, therefore increasing overall flow length and consequently reducing the overall slope of the flow path. The baffles would also provide areas of refugia for trout passing through the culvert. An alternative evaluation of the baffles was completed, to understand which configurations would be the most beneficial in providing fish passage, by comparing flow depths and velocities.

The hemispheres would also provide many benefits for fish passage. The hemispheres would replicate feature boulders in a natural channel and therefore should vary. The hemispheres would 'protrude' from the culvert floor providing additional roughness along the steep culvert. The hemispheres would also provide areas of refugia while the spawning trout passed through the long culvert. The hemisphere sizes evaluated had diameters of 12 inches and 18 inches, therefore protruding 6 inches and 9 inches, respectively. An alternative evalaution of the hemispheres was completed to understand which configurations would provide the greatest minimum flow depth and least maximum velocity and work in harmony with the baffles.

In coordination with Colorado Parks and Wildlife, Colorado Department of Transportation, and fishery biologist Ashley Ficke, Ph.D., the 13 alternatives were reduced to a few based on previous experience and research conducted as part of the "Assessment of Retrofitted Ramped Weirs to Improve Passage of Potamodromous Fish" (Amaral et al. 2019). An unimproved culvert scenario and an existing conditions scenario were also evaluated. The alternatives were modeled utilizing HEC-RAS 2D (U.S. Army Corps of Engineers, 2022). Digital elevation models for the existing conditions and five alternatives were developed. The alternatives evaluated were examined for velocity and depth of flow during low flow conditions of 17 cfs (Figure 6).

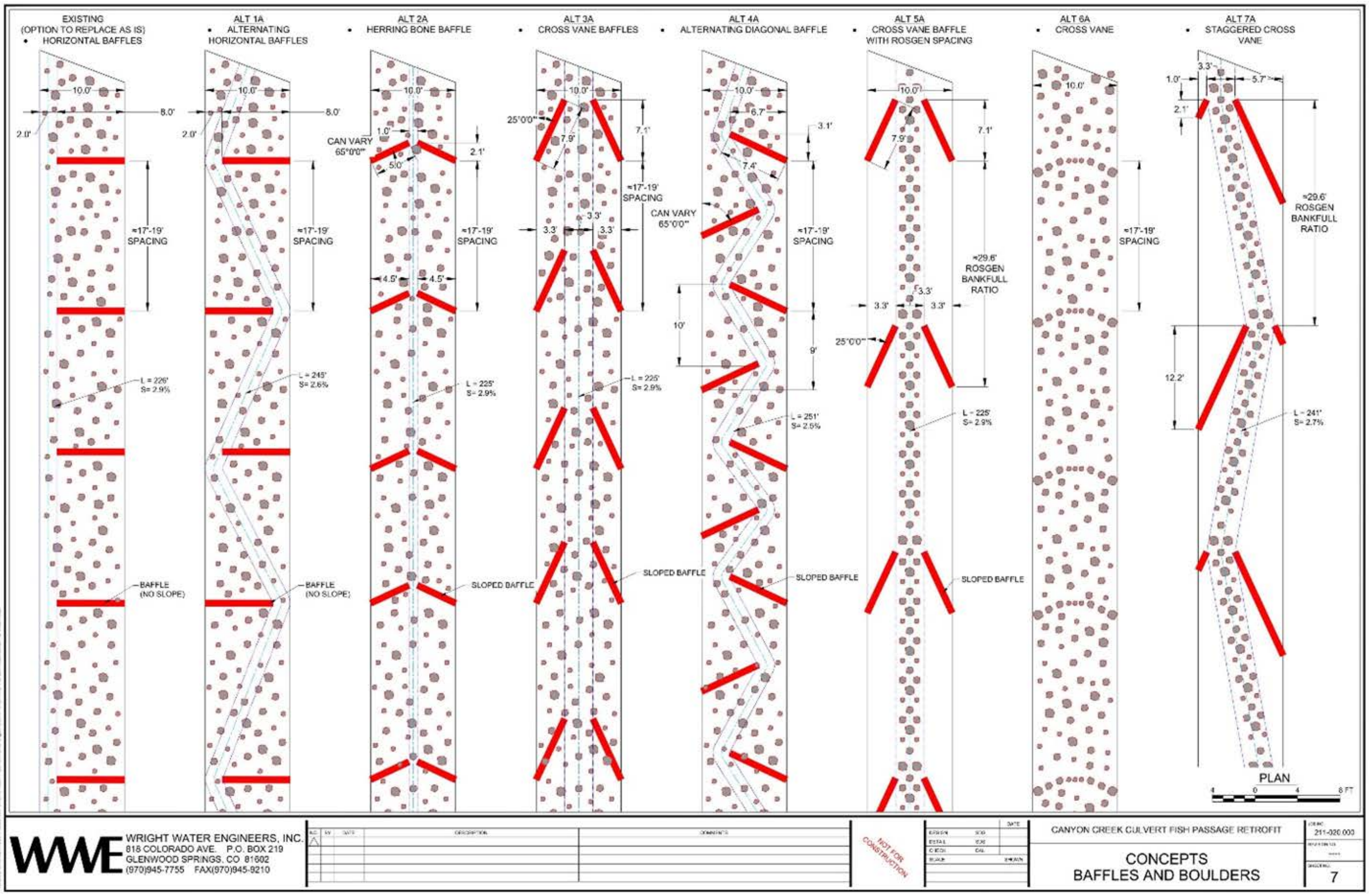

Figure 5. Various alternatives evaluated utilizing baffles and hemispheres that were considered for further evaluation. The thick red lines represent profiled baffles that concentrate flow and the grey circles represent concrete hemispheres to mimic boulders.

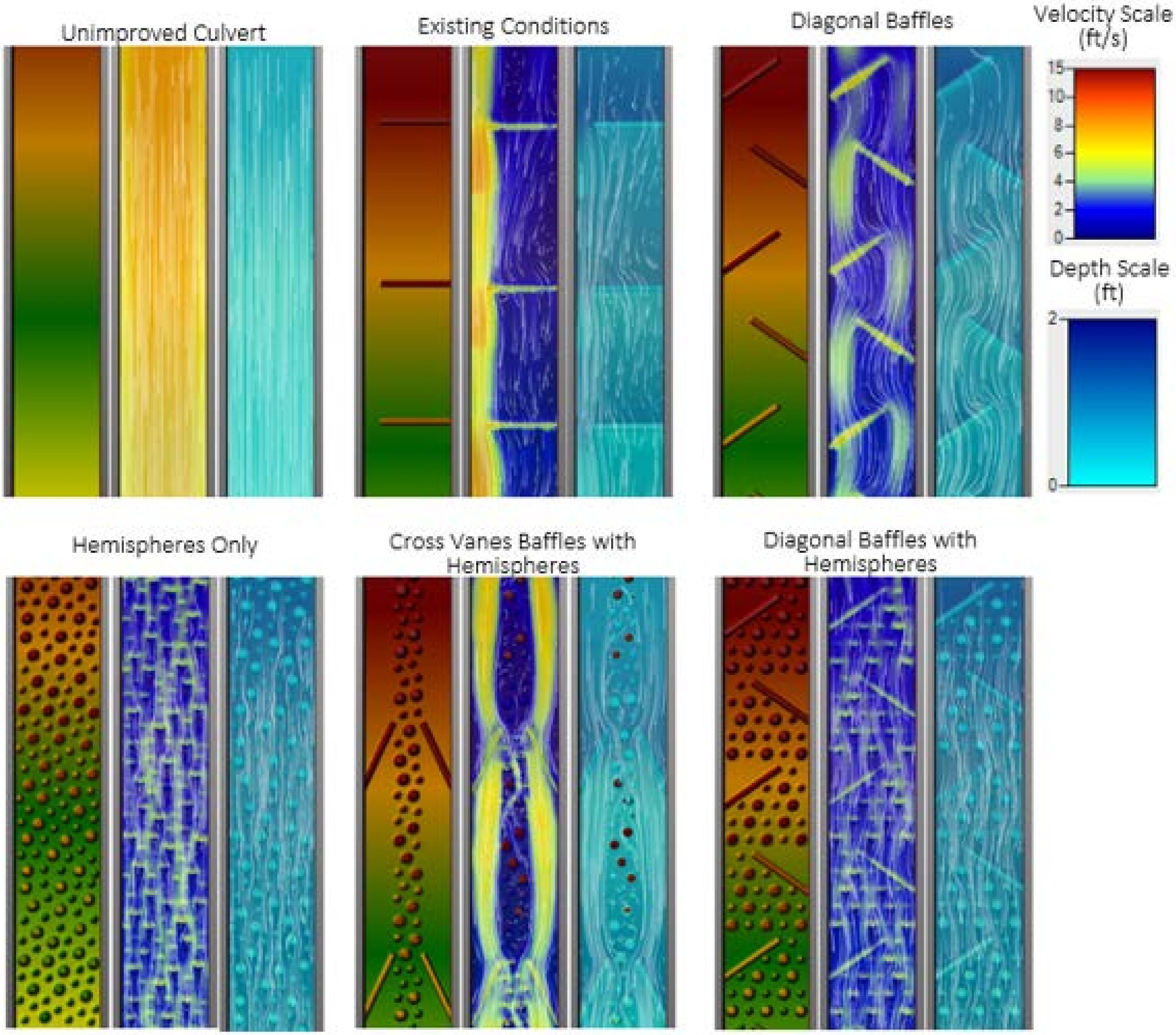

Figure 6. Digital elevation models and graphical representations of the velocities evaluated for six scenarios modeled in HEC-RAS 2D.

Minimum and maximum depth and velocities varied among scenarios (Table 1).

The two key variables examined as part of this evaluation were depth and velocity. The goal of this alternative evaluation was to determine the alternative that provided the greatest minimum depth and least maximum velocity at a discharge of 17 cfs. Through hydraulic modeling, it was determined that the diagonal baffles with hemispheres provided a minimum depth of 1 foot and a maximum velocity of 3.5 feet per second, which meets both goals of the alternative analysis. Based on analysis of the alternatives, the diagonal baffles with hemispheres was selected as the preferred alternative to be constructed.

Table 1: Modeled minimum and maximum water depth and water velocity at a discharge of 17 cubic feet per second for alternatives evaluated using 2D modeling.

Scenario	Depth [ft]		Velocity [ft/s]	
	Min	Max	Min	Max
Unimproved Culvert	0.2	0.3	6.0	7.5
Existing Conditions	0.6	1.1	0.2	8.4
Diagonal Baffles	0.7	1.1	1.0	5.3
Hemispheres Only	0.9	1.0	0.2	3.9
Cross Vane Baffles with Hemispheres	0.5	1.1	0.1	6.8
Diagonal Baffles with Hemispheres	1.0	1.1	0.5	3.5

The baffles and the hemispheres were pre-cast offsite using molds. Steel reinforcement bars were formed into the concrete baffles and hemispheres to allow them to be mounted in the culvert with epoxy in pre-drilled holes in the culvert floor. Flow in Canyon Creek was conveyed to the culvert cell where the baffles and hemispheres were not being installed to allow for construction in dry conditions (Figure 7). Equipment to install the baffles was lowered into the culvert with a crane. The existing culvert had a concrete apron that extended out beyond the exit of the culvert which allowed the equipment to be lowered and then 'swung' into the culvert. Additional instream work was performed upstream of the fish passage culvert to convey low flows predominantly into the cell where fish passage baffles and hemispheres were installed. The construction also included filling existing scour holes with reinforced steel bars and structural concrete. A video documenting the planning, design, and construction process was produced and is available online (https://www.youtube.com/watch?v=PxynHvflZmA).

The Project will continue to be monitored by WWE, Trout Unlimited, and Colorado Parks and Wildlife. Monitoring will include structural evaluation of the baffles and hemispheres, velocity, and depths investigations, and electroshocking during spawning periods.

Figure 7. Baffles and hemispheres after being installed in the Canyon Creek culvert under Interstate 70.

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Stuart Gardner, Hydraulics Engineer, Colorado Department of Transportation

Kyle Kissner, Contractor, Kissner General Contractors, Inc.

Matt Kondratieff, Aquatic Research Scientist, Colorado Parks, and Wildlife

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Resilience of Yellowstone Cutthroat Trout in Yellowstone Lake in the Face of Climate Change

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Extended Abstract

Yellowstone Lake supports the world's largest genetically unaltered assemblage of Yellowstone Cutthroat Trout *Oncorhynchus clarki bouvieri* (Gresswell and Varley 1988); however, the effects of climate change on this iconic assemblage is a major concern for wildlife managers, conservationists, and the public (Hostetler and Giorgi 1995; Koel et al. 2005; Kaeding 2020). Predation by introduced Lake Trout *Salvelinus namaycush* since the early 1990s has significantly altered the native assemblage (Koel et al. 2005), further confounding the future of the native trout (Koel et al. 2021). Recent questions concerning the effects of climate change on the reproductive capacity of the two species (Kaeding 2012) prompted us to undertake a modeling study to investigate the potential changes in thermal properties of Yellowstone Lake and a primary spawning tributary over the next 80 years.

We combined our recent Holocene regional climate simulations (Hostetler et al. 2021) and future projections drawn from the North American Coordinated Regional Climate Downscaling Experiment (Giorgi and Gutowski Jr 2015) to derive input for a lake temperature model for Yellowstone Lake and stream temperature and runoff model for Clear Creek, a primary Yellowstone Cutthroat Trout spawning tributary. The resulting simulations were used to explore how past and potential future climate change influence the thermal properties of the lake system, and thus the resilience of Yellowstone Cutthroat Trout to climate change. Projected future changes were compared with those of the early Holocene (12 ka, 9 ka, and 6 ka) when Yellowstone Cutthroat Trout experienced a wide range of climate conditions in the absence of Lake Trout.

To evaluate growth potential for two species in Yellowstone Lake, Kaeding (2012) assumed optimal temperature ranges in the thermocline of 13-17 °C for Yellowstone Cutthroat Trout and 8-12 °C for Lake Trout. Because we were focused on the effects of climate change on relative abundance and persistence of the native and introduced salmonids, we assumed broader lake water temperature ranges (7-22 °C for Yellowstone Cutthroat Trout and 5-20 °C for Lake Trout) for our thermal habitat assessment. These water temperatures were based on studies by NDEP (2015), Al-Chokhachy et al (2021), and Isaak and Hubert (2004) for the native trout and Johnson et al. (2001) for Lake Trout.

Lake simulations suggest that under a high greenhouse gas emission scenario (RCP8.5; Hostetler et al. 2021), the projected annual cycle of lake thermal characteristics (e.g., epilimnion temperature, ice cover, and open water period) at the end of the 21st century will yield an increase of approximately 26% in suitable thermal habitat for Yellowstone Cutthroat Trout (Figure 1). In contrast, thermal habitat for Lake Trout will increase approximately 15% by mid-century and then decline to a 5% increase by the end of the century. Projected future annual stream temperatures will increase 2-3 °C warmer than present, but seasonal comparisons suggest that early season temperatures will be 3-4 °C higher than present.

Field data collected from the 1970s through the 1990s suggest that the annual timing of Yellowstone Cutthroat Trout spawning in Clear Creek is tightly associated with the seasonality of runoff (i.e., peak numbers of fish arrive in late June just after peak flow; Gresswell et al. 1997). Our projections indicate that projected reductions in snow, snowpack, and early snowmelt will lead to future peak flows 1-2 months earlier than historic observations (Figure 2). Projected future annual stream temperatures will be 2-3 °C warmer than present, but seasonal comparisons suggest that early season temperatures will be 3-4 °C higher than present (Figure 3).

Our results suggest that the effects of potential changes in the annual cycle of thermal habitat in Yellowstone Lake will increase thermal habitat for both Yellowstone Cutthroat Trout and Lake Trout assemblages over the 21st century, but effects will be greater for the native trout. In order to adapt to projected changes in spawning stream temperatures and discharge, however, Yellowstone Cutthroat Trout will need to spawn earlier, and larvae will need to return to the lake sooner than they do at present. Assuming that they can adapt, the growing season in Yellowstone Lake will be longer than it is currently, and it appears that within the bounds of our future projections, Yellowstone Cutthroat

Trout in the Yellowstone Lake system should be resilient to climate change. At the same time, we found no evidence to suggest negative effects of climate change on Lake Trout, underscoring the importance of maintaining the Lake Trout suppression program to minimize predation by the nonnative species and ensure the persistence of Yellowstone Cutthroat Trout.

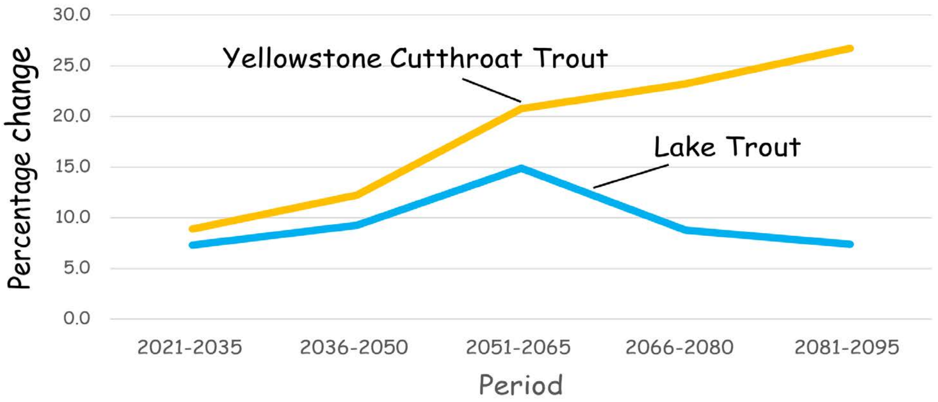

Figure 1. Projected changes in thermal habitat for native Yellowstone Cutthroat Trout (assuming a thermal tolerance of 7-22 °C) and Lake Trout (assuming a thermal tolerance of 5-20 °C) in Yellowstone Lake resulting from climate change during the 21st century.

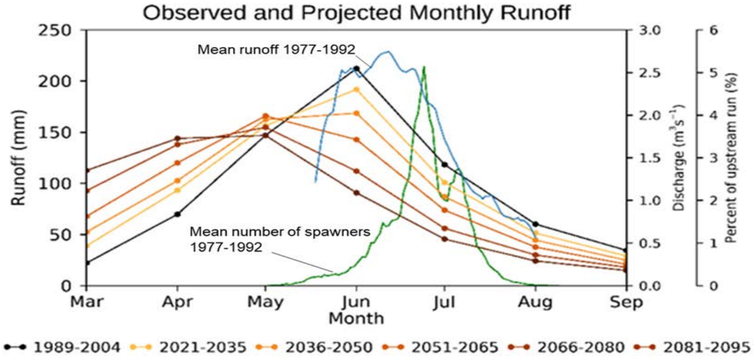

Figure 2. Projected changes in runoff for Clear Creek, a tributary to Yellowstone Lake, resulting from climate change during the 21st century.

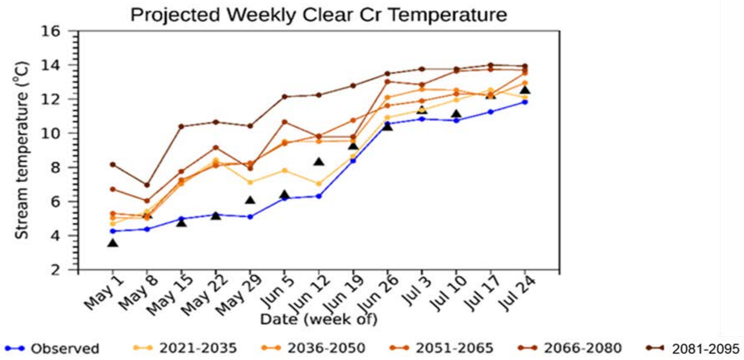

Figure 3. Projected changes in stream temperature for Clear Creek, a tributary to Yellowstone Lake, resulting from climate change during the 21st century.

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Session 4

Interactions Between Native and Non-native Trout: What Have We Learned? And Molecular Studies To Support Wild Trout Conservation

Give or Take: Role of Stream Habitat Rehabilitation in a Driftless Area Riverscape

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Abstract - Studies have documented increasing Brown Trout Salmo trutta abundance following stream rehabilitation projects, but few have quantified mechanisms responsible for increases, such as increasing survival or immigration from adjacent reaches. Alternatively, biologists have suggested that habitat projects produce excess individuals that emigrate and bolster populations in adjacent reaches. To better understand the role of habitat rehabilitation (HR) and stream connectivity on Brook Trout *Salvelinus fontinalis* and Brown Trout recruitment, survival and movement in a Midwestern riverscape, we PIT tagged trout and conducted a three year capture-recapture study. Trout populations were grouped based on three size and age groups: age-0 juveniles, adult age-1 (Brook Trout) or age 1-2 (Brown Trout), and older/larger adults. The 4.7-km riverscape included six reaches (two had been rehabilitated 20 years earlier) spanning three streams. We found little support for a HR benefit on recruitment or survival. Survival of only older (age-3+) Brown Trout was higher in one HR reach, (63%) annually, but was 35% in the other HR reach and 41% in a non-HR reach. Rehabilitated reaches contributed fewer age-0 emigrants (both species) to adjacent reaches, than received as immigrants. Conversely, HR reaches contributed slightly higher percentages of adult emigrants to adjacent reaches than received immigrants, but differences in total numbers were modest. Reaches with HR only contributed about 5-10 more adults to adjacent reaches than they received. Very few older/larger adults moved between HR and non-HR reaches. Our results indicate that ontogenetic immigration is an important mechanism explaining abundance increases following habitat rehabilitation and refute the notion that HR reaches provide substantial numbers of excess individuals to adjacent reaches.

Introduction

Large sums of public money have been invested in stream habitat restoration and improvement (hereafter termed habitat rehabilitation [HR]) since at least the 1950s with estimates exceeding billions of dollars worldwide. In just three states in the upper Midwestern United States, almost half a billion dollars were spent between 1990 and 2004 (Alexander and Allan 2006) and many more HR projects have been implemented since. Goals and objectives of these projects have been variable but generally include some combination of improving physical habitat for aquatic organisms, restoring hydrogeomorphological and ecological function, and increasing abundance of desired fishes and aquatic insects (Foote et al. 2020). Many individual, often smaller-scale projects, were evaluated (e.g., Avery 2004), which facilitated more comprehensive meta-analyses and reviews (e.g., Roni et al. 2008). These reviews and syntheses helped document changes in HR practices and expenditures (Alexander and Allan 2006), evaluate selected objectives, such as changes in salmonid abundance (Foote et al. 2020) and more broadly, engendered philosophical and policy discussions about the practice of restoration ecology as applied to streams and rivers (Palmer et al. 2004; Palmer et al. 2014).

Many of the earliest stream HR projects were found to significantly increase abundance or biomass of salmonids in smaller coldwater streams, but also led to subsequent debate about the biological mechanisms for those increases. White (1996) and Roni et al. (2008) provided extensive reviews of stream HR projects and noted studies as early as the 1930s but most had been completed after about

1970. Although results were variable, salmonid abundance increased after completion of many stream HR projects. Early studies thought abundance increases were primarily due to enhanced carrying capacity, including higher quality spawning areas, higher recruitment, and improved survival, in part because stream-dwelling salmonids were considered mostly sedentary. However, Gowan et al. (1994) documented substantial immigration of stream-resident trout into restored stream reaches in some inter-mountain west streams and more broadly challenged the earlier concept of restricted movement of stream-dwelling salmonids. Their paper stimulated others to examine the importance of fish movement in the context of evaluating stream restoration success. Roni (2019) summarized several of those findings and emphasized the importance of simultaneously examining fish recruitment, survival and movement to better disentangle the relative roles of each in assessing the effectiveness of stream restoration. The reviews of Roni et al. (2008) and Roni (2019) also highlighted several additional considerations, including the often short time frame of evaluations (< 5 years) immediately surrounding implementation of stream restoration projects. Few studies examined long-term trout population responses to HR or even responses decades later and none of the studies reviewed had been conducted in the groundwater-fed streams of the Driftless Area.

The Driftless Area in the upper Midwestern United States is an area with exceptional recreational fisheries for stream trout that followed a similar temporal pattern in development, evaluation and support for stream habitat restoration. The Driftless Area is a 62,000 km^2 area covering portions of southwest Wisconsin, southeast Minnesota, northeast Iowa, and northwest Illinois. Instream HR was first implemented by government agencies in the early to mid-20th century in Michigan and Wisconsin, including streams in the Driftless Area (White 1996). Stream HR practices expanded in the region with important evaluations conducted in Driftless Area streams in southwest Wisconsin (Hunt 1976; 1988) and southeast Minnesota (Thorn 1988; Thorn et al. 1997). Those evaluations similarly found increases in stream trout abundance and biomass, but did not fully assess biological mechanisms (i.e., recruitment, survival or movement) responsible for increases.

Based on increasing abundance following stream HR projects, public and financial support for implementing more projects increased, culminating in Trout Unlimited's Driftless Area Restoration Effort (Welter 2017) begun in 2004 as one of the first National Fish Habitat Partnerships. Trout Unlimited's Driftless Area Restoration Effort leverages funds from a variety of sources, such as Farm Bill conservation grants, U. S. Department of Agriculture's Natural Resources Conservation Service and Trout Stamp dollars leading to about $5 million dollars a year invested in stream habitat rehabilitation throughout the Driftless Area (Welter 2017). In 2008, the citizens of Minnesota passed the Clean Water, Land and Legacy amendment which directs 33% of sales tax revenue to the Outdoor Heritage Fund to be spent only to restore, protect, and enhance habitat for fish, game, and wildlife. About $1-2 million is annually appropriated from this fund to support stream HR projects throughout Minnesota, including in Minnesota's portion of the Driftless Area.

To acquire public funds, annual stream restoration proposals are prepared by a variety of conservation groups and government agencies and presented to policy makers for approval and funding. However, many proposals simply list general project objectives based on assumptions of what stream HR projects will accomplish. Objectives include increasing natural reproduction of trout and overall trout abundance in the whole stream, presumably under the belief that excess numbers of trout produced in restored sections will emigrate to adjacent reaches. Other objectives include increasing numbers of larger trout via improved survival, better habitat for aquatic invertebrates and non-game species, and increasing angler access and participation. Few proposals note the potential for immigration and fish concentration as mechanisms explaining increased trout abundance, as articulated by Roni (2019), likely because no one has examined these mechanisms in the groundwater-fed streams of the Driftless Area.

A series of studies were conducted in one set of three interconnected Driftless Area streams in southeast Minnesota in the late 2000s to better understand seasonal changes in growth, survival and movement of Brown Trout Salmo trutta and Brook Trout *Salvelinus fontinalis* among stream reaches (Dieterman and Hoxmeier 2011; Dieterman et al. 2012; Hoxmeier and Dieterman 2013). The study area included two stream reaches with past HR work, but study results were never emphasized nor presented in the context of how recruitment, survival and movement (immigration and emigration) varied as a function of the stream habitat work. Also, the study's main objective was to assess seasonal changes in these parameters, with less emphasis on annual changes. In the present paper, we re-calculated survival and immigration/emigration rates at an annual time step and re-examined these estimates to better understand how stream HR projects in the Driftless Area influenced these biological parameters.

Methods

A brief overview of the methods are presented here but more details can be found in Dieterman and Hoxmeier (2011) and Hoxmeier and Dieterman (2013). We used a capture-recapture study design with PIT tags to estimate seasonal survival and movement among six study reaches encompassing portions of Hemmingway, Pine, and Coolridge creeks. Hemmingway Creek is 3.2 km long and flows into Pine Creek, a larger 4th order stream that is 28 km in length. Coolridge Creek is 1.6 km long that also flows into Pine Creek, 0.4 km downstream from the mouth of Hemmingway Creek. Watersheds are primarily a mix of hardwood forests, pasture, and row crop agriculture. For these analyses, we defined reaches as those with and without completed habitat rehabilitation projects. Reaches with HR projects included Lower Hemmingway (LH) and Pine Creek, whereas upper Hemmingway (UH), and all three reaches in Coolridge Creek (LC, MC, UC) did not have HR projects. We did not have landowner permission to access the middle portion of Hemmingway Creek.

Trout were sampled on 10 occasions from September 2006 thru October 2008 by electrofishing the entirety of each stream reach. Brown Trout greater than 120 mm and Brook Trout greater than 100 mm total length were measured and tagged with a PIT tag and given an adipose fin clip to monitor tag loss in future sampling occasions. After tagging, trout were released back into the pool from which they were captured. Trout were marked on three occasions: September 2006, March 2007, and August 2007. Trout were resampled about every three months to calculate seasonal growth, survival, and movement for spring, summer, fall, and winter. We typically sampled in the months of March, May, August, and November.

Trout were divided into three age groups based on length frequency histograms and known age fish. Known age fish were those tagged at age-0 at the end of their first summer and followed through to older ages. Our age groups consisted of age-0, age-1, and age-2+ (those fish age-2 and older) for Brook Trout and age-0, age 1-2, and age 3+ for Brown Trout. Age groups were chosen based on ecologically important life stages. Age-0 juvenile trout typically use different stream habitat than adults, provide an index to recruitment and are usually sexually immature. Because age-0 trout were not vulnerable to our collection methods until fall, we could not calculate estimates of their growth, survival, and movement for their first spring (alevin stage) or summer. The middle age grouping comprises the bulk of the adult populations for each of these two species in most years. The oldest age groups are considered large Brook Trout and Brown Trout often desired by anglers.

We estimated survival and movement using a multistrata Cormack-Jolly-Seber model in Program MARK. Multistrata models were analyzed for each age group to estimate apparent survival (S), capture probability (p), and movement (Ψ). Effects of year (t) and stream reach (r) were tested for each parameter. Final models were selected based on lowest bias-corrected Akaike's Information Criteria scores (AICc). Models were ranked using AICc and determined to be supported if they had a delta AICc (Δi) value less than two (Burnham and Anderson 2002). We also calculated Akaike weights (wi) to examine the relative likelihood of each model. Pine Creek had very few Brook Trout and the low number of tagged and recaptured Brook Trout precluded us from making any estimates of movement and survival for Brook Trout in Pine Creek.

Results

If stream habitat rehabilitation was an important modifier of salmonid recruitment we expected recruitment of Brown Trout and Brook Trout to be higher in stream reaches with HR than in adjacent reaches without rehabilitation. Age-0 density of both Brown Trout and Brook Trout was spatially and temporally variable, with higher recruitment in 2006 than in 2007 (Figure 1). Lower recruitment in 2007 was likely the result of extreme spring and summer flooding. Age-0 density estimates for Brown Trout in 2006 ranged from a low of 44/ha (95% CI ± 4.5) in Pine Creek, a HR reach, to a high of 2,858/ha (95% CI ± 185) in middle Coolridge, a reach without rehabilitation. In 2007, age-0 Brown Trout density ranged from no recruitment (i.e., 0/ha) in upper Hemmingway to a high of 822/ha (95% CI ± 133), again in middle Coolridge. Age-0 density estimates were highest for Brook Trout in 2006 in the two most upstream reaches lacking HR and where Brown Trout recruitment was lowest. Age-0 Brook Trout density was 577/ha (95% CI ± 48) in upper Coolridge and 562/ha (95% CI ± 14) in upper Hemmingway and was lowest in rehabilitated Pine Creek where density was only 2/ha (no 95% CI because too few were collected). In 2007, age-0 Brook Trout were not captured in Pine Creek or in upper Hemmingway, and density was estimated to be 9/ha (too few captured to obtain a 95% CI) in lower Hemmingway. Brook Trout recruitment was estimated to be highest in 2007 in the most upstream reach in Coolridge Creek (91/ha ± 19).

From a total of 1,293 Brown Trout and 524 Brook Trout tagged with PIT tags and sampled over the course of 10 capture-recapture occasions over three years, there was almost no support for a beneficial effect of habitat rehabilitation on apparent survival of any age group of either species, but there was support for an effect on immigration/emigration (Table 1). Survival never varied as a function of stream reach except for large age 3+ Brown Trout, indicating that survival was similar regardless of whether a reach had HR or not. Instead, most support in these data were for models with time-varying survival estimates, meaning survival was more variable among seasons and years than among stream reaches for age-0 Brown Trout, adult Brook Trout (age-1), adult Brown Trout (ages 1-2) and larger age-2+ Brook Trout. Survival estimates for age-0 Brook Trout also did not vary by either season or stream reach.

Only survival of large age-3+ Brown Trout varied by stream reach, but results were equivocal among reaches with and without HR (Figure 2). Mean annual survival was highest in lower Hemmingway (LH; 63%), a reach with extensive HR, but 95% confidence intervals overlapped those for survival in middle Coolridge (MC; $\bar{x}$ =41%), a reach without HR. Further, survival in Pine Creek ($\bar{x}$ = 35%), the other rehabilitated reach, was similar to middle Coolridge. However, large Brown Trout survival in other reaches lacking HR was either zero, because no large Brown Trout were captured in them (upper Hemmingway, lower Coolridge), or suffered from wide confidence intervals because only three large trout were ever captured there (upper Coolridge).

Instead, most support in these data (86-100% depending on age group; Table 1) was for a potential habitat rehabilitation effect on movement among reaches. Age-0 Brown Trout emigration only varied by season (3% in fall, 6% over winter and 10% in spring = 18% overall; Dieterman and Hoxmeier 2011), meaning the same percentage emigrated from each reach. However, age-0 Brown Trout only emigrated from one HR reach, Pine Creek, and into only one adjacent reach without HR (lower Coolridge) (Figure 3). Conversely, age-0 Brown Trout emigrated from three non-HR reaches into both reaches with habitat rehabilitation. There were fewer age-0 Brook Trout movements and those varied by reach. Only 4% of age-0 Brook Trout emigrated from a HR reach, (i.e., from lower Hemmingway to upper Hemmingway), but lower Hemmingway received immigrants from two non-HR reaches, 15% of upper Hemmingway and 28% of lower Coolridge individuals (Figure 3).

Expressing movement in terms of percentages can be misleading if age-0 densities are large in a reach with a low percentage of emigrants versus another reach with a higher percentage of emigrants, but lower densities. Age-0 densities (#/ha) varied spatially (Figure 1), in part due to differences in surface area of study reaches. The two HR reaches comprised about 70% of the entire study area as Pine Creek encompassed 1.17 ha and lower Hemmingway 0.53 ha (1.70 ha total). Non-HR reaches comprised only about a third of the study area as upper Hemmingway encompassed 0.20 ha, upper Coolridge 0.09 ha, middle Coolridge 0.28 ha and lower Coolridge 0.15 ha (0.72 ha total). All non-HR reaches were on smaller tributaries to Pine Creek and included narrower headwater areas with less flow. Despite these size discrepancies, HR reaches had a net gain of 211 age-0 Brown Trout in 2006 and 63 in 2007, when recruitment was reduced by flooding (Table 2). Only one HR reach, lower Hemmingway, either contributed age-0 Brook Trout emigrants or received immigrants from other reaches and overall had a net gain of just 30 individuals in 2006 and two in 2007.

Movements of adult trout were similar to juveniles. Only one HR reach contributed small percentages of age 1-2 Brown Trout to adjacent non-HR reaches (4% into each non-HR reach; Figure 4). Conversely, that HR reach received larger percentages of adult Brown Trout from those reaches (11% and 52%) in addition to a third non-HR reach (15%). The other HR reach, lower Hemmingway, only received emigrants from one of the non-HR reaches; 28% of the adult Brown Trout population in upper Hemmingway. Adult age-1 Brook Trout movements were even more modest with 15% of adult Brook Trout emigrating from one downstream HR reach, lower Hemmingway, to upstream headwaters lacking habitat rehabilitation. Conversely, lower Hemmingway received a smaller proportion of emigrants from upper Hemmingway in addition to 4% of adult Brook Trout from one other non-HR reach. However, as with age-0 trout movement, adult densities, especially Brown Trout, were spatially variable and often higher in HR reaches (see Dieterman and Hoxmeier 2011). Thus, when actual numbers of trout moving among reaches were calculated, slightly more adult trout emigrated from HR reaches than immigrated into them (Table 2), suggesting that HR reaches did contribute “extra” individuals to adjacent reaches.

Lastly, there was little movement of larger adult trout between HR and non-HR reaches. Relatively small percentages of large age-3+ Brown Trout moved between the non-HR tributary reach and the HR reach downstream on the main stem of Pine Creek (Figure 5). When movement percentages were translated to actual numbers of large Brown Trout moving, there was a nearly equal exchange of a small number of big fish moving between these reaches. Three large Brown Trout emigrated from the HR reach on Pine Creek upstream to the non-HR reach on the tributary and five trout emigrated back downstream into Pine Creek in 2006. In 2007, the exchange was the same with two large Brown Trout emigrating from each reach to the other. Although larger age-2+ Brook Trout did move among selected reaches in this study area (see Hoxmeier and Dieterman 2013), none of those movements were between HR and non-HR reaches.

Conclusions

Our results provide some of the first observations of how native and non-native salmonids recruit, survive, and move across Driftless Area riverscapes in relation to instream habitat rehabilitation projects that have existed for more than a decade. There was no evidence showing recruitment of either native Brook Trout nor wild Brown Trout was higher in reaches where instream habitat had been rehabilitated than in adjacent reaches with no HR work. This suggests that these two HR projects did not increase recruitment, as has often been touted as a benefit of such projects; or, if recruitment had increased following project completion, those benefits have not persisted. Even if pre-HR-project data existed showing worse recruitment before the project was completed compared to current recruitment, the other reaches in this riverscape with no HR work and excellent recruitment suggest that general stream conditions were probably adequate to maintain Brown Trout and Brook Trout populations, regardless of HR. In addition, because numbers of age-0 trout immigrated into reaches with HR in this study, it is possible that in the absence of HR, age-0 trout could have supplemented recruitment in lower Hemmingway and Pine Creek anyway. We recognize that the current study design only provides a “snapshot” assessment of recruitment effects of HR projects and that a longer-term study with reaches subjected to HR and similar reaches without HR would be better. Dieterman et al. (2020) used results from a long-term monitoring program encompassing such a design and found long-term 40+-year trends in Brown Trout recruitment (7% annual increases) in other Driftless Area streams that were unrelated to HR projects, further supporting our observations here.

As with recruitment, we found little support for the notion that HR enhanced survival of either Brown Trout or Brook Trout. Survival of juveniles and adults of both species was influenced to a greater extent by temporal changes than by differences among reaches, including enhanced instream habitat in HR projects. Survival of adult Brook Trout was negatively affected by spring and summer floods during the study (Hoxmeier and Dieterman 2013) and age-1 & 2 Brown Trout consistently had lowest survival during the fall-spawning season (Dieterman and Hoxmeier 2011), perhaps due to spawning stress. Large age-3+ Brown Trout was the only life stage that may have higher survival due to the HR project on lower Hemmingway, but survival was not similarly high on the HR reach on Pine Creek. The reach on Pine Creek is in an open pasture, whereas Hemmingway Creek is less accessible with a riparian zone dominated by woods. It is possible that angler exploitation could explain lower survival of larger Brown Trout in the Pine Creek reach.

Lack of association between most Brook Trout parameters and HR projects was not unexpected. Additional studies in these streams found that Brook Trout survival and movement were associated with floods and competition with Brown Trout (Hoxmeier and Dieterman 2013; 2016). If the HR projects increased Brown Trout populations as in other streams (Thorn et al. 1997), it may have had a deleterious effect on native Brook Trout. Similar HR projects have been implicated in benefitting non-native Brown Trout populations to the detriment of native Brook Trout, and none of our findings refute that notion. Still, Brook Trout and Brown Trout movement was similar in the HR reach on lower Hemmingway. In particular, more juvenile Brook Trout immigrated into lower Hemmingway than emigrated from it and conversely, lower Hemmingway contributed a few more adult Brook Trout to other reaches than it gained from immigration. Because this pattern was isolated to a single HR reach, some other inherent factor in that reach may be responsible for these Brook Trout patterns. Summer water temperatures were colder in lower Hemmingway compared to Pine Creek and these differences may explain why Brook Trout persisted and behaved similarly to Brown Trout in lower Hemmingway and not in Pine Creek.

The most apparent effect of these HR projects was as modifiers of movement that may be partially explained by ontogenetic changes. For age-0 juvenile trout, these HR projects clearly do not produce excess individuals that emigrate to supplement populations in adjacent reaches lacking HR. Instead, HR reaches receive more immigrants than they produce and appear to concentrate juvenile trout, especially Brown Trout, during their first year. Both HR reaches in this study maintained higher abundances of age 1 and older adult Brown Trout than reaches lacking HR (see Dieterman and Hoxmeier 2011), which could be partially explained by concentration of age-0 juveniles during their first year. Subsequently, higher abundance of adult Brown Trout in HR reaches allows them to provide a small number of excess adult trout to adjacent reaches lacking HR. Such small numbers (i.e., 5-10 individuals) may not be important modifiers of overall population size but may be important to maintain genetic diversity in the broader population. Dieterman et al. (2020) found stream reaches with HR had an average of 30% higher abundance of larger age-3+ Brown Trout than reaches lacking HR in Driftless Area streams. Because the present study of mechanisms suggest little HR benefits on recruitment or survival, we hypothesize that Brown Trout abundance increases following HR are most likely a product of the HR project increasing overall carrying capacity of the reach that allows concentration of juveniles seeking higher quality adult habitat and to a lesser extent, perhaps improved survival of the largest sized adults.

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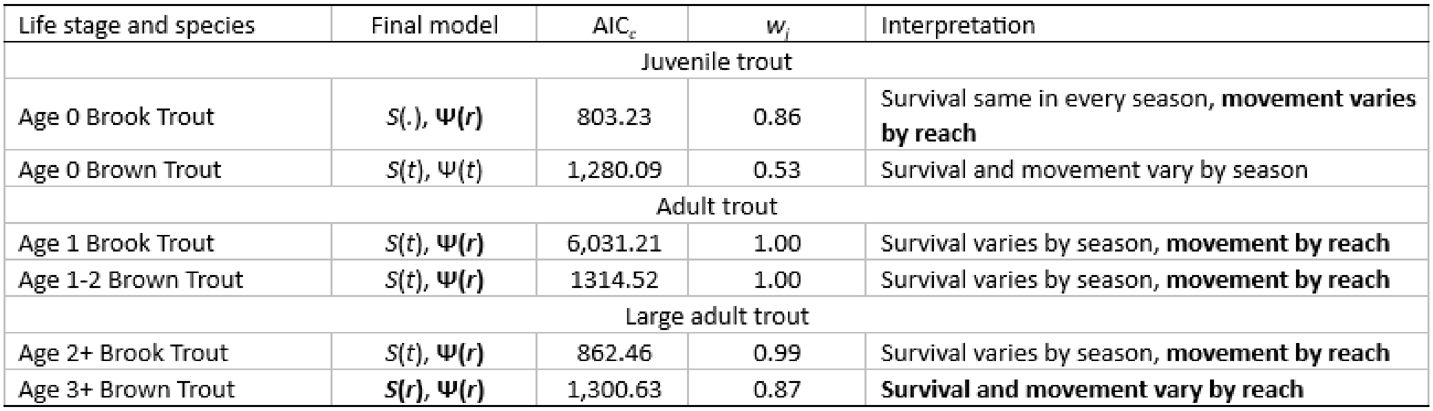
Table 1. Final multistrata Cormack-Jolly-Seber models selected by Dieterman and Hoxmeier (2011) and Hoxmeier and Dieterman (2013) for estimating survival (S), movement (Ψ) and capture probability (p; not shown here) for three age groups of Brown Trout and Brook Trout in three streams in southeast Minnesota 2006-2008. Final models were selected based on Akaike's Information Criterion (AICc), ΔAICc (not shown) and Akaike weights (wj). The t notation indicates if a parameter, such as Ψ varied by season in the model. Similarly, the r notation indicates that S or Ψ varied among reaches which could represent an effect of stream habitat projects and are highlighted in bold. The "." notation indicates the parameter was constant across seasons and reaches. Interactions between t x r were also tested but never present in final models selected.



Life stage and species	Final model	AIC_c	w_i	Interpretation
		Juvenile trout		
Age 0 Brook Trout	$S(.)$, **$\Psi(r)$**	803.23	0.86	Survival same in every season, **movement varies by reach**
Age 0 Brown Trout	$S(t)$, $\Psi(t)$	1,280.09	0.53	Survival and movement vary by season
		Adult trout		
Age 1 Brook Trout	$S(t)$, **$\Psi(r)$**	6,031.21	1.00	Survival varies by season, **movement by reach**
Age 1-2 Brown Trout	$S(t)$, **$\Psi(r)$**	1314.52	1.00	Survival varies by season, **movement by reach**
		Large adult trout		
Age 2+ Brook Trout	$S(t)$, **$\Psi(r)$**	862.46	0.99	Survival varies by season, **movement by reach**
Age 3+ Brown Trout	**$S(r)$, $\Psi(r)$**	1,300.63	0.87	**Survival and movement vary by reach**

Table 2. Estimated numbers of Brown Trout and Brook Trout emigrating from or immigrating into stream reaches with instream habitat rehabilitation (HR) in southeast Minnesota, 2006-2007. Numbers based on estimates of total numbers of trout present in each reach and annual movement rates.

Year	Number emigrating from HR reaches	Number immigrating into HR reaches	Net gain (+) or loss (-)	Interpretation
	Age-0 Brown Trout			HR reaches gain more juvenile trout than they lose
2006	82	293	+211	
2007	9	72	+63	
	Age-0 Brook Trout			
2006	8	38	+30	
2007	1	3	+2	
	Adult (age-1 & 2) Brown Trout			HR reaches contribute slightly more adult trout than they gain
2006	41	33	-8	
2007	56	51	-5	
	Adult (age-1) Brook Trout			
2006	8	2	-6	
2007	17	8	-9	

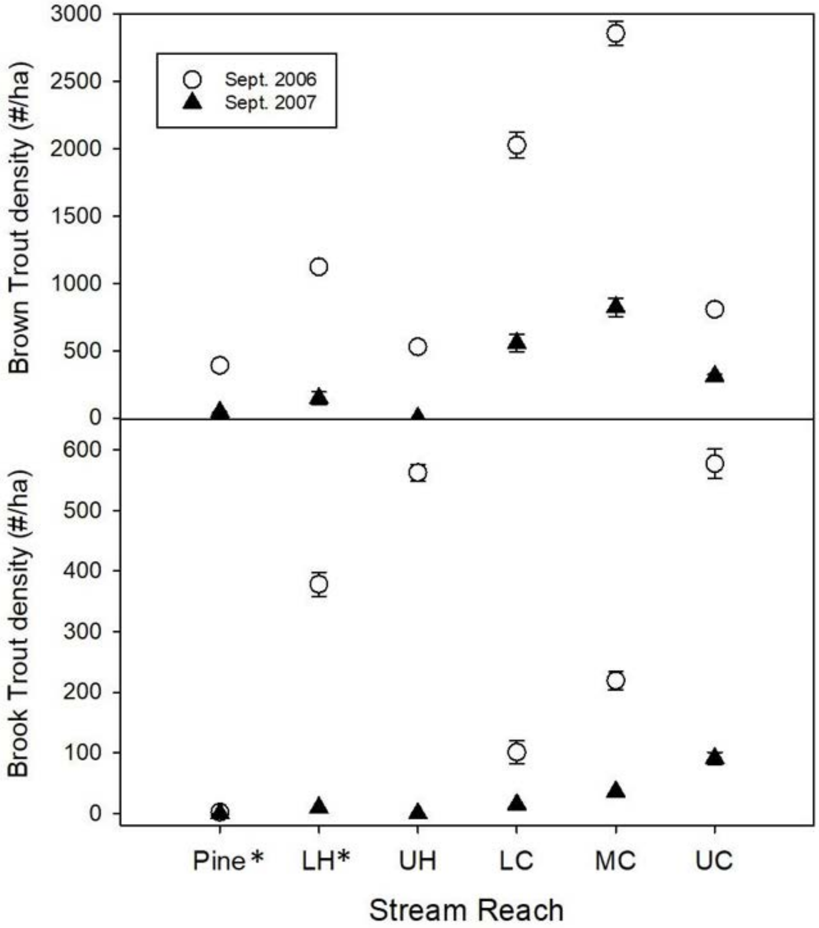


Figure 1. Spatial and temporal differences in density (± 1 SE) of age-0 Brown Trout and Brook Trout in six interconnected reaches in southeast Minnesota to assess the effect of stream habitat rehabilitation* on recruitment. Pine=Pine Creek (habitat rehabilitated); LH=lower Hemmingway (habitat rehabilitated); UH=upper Hemmingway; and LC, MC, and UC = lower, middle, and upper Coolridge Creek, respectively.

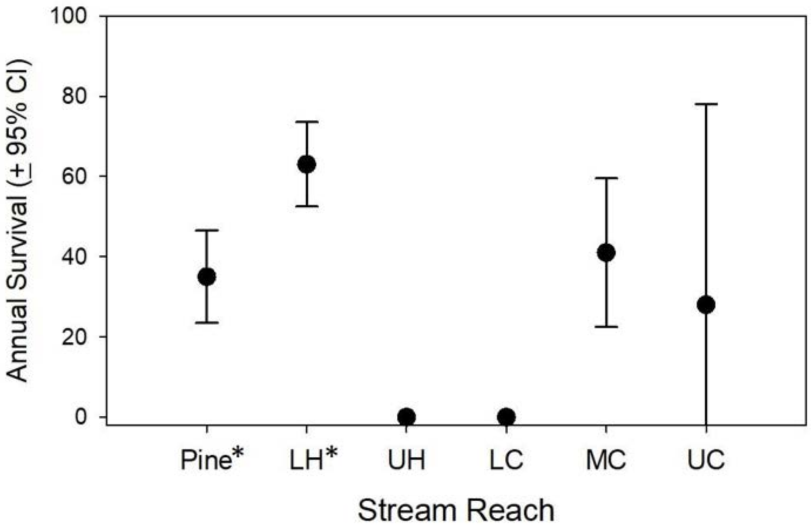


Figure 2. Annual survival of age-3+ large Brown Trout in six interconnected stream reaches in southeast Minnesota to assess the effect of stream habitat rehabilitation* on survival. Pine=Pine Creek (habitat rehabilitated); LH=lower Hemmingway (habitat rehabilitated); UH=upper Hemmingway; and LC, MC, and UC = lower, middle, and upper Coolridge Creek, respectively.

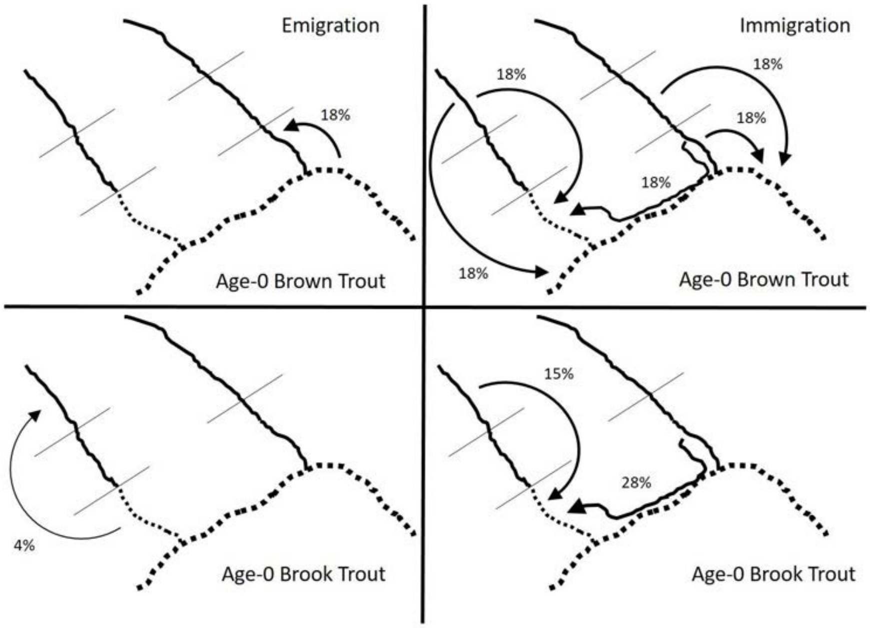


Figure 3. Annual emigration (left) and immigration (right) rates of age-0 juvenile Brown Trout and Brook Trout between stream reaches with habitat rehabilitated 20 years earlier (dashed lines) and reaches without habitat rehabilitation (solid lines) in three interconnected southeast Minnesota streams. Designated reach boundaries on one stream without habitat rehabilitation differed slightly between species because of differing study objectives. Only movements associated with immigration and emigration between rehabilitated and non-rehabilitated reaches are shown.

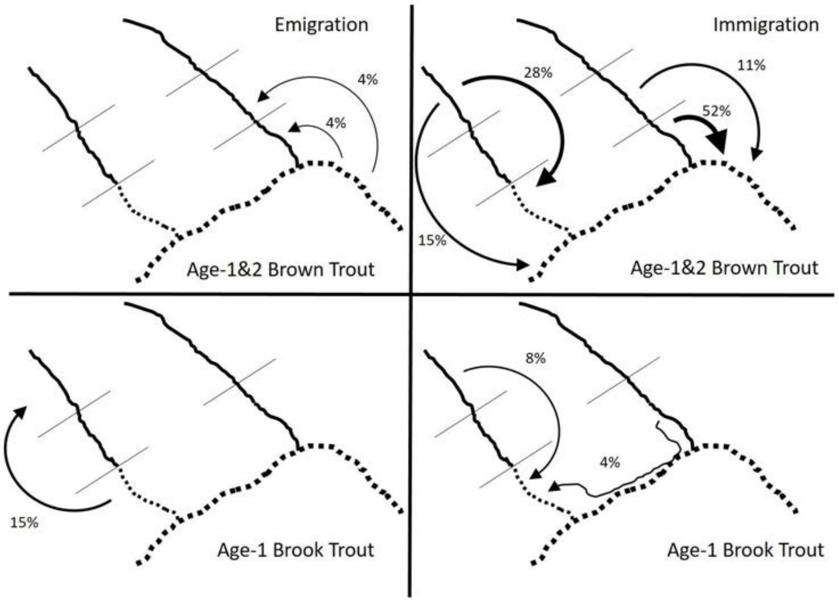


Figure 4. Annual emigration (left) and immigration (right) rates of adult Brown Trout and Brook Trout between stream reaches with habitat rehabilitated 20 years earlier (dashed lines) and reaches without habitat rehabilitation (solid lines) in three interconnected southeast Minnesota streams. Designated reach boundaries on one stream without habitat rehabilitation differed slightly between species because of differing study objectives. Only movements associated with immigration and emigration between rehabilitated and non-rehabilitated reaches are shown.

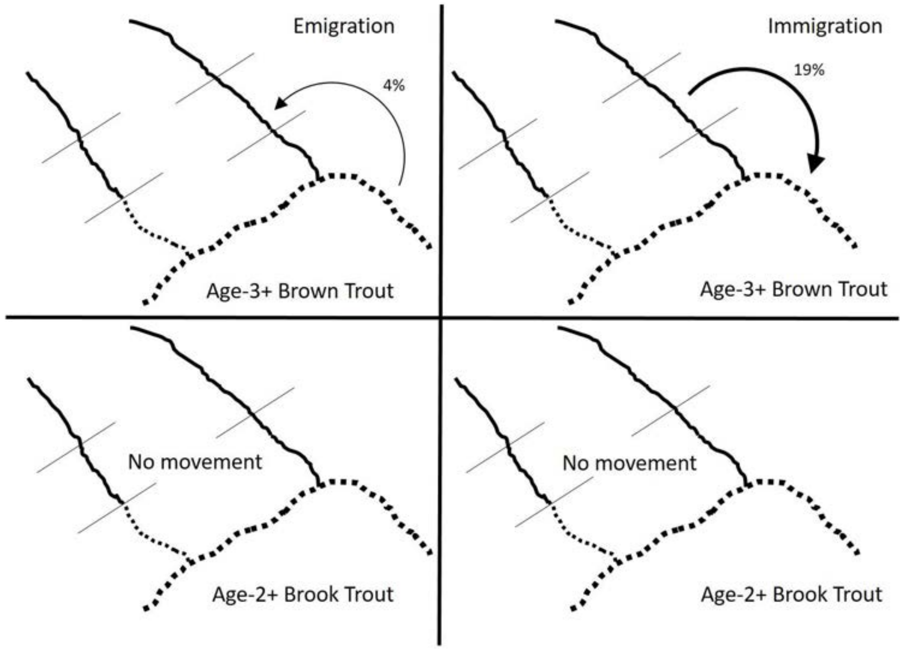


Figure 5. Annual emigration (left) and immigration (right) rates of large adult Brown Trout and Brook Trout between stream reaches with habitat rehabilitated 20 years earlier (dashed lines) and reaches without habitat rehabilitation (solid lines) in three interconnected southeast Minnesota streams. Designated reach boundaries on one stream without habitat rehabilitation differed slightly between species because of differing study objectives. Only movements associated with immigration and emigration between rehabilitated and non-rehabilitated reaches are shown.

Conservation Genetics and Wild Trout: Evolving Opportunities to Support Management

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Abstract - It is increasingly apparent that our planet is undergoing rapid, unprecedented environmental change. These changes are already impacting wild trout populations, leading to declines in occupancy and abundance across increasingly fragmented landscapes. Many changes are projected to intensify in the coming decades, which are likely to be crucial to the future of many wild populations. While there is some uncertainty in exactly how changes will manifest, we can be confident that many populations will need to rapidly adapt to change if they are to persist. The foundation of such rapid change is genetic diversity, and conservation strategies are most likely to be successful when they consider the role of genetics in the outlook for wild trout populations. While not a panacea, rapid advances in the field of conservation genetics allow longstanding questions to be addressed with greater rigor and present new opportunities to support management against a backdrop of accelerating change. Here, we review major advances in conservation genetics as they relate to wild trout and highlight potential opportunities to support conservation in the Anthropocene and key areas of continuing uncertainty.

Introduction

Over the last three centuries, humans have been responsible for environmental change at the global scale (Crutzen 2002), and freshwater habitats are among the most threatened ecosystems worldwide (Jelks et al. 2008). The health and function of freshwater systems have been negatively impacted by a multitude of anthropogenic threats (e.g., habitat fragmentation/loss, changes in flow regimes, invasive species, pollution, and overexploitation) and will be further altered by climate change (Arthington et al. 2016; Paukert et al. 2021). For example, it is expected that increases in global temperature will elicit range contractions in coldwater fish species (Eby et al. 2014); alter the timing of spawning migrations (Crozier et al. 2011); change patterns of abundance, growth and recruitment (Lynch et al. 2016); and increase the prevalence of certain aquatic diseases (Crozier et al. 2008). Given the fast rate of environmental change, fish populations will need to respond rapidly via plastic and/or genetic responses for long-term persistence. Yet, predicting how different species and populations will respond to climate changes is difficult (Rijnsdorp et al. 2009).

As environmental changes accumulate, conservation of coldwater fish species, including trout (members of the genera *Oncorhynchus, Salmo*, and *Salvelinus*), is predicted to become more difficult (Lynch et al. 2016). Historically, fisheries management has relied upon the manipulation of habitat, harvest, and hatcheries (and hydropower, especially in the western United States) to conserve and manage fisheries (e.g., Trushenski et al. 2018). However, as variability in air temperatures and stream flows increase, traditional management tools may be ineffective, especially for trout populations that presently occupy high-quality habitats free from harvest or hatchery supplementation. In response, fisheries managers may benefit from integrating novel forms of data and management strategies to ensure the long-term persistence of trout species.

Although not a panacea, rapid advances in the field of conservation genomics present new opportunities to support management against a backdrop of accelerating change. Relative to traditional genetic methods (e.g., microsatellites or restriction fragment length polymorphisms), genomic methods enable the identification and screening of a larger proportion of the genome, facilitating more powerful investigations of focal relationships (Ouborg et al. 2010). Additionally, genomic methods enable surveys of both neutral and adaptive regions of the genome, compared to traditional methods which typically target neutral genetic variation. Genomic methods thus make possible novel explorations of adaptive relationships, equipping managers with valuable information on genetic variation underlying the occurrence of adaptive traits, conservation units representing locally adapted groups, and the adaptive potential of populations relative to changing climatic conditions, among other possibilities (Goetz and MacKenzie 2008; Funk et al. 2012). However, unravelling these relationships first requires understanding of available techniques and their application. Therefore, a major goal here is to offer an accessible roadmap for non-geneticists to navigate the landscape of available genomic methods and their utility for addressing questions central to management decision-making.

Major Advances in Conservation Genetics

The genomic era was launched by the advent of technologies that allow vast amounts of DNA to be sequenced quickly and inexpensively. These advancements in sequencing have been accompanied by the development of methods to discover and genotype large swaths of the genome. A primary target of these sequencing technologies has been single nucleotide polymorphisms (SNPs) which are specific locations within the genome where nucleotides vary among individuals (Mardis 2011; Narum et al. 2013). The expansion of genomic methods into conservation and management applications has been fueled by quickly declining sequencing costs and increased access to equipment and expertise (Allendorf et al. 2010; Hohenlohe et al. 2021). These factors have driven the transition to SNPs as the molecular marker of choice for many applications (Ouborg et al. 2010; see Figure 1 for a comparison between SNPs and microsatellites). Historically, small numbers of microsatellite markers (<20 loci) were used to address many research questions now addressed by SNPs because microsatellite loci typically contain large numbers of alleles and offer a high degree of information content. In contrast, SNPs are biallelic and therefore hundreds to thousands of markers are often required to achieve a comparable level of statistical power (Morin et al. 2004; Morin et al. 2009). However, this limitation is overcome by genomic methods which yield thousands to millions of SNPs. Single nucleotide polymorphisms are also less prone to inconsistent genotype calls among studies, making standardization more feasible. Although microsatellites continue to offer a reliable tool for inferring demographic relationships, surveys of genome-wide SNPs have unlocked new applications that are already supplying vital information to guide management. The ability to characterize genetic variation across the genome using SNPs is significant in that it allows researchers to establish relationships between phenotypes and underlying genotypes at a level that was previously unavailable.

Genomic methodologies encompass a wide range of techniques for exploring the genome; however, two major approaches have emerged for surveying SNPs in management contexts. Reduced representation approaches employ restriction site-associated DNA sequencing (RADseq; Baird et al. 2008) to target a subset of the genome for SNP discovery and genotyping. Although several technical variations of RADseq exist (see review by Andrews et al. 2016), these methods are based on producing a reduced representation of the genome via fragmentation with one or more restriction enzymes, then preparing a subset of fragments for sequencing. Restriction site-associated DNA sequencing approaches are thus limited to the discovery of SNPs located near restriction enzyme recognition sites. In comparison, whole genome resequencing approaches (see review by Fuentes-Pardo and Ruzzante 2017) involve sequencing entire genomes to discover SNPs located anywhere in the genome. Whereas RADseq approaches typically uncover thousands to tens-of-thousands of SNPs, whole genome resequencing approaches are capable of yielding hundreds-of-thousands to millions of SNPs, enabling more comprehensive evaluation of focal relationships. However, while costs associated with whole genome resequencing are declining (e.g., Therkildsen and Palumbi 2017), they remain prohibitive for many applications.

In addition to SNP discovery methods, recently developed techniques for efficient genotyping make repeatable, targeted sequencing of previously identified markers across large numbers of individuals more practical than ever. These techniques include genotyping-in-thousands sequencing (GTseq; Campbell et al. 2015) and restriction site-associated DNA capture (also known as Rapture or RAD capture; Ali et al. 2016), both of which are used to target specific sets of SNPs, like those that have long been used in genetic monitoring efforts for some salmonid species. However, compared to traditional methods, GTseq and RAD capture enable high-throughput, cost-effective genotyping without requiring specialized equipment. Whereas GTseq is typically used to produce genotypes for hundreds of SNPs in thousands of individuals in a single sequencing run (Campbell et al. 2015), RAD capture is ideal for genotyping thousands of SNPs in hundreds of individuals in a single sequencing run (Ali et al. 2016). Another key distinction between these techniques is that RAD capture targets SNPs previously discovered using RADseq, whereas GTseq panels can target loci unassociated with restriction sites. Guidelines by Meek & Larson (2019) offer a valuable resource to help managers identify which approach to use depending on project goals and budget.

The genomic methods highlighted here have already proven instrumental in elucidating critical connections between genotypes and phenotypes in native trout and other salmonids, including in the context of a changing global climate. For example, studies of Redband Trout *Oncorhynchus mykiss gairdneri* that employed RADseq and whole genome resequencing have identified regions of the genome underlying thermal tolerance (Chen et al. 2018; Chen and Narum 2021). These methods have also facilitated the discovery of a major effect marker underlying premature migration in native steelhead O. mykiss and Chinook Salmon *O. tshawytscha* (Narum et al. 2018; Prince et al. 2017). Although most traits are presumably governed by many variants of small effect, knowledge of major effect markers is necessary to identify alleles required for specific traits to occur. Restriction site-associated DNA sequencing has also been used to characterize phenotypic impacts of hybridization between invasive Rainbow Trout *O. mykiss* and native Westslope Cutthroat Trout *O. clarkii lewisi* (Strait et al. 2021), building upon earlier microsatellite-based work that revealed expanding hybridization due to climate change (Muhlfeld et al. 2014). Additionally, GTseq and RAD capture panels recently developed for native trout (Bull Trout *Salvelinus confluentus,* Bohling et al. 2021; Lake Trout *S. namaycush*, Smith et al. 2020) offer resources that make exploring adaptive relationships in future studies more practical. Collectively, information on markers underlying climate-related traits from these and other studies is essential for understanding the capacity of wild trout populations to withstand changing climatic conditions.

Using Conservation Genomicto Address Key Questions in Conservation

In the past, conservation genetics focused on conserving existing diversity across the landscape. This remains a primary goal of the field; however, newer genomic approaches can provide a stronger characterization of genome-wide diversity (Fischer et al. 2017; Lemopoulos et al. 2019).

Current forecasts for environmental change clearly indicate that rapid adaptation to changing conditions will be critically important for many wild trout populations if they are to persist in the Anthropocene (Kovach et al. 2019). While conserving existing diversity is a key aspect of maintaining adaptive potential (Bernatchez 2016), the expected shifts underscore the importance of understanding evolutionary processes. Genomic advances are quickly improving our understanding of natural selection and adaptation. This new understanding coupled with rapid environmental change has accelerated conversations around more active genetic management measures such as genetic rescue, assisted migration, and direct gene editing (Ralls et al. 2018; Kovach et al. 2022). Captive breeding remains an important conservation tool to support and restore imperiled wild trout populations, and genomic advances can help us identify suitable stocks and understand the outcomes of captive rearing. We review these active management strategies, which are guided by recent advances in genomics, below.

Assisted migration

Human-assisted migration has traditionally been applied within the context of reintroduction, where individuals from captive or extant populations are translocated to formerly occupied habitats. In addition to restoring native communities, reintroduction can improve regional species resiliency by increasing the number of populations across the landscape and creating populations with redundant genetic diversity. Guidelines for the design and monitoring of reintroduction efforts often emphasize translocating individuals from populations with high genetic diversity, with the assumption that this will improve near-term population fitness and allow for retention of future adaptive capacity (Jamieson and Lacy 2012). However, genomic technologies now provide novel opportunities to test assumptions about the long-term viability of restored populations, including the ability to better monitor changes in genetic diversity through time, estimate effective population size, and predict future adaptive capacity under a range of environmental and demographic scenarios (Seaborn et al. 2021; Marshall et al. 2022).

Genomic data may be particularly informative in project planning, as higher-resolution datasets can better characterize source population genetic diversity and predict success in new environments (Malone et al. 2018).

In addition, recent advances in genomic technologies have improved our understanding of the heritable genetic variation associated with specific adaptive traits, which is making it increasingly feasible to translocate individuals that are pre-adapted to future environmental or climatic conditions (He et al. 2016; Chen et al. 2022). Future analyses which focus on connections between genotypes and phenotypes (i.e., quantitative trait locus analyses) are likely to continue to improve the success of reintroduction efforts, but also may increase the feasibility of assisted range expansion and species colonization, wherein individuals with adaptive genotypes are introduced to suitable habitats that occur outside of their native range (Butt et al. 2021). With increasing stream temperatures and limited capacity for large-scale movement, human-assisted range expansion and colonization may play a significant role in future wild trout conservation (Chen et al. 2022).

Genetic rescue

Genetic rescue is a special case of human-assisted migration where individuals are moved to an existing population, with the goal that admixture will result in an increase in genetic diversity and long-term adaptive potential (Whiteley et al. 2015). Because many trout populations lack effective connectivity and are experiencing erosion of genetic diversity and high rates of inbreeding, they are often ideal candidates for genetic rescue (Frankham et al. 2011; Ralls et al. 2018). Despite this, genetic rescue has been used sparingly in wild trout conservation, likely due to concerns about potential negative fitness effects of outbreeding depression (Bell et al. 2019). In addition, design guidelines for genetic rescue can be difficult to implement in wild trout. For example, guidelines stress that translocations should only occur between populations that have similar local adaptations and have been isolated for less than 500 years (Frankham et al. 2011); however, it can be difficult to correlate neutral genetic diversity to local adaptation and many wild trout populations have been isolated for thousands of years.

As before, genomic technologies are poised to improve the ability to design and monitor future genetic rescue attempts (Whiteley et al. 2015). However, one particularly exciting opportunity that genomic data may offer is an increased ability to identify populations that are optimal candidates for rescue. Specifically, through genomic analyses it may be possible to identify specific regions of the genome that are responsible for increased genetic loads (Allendorf et al. 2010), better understand the extent of inbreeding (Fitzpatrick and Funk 2019), reconstruct historic patterns of population connectivity through coalescent models (Saremi et al. 2018), and predict the adaptive potential of hybrid offspring prior to translocation. These analyses could be very helpful for identifying vulnerable populations that may benefit from rescue (Whiteley et al. 2015) and for further minimizing the negative effects of outbreeding depression.

Captive breeding

Captive breeding programs are increasingly being used to support the conservation of wild populations and, in extreme scenarios, may be a critical stopgap measure to avoid species extinction. Despite numerous success stories, the challenges associated with captive breeding and rearing are well-documented, with numerous studies documenting rapid loss of fitness, erosion of genetic diversity, and maladaptation to wild environments (e.g., Fraser 2008; Christie et al. 2012). Integration of genomic tools into the design and management of captive breeding programs provides increased opportunity to minimize the negative effects of captivity while also improving post-release survival of individuals in the wild (Bernos et al. 2020). For example, RADseq has been used to quantify divergence between wild and captive populations (Black et al. 2017), which has been useful for improving broodstock management plans to minimize artificial selection and identify ideal candidate source populations. In addition, SNP-based parentage analyses may be better at resolving familial relationships (Lemopoulos et al. 2019), which can be used to design breeding programs that minimize inbreeding and are more effective at preserving genome-wide diversity.

Future Outlook

Undoubtedly, the conservation of wild trout faces grand challenges. Selection pressures facing wild trout will change in the coming decades, sometimes in ways that are unprecedented in their evolutionary history. The tools we use to study genetics have also changed, providing much greater clarity into diversity and evolutionary processes. These tools are likely to become more powerful and more cost-effective in the future, affording greater opportunities to guide management. An ongoing paradigm shift towards more active intervention and a greater emphasis on conserving and/or augmenting adaptive potential may promote resilience in the face of rapid change, but also presents new potential risks to navigate. While many of the forecasted environmental changes are beyond the traditional scope of fisheries management, genomic-based strategies offer opportunities to support conservation objectives.

Collectively, these changes in our ecosystems and our science tools underscore the value of close engagement between trout managers and conservation geneticists in the years to come.

