EASTERN PLAINS NATIVE FISH RESEARCH

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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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COLORADO EASTERN PLAINS NATIVE FISH PROJECT SUMMARY

Period Covered: April 1, 2022 to March 31, 2023

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

PUBLICATIONS

Adams, C. M., D. L. Winkelman, P. A. Schaffer, D. L. Villeneuve, J. E. Cavallin, M. Ellman, K. Santana Rodriguez, and R. M. Fitzpatrick**.** 2022. Elevated Winter Stream Temperatures below Wastewater Treatment Plants Shift Reproductive Development of Female Johnny Darter *Etheostoma nigrum*: A Field and Histologic Approach. Fishes 7(6):1–22. https://doi.org/10.3390/fishes7060361

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Adams, C. M., D. L. Winkelman, and R. M. Fitzpatrick. 2023. Impacts of wastewater treatment plant effluent on the winter thermal regime of two urban South Platte Tributaries. *Frontiers in Environmental Science*.

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<u>RESEARCH PRIORITY</u>

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 and 2022 Progress Reports for additional details regarding Introduction, Methods, and previous Results.

INTRODUCTION

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 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

The first step in the metabarcoding process was primer selection and reference database development. 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. Metabarcoding libraries were prepared using a two-step PCR strategy similar to that used by Hopken et al. (2021). Once the database was developed, samples were collected to compare eDNA results to electrofishing and seining. 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. 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. 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). Extraction methods were modified from Spens et al. (2017) and Miya et al. (2015).

RESULTS AND DISCUSSION

Comparative eDNA samples collected in the field, just prior to electrofishing sampling, were extracted using a protocol previously optimized for this study. From all three site replicate and single control negative collected at a site (n=4), the 12S and 16S rRNA mitochondrial sub-units were amplified using PCR. Each PCR was conducted in triplicate producing twelve replicates per locus (i.e., 12S and 16S) to be sequenced from each survey site (n=24). Replicates from all sites were sequenced using the MiSeq next-generation sequencing platform (Illumina, San Diego, California, USA).

Raw data produced from the MiSeq platform was analyzed using a custom bioinformatics pipeline. Sequences were first trimmed to remove unnecessary nucleotides (e.g., primers) that may inhibit future taxonomic assignment. Trimmed sequences were passed through a quality filter that removed any reads that fell below a standard quality score determined by the MiSeq platform. Sequences were then limited to unique reads and clustered into groups of 98% similarity. Clustered sequences were assigned taxonomy using two reference databases based on 98% similarity. Multiple databases were used to validate assigned taxonomy from one another. The databases included were the previously developed local reference database built from fishes collected in Colorado by Colorado Parks and Wildlife and the Midori webserver (Leray et al. 2018). Due to the high computational resources required to perform this analysis, all computation was done on the Alpine high performance computing resource at the University of Colorado Boulder. Alpine is jointly funded by the University of Colorado Boulder, the University of Colorado Anschutz, Colorado State University, and the National Science Foundation (award 2201538). Output from the bioinformatics pipeline was finally cleaned and summarized for each site using a script in program R (R Core Team 2022). While comparative analyses between eDNA and paired-electrofishing samples awaits the receipt of electrofishing data, a general summary of the species detected across sites was generated (Table 1).

Table 1. Preliminary detections (species were detected in at least one replicate) of species from eDNA samples across all sites.

CPW Code	Common Name	Sequenced in Local Database	Preliminary eDNA Detection Across Sites
ARD	Arkansas Darter	x	x
BBH	Black Bullhead	x	x
BGL	Bluegill	x	x
BMS	Bigmouth Shiner	x	x
BMW	Brassy Minnow	x	x
BST	Brook Stickleback	x	x
BUR	Burbot	x	
CPP	Common Carp	x	x
CRC	Creek Chub	x	x
CSH	Common Shiner	x	x
DRM	Freshwater Drum	x	x
FHC	Flathead Chub	x	x
FLC	Flathead Catfish	x	
FMW	Fathead Minnow	x	x
IOD	Iowa Darter	x	x
JOD	Johnny Darter	x	x
LAC	Lake Chub	x	
LGS	Longnose Sucker	x	x
LMB	Largemouth Bass	x	x
LND	Longnose Dace	x	x
LOC	Brown Trout	x	x
MSQ	Western Mosquitofish	x	x
NPK	Northern Pike	x	
NRD	Northern Redbelly Dace	x	
ORD	Orangethroat Darter	x	
OSF	Orangespotted Sunfish	x	
PKF	Northern Plains Killifish	x	x
PMW	Plains Minnow	x	
PTM	Plains Topminnow	x	
QUI	Quillback	x	x
RDS	Red Shiner	x	x
SAH	Sand Shiner	x	x
SMB	Smallmouth Bass	x	x
SMM	Suckermouth Minnow	x	x
SNF	Green Sunfish	x	x
SRD	Southern Redbelly Dace	x	x
STP	Stonecat	x	x
STR	Central Stoneroller	x	x
WHS	White Sucker	x	x
YBH	Yellow Bullhead	x	

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PUBLICATIONS

Friebertshauser, R. J., A. Piaggio, M. Hopken, and R. M. Fitzpatrick. 2020. Colorado Plains Fishes Metabarcoding Protocol. Colorado Parks and Wildlife, Fort Collins, Colorado.

Friebertshauser, R. J. 2022. Environmental DNA (eDNA) extraction from 47 mm disc filters and Longmire's buffer using the DNeasy® blood and tissue kit (QIAGEN). National Wildlife Research Center Standard Operating Procedure. Fort Collins, Colorado.

<u>RESEARCH PRIORITY:</u> Design, construction, and hydraulic evaluation of a model rock ramp fishway

CITATION

Swarr, T., R., R. M. Fitzpatrick, and C. M. Myrick. 2023. Design, construction, and preliminary hydraulic evaluation of a model rock ramp fishway. *North American Journal of Fisheries Management*.

OBJECTIVES

The purpose of this study was to design and built a hydraulic research flume to specifically conduct controlled laboratory experiments on aspects of fish passage design and function such as fishway slope, roughness, and flow.

ABSTRACT

New fish passage structures are frequently designed using information gained from existing structures, particularly those that have been shown to allow passage of the target species. However, this process rarely involves a pre-installation design and testing phase. Developing an apparatus that allows rapid and relatively low-cost testing of potential fish passage structure designs prior to field installations could reduce the reliance on a "build it, monitor it, does it work?" approach. To meet this need, we developed an indoor research flume at the Colorado State University Foothills Fisheries Laboratory that housed a full-scale experimental rock-ramp fishway. The slope of the flume can be adjusted (0–10%) and integrates a set of four PIT tag antennas to allow fine resolution tracking of fish movements in the fishway. The flume can deliver variable flows (up to $0.082 m^3/s$) of 9–25°C water. Lessons learned during the design, construction, and initial operation of the flume are presented here. The basic system could be adapted for similar studies in other regions and provides a robust and flexible infrastructure that could be modified for other evaluations of instream structures in lotic systems.

INTRODUCTION

Fish passage structures or "fishways" are used to improve or restore longitudinal connectivity of streams and rivers on a global scale (Roscoe and Hinch 2010; Pennock et al. 2017; Silva et al. 2018). The design and construction of fishways dates back to at least 17th century France where regulations for their construction were already in place (McLeod and Nemenyi 1941). Early fishways were sometimes blasted out of bedrock to create primitive pool and weir structures to pass Atlantic Salmon *Salmo salar* over waterfalls (Berg 1973). Since then, multiple designs have been developed to accommodate a variety of fish species and project budgets (Katopodis and Williams 2012; Steffensen et al. 2013; Richer et al. 2020). These designs, including pool-and-weir, pool-and-weir-with-orifice, vertical slot, and rock ramp fishways, have been installed in waterways worldwide, with widely varying degrees of fish passage success (Katopodis and Williams 2012; Silva et al. 2018; Keefer et al. 2021).

A drawback to many past and contemporary fishway design efforts is that innovation in design often relies upon the evaluation of completed fishways, a process that requires the post-construction monitoring of passage success of the novel fishway using relevant metrics (Silva et al. 2018). This iterative design, construction, and monitoring process consumes a considerable amount of time, delaying the speed with which new innovations can be tested and widely adopted. Additionally, the metrics by which the success of existing fishways are evaluated are varied, though efforts such as that by Silva et al. (2018) are helping to create a widely-adopted set of criteria for evaluating passage success. This is then compounded by the need to adjust the designs using field- or laboratory-derived data on the expected passage performance (or swimming performance) of the target fish species, and the nature of those data (e.g., collected using large or small flumes) can affect the utility of the information. Modern fishways are frequently expensive, with cost estimates ranging widely ($10,000 to over $30,000 USD per vertical foot of dam) based on the specific project (Connecticut River Watershed Council and National Park Service 2000). Overall, this iterative process of build-and-evaluate is expensive, time consuming, and potentially risky when new approaches or innovations are being tested under field conditions or when the goal of the fish passage structure is to facilitate passage of species for which no performance or passage data currently exist.

An alternative approach to passage design is to use physical models of fishways to evaluate design features under controlled (laboratory) conditions using the target fish species before building them in the field, as was done for Rio Grande Silvery Minnow *Hybognathus amarus* (Bestgen et al. 2010), Longnose Dace *Rhinichthys cataractae* (Dockery et al. 2017), Flathead Chub *Platygobio gracilis,* Creek Chub *Semotilus atromaculatus*, White Sucker *Catostomus commersoni* (Ficke et al. 2011) and White Sturgeon *Acipenser transmontanus* (Cheong et al. 2006). Post-installation refinement of these laboratory-tested designs can then stem from further research into fish physiology, fish behavior, hydraulics, and, most importantly, from well-implemented monitoring of fishway performance under field conditions from fish, hydraulic, and operational points of view.

In response to the need for an experimental apparatus that would allow testing of a full-scale rock ramp fishway (i.e. an artificial, nature-like riffle) (Harris et al. 1998; Ficke 2015), we designed and built a hydraulic research flume to specifically conduct controlled laboratory experiments on aspects of fish passage design and function such as fishway slope, roughness, and flow. This Management Brief describes the design and construction process. Additionally, preliminary hydraulic data describing operational conditions within the fishway are presented.

METHODS

Flume Design

The development of a modular research flume and fishway was the first phase of a larger project that focused on identifying the ideal slope for rock ramp fishways for successfully passing a variety of small-bodied fishes of conservation concern, such as Arkansas Darters *Etheostoma cragini* and Flathead Chub, which are native to rivers flowing into the U.S. Great Plains. To complete this project, a variable slope (0–10%) flume that could hold a full-scale rock ramp bypass fishway was needed. In addition to variable slope, the ability to control as many environmental variables as possible, (e.g. water temperature, light level, and channel morphology) was desired, so that experimental conditions could approximate those of the target system (e.g., Great Plains warmwater stream or cold high elevation stream). This requirement, along with the projected high rates of water use in an arid region, made it advantageous to design a recirculating water system (Figure 1). The recirculating flume system consisted of a modular fiberglass flume box, a movable steel frame for the flume, a steel superstructure, a flume slope adjustment system, the water delivery system, and a temperature control system. These are described in greater detail below. A list of parts for the flume can be accessed at: https://warnercnr.colostate.edu/wp-content/uploads/sites/2/2023/01/FFL-Flume-Parts-List.pdf.

