EASTERN PLAINS NATIVE FISH RESEARCH

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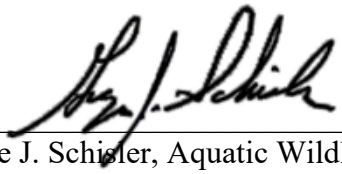
Approved by: 

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Date: May 4, 2022

The results of the research investigations contained in this report represent work of the authors and may or may not have been implemented as Colorado Parks & Wildlife policy by the Director or the Wildlife Commission.

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Period Covered: April 1, 2021 to March 31, 2022

PROJECT OBJECTIVE: To assist in the conservation of Colorado's eastern plains native fish species.

PUBLICATIONS

Adams, C. M., A. Ficke, and D. L. Winkelman. Review of current knowledge on CDPHE WS-II designated species: Temperature impacts and laboratory study feasibility. Colorado Cooperative Fish and Wildlife Research Unit. Fort Collins, Colorado.

Baum, C. M. 2021. Temperature and winter duration requirements for reproductive success in Johnny Darter *Etheostoma nigrum* in the South Platte River basin, Colorado. Master's thesis. Colorado State University. Fort Collins, Colorado.

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Fitzpatrick, R. M., D. Longrie, R. J. Friebertshauser, and P. Foutz. *Submitted.* Evaluation of the Longrie-Fecteau fish passage structure for Great Plains fishes. River Research and Applications.

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Swarr, T. R., C. A. Myrick, and **R. M. Fitzpatrick**. 2021. Tag retention in and effects of passive integrated transponder tagging on survival and swimming performance of a small-bodied darter. Journal of Fish Biology 100:705–714. https://doi.org/10.1111/jfb.14984

Swarr, T. R., C. A. Myrick, and **R. M. Fitzpatrick**. *In review*. Effects of slope on small-bodied fish passage success in an experimental rock ramp fishway. Transactions of the American Fisheries Society.

Swarr, T. R., C. A. Myrick, and **R. M. Fitzpatrick**. *In review*. Design, construction, and hydraulic evaluation of a model rock ramp fishway. North American Journal of Fisheries Management.

PRESENTATIONS

Adams, C., D. L. Winkelman, P. Schaeffer, and **R. M. Fitzpatrick**. Field examination of altered stream temperatures on reproductive development of Johnny Darter, *Etheostoma nigrum* in the Front Range of Colorado. National Meeting of the American Fisheries Society. Baltimore, Maryland. November 9, 2021.

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Fitzpatrick, R. M., D. Longrie, R. Friebertshauser, and P. Foutz. Evaluation of the Longrie-Fecteau fish passage structure for Great Plains fishes. Annual meeting of the Colorado/Wyoming Chapter of the American Fisheries Society. March 1, 2022.

Fitzpatrick, R. M. Research and Conservation of Great Plains Native Fishes in Colorado. Adams State Fish Management Class (BIO 469). December 8, 2021.

Myrick, C. A., T. R. Swarr, C. Brittain, and **R. M. Fitzpatrick**. Steep grade and bumps ahead? Optimizing fishway designs for small-bodied Great Plains fishes. Montana State Ecohydraulics Group. November 21, 2021.

PEER REVIEWS

Western North American Naturalist

RESEARCH PRIORITY

Evaluation of the Longrie-Fecteau fish passage structure and potentially use this structure as a relatively low cost template for other plains fish barriers.

OBJECTIVES

Determine the amount and timing of native fish movement through the Longrie-Fecteau fish passage structure.

ABSTRACT

Connectivity is critical for stream fish persistence, and fish passage structures are a useful conservation tool to increase connectivity in fragmented systems. The design of fish passage structures is a tradeoff between the space available for construction, slope, and costs associated with the structure. The Longrie-Fecteau fish passage structure was designed to be modular, readily deployable, nature-like, and adjustable to barrier-specific needs. To accomplish this, it was designed as two primary prefabricated portions (straight and curved) that can be quickly installed and accommodate various barrier heights by adding or removing sections. The sinuous passage design allows a low slope (2%) and passage over large structures with relatively short apron space. We evaluated fish passage through this structure in Fountain Creek, Colorado, USA, via a PIT tag mark-recapture study. We documented four native Great Plains fish species successfully ascending the passage structure, with most passage occurring at night. We estimated a 3% probability of a released fish entering the structure, but then 89% and 99% passage to the midpoint and exit of the 123-m structure respectively. Since passage success was high once fish entered the structure, we realize attraction flows are the limiting factor at this site. Fish ascended the structure quickly, with median time for successful ascent of 19 minutes, and minimum time of 6 minutes. While fish passage often requires site-specific engineering and design, the Longrie-Fecteau fish passage structure is a conservation tool that may reduce construction costs due to its modularity and simplicity.

INTRODUCTION

This study was conducted on Fountain Creek, Colorado, USA, which is a 120-km tributary of the Arkansas River that is representative of similar low-gradient reaches in the Great Plains ecoregion (Figure 1). In its lower reaches, Fountain Creek is characterized by wide, sandy channels with a high sediment load that are prone to morphological fluctuation within the flood plain due to an extremely flashy hydrograph (range over study period = 0.3–32.8 m^3/s (USGS gage station 07106500)) (Mau et al., 2007). Between its headwaters and confluence with the Arkansas River, Fountain Creek has 18 potentially impassable instream structures ranging from small, rock-fill dams to concrete diversion structures spanning the entire width of the channel. A motivating

factor for this project was to design a fish passage structure that could be used as a template for other barriers in the system, and hopefully in other systems where passage of small-bodied fishes is a conservation priority. Fountain Creek is a unique system due to its native species dominated fish assemblage, the high amount of sediment in the system, and the highly fluctuating flow regime that the structure needs to withstand and operate within.

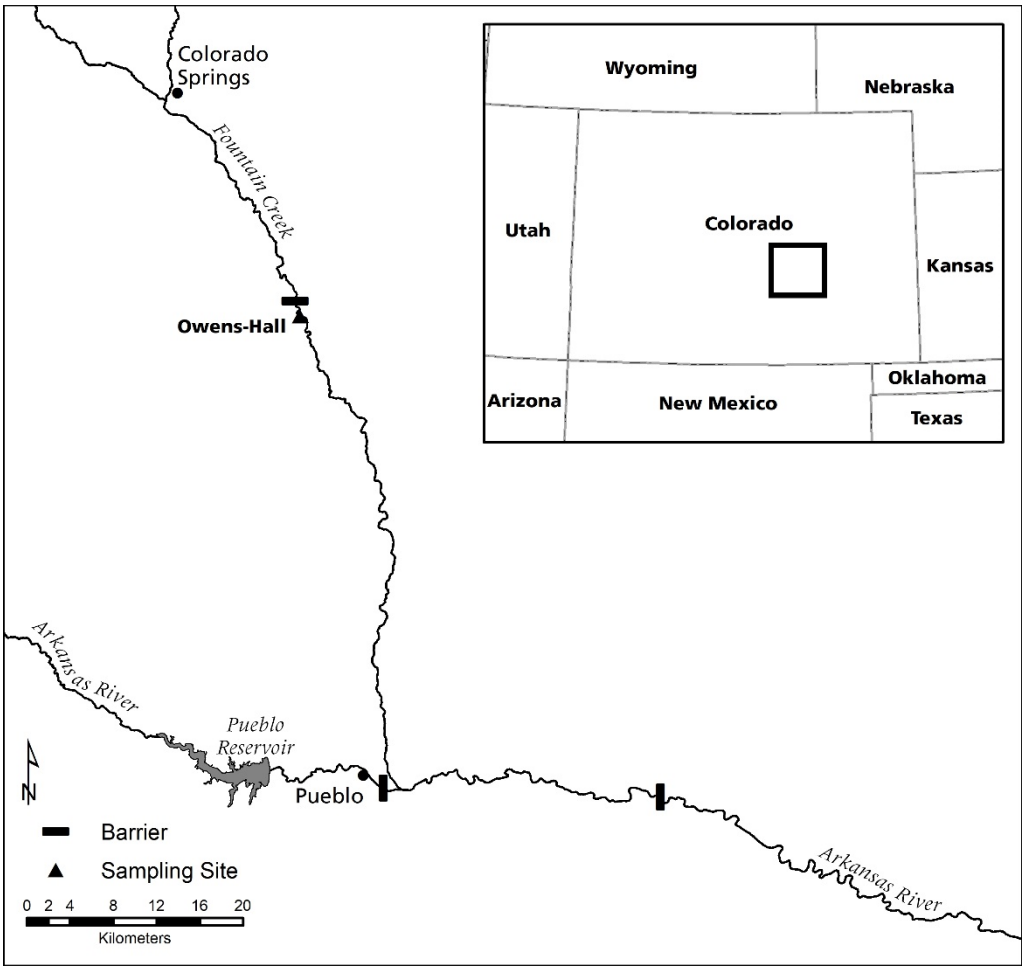

Figure 1. Fountain Creek is located in south-central Colorado, USA along the Front Range of the Rocky Mountains. The study site was between Colorado Springs and Pueblo (UTMs for Owens-Hall Diversion E: 526847 N: 4277740).

METHODS

The Longrie-Fecteau fish passage structure was designed to be repeatable, adjustable, and easy to deploy. To be repeatable, it was a prefabricated design that consisted of two main pieces, a straight section and a corner section that can be combined to accommodate various sizes of barriers (Figure 2). Each portion had small and large roughness elements to provide flow refuge and increase passage probability. To be economical, it was built in a prefabricated design that can be produced to barrier specifications. It is adjustable in that the height of the drop determines how many sections are required. In addition, the straight sections can be shortened to fit site-specific requirements. Through the use of a sinuous architecture, this passage structure additionally maintains a shallow grade (2%) over a short distance, lending to application at sites with minimal apron length. These characteristics allow this design to be readily-deployable and most importantly, adjustable to barrier specific heights.

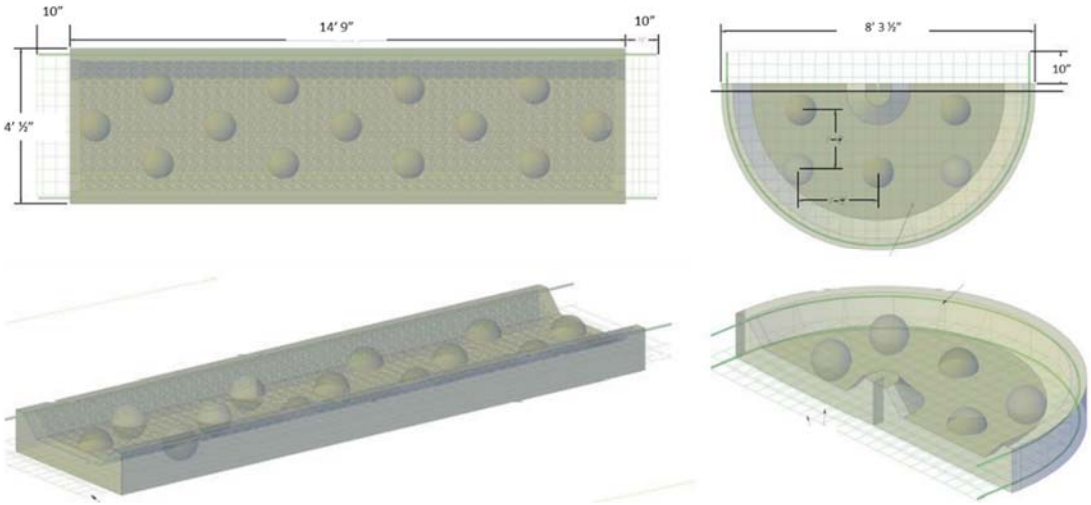

Figure 2. Straight and curved sections that make up the Longrie-Fecteau fish passage structure. By adding and removing the number of these prefabricated pieces, the fish passage structure can be adjusted to the height required for a particular barrier.

Program MARK (White and Burnham, 1999) was used to fit all models and obtain parameter estimates, and an information-theoretic approach was used for model selection (Anderson 2008; Burnham and Anderson, 2002). Parameters estimated included apparent survival (ϕ), which was used to estimate movement in this analysis, and detection probability (p) for each of the three arrays (Figure 3). Three movement parameters were estimated, including movement from release to A1 (ϕ_1), movement from A1 to A2 (ϕ_2), and movement from A2 to A3 (ϕ_3) (Figures 3 and 4).

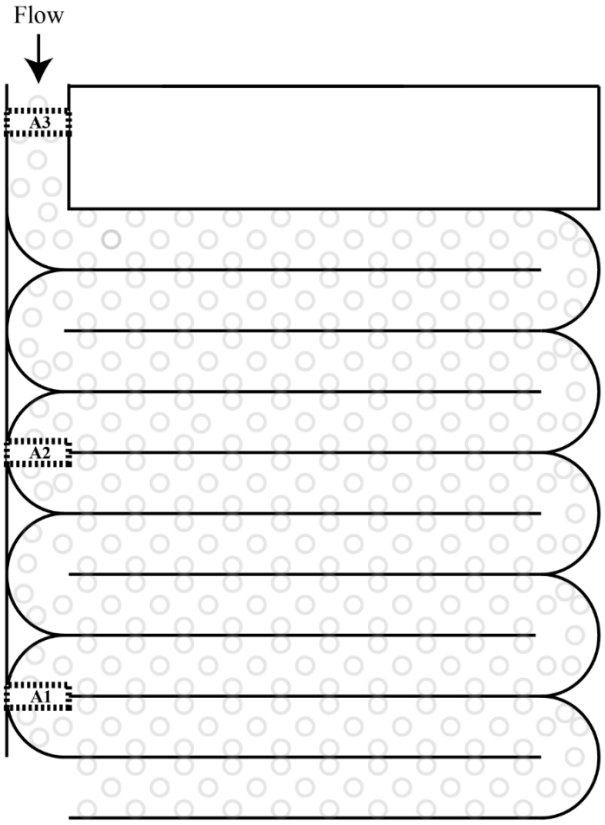

Figure 3. Schematic of PIT-tag antenna array within the Longrie-Fecteau fishway at Owens Hall diversion structure. Dashed boxes indicate PIT-tag antennas. Open circles indicate roughness elements. Total travel distance from the entrance of the fishway to its exit = 123.3 m (structure entrance to A1 = 22.3 m, A1 to A2 = 49.7 m, A2 to A3 = 49.7 m, A3 to structure exit = 1.6 m).

RESULTS

Weekly sampling efforts resulted in a total of 1,327 fishes from eight species being implanted with PIT tags (Table 1). The majority of these were Flathead Chub (n = 816), followed by White Sucker (n = 367). Average fish total length was 104 mm, and ranged 59–297 mm (Table 1). To date, four plains fish species have been documented successfully passing the structure, which were Flathead Chub, White Sucker, Central Stoneroller, and Creek Chub. There were two Flathead Chub detected moving downstream through the structure.

Table 1. Summary of Great Plains fishes PIT tagged and detected at the Longrie-Fecteau fish passage structure.

Species	# PIT tagged	# Detected	% Nighttime movement	Total length (mean (range); mm)
Flathead Chub (*Platygobio gracilis*)	816	24	85%	100 (63–177)
White Sucker (*Catostomus comersonii*)	367	10	80%	117 (70–297)
Central Stoneroller (*Campostoma anomalum*)	65	1	100%	84 (67–144)
Longnose Dace (*Rhinichthys cataractae*)	32	1	100%	73 (61–99)
Creek Chub (*Semotilus atromaculatus*)	1	1	0%	109
Longnose Sucker (*Catostomus catostomus*)	41	0	-	113 (83–187)
Fathead Minnow (*Pimephales promelas*)	4	0	-	62 (59–66)
Sand Shiner (*Notropis stramineus*)	1	0	-	63
Total	1,327	37	-	104 (59–297)

The top model from a Cormack-Jolly-Seber analysis was ϕ(array) p(total length), which indicates differences in passage based on arrays (Table 2). The lowest movement probability was ϕ_1 (0.03; SE = 0.02–0.04), which was expected as a released fish needed to find the 1.25-m entrance to the fish passage structure, and then, depending on the water level, ascend 22.3 m to reach A1. However, once fish were in the structure, there were high rates of successfully ascension with ϕ_2 = 0.89 (0.73–0.96) and ϕ_3 = 0.99 (0.87–1.03) (Figure 4). Specifically, there is a 3% probability of a released fish encountering the first array, but once in the structure, there is an 88% (0.89 x 0.99) probability that the fish will successfully ascend it. Therefore, total probability of fish passage success for this structure was 2.6% (0.03 x 0.89 x 0.99).

Detection probabilities for the three arrays were high, with the lowest detection probability being A2 at 0.97 (0.81–0.99) (Figure 4). Detection probability for the top model included the fish total length covariate. The beta for this estimate was positive (0.093), indicating detection probability for larger fish was greater than smaller fish. However, the confidence interval overlapped zero (-0.025–0.211), indicating this was a weak relationship.

Table 2. Cormack-Jolly-Seber models in Program MARK used to estimate movement (ϕ), and detection probability (p) for PIT tagged Great Plains fishes in Fountain Creek, Colorado. The top eight models selected by Akaike's information criterion (AIC_c) and model weights are shown for comparison. The maximized log-likelihood (-2log(L)), the number of parameters (K) in each model, and the small sample size-corrected AIC_c values (AIC_c) are shown. Models are ranked by their AIC_c differences (ΔAIC_c) relative to the best model in the set and Akaike weights (w_i) quantify the probability that a particular model is the best model in the set given the data and the model set.