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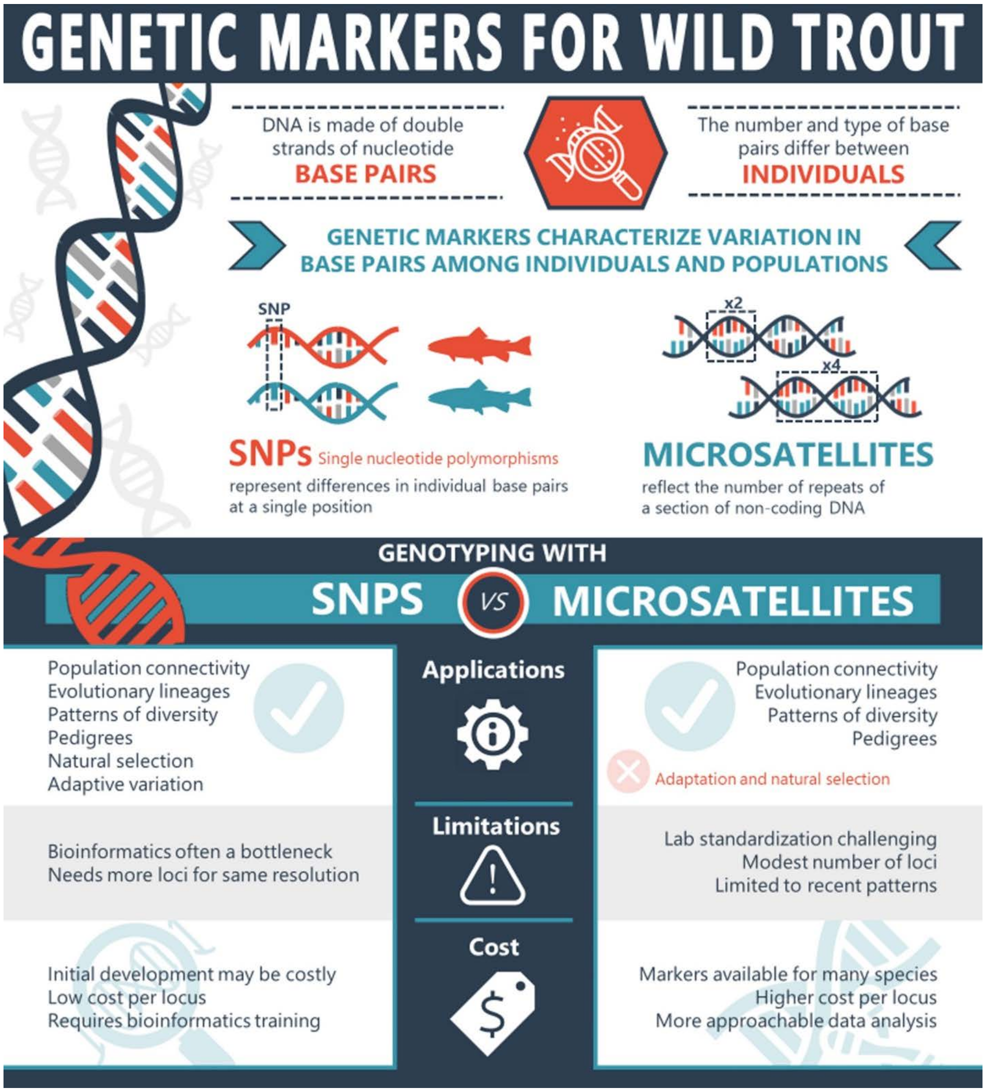

DNA is made of double strands of nucleotide **BASE PAIRS**

The number and type of base pairs differ between **INDIVIDUALS**

GENETIC MARKERS CHARACTERIZE VARIATION IN BASE PAIRS AMONG INDIVIDUALS AND POPULATIONS

SNPs Single nucleotide polymorphisms represent differences in individual base pairs at a single position

MICROSATELLITES reflect the number of repeats of a section of non-coding DNA

GENOTYPING WITH SNPS VS MICROSATELLITES

SNPS		MICROSATELLITES
Population connectivity Evolutionary lineages Patterns of diversity Pedigrees Natural selection Adaptive variation ✓	**Applications**	✓ Population connectivity Evolutionary lineages Patterns of diversity Pedigrees ✗ Adaptation and natural selection
Bioinformatics often a bottleneck Needs more loci for same resolution	**Limitations**	Lab standardization challenging Modest number of loci Limited to recent patterns
Initial development may be costly Low cost per locus Requires bioinformatics training	**Cost**	Markers available for many species Higher cost per locus More approachable data analysis

Figure 1. A comparison of single nucleotide polymorphisms (SNPs) and microsatellites for conservation genetic studies of wild trout.

Prevalence and Distribution of *Renibacterium salmoninarum*, the Causative Agent of Bacterial Kidney Disease, in Wild Trout Fisheries in Colorado

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Abstract - The prevalence of many fish diseases has declined in Colorado in recent years, but cases of bacterial kidney disease (BKD) seem to be increasing. We explored the prevalence of *Renibacterium salmoninarum* in Colorado's wild trout fisheries, investigated variables that influence the distribution of it, and evaluated common testing methods. We sampled wild trout across Colorado and tested kidney tissue with enzyme-linked immunosorbent assay (ELISA), quantitative polymerase chain reaction (qPCR), nested polymerase chain reaction (nPCR), and direct fluorescent antibody test (DFAT). Screening with ELISA showed high prevalence among fish populations, but antigen levels were low. No clinical disease was observed in any of the fish sampled despite *R. salmoninarum* being common. Antigen levels measured by ELISA increased in smaller streams with lower historic fish stocking rates. Brook Trout *Salvelinus fontinalis* had the highest prevalence of the bacterium among fish species and the highest ELISA antigen levels. The most effective assays for screening wild trout were qPCR and ELISA, DFAT was inconsistent at bacterial levels in wild trout and was generally uninformative. The bacterium *R. salmoninarum* is ubiquitous in Colorado trout fisheries but generally occurs at low levels. Active infections are rare and overt BKD appears more common in Colorado hatcheries than in wild fish.

Introduction

While the prevalence of many fish diseases has declined in Colorado in recent years, cases of bacterial kidney disease (BKD) seem to be increasing in fish hatcheries. The disease is caused by *Renibacterium salmoninarum,* a gram-positive intracellular bacterium. The disease is characterized by the presence of gray-white, necrotic abscesses in the kidney and causes mortality in both wild and cultured salmonids. The bacterium can be transmitted horizontally among fish and vertically from adult to egg due to its intracellular nature (Austin and Austin 2016). Overt bacterial kidney disease is most frequently observed in cultured salmonid fishes where it continues to be a serious concern worldwide in hatchery and aquaculture facilities (Austin and Austin 2016). The disease is less common in wild fish populations but has been reported (Mitchum et al. 1979; Mitchum and Sherman 1981; Austin and Austin 2016). Most of the documented disease outbreaks in wild fish are in anadromous Pacific salmonids. Resident trout are common carriers of the bacterium but are more resistant to disease than anadromous salmonids (Meyers et al. 1993; Guðmundsdóttir et al. 2017).

In Colorado, *R. salmoninarum* is a regulated pathogen and hatcheries that test positive are restricted from stocking fish into most state waters that support wild trout. The bacterium and associated disease outbreaks were a large problem in Colorado's hatchery system in the 1950s and 1960s. Annual fish health inspections did not detect the bacterium in Colorado hatcheries between 1997 and 2015, but since 2016, six hatcheries and a wild broodstock lake have tested positive for *R. salmoninarum* (Fetherman et al. 2020). Clinical bacterial kidney disease outbreaks have been documented at least twice in Colorado hatcheries since 2016. One outbreak cost over $2.1 million in depopulation and disinfection efforts, which affected fish management statewide with the loss of over 675,000 sport fish. That outbreak may have originated from bacteria in fertilized eggs brought into a hatchery from a wild spawn take. The bacterium can also be spread from hatcheries to wild fish. In Wyoming, a chronic BKD epizootic in wild trout was thought to have originated from hatchery plants from a facility with a history of BKD (Mitchum et al. 1979; Mitchum and Sherman 1981). Ongoing concern about the transfer of the bacterium between hatcheries and wild fish emphasized the need for more information on the prevalence and distribution of the bacteria in wild trout populations.

The objectives of this study were to document the distribution and prevalence of *R. salmoninarum* in Colorado's wild trout fisheries, investigate the influence of environmental variables and historic fish stocking practices on that distribution, and evaluate common testing methods under conditions found in wild trout populations.

Methods

To investigate the prevalence of *R. salmoninarum* in wild trout streams, we randomly selected third- to fifth-order streams from all major river basins that are managed solely with wild trout management strategies. Native Cutthroat Trout streams were excluded from the sampling frame to avoid terminal sampling of native fish, and instead, we focused on wild, self-sustaining populations of other trout species. Fish were collected using a backpack electrofisher and examined for signs of clinical disease. Tissue was collected from each fish from the anterior, middle, and posterior regions of the kidney. Individual fish tissue samples were combined into composite samples of five fish of single species either in the field or in the lab. Composite samples were made of only one age class (adult vs. sub-adult) of one species of fish, from a single water. We summarized data by three metrics: samples, lots, and waters. A sample was a single five-fish composite group, a lot was all the samples from one species from one water, and waters were all the lots from a single stream. Because the focus of this study was screening wild trout populations in the context of fish health inspections, we focused on comparing testing results among waters and lots rather than individual fish samples and used the sampling and testing protocols commonly used by state and federal fish health inspections.

Diagnostic Assays

Samples were tested with enzyme-linked immunosorbent assay (ELISA) at the Colorado Parks and Wildlife Aquatic Animal Health Laboratory. Quantitative polymerase chain reaction (qPCR), nested polymerase chain reaction (nPCR), and direct fluorescent antibody test (DFAT) screening occurred at the U.S. Fish and Wildlife Service Bozeman Fish Health Center. All assays followed standard operating procedures of American Fisheries Society Fish Health Section Blue Book (Elliott 2012). All samples were screened with ELISA, DFAT, and qPCR but only positive results from qPCR tests were tested with nPCR. We compared fish lots (single species from a single water) to evaluate the various assays and considered an individual water "positive" by a specific assay if any lots from that water were positive. To avoid false positive results with ELISA, we used a liberal negative-positive threshold for optical density values (OD) of 0.100 following Munson et al. (2010).

Statistical Analysis

We used correlation, multiple linear regression, and model selection to investigate how environmental variables and past stocking practices affect bacteria levels (Burnham and Anderson 2002). We identified five primary variables through literature review and professional judgment that may influence bacteria levels in inland trout: elevation, stream order, drainage area, stream temperature, and the number of hatchery fish historically stocked in each stream. To report the difference in ELISA OD values among species, we reported effect sizes (difference between means), standardized effect sizes (difference between means divided by the standard deviation), and 95% confidence intervals (95% CI)

The five variables were evaluated with Pearson's product moment correlation coefficient and then analyzed with multiple linear regression and the information-theoretic approach to identify the best predictive models and most influential explanatory variables (Burnham and Anderson 2002). Model assumptions of homogeneity of variance and normality were evaluated by examining residuals of the global model (additive combination of all individual variables). The response variable, average OD values from the ELISA assay, was transformed with the Box Cox procedure due to patterns observed in the residuals (Box and Cox 1964). Ten linear regression models were built with additive combinations of uncorrelated variables and interaction models were tested when they made biological sense. Model selection was completed with the small sample size version of Akaike's information criterion (AICc) following Burnham and Anderson (2002).

Results

Sixty-eight streams were sampled in all major river basins in Colorado from an elevation of 1,393 m to 3,078 m. A total 3,809 individual fish were sampled from June to October in 2016 and 2017. All waters (100%) had some fish that tested positive for *R. salmoninarum* by ELISA. Almost six percent (5.9%) of all waters had tissue samples test positive by DFAT, 23.5% tested positive by qPCR, and 11.8% were confirmed positive by nPCR (Figure 1).

While the prevalence of *R. salmoninarum* was high among wild trout waters, most of the samples had relatively low antigen levels measured by ELISA. Of the 104 lots tested, 12.6% were negative, 49.5% had low antigen levels (OD < 0.199), 30.1% had moderate antigen levels (OD 0.200-0.999), and 8.7% had high antigen levels (OD > 1.000). Within an individual water, prevalence of *R. salmoninarum* was moderate; less than half of samples from each water (48.7%) had OD values greater than the 0.100 threshold. Only five lots of fish from four different waters tested positive by DFAT, which is the screening assay generally used by Colorado Parks and Wildlife. The DFAT assay did a poor job of identifying cases with high DNA or antigen levels by other assays. Of the five lots that tested positive by DFAT, all of them had low ELISA OD values (average 0.120, range 0.074-0.186) and four of the five lots positive by DFAT were negative by qPCR. Sixteen waters (23.5%) tested positive by qPCR and eight of those waters were confirmed by nPCR. Of the 104 lots (single species, single water) tested, 15.4% were positive by qPCR with a Ct threshold of 35.

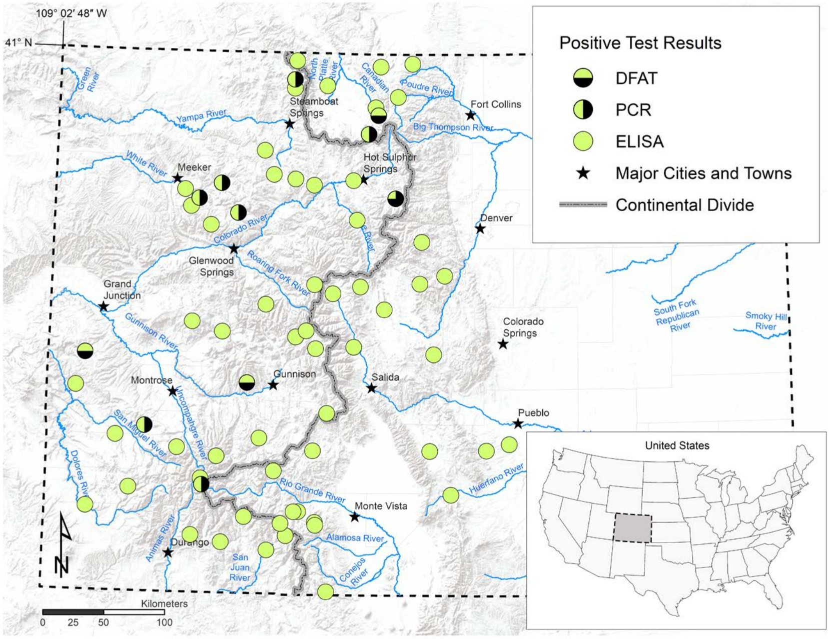

Figure 1. Wild trout sampling sites in Colorado 2016-2017 that tested positive for R. salmoninarum with DFAT, ELISA, and qPCR confirmed with nPCR.

Thirty-seven of the wild trout waters (54.4%) were stocked at some time historically, but the prevalence and average OD values for those waters were similar to wild trout waters with no stocking records. The difference between average ELISA OD values of historically stocked waters (0.134) and unstocked waters (0.130) was only 0.004 (95% CI 0-0.021), P = 0.743.

The correlation between fish stocking and inverse transformed OD values was relatively weak (R = 0.27) and stocking was negatively correlated with OD values: antigen levels were higher in waters with lower levels of fish stocking (Figure 2). The correlation analysis indicated that the variables explained the most variability in transformed OD values were drainage area (R = 0.35, P = 0.004), total trout stocked (R = 0.27, P = 0.027), and stream order (R = 0.26, P = 0.030).

The antigen of the *R. salmoninarum* was highest in trout from low order streams with a small drainage area that were historically stocked at lower levels. Of the explanatory variables, total stocked trout and drainage area were highly correlated (R = 0.79) as well as August stream temperature and elevation (R = -0.82). The collinear pairs of variables were not included in the same model to avoid problems with parameter estimation due to multicollinearity. Model

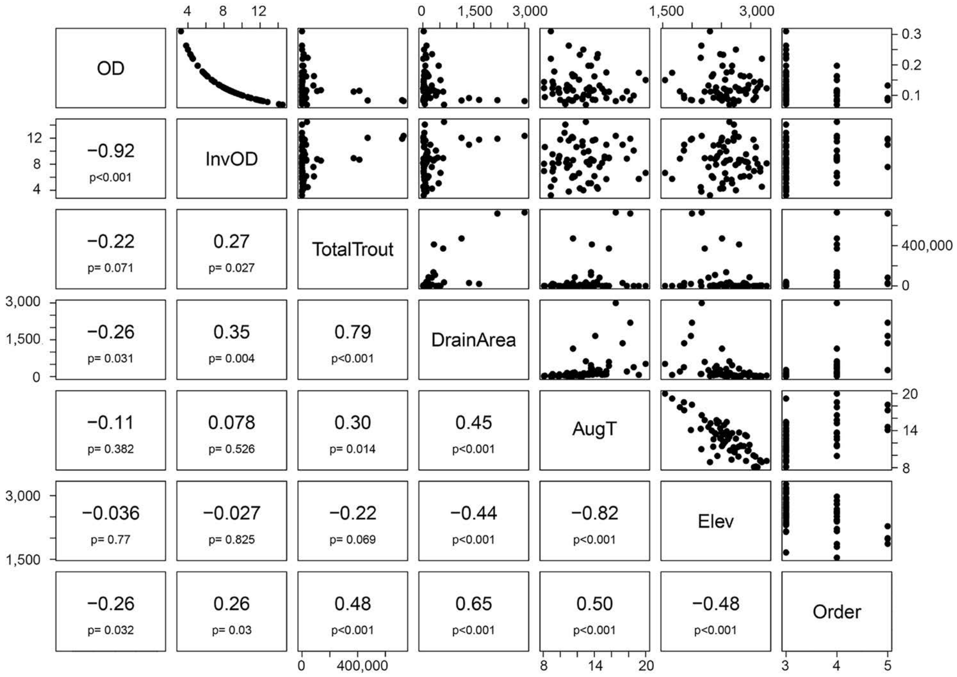

Figure 2. Pearson correlation matrix of explanatory variables and untransformed (OD) or inverse transformed ELISA optical density values (InvOD) from wild trout populations in Colorado 2016-2017.

selection results indicated that the single variable model with drainage area was the top model, with a model weight of 0.45. An additive combination of drainage area and August temperature was 1.65 AICc units behind the top model and explained 12% of the variation in OD values. Overall, the best models of stocking and environmental variables that we explored explained relatively little variation in OD values (0-13%), so more work is needed to investigate factors that are related to *R. salmoninarum* antigen levels in wild trout.

Species Trends

Brook Trout *Salvelinus fontinalis* lots had the highest average ELISA OD values followed by Brown Trout *Salmo trutta* and Rainbow Trout *Oncorhynchus mykiss* (Figure 3). Brook Trout had average ELISA OD values 0.032 (0.4 SD's) higher than Rainbow Trout (95% CI 0-0.065), P = 0.051. Brook Trout had average ELISA OD values 0.016 (0.2 SD's) higher than Brown Trout (0-0.051), P = 0.354, and Brown Trout had average ELISA OD values 0.016 (0.3 SD's) higher than Rainbow Trout (0-0.036), P = 0.101. Brook Trout also had the highest prevalence by qPCR as 26.5% were positive while 12.0% of Rainbow Trout lots, 9.5% of Brown Trout lots, and no lots of Mountain Whitefish were positive. Brook Trout occupied the smallest, highest elevation streams, with the coldest mean August temperature (Table 1).

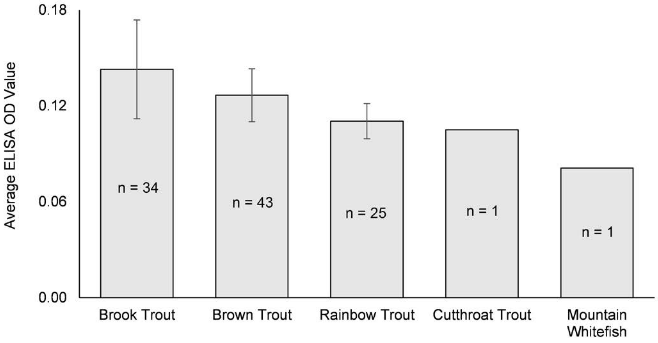

Figure 3. Average ELISA OD values and 95% confidence intervals of fish lots sampled from wild trout populations in Colorado 2016-2017.

Discussion

The causative agent of bacterial kidney disease, *R. salmoninarum*, is widespread in Colorado's wild trout fisheries. While common, bacteria levels are generally low, and clinical disease is rare. After sampling 3,809 individual fish from 68 waters in all major river basins (all of which showed evidence of the presence of soluble antigen of the bacterium), no cases of clinical BKD were observed. Our results support the hypothesis that *R. salmoninarum* is common in inland trout populations, generally occurs at low levels, and that resident trout are somewhat resistant to the bacterium and are generally refractory to clinical BKD (Meyers 1993). High prevalence of *R. salmoninarum* in wild, non-anadromous trout and char has been reported in Iceland (Guðmundsdóttir et al. 2017), Alaska (Meyers et al. 1993), Michigan (Eissa et al. 2007), and Wyoming (Mitchum et al. 1979).

Table 1. Summary of environmental and stocking variables of waters sampled in Colorado 2016-2017 by the dominant species present at each site.

Dominant Species	Elevation (m)	Drainage Area (km^2)	Mean August Temp (C)	Stream Order	Total Fish Stocked 1987-2008
Brook Trout	2,758	60	11.2	3.1	4,130
Brown Trout	2,509	384	13.3	3.6	103,850
Rainbow Trout	2,236	299	15.3	3.4	5,149
Mountain Whitefish	1,980	1,656	14.1	5.0	20,266

Environmental and fish stocking variables that we hypothesized to influence *R. salmoninarum* antigen levels in wild trout explained less than 13% of the variability in ELISA OD values. While the effect sizes were not large, there were influential correlations between antigen levels and stream order (P = 0.030), drainage area (P = 0.004), and historical stocking rates (P = 0.027). Antigen levels were highest in small streams (low stream order and small drainage area) that had low historical stocking rates. Differences in species distribution and the infection intensity of the trout species could explain these patterns. Brook Trout are more likely to occupy smaller high-elevation streams in Colorado due to colder temperature preferences than Brown Trout or Rainbow Trout (Behnke 2002, Table 1). Brook Trout had the highest average ELISA OD levels as well as the highest prevalence by DFAT and qPCR of the fish species we tested. Therefore, the trend of higher OD values in smaller streams could be an artifact of those streams being dominated by Brook Trout. Brook Trout are known to have higher prevalence of *R. salmoninarum* and have higher antigen levels than other resident trout species (Mitchum et al. 1979; Snieszko and Griffin 1955). The smaller, Brook Trout-dominated streams in our study were also less likely to be stocked, so the negative correlation between antigen levels and fish stocking could be a result of the species distribution on the landscape and unrelated to fish stocking. More work is necessary to explore species-related differences in bacterial levels as well as the environmental factors that may influence antigen and DNA levels of *R. salmoninarum* in trout fisheries in Colorado.

We expected the low sensitivity and lack of concordance between DFAT results and the results from other assays because the assays detect different macromolecules (antigens vs. DNA) and have different sensitivities. The fluorescent antibody test can detect the antigens of morphologically intact live or dead bacteria, ELISA detects soluble antigens, and PCR detects the DNA of live or dead organisms. The fluorescent antibody test is inconsistent at detecting low bacterial numbers and, under those conditions, DFAT results do not correlate well with other assays, especially in non-anadromous salmonids (Meyers et al. 1993; Elliott et al. 2013). In one of the larger studies of *R. salmoninarum* in resident trout, the DFAT assay did not detect bacteria in samples with ELISA OD values less than 0.17 and inconsistently detected the bacteria at OD values less than 0.98 (Meyers et al. 1993). Eighty-seven percent of our samples had ELISA OD values less than 0.17 and 99.6% were less than 0.98. The vast majority of fish samples in our study were unlikely to test positive by DFAT but actually had low levels of *R. salmoninarum* antigen. Using DFAT as a liberal screening method to identify only severe cases does not appear to be an effective strategy; it did a poor job of discerning high antigen or DNA cases identified by ELISA or qPCR. The results of DFAT testing were somewhat stochastic in which samples tested positive in comparison to other assays. Our results, as well as previous work, suggest that DFAT is not the optimal screening assay for the *R. salmoninarum* levels commonly observed in inland wild trout (Meyers et al. 1993).

Exact agreement between ELISA and PCR is not expected because they measure different macromolecules (antigen vs. DNA). These differences should not be interpreted as conflicting results but as useful information indicating different states of infection (Nance et al. 2010). Fish with various stages of infection would be expected to be present concurrently in wild salmonid populations (Nance et al. 2010; Elliott et al. 2013). The antigen of *R. salmoninarum* can be present in kidney tissue in fish recovering from infection and can be detected by ELISA up to three months after viable bacteria have been cleared from the fish (Pascho et al. 1997). Many lots and samples in our study were positive by ELISA but negative by qPCR, a pattern that reflects naturally resolving infections (Nance et al. 2010). Because *R. salmoninarum* is common in Colorado trout fisheries and resident trout are more resistant to the bacterium, it is likely that many trout in wild populations would have low level, resolving infections. Our results emphasize the importance of using both a molecular and immunological assay to reveal different stages of infection, especially in non-anadromous trout (Nance et al. 2010; Elliott et al. 2013).

Using DFAT to screen inland trout populations for *R. salmoninarum* is not recommended. Due to the lower bacteria levels of wild trout and the unreliable nature of the DFAT assay at these levels, it is an inappropriate screening assay at worst and uninformative at best. A quantitative tool that more reliably detects the DNA or antigen of R. salmoninarum at lower levels like qPCR or ELISA would be more useful. Using these tools to estimate bacteria levels and adopting a liberal threshold for "positive" waters such as ELISA OD values of 1.0 to 2.0 would be a logical strategy if waters must be classified for management or regulatory reasons. Managers should instead focus on using multiple assays (molecular and immunological) to quantify bacterial levels and interpret results with nuance. The overly simplistic paradigm of considering populations "positive" or "negative" is not an informative way of thinking about *R. salmoninarum* in resident trout.

The bacterium *R. salmoninarum* is common and widespread throughout Colorado's wild trout fisheries, but bacterial levels (indicated by antigen and DNA) are generally low and clinical disease is uncommon. Active infections (indicated by the detection of DNA) are rare, but the presence of lower levels of the bacteria's antigen is common and widespread, supporting the paradigm that resident trout are commonly resistant carriers of the bacterium. Clinical BKD is occasionally observed in spawning Brook Trout in small high-elevation streams in Colorado, but we have not documented any population-level effects. More work is necessary to investigate disease dynamics in wild trout populations. Bacterial kidney disease appears rare in wild trout populations in Colorado despite being an ongoing and potentially increasing problem in trout hatcheries.

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Utilization of Genetic Data to Inform Native Brook Trout Conservation in North Carolina

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Abstract - As North Carolina's only native salmonid, Brook Trout *Salvelinus fontinalis* is a fish of considerable ecological and cultural significance in the state, but anthropogenic alterations to the landscape and introductions of nonnative salmonids have fragmented and reduced its native range. As a result, the North Carolina Wildlife Resources Commission (NCWRC) has enacted numerous efforts to help conserve the species. Annual demographic surveys of self-sustaining Brook Trout populations have been on-going since 1978, which have also included successful efforts to document previously unidentified populations. Beginning in earnest during the 1990s, allozyme testing was used to assess patterns of hatchery introgression, with over 480 collections genotyped at the creatine kinase locus. In 2010, the NCWRC began using microsatellite markers to conduct an extensive survey of Brook Trout genetic diversity and variation. To date, 541 Brook Trout collections representing 11,090 individuals have been genotyped at 12 microsatellite loci. These data have provided insights into evolutionary relationships among populations, spatial patterns of genetic diversity, and the extent of hatchery introgression within populations. Ultimately, an increased understanding of genetic diversity and relatedness have been informative for determining that Brook Trout management in North Carolina may be best enacted at the level of individual populations. Moreover, we have used these data to actively guide stream restoration and population reintroduction activities. Over the last 15 years, NCWRC and its partners have used genetic data to prioritize habitat enhancement activities and guide 17 Brook Trout population reintroduction projects. In the future, we plan to continue expanding the microsatellite genetic baseline while also exploring the utility of phylogenomic analyses to inform Brook Trout conservation activities. Genetic and genomic approaches have great potential to improve the efficacy of conservation actions for Brook Trout in North Carolina and throughout its native range.

Background

As North Carolina's official freshwater fish, the only native salmonid, and a Species of Greatest Conservation Need (NCWRC 2015), Brook Trout *Salvelinus fontinalis* is a fish of considerable biological, social, and economic importance to the state. Introductions of nonnative salmonids (Rainbow Trout *Oncorhynchus mykiss* and Brown Trout *Salmo trutta*) and anthropogenic alterations to the landscape have fragmented and reduced the range of Brook Trout in the state and throughout its native range. Nonetheless, over 700 genetically isolated Brook Trout populations have been identified in North Carolina. Moreover, populations continue to be located within the state's rugged mountainous terrain, with 20−35 previously undocumented populations identified annually between 2016 and 2021. In addition to population discovery, the North Carolina Wildlife Resources Commission (NCWRC) has placed considerable effort into reassessing known Brook Trout populations, given the importance of the species (NCWRC 2013).

In conjunction with demographic monitoring efforts, the NCWRC has also been characterizing the genetic composition of Brook Trout within the state. This work was initially motivated by a desire to determine the amount of hatchery introgression due to legacy effects of intensive historical stocking activities (Rash et al. 2014), with concerns that hatchery introgression could have negative consequences for the long-term fitness and survival of wild Brook Trout populations. For this initial work, one allozyme locus (creatine kinase) was used to determine population origin (northern, southern, or mixed; Galbreath 2002; Cornelison et al. 2005) of wild Brook Trout populations. Based on studies of this single locus, it was determined that while the majority of populations examined showed evidence of hatchery introgression, others remained uninfluenced by domestic lineages.

This allozyme work was conducted at a time when the field of conservation genetics was rapidly advancing. It soon became apparent that newer molecular tools could play a greater role in supporting Brook Trout management in North Carolina, and the NCWRC was keen to apply these technologies to advance species conservation.

Pivoting to microsatellite genetics to guide management activities

In 2010, the NCWRC and U.S. Geological Survey Eastern Ecological Science Center initiated an ambitious and unique effort to identify and genotype Brook Trout populations using microsatellite markers. Microsatellite markers are highly variable neutral sites within the Brook Trout genome that can provide a wealth of information about patterns of diversity and gene flow within- and among-populations. To date, we have genotyped 11,090 Brook Trout at 12 or more microsatellite loci, representing 541 collections from across North Carolina. More details about database development, genotyping, and analyses can be found in Rash et al. (2014) and Kazyak et al. (2017, 2018, 2021, 2022).

From this database, we have been able to infer temporal and spatial patterns in Brook Trout genetic diversity and connectivity. Briefly, we found that most collections appear to represent single populations and, relative to Brook Trout populations at more northern latitudes, allelic diversity in North Carolina is generally lower (Kazyak et al. 2022). Effective population sizes (N_e) vary widely among populations but are often very small (32% and 78% of populations examined had an estimated $N_e < 10$ and 50, respectively; Kazyak et al. 2021) and suggest that many populations may be at risk of losing diversity through genetic drift. Remarkable levels of genetic differentiation exist among populations, indicating that little, if any, gene flow occurs among populations (Kazyak et al. 2021), and a substantial portion of the observed genetic variation can be attributed to differences among patches (44.8%). However, approximately 11.2% of genetic variation was found among collections within a single patch (Kazyak et al. 2021). Although low, this intra-patch variation is interesting given that patches (a group of contiguous catchments that contain self-sustaining trout) average only 800 ha in the southern Appalachian Mountains (Fesenmyer et al. 2007). In addition, the majority of populations show limited evidence of introgression by northern-origin hatchery strains (Kazyak et al. 2018; Figure 1). Taken together, these results suggest that the management of Brook Trout in North Carolina may be most appropriate at the level of individual populations.

This genetic baseline has also demonstrated the utility of fine-scale genetic data for stewarding Brook Trout populations into the future. For example, spatial patterns of genetic diversity and differentiation have been informative for prioritizing and designing Brook Trout restoration and reintroduction activities. The baseline has also highlighted the extent of genetic diversity among southern Appalachian Brook Trout populations, ultimately highlighting the region as a unique area of conservation concern within the species' range. Below we describe how the baseline has helped provide critical information to support our portfolio of Brook Trout management activities.

Applying results from microsatellite analyses to guide conservation

North Carolina maintains one of the more comprehensive genetic datasets to guide Brook Trout conservation, and research scientists at the U.S. Geological Survey Eastern Ecological Science Center engage in extensive communication with the NCWRC to interpret results in the context of management needs. In turn, the NCWRC works closely with its partners to use results from genetic analyses to identify, prioritize, and address conservation projects across western North Carolina. Given the breadth of opportunities and resource limitations, supporting information can be extremely beneficial to help guide conservation activities and increase the likelihood of project success.

Population Reintroduction

The NCWRC relies on genetic data to plan Brook Trout reintroduction activities (see White et al. 2022 for one such example). Specifically, during project planning, the genetic database is used to identify potential source populations with minimal evidence of hatchery introgression and high endemic genetic diversity and large effective population sizes (Figure 2). Translocating individuals from populations with high endemic genetic diversity and large effective

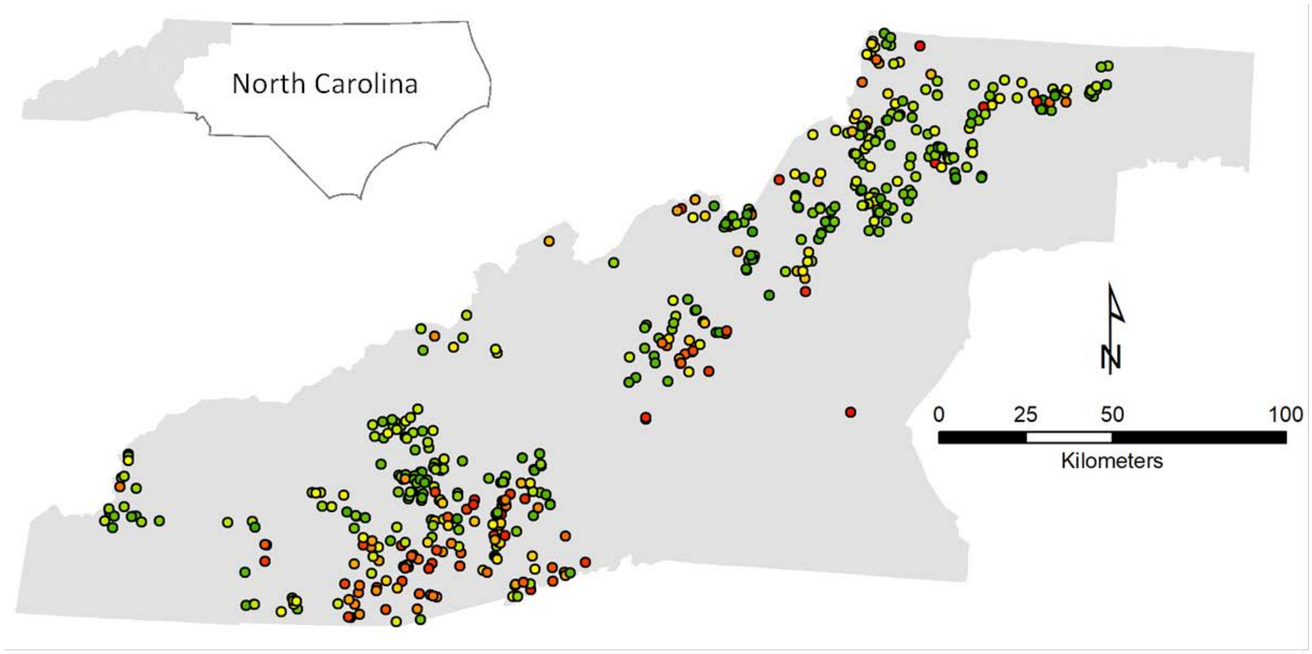

Figure 1.—Composite scores of three metrics (based on genetic distance, discriminant analysis of principal components, and Bayesian clustering analysis) to determine hatchery introgression in North Carolina's self-sustaining Brook Trout populations. Composite scores closer to 0 (red dots) represent greater levels of introgression, while scores approaching 1 (green dots) represent collections not influenced strongly by hatchery stockings.

population sizes provides the best chance for long-term survival and the potential to adapt (Jachowski et al. 2016).

Since 2007, NCWRC has initiated 17 Brook Trout reintroductions (Figure 3). Initial efforts were informed by allozyme data and propagules were selected from non-hatchery-influenced populations nearest to receiving waters (D. Besler, NCWRC, written communication). However, seven reintroductions have been completed since 2018 and were guided by results from microsatellite analyses, including an interstate effort in partnership with the Tennessee Wildlife Resources Agency. Numerous reintroduction opportunities remain (Figure 3) and highlight the future utility of the genetic baseline for continuing to support Brook Trout conservation in North Carolina.

Habitat Enhancement

The NCWRC works with multiple non-profit, state, and federal agencies to identify and initiate cold-water habitat enhancement projects. Efforts span the entire Brook Trout range in North Carolina, which includes waters on public and private lands. The NCWRC (in conjunction with partner input) maintains an extensive list of potential habitat enhancement projects and often uses the genetic baseline to prioritize, implement, and evaluate restoration activities. Addressing habitat issues can become costly, with expenditures scaling with project complexity and scope. Thus, all the habitat needs across the landscape cannot be addressed instantaneously, and so it is important to have tools that can assist in project prioritization. The genetic baseline provides one such tool, as information about regional population genetic diversity, differentiation, and hatchery introgression can help gauge the feasibility of reintroduction following habitat enhancement (see White et al. 2022). Alternatively, these measures of genetic integrity can also be used to identify contemporary populations that may be strongholds for genetic diversity, and thus, may merit increased habitat conservation.

Examples of recent projects include partnering with Trout Unlimited and the U. S. Forest Service to improve aquatic organism passage and water quality in Alarka

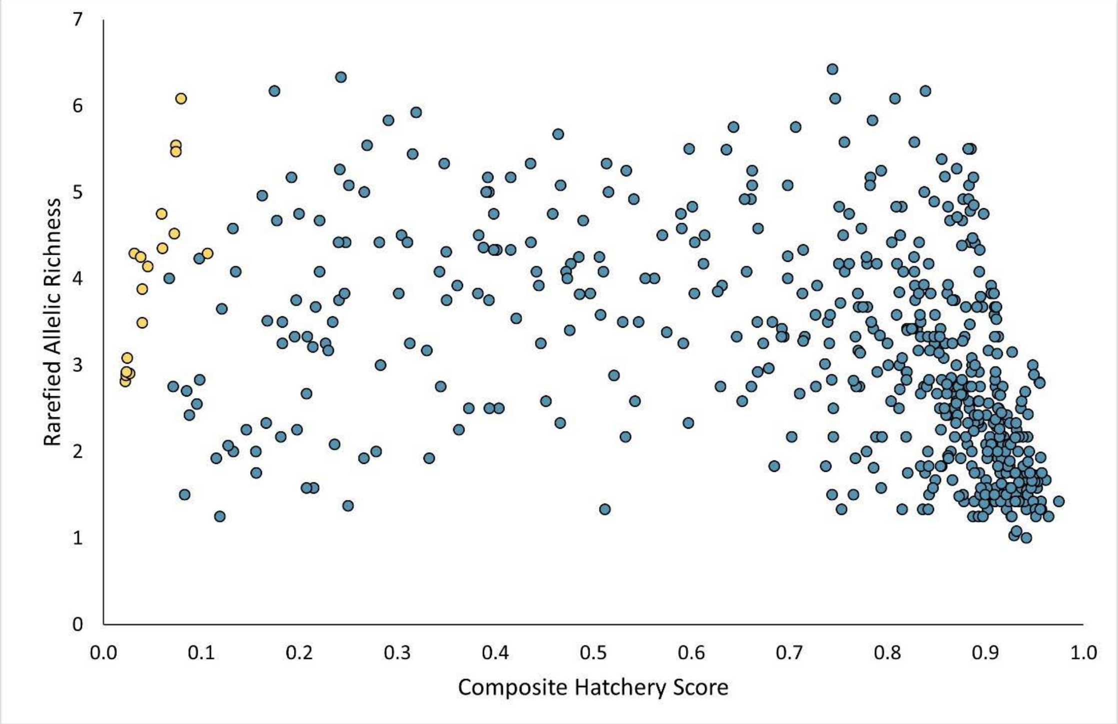

Figure 2.—Scatter plot representing genetic diversity (rarefied allelic richness) and hatchery-introgression (composite hatchery score) across all self-sustaining (blue dots) and hatchery (yellow dots) Brook Trout collections examined. Populations in the upper right quadrant exhibit higher genetic diversity values and lower levels of hatchery introgression, which make them favorable source populations in comparison to other collections.

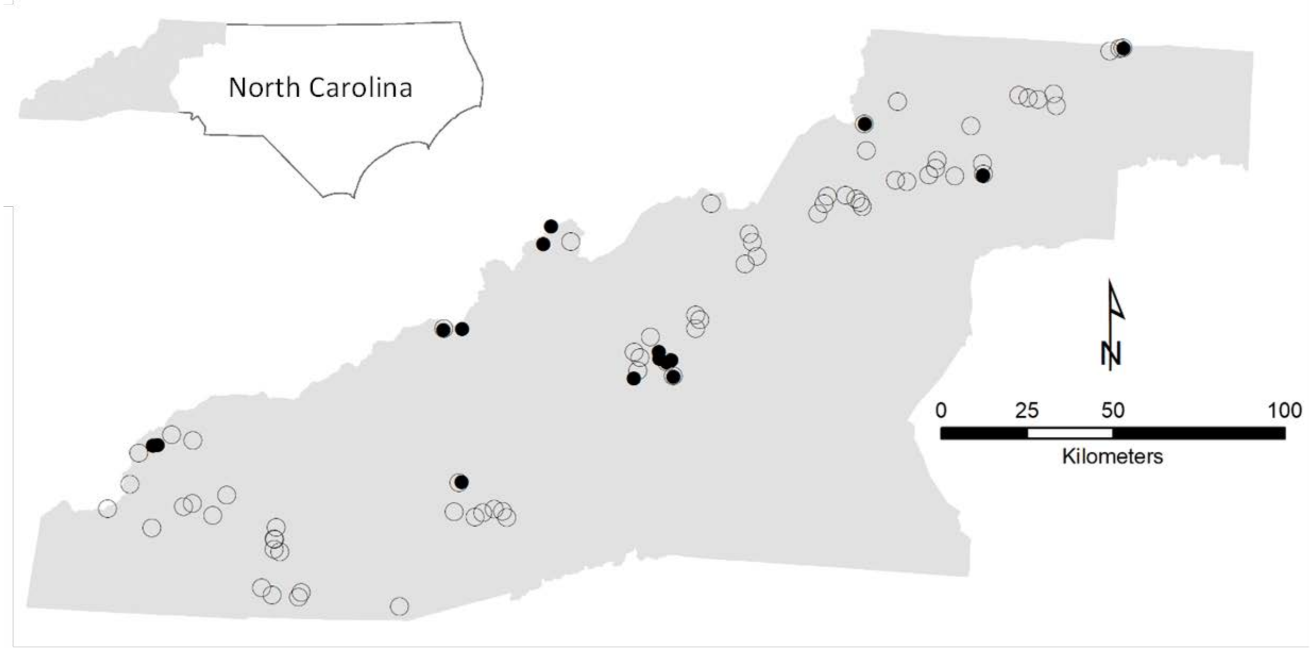

Figure 3.—Distribution of Brook Trout population restorations since 2007 (solid dots) and potential restoration opportunities (open circles) in North Carolina.

Creek and its tributaries and continuing that partnership to address sedimentation in Gragg Prong. The NCWRC also collaborated with Trout Unlimited and North Carolina State Parks to reconnect a fragmented Brook Trout population in Powdermill Creek. In addition, NCWRC is working with the Town of Beech Mountain to improve connectivity in Pond Creek via innovative culvert modifications.

Future directions

Although genetic data play an important role in North Carolina Brook Trout conservation, there are additional opportunities to continue refining our understanding of appropriate conservation and management activities. The genetic database has been expanded over time, and the addition of new collections (e.g., newly discovered or previously unsampled populations) will continue to enhance its utility for Brook Trout management. As the database has matured, so too has the ability of NCWRC to effectively plan and monitor habitat restoration and Brook Trout reintroduction activities. As the list of potential Brook Trout conservation projects has expanded, there is now a challenge of identifying the time and resources needed for project implementation.

NCWRC staff play essential roles by assisting in initial distribution assessments, identifying potential reintroduction sites, collecting tissue for genetic samples, participating in collection and translocation of propagules, and evaluating translocation success. Given the scope and complexity of ecological challenges which remain, such activities require careful allocation of limited resources. However, the genetic database is a tool that may help to prioritize efforts, reduce uncertainty, and improve conservation outcomes.

The North Carolina Brook Trout genetic baseline is an effective tool for assessing patterns of diversity among populations; however, because microsatellites are neutral genetic markers that are not under selection, they provided a limited ability to understand adaptive potential and deep evolutionary lineages among populations. In the future, a companion genomics database would help improve our understanding of questions such as *how long have populations been isolated* and *how are adaptive genes distributed across the landscape*. Genomics data would also improve the ability to more effectively perform Brook Trout translocations (i.e., reintroduction or genetic rescue) and evaluate success.

Conclusions

The genetic baseline has been foundational in our efforts to conserve native Brook Trout in North Carolina. However, the ultimate outcomes will not be realized for many decades, as management actions informed by contemporary genetic data have propagated through multiple generations of wild Brook Trout. Initial assessments of reintroductions are positive, but it will take many generations to determine whether present-day management activities were successful for conserving Brook Trout populations into the future.

Despite this uncertain future, genetic data are providing a synoptic view of current conditions and allowing managers to use the best available science to make informed decisions and prioritize limited conservation resources. While the baseline is specific to North Carolina Brook Trout, the information and patterns and processes that shape genetic diversity have been communicated to a wider audience. This information exchange has allowed other fisheries managers to remain informed of on-going efforts within North Carolina and advances in our understanding of range-wide patterns of genetic diversity (see Kazyak et al. 2022). Consideration of spatial scales of genetic diversity is important as Brook Trout conservation often spans administrative boundaries and can benefit from collaborative approaches (including long-term evaluation of management actions informed by genetic data).

The genetic baseline provides an important tool to assist in the conservation and management of a fish species that is beloved by the public. Modern anglers appreciate special opportunities to interact with this native fish, but ask anyone that has a generational connection to the North Carolina mountains and they have probably heard a story about Brook Trout or “specks” as they are known locally. That cultural significance cannot be overstated, and NCWRC looks forward to applying genetic information to help keep our native trout where it belongs: throughout the Appalachian Mountains.

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Session 5
Molecular Studies to Support Wild Trout Conservation

Avoiding the Telephone Pole Family Tree: A Management Strategy for Retaining Genetic Diversity in Utah's Wild Cutthroat Trout Broods

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Abstract - We conducted a review of all of the inland Cutthroat Trout *Oncorhynchus clarkii* subspecies conservation strategies in the intermountain west. This review revealed a common theme of the need to maintain genetically pure fish sources. Several of these strategies further postulate the importance to retain pure broodstock populations with which struggling populations may be supplemented or vacant habitats may be colonized via progeny from these broodstocks. With that in mind, maintaining genetic integrity and adequate allelic diversity is of paramount importance if broodstocks are to represent entire populations of fish.

The State of Utah continuously operates nine different Cutthroat Trout broodstocks for conservation and sportfishing purposes. While we typically operate these as wild broodstocks, we have recently introduced several into a hatchery environment. These broodstocks have been in continuous operations for 4 to 25 years with no outside genetic exchange. In this discussion, we provide examples of how the State of Utah operates native Cutthroat Trout broodstock sources, how we are utilizing the progeny created, how we plan to avoid inbreeding issues typical of hatchery selection and a lack of outside genetic exchange, and the challenges associated with operating and maintaining these broodstocks.

Introduction

Maintaining genetic integrity of a native species is of paramount importance when considering conservation actions (Belnap 1995), especially when considering genetic issues surrounding translocation from single sources (Hogg et al. 2020), and issues surrounding continued stocking in a fisheries management context (Marie et al. 2010). Within the realm of inland Cutthroat Trout *Oncorhynchus clarkii* conservation, genetic diversity and representation issues are further exasperated by population-level declines associated with habitat loss, disease, and drought, with nonnative fish species, competitive suppression, and hybridization arguably having the greatest impact (Young et al. 1996). The obvious consequence is that once a unique segment, genetic variation, or hereditary trait of a population is lost, it is lost forever and may not be regained.

There are currently five subspecies and/or lineages of inland Cutthroat Trout within Utah that are recognized by state and federal entities and by the Western Native Trout Initiative. These include the Yellowstone Cutthroat Trout *Oncorhynchus clarkii bouvieri*, Colorado River Cutthroat Trout *Oncorhynchus clarkii pleuriticus*, Bonneville Cutthroat Trout *Oncorhynchus clarkii* utah, Bear Lake Cutthroat Trout *Oncorhynchus clarkii* utah, and green lineage Colorado River cutthroat trout.

The Utah Division of Wildlife Resources (DWR) continuously operates nine distinct Cutthroat Trout broodstocks for conservation and sportfishing purposes. The majority of these broodstocks are maintained at local lakes to prevent domestication and encourage wild traits and survival. These broodstocks exist for Colorado River Cutthroat Trout, Bonneville Cutthroat Trout, and Bear Lake Cutthroat Trout subspecies. Yellowstone Cutthroat Trout and green lineage Colorado River Cutthroat Trout do not have substantial habitat in Utah where conservation needs have thus far been met with nearest neighbor translocations. The Utah DWR has recognized the importance of maintaining genetically pure brood populations (Oplinger 2020). Further, the Conservation Strategy for Colorado River Cutthroat Trout (CRCT Coordination Team 2006), of which the Utah DWR is a signatory, defines under Strategies 4 and 5 that broodstocks be developed for the purposes of protecting, propagating, and reintroducing populations. Thus, it stands to reason that broodstocks should represent localized adaptations and consist of the highest degree of genetic diversity represented of a population as possible.

Fish health issues present a very real problem for continued management of wild broods and introducing fresh genetics into existing brood populations. The whirling disease pathogen *Myxobolus cerebralis* has been detected in a majority of waters throughout the state of Utah. Additionally, certifying a water as pathogen free often takes up to a year, which prevents quick remediation of any potential problems.

This manuscript focuses on management surrounding three of the Utah DWR operated Colorado River Cutthroat Trout broodstocks within the northeast quadrant of the state. Each represents a sub-geographic management unit (GMU) of occupied species range. These include the North Slope Uinta, South Slope Uinta, and North Tavaputs Colorado River Cutthroat Trout (CRCT) sub-GMU's. The "ages" of each brood, defined as that period when no additional wild fish were added and were exclusively maintained by hatchery stocking, range from 4 to 20 years. We individually describe our future application of each brood below.

Management History and Future

North Tavaputs

In 2001, the Utah DWR purchased a 40-acre lake, named Lake Canyon Lake, for the purposes of creating a North Tavaputs broodstock of CRCT. This broodstock is exclusively made up of a population of CRCT that were residents of Timber Canyon Creek. No wild fish have been infused into the brood since its completion in 2007. The population consists entirely of fish reared for one year at a local hatchery. The lake is very productive and grows large individuals, however it experienced a winterkill situation over the winter of 2018-2019, causing the loss of approximately 85% of the brood population. A small tributary to the lake exists where CRCT were stocked in the past. This small tributary was vital to maintaining our ability to continue to collect sufficient gametes to maintain the population. Since the winterkill event of four years ago, we have successfully rebuilt the brood to pre-winterkill levels.

We will continue to operate Lake Canyon Lake as a broodstock for the North Tavaputs sub-GMU. We are currently planning a supplementation project in spring 2023 to infuse additional genetic diversity into the brood at Lake Canyon Lake. Over the course of at least two years, we will capture, segregate by gender, and temporarily hold CRCT in net cages on nearby Avintaquin Creek, collect fertilized gametes from that system, and rear these fish for one year at a local fish hatchery before stocking them into the brood. A wild fish transfer is not an option due to whirling disease being detected at Avintaquin Creek. Our annual fish stocking quota into Lake Canyon Lake will continue to be 1200 individuals. During the genetic supplementation phase, we plan to harvest gametes from at least 30 couplings from Avintaquin Creek. We are planning for 800 of the 1200 fish quota to come from the Avintaquin Creek fish. Given that we are typically able to host spawns per generation of fish, we feel this mixture appropriate to infuse fresh genetics while retaining most of the current characteristics from our extant brood. We plan to repeat this infusion cycle from wild stream sources every nine years.

North Slope Uinta

The North Slope Uinta brood is formed completely of wild fish from North Fork Sheep Creek. Due to limited quantities of adult fish that are small (max size = ~220mm with low fecundity), the Utah DWR elected to host this as a captive broodstock beginning in 2017. The brood currently resides at Mammoth Creek State Fish Hatchery. We were able to procure three brood year classes to form the base of the brood. We have had poor eye-up and survival of progeny of the original brood year classes since its second year of operation. Our F2 generation will begin maturing in the spring of 2023. We will continue to evaluate survival and success of our captive North Slope Uinta brood. We expect to begin infusing additional gametes into the brood in the spring of 2026 to ensure the continued population representation and viability of this broodstock. The genetic representation of this brood is particularly important as we continue to reclaim and repatriate CRCT in areas of this sub-GMU in the near future.

South Slope Uinta

The original South Slope Uinta brood was formed from wild fish transfers from the West Fork Duchesne River, finishing in 2002. These fish were stocked into Sheep Creek Lake, which the Utah DWR constructed in 1959 for the purposes of Cutthroat Trout propagation. No wild-origin fish have been added to this brood since that time. The typical annual hatchery quota for Sheep Creek Lake is 4,000 age-1 fish. We opted to run several genetics sampling tests between 2012-2016 to ensure that our broodstock was pure. The reason we ran multiple tests was because results indicated that our brood had a 4-6% non-native Yellowstone Cutthroat Trout component in their makeup.

Beginning in 2018, we elected to conduct an ambitious broodstock replacement project. We made this determination because of the genetic impurity detected as well as the fact that the brood had been through several population bottlenecks that likely led to some levels of inbreeding. A brood replacement project was deemed the easiest and most effective way to ensure that we would have a genetically pure and robust population that we could utilize to repatriate future reclamation areas. We elected to supplement this broodstock with CRCT from the West Fork Duchesne River after multiple genetics tests determined that population to be pure. The West Fork Duchesne contains the largest lotic population of CRCT within Utah and could withstand the pressure of collecting extensive numbers of fish and gametes. However, the stream also contains the whirling disease fish pathogen. From 2018 through 2022, we spawned between 65-130 couplings with streamside egg takes and achieved a typical green egg yield of about 60,000 to 90,000. In the future, we plan to continue to infuse wild-origin fish into the brood on a 9-year cycle. In order to achieve sufficient new fish contribution (in order to avoid inbreeding), we will select two consecutive years within this 9-year cycle. During these two years, a minimum of half of the quota for our brood source will be from the wild-origin source. The remaining quantity will be filled from brood replacement from within the brood, as is the case with 100% of the quota for the remaining 7 years.

Conclusions and Implications

The Utah DWR has assembled a timetable for how we plan to complete additional genetic supplementation into our wild broods in the future (Table 1). Generally, we will operate on a 9-year cycle. We will have to plan ahead to ensure that the source populations are disease-certified ahead of spawning operations. Due to the prevalence of the whirling disease pathogen, we will be opting for streamside egg-take operations to collect gametes that can be disinfected, and then stocked into brood waters, rather than actual live fish transfers. Generally we plan to conduct these operations across 2-year periods to ensure survival from multiple-year classes in order to reduce the risk of complete year-class failure or high mortality events. Consultation with several geneticists has indicated that our supplementation plans will be more than sufficient to replace genetic diversity loss that occurs due to artificial spawning and subjugation to hatchery conditions, given the number of fish we plan to contribute and the frequency of supplementation. Properly maintained broodstocks are a key element to our native Cutthroat Trout repatriation program in order to ensure that newly established populations have sufficient and rigid genetic integrity to be representative of wild populations.

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Table 1. Northeast Region Colorado River Cutthroat Trout broodstock genetic supplementation timetable. Adapted from Oplinger (2020).

	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040
NS Sheep Creek Lake																					
Disease Collection					Y	Y	Y							Y	Y	Y					
Fish Transfer Performed						Y	Y	Y							Y	Y	Y				
SS Sheep Creek Lake																					
Disease Collection (WF Duchesne)	Y							Y	Y	Y							Y	Y	Y		
Fish Transfer Performed	Y	Y							Y	Y	Y							Y	Y	Y	
Lake Canyon																					
Disease Collection (Avintaquin)		Y	Y	Y							Y	Y	Y							Y	Y
Fish Transfer Performed			Y	Y	Y							Y	Y	Y							Y

A New Dawn As Neuro-Endocrinology Meets Wild Fish Management: Fish Scale Cortisol To Quantify Chronic Stress Originating From Local And Broad Scale Environmental Constraints Contributing to Accurate Monitoring and Conservation of Wild Fish Populations

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Abstract - An ever-increasing human population makes the need for sustainable food production as well as wildlife conservation a global public, scientific, and political prime priority. Besides industrial aquaculture and recreational and commercial fisheries, fish are highly relevant in the framework of conservation biology and environmental protection efforts. Anthropogenic activities such as energy production, shipping, and pollution significantly impact ecosystems by inducing climate change (e.g., increasing water temperature, ocean acidification), through loss of fish nurseries, addition of novel predators, addition of novel animal-environment relationships, and in all compromising wild fish stocks and even entire ecosystems. Therefore, various international monitoring schemes aim to clarify their impact on the health status of oceanic as well as freshwater niches.

Fish faced with stressful stimuli launch an endocrine stress response through activation of the hypothalamic-pituitary-interrenal (HPI) axis to release cortisol, the dominant and highly pleiotropic glucocorticoid in teleost fish known to impact a plethora of metabolic pathways, into the blood. Cortisol elicits a suite of physiological and behavioral changes allowing fish to cope with altered situations. Where short-term cortisol-mediated actions are adaptive, long-term elevations of cortisol are detrimental to fish performance, reproduction, and survival as it makes individuals more susceptible to disease thereby increasing mortality. Therefore, cortisol in fish scales, a scientifically validated biomarker capturing systemic cortisol exposure over long periods of time, is of utmost importance to assess chronic stress in governmental, wildlife, aquaculture, and scientific settings.

Here we focus on how fish scale cortisol is applied as a powerful tool in wildlife monitoring and conservation by (i) quantifying chronic stress from (anthropogenic-induced) environmental constraints enabling accurate monitoring of gradual climate change, the loss of nurseries, the impact of pollution, etc. in wild fish and their ecosystem, as well as (ii) breeding fish for stress resilience enabling to replenish wild stocks with more stress resilient fish counteracting dwindling populations.

The Origin and Ancestry of *Oncorhynchus mykiss* in the Wood River basin of Central Idaho

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Abstract - The origin and taxonomic identification of trout within the isolated Wood River basin of central Idaho has been in question for more than 120 years. The earliest surveys described trout specimens from the Wood River as Cutthroat Trout *Oncorhynchus clarkii*. Later surveys described them as Rainbow Trout *O. mykiss* and based on meristic examination of a single museum specimen, it was suggested they were a relict form of Redband Trout. Genetic investigations conducted over the last 30 years, using a variety of genetic markers, assumed that the native trout in the Wood River Basin were Columbia River Redband Trout *O. m. gairdneri,* but had been extensively introgressed or replaced with hatchery coastal Rainbow Trout *O. m. irideus*. In an attempt to disentangle the various hypotheses of native contemporary, native relict, non-native, or some admixture between native and non-native forms, we greatly expanded the sampling and genetic screening that had been completed in previous studies. Our results suggest that *O. mykiss* are native to the Wood River Basin, have been isolated for a long period of time, and represent a unique, old, and previously undescribed lineage of *O. mykiss*. Surprisingly, despite extensive hatchery stocking throughout the basin, introgression from non-native hatchery Rainbow Trout of coastal origin appears limited. We discuss management and conservation implications for current populations within the basin.

***Oncorhynchus mykiss* from the Wood River, Idaho (fish caught and photographed by Shawn Narum).**

Introduction

The origin and taxonomic identification of trout within the isolated Wood River basin of central Idaho has been in question for more than 120 years. The earliest surveys described trout specimens from the Wood River as Cutthroat Trout *Oncorhynchus clarkii* (Gilbert and Evermann 1894). Later surveys described them as Rainbow Trout *O. mykiss* and based on meristic examination of a single museum specimen, it was suggested they were a relict form of Redband Trout (Behnke 1979). Genetic investigations conducted over the last 30 years, using a variety of genetic markers including allozymes (Leary 2001; Williams et al. 1996), mitochondrial DNA (Williams and Shiozawa 1993), and microsatellite DNA (Kozfkay et al. 2011), assumed that the native trout in the Wood River Basin were Columbia River Redband Rrout *O. m. gairdneri*, but they had been extensively introgressed or replaced with hatchery coastal Rainbow Trout *O. m. irideus.*

In an attempt to disentangle the various hypotheses of native contemporary, native relict, non-native, or some admixture between native and non-native forms, we greatly expanded the sampling and genetic screening that had been completed in previous studies. To examine the possibility of a non-native origin or intraspecific introgression we included the major lineages of Rainbow Trout and Cutthroat Trout that have been used in hatchery production and stocking purposes throughout the western United States. We included genetic markers that differentiate *O. clarkii* from these taxa from *O. mykiss* populations. Finally, in order to compare the genetic diversity and differentiation of Wood River *O. mykiss* in a broader phylogenetic context, we included samples of *O. mykiss* from throughout the Columbia River Basin.

Methods

Identifying reference populations for assessing inter- and intraspecific hybridization

To determine the reference populations to assess intra- and interspecific hybridization/introgression, we first queried Idaho Department of Fish and Game's (IDFG) historical stocking database from 1913 to the present (https://idfg.idaho.gov/ifwis/fishingplanner/stocking/). We queried all stocking that had taken place in the Wood River Basin during this period and summarized all identified strains of Rainbow Trout, Cutthroat Trout, or hybrids. Strains labeled with the designation "triploid" were excluded. We identified 18 strains of Rainbow Trout stocked in the Wood River Basin as well as one designation identified as "Unspecified Rainbow". In total, more than 80 million Rainbow Trout were stocked in the basin from 1913 until 2000, when stocking switched primarily to non-fertile triploid Rainbow Trout stocks (Kozfkay et al. 2006). We also identified the stocking of steelhead (anadromous Rainbow Trout) x Cutthroat Trout hybrids, Rainbow Trout x Cutthroat Trout hybrids, Westslope Cutthroat Trout *O. c. lewisi,* Yellowstone Cutthroat Trout *O. c. bouvieri* (Henrys Lake origin), and unspecified Cutthroat Trout. These additional species/strains totaled ~3 million stocked fish over this period.

Of the 18 strains of hatchery Rainbow Trout we identified, we obtained reference genetic samples (N = 1,249) for 17 (strain and collection details available from authors). These samples came either from collections that we already had archived or from requests from other labs. Although we did not find records of McCloud River redband *O. m. stonei* strain having been stocked in the Wood River Basin, we did obtain reference samples from three populations. In doing so, we had reference samples from four of the primary California *O. mykiss* lineages (coastal, Eagle Lake, Golden Trout and McCloud River) used for hatchery strain development and stocking purposes (Behnke 1992). We felt this was important, since the vast majority (~78%) of stocking in the Wood River basin came from "Unspecified Rainbow" sources. In addition to these hatchery reference populations, we also included samples of Rainbow Trout from two populations in the Henrys Fork Basin in the upper Snake River, Idaho. As with all waters above Shoshone Falls on the Snake River, the Henrys Fork drainage historically did not contain populations of *O. mykiss* (Behnke 1992). It was stocked with hatchery Rainbow Trout during the same period as the Wood River Basin and most of the *O. mykiss* stocking came from "Unspecified Rainbow". The Henrys Fork drainage now supports robust, self-reproducing Rainbow Trout populations. We assumed, given the close geographic proximity to the Wood River basin, that the same sources of hatchery Rainbow Trout would have been stocked in both watersheds and these samples would provide good references for comparison purposes.

O. mykiss samples from the Wood River

Samples were collected via backpack electroshocking from throughout the Wood River Basin upstream of a barrier waterfall (Malad Gorge; Figure 1). Samples were collected between 1999 and 2021 (47 collections, 1,767 samples; Table 1). Samples were stored in 100% non-denatured ethanol or on Whatman filter sheets.

DNA Extractions and Genetic Marker Panel

We extracted DNA from all samples using the nexttecTM

Table 1. Pedigree and collection name, sample size, total samples identified in Newhybrids as O.mykiss, Cutthroat Trout (CUT), F1, F2, Rainbow Backcross (RBT-BC), Cutthroat Trout Backcross (CUT-BCC), or FN hybrid (not assigning to other categories). Collection Names in bold exhibit interspecific hybridization. Also included are average "coastal" ancestries observed in each collection (Structure or DAPC). The three locations that showed evidence of coastal Rainbow Trout introgression with both analyses are shown italized.

Map	Collection Name	N	O. mykiss	CUT	F1	F2	RBT-BC	CUT-BC	F_N hybrid	Structure	DAPC MP
1	*Little Wood R. '03*	31	31	0	0	0	0	0	0	*0.278*	*0.139*
2	**Little Wood R. '99**	**103**	**73**	**4**	**10**	**1**	**4**	**4**	**9**	0.039	0.000
3	Little Wood R. '21	46	46	0	0	0	0	0	0	0.082	0.000
4	*Silver Cr. '05*	43	43	0	0	0	0	0	0	*0.243*	*0.044*
5	Cold Springs Cr. '20	40	40	0	0	0	0	0	0	0.062	0.000
6	Porcupine Cr. '20	30	30	0	0	0	0	0	0	0.016	0.000
7	Grays Cr. '99	13	5	0	0	**1**	**7**	0	0	0.010	0.000
8	**Little Wood R. '20**	**49**	**42**	**0**	**2**	**0**	**5**	**0**	**1**	0.029	0.000
9	Friedman Cr. '03	14	14	0	0	0	0	0	0	0.037	0.000
10	Friedman Cr. '21	15	15	0	0	0	0	0	0	0.043	0.000
11	Muldoon Cr. '04	24	24	0	0	0	0	0	0	0.057	0.000
12	Copper Cr. '05	9	11	0	0	0	0	0	0	0.057	0.000
13	**Mormon Gulch '20**	**46**	**30**	**0**	**3**	**2**	**6**	**0**	**5**	0.023	0.000
14	N.F. Soldier Cr. '20	4	4	0	0	0	0	0	0	0.022	0.000
15	Willow Cr. '03	41	43	0	0	0	0	0	0	0.034	0.000
16	W.F. Willow Cr. '20	50	50	0	0	0	0	0	0	0.004	0.000
17	W.F. Rock Cr. '21	20	20	0	0	0	0	0	0	0.063	0.000
18	Deer Cr. '99	59	59	0	0	0	0	0	0	0.025	0.000
19	Kinsey Cr. '20	50	50	0	0	0	0	0	0	0.040	0.000
20	Deer Cr. '21	50	50	0	0	0	0	0	0	0.034	0.000
21	Deer Cr. '20	50	50	0	0	0	0	0	0	0.042	0.000
22	Big Wood R. '03	46	46	0	0	0	0	0	0	0.052	0.000
23	Big Wood R. '21	179	179	0	0	0	0	0	0	0.056	0.000
24	Big Wood R. E.F. '03	30	30	0	0	0	0	0	0	0.039	0.000
25	Greenhorn Cr. '99	29	29	0	0	0	0	0	0	0.053	0.000
26	E.F. Big Wood R. '21	80	80	0	0	0	0	0	0	0.060	0.000
27	Hyndman Cr. '20	50	50	0	0	0	0	0	0	0.080	0.000
28	Elkhorn Gulch '21	17	17	0	0	0	0	0	0	0.092	0.000
29	Elkhorn Cr. '19	8	8	0	0	0	0	0	0	0.054	0.000
30	Trail Cr. '99	36	36	0	0	0	0	0	0	0.093	0.000
31	Warm Springs Cr. '03	26	26	0	0	0	0	0	0	0.093	0.000
32	Red Warrior Cr. '99	60	60	0	0	0	0	0	0	0.047	0.000
33	Rooks Cr. '21	28	28	0	0	0	0	0	0	0.100	0.000
34	Thompson Cr. N.F. '03	24	24	0	0	0	0	0	0	0.061	0.000
35	N.F. Thompson Cr. '21	50	50	0	0	0	0	0	0	0.069	0.000
36	Castle Cr. '99	27	27	0	0	0	0	0	0	0.030	0.000
37	Placer Cr. '20	49	49	0	0	0	0	0	0	0.020	0.000
38	Castle Cr. '20	48	49	0	0	0	0	0	0	0.056	0.000
39	S.F. Warm Springs Cr. '21	50	50	0	0	0	0	0	0	0.035	0.000
40	Adams Gulch '99	40	40	0	0	0	0	0	0	0.016	0.000
41	*Trail Cr. '03*	12	12	0	0	0	0	0	0	*0.236*	*0.155*
42	Trail Cr. '21	40	40	0	0	0	0	0	0	0.073	0.000
43	Wilson Cr. '20	9	9	0	0	0	0	0	0	0.011	0.000
44	Fox Cr. '21	50	50	0	0	0	0	0	0	0.027	0.000
45	**Baker Cr. '21**	**27**	**24**	**0**	**3**	**0**	**0**	**0**	**0**	0.041	0.000
46	E.F. N.F. Big Wood R. '20	45	45	0	0	0	0	0	0	0.048	0.000
47	Trail Cr. '20	17	17	0	0	0	0	0	0	0.021	0.000

Genomic DNA Isolation Kit from XpressBio (Thurmont, Maryland). All samples were genotyped with a panel of 379 loci described in Collins et al. (2020). We used custom scripts to identify loci within the panel that exhibited additional Single Nucleotide Polymorphisms (SNPs) not described by Collins et al. (2020) and then genotyped these loci for all identified SNPs while inferring phase from the sequencing reads. In total, we identified 229 microhaplotype loci that exhibited > 3 alleles and genotyped well in reference and study populations. This panel was genotyped following Polymerase Chain Reaction, barcoding, and library protocols described in Collins et al. (2020) and libraries were sequenced on an Illumina NextSeq 500 instrument.

Genetic Assessments of Inter- and Intraspecific Hybridization

We assessed interspecific hybridization (from Cutthroat Trout) using a subset of 17 loci in our panel that exhibit fixed or nearly fixed diagnostic allelic differences between *O. mykiss* and Cutthroat Trout (IDFG unpublished data). We used the software program NewHybrids (Anderson and Thompson 2002) to estimate the probability that a sample belongs to one of six *a posteriori* hybrid categories based on patterns in allelic inheritance. Fish were classified into the following categories: RBT (non-hybridized *O. mykiss*), CUT (non-hybridized *O. clarkii*), first generation hybrids (F_1), second generation hybrids (F_2), *O. mykiss* Trout backcrosses (RBT-BC) or Cutthroat Trout backcrosses (CUT-BC). Individuals that did not assign to a single category with a posterior probability >99% were classified as "FN hybrid" (i.e. belonging to a hybrid class not defined by NewHybrids and are likely later stage backcrosses). For these analyses, we included reference samples from various Idaho locations including Yellowstone Cutthroat Trout from Henrys Lake, Palisades Creek, Bonneville Cutthroat Trout from the Bear River (Cottonwood Creek), Westslope Cutthroat Trout from Ball Creek, and known F_1 hybrids from Henrys Lake along with all samples from Wood River Basin collections. NewHybrids was run with 50,000 burn-in iterations followed by 50,000 sample iterations, with reference individuals identified.

To assess the possibility of intraspecific hybridization/introgression, we included the baseline of reference hatchery *O. mykiss* strains discussed above and samples of *O. mykiss* from a wide geographic area of Idaho. This included sample collections of *O. mykiss* from the Boise, Salmon Falls, and Owyhee basins of southern Idaho, geographically proximate to the Wood River Basin. We also included native resident and anadromous *O. mykiss* from the Snake, Salmon, Clearwater and Kootenai rivers of Idaho. We used two methods to assess genetic population structure and to evaluate the ancestry of *O. mykiss* populations in the Wood River. We used the Bayesian model-based method implemented in the software Structure 2.33 (Pritchard et al. 2000) to identify genetic clusters and estimate ancestry coefficients (q-values) and the non-model based method of discriminate analysis of principle components (DAPC) using the R package Adegenet version 1.3-1 (Jombart et al. 2008) to provide individual membership probabilities for each sample.

To assess the genetic relationships of *O. mykiss* samples from our entire dataset (147 collections, 8,327 samples) we again used DAPC analyses using Adegenet. We also constructed an unrooted, bootstrapped (N = 1000) Neighbor-Joining (NJ) tree based on pairwise Cavalli-Sforza Edwards chord distance (Cavalli-Sforza and Edwards 1967) calculated in GENDIST (PHYLIP v3,5; Felsenstein 1993). The tree was produced using the NEIGHBOR method in PHYLIP v3.5 and subsequently visualized and edited using FigTree v.1.4.3 (Page 1996).

Results And Discussion

Interspecific Hybridization

Hybrids between Cutthroat Trout and *O. mykiss* were observed in four sample locations (5 collections): Baker Creek (2021), Grays Creek (1999), Little Wood River (1999, 2020) and Morman Gulch Creek (2020;Table 1). All sample locations identified with hybrids, except Baker Creek (2021), were from the Little Wood River drainage. First generation hybrids were detected in 3 of the 4 sample collections, indicating recent hybridization events. The highest number of F1 hybrids were identified in the Little Wood River-1999 sample collection (N = 10). This sample collection also contained four samples with genotypes indicative of pure Cutthroat Trout. These samples were visually identified in the field as putative Cutthroat Trout. Of the 1,831 samples from the Wood River drainage examined, 1,767 (96.5%) exhibited genotypes indicative of pure *O. mykiss*.

Intraspecific hybridization was assessed based on ancestry coefficients (q-values) reported from Structure and membership probabilities provided by DAPC (Table 1). For both methods, we used 10 genetic clusters (K) to summarize results. This was a smaller number than the most likely number of genetic clusters identified by either method but provided a sufficient number from which to efficiently summarize the data (Jombart and Collins 2015). In other words, higher numbers (15 and 20) of genetic clusters did not change ancestry levels observed in Wood River collections. Any genetic cluster that received assignments from hatchery reference populations were designated as "coastal" clusters. For Wood River sample collections we combined values observed in "coastal" clusters to provide an estimate of coastal ancestry. Both methods identified three collections as having ancestry likely from coastal origin hatchery fish. Trail Creek-2003, Little Wood River-2003 and Silver Creek exhibited q-values of 0.236, 0.278 and 0.243 respectively and membership probabilities of 0.155, 0.139 and 0.044, respectively. Bohling et al. (2013) argued that q-values observed from Structure > 0.2 usually indicate the real detection of non-native ancestry. Of the remaining sample collections, Structure identified a low level of coastal ancestry in all populations (range 0.004 - 0.0997; average 0.0465). These results differed from DAPC analyses, which did not identify coastal ancestry in any of the remaining sample collections.