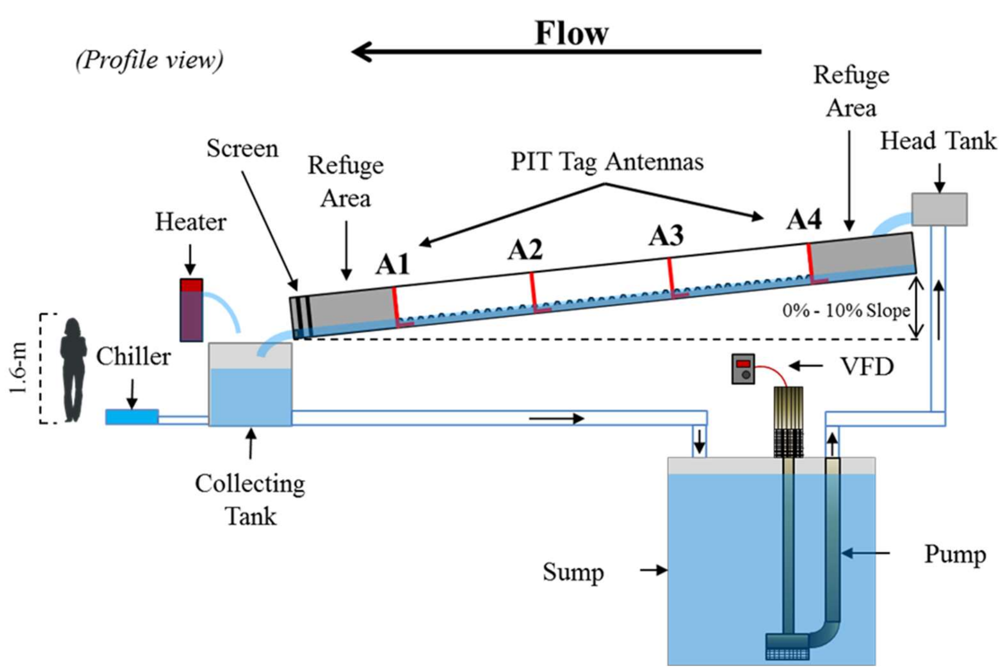

Figure 1. Diagram of the research flume built at the CSU Foothills Fisheries Laboratory. The flume is a closed recirculating system equipped with a 4,800-watt heater and large

chiller for temperature control. The flume can be adjusted from 0–10% slope by using two overhead chain hoists. A variable frequency drive (VFD) can adjust the output of the 11.19 kW pump to deliver flows of up to 0.082 m^3/s to the flume from a 22,700-l underground sump. Four evenly spaced swim-over PIT tag antennas (A1–A4) were installed in the flume to monitor fish progress as they navigated the fish passage structure.

Fiberglass flume

The flume consists of two identical OpenChannelFlow (Atlanta, Georgia) 4.57-m long, 1.22-m wide, and 0.61-m high fiberglass segments that bolt together to form a 9-m long flume. The segments include plastic reinforcing bars spaced at 1.55-m intervals to add structural rigidity. Each flume section was fabricated with a pair of 0.61 × 0.30-m Plexiglas (Darmstadt, Germany) windows to allow observations. In addition to the two primary flume segments, pre-fabricated curved sections of fiberglass flume (one with a 45° bend and two with 90° bends) were also acquired. The curved sections could be added individually or jointly to create a flume with a 45 to 180° curve in the middle, or, if desired, at either the flume entrance or exit, in case there was a need to test fishways with different entrance, exit, or mid-section geometries.

Flume frame and support structure

The flume was directly supported by a two-part metal frame (two 4.6 × 1.4-m) constructed of Unistrut (Harvey, Illinois) 12-gauge zinc-coated steel strut channel. The frame incorporated a wood-decked walkway (9 × 0.6-m wide) coated with a non-slip surface and integrated safety railing to allow easy access to the flume. The flume fit snugly onto the frame, which then acted as the load arm to which the flume height adjustment system was attached.

The flume and frame were supported by a two-section steel superstructure (14-gauge zinc coated steel strut channel) bolted together with 13-mm diameter steel bolts. Each section consisted of a 4.62 × 1.50 × 3.68-m (length × height × width) frame that held a flume segment, frame, and walkway assemblies. The design load of each section was 3,402 kg, with a maximum anticipated payload of 1,642 kg, giving the flume structure a safety margin of 49%. This safety margin allows the flume to safely accommodate a fish passage structure built inside of the flume box while still maintaining a high degree of safety for laboratory personnel operating the structure. The frame also included braces to adjust the flume slope. The downstream end of flume frame connected to the downstream end of the support structure and served as a pivoting fulcrum when the flume slope was being adjusted.

Flume hoisting system

The flume and frame were raised and lowered with a pair of Dayton (Lake Forest, Illinois) model 1VW51 907-kg capacity manual chain hoists suspended from an overhead

I-beam. Each hoist was positioned over the upstream end of one of the 4.55-m long flume segments, and were operated simultaneously to adjust the flume height and slope. The hoists were connected to the flume support brace with 1,088-kg capacity nylon cargo hoisting straps.

Water delivery system

Water was delivered to the flume by a 15-hp (11.19-kW) Vertiflo (Cincinnati, Ohio) pump located in a 22.7-m^3 (6,000-gallon) underground sump that pumped water through a 0.20-m diameter PVC pipe system to a 351-L covered polyethylene head tank (1.4 × 0.78 × 0.45-m) mounted at the upstream end of the flume. Water flowed through the flume into a 4,300-L welded aluminum collecting tank (3.04 × 1.22 × 1.18-m) fitted with a Coroplast (Minneapolis, Minnesota) flow deflector that reduced splashing and then drained back to the outdoor sump through 0.20-m diameter PVC pipe. The 0.20-m diameter pipe was then upgraded to a 0.30-m diameter pipe in 2018 after it became apparent that the return flows were restricted by the smaller pipe. Water level in the head tank and overall flume operation were monitored with a Sensaphone (Aston, Pennsylvania) FGD-0222 float switch connected to a Sensaphone Express II Monitoring System that both alerted project staff if water levels dropped below the setpoint and activated a small (4,542 liter per hour) recirculating pump to maintain a conservation pool of water at the downstream end of the flume to prevent the fish from becoming dewatered.

An 11.19-kW Vertiflo (Cincinnati, Ohio) model 832 variable speed vertical column pump mounted in the sump delivered water to the head tank. The pump was controlled by a Teco (Round Rock, Texas) model N3-415-C Variable Frequency Drive (VFD), giving it the capability to deliver a range of flows to the flume up to a maximum of 0.0823 m^3/s. A TCI (Germantown, Wisconsin) model KDRB2H drive reactor and a TCI model KRF0025ATB electromagnetic interference (EMI) filter were also installed in an effort to reduce electronic interference with the sensitive PIT tag detection system. The VFD, drive reactor, and EMI filter were all mounted inside of a Hoffman (Lake Forest, Illinois) model WF10LP steel enclosure, to minimize electromagnetic interference with PIT tag antennas and other laboratory systems. Magnesium sacrificial anodes were attached to the pump to reduce corrosion.

Temperature control system

The temperature control system was designed to provide the flume with 9–25°C water with an accuracy of ± 0.5°C. The target temperatures were achieved by counterbalancing the output of a 4.8-kW heater and a large chiller that recirculated water to the aluminum collecting tank. Both heater and chiller had their own temperature controllers and recirculating pumps in the collecting tank. An additional pump (13,600-L per hour) recirculated water from the outdoor sump to the collecting tank, allowing the system to regulate temperature even when the primary pump was not operating.

Fishway Design

An experimental rock ramp fishway was built inside of the research flume. The fishway was modelled after the one used successfully by Ficke (2015) to measure the effects of roughness element spacing on the passage success of Longnose Sucker *Catostomus catostomus*, Longnose Dace, and Johnny Darter *Etheostoma nigrum*, and was very similar in design and dimensions to one installed on the Cache la Poudre River in northeastern Colorado (see Richer et al. 2020 for a detailed description of that fishway).

The fishway was constructed with 6-mm thick PVC sheet supported by an epoxy-coated wood frame and was trapezoidal in cross-section with 30° side slopes and a 0.6-m wide center section (Figure 2). The downstream fishway entrance consisted of a small, sloped section of PVC sheet (0.30-m long at an approximately 30° angle) that connected the floors of the flume and fishway. A removable 3-mm mesh screen at the downstream end of the flume prevented fish from exiting the tailwater pool. Slots for flashboards were installed just downstream of the screen to regulate the water depth at the downstream end of the flume. We used the flashboards to help maintain consistent entrance conditions (i.e., water depth at the entrance) at the downstream entrance of the fishway, which meant that more flashboards were required at higher slopes.

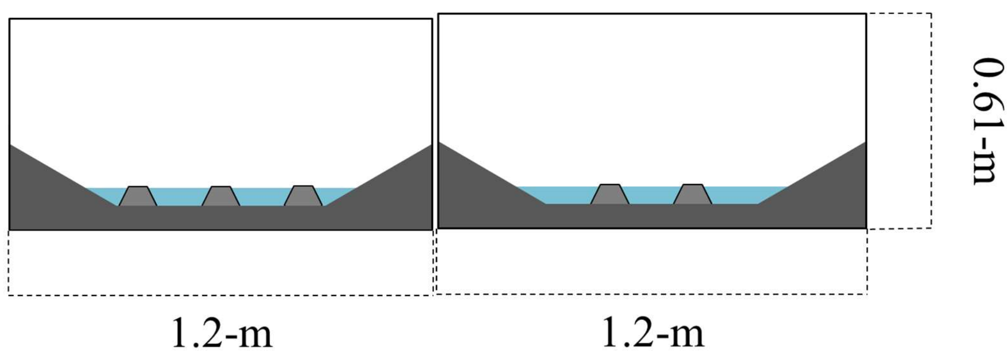

Figure 2. Cross-sectional view of the roughness elements in the trapezoidal rock ramp fishway. A cross-section of the fishway with three roughness elements is shown on the left and a cross-section of the fishway with two roughness elements on the right. Water depth is shown at the target depth of 51 mm throughout the fishway at each slope treatment. The fishway was made of epoxy coated wooden studs covered with PVC sheet. Fishway side slopes were 30° to allow the formation of a wetted margin. Diagrams are to scale.

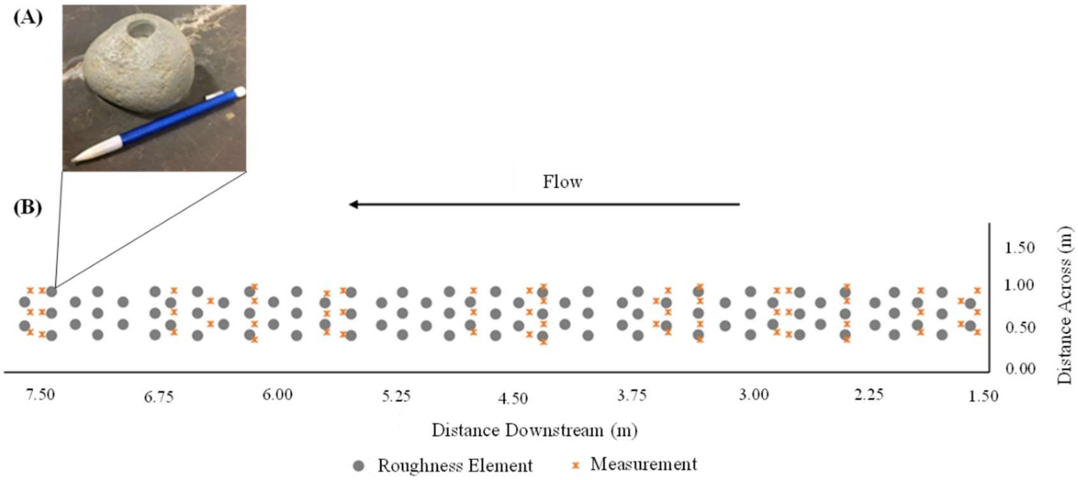

Figure 3. (A) Photograph showing the shape of the roughness elements used throughout the fishway. Roughness elements were identical moulded polyethylene rocks and could be rearranged by bolting the roughness elements to the floor of the PVC fishway. The sloped side of the roughness elements were oriented upstream to mimic the orientation of cobbles in streams. (B) Location of roughness elements in the rock ramp fishway in relation to the location of where water velocity and water surface elevation measurements were taken at each slope treatment (2–10% slope). Water velocity measurements were taken at approximately 60% depth. X-axis represents the distance downstream from the top of the flume. This diagram is not to scale to show needed detail.

The smooth floor of the fishway channel was fitted with 97 regularly-spaced roughness elements. They consisted of identical roughly-hemispherical Atomik Climbing Holds (Provo, Utah) polyethylene rock climbing holds (95-mm diameter; 55-mm high; 7,088-mm^2 area; Figure 3A) that attached to the PVC floor of the fishway with screws. Roughness elements were arranged in a chevron pattern using a spacing of one diameter (Figure 3B), following the perturbation boulder design recommended by Mooney et al. (2007). Average aerial density of roughness elements was 0.19 ([97 × 0.007088-m^2] / 3.6-m^2) and remained consistent through the study as water depth was kept constant. This design and spacing allowed passage of all fish species tested by Ficke (2015). The flume, PVC sheet, and roughness elements were all grey to provide a subdued substrate color to minimize behavioral changes or avoidance behaviors that could occur with a bright substrate color such as white (Casterlin and Reynolds 1977; Houtman and Dill 1994).

