

# EASTERN PLAINS NATIVE FISH RESEARCH

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Aquatic Wildlife Research Section

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# COLORADO EASTERN PLAINS NATIVE FISH PROJECT SUMMARY

Period Covered: April 1, 2019 to March 31, 2020

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

## **RESEARCH PRIORITY:**

Develop a framework to identify abiotic and biotic factors affecting native plains fish persistence and gaps in that information to focus future research. The first phase will focus on Flathead Chub, *Platygobio gracilis*, but other species can be added based on management priorities.

## **OBJECTIVES:**

The purpose of this project is to (1) provide a Flathead Chub conceptual model describing mechanisms affecting four life stages at multiple spatial and temporal scales in the Arkansas River basin, Colorado, and (2) use this conceptual life history model as a case study to better understand the ecology and conservation strategies for Great Plains native fishes.

## **INTRODUCTION:**

Effective conservation requires identifying abiotic and biotic factors affecting a species throughout its life cycle at various spatial and temporal scales. Spatial scales can range from small-scale microhabitats that are required for juvenile development, to very large, basin-wide scales where connectivity throughout the basin may be necessary for population persistence. Temporal scales can vary from within seasons, within the lifetime of a fish, and ultimately to long-term population persistence.

Structured methods to examine plains fish ecology and conservation are not well described for many species, life stages, or underlying mechanisms. Conceptual models can help identify important abiotic and biotic factors and temporal scales by providing a way to visualize the mechanisms affecting species persistence, identifying areas where information is lacking, and generating hypotheses. This ultimately leads to understanding what management actions are required for conservation.

There is great interest in conservation of stream fish in arid environments, but holistic ways to examine these systems and focus conservation efforts are not well understood. This project is producing a conceptual framework that incorporates multiple spatial and

temporal scales that influence an individual fish's survival and population persistence of Flathead Chub (*Platygobio gracilis*) in the Arkansas River basin, Colorado (Figure 1).

## **METHODS:**

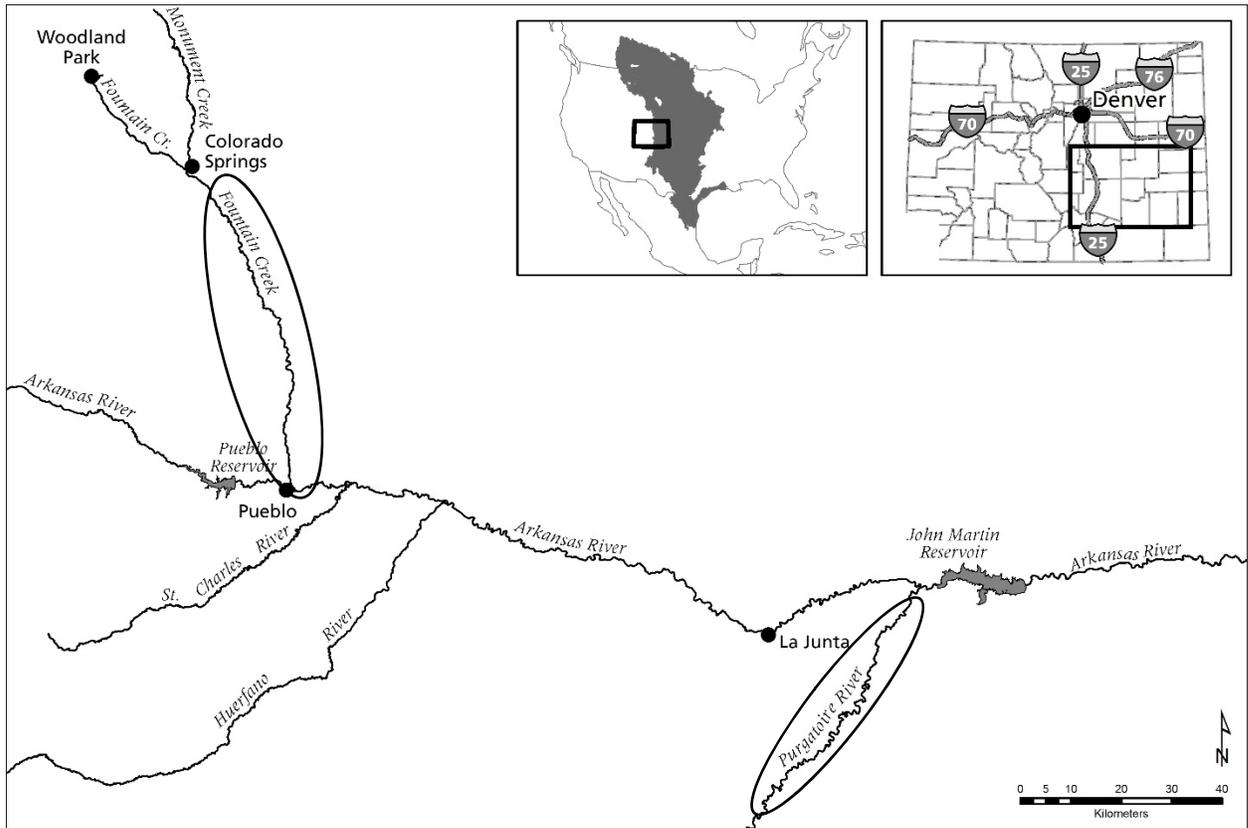
The process of selecting important drivers of Flathead Chub persistence followed the template provided by Worthington et al. (2018), which examined the pelagic guild of plains fishes as a whole. This paper provided some information about Flathead Chub, but there are large gaps in knowledge in Flathead Chub life history. Worthington et al. (2018) described Flathead Chub as a phylogenetically distinct taxa, but provided little information about its spawning mode or early life history. The current review sought to provide finer scale information about Flathead Chub and identify information that is lacking to guide future research.

A literature search was conducted using search engines Web of Science, GoogleScholar, and grey literature, such as reports from state agencies. Search terms included *Platygobio gracilis*, Flathead Chub, Arkansas River basin, pelgofil, and Great Plains fishes. Literature was also obtained from citations contained in papers from database search results. Historic Flathead Chub distribution data in Colorado were obtained from Colorado Parks and Wildlife's fish database. Data prior to 1979 were too sparse to make meaningful inference. Sampling periods focused on major sampling events in the basin, especially those conducted by Colorado Parks and Wildlife and the Larval Fish Laboratory, Colorado State University (Leoffler et al. 1982; Nesler et al. 1999). Literature was organized by life stage, and then important drivers of persistence at that stage were identified and summarized. This included assessing studies ranging from those describing the general Flathead Chub life cycle, to papers those that described specific important factors for long-term persistence of Flathead Chub populations. I aggregated the detailed information and synthesized repeatedly identified factors into a conceptual framework that describes mechanisms critical for Flathead Chub persistence.

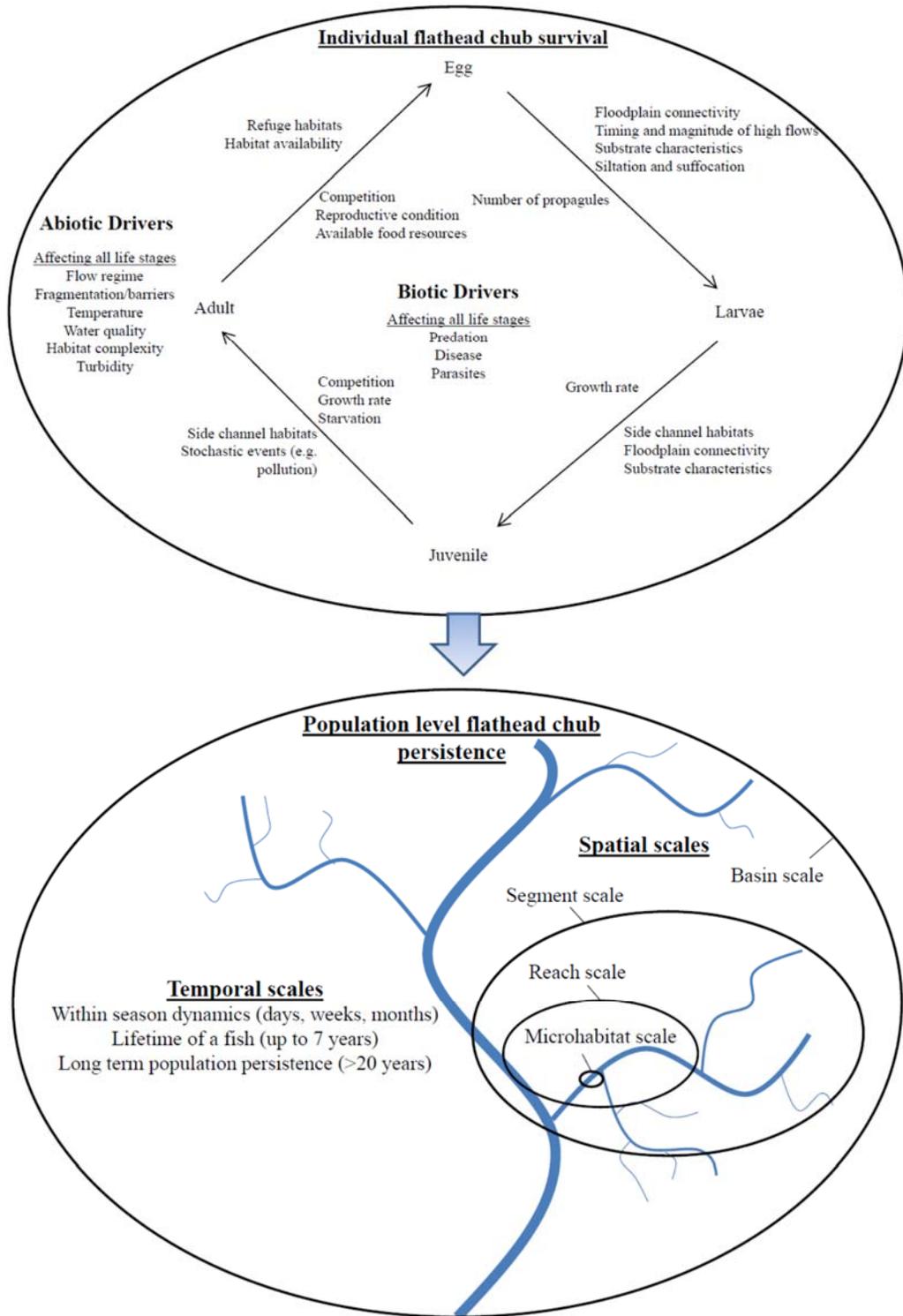
## **RESULTS AND DISCUSSION:**

Regression analysis of current and historic Flathead Chub in Colorado indicate the importance of the location of headwaters and proximity to the mainstem Arkansas River. This species and study area provides a case study of mechanisms affecting an understudied ecoregion that is of great conservation concern. Flathead Chub long-term population persistence requires connectivity, habitat complexity, and an appropriate flow regime for each life stage. Gaps in knowledge that should be the focus of future research efforts include quantifying life history metrics (including survival rates and movement patterns), further elucidating spawning mode, juvenile feeding requirements, and disease and parasitic effects on all life stages. Quantifying life history metrics will allow rigorous testing of abiotic and biotic effects—including flow regimes and seasonal effects—which can focus management efforts.

