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, 2020 to May 31, 2021

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

RESEARCH PRIORITY:

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

OBJECTIVES:

1) Assess effects of annual and seasonal flow variability on apparent survival, 2) quantify Flathead Chub movement at both large and small spatial scales and test mechanisms related to flow magnitude and timing, distance and direction between sites, and seasonal variability, and 3) provide gear and field protocol recommendations for future studies by quantifying detection probability of PIT tags using three gear types.

See 2020 Progress Report for Introduction and Methods.

RESULTS AND DISCUSSION:

Objective 1. Estimate monthly survival

Additional details regarding the effects of annual and seasonal flow variability on apparent survival were provided in the 2020 Progress Report. Multistate analysis resulted in seven models with ΔAIC_c values within two, which means parameter estimates needed to be model averaged. Overall mean monthly apparent survival rate was 0.75 (0.68–0.80), and varied seasonally, with the highest rate in winter (mean = 0.91; range 0.88–0.93), then summer (mean = 0.76; range 0.66–0.84), and then the transition seasons of spring and fall (mean = 0.63; range = 0.55–0.70).

Seasonal differences in apparent survival included positive betas for summer and winter (Figure 1; Table 1). An initial hypothesis was that summer would have lower survival than other seasons due to the stresses of spawning and high water temperatures. However, these negative effects seem to be countered by greater food availability during this season.

The positive beta for winter survival was also surprising (Figure 1; Table 1). The initial for this effect was that winter would be stressful due to low abundance of food. However, one potential explanation is that fish may be able to find a wintering area without many stressors (such as low flow pools without predators) and survive the winter. A management implication for the increased survival is that if these wintering areas can be found they should be protected.

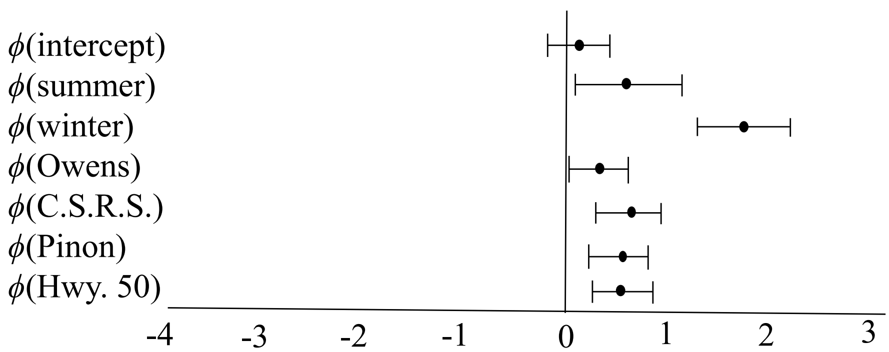

Figure 1. Beta estimates (95% confidence intervals), which indicate direction and magnitude of effects, for covariates affecting apparent survival (ϕ), from the top model of a multistate analysis in Program MARK. Note: C.S.R.S = Clear Springs Ranch South.

An interesting trend within each year was that apparent survival increased at sites farther downstream (Figure 2). A possible explanation for this is as Fountain Creek flows downstream, you would expect flows to be higher and more stable due to tributary inputs. These higher flow levels and potential to find refuge in the mainstem Arkansas River may explain these higher survival rates lower in the basin.

An important consideration is that these monthly survival rates are conservative, which means survival rates and lifespan of Flathead Chub is likely longer than these survival rates would indicate. This is due to the term “apparent” in apparent survival taking into account the fish being available to our gears. Although this was a very labor intense and long-term study, field activities only sampled 3% of the habitat spatially and <1% temporally during the course of the study, which makes the probability of encountering fish extremely low. Fish likely moved out of our study area into the Arkansas River, or moved between sites and were not available for capture. Therefore, these survival rates are a conservative minimum rate for this species, and they likely are longer lived than these rates indicate. However, for conservation purposes it is better to be conservative in life spans rather than over-estimating survival rates.