The system used four custom-made pass-over PIT tag antennas spaced at 2.03-m intervals in the fishway (entrance and exit, and at two other evenly spaced locations within the fishway) to track partial or complete passage success of individual fish, and to gain more information on the rates and timing of movements. Antennas were oriented with their long axis perpendicular to the direction of flow in the flume in a pass-over configuration. This configuration had detection ranges greater than the depth of the water used in the trials. The antennas consisted of ten wraps of 20-gauge copper wire inserted into strips of Coroplast sheet to maintain constant wire spacing, housed in air-tight 60-mm diameter schedule 40 PVC pipe enclosures (1.0 × 0.25-m) that fit under the fishway floor.

As a proof of concept for future fish movement studies, PIT tag antennas were connected to Oregon RFID (Portland, Oregon) dual mode (full and half duplex) long range readers. The PIT tag readers were wired in a "primary-secondary" configuration with the most upstream antenna being the "primary" to prevent the readers from interfering with each other. Each PIT tag type had its own unique read range due to the size of the tag (larger tag, larger read range). Full-duplex (FDX) tags had a shorter read range than that of half-duplex (HDX) tags because they are much more impacted by electromagnetic interference and vibration (Warren Leach, personal communication). Small-bodied fishes such as Arkansas Darters can be safely tagged with 8-mm PIT tags that were only available in FDX at the time of this study (Swarr et al. 2021), but they, and larger FDX tags are more susceptible to electromagnetic interference (W. Leach, Oregon RFID, personal communication). However, the read ranges of the larger (12–23 mm) HDX tags were less affected by EMI caused by the variable frequency drive (VFD), so we recommend using larger tags when possible, provided they do not adversely affect survival or swimming performance (Swarr et al. 2021). The PIT tag antenna read ranges were determined by orienting the tag perpendicular to each antenna's field. This was the orientation that received the farthest read range and also mimicked the orientation that the tags would be crossing the field while implanted in fish. Read ranges discussed in the publication were measured while the vertical column pump was on and the VFD was in operation. This produced the highest amount of electromagnetic interference possible in our setup, and reduced the read range of the antennas as opposed to when the pump and VFD were off. This also was the condition that the antennas would experience during the fish passage trials, as the pump and VFD both needed to be on while fish passage trials were underway. As part of the evaluation, Flathead Chub *Platygobio gracilis*, Stonecat *Noturus flavus*, and Arkansas Darter *Etheostoma cragini* were tagged and those results were reported in Swarr (2018).

Basic Hydraulic Measurements

Mean water column velocity (m/s) in the flume was measured with a Marsh McBirney (Frederick, Maryland) Flo-Mate 2000 current velocity meter (accuracy of ± 0.025 m/s; minimum reading = 0.01 m/s) and mean water surface elevations were measured with a point gauge at 58 points in 19 cross-sections throughout the fishway at each of five gradients (2–10% in 2% increments; Figure 3B) to give a coarse approximation of the fishway hydraulics. Other attempts to characterize the flow with a pitot tube and an acoustic doppler velocimeter (ADV) were unsuccessful due to the shallow, aerated nature of the flow, and the constraints of the ADV we had access to. Due to logistical reasons, we were not able to obtain an ADV that worked at shallower depths. Measurements were taken upstream, downstream, and between roughness elements to characterize the hydraulic conditions inside the fishway. Water velocity was measured at approximately 60% depth following protocol set forth in Turnipseed and Sauer (2010). Flows to the fishway at each slope treatment (2, 4, 6, 8, and 10%) were set to 0.0074, 0.0096, 0.0229, 0.0316, and 0.0363 m^3/s to maintain a nominal average water surface elevation (a surrogate for depth) of approximately 51 mm at each slope treatment, as measured at two transects (1.58 and 5.69-m below the upstream end of the flume). These transects were chosen to characterize depth conditions at upstream and downstream portions of the fishway. Water surface elevations and velocities of the fishway at different slopes were compared using one-way ANOVAs in JMP Pro 16 (Cary, North Carolina). When the ANOVA indicated statistically significant differences ($p < 0.05$), a Tukey-HSD test was used to identify the different treatments.

RESULTS

The process of designing, building, and testing a recirculating research flume and full-scale rock ramp fishway proved challenging, yet ultimately was successful. The preliminary hydraulic data collected showed that consistent and repeatable conditions could be generated as the flume slope was changed, and provided conditions that were similar to those observed in some existing fishways that have been installed on river systems flowing from the Rocky Mountains out onto the Great Plains in Colorado.

Flume Assembly and Operation

The slope of the fishway could be easily adjusted from 0–10% slope and operated under a variety of discharges. The two flume pieces could be separated in the middle to perform maintenance or to install the curved sections. Technicians conducting velocity measurements, adding or removing fish, or changing the flume substrate could easily access the flume via the walkway. The temperature control system worked effectively from 9–25°C with an accuracy of ± 0.5°C.

While the pump had the capacity to deliver 0.082 m^3/s to the system at full power (60 Hz on the VFD), output for this project was limited to 0.036 m^3/s because of insufficient static head between the collecting tank and the sump to maintain return flows greater than 0.036 m^3/s through the 0.20-m diameter pipe. To resolve this issue, the size of the return line was subsequently increased at the end of the study to 0.30-m diameter as described in the methods section, allowing the pump to be operated at full capacity. During pilot trials with the pump, it became apparent that it was important to maintain a constant head in the sump by regulating the water level to maintain steady state in the system. This was done by manually checking the sump water level prior to operating the system, but could be automated by adding a water level control system to the sump, combined with a secondary water supply.

Fishway Assembly and Operation

The general fishway structure was simple to assemble, but installing the PIT tag antennas proved challenging because of the EMI emitted by the VFD. The mean antenna read range for the 8-mm FDX tags was 6.4 cm, while read ranges for the 12- and 23-mm HDX tags were 12.7 cm and 30.5 cm, respectively. These detection ranges were sufficient to sample the entire water column for the associated study, but if higher flows and deeper water were used, the detection probability of 8-mm FDX tags would be reduced.

Fishway Hydraulic Measurements

Water surface elevation was relatively consistent between each slope treatment (Figures 4 and 5), with most differences between slopes being driven by variation in tailwater effect in the lowest 1 to 2-m of the flume. Otherwise, water surface elevations were largely uniform throughout the fishway for a given slope. On average, observed water surface elevations were slightly greater at higher slope configurations due to fluctuations caused by turbulence amongst the roughness elements and because more flashboards were needed at the downstream end of the flume to maintain consistent fishway entrance conditions (Figure 4). A hydraulic jump developed approximately 6.4 m downstream from the top of the flume at slopes of 6–10% (Figure 6). As expected, mean water velocities were significantly different between 10, 8, 6, and 4% slope treatments, with greater velocities at higher slopes. There was no significant difference in mean water velocity between the 2 and 4% slope settings (Figure 7). Although mean velocities were higher for the higher slopes, the velocity field at each slope was heterogeneous, providing fish with a range of velocities to negotiate (Figures 6 and 7). The system used to characterize the velocities in the flume lacked the resolution to create an x-y velocity map that could be used to show low-velocity pathways the fish might use; this is the subject of some current modeling and measurement studies using a modified version of the flume and a higher resolution acoustic doppler velocimeter.

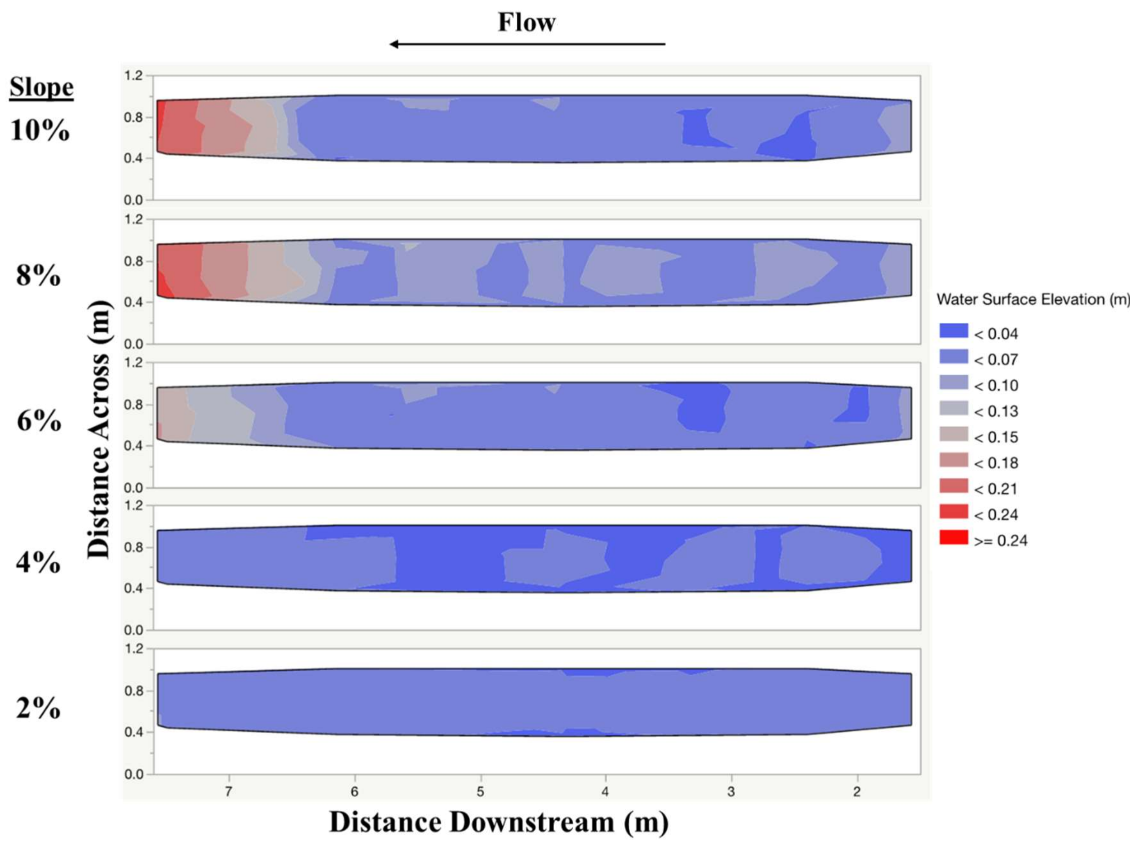

Figure 4. Contour plots of water surface elevations throughout the fishway at five slopes. Water surface elevations were taken using a point gauge at 58 points in 19 cross-sections.

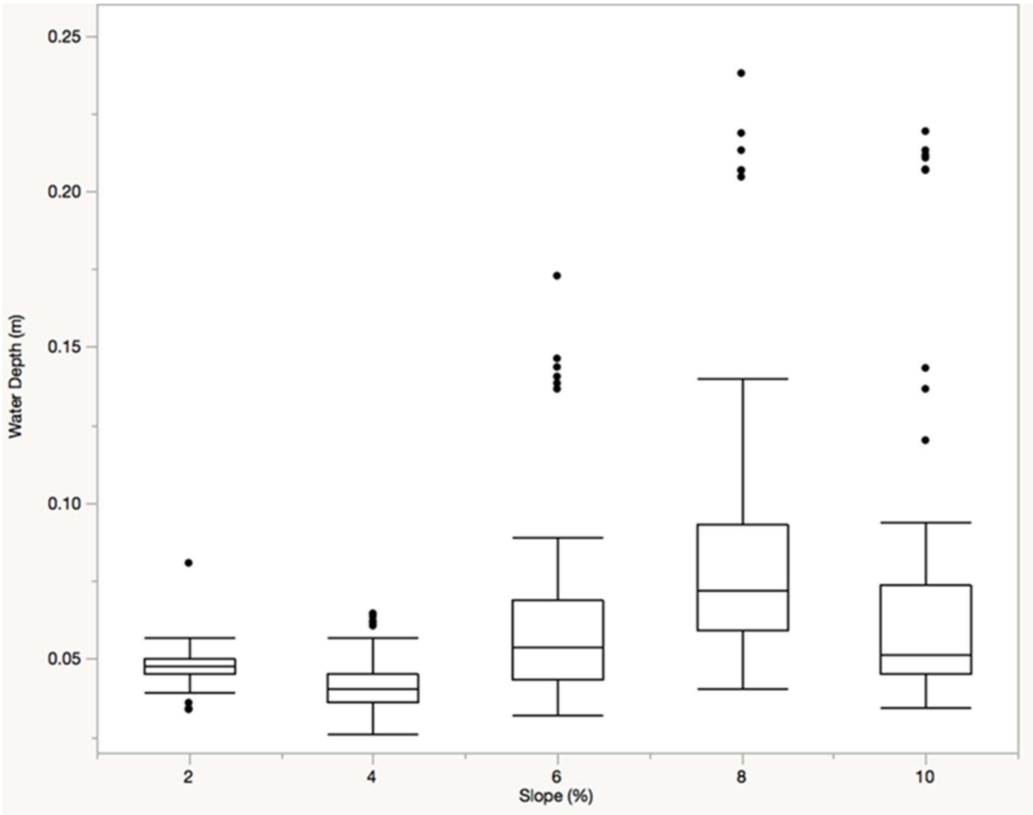

Figure 5. Box-and-whisker plots of slope and water surface elevation for the Colorado State University Foothills Fisheries Laboratory rock ramp fishway. Letters indicate statistically significant differences at $\alpha < 0.05$.