This project is examining a case study of a plains fish species with documented reduced ranges tied to a loss of connectivity. Unfortunately, these effects are not unique to Flathead Chub. Therefore, this conceptual life history model and associated mechanisms can be used as a template for other pelagic spawning Great Plains cyprinids, and be expanded to incorporate each species unique life history (Figure 2).



**Figure 1.** The Great Plains ecoregion (A) extends from Canada to Mexico, with Colorado outlined in the rectangle. The Arkansas River basin is located in southeast Colorado (B), and flows in an easterly direction from the Rocky Mountains onto the Great Plains near Pueblo, Colorado, until its confluence with the Mississippi River in Arkansas. Ovals indicate two remaining robust populations of Flathead Chub in Colorado.



**Figure 2.** Conceptual model of Flathead Chub life history from an individual's life to population persistence. The spatial scales, based on Fausch et al. (2002), are: microhabitat  $10^{-1}$ - $10^0$ m; reach  $10^1$ - $10^3$ m; segment  $10^3$ - $10^5$  m; basin  $10^5$ - $10^6$  m.

## **ACKNOWLEDGEMENTS:**

I would like to thank my coauthor, Dr. Kevin Bestgen.

## **REFERENCES:**

- Fausch, K. D., C. E. Torgersen, C. V. Baxter, and H. W. Li. 2002. Landscapes to riverscapes: bridging the gap between research and conservation of stream fishes. *BioScience* 52:483–497.
- Loeffler, D., D. Miller, R. Shuman, D. Winters, and P. Nelson. 1982. Arkansas River threatened fishes survey. Performance Report. Federal Aid Project SE\_8-1-2. Colorado Division of Wildlife, Denver.
- Nesler, T. P., C. Bennett, J. Melby, G. Dowler and M. Jones. 1999. Inventory and status of Arkansas River native fishes in Colorado. Fort Collins: Colorado Division of Wildlife.
- Worthington, T. A., A. A. Echelle, J. S. Perkin, R. Mollenhauer, N. Farless, J. J. Dyer, D. Logue, and S. K. Brewer. 2018. The emblematic minnows of the North American Great Plains: A synthesis of threats and conservation opportunities. *Fish and Fisheries* 19:271–307.

## **RESEARCH PRIORITY:**

Quantify life history metrics of survival and movement of a Great Plains cyprinid to guide future management and conservation.

## **OBJECTIVES:**

- 1) Estimate seasonal survival rates of Flathead Chub, *Platygobio gracilis* through the lower section of Fountain Creek, Colorado
- 2) Quantify seasonal Flathead Chub movement through the study area
- 3) Examine mechanistic effects on these metrics, especially related to flow and
- 4) Provide gear and field protocol recommendations for future studies by quantifying detection probability of PIT tags using three gear types.

## **INTRODUCTION:**

Effective conservation requires understanding the life history traits of the species of interest and mechanisms affecting those traits. Unfortunately, there is a lack of life history information—particularly quantified life history metrics—for many species. Quantifying life history metrics allows rigorous testing of mechanisms affecting species' persistence, which can focus management efforts on the most effective actions for conservation.

The native fishes of the North American Great Plains ecoregion are an assemblage of conservation concern with gaps in knowledge of many species' life history traits. I report here, for the first time, seasonal apparent survival rates, transition probabilities, individual detection probabilities, and temporary emigration rates for a Great Plains cyprinid.

## **METHODS:**

Flathead Chub in Fountain Creek were selected as the study organism and site for three reasons. First, compared to other plains stream fishes, Flathead Chub are relatively well studied, including a mark-recapture study in this study area (Walters et al. 2014; Haworth and Bestgen 2016; Haworth and Bestgen 2017). Second, for conservation purposes, it is important to determine the maximum amount of movement that fish within an assemblage will undertake. Therefore, it makes sense to select an active swimming species within a relatively long, unimpeded section of river. Flathead Chub in Fountain Creek fit this criterion. Third, Flathead Chub are relatively large-bodied compared to many other Great Plains fishes. This makes deploying PIT tags easier and reduces the effect of PIT tags on swimming performance.

PIT tag detection occurred with three gear types: a 12-m mobile array; a 2-m mobile array; and scanning fish, collected via electrofishing, with an Oregon RFID handheld PIT tag reader (Figure 3). Apparent survival ( $\phi$ ), transition probabilities ( $\psi$ ), and detection probability ( $p$ ) were estimated using multi-state models in Program MARK (White and Burnham 1999; White et al. 2006). Apparent survival covariates included fish total length (mm), site, season (summer, winter, and transition seasons (spring and fall)), and multiple high flow covariates. Transition probability covariates included high flow events, distance between sites, direction of movements, seasons, and fish total length. Detection probability covariates included fish total length, season, and mean daily discharge. Five site-specific robust design analyses were conducted to obtain finer scale information on gear efficiency ( $p$ ) and temporary emigration ( $\gamma$ ) (Kendall et al. 1997).



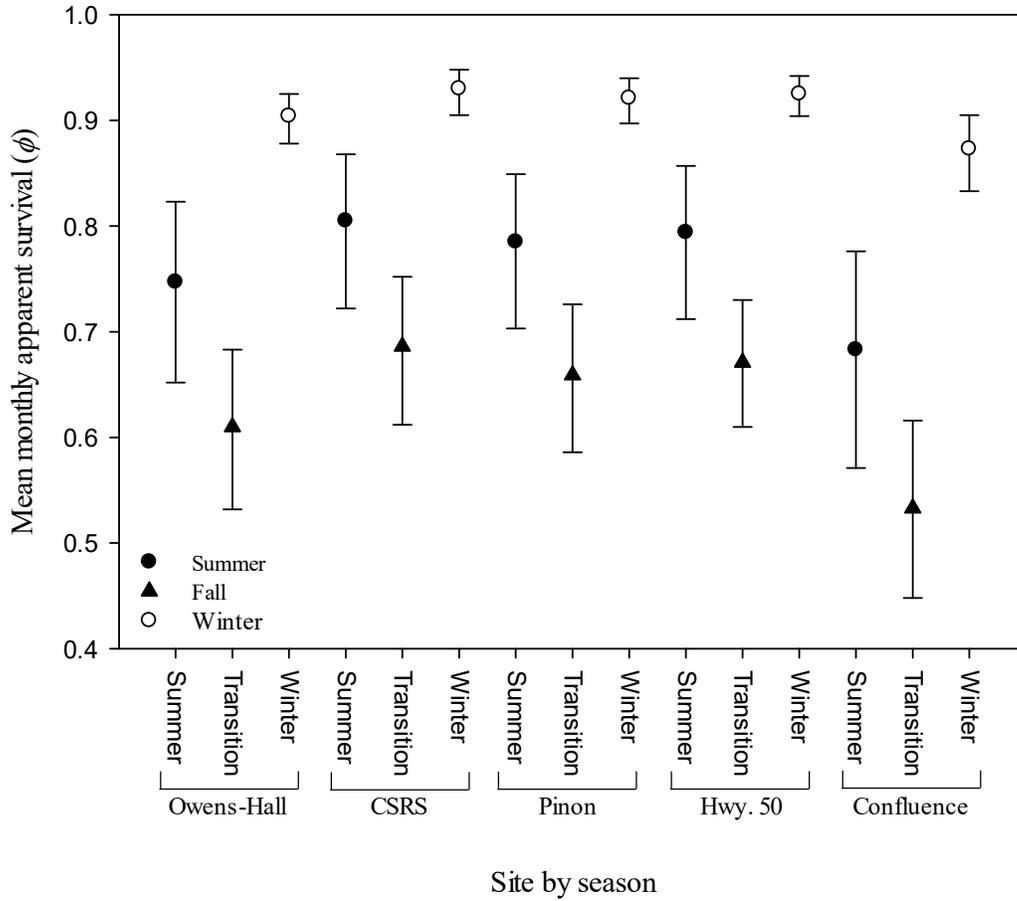
**Figure 3.** A. 12-m mobile array deployed at Owens Diversion. This array specialized in detecting tagged fish in the main channel. B. 2-m mobile array that was used to detect tagged fish in shoreline habitat.

### **RESULTS AND DISCUSSION:**

From 2011 to 2015, 22,060 passive integrated transponder (PIT) tags were deployed in Flathead Chub *Platygobio gracilis* in a 58-rkm study area of Fountain Creek, Colorado. The overall recapture rate for individual fish was 11.6%, but increased to 14.8% when multiple detections of individual fish were included.

**Objective 1. Estimate monthly survival**

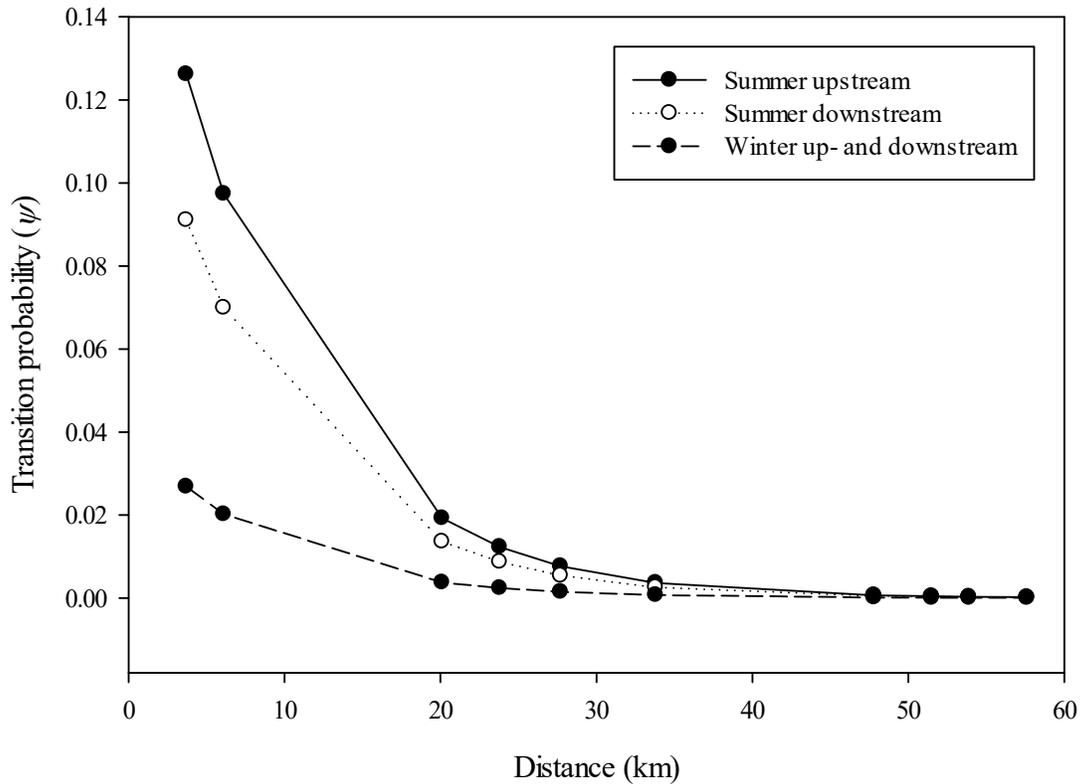
A subset of 13,108 fish were analyzed in a closed multi-state model, resulting in a mean monthly apparent survival rate ( $\phi$ ) of 0.75 (0.68–0.80). Apparent survival varied seasonally, with the highest rate in winter, then summer, and then the transition seasons of fall and spring respectively (Figure 4). Survival also varied among sites, likely due to habitat differences (Figure 4).