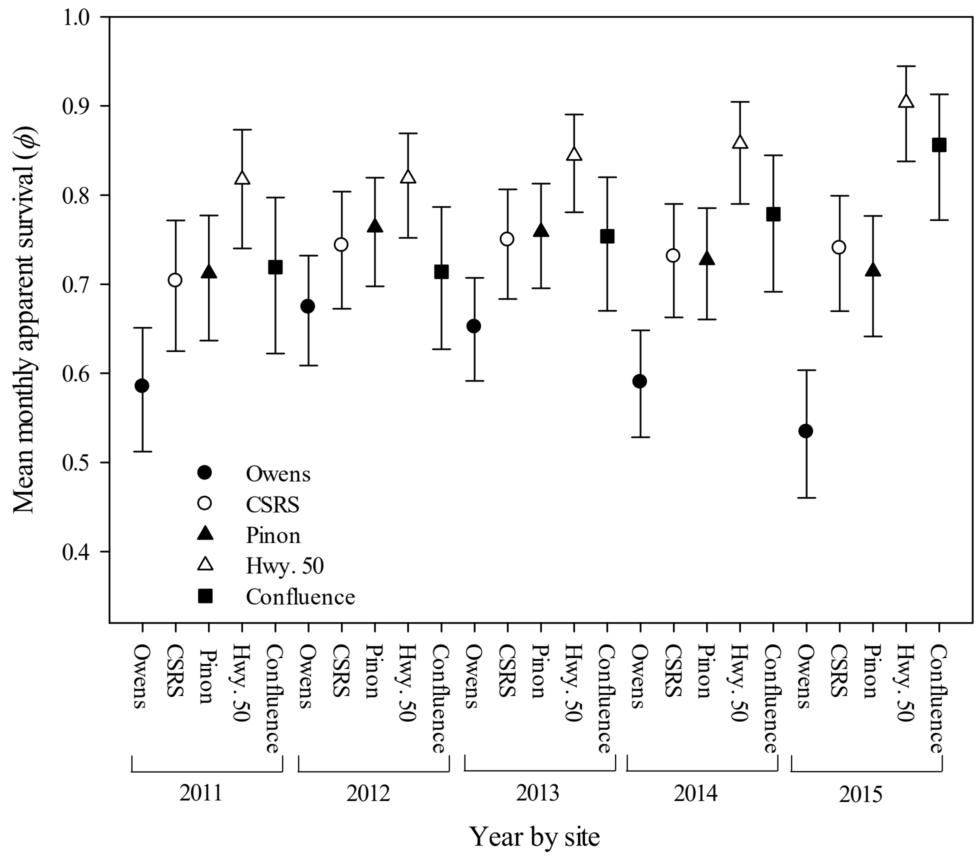

Figure 2. Mean monthly apparent survival (ϕ; standard error bars) by year and site from a closed multistate model in Program MARK. Sites are arranged from the most upstream (Owens) to the most downstream (Confluence). Note: CSRS = Clear Springs Ranch South.

Table 1. Results of a closed multistate analysis in Program MARK that includes the specific covariate based on ΔAIC_c value. Cumulative weights were calculated from all models having any weight. If a covariate was not included in any models that had weight, the estimates from the top model including that covariate are included. Beta directions were obtained from the top model that included the covariate. Note: * indicates that covariate was included in top model; MDF = mean daily flow (cms).