Intraspecific Hybridization

To assess overall genetic relationships among *O. mykiss* collections throughout the Columbia River Basin, Idaho,

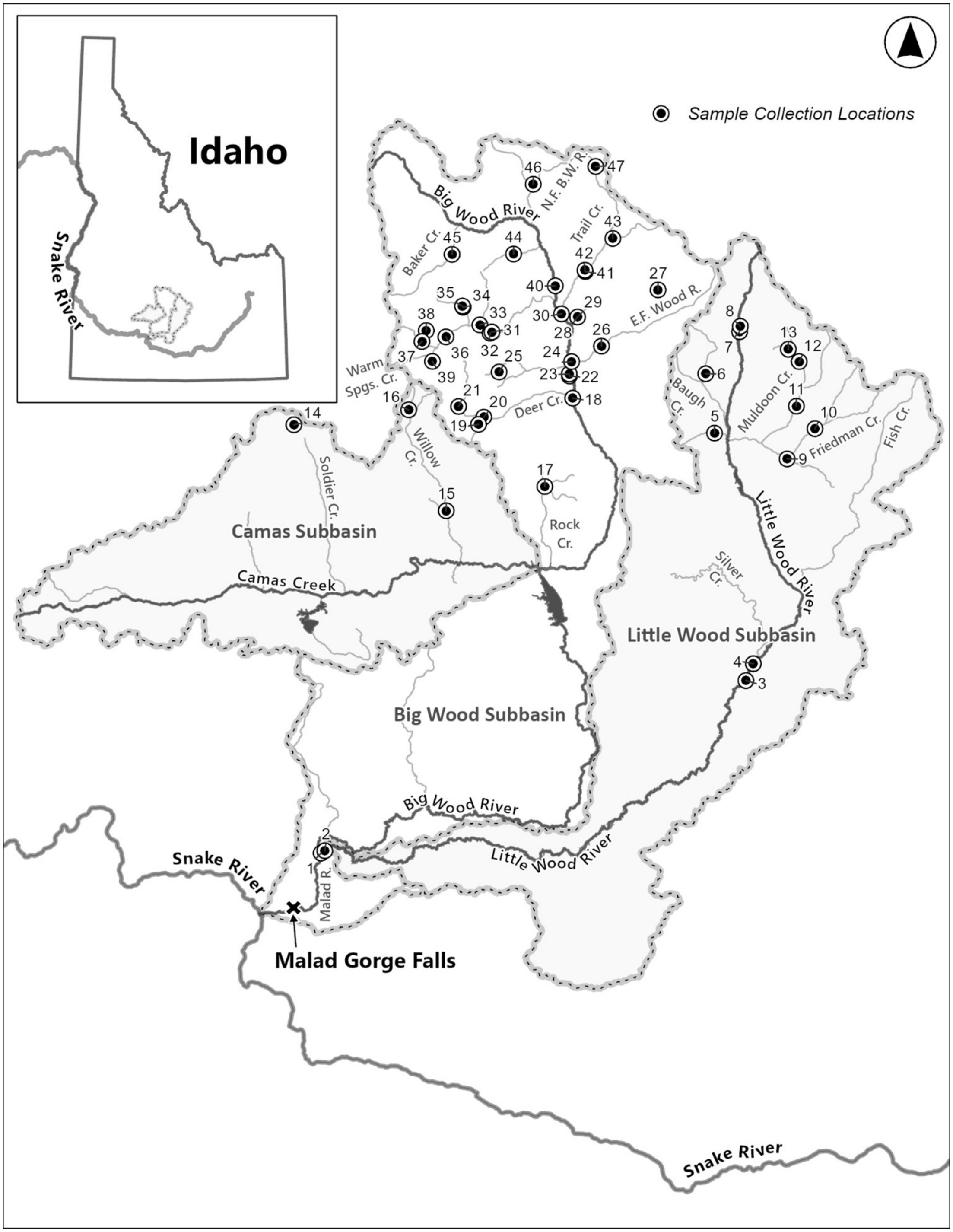

Figure 1. Map of sample collections within the Wood River Basin. Sample site numbers correspond to numbers in Table 1. Location of the Malad Gorge Falls is shown, which is an upstream migration barrier.

Great Basin Redband Trout, and California hatchery Rainbow Trout stocks, we included all collections/samples (147 collections, 8,327 samples) in DAPC and Phylip NJ tree analyses (Figure 2). In the DAPC analyses, the first discriminant separated Wood River *O. mykiss* collections (Cluster 9) and hatchery coastal Rainbow Trout strains *O. m. irideus* (Cluster 10) from all Columbia River Redband Trout *O. m. irideus* collections (Clusters 1,2,4,5, and 7). Known non-native Rainbow Trout from the Henrys Fork drainage in Idaho all assigned to Cluster 10 with hatchery coastal Rainbow Trout strains. The second discriminant separated all Wood River collections from all the hatchery coastal Rainbow Trout strains. Several collections of Great Basin Redband Trout and collections from the lower Columbia River, while showing strong individual clustering, yielded intermediate positions in the DAPC plot.

The NJ Tree topology and bootstrap support results were consistent with the relationships observed in the DAPC plot. For example, all collections of Columbia River Redband Trout clustered together (749 support). This included all collections in Idaho outside the Wood River Basin, including samples from the Kootenai River Basin, steelhead from the Clearwater, Grande Ronde, and Salmon River basins, and resident Redband Trout populations from the Dry Creek, Boise, Salmon Falls, and Owyhee River drainages. Collections of *O. mykiss* from the Wood River drainage all clustered together (768 support) and were diverged from hatchery coastal Rainbow Trout collections. All hatchery coastal Rainbow Trout strains clustered together (953 support) and were most similar to collections of *O. mykiss* from the Chewaucan, Goose, and Warner Lake drainages. Similar phylogenetic results were previously reported by Currens et al. (2009) using allozyme analyses. Also similar to Currens et al. (2009) study, we observed that collections from Malheur Lake appeared to be more genetically similar to Columbia Basin Redband Trout than to other Great Basin Redband Trout populations.

The isolated Wood River Basin has been noted previously as having "faunal peculiarities" (Hubbs and Miller 1948). The basin contains an endemic species of sculpin *Cottus leiopomus,* and genetically divergent populations of Bridgelip Sucker *Catostomus columbianus* (Smith 1966) and Mountain Whitefish *Prosopium williamsoni* (Miller 2006). Results from this study indicate that *O. mykiss* in the Wood River Basin appear genetically diverged from all other sampled *O. mykiss* populations in Idaho. This divergence is surprising given the speculation that the colonization timing of *O. mykiss* in the Snake River may be constrained by the Bonneville flood (14,500 years before present) and presumably the formation of Shoshone Falls, which marks the upstream range of *O. mykiss* in the Snake River (Behnke 1992). This divergence does not appear to be due to inter- or intraspecific hybridization despite an extensive history of stocking within the basin. We observed hybridization from Cutthroat Trout in only a limited number of sites and analyses suggest recent hybridization (pure CUT, F1 and backcrosses detected). These results can be explained by the previous stocking of diploid Westslope Cutthroat Trout in adjacent high mountain lakes to provide fishing opportunities. We ran two analyses to assess whether populations showed evidence of hatchery coastal Rainbow Trout ancestry. While both methods identified three sample collections that showed moderate levels of non-native introgression, the remaining sites showed low (avg.~5% from Structure) or no evidence of coastal Rainbow Trout ancestry (from DAPC).

Despite the fact that *O. mykiss* in the Wood River show limited evidence of hatchery coastal Rainbow Trout introgression, they appear to share a more recent (but distant) common ancestor with *O. mykiss* lineages found outside the Columbia River Basin. The ancestral Snake River was not connected to the Columbia River until the draining of ancient Lake Idaho during the late Pliocene (~2 million years ago; Gillerman et al. 2006). Instead, the Snake River was believed either to have flowed southeast through Oregon to California into the Klamath or Sacramento Basins, or south, near Hagerman, Idaho through the Humboldt River and Lahontan Basin (reviewed in Hershler and Liu 2004). These connections provided opportunities for faunal exchange between these southern basins and the Wood River. Multiple periods of basaltic volcanism and megaflooding (Lamb et al. 2013) throughout the Pleistocene near the vicinity of the Wood River and its connection with the Snake River (Malde 1971) provided numerous opportunities to isolate the Wood River Basin and the fish species in it. We believe this is the most likely explanation for the shared pattern of divergence observed across taxa.

We argue that this study provides evidence that *O. mykiss* in the Wood River Basin represent a distinct, previously undescribed lineage, unique from populations throughout the species range. The obvious caveat to our findings, is that our study is limited to 229 nuclear DNA loci. We suggest that ongoing work examining, mitochondrial DNA, morphology, and karyotyping be completed before a taxonomic assessment can be performed. In the meantime, we believe this finding is significant and deserves continued conservation and management attention.

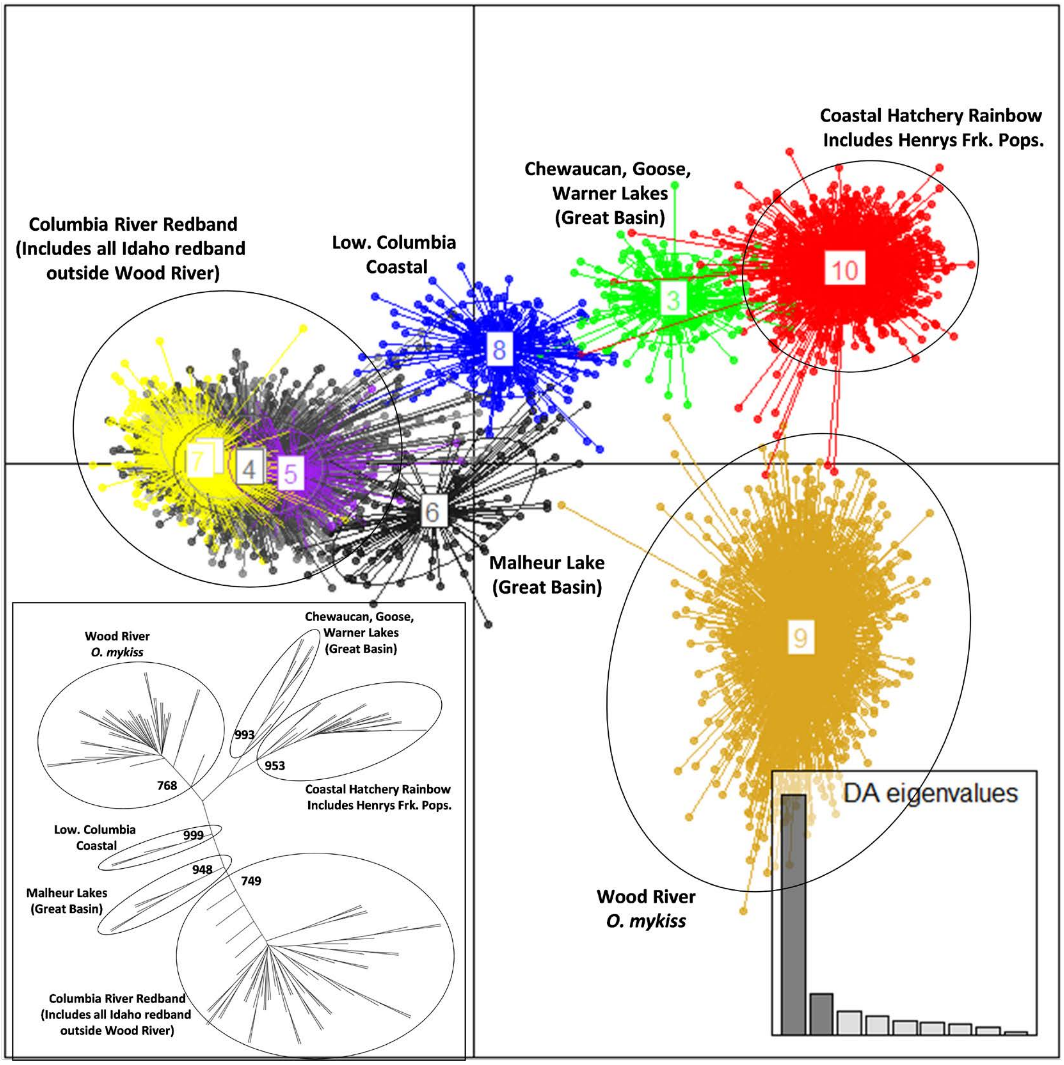

Figure 2. Scatterplot of all individuals on the two principal components of DAPC. Lower Right inset are DA eigenvalues. Left inset is a NJ tree based on pairwise Cavalli-Sforza Edwards chord distance showing similar patterns of genetic clustering among sample collections (>50 bootstrap support).

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Benefits of Genetic Data in Design of Brook Trout Translocation Efforts

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Abstract - With wild trout populations in decline, many conservation practitioners are evaluating the feasibility of incorporating reintroduction and genetic rescue into management frameworks. As interest in these conservation tools continues to grow, so too has the need for rigorous science to evaluate translocation success and improve the efficacy of future efforts. From this, it has become increasingly apparent that approaches that consider both demographics and genetics are most likely to result in successful translocations. In particular, while demographic data are often a central component of project designs, they are insufficient for diagnosing genetic threats such as low diversity, maladaptation, and introgression that characterize many wild trout populations. Consideration of these genetic characteristics is important for long-term project success and to reduce the unintended spread of domestic lineages across the landscape. Using a case study of reintroduction of Brook Trout *Salvelinus fontinalis* into a restored stream in the state of North Carolina, USA, we show how a combined demographic and genetic approach can be used throughout all stages of project design. In particular, we highlight how genetic data were informative for identifying source populations that had the greatest potential to establish a population with the genetic diversity needed for future adaptation. We also discuss how genetic monitoring of the reintroduced population provided insights into reproductive success and genetic diversity that could be indicative of long-term population persistence. While monitoring is ongoing, this combined genetic and demographic approach provides a promising framework for helping to meet reintroduction goals and provides more opportunities for adaptive management following translocation.

Introduction

The combined effects of habitat loss, climate change, and competition with nonnative species have led to rapid declines in native trout populations over the last century (Hudy et al. 2008; Roberts et al. 2017). Reports of local extirpations have become commonplace, with several long-term monitoring and modeling efforts noting an increased rate of population loss in recent decades (Stranko et al. 2008; Merriam et al. 2019). Future viability of many remaining populations is uncertain, as anthropogenic movement barriers, rising stream temperatures, and expanding ranges of interspecific competitors have relegated many wild trout populations to small, isolated habitat patches of declining suitability (Wagner et al. 2013). Within these environments, the interactive effects of genetic drift and inbreeding, combined with the loss of genetic connectivity, often lead to trout populations that are genetically depauperate with low effective population sizes and higher incidences of maladapted gene complexes (Whiteley et al. 2013; Zeigler et al. 2019; Kazyak et al. 2022).

Loss of genetic diversity and connectivity has significant potential to accelerate extirpation rates and reduce the long-term viability of populations under predicted climate change scenarios (Willi et al. 2006). On more contemporary time scales, decreases in genetic diversity and an absence of effective migration can increase genetic loads and reduce the efficiency of natural selection, ultimately leading to a reduction in population fitness (Bijlsma and Loeschcke 2012). While it is possible for genetically depauperate populations to persist, particularly in stable environments, future disturbance and climate change projections will likely require populations to be able to rapidly adapt to novel conditions. Because genetic diversity is the foundation for adaptation, lack of contemporary diversity can place even demographically robust populations at heightened risk of future extirpation (Willi and Hoffman 2009).

To offset recent population loss and improve the long-term viability of extant populations, wild trout management plans are increasingly considering the use of reintroduction (movement of individuals to an unoccupied site with the goal of population reestablishment) and genetic rescue (movement of individuals into an occupied site with the goal of increasing genetic diversity and adaptive potential). To date, both of these tools have been used somewhat sparingly in wild trout conservation, particularly genetic rescue, which has only a few documented incidences in the United States (see Robinson et al. 2017 for one such example). However, as interest in reintroduction and genetic rescues grows, so has the need for more rigorous design guidelines and monitoring programs that can better quantify translocation success and improve the efficacy of future efforts. From this, it has become increasingly apparent that considering both demographic and genetic data throughout all phases of project planning and evaluation is likely to improve translocation success. For example, census population size is an important metric to consider when identifying ideal source populations for translocation and is informative for documenting demographic stability of the restored population. However, census population size is not always a reliable predictor of effective population size (Willi et al. 2006). Thus, demographic data may provide little insight into patterns of genetic diversity, inbreeding, and genetic drift, which are better predictors of near- and long-term viability. Additionally, demographic data are often unable to detect patterns of hybridization and introgression with domestic lineages, which are often difficult to discern given incomplete stocking histories and complex fish behavior (White et al. 2018; Kazyak et al. 2021). For many wild trout populations, considering hybridization and introgression in project planning may be critical for preventing the unintended spread of maladapted alleles throughout the landscape, which could reduce fitness of the restored population and degrade endemic diversity. Taken together, this highlights how a combined demographic and genetic approach may improve translocation success by increasing the probability that the restored population is not only self-sustaining, but also has sufficient genetic diversity for future adaptation.

Here we highlight a case study that used a combined demographic and genetic approach to design and monitor a Brook Trout *Salvelinus fontinalis* reintroduction effort in North Carolina, USA. In particular, we discuss how genetic data were informative for identifying ideal source populations for translocation, testing hypotheses about reproductive success, and identifying potential obstacles to long-term population viability. As we discuss, use of genetic data in this case study was particularly informative as it required translocation of relatively few individuals from genetically depauperate sources and into a habitat with low carrying capacity. This scenario violates many theoretical and empirical guidelines that are prognostic of reintroduction success (Jachowski et al. 2016) and diverges from methods used in other Brook Trout reintroduction efforts (e.g., Kanno et al. 2016). However, many future wild trout reintroductions are likely to require translocation under similar constraints, and we contend that use of genetic data can help improve project success despite these challenges.

Role of Genetics in Project Design and Monitoring

This case study follows a reintroduction of Brook Trout to Purlear Creek, a second-order stream located in northwest North Carolina (Figure 1). Following historical logging and cattle grazing, habitat in Purlear Creek was restored in 2006, eventually resulting in increased pool habitat and decreased substrate embeddedness and temperature typical of Brook Trout streams in the region (Gerow 2012). By the late 2010s, habitat in Purlear Creek had stabilized and the North Carolina Wildlife Resources Commission became interested in reintroducing Brook Trout. However, because Purlear Creek is a relatively small stream (approximately 3 m wetted width) with low habitat availability and thus low trout carrying capacity, there was concern that slow growth in census and effective population size could increase the prevalence of inbreeding and lead to loss of fitness and genetic diversity, ultimately resulting in extirpation of the reintroduced population. These concerns were further compounded by the fact that reintroduction success is often predicated on the transfer of large numbers of individuals from genetically diverse source populations (Jachowski et al. 2016), which was not possible given the demographic and genetic characteristics of regional Brook Trout populations (Kazyak et al. 2021).

Given the demographic and genetic challenges with reintroducing Brook Trout to Purlear Creek, we used a multi-step selection process to help identify source populations that had the greatest potential to transfer endemic genetic

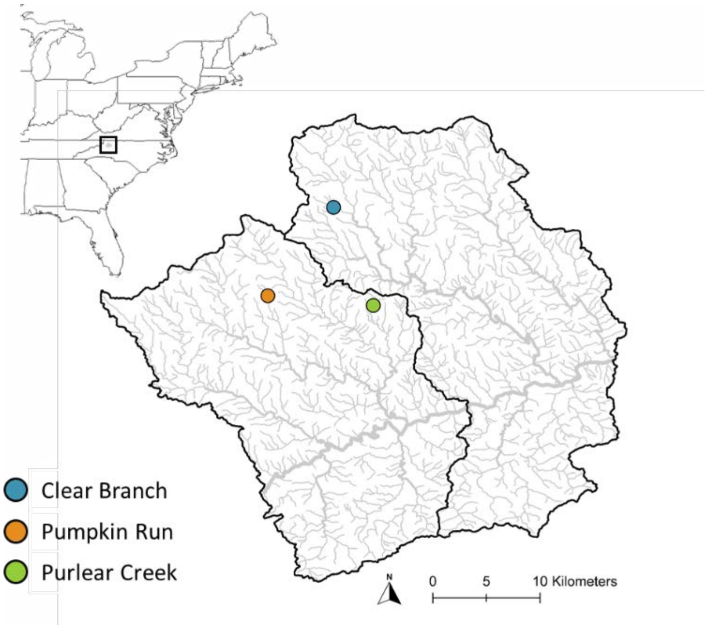

Figure 1. Location of two source populations Clear Branch (blue) and Pumpkin Run (orange) relative to the Brook Trout reintroduction site at Purlear Creek (green) in the state of North Carolina, USA. Black outline indicates watershed boundaries (ten-digit hydrologic unit code). Map modified from White et al. (2022).

diversity and were large enough to support removal of individuals without considerable risk of demographic and/or genetic bottlenecks. This process is described in more detail by White et al. (2022) and in Figure 2. Briefly, in our selection process, we sought to identify a potential source population that had low hatchery introgression, high census and effective population size, high genetic diversity, and was located in the same subdrainage as Purlear Creek to minimize potential maladaptation to the local environment.

At the conclusion of our filtering process, we determined that no single source could support the establishment of a population with high genetic diversity. Specifically, all populations that were in the same subwatershed and were not introgressed with hatchery lineages had low census size and genetic diversity. Therefore, we chose to translocate fish from two source populations, Pumpkin Run and Clear Branch (Figure 1; see White et al. [2022] for additional discussion on source stream selection), with the goal that admixture would result in an increase in genetic diversity relative to solely using either source population. Use of two sites also reduced the risk that removal of individuals would result in negative impacts to source populations.

We translocated sexually mature Brook Trout from Pumpkin Run and Clear Branch to Purlear Creek in mid-September with 11 and 10 fish translocated from each source population in 2018 and 2019, respectively. In each year, we attempted to transfer an approximately equal ratio of males and females, with sex being determined using external morphological traits. Translocating fish in two consecutive years was intended to further maximize transfer of genetic diversity and spread the demographic risk to source populations over multiple years while also minimizing the risk of failure due to stochastic environmental conditions.

Post-translocation monitoring efforts have been on-going since 2019. In that time, we have documented consistent recruitment and population expansion outside the original stocking area. Additionally, we collected age-1 and older individuals throughout the study reach, indicating subsequent survival and growth of offspring that were produced in Purlear Creek in 2018 and 2019. Post-translocation demographic monitoring of the two source sites, Pumpkin Run and Clear Branch, suggests that removal of individuals had no enduring effect to population size and that the size of the Purlear Creek population is now comparable to that of the two sources (see White et al. [2022] for population estimates in source and restored sites).

For post-translocation genetic monitoring, we genotyped young of year collected in 2019 and 2020, representing individuals produced in the first two years after the start of translocation efforts. Genetic diversity varied across years; however, we consistently found that diversity in Purlear Creek was equal to, or higher than, the two source streams. Results from parentage and admixture analyses suggest that the increase in diversity was due to high levels of interbreeding between fish translocated from Pumpkin Run and Clear Branch. However, we also found significant bias in individual reproductive success. Together, this suggests that our strategy was successful for increasing genetic diversity in the restored population. However, genetic diversity in Purlear Creek is likely lower than would be theoretically possible with complete and equal admixture between translocated fish (see White et al. [2022] for more detailed discussion and estimates of genetic diversity parameters).

Discussion

Genetic data provided two significant benefits in the design and analysis of the Purlear Creek reintroduction. First, in source population selection, genetic data enabled us to identify populations that were not ideal candidates due to hatchery introgression and/or low genetic diversity. Ultimately, we were unable to identify a single source

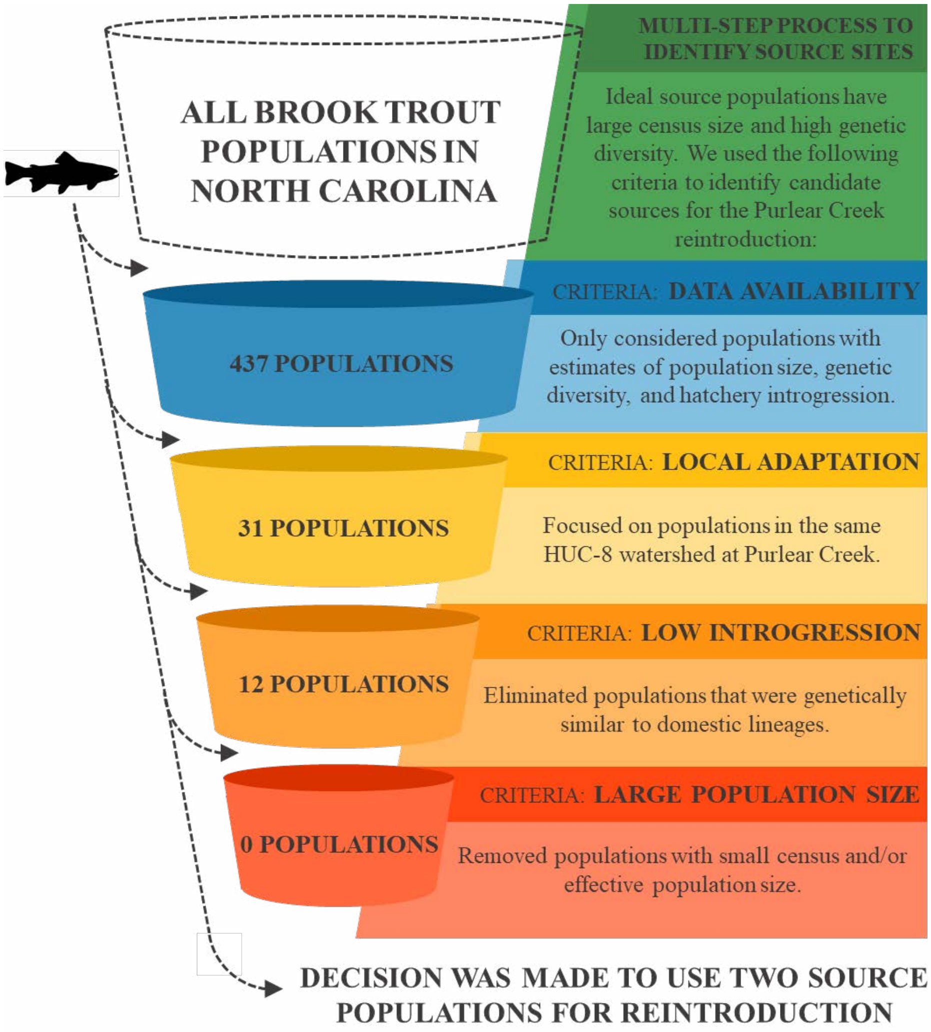

Figure 2. Multi-step filtering process used in the design of a Brook Trout reintroduction effort in North Carolina based on demographic and genetic data.

population that met both our genetic and demographic criteria, which prompted us to translocate fish from two sources to maximize diversity and minimize potential impacts to source populations. Although using multiple source populations can result in outbreeding depression at the restored site (e.g., Huff et al. [2011], but also see Frankham et al.[2011]), it also has the benefits of reducing the demographic risk to extant populations. In addition, provided interbreeding occurs, use of multiple source populations can increase the amount of genetic diversity in the restored population with less demographic risk to source populations. Our post-translocation genetic monitoring did suggest that there was higher genetic diversity in Purlear Creek than would be expected if a single source population had been used. However, while our strategy met most of the management objectives, genetic diversity in Purlear Creek is still low and could threaten long-term population persistence. Ultimately, the results of on-going monitoring will help determine the viability of the restored population, as well as the efficacy of our approach.

The second advantage that genetic data provided was the ability to better evaluate the reproductive success of fish translocated to Purlear Creek. Our demographic assessments showed establishment of a mixed-aged population with expanding occupancy. However, it was only through genetic analyses that we were able to quantify patterns of admixture and reproductive bias that could drive longer-term changes in genetic diversity and effective population size. Long-term genetic monitoring of restored populations may be particularly important when source populations are genetically depauperate, relatively few individuals are translocated, and/or population growth rates are low due to life history or habitat constraints. In such circumstances, the lack of successful (inter)breeding among even a few translocated individuals could significantly reduce the genetic diversity of the restored population and result in extirpation after relatively few generations. As such, early detection of genetic diversity loss can be valuable for determining whether additional intervention is needed to support the population and prevent extirpation.

Although we highlighted the value of genetic data in a reintroduction effort, use of genetics is likely to be equally, if not more, important in the design and monitoring of genetic rescue – which is another application of translocation with many similar considerations as those presented in our case study. Although it has been used sparingly in wild trout conservation, more are advocating for increased consideration for genetic rescue for the management of isolated trout populations (e.g., Kovach et al. 2022). However, ideal conditions for genetic rescue require not only an assessment of genetic diversity, inbreeding, and introgression of source and recipient populations, but minimizing among-population genetic differentiation (Frankham et al. 2011; Ralls et al. 2018). Moreover, by definition, genetic rescue, which aims to increase population adaptive potential via an increase in genetic diversity (Whiteley et al. 2015), cannot be properly evaluated in the absence of genetic monitoring.

The demonstrated utility of genetic data, along with the prospective increase in the number of reintroduction and genetic rescue attempts in the future, highlights the critical role of baseline genetic information. Although the highlighted case study was made possible by the availability of a robust baseline dataset which included over 500 Brook Trout populations in North Carolina (Kazyak et al. 2021), similar data may exist for Brook Trout in other regions and species. Ultimately, as genetic data become increasingly affordable and available, their role in translocation efforts will likely increase.

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Inbreeding Depression Reduces Fitness in Colorado's Last Remaining Greenback Cutthroat Trout: Consequences for Management

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Abstract - Recent molecular studies of historical and modern trout specimens from the southern Rocky Mountains revealed Colorado's state fish, the Greenback Cutthroat Trout *Oncorhynchus clarkii ssp*. (GBCT), was only represented by a single relict population. That revelation spawned an intense recovery effort to conserve the taxon. However, hatchery propagation has been complicated by poor survival of offspring. High prevalence of physical deformities and very low heterozygosity suggests what remains of the taxon may carry a high inbreeding load. Outcrosses with individuals from a sister subspecies in a common garden experiment showed a strong effect of genotype on fitness, doubling both survival and growth. Such pronounced hybrid vigor suggests genetic restoration by outcrossing may be a viable strategy for improving individual fitness against a background of inbreeding depression. This presents an interesting dilemma for managers, where the conservation focus has rightly been on repatriating pure individuals that best represent the trout that occupied these streams prior to European settlement. Unfortunately, this can mean the removal of extant robust populations of Cutthroat Trout in the South Platte basin that are native to western Colorado, and replacing them with the native trout that now appears compromised by reduced genetic diversity. With only a single source population of GBCT remaining, any efforts at genetic rescue would necessarily involve outcrossing with a different taxon, intentionally introducing nonnative admixture which then fails to preserve what little remains of this lineage (extinction through hybridization). We advocate a multi-pronged strategy where the pure population is replicated in disparate locations to mitigate loss from stochastic events, but also promote genetic rescue for other populations using a closely related sister taxon to help reset the evolutionary trajectory of these fish should fitness deficiencies make establishing robust wild populations impossible.

Introduction

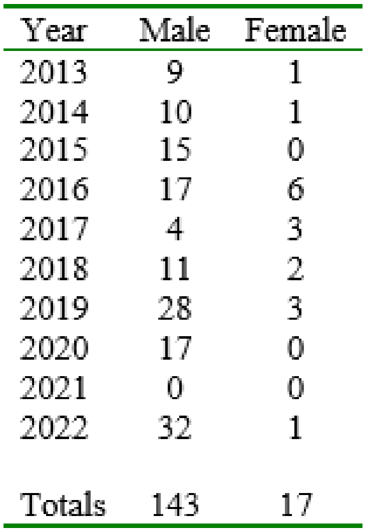
Loss of genetic diversity can pose a serious threat to small populations (Vucetich and Waite 1999; Hedrick and Kalinowski 2000), and is an important component of extinction risk (Frankham 1998; Frankham and Ralls 1998). When populations lose alleles, increases in individual homozygosity can reduce fitness (Markert et al. 2010), often manifested in lower survival rates (Westemeier et al. 1998; Slate et al. 2000; Fritzsche et al. 2006). This problem is particularly relevant to the conservation of native Cutthroat Trout *Oncorhynchus clarkii* in the southern Rocky Mountains. Here, remaining populations only occupy a small fraction of their historical ranges (Alves et al. 2008; Hirsch et al. 2013; Penaluna et al. 2016), usually in small isolated headwater habitats protected from nonnative invasions by impassable barriers to fish movement (Fausch et al. 2009). With median occupied habitat patch lengths of 6 km (Roberts et al. 2013; Zeigler et al. 2019), many populations simply do not occupy large enough stream reaches to support large populations (Hilderbrand and Kershner 2000; Young et al. 2005) needed to maintain robust effective population (N_e) sizes and adaptive potential (Franklin 1980). With low Ne, populations can then become more vulnerable to inbreeding depression (Rieman and Allendorf 2001; Allendorf and Luikart 2007).

Nowhere is this problem more pronounced than with the recently rediscovered Greenback Cutthroat Trout *Oncorhynchus clarkii stomias* (GBCT), Colorado's state fish. The subspecies has persisted in one locality as a single isolated population outside its native range for the last 130 years (Metcalf et al. 2012; Rogers et al. 2018; Bestgen et al. 2019). Apparently founded from stocked trout escaping a constructed headwater pond in the Bear Creek drainage (Kennedy 2010), this population occupies just 7 km of first-order stream habitat protected by a natural waterfall barrier. Their discovery spurred the rapid development of both captive and wild populations as sources for producing progeny as part of a large recovery effort coordinated by the GBCT Recovery Team (USFWS 2019). After the discovery, 66 individuals were brought into captivity in 2008, of which 16 females produced eggs in 2010 that were fertilized with 37 males to develop the initial broodstock. This stock has been supplemented in subsequent years with milt obtained from wild males, and fertilized eggs from an occasional wild ripe female (Table 1). Hatchery-reared progeny have been introduced into six isolated and geographically distinct reclaimed waters to date, with the goal of establishing multiple viable populations throughout the headwaters of the South Platte basin, the putative native range of GBCT (Metcalf et al. 2012).

While supplemental infusions of milt have helped ensure the broodstock represents the source population well, the source itself has likely faced significant bottlenecks over the past century, first at founding with only a portion escaping their headwater pond confines, and likely subsequently in response to drought or flood events. They currently display the least heterozygosity of any Cutthroat Trout population we have studied (A. Martin, unpublished). In addition, they are extremely challenging to raise in captivity, plagued by poor survival and growth even when cultured in small lots (Rogers et al. in press), and often possess unusual physical deformities.

Large repatriation projects are planned, but apparent inbreeding depression may serve to complicate those efforts (Ralls et al. 1988; Lacy et al. 1996; Hedrick and Kalinowski 2000; but see Visscher et al. 2001; Johnson et al. 2009). Here we explore whether genetic rescue would be a viable method to improve fitness in these last remaining relicts of Cutthroat Trout diversity by comparing performance of the GBCT from Bear Creek with a population representing a sister taxon, the Colorado River Cutthroat Trout (CRCT), as well as their hybrid crosses in a controlled "common garden" setting. We examined four fitness measures on fry: 1) survival to 60-d post-hatch 2) growth of 60-d old fry, along with 3) low dissolved oxygen tolerance, and 4) high temperature tolerance in three-month-old fingerlings, to provide insight into the potential consequences of inbreeding depression on this small population, and whether genetic rescue should be considered (Tallmon et al. 2004; Johnson et al. 2010; Whiteley et al. 2015).

Table 1. Number of ripe male GBCT captured during annual electrofishing efforts on Bear Creek. Milt was stripped from each and used to fertilize eggs from the captive brood stock housed at the Leadville National Fish Hatchery. Milt was also used to fertilize eggs from wild ripe females captured incidentally.



Year	Male	Female
2013	9	1
2014	10	1
2015	15	0
2016	17	6
2017	4	3
2018	11	2
2019	28	3
2020	17	0
2021	0	0
2022	32	1
Totals	143	17

Methods

Spawn timing of the GBCT broodstock derived from Bear Creek was synchronized with broodstock derived from the Carr Creek population of CRCT by raising both stocks on a common water supply at the Leadville National Fish Hatchery. Carr Creek lies on the Roan Plateau, Colorado, and is home to a "green lineage" population of CRCT that appears to be a closely related sister taxon to GBCT (Metcalf et al. 2012; Rogers et al. 2018). We mitigated the chance of an infertile male compromising the experiment by blending stocks of milt from each of two brood sources to fertilize eggs. Milt from each of four ripe Bear Creek males was extruded into a dry glass bowl from which 400 ul was pooled into a flask containing 8 mL of extender (Rogers 2010), oxygenated and stored on ice. This process was then repeated with four Carr Creek males. Each of four gravid Bear Creek females were stripped into two bowls, then fertilized with 1 mL of pooled extended milt from either the four Bear Creek males or the four Carr Creek males. A similar procedure was used with four gravid Carr Creek females to provide a total of 16 distinct families from 16 parents comprising four treatments (Figure 1). Fertilized eggs were water hardened for an hour in 3.8 L drink coolers, randomly assigned a code so that those caring for the eggs would be blind to treatment, then transported to the Colorado Parks and Wildlife Aquatic Toxicology Lab in Fort Collins, Colorado where each family was reared under blind common garden conditions. Upon arrival, eggs were treated with 1600 ppm formalin for 15 minutes (Piper et al. 1982). Two hundred eggs from each clutch were transferred into discrete egg cups and incubated at 10ºC. Egg cups were constructed of 53 mm ID X 75 mm PVC pipe with 1000 µm nylon mesh affixed to the PVC pipe with aquarium-grade silicone adhesive (Brinkman et al. 2013; Ziegler et al. 2013). Each egg cup was suspended in a 2 L glass tank (18.5 x 9 x 12 cm) and received a flow of 40 mL/min dechlorinated Fort Collins municipal tap water (Brinkman et al. 2013). Egg cups were arranged in a randomized complete block design with four replicates per treatment. Families were randomly assigned numbers 1-16 so identity of parents could be kept blind and then placed in 16 tanks randomized by block.

Survival

Fertilized eggs were monitored daily, with onset of the eyed egg stage, hatch, and swim up being recorded. Hatched fry were carefully removed from each egg cup and released into their respective glass tanks, where they were allowed to develop to swim-up stage. Feeding was initiated once the yolk sacs were absorbed by introducing live *Artemia* nauplii into the tanks and increasing the water temperature to 13.1ºC. Fry were transitioned over to Starter feed (Rangen Inc., Buhl, Idaho) by supplementing with Cyclop-eeze (Argent Chemical Labs, Redmond, Washington). Fry were fed *ad libitum* four times daily with automatic feeders. Feces and uneaten food were siphoned from the tanks daily, along with any egg, larvae, or fry mortalities. Siphoned trout were assigned a sample number and date and preserved in individual vials of 80% ethanol.

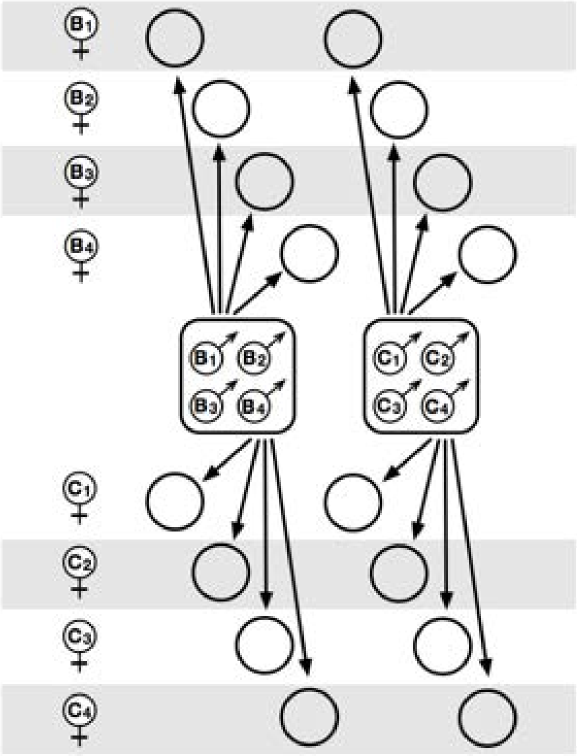


Figure 1. Four gravid females from each of two broodstocks housed at the Leadville National Fish Hatchery were stripped into two bowls each that were then fertilized with either pooled milt from four male Greenback Cutthroat Trout from Bear Creek (B) or four male Colorado River Cutthroat Trout from Carr Creek (C), to generate 16 families.

Growth

At 60 days post-hatch, ten fish from each family were placed in one of sixteen 2.7 L tanks arranged in a randomized block design where each treatment was represented in each block but blind to those caring for the fish. A water flow rate of 40 mL/min was maintained throughout the study and temperature was maintained at a constant13.1ºC. For the first 8 days, fish were fed 3% of their average weight at Day 0 (60 d post-hatch) based on a batch weight when they were transferred to the test tanks. On Day 8, another batch weight was made and the feeding rate was adjusted to 3% of the average weight from Day 8. The growth experiment ended after 15 days, and another batch weight was obtained.

Hypoxia tolerance

At 14 weeks post-hatch, eight fish from each family were subjected (individually, still blinded) to hypoxia trials to measure when loss of equilibrium occurred with dropping dissolved oxygen levels. Individual fish were placed in 1.75 L glass aquaria fitted with an airstone, a titanium cooling loop, and a temperature probe. The airstone supplied pure nitrogen which served two functions; decreasing the partial pressure of oxygen in the tank in order to remove oxygen from the water, and circulating the water within the tank. Temperature in the tank was maintained at 12.0°C using a temperature probe and temperature controller (Love B-series, Dwyer Instruments, Michigan City, Indiana) which supplied power to a peristaltic pump that supplied ice water through a titanium heat exchanger submerged in the tank. Oxygen levels in the tank were measured using an optical dissolved oxygen probe (ProODO, YSI Inc., Yellow Springs, Ohio). For each trial, fresh 12.0°C water was first added to the experimental chamber, then a fish was introduced and allowed to acclimate for 5 min before the supply of nitrogen was initiated. Oxygen concentrations were monitored continuously while fish were carefully observed to determine when loss of equilibrium (failure to maintain a dorsal-ventral vertical orientation) would occur. The oxygen concentration was recorded when sustained loss of equilibrium exceeded 30 s, at which point the fish was placed in a recovery tank.

Thermal tolerance

Fish used in the hypoxia test were allowed to recover for 7 days before being subjected to a Critical Thermal Maximum (CTM) challenge (Becker and Genoway 1979). These trials occurred in the same 1.75 L tanks described above fitted with the same programmable temperature controller which regulated a submersible aquarium heater to heat the water at a rate of 0.3°C/min, as is standard (Becker and Genoway 1979; Wagner et al. 2001; Underwood et al. 2012). Aeration of the tank maintained saturated dissolved oxygen levels and ensured homogenous temperatures throughout the chamber. Water temperatures were increased until sustained (≥10 s) loss of equilibrium was observed in the fish being tested, at which point the temperature was recorded. Following the test, fish were removed from the experimental tank and allowed to recover.

We used analysis of variance (ANOVA) to test for differences among treatment groups for each fitness measure in R (R Core Team 2020). Treatment means were compared using Tukey's honest significant difference. Genetic diversity of the source populations used to develop the broodstocks was measured using Amplified Fragment Length Polymorphisms (AFLP) on archived DNAs (Rogers 2008; Bestgen et al. 2019) and the program AFLP-SURV (Vekemans et al. 2002). The AFLP selective amplification procedure followed Vos et al. (1995), using the restriction enzymes EcoRI and MseI, with three base selective primers (RI-ACT and MseI-CAG). Amplified fragments were run on an ABI3130 Genetic Analyzer (36cm array, POP7 polymer) with GeneScan ROX 500 as the size standards. The fragments present in each sample were scored in GeneMapper 4.0 using a binset of 119 fragments previously used for Cutthroat Trout AFLP analyses (Metcalf et al. 2007; Bestgen et al. 2019). Data files were configured for AFLP-SURV and expected heterozygosity (Hj) was calculated using the Bayesian method with non-uniform prior distribution of allele frequencies assuming Hardy-Weinberg equilibrium (inbreeding coefficient Fis = 0.0).

Results

When compared to other native Cutthroat Trout populations across Colorado (Figure 2), our study populations harbored either more genetic diversity (Carr Creek) or much less (Bear Creek). Not surprisingly, expected heterozygosity calculated from AFLP markers was three-fold lower in the Bear Creek source population than the Carr Creek population.

Survival was monitored daily up to 60-d post-hatch when the growth study was initiated, though the last mortality was recorded at 50 days. Survival was not equal among groups (P = 0.036) through 60 d post-hatch. While mean survival for the Carr Creek fish (47.1%) and the hybrids (45.6% and 47.7%) were not different (P > 0.995), survival was markedly reduced in the Bear Creek fish (Figure 3a; 20.7%; P < 0.082).

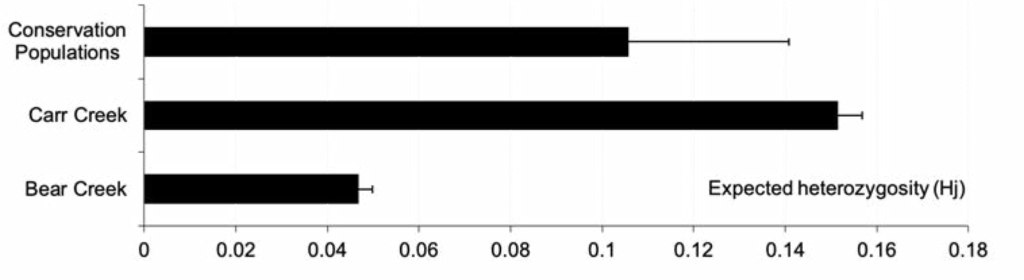


Figure 2: Expected heterozygosity from both Carr Creek and Bear Creek compared to an average Hj value generated from nine conservation populations of native Cutthroat Trout in Colorado. Error bars represent the 95% confidence interval on mean Hj values for the conservation populations (top bar), or around Hj calculated for each population (Carr Creek and Bear Creek).

A similar trend was observed for growth after 60-d post-hatch where again groups were not equal (P = 0.001). Average growth of Bear Creek individuals was two-fold lower (0.014 g/g/d) than the Carr Creek and hybrid fish (Figure 3b; 0.028 g/g/d in all cases; P < 0.003). Hypoxia tolerance (Figure 3c) and CTM (Figure 3d) were not different among groups (P = 0.705, 0.744 respectively).

Some additional observations were made while raising these fish for the fitness challenges discussed above. First, all four families with progeny from two of the Bear Creek mothers contained some albino fry. This occurred regardless of whether eggs were fertilized with Bear or Carr Creek milt, suggesting maternal influence for this trait. In fact, the highest proportion of albino fry were recorded for progeny from Carr Creek milt (Tank 13). Additionally, technicians responsible for raising the fish (and blind to the provenance of the fish in each tank) noted that "tanks 1, 4, 5, 6, 9, 11, and 14 appear to behave differently than the other tanks," and that "the fry seem to avoid light to a greater extent." While these additional comments were unsolicited, indeed these tanks were all progeny from Bear Creek mothers, regardless of whether they were sired by Bear or Carr Creek fathers.

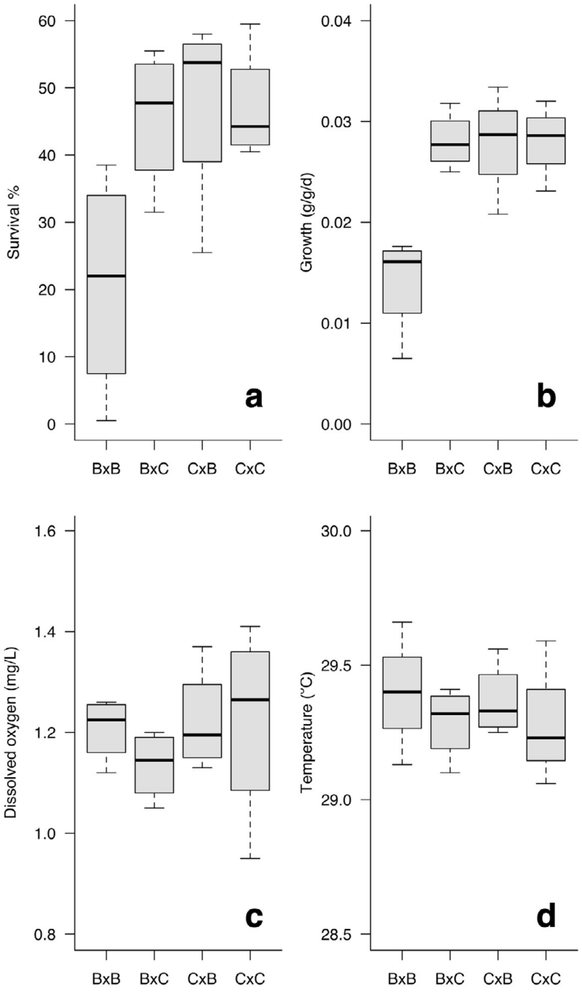


***Figure 3:** The X-axis labels represent parental fish from Bear Creek (B) or Carr Creek (C) where the first letter represents the female and the second the male in each group of family crosses. Survival to 60-d post-hatch by cross type (a), growth rate at 60-d post-hatch (b), low dissolved oxygen tolerance at 14 weeks (c), and critical thermal maxima at 15 weeks of age (d).*

Discussion

Observed survival in Bear Creek fish was only half of what Carr Creek or the hybrid trout were able to achieve. Differences in growth were even more pronounced, with mean values in the Bear Creek trout again being half of what the hybrids and Carr Creek trout displayed. These results provide compelling evidence for an effect of parental relatedness on survival and growth (Figure 3). Offspring survival from the hybrid crosses were identical to that seen in the Carr Creek trout, suggesting that the observed reduction in fitness of the Bear Creek fish is best explained by the effects of recessive, deleterious alleles. Poor survival and growth seen here is consistent with early life history consequences of inbreeding registered in other vertebrate studies on birds (Westemeier et al. 1998; Bensch et al. 1994; Spottiswoode and Møller 2004) and mammals (Slate et al. 2000; Bensch et al. 2006; Johnson et al. 2010).

Heterosis did not increase individual tolerance of hypoxia or CTM, perhaps because the response in parental stocks were also similar (Figure 3). Although others have demonstrated variation in CTM even within subspecies (Underwood et al. 2012), some have suggested that upper thermal tolerance limits are governed by molecular pathways that may not be very plastic (Chown et al. 2010; Logan and Buckley 2015; Ooman and Hutchings 2017). These trout may already be operating at close to the maximum attainable level of thermal and hypoxia tolerance, and increased heterosis may not be able to change that.

Fragmented populations are at high risk for inbreeding depression (Hedrick and Kalinowski 2000) that can lead to local extinction (Lande 1988; Frankham and Ralls 1998; Johnson et al. 2010). In the absence of pedigree data, measures of heterozygosity have long been used as a proxy for inbreeding coefficients in order to identify the costs of inbreeding, and are strongly correlated with fitness measures (Bensch et al. 2006). As anticipated, expected heterozygosity was extremely low in the Bear Creek trout (Figure 2), suggesting that the population has endured at least one substantial bottleneck, and that many more might have been possible. Despite some level of inbreeding depression in the Bear Creek trout, they have been able to persist in their isolated headwater habitat for over 130 years. In stable systems, inbreeding depression-like effects may not manifest themselves, particularly over the near term (Markert et al. 2010). However, the goal for the Bear Creek broodstock is to use their progeny to repatriate GBCT across their former range in the South Platte River basin. How well these fish perform in more challenging environments has yet to be determined, though other studies on inbred organisms suggest this may be problematic (Spielman et al. 2004; Frankham 2015).

Genetic rescue has been proposed as a way to mitigate the negative effects of inbreeding (Allendorf et al. 2001; Tallmon et al. 2004). It can be especially useful for management and conservation because it induces population-level demographic responses with the introduction of new, beneficial alleles (Whiteley et al. 2015; Fitzpatrick et al. 2020). Importantly, one only need introduce a few individuals to a population to see a strong positive response (Mills and Allendorf 1996; Frankham 2015). While genetic rescue remains controversial and is seldom implemented (Tallmon et al. 2004; Whiteley et al. 2015), reticence is usually centered around whether outbreeding depression will make the target population less fit (Edmands 2007; Frankham et al. 2011). Our data provide evidence that this would likely not be the case for GBCT. Rather, the concern here lies squarely on the fact that rescue in this case would necessarily cross alleles from a different subspecies into the population, thereby setting the stage for extinction through hybridization (Rhymer and Simberloff 1996). This scenario is similar to the high-profile dilemma that faced managers trying to save the Florida Panther in the 1990s (Johnson et al. 2010), although the panther subspecies diverged much more recently (Ochoa et al. 2017) than the Cutthroat Trout subspecies (Shiozawa et al. 2018). The stakes are high, as one would not just be resetting the evolutionary trajectory of a population, but also of an entire subspecies as the Bear Creek stock is all that remains of the GBCT.

While recovery and conservation efforts have generally focused on repatriation of indigenous fish free of nonnative alleles (UDWR 2000; Allendorf et al. 2001, 2004), the Bear Creek situation forces us to at least consider alternatives like genetic rescue. Managers should address several questions when contemplating intentional hybridization in this case: 1) How well do Bear Creek fish represent the native trout of the South Platte basin? Backing up the evolutionary trajectory of these fish with genetic rescue might be more palatable if genetic drift following substantial past population bottlenecks has made them poor representatives of the subspecies. 2) Are the detrimental effects of inbreeding depression readily apparent (e.g. reduced viability or an increased proportion of deformed or asymmetric individuals; Allendorf et al. 2001)? 3) Would donor populations offer ecological exchangeability (Crandall et al. 2000), serving the same function in a similar environment?

When deliberating implementation of a rescue program, we believe it is also important to acknowledge that management actions do not have to reflect a binary outcome. The Bear

Creek population could be replicated a number of times to secure against further loss, while rescue could be considered in additional populations where environmental conditions are particularly challenging and population persistence would be more likely with additional heterosis (Markert et al. 2010). These two management strategies could even be implemented in the same reclamation project if the system were large enough to accommodate a replicated Bear Creek trout population above barriers to upstream passage, but allow genetic rescue to proceed down below to help foster a more robust population. These scenarios would serve as ideal in situ experiments for comparing population growth, individual growth and survival, recruitment, and other key demographic factors. Careful monitoring of these key demographic traits would then help inform whether additional intentional hybridization events should be considered in other reclaimed and repatriated populations of the iconic GBCT.

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Session 6
Global Perspectives, Special Regulations, and Wild Trout Monitoring Techniques

Is the Energy from Run-of-River Hydropower Plants Renewable if it Threatens Indigenous Fish Communities in Montane Stream Ecosystems in Serbia? A Long-Pole Mechanism of Questioning

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Abstract - Molecular diversity of Brown Trout *Salmo trutta* is reflective of biodiversity in the Balkans. In recent years, various threats have been augmented, including run-of-river hydropower plants (RORs) that are rapidly installed on pristine montane streams, whose steep slopes are most convenient for hydropower energy production at minimal costs. Records from both fishery management plans and recent research for highland stream fisheries with RORs revealed reduced Brown Trout biomass compared both with streams without RORs and upstream sections of the dammed ones. Six streams with operational RORs revealed severe deterioration of habitat, e.g., increased water temperature, reduced dissolved oxygen, and increased nutrient contents. In these streams, fish community structure changed, with Brown Trout as the most affected species and experiencing steep declines in abundance, biomass, productivity, and reduced longevity. Explaining RORs adverse effects on Brown Trout brought closer scrutiny by the general public, who was informed about the indigenous and native character of Brown Trout along with the difficulties of restoring them by stocking hatchery fish and the ineffectiveness of fish passage facilities commonly supplied to compensate for disruption in access to Brown Trout habitat. Increased public awareness and rapid spread of the Rights of Nature movement led to organization of a Tribunal for the Defense of Aquatic Ecosystems of Europe by European non-governmental organizations in cooperation with the local communities. The State of Serbia and the European Commission were recognized as the perpetrators. They were tried during April 2021 and sentenced for ecocide as a violation of the Rights of Nature, for breach of the Universal Declaration of the Rights of Mother Earth and the European Union's Water Framework Directive. Sentencing provided an opportunity to demand restorative justice, which will comprise mitigation of harm to both ecosystem and social components.

Introduction

Despite the allegedly favorable features of small, run-of-river (RORs) hydropower plants as a renewable energy source, they have substantial adverse effects, including: habitat degradation and fragmentation, disruption of the hydrological regime, negative impact on groundwater, forest removal and fragmentation, intensified erosion and change of rivers' hydrological and geomorphological features (Nilsson et al. 2005; Csiki and Rhoads 2010; Liermann et al. 2012; Carvajal-Quintero et al. 2017; Eichelman and Scharl, 2017).

Serbia is in the center of the Balkans, with its climate influenced by its geographical position. According to the Koppen-Geiger climate classification system (Peel et al. 2007), most of Serbia is in the C, i.e., temperate/mesothermal type, with the most north-western and south-eastern parts featuring the D, i.e., continental/microthermal type. It is predominantly a montane country of moderately continental climate.

The diversity of trout species *Salmo* spp. reflects the Balkans' status as a World and Mediterranean biodiversity hotspot (Blondel and Aronson 1999; Médail and Quézel1999), both by endemism of nominal taxa and their molecular diversity. Simonović et al. (2017a) reported four novel mitochondrial DNA control region haplotypes of the Danubian phylogeographic lineage and three of the Adriatic lineage (after Bernatchez 2001) for Brown Trout *Salmo trutta* in Serbia. This is extraordinary considering Serbia's southerly position in Europe, low altitude and corresponding high annual temperature, and scarcity of water. These factors limit habitat for exclusively resident, stream-dwelling Brown Trout (Simonović et al. 2020). The majority of streams inhabited by Brown Trout in Serbia are freestone type, and include few spring creeks. Only a few of the largest freestone streams and spring creeks contain Brown Trout fisheries and all feature either conditional, or unconditional, catch-and-release (C&R) regulations (Barnhart 1989), barbless hook requirements (Jenkins 2003; Pope et al. 2007), and are stocked with hatchery-reared Brown Trout (Simonović et al. 2017b). Many fly-fishermen support C&R as a means of maintaining the native character of Brown Trout stocks, but others remain resistant to C&R over fears that the lack of stocking would decrease the quality of the fisheries (Simonović et al. 2014).

Although ROR construction began in Serbia as early as 2010, their numbers have increased substantially since 2015 in the steep-sloped montane streams that host fish communities containing Brown Trout. Diversion pipelines, typically 1–3 km long but sometimes up to 5 km in length, were often installed in the streambed and the heavy machinery required for construction negatively affected the streambed and riparian area. Such construction changed stream sections along the diversion pipes to uniform runs that resemble shallow (up to 50 cm deep) canals, without any diversity or natural montane stream habitats that are present upstream of the RORs' dams. During low water periods, RORs' operators divert almost all water into the pipelines.

Opposition to RORs emphasizes their impacts on the availability of other natural resources (e.g., ground and surface water regimes, forest health), the rural socio-economics they support (e.g., water supply, feeding trout farms, watering crops, orchards, and livestock, etc.), local politics (e.g., basic human rights, private property issues, etc.), and on the local trout fisheries. On the rare occasions when preparation of an Environmental Impact Assessment Study (EIAS) was mandatory, the RORs' studies listed expected effects on fish stocks, especially Brown Trout, to be negligible or even positive (e.g., Josimović and Crnčević 2012; Simić and Simić 2016). In some instances, EIAS documents stated that fish benefits included: less energy expended by fish in reduced flow, increased productivity, and enhanced spawning. Two main compensatory measures proposed in EIASs were stocking and installation of fish passage.

After initial disclosure, citizens from several municipalities organized to oppose the threat that RORs posed to the streams. The River Jošanica and its tributaries were dammed with 16 dams and it was the stream most affected by RORs in Serbia. In the Jošanička Spa at the foothills of the Mt. Kopaonik, where the National Park is situated, citizens networked with academic and non-governmental sectors to provide documentation on the adverse effects of RORs on the montane streams' ecosystem in an effort to halt further occurrence of RORs and to restore aquatic ecosystems destroyed by RORs. This inspired the case on the ecocide over the Balkans rivers focusing on the rights of fish impacted by the RORs. It went to trial at the European Tribunal in Defense of Aquatic Ecosystems that was heard at the end of April 2021. This paper reports this specific case study as a possible way of alarming and presenting to the international public the ecological threat that Serbian and European institutions ignored despite the warnings and calls of citizens.

Methods

The increase in public awareness was accomplished during 2018 and 2019 by introducing people to the impact of RORs on the fish communities in several montane streams, including the River Jošanica. In brief, using the longitudinal approach, Brown Trout biomasses were compared for the same sampling spots of streams (Figure 1) in years before construction of RORs (2003 and 2008) and after RORs had become operational in 2018 (see Simonović et al. 2021). Data obtained were compared to those available for streams under similar management regimes that were devoid of RORs through the whole period under investigation. This comparison conformed to before-after-control-impact analysis (Green 1979) using a fixed-effect two-way ANOVA model, with the years from start of data recording and occurrence/absence of RORs as fixed effects.

The field experiment was designed as simultaneous pairwise single-pass depletion sampling (Edwards et al. 2003; Foley et al. 2015) in each of the six streams (Figure 1, numbered 1 – 6): one sampling spot was immediately upstream of the water intake facility and the counter spot was downstream of it. Sampling encompassed all habitat types to sample trout of all available size- and age-classes. Age of Brown Trout was assessed based on length and

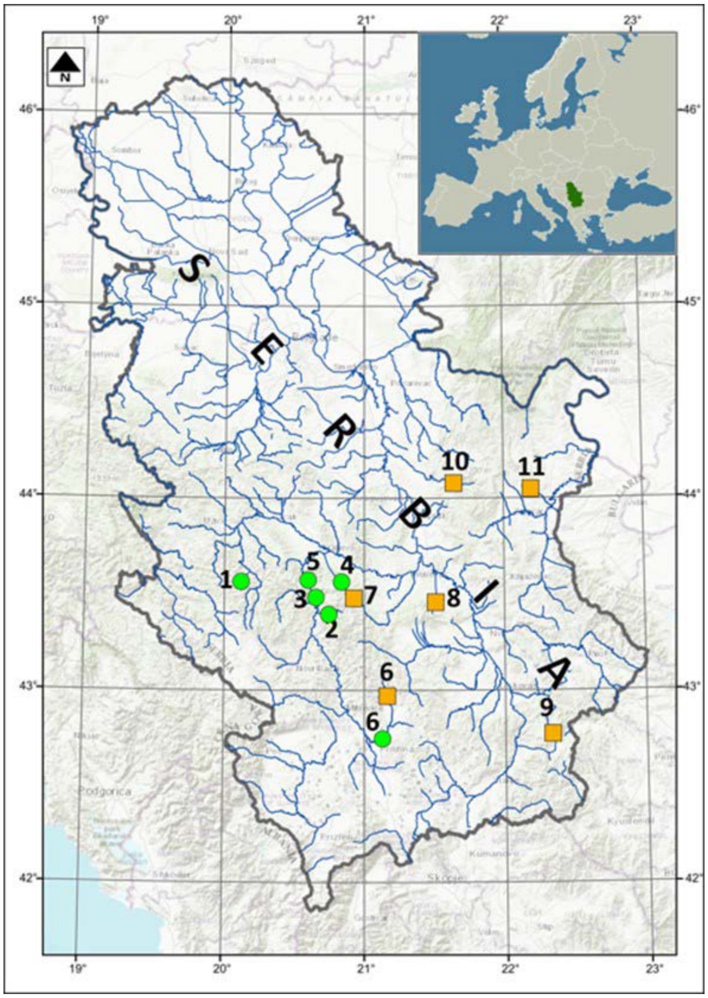

Figure 1. Map of Serbia with the position of Brown Trout streams (1, Panjica River; 2, Brezanska River; 3, Gokčanica River; 4, Sokoljska River; 5, Kolska River; 6, Jošanica River; 7, Rasina River; 8, Lomnica River; 9, Vlasina River; 10, Resava River; 11, Zlotska River). Circles denote streams where the correlational natural field experiment was conducted in 2019 and squares denote streams analysed using the correlational longitudinal approach).

weight frequency distribution curves and from scales taken from Brown Trout. Biomass and abundance were calculated based on catch-per-unit-of-effort (Ricker 1958) recorded on the sites and extrapolated to 1 ha stream surface. This extrapolation was done because of the difference in size, habitat structure, and duration of sampling at each site. Other species captured included Danube Barbel *Barbus balcanicus*, European Chub *Squalius cephalus*, and Spirlin *Alburnoides bipunctatus*. All data for change of water quality variables (water temperatures, dissolved oxygen and conductivity) along streams were transformed into change per length of diversion pipe, as there were large variances in both altitude and length of diversion pipes in RORs. Streams where diversions were operational were compared with those where all water volume remained in the streams' channel.

Rights of Nature is one of the most rapidly spreading environmental and legal movements in the world. It relies on the Universal Declaration of the Rights of Mother Earth. This declaration was adopted by representatives of 130 countries at the World Climate Changes and Mother Earth's Rights Conference held in Bolivia in 2010 and was included in the International Union of Conservation of Nature's World Congress in Hawaii during 2016. Since then, it was implemented worldwide by treaties, constitutional acts, court verdicts, and national and local legal acts. Currently, it is still a quasi-legal instrument without mandatory legal power, though with the perspective for adoption at the United Nations level.

Results

Brown Trout abundance and biomass in streams without RORs generally increased over the sampling time period but dropped in streams where RORs were installed (Figure 2). The adverse effect of RORs on Brown Trout was evidently stronger in streams where several RORs were installed than in streams where only one ROR operated. The Fixed-effect Two-way ANOVA model on the before-after-control-impact design revealed the overall significance of the common treatments' (years and RORs occurrence) fixed effect. The occurrence of RORs was the single significant fixed effect, but Brown Trout biomass was also significant as a fixed effect.

Data from the 2019 correlational field experiment showed the direct effects of RORs on montane stream habitat features, fish community structure, and particularly relative abundance, biomass, natural production, and age-structure of Brown Trout stocks (Table 1). In five streams with operating RORs, water temperature and conductivity increased, and in four of them dissolved oxygen content decreased. Sampling in areas without operable water diversions showed that water temperature increased and dissolved oxygen content decreased in the stream section along the pipeline at much lower rates than in the remaining streams. Water conductivity increased in all streams, but at a significantly lower rate of increase in the two streams without water diversions than in those where water was diverted. The structure of fish communities changed substantially in sections along diversions compared with upstream sections in all streams, demonstrating the lack of connectivity between the two

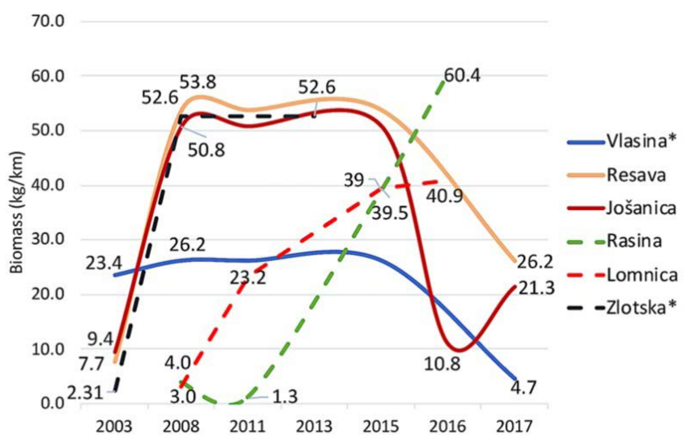

Figure 2. Relative biomass of Brown Trout populations important for conservation in streams with (solid line) and without (dashed line) RORs. The construction of RORs started in 2015).

Table 1. Species presence and fish population characteristics (for Brown Trout only) in sections upstream of water intake facilities (1) and along diversions (2).

Stream	Sample	Fish species	Relative abundance (ind. ha^{-1})	Relative biomass (kg ha^{-1})	Annual natural production (kg ha^{-1})	Age classes
Jošanica	1	Brown Trout	735	26	12.97	0-3
		Danube Barbel				
	2	Brown Trout	286	2.12	0	0
		Danube Barbel				
Brezanska	1	Brown Trout	90	3.84	1.04	1-5
		Danube Barbel				
		European Chub				
		Spirlin				
	2	Danube Barbel				
		Spirlin				
Gokčanica	1	Brown Trout	2250	63.56	0.57	1-2
	2	Brown Trout	67	0.8	0	1
		Danube Barbel				
		European Chub				
		Spirlin				
Kolska	1	Brown Trout	289	2.98	0.31	1-2
	2	Brown Trout	50	0.45	0	1
Sokoljska	1	Brown Trout	39	20.66	11.94	1-5
		Danube Barbel				
	2	Brown Trout	20	0.58	0	1
		Danube Barbel				
Panjica	1	Brown Trout	533	24.85	13.21	0-3

stream sections and indicating a drop in abundance and in age structure of Brown Trout occurring in sections along the diversions. Relative biomass of Brown Trout and Danube Barbel revealed variability determined predominantly by Brown Trout for upstream sections (at PC1) and by Danube Barbel for downstream sections (at PC2), with the right angle between their eigenvectors suggesting the complete lack of correlation, i.e., impact occurring between those two species (Figure 3). Hierarchical clustering of PCA scores showed upstream sections of particular streams (rivers Jošanica, Gokčanica, Sokoljska, and Panjica) to be sharply distinguishable from their downstream counterparts (Figure 3).

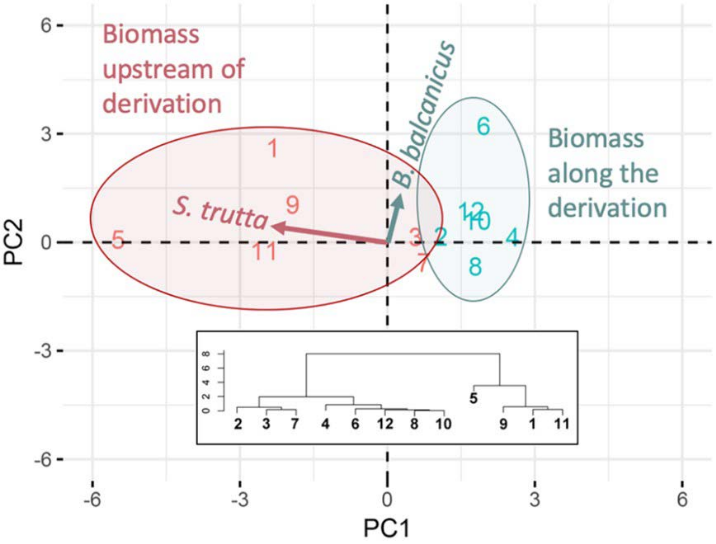

Figure 3. Biplot of the first two Principal Components' loadings (vectors) and scores revealing their determination by particular fish species, grouped by the sections of streams (odd numbers denote section upstream of, and even numbers the downstream ones along the diversion). The relationship between sections was assessed by theirs Complete Linkage clustering using the Euclidean distances between them (1 and 2, Jošanica River; 3 and 4, Brezanska River; 5 and 6, Gokčanica River; 7 and 8, Kolska River; 9 and 10, Sokoljska River; 11 and 12, Panjica River).

of fish communities changed substantially in sections along diversions compared with upstream sections in all streams, demonstrating the lack of connectivity between the two stream sections and indicating a drop in abundance and in age structure of Brown Trout occurring in sections along the diversions. Relative biomass of Brown Trout and Danube Barbel revealed variability determined predominantly by Brown Trout for upstream sections (at PC1) and by Danube Barbel for downstream sections (at PC2), with the right angle between their eigenvectors suggesting the complete lack of correlation, i.e., impact occurring between those two species (Figure 3). Hierarchical clustering of PCA scores showed upstream sections of particular streams (rivers Jošanica, Gokčanica, Sokoljska, and Panjica) to be sharply distinguishable from their downstream counterparts (Figure 3).

The findings regarding adverse impacts of RORs were presented to the Tribunal in extenso. The Tribunal accepted the Mt. Kopaonik rivers' case as a potential violation of the rights of aquatic ecosystem under the Declaration of Mother Earth, as a potential case of ecocide, under the currently proposed definition, and as a contravention of the requirements of the European Water Framework Directive and other legislation for the protection of Nature. Serbian ministers in charge of energetics and environment protection and the relevant representatives of the European Commission (EC) were invited to participate in the Tribunal as the defendants in order to present their reply to the Tribunal's charges. Only the EC representatives responded, by declining the invitation due to the conflict with their schedules. Though they did re-direct the invitation to another, more relevant EC official. The Tribunal set the COVID-dependent virtual hearing for the Balkan rivers case for April 24, 2021. The full verdict, promulgated by the team of judges on September 4, stated that in the Mt. Kopaonik Rivers vs. the State of Serbia case there was a clear violation of the Rights of Nature. They found the Serbian State and the EC liable for wrongful failure to act and for ecocide, enjoining the Serbian State to adopt deconstruction of dams and putting a halt on all new ones. They also directed the EC to exclude new hydropower plants from the renewable energy category within the framework of the objectives set by the Renewable Energy Directive and to eliminate the subsidies for small hydropower plants, because they degrade aquatic ecosystems.