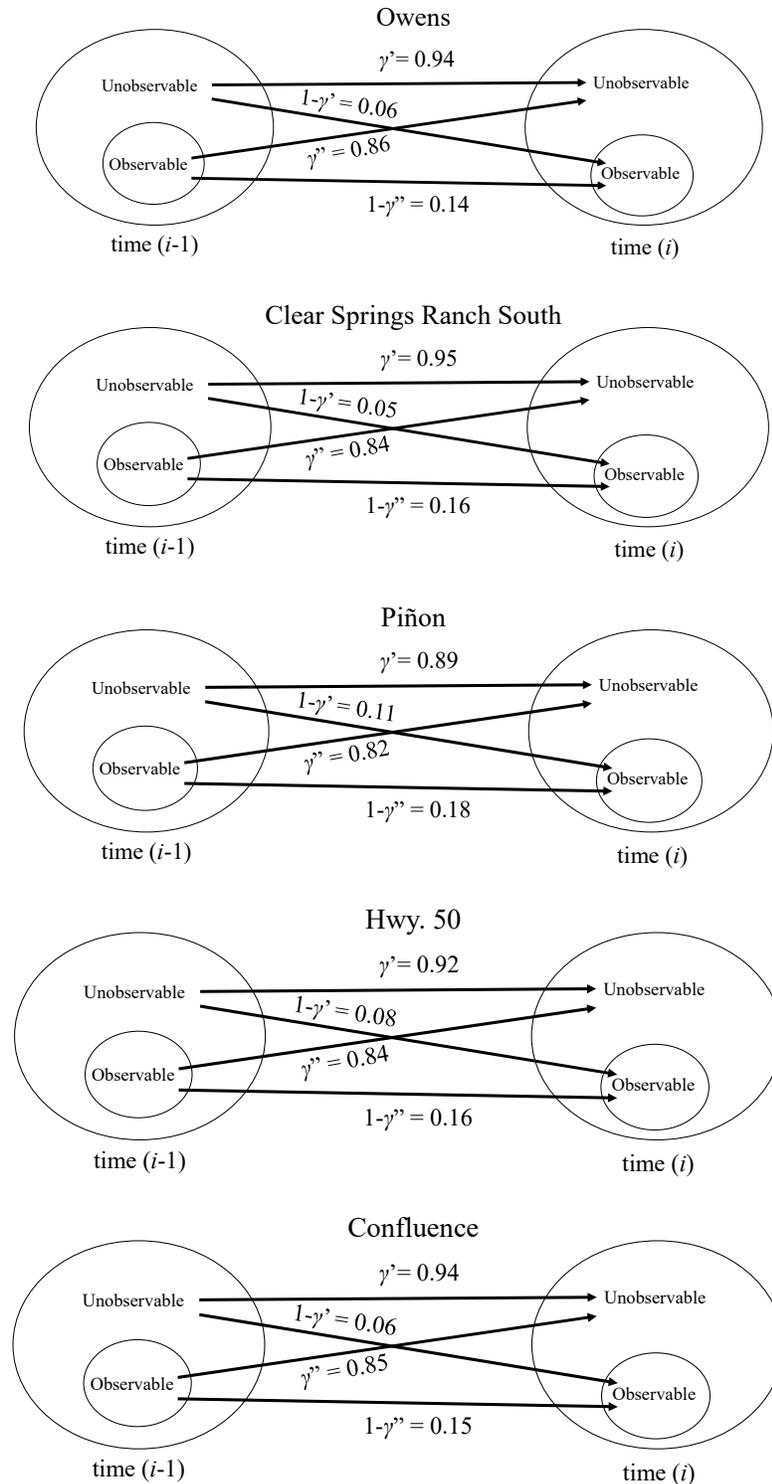
**Figure 4.** Mean monthly apparent survival ( $\phi$ ) by site and season  $\phi(3\_Seasons + Site)$  from a closed multi-state analysis in Program MARK. Note: CSRS = Clear Springs Ranch South.

**Objective 2. Quantify movement through the study system**

The maximum documented distance moved was an upstream 58-rkm movement through the entire study area. Transition probabilities ( $\psi$ ) ranged from 0.134–3.67x10<sup>-5</sup> with corresponding distances between sites of 3.7–57.6-rkm. Flathead Chub moved most in summer and very little in winter, with higher summer transition probabilities for upstream movements than downstream movements (Figure 5). Overall mean temporary emigration rates were  $\gamma''=0.84$  (0.81–0.87) and  $\gamma'=0.93$  (0.90–0.95), indicating extremely high levels of Flathead Chub movement (Figure 6).



**Figure 5.** Summer and winter estimated transition probabilities of Flathead Chub in Fountain Creek, Colorado. Summer transition probabilities were separated into upstream and downstream directions. Winter directions were very similar, so they were averaged. Transition probabilities were calculated in time-periods as short as 11 days, indicating Flathead Chub in this portion of the system are capable of moving long distances in short periods of time. Summer estimates were calculated using the most parsimonious model,  $\psi(\text{Distance} + \text{Summer} \times \text{Direction})$  from a closed multi-state model in Program MARK. The winter estimates were from the top rated model that included winter transition probabilities  $\psi(\text{Direction} + \text{Winter})$ .



**Figure 6.** Temporary emigration ( $\gamma$ ) estimates derived from site-specific robust design analyses in Program MARK. Primary periods were the days sampled, and secondary periods were five passes made through the site with three gear types (12-m mobile array, 2-m mobile array, and handheld reader). These results indicate extremely high rates of Flathead Chub movement in short periods of time.

**Objective 3. Test mechanistic effects on these life history metrics.**

Apparent survival was most affected by seasons and site (Table 1). One surprising result was that none of the flow covariates carried much weight (Table 1). This could be due to several reasons. First, these covariates are confounded with season, which were included in top models. In Fountain Creek, most high flows events are the result of summer, monsoonal thunderstorms. To separate the effect of high flows from seasons, there would have had to be high flow events in the winter, which did not occur. Second, this could be because our study organism was the adult life stage of a very strong swimming species, which is likely better able to withstand the high flow events. If we had selected a poorer swimming plains fish species, or examined an earlier life stage, I expect high flow events to have a large effect on survival. Third, the fact that there is still a robust population in Fountain Creek, with its extremely flashy hydrograph, provides evidence that these adults are able to withstand the high flows. If they weren't, obviously, they would have been extirpated from this reach. As an exploratory exercise to guide future research, I reran the top model comparing the flow covariates to each other. This allowed an examination of flow effects in absence of year and isolate flow from other effects. The post hoc comparison of the effect of high flow events indicated that a delayed effect of approximately one month more negatively affected apparent survival than immediate or annual high flow events. This indicates that high flow events have a negative effect on survival, but the effect is not immediate—such as with stranding—but more likely due to increased stress on fish that results in delayed mortality.

The transition probability covariates with the most weight were distance, season (especially summer), and direction of movement. Distance makes intuitive sense, as the greater the distance between sites, the less likely fish are to move between them. The summer and direction interaction in the top model is interesting because this indicates Flathead Chub move upstream in the summer to spawn, and downstream in other seasons to seek out refuge habitats. The robust design analysis at Owens indicated that high flow events during summer increase temporary emigration from study sites (Table 2).

**Table 1.** Closed multi-state models with weight in Program MARK used to estimate apparent survival ( $\phi$ ), detection probability ( $p$ ), and transition probability ( $\psi$ ) for PIT tagged Flathead Chub *Platygobio gracilis* in Fountain Creek, Colorado. The maximized log-likelihood ( $\log(L)$ ), the number of parameters (K) in each model, and the small sample size-corrected AIC<sub>c</sub> values (AIC<sub>c</sub>) are shown. Abbreviations are as follows: SE = season; SI = site; F\_IM = flow events immediate effect; F\_D = high flow events, delayed effect; F\_AN = high flow events, annual effect; SU = summer; WI = winter; TR = transition seasons of fall and spring; OT = other seasons (long break between years); MDF = mean daily flow (cms); TL = fish total length in mm; DS = distance in km; DR = direction (upstream or downstream).