Model	AIC_c	ΔAIC_c	w_i	Likelihood	K	-2Log(L)
ϕ(array) p(TL)	389.1491	0.0000	0.36302	1.0000	5	379.1058
ϕ(array) p(.)	390.5102	1.3611	0.18381	0.5063	4	382.4815
ϕ(array + TL) p(TL)	391.1536	2.0045	0.13325	0.3671	6	379.0930
ϕ(array) p(array)	391.6376	2.4885	0.10461	0.2882	6	379.5770
ϕ(array + TL) p(.)	392.5174	3.3683	0.06738	0.1856	5	382.4742
ϕ(array + TL) p(array + TL)	392.5990	3.4499	0.06468	0.1782	8	376.4949
ϕ(array) p(array + TL)	393.3235	4.1744	0.04503	0.1240	7	379.2427
ϕ(array + TL) p(array)	393.6507	4.5016	0.03823	0.1053	7	379.5699

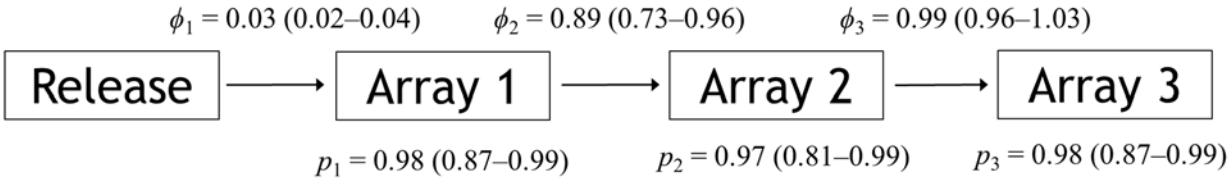

Figure 4. Model averaged parameter estimates from a Cormack-Jolly-Seber model to estimate movement (ϕ) and detection probabilities (p) on a conceptual diagram of the Longrie-Fecteau fish passage structure.

Most movement through the Longrie-Fecteau fish passage structure took place at night, with 85% of Flathead Chub movements and 80% of White Sucker movements occurring at night (Table 1; Figure 5). Although it was only one detection per species, both the Central Stoneroller and Creek Chub successful ascents took place at night. Median time for successful ascent was 19 minutes, while the maximum time was 12 hours, and minimum time was 6 minutes.

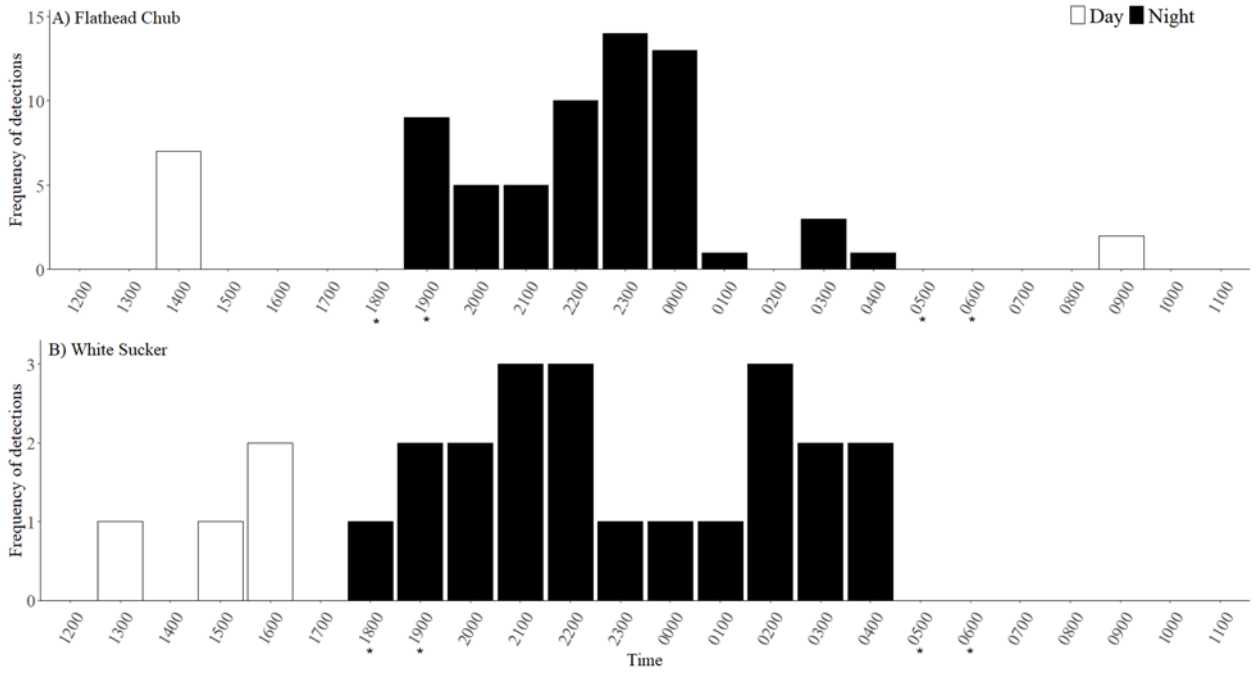

FIGURE 5. Frequency of PIT tag detections (pooled across all antennae) for A) Flathead Chub and B) White Sucker moving through the Longrie-Fecteau fish passage structure on Fountain Creek, Colorado, USA. Asterisks indicate hours that differentiated in light due to seasonality. During the study period, sunrise and sunset ranged from 0554–0714 and 1811–2015 respectively. All detections occurring during hours of variable light (*) were at night.

DISCUSSION

The Longrie-Fecteau fish passage structure was successful at passing small-bodied Great Plains fishes, transporting sediment, and withstanding an extreme flow regime of 0.3–523.9 m^3/s (USGS gage station 07106500) since initial construction. While the probability of tagged fishes encountering the entrance to the fishway was low (3% to A1), once in the structure, the probability of full passage was high (89% and 99% to A2 and A3 respectively), with most movement occurring at night. Detection probability was high for each array, indicating interference from metal in the structure was not an issue. Maintenance has been minimal, but occasionally large wood was impinged on the structure due to high flows.

ACKNOWLEDGEMENTS

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PUBLICATION

Fitzpatrick, R. M., D. Longrie, R. J. Friebertshauser, and P. Foutz. *Submitted.* Evaluation of the Longrie-Fecteau fish passage structure for Great Plains fishes. *River Research and Applications*.

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RESEARCH PRIORITY

Incorporating environmental DNA metabarcoding into the plains fish monitoring protocol.

OBJECTIVES

This project will incorporate environmental DNA metabarcoding into CPW's plains sampling protocol to detect threatened and endangered fish, detect aquatic invasive species, and guide future sampling efforts.

See 2021 Progress Report for additional details regarding Introduction, Methods, and previous Results.

INTRODUCTION

Fed by the eastern slope of the Rocky Mountains in its headwaters and maintained by groundwater and precipitation in lower reaches (Fausch and Bramblett 1991), rivers and streams in the western Great Plains offer a broad diversity of geomorphology, hydrology, and ultimately habitat for aquatic taxa (Fausch and Bestgen 1997). This diversity in habitat, and thereby fish communities, is largely apparent in the eastern Great Plains of Colorado where assemblage shifts can be seen longitudinally from the Rocky mountain headwaters to the eastern border and the mountain-plains transition zone occurring between them (Rahel and Hubert 1991; Haworth et al. 2020). Despite this unique zonation of fish assemblages, species richness in this ecoregion is less than that of more mesic drainages (Fausch and Bestgen 1997) likely due to relatively simple habitat, harsh physiochemical attributes (Matthews 1987), and historical, intermittent flow during the dry season (Magoulick and Kobza 2003). While adaptations to this dynamic environment may suggest resiliency, contemporary, anthropogenic disturbances such as groundwater pumping (Falke et al. 2011), introduction of nonnative taxa, and stream fragmentation due to the installation of instream structures (Walters et al. 2014; Richer et al. 2020) greatly imperil taxa native to this ecoregion. Among the 30 extant species native to the South Platte, Arkansas, and Republican River drainages in Colorado, 13 are currently listed as threatened or endangered by Colorado Parks and Wildlife (Colorado Parks and Wildlife, 2022).

An alternative method to conventional sampling techniques in lotic systems has emerged through the ability to accurately collect and assign taxonomy to extra-organismal DNA (eDNA) (Taberlet et al. 2018). Through successful collection and amplification of genetic material, emanating from shed scales, slime, feces, etc. (Jerde et al. 2011; Rees et al. 2014), researchers and managers alike have expanded distribution knowledge (Schmelzle and Kinziger 2016; Janosik and Johnston 2015), improved the ability of early detection of invasives (Goldberg et al. 2013; Xia et al. 2018; Whitaker et al. 2021), and increased detection ability for rare or elusive species (Johnston and Janosik 2019) across a wide range of aquatic taxa. Multiple comparative studies have observed

eDNA performing equally as well or better than conventional, aquatic sampling methods in terms of detection (Evans et al. 2016; Shaw et al. 2016; Valentini et al. 2016; McColl-Gausden et al. 2021). Further, sampling through eDNA not only permits sites to be surveyed that are normally inacessible by traditional methods (McColl-Gausden et al. 2021) but requires less time, effort, and equipment as well (Pfleger et al. 2016). While eDNA studies have historically focused on single-species detection (McColl-Gausden et al. 2021), an emerging technique, termed metabarcoding, expands upon the method through it's ability to produce species-richness data (Tsuji et al. 2019); a metric paramount to freshwater conservation (Su et al. 2021).

Through the use of high-throughput sequencing and clade-, as opposed to species-specific primer sets, metabarcoding can reveal species compositions from single collection event (Deagle et al. 2014; Miya et al. 2015; Deiner et al. 2017; Yamamoto et al. 2017). Rapid biodiversity assessments of this nature can be designed to not only identify native communities but invasive taxa as well which can be crucial in the early detection and management of previously unknown invaders (Brown et al. 2016; Borrell et al. 2017). While the preparation and laboratory processes associated with metabarcoding are far more in depth than single-species eDNA work (McColl-Gausden et al. 2020), the information produced ultimately leads towards a less time consuming, and potentially more sensitive survey method thereby reducing the strains of empirical sampling listed above and ultimately expanding the reach of biologists and managers working in eastern plains systems.

In order to validate the use of eDNA as a complimentary survey method, we are conducting a comparative study investigating the efficacy of eDNA and conventional methods at paired sites across the eastern plains of Colorado. While most comparative studies of this nature have taken a single-species approach across temporally disparate sampling events (McColl-Gausden et al. 2020), the current work remedies this by taking temporally paired metabarcoding samples. We hypothesized that eDNA-metabarcoding samples will perform equally as well or better than conventional sampling in regards to detection probability (sensitivity) and measurements of alpha diversity. We additionally developed a sampling protocol for the field designed to be accessible, repeatable, and accurate regardless of a collector's background in molecular ecology (Friebertshauser et al. 2020). Our primary aim was to develop and validate an alternative and complimentary survey technique that will assist in the limited effort conservation biologists and managers have to monitor and conserve fishes native to the eastern plains ecoregion of Colorado.

METHODS

Primer Selection and Local Reference Database Development

Taxonomic identification of multiple species from eDNA relies on the ability to compare unidentified reads from next-generation sequencing efforts to a reference database of known sequences (Taberlet et al. 2018). Therefore, not only the completeness of a reference database but the primer(s) chosen when metabarcoding will have an impact on the accuracy and coverage of a protocol. Due to its variation among species and taxonomically expansive reference library (iBOL, International Barcode of Life Consortium 2016), the mitochondrial cytochrome oxidase subunit I (COI) has become

widely used in metabarcoding studies (Deagle et al. 2014). However, certain drawbacks apparent in the COI subunit (Deagle et al. 2014) have lead many studies focused on metabarcoding of fish communities towards amplifying fragments from the 12s and 16s regions of mitochondrial rRNA (Miya et al. 2015; Evans et al. 2016; Lecaudey et al. 2019). Additionally, the use of multiple primers has been observed to increase the taxonomic breadth of detection (Evans et al. 2016; Schenekar et al. 2020), therefore, the current study used both a 16s (16S fish-specific, Shaw et al. 2016) and 12s (MiFish-U, Miya et al. 2015) region of mitochondrial rRNA. These primers were designed to amplify fragments of ~100 bp (base pairs) and 163-185 bp respectively. These relatively short amplicon lengths are ideal for eDNA application as longer fragments will degrade more quickly in an extraorganismal environment than shorter ones (Taberlet et al. 2018). Since databases for these regions are relatively less complete than those for COI regions (Weigand et al. 2019), we compiled a local reference database containing 12s and 16s sequences from species of interest within the eastern plains of Colorado.

Up to five fin clips per target species were collected across the South Platte and Arkansas River basins with a portion being collected throughout Kansas by the Kansas Department of Wildlife, Parks and Tourism. Target species fell into one of three distribution statuses: Native, Invasive, or Potentially Invasive. Fin clips were collected using sterilized dissection scissors and stored in 100% molecular grade ethanol (EtOH) at room temperature until extraction. Tissues were extracted using a DNeasy Blood and Tissue Kit in conjunction with a QIAcube Automated DNA Isolation and Purification system (QIAGEN, Hilden, Germany). Prior to purification via the QIAcube, tissues were digested in a lysis solution of 180 µl Buffer ATL and 20 µl proteinase K for 24 hr on a dry bath at 56°C.

PCR for the 16S1 primer was carried out using a 20 µl reaction containing 10.3 µl molecular grade H_2O, 4 µl 5x PCR buffer (Promega Corporation, Madison, Wisconsin, United States), 0.2 µl of $MgCl_2$ (25mM), 2 µl dNTPs (10mM), 1 µl forward and reverse primer (10 µM), 0.5 µl GoTaq polymerase (5 U/µl) (Promega Corporation, Madison, Wisconsin, United States), and 1 µl template. The 35 cycle thermal cycling profile after an initial 4 min denaturation at 95°C was as follows: denaturation at 95°C for 15 s; annealing at 55°C for 30 s; and extension at 72°C for 30 s. A final extension step occurred at 72°C for 5 min.

PCR for the MiFish-U primer was carried out using a 15 µl reaction containing 7.6 µl molecular grade H_2O, 3 µl 5x PCR buffer, 0.9 µl of $MgCl_2$ (25mM), 0.5 µl dNTPs (10mM), 0.75 µl forward and reverse primer (10 µM), 0.5 µl GoTaq polymerase (5 U/µl), and 1 µl template. The 30 cycle thermal cycling profile after an initial 2 min denaturation at 94°C was as follows: denaturation at 98°C for 5 s; annealing at 50°C for 10 s; and extension at 72°C for 10 s. A final extension step occurred at 72°C for 5 min.

Prior to cycle sequencing, unincorporated primers and dNTP's were removed from PCR products with Exo SAP-IT (Applied Biosystems, Waltham, Massachusetts, United States). Cycle sequencing was conducted in both directions using BigDye Terminator following the manufacturer's protocol (v3.1, Applied Biosystems, Waltham, Massachusetts, United States) and run in a 10 µl reaction: 5.475 µl H_2O, 2.275 µl 5x sequencing buffer, 0.25 µl BigDye Terminator, 1 µl primer, and 1 µl template. The 35 cycle thermal cycling profile after an initial 1 min denaturation at 96°C was as follows: denaturation at 96°C for 10 s; annealing at 50°C for 30 s; and extension at 60°C for 4

min. In order to remove unincorporated dye-terminators, products were then passed through a UNIFILTER microplate (Cytiva, Marlborough, Massachussetts, United States) filled with a Sephadex (Cytiva, Marlborough, Massachussetts, United States) preparation. Purified products from both forward and reverse strands were then Sanger sequenced on a 3500xL genetic analyzer (Applied Biosystems, Waltham, Massachussetts). Sequences were edited and aligned using Sequencher (version 5.4.6, Gene Codes Corporation) and uploaded to a custom, relational database. Species identification was verified against reference sequences in GenBank using the Basic Local Alignment Search Tool (BLAST, Altschul et al. 1990). In total, 361 sequences (both 12s and 16s) were uploaded to the reference database comprising 8 orders, 13 families, and 39 species (Sequenced taxa, S3).

Sampling Sites

Comparative sampling sites were chosen based on conventional fish community sampling conducted by Colorado Parks and Wildlife during the fall of 2021. All sampling sites (n=11) occurred east of the continental divide in Colorado and within the South Platte and Arkansas River basins. Comparative sites were separated by >/= 5 river km in order to increase the probability of detecting novel, genetic material in eDNA samples (Wilcox et al. 2016; Wacker et al. 2019; Bedwell and Goldberg 2020). Sampling sites in South Platte River basin (n=7) took place across two of the three major physiographic regions. These regions consist of montane streams, eastern plains streams and the transition zone that divides the two. Montane and eastern plains streams are largely differentiated by gradient, channel morphology, temperature (Fausch and Bestgen 1997), and species assemblage (Rahel and Hubert 1991) while the transition zone describes an ecotone between them (Propst 1982) thereby supporting unique fish assemblages (Bestgen et al. 2017; Haworth et al. 2020). Three sites were sampled within the transition zone of the South Platte River basin: West Plum Creek (WP1 and WP2, Douglas County), St. Vrain Creek (SV1, Boulder County), and Left Hand Creek (LH1, Boulder County). Sample reaches in the transition zone were characterized by narrow channels, cobble-gravel substrate, and relatively cooler water. The remaining three sites sampled in this drainage occurred in the eastern plains physiographic region: Lodgepole Creek (LP1, Sedgwick County), South Platte River (SP1, Morgan County and SP2, Logan County). With the exception of the site on Lodgepole Creek (a tributary to the South Platte River with a relatively narrow channel), reaches in this physiographic region are defined by wide, braided channels, sandy substrate, and low gradient. Four sites were sampled along Fountain Creek (FC1, FC2, and FC3, El Paso County; FC4, Pueblo County); a tributary to the Arkansas River. These site occur at the eastern terminus of the transition zone and accordingly resemble eastern plains streams in their hydrology and geomorphology. Fountain Creek sites historically contain a reduced species assemblage compared to reaches within the South Platte River basin (Colorado Parks and Wildlife, 2021).