Discussion

In contrast to Scandinavian and Alpine countries where annual precipitation varies from 1110 mm in Austria to 1540 mm in Switzerland, Serbia has only 686 mm of annual precipitation (https://www.indexmundi.com/facts/indicators/AG.LND.PRCP.MM/map/europe). Its runoff of only 5.7 L sec^{-1} km^{-2} (Anonymous 2015) is the lowest in the Balkans. It is one of Europe's poorest areas regarding availability of surface water resources and those water resources are located mostly in networks of small streams of uneven hydrologic regime in montane region (Ristić et al. 2012) and are "carriers" of biodiversity.

Citizens of municipalities, as well as the judges from the Tribunal, realized that both approaches we employed confirmed the hypothesis that Simonović (2020) stated about adverse effects of RORs on montane streams' fish communities. The reduction of water level, water velocity, and flow rate in downstream sections parallel to diversions resulted in warming of the water, decreases in oxygen content, and increases in mineral dissolution. These changes reduce the diversity of habitat and its suitability for various age classes of Brown Trout and other fish species. Numerous studies have revealed large-scale trends of fish species loss and reduced abundance due to loss of habitat, habitat fragmentation, and disruption of the hydrological regime (Nilsson et al. 2005; Liermann et al. 2012; Carvajal-Quintero et al. 2017). In streams of greater order *sensu* Horton (1945) and Strahler (1957), changes in habitat features and close vicinity of next-order streams favored fish species that tolerate warmer water with lower oxygen content (e.g., Spirlin and European Chub). These fish then spread upstream into the sections along the diversion pipes where they displace Brown Trout (Table 1).

The effects of RORs that we detected differed from those detected by Bilotta et al. (2016) for British streams. They observed similar Brown Trout population sizes before and after installation of RORs equipped with fish passage. They attributed this lack of change in fish populations to the migratory instinct in Brown Trout and to operational changes that stopped water abstraction during low-water periods. Weiss et al. (2018) stated that if there is generous residual flow in the stream section along the diversion, environmental impacts can be considerably reduced. Stream-dwelling, resident Brown Trout in Serbia lack a migratory instinct, which is a precondition for the functionality of fish passage structures (Armstrong et al. 2010). It is unlikely that Brown Trout would use the fish ladders already installed in montane streams of Serbia, especially during the year-round low-water periods when flow down the fish ladder is reduced giving a weak "fishway entrance" signal (Silva et al. 2018). These conditions likely negate the benefit of constructing fish passage facilities on montane streams of

Serbia, even under the hypothetic best possible surveillance of ROR operating regimes by authorities.

The stocking of hatchery-reared Brown Trout fry that serve as the usual compensatory measure for decreased natural productivity in EIASs in streams with RORs has no effect in sections along diversions where water is already scarce and of poor quality. If applied, the stocking might only cause harm to the genetic integrity of native Brown Trout populations (White 1989; Laikre and Ryman 1996; Ferguson 2006), which has already been observed in some Serbian streams (Simonović et al. 2015). The verdict of the Tribunal and measures it issued to the State of Serbia, led to the proposal for the implementation of various mechanisms of the Restorative Justice, a form of the holistic community-based justice that aims to repair and care for relationships within the whole of the community, both human and non-human living beings.

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Small Giants: Tributaries Rescue Japanese Native Salmonid Metapopulations from Habitat Fragmentation

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Abstract - We studied dynamics of spatially structured populations of Japanese landlocked salmonids, White-spotted Char *Salvelinus leucomaenis japonicus* and Red-spotted Masu Salmon *Oncorhynchus masou ishikawae*, persisting for > 30 years in a headwater stream network that is highly fragmented due to low-head dams in the mainstem (i.e., six impassable infrastructures in a < 500 m stream segment). We parameterized and analysed spatial matrix population models using 9-year annual mark-recapture data. Tributaries supported higher survival rates in some life stages, and movement was asymmetrical from the tributaries to the mainstem. Accordingly, the tributaries maintained higher population growth than the mainstem and made disproportionate demographic contributions to the metapopulation persistence in both species, despite the tributaries occupying only 12 or 18% of the study stream network by surface area. The tributaries harboured more complex instream habitats (i.e., higher wood density and flow refugia), indicating that habitat quality was more important than habitat size in determining the dynamics of these spatially structured populations. We conclude that small tributaries have rescued the spatially structured populations from extirpation due to habitat fragmentation. Fisheries and habitat management underestimating the demographic value of small tributaries will likely fail to conserve trout populations in headwaters, where coldwater conservation is critical worldwide.

Introduction

White-spotted Char *Salvelinus leucomaenis japonicus* and Red-spotted Masu Salmon *Oncorhynchus masou ishikawae* are native to central and southern parts of the main island of Japan, called Honshu (Figure 1). Their native populations have declined due to common culprits such as habitat loss, invasive species, and hybridization, and genetically pure land-locked populations typically persist in small headwaters. Habitat fragmentation is a grave threat to these isolated populations because Japanese streams are intensely managed for erosion control. This has resulted in ubiquitous occurrences of low-head dams in Japanese headwaters and consequently local extirpations of native salmonid populations (Morita and Yamamoto 2002).

Despite well-documented effects of low-head dams on population persistence, some native salmonid populations appear to persist in highly fragmented headwater streams in Japan. Sabusawa Creek is an example of such streams (Figure 2). It is a mountainous headwater stream (880 - 1,360 m in altitude) in the Fuji River system, central Japan (35°48’28” N, 138°34’13” E). Five impassable low-head dams and a passable culvert exist in a < 500 m mainstem segment. However, populations of White-spotted Char and

Red-spotted Masu Salmon have persisted for > 30 years after the dam construction.

We conducted a 9-year mark-recapture study to characterize metapopulations in this tributary-mainstem headwater network. Our analysis showed that small tributaries have made disproportionate contributions demographically and have likely rescued these spatially structured populations from extirpation in the highly fragmented stream. Below we provide a summary of this work and more details can be found in Tsuboi et al. (2020, 2022).

Figure 1. White-spotted Char (top) and Red-spotted Masu Salmon (bottom) in Sabusawa Stream

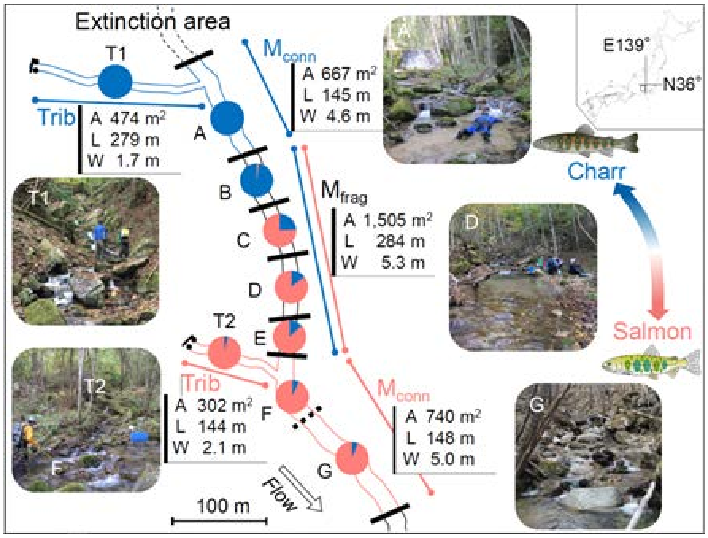

Figure 2. Map of Sabusawa Stream in central Japan. Dams and culverts (solid lines) block upstream fish movement, except between Section F and G (dotted line). Pie charts show the proportion of charr (blue) and salmon (red) count in each section (A-G, T1 and T2) averaged between 2009-2017. Patches are defined as T1, A, and B-E for charr, and T2, F-G, and B-E for salmon for matrix population analysis. Surface area (A), stream length (L) and width (W) are shown.

Materials and Methods

Study Area

This study was conducted in the Sabusawa Stream, which was characterized by two small tributaries and a highly fragmented main stem (Figure 2). The mainstem is divided into seven sections (A–G) by barriers including low-head erosion control dams and a culvert, which were installed between 1987 and 1992. All barriers impede upstream movement of fish, except the low-head dam (circa 1.4 m high) between Section F and G. Fish can move between the tributaries and the mainstem (i.e., T1-A and T2-F). Environmental characteristics differ between the tributaries and the mainstem, with the former having slower water velocity and more abundant physical shelters for fish created by undercut banks and coarse woody debris than the mainstem. Given these environmental characteristics, we defined three spatial patches for demographic analysis of each species: *tributary* (Section T1 for char and Section T2 for salmon), mainstem sections with unimpeded connectivity to tributaries ("*mainstem connected*" hereafter; Section A for char and Section F and G for salmon), and a set of mainstem sections without connectivity to tributaries ("*mainstem fragmented*" hereafter; Section B through Section E for both species) (Figure 2). Stream width was approximately 2 m in the tributaries versus 5 m in the mainstem, and the tributary patches were shorter than the mainstem patches. Consequently, the tributary patches occupied only 18% (char) and 12% (salmon) of the total habitat by surface area (Figure 2).

The Sabusawa Stream harbors two landlocked salmonid species, White-spotted Char ("char" hereafter) and Red-spotted Masu Salmon ("salmon" hereafter), with relative abundance of char increasing upstream and that of salmon increasing downstream (Figure 2). No other fish species are present in the study area. Char and salmon are short-lived (< 5 years) and exhibit restricted movement.

Field Sampling

A mark-recapture survey of the two species was conducted by backpack electrofishing annually in the third weekend of October in 2009–2017. Sampling occurred immediately before the spawning season, which overlaps between the two species. All captured fish with fork length > 43 mm, the smallest fish captured by electrofishing, were individually marked with a unique combination of four elastomer colors injected subcutaneously on the forehead (Visible Implant Elastomer Tags, Northwest Marine Technology, Inc., Anacortes, WA, USA). The timing of the survey allowed us to confidently determine maturity status of individuals by the presence of eggs and milt and other external characteristics. We recorded maturity status of individuals as age-0 (young-of-the-year), immature, and mature. A total of 1,372 char and 1,335 salmon were captured and marked uniquely during 2009-2017.

Statistical Analyses

We constructed metapopulation matrix models for char and salmon representing dynamics of three habitat patches and three life stages. Three habitat patches (i.e., tributary, mainstem connected, and mainstem fragmented) were defined differently for each species because of their different longitudinal distributions (Figure 2). Metapopulation matrix models were constructed using pre-breeding census representation. The three life stages defined for each species were age-0, immature, and mature fish (Figure 3). In both species, a proportion of age-0 reach sexual maturity in the following year, and transition probabilities to the next stages were denoted by Ω (maturation), given survival (Φ). Fecundity (F), or number of eggs, was based on body size of mature females. Vital rates were modeled to differ by patch, and movement among patches was modeled for the three life stages in char but not in salmon. These vital rates were estimated using multi-state Cormack-Jolly-Seber analysis of mark-recapture data with a Bayesian approach (Kéry and Schaub 2012), except that fecundity (F) and annual survival probability of eggs (ϕ_0) were inferred using other field data (Figure 3). We used posterior samples from the Cormack-Jolly-Seber analysis in the metapopulation matrix models to account for parameter uncertainty.

We followed the vec-permutation matrix approach (Hunter and Caswell 2005) to construct metapopulation matrix models. During annual census steps, movement between patches was modeled to occur first, followed by the demographic processes of survival and transition to the next stages. To model movement of age-0, immature, and mature fish between patches, a block diagonal matrix $\mathbf{M}_j$ for species j was constructed, in which diagonal element $\mathbf{M}_{h,j}$ represents movement of stage h (1 = age-0, 2 = immature, and 3 = mature) of species j among the three patches;

$$M_j = \begin{pmatrix} M_{1j} & 0 & 0 \\ 0 & M_{2j} & 0 \\ 0 & 0 & M_{3j} \end{pmatrix}.$$

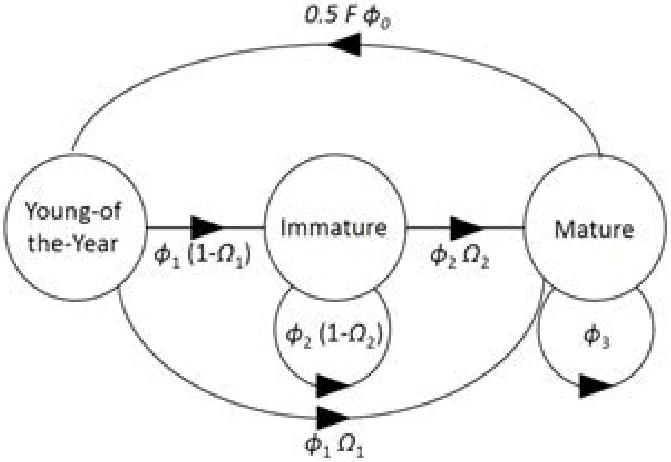

Figure 3. Life cycle graph of char and salmon that represent stage-structured demographic parameters in a local patch. Symbols indicate: F = fecundity, ϕ_0 = survival probability of eggs, ϕ_1 = survival probability of age-0 fish, ϕ_2 = survival probability of immature fish, ϕ_3 = survival probability of mature fish, Ω_1 = maturation probability of age-0 fish, and Ω_2 = maturation probability of immature fish.

For each stage (h = 1,2,3) of char in $\mathbf{M}_{h,1}$, their elements $\Psi_{h,j,p,q}$ represented the probability that an individual in stage h moves from patch p to patch q (1 = tributary, 2 = mainstem connected, and 3 = mainstem fragmented) (Figure 2). For example, $\Psi_{1,1,1,2}$ represented the movement probability of char (j = 1) in the age-0 stage (h = 1) moving from the tributary (p = 1) to the mainstem connected patch (q = 2). Individuals stayed in the same patch when $p = q$. For char ($\mathbf{M}_{h,1}$), this was represented by;

$$M_{h,1} = \begin{pmatrix} \Psi_{h,1,1,1} & \Psi_{h,1,2,1} & 0 \\ \Psi_{h,1,1,2} & \Psi_{h,1,2,2} & 0 \\ \Psi_{h,1,1,3} & \Psi_{h,1,2,3} & 1 \end{pmatrix}.$$

Char in the fragmented mainstem could not move upstream due to habitat fragmentation (Figure 2). Therefore, all individuals were locked in this patch (i.e., $\Psi_{h,1,3,3} = 1$).

For salmon ($\mathbf{M}_{h,2}$), elements Ψh,j,p,q represented the probability that an individual in stage h of salmon (j = 2) moved from patch p to patch q;

$$M_{h,2} = \begin{pmatrix} \Psi_{h,2,1,1} & \Psi_{h,2,2,1} & \Psi_{h,2,3,1} \\ \Psi_{h,2,1,2} & \Psi_{h,2,2,2} & \Psi_{h,2,3,2} \\ 0 & 0 & \Psi_{h,2,3,3} \end{pmatrix}.$$

Immigration into the mainstem fragmented patch could not happen in salmon (Figure 2), so that $\Psi_{h,2,1,3} = \Psi_{h,2,2,3} = 0$. In salmon, movement was modeled to be constant across life stages such that $\mathbf{M}_{1,2} = \mathbf{M}_{2,2} = \mathbf{M}_{3,2}$, due to a small sample size which caused the model not to converge.

Demography was represented by a block diagonal matrix $\mathbf{B}_j$ for species j, which included 3 × 3 local patch demographic matrices diagonally and 0 elsewhere. Let $\mathbf{B}_{i,j}$ be a 3 × 3 demographic projection matrix for patch i (1 = tributary, 2 = mainstem connected, and 3 = mainstem fragmented) and species j (1 = char, and 2 = salmon);

$$\mathbb{B}_j = \begin{pmatrix} B_{1j} & 0 & 0 \\ 0 & B_{2j} & 0 \\ 0 & 0 & B_{3j} \end{pmatrix}.$$

Each demographic projection matrix $\mathbf{B}_{i,j}$ was represented by patch-specific vital rates, where $F_{i,j}$ is fecundity, or number of eggs that a mature female of average size will produce in patch i for species j, $\Phi_{h,i,j}$ is the probability of annual survival of stage h of species j in patch i, and $\Omega_{h,i,j}$ is the probability of maturation (i.e., transition to mature fish) of stage h of species j in patch i. The sex ratio approximates 1:1 in landlocked char (Yamamoto et al. 1999) and salmon (Morita et al. 2018), so that fecundity was multiplied by 0.5. Because our projection matrices are represented by pre-breeding census formulation, fecundity was multiplied by egg-to-YOY survival probability, $\phi_{0,j}$, for each species j;

$$B_{ij} = \begin{pmatrix} 0 & 0 & 0.5F_{ij}\Phi_{0j} \\ \Phi_{1,ij}(1-\Omega_{1,ij}) & \Phi_{2,ij}(1-\Omega_{2,ij}) & 0 \\ \Phi_{1,ij}\Omega_{1,ij} & \Phi_{2,ij}\Omega_{2,ij} & \Phi_{3,ij} \end{pmatrix}.$$

Metapopulation projection matrix $\mathbf{A}_j$ for species j is then $\mathbf{A}_j = \mathbf{B}_j\ \mathbf{P}^{T}\ \mathbf{M}_j\ \mathbf{P}$, where $\mathbf{P}$ is the vec-permutation matrix (Hunter and Caswell 2005). For a metapopulation composed of s life stages and t patches, $\mathbf{P}$ has a $st \times st$ dimension and is derived by;

$$P(s,t) = \sum_{i=1}^{s} \sum_{j=1}^{t} E_{ij} \otimes E_{ij}^{T},$$

where E_{ij} is an $s \times t$ matrix with 1's in the (i,j) positions and 0's elsewhere, and $\otimes$ denotes the Kronecker matrix product. The resulting $\mathbf{A}_j$ for species j is a 9×9 matrix representing diagonally demography of patch i ($A_{i,j}$; a 3×3 matrix) after emigration from the patch was accounted for, and a combination of demography and movement dynamics off-diagonally;

$$\mathbb{A}_j = \begin{pmatrix} A_{1j} & a_{2,1j} & a_{3,1j} \\ a_{1,2j} & A_{2j} & a_{3,2j} \\ a_{1,3j} & a_{2,3j} & A_{3j} \end{pmatrix},$$

where $a_{p,q,j}$ denotes demography and emigration of individuals in species j from patch p to patch q (1 = tributary, 2 = mainstem connected, and 3 = mainstem fragmented).

Eigen analyses of the project matrix models were used to characterize metapopulation dynamics. Metapopulation growth rate (λ_M) was inferred for species j based on metapopulation projection matrix A_j. Population growth rate was inferred for patch i for species j before loss of emigrants was accounted for ("pre-emigration" lambda, λ_{Pre}) based on matrix $B_{i,j}$ and after loss of emigrants was accounted for ("post-emigration" lambda, λ_{Post}) based on matrix $A_{i,j}$. We interpreted the difference in patch growth rates between the two ($\Delta\lambda = \lambda_{Pre} - \lambda_{Post}$) as a measure of demographic contribution of the patch to the metapopulation growth rate because emigrants from a patch ended up elsewhere in our isolated study area. Analysis of population growth rates was based on posterior samples of movement and demography in the multi-state Cormack-Jolly-Seber models, so uncertainties about population growth rates were quantified using 95% credible intervals (CI). We interpreted population growth to be positive if the 95% CI of λ_{Pre} or λ_{Post} was > 1 and negative if it was < 1.

Results

Movement

Fish movement among habitat patches was limited overall. When movement occurred, it was asymmetrical from upstream to downstream patches even when physical barriers were absent (Figure 4). In char, movement was most common from the mainstem connected (Section A) to the mainstem fragmented patch (Section B-G) at the age-0 stage (mean probability = 0.26; 95% CI = 0.16-0.39). Movement of mature fish from the tributary (Section T1) to the mainstem fragmented patch was the next most frequent (mean probability = 0.17; 95% CI = 0.01-0.55).

Salmon similarly emigrated to the lowermost, mainstem connected patch (Section F and G) from upstream (Figure 4). The most common movement occurred from the tributary (Section T2) to the mainstem connected patch (mean probability = 0.29; 95% CI = 0.15-0.48), followed by movement from the mainstem fragmented (Section B-E) to the connected patch (0.12; 95% CI = 0.07-0.19). In both species, immigration into the tributary patch was infrequent.

Demography

In general, tributaries supported higher annual survival rates than mainstem patches (Figure 5). In char, survival of mature fish was higher in the tributary patch (mean probability

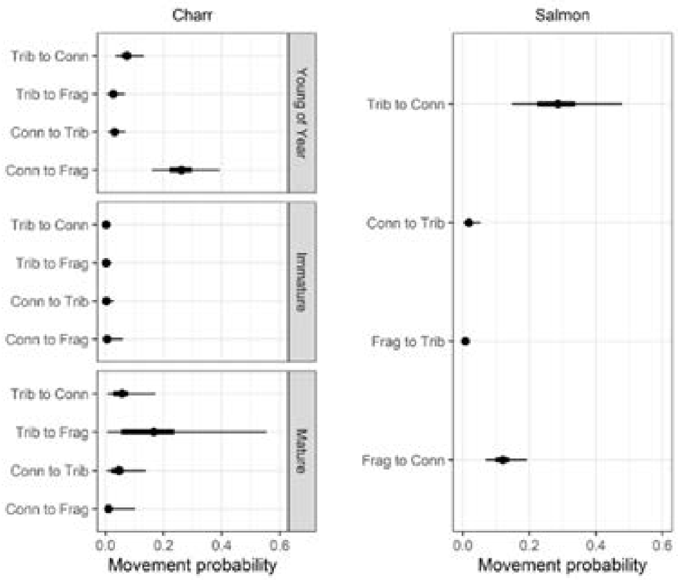

Figure 4. Posterior distributions of movement probabilities between three patches. Movement was modelled to differ by life stage for char but was constant among stages for salmon due to low sample size. Posterior mean values are shown by dots with 50% (thick line) and 95% (thin line) credible intervals.

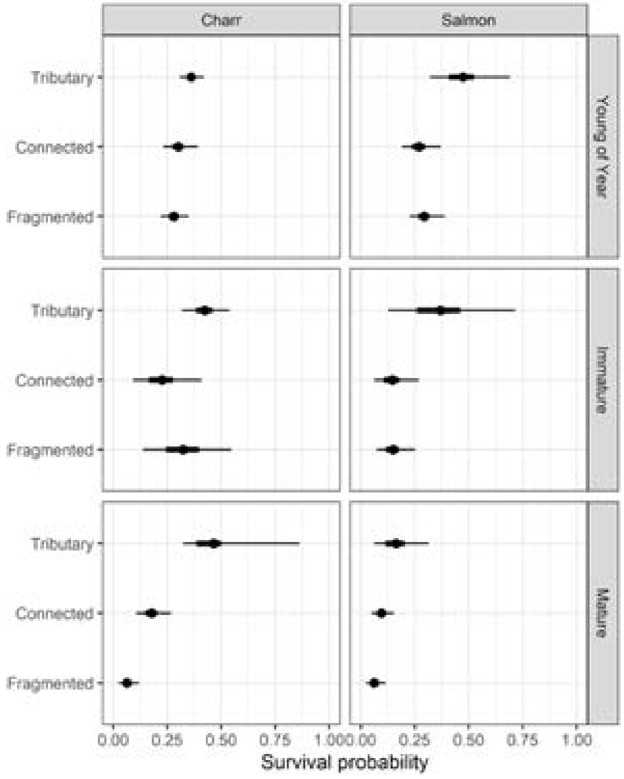

Figure 5. Posterior distributions of annual survival probabilities of young-of-the-year, immature and mature fish in three habitat patches. Posterior mean values are shown by dots with 50% (thick line) and 95% (thin line) credible intervals.

= 0.47; 95% CI = 0.32-0.86) than in the mainstem connected (0.18; 95% CI = 0.11-0.27) and fragmented (0.06; 95% CI = 0.02-0.12) patches. In salmon, the tributary supported higher survival rates of age-0 (0.47; 95% CI = 0.32-0.69) and immature stage (0.37; 95% CI = 0.13-0.71), compared to the mainstem connected and fragmented patches.

Maturation probability was similar among habitat patches, except that a smaller proportion of age-0 char matured in the tributary patch (mean = 0.69; 95% CI = 0.61-0.79), compared to the mainstem connected (0.83; 95% CI = 0.74-0.90) and fragmented patch (0.90; 0.83-0.95). Across patches, the mean maturation probability ranged 0.86-0.97 in immature char, 0.62-0.73 in age-0 salmon, and 0.64-0.73 in immature salmon.

Metapopulation Stucture

Mean metapopulation growth rate (λ_M) was positive in both species, reaching 1.05 (95% CI = 0.95-1.15) in char and 1.03 (95% CI = 0.89-1.21) in salmon. The tributary patches had the highest pre-emigration population growth rate in both species, with a mean λ_{Pre} of 1.22 (95% CI = 1.10-1.48) in char and mean λ_{Pre} of 1.30 (95% CI = 1.06-1.60) in salmon (Figure 6). The lower bound of the 95% CI was > 1 in both cases, indicating that population growth rates were positive in the tributaries, before emigration was accounted for. The posterior samples of λ_{Pre} were mostly > 1 for the mainstem connected patch for char (mean = 1.07; 95% CI = 0.94-1.23) but were about equally positive and negative in the mainstem fragmented patch for char, and the mainstem connected and fragmented patches for salmon.

Once emigration was accounted for, population growth rates decreased most in the tributary patches for both species (Figure 6). The mean λ_{Post} of the tributary patch was 1.00 (95% CI: 0.78-1.13) for char and 0.92 (95% CI: 0.74-1.12) for salmon. The mean difference between pre- and post-emigration population growth rates ($\Delta\lambda$) was 0.22 in char and 0.38 in salmon, and $\Delta\lambda$ of the tributary patch was larger than those of the mainstem connected and fragmented patches in both species, demonstrating that the tributaries made disproportionate demographic contributions to metapopulation persistence. The mean λ_{Post} of the mainstem connected patch decreased to 0.89 in char; its 95% CI ranged below 1 (0.77-0.99) whereas 95% CI of λ_{Pre} was mostly above 1 when emigration was not accounted for (Figure 6). This indicated that the mainstem connected patch of char relied on immigration from the tributary for population persistence because immigration could not happen from the mainstem fragmented patch due to dams (Figure 2). In salmon, the mean λ_{Post} of the fragmented mainstem patch was 0.88,

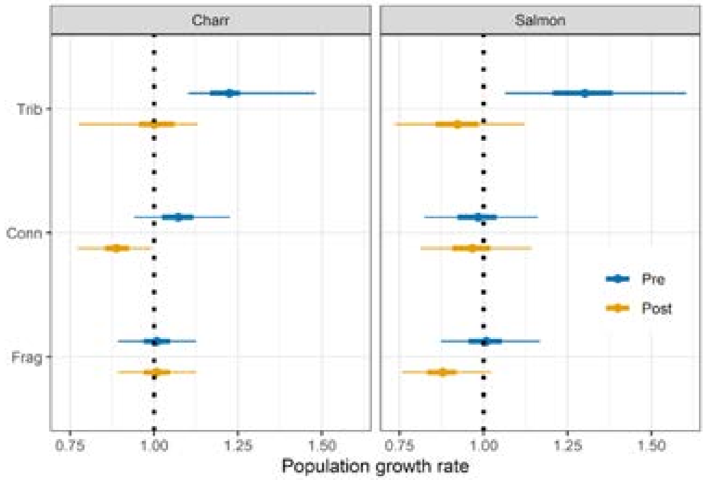

Figure 6. Posterior distributions of population growth rates before (λ_{pre}) and after (λ_{post}) loss of emigrations was accounted for. Posterior mean values are shown by dots with 50% (thick line) and 95% (thin line) credible intervals. Vertical dotted lines indicate where populations growth rates are 1 (i.e., populations are neither increasing nor decreasing).

and 95% of the posterior λ_{Post} samples barely overlapped 1 (0.76-1.02). This indicated that the salmon population in the fragmented mainstem patch could not maintain a positive population growth. At the time of this writing (2022), salmon have been extirpated in Section B and C.

Discussion

The small tributaries were important for the metapopulation persistence of native salmonids in this highly fragmented tributary-mainstem network. Tributaries supported higher survival rates in certain life stages, and movement was asymmetrical from the tributaries to the mainstem when it occurred. Pre-emigration population growth rates (λ_{Pre}) were the highest in the tributaries in both species, but after accounting for emigration, population growth rates (λ_{Post}) were similar among the habitat patches. These demographic results showed that the spatially structured populations in our study system were characterized by source-sink dynamics in which tributaries were critically important in sustaining the metapopulations that occupy the mainstem fragmented by a series of dams. Notably, the tributaries (circa 2 m wide) were less than half as wide as the mainstem (circa 5 m) and occupied only 18% (char) and 12% (salmon) of the study system by surface area.

We reason that the tributaries made large demographic contributions due to patch quality, not patch size. The tributaries were more physically complex, characterized by higher densities of large wood and more heterogeneous hydraulic patterns that created slower-moving eddies (Tsuboi et al. 2020). These habitat features were less common in the mainstem due presumably to the series of dams altering hydrological and sediment transport regimes. Habitat complexity coincided with higher survival rates of some life stages in each species in the tributaries, which led to their higher population growth rates relative to the mainstem before loss of emigrants was accounted for.

The persistence of char and salmon for over 30 years after dam construction is noteworthy. The total stream length of the three habitat patches is < 1 km for each species and includes five impassable dams. The basin size was only 0.41 km2 for T1 and 0.64 km2 for T2 in this study. Our analysis shows that the tributaries have prevented the metapopulation extirpation of char and salmon.

In conclusion, this study highlights the importance of conserving small headwaters. Headwaters are often overlooked in aquatic conservation planning. In Japan's River Act, headwaters are not considered an aquatic habitat, which has resulted in widespread constructions of dams in Sabusawa Stream and nationwide. Under the Clean Water Act of the U.S.A., waterways are afforded with legal protection only when they are deemed to have "significant nexus" with seemingly larger, more productive habitats downstream (Colvin et al. 2019). Our study shows that headwaters serve as fish habitat per se and provides scientific support for the ecological importance of headwater habitats based on a long-term demographic study. Our findings offer guidance on native trout conservation in headwaters, such as habitat connectivity restoration and spatially structured fishery regulations.

Acknowledgements

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Effects of Elevated Water Temperatures on Trout Angler Catch Rates and Catch-And-Release Mortality

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Abstract - During catch-and-release angling, some released fish do not survive, and there is growing concern that as climate change increases summer water temperatures in streams, occasional cessation of angling may be needed to protect fish populations. The objectives of the present study were to evaluate whether relative survival of fish caught by anglers was reduced when water temperature was elevated at the time of landing, and to evaluate the effect of temperature on angler catch rates. Anglers caught, marked, and released Cutthroat Trout *Oncorhynchus clarkii* (17 – 37 cm in length) in streams at temperatures from 13.5 to 25.7°C. Recapture rate of marked fish (i.e., relative survival) declined from 0.58 for fish landed at water temperatures <19°C to 0.30 for fish landed at temperatures >21°C, but angler catch rate declined similarly, with mean catch rates of 5.3 fish/h at temperatures <19°C and 3.4 fish/h at temperatures >21°C. Considering both declines, the number of fish mortalities/angler/h might be higher at cooler temperatures than at warmer temperatures, thus inhibiting fishing at elevated temperatures would likely have no more benefit to a trout population than it would at lower temperatures. Moreover, such temperatures are currently rare in Idaho's most popular trout fisheries. Consequently, we urge caution on implementing temperature-induced angling closures until population-level benefits are shown.

Introduction

Climate change models predict large reductions in salmonid occupancy of flowing waters during the 21st century as some streams become too warm to support coldwater fish populations (Isaak et al. 2015). In addition to restricting the ability of salmonids to occupy warmer sections of streams, elevated stream temperature will also likely impact their ability to tolerate and recover from human-induced stressors they are subjected to in reaches where they can persist (reviewed in McCullough et al. 2009). One stressful event that salmonids are commonly exposed to is handling when they are caught and released by anglers.

Catch-and-release angling in recent decades has become very popular among anglers of all types, especially trout anglers (Policansky 2002). While catch-and-release angling – whether voluntary or mandatory – can be an effective tool to limit fishing-related mortality in recreational fisheries, not all fish that are released by anglers survive (e.g., High and Meyer 2014). In general, the level of fishing mortality induced by anglers during the catch-and-release process is directly related to the physical injury and level of stress a fish experiences while being hooked, landed, and handled prior to release (reviewed by Muoneke and Childress 1994). Some stress factors, such as fight duration and air exposure duration during the landing and releasing process (Lamansky and Meyer 2017), and the terminal tackle used (High and Meyer 2014), are within the control of anglers. Other factors, such as the water temperature that fish are experiencing while being hooked and landed, cannot be controlled by anglers unless they cease fishing when the temperature becomes elevated.

Temperature-related angling restrictions on trout and salmon in North America have been implemented in some Canadian provinces (Dempson et al. 2001) and some U.S. states, such as Montana as part of their drought fishing closure policy (Boyd et al. 2010). The Montana policy states that angling is closed (for all or part of the day) in waters containing salmonids when daily maximum water temperature is ≥ 23°C for three consecutive days. This policy was based on a study in Montana which found that catch-and-release angling on days in which maximum water temperature exceeded 23°C resulted in 13% mortality for Rainbow Trout *Oncorhynchus mykiss* and 3% mortality for Brown Trout *Salmo trutta* that were held in cages for three days, compared to zero mortality for both species for fish caught and held on days in which maximum water temperatures never exceeded 20°C (Boyd et al. 2010). However, differential mortality of free-ranging trout in relation to water temperature at the time of capture has not been investigated. Moreover, angler catch rates for stream-dwelling salmonids may decline at higher water temperature (McMichael and Kaya 1991; Van Leeuwen et al. 2021), dampening the impact that elevated water temperature may have on lotic fish populations by reducing the number of fish landed by anglers when temperatures are warmer. We are unaware of any studies simultaneously investigating angler catch rates and the survival of stream-dwelling trout caught and released by anglers in relation to relatively warm summer stream temperatures. Consequently, our objective was to quantify the effect that elevated water temperature had on catch rates and catch-and-release mortality in stream-dwelling trout populations.

Methods

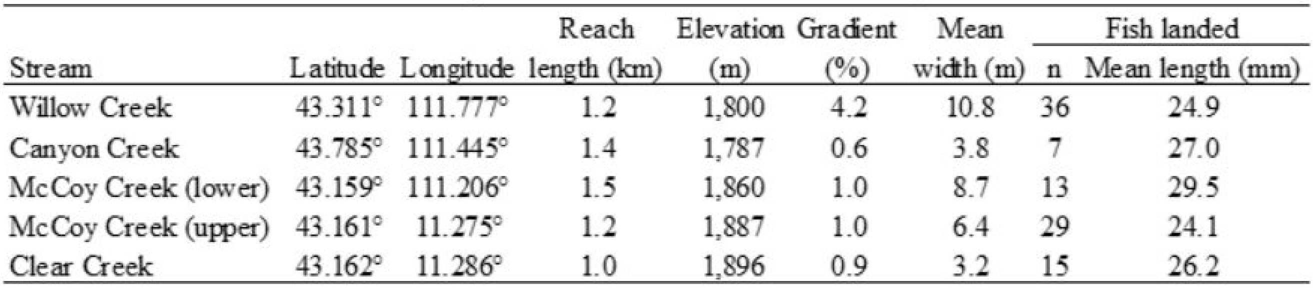
We conducted our study in four streams in eastern Idaho with summer water temperatures that were relatively high but that nonetheless maintained relatively abundant populations of stream-resident Cutthroat Trout *O. clarkii* (Table 1). Angling regulations prohibited the harvest of Cutthroat Trout in all study streams. Brook Trout *Salvelinus fontinalis* were also occasionally encountered, they were not included in the survival portion of the study because only a few were landed (n = 5), and it has been previously shown that survival of caught-and-released salmonids at elevated temperatures can differ among species (Boyd et al. 2010).

Angling occurred from July 27 to August 12, 2020, during some of the warmest days of the year. Anglers fished from about 0900 to 1800 hours each day as water temperatures increased from an overnight low and reached a peak for the day in late afternoon (Figure 1). One or two anglers fished each reach over part or all of any given day, but no reach was fished more than three times over the course of the study. Anglers recorded start and end times for each period of angling, and time recording was halted throughout the day for any nontrivial interruptions in angling effort (e.g., lunch break). Anglers used artificial flies exclusively to capture fish, and a landing net was used to minimize handling stress during data collection.

For each fish caught, species was recorded, and total length (to the nearest cm) was measured in the landing net underwater using a tape measure. Time of capture was also recorded, and instantaneous water temperature at the time of capture was measured with a digital thermometer. Fight time was minimized to the extent possible but was not recorded explicitly. Landed fish were tagged with an individually-numbered anchor tag inserted just below the base of the dorsal fin. We assumed that tagging mortality was inconsequential. An adipose fin clip was used to evaluate whether any anchor tags were shed prior to recapture efforts. No fish were landed by anglers more than once. Fish were released at the point of capture, having received no air exposure during the catch-and-release process. Processing time from the point of landing the fish to releasing it was not measured but generally took 1-2 minutes.

Post-release relative survival was evaluated by recapturing tagged fish on August 25-27, 2020, using a single backpack electrofishing pass through each stream reach where angling occurred. Electrofishers were set at 60 Hz, 25% duty cycle, and enough volts to emit about 100 Watts of average power output. Captured fish were examined for anchor tags and adipose fin clips (none of the recaptured fish had lost their tag), measured for length (nearest cm), and released after recovering from being handled. Because recapture efficiency

Table 1. Characteristics of streams in eastern Idaho where Cutthroat Trout and Brook Trout were landed to evaluate the effect of elevated summer water temperatures on relative survival and angler catch rates.



Stream	Latitude	Longitude	Reach length (km)	Elevation (m)	Gradient (%)	Mean width (m)	Fish landed n	Fish landed Mean length (mm)
Willow Creek	43.311°	111.777°	1.2	1,800	4.2	10.8	36	24.9
Canyon Creek	43.785°	111.445°	1.4	1,787	0.6	3.8	7	27.0
McCoy Creek (lower)	43.159°	111.206°	1.5	1,860	1.0	8.7	13	29.5
McCoy Creek (upper)	43.161°	11.275°	1.2	1,887	1.0	6.4	29	24.1
Clear Creek	43.162°	11.286°	1.0	1,896	0.9	3.2	15	26.2

of fish landed by anglers was clearly not 100% with backpack electrofishing in our study streams, and some emigration of fish out of each study reach may have occurred, our analyses on relative survival in no way represent actual survival and is only meaningful in a comparative sense.

The effect of water temperature on catch-and-release relative survival was examined using logistic regression. Each landed fish was the experimental unit, with fish landed and tagged by anglers receiving dummy response variables for whether they were subsequently recaptured by electrofishing (0 = not recaptured, 1 = recaptured). Because sampling efficiency likely differed between streams, stream was included as a random effect in all models. Fish length was included as a fixed effect because relative mortality could be dependent on fish length, and because capture efficiency for stream-dwelling salmonids using backpack electrofishing is size dependent (Chiaramonte et al. 2020). Angler and instantaneous water temperature at the time of landing were also included as fixed effects; angler was included to account for potential differences in handling stress for landed fish among anglers. Finally, fish length × temperature and angler × temperature interaction terms were included to evaluate whether any effect of water temperature on relative survival of caught and released fish was mediated by fish length or the angler.

The effect of water temperature on catch rate was examined using general linear models. Each landed fish (including all salmonids caught) was the experimental unit. Catch rate (fish landed/h) for each landed fish (i.e., the response variable) was calculated by dividing 60 by the number of minutes since the last fish was landed; thus, for a fish that was landed 25 minutes after the previous fish, catch rate for that fish was calculated as 60/25 = 2.4 fish/h. For each angler's last fish caught on each day, if fishing effort did not end at the time a fish was landed, then any extra fishing time that resulted in no fish landed was added to the time recorded for the last fish; this extra time averaged 18 minutes. Predictor variables included a random effect for stream and fixed effects for the angler and water temperature at the time of landing. An angler × temperature interaction term was included to evaluate whether any effect of water temperature on catch rate was mediated by the angler.

Candidate models included all combinations of predictive factors, and the random effect of stream was included in all models. Models were ranked using Akaike's information criterion corrected for small sample size (AICc), and we considered the most plausible models to be those with AICc scores within 2.0 of the best model (Burnham and Anderson 2004). We used AICc weights (wi) to assess the relative plausibility of each model. Coefficients were only considered influential if their 90% confidence intervals (CIs) did not overlap zero. This more lenient interpretation of CIs was used to balance type I and type II errors, considering our relatively small sample size.

Results

In total, we landed 100 Cutthroat Trout and 5 Brook Trout *Salvelinus fontinalis*. Total length for Cutthroat Trout ranged from 17 to 37 cm, whereas Brook Trout ranged from 20 to 26 cm. The size of landed fish was similar for all streams (Table 1). Instantaneous water temperature at the time that fish were landed ranged from 13.5°C to 25.7°C (Figure 1). During electrofishing, 50 tagged Cutthroat Trout were recaptured (Brook Trout were not tagged).

Relative survival of angled trout declined as water temperature increased, with a recapture rate (all streams combined) of 0.58 for Cutthroat Trout caught at water temperatures <19°C compared to 0.30 for those caught at temperatures ≥21°C and 0.17 for those caught at temperatures ≥23°C (Figure 2). The best model explaining the variation in catch-and-release relative survival included fish length, water temperature, and angler, as well as the random effect of stream (Table 2). There was also some support for models without some combination of water temperature, fish length, and angler, and for a model that included an

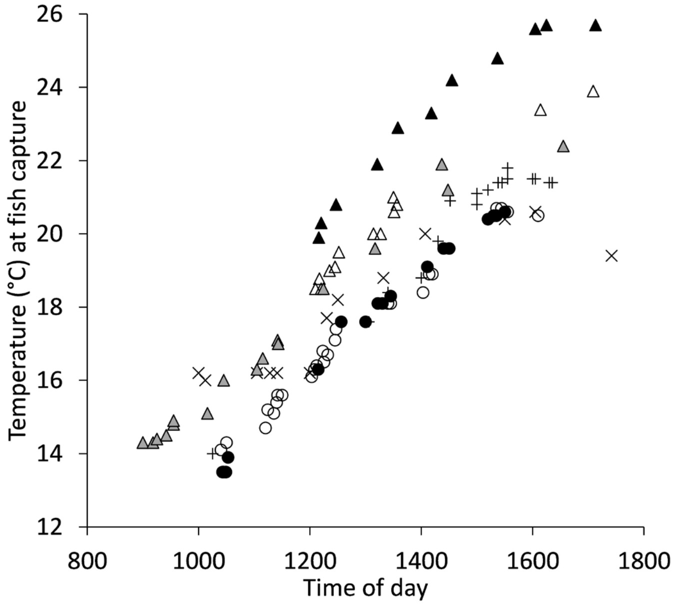


Figure 1. Instantaneous water temperatures at the time that Cutthroat Trout were landed and tagged by fly anglers in eastern Idaho streams. Each symbol type depicts data at one stream, with any change in symbol color indicating different days at that same stream.

Table 2. Comparison of models relating relative survival of Cutthroat Trout to water temperatures in eastern Idaho streams. Akaike's information criteria (AICc), change in AICc (ΔAICc), and AICc weights (wi) were used to assess models plausibility.

Model	AIC$_c$	ΔAIC$_c$	w_i
Length + temperature + angler + stream	136.94	0	0.28
Length + angler + stream	137.98	1.04	0.17
Length + temperature + length*temperature + angler + stream	138.82	1.88	0.11
Temperature + stream	138.85	1.91	0.11
Temperature + angler + stream	139.13	2.19	0.1
Temperature + length + stream	139.58	2.64	0.08
Null (stream only)	140.28	3.34	0.05
Angler + stream	140.55	3.61	0.05
Length + stream	140.79	3.85	0.04
Length + temperature + angler + temperature*angler + stream	142.95	6.01	0.01

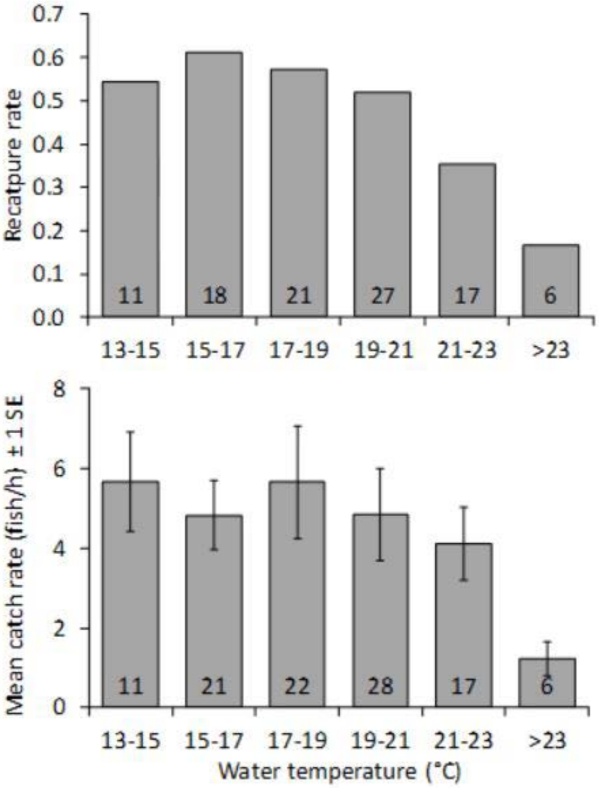


Figure 2. Recapture rate of Cutthroat Trout landed and marked by anglers, and angler catch rate of trout, in relation to summer instantaneous water temperature at the time that fish were landed. Sample size is provided inside the bars.

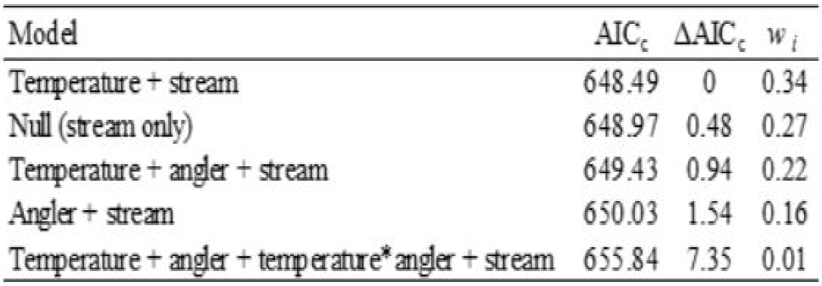
Table 3. Coefficient estimates and 90% confidence intervals (CIs) for the most plausible models constructed to evaluate the relative survival of Cutthroat Trout in relation to elevated summer water temperatures in eastern Idaho streams.

Coefficient	Estimate	90% CI
Best relative survival model		
Intercept	1.23	-2.38 - 4.84
Fish length	0.08	0.00 - 0.16
Water temperature	-0.16	-0.31 - -0.01
Angler 2	-1.14	-2.85 - 0.57
Angler 3	-1.33	-2.47 - -0.19
Angler 4	0.49	-0.56 - 1.53
Stream	0.32	-0.53 - 1.16
Second best relative survival model		
Intercept	-1.99	-4.12 - 0.13
Fish length	0.08	0.01 - 0.16
Angler 2	-1.13	-2.77 - 0.51
Angler 3	-1.17	-2.25 - -0.09
Angler 4	0.64	-0.35 - 1.63
Stream	0.32	-0.41 - 1.05
Third best relative survival model		
Intercept	6.78	-7.19 - 20.74
Fish length	-0.14	-0.67 - 0.40
Water temperature	-0.46	-1.20 - 0.28
fish length * water temperature	0.01	-0.02 - 0.04
Angler 2	-1.02	-2.73 - 0.70
Angler 3	-1.3	-2.43 - -0.17
Angler 4	0.43	-0.62 - 1.49
Stream	0.32	-0.51 - 1.15
Fourth best relative survival model		
Intercept	3	0.19 - 5.82
Water temperature	-0.16	-0.30 - -0.01
Stream	0.34	-0.43 - 1.11

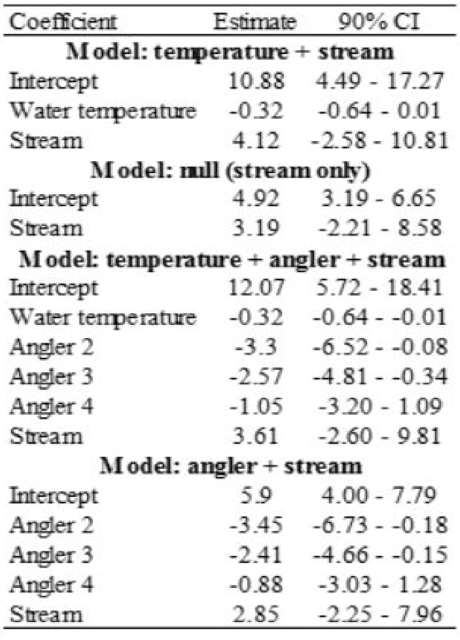
interaction between length and water temperature. In the most parsimonious model, based on parameter estimates with 90% CIs that did not include zero, relative survival was reduced at higher water temperatures, for smaller fish, and for fish caught and released by angler 3 compared to angler 1 (Table 3). The effects of fish length and angler were also considered influential (i.e., 90% CIs did not include zero) in the second best model. Interaction terms for fish length × temperature and angler × temperature were not considered influential in any model based on 90% CIs.

Catch rate also declined as water temperature increased, with a mean catch rate of 5.3 fish/h (SE = 0.7 fish/h) at temperatures <19°C compared to 3.4 fish/h (SE = 0.7 fish/h) at water temperatures ≥21°C, or 1.2 fish/h at ≥23°C (Figure 2). The best model explaining the variation observed in angler catch rate included water temperature and the random effect of stream (Table 4). There was also support for the null (random effect only) model, a model with water temperature, angler, and stream, and a model with only angler and stream. Based on parameter estimates with 90% CIs that did not include zero, water temperature was not considered influential in the best model but was considered influential in the 3rd best model (Table 5), and indicated that catch rates declined at higher water temperatures. Catch rates also varied among anglers, but an angler × temperature interaction term was not included in any of the plausible models (Table 5).

Table 4. Comparison of linear regression models constructed to evaluate catch rates of trout in relation to elevated summer water temperatures in eastern Idaho streams. Estiamtes of log-likelihood, Akaike's information criteria (AICc), change in AICc (ΔAICc), and AICc weights (wi) were used to assess plausible models.



Model	AIC_c	ΔAIC_c	w_i
Temperature + stream	648.49	0	0.34
Null (stream only)	648.97	0.48	0.27
Temperature + angler + stream	649.43	0.94	0.22
Angler + stream	650.03	1.54	0.16
Temperature + angler + temperature*angler + stream	655.84	7.35	0.01



Coefficient	Estimate	90% CI
Model: temperature + stream		
Intercept	10.88	4.49 - 17.27
Water temperature	-0.32	-0.64 - 0.01
Stream	4.12	-2.58 - 10.81
Model: null (stream only)		
Intercept	4.92	3.19 - 6.65
Stream	3.19	-2.21 - 8.58
Model: temperature + angler + stream		
Intercept	12.07	5.72 - 18.41
Water temperature	-0.32	-0.64 - -0.01
Angler 2	-3.3	-6.52 - -0.08
Angler 3	-2.57	-4.81 - -0.34
Angler 4	-1.05	-3.20 - 1.09
Stream	3.61	-2.60 - 9.81
Model: angler + stream		
Intercept	5.9	4.00 - 7.79
Angler 2	-3.45	-6.73 - -0.18
Angler 3	-2.41	-4.66 - -0.15
Angler 4	-0.88	-3.03 - 1.28
Stream	2.85	-2.25 - 7.96

Table 5. Coefficient estimates and 90% confidence intervals (CIs) for the most plausible models constructed to evaluate catch rates of trout in relation to elevated summer water temperatures in eastern Idaho streams. All parameters were fixed effects except stream, which was a random effect included in all models.

Discussion

Myriad studies have been conducted on the effects of catch-and-release angling in recreational fisheries. Most of the work from the 1960s to the 1980s focused on the benefits of catch-and-release angling, and generally showed that in waters with high exploitation, both population abundance and angler catch rates increased when anglers switched to releasing most of their catch (see Barnhart 1989). In recent decades, the focus of most catch-and-release research has shifted to concerns that individual released fish may experience sub-lethal or lethal negative impacts after release due to stressful handling practices by anglers (reviewed in Cooke and Schramm 2007). Since water temperature can be a major stressor for coldwater species such as trout, as summer stream temperatures continue to rise due to climate change (Isaak et al. 2015), concern regarding the stress that catch-and-release angling may pose to stream-dwelling salmonid populations will also continue to rise in both the scientific literature (e.g., Isaak et al. 2015) and in popular articles and social media (Painter 2021; Peterson 2021).

In the present study, there was equivalent evidence that both relative survival and catch rate of stream-dwelling trout declined as water temperature increased. Both findings concur with previous research. Indeed, while elevated water temperatures have been shown to be negatively related to catch-and-release survival for Rainbow Trout, Brown Trout, Mountain Whitefish *Prosopium williamsoni* (Boyd et al. 2010), and Atlantic Salmon S. salar (Van Leeuwen et al. 2021), elevated temperature has also been shown to be negatively related to angler catch rates for Rainbow Trout (McMichael and Kaya 1991), Brown Trout (Taylor 1978), and Atlantic Salmon (L'Abée-Lund and Aspås 1999; Dempson et al. 2002; Van Leeuwen et al. 2021). The decline in angler catch rate at higher water temperatures is important because anglers presumably will either limit their fishing effort due to lack of success, or they will handle fewer fish at warmer water temperatures due to lower catch rates.

To scale this to actual fish mortality, let's assume that fly fishing catch-and-release mortality rate for stream-dwelling trout at non-elevated water temperatures averages about 0.05 (see High and Meyer 2014 and citations therein). Relative mortality (i.e., 1-relative survival) in the present study was 0.42 at cool temperatures (<19°C) and 0.87 at high temperatures (≥23°C). Let's therefore assume that mean fly-fishing mortality rate at high temperature is about $0.05 \times (0.87/0.42) = 0.10$, or about double the mortality rate at cool temperatures. Catch rate was 5.3 fish/h at cool temperatures and 1.2 fish/h at high temperatures. So when anglers fish at cool temperatures, they can be expected to incidentally cause mortality to 5.3 fish/h × 0.05 = 0.27 fish/h. In contrast, when anglers fish at high temperatures, they can be expected to incidentally cause mortality to 1.2 fish/h × 0.10 = 0.12 fish/h. This simple thought experiment suggests that, because angler catch rates are so much higher at cooler temperatures, inhibiting fishing at cooler water temperatures would actually be more beneficial to the trout populations than would fishing closures at high water temperatures. While we certainly do not recommend such closures, this highlights the need for caution in implementing temperature-related fishing closures until population-level benefits can be demonstrated in the trout populations they are purportedly protecting. Indeed, seasonal angling closures restrict access to a public resource, so ideally they should be based on evidence of biological benefits for the fishery at the population scale.

How prevalent such high water temperatures actually are in Idaho's trout streams can also be considered. Here we only considered the main stems of the most popular trout fisheries where water temperature data was available (Table 6), assuming that if the main stems do not experience high water temperatures, neither do the tributaries (regardless of whether they also are popular trout fisheries). From 2014 to 2021, we gathered >300,000 hours of summer (Jun-Aug) hourly water temperature data at these waters and found that summer water temperatures reached or exceeded 23°C for only about 4,000 combined hours, or just over 1% of all the hours for which we had data. High water temperature occurrence was restricted to a few of the streams, usually at the lowest elevations in that stream.

The present study has a major limitation, that being a relative small sample size. This resulted in wide confidence bounds on model parameter estimates, even when using more liberal 90% CIs, thus there is less certainty that the reductions we observed in relative survival and catch rate at higher water temperatures are reliable. Additional studies are clearly needed to confirm or refute our preliminary findings. In the meantime, as climate change increasingly leads to warmer water temperatures, concern is likely to accelerate on the part of fisheries managers as well as anglers with regard to potential impacts that warmer temperatures may have on the survival of caught-and-released fish. Research to date has largely focused on the impact of elevated water temperatures on the growth and survival of released fish, but the effect of increased temperature on angler catch rates should be given equal attention because if anglers' ability to land fish is diminished, so is their likelihood of causing incidental catch-and-release mortality. We expect that this topic will be hotly debated in the coming decades, but until there is evidence that trout populations are being negatively impacted by catch-and-release practices in areas where fishing is permitted at elevated water temperatures, we urge caution with the proliferation of angling restrictions at elevated water temperatures (often termed "hoot owl" regulations).

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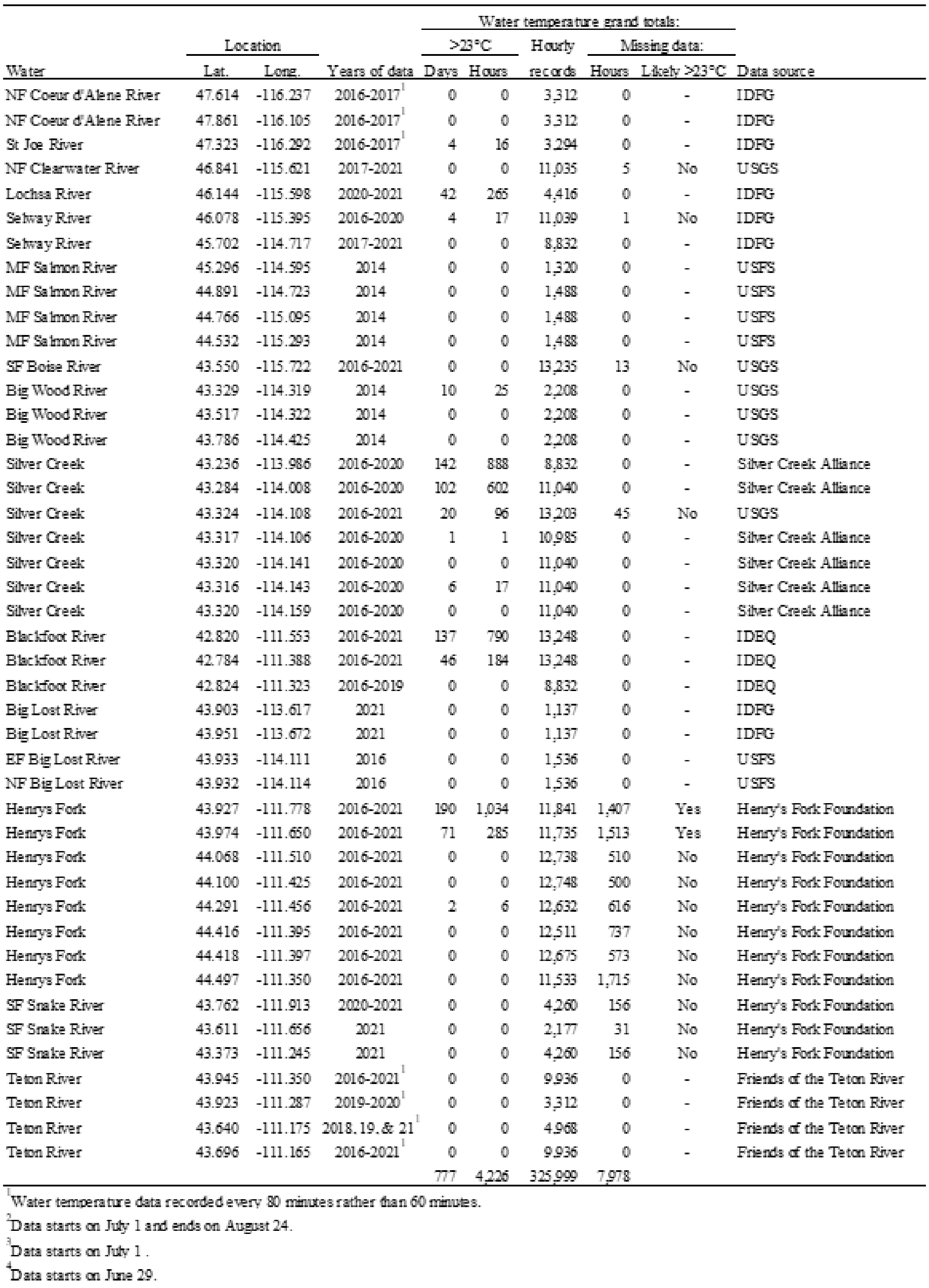
Table 6. Summer (June-August) water temperature data from several of Idaho's most popular wild trout fisheries.



				Water temperature grand totals:					
	Location			>23°C		Hourly	Missing data:		
Water	Lat.	Long.	Years of data	Days	Hours	records	Hours	Likely >23°C	Data source
NF Coeur d'Alene River	47.614	-116.237	2016-2017[1]	0	0	3,312	0	-	IDFG
NF Coeur d'Alene River	47.861	-116.105	2016-2017[1]	0	0	3,312	0	-	IDFG
St Joe River	47.323	-116.292	2016-2017[1]	4	16	3,294	0	-	IDFG
NF Clearwater River	46.841	-115.621	2017-2021	0	0	11,035	5	No	USGS
Lochsa River	46.144	-115.598	2020-2021	42	265	4,416	0	-	IDFG
Selway River	46.078	-115.395	2016-2020	4	17	11,039	1	No	IDFG
Selway River	45.702	-114.717	2017-2021	0	0	8,832	0	-	IDFG
MF Salmon River	45.296	-114.595	2014	0	0	1,320	0	-	USFS
MF Salmon River	44.891	-114.723	2014	0	0	1,488	0	-	USFS
MF Salmon River	44.766	-115.095	2014	0	0	1,488	0	-	USFS
MF Salmon River	44.532	-115.293	2014	0	0	1,488	0	-	USFS
SF Boise River	43.550	-115.722	2016-2021	0	0	13,235	13	No	USGS
Big Wood River	43.329	-114.319	2014	10	25	2,208	0	-	USGS
Big Wood River	43.517	-114.322	2014	0	0	2,208	0	-	USGS
Big Wood River	43.786	-114.425	2014	0	0	2,208	0	-	USGS
Silver Creek	43.236	-113.986	2016-2020	142	888	8,832	0	-	Silver Creek Alliance
Silver Creek	43.284	-114.008	2016-2020	102	602	11,040	0	-	Silver Creek Alliance
Silver Creek	43.324	-114.108	2016-2021	20	96	13,203	45	No	USGS
Silver Creek	43.317	-114.106	2016-2020	1	1	10,985	0	-	Silver Creek Alliance
Silver Creek	43.320	-114.141	2016-2020	0	0	11,040	0	-	Silver Creek Alliance
Silver Creek	43.316	-114.143	2016-2020	6	17	11,040	0	-	Silver Creek Alliance
Silver Creek	43.320	-114.159	2016-2020	0	0	11,040	0	-	Silver Creek Alliance
Blackfoot River	42.820	-111.553	2016-2021	137	790	13,248	0	-	IDEQ
Blackfoot River	42.784	-111.388	2016-2021	46	184	13,248	0	-	IDEQ
Blackfoot River	42.824	-111.323	2016-2019	0	0	8,832	0	-	IDEQ
Big Lost River	43.903	-113.617	2021	0	0	1,137	0	-	IDFG
Big Lost River	43.951	-113.672	2021	0	0	1,137	0	-	IDFG
EF Big Lost River	43.933	-114.111	2016	0	0	1,536	0	-	USFS
NF Big Lost River	43.932	-114.114	2016	0	0	1,536	0	-	USFS
Henrys Fork	43.927	-111.778	2016-2021	190	1,034	11,841	1,407	Yes	Henry's Fork Foundation
Henrys Fork	43.974	-111.650	2016-2021	71	285	11,735	1,513	Yes	Henry's Fork Foundation
Henrys Fork	44.068	-111.510	2016-2021	0	0	12,738	510	No	Henry's Fork Foundation
Henrys Fork	44.100	-111.425	2016-2021	0	0	12,748	500	No	Henry's Fork Foundation
Henrys Fork	44.291	-111.456	2016-2021	2	6	12,632	616	No	Henry's Fork Foundation
Henrys Fork	44.416	-111.395	2016-2021	0	0	12,511	737	No	Henry's Fork Foundation
Henrys Fork	44.418	-111.397	2016-2021	0	0	12,675	573	No	Henry's Fork Foundation
Henrys Fork	44.497	-111.350	2016-2021	0	0	11,533	1,715	No	Henry's Fork Foundation
SF Snake River	43.762	-111.913	2020-2021	0	0	4,260	156	No	Henry's Fork Foundation
SF Snake River	43.611	-111.656	2021	0	0	2,177	31	No	Henry's Fork Foundation
SF Snake River	43.373	-111.245	2021	0	0	4,260	156	No	Henry's Fork Foundation
Teton River	43.945	-111.350	2016-2021[1]	0	0	9,936	0	-	Friends of the Teton River
Teton River	43.923	-111.287	2019-2020[1]	0	0	3,312	0	-	Friends of the Teton River
Teton River	43.640	-111.175	2018, 19, & 21[1]	0	0	4,968	0	-	Friends of the Teton River
Teton River	43.696	-111.165	2016-2021[1]	0	0	9,936	0	-	Friends of the Teton River
				777	4,226	325,999	7,978		

[1] Water temperature data recorded every 80 minutes rather than 60 minutes.

[2] Data starts on July 1 and ends on August 24.

[3] Data starts on July 1 .

[4] Data starts on June 29.

Evaluation of a New Harvest Slot Limit Regulation for Wild Brown Trout in Penns Creek: Merging Science and Politics to Meet Social Preferences and Biological Objectives on one of the Most Popular Wild Trout Fisheries in the Eastern U.S.

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Abstract - Penns Creek is a large, limestone stream in the mountains of central Pennsylvania known for its prolific insect hatches and is one of the most popular wild Brown Trout *Salmo trutta* fisheries in the eastern U.S. From 1995-2013, a 7-mile-long reach was managed under all-tackle trophy trout regulations [two trout/day > 14 inches from opening day of trout season (mid-April) through Labor Day, with no harvest for the remainder of the year]. During 2012-2013, the trophy trout regulation program was reviewed, as the size restriction no longer represented a "trophy trout" and was possibly limiting the abundance of large trout. An angler use, harvest, and opinion survey was implemented during 2013 and results indicated moderate harvest of large (> 14 inches) Brown Trout was occurring. Additionally, results of the angler survey and a landowner survey indicated a continued interest in having the option of harvesting trout, the opportunity to use all tackle types, and support for a regulation change if it could meet social preferences. A new harvest slot limit (HSL) regulation was established in 2014 to best meet social preferences and biological objectives. The HSL allowed year-round fishing, use of all tackle types, and harvest of two trout/day > 7 and < 12 inches from opening day of trout season (mid-April) through Labor Day, with no harvest for the remainder of the year. The Brown Trout population was monitored in treatment and control reaches over a 10-year period before (2004-2013) and 7-year period after (2014-2019) implementation of the HSL regulation. Results showed a significant (p=0.01) increase in electrofishing catch rates of large (≥ 18 inches) Brown Trout during the post-HSL period compared to the pretreatment period. An adjacent section of Penns Creek managed under catch-and-release regulations served as a control, and no change in the abundance of large (≥ 18 inches) Brown Trout was observed. These results suggest the HSL regulations played an important role in the increased electrofishing catch rates of large (≥ 18 inches) Brown Trout in the treatment reach. A follow-up angler survey in 2019 indicated strong support for the HSL regulations. This approach merged social interests and biological objectives to achieve a positive change for the management of Penns Creek.

Introduction

Special regulations play social and biological roles in wild trout management, especially on high use fisheries. Often the objectives of special regulations include increasing trout abundance, size structure, and angling quality (Carline et. al 1991). Penns Creek is a large, limestone-influenced stream that supports one of the most popular and renowned wild Brown Trout *Salmo trutta* fisheries in Pennsylvania and the Eastern U.S., and has multiple reaches managed under special regulations. Penns Creek supports a strong wild Brown Trout population for over 30 miles from its source at Penns Cave downstream to near the community of Weikert, Pennsylvania. The 7.0-mile-long reach of Penns Creek from the confluence with Elk Creek in Coburn to 0.3 miles downstream of the confluence with Swift Run (hereafter, Section 03) was the primary focus of this project (Figure 1).

Section 03 has a long management history that includes trout stocking, wild trout management, special regulations, and varying public perspectives. Until 1992, Section 03 was managed as a stocked trout fishery when it was designated by the Pennsylvania Fish and Boat Commission (PFBC) as a

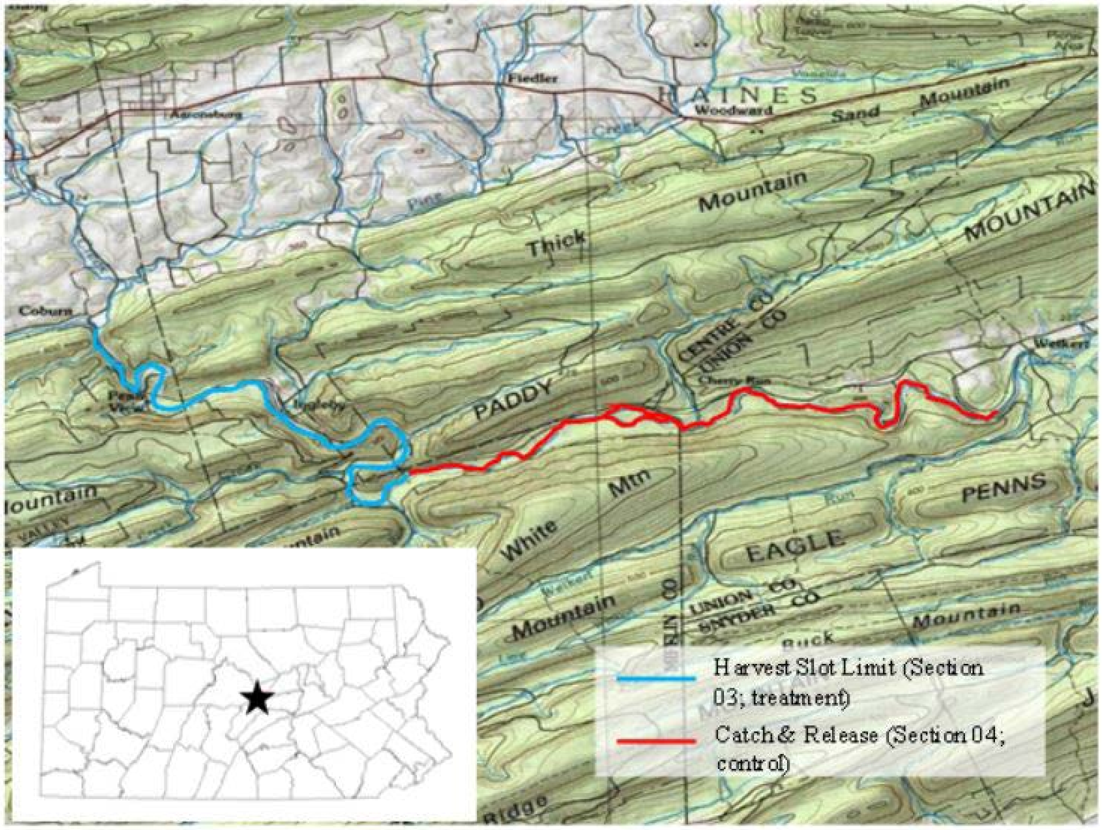

Figure 1. Location of the Penns Creek study area. Section 03 (harvest slot limit regulations; treatment) extends for 7.0 miles from the confluence with Elk Creek in Coburn downstream to 0.3 miles downstream of the confluence with Swift Run. Section 04 (catch-and-release regulations; control) extends for 7.5 miles from 0.3 miles downstream of the confluence with Swift Run downstream to the lower end of the island at Jollys Grove Lane.

Class A wild trout stream and the stocking of hatchery trout was discontinued in favor of wild trout management. During 1993, landowner and angler surveys were conducted to inform management decisions. Based on the results of these surveys and biological data collected during 1991 and 1992, miscellaneous special regulations (later renamed all-tackle trophy trout regulations) were developed for Section 03 and implemented in 1995. The all-tackle trophy trout regulations allowed for year-round fishing, use of all tackle types, and the harvest of two trout/day ≥ 14 inches from opening day of trout season (mid-April) through Labor Day, with no harvest permitted during the remainder of the year. Section 03 was managed with all-tackle trophy trout regulations for 19 years from 1995-2013. Fishery surveys conducted between 1995 and 2013 documented a positive response in the wild Brown Trout population following stocking cessation and implementation of all-tackle trophy trout regulations and subsequently maintained a higher total abundance of Brown Trout and higher abundance of large (14-16 inch) Brown Trout in the population. However, there was no significant change in the abundance of Brown Trout > 17 inches (Detar and Hollender 2007).

During 2012, PFBC staff and Commissioners met to discuss whether Section 03 of Penns Creek could be further improved by implementing more restrictive angling regulations. The proposed regulations would provide increased protection to the wild Brown Trout population from harvest, particularly the largest fish, and limit the use of tackle types to artificial lures only. Subsequently, staff requested feedback from landowners and anglers via letters and a public meeting to determine potential support for implementing catch-and-release artificial-lures-only regulations. Additionally, staff conducted an angler use, harvest, and opinion survey from the opening day of trout season through Labor Day in 2013 to help inform a regulation change. The overwhelming feedback received from landowners at that time was they did not support implementing catch-and-release artificial-lures-only regulations. Responding landowners wanted to continue to have the opportunity to harvest a few trout and utilize all tackle types. Anglers showed support for more restrictive regulations but also expressed concern for loss of public access to private properties if regulations were changed to catch-and-release artificial-lures-only. Both anglers and landowners frequently expressed an interest in conservation of Penns Creek and many anglers also supported increased protection of larger trout by limiting harvest of that segment of the population.