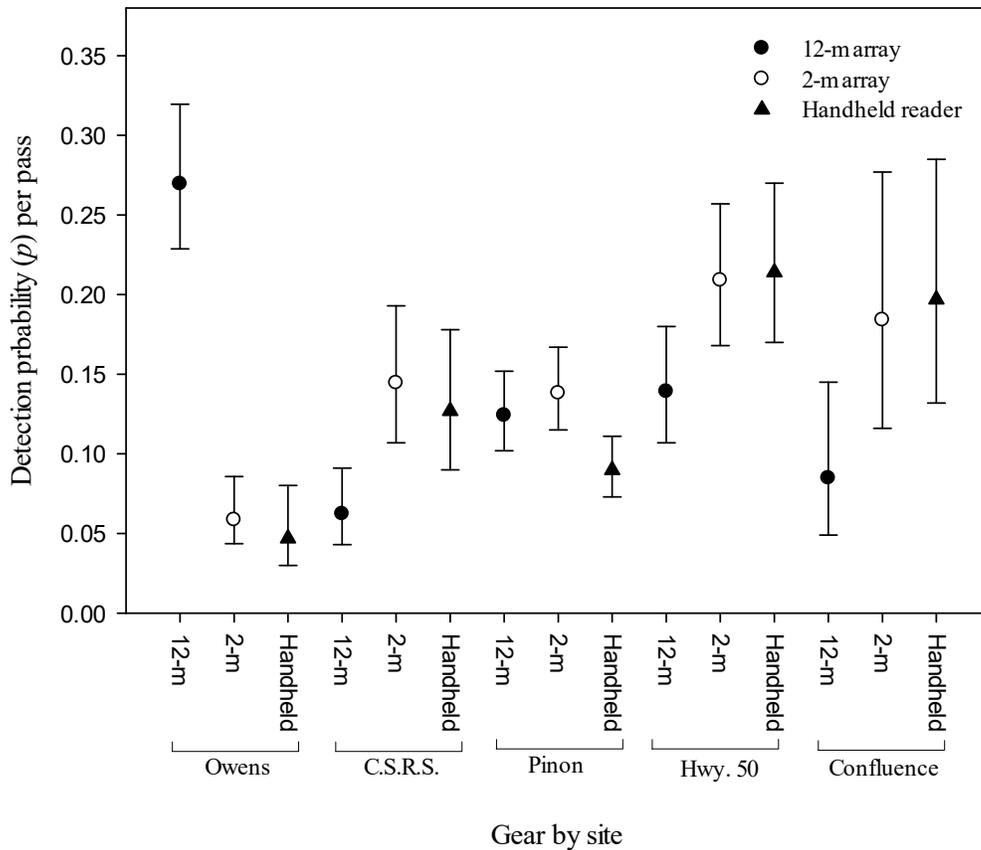
| Model  | AIC <sub>c</sub> | $\Delta$ AIC <sub>c</sub> | w <sub>i</sub> | K  | -2log(L) |
|--|------------------|---------------------------|----------------|----|----------|
| $\phi(3\_SE + SI) p(MDF + TL + 3SE \times SI) \psi(DS + SU \times DR)$   | 10804.917        | 0.000                     | 0.22270        | 29 | 10746.79 |
| $\phi(3\_SE + SI) p(MDF + TL + 3SE \times SI) \psi(DS + SU)$             | 10804.924        | 0.007                     | 0.22195        | 27 | 10750.82 |
| $\phi(F\_IM + 3\_SE + SI) p(MDF + TL + 3\_SE \times SI) \psi(DS + SU)$   | 10805.27         | 0.35                      | 0.18697        | 28 | 10749.15 |
| $\phi(F\_AN + 3\_SE + SI) p(MDF + TL + 3\_SE \times SI) \psi(DS + SU)$   | 10805.99         | 1.07                      | 0.13038        | 28 | 10749.87 |
| $\phi(3\_SE + SI) p(MDF + TL + 3\_SE \times SI) \psi(DS + SU + DI)$      | 10806.15         | 1.23                      | 0.12012        | 28 | 10750.04 |
| $\phi(F\_D + 3\_SE + SI) p(MDF + TL + 3\_SE \times SI) \psi(DS + SU)$    | 10806.81         | 1.89                      | 0.08635        | 28 | 10750.70 |
| $\phi(WI + SI) p(MDF + TL + 3\_SE \times SI) \psi(DS + SU)$              | 10809.36         | 4.44                      | 0.02416        | 26 | 10757.26 |
| $\phi(3\_SE + SI) p(MDF + TL + 3\_SE \times SI) \psi(DS + OT)$           | 10814.12         | 9.20                      | 0.00223        | 27 | 10760.01 |
| $\phi(3\_SE + SI) p(MDF + TL + 3\_SE \times SI) \psi(DS + OT + DR)$      | 10814.48         | 9.56                      | 0.00187        | 28 | 10758.36 |
| $\phi(TR + SI) p(MDF + TL + 3\_SE \times SI) \psi(DS + SU)$              | 10814.63         | 9.71                      | 0.00173        | 26 | 10762.53 |
| $\phi(3\_SE + SI) p(MDF + TL + 3\_SE \times SI) \psi(DS + OT \times DR)$ | 10815.82         | 10.91                     | 0.00095        | 29 | 10757.70 |
| $\phi(3\_SE) p(MDF + TL + 3\_SE \times SI) \psi(DS + SU)$                | 10818.34         | 13.42                     | 0.00027        | 23 | 10772.26 |
| $\phi(TR \times SI) p(MDF + TL + 3\_SE \times SI) \psi(DS + SU)$         | 10818.77         | 13.85                     | 0.00022        | 30 | 10758.64 |
| $\phi(WI) p(MDF + TL + 3\_SE \times SI) \psi(DS + SU)$                   | 10823.61         | 18.69                     | 0.00002        | 22 | 10779.54 |
| $\phi(3\_SE + SI) p(MDF + TL + 3\_SE \times SI) \psi(DS + TR + DR)$      | 10823.85         | 18.93                     | 0.00002        | 29 | 10765.73 |
| $\phi(3\_SE + SI) p(MDF + TL + 3\_SE \times SI) \psi(DS)$                | 10825.09         | 20.17                     | 0.00001        | 26 | 10772.99 |
| $\phi(3\_SE + SI) p(MDF + TL + 3\_SE \times SI) \psi(DS + TR + DR)$      | 10825.17         | 20.25                     | 0.00001        | 28 | 10769.05 |
| $\phi(3\_SE + SI) p(MDF + TL + 3\_SE \times SI) \psi(DS + TR)$           | 10825.34         | 20.42                     | 0.00001        | 27 | 10771.23 |
| $\phi(TR) p(MDF + TL + 3\_SE \times SI) \psi(DS + SU)$                   | 10825.87         | 20.95                     | 0.00001        | 22 | 10781.80 |

**Table 2.** Weighted model selection results for robust design models fit to estimate apparent survival ( $\phi$ ), temporary emigration ( $\gamma''$ = observable at  $i-1$  and  $\gamma'$ =unobservable at  $i-1$ ), detection probability ( $p$ ) and recapture probability ( $c$ ) for PIT tagged Flathead Chub, *Platygobio gracilis* at Owens-Hall Diversion, Fountain Creek, Colorado. Models are ranked by their AIC<sub>c</sub> differences ( $\Delta$ AIC<sub>c</sub>) relative to the best model in the set and Akaike weights ( $w_i$ ) quantify the probability that a particular model is the best model in the set given the data and the model set. Gamma'' and gamma' were estimated using Markovian movement. Note: SU = summer; SI = site; W\_O = winter other (time-period between sampling one year and the next); MDF = mean daily flow (cms); TL = fish total length in mm.

| Model   | AIC <sub>c</sub> | $\Delta$ AIC <sub>c</sub> | $w_i$   | K  | -2log(L) |
|---|------------------|---------------------------|---------|----|----------|
| $\phi(3\_SE) M\_ \gamma''(HF + SU) \gamma'(HF + SU) p=c(3\_SE \times G + TL)$                     | 7165.67          | 0.00                      | 0.56929 | 19 | 7127.55  |
| $\phi(3\_SE) M\_ \gamma''(HF \times SU) \gamma'(HF \times SU) p=c(3\_SE \times G + TL)$           | 7168.84          | 3.17                      | 0.11691 | 21 | 7126.68  |
| $\phi(3\_SE) M\_ \gamma''(HF + SU + TL) \gamma'(HF + SU + TL) p=c(3\_SE \times G + TL)$           | 7169.04          | 3.36                      | 0.10598 | 21 | 7126.88  |
| $\phi(3\_SE) M\_ \gamma''(SU) \gamma'(SU) p=c(3\_SE \times G + TL)$                               | 7169.26          | 3.59                      | 0.09479 | 17 | 7135.16  |
| $\phi(3\_SE) M\_ \gamma''(F\_IM + SU + TL) \gamma'(F\_IM + SU + TL) p=c(3\_SE \times G)$          | 7170.89          | 5.22                      | 0.04184 | 20 | 7130.75  |
| $\phi(3\_SE) M\_ \gamma''(F\_IM+SU+TL) \gamma'(F\_IM+SU+TL) p=c(3\_SE \times G + MDF)$            | 7171.53          | 5.86                      | 0.03038 | 21 | 7129.38  |
| $\phi(3\_SE) M\_ \gamma''(HF \times SU + TL) \gamma'(HF \times SU + TL) p=c(3\_SE \times G + TL)$ | 7172.34          | 6.67                      | 0.02035 | 23 | 7126.15  |
| $\phi(3\_SE) M\_ \gamma''(SU + TL) \gamma'(SU + TL) p=c(3\_SE \times G + TL)$                     | 7172.74          | 7.07                      | 0.01661 | 19 | 7134.61  |
| $\phi(3\_SE) M\_ \gamma''(HF) \gamma'(HF) p=c(3\_SE \times G + TL)$                               | 7176.60          | 10.93                     | 0.00242 | 17 | 7142.49  |
| $\phi(3\_SE) M\_ \gamma''(TR) \gamma'(TR) p=c(3\_SE \times G + TL)$                               | 7178.34          | 12.67                     | 0.00101 | 17 | 7144.24  |
| $\phi(3\_SE) M\_ \gamma''(OT) \gamma'(OT) p=c(3\_SE \times G + TL)$                               | 7181.41          | 15.74                     | 0.00022 | 17 | 7147.31  |
| $\phi(3\_SE) M\_ \gamma''(.) \gamma'(.) p=c(3\_SE \times G + TL)$                                 | 7182.00          | 16.33                     | 0.00016 | 15 | 7151.92  |
| $\phi(3\_SE) M\_ \gamma''(TL) \gamma'(TL) p=c(3\_SE \times G + TL)$                               | 7185.93          | 20.26                     | 0.00002 | 17 | 7151.82  |
| $\phi(3\_SE) M\_ \gamma''(F\_IM + SU + TL) \gamma'(F\_IM + SU + TL) p=c(WI + TI)$                 | 7188.39          | 22.72                     | 0.00001 | 17 | 7154.28  |
| $\phi(3\_SE) M\_ \gamma''(F\_IM + SU + TL) \gamma'(F\_IM + SU + TL) p=c(WI \times G)$             | 7188.67          | 23.00                     | 0.00001 | 17 | 7154.57  |

**Objective 4.** Provide gear and field protocol recommendations for future PIT tag studies.

Five site-specific robust design analyses were conducted to obtain more detailed information on gear efficiency and temporary emigration (Kendall et al. 1997). Although there was no difference in the overall mean detection probability of gear types (two mobile arrays and a handheld PIT tag reader), there were significant differences seasonally and within sites (Figure 7). Mobile array detection probabilities were higher at lower flows, but were effective in flows up to 5.3-cms (187-cfs). Future studies, especially if conducted in complex habitats over multiple seasons, should use all three PIT tag detecting gears and conduct multiple passes with each mobile array gear type as different fish were detected on different passes.



**Figure 7.** Detection probability ( $p$ ) by site for the 12-m and 2-m mobile arrays for PIT tagged Flathead Chub at Owens-Hall Diversion, Fountain Creek, Colorado. Estimates were derived in a robust design models in Program MARK.

## **ACKNOWLEDGEMENTS:**

I would like to thank my coauthors: Dr. Kevin Bestgen and Dr. Larissa Bailey. I thank Paul Foutz for his field assistance in this project, Eric Fetherman with his assistance on modelling, and Andrew Treble for assistance on database management. I would also like to thank the army of technicians and volunteers who assisted in field work and data organization.

## **REFERENCES:**

- Haworth, M. R. and K. R. Bestgen. 2016. Daily increment validation and effects of streamflow variability and water temperature on growth of age-0 flathead chub. *North American Journal of Fisheries Management* 36:744–753.
- Haworth, M. R. and K. R. Bestgen. 2017. Flow and water temperature affect reproduction and recruitment of a Great Plains cyprinid. *Canadian Journal of Fisheries and Aquatic Sciences* 74:853–863.
- Kendall, W. L., J. D. Nichols, and J. E. Hines. 1997. Estimating temporary emigration using capture-recapture data with Pollock’s robust design. *Ecology* 78:563–578.
- Walters, D. M., R. E. Zuellig, H. J. Crockett, J. F. Bruce, P. M. Lukacs, and R. M. Fitzpatrick. 2014. Barriers impede upstream spawning migration of flathead chub. *Transactions of the American Fisheries Society* 143:17-25.
- White, G. C., and K. P. Burnham. 1999. Program MARK: Survival estimation from populations of marked animals. *Bird Study* 46 Supplement, 120-138.
- White, G. C., W. L. Kendall, and R. J. Barker. 2006. Multistate survival models and their extensions in Program MARK. *Journal of Wildlife Management* 70:1521-1529.

## **RESEARCH PRIORITY:**

Obtain quantifiable life history metrics for a Great Plains cyprinid.