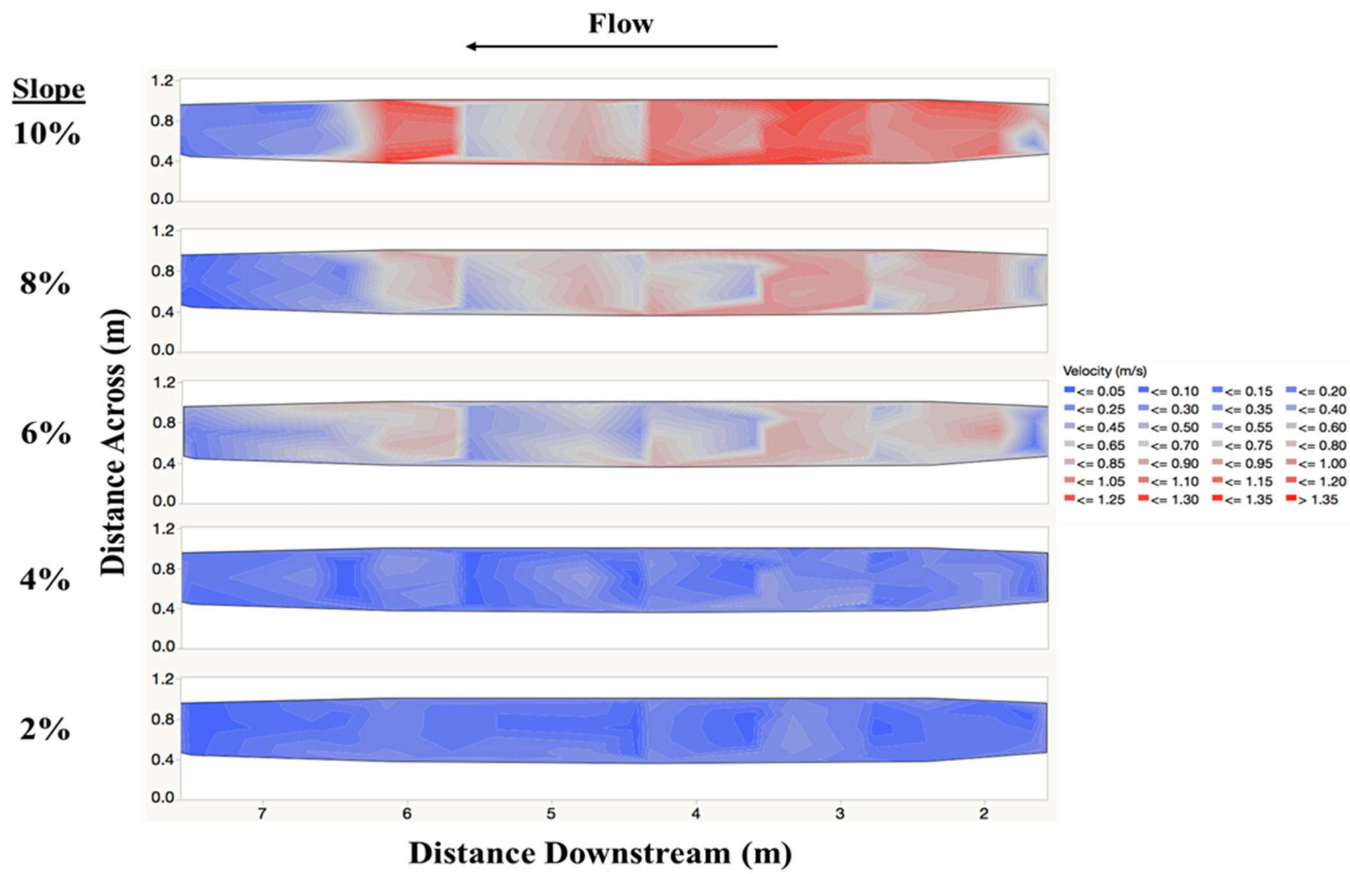

Figure 6. Contour plots of the velocity measurements taken throughout the rock ramp fishway at five slopes. Depth-averaged velocity measurements were taken using a Marsh McBirney flowmeter at 58 points in 19 cross-sections.

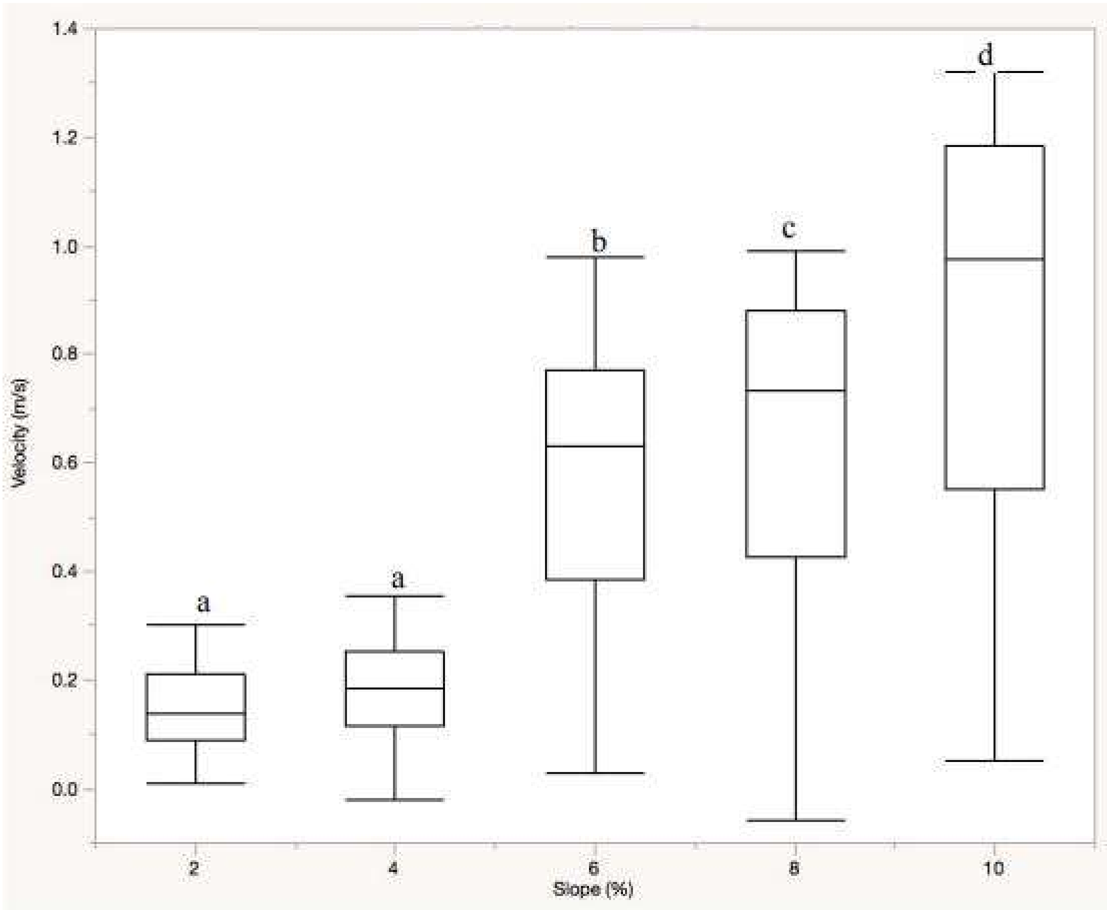

Figure 7. Box-and-whisker plots of slope and velocity for the Colorado State University Foothills Fisheries Laboratory rock ramp fishway. Letters indicate statistically significant differences at $\alpha < 0.05$.

DISCUSSION

The modular adjustable flume proved through this pilot study that it will be useful for measuring fish performance in a full-scale rock-ramp fishway. The ability to change the geometry and configuration of both the flume and fishway should provide researchers with the flexibility needed to test various fishway innovations under controlled laboratory conditions prior to employing them in the field.

Evaluation of Fishway Design

The steel strut channel was a useful material for constructing the flume superstructure and the flume brace. Available in a range of sizes and strengths, it allowed rapid assembly and reconfiguration of the support structures without requiring welding. Constructing the whole flume, fishway, and support structure apparatus as two separate "halves" made it easier to change configurations within limited laboratory space and would simplify transportation over a single-piece structure. The chain hoists used to lift the flume performed acceptably for raising and lowering the flume, though careful coordination between two operators (one per hoist) was required for efficient operation and to avoid placing undue stress on the supporting brace and fiberglass flume. Alternatives to the chain hoists, particularly in situations without overhead support structures for mounting the hoists could include hydraulic jacks or motor-driven gear systems.

Although the fishway was made of smooth PVC sheet, Baker and Boubée (2006) showed that increasing roughness of an artificial ramp increases passage success of fishes. Future fishway studies should consider testing a rough substrate as opposed to smooth plastic or metal to evaluate passage success, as fishways in the field are often not smooth and may produce more realistic estimates of fish passage. The current fishway could be easily modified to test different levels of roughness by unbolting the roughness elements, installing a thin, flexible PVC sheet on top of the smooth PVC sheet, and covering the upper sheet with substrate of the desired size (e.g., sand or gravel) (Bestgen et al. 2010). The large roughness elements could then be reinstalled and the effect of substrate texture determined.

Collecting accurate hydraulic measurements in the fishway was challenging due to the shallow depth in the flume and the turbulent and aerated nature of the flow, especially at higher slopes. It may be possible to combine the water surface elevations and known floor geometry to develop a 3-dimensional model of the flume and fishway that could be used to predict flow fields using hydrodynamic modeling software such as HEC-RAS or Flow3D. However, in order to collect more accurate velocity field data, higher flows and the resulting deeper water are needed. Alternately, a high-resolution acoustic Doppler velocimeter that could operate at depths of less than 50-mm could also be used to collect higher resolution 2-D or 3-D velocity data. There are current modeling and measurement efforts underway using such approaches and a modified version of the flume.

It is important to point out that while the fishway was operated at shallow depths in this study, these depths are not out of character with those seen in operational fishways, particularly during low flow periods or when a large proportion of the water in a stream or river is being diverted (Richer et al. 2020). By evaluating fish passage under low flow conditions, we can provide fisheries biologists with fish passage estimates even if available flows are less than optimal.

Future Modifications

The electronic interference emitted from the VFD was not eliminated but was sufficiently mitigated to allow data collection. Adding a drive reactor, EMI filter, steel enclosure, and steel conduit around the power lines did not completely eliminate interference with the PIT tag antennas in the flume when the VFD and pump were operational. Electrical engineers raised the possibility that the interference was traveling back through the power lines and into the stainless-steel breaker box and being emitted throughout the building. The line reactor and EMI filter were designed to filter out high frequency interference (150 kHz–30 MHz); however, the PIT tag arrays operate on a low frequency (134.2 kHz), and low frequency interference was likely the issue for the installed arrays (Zydlewski et al. 2006; Weis 2007; Johnston et al. 2009). Most electronic filters tend to eliminate high frequency interference, while filters that eliminate low frequency interference are more expensive. Where possible, working with electrical engineers to review the electrical system where such a flume is being installed prior to the construction and installation of the flume would be beneficial.

The read ranges within the fishway were short enough to ensure that the fields from the antennas did not overlap horizontally, allowing for higher resolution fish tracking in the fishway (i.e., at four discrete points) for future fish movement studies, as opposed to just recording fish entering/exiting the fishway. Increasing the number of PIT tag antennas in the fishway would provide finer resolution in fish movements throughout the fishway, provided that the antenna spacing could be optimized to prevent overlap and inter-antenna interference. Given the 30.5-cm read range of the larger tags, it would be theoretically possible to install up to nine antennas at 0.68-m intervals without overlapping read ranges. This would give much better resolution of fish movements which could help with understanding fish behavior in discrete zones of the flume. Regardless of the number of antennas used, it is important to have an antenna at the flume entrance to generate an estimate of the attraction efficiency (Castro-Santos and Haro 2003; Silva et al. 2018).