To address landowner and angler feedback regarding the opportunity to continue to use all tackle types and have the opportunity to harvest a few trout along with attempting to increase the abundance of large wild Brown Trout, regulatory options other than catch-and-release artificial-lures-only were considered. Pennsylvania has a 7-inch minimum length limit for trout; therefore, a maximum size limit was not a viable option. A slot limit that would allow limited harvest of intermediate-size trout which are abundant in Section 03, while also protecting the largest trout in the population from harvest, was identified as the preferred option to meet social and biological objectives.

The PFBC approved establishment of a harvest slot limit (HSL) regulation for a 7-year period from January 1, 2014 through December 31, 2020, during which time the trout population would be monitored to determine the effectiveness of the regulation at meeting biological and social objectives. The HSL regulation allowed for year-round fishing, use of all tackle types, and the harvest of two trout/day > 7 and < 12 inches from opening day of trout season (mid-April) through Labor Day, with no harvest for the remainder of the year.

Methods

The Penns Creek wild Brown Trout population was monitored over a 10-year period before (2004-2013) and 7-year period after (2014-2019) implementation of the HSL regulation in the treatment reach (Section 03) as well as in the adjacent (downstream) control reach (Section 04; Figure 1). The treatment reach (Section 03) was 7.0-miles in length and sampled during five years in the pretreatment period and four years during the post-treatment period. Section 04 served as a control and was 7.5-miles in length and managed with catch-and-release artificial- lures-only regulations throughout the entire pre- and post-treatment time periods. The control reach (Section 04) was sampled during three years in the pretreatment period and four years during the post-treatment period. Towed-barge electrofishing gear was used for trout population monitoring at a fixed 470-yard-long station in Section 03 and, due to increased pool depth, flat-bottom boat electrofishing gear was used to monitor a fixed 570-yard-long station in Section 04. All sampling occurred in early summer under base-flow conditions. A combination of single- and multiple-pass electrofishing was conducted; therefore, electrofishing catch was standardized as the number of trout captured per mile during the first electrofishing pass. Electrofishing catch data was analyzed as a before-after-control-impact design using a linear mixed-effects model where year was a random effect using the "lmer" function in the R package "lmerTest". Overall treatment effects (HSL versus catch and release) and treatment by time period interactions were examined to determine if changes in electrofishing catch rates occurred following the implementation of the HSL regulations.

In addition to biological monitoring, an angler use, harvest, and opinion survey was conducted during 2019 to estimate angler harvest and determine the effectiveness of this new regulation in meeting social objectives for the Section 03 wild trout fishery.

Results

The mean electrofishing catch of large Brown Trout ≥ 18 inches significantly increased ($p = 0.01$) in the treatment reach (Section 03) post-HSL implementation to similar levels as the control reach (Section 04; Figures 2 and 3). Brown Trout ≥ 18 inches were rarely captured in the treatment reach (1 out of 5 surveys) prior to the HSL regulation, whereas they were captured in all four post-HSL implementation surveys. Additionally, there was no change in the number of Brown Trout ≥ 18 inches in the adjacent control reach (Section 04), which suggests that implementation of the HSL regulations likely played an important role in the increased electrofishing catch of large (≥ 18 inches) Brown Trout in the treatment reach (Section 03). There was not a significant change in the electrofishing catch rate of Brown Trout 14-17 inches in length.

Results of the 2019 angler use, harvest, and opinion survey to evaluate the social aspects of the HSL regulation and estimate angler harvest showed low harvest of trout occurred. As such, there was no change in the electrofishing catch rates of Brown Trout ≥ 7 and < 12 inches (harvestable-size trout) following the implementation of HSL in Section 03. These results suggest that angler harvest is currently having minimal influence on the population size structure. The survey also documented high angler support for continuing the HSL regulations on Penns Creek, Section 03, and potentially expanding the use of the HSL regulations to other productive waters with similar management objectives.

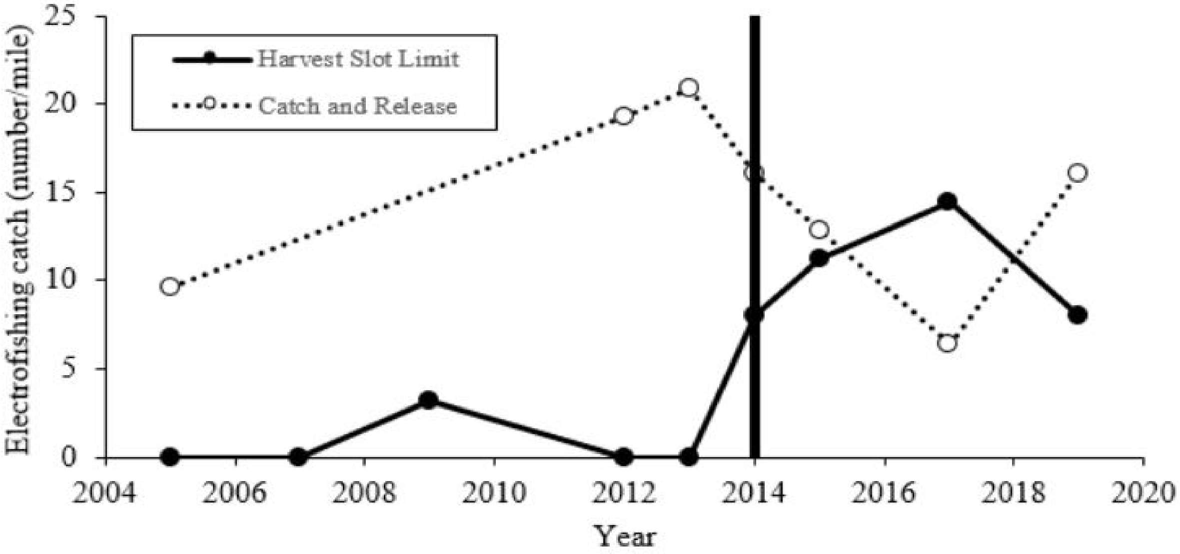

Figure 2. Electrofishing catch (number/mile) of Brown Trout > 18 inches pre- and post-harvest slot limit (HSL) regulation implementation in treatment (Section 03; solid line) and control (Section 04; dashed line) reaches of Penns Creek. The vertical bar denotes establishment of the HSL regulations in 2014.

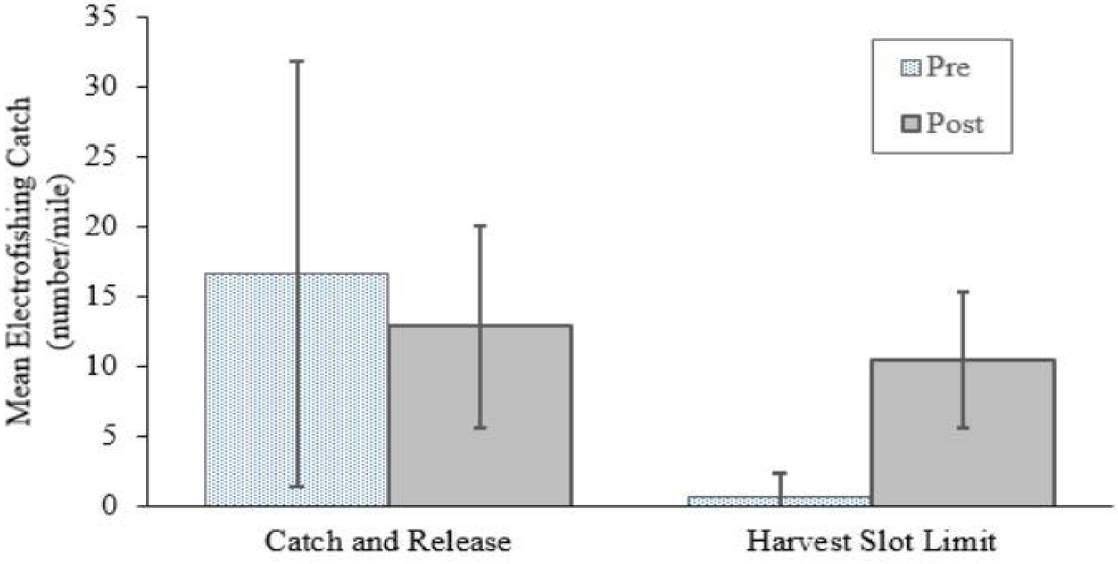

Figure 3. Mean electrofishing catch (number/mile) with 95% confidence intervals of Brown Trout > 18 inches pre and post-harvest slot limit (HSL) regulation implementation in treatment (Section 03; HSL) and control (Section 04; catch-and-release) reaches of Penns Creek.

Discussion

Penns Creek provided a unique opportunity to evaluate a new special regulation on a popular wild trout fishery. The productive nature of Penns Creek, along with moderate angler harvest of large (≥ 14 inches) Brown Trout under the previous regulations (all-tackle trophy trout), provided ideal conditions for a favorable response of the wild Brown Trout population to trend toward larger sizes under the HSL regulations. Despite low angler harvest currently occurring under the HSL regulations, the regulations provide the opportunity for anglers to harvest a trout if they desire, which was an important social consideration when the regulations were being considered for implementation. Maintaining an opportunity for harvest was a primary reason the public did not support implementing catch-and-release regulations on this reach.

A limitation of this study is that it was only conducted at one location. However, results from Penns Creek demonstrated the success of implementing HSL regulations on a high-profile fishery, which was needed to gain support prior to considering implementation at other suitable locations. Given the public support for and biological response to HSL regulations on Section 03 of Penns Creek, and potential opportunities to utilize this regulation at other suitable wild trout streams in the future, the HSL was renamed the trout slot limit regulation program and formally adopted as a special regulation program by the PFBC in 2022. The trout slot limit regulation program has two subprograms: all-tackle trout slot limit and artificial-lures-only trout slot limit. The two subprograms provide the PFBC with the ability to select the best tackle option to achieve biological and social objectives for each water considered for inclusion in the program in the future. If additional locations are added to the trout slot limit program, monitoring will occur at those locations to further evaluate the regulations.

Acknowledgements

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The Undiscovered Contraception: Does Post-Ejaculatory Cryptic Female Choice Reduce Hybridization in Salmonids?

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Abstract - The presence of hybrids requires many successful events (hetero-specific mating, fertilization, incubation, juvenile survival). A bottleneck anywhere in this process removes hybrids, and thus the frequency of hybrids tells us little about the rate of hetero-specific matings. There is often strong selective pressure against hybridization, as offspring or grand-offspring may be incapable of reproducing. Females have more to lose from each hybrid fertilization than males, and thus should avoid it. Pre-mating sexual selection via courtship behavior reduces hetero-specific matings. However, in external fertilizers, females have no control over which males release sperm along with her chosen mate. Post-mating sexual selection continues via sperm competition among males, and cryptic female choice, which biases paternity towards certain males. When males of different species are in sperm competition, a type of cryptic female choice, known as con-specific sperm preference, can reduce the rate of hybrid fertilizations. This occurs in *Salmo spp*. from Europe, where ovarian fluid released with eggs changes sperm swimming behavior, and gives a greater boost to con-specific over hetero-specific sperm. Many unknowns remain, including the influence of historical and contemporary allopatry/sympatry in influencing con-specific sperm preference, and whether it occurs in *Salvelinus* and *Oncorhynchus*. A case study from Newfoundland is presented on con-specific sperm preference in native Atlantic Salmon *Salmo salar*, native Brook Trout *Salvelinus fontinalis*, and non-native Brown Trout. Sperm swimming data suggest little ability of native salmonids to resist hybrid fertilizations by Brown Trout *Salmo trutta*, and we await results to directly test this through in vitro sperm competitions. I present desirable study systems to explore this concept further, including those under natural sympatry (e.g., Pink Salmon *O. gorbuscha* / Chum Salmon *O. keta*; coastal Rainbow Trout *O. mykiss* / Coastal Cutthroat Trout *O. clarkii clarkii*), and natural allopatry with contemporary sympatry (introduced coastal Rainbow Trout / Yellowstone Cutthroat Trout O*ncorhynchus clarkii bouvieri* or Westslope Cutthroat Trout *Oncorhynchus clarkii lewisi*).

Introduction

Hybridization can be thought of as an outcome (the occurrence of hybrid individuals) or a process (the series of events that are required to produce a hybrid individual). Many steps are required to produce a hybrid (Figure 1). Although the presence of hybrid individuals indicates that hybrid matings must have occurred, their absence tells us nothing about mating. The frequency of hybrids and hybrid matings are not necessarily correlated, as a bottleneck anywhere in the steps (Figure 1) required after mating will prevent hybrids from occurring.

The outcome of a hybrid mating is often maladaptive, as it can result in failed fertilization, high embryo mortality, or the production of sterile offspring. However, the cost is asymmetrical between sexes. Females invest more into individual gametes than males, whereas males invest more into creating mating opportunities. Females thus have more to lose from each hybrid mating than males, and thus should avoid it (Lantiegne and Purchase 2021). Pre-mating sexual selection enabled by male-male fighting and female choice of males (courtship) reduces the likelihood of hybrid matings if two species are reproductively active in the same place at the same time (Figure 1). Once a female releases eggs, there is no benefit to her to not have them fertilized and survive, but there is benefit in having them fertilized by her own (con-specific) species as opposed to a hetero-specific male. In externally fertilizing fish, despite potential careful mate choice, females have no control over which additional males may release sperm along with her chosen (con-specific) mate. However, pre-zygotic post-mating sexual selection occurs as sperm competition, where ejaculates from two or more males compete to fertilize the same set of eggs (ubiquitous in salmonids), and cryptic female choice that biases paternity towards particular males. Under threat of hybridization when sperm competition occurs between males of different species that are either the same (con-specific) or different (hetero-specific) to the female, cryptic female choice should manifest as con-specific sperm preference, which helps bias paternity towards her own species (Lantiegne and Purchase 2021).

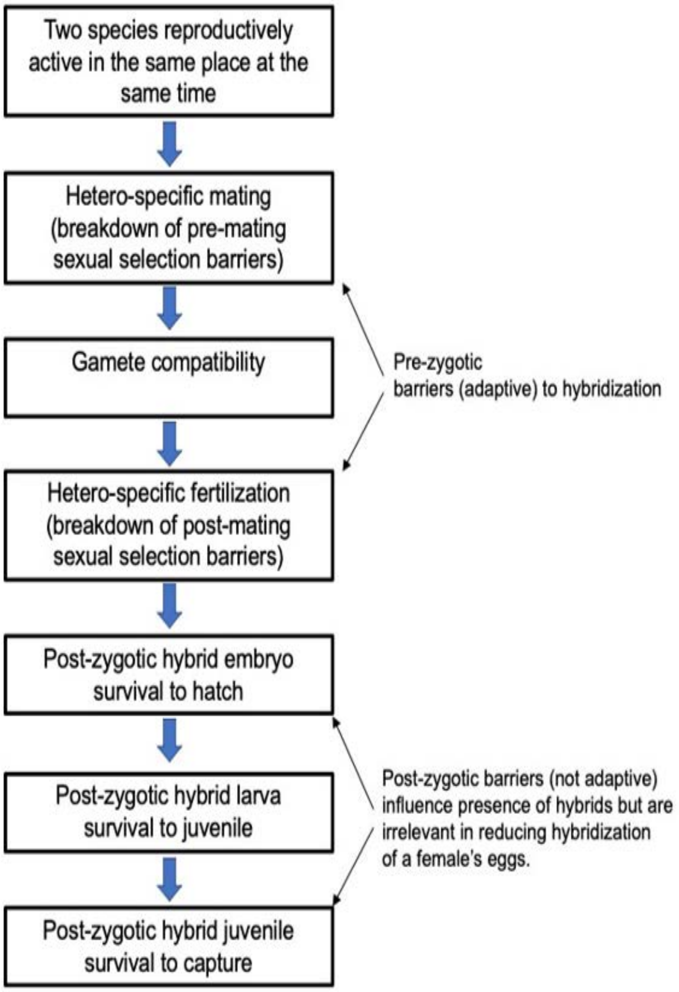

Figure 1. Successful reproductive steps that must have occurred when hybrid fish are found in the wild (internal fertilizers aslo requre successful insemination). Note that pre-mating sexual selection is typically enabled by male-male competition and femal mate choice for males, and post-mating sexual selection is typically enabled by sperm competition and cryptic female choice (including con-specific sperm preference).

In Europe, native Atlantic Salmon *Salmo salar* and Brown Trout *Salmo trutta* often occur in the same watersheds, have habitat overlap and are phenotypically similar, but are very different genetically (Jonsson and Jonsson 2011). Both species have two male phenotypes, where large dominate males fight for access to females, and precocial parr (much more common in salmon) sneak fertilizations and steal paternity from the female's chosen mate. Trout generally spawn earlier in the season than salmon, but there is some overlap (Jonsson and Jonsson 2011). Although unquantified, hybrid matings obviously occur as hybrid juveniles are often found in rivers (Álvarez and Garcia-Vazquez 2011), but their frequency varies widely (Poulos 2019). First generation hybrids are fertile, but hybridization of a female's eggs is an evolutionary dead end, as long-term introgression does not occur (Álvarez and Garcia-Vazquez 2011; Yeates et al. 2013). Females would therefore benefit by preventing hybrid fertilization of eggs. Lab work indicates that gametes of the two species are fully compatible, producing high fertilization success and early embryo survival (reviewed by Poulos 2019).

One thorough study has examined sperm competition using a single population of each species from Norway. When eggs are exposed to sperm competition between the species, paternity is biased towards con-specific sperm. The egg itself does not control paternity. This is enabled by ovarian fluid (Yeates et al. 2013), which is released with the eggs. It up-regulates sperm swimming behavior, and may do so more for con-specific than hetero-specific sperm. Thus, female Atlantic Salmon exert con-specific sperm preference over Brown Trout in sperm competition, and female trout exert it over salmon. How robust and widespread this phenomenon is in other populations of these species, and in other salmonids is not known.

North American Atlantic Salmon have been isolated from con-specifics from Europe for >600,000 years (Cauwelier et al. 2012) and contain different numbers of chromosomes (Hartley 1987). They presumably have been isolated from Brown Trout for at least that long, before human induced contact starting in the 1880s. There is some evidence suggesting the frequency of hybrid juveniles is higher in North America than in Europe (reviewed by Poulos 2019), but what part of the hybridization process (Figure 1) results in this is not known. North America is thus ripe for detailed studies of the potential for con-specific sperm preference in this and other genera of salmonids.

Case-Study

The island of Newfoundland became surrounded by salt water before deglaciation, and the only fishes to reach its fresh waters were euryhaline (e.g., sticklebacks). Many eastern North American fishes are absent, e.g., there are no Cypriniformes, Esociformes, Siluriformes, Perciformes, or Coregoninae (Scott and Crossman 1964). Salmonids dominate but there are no Lake Trout *Salvelinus namaycush*. There are several hundred anadromous Atlantic Salmon populations, along with hundreds/thousands resident Atlantic salmon populations in lakes (ouananiche, which is locally pronounced winnish). Dwarf non-anadromous Arctic Char *Salvelinus alpinus* are in many lakes deeper than >20 m, and native Brook Trout *Salvelinus fontinalis* (aka Brook Char) occur everywhere. Due to the lack of typical lake specialists, Brook Trout and Atlantic Salmon use pond/lake habitat much more extensively than elsewhere. High relief and short watersheds, create tens of thousands of unique populations. No measurable stocking for these species ever occurred, hence these populations are genetically pure.

There is no current fish stocking in Newfoundland for any species. Starting in 1883, a few decades of non-native salmonid stocking did occur (Scott and Crossman 1964; Hustins 2007). Rainbow Trout *Oncorhynchus mykiss* were brought from California, have naturalized, but have not spread. They occur as resident forms in only a few small lakes, mostly in eastern Newfoundland near the city of St. John's. Brown Trout were brought from Europe and stocked near the city. They have become established in numerous watersheds on the Avalon Peninsula and continue to spread west (Hustins 2007; Westley and Fleming 2011), albeit at a very slow rate (MacDonald et al. 2022).

These invasive Brown Trout hybridize with native con-generic Atlantic Salmon, and with native hetero-generic Brook Trout (Poulos 2019) – hereafter called Brook Char to more clearly distinguish from Brown Trout. Although based on very limited data, frequency of hybrid salmon-brown trout juveniles seems higher than in their native Europe (McGowan and Davidson 1992), and naturally produced "tiger trout" (hybrid Brown Trout and Brook Char) are caught by anglers. However, there is no information available on frequency of spawning between Brown Trout and Atlantic Salmon, or Brown Trout and Brook Char.

When females spawn with both a con-specific male and a hetero-specific male, the outcome of sperm competition will determine if no offspring, a hybrid offspring, or a pure offspring is produced. European salmonids have been shown to be able to bias paternity towards the female's own species via con-specific sperm preference. Ovarian fluid from North American salmonids is therefore predicted to up-regulate (improve) swimming performance of con-specific sperm more than hetero-specific sperm, thus giving them a fertilization advantage. This was examined for Atlantic Salmon, Brook Char, and Brown Trout from Newfoundland (Lantiegne and Purchase 2021). Contrary to prediction, the results suggest that these native species cannot reduce hybridization by invasive Brown Trout in this way (Figure 2). Atlantic Salmon and Brook Char females do not up-regulate the swimming performance of con-specific sperm more than Brown Trout sperm. Further research is required to confirm that sperm competition is indeed not biased towards con-specific males.

Figure 2: Ratio of sperm curvilinear swimming velocity (VCL μm/s) in specific ovarian fluid (X-axis) compared to water from 6.0-6.5s post-activation – any value above 1.0 indicates ovarian fluid up-regulated sperm swimming performance. Plotted are the average (circles are con-specific sperm to the ovarian fluid, triangles are hetero-specific sperm), and 2StErr among 12 males and females within a species. Ovarian fluids strongly up-regulated sperm swimming, but they did not do so more for con-specific vs hetero-specific sperm, and thus these populations cannot enable con-specific sperm preference via this mechanism. Modified from Lantiegne & Purchase (2021).

Discussion

The ability of females to exert con-specific sperm preference is adaptive if her eggs are under threat of hybridization. However, the ability probably has some unknown costs, and thus would not be expected to evolve or might be secondarily lost if not needed. The former would be predicted if pre-mating sexual selection is strong enough to prevent hybrid spawnings, and the later if populations become allopatrically separated for a long enough time. If patterns hold as predicted, it has implications for hybridization resulting from invasive/translocated species and/or locally adapted populations.

Limited available research implies that in their native Europe, Brown Trout and Atlantic Salmon can exert con-specific sperm preference. North American Atlantic Salmon have been isolated from European salmon for >600,000 years (Lehnert et al. 2020), and our limited work from Newfoundland indicates these salmon cannot bias sperm swimming towards their own species, implying no ability to exert con-specific sperm preference. Whether this is indicative of wider patterns of allopatry/sympatry gradients is unknown. The work in Newfoundland also suggests that Atlantic Salmon and Brook Char cannot exert con-specific sperm preference over each other. These species do not produce hybrids in the wild (reviewed by Lantiegne 2021), perhaps pre-mating sexual selection is strong enough to avoid hybrid matings (and thus con-specific sperm preference would have no added benefit), but rates of hybrid spawning are unknown.

To my knowledge, no other *Salvelinus* and no *Oncorhynchus* species has been examined in its ability to exert con-specific sperm preference. Relatively well-studied *Oncorhynchus* provide good opportunities to test questions related to the importance of allopatry/sympatry gradients. Here I provide a short list of useful examples to illustrate the point.

(1) Naturally sympatric populations that spawn in close proximity of time and space. Con-specific sperm preference as a form of cryptic female choice might be predicted to be most pronounced under such conditions, as reinforcement would occur on a continual basis.
   a. Pink Salmon *O. gorbuscha* and Chum Salmon *O. keta* from the same stream. These fish spawn at high densities, and hybrids do occur ("chumpies"), indicating that gametes are capable of fertilizing and post-zygotic mortality is not absolute. Con-specific sperm preference might dramatically reduce the rate of hybridization.
   b. Other combinations among the different species of Pacific salmon. If post-zygotic mortality is high, the absence of hybrid juveniles does not inform on barriers to prevent hybrid fertilizations.
   c. Coastal Rainbow Trout *O. mykiss* and coastal Cutthroat Trout *O. clarkii* are naturally sympatric and have a very low rate of hybrid juveniles in the wild. If sperm competition between both species occurs (likely), females would benefit if they evolved ability to exert con-specific sperm preference. This could be quantified.

(2) Naturally allopatric populations that are now sympatric due to human introductions. In allopatric populations con-specific sperm preference is not needed, and thus might not evolve or might be secondarily lost.
   a. Inland lineages of Cutthroat Trout (Westslope and Yellowstone) have long been isolated from each other, and from coastal Cutthroat Trout, and are genetically very unique (Behnke 2002; Kershner et al. 2019). They evolved in the absence of Rainbow Trout and thus female Cutthroat Trout (Westslope and Yellowstone) would not have needed any barriers to prevent hybridization of their eggs. With stocking of coastal Rainbow Trout into Westslope/Yellowstone Cutthroat Trout habitat, hybridization is much higher than with coastal Cutthroat Trout (Behnke 2002; Kershner et al. 2019), as would be predicted if coastal Cutthroat Trout exert con-specific sperm preference but Westslope/Yellowstone Cutthroat Trout do not (or it is less effective).
   b. Anadromous populations of Sockeye Salmon *O. nerka* would generally be continually exposed to potential hybridization from other species of *Oncorhynchus*. Reinforcement against wasted eggs would predict strong barriers to reduce hybrid fertilizations. Some populations of isolated kokanee, a resident form of Sockeye Salmon, however may have lost this, and thus might have high hybrid fertilization rates if other species have been stocked into their habitats.

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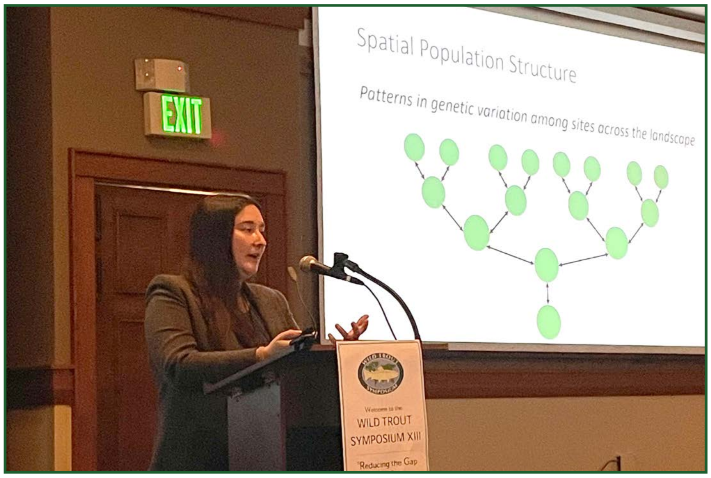
Session 7
Brook Trout Research and Management Across the Species' Historic and Introduced Range



Brook Trout Management in Iowa

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Abstract - Although Brook Trout *Salvelinus fontinalis* were likely native to northeast Iowa, early degradation of streams caused widespread extirpations leading to regulations and stocking to restore and maintain populations. Brook Trout stockings began in 1875, and were common, but disappeared from Iowa stocking records by 1956. Fingerling and catchable Brook Trout stockings resumed in 1993. The ancestry of Brook Trout used for early stockings is generally unknown; however, Brook Trout used for fingerling and catchable trout production that began in 1993 were acquired as eggs from the St. Croix Falls Fish Hatchery. In 1994, the Iowa Department of Natural Resources purchased a portion of South Pine Creek, the only stream in Iowa known to have reproducing Brook Trout. A subsequent genetic evaluation of the fishery suggested the South Pine population was a unique population, but with low genetic diversity. As a result, South Pine fingerlings were stocked into streams to restore Iowa Brook Trout fisheries beginning in the mid-1990s. Since the mid-1990s, 45 streams have received restoration stockings totaling 117,388 fingerlings; however, only 16 of those streams developed Brook Trout populations. Today, work continues to expand Brook Trout populations in Iowa to protect the unique South Pine ancestry from a catastrophic loss.

Introduction

Iowa is known for its rolling hills and rich, deep prairie soils that support some of the most productive farms in North America. Northeast Iowa, however, is unique with abundant rock outcroppings, many narrow valleys with cool fast-flowing streams, woodlands, and few glacial deposits. Also known as the Driftless Area of Iowa, the influence of glacial drift and loess is minimal, resulting in a landscape that is heavily influenced by bedrock (Figure 1; Prior 1976). This limestone and dolomite bedrock has been dissolved over time by surface and ground water, creating sinkholes and underground crevices, joints, and fractures in a landscape commonly referred to as karst. These rock conduits direct groundwater towards the surface creating seeps and springs.

The influence of karst on northeast Iowa's streams is appreciable, creating suitable habitat conditions for trout. Seeps and springs supply many surface waters with an abundance of groundwater, moderating water temperatures year-round (Figure 2). As a result, the streams of northeast Iowa are home to many plant and animal species unique to the Driftless Area. Today, over 474 miles of Iowa streams are officially recognized as coldwater under the state's water quality standards (Iowa Department of Natural Resources 2015). Ongoing research suggests that the actual amount of coldwater streams in Iowa likely exceeds 1,000 miles,

Figure 1. The Driftless Area covers parts of Iowa, Minnesota, Wisconsin, and Illinois. Provided by the Driftless Area Restoration Effort

providing more opportunities for native Brook Trout *Salvelinus fontinalis* restoration than previously thought.

Figure 2. Springs like this one on Grannis Creek provide cold water to northeast Iowa streams, allowing them to support native Brook Trout

History of Brook Trout Management

Trout and trout fishing have a long and active history in Iowa. Trout are now widely distributed across Iowa as a result of fish culture and stocking efforts of the Iowa Department of Natural Resources (Iowa DNR). Wild self-sustaining populations of Brook Trout and Brown Trout *Salmo trutta* are now present in more than 50 streams across nine counties of northeast Iowa following decades of fish management aimed at increasing abundance and availability of the popular sport fishes.

Given how ubiquitous trout are in Iowa today, it is noteworthy that there is some uncertainty about the presence and distribution of native Brook Trout in Iowa at the time of European settlement (early 1800s). There are several unscientific first-person accounts of trout by Europeans first settling in northeast Iowa (Alexander 1882; Bailey 1913; Faldet 2009). One of the earliest accounts from an 1836 issue of the Du Buque Visitor read “every stream is filled with them; and among them may be found the pike, the pickerel, the catfish, the trout, and many other varieties” (Du Buque Visitor 1836). Henry Rice, a local trader, was brought Brook Trout by the Winnebago, and “he was not long in finding out where they could be caught.” He was probably the first European settler to fish for trout in Trout Run near present-day Decorah (Decorah Republican 1894). Further, a manuscript written by frontier doctor Fredrick Andros describes traveling in 1845 to Siewers Spring near Decorah to trout fish (Faldet, unpublished writings). Later in 1854, Elisabeth Koren, an early Decorah area settler, wrote about receiving trout as a gift (Koren 1955). The first fishing regulations in Iowa, enacted in 1862, pertained to gear restrictions and seasons for trout fishing (Iowa General Assembly 1862; Harlan et al. 1987), which strongly supports the presence of native Brook Trout populations more than a decade before the first recorded stockings by the State of Iowa. In 1875, B. F. Shaw provided additional support for native Brook Trout when he wrote “He is a native of the northeastern part of Iowa, originally found in the tributaries of the Upper Iowa River, and some other streams, in large numbers; but the perseverance of fishermen, and improved appliance of civilization used for destroying them, even during their spawning season, have so diminished their numbers that stories of large strings of trout are quite mythical” (State of Iowa 1876). Unfortunately, the first scientific fish collections occurred in Iowa during the late 1800s at a historical point well past the time of settlement when trout stockings had already been conducted for several years. Meek (1890) observed Brook Trout in some Iowa streams but attributed their presence to stocking. Based on early accounts, it seems likely that Brook Trout were part of northeast Iowa’s native fish communities.

Stocking records from biennial reports of the Iowa Fish Commissioner show that stocking of Brook Trout occurred in Iowa in an official capacity as early as 1875 (State of Iowa 1876). Trout were common among the species distributed by B.F. Shaw from the first Iowa fish hatchery built near Anamosa in 1874. Stocking records from 1876 and 1877 report that more than 80,000 Brook Trout were distributed across Iowa, followed by more than 66,000 Brook Trout during 1879 (State of Iowa 1878, 1880). It is probable that early stockings of trout in Iowa did not all originate from native Iowa populations, with early records suggesting a mixture of potentially native stocks as well as Brook Trout originating from Wisconsin. Brook Trout produced at Anamosa in 1877 originated from the farm of H. Dousman, a privately-operated fish hatchery in Waukesha County, Wisconsin about 55 miles east of Madison (State of Iowa 1876). Brook Trout eggs received at the Anamosa Hatchery during 1884 and 1885 included Brook Trout from Plymouth Rock, Massachusetts and Northville, Michigan. The Anamosa State Fish Hatchery was short-lived with a period of operation from 1874 to 1887 before production of most species other than trout shifted to the new Spirit Lake Fish Hatchery in 1880.

Early stockings of Brook Trout from Anamosa State Fish Hatchery resulted in several first-person accounts of quality fishing recorded in biennial reports. For example,

S. G. Van Anda of Manchester wrote to B. F. Shaw during April 1879 that, "We are catching some fine, beautiful Brook Trout in Spring Branch. I think you put them in from Delaware Station two years ago. I have seen them caught from eleven to sixteen inches long" (State of Iowa 1880). The Spirit Lake Hatchery had difficulty raising fish during the early years, and trout were never raised there to any great extent (Vance Polton, Iowa DNR, personal communication November 2018). Few trout for stocking were produced by the State of Iowa during this period, until 1918, when trout culture activity in Iowa was transferred back to northeast Iowa at Lansing (Cooper 1949). When trout production commenced at Lansing there had already been more than 40 years of largely unsuccessful trout stocking in areas beyond northeast Iowa. This led trout stocking efforts for the next 60 years to focus on northeast Iowa. During this period of limited state trout production, fish were obtained from federal hatcheries, including the Manchester Federal Fish Hatchery in Manchester, Iowa. Trout production at Lansing was eventually shifted to Backbone Fish Hatchery when Lansing closed in 1974 (Vance Polton, Iowa DNR, personal communication November 2018).

Brook Trout were a common part of early Iowa fish stockings but disappeared from state stocking records by 1956. From 1977 to 1979, fingerling Brook Trout were stocked in North Cedar and the South Fork of Big Mill creeks as part of a Brook Trout special management effort. The stockings were unsuccessful and Brook Trout remained largely absent from the trout program until 1993 when fingerling and catchable Brook Trout stockings resumed in earnest. Brook Trout used for fingerling and catchable trout production that began in 1993 were acquired as eggs from the St. Croix Falls Fish Hatchery in Wisconsin (Dave Marolf, Iowa DNR retired, personal communication 2019). The St. Croix Falls population originated from the Nashua Fish Hatchery, New Hampshire (Hoxmeier et al. 2015). Between 1993 and 2006, nearly 319,000 fingerling domestic Brook Trout were stocked into northeast Iowa streams. From 2007 to 2018, an additional 5,000 domestic fingerlings were stocked into Dunning Spring near Decorah. Thousands of domestic St. Croix Falls Brook Trout were also stocked into Iowa streams each year as catchable-sized trout until the program ended after the 2019 stocking season. Although thousands of domestic Brook Trout have been stocked into Iowa streams, there is little information on how these stockings may have influenced the genetic status of Iowa's wild Brook Trout populations.

Brook Trout management in Iowa was forever changed in June 1994 when the Iowa DNR purchased land that would become the South Pine Creek Wildlife Management Area. South Pine Creek was known locally as one of the last streams in Iowa with reproducing Brook Trout. The stream was sampled by Iowa DNR staff in the 1980s and again in 1994 (Scott Gritters, Iowa DNR, personal communication 2019); Brook Trout were found during both surveys. Some history on South Pine Creek was provided in 1994 when DNR staff received a letter from Mr. Gavin Sampson of Decorah, Iowa. Mr. Sampson wrote that he had been "intimately associated with this (South Pine) creek all my 72 years" and could remember Brook Trout being backpacked into the stream around 1955 by a state game warden. No other information is available related to the stocking in the 1950s. Mr. Sampson also mentioned Brook Trout reproducing in South Pine Creek around 1970. Regardless of how the trout came to thrive in South Pine Creek, it was the only stream in Iowa with known Brook Trout reproduction by the mid-1990s.

In 1996 a joint management and hatchery initiative began to experimentally introduce wild Brook Trout fingerlings of South Pine Creek origin into other coldwater streams (Iowa DNR 1996). The primary goal of the initiative was to introduce the unique South Pine genetic stock into other coldwater streams to prevent a catastrophic loss. Unfortunately, a reduction in mature Brook Trout in South Pine Creek by the early 2000s was thought to have been caused by the repeated use of South Pine Creek as an egg source for restoration efforts. An earlier genetic evaluation further complicated matters by recommending South Pine Brook Trout be managed as a relict population and not transferred to other streams (Fields and Philipp 1998). As a result, Brook Trout eggs collected from Wisconsin's Ash Creek were requested for stockings in 2006, 2009, and 2010. During those three years, 15,089 Ash Creek strain Brook Trout were stocked into Middle Bear Creek, Pine Creek near Sattre, and North Cedar Creek. Those streams now have self-sustaining wild populations of Brook Trout that are genetically distinct from native Iowa Brook Trout based on a recent genetic evaluation.

From 2016 to 2019, genetic samples were collected from known Iowa wild Brook Trout populations as well as the Iowa domestic Brook Trout housed at the Manchester Fish Hatchery. A total of 920 samples were collected and sent to Wes Larson at UW-Stevens Point for analysis as part of a larger Brook Trout genetic analysis (Erdman et al. 2022). Although the analysis does not provide concrete evidence that South Pine Creek Brook Trout are native to Iowa, it also does not provide strong evidence that they are not native. Similar to other studies, Larson (2020) placed South

Pine Creek in a native node along with other Driftless wild populations. Nearly all streams restored with South Pine Creek stockings showed South Pine ancestry with moderate to high diversity; however, a few have low diversity and could benefit from future supplemental stockings. Two streams had self-sustaining populations of Brook Trout consistent with Manchester domestic ancestry. Ash Creek ancestry was also confirmed for three streams (Pine, Middle Bear, and North Cedar creeks) that received stockings of Ash Creek Brook Trout beginning in 2006. Larson (2020) supports South Pine Creek Brook Trout as a native Iowa ancestry and several restored Brook Trout fisheries exist on private and publicly accessible streams, helping to protect this unique Brook Trout lineage from a catastrophic loss event.

Brook Trout Management Today

Brook Trout are now considered a priority for the Iowa Trout Program as outlined in "A Plan for Iowa Trout Management" (Kirby et al. 2020). For example, Goal 1 of the plan guides program staff to maintain existing populations of native Brook Trout in Iowa by investigating the genetic status of existing populations and to phase out stockings of non-native strains, both of which have already been completed. Goal 2 proposes the restoration of Brook Trout in eight new streams, stream surveys to locate two extant populations of Brook Trout, and the development of a Brook Trout restoration plan. Further, Goal 6 of the plan suggests staff determine how certain Brown Trout populations could negatively affect Brook Trout recovery efforts. To this end, Iowa Trout Program staff are increasing management and hatchery emphasis on Brook Trout in several ways.

Work to locate extant populations of Brook Trout in Iowa has begun. Kelly et al. (2021) examined the distribution of Brook and Brown Trout in Iowa by sampling 138 headwater stream segments in northeast Iowa. Brook Trout occupancy was low (19 sites) and usually consisted of a small number of adults within an area that had already received restoration stockings. The Iowa DNR Streams Research Team continues to sample headwater streams following the approach of Kelly (2020), and focusing on headwater sections of coldwater streams that have not previously been sampled. This work continues to fill gaps in fish community information pertaining to headwater reaches of coldwater streams in northeast Iowa.

Only 16 streams in Iowa have self-sustaining populations of restored native Brook Trout (Figure 3); therefore, expanding native Brook Trout restoration stockings continues to be a priority of the Iowa Trout Program. The domestic Manchester Brook Trout are no longer stocked in Iowa after Larson (2020) showed mixing with local native populations. Today, only South Pine Brook Trout are reared for restoration stockings throughout northeast Iowa. The number of streams stocked each year is dependent on the number of fingerling Brook Trout available for stocking. In the past, South Pine Brook Trout were spawned along the stream with no adults transported to the hatchery. While this did reduce mortalities of adults, it also limited the number of gametes collected each year. Prior to 2020, up to 3,000 fingerling Brook Trout would be available annually for restoration stockings, limiting the number of sites stocked.

In an effort to increase the number of fingerling Brook Trout available each year for restoration stockings, an expanded spawning process was adopted in autumn 2019. Stream-side spawning is still conducted annually, but 500 fingerling South Pine Brook Trout progeny are retained at the Manchester Hatchery each year for broodstock (D. Rosauer, Iowa DNR, personal communication). Two-year-old females are routinely checked and spawned when ripe. Milt from wild South Pine males is used to fertilize the eggs to limit the hatchery influence on fingerlings. Females are only spawned once and then released along with the two-year-old males as part of restoration stockings. Using this approach in addition to streamside spawning of wild Brook Trout has increased the total number of fingerlings available for stocking to over 30,000 each year, allowing 45 streams to receive restoration stockings totaling 117,388 fingerlings since the program began in the mid-1990s.

Figure 3. Sixteen self-sustaining populations of native Brook Trout have developed from restoration stockings in Iowa.

Brook Trout restoration site selection can be challenging. In rare cases, established relationships with landowners have allowed for long-term water temperature or fish community datasets that could be used to determine if the site is a high-quality location where restoration stockings may be successful. Typically, little is known about the many springs and headwater stream segments found in northeast Iowa. Few have had fish community assessments completed and water temperature data seldom exists for headwater sites. Although Iowa has over 475 miles of streams currently classified as coldwater (Iowa Department of Natural Resources 2019), many of the reaches considered coldwater do not include headwater segments where Brook Trout restoration stockings are likely to occur. Current research by the Iowa DNR and the University of Wisconsin-LaCrosse using high-resolution winter imagery to locate springs and segments of streams with high groundwater inputs shows promise for helping to locate potential restoration sites. Only sites with high levels of groundwater input remain ice-free during the winter months and are easily visible during periods of snow cover. These same sites may be good candidates for Brook Trout restoration stockings; however, additional research will be needed to confirm the usefulness of this approach.

Although Iowa is probably not a state that comes to mind when people think of wild trout, it was likely home to a native population of Brook Trout prior to European settlement. Soon after, rapid landscape conversions decimated trout numbers to the point that stocking has been required for decades to maintain trout populations. Only recently has the focus shifted towards wild Brook Trout management. Today, a renewed focus on Brook Trout management has initiated several research projects in addition to adjusted management and hatchery priorities. Together, these changes should expand wild trout conservation successes in Iowa that will increase the number of streams with thriving Brook Trout populations beyond the 20 streams where they exist today.

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Advancing Brook Trout Restoration in Iowa using a Multi-Faceted Approach

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Abstract - Brook Trout *Salvelinus fontinalis* are the only native salmonid to Iowa, and substantial effort has been directed toward protecting populations. Culture of the Brook Trout from South Pine Creek, the only wild population in Iowa, began in 1996 and continued annually to restore additional populations. Recent genetic evaluations confirmed that the South Pine Creek strain was unique and suitable for use as a brood source. The evaluations also identified five streams that had non-native Brook Trout ancestries. Of these, one population of domestic hatchery Brook Trout was identified to be removed using electrofishing. To date, three removal passes have been completed with over 350 Brook Trout removed. Naturalized Brown Trout *Salmo trutta* are another threat to wild Iowa Brook Trout, with populations established in most quality coldwater streams. Two streams with barriers to prevent Brown Trout recolonization were selected for Brown Trout removal. The effectiveness of trout removal via stream electrofishing is reviewed and the use of fish culture to improve native Brook Trout restoration efforts is also discussed.

Introduction

Brook Trout *Salvelinus fontinalis* have a long management history that includes widespread stocking in the Driftless Area of northeast Iowa going back as far as 1875. In the early 1990's domesticated Brook Trout, received from the St. Croix Fish Hatchery and maintained at the Manchester Fish Hatchery, were being stocked into Iowa streams as catchable-size fish (254 to 305 mm TL) or fingerlings (51 mm TL) (Dave Marolf, Iowa DNR retired, personal communication, January 2019). Meanwhile, in the mid-1990s, a population of naturally reproducing Brook Trout was identified in South Pine Creek. An early genetic evaluation of the population suggested it was unique and likely a native strain but with low genetic diversity (Fields and Philipp 1998). The strain was then used to develop more populations to protect the unique Iowa ancestry from a catastrophic loss. During that same period, adult Brook Trout numbers in South Pine Creek experienced a drastic decline, resulting in the importation of Brook Trout fingerlings originating from another Driftless Area stream, Ash Creek. Domestic Brook Trout from Manchester Fish Hatchery were stocked into many streams, but no populations of Brook Trout were known to have been established from those stockings. Ash Creek Brook Trout were stocked into three Iowa streams in the mid-2000s, creating successful populations. A more recent genetic evaluation confirmed the South Pine Creek population was unique and that it contained adequate genetic diversity (Larson 2020), supporting its preservation as the preferred strain for the restoration of Brook Trout populations in northeast Iowa. Larson (2020) also confirmed Ash Creek ancestry in three streams and Manchester Fish Hatchery domestic influence in one stream (Falcon Springs). The Iowa Trout Management Plan calls for the continued restoration of native Brook Trout and to increase the number of restored Iowa Brook Trout populations (Kirby et al. 2020). Ultimately, restoration stockings should create high-quality Brook Trout populations that can be used as additional brood fish sources in the future.

The restoration of Brook Trout populations in Iowa is challenging. Factors such as population genetics and competition with other species may limit the success of restoration stockings. Four Iowa streams currently have Brook Trout populations with domestic or Ash Creek ancestries not originally found in Iowa. Casey Springs was the only stream where the domestic Brook Trout persisted with limited natural reproduction. The inability of the domestic Brook Trout to establish populations throughout northeast Iowa after years of stockings suggests that they are not adapted to local streams. To maximize the likelihood of success during restoration efforts, the removal of Brook Trout of domestic or Ash Creek ancestries before Iowa native stockings occur was recommended by Siepker (2020).

The management of Iowa trout streams has changed dramatically over the last 147 years, producing some great success stories while also making it more challenging to restore native populations. Naturalized Brown Trout *Salmo trutta* populations have expanded rapidly due to stocking and successful colonization. Brown Trout populations are now established in many of the quality coldwater streams in northeast Iowa (Kelly et al. 2021). Non-native Brown Trout grow faster than native Brook Trout (Carlson et al. 2007; Hoxmeier and Dieterman 2013), compete with them for preferred resting areas (Fausch and White 1981), and prey on their young (Alexander 1977). DeWald and Wilzbach (1992) found that Brown Trout had higher prey capture rates than Brook Trout in a laboratory stream channel when compared together and separately. They also found that, when evaluated separately, both Brown Trout and Brook Trout maintained their weight; but in the presence of Brown Trout, the Brook Trout lost weight. This resulted in positive instantaneous growth rate for Brown Trout in the mixed trials. In 1965, Brook Trout made up 100% of the Valley Creek, Minnesota fishery; but in 1980, following the introduction of Brown Trout, Brook Trout made up only 15% of the fishery. This reduction was attributed, in part, to competition with Brown Trout (Waters 1983). If streams have barriers to movement that isolate Brook Trout populations and limit future Brown Trout immigration, those Brook Trout populations will likely benefit from the removal of Brown Trout.

In this study, we took a multi-faceted approach to protecting and restoring Brook Trout populations in the northeastern Iowa portion of the Driftless Area. First, we attempted to remove, via electrofishing, a population of Brook Trout developed from the non-native domesticated Brook Trout previously stocked in Iowa. Second, we removed Brown Trout from two stream segments supporting restored populations of native Brook Trout that are disconnected from other coldwater streams by physical or thermal barriers. By protecting the Iowa ancestry of Brook Trout while also reducing the competition between Brook Trout and Brown Trout, we hope to develop resilient Brook Trout populations in many more streams.

Study Area and Methods

Study streams are located in northeast Iowa within an area known as the Driftless Region. This geographical area is known for its karst limestone topography with abundant springs, rock outcroppings, many narrow valleys with cold fast-flowing streams, woodlands, and few glacial deposits. One headwater stream (Falcon Springs) was chosen for Brook Trout removal. Falcon Spring, located just north of Decorah, starts on the Falcon Springs Wildlife Management Area and flows 3.33 km to its confluence with the Upper Iowa River. The stream flows through a mix of public and private land. It has a long history of stocking and reproduction of Brown Trout, domestic Brook Trout, and occasional hybrids, commonly referred to as tiger trout (Brook Trout × Brown Trout). Two streams were chosen for Brown Trout removal, including Lansing Creek and Brownfield Creek. Lansing Creek is located north of Lansing, Iowa and flows 3.97 km, mostly on public land, and drains directly into the Mississippi River. Both Brown Trout and restored native Brook Trout successfully reproduce in this stream with occasional hybridization as described above. Brownfield Creek (Figure 1) is a private stream located northwest of Colesberg, Iowa in the Turkey River drainage. This creek flows 700 m before it drains into a 4.96-hectare lake. This stream supports a quality Brown Trout population with successful annual reproduction. Other species typically found in these creeks include Johnny Darter *Etheostoma nigrum* (Falcon Springs), White Suckers *Catostomus commersonii* (Falcon Springs), Western Blacknose Dace *Rhinichthys obtusus* (Lansing Creek), Brook Stickleback *Culaea inconstans* (Lansing Creek), and Grass Pickerel *Esox americanus* (Lansing Creek). All work on private property was completed with landowner permission.

Three- or four-person crews using backpack electrofishers (ETS Electrofishing Systems, LLC., Model ABP-4, 110 V, 60 Hz, 25% PDC) working in tandem in an upstream direction removed Brook Trout and their hybrids (hereafter referred to as Brook Trout) from Falcon Springs and all Brown Trout and their hybrids (hereafter referred to as Brown Trout) from Lansing Creek and Brownfield Creek. Age-0 trout (typically less than 75 mm) were not targeted during spring passes because they were difficult to collect from the stream and, in some cases, difficult to identify to species at that time of year. Multiple single-passes were completed on all study streams during summer 2020 and summer 2021. On Brownfield Creek, a modified trap net was also placed at the confluence of the incoming stream and the lake to capture large individuals as they moved up from the lake in the fall to spawn. All trout were captured and identified to species or as a hybrid and total length was recorded. A linearized catch curve was completed to evaluate removal efficiency by year or pass. We used the instantaneous mortality rate (Z) from the catch curve as a substitute for removal efficiency percentage from year to year or from pass to pass.

Results

Brook Trout were removed from Falcon Springs during two single-pass collection events in both 2020 and 2021. In 2020, 315 Brook Trout (range = 125-279 mm; Table 1) were removed followed by another 50 individuals (range = 163-241 mm) in 2021. A total of 365 Brook Trout were removed, resulting in an 84% removal efficiency of fish between years.

Brown Trout were removed from Lansing Creek during nine single-pass collection events completed over two years. The first three complete passes took two days each to complete because of the high number of Brown Trout collected and the length of the creek. In 2020, 1,682 Brown Trout (range = 115-533 mm; Table 2) were removed with another 413 Brown Trout (range = 160-343 mm) removed in 2021. Across both years, a total of 2,095 Brown Trout were removed with nine passes resulting in a 75.4% removal efficiency of fish between years and a 51% removal efficiency between passes.

Brown Trout removal in Brownfield Creek included fourteen single-pass collection efforts and three net nights. A total of 7,119 trout were removed over the two years, with 5,964 trout (range = 111-508 mm; Table 2) removed in 2020, resulting in an 80.6% removal efficiency of Brown Trout between years.

Discussion

Brook Trout removal from Falcon Springs has been difficult because access to the stream is prohibited on a reach that flows through private land. Although the landowner has not allowed trout removal on their property, this lack of removal did not result in a large number of adult fish or extensive reproduction being found in 2021. No fish larger than 254 mm were captured in the second pass and a substantial reduction in fish less than 152 mm also occurred. The Brook Trout removal from Falcon Springs was more efficient than Brown Trout removal at Lansing Creek. More passes were needed at Falcon Springs to reduce the number of domestic Brook Trout, but total removal may be impossible because of the lack of access to private land. To completely eradicate the domestic Brook Trout, an additional tool maybe needed such as introducing YY Brook Trout (Schill et al. 2016).

Since we began our work on Lansing Creek, there has been a dramatic change in the Brown Trout population. After the second pass, only two fish larger than 305 mm have been captured. Very few age-0 fish were found in 2021, and all of them were found in a 50 m stretch of stream. The trout removal efficiency has been constant with an average reduction of 51% between passes (Figure 2). The only pass that deviated from this was pass five, which was completed only seven days after the previous pass. It may be important to allow more time between sample passes to allow fish to re-disperse into areas that are easier to sample. The Mississippi River has been an effective barrier for Lansing Creek and does not seem to be a refuge or reservoir for adult Brown Trout, increasing the likelihood that we will be able to successfully remove all Brown Trout from Lansing Creek.

Trout removal in Brownfield Creek has been more challenging than the other two streams because of the downstream reservoir. Reproduction continues to be high and continued sampling results in a large number of large Brown Trout immigrating into the stream from the reservoir in autumn. Despite the challenge of removing Brown Trout from the reservoir, we have reduced the population by 80.6 % from year to year. This creek is very small and short, as it only flows 700 m before entering the lake. Although the reservoir forms an impassable barrier to the upstream movement of Brown Trout found downstream of the reservoir, it also serves as a refuge for adult Brown Trout that inhabit the reservoir for most of the year and only migrate into the creek to spawn. A different strategy may have to be developed to address the adult Brown Trout in the reservoir in addition to the continued removal efforts in the stream.

The two streams where Brown Trout removal is being completed have a good chance of success. It will take several more years and many more staff hours to completely remove them. Shepard et al. (2014), using similar methods, was able to eradicate Brook Trout from six different streams and it took 4 to 8 years to complete. This project will help meet one of the goals in the Iowa Trout Management Plan which was to “Create high-quality Brook Trout populations that can be used as additional brood fish sources in the future.” Domestic Brook Trout removal from Falcon Springs will be much more difficult because, like most Iowa streams, the majority of it is on private land, and it is difficult to gain access from private landowners. Alternative strategies may need to be implemented if we hope to be successful at removing non-native populations of salmonids.

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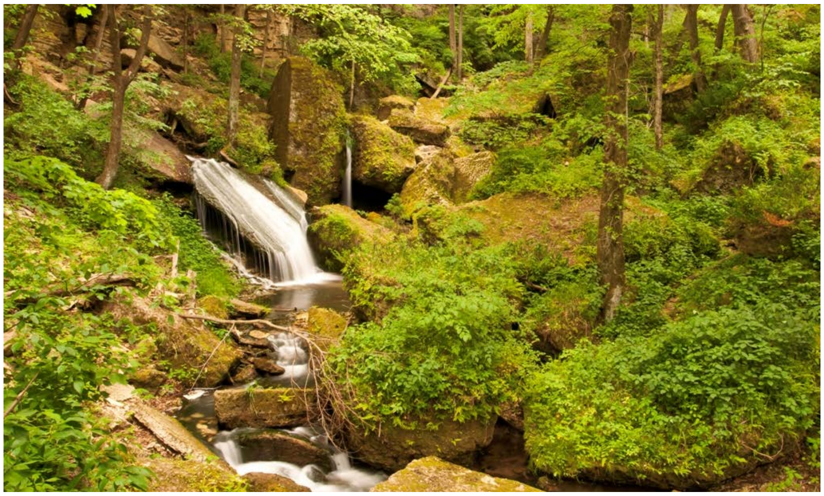

Figure 1. Brownfield Creek a Brown Trout removal site.

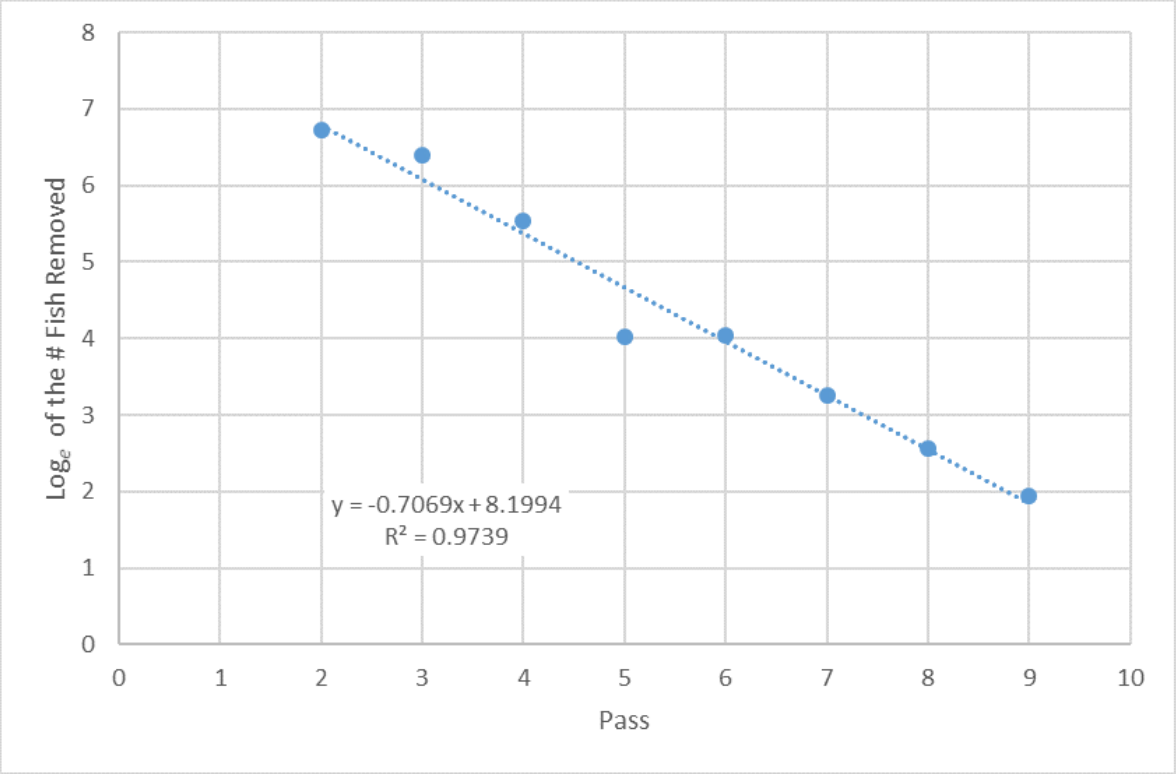

Figure 2. Catch-curve for Brown Trout removed from Lansing Creek by pass. The absolute value of the dashed line is the estimate of trout removed between passes and the exponentiated negative value of the estimated slope gives the efficiency of removal between pass estimate of 51%.

Table 1. Combined number of Brook Trout removed by length category from Falcon Springs.

Year	< 152 mm	152 - 254 mm	254 - 305 mm	> 305 mm	Total
2020	242	65	8	0	315
2021	9	41	0	0	50
Total	251	106	8	0	365

Table 2. Combined number of Brown Trout removed from Lansing Creek and Brownfield Creek.

Stream	Year	< 152 mm	152 - 254 mm	254 - 305 mm	> 305 mm	Total
Lansing Creek	2020	1383	261	19	19	1682
	2021	150	253	8	2	413
Total		1533	514	27	21	2095
Brownfield Creek	2020	5079	690	66	129	5964
	2021	926	210	4	15	1155
Total		6005	900	70	144	7119

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Gene Flow and Spatial Population Structure of Brook Trout in a Large Headwater Stream Network in Colorado

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Abstract - We studied gene flow of non-native Brook Trout *Salvelinus fontinalis* in a 60-km section of continuous stream network in the upper Cache la Poudre River basin, where a large-scale reclamation effort to restore federally threatened Greenback Cutthroat Trout *Oncorhynchus clarkii stomias* (GBCT) is taking place. This effort—the Poudre Headwaters Project—represents the most important recovery effort of the state fish of Colorado and could result in a fivefold increase in total occupied GBCT habitat. However, the reclamation area is currently dominated by non-native Brook Trout; key steps to ensure the success of the Poudre Headwaters Project include removing non-native Brook Trout and physically isolating the area from reinvasion. We examined existing genetic population structure in the reclamation area to provide science-based guidance for effective Brook Trout removal and subsequent GBCT reintroduction. During 2018 and 2019, tissue samples were collected from 23 sites in the Long Draw region. We genotyped 792 Brook Trout using 12 microsatellite loci to examine genetic population structure within the reclamation area. Our results show that fine-scale genetic population structure is present among sites (F_{ST} = 0.038) and overall genetic diversity is comparable to previously published estimates throughout the native range of Brook Trout (H_O = 0.610; HE = 0.618). Analysis of genetic differentiation among sites indicates that sites in the reclamation area may function as a metapopulation, with fine-scale genetic structure present among tributaries that are connected by gene flow. Understanding how the landscape influences connectivity and population persistence will provide science-based guidance for Brook Trout removal efforts and allow managers to release GBCT in stretches of key habitat that ensure the highest probability of reintroduction success.

Introduction

The Brook Trout *Salvelinus fontinalis* is a cold-water salmonid native to eastern North America, ranging from northern Quebec to the southern Appalachian Mountains. However, due to habitat loss and fragmentation, stream acidification, and introduction of exotic salmonids, Brook Trout populations are in decline throughout much of their native range, particularly in the Southeastern United States (Hudy et al. 2008). The majority of remaining populations are small and isolated in fragmented headwater streams. Brook Trout populations that persist in fragmented watersheds are highly susceptible to deleterious effects of genetic drift, including decreased genetic diversity and increased extinction risk. While patch size and connectivity may buffer populations from effects of genetic drift, stream patches that harbor Brook Trout are increasingly small and fragmented (Whiteley et al. 2013).

Currently, few studies examining spatial population structure and metapopulation dynamics of Brook Trout in unfragmented watersheds exist (though see Aunins et al. 2015; Kelson et al. 2015; White et al. 2020), and we know little about how populations are spatially structured in large, connected headwater stream networks. However, Brook Trout have been introduced throughout much of the intermountain west, where they continue to invade new habitats and displace native Cutthroat Trout Oncorhynchus clarkii (Fausch 2008). Many studies have investigated the adverse effects of non-native Brook Trout on native Cutthroat Trout (Peterson and Fausch 2003; Benjamin and Baxter 2012), but to our knowledge, Brook Trout genetic population structure has not been studied outside their native range. Thus, the naturalized range of Brook Trout may provide an opportunity to better understand genetic population structure in large, unfragmented headwater stream networks.

We studied gene flow of non-native Brook Trout in a 60-km section of continuous stream network in the upper Cache la Poudre River basin, Colorado, where a large-scale reclamation effort to restore a metapopulation of federally threatened Greenback Cutthroat Trout *O. c. stomias* is taking place. This reclamation effort—the Poudre Headwaters Project—represents the most important recovery effort of the state fish of Colorado and could result in a fivefold increase in total occupied Greenback Cutthroat Trout habitat. The reclamation area is currently dominated by non-native Brook Trout, and key steps to ensure the success of the Poudre Headwaters Project include removing non-native Brook Trout and physically isolating the area from reinvasion. Existing Brook Trout genetic population structure in the reclamation area will provide key scientific information to inform upcoming management actions, including Brook Trout removal and Cutthroat Trout reintroduction. However, the Poudre Headwaters Project reclamation area also provides a much-needed opportunity to advance our understanding of trout spatial population structure in large, unfragmented headwater stream networks.

Methods

Study Area

Our study took place in the headwaters of the Cache la Poudre River within Rocky Mountain National Park and Arapaho and Roosevelt National Forests (Figure 1). The dendritic stream network has several major tributaries—Corral Creek, Willow Creek, the upper Poudre River, Chapin Creek, Hague Creek, and La Poudre Pass Creek (Figure 1). Long Draw Reservoir was built on La Poudre Pass Creek in 1930, and the dam at the terminal end of the reservoir blocks upstream fish passage. Water is typically released from Long Draw Reservoir between mid-May and mid-September, and the section of La Poudre Pass Creek below the reservoir experiences flow intermittency and stream dewatering when reservoir releases cease each winter. According to historical stocking records, Brook Trout were stocked in waters near the study area beginning in 1892, and stocking ceased in 1955 (Andrew Treble, Colorado Parks and Wildlife, unpublished data, 2021).

Sample Collection and Laboratory Analysis

We collected Brook Trout tissue samples from 23 sites across nine streams (Figure 1) in summer and fall of 2018 and 2019 via backpack electrofishing surveys. Multiple sites were sampled along each major tributary to capture potential longitudinal differences among sites within a stream and ensure broad spatial coverage across the reclamation area. We measured each fish for total length, collected an anal or caudal fin clip for genetic analysis, and released fish alive after processing. Fin clips were dried on Whatman chromatography paper and stored individually in coin envelopes. To reduce the risk of sampling related individuals (Whiteley et al. 2012), we did not collect fin clips from age-0 individuals, which were easily distinguished by their small size.

We used 12 microsatellite markers to characterize Brook Trout genetic population structure in the Poudre Headwaters Project reclamation area: *SfoC113, SfoC115, SfoC129, SfoC38, SfoC88, SfoD91, SfoB52, SfoC24, SfoC28, SfoC79, SfoC86,* and *SfoD75* (King et al. 2012). We randomly selected 35 individuals per site across size classes for genetic analysis; if fewer than 35 individuals were captured, all individuals were genotyped. Genomic DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit according to the manufacturer's protocol. Loci were combined in two multiplexes for polymerase chain reaction (PCR) amplification. Each 10 µl PCR multiplex consisted of 2 µl of extracted genomic DNA, 0.04-0.10 µl of each forward dye-labeled and reverse unlabeled primer, 5 µl of 2x Qiagen Multiplex PCR Mastermix, and 2.18-2.20 µl of nuclease-free water. The thermocyler protocol for multiplexed PCRs consisted of an initial denaturation at 95°C for 15 min; 35 cycles of denaturation at 95°C for 45s, annealing at 56°C for 45s, and extension at 72°C for 2 min; and a final extension of

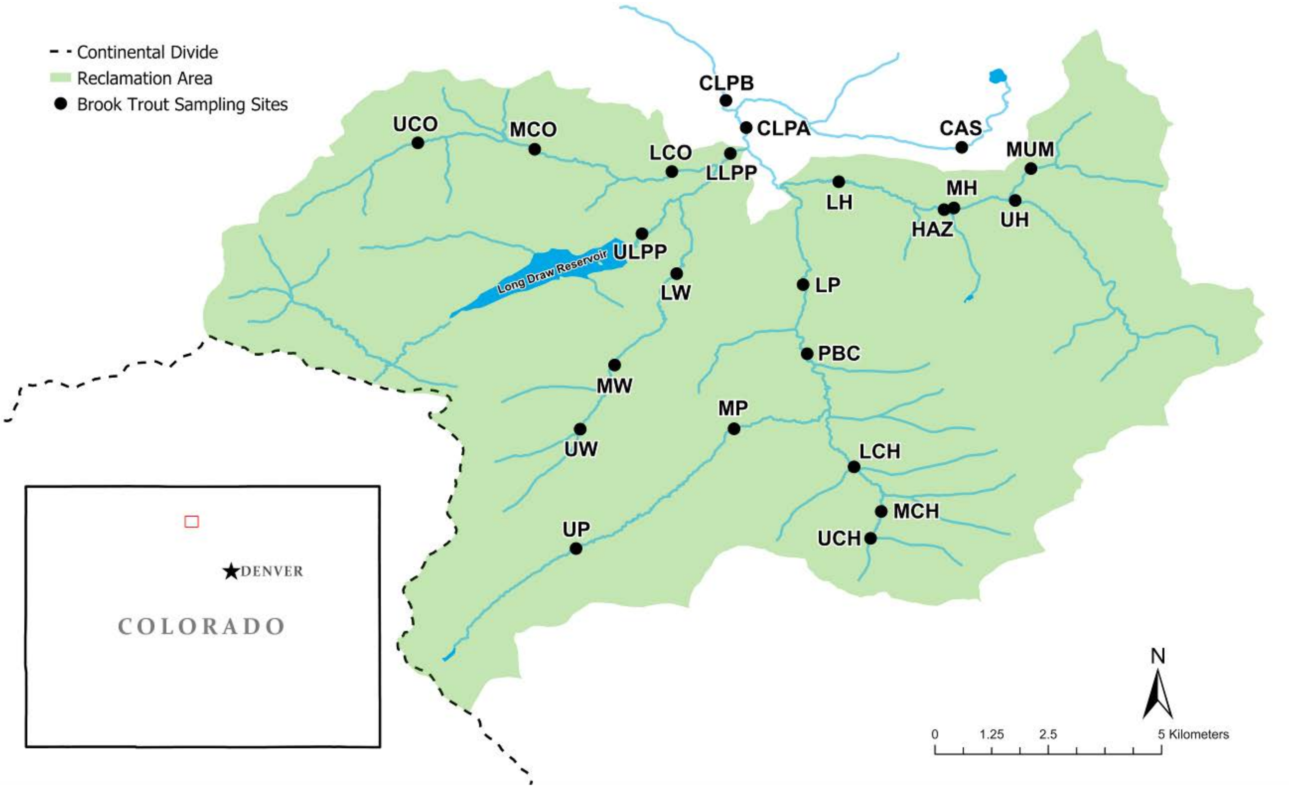

Figure 1. Map of the study area showing sites (black dots) where Brook Trout were sampled in Arapaho and Roosevelt National Forests and Rocky Mountain National Park in northern Colorado, USA. Streams are shown as light blue lines, and the Greenback Cutthroat Trout reclamation area is shaded in green. Sites on major tributaries are as follows—Corral Creek: UCO, MCO, and LCO; Willow Creek: UW, MW, LW; Hague Creek: MUM, HAZ, UH, MH, LH; upper Poudre River: UP, MP, PBC, LP; Chapin Creek: UCH, MCH, LCH; La Poudre Pass Creek: ULPP, LLPP; Mainstem Cache la Poudre River: CLPA and CLPB; Cascade Creek: CAS.

60°C for 30 min. Fragment analysis was performed using an Applied Biosystems 3500 genetic analyzer, and alleles were binned and scored using GeneMapper version 6 and checked by eye.

Genetic Diversity and Differentiation

We assessed potential deviations from Hardy-Weinberg equilibrium (HWE) with 1,000 Monte Carlo permutations using the package pegas (Paradis 2010) and applied a Bonferroni correction for multiple comparisons across 276 HWE tests in R (R Core Team 2022). To calculate overall within-population gene diversity (H_E), observed heterozygosity (H_O), inbreeding coefficient (F_{IS}), and rarefied allelic richness (A_R; rarefied to 58 alleles), we used the R package hierfstat (Goudet 2005). Pairwise FST was calculated following Weir and Cockerham (1984) with hierfstat using 1,000 bootstrap replicates for 95% confidence intervals. We considered an estimate of pairwise F_{ST} significant if 95% confidence intervals did not overlap zero. Estimates of pairwise F_{ST} were visualized as a heatmap using the lattice R package (Sarkar 2008).

Clustering Analyses

To understand gene flow and population structure within the Poudre Headwaters Project reclamation area, we used two clustering methods. The first, STRUCTURE version 2.3.4 (Pritchard et al. 2000), is a model-based Bayesian clustering method for multilocus genetic data. We performed STRUCTURE runs with the admixture model, correlated allele frequencies, and no location prior. Each STRUCTURE run consisted of 20,000 burn-in iterations, 100,000 subsequent iterations, and five replicates of each K. We examined K = 1–23 and determined the number of genetic clusters likely in the data using the highest values of the likelihood of K (L(K); Pritchard et al. 2000) and ΔK (Evanno et al. 2005). Within each STRUCTURE run, we merged replicates of each K using the R package pophelper (Francis 2017) and visualized individual cluster assignment probabilities using ggplot (Wickam 2016). The second clustering method, discriminant analysis of principal components (DAPC; Jombart et al. 2010), uses a principal components analysis to transform genetic data before applying discriminant functions to minimize within-group variance and maximize between-group variance. Because DAPC is sensitive to the number of retained principal components, we used the *optim.a.score* function from adegenet (Jombart 2008) to determine the optimum number of principal components to retain for DAPC. We evaluated de novo genetic clusters using the *find.clusters* function from adegenet (Jombart et al. 2010) for K = 1–23. The optimal number of clusters was chosen as the point at which the rate of change for the Bayesian information criterion plateaued. All discriminant functions were retained, and clusters were visualized in an ordination plot along axes of the first and second discriminant functions. The DAPC posterior assignment probabilities for individuals were visualized using ggplot (Wickam 2016). To further visualize spatial structure, we calculated site-level cluster probabilities as the mean probability across individuals for each cluster within a site and plotted resulting pie charts on a map of the study area for both STRUCTURE and DAPC.