## **OBJECTIVES:**

Quantify age and growth rates of Flathead Chub, *Platygobio gracilis*.

## **INTRODUCTION:**

Understanding the age structure of a population of interest is critical for effective management. This can be difficult for small-bodied organisms, such as the fishes of the Great Plains ecoregion in central North America. This ecoregion is home to a unique assemblage of fishes whose reproductive strategy utilizes nonadhesive, semibuoyant eggs that are released into the water column and then are passively transported downstream by the current (Fausch and Bestgen 1997). This assemblage has seen large declines in

distribution due to anthropogenic effects, including fragmentation, altered flow regimes, and nonnative species (Hoagstrom 2015; Worthington et al. 2018; Perkin et al. 2019).

This study is using multiple lines of evidence to estimate the age and growth of Flathead Chub, *Platygobio gracilis*. Growth rates are being calculated from an ongoing mark-recapture study. Flathead Chub were individually marked, allowing calculation of growth rate between release and recapture dates. Age estimates are being obtained from two bony structures, otoliths and fin rays, as well as using growth rates to calculate length at age relationships.

## **METHODS:**

Otoliths and fin rays were collected and aged from 161 flathead chub collected August 22–23, 2016 from five sites Fountain Creek, Colorado. Fish were collected throughout the study area to try to control for any differences among sites. Fish were preserved in 5-mm size class bins to represent the size range characteristic of the adult population. Fish were euthanized with a fatal dose of MS-222, preserved in ethanol, and then returned to the laboratory for otolith extraction. Left and right sagittal otoliths were dissected from fish and mounted on a standard microscope slide in a drop of cyanoacrylate glue and allowed to harden for at least 48-h. Otoliths were then polished, covered with a drop of immersion oil, and examined for annuli. Two readers independently aged otoliths.

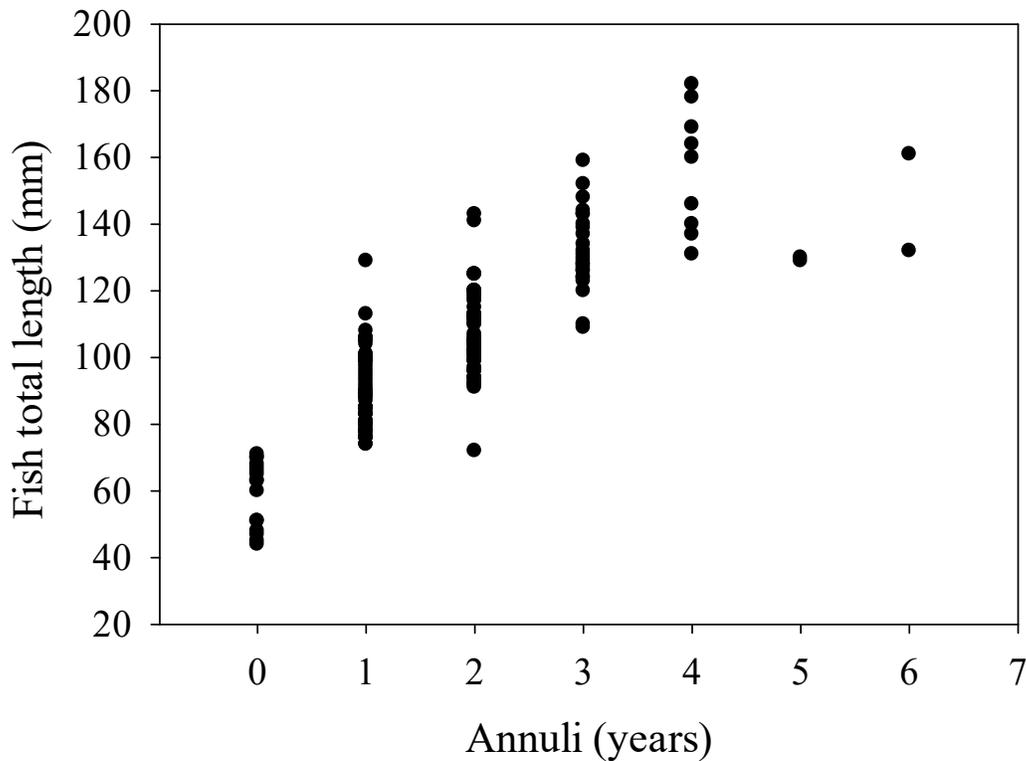
A requirement of age and growth studies is to validate the accuracy and precision of the techniques used for aging (Beamish and McFarlane 1983). Therefore, we validated aging techniques using age-0 fish as our baseline. A 500- $\mu\text{m}$  line was made on images, which acted as an age-0 baseline. From that baseline, annuli were counted outward. Eleven of the preserved fish were previously PIT tagged, which allowed comparison of age with growth from release date. Age was determined directly for each specimen independently by two investigators using otoliths and fin rays.

## **RESULTS AND DISCUSSION:**

Individual growth rates were obtained from 285 individuals, and growth rates were very low for adult flathead chub. The growth equation for all seasons was  $y = -0.36\ln(x) + 1.61$  ( $R^2 = 0.018$ ). The summer growth curve was  $y = -0.07\ln(x) + 0.35$  ( $R^2 = 0.12$ ) and the winter growth curve was  $y = -0.025\ln(\text{total\_length}) + 0.12$  ( $R^2 = 0.020$ ). Flathead Chub ages ranged from less than one year to greater than six years (Figure 8). Otoliths provided more consistency among readers regarding age, though they were difficult to read as age increased. Females tended to be larger bodied than males.

The flathead chub subspecies *P. gracilis gulonella* appears to be older and slower growing than previously thought. Management implications of this is that, although they are able to live through poor conditions and reproduce when conditions are more suitable,

they are susceptible to a catastrophic event as it would eliminate several years of reproductive output. Further analysis of these data are ongoing.



**Figure 8.** Otolith ages compared to fish lengths of Flathead Chub collected 2015 in Fountain Creek, Colorado. These ages are older than previously reported ages of the *P. gracilis gulonella* subspecies.

**ACKNOWLEDGEMENTS:**

I would like to thank my coauthor: Dr. Kevin Bestgen as well as field and laboratory staff who assisted in data collection.

**REFERENCES:**

Beamish, R. J., and G. A. McFarlane. 1983. The forgotten requirement for age validation in fisheries biology. *Transactions of the American Fisheries Society* 112:735–743.  
Fausch, K. D., and K. R. Bestgen. 1997. Ecology of fishes indigenous to the central and southwestern Great Plains. Pages 131-166 in F. L. Knopf and F. B. Samson, editors. *Ecology and conservation of Great Plains vertebrates*. Springer-Verlag, New York.

- Hoagstrom, C. W., J. E. Brooks, and S. R. Davenport. 2011. A large-scale conservation perspective considering endemic fishes of the North American plains. *Biological Conservation* 144:21–34.
- Perkin, J. S., S. P. Murphy, C. M. Murray, W. K. Gibbs, and A. E. Gebhard. 2019. If you build it, they will go: A case study of stream fish diversity loss in an urbanizing riverscape. *Aquatic Conservation – Marine and Freshwater Ecosystems* 29:623–638.
- Worthington, T. A., A. A. Echelle, J. S. Perkin, R. Mollenhauer, N. Farless, J. J. Dyer, D. Logue, and S. K. Brewer. 2018. The emblematic minnows of the North American Great Plains: A synthesis of threats and conservation opportunities. *Fish and Fisheries* 19:271–307.

### **RESEARCH PRIORITY:**

Enhancing rock ramp designs for small-bodied fishes

### **OBJECTIVES:**

Design and test a large flume to estimate slope and distance combinations that allow small-bodied fish passage through a rock ramp fishway.

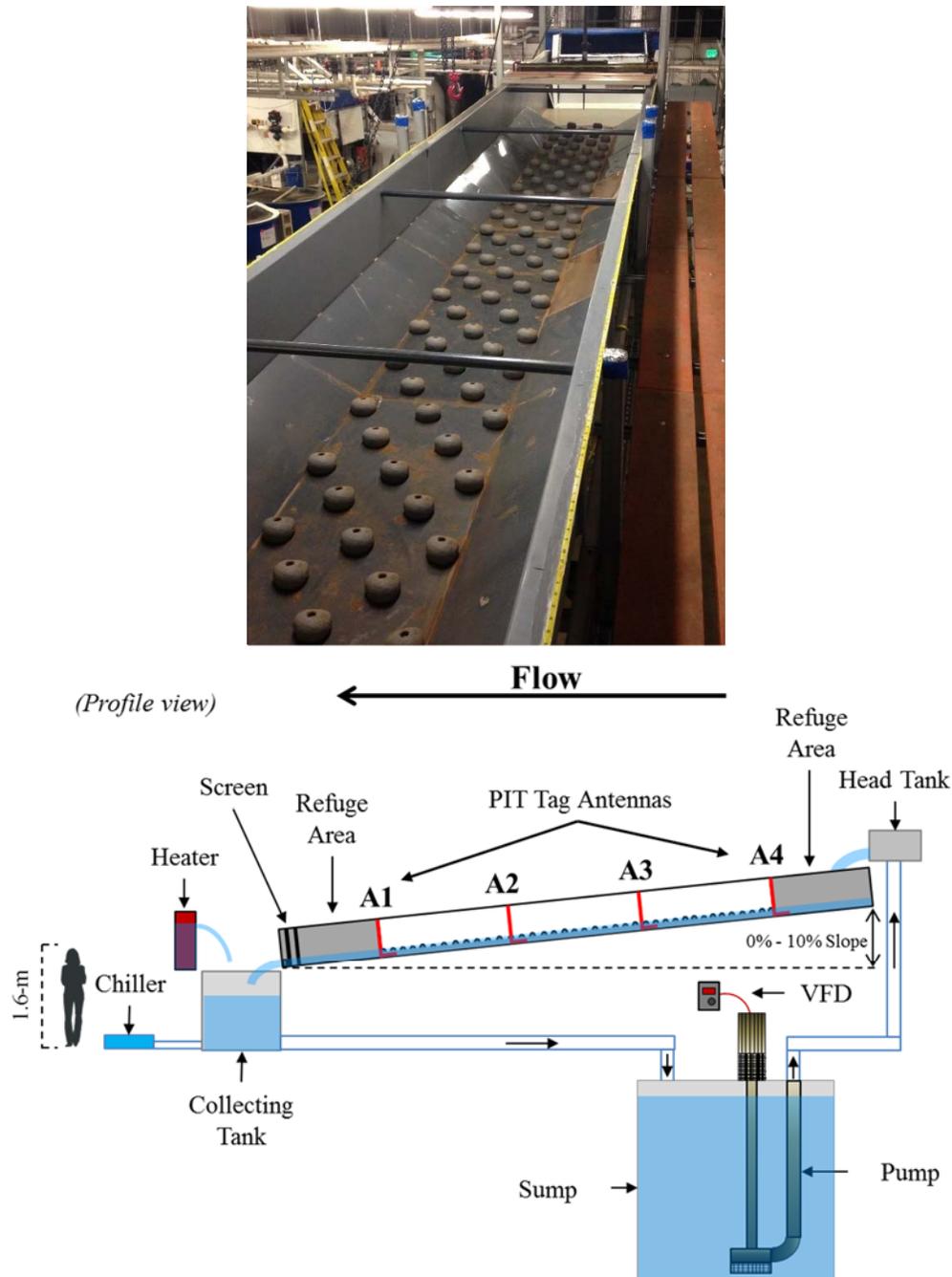
### **INTRODUCTION:**

The growing global need to improve the longitudinal connectivity of lotic systems is often met by using fish passage structures (fishways). When designing fishways in the past, biologists and engineers focused primarily on strong swimming species such as salmonids. However, the majority of riverine species in the interior United States are not salmonids and may be excluded by fishways built using salmonid criteria due to lower swimming abilities and/or behavioral differences. Rock ramp fishways (sometimes referred to as nature-like fishways) are comprised of a sloped section of channel with roughness elements installed on the bed to provide velocity refuges and decrease water velocity for fishes as they ascend the fishway. This design is widely recognized as a good choice to allow passage of small-bodied fishes because they can be built without vertical drops, high velocity sections, and provide heterogeneous hydraulic conditions to accommodate a diversity of swimming behaviors. This study was designed to improve the design of rock ramp fishways by identifying the ideal slope and length combinations for successful passage of small-bodied Great Plains fishes. A custom-made adjustable full-scale fishway was used to test fish passage success at slopes of 2 to 10%. This range of slopes, and associated water velocities, encompasses the range of slopes of existing or proposed fishways used along Colorado's Front Range.