Environmental DNA Sampling and Filter Extraction

Environmental DNA samples were collected at comparative sites on the same day, just prior to conventional fish sampling or any disturbance of the sampling reach (Figure 6). Sampling occurred during September and October of 2021. While a variety of methods for the collection of aquatic eDNA exist (Tsuji et al. 2019), samples were filtered *in situ* using the Smith-Root eDNA Sampler (Smith-Root, Inc., Vancouver,

Washington, United States) (Figure 6). This unit not only allows for on-site filtration, which has been shown to increase detection, (Yamanaka et al. 2016) but decreases sampling time and limits contamination potential through its design and single-use filter housings (Thomas et al. 2018). Through the use of the Smith-Root telescopic sampling pole and trident head attachment, 3, 2, and l samples were able to be collected simultaneously (Figure 6). Filtration parameters of the unit were based on a comparative study (Thomas et al. 2018): flow rate of 1 l/min, maximum pressure of 10 PSI, and use of a 5µm, 47 mm diameter filter. Immediately following on-site filtration, filter discs were placed in a 2 ml cryovial filled with Longmire's buffer (Longmire et al. 1997) and stored at 4°C until extraction. One field negative per site was filtered and stored in the same manner as above using distilled water. Filtration was conducted at the downstream-most point of each paired, traditional sampling reach.

Extraction methods were modified from Spens et al. (2017) and Miya et al. (2015). The filter was first cut in half with each half being placed into an individual 2 ml safe-lock microcentrifuge tube. Remaining Longmire's buffer (~1 ml) was then transferred equally among two, 2 ml safe-lock microcentrifuge tubes. Half of the filter disc and volume of Longmire's buffer was archived prior to extraction. Unarchived Longmire's buffer was then centrifuged at 6,000 x g for 45 min in order to pelletize genetic material. After removing the supernatant, each pellet and filter half was submerged in 100 µl and 300 µl of lysis working solution (90% ATL buffer, 10% Proteinase K) respectively. Filters and pellets were digested overnight in independent vessels on a shaking dry bath at 56°C rotating at 80 rpm. The following day, contents from the digested filters were transferred to a modified mini spin column (QIAGEN, Hilden, Germany) with the filter membrane removed. Spin-columns were then centrifuged at 6,000 x g for 5 min into the collection tubes containing the digested pellet in order to concatenate DNA from both sources. Half of the digested solution (200 µl) was purified with the Dneasy Blood and Tissue kit in conjuction with the QIAcube Automated DNA Isolation and Purification system. The remaining volume was archived.

Conventional Fish Sampling

Conventional fish sampling was conducted as part of Colorado Parks and Wildlife's annual stream monitoring and occurred immediately following filtration of eDNA samples. Sites within the South Platte River basin were sampled using a three-pass removal technique where the first two passes consisted of electrofishing with either a Smith-Root VVP-15B electrofishing barge or three, LR-24 electrofishing backpacks depending on the water depth at each site. The third pass consisted of multiple seining efforts with a 4.7-mm mesh size seine. Fishes were held in live wells between passes. Sampling within the Arkansas River drainage consisted of single-pass electrofishing with two, Smith-Root LR-24 electrofishing. All fishes collected during traditional sampling were enumerated and identified to species. Sampling reaches ranged in length from 81.7 to 211.8 m ($\bar{x}$ = 148.9 m). Habitat measurements were additionally collected during each sampling event (e.g. pH, turbidity, water temperature, and measurements of channel morphology).

Library Preparation and Sequencing

Metabarcoding libraries were prepared using a two-step PCR strategy similar to that used by Hopken et al. (2021). The first round PCR targeted fragments using the 16s fish-specific MiFish-U primers (Table 3). Both primers were modified to include heterogeneity spacers, in order to improve sequencing quality, and Illumina sequencing primers were added at the 5' end in order to add indexes and Illumina sequencing adaptors in the second PCR (Illumina, Sand Diego, CA, United States). Primers were unable to be multiplexed due to divergent annealing temperatures. PCR using the 16s fish-specific primers was carried out using a 25 µl reaction containing 3.5 µl molecular grade H_2O, 12.5 µl 2x QIAGEN Multiplex PCR Master Mix (QIAGEN, Hilden, Germany), 2.5 µl of 2 µM forward and reverse primer, and 4 µl template. The 40 cycle thermal cycling profile after an initial 15 min denaturation at 95°C was as follows: denaturation at 94°C for 30 s; annealing at 52°C for 90 s; and extension at 72°C for 120 s. A final extension step occurred at 72°C for 10 min.

Table 3. Primers for database development and first round PCR.

Primer Name	Sequencing Primer	Heterogeneity Spacer	Region of Interest
16S1 F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG	NNNNNN	GTCGGTAAAACTCGTGCCAGC
16S1 R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG	NNNNNN	CATAGTGGGGTATCTAATCCCAGTTTG
MiFish-U F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG	NNNNNN	GGTCGCCCCAACCRAAG
MiFish-U R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG	NNNNNN	CGAGAAGACCCTWTGGAGCTTIAG

PCR using the MiFish-U primers was carried out using a 25 µl reaction containing 3.5 µl molecular grade H_2O, 12.5 µl 2x QIAGEN Multiplex PCR Master Mix (QIAGEN, Hilden, Germany), 2.5 µl of 2 µM forward and reverse primer, and 4 µl template. A touchdown PCR method, modified from Gold et al. (2021), was employed: initial 15 min denaturation at 95°C followed by 13 cycles of denaturation at 94°C for 30 s, annealing for 39 s beginning at 69.5°C and decreasing by 1.5°C every cycle, and extension at 72°C for 60 s. Thirty additional cycles were carried out with an annealing temperature at 50°C followed by a final extension at 72°C for 10 min.

Dual-indices and Illumina sequencing adaptors were added to first-round PCR products (Table 4) through a 15 µl reaction containing 2.9 µl molecular grade H_2O, 7.5 µl 2x QIAGEN Multiplex PCR Master Mix, 1.8 µl of forward and reverse, indexed primers, and 1 µl of undiluted product from the first-round PCR. The 8 cycle thermal cycling profile after an initial 15 min denaturation at 95°C was as follows: denaturation at 95°C for 15 s; annealing at 55°C for 45 s; and extension at 72°C for 60 s. A final extension step occurred at 72°C for 10 min.

Table 4. Primers for second round of PCR.

Primer name	Flowcell Adaptor	I5/I7 Indexes	Sequencing primer
PCR2-P5	AATGATACGGCGACCACCGAGATCTACAC	XXXXXXXX	TCGTCGGCAGCGTC
PCR2-P7	CAAGCAGAAGACGGCATACGAGAT	XXXXXXXX	GTCTCGTGGGCTCGG

Following each PCR step, products were checked for successful amplification using a QIAxcel fragment analyzer (QIAGEN, Hilden, Germany) and cleaned (removal of unincorporated primers, DNTP's, and primer dimers) using 1.4x Mag-Bind TotalPure NGS magnetic beads following the manufacturer's protocol (Omega Bio-Tek Inc., Norcross, Georgia, United States). Concentrations of second-round PCR products were calculated using a Qubit dsDNA HS Assay Kit (Invitrogen, Carslbad, CA, United States) and then pooled in equimolar volumes. Final library quantification was conducted using a KAPA Library Quantification Kit (Roche Holding AG, Basel, Switzerland). The 20 µl reaction volume consisted of 12 µl KAPA SYBR FAST qPCR master mix/primer premix, 4 µl molecular grade H_2O, and 4 µl of the library diluted to both 1:1000 and 1:10000. The 35 cycle thermal cycling profile after an initial 5 min denaturation at 95°C was as follows: denaturation at 95°C for 30 s and annealing at 60°C for 45 s. Libraries were then run on an Illumina MiSeq System using the xx-cycle MiSeq Reagent Kit v2 (Illumina, San Diego, CA, United States).

Figure 6. ANDe environmental DNA collection system. Note the triplicate collection system. Water is collected and run through filters in the blue backpack seen at the feet of the technician. Photo credit: Boyd Wright.

RESULTS AND DISCUSSION

The focus of 2021–2022 work was sequencing tissues collected during previous years (Table 5). To date, approximately 370 sequences from two, target loci (12s and 16s fragments) have been produced (Table 5). These sequences represent 8 orders, 15 families, and 45 species. All taxa native to Colorado have been covered in these sequencing efforts. These sequences, respective taxonomy, and metadata relevant to sequenced fin clips are currently stored in a custom, relational database hosted on a SQL server accessible to CPW staff. All sequences were verified to taxonomic identity using the BLAST tool against the National Center for Biotechnology Information (NCBI) nucleotide database.

A formalized and novel USDA SOP (standard operating procedure) has been developed in order to standardize the extraction of DNA from the 47-mm disc filters used in the Smith-Root eDNA backpack sampler. This SOP is currently in the process of being 'published' by the USDA National Wildlife Research Center (NWRC).

During the fall of 2021, eDNA samples were collected alongside traditional sampling efforts in order to compare species richness indices across the two methods. Comparative sampling was completed at 14 sites across the South Platte and Arkansas River drainages. Streams/rivers samples include: St. Vrain Creek, Lefthand Creek, South Platte River (mainstem), Lodgepole Creek, West Plum Creek, and Fountain Creek. Sites were chosen based on historical diversity and the presence of imperiled taxa. Through this process, field protocols surrounding the collection of eDNA through the use of the Smith-Root eDNA backpack sampler were optimized.

One comparative site has been completed, which was Spring Creek behind the Colorado Parks and Wildlife Fort Collins office. The metabarcoding data showed the same fish assemblage as historical sampling, which is encouraging. Work in 2022–2023 will focus on sequencing more of these field sites to make a robust comparison between these two methods.

Table 5. Tissue samples that have been collected, **extracted, and sequenced** for the plains fish eDNA study.

Common Name	**Scientific Name**	#		
Arkansas Darter	*Etheostoma cragini*	5	Native	Threatened
Bigmouth Shiner	*Notropis dorsalis*	5	Native	
Black Bullhead	*Ameirus melas*	1	Native	
Bluegill	*Lepomis macrochirus*	5	Nonnative	
Brassy Minnow	*Hybognathus hankisoni*	8	Native	Threatened
Brook Stickleback	*Culaea inconstans*	5	Nonnative	
Brown Trout	*Salmo trutta*	5	Nonnative	
Burbot	*Lota lota*	5	Nonnative	
Central Stoneroller	*Campostoma anomalum*	8	Native	
Common Carp	*Cyprinus carpio*	5	Nonnative	
Common Shiner	*Luxilus cornutus*	5	Native	Threatened
Creek Chub	*Semotilus atromaculatus*	5	Native	
Fathead Minnow	*Pimephales promelas*	5	Native	
Flathead Catfish	*Pylodictis olivaris*	1	Nonnative	
Flathead Chub	*Platygobio gracilis*	5	Native	SOC
Freshwater Drum	*Aplodinotus grunniens*	1	Nonnative	
Green Sunfish	*Lepomis cyanellus*	5	Native	
Iowa Darter	*Etheostoma exile*	1	Native	SOC
Johnny Darter	*Etheostoma nigrum*	5	Native	
Largemouth Bass	*Micropterus salmoides*	5	Nonnative	
Longnose Dace	*Rhinichthys cataractae*	8	Native	
Longnose Sucker	*Catostomus catostomus*	5	Native	
N. Redbelly Dace	*Chrosomus eos*	5	Native	Endangered
Orangespotted Sunfish	*Lepomis humilis*	2	Native	
Orangethroat Darter	*Etheostoma spectabile*	5	Native	SOC
Plains Killifish	*Fundulus kansae*	8	Native	
Plains Minnow	*Hybognathus placitus*	6	Native	Endangered
Plains Topminnow	*Fundulus sciadicus*	5	Native	
Red Shiner	*Cyprinella lutrensis*	5	Native	
S. Redbelly Dace	*Chrosomus erythrogaster*	5	Native	Endangered
Sand Shiner	*Notropis stramineus*	5	Native	
Smallmouth Bass	*Micropterus dolomieu*	2	Nonnative	
Stonecat	*Noturus flavus*	4	Nat ive	SOC
Suckermouth Minnow	*Phenacobius mirabilis*	6	Native	Endangered
W. Mosquitofish	*Gambusia affinis*	6	Nonnative	
White Sucker	*Catostomus commersonii*	8	Native	

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PUBLICATION

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RESEARCH PRIORITY: Investigate interactions between native Great Plains species and invasive species.

OBJECTIVES: Evaluate behavioral interactions between the state endangered Northern Redbelly Dace, *Chrosomus eos* and the invasive Northern Mosquitofish, *Gambusia affinis*.

ABSTRACT

Mechanisms of the displacement of native fish by nonnative fish can include agonistic behaviors that push native fish species out of their preferred habitat, including their thermal optima. To examine these interactions, we built an experimental thermal preference chamber to evaluate: (1) the thermal preference of native, glacial relict northern redbelly dace *Chrosomus eos*; (2) if the thermal preference and movement changed in the presence of the invasive western mosquitofish *Gambusia affinis*; and (3) the direction of agonistic interactions. We hypothesized that *G. affinis* would express agonistic behavior toward *C. eos*, because *G. affinis* is widely recognized as an aggressive invader. Given the temperature range of the experimental chamber, i.e., 20–30 °C, *C. eos* selected an average of 24.3 °C as its thermal preference. After *G. affinis*' introduction, the thermal preference of *C. eos* increased by 1.7 °C and the movement, given by distance (cm) travelled, increased by 21%. Contrary to our prediction, more agonistic interactions were observed in *C. eos* toward *G. affinis*. These results indicate that agonistic behavior of *G. affinis* toward native fish species may be species- and condition-specific, and may not always be the primary mechanism of native species' displacement. Biological invasions are a global issue and altered thermal regimes are expected to continue. This study provided the novel approach of using a thermally heterogeneous thermal chamber to examine thermal preferences and aggressive interactions between a native and an invasive species. Future research should examine other life history traits that may be conveying the competitive advantage to *G. affinis*.

INTRODUCTION

Nonnative fish are implicated in the demise of native fish globally, but a mechanistic understanding of how displacements take place is often lacking [1–3]. Mechanisms of displacement may include agonistic interactions, where aggressive behaviors by nonnative species push native species out of their preferred habitat, including their thermal optima (van Snik Gray et al 2001; Hawgawa et al. 2004; McMahon et al. 2007). Western mosquitofish *Gambusia affinis* (Baird and Girard 1853) is listed as one of the 100 worst invasive species by the International Union for Conservation of Nature and has been implicated in the decline of freshwater fish (Courtenay et al. 1989; Lowe et al. 2021; Schumann et al. 2015), amphibians (Segev et al. 2008; Schulse et al. 2013), and invertebrates (Peck and Walton 2008; Preston et al. 2017; Harmon et al. 2020). A suite of ecological traits confer competitive advantages to *G. affinis*, including broad physiological tolerances (i.e., eurythermic) and high fecundity (Otto 1973; Pyke 2005),

but their aggressive behaviors are considered a key mechanism in native fish displacement (Mills et al. 2004; Laha and Mattingly 2007; Sutton et al. 2013). These agonistic interactions can result in an increased movement, which is an energetic cost that can require fish to consume more food (Boisclair and Leggett 1989), increase predation risk (Hulthén et al. 2017), and reduce growth (Rennie et al. 2004). To inform conservation actions, we hypothesized that the displacement from preferred thermal range occurs due to agonistic behavior of *G. affinis* toward *Chrosomus eos,* a species designated as endangered by the State of Colorado. Trials were conducted in a thermal preference chamber to determine: (1) the thermal preference of *C. eos*, (2) if the thermal preference changed with the addition of the invasive *G. affinis*, and (3) the direction of agonistic interactions.

METHODS

We used a modified Myrick-type thermal preference chamber to quantify the thermal and behavioral response of *C. eos* to the introduction of *G. affinis* (Figure 7) (Myrick et al. 2004). Switching between cool and warm water delivered to the top mixing ring reversed the orientation of the thermal gradient and allowed us to randomize the thermal gradient orientation during trials. When cool water was delivered to the top mixing ring, the water temperature decreased from 30 °C to 19 °C as it traveled from the center point to the two capped ends. The gradient was reversed when warm water was delivered to the top mixing ring.