Results

Genetic Diversity and Differentiation

We did not detect any patterns of deviation from HWE that warranted excluding markers, though *SfoD91* deviated from HWE in a single site on Hague Creek. Across the reclamation area, estimates of genetic diversity (mean H_E = 0.618, mean H_O = 0.610, mean F_{IS} = 0.007, mean A_R = 5.699) were comparable to previously published estimates throughout the native range of Brook Trout (Pregler et al. 2018; Kazyak et al. 2022). Estimates of pairwise FST ranged from -0.0075 for MW-LW to 0.1175 for MCO-UW, indicating that fine-scale genetic structure is present in the study area. Pairwise F_{ST} values were highest for comparisons of sites on Corral Creek (UCO, MCO, and LCO) to sites on other tributaries in the reclamation area (Figure 2), suggesting that Corral Creek is somewhat isolated from the rest of the stream network.

Clustering Analyses

Our STRUCTURE analyses indicated four genetic clusters (K = 4) in the reclamation area, with concordant results from both L(K) and ΔK estimators. STRUCTURE clusters roughly corresponded to major tributaries (Figure 3A), with distinct clusters for Corral Creek (cluster 1), Willow Creek (cluster 2), Hague Creek and its tributaries (cluster 3), and Chapin Creek and the Upper Poudre River (cluster 4). Though major tributaries strongly influenced genetic structure, some individuals in each major tributary were admixed, and sites further downstream on La Poudre Pass Creek and the mainstem Poudre River showed increased levels of admixture (Figure 3B). We also performed hierarchical STRUCTURE analyses for each distinct cluster, but we did not find evidence of hierarchical structure in any case.

The *optim.a.score* function indicated the optimum number of PCs to retain for DAPC was 51, which conserved 93.9% of the observed variance. Our DAPC analysis showed evidence of six distinct genetic clusters (K = 6), and the majority of variance in the discriminant analysis was explained by discriminant functions 1 and 2 (Figure 4A inset). Discriminant function 1 (x-axis; eigenvalue = 423.6) and discriminant function 2 (y-axis; eigenvalue = 213.7) demonstrated overlap between most genetic clusters, indicating a large degree of movement and admixture among some tributaries. In contrast, cluster 4 was largely composed of individuals from Corral Creek and distinctly segregated from other clusters along discriminant function 1 (Figure 4), again suggesting that Corral Creek is largely isolated from the rest of the stream network.

The STRUCTURE and DAPC anlaysis identified different numbers of *de novo* genetic clusters (K = 4 for STRUCTURE and K = 6 for DAPC) within the reclamation area, which is unsurprising given that STRUCTURE can have difficulty identifying hierarchical structure (Evanno et al. 2005) and that STRUCTURE and DAPC have different underlying assumptions. However, we argue that both K = 4 and K = 6 are biologically relevant and, when considered together, represent a nuanced view of genetic structure in the Poudre Headwaters Project area. Our STRUCTURE analysis shows fine-scale genetic structure at the major tributary level, with distinct genetic clusters for each major tributary complex in the reclamation area (Figure 3). Conversely, DAPC shows evidence of higher levels of gene flow between most clusters and a stronger signature of admixture across the reclamation area (Figure 4).

Discussion

Though STRUCTURE and DAPC identified different numbers of genetic clusters, both clustering methods demonstrate that tributaries in the reclamation area influence genetic population structure but are also connected by gene flow. Estimates of pairwise F_{ST} also reinforce this conclusion, with lower F_{ST} values for within-tributary comparisons. However, connectivity is not uniform across the stream network. Pairwise F_{ST}, STRUCTURE, and DAPC suggest that Corral Creek is an isolated patch, somewhat separated from the rest of the study area. We hypothesize that this isolation is potentially driven by a hydrological barrier caused by seasonal water releases from Long Draw Reservoir and resulting intermittent flows on La Poudre

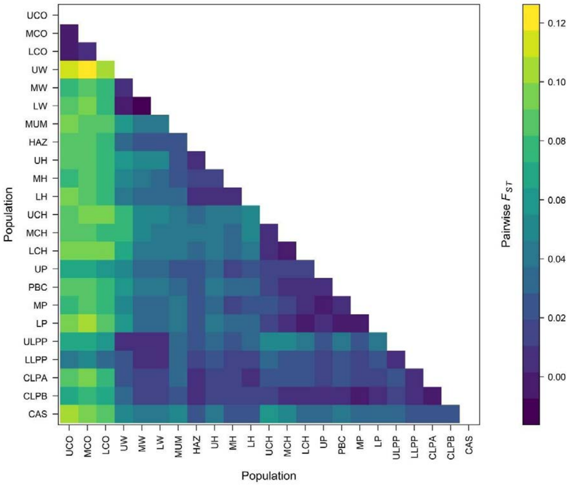

Figure 2.

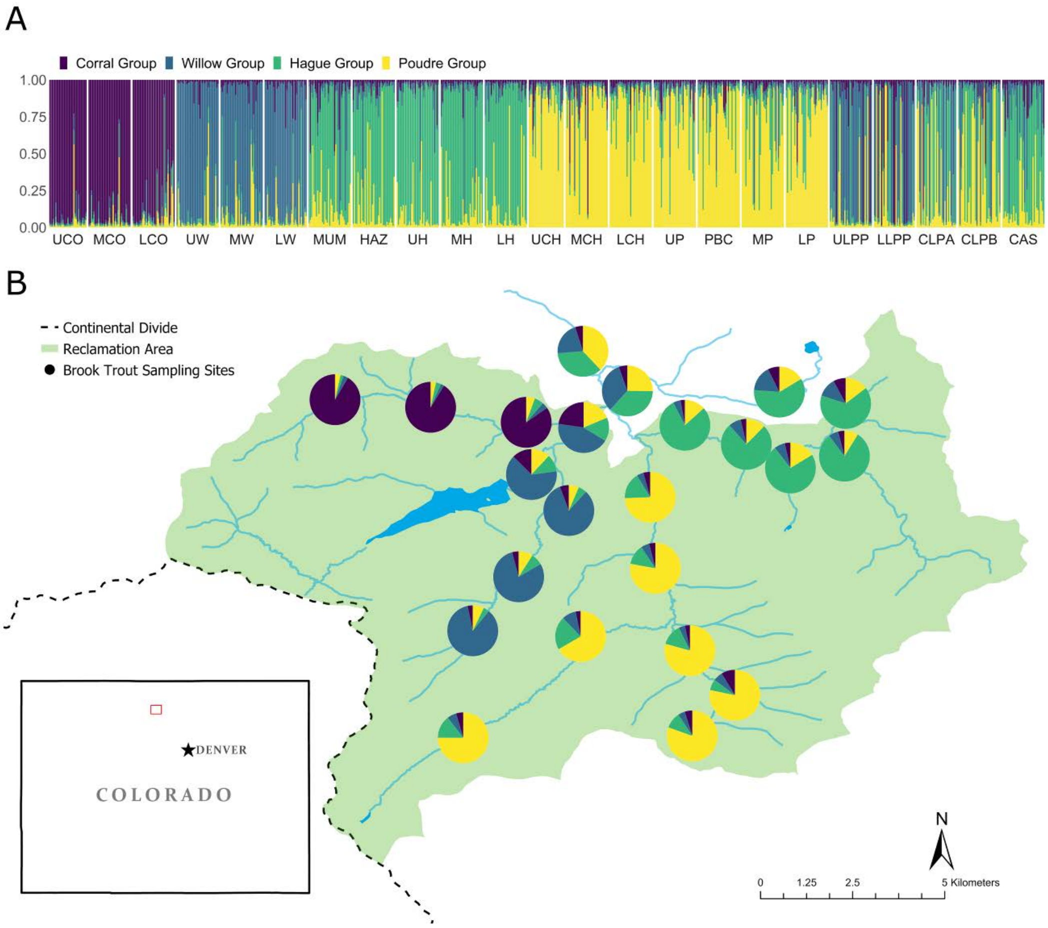

Figure 3. A) STRUCTURE analysis (K = 4) of Brook Trout. Each individual is represented as a vertical bar whose colors correspond to the probability of assignment to different clusters. B) Map of the study area showing mean cluster assignment probabilities for each site under the K = 4 scenario.

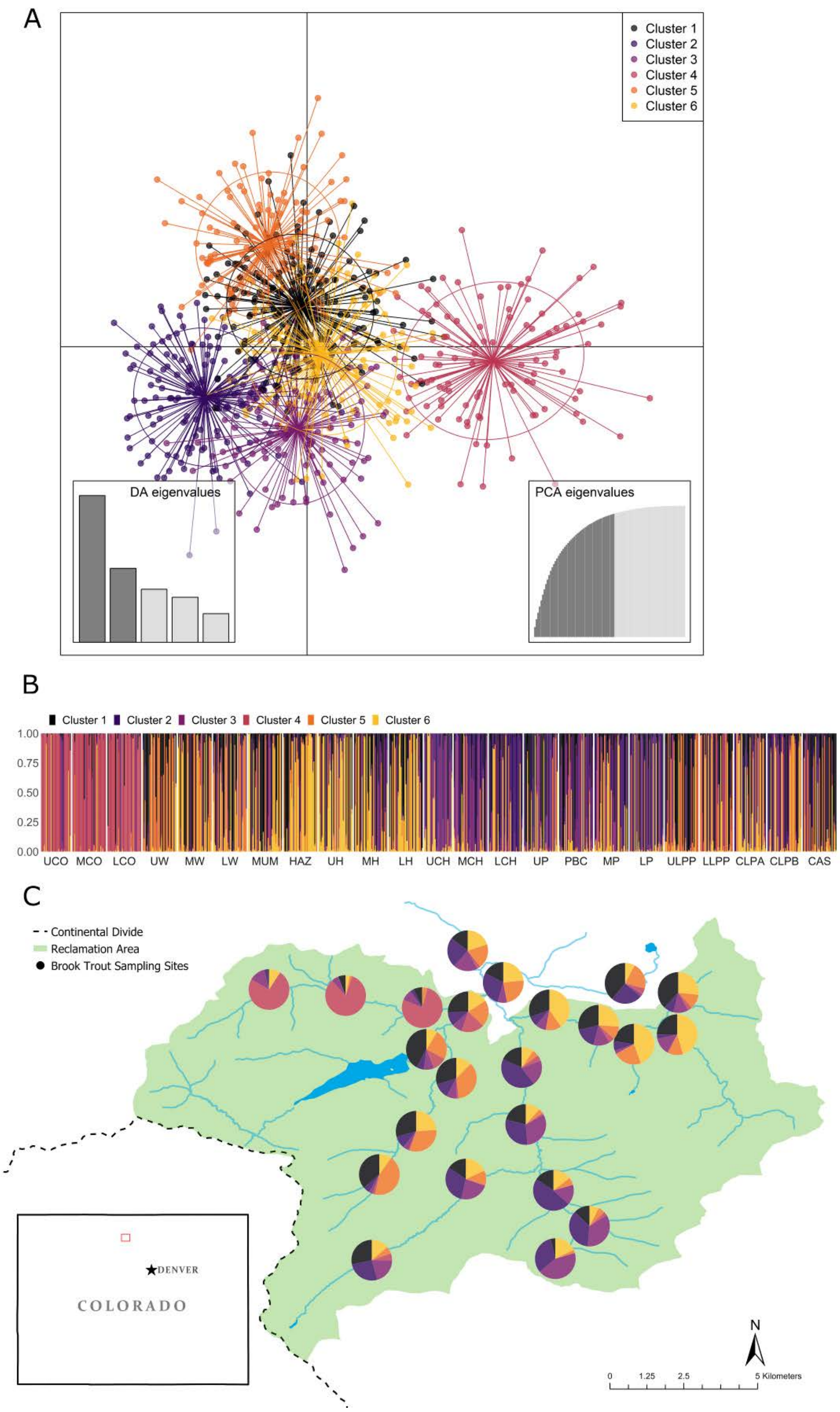

Figure 4. A) STRUCTURE analysis (K = 4) of Brook Trout. Each individual is represented as a vertical bar whose colors coA) Discriminant analysis of principal components (DAPC) of Brook Trout (K = 6). Each individual is represented by a point plotted along discriminant function 1 (x-axis) and discriminant function 2 (y-axis). Colors of individual points and inertia ellipses correspond to genetic clusters. B) DAPC posterior assignment probabilities for the K = 6 scenario. Each individual is represented as a vertical bar whose colors correspond to the probability of assignment to different clusters. C) Map of the study area showing mean cluster assignment probabilities for each site under the K = 6 scenario.

Pass Creek.

Overall, our genetic analyses with Brook Trout indicate that sites within the Poudre Headwaters Project area may function as a metapopulation, with fine-scale genetic structure present among tributaries that are connected by gene flow. For Poudre Headwaters Project managers, this indicates that after Brook Trout eradication is complete, reintroduced Greenback Cutthroat Trout will likely be able to move among tributaries within the study area and function as a robust metapopulation. As a result of this study, managers can also use our genetic analyses to understand how subpopulations interact and help guide Brook Trout removal efforts. In addition, we plan to implement a riverscape genetics framework in the future (White et al. 2020) to assess how environmental variables influence connectivity and population persistence. This riverscape genetic analysis will help managers identify stretches of critical habitat that ensure the highest probability of reintroduction success for Greenback Cutthroat Trout.

More broadly, our work has implications for Brook Trout conservation in their native range, where many populations are in decline. Eastern Brook Trout populations are often highly fragmented, and few studies have looked at Brook Trout spatial population structure in continuous stream networks. Our work indicates that when given the opportunity, Brook Trout can form metapopulations in unfragmented stream networks. While native and non-native Brook Trout populations may not be directly comparable, our work provides evidence that habitat restoration may be a promising avenue for Brook Trout conservation. Our study also provides an example for managers who seek to understand spatial population structure without investing the time and resources needed in a traditional mark-recapture approach. This example could be used for other inland salmonids across the globe, especially in remote areas where operating PIT tag arrays is not feasible. Finally, our work shows that widespread invasive species, like Brook Trout in the intermountain west, may be used in a surrogate context to help achieve conservation goals for threatened native species.

Acknowledgements

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The use of M_{YY} fish to eradicate non-native Brook Trout populations in Idaho

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Abstract - Brook Trout *Salvelinus fontinalis* (BKT) in the west have established self-sustaining populations that threaten native salmonids and are difficult to eradicate. One novel eradication approach uses hatchery-produced, genetically YY male fish (M_{YY}) created by feminizing XY males and crossing with normal XY males. All progeny of M_{YY} × wild female crosses are male, thus successful stocking and reproduction by M_{YY} fish could potentially shift sex ratios of wild populations to 100% male, causing extirpation of undesirable populations. The Idaho Department of Fish and Game is evaluating such an approach in streams and alpine lakes, which previously has included: 1) M_{YY} BKT hatchery broodstock development; 2) demonstration of successful M_{YY} post-release survival and reproduction in streams; and, 3) population simulations predicting that, with realistic rates of wild fish suppression and M_{YY} stocking, survival, and reproductive success, complete eradication of wild BKT populations could occur in reasonable management timelines. Here we present results to date from the culminating M_{YY} BKT stocking field trial ongoing in several streams and alpine lakes. In one stream, the sex ratio has shifted from 28% males in 2016 to 77% male in 2021. Growth rates and body condition appear to be equivalent between M_{YY} and wild BKT. Preliminary findings indicate that M_{YY} offspring production has been higher in streams than in lakes, when stocking fingerlings instead of catchables, and when wild fish are suppressed annually. Whether complete eradication occurs in any waters remains to be seen.

Introduction

Brook Trout *Salvelinus fontinalis* (BKT) in the west have established self-sustaining populations in streams and alpine lakes that threaten myriad native salmonid populations via hybridization and competition for space and resources (reviewed in Dunham et al. 2004). Once established, eradication of non-native BKT populations is difficult with standard techniques such as electrofishing, gill netting, chemicals (such as rotenone), and biological control (such as predator stocking).

A novel eradication approach introduced by Gutierrez and Teem (2006) suggested the use of hatchery produced male fish with an YY genotype (known as "supermales", herein referred to as M_{YY} fish) to shift the sex ratio of the wild population. A M_{YY} broodstock must first be created using the following steps: converting normal M_{XY} males to F_{XY} fish by exposing them to estrogen; crossing F_{XY} fish with normal M_{XY} males and retaining all YY offspring, using genetic sex markers to differentiate fish (see below); and, converting ½ of the M_{YY} offspring from M_{YY} to F_{YY} by exposing them to estrogen (Teem and Gutierrez 2010). Annual stocking of the offspring of this M_{YY} and F_{YY} broodstock theoretically could shift the sex ratio of the wild population to 100% male, thus collapsing the population.

The Idaho Department of Fish and Game (IDFG) developed a "recipe" to successfully create a M_{YY} and F_{YY} BKT broodstock in 2012 (Schill et al. 2016), which annually produces 20,000 – 30,000 M_{YY} BKT for stocking needs.

Population models considering possible eradication of BKT populations indicated that eradication is theoretically feasible if the fitness of hatchery M_{YY} individuals approaches that of individuals in the wild population (Schill et al. 2017). A detailed review of past IDFG M_{YY} studies is provided by Kennedy et al. (2017).

Due to the novelty of using M_{YY} vertebrates as an eradication method, almost nothing is known about the fitness of M_{YY} individuals once released into the wild. In the only such study ever conducted, hatchery M_{YY} BKT were reared to about 225 mm total length and stocked in four Idaho mountain streams; these fish survived and spawned successfully with wild conspecifics, and produced all-male progeny, though reproductive success was lower for M_{YY} fish than for their wild counterparts (Kennedy et al. 2018). While those preliminary results were insightful, additional evaluations of M_{YY} fitness are clearly needed.

To more thoroughly evaluate the practical use of M_{YY} BKT as an eradication tool, a broad-scale field study was initiated in 2015 in several Idaho streams and alpine lakes containing wild BKT. The objectives for this paper are to: 1) further evaluate M_{YY} BKT fitness relative to wild fish by comparing growth rates and body condition; and, 2) present preliminary sex ratio changes and M_{YY} BKT offspring production at study waters being annually stocked with M_{YY} BKT.

Methods

For complete details on YY broodstock production see Schill et al. (2016). Offspring are annually produced by crossing F_{YY} and M_{YY} broodstock at the IDFG Hayspur Hatchery. All M_{YY} BKT are adipose fin clipped prior to stocking to differentiate between wild and M_{YY} BKT in the field. Offspring are reared to either fingerling-size (~120 mm total length, at eight months of age) or catchable-size (~225 mm, at 20 months of age) for stocking purposes.

This study was initiated in 2015, but not all waters were sampled or stocked in the first year, and some waters were not included in the study until 2017. A total of 15 waterbodies comprise the entire study, most of which receive annual stocking of either fingerling- or catchable-sized M_{YY} BKT (Figure 1; Table 1). All study waters have self-sustaining wild BKT populations which comprise > 80% of the original fish species composition.

At several study waters, wild BKT are suppressed annually, prior to stocking, to evaluate whether suppression of wild fish improves the post-release performance of M_{YY} BKT. Suppression streams undergo annual suppression of the wild BKT population using backpack electrofishing, whereas suppression of wild fish in lakes relies on boat electrofishing and gill netting. All study stream treatment reaches have both a downstream and upstream passage barrier with a total stream length not exceeding 10 km between the barriers. Study lakes also have passage barriers. Passage barriers provide isolation from potential recolonization by wild BKT. To assess barrier passage, fish are marked with double maxillary clips below each downstream barrier in every stream and alpine lake; to date no recolonizing fish have been observed above passage barriers. Two streams and two lakes receive no stocking or wild suppression and serve as controls.

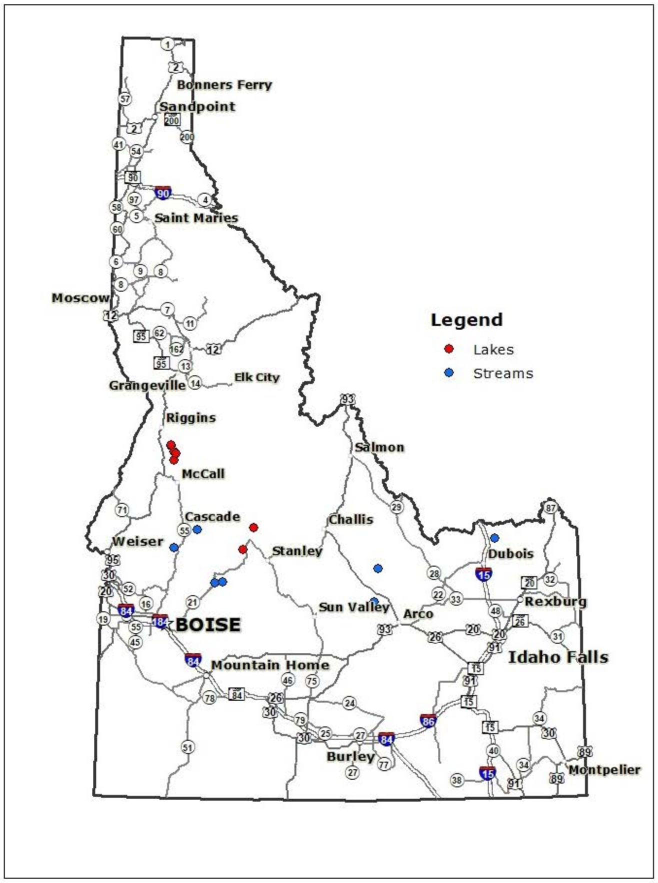

Figure 1. Location of M_{YY} Brook Trout study waters in Idaho.

Stocking Rates

Fingerling and catchable M_{YY} BKT are stocked annually in a single event. Fish are stocked by hand using buckets and in backpacks for streams and via helicopter and bucket (90–100 gal capacity SEI Industries Bambi bucket or 208 L barrel) for alpine lakes, except Martin and Seafoam Lake #4 which are stocked directly by hatchery truck.

In streams, stocking rate was set at 125 catchables/km because that is a typical stocking rate for Idaho streams. However, once abundance estimates could be made at each water, stocking rate was adjusted to be 50% of the original adult wild BKT population size. This rate was selected because earlier research indicated that a 50% stocking rate could skew the sex ratio of wild BKT populations in a reasonable amount of time (Schill et al. 2017). This adjustment resulted in a 46% reduction in East Threemile Creek and 27% reduction in Pikes Fork Creek from the original catchable stocking rate. The fingerling stocking rate in streams was set at four times the catchable stocking rate (500 fingerlings/km) based on the ratio of juvenile to adult fish presented by McFadden (1961) for a stream in Wisconsin, but at narrow study streams (East Fork Clear and Tripod Creeks; Table 1), we reduced stocking densities to 250 fingerlings/km. Stocking rates in fingerling streams were also adjusted once abundance estimates were available which resulted in an increase of 34% in Dry Creek and 116% in Tripod Creek while East Fork Clear Creek was reduced by 92%.

In alpine lakes, stocking rate was set at 175 fingerlings/ha because that is a typical stocking rate for such waters in Idaho. Because the weight of catchables is five times heavier than fingerlings, we stocked 1/5 as many catchables (35/ha) in alpine lakes receiving catchable fish. Since abundance estimates were not available for most alpine lakes, no adjustment to the original stocking rate was made for any lakes.

Abundance Estimates

Mark-recapture abundance estimates of wild and M_{YY} BKT ≥ 100 mm TL have been conducted annually at each suppression stream and alpine lake once incorporated into the study. All data are pooled over the entire study reach by year and total BKT abundance is estimated using the modified Peterson estimator from the FSA package in statistical package R (R Core Team 2022). At non-suppression streams, we complete multiple-pass depletion abundance surveys every 3 years and estimate abundance with the maximum-likelihood model in the MicroFish software package (Van Deventer and Platts 1989). No such estimates are possible at non-suppression alpine lakes.

Sex Ratio Monitoring and Genetic Assignment

Prior to the first stocking event, sex ratios were obtained for the wild BKT population at each study water to obtain baseline sex ratios. In subsequent years, genetic samples have been obtained annually from all suppression waters and tri-annually from non-suppression and control waters. Tissue samples were collected from approximately 100 BKT fry (≤ 100 mm) and 100 BKT adults (≥ 100 mm) from each waterbody during July–September to estimate sex ratios and reproductive success. Tissue samples are caudal fin clips preserved on Whatman™ 3MM chromatography paper (Thermo Fisher Scientific, Inc., Pittsburgh, Pennsylvania). Samples are screened by the IDFG Eagle Genetics Lab using two genetic markers that differentiate sex in BKT: SexY_Brook1 (Schill et al. 2016) and the master sex-determining gene sdY (Yano et al. 2013). For detailed information on primer sequencing, amplification, and sex markers, see Roth et al. (2021). We calculate 95% confidence intervals (CIs) around the estimated male proportions, following Fleiss (1981).

Growth and Body Condition

To assess whether growth and body condition was comparable between hatchery M_{YY} and wild BKT, some fish were collected from two study streams (Dry and Tripod Creeks; Table 1) using backpack electrofishing and from two lakes (Seafoam Lake #4 and Lloyds Lake; Table 2) using either raft electrofishing or gillnetting. A minimum of two hatchery M_{YY} BKT and two wild BKT were selected from every 10 mm length-bin, when present. Each selected fish was euthanized, measured for length and weight, and had the sagittal otoliths removed. One otolith from each fish was embedded in epoxy and, using a low-speed saw, a 0.55-mm section of each otolith was cut through the transverse plane of the otolith to expose a cross-section of the nucleus. Sectioned otoliths were polished and then photographed in immersion oil using reflected light at 40x magnification with a Leica (model DFC450 C) digital camera and a Leica (model DM 4000 B) compound light microscope. Photographs were reviewed by two independent readers who were unaware of fish length, and age was estimated by enumerating presumptive annuli. In cases where the readers did not agree on the age of the fish, fish length was considered to determine a consensus age.

Comparisons of growth rate and body condition between hatchery M_{YY} and wild fish were conducted using linear regression and von Bertalanffy growth models (von Bertalanffy 1938) in statistical software R (R Core Team 2021) because preliminary analysis indicated that growth was asymptotic in one water (i.e., Dry Creek) but linear in the remaining three waters. Within the asymptotic growth model, the effect of hatchery M_{YY} and wild BKT strain on growth was evaluated by estimating the theoretical maximum average length fish in the population could achieve (L_∞), the Brody growth coefficient (K), and the theoretical age when length equals zero (t_0) for each strain. We estimated 95% CIs for all parameters, and estimates were considered statistically different between hatchery M_{YY} and wild BKT strains if the CIs did not overlap (Ogle et al., 2017).

Linear growth models were developed with length at capture as the response variable; predictor variables included the estimated age of the fish at capture (age), a categorical variable that designated the fish as either hatchery M_{YY} or wild (strain), and an age × strain interaction term. By constructing the models in this manner, the slope of the line was the estimated growth rate for wild fish (which were the reference strain in the model), and the interaction term was the estimated difference in growth rate between hatchery M_{YY} fish and wild fish. Ninety-five percent CIs were constructed for each parameter estimate, and growth was considered significantly different between hatchery M_{YY} and wild BKT if the interaction term in the model produced 95% CIs that did not overlap zero (Johnson, 1999).

Body condition models were linearized with $\log_e$ transformed weight as the response variable, $\log_e$ transformed length as the predictor variable, and a length × strain interaction term (Quinn and Deriso, 1999). As with linear growth models, the interaction term was the estimated difference in condition for hatchery M_{YY} fish compared to wild

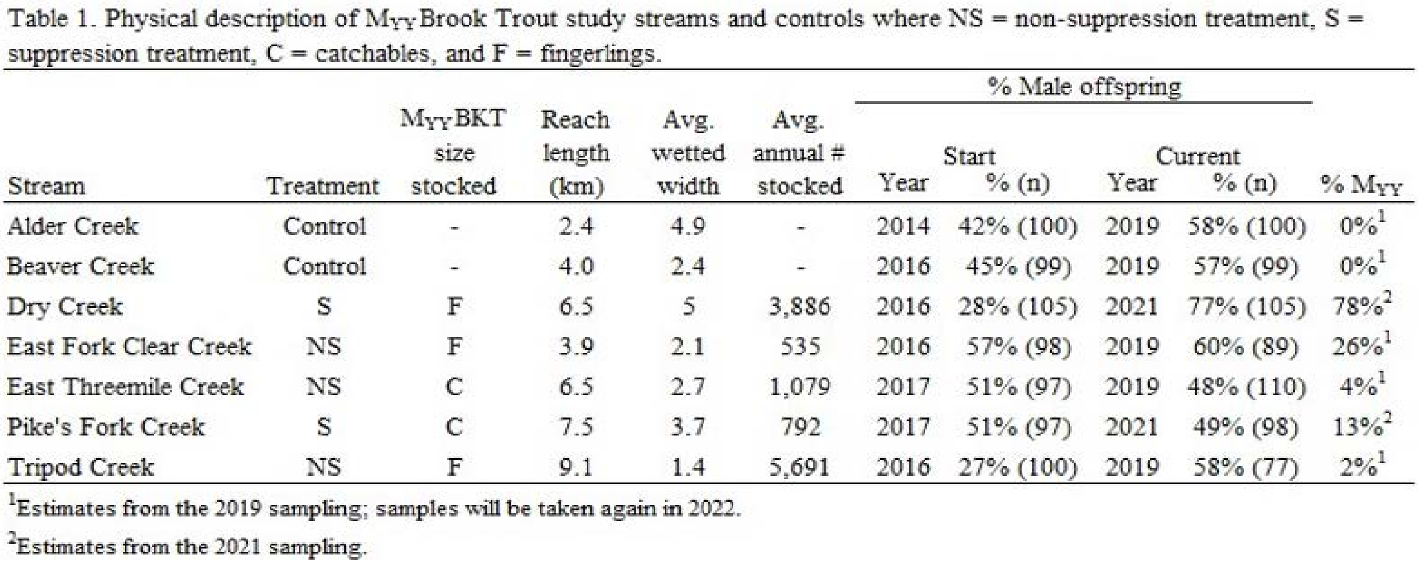

Table 1. Physical description of M_{YY} Brook Trout study streams and controls where NS = non-suppression treatment, S = suppression treatment, C = catchables, and F = fingerlings.

						% Male offspring				
						Start		Current		
Stream	Treatment	M_{YY} BKT size stocked	Reach length (km)	Avg. wetted width	Avg. annual # stocked	Year	% (n)	Year	% (n)	% M_{YY}
Alder Creek	Control	-	2.4	4.9	-	2014	42% (100)	2019	58% (100)	0%[1]
Beaver Creek	Control	-	4.0	2.4	-	2016	45% (99)	2019	57% (99)	0%[1]
Dry Creek	S	F	6.5	5	3,886	2016	28% (105)	2021	77% (105)	78%[2]
East Fork Clear Creek	NS	F	3.9	2.1	535	2016	57% (98)	2019	60% (89)	26%[1]
East Threemile Creek	NS	C	6.5	2.7	1,079	2017	51% (97)	2019	48% (110)	4%[1]
Pike's Fork Creek	S	C	7.5	3.7	792	2017	51% (97)	2021	49% (98)	13%[2]
Tripod Creek	NS	F	9.1	1.4	5,691	2016	27% (100)	2019	58% (77)	2%[1]

[1] Estimates from the 2019 sampling; samples will be taken again in 2022.

[2] Estimates from the 2021 sampling.

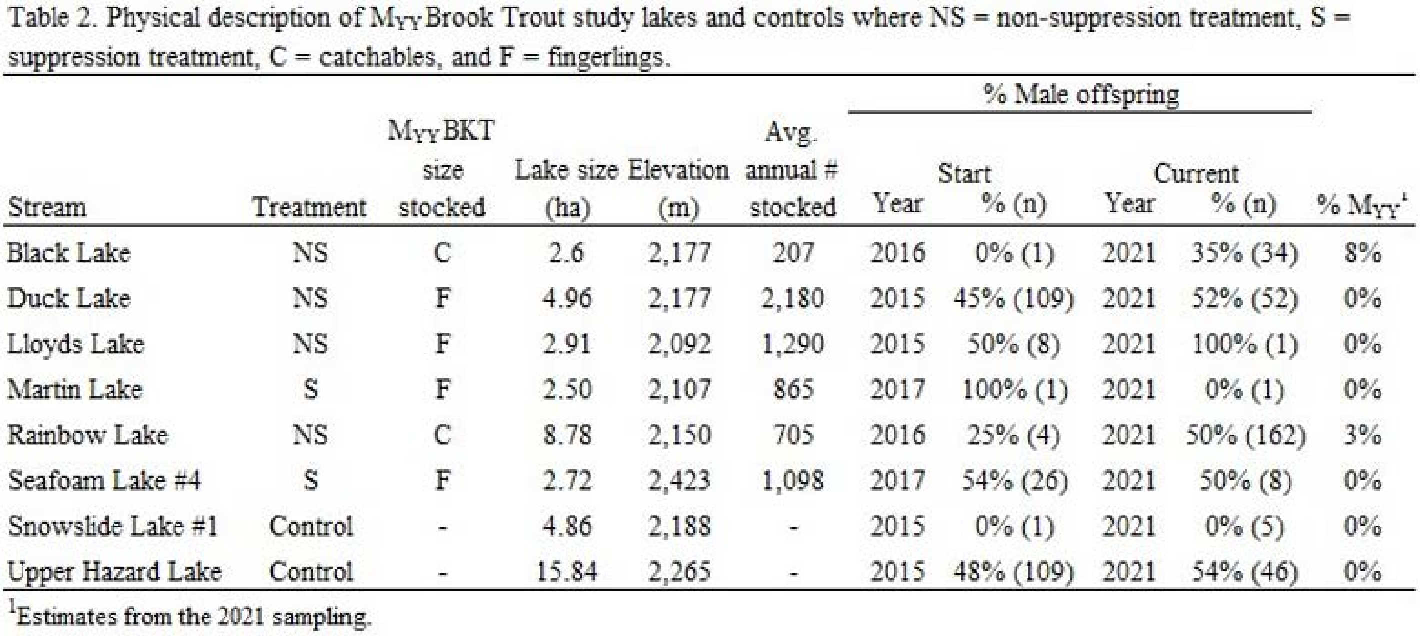

Table 2. Physical description of M_{YY} Brook Trout study lakes and controls where NS = non-suppression treatment, S = suppression treatment, C = catchables, and F = fingerlings.

						% Male offspring				
						Start		Current		
Stream	Treatment	M_{YY} BKT size stocked	Lake size (ha)	Elevation (m)	Avg. annual # stocked	Year	% (n)	Year	% (n)	% M_{YY}[1]
Black Lake	NS	C	2.6	2,177	207	2016	0% (1)	2021	35% (34)	8%
Duck Lake	NS	F	4.96	2,177	2,180	2015	45% (109)	2021	52% (52)	0%
Lloyds Lake	NS	F	2.91	2,092	1,290	2015	50% (8)	2021	100% (1)	0%
Martin Lake	S	F	2.50	2,107	865	2017	100% (1)	2021	0% (1)	0%
Rainbow Lake	NS	C	8.78	2,150	705	2016	25% (4)	2021	50% (162)	3%
Seafoam Lake #4	S	F	2.72	2,423	1,098	2017	54% (26)	2021	50% (8)	0%
Snowslide Lake #1	Control	-	4.86	2,188	-	2015	0% (1)	2021	0% (5)	0%
Upper Hazard Lake	Control	-	15.84	2,265	-	2015	48% (109)	2021	54% (46)	0%

[1] Estimates from the 2021 sampling.

fish, and condition was considered significantly different if the interaction term in the model produced 95% CIs that did not overlap zero (Johnson, 1999).

Results

Annual stocking has occurred at all waterbodies from inception of the study (2015–2017; Table 1 and 2) and is scheduled to continue until 10 years of stocking M_{YY} BKT has been completed at each waterbody. On average we annually stock between 535 and 5,691 fingerling M_{YY} and 792 to 1,079 catchable M_{YY} BKT into streams and 865 to 2,180 fingerling and 207 to 705 catchable M_{YY} BKT into lakes.

The proportion of adult (≥ 100 mm) BKT ranged from 0 to 67% M_{YY} BKT across all streams and suppression lakes following M_{YY} stocking. We were unable to estimate abundance in non-suppression lakes due to limitations of lethal sampling methods for alpine lakes (i.e. gillnets). The proportion of M_{YY} BKT was highest in streams stocked with fingerlings (19% to 67%) compared to streams stocked with catchables (0% – 10%) and suppression lakes (3% – 39%).

On average there has been a 16% increase in male sex ratio across all study streams with the highest increase at Dry Creek (49%) and lowest at East Threemile Creek (-3%, Table 1). Genetic assignment analyses indicate the proportion of offspring produced by M_{YY} BKT stocked into study streams has varied from 0% to 78% (Table 1). Although sample sizes have been low for some lakes, results show that sex ratios have changed very little (Table 2), and M_{YY} BKT offspring have only been detected at two of the six study lakes in 2021.

Growth and Body Condition

For the 381 BKT sampled across four waters, maximum age was age 6 at Dry Creek and age 4 or 5 at other waters for wild BKT, and age 5 at Dry Creek and age 4 at other waters for hatchery M_{YY} BKT. Total length ranged from 103 – 359 mm for wild BKT and 115 – 353 mm for hatchery M_{YY} BKT.

Growth did not differ between hatchery-reared M_{YY} and wild BKT in any stream or lake we sampled. In Dry Creek, where growth was asymptotic, K was 0.37/year (95% CI = 0.17 – 0.59/year) and L∞ was 357 mm (311 – 500 mm) for hatchery M_{YY} BKT, while K was 0.51/year (0.28 – 0.81/year) and L∞ was 306 mm (273 – 378 mm) for wild BKT. In other waters, where growth was linear, hatchery M_{YY} BKT grew an estimated 24 – 43 mm per year, whereas wild BKT grew an estimated 36 – 42 mm per year, although differences in growth rate were not significant (Figure 2). In two waters where growth was linear (i.e., Seafoam Lake #4 and Tripod Creek), age 0 M_{YY} fish were significantly larger than their wild counterparts, but this did not translate into different growth rates. Body condition also did not differ significantly between wild and hatchery M_{YY} BKT (Figure 3).

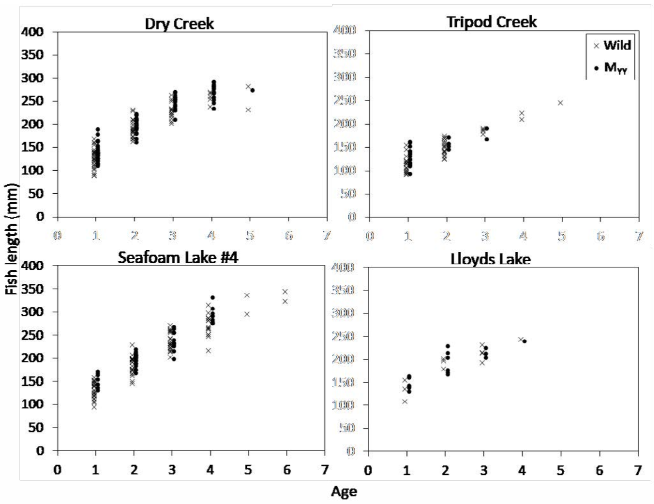

Figure 2. Back-calculated length-at-age for hatchery M_{YY} and wild brook trout sampled in four Idaho waters. Each data point represents an individual fish at its age when captured.

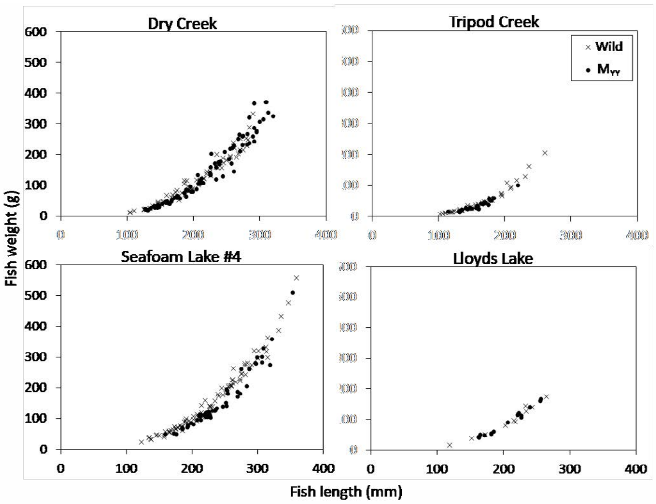

Figure 3. Length-weight relationships for hatchery M_{YY} and wild brook trout sampled in four Idaho waters. Each data point represents an individual fish.

Discussion

This study is now in its 7th survey year for some waters, and clearly there are greater shifts in sex ratio and M_{YY} BKT offspring (fry production) in streams compared to lakes, and in streams stocked with fingerlings compared to streams stocked with catchables. It is not surprising to see streams exhibiting faster and more promising results over lakes as prior simulations suggested the need for much longer time frames to reach eradication in lakes (Schill et al. 2017). This is likely due in part to later maturity and longer life spans in lakes compared to streams. Moreover, the use of lethal gillnet sampling methods in lakes removes both M_{YY} and wild BKT from the system, whereas stream electrofishing allows the release of M_{YY} fish (Schill et al. 2017). The superior performance of M_{YY} fingerlings compared to catchables is likely due to greater longevity of fingerlings. Indeed, catchable trout rarely survive more than a year after being released in Idaho waters (High and Meyer 2009; Cassinelli and Meyer 2018), and while fingerlings are also known to generally have poor survival, our results have documented M_{YY} fingerlings surviving for many years (Figure 2), providing numerous opportunities to spawn. Stocking rates are also inherently much higher for fingerlings than catchables, so even if survival of fingerlings is lower than survival for catchables, the total number of spawning M_{YY} fish could be higher for fingerlings.

Dry Creek currently exhibits the most promising results, with the highest increase in sex ratio towards males and highest proportion of M_{YY} offspring. Tripod Creek also exhibited an increase in male sex ratio by 31%, but unlike Dry Creek, only a small proportion of the sampled males were M_{YY} offspring. The main treatment difference between these two streams is the use of manual suppression of the wild BKT population at Dry Creek and no suppression in Tripod Creek. Manual suppression through electrofishing has been used for decades to reduce the density of non-native trout and lead to an increase in native salmonids (Moore et al. 1983) and more specifically to reduce non-native BKT populations (Shepard et al. 2014). Suppression of wild trout populations has contributed to increased survival of both stocked Rainbow trout *Oncorhynchus mykiss* (Horner 1987) and fingerling Cutthroat Trout *Oncorhynchus clarkii* (Miller 1955). As such, manual suppression has likely increased survival of our stocked M_{YY} BKT in this study.

Results of this study indicate that hatchery M_{YY} BKT stocked into mountain streams and alpine lakes as age-0 fingerlings grew at a similar rate and maintained a similar body condition as wild BKT. Our growth results are contrary to much of the existing literature demonstrating poorer performance for hatchery salmonids relative to their wild counterparts (reviewed in Araki et al. 2008). For example, hatchery salmonids generally demonstrate poorer survival (Miller 1954; Jonsson et al. 2003), slower growth (Finstad and Heggberget 1993; Bohlin et al. 2002), and reduced reproductive fitness (reviewed in Christie et al., 2014) compared to wild salmonids in the same environments. Kennedy et al. (2018) reported slightly reduced reproductive fitness for hatchery M_{YY} BKT relative to wild conspecifics in several mountain streams, though their study was conducted on catchable-sized fish (as compared to fingerlings in the present study), and they did not compare growth or condition between M_{YY} and wild fish. Taken together, the results of Kennedy et al. (2018) and the present study suggest that hatchery M_{YY} fish stocked in lentic and lotic waters may survive and grow similarly to wild fish, but once they reach maturity, they may have comparatively lower reproductive fitness. However, since these are the first studies ever to evaluate M_{YY} vertebrates liberated into the wild, more research is clearly needed on all aspects of their post-release performance.

There was no evidence that growth or condition differed in suppression and non-suppression waters for either wild or hatchery fish. The lack of a suppression effect on fish growth and condition in our study may be related to the well documented ability of BKT to undergo compensatory responses to population changes (McFadden 1961; Meyer et al. 2006). Additionally, the wild components of the BKT populations were composed of both male and female individuals, whereas the hatchery M_{YY} components of the populations were inherently composed of only males. In wild BKT populations, male BKT often grow faster than females (McFadden 1961), so had we assessed fish sex, we could have compared the growth of hatchery males to wild males. However, male BKT do not always grow faster than females (Curry et al. 2003), and even when they do, the growth difference between sexes for BKT is usually only a few millimeters at each age, so we consider this limitation minor.

Our results clearly indicate that hatchery M_{YY} fingerling BKT can survive for several years, grow at an equivalent rate, maintain an equivalent body condition relative to wild BKT in both alpine lakes and mountain streams, and can successfully reproduce with wild BKT. In contrast, survival and successful reproduction by catchable M_{YY} BKT appears to be diminished, and thus they are failing to shift the sex ratio of wild BKT toward 100% male. Whether the use of M_{YY} BKT stocking can be used to successfully eradicate any wild BKT populations remains to be seen, but promising results are apparent when fingerling M_{YY} BKT are stocked into streams that receive annual suppression.

Acknowledgements

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Brook Trout Seasonal Movement, Northeast Wisconsin

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Abstract - Salmonid movement behaviors have broad ecological and evolutionary impacts that affect individual fitness, metapopulation dynamics, distribution, abundance, and gene flow. Effective management also requires data on population size, survival and size structure, all of which are linked to movement. For Brook Trout *Salvelinus fontinalis*, their movement patterns are frequently shaped by temperature regimes and riverine connectivity. Changing temperatures and widespread connectivity issues, coupled with the push for more passage improvements, habitat projects and the rise in popularity of beaver *Castor canadensis* as restoration collaborators, have made describing regional movement patterns the first crucial step for supporting management of inland trout fisheries in Wisconsin. Given this context, we ask: (1) What is the timing and extent of Brook Trout seasonal movement in northeastern Wisconsin? And (2) How might observed movement patterns direct future decisions about stream restoration and management in this region? We use tag-recapture methods to detect seasonal movement. We observed most (77.3%) individuals making season movements less than 1 km, while a smaller portion of the tagged population (22.7%) moved recorded distances of up to 12.1 km. Our results align with instream Brook Trout movement trends described in other regions, where both site fidelity and long-distance movements are observed, often coinciding with changes in environmental conditions and spawning season.

Introduction

Freshwater scientists have invested decades of research into better understanding movement behaviors of instream salmonids (Gowan et al. 1994; Burrell et al. 2000; Curry et al. 2002; Peterson et al. 2005; Petty et al. 2012; Kanno et al. 2021). For salmonids, movement patterns have broad ecological and evolutionary impacts, affecting individual fitness (White and Wagner 2020), metapopulation dynamics (e.g., population size, population survival) (Rieman and Dunham 2000), distribution and abundance (Kanno et al. 2021), adaptation and speciation (Timm et al. 2016) and the success of management practices (Pine et al. 2003; Ousterhout and Semlitsch 2013). Seasonal movement patterns are particularly important in regions with harsh winter environments where ice conditions may limit access to necessary habitats (Chisholm et al. 1987; Cunjak 1996; Lindstom and Hubert 2004). Like other regions, coldwater streams in Wisconsin are facing unprecedented changes in hydrology and increasing water temperatures (WICCI 2011), which may lead to future seasonal declines in available trout habitat (Mitro et al. 2019). Changes in hydrology and temperature coupled with concerns about riverine connectivity have made the seasonal movement behaviors of coldwater obligate species, like the native Brook Trout *Salvelinus fontinalis*, a high priority (WNDR 2019). Adding a layer of complexity to the intersecting forces of hydrology, habitat, and movement dynamics of Brook Trout is the increased interest in co-management of beaver *Castor canadensis* and salmonid populations in the United States (Johnson-Bice et al. 2018; Charnley et al. 2020; Renik et al. 2020), and specifically in Wisconsin (WDNR 2015; WDNR 2019). Beavers are popularly known as 'ecosystem engineers' known for shaping riverine ecosystems (Collen and Gibson 2000; Curran et al. 2014; Johnson-Bice et al. 2018), all of which may influence Brook Trout populations and their movements in northeastern Wisconsin (McRae and Edwards 1994; Avery 2002).

During previous fieldwork for a trout age and growth study in Wisconsin, the Department of Natural Resources (WDNR) trout research crew described capturing few trout in the early spring versus during the later summer months in the northeastern region. The field crew found that trout tagged one summer were rarely recaptured the following year but were rather replaced by untagged individuals. These field observations suggested that trout in northeastern Wisconsin might seasonally move and randomly redistribute during the late-spring and early summer months. Beyond the field observations that spurred this research, northeastern Wisconsin is considered a hub for historic beaver-trout research with active efforts to maintain free-flowing conditions (e.g., beaver removal) on coldwater trout streams. Extensive beaver and dam removal has been a common practice in this region of Wisconsin since the 1930s (Johnson-Bice et al. 2018) and is based partly on the findings of historic research on the impacts of beavers and dams on trout streams in the region (WDNR 2015). Beavers are considered a challenge to trout management throughout the state, partly due to the relatively low gradient common across most Wisconsin streams, especially in northern regions where beavers are considered an acute concern for trout streams (WNDR 2015). As such, this study is nested within the larger WDNR led statewide effort to reassess beaver, trout and habitat interactions and is discussed by Dr. Matthew Mitro in a separate conference proceeding piece (Mitro 2022).

Given the importance of stream connectivity, the critical need to characterize movement behaviors in the context of stream management practices and the increasing emphasis on ecosystem rather than single-species management, we asked: (1) what is the timing and extent of Brook Trout seasonal movement in northeastern Wisconsin? And (2) how might observed movement patterns influence decisions about stream restoration and beaver management? We used single-pass electrofishing to capture-mark-recapture individuals and implanted passive integrated transponder (PIT) tags to monitor seasonal movement in our study system, Upper Middle Inlet (UMI), in northeastern Wisconsin. We installed two stationary PIT arrays to continuously monitor movement and record the timing and duration of seasonal movement. With these data, we describe movement behaviors of Brook Trout on a subwatershed-scale that may be disrupted by changes in beaver management and the current role that connectivity plays in northeastern Wisconsin.

Methods

Study Area

Our study system is part of the Northeast Sands Ecological Landscape, which encompasses 987,176 acres of land in northeastern Wisconsin and is characterized by extensive forest cover, conifer swamps and pine barrens (WDNR 2020). Upper Middle Inlet is a 23.1 km long coldwater stream that drains 17,539 acres at the subwatershed level (HUC-12) into the Middle Inlet-Lake Noquebay Watershed (HUC-10) (97,280 acres) in Marinette County, Wisconsin (WDNR 2010) (Figure 1). Land cover in the watershed is predominantly forested (46%) and wetlands (29%), with some agricultural use (14%), established grasslands (10%) and minimal urban or suburban development (5%) (WDNR 2010). Upper Middle Inlet is classified as a Class I trout stream in the headwaters (WDNR: *high quality trout waters; sufficient natural reproduction to sustain wild trout populations at or near capacity; often small contain small or slow-growing trout*) and a Class II trout stream downstream near the confluence with Middle Inlet (WNDR: *some natural reproduction but not enough to utilize habitat and food; stocking required to maintain a desirable fishery; good survival and carryover of adult trout*) (WDNR 2002).

During summer baseflow conditions, we collected habitat data at five study sites following the *Guidelines for Evaluating Fish Habitat in Wisconsin Streams* (WDNR 2002). Upper Middle Inlet is a sand dominated system, with more silt and detritus in the headwater area (W-A) and the downstream sites (Creek Road, Nejedlo Road downstream crossing) than in the middle reaches. The middle reaches are slightly higher gradient, characterized by coarse gravels, boulders and rubble-cobble, interspersed with sand and minimal detritus and silt (Figure 4). Fish cover includes woody habitat, overhanging vegetation, undercut banks, exposed root systems and small areas of submerged macrophytes. The middle reaches of UMI are distinct with a wider diversity of habitat types and complexity, including boulders, gravels and cedar swamp conditions with flow-through root systems. Stream width ranged from approximately 3 m in the headwater reaches to 7 m in the downstream reaches, with canopy cover typically between 80 to 100%. The riparian area (20 m out from the bank) is largely intact, with minimal impacts occurring along the roadside at stream crossings. The riparian area of the lower reaches of the system is composed of woodland and shrubs with some associated meadow and wetland habitat. The riparian area of the middle reaches of UMI is predominantly cedar swamp, while the headwaters are woodland interspersed with meadow.

Study Design

Study sites were located at all eight road crossing locations (Figure 1). At each crossing, we captured and tagged Brook Trout from late spring to early winter between August 2019 to December 2021 at monthly intervals, excluding the ice

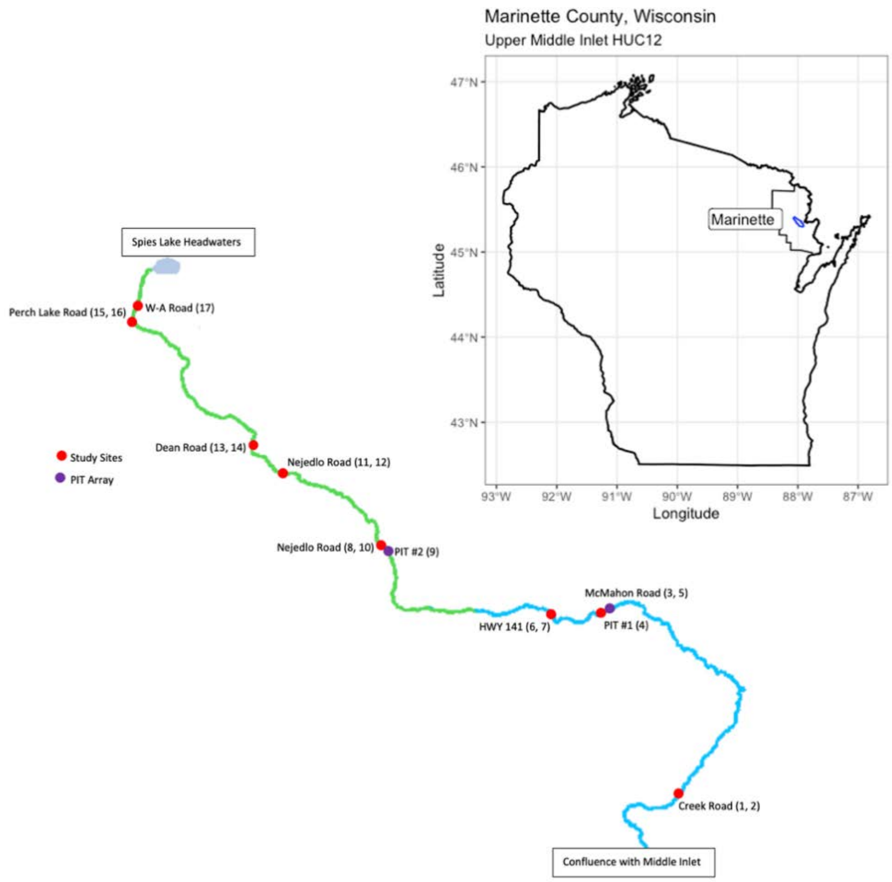

Figure 1. Simple map of Wisconsin with Marinette County outlined in the northeast of the state, and the UMI subwatershed highlighted in blue. To the left of Wisconsin, is the Upper Middle Inland flowline with survey sites denoted by red dots and the PIT arrays with purple dots. The green flowline segment is classified as a Class I trout stream and the blue segment is classified as Class II.

over period. We used a combination of active (single-pass electrofishing) and passive (stationary PIT arrays) sampling techniques to capture or detect seasonal movement data. For individual fish, we collected total length (mm), weight (grams), gill lice data (i.e., presence of infection, number of lice, location on the body) and recorded sex during spawning season. We implanted individuals >100 mm with Oregon RFID (12.0 mm x 2.12 mm, 0.01 g) half-duplex PIT tags. When feasible, we extended the standard survey sites to recapture tagged individuals that had moved beyond the site boundaries.

We installed two Oregon RFID PIT arrays (Figure 2, Figure 3) to continuously monitor bidirectional movement of tagged trout. These arrays operated from June through December of 2020, were reinstalled in March 2021, and were maintained over the winter months into 2022. The antenna detection distance ranged from 40 to 66 cm, per antenna. We used a combination of 12-volt deep-cycle marine batteries and 130-watt solar panels (without solar controllers) to power the arrays. The battery-solar panel combination effectively powered our systems for up to 21 days in the summer before requiring a battery exchange. One array was installed on public land (downstream PIT array), while the other was installed on private land (upstream PIT array).

Figure 2. Downstream PIT array at McMahon Rd crossing, downstream of the crossing. The multi-reader, auto-tuners and deep-cycle marine batteries are located within the black locking box.

Figure 3. Upstream PIT array at Lower Nejedlo Rd crossing, installed downstream of crossing. Image of the Nejedlo PIT monitoring system from the right bank (top) and the left bank (bottom).

Analyses

Data analyses were completed in RStudio version 1.4.1717 (Rstudio Team 2021), using the PITR (v1.2.0, Harding et al. 2018) and riverDist (v0.15.4, Tyers 2021) packages and visualized using the ggplot2 package. We plotted length-frequency histograms and catch-per-unit-effort, standardized to 100 m, across sites and sample occasions to gain a better understanding of the movement trends we observed with the mark-recapture data. Length histograms provide basic insight into population size structure, which reflects key dynamics such as growth, trends in year-classes and recruitment, and potential movement (Ogle 2018). We analyzed the passively collected PIT array dataset and the electrofishing-based mark-recapture data separately, then combined these data to describe the full extent of movement patterns observed through the duration of the study.

Results

Over the study period (August 2019 – December 2021) we recorded data from 5,895 captured individuals across 15 discrete sampling events occurring at monthly intervals. We tagged 1,491 individuals >100 mm with 12 mm PIT tags. From the total 1,491 tagged individuals and 2,348 recapture observations, 1,343 individuals were Brook Trout (90%) accounting for 2,078 recapture observations. The 2,078 total recapture observations include recapture by electrofishing (1760 recapture observations) and passive detection through the PIT array systems (318 unique detections). Only Brook Trout data were used for these analyses. Of the total tagged Brook Trout, we identified 182 males, 168 females and were unable to determine the sex of 1,081 individuals. Total lengths (mm) of tagged individuals ranged from 100 mm to 405 mm, with 67% of the sample population between 125 mm and 220 mm (1,000 individuals).

We recorded 70% (1,462 observations) of recaptures between the months of August and November, which aligns with our fieldwork effort that began in August 2019 and the increase in observed movement during spawning season. The total number of observations per individual ranged from 0 to 30, with 27.6% of individuals (371) recaptured between one to four occasions (excluding the initial capture). Of those fish captured on more than one occasion, 222 (16.5%) were recaptured once, 102 (7.5%) were recaptured twice, and 65 (5%) were recaptured three times throughout the study. The proportion of non-recaptured individuals, including both electrofishing and PIT array detections, was 71% (945 individuals).

PIT Array Detections

We detected 156 unique tags moving through our PIT array systems. Detections per individual ranged from 1 detection on one antenna at one array location, to 28,106 detections across all antennas on both arrays. We split up- and downstream detections by year and season intervals (i.e., *Spring*: March, April, May; *Summer*: June, July, August; *Fall*: September, October, November; *Winter*: December, January, February) and found most bidirectional detections occurred during the fall (Figure 5), with no downstream movement detected in winter 2021.

Combined PIT Array and Recapture Data

We combined PIT detections and recapture data to gather a better understanding of seasonal movement behaviors

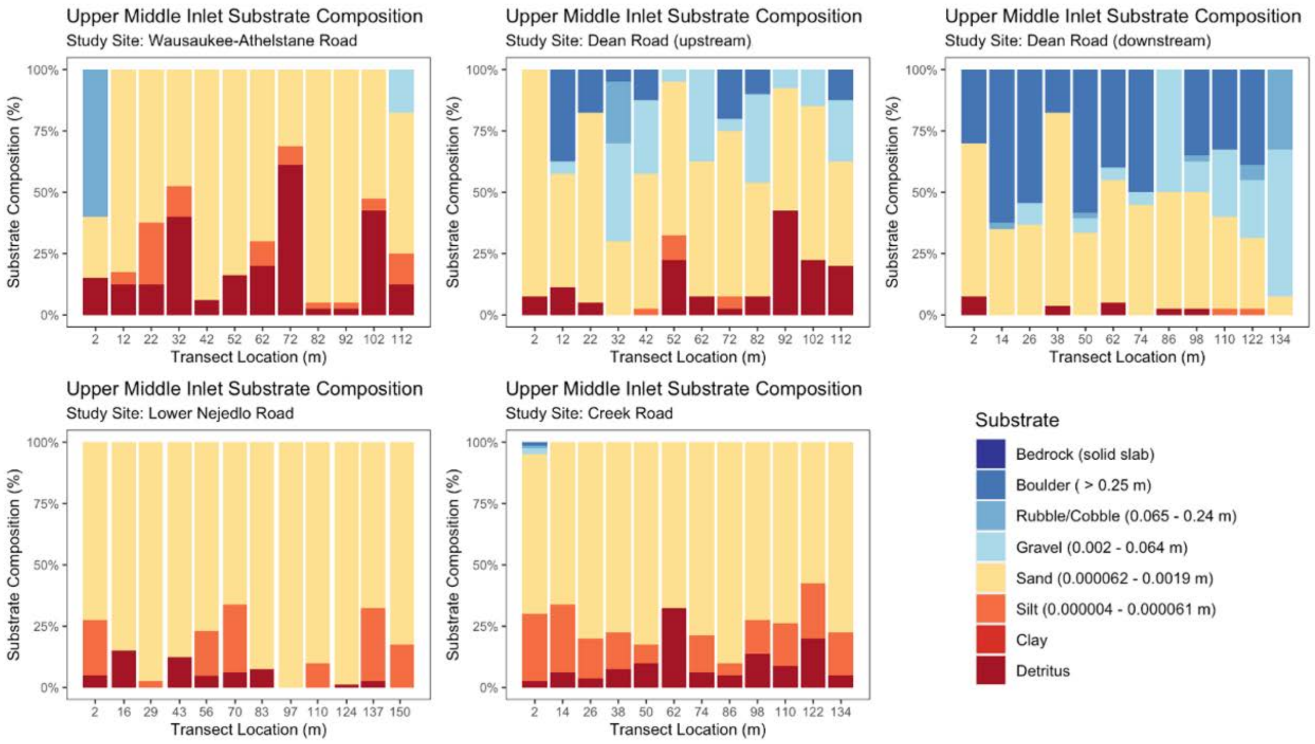

Figure 5. The UMI substrate plots showing the range of available substrate throughout the study system. The uppermost site is shown in the top left and the bottommost site on the bottom right.

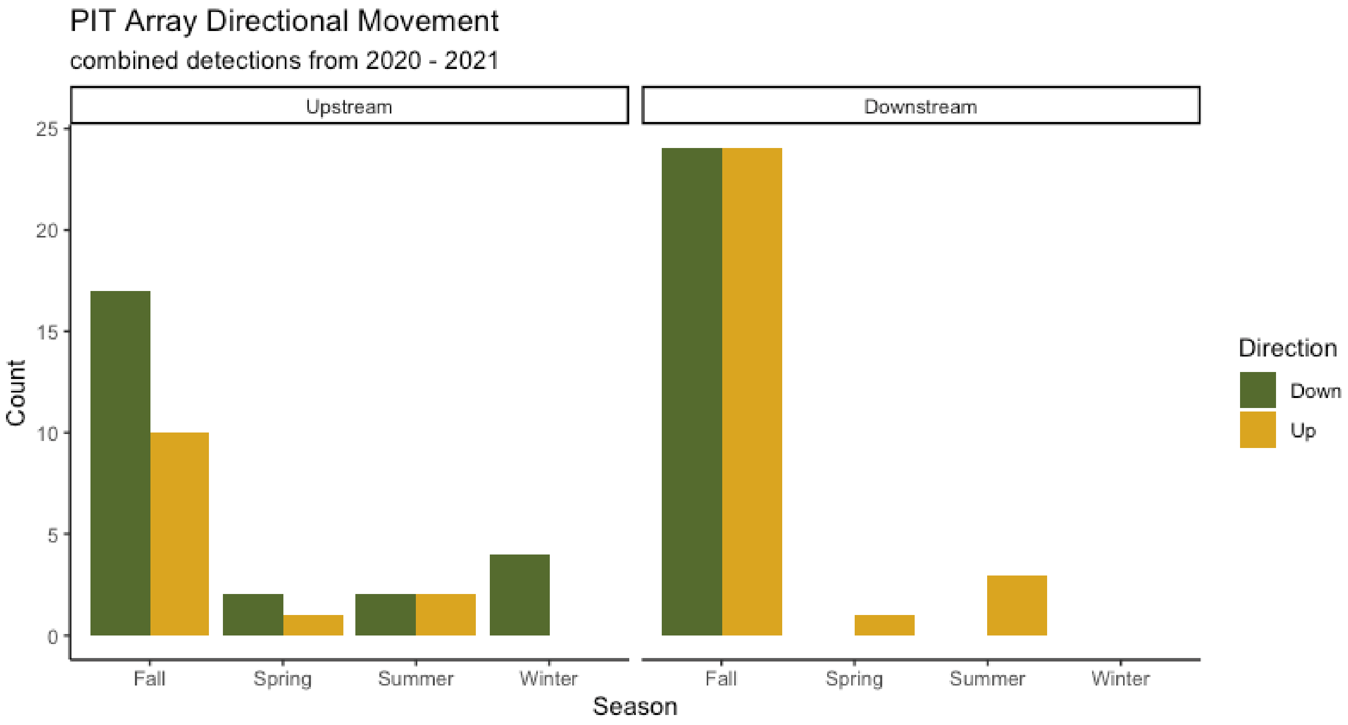

Figure 6. PIT array directional movement, showing bidirectional detections across seasons in 2020 and 2021. Most bidirectional movements were detected during the fall season, corresponding with spawning season. Few Individuals were detected in spring, summer and winter; however, equipment failures likely limited continuous coverage resulting in missed detections.

across the UMI system. We plotted these data with each tag location color-coded and fall spawning season highlighted in burnt orange (Figure 6). We observed an increase in upstream movement occurring in the fall and downstream movement detected in the winter months following the spawning season. We detected two distinct movement patterns: (1) a larger resident population with minimal movement detected and (2) a smaller portion of the population seasonally making longer-distance movements. Recapture sampling did not occur during the ice-over period and the PIT arrays were not maintained during winter 2020, leaving a temporal gap in the movement data we were able to collect.

We calculated the total recorded distances per individual fish (Figure 7). Most individuals (77.3%) with consecutive detections were recorded moving distances < 1,000 m, while 22.7% of tagged fish were recorded moving distances >1,000 m over the duration of the study.

Catch-per-unit-effort and Length-Frequency Histograms

In an effort to look more broadly at movement patterns across the adult UMI Brook Trout population, we calculated the catch per unit effort (CPUE) per sampling event and plotted per site (Figure 8). To calculate CPUE, we used total number of adult Brook Trout (tagged and untagged) per sampling distance, standardized by 100 m. The CPUE data show noticeable peaks in the middle watershed during the early fall season, while the lower watershed shows an increase in CPUE during the early winter months, suggesting Brook Trout movement upstream to the mid-watershed reaches during the fall spawning season, followed by downstream movement toward the lower watershed at the onset of winter conditions. We coupled CPUE with length-frequency histograms across sample occasions to provide a qualitative assessment of movement behaviors throughout UMI. For brevity, we focused on the CPUE and length-

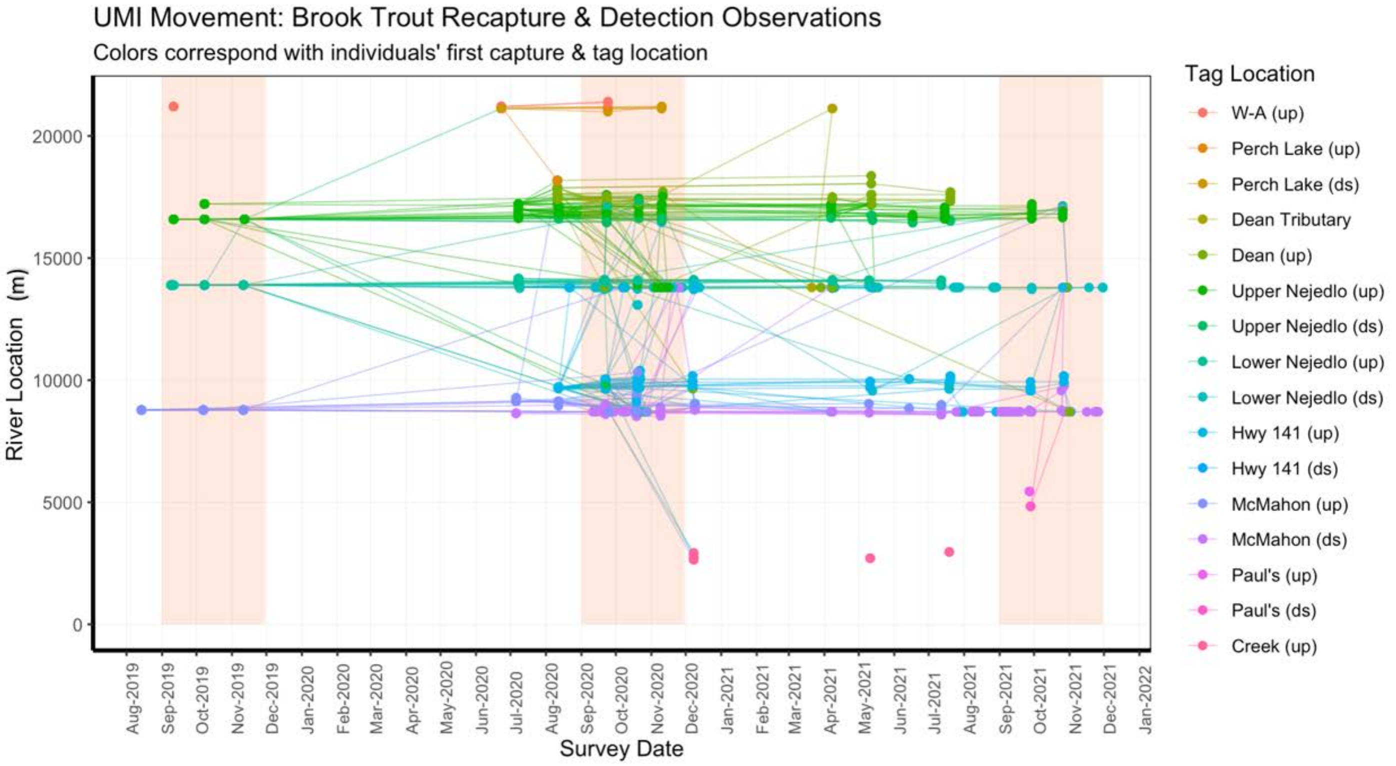

Figure 7. Movement plot showing individual tags (points) connected by lines across the study duration. Each tag with the connecting line is color-coded by the original tag location. The downstream PIT array was located at approximately 9,000 m from the confluence with Middle Inlet and the upstream PIT array was located at approximately 14,000 m from the confluence.

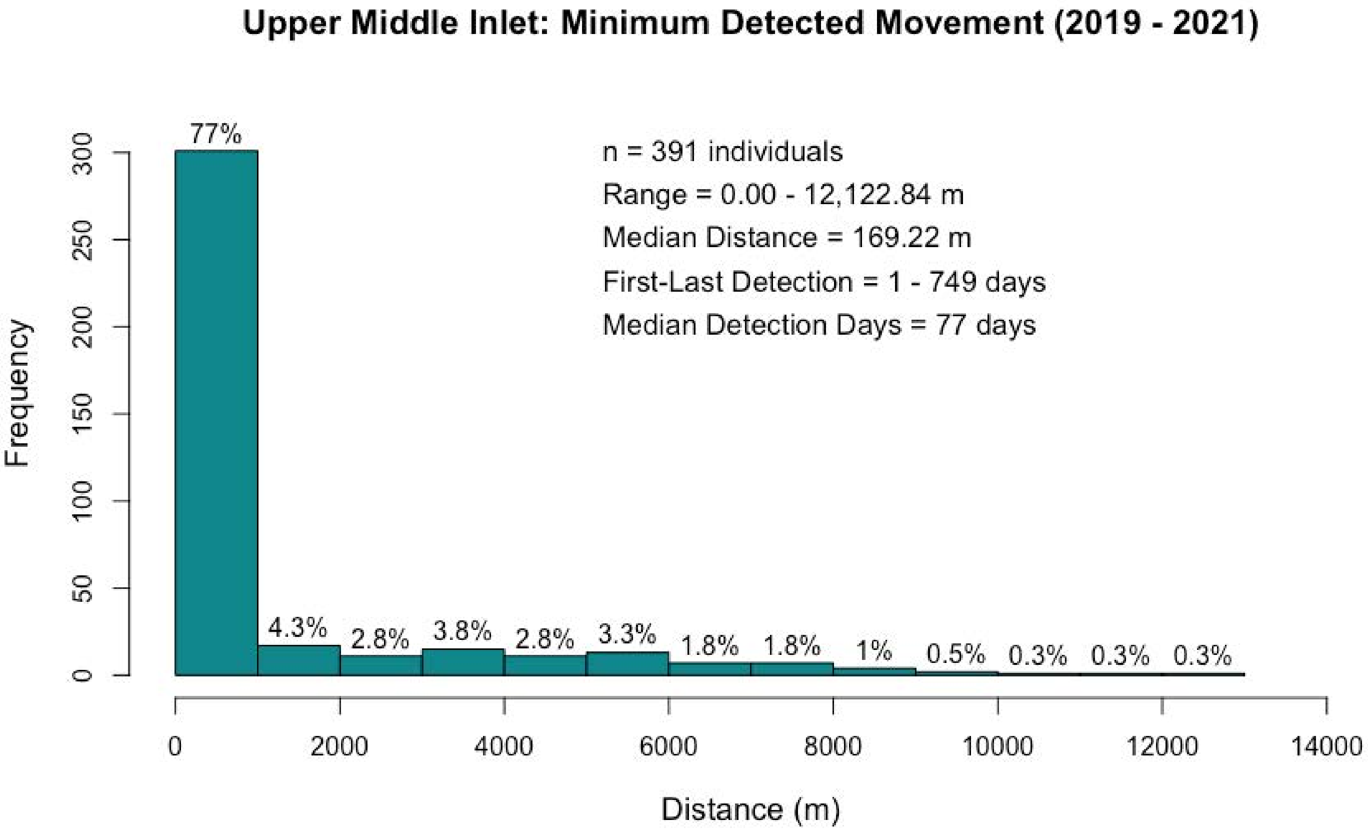

Figure 8. Figure displays the minimum detected movement of tagged fish. Movement ranged from no movement detected to 12.1 km. First-last detections per individual fish ranged from 1 to 749 days, with a median of 77 days between first and last detection.

histogram patterns from one site in the mid-reaches of UMI, Upper Nejedlo, due to the unique available habitat (Figure 4) and interesting seasonal movement patterns detected in this segment of the system.