### **METHODS:**

We designed and built a 9.1-m long adjustable hydraulic research flume at the Colorado State University Foothills Fisheries Laboratory (FFL) to test fish passage and evaluate the effects of grade (slopes of 2 – 10%, in 2% increments) on the passage success of three Great Plains fish species: Flathead Chub *Platygobio gracilis*, Stonecat *Noturus flavus*,

and Arkansas Darter *Etheostoma cragini*. A 6.1-m long rock ramp fishway was installed in the flume and four PIT tag antennas were used to detect full or partial passage success.



**Figure 9.** Diagram of the research flume built at the CSU Foothills Fisheries Laboratory. The flume can be adjusted from 0 – 10% slope by using two overhead chain hoists. 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. This diagram is to scale for what a person who is 1.6-m tall would look like standing next to the structure.

## **RESULTS AND DISCUSSION:**

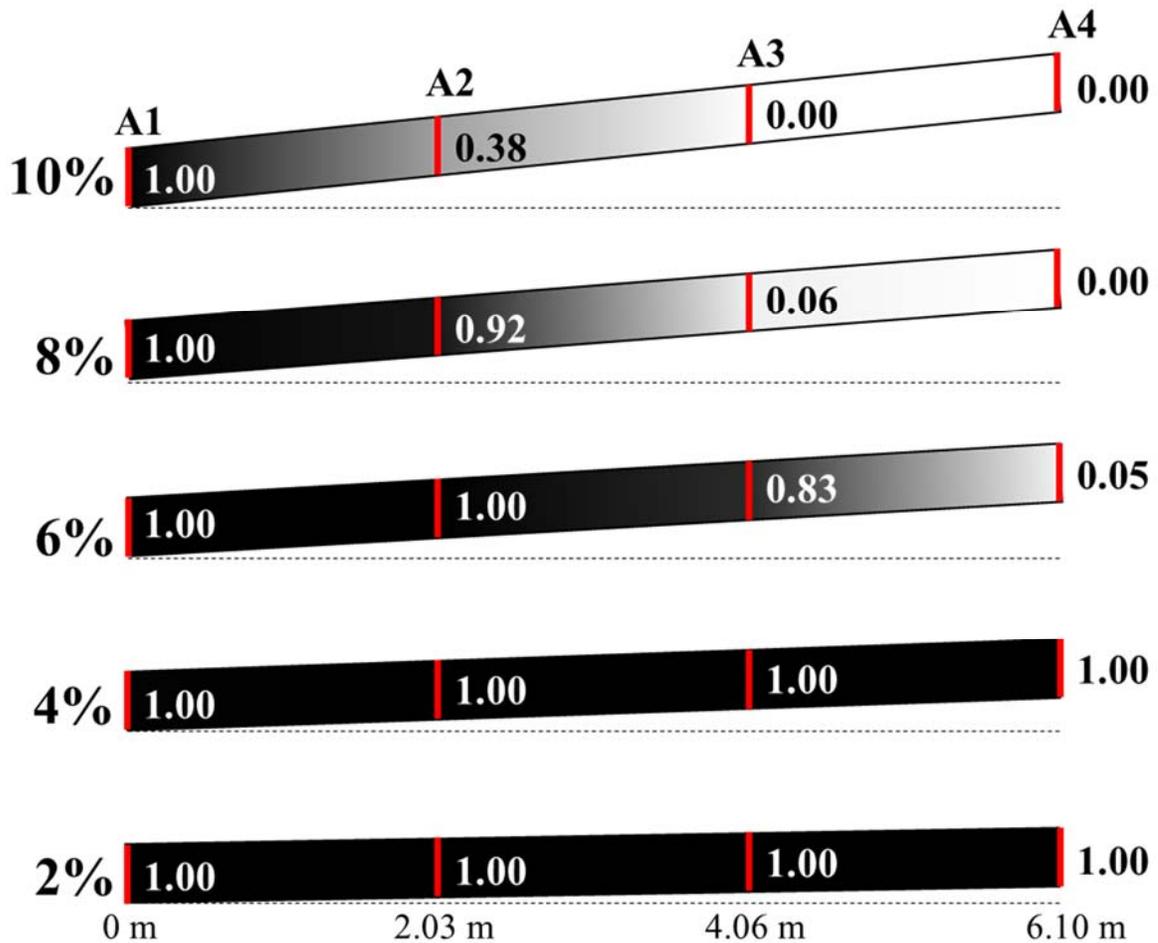
We used the Cormack-Jolly-Seber (CJS) model in Program MARK to determine the probability of full and partial passage success over the fishway based on the PIT tag detection history of each fish at each antenna. Passage success to upstream antennas was highest at shorter distances and at lower slopes for all species. Probability of passage success was highest for Flathead Chub, followed by Stonecat, and then Arkansas Darter.

The probabilities of Flathead Chub successfully ascending a 6.1-m rock ramp fishway at slopes of 2, 4, and 6% were 1.0. Probability of Flathead Chub passage success was very high (0.96) for a 4.06-m, 8% slope fishway. Flathead Chub were unable to ascend 4.06-m of a 10% slope fishway.

Stonecats had a passage probability of 1.0 for a 6.1-m fishway at 2 and 4% slope, and a passage probability of 0.83 for a 4.06-m, 6% slope fishway. No passage was predicted for 10% slope fishways greater than 4.06-m and 8% fishways greater than 6.1-m.

Arkansas Darters never achieved a probability of 1.0 for ascending a 6.1-m fishway. However, their probability of partial passage success was moderate for a 2.03-m, 4% slope fishway with a probability of 0.43, and for a 4.06-m, 2% slope fishway with a probability of 0.54. Passage probabilities for Arkansas Darters were 0.00 for 10% slope 4.06-m, 8% slope 4.06-m, and 6% slope 6.10-m fishways.

Based on the results of this study, it is clear that fishway designs should consider the passage requirements of the species with the lowest performance both in terms of fishway slope and fishway length. For example, a rock ramp fishway with a slope of 4% and a length of 2.03 m would be passable by some Arkansas Darters and all of the Stonecats and Flathead Chub in the size ranges tested. The results of this study provide valuable design criteria by identifying fishway slope and length combinations that allow passage of this representative suite of small-bodied Great Plains fishes.



**Figure 10.** True estimates of the probability that an adult Stonecat can successfully ascend to one of the PIT tag antennas (A1 – A4) in the rock ramp fish passage structure at slopes of 2 to 10%. PIT tag antennas were evenly spaced (every 2.03 m) in the fishway to detect fish movements. Dashed lines indicate 0% slope for reference. Each flume diagram is tilted to scale for its respective slope treatment. Darker fill indicates higher probability of passage success. Estimates were determined by multiplying the apparent rates of fish movement between antennas.

#### ACKNOWLEDGEMENTS:

I thank my collaborators on this project: Tyler Swarr and Dr. Chris Myrick. We thank staff at Colorado Parks and Wildlife, specifically Boyd Wright, and staff at Wyoming Game and Fish, specifically Bobby Compton, for assistance collecting wild fish. We thank the staff at CPW’s Native Aquatic Species Restoration Facility for providing Arkansas Darters.

## **PUBLICATIONS:**

- Swarr, T. R. 2018. Improving rock ramp fishways for small-bodied Great Plains Fishes. Master's Thesis. Colorado State University. Fort Collins, Colorado.
- Swarr, T. R., C. M. Myrick and R. M. Fitzpatrick. *In review*. PIT tag retention, survival, and effects on swimming performance in a small-bodied fish, the Arkansas darter, *Etheostoma cragini*. Journal of Fish Biology.
- Swarr, T. R., C. M. Myrick and R. M. Fitzpatrick. *In review*. Effects of slope on small-bodied fish passage success in an experimental rock ramp fishway. Journal of Ecohydraulics. Breiman, L. 2001. Random forests. Machine Learning. 45:5-32.

## **RESEARCH PRIORITY:**

Evaluation of the Owens-Hall fish passage structure and potentially use this structure as a template for other plains fish barriers.

## **OBJECTIVES:**

Determine the amount and timing of native fish movement through the Owens-Hall fish passage structure.

## **INTRODUCTION:**

Fountain Creek, Colorado, has a relatively intact native fish community that is dominated by the Species of Concern Flathead Chub, *Platygobio gracilis* and is also home to the state threatened Arkansas Darter, *Etheostoma cragini*. The native species community is strongest in the lower section of Fountain Creek, which is also the longest stretch of stream without a barrier (58-km). The first barrier that fish can encounter in this reach is the Owens-Hall diversion. To increase the stream reach available to native plains fishes, Colorado Springs Utilities installed a rock ramp fish passage structure on the diversion. This fishway was designed to act as a template to be used for barriers farther upstream. If this structure is effective at fish passage, it can be used as a relatively inexpensive template to increase connectivity within Fountain Creek and along the Front Range of Colorado. The purpose of this project is to evaluate the effectiveness of the Owens-Hall fish passage structure.

## METHODS:

Three PIT tag reading arrays were used to be able to detect partial movement and direction of movement (Figure 11). Flathead Chub were collected by electrofishing below the fish passage structure. All fish were checked for a PIT tag. Deploying of PIT tags consisted of sanitizing all scalpels, tags, and sutures in ethanol. An incision was made ventrally into the fish's body cavity just posterior to the left pectoral fin. The 12-mm half-duplex Oregon RFID PIT tag was inserted and one suture (Braunamid suture with needle, 3/8 circle, 3/0) was applied with two half-hitch knots. Fish were allowed to recover equilibrium before being released at the tagging location. All mortalities were recorded and those individuals were removed from the study.



**Figure 11.** Fish passage structure on the Owens-Hall Diversion, Fountain Creek, Colorado.

## **RESULTS AND DISCUSSION:**

Evaluation of the fish passage structure is ongoing. Flathead Chub have been documented swimming through the fish passage structure. Future research will PIT tag additional species of fish focusing on the area directly downstream of the fish passage structure.