Under higher flow conditions, the read ranges of smaller PIT tags may not exceed the depth of the water column, indicating a need to quantify passage and detection probability. Changing the antenna configuration and orientation might address this issue. If such changes are ineffective, then careful measurements of detection ranges and detection probability should be made. Program MARK uses capture histories of organisms to determine survival, or in this case, estimates of fish passage (White and Burnham 1999). Even if a fish is not detected at an individual PIT tag antenna, but is at a subsequent antenna, passage estimates can still be calculated for that fish. Data analysis software such as Program MARK can provide estimates of fish passage, even when detection probability is not 1.0, therefore it is not necessary for the PIT tag antenna read range to exceed the water column height in all cases, though it is the preferable situation. An alternative approach would be to use a series of video cameras with overlapping fields of vision, as described by Dockery et al. (2017). Perhaps the most powerful approach would be to combine the two, using both PIT tags and video so that not only could fish movements be detected, but some record of fish behavior could also be recorded, because understanding how fish behave within a fishway is an important component of sound fishway design (Silva et al. 2018).

The construction of a hydraulic research flume and fishway cost approximately $130,000 USD in 2015, but the resulting apparatus provided a valuable tool for evaluating instream structures under controlled conditions and at a realistic scale. When combined with a facility that can hold or raise a variety of fishes, a research flume and modular fishway make it possible to test realistic fishway designs for a variety of species relatively rapidly (approximately two months to test slopes of 2–10% for a single species, with nine replicate trials per slope, and 20-hour passage trials).

There are other fish passage research flumes in existence, including the large flumes at the USGS LSC Conte Anadromous Fish Center (Castro-Santos et al. 1996; Haro et al. 2004) and those at the U.S. Fish and Wildlife Service Bozeman Fish Technology Center (Dockery et al. 2017; Plymesser et al. 2022) in the United States, and similar facilities in Europe (see Romão et al. 2018), Asia, South America, and Australia (Boys et al. 2013 and Stocks et al. 2018). Each research center and the associated flumes have unique capabilities based on the original design purposes and their ability to hold or import fish of different species. Being located in the Great Plains and southern Rocky Mountain region makes the flume and fishway described here ideal for developing passage guidelines of small- and medium-sized cold-, cool-, and warmwater fishes found within this area. Overall, the flume designed in this study is a flexible apparatus that can be used for a variety of fisheries and hydraulic research projects that could be easily adapted to meet site-specific research needs at other institutions. While relatively small in size (e.g., compared to the large flumes at the U.S. Bureau of Reclamation Technical Service Center in Denver, Colorado), flumes such as this one are useful tools for developing and evaluating fish passage solutions for small-bodied fishes.

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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 more detailed Introduction and Methods. This project is scheduled to be an ongoing, annual site selection tool.

INTRODUCTION:

Due to logistical, financial, and time constraints on staff, it is important that field activities are conducted as efficiently as possible and result in data that are statistically rigorous and defensible. This project provides a site selection tool for eastern plains native fishes that is adaptable to changing management priorities, and can be accomplished within the logistical parameters set by CPW staff.

METHODS:

The five major components of an optimal adaptive sampling design are 1) organizing the data, 2) finding a best-predicting model, 3) setting the design criterion, 4) selecting sites for future sampling, and 5) collecting more data and repeating the process (Figure 13).

1. The data. The data provide structure for the model, the desired inference, and the design criterion. Defining the data includes setting the boundaries, scale, and resolution of the area of inference, checking and cleaning the data that have been collected, and obtaining potential covariates. If the data change, for example a new covariate becomes available or a different resolution is considered, it may affect which model is chosen, subsequently altering the design criterion and the sites selected for future sampling. The scale and resolution of the covariates need to match the scale and resolution of the collected data and the research questions being asked. There may be sites and variables that are important ecologically but that cannot be incorporated into the design framework. This step explores the potential and limitations of the monitoring program.

2. The model. The model structure and output make the inference associated with the monitoring efforts explicit and concrete. The model parameter estimates and standard errors (or posterior distributions if one fits a Bayesian model) will define the design criterion, which is how the model connects the data to the design criterion and hence to

the future sampling. Therefore, one should be confident that the model meets its assumptions and fits the data.

3. The design criterion. The design criterion is a formal connection between monitoring and the model and is the quantity of interest about which improved inference is desired. It is a single statistic that summarizes the uncertainty associated with the study and is used to compare the efficiencies of sampling at various sets of locations in the future. Generally, it is a quantity to be minimized through the selection of an optimal set of future sampling locations, although there are design criteria that should be maximized for optimization. Common choices for the criterion are the average prediction variance, maximum prediction variance, or variance of the regression parameters (Wikle and Royle 1999). Fanshawe and Diggle (2013) used a threshold function for an environmental monitoring program where the goal was to find areas with high pollutant concentrations. The design criterion may include multiple goals and components.

4. Selecting sites for future sampling. This step involves finding the set of sites that minimizes the design criterion. The logistical constraints of limited time, money, and resources to commit to the sampling must also be taken into account. These constraints can be incorporated into the design criterion or the optimization algorithm. Management must decide the number and types of future sampling locations. However, several optimal sets of future sampling locations of various sizes can be selected to determine the extra utility of sampling more sites.

5. Collect more data and repeat. After future sites are selected and sampled, the model is re-fit with the new data and modified as necessary. Because the design criterion is based on the parameter estimates or posterior distributions, the next set of optimal sites will change with the newly fitted model.

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.

2022 Results

Site selection in 2022 resulted in ten sites selected in the South Platte River basin. Of which, six were on the main stem South Platte River and the remaining sites were located in the Big Thompson River, the Little Thompson River, and two sites on Pawnee Creek.

The sites on the South Platte River were concentrated in the middle section of the river, which was due to this area being the edge of both the Suckermouth Minnow *Phenacobius mirabilis* and Brassy Minnow *Hybognathus hankinsoni* distribution. These two species accounted for 40% of the species weighting. Since they account for a high proportion of the species weight, and this area is an area of high uncertainty due to being the edge of both species distributions, sites were focused on this area to determine the edge of these species' distributions. Sampling in previous years has slowly been narrowing in on this area as the edge of both of these species distributions. If sites continue to be focused on a narrower and narrower area, management may need to decide how important this area is as a priority. Species weightings could be changed to reduce this importance of this area for future site selection.

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Broms, K. M., M. B. Hooten, and R. M. Fitzpatrick. 2016. Model selection and assessment for multi-species occupancy models. *Ecology* 97:1759–1770.

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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.

CITATION

Baum, C. M., D. L. Winkelman, and R. M. Fitzpatrick. 2023. Temperature and winter duration requirements for reproductive success in johnny darter *Etheostoma nigrum* in the South Platte River Basin, Colorado. *Freshwater Biology*.

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.

See 2021 and 2022 Annual Reports for Introduction, Methods, Results and Discussion.

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.

ACKNOWLEDGEMENTS

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OTHER ASSOCIATED PUBLICATION

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.

CITATION

Adams, C. M., D. L. Winkelman, P. A. Schaffer, D. L. Villeneuve, J. E. Cavallin, M. Ellman, K. Santana Rodriguez, and R. M. Fitzpatrick**.** 2022. Elevated Winter Stream Temperatures below Wastewater Treatment Plants Shift Reproductive Development of Female Johnny Darter *Etheostoma nigrum*: A Field and Histologic Approach. Fishes 7(6):1–22.

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 and 2022 Annual Reports for Introduction, Methods, and previous Results.

ABSTRACT

River water temperatures are increasing globally, particularly in urban systems. In winter, wastewater treatment plant (WWTP) effluent inputs are of particular concern because they increase water temperatures from near freezing to ~7–15 ◦C. Recent laboratory studies suggest that warm overwinter temperatures impact the reproductive timing of some fishes. To evaluate winter water temperature's influence in the wild, we sampled Johnny Darter *Etheostoma nigrum* from three urban South Platte River tributaries in Colorado upstream and downstream of WWTP effluent discharge sites. Fish were collected weekly during the spring spawning season of 2021 and reproductive development was determined from histological analysis of the gonads. Winter water temperatures were approximately 5–10 ◦C greater ~300 m downstream of the WWTP effluent compared to upstream sites, and approximately 3 ◦C warmer at sampling sites ~5000 m downstream of the effluent discharge. Females collected downstream of WWTP effluent experienced accelerated reproductive development compared to upstream by 1–2 weeks. Water quality, including total estrogenicity, and spring water temperatures did not appear to explain varying reproductive development. It appears that small increases in winter water temperature influence the reproductive timing in *E. nigrum*. Further

investigations into how shifts in reproductive timing influence other population dynamics are warranted.

ACKNOWLEDGMENTS

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OTHER ASSOCIATED PUBLICATIONS

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.

Adams, C. M., D. L. Winkelman, P. Schaffer, and R. M. Fitzpatrick. 2022. Elevated stream temperatures below wastewater treatment plants influence reproductive development of Johnny Darter *Etheostoma nigrum* in the Front Range: A field and histologic approach. Final Report to Colorado Parks and Wildlife, Fort Collins, Colorado.

RESEARCH PRIORITY

Examine the impact of wastewater treatment plant effluent on the winter thermal regime of two urban Colorado South Platte tributaries

OBJECTIVES

The main goal of our study was to create a model to investigate the magnitude and spatial impact of warm effluent on water temperature in winter (December–February) using linear regression for two urban Colorado South Platte tributaries, the Big Thompson River, and St. Vrain Creek.

CITATION

Adams, C. M., D. L. Winkelman, and R. M. Fitzpatrick. 2023. Impacts of wastewater treatment plant effluent on the winter thermal regime of two urban South Platte Tributaries. *Frontiers in Environmental Science*.

ABSTRACT

Wastewater treatment plant effluent can increase stream water temperature from near freezing to 5–12˚C in winter months. Recent research in the South Platte River Basin in Colorado showed that this warming alters the reproductive timing of some fishes. However, the spatial extent and magnitude of this warming are unknown. Thus, we created winter water temperature models both upstream and downstream of effluent inputs for two urban tributaries of the South Platte River, the Big Thompson River, and St. Vrain Creek. We examined the influence of air temperature, discharge, effluent temperature, and distance downstream on water temperature over the winter period (December–February). The models were also used to predict water temperature in the absence of effluent and based on air temperature predictions in 2052 and 2082. Effluent temperature was the largest driver of water temperature downstream of the effluent, while the impact of air temperature was comparatively small. Streams cooled after an initially sharp temperature increase, though were still predicted to be ~2˚C greater than they would be in the absence of effluent at ~0.5 km. Predicted air temperatures in 2052 and 2082 had a negligible effect on water temperature, suggesting that mitigating effluent temperature is key to protecting the winter thermal regimes of effluent-impacted rivers. Our models can be used to gain insight into the magnitude and downstream extent of the impact of effluent temperature on small urban streams in winter and provide a baseline for models in other watersheds and at larger scales.

INTRODUCTION

Anthropogenic modification of river systems is ubiquitous, resulting primarily from population growth and subsequent urbanization and industrialization. Modifications typically have impacts on geomorphological processes that in turn influence river health. Water temperature is particularly sensitive to human influence and anthropogenically caused deviations from natural thermal regimes are well documented, including increased overland flow (Nelson and Palmer 2007), deforestation (Burton and Likens 1973), rising air temperatures and declining streamflow due to climate change (Issak et al. 2017; Pankurst and Munday 2011). Most anthropogenic alterations increase river water temperature, though the magnitude, duration, and timing of these thermal increases vary (Cassie 2006). Even small changes in temperature, however, can have measurable impacts on aquatic communities.

Water temperature is an environmental driver of ectotherm biology. It controls and regulates important biological functions and behaviors including activity, metabolism, development, and reproduction (Brett 1956; Bestgen and Williams 1994; Hester and Doyle 2011). It has been well documented that abnormal thermal regimes resulting from changes in water temperature can alter fish life history processes, reproductive timing, and hatching success (Bestgen and Williams 1994; Pankhurst and Munday 2011; Starzynski and Lauer 2015; Fraser et al. 2019; Adams et al. 2022). Human induced alterations to water temperature have been documented to have negative impacts on fish populations (Farmer et al. 2015; White et al. 2020). For example, bluegill sunfish populations in lakes warmed by electrical power plant cooling systems had shorter life spans than those in nearby ambient lakes (White et al. 2020). Increased winter water temperature also affects the reproductive biology of percid fishes (Farmer et al. 2015; Baum 2021; Adams et al. 2022). This has led federal and state agencies to create river water temperature standards for protection of fishes and river ecosystems. Mitigation of thermal pollution is usually complex and expensive and requires managers to have accurate information to implement management options. Water temperature models can provide managers with broader temporal and spatial information to prioritize management actions more efficiently.