The apparatus created a continuous 10 °C circular thermal gradient (mean segment temperature ± 95% CI; 21.2 ± 0.7 to 29.4 ± 0.6 °C) with the capacity to control flow direction (Figure 8). We randomized the thermal gradient orientation between trials to ascertain whether fish were selecting a temperature and not a location in the chamber (Figure 8). The thermal chamber was enclosed with a curtain, and a 122 cm × 122 cm sheet of white Plexiglas was mounted above the chamber to eliminate external disturbances during trials and allow for a uniform lighting of the chamber.

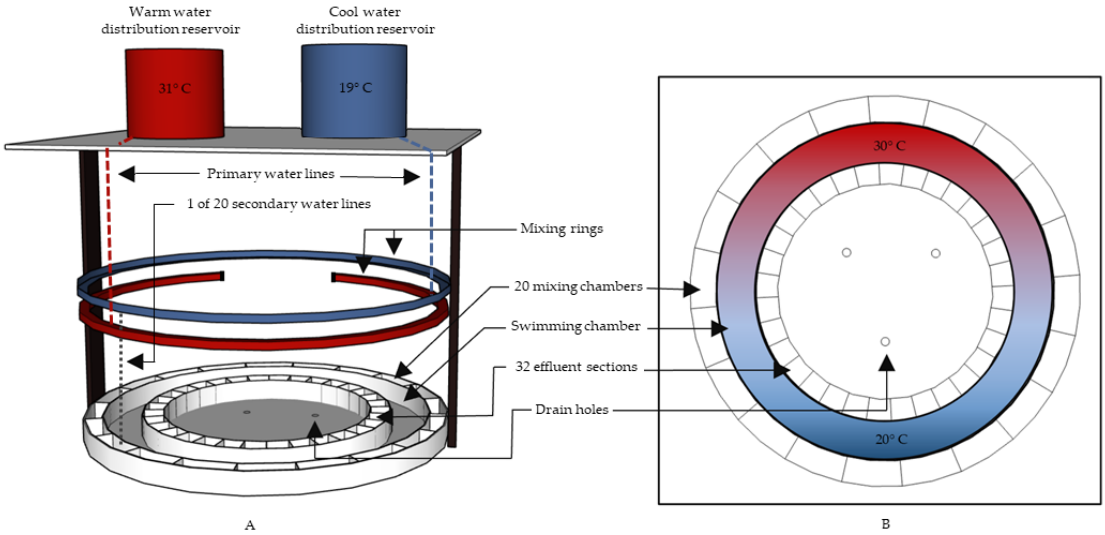

Figure 7. Lateral (**A**) and overhead (**B**) views of the thermal preference chamber that was used to quantify fish's thermal preference and movement (modified from Myrick et al. 2004). One of the 20 secondary water lines is shown to provide an

example of the water distribution from the top mixing ring to the bottom mixing ring and finally into the mixing chamber. The remaining 19 secondary water lines were equally spaced around the mixing rings, with their location corresponding to the center of each mixing section.

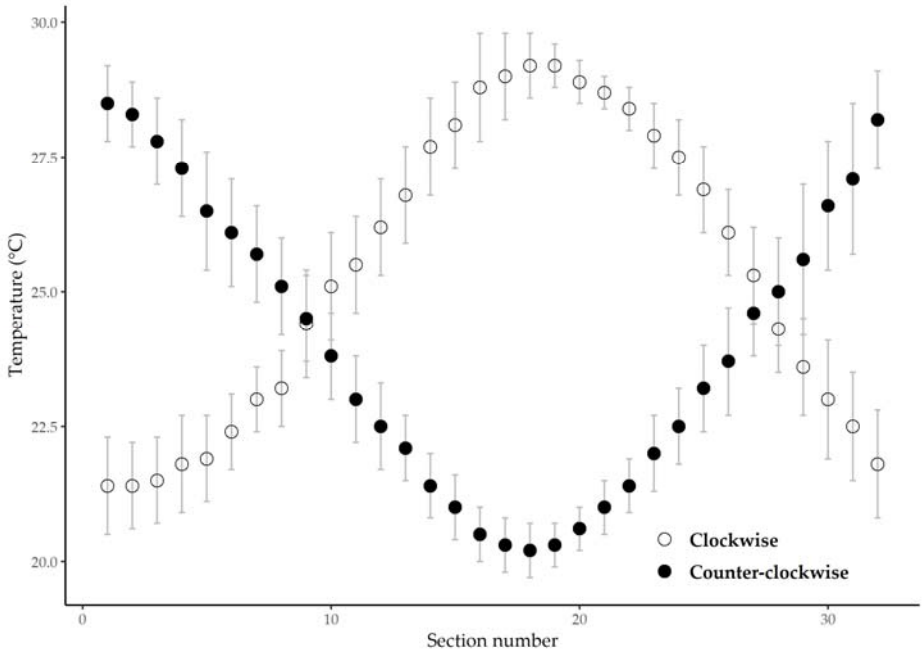

Figure 8. Mean (± one standard deviation) water temperature recorded immediately prior to initiating trials assigned to one of two flow directions, clockwise (open circles) or counter-clockwise (closed circles). The water temperature was measured in the swimming chamber at the center of 32 equidistant visual sections.

During trials we video-recorded fish's movement and behavior with four web-cameras mounted 1.5 m above the surface of the water (Figure 9). Collectively, the four cameras were positioned to capture the entirety of the swimming chamber (Figure 9). Post-trial we used the video files to note the location, and thus temperature, of all fish at the start of every minute during reference condition and treatment periods, for a total of 60 temperatures for each fish. We noted fish's location (nose position) in relation to the 32 effluent sections. To estimate *C. eos*' movement travel, we visually tracked and recorded the number of sections each *C. eos* travelled through in the first 10 s of each minute during the reference condition and treatment periods and multiplied the number of sections traveled by the center width of each section (9.6 cm). Finally, to quantify the species interactions we counted the number of chases that occurred during the first 10 s of each minute in the treatment period.

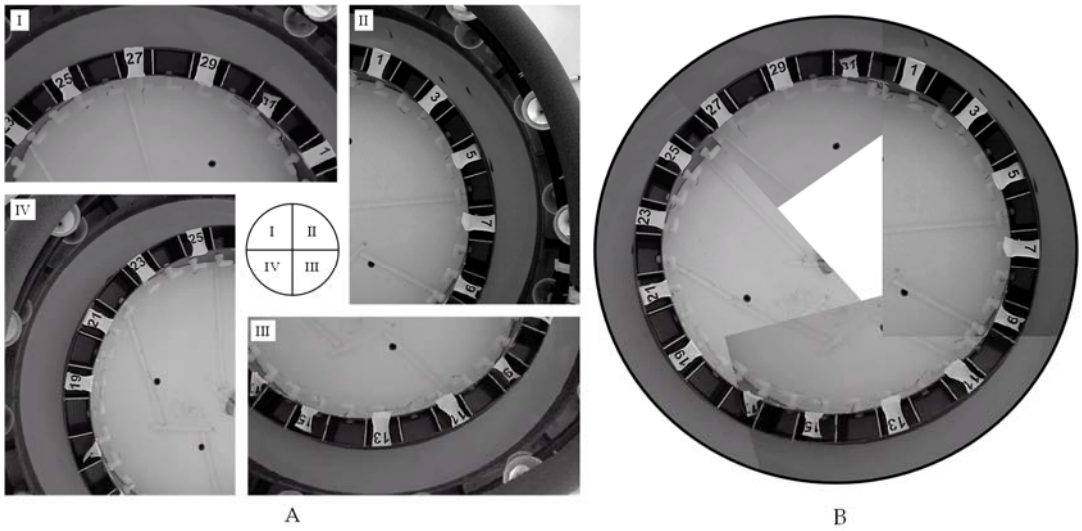

Figure 9. Photographs of the thermal preference chamber. (**A**) Display of the four camera angles. (**B**) Display of when the four images are merged into one. Note: There are four fish in Figure A (II) and in the upper right corner of Figure B.

RESULTS

Thermal preference

Prior to *G. affinis* introductions, *C. eos* occupied an average temperature of 24.3 °C ($\mu_{\alpha.temp}$) that varied between trials ($\sigma_{\alpha.temp}$: mean = 2.5 °C). After *G. affinis* were introduced, *C. eos* selected significantly (PPS = 0.98) higher temperature ranges (mean = 1.7 °C higher ($\mu_{\beta.temp}$); Figure 10). The magnitude of changes in temperature selection varied by trial, ranging from −1.3 °C to 4.7 °C.

Body length of *C. eos* did not explain the inter-trial variation in its temperature selection ($\gamma_1.temp$: mean = −0.38, PPS = 0.69) or movement distance ($\gamma_1.move$: mean = 0.70, PPS = 0.63) before *G. affinis*' introduction, or changes in its temperature selection ($\delta_1.temp$: mean = −0.09, PPS = 0.53) or movement ($\delta_1.move$: mean = 0.07, PPS = 0.56) in response to *G. affinis* introduction.

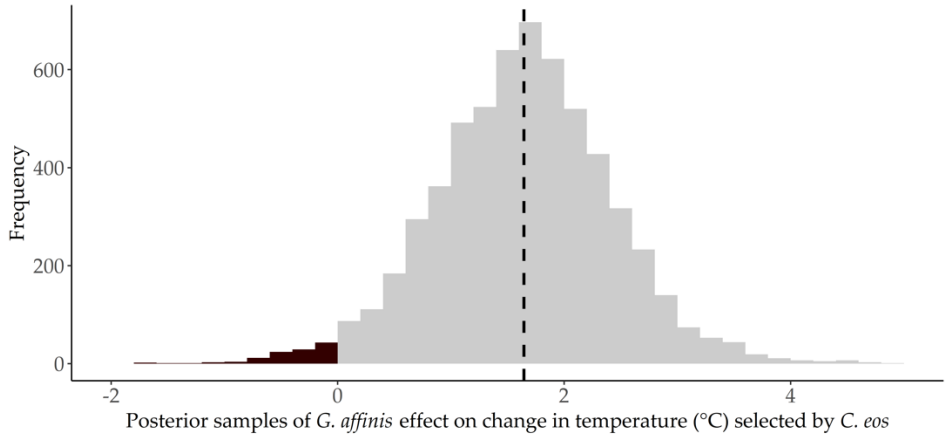

Figure 10. Proportion of posterior samples (PPS) of *Gambusia affinis* introduction effects on the change in temperature selected ($\mu_{\beta.temp}$) in $\beta.temp_j \sim Normal\ (\mu_{\beta.temp}, \sigma^2_{\beta.temp})$ by *Chrosomus eos*. Posterior samples that are positive are shown in gray and those that are negative are shown in black. The vertical black dotted line shows the mean posterior value.

Movement

C. eos' movement distance increased 21% after *G. affinis* were introduced. Without *G. affinis*, *C. eos* moved an average of 10 sections (96 cm) per 10 s across minutes and trials. After *G. affinis* introductions, *C. eos* moved an average of 12 sections (116 cm), and this effect was statistically significant ($\mu_{\beta.move}$: mean = 0.36 (log scale), PPS = 0.96) (Figure 11). Again, the change in the movement distance of *C. eos* varied by trial, ranging from two fewer sections moved to nine more sections of movement after *G. affinis*' introduction.

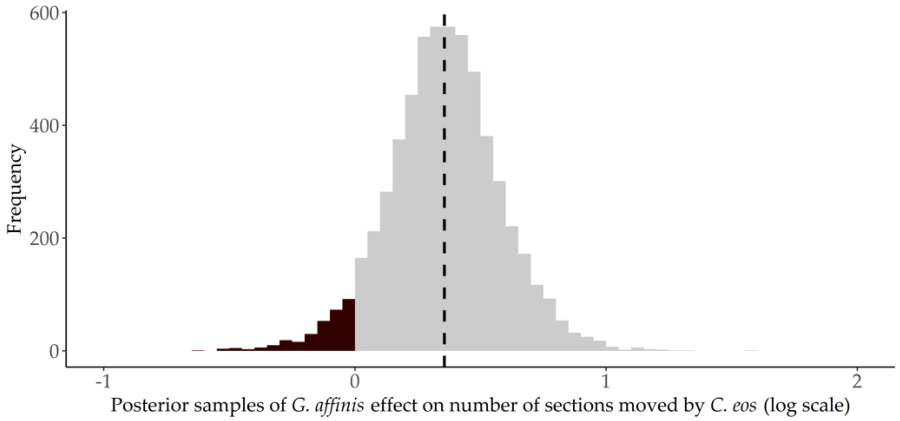

Figure 11. Proportion of posterior samples (PPS) of *Gambusia affinis*' introduction effects on the distance moved ($\mu_{\beta.move}$) in $\beta.move_j \sim Normal\ (\mu_{\beta.move}, \sigma^2_{\beta.move})$ by *Chrosomus eos*. Posterior samples that are positive are shown in gray and those that are

negative are shown in black. The vertical black dotted line shows the mean posterior value.

Agonistic interactions

Contrary to our prediction, more agonistic interactions were initiated by *C. eos* toward *G. affinis* than the opposite. We recorded interspecific interactions (i.e., chases) in 2,562 out of 6,600 total seconds of observations (39%). Of these, 1,976 observations (77%) were chases by *C. eos* of *G. affinis*, and 348 observations (14%) were chases by *G. affinis* of *C. eos* (Figure 12). In the remaining 238 observations (9%), *G. affinis* and *C. eos* chased each other, and we could not determine which species initiated the interactions.

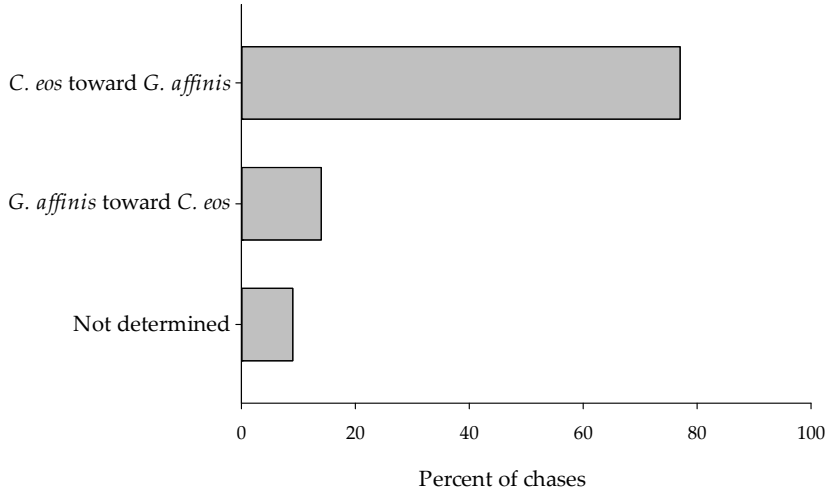

Figure 12. Direction of agonistic interactions between *C. eos* and *G. affinis*.

DISCUSSION

Our results indicated a temperature preference of *C. eos* (24.3 °C) slightly lower than the temperature preference of 25.3 °C estimated by Stauffer et al. (1980) when integrating temperature preferences of fish acclimated to five different temperatures (6, 18, 24, 30, and 33 °C). However, the preferred temperature of 24 °C-acclimated *C. eos* reported by Stauffer et al. (1980) (24.0 °C) was similar to the preferred temperature of the 25 °C-acclimated *C. eos* in this study (24.3 °C). The similarity in the preferred temperatures between these two studies was interesting given the differences between the *C. eos* source populations used in each study. The source population of the hatchery-propagated *C. eos* used in this study was West Plum Creek (a tributary to the South Platte River, CO, USA), which is at approximately 2,000 m in elevation, whereas the source population of *C. eos* used by Stauffer et al. (1980) was Spratt Creek (a tributary of the Thunder Bay system, Lake Huron, Michigan, USA), which is at an approximate elevation of 220 m. As a glacial relict species in Colorado, *C. eos* may have experienced cooler temperatures than other populations in *C. eos*' native range, however our results indicate that *C. eos*'

preferred temperature remains stable across geographic regions when acclimated to similar temperatures.

CONCLUSION

In this study, we observed a native fish shifting its temperature preference in the presence of *G. affinis* in an experimental chamber simulating a thermally heterogeneous habitat, but aggressive behaviors were more frequently observed in the native fish toward *G. affinis* rather than the other direction. We hypothesize that indirect mechanisms (e.g., reduced growth via a thermal displacement and increased movement), not direct agonistic behaviors, may negatively affect *C. eos* in the presence of *G. affinis*. Conservation actions that may help conserve remaining glacial relict populations of *C. eos* include: protecting thermal refugia; promoting habitat enhancement strategies for *C. eos*; the hatchery stocking of *C. eos* into suitable habitats, with priority given to areas that do not include *G. affinis*; and establishing genetic refugia in hatcheries that can be used as source populations for hatchery releases. The thermal experimental chamber used in this study is a promising tool to study the thermal preference, and changes in preference and behavior, of native fish in the presence of invasive species. Additional research is needed to identify which native species are vulnerable to the direct and indirect effects of *G. affinis*' presence, especially given the likelihood of increasing temperatures, which would favor *G. affinis*. Future studies should examine temperatures outside of those included in this study, especially higher temperatures, to test the hypothesis that behaviors change at elevated temperatures. Additional research may also include further investigation into the impacts of *G. affinis* on native fish populations relative to the other ecological traits, especially its reproductive output, of this highly successful invasive species.

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PUBLICATION

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RESEARCH PRIORITY: Tag retention in and effects of passive integrative transponder tagging on survival and swimming performance of a small-bodied darter

OBJECTIVES

Investigate effects of PIT tags on small-bodied fish swimming performance.