## **ACKNOWLEDGEMENTS:**

I would like to thank David Longrie and Kirsta Scherff-Norris at Colorado Springs Utilities for their financial support of this project. I would also like to thank Paul Foutz for his assistance with field work. I thank Harry Crockett, Matt Nicholl, Josh Nehring, and Jeff Spohn for the ongoing funding of this project.

## **RESEARCH PRIORITY:**

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

## **OBJECTIVES:**

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

## **INTRODUCTION:**

Johnny Darter are native to the South Platte River Basin and are regularly exposed to elevated winter temperatures from wastewater treatment plant (WWTP) effluent flows along the Colorado Front Range. Studies have been conducted on the effects of elevated winter water temperatures on Johnny Darter reproduction (Firkus et al. 2017). However, less is known about how duration of winter temperatures influences their reproductive success in the spring. This study will evaluate the effects of both winter stream temperature and duration of winter period on Johnny Darter reproductive success and development. This project will contribute to potential explanations of this observed decline in Johnny Darter abundance and could provide valuable life history information for this species.

The study area will be the South Platte River Basin along the Colorado Front Range (CFR). The CFR is an area of increasing research interest due to its unique native fauna, dynamic environmental conditions, and accelerated rate of human development within the last few decades. The population of Denver, Colorado has increased by an estimated 48.1% from 1990 to 2016 (United States Census Bureau 2016). Fort Collins, another Colorado city hosting South Platte River tributaries, has experienced an estimated 88.7% increase in human population within the same 26-year period. The CFR supports a high density of people in a relatively small range: 90% of the people in the South Platte River Basin are residing within 10% of the basin's area. All of this human activity has led to major modifications of the natural river systems and disrupted biological processes of native fishes (Vajda et al. 2008; McGree et al. 2010; Schwindt et al. 2014). When taking into account the high concentration of WWTPs, limited water sources, arid environment, and fluctuating flow regimes of the CFR, the local fish community is under a substantial amount of stress. This rapid and expansive change exacerbates the need to study how the assemblages of native fishes within this area are responding to the local urban conditions. The Colorado Front Range is an area worth protecting because it is home to a native fish assemblage that is exposed to a high level of anthropogenic influence. This emblematic suite of warm water fishes could serve as a relevant platform for future human impact studies therefore research regarding the management of these species is needed.

## **METHODS:**

This project is evaluating the effects of over-winter temperatures and winter duration periods on Johnny Darter reproductive success. We will expose wild Johnny Darters to six laboratory treatments of two different temperature regimes and three duration periods to simulate varying winter conditions. After winter simulations, we will replicate a spring transition period to induce spawning and evaluate the reproductive success of the Johnny Darters. We will be examining these components of reproductive success for each of the six treatments: 1) fecundity, 2) hatching success, and 3) larval condition. With this approach, we will estimate acceptable winter temperature and duration conditions for reproductive success in Johnny Darters within the South Platte River Basin of Colorado.

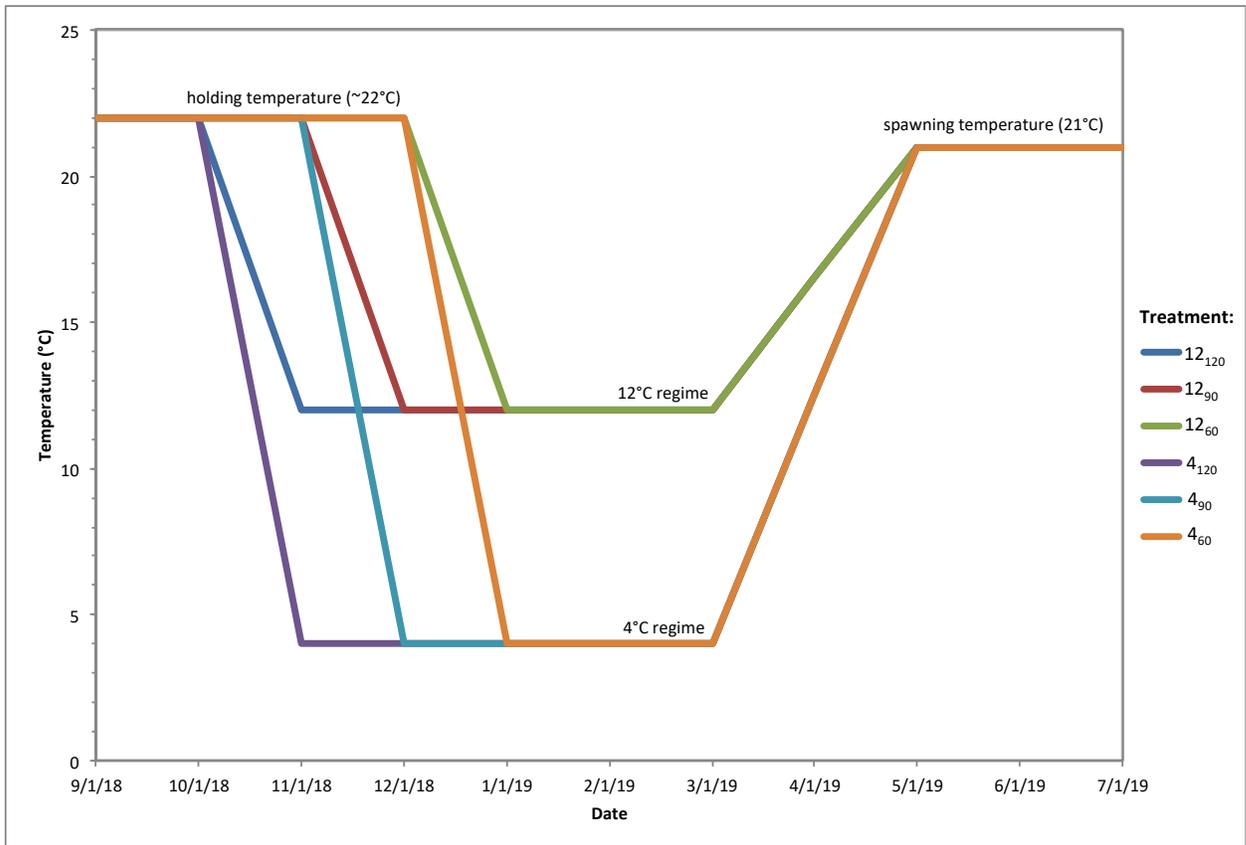
*Treatments.* We will hold Johnny Darters at two winter temperature regimes (12°C and 4°C) for three winter duration periods (60-, 90-, and 120-day durations), which equates to six total treatments (Table 1):

- 1) Regime 12<sub>60</sub>: 60-day winter duration period at 12°C.
- 2) Regime 12<sub>90</sub>: 90-day duration period at 12°C = baseline treatment. CDPHE Warm Stream Tier I winter water temperature standard for the state of Colorado.
- 3) Regime 12<sub>120</sub>: 120-day duration period at 12°C.
- 4) Regime 4<sub>60</sub>: 60-day duration period at 4°C.
- 5) Regime 4<sub>90</sub>: 90-day duration period at 4°C.
- 6) Regime 4<sub>120</sub>: 120-day duration period at 4°C.

The 12°C winter temperature regime is representative of the current Colorado Department of Public Health and Environment (CDPHE) Warm Stream Tier I winter water temperature standard for the state of Colorado: 12.1°C from December 1 to February 28 (Colorado Department of Public Health and Environment 2016). The 4°C winter temperature regime mimics natural winter temperatures of streams minimally impacted by WWTP. We chose our winter duration periods of 60 and 120 days to simulate a short winter and a long winter within a 30-day bracket of the CDPHE standard of a 90-day winter period.

**Table 3.** Specifications of the six laboratory treatments of Johnny Darters. Regime 12<sub>90</sub> is representative of the current CDPHE Warm Stream Tier I winter water temperature standard. The 4°C regime represents the natural winter temperature regime of streams minimally impacted by WWTP effluent.

|                                | 60-day Winter Duration | 90-day Winter Duration | 120-day Winter Duration |
|--------------------------------|------------------------|------------------------|-------------------------|
| Winter Temperature Regime 12°C | 12 <sub>60</sub>       | 12 <sub>90</sub>       | 12 <sub>120</sub>       |
| Winter Temperature Regime 4°C  | 4 <sub>60</sub>        | 4 <sub>90</sub>        | 4 <sub>120</sub>        |



**Figure 12.** Winter duration period timeline of JD exposed to regimes 12°C and 4°C.

## RESULTS AND DISCUSSION:

Experiments are ongoing. Preliminary results of this study include Johnny Darter successfully spawning in the laboratory. The field portion of this study is just beginning, but plans for the upcoming field season include deploying temperature loggers at various sites and preliminary histological analysis of gonadal development.

## ACKNOWLEDGEMENTS:

Collaborators on this project include: Dr. Dana Winkelman, Carli Baum, Catherine Adams, Melynda May, and Patrick Bachmann.

## REFERENCES:

- Firkus, T., F. J. Rahel, H. L. Bergman, and B. D. Cherrington. 2017. Warmed winter water temperatures alter reproduction in two fish species. *Environmental Management* 61:291-303.
- McGree, M. M., D. L. Winkelman, N. K. Vieira, and A. M. Vajda. 2010. Reproductive failure of the red shiner (*Cyprinella lutrensis*) after exposure to an exogenous estrogen. *Canadian Journal of Fisheries and Aquatic Sciences* 67:1730–1743.

- Schwindt, A. R., D. L. Winkelman, K. K. Keteles, M. Murphy, and A. M. Vajda. 2014. An environmental oestrogen disrupts fish population dynamics through direct and transgenerational effects on survival and fecundity. *Journal of Applied Ecology* 51:582–591.
- Vajda, A. M., L. B. Barber, J. L. Gray, E. M. Lopez, J. D. Woodling, and D. O. Norris. 2008. Reproductive disruption in fish downstream from an estrogenic wastewater effluent. *Environmental Science and Technology* 42:3407–3414.

### **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.

### **INTRODUCTION:**

Due to financial, logistical, 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. The model output should match the type of inference desired from the monitoring program. For the South Platte River basin, we fit the data to occupancy models because inference on occupancy was desired.

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.

Because the criterion quantifies the efficiency of the sampling, it is important to keep in mind that different objectives will lead to different designs. The chosen criterion will depend on the goals of the monitoring program. Our goal was to minimize prediction uncertainty across the area of inference. Therefore, we used the average prediction variance as our design criterion.