The Upper Nejedlo site CPUE (Figure 8) and the length histogram data across 2019, 2020 and 2021 (Figure 9) suggests seasonal movement into this river segment, largely concentrated during the fall, suggesting seasonal spawning-related movement. This makes sense, as spawning behavior was observed in the mid-reaches of UMI, specifically within the Upper Nejedlo site reach. This segment of UMI is characterized by unique habitat (e.g., higher gradient, bounders, channel complexity) and available spawning substrate (e.g., coarse gravels) not observed in many other areas sampled in the system. While the CPUE data includes only adult Brook Trout, the length histograms include both young-of-the-year and adults.

While the length-histograms show an increase in number and length of individuals captured in the fall surveys, they also show a decline in numbers following the fall spawning season, suggesting post-spawn downstream movement. Coupled with these data, we observed a group of approximately 30 to 40 adult Brook Trout at the downstream most Creek Road site in 2020, including multiple individuals tagged in upstream reaches earlier that year.

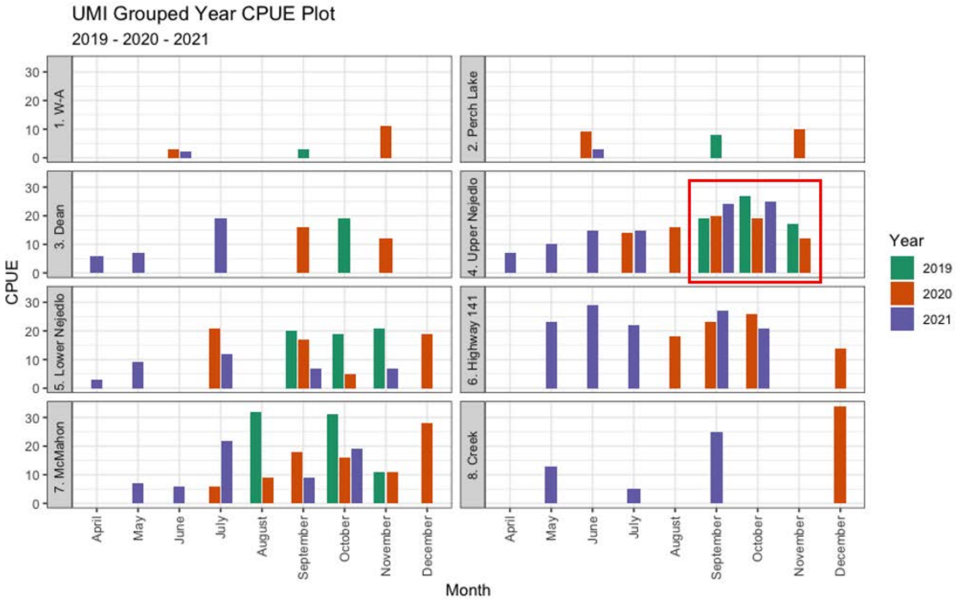

Figure 9. Grouped CPUE grid, showing CPUE for all sample events throughout the duration of the study. Catch-per-unit-effort is plotted by site, grouped by sampling month and color-coded by year. Site 1. W-A is the most upstream site (upper left corner) and Site 8. Creek is the most downstream site (bottom right corner). Site 4. Upper Nejedlo has a red rectangle around the fall season CPUE (September-November) to highlight a key segment of UMI with unique habitat features, slightly higher gradient and an increase in Brook Trout CPUE during the early fall and decrease in late fall post-spawn and at the onset of cooler temperatures.

Figure 10. Length-frequency histograms for Upper Nejedlo across 2019, 2020 and 2021 organized in chronological order of survey events. Fall 2020 and 2021 show higher numbers of individuals relative to the summer and spring surveys.

Discussion

Fisheries researchers have invested decades into understanding movement behaviors of instream salmonids (Kahler and Quinn 1998; Burrell et al. 2000; Curry et al. 2002; Peterson et al. 2005; Petty et al. 2012; Kanno et al. 2021). Seasonal movement is especially important in regions, like northeastern Wisconsin, with harsh winter environments where ice conditions may limit access to necessary habitats (Chisholm et al. 1987; Cunjak 1996; Lindstom and Hubert 2004). Movement patterns have broad ecological and evolutionary impacts, affecting individual fitness (White and Wagner 2020), metapopulation dynamics (e.g., population size, population survival) (Rieman and Dunham 2000), distribution and abundance (Kanno et al. 2021), gene flow (related to adaptation and speciation) (Timm et al. 2016) and the success of management practices (Pine et al. 2003; Ousterhout and Semlitsch 2013). Previous research on instream movement of many trout species across various regions suggests movement patterns are highly variable, with some individuals showing high site fidelity while others demonstrate preference for long-range movements (Gowan and Fausch 1996). In our study system, Upper Middle Inlet, we used mark-recapture techniques between August 2019 and December 2021 to detect the timing and minimum extent of long-distance seasonal Brook Trout movements, which may offer insight into regional movement patterns more broadly.

With the PIT array systems, we were able to detect pass-through movements, where an individual was only detected once moving either up- or downstream, and daily short movements up- and downstream through the antennas on multiple occasions throughout the duration of the study. We detected most tagged Brook Trout (77.3%) within 1 km of their original tag location, while a smaller proportion of the tagged population (22.7%) was detected moving distances greater than 1 km up to 12.1 km. The timing of these movements largely coincided with biologically significant seasonal events, including spawning-related upstream movement (late-summer, early-fall) and downstream post-spawning movements. Due to the nature of field-based work, it is likely we were unable to capture the full extent of long-distance movements. As such, the distances we recorded can be thought of as the minimum recorded distances. Additionally, we were unable to recapture approximately 71% of tagged individuals, leaving questions about where these trout may have moved or redistributed to within the UMI system and beyond.

In an attempt to design a study capable of characterizing long-distance movements as recommended by Kanno et al. (2020) and Gowan et al. (1994), we established as many study sites along UMI as possible, taking labor, time, equipment and land access constraints into consideration. Our study results align with established literature on trout movement, pointing toward both seasonal movement patterns and multiple life histories (e.g., resident, highly-mobile), including recent work from central Wisconsin that describes an increase in upstream movement from late-summer into early-fall, coinciding with the timing of Brook Trout spawning (Schleppenbach et al. 2021). This upstream pattern is followed by downstream movement in the early winter post-spawning season. The downstream movement that occurs post-spawn is likely related to movement toward biologically important overwintering habitat in the early winter, which is an important resource for Brook Trout populations in northern, harsh winter climates (Lindstom and Hubert 2004). Another explanation for this downstream movement could be trout moving back downstream toward the individual's home range following the upstream spawning migration. The largest amount of bidirectional movement we detected was between September to November each year (Figure 5), which makes sense as this is the spawning season.

Though we detected movement patterns in our system, there are limitations worth mentioning. First, our study design likely created an inherent bias due to our sampling and tagging locations established in the study (as described in Gowan et al. 1994), as we had established sites up- and downstream of road crossings due to land access, labor, time and equipment limitations. We are also aware of equipment limitations, including missed detections on our PIT arrays for individuals we know moved through the system which may have been due to dead batteries or reduced read range following high flow events. There were also environmental limitations, including flood conditions that reduced capture efficiency during monthly sampling events. We did not collect flow data for each sampling event, which would have been useful for assessing the relationship between water level and capture efficiencies and flow and movement, as previous research has reported high-flow events contribute to relatively high levels of up- and downstream trout movement (Connelly et al. 2008). Due to interannual variability in flow, it is possible our capture efficiency differed across years. It is likely that we missed opportunities to recapture tagged individuals due to labor, land access, equipment and time constraints.

Future research into Brook Trout movement in northeastern Wisconsin would benefit from an increase in monitoring efforts of the lower reaches of study systems, especially if these systems are connected to lakes and spring ponds that may be utilized for overwintering habitat by Brook Trout. For example, in our UMI study system, we would have benefited from monitoring at the inlet to Lake Noquebay (Figure 1) to assess whether Brook Trout are migrating from and to the lake for spawning and overwintering habitat. This is especially interesting in the context of the large-bodied individuals we captured in the fall of 2020 but did not recaptured thereafter.

Management Implications

Overall, the movement patterns we detected in UMI demonstrate the important role of riverine connectivity for Brook Trout populations in this region in northeastern Wisconsin, under the current environmental and connectivity regimes. In order to conserve and maintain recreationally important Brook Trout populations that are comprised of resident and migratory variants, thinking about the importance of seasonal movement behaviors throughout the riverine system should be taken into management consideration (Meyers et al. 1992). Echoing the concept of system-wide approaches to Brook Trout management described by Meyers et al. (1992), conceptualizing northeastern river systems as a riverscape with diverse movement behaviors connecting populations, as opposed to discrete populations, may offer an important perspective on future management decisions that are working toward balancing the needs of recreational anglers and dynamic trout populations. It is also critical to discuss the important point that these movement behaviors are occurring under current, free-flowing conditions that likely are not historically representative of this region (WDNR 2015), where beavers were historically abundant and played a large role in shaping the regional landscape.

Since 2001, the UMI system has been managed with extensive beaver management practices (control and removal) (WDNR 2010). As stated in the Wisconsin Watersheds overview of the Middle Inlet-Lake Noquebay watershed, many of the concerns with water quality impairments and fragmentation have largely been alleviated following beaver removal and the maintenance of free-flowing conditions. Due to these changes and perceived improvements, the beaver management program will likely remain a management priority into the future (WDNR 2010). While fragmentation may have decreased due to the beaver management program on UMI, it is difficult if not impossible to assess whether the removal of beavers and dams significantly altered seasonal movement patterns or greatly improved access to biologically significant habitats. Under the current conditions, connectivity and movement are important in this region, but it is even more important to recognize the artificial context in which these patterns exist. Historically, these systems were discontinuous beaver-mediated environments offering substantially different levels of complexity, which likely shaped movement in very different ways. Moving into the future, a stronger focus on movement in beaver-mediated systems in this region is important to more fully inform emerging trends in river restoration and management that centrally focus on restablishing beavers to support ecological restoration.

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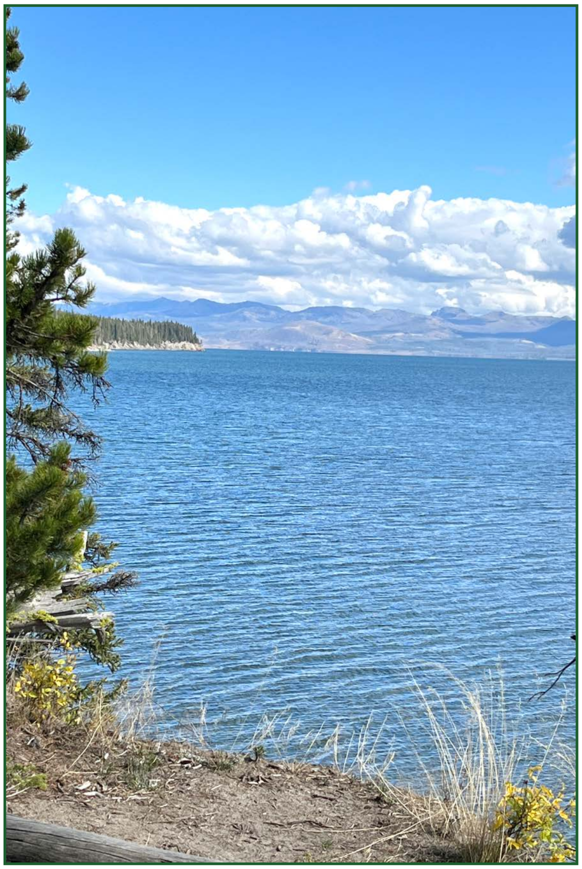
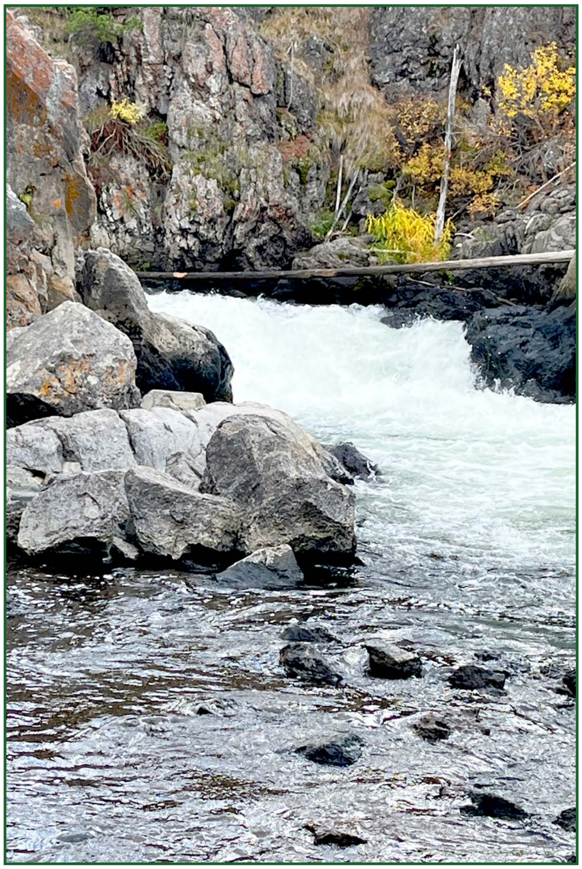
Session 8

Brook Trout Research and Management, and Wild Trout Population Monitoring Techniques





Investigating the Use of eDNA Monitoring to Improve Management of Wild Brook Trout in Virginia.

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Abstract - The Virginia Department of Wildlife Resources (VDWR) has managed wild Brook Trout *Salvelinus fontinalis* populations using a standard backpack electrofishing (EF) protocol for over 40 years. In 2019 and 2020, with the advent of environmental DNA (eDNA), the VDWR and United States Forest Service (USFS) explored using eDNA to monitor Brook Trout populations. Streams were sampled across the native range of Brook Trout in Virginia using both the standard electrofishing protocol and eDNA sampling to determine if the electrofishing protocol was accurate. In 2020, we sampled 56 streams where eDNA sampling and standard electrofishing were paired. Environmental DNA and standard electrofishing sampling presence/absence results matched at 52 of these streams. Additionally, data from the VDWR Coldwater Stream database was examined to determine where Brook Trout populations may have been extirpated. There were 36 streams identified based on the electrofishing protocol that indicated Brook Trout populations may be extirpated. In 2019, eDNA sampling indicated 8 of 36 streams thought to be extirpated were positive for Brook Trout DNA. Results indicated that determining presence or absence of Brook Trout using eDNA is more accurate and efficient than VDWR's standard electrofishing protocol. The Virginia Department of Wildlife Resources plans to incorporate eDNA as a component of the standard electrofishing protocol. We found that eDNA sampling is a useful tool to identify streams where populations may have been extirpated and therefore where repatriation could be useful to increase resiliency of Brook Trout across their native range. The sampling efficiency of eDNA will allow agencies to more effectively monitor potential population loss due to pending climate change. Goals of both the Eastern Brook Trout Joint Venture (EBTJV) and Chesapeake Bay Program (CBP) are focused on conserving and increasing stream habitat occupied by Brook Trout. Using eDNA detection methods will allow for more accurate assessments toward reaching the goals in conserving Brook Trout populations.

Introduction

Wild populations of Brook Trout *Salvelinus fontinalis* currently inhabit over 620 individually-named steams across western Virginia. The spatial distribution of these resources is estimated to be >3,200 stream kilometers (Reeser 2019). There is not sufficient information to accurately identify where Brook Trout populations have been reduced or extirpated from historical habitats throughout Virginia. The first statewide inventory of wild trout resources was conducted by The Virginia Department of Wildlife Resources (VDWR) in the late 1970's (Mohn and Bugas 1980). The agency has used this original census to document any changes in Brook Trout distribution. However, we wanted to investigate if environmental DNA (eDNA) monitoring methods could improve the accuracy of our assessment of Brook Trout distribution in Virginia. Brook Trout is identified as a species of greatest conservation need in Virginia's Wildlife Action Plan (VWAP 2015). In addition, goals of both the Eastern Brook Trout Joint Venture (EBTJV 2018) and the Chesapeake Bay Program (CBP 2015) are centered on maintaining/increasing stream habitat occupied by Brook Trout. The Commonwealth of Virginia is an active member of both the EBTJV and CBP. Understanding the spatial distribution of wild Brook Trout in Virginia is essential to effectively managing and conserving the species.

Environmental DNA sampling has been an effective method to detect the presence of aquatic animals in lotic habitats (Jerde et al. 2011; Laramie et al. 2015). This monitoring method has also been successful in detecting the presence of Brook Trout in stream habitats (Wilcox et al. 2016; Baldigo et al. 2017; Schumer et al. 2019). Specifically, researchers have compared electrofishing and eDNA monitoring in assessing Brook Trout presence in streams in New York (Baldigo et al. 2017) and Wisconsin (Evans et al. 2017).

The objectives of this project were: 1) Examine the accuracy of VDWR's current wild trout stream survey protocol; 2) Conduct eDNA monitoring in Virginia streams where Brook Trout may have been extirpated, as indicated by past backpack electrofishing surveys; 3) Evaluate the published eDNA detection methods for Brook Trout presence/absence monitoring in Virginia streams.

Methods

The detailed protocol that VDWR developed in the late 1970s to inventory stream habitat occupied by Brook Trout is described in the F-32 Federal Aid Report (Mohn and Bugas 1980). The summarized methods from the 1970s project entailed measuring water temperature and pH during the summer months in all streams that exhibited at least one mile of perennial flow or which originated from a ground-water spring. If water temperature and pH were in the preferred range for Brook Trout (maximum summer temperature 20°C; pH 5-8), a single-pass with a backpack electrofishing unit was conducted. Sampling effort included a minimum of three representative pool habitats and three representative riffle habitats for each stream. Sampling lengths varied considerably between streams. If numerous trout were collected, sampling did not exceed 70 m of stream and may not have included three representative pools or riffles. If zero trout were collected in 70 m, electrofishing was continued upstream an additional 400-600 m. All fish netted were identified to species, counted, measured and weighed. Flow and stream temperature data was used to determine the upstream and downstream limits of coldwater habitat suitable for trout. The upstream extent of trout habitat was considered to be at the point where the United States Geological Survey topographic map indicated the end of perennial flow. The downstream extent of trout habitat was determined based on water temperature measurements. Since the initial statewide trout stream survey, VDWR has slightly modified the coldwater stream electrofishing sampling protocol over the past four decades. Currently, when surveying suspected wild trout streams that have not been previously sampled, biologists select a stream reach with adequate physical habitat and make a single-pass with one backpack electrofishing unit moving upstream for a minimum of 100 m. This sample reach must contain a minimum of one pool-riffle-run sequence. If zero trout are collected in the first 100 m, electrofishing is continued upstream for an additional 300 m. If pool habitat is underrepresented, it is the biologist's discretion on the distance electroshocked beyond 400 m. The Virginia Department of Wildlife Resources' current wild trout stream sampling scheme for classified streams in the Coldwater Stream Database is to return to the same location on each classified wild trout stream every 5-10 years and electrofish a minimum of 100 m of stream.

In 2020, we examined the accuracy of VDWR's current wild trout stream survey protocol to detect Brook Trout presence, compared to eDNA monitoring. Electrofishing and eDNA monitoring was conducted on the same day at the same location on multiple streams. All streams surveyed were included in VDWR's original wild trout stream inventory from the 1970s. However, some streams from the original inventory did not meet the minimum flow and temperature requirements (at that time) and thus were not electrofished. In our study, all streams were first or second order and sampled during low flow conditions in June, July, or August. Each 100 m sample reach contained a minimum of one pool-riffle-run complex. At each sampling location biologists filtered a minimum of 5 L of stream water following the protocol described in the procedures recommended by the U.S. Forest Service Rocky Mountain Research Station (Carim et al. 2015). Immediately following the collection of the eDNA sample, two technicians entered the stream at the eDNA collection point and electrofished for 100 m upstream netting as many fish as possible as described by Bateman et al. (2005). Our main target species was Brook Trout. We used a single Smith Root LR24 backpack electrofishing unit employing a pulsed direct current ranging from 300-800 v. Water temperature (°C) and pH were also recorded using a Hanna pHep pH meter at each sampling location.

Additional eDNA sampling was conducted at multiple locations for other objectives of this study. In 2019 thirty-six streams were sampled at a single location where VDWR had collected Brook Trout via electrofishing pre-1990 and then failed to capture Brook Trout in electrofishing surveys conducted post-1990. These 36 streams were considered potentially extirpated. Also during summer 2019, VDWR conducted eDNA monitoring at single locations on forty-seven additional streams. Some of these streams were known to contain Brook Trout, while many of these streams had never been surveyed before. In 2020, staff from the U.S. Forest Service Center for Aquatic Technology Transfer (CATT) conducted eDNA sampling on 67 of the streams sampled by VDWR in 2019. Of these, 36 streams were part of the extirpation investigation initiated in 2019 and discussed above previously. Single samples were collected at these locations in 2019, and additional samples were collected in 2020 at 1 km intervals upstream from the locations sampled in 2019 until stream flow became inadequate for sampling. Upstream tributaries were included in this sampling. In several cases, private property prevented access upstream from the initial starting location.

All samples were analyzed by the USDA Forest Service's National Genomic Center for Wildlife and Fish Conservation. DNA was extracted from one-half of each filter using the DNeasy Blood and Tissue Kit and QIAshredder columns with modifications from the manufacturer's protocol as described in Carim et al. (2016). Each extraction was assayed for mitochondrial DNA from Brook Trout using species-specific quantitative PCR (qPCR) marker BRK2 described by Wilcox et al. (2013). Each sample was analyzed in triplicate on a StepOne Plus qPCR Instrument (Life Technologies) or a QuantStudio 3 qPCR System (Life Technologies) using the protocol described by Franklin et al. (2019). The internal positive control template and assay were used to test for the presence of PCR inhibitors (indicated by a > 1 CT shift in amplification relative to the control samples. On the PCR plate with environmental samples, a triplicate no-template control was included to test for contamination.

Results

The VDWR and CATT conducted eDNA monitoring on 137 different streams during this study (Figure 1). Brook Trout DNA was detected in 67 of these streams and incorporated into the USDA Forest Service's aquatic eDNAtlas project. Environmental DNA samples were collected at the same locations in 2019 and 2020 on 67 streams. Results of Brook Trout DNA detection matched at 56 streams, and did not match for 11 streams. Brook Trout were detected in both 2019 and 2020 at the same location 84% of the time.

We compared the accuracy of Brook Trout detection using VDWR's standard stream electrofishing protocol compared to eDNA monitoring. The VDWR tested these two sampling methods on 56 streams and found that our results "matched" for 52 streams and did not "match" at four (Figure 2). We defined a "match" as Brook Trout being detected with electrofishing and eDNA monitoring at the same site. The four unmatched streams were positive for Brook Trout DNA, but no trout were collected during electrofishing. This suggest that VDWR may be failing to detect Brook Trout with electrofishing 7% of the time in streams with very low Brook Trout densities.

In 2019, eDNA sampling indicated 8 of 36 streams thought to have extirpated populations based on electrofishing were positive for Brook Trout DNA (Figure 3). All 36 of these streams were resampled in 2020 at the same location. Brook Trout DNA discrepancies occurred at 3 locations. These were either a negative DNA in 2019 with a positive in 2020 or the opposite. For all three locations with discrepancies, hatchery-reared Brook Trout were stocked several times in the months prior to collecting eDNA samples within 5.6 kilometers of the eDNA collection points. This suggests that these three results could be positives for hatchery-reared Brook Trout.

Discussion

Our study showed that eDNA monitoring could be useful in increasing the accuracy of determining the amount of stream habitat occupied by Brook Trout in Virginia's headwater streams. When implemented correctly, there are advantages of using eDNA monitoring to complement our traditional electrofishing sampling methods when evaluating wild Brook Trout resources in Virginia. Virginia biologists have often questioned the effectiveness of our current electrofishing methods to validate the presence of Brook Trout, particularly in streams exhibiting extremely low population densities. Brook Trout densities in Virginia headwater streams have been recorded as low as one fish per 100 m at some sampling locations (B. Fink, VDWR personal communication). Advantages of implementing eDNA monitoring include situations where there is limited stream access on private property; however, access to streams to conduct eDNA monitoring may be possible at public road crossings. Electrofishing is often limited to periods of lowest stream flows to improve capture efficiency. Therefore, we conduct our coldwater stream surveys during the June-August time period in Virginia. We also typically do not see Age-0 Brook Trout efficiently recruit to our electrofishing gear until they reach 50 mm total length, which does not occur until mid-June in Virginia. Every summer, our biologists travel to electrofish coldwater streams and encounter sites with dry streambeds. Therefore, our detection of Brook Trout could also increase if we were to conduct eDNA monitoring from fall through spring. Additionally, eDNA monitoring is safer for field staff than electrofishing (Evans et al. 2017) and

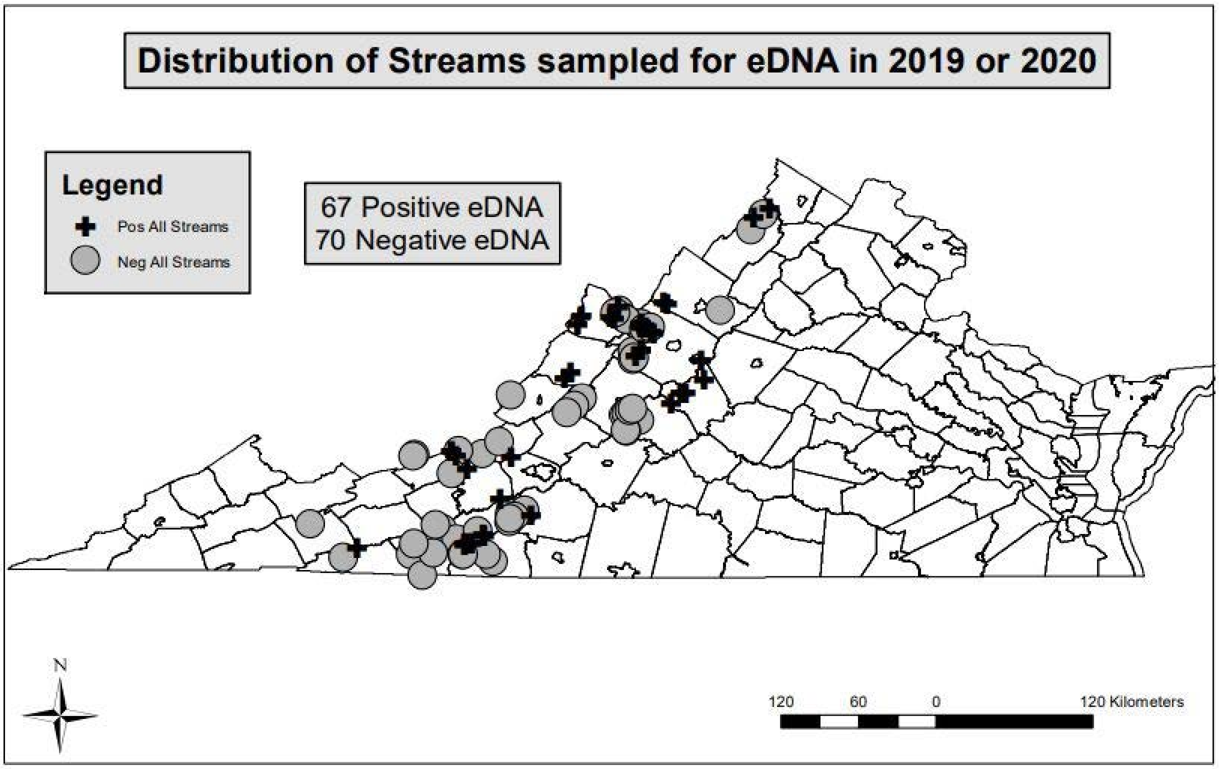

Figure 1. Locations of 137 total streams sampled for eDNA in 2019 or 2020 throughout the Brook Trout native range in Virginia.

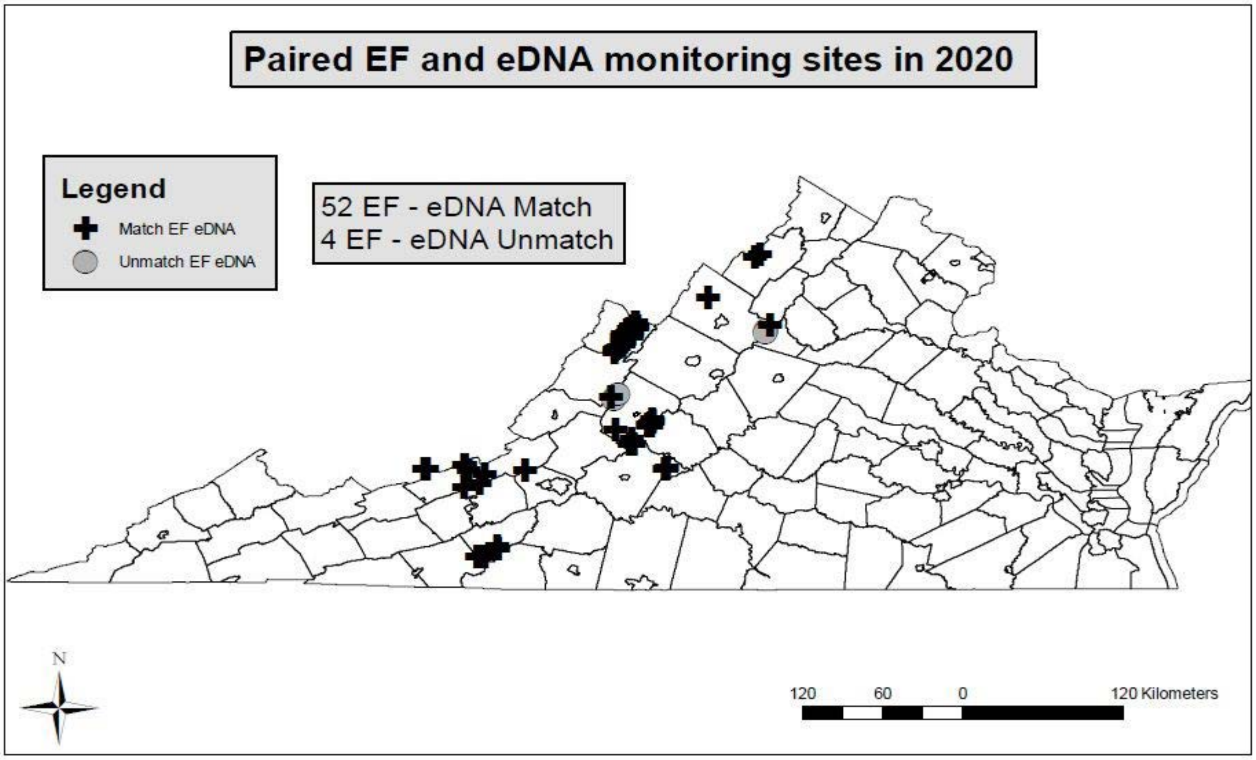

Figure 2. Results of the 2020 eDNA and electrofishing paired samples.

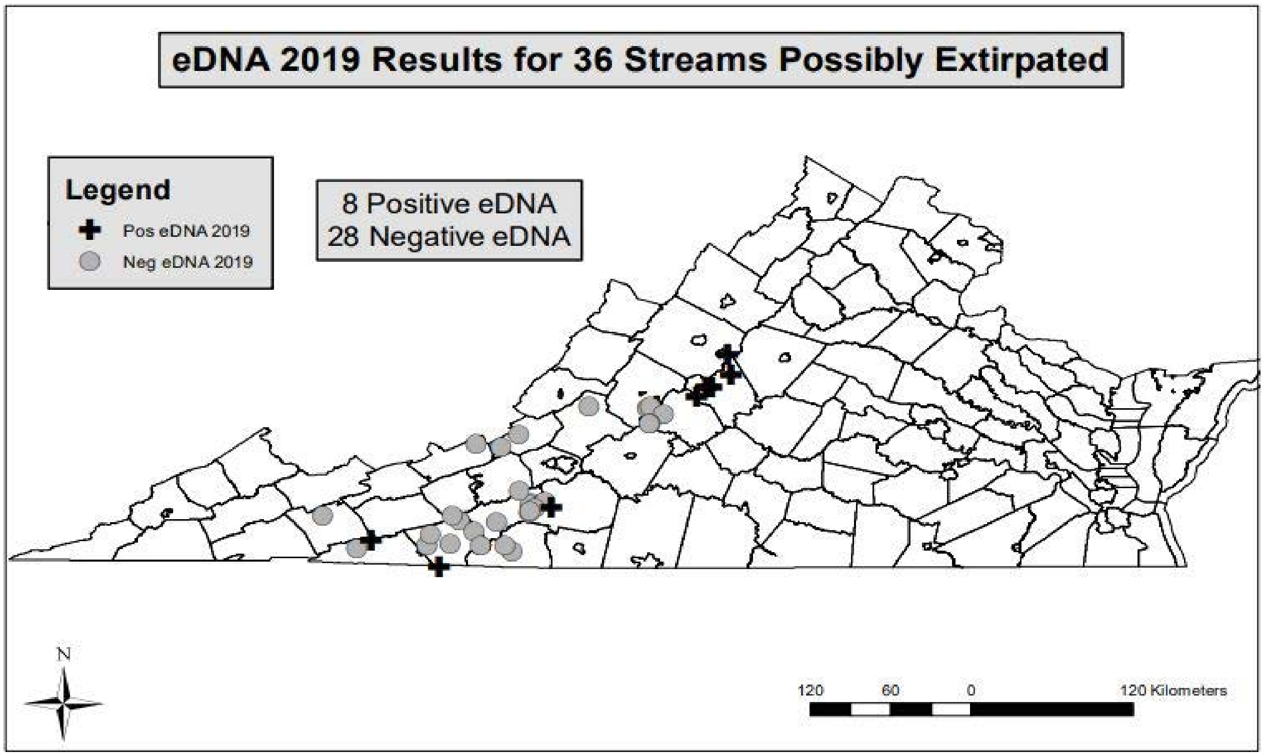

Figure 3. Results of 2019 sampling of the 36 streams thought to have extirpated Brook Trout populations based on electrofishing data. Brook Trout were collected prior to 1990, but not after 1990, using electrofishing.

less invasive to wild Brook Trout than electrofishing (Hollender and Carline 1994). Eliminating some electrofishing may also contribute to lessening the impact on threatened or endangered aquatic species.

Of the 36 streams, we theorized Brook Trout populations to be extirpated (lack of detection with VDWR electrofishing protocol since 1990), eight streams indicated a positive eDNA sample in 2019. Based on the proximity (5.6 km or less) of two of those eight streams to stocked trout water, we believe those detections represent stocked (hatchery) Brook Trout rather than a change in stream occupancy of wild populations. We encountered the same potential results in 2020 on a different stream (i.e. negative in 2019 positive in 2020). This would indicate there may be 31 streams with extirpated populations with negative results for Brook Trout with both traditional and eDNA methods.

The current results indicate that utilization of eDNA to monitor wild Brook Trout populations and other species could be advantageous. Of the 137 different streams sampled during this study over two years, Brook Trout DNA was detected in 67 of these streams. Eleven of these streams did not match from 2019 to 2020. There are a few explanations for these discrepancies. Authorized and unauthorized Brook Trout stocking occurs annually in Virginia streams. Although VDWR strives to regulate fish stocking in public waters, there is always the possibility of unauthorized stockings or "baitbucket biologists" attempting to restore or enhance their local stream. In addition, some of the streams that were sampled during the study exhibit marginal habitat and flow with very low densities of wild Brook Trout. Year-to-year and seasonally, these low flow streams may be utilized for feeding, spawning, or seasonal habitat (Petty et al. 2012), leading to a positive or negative eDNA detection based on time of sampling. Our study indicated that the lower reaches of a stream may not always yield a positive detection for wild Brook Trout DNA, even when there are documented positive eDNA samples at upstream sites. We collected a single 5 L filtered sample at each site. Schumer et al. (2019) suggests that increasing the number of samples at individual sites may increase detectability of eDNA. We suspect some of our discrepancies were due to the presence of hatchery Brook Trout. Based on our findings, we recommend investigating the potential existence of hatchery stocked Brook Trout in relation to sampling locations. On numerous occasions, VDWR has documented the movement of hatchery Brook Trout from stocked locations into upstream reaches and adjacent watersheds. The VDWR differentiates hatchery trout from wild trout based on total length and eroded or underdeveloped pectoral, pelvic, and caudal fins (B. Fink and S. Reeser, VDWR personal communication).

There may be additional applications for eDNA sampling beyond determining presence or absence of Brook Trout. Utilization of eDNA to determine if Brook Trout or other native species have been extirpated could be a very useful tool to decide if repatriation is warranted. Another application we intend to investigate will be using eDNA to document movements of hatchery trout. This would entail eDNA monitoring upstream of locations stocked with hatchery trout in watersheds that do not have wild trout present. This will better inform VDWR to determine where to authorize trout stocking in public waters while still maintaining the goals of conserving wild Brook Trout populations (Reeser 2019). Environmental DNA monitoring would also help identify stream reaches utilized by Brook Trout that could receive more regulatory protection. The VDWR intends to incorporate eDNA sampling into their current Brook Trout monitoring protocol to improve the understanding of Brook Trout distribution.

Acknowledgements

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Redd Superimposition Mediates the Accuracy, Precision, and Significance of Redd Counts for Yellowstone Cutthroat Trout

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Abstract - For salmonid fish, redd counts are commonly applied to estimate spawning population size and allow for broad coverage in monitoring. However, the validity of redd counts may be compromised due to observation error, particularly with respect to superimposition, where later spawners construct redds overlapping existing redds. Redd counts are used to monitor Yellowstone Cutthroat Trout *Oncorhynchus clarkii bouvieri* within the Snake River watershed, Wyoming, where high rates of superimposition may limit the value of redd count data. We used a Bayesian framework to parse observation error into three components: (1) redd cluster detection was low but comparable among observers; (2) rates of false identification were low and had little effect on total counts; and (3) the number of redds per cluster was overestimated and varied among observers. However, error components acted antagonistically, such that observed counts were accurate. A saturating relationship between redd counts and spawner abundance indicates that counts are best interpreted as effective reproductive effort. Our results indicate that redd counts are well suited for population monitoring; however, management programs should be designed with the flexibility to account for the uncertainty associated with redd count data.

Introduction

For salmonids, annual estimates of spawning population size often serve as the basis for species management plans (Alves et al. 2004). However, the implementation of a specific methodology to monitor population size is often based on logistical considerations rather than statistical accuracy, precision, and the ability to meet assumptions (Parsons and Skalski 2010). Multiple types of sampling error may therefore limit the power of monitoring to detect long-term trends (Dauwalter et al. 2009). As a result, monitoring programs may lack the ability to inform corrective actions designed to prevent local extirpations (Ham and Pearsons 2000).

Redd counts are commonly applied to provide indices of population size for salmonids. Redd counts allow for greater coverage in monitoring than can be attained through traditional approaches such as mark-recapture (Chasco et al. 2014). The validity of redd counts relies on the assumption that observed counts are representative of true redd numbers. There is increasing recognition that this assumption is often not met in practice (Dunham et al. 2001; Muhlfeld et al. 2006). A second assumption regarding the validity of redd count data is that counts accurately reflect population status. While redd counts may often be correlated with spawner abundance (Hay 1984), variation in the sex ratio may limit the validity of this interpretation (Dauble and Watson 1997). Alternatively, redd counts can also be used as indices of recruitment, as redd counts and juvenile abundance are often related (Beard and Carline 1991). Therefore, the use of redd counts as a cost-effective monitoring tool may be limited due to observation error and unknown biological relevance.

When spawning densities are high, competition for suitable spawning habitat is mediated through redd superimposition, where later spawning females construct redds overlapping those constructed previously (Quinn 2005). Previous work suggests that superimposition reduces the accuracy and precision of redd count data (Murdoch et al. 2019). However, the precise effects of superimposition have been ignored as discriminating redds within superimposed redd clusters can be challenging even for the most experienced observer. The effect of superimposition on the error structure of redd count data represents a considerable knowledge gap that must be addressed if we are to continue to use redd counts to identify, justify, and evaluate management actions.

In this study, we provide a mechanistic understanding of the error structure of redd count data and how redd counts can be interpreted with respect to population status for Yellowstone cutthroat trout *Oncorhynchus clarkii bouvieri* (YCT) spawning in spring-fed tributaries of the upper Snake River watershed, Wyoming. First, we quantified the magnitude of discrete error components in redd count data and the extent of variation among observers, streams, and years. Second, we summarized the net accuracy and precision of redd count data at multiple spatial scales. Third, we explored the biological significance of redd counts in terms of spawning population abundance by leveraging multiple types of long-term monitoring data. Our research provides insight into the value and limitations of redd counts as a monitoring tool.

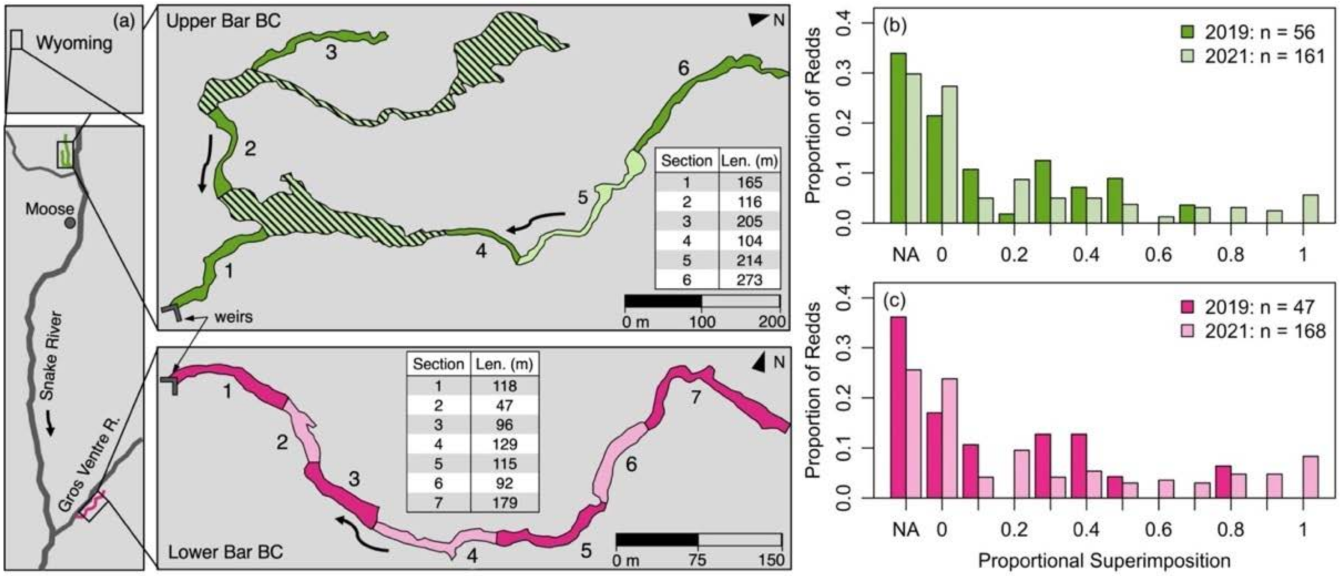
Methods

Study System and Design

Since 1965, the Wyoming Game and Fish Department (WGFD) has used redd counts to monitor populations of YCT spawning in spring-fed streams within the upper Snake River watershed, Wyoming. There is concern that the value of YCT redd count data may be limited due to sampling error associated with high rates of superimposition and inter-observer variability. We assessed the error structure of redd count data on two spring-fed streams (Figure 1). Lower Bar BC (LBBC) is a spring-fed tributary of the Gros Ventre River. Upper Bar BC (UBBC) is a spring-fed tributary of the mainstem Snake River within Grand Teton National Park. We conducted our study over the course of the spawning period (May-July) in 2019 and 2021. As our objective was to evaluate error structure over the historical range of redd densities, we divided each stream into reaches; thus, the unit of analysis was the reach. However, we considered the net error in redd count data at both reach and stream scales as WGFD monitoring includes both index reaches and census redd counts. We delineated approximately equidistant reach boundaries according to geomorphic features that separated primary spawning areas (Figure 1).

Data Collection

We conducted spawning ground surveys (i.e., census surveys) twice weekly to monitor redd construction and the condition of redds already constructed. We mapped redds with a handheld GPS device and assigned a categorical age. Categorical ages are complementary to discrete age (days since construction) as local flow hydraulics "age" some



Section	Len. (m)
1	165
2	116
3	205
4	104
5	214
6	273

Section	Len. (m)
1	118
2	47
3	96
4	129
5	115
6	92
7	179

Figure 1. Map (a) showing Upper Bar BC and Lower Bar BC spring creeks in the Snake River watershed, Wyoming. Arrows indicate direction of flow. Striped polygons indicate slack water areas lacking spawning gravel where spawning ground surveys were not performed. Proportion of redds not superimposed ("NA", redds per cluster = 1) and the distribution of proportional superimposition for redds within clusters (redds per cluster > 1) for (b) Upper Bar BC and (c) Lower Bar BC.

redds more quickly than others; therefore, categorical age can be thought of as a metric of visual identifiability. We also measured the length and width of the pot and tail-spill to the nearest cm, visually estimated the proportion of the redd surface area (0-100%) disturbed due to superimposition, and assigned a binary cover score (1 if habitat features obscured the redd as viewed by an observer standing on the bank; 0 for all other cases). Finally, we used an electronic tablet to photograph each redd and annotate distinctive features with a drawing application, particularly superimposition of nearby redds (Figure 2). We used photographs taken during previous surveys to identify redds under construction and resolve difficulties in redd identification associated with superimposition. Counts from census surveys serve as the best estimate of the "true" number of redds in any given stream reach.

To assess the degree to which observer bias and variability affect the error structure of redd count data, we compared census surveys to redd surveys conducted independently by three senior WGFD fisheries biologists. We provided WGFD observers with detailed maps on which they recorded the number and location of redds in a two-step process. First, redd clusters (single redds or multiple redds superimposed) were identified. Second, observers estimated the minimum and maximum number of redds in each cluster based on the number of pots and total area of disturbance as compared to the average footprint of a single redd.

We assessed the biological significance of redd count data by comparing redd counts to direct measures of spawner abundance. Beginning in 1971, the WGFD has conducted redd surveys while simultaneously operating a weir during the spawning migration (late May – early July) to enumerate the number and size structure of YCT returning to spawn in LBBC. Each day, WGFD biologists counted and determined the sex of all YCT captured. Historical redd counts were conducted once annually following the date of peak spawning activity (early July).

Statistical Analysis

We used a Bayesian framework to parse counting errors into three components: (1) imperfect detection of redd clusters, (2) false identifications, and (3) imperfect assignment of the number of redds per cluster. We used a crossed random effects structure to investigate variability among the three grouping variables. The most parsimonious explanation regarding cluster detection is that each cluster has an equal chance of detection, implying that clusters are binomially distributed (Muhlfeld et al. 2006). The most parsimonious explanation regarding false identifications is

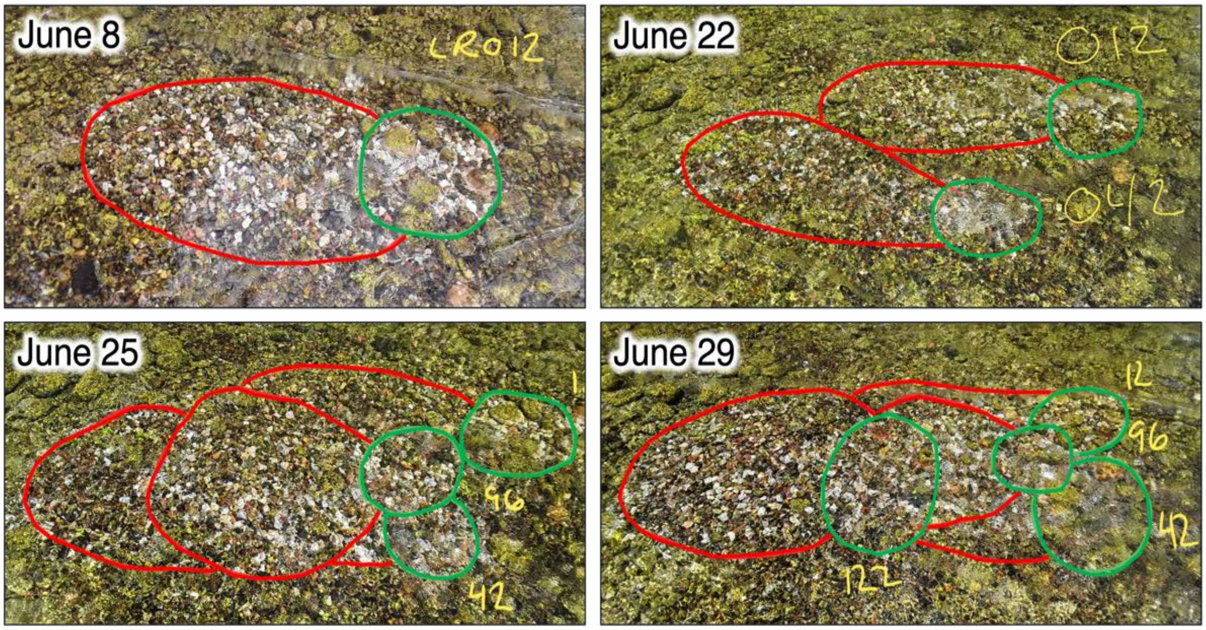


Figure 2. Sequential superimposition of redds from the same cluster within Lower Bar BC during the 2021 spawning season. Dates of photos/redd surveys are noted in the upper left-hand corner of each panel. Green and red polygons represent the pot and tailspill for each redd, respectively.

that each increment of stream has an equal chance of holding a false identification, implying that false identifications will be Poisson distributed (Muhlfeld et al. 2006). Finally, we transformed the average observed number of redds per cluster into an error rate (observed – census / census) and modeled following a normal distribution. All error components were modeled within a generalized linear model framework to assess the effects of stream and redd features on mean error rates.

To determine the combined effect of error components on the accuracy and precision of redd counts, we considered the linear relationship between observed and true redd density and how this relationship varied among observers, streams, and years. We fit the net accuracy model with variable intercepts and slopes. Comparing slopes relative to 1 provides insight into the degree to which bias in redd counts occurs at a fixed rate. Comparing intercepts relative to 0 provides insight into the degree to which the bias in redd count data occurs as a fixed offset. To determine how the precision of redd count data changes with redd density, we used simulations to explore the effect of true redd density on the standard deviation (SD) and coefficient of variation (CV) of observed redd densities. We used locally weighted scatterplot smoothing to describe how the SD and CV of simulated observed densities change with true density.

We compared historical redd counts and direct measures of female spawner abundance (weir data) to explore the biological significance of redd counts. We modeled redd count as a function of female spawner abundance using linear, logarithmic, exponential decay (increasing form), and broken stick functions. A linear relationship implies that female spawners construct a proportional number of redds. A logarithmic relationship implies that spawners construct proportionally fewer redds as spawner abundance increases. An exponential relationship suggests that there is a point at which streams become saturated with redds. A broken stick relationship has a similar interpretation as the exponential relationship, but instead suggests that below the saturation point, the relationship between spawner abundance and redd counts is linear (rather than concave).

Model Fitting, Evaluation, and Significance Testing

We evaluated candidate models using leave-one-out cross-validation. We did not use model selection to test for differences among groups, as this was our primary objective. Instead, we evaluated variability among groups post-hoc by examining distributions of credible differences (Kruschke 2014). For example, for each Bayesian Markov chain Monte Carlo iteration, we subtracted the estimated value for observer one from that of observer two, then analyzed the distribution of all iterations. We calculated the mean, probability of direction (pd: the proportion of the distribution that is of the median's sign and represents the probability that a difference exists), and probability within the region of practical equivalence (p-ROPE: the proportion of the distribution that lies within a range of a null value, where values within that range are considered irrelevant). These metrics provide insight into the existence and relevance of differences among groups.

Results

In 2019, 48 and 59 redds were constructed in LBBC and UBBC, and 168 and 162 redds in 2021. 65% and 63% of redds were superimposed in LBBC and UBBC in 2019 and 74% and 69% were superimposed in 2021. Of the superimposed redds, the proportion of redd surface area disturbed due to superimposition ranged from 0-0.8 and 0-0.7 in LBBC and UBBC in 2019 and 0-1 in 2021 (Figure 1). Long-term LBBC weir sampling captured as few as 39 YCT in 1973 (12 females) and as many as 624 YCT in 2017 (337 females). A single annual redd count was conducted in mid-July of 33 years (mostly 1985-2021). These counts ranged from 30 to 379 total redds.

We found detection probabilities of redd clusters were low (global mean = 0.64). While we observed differences in detection among groups, post-hoc pairwise comparisons indicate that these differences were small and irrelevant. For example, detection for observer three (mean = 0.68) was 0.05 and 0.06 greater than that of observers one (mean = 0.63) and two (mean = 0.62), respectively. While there was a ~95% chance (pd) that these differences existed, there was a 45% and 39% chance that they were equivalent to 0 (p-ROPE). Differences in detection among streams and years were small, uncertain (pd = 79% and 75%), and equivalent to 0 (p-ROPE = 80% and 70%). In general, detection decreased with cover and increased with the proportion of clusters ≤ categorical age-2 (redd features crisp and well-defined). False identifications were committed infrequently (global mean = 0.130 per 100 m) and differences among groups were irrelevant (p-ROPE > 85%). Lastly, the number of redds per cluster was overestimated (global mean error rate = 38%). Mean error rates differed considerably among observers: 37%, 12%, and 64% for observers one, two, and three, respectively (pd = 100%, p-ROPE < 1%). In contrast, differences in error rates between streams and years were smaller and likely equivalent to 0 (p-ROPE = 47% and 29%). We found that error rates in the number of redds per cluster declined with mean proportional superimposition and increased with minimum redd age in days.

We assessed the net accuracy of observed redd count data by exploring the relationship between observed and true redd densities, which we found to approximate 1:1 (Figure 3). There was a 44% chance (p-ROPE) that the global slope (0.895) was equivalent to 1, suggesting minimal relative bias. Similarly, there was a 79% chance that the global intercept (0.006) was equivalent to 0 suggesting minimal bias as an offset. Differences in group-level slopes and intercepts were small and practically equivalent to 0. Analysis of the net error in redd count data at the stream scale supported results at the reach scale (Figure 3): observed counts underestimated true counts by 14.7%, but there was a 21% chance that this difference is practically equivalent to 0 (p-ROPE).

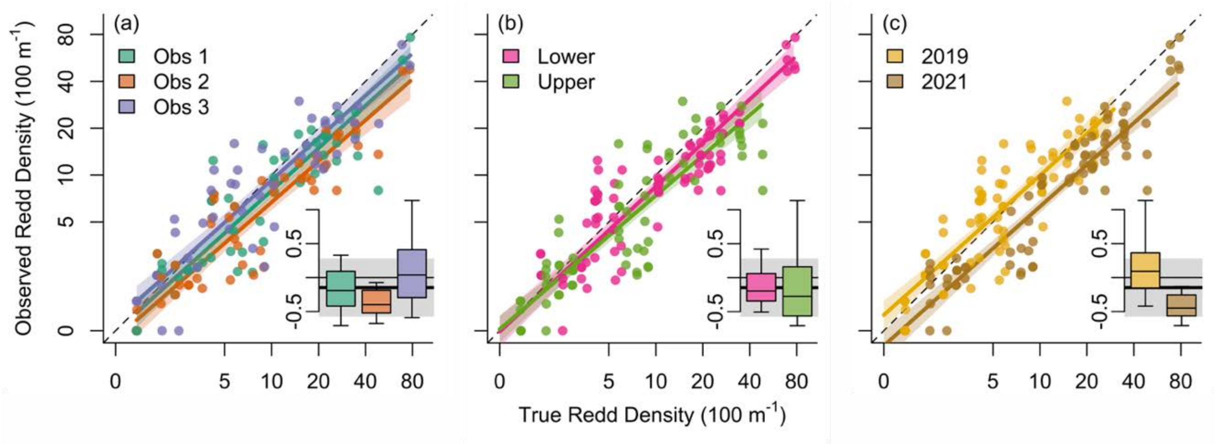


Figure 3. Hierarchical Bayesian linear model output of the relationship between observed and census redd densities (log scale, redds per 100 m) faceted by (a) observers, (b) streams, and (c) years. Dashed lines denote 1:1 for visual reference. Inset boxplots summarize the relative error in redd counts at the scale of the entire stream, where 0 indicates no differences between observed and true counts. Thick horizontal lines and grey polygons represent the global mean ⍰ SD relative error among groups (-0.15 ⍰ 0.43). Thin horizontal lines denote 0 (no difference) for visual reference.

Our simulation analysis of the precision of redd count data shows that the SD of observed redd densities increases with true density, but the CV is constant. This finding is consistent with our net accuracy model using log-transformed data (mean and variance scale positively). Variation in the effect of redd density on the SD of predictions among groups is consistent with our net accuracy model, whereas variation in the CV of predictions is minimal.

Model evaluation and selection supported a saturating (exponential decay, increasing form) relationship between female spawning abundance and redd count (Figure 4). The asymptotic redd count was estimated at 228 redds (posterior mean). Assuming each female constructs a single redd, increases in female spawner abundance above 228 do not yield corresponding increases in redd counts.

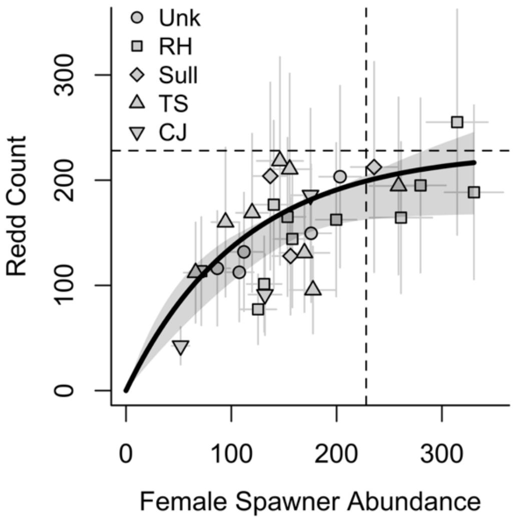


Figure 4. Redd count corrected for observation error as a function of the female spawner abundance corrected for the timing of weir operation. Error bars represent standard deviations. Black line and grey polygon represent the top model output and 95% credible interval (exponential decay, increasing form). Dashed lines represent the asymptotic limit of the exponential function. Point shape denotes unique observers.

Discussion

Redd counts are commonly used to monitor the status and trends of salmonid populations at broad spatial and temporal scales. While effects of inter-observer variability and bias on the error structure of redd count data are well documented (Dunham et al. 2001, Muhlfeld et al. 2006), how superimposition mediates the accuracy and precision of redd counts is less well understood. We found that redd counts are prone to multiple types of error, and redd superimposition is the leading cause for variability in error rates among observers. However, discrete error components act antagonistically, such that observed redd counts accurately reflect true redd abundance (Figure 3). While precision declined with redd density, the CV was constant, indicating that redd counts are well suited for long-term monitoring. Finally, we found that estimates of spawner abundance derived from redd count data may be misleading, especially under high redd density conditions. Instead, we argue that redd count data is best interpreted as effective reproductive effort.

Our investigation into error components revealed that superimposition is the primary cause of inter-observer variability as error rates in the number of redds assigned per cluster (i.e., areas of superimposition) varied considerably among observers, and mean error rate was primarily driven by proportional superimposition. Our results provide explicit evidence that superimposition has important effects on the ability of observers to survey accurately (Murdoch et al. 2019). Because the effect of superimposition was apparent among years in which redd numbers differed three-fold, our results suggest that superimposition should be considered even when surveying populations that spawn at low densities, unlike what has been suggested previously (Lestelle and Weller 2002). While our models of the drivers of error were simple, our results highlight the importance of observer experience to the validity of redd counts (Howell and Sankovich 2012).

Despite variation in the magnitude of discrete error components among observers, we found that error components offset each other such that the relationship between observed and true counts approximated 1:1. It is possible that error components would not offset each other for un-tested observers. In this regard, low sample size may limit our ability to characterize the full range of net error. However, the observers tested in our study trained each other, these individuals were themselves trained by former observers, and these individuals will train future observers. Therefore, we believe the variability we describe is representative of a more complete set of observers. We also found that precision declined as redd density increased, but the CV in observed redd count data was constant. In a monitoring context, this error structure may result in reduced ability to detect declines that would otherwise precipitate management intervention and reduced ability to detect whether recovery targets are being met (Wagner et al. 2013). However, because the overall bias is minimal, our results imply that redd counts can be an efficient and effective monitoring tool given that management plans are robust to uncertainty. For example, caution should be taken when comparing means to thresholds demarcating when interventions should or should not be implemented (Ham and Pearsons 2000; Dauwalter et al. 2009).

Our analysis of the biological significance of redd count data revealed a saturating relationship between female spawner abundance and redd count, suggesting redd counts may be useful for inferring spawning population abundance only at low to moderate abundances. Underestimates of redd numbers at high spawner abundances can be attributed to the fact that many redds have been destroyed due to superimposition and are not visible to observers. It is widely appreciated that superimposition reduces egg-to-fry survival (McNeil 1964; Hayes 1987). Therefore, underestimates of redd numbers due to superimposition are irrelevant. Instead, redd counts should be interpreted as effective reproductive effort: a metric of fry production. Direct measures of population abundance or density may be only marginally correlated with recruitment, given the effects of superimposition on spawning success. Redd counts thus provide conservation efforts with more relevant information regarding population status, as fry production and recruitment underlie long-term population dynamics in many trout species (Elliot 1994).

In this study, we provide a mechanistic understanding of the error associated with redd counts for YCT, how discrete error components sum to drive net accuracy and precision of redd counts, and how redd counts can be interpreted with respect to spawner abundance. We show that observation error in redd count data is considerable and driven by superimposition, but antagonistic error components offset such that observed counts are representative of true redd abundance. Bayesian approaches allowed us to express results in terms of probability and biological relevance rather than p-values and effect sizes that can be difficult to interpret. Such flexibility is valuable for fisheries biologists that must design and implement management plans that are robust to uncertainty in monitoring data (Dauwalter et al. 2009). Our results illustrate the need for mechanistic evaluations of the accuracy and precision of population monitoring programs.

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Life History Strategies and Movement Patterns of Anadromous Coastal Cutthroat Trout in South Puget Sound

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Abstract - Anadromous Coastal Cutthroat Trout *Oncorhynchus clarkii clarkii* fuel an economically important recreational fishery in the marine waters of Puget Sound. While anadromous Coastal Cutthroat Trout are highly valued by anglers, information regarding biological traits such as life history strategies, age and size structure, and seasonal movement patterns is lacking relative to other native salmonids in Puget Sound. Individuals were PIT tagged and sampled for scales, length, and weight and this was paired with the installation of stationary PIT arrays in key spawning tributaries. These tools along with earlier genetic stock identification work, have provided insight into marine and freshwater movements, iteroparity, size and age structure, and supported previous conclusions that anadromous Coastal Cutthroat Trout in South Puget Sound display a diverse range of life histories and migration patterns. Some of the unique life histories include large spawning migrations across inlets to natal spawning tributaries, repeat spawning migrations, and multiple freshwater migrations in different months of the year. The results from this study will help inform decisions made by fisheries managers to maintain healthy populations of anadromous Coastal Cutthroat Trout in Puget Sound.

Introduction

The Cutthroat Trout *Oncorhynchus clarkii* has been described as the ancestral salmonid in the Pacific Northwest (Trotter 2008), and through thousands of years, this species has evolved into at least 11 other subspecies and more than five life history types, including anadromy (Behnke 1979). The subspecies Coastal Cutthroat Trout *O. clarkii clarkii* is not an important commercial species and so is understudied relative to other salmonids on the west coast of North America. Although general life cycle information has been documented for anadromous Coastal Cutthroat Trout (Wenburg 1998; Trotter 2008), their migration patterns, life, history strategies, and other biological traits are not well understood. Without this information, biologists may be unable to evaluate management plans or ensure the long-term stability of a population. In the absence of definitive information on population attributes of anadromous Coastal Cutthroat Trout in Puget Sound, managers have relied on conservative management approaches to protect and conserve anadromous populations. While harvest is permitted in select rivers in Western Washington, current sport fishing regulations for Coastal Cutthroat Trout in the marine waters of Puget Sound require barbless hooks and prohibit harvest year-round.

In Puget Sound, Coastal Cutthroat Trout are managed assuming a mixed-stock management model, but the degree of mixing and general migrations patterns are unknown. Coastal Cutthroat Trout exhibit high site fidelity during spawning (Wenburg and Bentzen 2001) forming genetic stock structure organized at the stream level. Results from tagging studies in Hood Canal, a large fjord of Puget Sound, suggest that, Cutthroat Trout rarely migrate far from their natal stream in the marine environment (Moore et al. 2010). It is unknown, however, if the fidelity Coastal Cutthroat Trout exhibit to their natal inlet in Hood Canal is characteristic of Coastal Cutthroat Trout throughout Puget Sound. If so, popular sport fisheries concentrated around estuaries of natal streams anadromous Coastal Cutthroat Trout spawn in may be best managed as a series of inlet-specific terminal fisheries. In that scenario, angling regulations could be applied to marine waters based on the status of the associated population. Conversely, if longer distance migrations, that are common for other species of anadromous trout (i.e., Bull Trout *Salvelinus confluentus*, Brown Trout *Salmo trutta*, Arctic Char *Salvelinus alpinus*, etc.; Quinn and Myers, 2004), are observed for Coastal Cutthroat Trout, inlets of Puget Sound may be best managed as mixed stock fisheries. In this case, angling regulations applied across a broad geographic region may be appropriate to protect small, independent populations mixed with larger ones.

The goal of this work was to improve the knowledge of migration patterns, life-history strategies, and other important biological traits of anadromous Coastal Cutthroat Trout populations in South Puget Sound and provide fisheries managers with tools to improve monitoring and management activities. Specifically, we sought to describe the (1) life-history strategies, (2) size and age, and (3) migration patterns of anadromous Coastal Cutthroat Trout in South Puget Sound, Washington. Combined, this new information may assist managers in designing adequate management plans that protect weak populations while providing recreational opportunity and promote the long-term viability of anadromous Coastal Cutthroat Trout.

Methods

Study Area

Puget Sound, Washington is characterized by numerous fjord-like inlets, each fed by one or more streams draining into it. As a whole, Puget Sound has water chemistry properties resembling partially mixed estuaries (Sutherland et al. 2011). The current study was conducted in freshwater and nearshore marine waters of South Puget Sound that represent high-use fishing areas for those targeting Coastal Cutthroat Trout (Lothrop and Losee 2016). The marine study areas comprised Skookum, Totten, and Eld inlets (Figure 1). The aspects of the study carried out in freshwater included the three major streams draining into the marine study area, Skookum creek, Kennedy Creek, and McLane Creek.

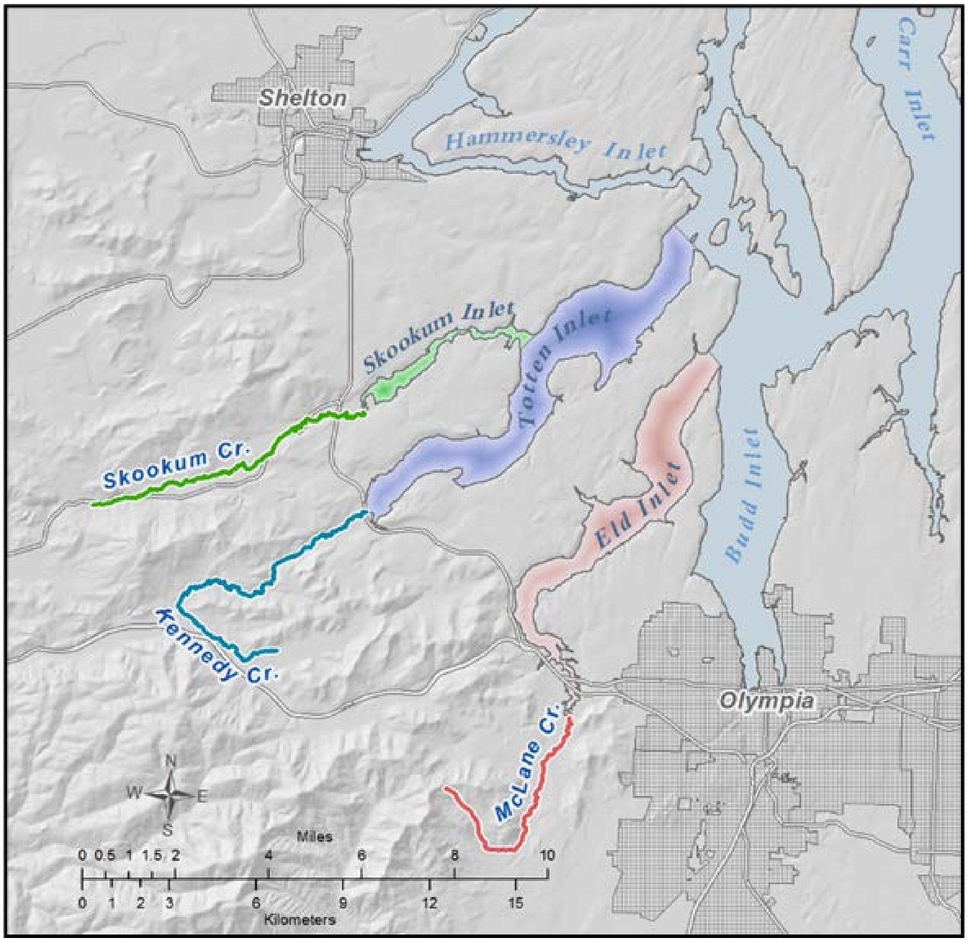

Figure 1. Study area in South Puget Sound, Washington, U.S.A. Tributaries are color coded to match the inlets they feed.

Collection of Fish and Age Analysis

Fork lengths, weight, scales, and tissue samples were collected from Coastal Cutthroat Trout captured throughout the study area using beach seines and angling. To determine age, scales collected from the preferred area above the lateral line midway between the dorsal and adipose fins were analyzed for age and saltwater and freshwater entry at the Washington Department of Fish and Wildlife marine aging lab. The scales were mounted on scale cards, lightly dyed for visibility, and analyzed under 40× magnification. We defined juveniles and adults as fish with no annuli or ≥1 annuli after marine entry respectively.

Marine Movements

To investigate both broad and fine-scale movements, we tagged all anadromous Coastal Cutthroat Trout ≥ 200 mm captured during the study with Passive Integrated Transponder (PIT) tags. Each captured individual was scanned with a handheld PIT tag reader prior to tagging to ensure it had not been previously tagged, and all recaptures were recorded. Multiple PIT tag arrays were installed in both Skookum Creek and McLane Creek to detect anadromous Coastal Cutthroat Trout entering natal tributaries on spawning migrations, for foraging opportunities, seeking cool water refugia, etc. Each array was composed of paired antennas to describe directionality of movements.

The PIT tagging efforts were paired with earlier genetic methodologies as described by Losee et al. (2017, 2018) to help identify migration patterns in the marine environment. To document broad patterns of stock-specific movements and identify the degree of "mixing" of various populations, Coastal Cutthroat Trout were sampled throughout the study area using hook and line and individuals were assigned to their population of origin using genetic stock identification (Losee et al. 2017). To describe fine-scale movements of Coastal Cutthroat Trout, we evaluated site fidelity by sampling the same location in Eld Inlet monthly using a beach seine while recording the number of times individual Coastal Cutthroat Trout were recaptured at this location. Recaptures were identified using genetic tags; samples with matching genotypes were assumed to be the same individual (Losee et al. 2018).