ABSTRACT

Darters, a group of North American small-bodied percid fishes, include many imperiled species that could benefit from the development of passive monitoring methods. Passive integrated transponder (PIT) technology is often used to monitor larger fish species under field conditions, however, fisheries biologists have been hesitant to use PIT tags on darters because of the fishes' small size (40 – 200 mm TL) and relatively small peritoneal cavity. No study has determined the effect of PIT tags on the swimming performance of a darter species and few darter species have been PIT tagged. To address this lack of information, constant acceleration trials (CAT) were used to study the swimming responses of Arkansas Darters *Etheostoma cragini* from three treatments: control (no incision or tag), sham (incision and suture), or PIT tagged (surgically implanted 8 x 1.4-mm intra-peritoneal PIT tag and suture). Swimming performance was measured immediately before tagging, 1 day post-tagging, and 7 to 8 days post-tagging. Retention and survival were monitored for up to 199 days post-tagging. Maximum swimming speeds in body lengths per second (BL/s) did not differ between control, sham, and PIT tag treatments (repeated measures ANOVA, $P > 0.05$), nor was maximum swimming speed affected by the tagging procedure. Tag retention was 100% and the overall survival of tagged fish was 100%. An additional comparison of surgical techniques was conducted to determine if suturing the fish improved tag retention and survival. There were no differences in tag retention (100%) and survival (100%) for both groups, however, not suturing the fish reduced handling time by 30 - 45 seconds. Based on these results, 8-mm PIT tags appear acceptable as an individual-based tracking method for darters when combined with the appropriate PIT tag readers or antenna arrays.

INTRODUCTION

Conservation and management of native fishes requires an understanding of their life history, ecology, and habitat preferences, and population status (Fausch et al. 2002; Bestgen et al. 2007), information that can often be gleaned through mark-recapture or mark-resight studies. However, such studies are challenging for species whose body size or morphology precludes them from consideration for most mark or tag types, such as darters (Percidae: *Etheostomatinae*) and topminnows (Fundulidae; Clark 2016). Darters are disproportionally imperiled compared to other groups of North American fishes (Helfman 2007). Of the 203 recognized species of darters, 54 (26%) are designated as critically Endangered, Endangered, or Vulnerable to extinction (IUCN 2019). Because

the group is of growing conservation concern, the development of effective monitoring methods is crucial

Determining whether PIT tags impact darter health and behavior is an important step in their potential adoption as a monitoring method—key assumptions of any tagging effort are that the tags do not affect the survival or behavior of the tagged organisms (Guy et al. 1996). We used this study to develop a surgical technique for PIT tagging *E. cragini* and evaluated tag retention, fish survival post-tagging, and the effects of PIT tags on swimming performance. Additionally, we evaluated survival and tag retention in fish whose incisions were sutured following tag insertion when compared to fish with un-sutured incisions

METHODS

Hatchery–reared *E. cragini* (mean ± SE TL: 51 ± 3 mm; mean wet weight: 1.40 ± 0.28 g) from the Colorado Parks and Wildlife Native Aquatic Species Restoration Facility (Alamosa, CO) were held in a 340-L round polyethylene tank receiving 5 - 10 L/min of air-saturated water at 20 ± 0.5°C through a spray bar that produced a current of 0.05 - 0.10 m/s along the periphery of the tank. The laboratory was kept under a natural photoperiod for Fort Collins Colorado, USA (40.581°N, 105.138°W). Cover was provided in the form of PVC pipe, PVC sheets, and artificial aquatic plants. Fish were fed daily satiation rations of thawed bloodworms.

Individual fish were taken from the holding tank and placed in a 0.9-L tank for 24 h prior to the first measurement of swimming performance and treatment application. Fish were randomly assigned to one of three treatments: control (handled, but no surgery or tag); sham (surgery and suture without a PIT tag), and; PIT tagged (8-mm PIT tag surgically implanted into the fish's body cavity and sutured closed) with a sample size of 15 darters per treatment.

Individual fish V_{max} was measured at three time points to evaluate the short-term effects of PIT tagging on fish swimming ability.

T_0: Immediately prior to surgical treatment application to determine baseline swimming ability;
T_1: One day following the surgical treatment, and;
$T_{7\text{-}8}$: Seven to eight days after the treatment.

Fish were swum in a Loligo Model 32 swim flume (32-L volume, 55 cm × 14 cm × 14 cm test section; velocity range of 3-110 $cm \cdot s^{-1}$; Loligo Systems, Denmark). Fish were given 1 h to become familiar with the flume with a 11-$cm \cdot s^{-1}$ current for rheotaxis. At the beginning of the constant acceleration trial, water velocity increased from the starting velocity by 5 $cm \cdot s^{-1}$ every 5 s until exhaustion, defined as partial or full-body impingement for more than 5 s on the rear screen of the swimming chamber. The velocity at exhaustion was defined as the maximum exposure velocity (V_{max}), and was

recorded in both $cm \cdot s^{-1}$ and body lengths per second ($BL \cdot s^{-1}$). If fish were found to be "cheating", defined as resting on the rear screen of the flume or resting in a low velocity area of the flume, the current was momentarily reversed to encourage swimming behaviors. Non-performing fish, or fish that refused to swim in one of the trials were removed from the study (Table I). Tag retention and survival were monitored daily for up to 221 additional days.

RESULTS

Difference in total lengths and wet weights of *E. cragini* used in the various treatments were not statistically significant (Table I). Neither the full PIT tag insertion procedure nor the sham surgery affected *E. cragini* swimming performance (RMANOVA; p = 0.12; d.f.= 2), nor were there differences between pre-surgery and either post-surgery swimming performance measurements (RMANOVA; p = 0.08; d.f. = 2) (Table 6). PIT tag retention was 100% and survival rates were 100% for control and sham groups, and 88% for the tag group in the swimming portion of the study. The lower survival was caused by two tagged fish that died 7 and 12 days post-tagging; these fish were recorded as non-performers in the swimming study. The mortalities were thought to have resulted from injuries incurred during suturing.

Tag retention and survival were 100% for both sutured and un-sutured fish in the second study (Table I). Sutures were expelled 7 – 14 days post-surgery for sutured fish; incisions of non-sutured fish closed 3 – 5 days after surgery. The process of weighing, measuring, and surgically inserting a PIT tag in an anesthetized fish took 40 – 60 seconds depending on the experience of the tagger; suturing the incision added an additional 30 – 45 seconds of handling time.

Table 6. Effects of tagging procedure on the survival, tag retention rate, and baseline swimming ability (T_0), and 1- and 7-day post-tagging swimming performance (T_1 and T_{7-8}) of *E. cragini*. Tagged fish had an 8.0 × 1.4-mm PIT tag surgically implanted in their peritoneal cavity. Survival and tag retention were monitored for 199 days post-treatment application for control, sham, and tag groups. Values are means with SD in parentheses. Incisions of PIT tagged fish were sutured for this portion of the study. There were no statistically significant differences in maximum swimming velocity within or between treatments (RMANOVA; P > 0.05). Additionally, survival and tag retention were monitored for 243 days for *E. cragini* tagged with 8-mm PIT tags where incisions were sutured closed or left open. There were no statistically significant differences between suture and no suture treatments (X^2; P > 0.05).

Treatment	*n*	TL (mm)	Wt (g)	Survival Rate (%)	Tag Retention Rate (%)	Maximum Swimming Velocity (BL/s)			Non-performers
						T_0	T_1	T_{7-8}	
Control	15	51 (3)	1.40 (0.26)	100	-	13.1 (1.8)	12.2 (1.4)	12.1 (1.4)	1
Sham	15	52 (3)	1.41 (0.28)	100	-	11.9 (1.8)	11.6 (1.1)	11.6 (0.9)	4
Tag	17	52 (3)	1.41 (0.26)	88	100	12.8 (1.6)	12.1 (1.2)	12.3 (2.1)	3
Suture	16	51 (3)	1.42 (0.31)	100	100	-	-	-	-
No Suture	29	53 (3)	1.50 (0.20)	100	100	-	-	-	-

DISCUSSION

Our results show that it is possible to tag *E. cragini* ≥ 48 mm TL with 8-mm PIT tags without significantly affecting swimming ability or survival. Despite the relatively large size of the PIT tags (up to 16% of the fish's TL, but < 2% of their weight), the surgical approach allowed us to successfully tag the fish and, importantly, did not violate two of the key assumptions of any marking or tagging operation—there was no significant difference in survival between tagged and untagged individuals and that the tags did not affect the physical performance of the fish. Indeed, the continued growth of some individuals of *E. cragini* (up to 12 mm during 199-d post-tagging period) and the sexual maturation of male and female darters were further evidence that the tags had little impact on the fish.

The ability to use PIT tags in small-bodied fishes could improve monitoring and conservation efforts for these smaller species by allowing fisheries biologists to monitor their movements with passive or mobile antenna arrays placed in stream networks, at fish passage structures, in laboratory studies, or by allowing rapid broodstock identification in conservation hatcheries. For smaller fish, it may be possible to use alternative technologies, such as the p-Chips evaluated by Moore and Brewer (2021), though that technology may not be suitable for remote detection of fish in the field, at least using current techniques.

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PUBLICATION

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RESEARCH PRIORITY: Otolith microchemistry to estimate an invasive species natal origin

ABSTRACT

Otolith microchemistry has emerged as a powerful technique with which to identify the natal origins of fishes, but it relies on differences in underlying geology that may occur over large spatial scales. An examination of how small a spatial scale on which this technique can be implemented, especially in water bodies that share a large proportion of their flow, would be useful for guiding aquatic invasive species control efforts. We examined trace isotopic signatures in Northern Pike *Esox lucius* otoliths to estimate their provenance between two reservoirs in the Upper Yampa River Basin, Colorado, USA. This is a challenging study area as these reservoirs are only 11-rkm apart on the same river and thus share a high proportion of their inflow. We found that three isotopes (^{86}Sr, ^{137}Ba, and ^{55}Mn) were useful in discriminating between these reservoirs, but their signatures varied annually, and the values overlapped. Strontium isotope ratios ($^{87}Sr/^{86}Sr$) were different between sites and relatively stable across three years, which made them an ideal marker for determining northern pike provenance. Our study demonstrates the usefulness of otolith microchemistry for natal origin determination within the same river over a relatively small spatial scale when there are geologic differences between sites, especially geologic differences underlying tributaries between sites.

INTRODUCTION

Invasive species can have a strong negative influence on aquatic communities, and invasive piscivores are a contributing factor in the decline of native fish populations worldwide (Leprieur et al. 2009; Vitule et al. 2009; Gallardo et al. 2016). The control of nonnative species is critical for conservation, and piscivore management typically focuses on the system-wide removal of nonnative fishes accompanied by management efforts to reintroduce or enhance populations of those that are native (Vander Zanden et al. 2016; Rahel et al. 2018). However, controlling established nonnative piscivores is problematic and is frequently unsuccessful (Vitule et al. 2009; Britton et al. 2011; Cucherosett et al. 2011). Ultimately, the success or failure of removal efforts depends on the probability of reinvasion and an understanding of the sources of established nonnative piscivores.

Northern Pike *Esox lucius* are a widespread nonnative piscivore that are responsible for reductions in native fish populations, especially in the arid western United States (Aguilar et al. 2005; Muhlfeld et al. 2008; Dunker et al. 2020). Northern Pike are not native in the Colorado River Basin and are a major predatory threat to four U.S. federally listed species: the Colorado Pikeminnow *Ptychocheilus lucius*, Humpback Chub *Gila cypha*, Razorback Sucker *Xyrauchen texanus*, and Bonytail *Gila elegans* (Nesler 1995; Zelasko et al. 2016). Johnson et al. (2008) estimated that Northern Pike consumed more fish on a per-capita basis than any other fish species in the Yampa River. Control efforts for Northern Pike are ongoing in the Yampa River, which would benefit from more

information regarding sources of recruitment (Zelasko et al. 2016; Rogers et al. 2005). The Yampa River is located in northwest Colorado and flows primarily to the west from its headwaters in the Flat Tops Wilderness to the Green River near the Colorado/Utah border (Figure 13). Geologic composition underlying the Yampa River is diverse (Figure 13), and this may be reflected in water chemistry which ultimately affects the chemical composition of fish otoliths (Bauch et al. 2012; Walther et al. 2006; Feyrer et al. 2007). It is largely free flowing, with extreme changes in flow being driven by snowmelt (Bauch et al. 2012). Flows generally begin increasing in April, with maximum flows in May and June, and then decreasing flows beginning in July. Two reservoirs in the upper Yampa River have established populations of northern pike: Stagecoach Reservoir and Lake Catamount.

Stagecoach Reservoir and Lake Catamount are both located on the mainstem Yampa River and are separated by 11 rkm (Figure 13). Stagecoach Reservoir is 316 ha with a mean depth of 13 m and has a substantial amount of seasonally inundated terrestrial vegetation that northern pike can use for spawning (Otrautt 2006). Lake Catamount is 228 ha with a mean depth of 4 m. The southern portion of Lake Catamount is relatively shallow with extensive vegetation, making it a highly productive northern pike habitat. These reservoirs are likely major sources of northern pike recruitment into the Yampa River, and Northern Pike eradication efforts are ongoing in Lake Catamount and other areas in the Upper Colorado River Basin (Hill 2004; Martin 2005; Orabutt 2006). Active control efforts have not been enacted on Stagecoach Reservoir because it is logistically infeasible. Therefore, Stagecoach Reservoir will likely remain a source of Northern Pike recruitment to Lake Catamount and the Yampa River for at least the near future. A clearer understanding of Northern Pike recruitment dynamics and sources would help control efforts in the basin.

The purpose of our study was to examine the usefulness of isotopic data derived from otolith microchemistry to inform us of the natal origins of Northern Pike in the Upper Yampa River, Colorado. The specific objectives of our study were to (1) compare isotopic signatures from two reservoirs in the Upper Yampa River that are potential sources of Northern Pike recruitment in the Yampa River and (2) estimate temporal variation in isotopic data.

METHODS

Isotopic concentrations of otoliths was analyzed using Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) at the U.S. Geological Survey (USGS) laboratory in Lakewood, Colorado, USA, using a Perkin Elmer ALAN6000 ICP-MS and a CETAC Technologies LSX-5 laser system with a 25 μm spot size, 10 HZ pulse frequency, and 8–9 J energy. The particles ablated during the analyses were entrained in a carrier gas (Ar) and transported directly to the ICP-MS. With the use of standard reference materials specifically designed for in situ analyses (e.g., Wilson et al. 2002), the raw LA-ICP-MS data could be converted to quantitative concentrations. The raw signals were qualitatively evaluated for distinct changes in elemental response. Once the

integration area was selected, data were converted to concentration using the methods of Longerich et al. 1996. Calcium (40% in $CaCO_3$) was used as the internal standard (USGS calcium carbonate reference material MACS-1). Drift was monitored using periodic analyses with MACS-1. Internal detection limits were 4.9034 ppm for ^{86}Sr, 1.870 ppm for ^{137}Ba, and 1.3682 ppm for ^{55}Mn. No indications of vaterite were observed, so we were confident that the otoliths consisted of aragonite and that the elemental concentrations reflected those in the environment (Pracheil et al. 2019).

The isotopic ratios of the otoliths were analyzed at the Woods Hole Oceanographic Institution Plasma Mass Spectrometry Facility, Woods Hole, Massachusetts, USA, using a Thermo Finigan Neptune multicollector ICP-MS coupled with a New Wave Research UP 193 nm excimer laser ablation system. The laser was configured to run at 80% intensity, 10 Hz pulse rate, 35 μm laser beam spot size, 5 μm per second laser scan speed, and 550 μm laser ablation distance. Data normalization and standardization followed the protocol set forth in Wolff et al. 2012. Otoliths and standards were normalized to a daily mean of the National Institute of Standards and Technology (NIST) standard reference material 987 (SRM 987; accepted $^{87}Sr/^{86}SR$ of 0.71024) using the following formula: $^{87}Sr/^{86}Sr_{normalized} = (0.71024 \div SRM\ 987_{measured}) \times {}^{87}Sr/^{86}Sr_{sample}$ (Wolf et al. 2012). Dissolved otolith certified reference material (CRM, [65]; accepted $^{87}Sr/^{86}Sr$ of 0.70918); and SRM 987 produced daily mean (±1 standard deviation, SD; sample size) $^{87}Sr/^{86}Sr$ of 0.70916 (±0.00001; $n = 5$), and 0.71029 (±0.00006), respectively, and ablations of marine sclerosponge produced a daily mean $^{87}SR/^{86}Sr$ of 0.70918 (±0.00003; $n = 4$) (Wolff et al. 2012).

RESULTS

From 2005–2007, we collected 141 age-0 Northern Pike from Lake Catamount and 96 from Stagecoach Reservoir. Strontium isotopic concentrations in Lake Catamount were not stable, with increasing concentrations from 2005 (mean ± 95% CI; 548 ± 52 ppm), 2006 (893 ± 46 ppm), and 2007 (1216 ± 53 ppm) (Figure 14). Strontium isotopic signatures were more stable in Stagecoach Reservoir, though the concentration did increase in 2007 (896 ± 28 ppm) compared to 2005 (750 ± 34 ppm) and 2006 (787 ± 44 ppm). Barium signatures in Stagecoach Reservoir were fairly stable, but the concentration decreased in 2007 (24 ± 3 ppm) compared to 2005 (29 ± 3 ppm) and 2006 (33 ± 4 ppm). Lake Catamount barium concentration was highest in 2006 (48 ± 4 ppm), versus 2005 (38 ± 3 ppm) and 2007 (41 ± 3 ppm). Manganese isotopic concentrations were similar in Stagecoach Reservoir, though concentration and variation increased each year from 2005 (31 ± 4 ppm) to 2006 (40 ± 6 ppm) and 2007 (59 ± 17 ppm). Manganese isotopic concentration in Lake Catamount was similar in 2005 (55 ± 12 ppm) and 2006 (54 ± 8 ppm), but it increased in 2007 (123 ± 15 ppm).