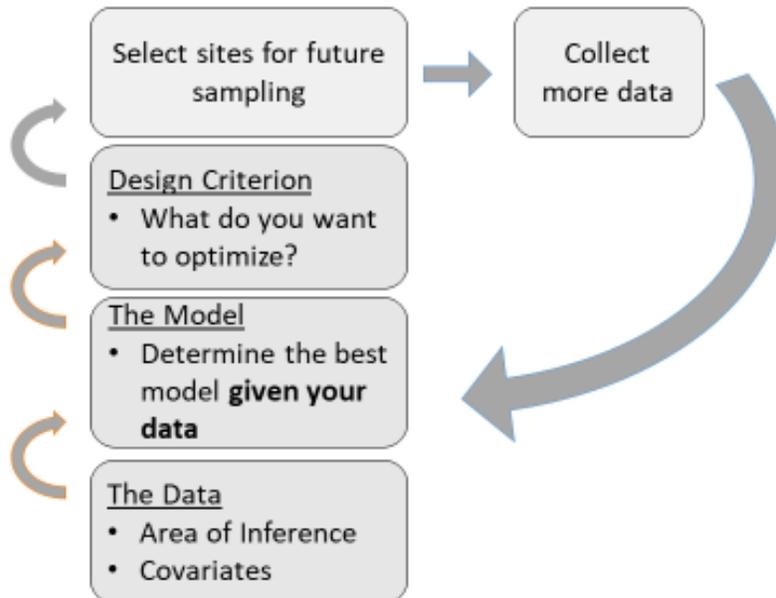
4. Selecting sites for future sampling. This step involves finding the set of sites that minimizes the design criterion. There are three components to this step: creating a finite set of potential sampling locations, incorporating logistical constraints, and implementing an optimization algorithm to find the optimal set of sampling locations. One may need to approximate a continuous area of inference into a discrete set of potential sampling locations. After being discretized, the set of potential sampling locations may need to be further refined to remove any sites that are not suitable for monitoring. The optimization algorithm depends on this set of potential sites. If there are too many potential locations, it will be computationally challenging to find an optimal design. A relatively small, finite set of locations eases computation. We followed the guidance of Diggle and Lophaven (2006) who suggested that the general spatial structure of a design is more important than the precise location of each point within it.

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.

A search algorithm is used to find the best combination of future sampling locations. It is important to find an efficient optimization algorithm because this step can be computationally burdensome as it is likely that there are a large number of combinations of sites among which to choose. There are three options commonly used in the literature: a simple search algorithm, simulated annealing, or an exchange algorithm. For the simple search and exchange algorithms, a set of sites is selected and the design criterion is calculated based on those sites. If the resulting design criterion is lower than for the previously selected set of sites, the new set of sites is retained as optimal, otherwise the previous set of sites are retained. This series of steps is continued for many iterations to find an optimal or quasi-optimal design. The simulated annealing algorithm retains the new set of sites if it leads to a lower design criterion, and it also retains the new sites with a probability that is a function of how many iterations have already been completed and how inferior the new set is compared to the last (Dixon et al. 1999; Fuentes et al. 2007).

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. **It is this responsiveness to new data that makes the procedure ideal for optimal long-term monitoring.**



**Figure 13.** The building blocks of an optimal adaptive sampling design.

## **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. This procedure has been used by biologists for the previous three field seasons, and is scheduled to be an ongoing, annual site selection tool.

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## **PUBLICATIONS:**

Broms, K. M., M. B. Hooten, and R. M. Fitzpatrick. 2015. Accounting for imperfect detection in Hill numbers for biodiversity studies. *Methods in Ecology and Evolution* 6:99–108.

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

Broms, K. M. and R. M. Fitzpatrick. 2016. A procedure manual for optimal adaptive sampling design for multiple species of plains fishes in eastern Colorado.

## **REFERENCES:**

Diggle, P., and S. Lophaven. 2006. Bayesian geostatistical design. *Scandinavian Journal of Statistics* 33:53–64.

Dixon, W., G. K. Smyth, and B. Chiswell. 1999. Optimized selection of river sampling sites. *Water Research* 33:971–978.

Fanshawe, T. R., and P. J. Diggle. 2013. Adaptive sampling design for spatio-temporal prediction, Chapter spatio-temporal design: *Advances in efficient data acquisition*. Pages 249–268. Wiley Online Library.

Fuentes, M. A., Chaudhuri, and D. M. Holland. 2007. Bayesian entropy for spatial sampling design of environmental data. *Environmental and Ecological Statistics* 14:323–340.

Wikle, C. K., and J. A. Royle. 1999. Space-time dynamic design of environmental monitoring networks. *Journal of Agricultural, Biological, and Environmental Statistics* 4:489–507.

### **RESEARCH PRIORITY:**

Environmental DNA metabarcoding for plains fishes and mollusks

### **OBJECTIVES:**

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

### **INTRODUCTION:**

Distribution and abundance of Colorado's eastern plains native fishes have declined since 1900 such that many are state-listed and in need of conservation. Anthropogenic changes including stream barriers, altered flow regime, siltation, channelization, changes in water quality, and introduced species have been implicated in the demise of native fishes. Understanding the distribution of these species and nonnative species affecting them is essential to promoting conservation and the potential expansion of remaining populations.

There are 33 native plains fish species in the South Platte and Arkansas River basins in Colorado. Of these, 12 currently are currently listed as endangered, threatened, or species of concern by the State of Colorado (Table 4). Effective conservation of these species requires information about species distributions and identification of threats. One threat to this suite of species are aquatic invasive species. Establishing an early detection system for invasive species will help management control these populations as early as possible.

Freshwater mollusks have become the most endangered group of animals in North America (Williams et al. 1993). Of the approximately 300 species and subspecies of unionids in the United States and Canada, approximately 72% are considered endangered, threatened, or of special concern. Information regarding mollusks on Colorado's eastern slope is lacking, even though nine species are currently listed in the Colorado State Wildlife Action Plan (Harrold et al. 2010) (Table 5). In order to effectively manage these species, a greater understanding of their distribution is required.

Detecting small-bodied organisms in large river systems is difficult. Species monitoring using environmental DNA is a powerful new technique for wildlife detection that may improve the efficiency of these sampling efforts (Deiner et al. 2017; Thomas et al. 2017). The ANDe aquatic eDNA sampling system was designed by a team of molecular ecologists and engineers for high-throughput eDNA sample collection (Thomas et al.

2010). The system is optimized for sampling speed and replicability, while minimizing the risk of contamination (Thomas et al. 2017). It is designed to sample larger volumes of water compared to other eDNA sampling methods, which reduces the risk of contamination among sampling sites. Therefore, this system could readily be incorporated into CPW’s existing sampling protocol and provide additional information regarding species distributions, especially for hard to detect species (Mariac et al. 2018).

**Table 4.** Special status plains and transition zone fish species to be included in the plains fish eDNA metabarcoding project.

| Common Name                | Scientific Name               | State Status       |
|----------------------------|-------------------------------|--------------------|
| Northern Redbelly Dace     | <i>Phoxinus eos</i>           | Endangered         |
| Southern Redbelly Dace     | <i>Phoxinus erythrogaster</i> | Endangered         |
| Lake Chub                  | <i>Couesius plumbeus</i>      | Endangered         |
| Plains Minnow              | <i>Hybognathus placitus</i>   | Endangered         |
| Suckermouth Minnow         | <i>Phenacobius mirabilis</i>  | Endangered         |
| Arkansas Darter            | <i>Etheostoma cragini</i>     | Threatened         |
| Brassy Minnow              | <i>Hybognathus hankinsoni</i> | Threatened         |
| Common Shiner              | <i>Luxilus cornutus</i>       | Threatened         |
| Flathead Chub              | <i>Platygobio gracilis</i>    | Species of Concern |
| Iowa Darter                | <i>Etheostoma exile</i>       | Species of Concern |
| Plains Orangethroat Darter | <i>Etheostoma spectabile</i>  | Species of Concern |
| Stonecat                   | <i>Noturus flavus</i>         | Species of Concern |

**Table 5.** List of mollusk species included in the Colorado State Wildlife Action Plan, their priority tier, state status (SC= Species of Concern), and if the species is listed as a USFS Sensitive Species.

| Common Name             | Species                           | Priority Tier | State Status | USFS Sensitive Species |
|-------------------------|-----------------------------------|---------------|--------------|------------------------|
| Rocky Mountain capshell | <i>Acroloxus coloradensis</i>     | Tier 2        | SC           | X                      |
| Cylindrical papershell  | <i>Anodontoides ferussacianus</i> | Tier 2        | SC           |                        |
| Cloche ancylid          | <i>Ferrissia walkeri</i>          | Tier 2        |              |                        |
| Cockerell               | <i>Promenetus umbillicatellus</i> | Tier 2        |              |                        |
| Fragil ancylid          | <i>Ferrissia fragilis</i>         | Tier 2        |              |                        |
| Hot springs physa       | <i>Physa cupreonitens</i>         | Tier 2        |              |                        |
| Pondhorn                | <i>Uniomerus tetralasmus</i>      | Tier 2        |              |                        |
| Sharp sprite            | <i>Promenetus exacuous</i>        | Tier 2        |              |                        |
| Utah physa              | <i>Physa gyrina utahensis</i>     | Tier 2        |              |                        |

## **METHODS:**

The first step of this process will be to collaborate with biologists and senior biologists to develop a prioritized list of species of interest. This list will include threatened and endangered species, as well as invasive species. Once this list is developed, genetic samples from each species will be collected, analyzed, and added to a database.

Once the eDNA database is built, two types of sites will be sampled. The first are sites associated with the plains fish sampling protocol. Before the standard sampling begins, multiple eDNA samples will be taken. Sampling with these two techniques will allow comparison among them. This will inform biologists of the most efficient combination of conventional methods and eDNA sampling moving forward. The second site type will be exploratory sites. Biologists will be able to visit areas at the edges of known species ranges, take samples, and determine if the species occurs further up the drainage. This will be particularly useful for rare species, such as northern and southern redbelly dace, common shiner, and suckermouth minnow.

## **RESULTS AND DISCUSSION:**

This project is just beginning. Sample collection and database building are currently scheduled for 2020.

## **ACKNOWLEDGEMENTS:**

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## **REFERENCES:**

- Colorado Parks and Wildlife (CPW). 2015. *State Wildlife Action Plan*. Colorado Parks and Wildlife, Denver, Colorado, 448 pp.
- Deiner, K., H. M. Bik, E. Machler, M. Seymour, A. Lacaousiere-Roussel, F. Altermatt, S. Creer, I. Bista, D. M. Lodge, N. deVere, M. E. Pfrender, and L. Bernatchez. 2017. Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Molecular Ecology* 26:5872-5895.
- Harrold, M. N. and R. P. Guralnick. 2010. A field guide to the freshwater mollusks of Colorado. Second edition. Colorado Division of Wildlife. Denver, Colorado. 126-pp.
- Mariac, C., Y. Vigouroux, F. Duponchelle, C. Garcia-Davila, J. Nunex, E. Desmarais, and J. F. Renno. 2018. Metabarcoding by capture using a single COI probe (MCSP) to identify and quantify fish species in ichthyoplankton swarms. *PLoS ONE* 13(9): e0202876. <https://doi.org/10.1371/journal.pone.0202976>
- Thomas, A. C., J. Howard, P. L. Nguyen, T. A. Seimon, and C. S. Goldberg. 2017. ANDe: A fully integrated environmental DNA sampling system. *Methods in Ecology and Evolution* 2018;9:1379-1385.

Williams, J. D., M. L. Warren, K. S. Cummings, J. L. Harris, and R. J. Reeves. 1993.  
Conservation status of freshwater mussels of the United States and Canada. *Fisheries*  
18:6-22.