Results

Age and Size Distribution

Based on scale analysis, mature Coastal Cutthroat Trout sampled in the marine environment were dominated by age-2 and -3 fish (49% and 33%, Figure 2). Ten separate life-history strategies were identified with juveniles entering marine water at age-1, -2, and -3 and adults spending from one to three years in the marine environment. Rearing for one or two years in natal tributaries were the dominant freshwater life histories for juveniles (31% and 64%, Figure 3), while spending one to two years in Puget Sound were the dominant life history strategies for anadromous adults (80% and 19%; Figure 3). Few individuals spent more than two years in freshwater or two years in marine water (5% and 1%). There were also only a small number of fish that demonstrated a spawning check (N=18) at the time of capture in marine water. Of those, the majority (N=16/18) of Coastal Cutthroat Trout had undergone only one spawning migration, with two individuals spawning in repeat years before being captured. Individuals that had migrated into natal tributaries to spawn spent either one or two years in the marine environment before making their first spawning migration (56% and 44%). Anadromous individuals included in the age analyses ranged in size from 185- 454 mm (meant ± SD = 281 ± 56; Figure 2).

Marine Movements

Over the course of the study, 1,234 anadromous Coastal Cutthroat Trout were captured and PIT tagged to examine marine migrations, with 166 individuals being recaptured during subsequent sampling events. The overall recapture rate for the study was 14% across the marine locations that were sampled. Data from PIT tagged individuals support the high recapture rates observed in Eld Inlet in South Puget Sound using genetic mark-recapture techniques.

Based on genetic analyses of samples from Eld Inlet, 21% (64/305) of Coastal Cutthroat Trout sampled were recaptured during successive sampling events. Highest recapture rates occurred on March 26th (Figure 4). On this sampling event, 100% of adults captured had been sampled previously (N=24) and 86% of total catch had been captured previously (25/29). At the study site in Eld Inlet, 13.1% (21/160) of known juvenile Coastal Cutthroat Trout were captured more than once, and 30.8% (37/120) of adult Coastal Cutthroat Trout were captured more than once.

Inlets of South Puget Sound were comprised of multiple genetically distinct populations in all months. The majority (71.6%) of Coastal Cutthroat Trout captured using hook and line in the marine environment were less than 15 km from the mouth of their natal stream, while 14.1% were captured greater than 30 km from their natal stream. Average migration distance was greatest in summer months when marine water temperatures were highest and the spawning season had ended.

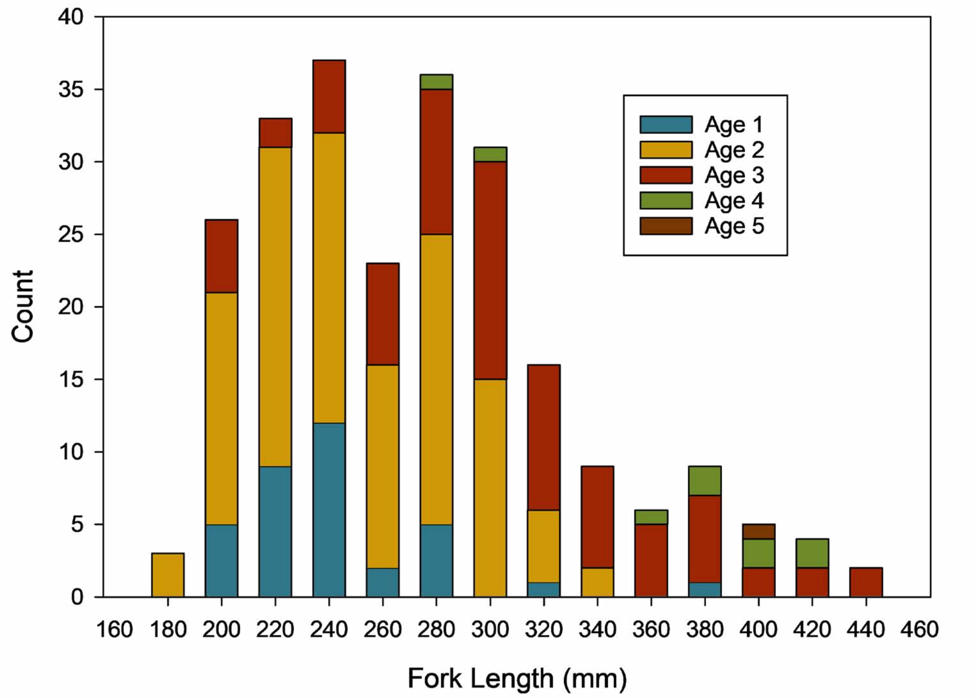

Figure 2. Length frequency distribution and age composition (stacked bars) of Coastal Cutthroat Trout captured in marine water of South Puget Sound, Washington

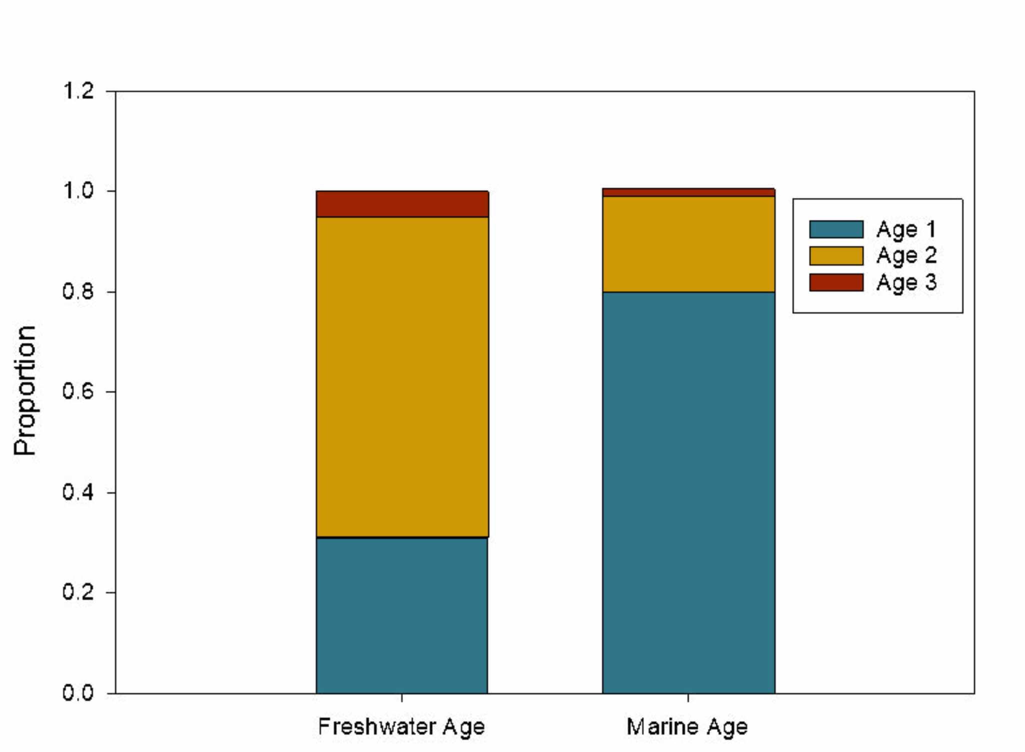

Figure 3. Proportional age composition for the freshwater (juvenile) and marine (adult) phases of anadromous Coastal Cutthroat Trout in South Puget Sound.

Detections from PIT tag arrays revealed that anadromous Coastal Cutthroat Trout utilize a diverse range of unique life history strategies and migratory patterns. To illustrate some of the life history strategies and marine movements that were observed, we highlight the detection histories of unique Coastal Cutthroat Trout below. The first example is a Coastal Cutthroat Trout that was tagged in Totten Inlet in September, 2018 and then detected on the lower Skookum Creek array in July, 2019 and stayed in freshwater until October before entering marine water again. That adult then migrated back into Skookum Creek in March 2020, undergoing both seasonal freshwater movements in the summer/fall and a spawning migration the following spring. While the temporal and spatial aspects of the migration were unique to this Coastal Cutthroat Trout, there was a relatively large number of anadromous Coastal Cutthroat Trout that underwent seasonal freshwater movements in the summer and fall that varied in timing and duration. The second example is a life history pattern that was observed for multiple individuals that were tagged in the early spring in Eld Inlet and then quickly moved across multiple inlets and were detected on the lower Skookum Creek PIT array on spawning migrations (the quickest migration from Eld Inlet to Skookum Creek was ten days). There were also Coastal Cutthroat Trout that were detected making multiple spawning migrations across years, and individuals that made up to three separate trips into freshwater during the summer and fall months.

Discussion

Using PIT tag technology and genetic stock identification, we showed that anadromous Coastal Cutthroat Trout regularly made marine migrations outside of natal inlets. Anadromous trout have been shown to exhibit a variety of different migration patterns from transoceanic migrations of steelhead *Oncorhynchus mykiss* (Quinn and Myers 2004) to short inter-basin migrations of Dolly Varden *Salvelinus malma* (Spares et al. 2015) and Brown Trout (Eldøy et al. 2015) as well as partial expression of anadromy in Dolly Varden and Rainbow Trout (Bond et al. 2015). While studies specifically focused on Coastal Cutthroat Trout in the marine environment are limited, the majority of what is known suggests that Coastal Cutthroat Trout make relatively short distance marine migrations (Goetz et al. 2013), do not migrate across large bodies of water (Jones and Seifert 1997) and are unlikely to leave their natal inlet (Moore et al. 2010; however, longer migrations have been documented, e.g., Pearcy et al. 1990). In the current study, the majority of fish were assigned to their nearby natal streams <15 km away from the capture location, however, a second mode of longer migrating fish was observed. Anadromous Coastal Cutthroat Trout made migrations across multiple inlets, and individuals were captured in non-natal inlets throughout the year at sampling sites. These results are consistent with

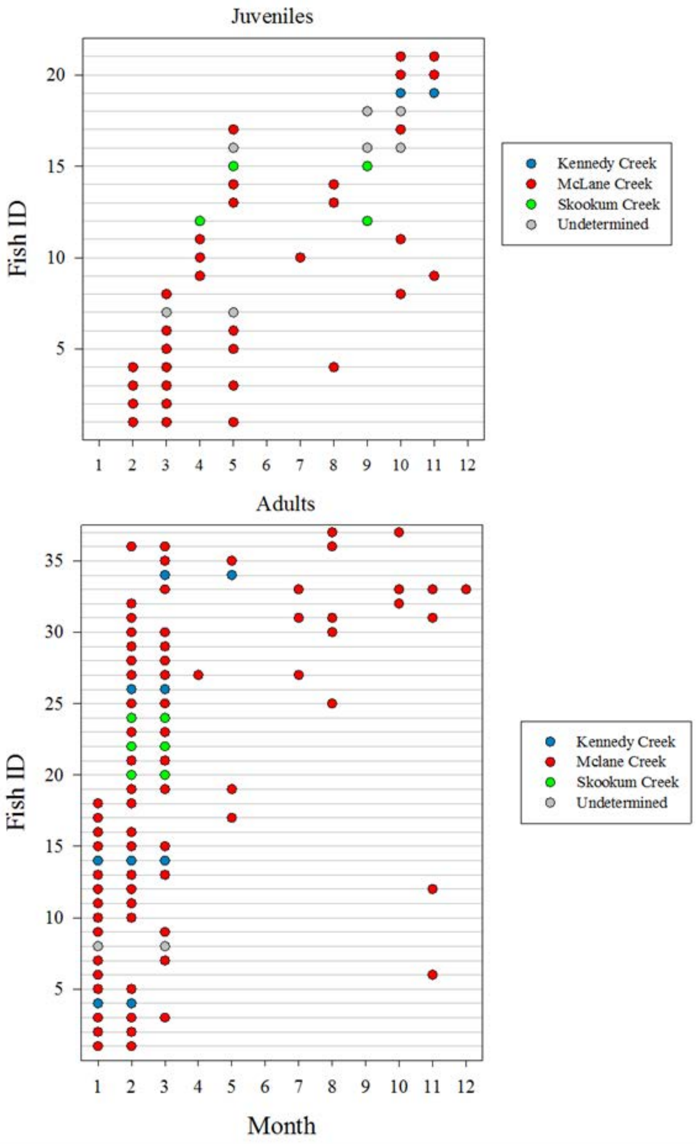

Figure 4. Month of recapture for Coastal Cutthroat Trout. Each horizontal line represents an individual Coastal Cutthroat Trout (Fish Identification [ID]) captured more than one time in Eld Inlet, South Puget Sound Washington in 2015. Dots indicate months of capture (x-axis). Colors indicate genetically assigned stream of origin.

those of Goetz et al. (2013) where most fish underwent short marine migrations (residents) while others exhibited longer migrations (migrants). Overall, information reported here suggests that, unlike Coastal Cutthroat Trout observed in Hood Canal (Moore et al. 2010), Coastal Cutthroat Trout in South Puget Sound regularly leave their natal inlet, cross multiple inlets on spawning migrations, and exhibit a high degree of variability in migration distance.

The PIT tag arrays installed in natal tributaries revealed that anadromous Coastal Cutthroat Trout in South Puget Sound utilize a suite of life history strategies and migration patterns. Anadromous Coastal Cutthroat Trout were observed migrating into freshwater in spring, summer, fall, and winter months which are likely tied to spawning, cool water refugia, or foraging events. It is also possible that anadromous Coastal Cutthroat Trout move into freshwater to shed marine parasites (argulids and copepods) that have been documented on Coastal Cutthroat Trout in South Puget Sound in varying levels of parasite infections (Losee et al. 2022 in review), or for other biological reasons that are not currently understood. Anadromous Coastal Cutthroat Trout also moved into freshwater multiple times throughout the year, and the duration was sometimes as short as one day, highlighting the ability of anadromous Coastal Cutthroat Trout to deal with changes in salinity and move quickly between marine and freshwater environments.

Along with inter-estuarine migrations, we found that Coastal Cutthroat Trout exhibited high site fidelity in an area where they are easily accessible to anglers. Recent work by Washington Department of Fish and Wildlife has identified challenges in management of anadromous Coastal Cutthroat Trout due to their mixed stock composition in marine water (Losee et al. 2017), unpredictable migratory patterns (Moore et al. 2010), variability in spawn timing (Losee et al. 2015) and increasing effort by sport anglers targeting them. Results of the current and past research provide insight on movement patterns of this species and add additional support for conservative regulations to protect Coastal Cutthroat Trout from over-harvest in areas where the remaining nearshore habitat overlaps with fishing access sites. Additionally, catch-and-release regulations most likely provide the greatest economic benefit by maximizing catch rates over the long term for relatively small population sizes (Schill et al. 1986). The high site fidelity to important nearshore habitats and the migration into freshwater tributaries throughout the year emphasizes the importance of protecting the remaining intact nearshore and freshwater habitat that anadromous Coastal Cutthroat Trout depend on.

It is now understood that sport anglers targeting Coastal Cutthroat Trout in marine waters of South Puget Sound encounter a variety of distinct stocks composing mixed stock fisheries. The age and size structure data collected from anadromous Coastal Cutthroat Trout indicates that older (≥ age-4), larger bodied (> 350 mm), adults that had spawned previously were uncommon. Depending on the goals of fish managers, considerations for more fecund, larger females and those stocks that are limiting should be made when designing regulations consistent with a mixed-stock management strategy. By gaining additional information on the movements, age and size structure, and life histories of anadromous Coastal Cutthroat Trout relative to historical information, managers may be better able to evaluate the impact sport fisheries have on Coastal Cutthroat Trout in the marine and freshwater and design fisheries to maximize long-term fishing opportunity on abundant stocks.

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Description of Movement Patterns and Mortality Hotspots of Post Spawn Steelhead *Oncorhynchus mykiss* in a Coastal Stream of Washington State Using Acoustic Telemetry

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Abstract - Following initial spawning, anadromous steelhead trout *Oncorhynchus mykiss* exhibit relatively low yet variable rates of survival and iteroparity. However, on subsequent spawning migrations, adult fecundity and juvenile fitness is often higher than on previous spawning runs. This project explores factors limiting post spawn survival by assessing behavior and movement patterns exhibited by 86 steelhead trout (49 wild and 37 hatchery origin) in a coastal tributary of Willapa Bay, Washington. Each fish was captured, tagged, released, and then tracked using acoustic telemetry receivers placed in river, estuary, and near coastal locations. These receivers were positioned to facilitate the assessment of potential mortality hotspots along the migration route as well as spatiotemporal patterns of post spawn movement more generally. Most wild fish tagged prior to spawning (73%) were not detected following spawning. Of hatchery and wild kelts detected moving downstream, 23% (3/13) of wild fish and 14% (5/37) of hatchery fish successfully returned to the ocean after spawning. Movements of wild fish did not show circular uniformity over a 24-hr period, with 86% of spawning movements occurring between 3:00 pm and 1:00 am Pacific Standard Time (PST) ($P < 0.001$) and 82% of wild out-migration occurring between 8:00 pm and 7:00 am PST ($P < 0.001$). Additional information regarding behavior and survival through various migration segments will be discussed further. This analysis provides fisheries managers with improved tools to maintain healthy populations of anadromous *O. mykiss* across their range.

Introduction

In the context of widespread abundance declines, fisheries managers have increasingly prioritized research on iteroparity among anadromous steelhead trout *Oncorhynchus mykiss* (Narum et al. 2008; Hatch et al. 2013) because repeat spawners typically have higher productive capacity than first time spawners (Seamons and Quinn 2010; Halttunen 2011; Copeland et al. 2019). In fact, Seamons and Quinn (2010) found that steelhead repeat spawners produced twice as many progeny during their lifetimes as one-time spawners. Additionally, iteroparity bolsters population resilience in anadromous salmonids (Crespi and Teo 2002; Moore et al. 2014; Trammel et al. 2016) by providing plasticity in reproductive strategy. However, anthropogenic climate change (Scott and Gill 2008), exploitation of spawning populations, and other human influences may contribute to declining rates of salmonid iteroparity, which vary widely (0-79%) across both spatial and temporal scales (Withler 1966; Savvaitova et al. 1996; Narum et al. 2008; Nielsen and Turner 2011). Regardless, relatively little research has focused on steelhead iteroparity, making it difficult for managers to account for when setting management objectives (Halttuten 2011, Nielson and Turner 2015, Copeland et al. 2019). Gaining a better understanding of the migratory behaviors of post spawn steelhead using acoustic telemetry provides progress towards filing these data gaps.

Acoustic telemetry is commonly used to describe patterns of inter-habitat migrations between freshwater and marine environments among anadromous salmonids (Voegeli et al. 1998; Welch et al. 2004; Kristianson and Welch 2007; Nielsen and Turner 2011). Most of that work has focused on juvenile life stages (Welch et al. 2004; Melnychuk et al. 2007; Harnish et al. 2012; Goetz et al. 2015), however, a growing number of studies have described the behavior and mortality of iteroparous salmonids immediately after spawning (Halttunen et al. 2009; Nielsen and Turner 2011). The descriptive power of acoustic tagging studies across multiple spatial scales is enhanced when widespread receiver networks are available across rivers, estuaries, and the coastal ocean (Teo et al. 2011). In this study, we utilized a network of receivers to track post spawn movements of wild and hatchery origin steelhead from the Willapa River to the near coastal Pacific Ocean. The primary goals of the study were to 1) describe the spatial, temporal, diurnal, and tidal patterns of habitat use and freshwater-marine migration of steelhead kelts, 2) compare biometrics between fish that survived the observed migration period to those that did not, and 3) identify habitat "hotspots" for kelt mortality.

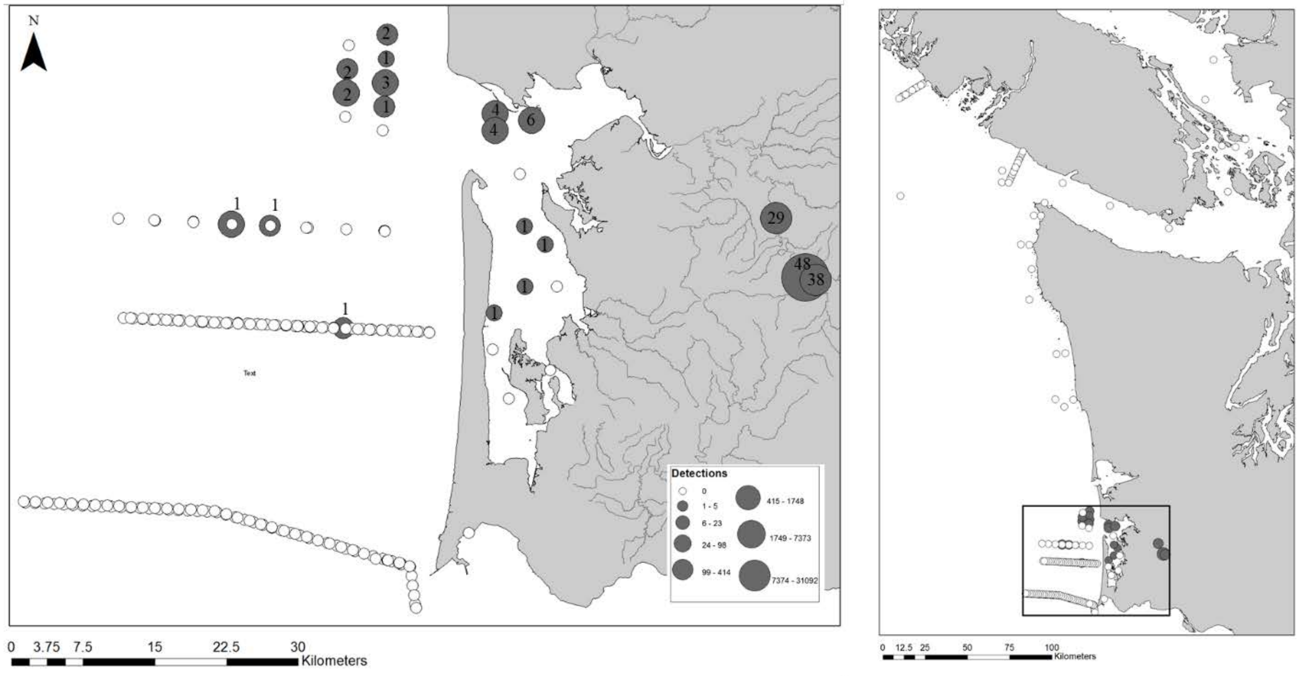

Figure 1: Study area map depicting receiver locations and scaled detections by receiver. The hollow circles represent receiver locations, and the solid circles represent receivers that registered detections. The size of the solid circle and the numbers associated with each of those circles represent the number of fish detected at each receiver location.

Methods

Study Area

Adult winter-run steelhead were intercepted during their upstream spawning migration in Forks Creek, a tributary of the Willapa River, in Pacific County. Here, Washington Department of Fish and Wildlife operates a permanent weir used for hatchery operations at Forks Creek Hatchery. Post spawn kelt migration was tracked from Forks Creek through Willapa Bay, Washington, and the near coastal zone of southwest Washington along the continental shelf (Figure 1). With a long-term mean daily discharge of 62 ft3s-1 (United States Geological Survey), the Willapa River runs approximately 32 km to Willapa Bay, which is the second largest estuary on the Pacific coast of the United States, covering 670 km^2. The bay is made up of three, 10-20 m deep channels surrounded by extensive tidal flats (Banas et al. 2004). A mixed-semidiurnal tide cycle influences the bay, with a mean daily tidal exchange of 2.7 m. Approximately 50% of the estuary's surface area and volume lie in the intertidal zone (Andrews 1965), and brackish water reaches ~29.1 km up the Willapa River (Ashbrook et al. 2007). The coastal zone off southwest Washington is characterized by a relatively narrow continental shelf and is subject to the California Current, a cold-water eastern boundary current associated with strong upwelling.

Acoustic Array

A network of 281 acoustic receivers were utilized in this project, including 3 VR2Tx receivers (https://www.innovasea.com) in the Willapa River (located in Forks Creek near wild steelhead spawning grounds, Forks Creek Hatchery, and in the mainstem Willapa River), 12 in Willapa Bay, two north-south oriented multi-receiver arrays located to the northwest of the mouth of the bay, and three east-west multi-receiver arrays to the southwest of the mouth of the bay (Figure 1). Additional receivers were located along the coastal Olympic Peninsula and Vancouver Island as well as in the Strait of Juan de Fuca and Puget Sound.

Sampling and Tagging

Between January 1st and June 30th, 2021, female steelhead were collected during their upstream migration, identified in the field as hatchery or wild based on adipose fin status (clipped vs. unclipped), and placed in separate holding ponds. Throughout the spawning migration, wild fish were sampled, tagged, and placed upstream on the day of capture. A subsample of the hatchery fish captured was live spawned using electronarcosis gloves (Smith-Root Electric Fish Handling Gloves) two weeks prior to sampling and tagging. At the time of tagging, 49 wild and 37 hatchery steelhead were anesthetized with tricaine methane sulfonate (MS-222; 70 mg/L), measured (FL) and sampled for scales from the preferred area (posterior to the dorsal fin and approximately four scale rows above the lateral line; Davis and Light 1985) to estimate age and validate origin assignments (hatchery vs. wild).

Steelhead were then placed in a polyvinyl chloride surgery cradle filled with water recirculating from an external source to continuously irrigate the gills. An incision was made on the ventral side of each fish just posterior to the pectoral fin and approximately 1 inch from the ventral midline. Acoustic Transmitters (VEMCO V-9) set at low power to ping every two minutes and PIT tags were then inserted into the incisions, which were closed with two sutures of absorbable material. Following surgery, fish were placed in a 500-L tank with oxygenated water for at least 10 minutes to ensure that equilibrium was achieved. After recovery, hatchery fish were returned to the hatchery pond and monitored for an additional week, then released below the weir downstream of capture location on Wednesday February 10th. Wild fish were released upstream immediately after capture between February 10th and May 7th. No mortalities occurred between the time of tagging and release for hatchery or wild fish. Following release, adult steelhead were tracked in the freshwater, estuary and marine environment using VEMCO VR2Tx receivers deployed prior to tagging in locations described above. Data was offloaded from the acoustic telemetry receivers in early May and late June 2021. The receivers in the Willapa River were removed from the field following the final data upload.

Scale Analysis

In the lab, acetate impressions of each scale card were made using a heated hydraulic press for approximately one minute. Acetate impressions were examined by one reader using a Realist Vista microfiche reader (magnification, 48X). The age notation for steelhead we used is described in Loch and Miller (1988) and describes total age, life history and origin (hatchery vs. wild). Briefly, total age was defined as the total number annuli on the scale given a birthday of January 1st. Freshwater annuli are enumerated at the point of marine entry (defined as the discernable and constant increase in circuli spacing).

Data Analysis

The steelhead migration pathway was separated into four segments 1) hatchery to spawning grounds and back (wild origin fish only) 2) hatchery to mainstem 3) mainstem to bay and 4) bay to ocean. The mean duration that hatchery and wild fish spent in Willapa Bay and the detectable near coastal area was calculated. The first and last detections of each fish at each receiver were isolated to identify individual movement events, excluding initial detections at the hatchery release site. Movement events by segment were defined as the duration of time between the last detection at one receiver to the first detection at the next receiver that marked the end of the segment. To estimate migration velocity by segment, the duration of each movement event (in days) was divided by linear segment length (in km) for each fish. Mean migration velocity by segment was then calculated for hatchery vs. wild steelhead and for fish that survived to reach the ocean and those that did not. Next, the percentage of hatchery and wild fish that survived each segment was calculated by dividing the number of fish that survived each segment by the total number of fish that entered each segment.

First and last detections at each site along the outmigration path (excluding initial detections at the hatchery release site and spawning ground detections) were then assessed in relationship to diurnal and tidal cycles, separated by hatchery and wild fish. Rayleigh's test of circular uniformity (Mardia 1972) was used to analyze diel activity patterns. First and last detections were binned by hour for this analysis. The chi-square test for goodness-of-fit was used to test whether fish movements were independent of the tidal phase or the crepuscular period. Finally, biometric attributes of sampled fish were assessed, comparing hatchery vs. wild steelhead and fish that survived to reach the ocean to those that did not.

Results and Discussion

Of the wild fish that were intercepted and tagged during their upstream migration, 73.5% died on or near the spawning grounds. Those fish that survived spawning and migrated downstream took an average of 9.94 days to travel from the release location (hatchery) to the spawning ground and back to the hatchery (a 2.23 km trip), with a mean migration velocity of 0.22 km/d, including time spent on the spawning grounds (Figure 2). Spawning movements were disproportionately oriented toward dusk and nighttime hours and flood and slack tides. Specifically, arrivals and departures from the spawning grounds (n = 50; excluding last detections for fish that did not survive the spawning grounds), did not show circular uniformity, with 86% of those arrivals and departures occurring between 3:00pm and 1:00am PST ($P < 0.001$, Rayleigh's test, Figure 3). A greater proportion of spawning ground movements than expected occurred during flood (0.54 observed; 0.43 expected) and slack (0.18 observed; 0.15 expected) tides, whereas fewer than expected occurred during the ebb tide (0.28 observed; 0.42 expected) ($P = 0.005$, chi-square test). Johnson et al. (2010) also found that steelhead tended to move upstream during nighttime flood tides, although that study included only hatchery fish.

Between the hatchery and the ocean, mean linear migration velocity increased in each successive segment moving towards the ocean for hatchery and wild kelts. Wild fish out-migrated more quickly than hatchery fish on average between the hatchery and the bay, while hatchery fish out-migrated more quickly on average between the bay and the ocean (Figure 2). The average time elapsed between the first and last detections in the bay was greater for wild fish than for hatchery fish, at 0.54 days and 0.11 days, respectively. However, average time elapsed between the first and last detections in the ocean was greater for hatchery fish (0.99 days) than for wild fish (0.02 days).

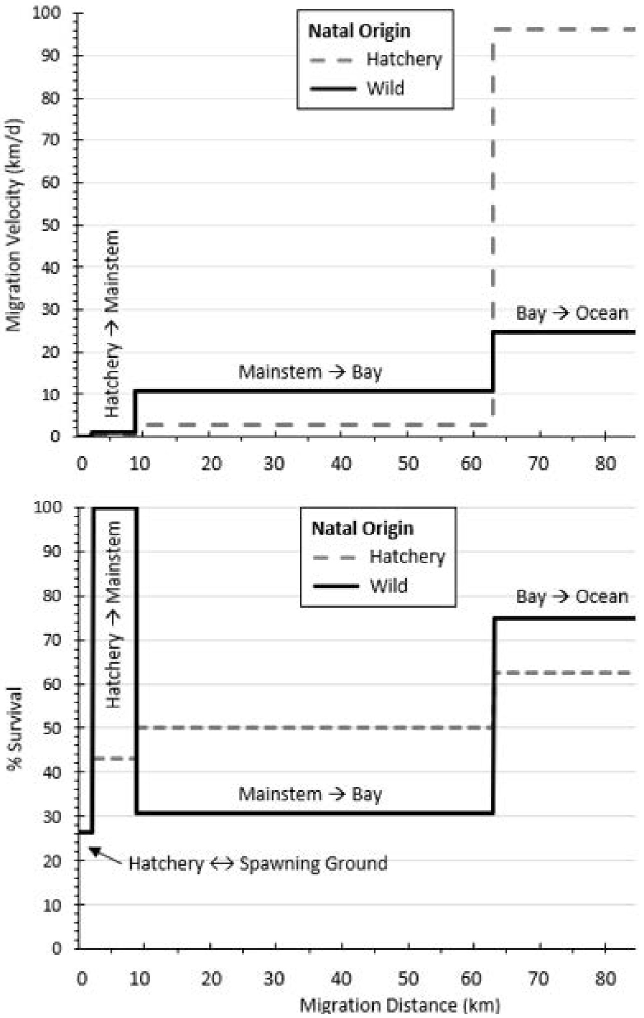

Figure 2: Migration velocity (top panel) and percent survival (bottom panel) by segment from hatchery and wild steelhead. Wild steelhead are tracked from their release point above the hatchery to the hatchery and back towards the ocean, whereas hatchery fish are only tracked during outmigration.

Overall, the proportion of tagged steelhead that were detected during outmigration and survived to reach the ocean was 23.1% (3/13) of wild fish and 13.5% (5/37) of hatchery fish (Figure 4). Of out-migrating wild fish that survived the spawning grounds (13/49), average mortality was 0%/km for the Hatchery-Mainstem segment, 1.21%/km for the Mainstem-Bay segment, and 1.08%/km between the bay and ocean. Of the out-migrating hatchery kelts, average mortality was 8.24%/km for the Hatchery-Mainstem segment, 0.89%/km for the Mainstem-Bay segment, and 1.62%/km between the bay and ocean. The relatively high rate of mortality among hatchery fish in the Hatchery-Mainstem segment could mimic natural spawning mortality, where post-spawn fish are unable to meet the energetic requirements necessary to feed and out-migrate. Mortality among wild fish that occurred immediately after spawning was accounted for in this study, whereas hatchery fish were kept in an artificial environment (hatchery pond) with adequate water supply, temperature and free of predators for

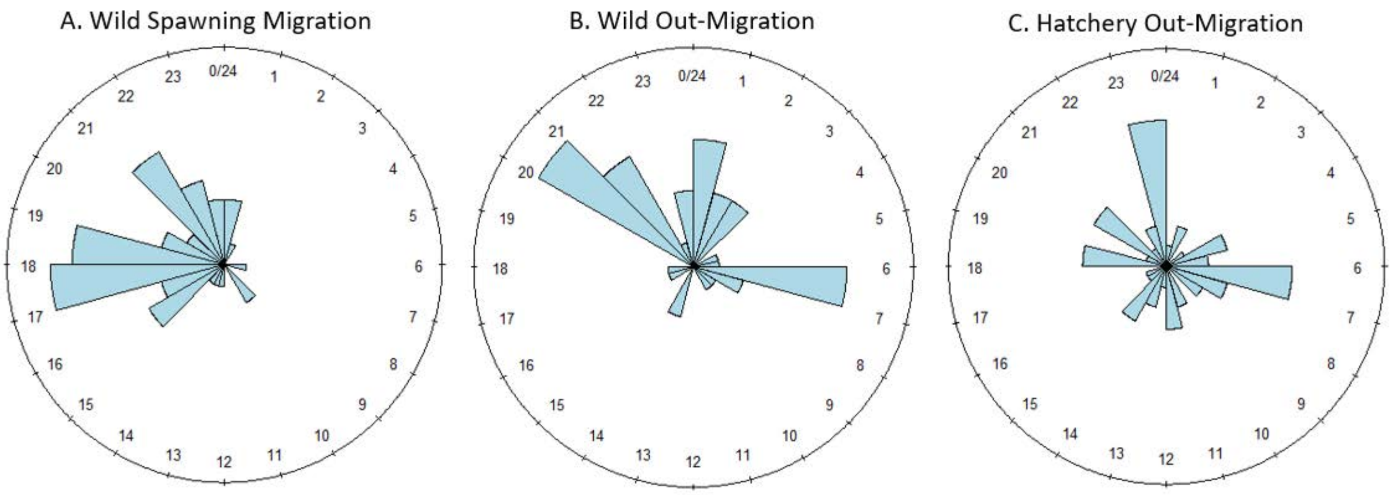

Figure 3: Circular distribution of steelhead movements over a 24-hr period. Movements were defined as first or last detections at a given receiver, excluding first detections at the hatchery release site at the time of release. Wild spawning migration and wild out-migration did not show circular uniformity (P < 0.001 in both cases), whereas circular uniformity cannot be rejected for hatchery out-migration movements (P = 0.65).

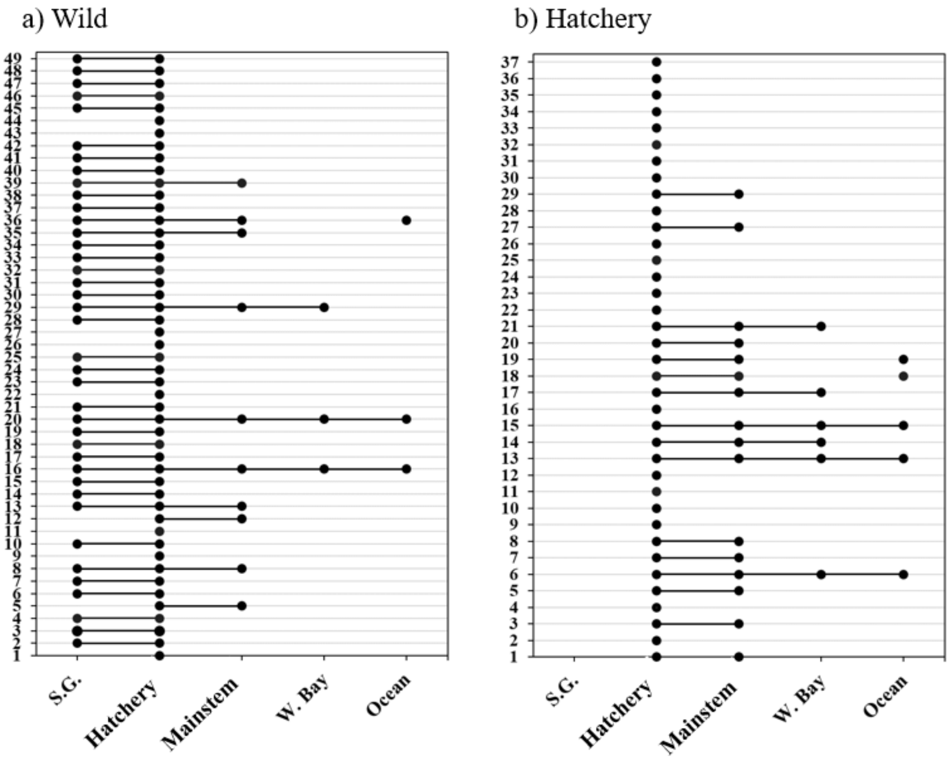

Figure 4: Wild and hatchery steelhead detections between the spawning grounds (wild only) and hatchery-ocean (wild and hatchery fish).

one week prior to release into the river. Variation in migration patterns between wild and hatchery fish may also be linked to differences in fitness, which manifest through differences in genetics, physiology, size, and behavior (Goetz et al. 2015). Assessment of biometric data showed little difference between the attributes of fish that did or did not survive to reach the ocean. However, the average time spent in each segment was higher for survivors vs. mortalities in all segments. Only three missed detections were documented; one wild steelhead and two hatchery steelhead were detected in the ocean but not the bay (Figure 4).

Wild Steelhead movements showed circular non-uniformity over 24-hour cycles during out-migration ($P < 0.001$), but hatchery steelhead movements did not ($P = 0.65$). Among wild out-migration movements between the hatchery and the ocean, 81.8% occurred between 8:00 pm and 7:00 am PST (Figure 3). This adds to a growing body of literature documenting the variations in diel behavior among steelhead (*see* Reeves et al 2010; Keefer et al. 2013; Goetz et al. 2015), although much of that work has focused on smolt migration. Anadromous salmonid smolts predominantly migrate down river after dusk or during the night (Godin 1982; Moser et al. 1991; Crittenden 1994; Ibbotson et al. 2006; Melnychuck 2007; Johnson et al. 2010; Goetz 2015), but transition to diurnal activity in estuaries (Ledgerwood et al. 1991). In this study, analysis of movements relative to tidal phase indicates that there is no significant variation between the expected and observed proportion of movements by adult wild of hatchery steelhead during ebb, flood, and slack tides.

Given declining population abundance and rates of iteroparity among steelhead coupled with the increased productive capacity of repeat spawners relative to first-time spawners, fisheries managers have recently included measures to protect steelhead kelts in fisheries management plans. This study provides information on the migration behaviors of steelhead kelts that can be used to inform management as well as progress towards filing the existing data gap pertaining to steelhead iteroparity on the southwest Washington coast.

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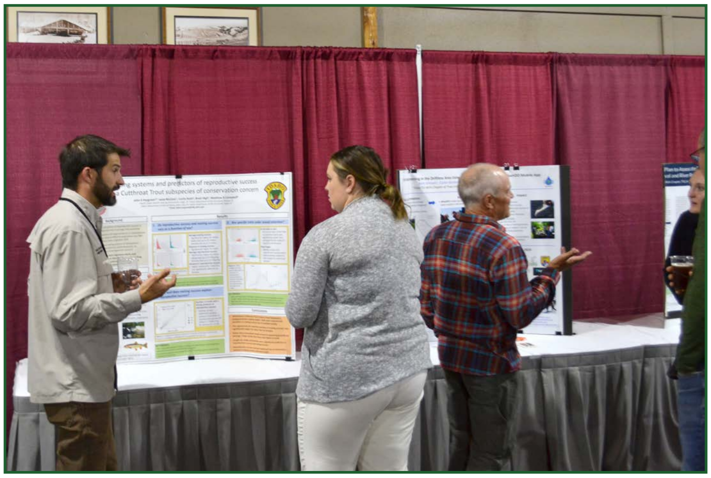
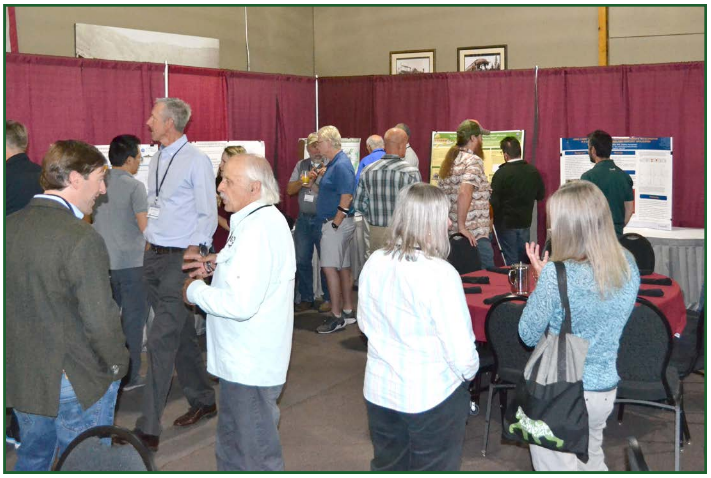
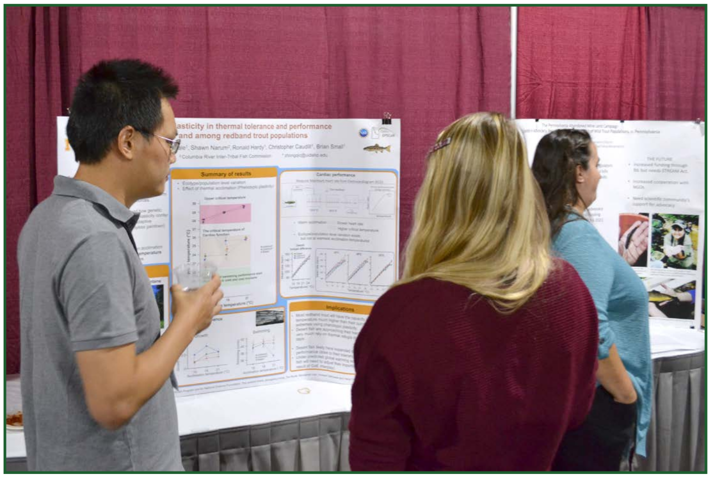
Poster Presentation







Poster Presentation Abstracts (in alphabetical order)

Presenting Author	Poster Title and Abstract
Batchelor, Sarah	**A Landscape Transcriptomics Approach to Evaluating Thermal Stress in Wild Populations of *Salvelinus fontinalis*** **Abstract:** Brook Trout *Salvelinus fontinalis* are a high conservation priority within its native range in the eastern USA and Canada. Climate change is a primary threat to the persistence of this ecologically important species and popular game fish. Its distribution is highly influenced by temperature; Brook Trout are rarely found in streams that reach average summer water temperatures >22 °C. It is at these elevated water temperatures where known physiological responses to thermal stress begin to occur, such as increases in expression of heat shock protein 70 and plasma glucose concentration. Although much is known about the thermal tolerance of Brook Trout and select physiological indicators, we lack a thorough mechanistic understanding of the molecular and physiological pathways underlying heat stress. Therefore, the purpose of this study was to utilize a whole-genome approach called landscape transcriptomics that identifies in an unbiased way all genes whose expression are related to current and/or past habitat conditions. This may reveal additional pathways associated with thermal stress physiology, and variation in response by different populations. Fifteen individuals were collected from nine populations in the summer of 2021. The streams sampled vary in their summer temperature profiles (i.e., mean and temporal variability), with some streams occasionally exceeding 25 °C. Expression levels of all genes (transcriptomes) were quantified using 3’ TagSeq for six individuals from each population. Additionally, the sex of each individual was determined with a genetic marker. We present results relating sex and current and past temperature to gene expression. Results from this study may add critical information to what is already known about Brook Trout thermal stress response and associated biomarkers in wild Brook Ttrout populations, and may reveal applications for fisheries conservation and management regarding the response of organisms to rapidly changing environments.
Chen, Zhongqi	**Phenotypic Plasticity in Thermal Tolerance and Performance Within and Among Redband Trout Populations** **Abstract:** Fishes respond to environmental changes using phenotypic plasticity and evolutionary adaptation. Phenotypic plasticity can buffer organisms from diel and seasonal environmental fluctuations within a lifetime, while evolutionary adaptation occurs over a longer timescale across generations. To understand the role of phenotypic plasticity in thermal adaptation, we reared Redband Trout *Oncorhynchus mykiss gairdneri* from contrasting climates (i.e., ecotypes of the desert, cool montane and cold montane) under 15 °C, 18 °C and 21 °C in a common garden setting, and examined several ecological important traits including critical thermal maximum (CTMAX), maximum growth rate, critical swimming speed and cardiac performance. We found plasticity existed in all traits, but it was trait-specific and varied among ecotypes. Although CTMAX showed limited variation among ecotypes, it is plastic and positively correlated with acclimation temperature. Maximum growth rate, in contrast, had a strong genetic-by-environment interaction. The result from the desert Redband Trout suggested that adaptation to warmer environments may lead to a slower growth rate. Critical swimming speed showed less phenotypic plasticity, at least at the population level. However, the swimming performance of cold montane fish was severely impaired at 21 °C acclimation. In addition to the whole organism level performance, we also investigated the organ level performance by examining cardiac function. It is not surprising that warm acclimation reduced heart rate. Interestingly, ecotype level variation in cardiac function exists, but not at the warmest acclimation. These findings indicate that plastic responses differed by trait and ecotype; further niche modeling that integrates phenotypic plasticity needs to consider its complexity for a better predictive power for population-level adaptive capacity under future climate change scenarios.
Dauwalter, Daniel	**Water Quality Screening in the Driftless Area Using Community Scientists and the WiseH2O Mobile App** **Abstract:** We have been encouraging anglers and other community scientists to collect water quality information in the Driftless Area using the WiseH2O mobile application. Since the pilot program in 2019, 607 total water quality observations have been made, with 547 observations being made during 2020-2021 and most in the northern half of the Driftless Area. Of the 2020-2021 observations, 95% have been on state designated trout

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	streams and 42% have been made on Brook Trout *Salvelinus fontinalis* streams. Observations have been made by 102 unique observers (participants), and 53 unique observers (42 new) submitted an observation in 2021. Of the 403 observations made in 2021, 88 occurred during the "September Sampling Blitz" contest used to encourage participants to make observations during the last part of the fishing season. Three Trout Unlimited Chapters have set up their own monitoring programs. Enhancements to the WiseH2O app and program infrastructure continue to be made, and 2022 will include development of an actionable data framework for water quality screening information collected using the WiseH2O app. More information can be found at: https://www.mobileh2o.com/driftlessprogram.
Hargrove, John	**Mating Systems and Predictors of Relative Reproductive Success in a Cutthroat Trout Subspecies of Conservation Concern** **Abstract:** Mating systems and patterns of reproductive success in fishes play an important role in ecology and evolution. While information on the reproductive ecology of many anadromous salmonids *Oncorhynchus spp.* is well-detailed, there is less information for non-anadromous species including the Yellowstone Cutthroat Trout *O. clarkii bouvieri,* a subspecies of recreational angling importance and conservation concern. Using data from a parentage-based tagging study, we described the genetic mating system of a migratory population of Yellowstone Cutthroat Trout, tested for evidence of sexual selection, and identified predictors of mating and reproductive success. The standardized variance in mating success (i.e., the opportunity for sexual selection) was significantly greater for males relative to females, and while the relationship between mating success and reproductive success (i.e., Bateman gradient) was significantly positive for both sexes, a greater proportion of reproductive success was explained by mating success for males ($R^2 = 0.80$) than females ($R^2 = 0.59$). Overall, the population displayed a polygynandrous mating system, whereby both sexes experienced variation in mating success due to multiple mating, and sexual selection was variable across sexes. Tests for evidence of sexual selection indicated the interaction between mating success and total length best predicted relative reproductive success. We failed to detect a signal of inbreeding avoidance among breeding adults, but the group of parents that produced progeny were on average slightly less related than adults that did not produce progeny. Lastly, we estimated the effective number of breeders (Nb) and effective population size (Ne) and identified while Nb was lower than Ne, both are sufficiently high to suggest Yellowstone Cutthroat Trout in Burns Creek represent a genetically stable and diverse population.
Hering, Dave	**Salmonid Response to Restored Stream Connectivity in Oregon's Upper Klamath Basin** **Abstract:** Many tributaries in Oregon's Upper Klamath Basin were modified and diverted for flood irrigation during the late 19th and early 20th centurys. Habitat alteration blocked migratory pathways and reduced rearing potential for the basin's native salmonids, Bull Trout *Salvelinus confluentus* and Great Basin Redband Trout *Oncorhynchus mykiss newberrii*. In 2017 federal, state, and private partners restored 4.3 km of free-flowing stream through a working cattle ranch to re-connect historically altered Sun Creek to the Wood River. The project transferred a portion of the ranch's irrigation rights to in-stream use and replaced a dendritic network of ditches with year-round cold-water habitat that approximates the stream's historical alignment. From 2017 to 2022, we conducted repeated electrofishing surveys to assess the response of the fish community to restoration. Redband Trout rapidly colonized the restored stream reach and now rear in the reconnected habitat at age-0 and age-1 before migrating downstream to contribute to an adult fishery in Upper Klamath Lake. Bull Trout first occupied the project area in 2020 and increased in abundance during subsequent surveys. Bull Trout that were PIT-tagged have moved between the restored tributary and mainstem river, and several Bull Trout also moved upstream through a fish trap in the summer of 2021, suggesting restored connectivity may be promoting migratory behavior in the ESA-listed char population. One tradeoff of increased connectivity is that nonnative Brook Trout *S. fontinalis* and Brown Trout *Salmo trutta* continually invade the restored habitat from downstream, requiring management agencies to suppress nonnatives through annual mechanical removal. Although still in the early stages of post-restoration development, this project exemplifies successful collaboration among multiple stakeholders and demonstrates that restored stream connectivity can yield immediate benefits to potamodromous native trout.
Jimmo, John	**Using eDNA to Determine the Effectiveness of Non-Native Brook Trout Removal in Hidden Lake in Banff National Park** **Abstract:** Alberta populations of Westslope Cutthroat Trout *Oncorhynchus clarkii lewisi* (WSCT) are listed

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	as Threatened under Schedule 1 of Canada's Species at Risk Act and are threatened by hybridization and competition with non-native trout. In Banff National Park, WSCT were historically present within many headwater systems, including the Hidden Lake area, near Lake Louise, Alberta. Here, a population of WSCT persisted above a barrier among creeks and a subalpine lake until it was extirpated following the introduction of non-native Brook Trout *Salvelinus fontinalis* (BKTR). Due to the isolation of the system and quality fish habitat, Parks Canada Agency undertook a BKTR removal project to facilitate future WSCT reintroduction in the Hidden Lake area. Ensuring complete removal of BKTR from the system was an essential component of the Hidden Lake restoration project. Following unsuccessful efforts to eradicate BKTR at Hidden Lake through physical removal (e.g., angling, electrofishing and gill netting), Parks Canada applied a fish toxicant, Prentox Nusyn-Noxfish, to waterbodies upstream of a waterfall in 2018 and 2019. Assessing physical fish presence was the primary management effectiveness monitoring approach, and no presence of non-native BKTR was detected following the 2018 fish toxicant application using this method. To supplement management effectiveness monitoring of the non-native BKTR removal, environmental DNA (eDNA) samples were collected from 12 locations within the treatment area. As expected, significant sources of BKTR eDNA were detected throughout the headwater system following the initial fish toxicant treatment in 2018. In 2019, trace amounts of BKTR eDNA were found both pre- and post- fish toxicant application but were absent in August 2020. Environmental DNA proved to be a useful tool in determining the effectiveness of fish toxicant applications when compared to physical presence monitoring methods such as gill netting and electrofishing.
Johnson, Kent	**A Monitoring Plan to Assess the Ecological Benefits of Kinnickinnic River Dam Removal and River Restoration in River Falls, Wisconsin** **Abstract:** The Kinnickinnic River (Kinni) in western Wisconsin is a Class I trout stream and an Outstanding Resource Water, according to the Wisconsin Department of Natural Resources (WDNR). In 2018, the River Falls City Council authorized the removal of both hydropower dams (Junction Falls and Powell Falls) on the Kinni. Dam removal will return the Kinni to a free-flowing river for the first time since 1902, providing a significant opportunity for river restoration through aquatic and riparian habitat creation and fish passage barrier removal. The 2018 Council resolution stated that the dam removal and river restoration process should be documented to evaluate ecological restoration outcomes. To meet the intent of the City's resolution, Inter-Fluve and the local Kiap-TU-Wish Chapter of Trout Unlimited, prepared "A Monitoring Plan to Assess the Ecological Benefits of Kinnickinnic River Dam Removal and River Restoration in River Falls, Wisconsin" (Kinni Monitoring Plan) in May 2021. The plan was crafted with input from multiple project partners, including the City of River Falls, Kinni Corridor Collaborative (KinniCC), Kinnickinnic River Land Trust, The Prairie Enthusiasts (TPE), Kiap-TU-Wish, University of Wisconsin-River Falls (UWRF), United States Geological Survey, and Wisconsin Department of Natural Resources. The Kinni Monitoring Plan features five key monitoring components: aquatic biology, riparian biology, photo documentation, water quality, and fluvial geomorphology. Plan implementation will require coordination amongst a diverse group of professional scientists and volunteers from local organizations. A matrix of community volunteer monitoring opportunities was created to inform volunteer and student participation by KinniCC, TPE, Kiap-TU-Wish, and University of Wisconsin-River Falls. Monitoring of baseline Kinni conditions prior to Powell Falls Dam removal and river restoration is scheduled for April-October 2022. Future post-restoration monitoring will evaluate the ecological and community benefits. The 10-year budget for the monitoring plan is approximately $80,000.
Lavelle, Kathleen	**Determining Aquatic Passability Through Road Stream Crossings Post Policy Change in Pennsylvania** **Abstract:** Road stream intersections are a leading factor of habitat fragmentation and barriers to aquatic organism passage (AOP). Pennsylvania has approximately 180,000 road miles overlapping over 86,000 stream miles. Limited AOP impacts species, like salmonids, that rely on cold contiguous habitat and the ability to move

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	upstream to spawn. Frequently when a culvert presents a barrier to AOP it is undersized/lacks flood resiliency. Undersized crossings offer a smaller area for water flow, increasing flow velocity through the structure and limiting fish passage, debris transport, and threaten roadway infrastructure. Trout Unlimited has been a lead partner in Pennsylvania to assess crossings for AOP through North Atlantic Aquatic Connectivity Collaborative (NAACC). Trout Unlimited has strategically replaced crossings to reconnect crucial Brook Trout *Salvelinus fontinalis* habitat while encouraging smart design for increasing flows/storm events and a changing climate. A 2015 policy update in Pennsylvania dictated crossings on dirt/gravel roads to be built to bankfull. Between 2015-2017, 229 crossings were replaced. Trout Unlimited randomly selected 46 of these crossings of various structure types to evaluate and quantify the effectiveness of completed road/stream crossing projects in terms of design specifications, project cost, and AOP. The NAACC data was incorporated with longitudinal profiles of both constructed and reference reaches at each site. The NAACC scores and United States Forest Service FishXing models were used to determine AOP/flood resiliency through crossings. In brief, the results of this study revealed that round culverts were the most limiting structure type, were on average less than bankfull width, and lacked continuous substrate throughout the constructed reach. The results of this study provide technical guidance and recommendations on practical approaches to maximize project success with respect to AOP as well as providing geomorphic continuity between the upstream and downstream segments.
Masingale, Jonathan	**The Heart of the Issue: What Drives Inter-Population Differences in Behavioral Plasticity?** **Abstract:** Temperature is a crucial environmental factor that governs physiology and behavior in ectotherms and limits the distribution of coldwater fishes such as trout. In response to warming conditions, trout display traits reflecting genetic adaptation and phenotypic plasticity. Trout exhibit thermal habitat selection behaviors that mitigate losses in physiological performance as temperatures become physiologically stressful. Cardiac function is a limiting factor in thermal performance and tolerance. In fishes, the heart must work harder to meet elevated O_2 demands of increased metabolic rate and decreased O_2 availability caused by warmer temperatures. We are evaluating how a suite of genetic and plastic traits may influence the adaptive capacity of Redband Trout *Oncorhynchus mykiss gairdneri* (RBT). As stream temperatures increase across the intermountain west, fluvial ectothermic species like RBT are especially vulnerable because dispersal is limited to the extent of stream networks. We collected newly hatched RBT from nine streams spanning a thermal gradient from desert to cold montane forest; we reared fish in a common garden setting using three constant temperature regimes (15, 18, and 21 °C) and three diel fluctuating temperature regimes (±2 °C). Thermal preference data were collected using video tracking software that allowed a fish to determine its preferred temperature. Cardiac function data was collected by monitoring heart rate response to acute warming until peak heart rate was reached. We hypothesized that optimum cardiac performance is positively correlated with natural thermal regime, and that the physiological optimum window shifts with acclimation temperature, causing thermal preference to positively correlate with acclimation temperature. We also hypothesized that diel fluctuating temperatures will widen the scope of thermal tolerance, increasing the upper thermal range of optimum physiological performance. These data will assess the relative contribution, importance, and interactions among genetic and plastic traits and feed into climate change models to predict the adaptive capacity of RBT.
McAllister, Andrew	**The Pennsylvania Abandoned Mine Land Campaign: Cooperation in Advocacy Resulting in the Restoration of Wild Trout Populations in Pennsylvania** **Abstract:** In Pennsylvania, highly acidic and metals-laden discharges caused by abandoned coal mines have rendered over 5,500 miles of streams in the state uninhabitable by aquatic life. For over 20 years, volunteers, non-profit organizations, and state and local agencies have been working together to restore Pennsylvania's wild trout waters impaired by Abandoned Mine Drainage (AMD). To a great extent, Pennsylvania's success in reclaiming abandoned mines is due to cooperation between state agencies and Non-Government Organizations (NGOs). In headwater streams, there are numerous demonstrations of how this partnership is improving water quality and bringing wild trout back. In the Upper West Branch of the Susquehanna River watershed, data presented shows how cooperative AMD restoration activities have resulted in wild trout restoration or enhancement.

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	The majority of funding for the AMD treatment activities for land and water projects in Pennsylvania stems from federal grants to the states originating from fees collected from the active mining industry under the Surface Mining Control and Reclamation Act (SMCRA). The Pennsylvania Abandoned Mine Land (PA AML) Campaign, a loosely-organized group of organizations and individuals advocating for policies and programs that benefit coal-impacted communities, was instrumental in bringing about reauthorization of SMCRA in 2006 and again in 2021 and assisted in efforts to include AMD funding in the Infrastructure Investment and Jobs Act (IIJA) passed by Congress in 2021. Currently, the PA AML Campaign is partnering with state and tribal agencies across the nation to advocate for more flexibility in how the states can use the IIJA funding to address long-term Operation, Maintenance, and Replacement (OM&R) of AMD treatment systems. This poster highlights how the PA AML Campaign's advocacy for the reauthorization of SMCRA is an example of cooperation among NGOs and government resulting in the restoration of wild trout populations in Pennsylvania.
Oliveira, Michael	**Using CABIN to Monitor Benthic Macroinvertebrate Recolonization in a Lotic System Following Fish Toxicant Application** **Abstract:** The Canadian Aquatic Biomonitoring Network (CABIN) is a national program developed by Environment and Climate Change Canada to measure freshwater ecosystem health. The CABIN provides a standardized sampling protocol that can be used to conduct consistent, comparable, and scientifically credible biological assessments of lotic systems. Site-specific information including channel data, geology, local climate, hydrology, benthic macroinvertebrates (BMI), and water chemistry are collected at each test site and entered into an area specific (i.e. Canadian Mountain National Parks) reference condition approach (RCA) model. Aquatic ecosystem health and condition from each sampled location can then be analyzed and interpreted. The BMI taxonomic results were then used to compare community composition, species richness, and abundance against a selection of reference sites that comprise the RCA Model. In 2018 and 2019, Parks Canada completed a conservation project involving the use of a fish toxicant (Prentox Nusyn-Noxfish) to remove non-native Brook Trout *Salvelinus fontinalis* (BKTR) from a small headwater system in Banff National Park. Consecutive years of fish toxicant applications on Hidden and Corral Creeks resulted in a temporary collapse in the BMI community within this headwater system. The CABIN was used to monitor the changes and subsequent recolonization of the BMI communities in these streams following non-native BKTR removal with a fish toxicant. Using the RCA approach to analyze the composition of the benthic macroinvertebrate communities from Hidden and Corral Creek, we were able to quantify the change in community composition and identify the recolonization of these systems in the subsequent years following fish toxicant applications. Sampled locations within the Hidden Lake headwater system displayed recolonization of BMI and a return to reference conditions one year following applications.
Platis, Nitsa	**Using Fin Clips as a Non-Lethal Alternative for Stable Isotope Analysis in Juvenile and Small-Bodied Fish: An Evaluation of the Relationship Between Fin and Muscle Isotopic Signatures (15n and 13c)** **Abstract:** Stable isotope analysis (SIA) of muscle tissue is an increasingly popular method used to evaluate trophic characteristics (diet composition and trophic positioning) of fish, however attaining muscle tissue samples from juvenile or small bodied fish can be detrimental, if not lethal for the individual. Fin samples have been suggested as a non-lethal alternative for SIA, but a primary concern is whether fin and muscle tissue vary in isotopic signatures due to differences in composition or turnover rates. To assess the viability of using fin samples as a non-lethal alternative for SIA, we examined the relationship between caudal fin and dorsal muscle tissue isotopic signatures (15N and 13C) of juvenile Brown Trout *Salmo trutta* and Mottled Sculpin *Cottus bairdii*. We collected samples from 272 juvenile Brown Trout and 215 Mottled Sculpin ranging in size (80 - 200mm; 80 - 135mm) in the spring, summer, and fall of 2022, to evaluate interspecific variation, as well as the potential effects of body size and temporal variation on isotopic relationships. We expect our results will indicate fin clips are a viable non-lethal substitute for stable isotope analysis, providing an alternative approach that can reduce the mortality of juvenile and small-bodied fish in trophic studies.

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Rogers, Karli	**Using Crowdsourced Data to Estimate Stream Fish Abundance** **Abstract:** Technological innovations provide new opportunities to generate large datasets and engage the public in ecological research with crowdsourcing and citizen science platforms. We collected underwater 4K video samples from stream pools in Shenandoah National Park, Virginia and provided these samples online for participants to visually estimate the abundance of Brook Trout *Salvelinus fontinalis*. Counts of adult Brook Trout were compared among student age groups and regions of residency. Participant-generated counts were also compared to expert observations. We found that the accuracy and precision of counts were not affected by the age of the participant but increased with the number of observations. Bootstrap resampling of participant counts indicated among-site variation in accuracy and precision that can inform future monitoring designs.
Ruetz, Carl	**Remote Site Incubators: Recommendations for Managers** **Abstract:** Remote site incubators (RSIs) have been used successfully to establish salmonids in streams and are currently under consideration for use in the reintroduction effort of Arctic Grayling *Thymallus articus* in Michigan. To support fish conservation efforts, we developed a simple model to predict fry yield and uncertainty from RSIs. Specifically, we explored the dependence of fry yield (number of fry produced from a given number of eggs in a RSI) on egg hatching success, RSI failure rate, and number of RSIs employed. Our objectives were to: (1) explore whether managers should divide eggs among more than one RSI at a site, and (2) understand how factors that managers control (e.g., initial number of eggs, number of RSIs, RSI failure rate, and egg hatching success) should be adjusted to increase the expected value (mean) and decrease uncertainty (coefficient of variation; CV) of fry yield. Our model showed that using additional RSIs at a site had no effect on the expected value of fry yield, but CV exhibited a pattern of diminishing returns as the number of RSIs increased. Ideally, reintroduction efforts should be designed so that the expected value of fry yield is high and CV is low. We found that managers are most likely to increase the expected value of fry yield by increasing the initial number of eggs, RSI success, and egg hatching success. Moreover, managers can most effectively decrease the CV of fry yield by increasing the number of RSIs, increasing RSI success, and decreasing variation in egg hatching success. Given that using multiple RSIs adds costs and complexity to a project as well as the pattern of diminishing returns in the CV as more RSIs are added, we recommend using two to three RSIs at a site depending on the number of eggs available for incubation.
Siepker, Michael	**Using Aerial Imagery to Assess the Distribution of Coldwater Streams in the Iowa Driftless Region** **Abstract:** Iowa coldwater streams are important habitats for several species of greatest conservation need, including Brook Trout *Salvelinus fontinalis* and two sculpin species *Cottus spp*. To effectively manage coldwater streams, we must first know where they exist on the landscape. During winter, coldwater streams, with warmer groundwater input, remain free from ice cover whereas warmwater streams become ice covered. Winter imagery for several watersheds in the Driftless region of Iowa was used to examine (i) the potential of high-resolution winter imagery for detecting coldwater streams and (ii) the accuracy of existing Iowa surface water classifications for coldwater streams. Multi-temporal winter imagery was visually interpreted over the entire study area to designate coldwater habitat at two confidence levels. At level 1, streams were clearly open water with few to no obstacles complicating image interpretation and at level 2, stream status was not easily determined due to image limitations (image angle, clouds, tree cover). Detected stream reaches were compared to the existing Iowa coldwater stream designations and spatial agreement and disagreements were examined. Existing designations excluded significant stretches of coldwater streams that are currently classified as warmwater streams. Existing fish population data and water temperature data should be used to support the reclassification from warmwater to coldwater of several stream segments detected during this study.
Smith, Rebecca	**Native Brook Trout Reintroductions: Do a Source's Population Genetics Influence Reintroduction Success?** **Abstract:** Wildlife reintroductions are a conservation tool to re-establish native species to their historical ranges. However, identifying appropriate source populations for reintroductions can be a challenge because introduced genotypes may not be well suited for the target environment. In practice, reintroductions are rarely preceded by detailed genetic evaluation. A threatened species in Southern Appalachia is the Southern Appalachian Brook Trout *Salvelinus fontinalis*, which has been extirpated from its historical range due to anthropogenic impacts

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	and competition with non-native species. In the Great Smoky Mountain National Park (GRSM), Brook Trout are the only native salmonid but have been isolated in remote headwater systems since the early 1900s, exchanging little to no genetic information between populations. State and federal agencies have been strategizing to restore the genetically unique Southern Appalachian Brook Trout to their native range and reintroduction efforts are ongoing in GRSM. Currently, there are over 30 miles of reintroduced Brook Trout habitat in the National Park, which encompasses 13 different streams. To prevent depleting source populations, managers use multiple native Brook Trout populations as sources in one reintroduced habitat. Recent studies suggest multiple source populations do not readily admix as a consequence of being too genetically differentiated. This suggested nonrandom admixture raises concerns about assortative mating or outbreeding depression. My research addresses admixture concerns using high-throughput sequencing to assess the genetic composition of reintroduced populations. My research aim is to contribute and guide management's selection of source populations in a single reintroduction site.
Stitt, Brad	**Paying Forward: International Conservation and Restoration** **Abstract:** Endangered species of Argentina are facing threats from non-native and invasive species, similar to Canadian freshwater fishes. Action is required to ensure that these species are not extirpated from their historical occupancy or lost altogether. The persistence of the Naked Characin *Gymnocharacinus bergii* and the rincon-stream frog *Pleurodema somuncurense* are in jeopardy due to the non-native Urugauy Tetra *Cheirodon interruptus* and Rainbow Trout *Oncorhynchus mykiss*. Argentina has attempted to quell the pressure on endangered species through active management activities such as removal of non-native species, isolation of endangered species, and prevention of non-native species migration. Efforts had not been successful and alternatives needed to be investigated. Piscicides were identified as the most efficient and effective tool. An action plan to implement a piscicide application was initiated. Parks Canada Agency (PCA) had experienced the same revelation within the past 10 years and had implemented piscicide applications in multiple headwater systems of the Bow River to facilitate conservation and restoration of Westslope Cutthroat Trout *Oncorhynchus clarkii lewisi*, a threatened species-at-risk. While PCA was in the initial stages of planning and building our piscicide program we were assisted by government counterparts in Montana, USA. Their knowledge, experience, and expertise sharing was instrumental in allowing PCA to successfully plan then execute operational activities. Participating and sharing experience on the initial Argentine project provided a great opportunity to 'pay-forward' knowledge and experience within the international community. Piscicides can be contentious due to their non-selective nature. Used incorrectly, there may be ramifications to government and public interest in future use of this management tool. This poster highlights the planning and execution of international collaboration for the conservation and restoration of endangered species.
Valentine, George	**A New Dawn As Neuro-Endocrinology Meets Wild Fish Management: Hypothalamic-Pituitary-Interrenal Axis Key Gene Expression, Uplc-Ms/Ms Glucocorticoid Quantification and Biochemical Profiling Enabling Accurate Monitoring and Restocking of Wild Fish Populations with Stress Resilient Bred Fish** **Abstract:** Brook Trout *Salvelinus fontinalis* are a culturally and economically important native species in the southeastern USA. Populations of these cold water obligates are affected by local-scale factors such as stream temperature and flow. Current and future climatic changes in their native range mean that Brook Trout are increasingly threatened, and that habitats with stable, buffered temperatures will act as important refugia. However, thermal regimes differ among streams because they are mediated by landscape characteristics such as elevation, gradient, landcover, and groundwater. Understanding how stream temperature interacts with local landscape features to influence spatiotemporal dynamics in Brook Trout populations is critical to developing forward-thinking management. Here, we use paired air-water temperatures at 204 representative sites throughout the southeastern USA to calculate several metrics of thermal stability. We use machine learning techniques to link these metrics to publicly available landscape variables with the goal of predicting resilient Brook Trout habitat across the southeast USA. Preliminary findings suggest that stream temperatures are most

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	heavily influenced by watershed geometry and landcover and by local groundwater input. This broad-scale investigation of drivers of thermal stability will directly inform Brook Trout management through the ability to identify both at-risk populations and climate refugia.
Vanhomwegen, Marine	**A New Dawn As Neuro-Endocrinology Meets Wild Fish Management: Hypothalamic-Pituitary-Interrenal Axis Key Gene Expression, Uplc-Ms/Ms Glucocorticoid Quantification and Biochemical Profiling Enabling Accurate Monitoring and Restocking of Wild Fish Populations with Stress Resilient Bred Fish** **Abstract:** An ever-increasing human population makes the need for sustainable food production as well as wildlife conservation a global public, scientific, and political prime priority. Besides industrial aquaculture and recreational and commercial fisheries, fish are highly relevant in the framework of conservation biology and environmental protection efforts. Anthropogenic activities such as energy production, shipping, and pollution significantly impact ecosystems by inducing climate change (e.g., increasing water temperature, ocean acidification), through loss of fish nurseries, addition of novel predators, addition of novel animal-environment relationships, and in all compromising wild fish stocks and even entire ecosystems. Therefore, various international monitoring schemes aim to clarify their impact on the health status of oceanic as well as freshwater niches. Fish faced with stressful stimuli launch an endocrine stress response through activation of the hypothalamic-pituitary-interrenal (HPI) axis to release cortisol, the dominant and highly pleiotropic glucocorticoid in teleost fish known to impact a plethora of metabolic pathways, into the blood. Cortisol elicits a suite of physiological and behavioral changes allowing fish to cope with altered situations. Where short-term cortisol-mediated actions are adaptive, long-term elevations of cortisol are detrimental to fish performance, reproduction, and survival as it makes individuals more susceptible to disease thereby increasing mortality. Therefore, cortisol in fish scales, a scientifically validated biomarker capturing systemic cortisol exposure over long periods of time, is of utmost importance to assess chronic stress in governmental, wildlife, aquaculture, and scientific settings. Here we focus on how fish scale cortisol is applied as a powerful tool in wildlife monitoring and conservation by (i) quantifying chronic stress from (anthropogenic-induced) environmental constraints enabling accurate monitoring of gradual climate change, the loss of nurseries, the impact of pollution, etc. in wild fish and their ecosystem, as well as (ii) breeding fish for stress resilience enabling to replenish wild stocks with more stress resilient fish counteracting dwindling populations.
Zorn, Troy	**Quantitative Ecology of Brook Trout in a Michigan Stream Over a 55-Year Period** **Abstract:** Long-term monitoring data sets provide the foundation for understanding population dynamics and modeling population response to change. Long-term data on stream trout populations are useful for quantifying population-level processes (survival, growth, etc.), providing baseline estimates of mean and variation, and depicting temporal trends. Annual sampling of brook trout between 1949 and 2004 in a reach of Hunt Creek, Michigan represents one of the longest quantitative population records for a vertebrate species. The brook trout population, recreationally fished during 1950-1965 and closed to fishing since, was sampled annually via electrofishing to generate population estimates. The age-specific estimates of fish density, annual survival, and growth provided baseline data for Hunt Creek and experimental "control" values for comparison with similar data nearby reaches subjected to various experimental treatments. Using this dataset, we quantify long-term mean and variation in these parameters for brook trout and explore relationships among them. This effort provides information useful to anglers, fishery managers and researchers, and enables dissemination of a rare, long-term dataset to the scientific community.

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