Statistical models have been successfully utilized to evaluate anthropogenic impacts on stream water temperature and to predict water temperatures under a variety of hypothesized management strategies or future conditions (Neumann et al. 2003; Isaak et al. 2017). These models suggest air temperature, discharge, and riparian cover have substantial effects on water temperature. Model predictions have the added benefit of providing guidance to managers on how to mitigate these impacts and what to expect in future scenarios. For example, restoring riparian vegetation may mitigate water temperature increases predicted due to increasing air temperatures and declining streamflow (Justice et al. 2017) caused by climate change. Impacts of point source thermal pollutants on rivers have been less investigated and mainly focus on water releases below dams (Daniels and Danner 2020; Ahmad et al. 2021). Most water temperature models also focus on large river networks over the summer period (Isaak et al. 2017; Mandeville et al. 2019) when aquatic ectotherms are often already near their critical thermal maxima (Magnunson and DeStasio 1997). Most other predictive modeling efforts have not prioritized other seasons, particularly winter. This is despite considerable documentation that discharge from wastewater treatment plant (WWTP) effluent increases river water temperatures during the winter months (Kinouchi et al. 2007; Rice et al. 2011; Graham et al. 2014), and evidence that abnormally warm winters impact aquatic communities (Farmer et al. 2015; Firkus et al. 2018; Baum 2021; Adams et al. 2022). Models investigating the magnitude and spatial extent of WWTP effluent on the thermal regime of streams are needed to make efficient management strategies to mitigate thermal impacts in winter and meet local water temperature standards.

The main goal of our study was to create a model to investigate the magnitude and spatial impact of warm effluent on water temperature in winter (December–February) using linear regression for two urban Colorado South Platte tributaries, the Big Thompson River, and St. Vrain Creek. We chose these tributaries for our study because they have low winter baseflows, experience WWTP effluent discharge, and are of specific conservation concern due to their recreational importance and high concentration of native species (Woodling 1985). In addition, we have shown that warm effluent can substantially increase winter water temperature and influence the timing of fish reproduction (Adams et al. 2022). Specifically, we hypothesized that air temperature, river discharge, effluent temperature, and distance from the effluent would influence the spatial pattern of water temperature downstream of the WWTP effluent. In contrast, air temperature, discharge, and distance from the effluent would influence the spatial pattern of water temperature upstream of the effluent. We also used our upstream model to predict water temperature downstream of the WWTP in the absence of effluent. Finally, we used our models to anticipate the impact of predicted winter air temperatures on stream water temperatures 30 and 60 years in the future to compare the threat of climate change with that of effluent discharge on winter water temperature of these streams. The results of our study will provide management agencies with additional information necessary to understand the spatial and temporal extent of WWTP effluent thermal impacts on water temperature during the winter period and provide a framework for future assessment of the influence of wastewater effluents on stream ecosystems.

METHODS

Study Area

The Big Thompson River is a 125.5 km South Platte tributary that begins in Rocky Mountain National Park (RMNP) near Estes Park, CO, flows east through the city of Loveland, transitioning to a plains stream as it converges with the main stem South Platte near Greeley, CO (USGS 1981a). St. Vrain Creek is a 51.8 km tributary that begins further south in RMNP and flows east through the city of Longmont transitioning to a plains stream as it converges with the main stem South Platte near Milliken, CO (USGS 1981b). Both rivers have WWTP effluent inputs in the cities they flow through. The urban sections of these rivers sustain populations of multiple Colorado native warmwater fishes and are classified as WS–I streams by Colorado Department of Public Health and the Environment due to the presence of Johnny Darter *Etheostoma nigrum* (maximum weekly water temperature of 12.1˚C permitted during December-February). Both rivers decline ~ 30 m elevation through our study site that ends in natural or wildlife areas at the east edge of each town (USGS 1981a; USGS 1981b; Figure 8). Finally, the annual hydrographs of these streams are characterized by low baseflow discharge from September-April and peak/high flows from May-August due to montane snowmelt runoff.

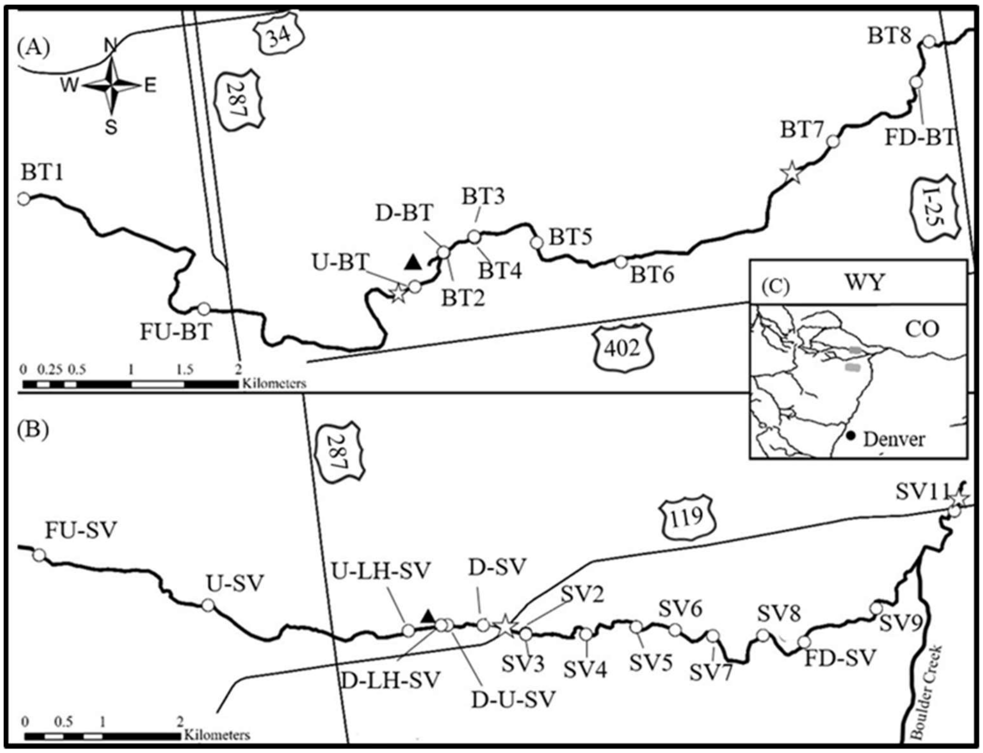

Figure 8. Map of temperature logger locations (circles) in the (A) Big Thompson River (BT) through Loveland, (B) St. Vrain Creek (SV) through Longmont, and (C) their location relative to the South Platte River Basin on the Front Range of Colorado (grey boxes). Loveland and Longmont are 74 and 48 km northwest or Denver respectively. Lines in panel (C) show the South Platte River and its major tributaries on the Front Range of Colorado. Black triangles indicate WWTPs and white stars indicate discharge gage locations. The discharge gage used for sites upstream of the WWTP on the St. Vrain is in Hygiene, CO further upstream than the maps extent. See text for explanation of monitoring sites.

Our study area centers around the WWTP effluent inputs in the Big Thompson River and St. Vrain Creek. Our most upstream temperature monitoring sites are 6.2 and 5.9 km upstream of the WWTP on the Big Thompson River and St. Vrain respectively, while our furthest downstream sites are 6.2 and 9.1 km respectively. Left Hand Creek, a small St. Vrain tributary, flows into the St. Vrain ~ 50 m upstream of the Longmont WWTP. Another St. Vrain tributary, Boulder Creek, flows into St. Vrain Creek ~30 m upstream of our furthest downstream monitoring site in that system. No tributaries converge with the Big Thompson River through our study reach. Within our study reaches these rivers both have sand, cobble, and gravel substrate with gradual increases in the percentage of finer sediment and wider more meandering channels in the downstream direction. All Big Thompson River sites have moderate overhead cover, while St. Vrain sites have less cover overall with a few exceptions that have similar overhead cover as sites in the Big Thompson River (FU-SV, SV2, SV3, and SV4; Figure 8).

The WWTPs in both streams have similar impact on streamflow. The WWTP in Loveland, CO, (Loveland Wastewater Reclamation Facility) has the capacity to treat 38 million gallons per day (MGD) and is discharged to the Big Thompson River at an average daily flow of 13.45 MGD (City of Loveland 2020). The WWTP in Longmont, CO (Longmont Wastewater Treatment Plant) has a capacity of 17 MGD and is discharged to St. Vrain Creek at an average daily flow of 8.0 MGD (City of Longmont 2023). Based on nearby stream discharge gages, the WWTP effluent of the Big Thompson River and St. Vrain Creek account for 42% and 33.6% of the average daily discharge during the winter baseflow period (USGS 2022a; CDNR 2022a). Effluent discharges into both streams during the winter period are continual, with daily fluctuations that peak between the afternoon and midnight and are at their lowest in the early–mid-morning (Adams, unpublished data).

Temperature monitoring

Hobo temperature loggers were initially placed at fish sampling locations as part of another project examining temperature impacts on the reproductive development of Johnny Darters (Adams et al. 2022). The naming of these monitoring sites is in relation to their initial position relative to the WWTP effluent, far upstream (FU), upstream (U), downstream (D), and far downstream (FD), and the river they are located, either the Big Thompson (BT) or the St. Vrain (SV). Loggers at FU–SV, FD–SV, U–BT, and D–BT were launched in the spring of 2020, FD–BT in February 2021, and at FU–BT in August of 2021 (Figure 8). All deployed temperature loggers collected water temperature every hour and data were downloaded multiple times a year. Additional effluent and water temperature data for the St. Vrain were obtained for 2020–2022 from the City of Longmont including from sites upstream of Lefthand Creek (U–LH–SV), downstream of Lefthand Creek (D–LH–SV), immediately downstream of the WWTP effluent (D–U–SV), and downstream of the WWTP effluent (D–SV; Figure 8). Winter water temperature data for D–SV were only available for winter 2020– 2021 due to logger displacement during 2021 high spring flows.

We deployed an additional 11 loggers in the St. Vrain (SV1–11) and 8 loggers in the Big Thompson (BT1–8) in late January of 2022 to increase our fine-scale spatial monitoring of stream temperature (Figure 8). Of the additional loggers deployed, one was placed upstream of the effluent on both rivers to investigate natural stream warming in the downstream direction before the impact of the warm effluent. We could only place 8 loggers in the Big Thompson due to river access restrictions. Loggers collected temperature data every 15 minutes and the data were downloaded in early March 2022.

Discharge and air temperature data

Continuous discharge data were obtained from the U.S. Geological Survey (USGS) and the Colorado Department of Natural Resources (CDNR) at sites upstream and downstream of the WWTP effluent on the Big Thompson and St. Vrain (USGS 2022a–b; CDNR 2022a–c; Figure 8). Temperature monitoring sites were matched to the closest discharge gauges that also incorporated nearby sources of additional discharge. For example, all sites downstream of the effluent on the Big Thompson were assigned to discharge data collected at the "BIG THOMPSON RIVER AT HILLBOROUGH DIVERSION (BIGHILL)" to incorporate additional discharge from the WWTP effluent, despite sites D–BT and BT2 being closer to the USGS BIG THOMPSON AT LOVELAND, CO site just upstream (Figure 8). Data from USGS discharge gauges were downloaded using the dataRetrieval package in Program R (R Core Team 2022; De Cicco et al. 2022). Discharge data were manually downloaded from the CDNR website (CDNR 2022a–c).

Air temperature data were obtained for Loveland (1997–current) and Longmont (1997–current) from CoAgMet, a network of agricultural weather stations around Colorado maintained by Colorado State University (CoAgMet 2022a–b). Data were downloaded from CoAgMet using the package rvest in Program R (Wickham 2021). Water temperature monitoring sites on the Big Thompson were matched with air temperature data from Loveland, while those on the St. Vrain were matched with air temperature data from Longmont. All available discharge and air temperature records were downloaded. Only data collected during December, January, and February of the 2020–2021 and 2021–2022 winters were used to create the model. All data were formatted, organized, and prepared for analysis using Program R.