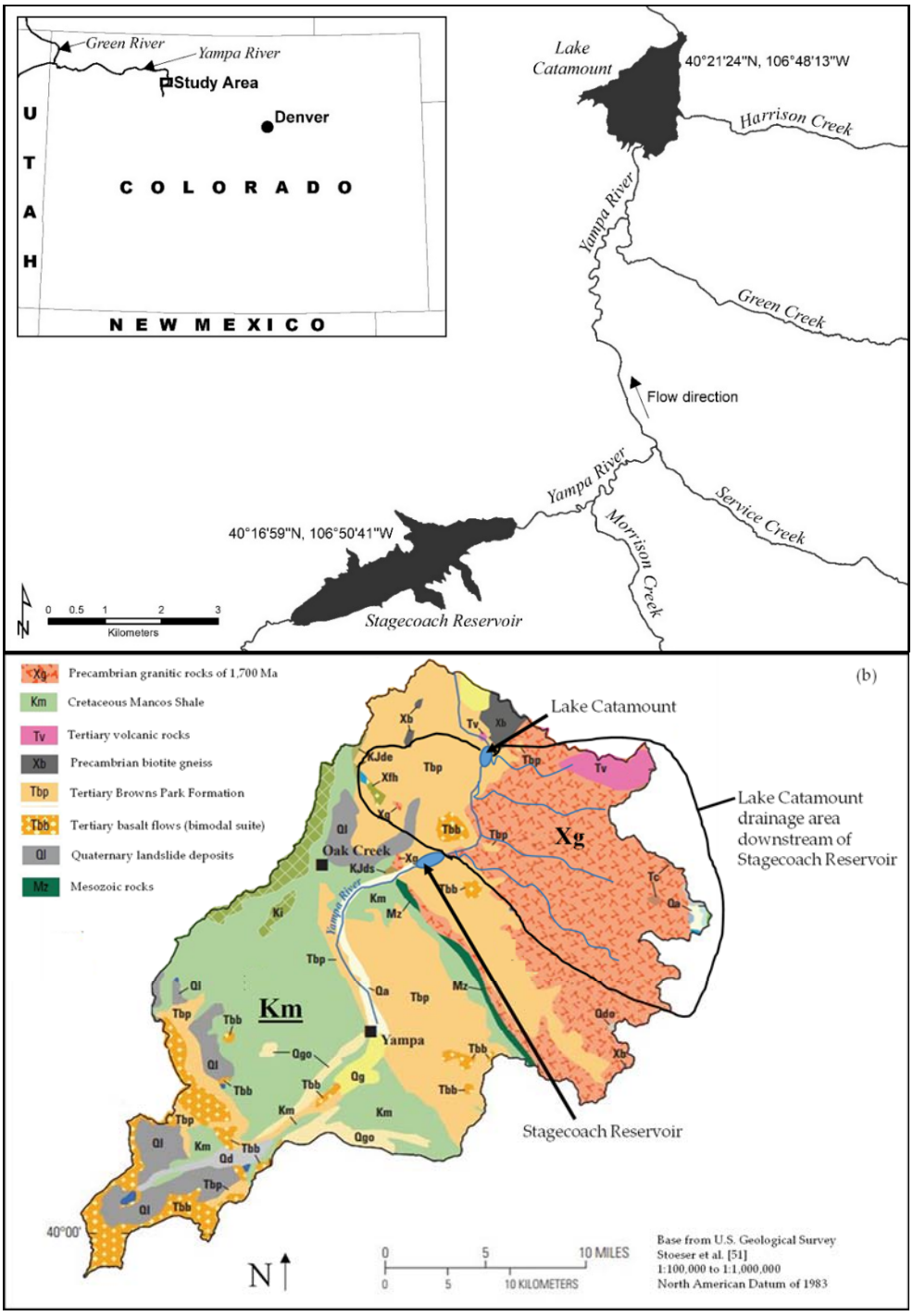

Figure 13. The study area (**a**) was located in northwest Colorado, USA between Stagecoach Reservoir and Lake Catamount. Geological differences (**b**) in the upper Yampa River Basin, Colorado (revised from Bauch et al. 2012). The Yampa River flows north from Stagecoach Reservoir to Lake Catamount. The high gradient tributaries that flow from the east into Lake Catamount and into the Yampa River between Stagecoach Reservoir and Lake Catamount have an underlying geology of Precambrian granite rocks of 1,700 Ma (red area labeled Xg). The area south of Stagecoach Reservoir has lower gradient tributaries that flow over Cretaceous

Mancos Shale (green area labeled Km). Definitions of other colors and abbreviations are provided in Bauch et al. (2012). Base map obtained from Stoeser et al. (2007).

Isotopic concentration classification accuracy averaged 86% with a range of 73–100% (Figure 15). Multivariate discriminant function analysis indicated that strontium had the highest canonical correlations in 2005 and 2007 and barium had the highest in 2006 (Table 7). All canonical axes were significant ($p < 0.001$). MANOVA using strontium, barium, and manganese indicated signatures within sites varied among years (Pillai's trace statistic; $p < 0.01$), with the exception of Stagecoach Reservoir between 2005 and 2006 ($p = 0.11$).

Strontium isotopic ratios ($^{87}Sr/^{86}Sr$) ranged from 0.7091–0.7122 and were relatively stable among years (Figure 14). The ratios did increase slightly from 2005 to 2007, when the mean increased from 0.7109 to 0.7111 and 0.7113, respectively. However, this small increase in isotopic ratio is relatively stable compared to the isotopic concentrations, and its value did not approach the value for the Lake Catamount signature. The top model comparing $^{87}Sr/^{86}Sr$ ratios (no isotopic concentration data were included in this analysis) included an intercept, site, year, and the interaction site by year (Table 8). The top three models accounted for all of the model weight and no other models were supported. Site was included in all weighted models, indicating that the two reservoirs differ in terms of strontium isotopic ratios. The model that contained only year had no weight, which indicates that the signatures are temporally stable. The year and site interaction indicates what little variation there is in the year covariate, and the effect is not consistently positive or negative. Box plots of the $^{87}Sr/^{86}Sr$ ratio from Stagecoach Reservoir and Lake Catamount indicate that these signatures are consistent within a site, different between sites, and relatively stable across years (Figure 16). Therefore, isotopic ratios ($^{87}Sr/^{86}Sr$ ratios) differed between the two investigated reservoirs and were relatively stable among years.

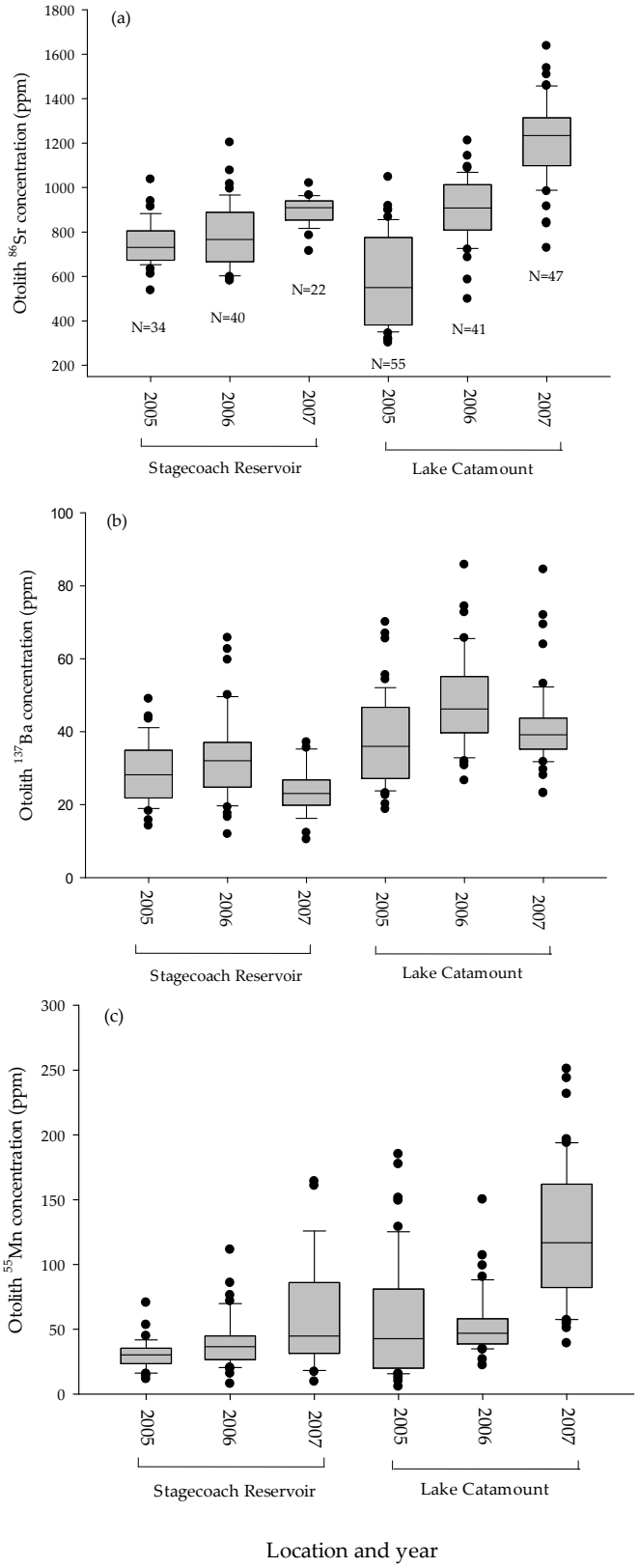

Figure 14. Box plots of northern pike otolith isotopic concentrations ((**a**) ^{86}Sr, (**b**) ^{137}Ba, and (**c**) ^{55}Mn) collected from Lake Catamount and Stagecoach Reservoir.

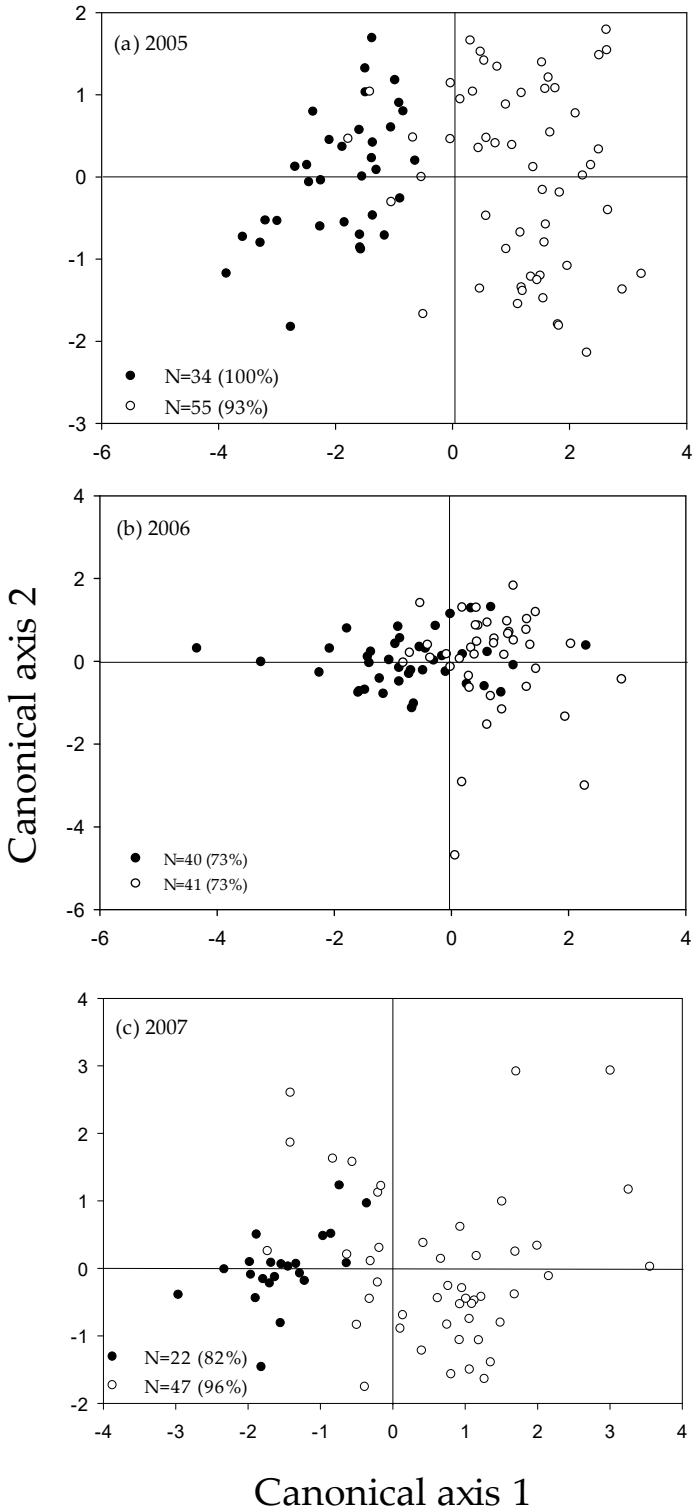

Figure 15. Canonical correlations of age-0 northern pike otolith isotopic concentrations collected in (**a**) 2005, (**b**) 2006, and (**c**) 2007 from Stagecoach Reservoir (closed circles) and Lake Catamount (open circles), Colorado, USA. The legend indicates sample size and correct classification percent for each year. Isotopic concentrations analyzed included barium (^{137}Ba), strontium (^{86}Sr), and manganese (^{55}Mn).

Table 7. Results of multivariate discriminant function analysis of isotopic concentrations of strontium (^{86}Sr), barium (^{137}Ba), and manganese (^{55}Mn) from northern pike otoliths collected in the Yampa River Basin, Colorado, USA. Only canonical axes explaining greater than 10% of the variation were included. All canonical axes shown were significant ($p < 0.001$). The element with the highest correlation for each analysis is shown in bold.

		Canonical Correlations		
Year	**Eigenvalue**	**^{86}Sr**	**^{137}Ba**	**^{55}Mn**
2005	2.24	**0.55**	−0.46	−0.28
2006	0.50	0.37	**0.76**	0.66
2007	1.11	**0.81**	0.54	0.34

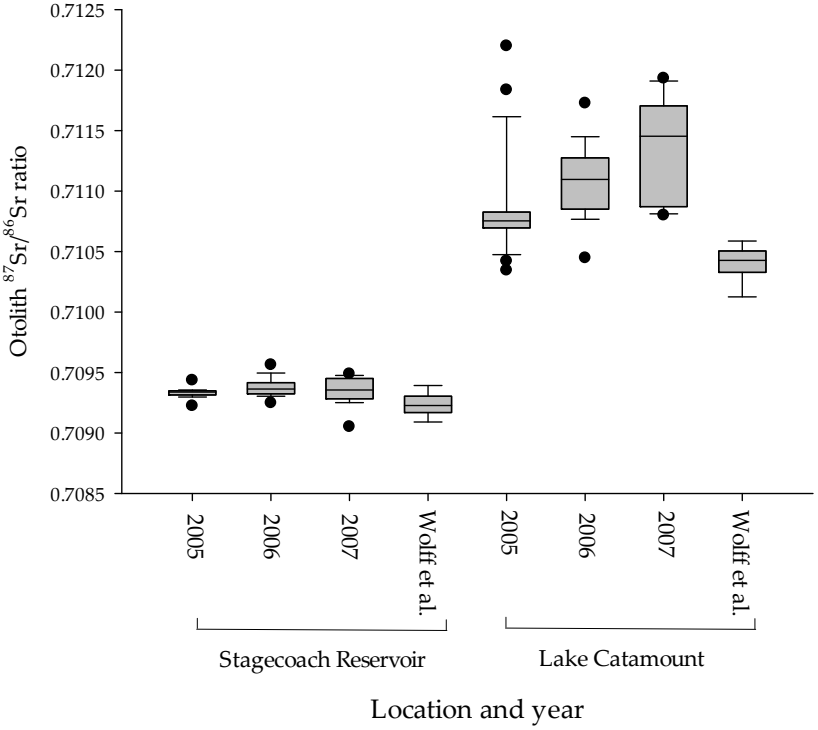

Figure 16. Box plots of the ^{87}Sr/^{86}Sr ratio from northern pike otoliths collected from Lake Catamount and Stagecoach Reservoir, Colorado, USA. Wolff et al. 2012 data were collected as part of a broader spatial scale study and included multiple age classes of fish.

Table 8. Results of model selection using Akaike's information criterion (AIC) for five models examining the effects of site, year, and interaction between site and year on $^{87}Sr/^{86}Sr$ ratios from northern pike in the Yampa River, Colorado, USA.