Model creation

Average daily winter water temperatures downstream of the WWTP were analyzed using multiple linear regression with air temperature, effluent temperature, river discharge, the interaction between river discharge and effluent temperature, and a 2nd -degree polynomial relationship with the distance from the effluent input as predictor variables. We believe the relationship with distance from the effluent is a polynomial because temperatures rapidly decline after initial effluent mixing with cool river water followed by a more gradual temperature decline further from the initial effluent input. An interaction effect between effluent temperature and river discharge was modeled because higher river discharge dilutes effluent and subsequently its thermal impact (Miara et al. 2018). We chose not to include effluent discharge in the model because it varies little through the winter period (Adams, unpublished data). Thus, the regression model used to investigate downstream winter water temperature in relation to the WWTP effluent was:

$$T_w = \beta_0 + \beta_1 T_a + \beta_2 D + \beta_3 T_e + \beta_4 D T_e + \beta_5 D_e + \beta_6 D_e^2$$

where T_w is water temperature, T_a is the air temperature (˚C), D is the discharge (cfs), T_e is the effluent temperature (˚C) and D_e is the distance (m) from the WWTP effluent. Preliminary results revealed the effluent discharge did not mix fully with the stream until ~390 m downstream of its input into the Big Thompson (BT3 and BT4; Figure 8). Because of this, we averaged the water temperatures at D–BT and BT2 for the model, which were both ~50 m from the effluent input (Figure 8). We refer to these models for both rivers as the downstream models.

Water temperature upstream of the effluent was also analyzed using multiple linear regression with distance from the effluent, discharge, and a 2nd–degree polynomial relationship with air temperature as predictor variables. Distance from the effluent was used in the model to account for water temperature spatial variation in the downstream direction if present. A 2nd–degree polynomial of air temperature was used because streams typically reach an asymptote near 0˚C due to freezing (Mohseni & Stefan 1999). Thus, to model water temperature upstream of the effluent we used:

$$T_W = \beta_0 + \beta_1 T_a + \beta_2 T_a^2 + \beta_3 D + \beta_4 D_e$$

where T_w is water temperature, T_a is the air temperature (˚C), D is the discharge (cfs), and D_e is the distance (m) from the WWTP effluent. We refer to these models for both rivers as the upstream models. Regression analyses were conducted in Program R using the lm function in base R in conjunction with the dyplr and broom packages (Wickham et al. 2021; Robinson et al. 2022).

Predicted scenarios

We wanted to estimate winter water temperature downstream of the effluent input as if the effluent did not exist to understand the magnitude of effluent temperature on winter thermal regimes. Thus, we used the upstream model coefficients along with average air temperature and discharge values from the 2020–2021 and 2021–2022 winter seasons to predict unimpacted water temperatures downstream of the effluent. Discharge values used for predictions were additionally modified to exclude any inputs attributed to the effluent. For example, average discharge downstream of the WWTP was considered to be the same as upstream. One exception to this was the discharge used for predictions >9,150 m from the effluent on the St. Vrain (approximate location of confluence with Boulder Creek), which was calculated as the average measured discharge at that location subtracted from the discharge attributed to the effluent. The differences between our model predictions and our measured thermal data provided estimates of the magnitude and spatial extent of the thermal impact of the WWTP effluent.

Predicting winter thermal regimes 30 and 60 years in the future required collecting historical air temperature data from the region and estimating average effluent temperatures. The National Oceanic and Atmospheric Administration (NOAA) provided air temperature data from the U.S. Climate Divisional Dataset for Colorado's Platte drainage from 1895 to the present day (NOAA 2022). For our predictive model, we used the average 3-month winter (December–February) temperature from the last 20 years for our baseline current air temperature. For our predictions of average air temperature 30 and 60 years in the future (2052 and 2082), we used the 3-month winter average air temperature trend in Colorado's Platte drainage over the last 50 years (1972–2022). Effluent discharge temperature for winter 2020–2021 and 2021–2022 ranged between 12–18˚C and averaged ~14.5˚C in both rivers (14.48˚C on the Big Thompson and 14.62˚C on the St. Vrain). For simplicity, we used 14.50˚C as the effluent temperature when making our predictions. Using this and forecasted winter air temperatures, we modeled current, 2052, and 2082 winter water temperatures for both streams. We chose not to make predictions about discharge because current winter conditions are near or at base flow, discharge in these rivers is highly managed, and future conditions are uncertain.

RESULTS

Model fit

Average winter water temperature in 2020–2021 and 2021–2022 ranged from 7.14˚C to 2.96˚C from 241–9,181 m downstream from the effluent on the St. Vrain and 8.04˚C to 4.31˚C from 52–6,185 m downstream from the effluent on the Big Thompson (Figure 9; Figure 10). Regression analysis showed all coefficients were significant in the downstream models, suggesting air temperature, discharge, effluent temperature, and distance from the effluent were correlated with water temperature (Table 2). The effluent temperature (β_3) had the greatest influence on water temperature, followed by discharge (β_2), and air temperature (β_1; Table 2). Only two variables, the interaction of discharge and effluent temperature (β_4) and distance from the effluent (β_5) had negative impacts on water temperature, though the magnitude of these effects were relatively small (Table 2). All variables except for air temperature appeared to have a greater effect on water temperature in the Big Thompson than the St. Vrain, most notably effluent temperature (2.67˚C vs 0.76˚C respectively) and the 2nd order polynomial distance from the effluent, which was an order of magnitude greater in the Big Thompson model than the St. Vrain model (Table 2). The R^2 values were high for both the St. Vrain (0.79) and Big Thompson (0.76) models suggesting they fit the data well (Møller and Jennions 2002).

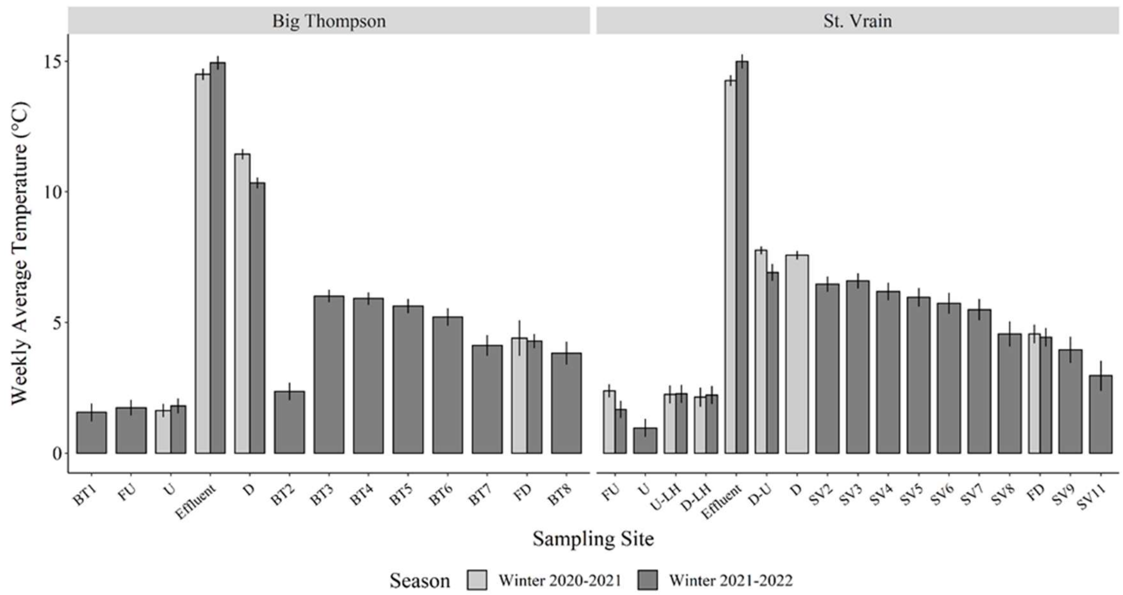

Figure 9. Average water temperatures at each temperature monitoring site for winter 2020–2021 and 2021–2022. BT1–BT8 and SV2–SV11 were deployed for a month in 2022 to collect more fine-scale spatial temperature data while other loggers were deployed prior to winter 2020–2021. D–SV was lost during the spring of 2021 and not replaced for the following winter. Sites are organized from upstream to downstream from left to right for each river.

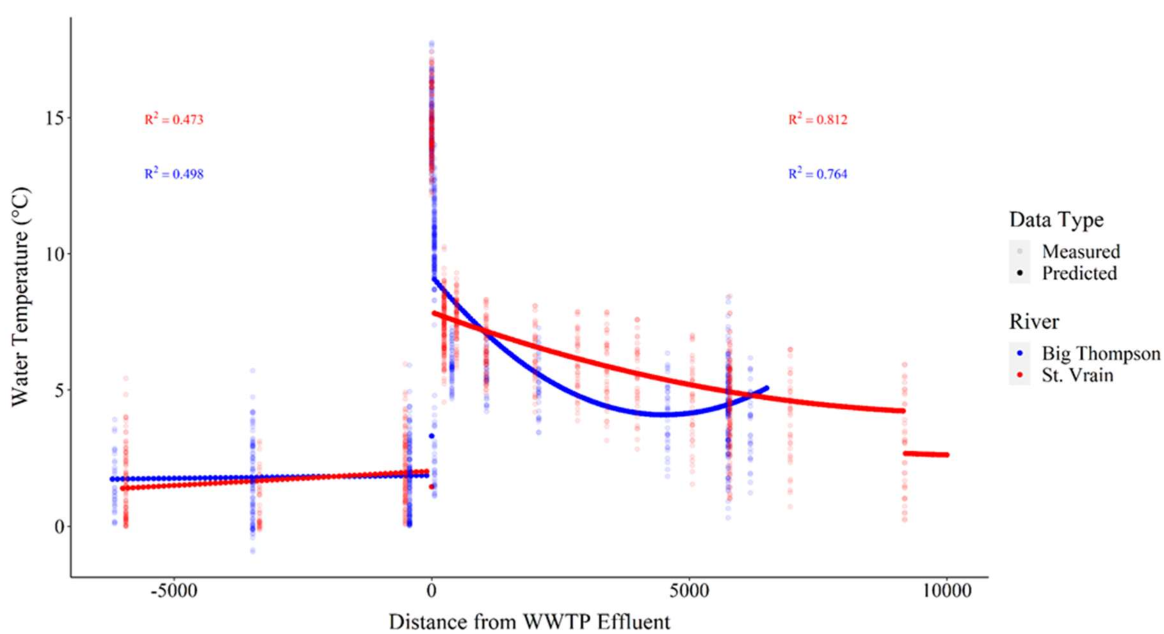

Figure 10. Daily average water temperatures at each monitoring site on the Big Thompson and St. Vrain for winters 2020–2021 and 2021–2022 in relation to distance from the WWTP effluent input and predicted water temperatures from the upstream and downstream models (Table 2; Table 3). Measured effluent temperatures are represented

at point 0. Predictions were calculated using model coefficients along with average air temperature, discharge, and effluent temperature (14.5˚C). The black lines (solid for Big Thompson and dashed for St. Vrain) show predicted daily average water temperature calculated with upstream model coefficients, average daily air temperature and average daily discharge for comparison of water temperature estimates if no effluent input was present.

Table 2. Model coefficients and summaries for winter water temperature in the Big Thompson and St. Vrain downstream of the WWTP effluent. All coefficients in the models were significant predictors of water temperature ($p < 0.05$). T_w is water temperature, T_a is the air temperature (˚C), D is the discharge (cfs), T_e is the effluent temperature (˚C) and D_e is the distance (m) from the WWTP effluent.

Model

$$T_w = \beta_0 + \beta_1 T_a + \beta_2 D + \beta_3 T_e + \beta_4 D T_e + \beta_5 D_e + \beta_6 {D_e}^2$$

Model Coefficients

River	β_0	β_1	β_2	β_3	β_4	β_5	β_6
Big Thompson	–30.636	0.119	0.792	2.670	–0.052	–0.002	2.490E–07
St. Vrain	–2.024	0.138	0.223	0.755	–0.018	–0.007	3.240E–08

Model Summaries

River	R^2	Adjusted R^2	sigma	statistic	df	logLik	AIC	BIC	Deviance	Residuals
Big Thompson	0.764	0.761	1.400	309.279	6	–1,018	2,052.27	2087.200	1127.025	575
St. Vrain	0.795	0.793	0.873	476.254	6	–953.4	1922.82	1959.738	562.791	739

Upstream of the effluent, the average winter water temperature ranged from 1.56˚C–1.74˚C on the Big Thompson and 0.96˚C–2.26˚C on the St. Vrain (Figure 9). Only FU–SV in winter 2020–2021 was significantly different and slightly higher, than other upstream sites. All other upstream sites had similar average winter water temperatures. All upstream model coefficients were significant except distance from the effluent for the Big Thompson (β_4; Table 3). This suggests that air temperature and discharge are correlated with water temperature, and on the St. Vrain, distance is also correlated with water temperature. The latter suggests a spatial relationship with St. Vrain water temperature as the river flows downstream. In the upstream models, air temperature (β_1) had the greatest influence on water temperature (Table 3). The R2 values were 0.51 and

0.52 for both the St. Vrain and the Big Thompson respectively suggesting a good model fit (Møller and Jennions 2002).

Table 3. Model coefficients and summaries for winter water temperature in the Big Thompson and St. Vrain upstream of the WWTP effluent. All coefficients in the models, except for the distance from the WWTP effluent coefficient (β_4; in bold), were significant predictors of water temperature ($p < 0.05$). Tw is water temperature, T_a is the air temperature (˚C), D is the discharge (cfs), and D_e is the distance (m) from the WWTP effluent.

Model

$$T_w = \beta_0 + \beta_1 T_a + \beta_2 T_a{}^2 + \beta_3 D + \beta_4 D_e$$

Model Coefficients

River	β_0	β_1	β_2	β_3	β_4
Big Thompson	1.322	0.190	0.005	0.058	**2.19E–05**
St. Vrain	2.359	0.188	0.007	–0.030	1.06E–04

Model Summaries

River	R^2	Adjusted R^2	sigma	statistic	df	logLik	AIC	BIC	Deviance	Residuals
Big Thompson	0.523	0.517	0.986	78.197	4	–404.96	821.92	843.94	277.223	285
St. Vrain	0.513	0.505	1.086	63.979	4	–369.84	751.69	772.77	286.62	243

Model predictions

The predicted downstream unimpacted winter water temperatures were cooler than all measured average winter water temperatures within our study reaches on the Big Thompson and the St. Vrain (Figure 11). Predicted unimpacted water temperatures at BT7 (4,578 m), FD–BT (5,755 m), and BT8 (6,185 m) were 1.97˚C, 1.99˚C, and 2.00˚C respectively while the actual average measured temperatures were 4.12˚C, 4.25˚C, and 3.82˚C (a difference of 2.15˚C, 2.26˚C, 1.82˚C respectively). Predicted unimpacted water temperatures at SV8 (5,060 m), FD–SV (5,786 m), SV9 (6,959 m), and SV11 (9,181 m) were 2.56˚C, 2.64˚C, 2.76˚C, and 1.71˚C respectively while the actual average measured temperatures were 4.57˚C, 4.45˚C, 3.95˚C, and 2.96˚C (a difference of 2.01˚C, 1.81˚C, 1.19˚C, and 1.26˚C respectively; Figure 10).

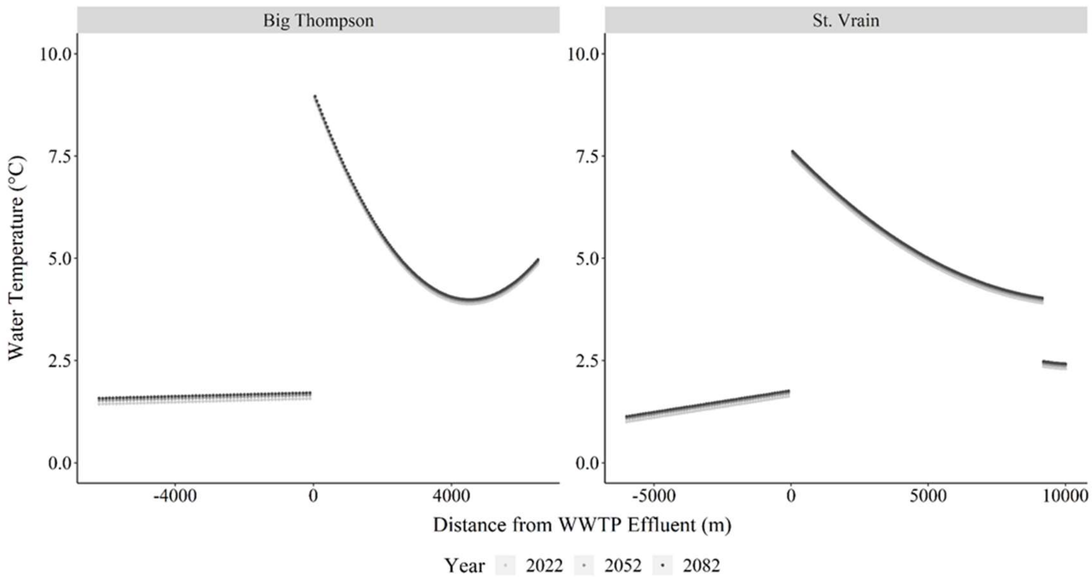

Figure 11. Predicted winter water temperatures in the Big Thompson and the St. Vrain under 2022, 2052, and 2082 winter air temperature conditions from coefficients in the upstream and downstream winter water temperature models (Table 2; Table 3).

The average winter air temperature from 2002 to 2022 in Colorado's South Platte drainage was -2.67˚C. The air temperature trend from 1972 to 2022 was +0.17˚C/year resulting in predicted average winter air temperatures of -2.16˚C in 2052 and -1.82˚C in 2082. Estimated changes in air temperature in 30 and 60 years had little influence on winter water temperature both upstream and downstream of the effluent input in our models (0.06–0.10˚C increase; Figure 11).

DISCUSSION

Effluent temperature had a measurable effect on winter water temperature within our Big Thompson River and St. Vrain Creek study reaches. Our models indicated that effluent temperature had the greatest effect on water temperatures downstream of the effluent compared to all other factors, including air temperature. Our models also showed that the interaction of river discharge with effluent temperature negatively influenced water temperature, suggesting that higher flows may help offset the influence of effluent temperature, though generally river discharge alone increased water temperature in the winter period. In the downstream models, the coefficient of air temperature had a comparatively small effect on water temperature, indicating that effluent temperature is of greatest concern to managing winter stream warming downstream of effluent inputs. The effect of air temperature was greatest among coefficients in the upstream models; however, the magnitude of the effect was similar to downstream models. Distance from the effluent had a small effect, albeit significant, in all models except the upstream Big Thompson model.

We show that WWTP effluent can warm winter stream water temperature by as much as 12˚C. This temperature increase has been shown to affect the timing of fish reproduction in our study reaches (Adams et al. 2022). Laboratory studies also indicate that warming of this magnitude can substantially influence the timing of reproduction (Baum 2021). However, by 0.5 km downstream the water temperature was about only 2˚C above predicted temperatures and declining, indicating that these tributaries can recover from warming during the winter over relatively short distances. In other effluent dominated rivers, where effluent discharge accounts for greater than 50% of baseflow, effluent likely has greater impacts on winter water temperature and has been documented to increase water temperature by 3–10˚C as far as 27 km from the source (Kinouchi et al. 2007; Lewis and McCutchan 2011; Graham et al. 2014). This is likely not the case in our smaller non-effluent dominated South Platte tributaries.

Air temperature is generally considered to be the most influential variable determining stream temperature (Ward 1985) and winter air temperatures have been increasing globally (NOAA 2022). Thus, we incorporated air temperature into our models to understand its role in winter water temperature in impacted systems. Our models revealed that downstream of the effluent input air temperature has a minimal effect in comparison to effluent temperature. This was additionally apparent in our predictions of water temperature using projected air temperatures 30 and 60 years in the future. Despite increasing winter air temperatures, the predicted changes in water temperatures increased by a maximum of only 0.06˚C in 2052 and 0.10˚C in 2082. While climate change poses a serious threat to aquatic communities worldwide, warm water inputs like WWTP effluent are potentially a more serious threat to urban stream ecosystems and may affect these streams in rural areas as well. Thus, winter thermal regimes in South Platte tributaries on the Front Range, and likely other urban streams, require prioritization to mitigate the effect of point source thermal pollutants, like WWTP effluent.

Our models suggest that predicted changes in air temperature due to climate warming will have only slight effects on winter water temperature increases in 30–60 years in Front Range South Platte streams. This conclusion is notably opposite of most water temperature models where predicted air temperature increases have significant influence on water temperature, though these models generally incorporate warmer seasons (Stefan and Sinokrot 1993; Mohseni et al. 1998; Isaak et al. 2017). This is likely due to unimpacted streams in the Front Range of Colorado having winter water temperatures that are already near zero. Additionally, it is possible for impacted reaches to reach near freezing temperatures as effluent cools as it flows downstream (Mohseni and Stefan 1999). Air temperature may have larger impacts on winter thermal regimes of streams and rivers in areas with warmer winters than our study reaches. This contrasts with the impact of effluent temperature on river thermal regimes in these areas, possibly dampened due to the smaller difference in basal water temperature and effluent temperature. Increased winter air temperatures due to climate change may have the greatest impact on streams and rivers in areas that currently have average winter air temperatures near 0˚C. In these areas, small increases in air temperature may prevent streams from returning to near freezing water temperatures, potentially impacting the aquatic ecosystems which evolved with this overwintering environment. Investigations on the winter thermal regime of streams in warmer climates are warranted to determine the impact of current and future predicted winter air temperatures.

When we predicted downstream winter water temperature without the influence of WWTP effluent, the predicted temperatures were slightly lower than those we measured by about 2˚C approximately 0.5 km downstream of the effluent. The difference between the predicted and actual temperature declines as water moves downstream. At the furthest downstream point in our study reaches, 6.2 km from the effluent on the Big Thompson and 9.3 km on the St. Vrain, the difference between actual and predicted was 1.8˚C and 1.3˚C respectively. While the full impact of this relatively small thermal increase is unknown, it is known that even small increases in water temperature can have measurable effects on fish populations in all seasons (Pankurst and Munday 2011; Heggenes et al. 2018; White et al. 2020). Warmer overwintering temperatures are known to decrease overwinter survival (Cunjak and Power 1987) and impact the spring reproduction of some fishes (Ficke et al. 2007; Im et al. 2016), including accelerating the reproductive timing of Johnny Darter in our study reaches (Adams et al. 2022). However, most studies that investigate the impact of warm winter water temperatures evaluate increases greater than we observed or predicted in our study system (Firkus et al. 2018; Baum 2021). Additional studies are needed to understand how these small increases in overwinter temperature impact all local fish populations.

Predicting winter water temperatures or attempting to predict them without the influence of WWTP effluents aids in understanding and subsequently mitigating the impact of effluent temperature. However, predictions assume that the factors we included in the model will not change significantly over time. For instance, we assume that the average contribution of WWTP effluent will not increase over time and that winter air temperature will remain near freezing. Our unimpacted predictions were based on models that incorporated air temperature, discharge, and distance downstream. A more accurate model would include other natural or anthropogenic warm water sources (i.e. overland flow and tributary or groundwater inputs) that increase in the downstream direction (Nelson and Palmer 2007; Brown et al. 2011). The rivers in our study also both pass through urban areas, a known cause of increased stream temperatures (Somers et al. 2013). Another factor specific to our study area that complicates our predictions of effluent free stream temperature is that it lies in the transition zone between cold–high–elevation–mountain and warm–open–plains reaches (Ward 1985). The downstream reaches of our study sites may be expected to be slightly warmer than our upstream sites because they are shallow, wide-open, and low gradient, though the effect of stream morphology on temperature is not well documented in these streams. Thus, it is possible that the water temperature we measured is similar to what it might be without effluent inputs, although our models suggest otherwise. Research incorporating more comprehensive variables including land use and geomorphology may provide further insight into factors that mitigate or aggravate thermal recovery from point source pollutants like WWTP effluent.

Mitigating the impact of warm WWTP effluent on urban streams is not straightforward and will likely require situational mitigation to address unique stream characteristics.

However, understanding the magnitude and extent of WWTP effluent warming is a critical first step in identifying and mitigating warming winter water temperatures. Our models provide a fine-scale view of the magnitude and spatial extent of WWTP effluent impact on winter water temperature in South Platte tributaries that are economically important, have high fish biodiversity and should be conserved for those reasons. We believe the models presented in our study can be used to gain insight into how best to manage and conserve these smaller urban streams. Additionally, our models provide a baseline for the creation of larger models to examine the thermal impact of effluent inputs on a watershed scale that will ultimately be necessary for conserving and managing thermal effluent on a watershed scale.

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OTHER ASSOCIATED PUBLICATION

Adams, C. A., D. L. Winkelman, and R. M. Fitzpatrick. 2022. Impacts of wastewater treatment plant effluent on the winter thermal regime of two urban South Platte tributaries. Final Report to Colorado Parks and Wildlife. Fort Collins, Colorado.