Model	AIC_c	ΔAIC_c	w_i
Intercept, site, year, and site x year	−2041.8	0	0.823
Intercept, site, year	−2038.2	3.6	0.136
Intercept and site	−2035.8	6.0	0.041
Intercept and year	−1315.0	726.8	0.000
Intercept	−1299.2	742.6	0.000

DISCUSSION

Strontium isotopic ratios ($^{87}Sr/^{86}Sr$) differed between the two investigated reservoirs and were relatively stable among years. This indicates that strontium ratios can be used as a reliable signature for the reservoirs in the study area. Isotopic concentrations varied annually and between sites. This indicates that if isotopic concentrations were to be used to classify northern pike origins, a bank of isotopic signatures from each reservoir would need to be collected annually. However, this would be an expensive management option that may not be feasible. Therefore, future examination of northern pike and other invasive species origins and movement between the investigated reservoirs would be best served to only focus on strontium isotopic ratios since they are spatially distinct and temporally stable (Ciepiela et al. 2019; Wolff et al. 2012; Whitledge et al. 2007). Studies in other areas, including other areas of the Yampa River system beyond the investigated reservoirs, may examine annual variation in isotopic signatures if they are attempting to use them to estimate natal origins.

The long-term efficacy of northern pike control efforts in Lake Catamount depends on the rate of northern pike movement from Stagecoach Reservoir to Lake Catamount. If reinvasion rates are low, then a large, focused effort to remove northern pike may be effective and control northern pike for many years. However, if reinvasion rates are high, continual control efforts will be necessary to keep northern pike numbers low. A greater understanding of these reinvasion rates will help guide management to the appropriate level of control efforts. Strontium isotopes ($^{87}Sr/^{86}Sr$) are different between sites and stable among years. Future studies interested in using otolith microchemistry may examine geological maps of their area of interest. If differences are identified in underlying geology between the study sites, especially in tributaries between sites, then ($^{87}Sr/^{86}Sr$) measurements may be useful for determining natal areas of fishes in those systems despite being in relatively close proximity.

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PUBLICATION

Fitzpatrick, R. M., D. L. Winkelman, and B. M. Johnson. 2021. Using elemental and isotopic data to evaluate *Esox lucius* (Linnaeus, 1758) natal origins in a hydrologically complex river basin. *Fishes*, *6(4)*, 1–14. https://doi.org/10.3390/fishes6040067

RESEARCH PRIORITY

Maintain up to date, statistically defensible knowledge regarding the distribution of native Great Plains fishes in Colorado.

OBJECTIVES

To guide biologists to the most efficient sampling locations to reduce uncertainty given logistical and financial constraints.

See previous Progress Reports for Introduction and Methods. This project is scheduled to be an ongoing, annual site selection tool.

RESULTS AND DISCUSSION

This protocol results in a sampling design that is statistically rigorous and biologist friendly. Biologists tell the model how many sites they are able to sample, and the model optimizes on those constraints. Sampling other locations can be incorporated, as long as sampling protocol is maintained. This protocol is optimal in that it optimizes on one metric—uncertainty. Uncertainty across the species and weights selected according to management priorities. The protocol is adaptive in that it incorporates new data learning—as management objectives change, this protocol can change with them.

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RESEARCH PRIORITY

Laboratory examination of the effects of temperature and winter duration periods on reproductive success of Johnny Darter, *Etheostoma nigrum* (Percidae), in the South Platte River Basin, Colorado.

OBJECTIVES

The ultimate goal of this project is to estimate the combination of winter stream temperature and winter duration period that ensures Johnny Darter reproductive success. The results of this project will provide CPW and CDPHE with insight regarding biologically appropriate winter water temperature standards for the South Platte River Basin. These results can also be implemented into management strategies for the conservation and recovery of other native warm water fishes.

ABSTRACT

Changes in water temperature and its seasonal timing influences the physiological processes of many aquatic ectotherms. Wastewater treatment plants (WWTP) along Front Range streams of Colorado have contributed to warmer and more consistent water temperatures throughout the year, particularly in winter months. Reduced variation in seasonal temperatures may have adverse effects on fishes that rely on temperature fluctuations or sustained periods of specific over-winter temperatures for proper reproductive development. Assessing thermal requirements for reproduction is a necessary step towards the conservation of native warm water fishes residing in WWTP effluent-impacted streams. Johnny Darter *Etheostoma nigrum* are being used as a sentinel species for winter water temperature regulations in Colorado because they are a thermally sensitive native species; however, their winter temperature requirements for successful reproduction are not known. Therefore, we evaluated the effects of winter stream temperature and winter duration on Johnny Darter reproductive success in the laboratory. Winter duration and temperature treatments simulated warmed effluent-impacted streams as well as streams with a natural thermal regime. Data indicated winter temperature and duration influenced timing of reproduction and egg development. Earlier spawning initiation was observed in fish exposed to warm winters and along with longer development time of eggs spawned at cooler water temperatures. Egg and larval production was similar among treatments and indicates that the current winter water temperature standard may be adequate. However, reproductive output needs to be evaluated in the context of seasonal timing because spawning timing has the potential to effect overall production, egg development and survival.

See 2021 Progress Report for Introduction and Methods.

RESULTS

Egg production in Experiment 1 was not statistically different among winter durations (p = 0.332; Figure 17). In Experiment 2, egg production did not differ among winter temperature (p = 0.577) or winter duration (p = 0.374), and no interaction was present (p = 0.450). The larvae production in Experiment 1 did not differ among winter durations (p = 0.587). In Experiment 2, larvae production did not differ among winter temperatures (p = 0.658) or durations (p = 0.287) and there was no interaction (p = 0.596).

Figure 17. Photograph of a spawning tile with Johnny Darter eggs.

In Experiment 1, the spawning temperature of all 4ºC treatments ranged from 15.7ºC to 21ºC and spawning temperature did not influence the days to hatch (Figure 18). The mean days to hatch on each end of the spawning temperature range were relatively similar: 7.5 days (range = 7–9 days) for eggs spawned from 15.7–16.7ºC and 7.1 days (range = 5–10 days) for eggs spawned at the warmest temperatures 20-21ºC (Figure 18). In Experiment 2, days to hatch was negatively related to spawning temperature. Five of the six winter treatments in Experiment 2 displayed a significant negative relationship between days to hatch and spawn temperature. Treatment 4_{90} had a positive slope, likely due to the lower numbers of spawning events across a narrow temperature range (Figure 18). The spawning temperature of all Experiment 2 treatments ranged from 12ºC to 21ºC. The mean days to hatch was 18.4 days (range = 12–24 days) for eggs spawned at the coldest temperatures 12-13°C and 6.7 days (range = 4–9 days) for eggs spawned at the warmest temperatures 20-21°C (Figure 18).

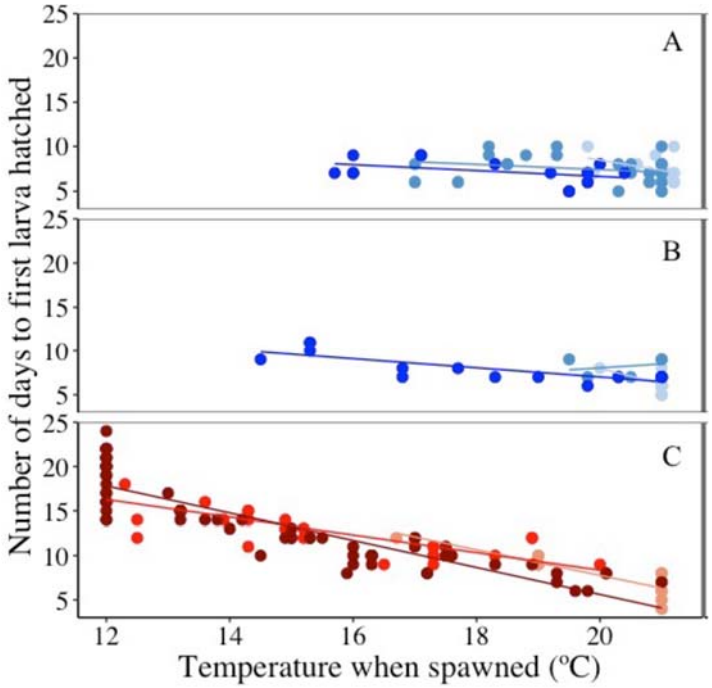

Figure 18. Relationship between the number of days to hatch and spawn water temperature (°C). Each data point represents a tile that had eggs. The color of the points refers to winter temperature treatment: blue are the 4°C treatments and the red are the 12°C treatments. Shade of the color refers to the winter duration treatment: lightest shade is the 60-day winter duration and the darkest shade is the 120-day winter duration. A) 4°C treatments of Experiment 1 at 60, 90, and 120 days; B) 4°C treatments of Experiment 2 at 60, 90, and 120 days; and C) 12°C treatments of Experiment 2 at 60, 90, and 120 days.

Spawning Initiation Date.— In Experiment 1, spawning initiation date did not differ among winter duration treatments ($p = 0.405$), likely due to the high variability in treatment 4_{60}. Treatments 4_{90} and 4_{120} both had a narrow range of days between their earliest and latest 16 spawning initiation date among tanks (11 and 9 days respectively), however treatment 4_{60} had the widest range of 92 days between spawning initiation dates of tanks (March 29–June 29, 2019; Figure 19).

In Experiment 2, spawning initiation date of fish in the three 12ºC treatments was significantly earlier than the 4ºC treatments ($p < 0.001$; Figure 19). The spawning initiation date did not differ among winter duration treatments in Experiment 2 ($p = 0.445$). Winter duration and temperature showed a significant interaction and spawning initiation date depended on both effects ($p < 0.001$; Figure 19). Pairwise comparisons suggested winter duration had a clear sequential effect on the spawning initiation date of fish in the 4°C treatments with the 60-day winter spawning earliest, followed by the 90-day duration, and the 120-day duration was the last to initiate spawning ($0.0002 < p < 0.040$). However, pairwise comparisons showed no evidence that winter duration affects spawning initiation date among the 12°C treatments ($0.067 < p < 0.984$), resulting in the observed interaction (Figure 19).

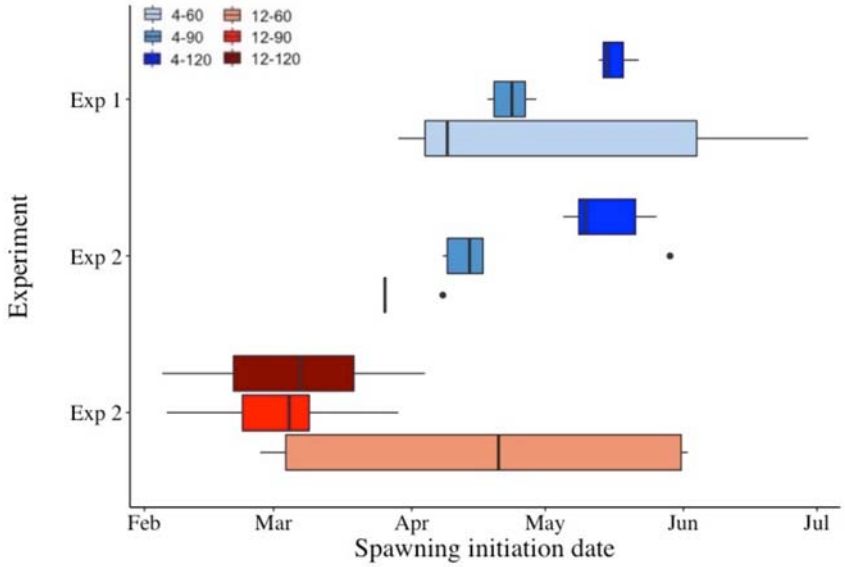

Figure 19. Box and whisker plot of spawning initiation in each tank of Experiment 1 and 2. The color of the boxes refers to winter temperature treatment: blue are the 4°C treatments and red are the 12°C treatments. Shade of the color refers to the winter duration treatment: lightest shade is the 60-day winter duration and the darkest shade is the 120-day winter duration. The vertical bars represent the median, the box is the interquartile range, and the horizontal bars are the minimum and maximum. Dots represent outliers.

Date of First Egg Observation.— In Experiment 1, fish in the 4_{60} treatment spawned first on March 29, 2019, when the water temperature was 19.9ºC, followed by treatment 4_{90} on April 18 at 17.0ºC, and 4_{120} fish were the last to spawn on May 13 at 15.7ºC (Figure 20). In Experiment 2, fish in the 12_{120} and 12_{90} treatments spawned first in early February 2020, when they were still experiencing winter temperatures of 12°C (Figure 20). The first eggs were observed in 12_{120} and 12_{90} on February 5 and 6, respectively. First eggs in the 12_{60} treatment were spawned later on February 27 when fish were approximately halfway through their spring transition and the water temperature was 16.4°C. Fish in the three 4°C treatments all spawned first eggs later in their spring transition: 4_{60} on March 26 at a water temperature of 20°C, 4_{90} on April 8 at 15.3°C, and 4_{120} on May 5 at 14.5°C (Figure 20).

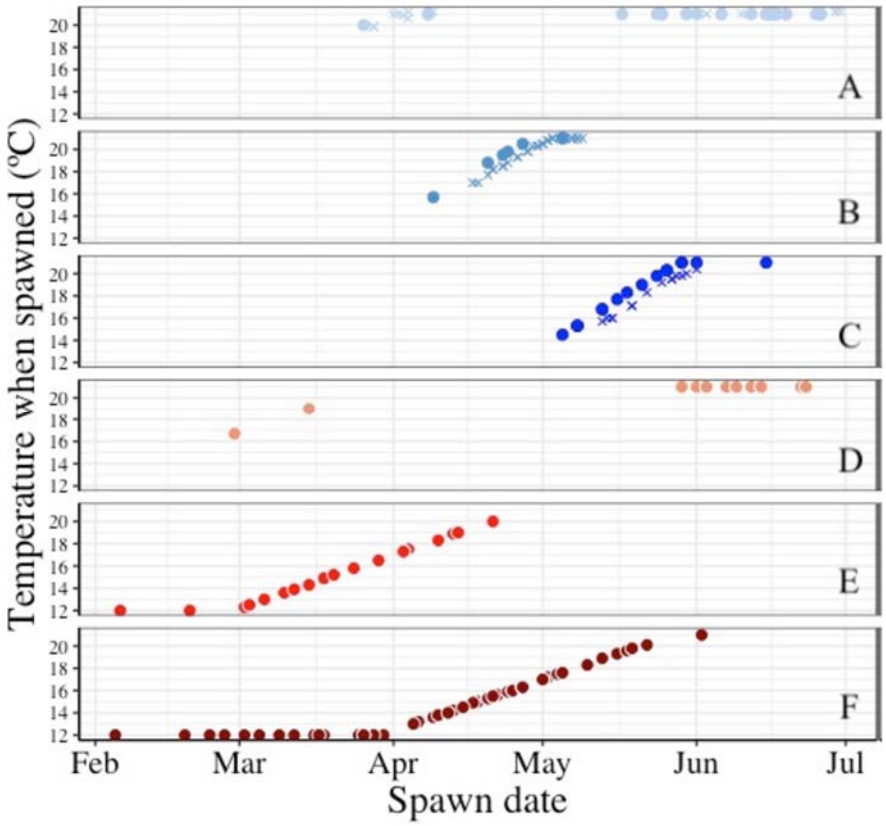

Figure 20. Faceted scatterplot of the spawning timing of all treatments in Experiment 1 (indicated by x) and 2 (indicated by dots). Each data point represents a tile that had eggs. The color of the points refers to winter temperature treatment: blue are the 4°C treatments and are the 12°C treatments. Shade of the color refers to the winter duration treatment: lightest shade is the 60-day winter duration and the darkest shade is the 120-day duration. A) treatment 4_{60} of Experiment 1 and 2; B) treatment 4_{90} of Experiment 1 and 2; C) treatment 4_{120} of Experiment 1 and 2; D) treatment 12_{60} of Experiment 2; E) treatment 12_{90} of Experiment 2; and F) treatment 12_{120} of Experiment 2. All data from both experiments are shown: Experiment 1 treatments are represented by the X's and are from the 2019 spawning season, whereas Experiment 2 treatments are represented by the circles and are from the 2020 spawning season.

DISCUSSION

Water temperature strongly influences ectotherm biology and is considered a master variable regulating fish physiology (Brett 1956; Nelson and Palmer 2007; Isaak et al. 2010; Hester and Doyle 2011). Our data indicated that winter conditions significantly influenced spawning initiation date and the rate of egg development. However, Johnny Darter egg and larvae production in the laboratory were not substantially different among the winter conditions examined, indicating that production is not critically dependent on winter temperature or duration. Although winter conditions did not appear to influence egg and larvae production in the laboratory, spawning timing has the potential to affect overall production in the wild through temperature mediated effects on egg development and survival. Therefore, it is crucial that production results be evaluated within the context of seasonal timing of spawning.

This study suggests that the current CDPHE winter water temperature standard of 12ºC and a duration of 90 days appears to be adequate for egg and larval production in Johnny Darter. However, it is important to recognize that egg and larval production are influenced by spawning timing through its effects on egg development. Adverse effects on reproduction from early spawning in effluent-impacted streams could reduce overall production and lead to recruitment failure and affect population sustainability (Farmer et al 2015; Firkus et al. 2018). Therefore, spawning timing must be considered during criteria evaluation and management decision-making for water temperature standards in the South Platte River Basin. We would also argue against shortening the duration of the winter standard because short winters could be disadvantageous to reproduction based on the long cessation of spawning seen in all 60-day winter treatments.

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PUBLICATIONS

Baum, C. M. 2021. Temperature and winter duration requirements for reproductive success in Johnny Darter *Etheostoma nigrum* in the South Platte River basin, Colorado. *Thesis*. Department of Fish, Wildlife, and Conservation Biology. Colorado State University, Fort Collins, Colorado.

RESEARCH PRIORITY

Field examination to determine if elevated stream temperatures from wastewater effluent alter natural reproductive development in Johnny Darter to help guide temperature standards.

OBJECTIVES

The goal of this study is to evaluate the reproductive condition of wild Johnny Darter to determine the effects of elevated water temperature on reproductive development, focusing on areas surrounding (WWTP) effluent discharge locations.

See 2021 Progress Report for Introduction, Methods, and previous Results.

RESULTS AND DISCUSSION

Temperature

Winter water temperatures were collected at our established 2021 sampling locations supported our hypothesis of the coldest winter temperatures at the FU site, followed by the U site, the FD site, and finally the D site, which had the warmest winter weekly average water temperatures (WAT) in both the Big Thompson River and St. Vrain Creek. In the winter of 2020-2021 (December, January, and February) WAT in the Big Thompson River was significantly different between sampling sites (Welch's ANOVA; $p<0.01$). During this time the WAT was 1.8˚C at the U site, 11.48˚C at the D site, and 4.45˚C at the FD site and all significantly differed (Games-Howell Post-Hoc pairwise comparison; $p<0.01$; Figure 21). The WATs in the St. Vrain similarly were different between sites during the winter period (Welch's ANOVA; p-value<0.01).

The winter WAT was 1.5˚C at the FU site, 2.4˚C at the U site, 7.0˚C at the D site, and 4.6˚C at the FD site and all significantly different from one another (Games-Howell post-hoc pairwise comparison; $p<0.05$; Figure 21). Notably, WAT during the winter of 2020-2021 did not exceed CDPHE WS-I water temperature standards at all sites most of the time (CDPHE 2020). However, the WAT on the first week of December at the D site on the Big Thompson did exceed this threshold (12.8˚C). Logger error at the Poudre River site (C) resulted in a loss of winter water temperature data after the first two weeks in December of 2020 so it was excluded from comparisons. Weekly average water temperature during this time was 2.9˚C.

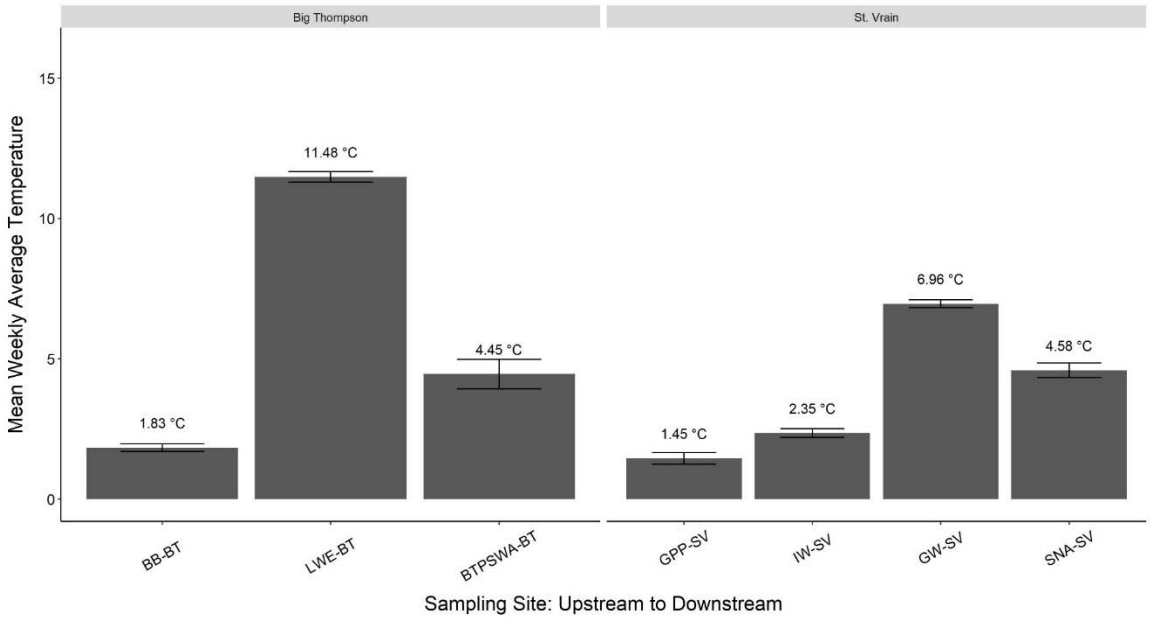

Figure 21. Mean weekly average temperatures in the winter season (December–February) at sampling sites on the Big Thompson River and St. Vrain Creek in 2021. FP–BT, the far upstream site on the Big Thompson, was not established for temperature monitoring until spring 2021 and thus no temperature data for winter 2020–2021 exist. Error bars represent 95% confidence intervals.

In March, April, and May when spring sampling was occurring WATs were similar between all sampling sites within each river, except for the D site on the Big Thompson which was significantly greater than both U and FD sites until week 17 of 2021 (Figure 22). During the sampling period, WATs did not exceed previously documented Colorado spawning temperature for Johnny Darter (17˚C; Propst and Carlson 1989). However, the D site on the Big Thompson always exceeded 11.7˚C, a documented spawning temperature of Johnny Darters elsewhere in its range (Becker 1983). On the Big Thompson the FD site also exceeded this threshold on week 14, and by week 17 all sites exceeded 11.7˚C. On the St. Vrain all sites were near 11.7˚C on week 14, with the D site slightly warmer, though it was not significant. A snowstorm and cold weather occurred during weeks 15 and 16, lowering the water temperature for most sites. By weeks 17 and 18 all sites on the St. Vrain were close to the 11.7˚C threshold. Water temperatures in the Poudre River were similar to the St. Vrain, though were slightly warmer in week 17 and slightly cooler in week 18 (Figure 22). Logger error resulted in no temperature data being recorded in week 12 or 13 of 2021 in the Poudre River.

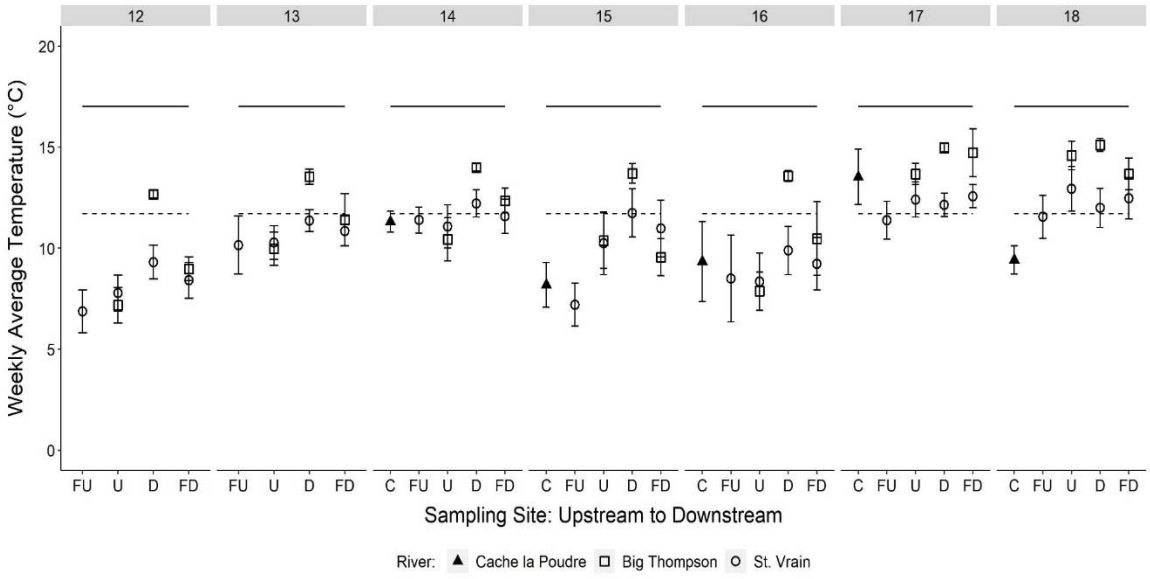

Figure 22. Weekly average temperatures for each week sampling occurred in spring of 2021 at the Control (C) site on the Poudre River, and the Far Upstream (FU), Upstream (U), Downstream (D), and Far Downstream (FD) sites on the Big Thompson and Saint Vrain. The solid line represents previously documented spawning temperatures for Jonny Darter in Colorado (17˚C; Propst and Carlson 1982) and the dashed line represents the coolest spawning temperature recorded elsewhere in their range (11.7˚C; Becker 1983). Fish samples were not collected from the Big Thompson on week 13 or from the St. Vrain on week 12 or 18. Spring 2021 temperatures were not recorded for the far upstream site on the Big Thompson due to its recent site establishment. Weeks 12-18 of 2021 occur during the last two weeks in March through the first week in May. Error bars represent 95% confidence intervals.

Female Gonad Histology

Overall, assigned developmental stages of females and presence of progressively more developed phases of oocyte follicles were similar (Figure 23), though in some cases staging failed to acknowledge females in transition from one stage to another. For example, a female in stage 2 may have a few late vitellogenic follicles present and within a short period of time transition to stage 3. Because histological staging and developmental oocyte phase presence are similar, and the latter is more descriptive, we chose to focus on presence of each developmental phase of oocyte follicle to describe the female reproductive histology.

In 2020-2021 all-female Johnny Darters collected from October-February had no vitellogenic follicles, suggesting none of these females had started vitellogenesis, the point which a female has committed to spawning that season (Óskarsson et al. 2002; Leino et al. 2005). One exception to this was at the D site on the Big Thompson on February 4, 2021 (week 6), where one female out of four sampled had an ovary which contained 10% early vitellogenic follicles.

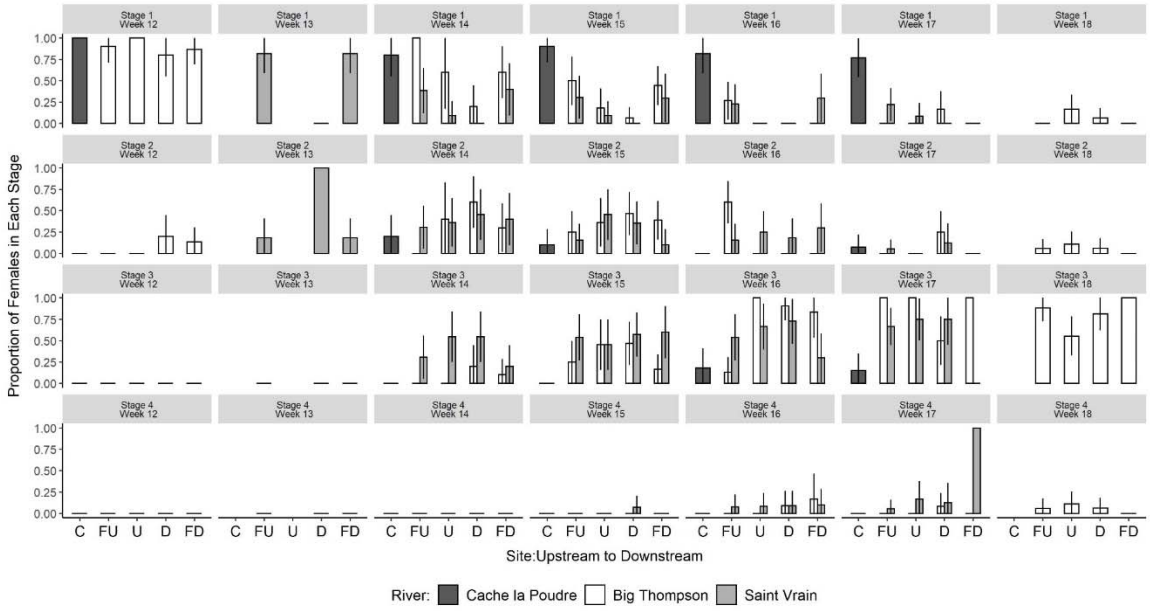

Figure 23. Proportions of females in each reproductive stage sampled each week during the spawning season in 2021 at the Control (C) site on the Poudre River, and the Far Upstream (FU), Upstream (U), Downstream (D), and Far Downstream (FD) sites on the Big Thompson and St. Vrain Creek. Stages were assigned using OECD guidelines (Johnson et al. 2009). Error bars represent 95% confidence intervals. Horizontal lines at 0 represent a sampling occasion in which no females met the criteria. The St. Vrain was not sampled on week 12, the Big Thompson and Poudre Rivers were not sampled week 13, and only the Big Thompson was sampled on week 18 due to high flows elsewhere. The upstream site on St. Vrain Creek was not established until week 14.

Fish health

Histological pathology identified numerous parasitic and non-parasitic diseases afflicting the Johnny Darters we sampled. Identified diseases and infections are listed in Appendix C. Most fish sampled were diseased. The most common ailments included parasitic infections in the brain and the oropharynx (throat), trematode infections in the coelom (main body cavity) and the pericardium (heart), and the disease pericarditis (inflammation of the heart). Gonadal parasites were present in some fish, but overall percentages of afflicted fish were low (0% in the Poudre River, 1.8% in the Big Thompson, and 0.9% in the St. Vrain). Interestingly, pericarditis was significantly greater in the Poudre River than in the Big Thompson and St. Vrain (81.9%, 39.9% and 14.8% respectively; $p<0.01$; Figure 24). Pericardial trematodes were noted in 27.4% of fish with pericarditis in the Poudre River, 3.2% in the Big Thompson River, and 12.1% in the St. Vrain. Though only seen in 27.4% of fish with pericarditis in the Poudre River, it is thought that pericardial trematodes may be the main cause of pericarditis in Poudre River fish, having been omitted during the histological slide processing. Parasitic infection in general was significantly higher in the Poudre River than in the Big Thompson and St. Vrain (85.7%, 52.1%, and 63.4% respectively; $p<0.01$), particularly trematode infections (73.2%, 28.6%, and 24.6% respectively; $p<0.01$; Figure 24).

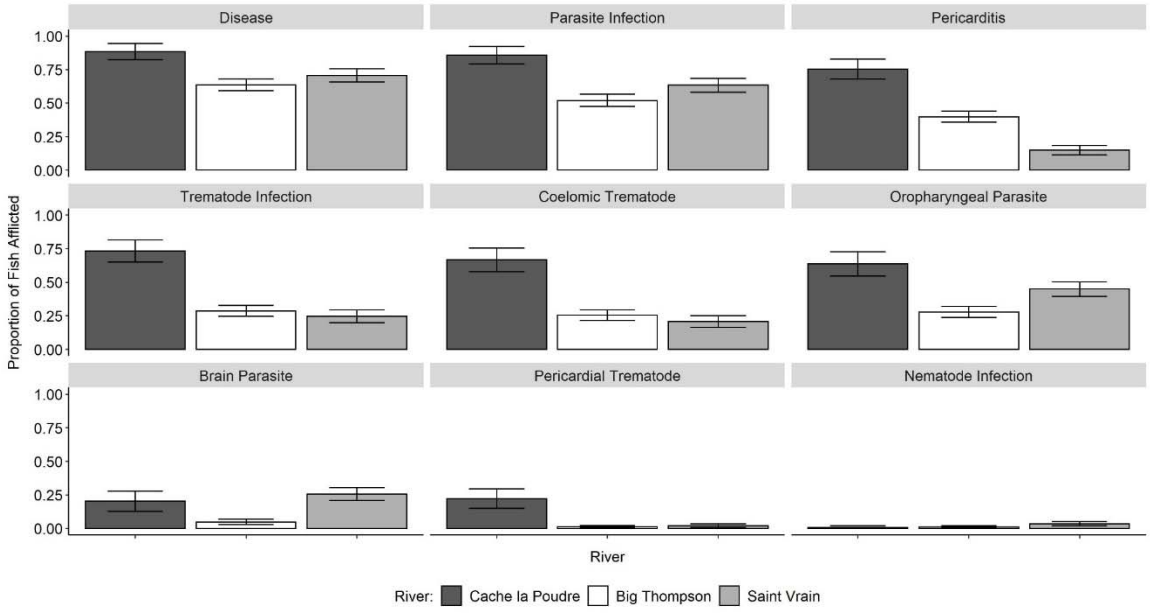

Figure 24. Proportion of fish sampled from each river afflicted with most common ailments noted through histological pathology. Disease refers to fish afflicted with any parasitic or non-parasitic disease. Parasite infection refers to fish infected with any parasite in any tissue, including trematodes, nematodes, and protozoa, as opposed to a brain or oropharyngeal parasite which refer to parasite infections particularly on the brain or oropharynx (throat). Trematode and nematode infection refer to fish infected with a trematode or nematode on any tissue, as opposed to pericardial or coelomic trematodes, which refer to trematodes specifically in the pericardium or coelom. Error bars represent 95% confidence intervals.

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PUBLICATION

Adams, C. M., A. Ficke, and D. L. Winkelman. 2021. Review of current knowledge on CDPHE WS-II designated species: Temperature impacts and laboratory study feasibility. Colorado Cooperative Fish and Wildlife Unit, Colorado State University, Fort Collins, Colorado.