Parameter and covariate	Cumulative weight	Beta Direction	Expectation	Implications
Survival (ϕ)				
*Winter	0.99988	+	-	Not expected to be weighted so heavily.
*Summer	0.98513	+	-	Not expected due to spawning being a negative effect on survival
*Site	0.99984			Expected due to differences in habitat
Owens		+	-	Simple habitat did not hold fish
C.S.R.S.		+	+	Large root wad important habitat
Pinon		+	+	Willows root wads important habitat
Hwy. 50		+	-	Lower in basin so gaining flows may help survival
Confluence		Negligible	-	FHC likely moved out of site into Arkansas River
High flows (immediate effect)	0.10637	Negligible	-	High flows confounded with other effects
High flows (annual effect)	0.07418	Negligible	-	High flows confounded with other effects
High flows (delayed effect)	0.04913	Negligible	-	High flows confounded with other effects
Transition seasons (spring/fall)	0.00111	-	+	Transition periods are stressful on FHC
Fish length	0.00000	+	+	Larger FHC have higher survival rates.
Detection (p)				
*MDF	1.00000	-	-	Expected
*Summer	1.00000	+	+	Expected as FHC are most active in summer
Site	1.00000			Expected due to differences in habitat
Owens		-	+	Simple habitat did not hold fish outside of summer when trapped below barrier
C.S.R.S.		-	+	Had hole too deep to sample effectively
Pinon		Negligible	-	Willow
Hwy. 50		Negligible	-	Willow root wads concentrated fish
Confluence		Negligible	+	Expected moderate depth pool to concentrate FHC
Winter	1.00000	-	-	Expected as FHC move to wintering areas that are likely not our sampling sites
Fish length	1.00000	-	-	Expected because larger fish are more able to avoid gears.
Transition probability (ψ)				
*Distance	1.00000	-	-	Expected
*Direction	0.54705	Negligible	+	Detected slightly more upstream than downstream movements
*Summer	0.9971	Negligible	+	More movement in summer was expected
Winter	0.43109	Negligible	-	Negative beta in winter was expected
Transition (spring and fall)	0.43111	Negligible	+	Expected.
High flows (immediate effect)	0.11038	Negligible	+	Expected positive effect of high flows
Fish length	0.00000	Negligible	+	Expected as there is not a lot of variation in FHC length

Objective 2. Quantify movement through the study system

Large-scale Flathead Chub movement results were provided in the 2020 Progress Report. Regarding small spatial scale movements, temporary emigration (γ) rates derived in a robust design analysis were very high (γ''=0.81–0.87; γ'=0.87–0.95), indicating a high amount of Flathead Chub movement at the site-level spatial scale (Table 2). All sites had high flows in the top model, and all five betas were positive with confidence intervals that did not overlap zero (Figure 3). This indicates high flow events act as a trigger for Flathead Chub movement.

Fish total length was in the top model for four of the five sites (Figure 3). All four betas were positive, with three of the four confidence intervals not overlapping zero (Figure 3). This indicates that larger Flathead Chub are more likely to move than smaller Flathead Chub. A plausible explanation for this is that larger fish are more likely to move long distances to spawn or feed than smaller fish. Maintaining connectivity is an important to ensure Flathead Chub can take advantage of dispersed food habitats, find refuge habitats, and complete their life cycle.

An important implication of temporary emigration rates is inference regarding overall population health. For example, if sampling at a site typically results in about 500 Flathead Chub collected, then it may appear that those are the same approximately 500 fish that have not moved from the area. However, this study shows that the vast majority of those Flathead Chub are different individuals than those that were collected only a week or two prior. The proportion of Flathead Chub that are the same at a site was estimated to be only 14–18%, depending on the site. Most tagged fish were never seen again, which is likely due to them moving out of the site and moving at an intermediate spatial scale between sites, or longer distance movements out of the study area and into the mainstem Arkansas River. High temporary emigration rates is an indication of a healthy population in a larger spatial scale because of the continual influx of new individuals at a site.

Table 2. Temporary emigration rates from an observable state (i.e. fish was at the site at t-1; γ'') and temporary emigration from an unobservable state (i.e. fish was not at the site at t-1; γ') from the top model from each site of a robust analysis of PIT tagged Flathead Chub *Platygobio gracilis* at five sites on Fountain Creek, Colorado. Temporary emigration probabilities were coded as Markovian movement.

Site	# tagged fish	γ''	γ'
Owens	5,234	0.86 (0.83–0.89)	0.94 (0.91–0.96)
C.S.R.S.	3,704	0.81 (0.73–0.87)	0.94 (0.90–0.96)
Piñon	3,239	0.82 (0.75–0.84)	0.87 (0.82–0.90)
Hwy. 50	3,389	0.84 (0.80–0.87)	0.92 (0.89–0.94)
Confluence	2,305	0.87 (0.80–0.90)	0.95 (0.91–0.97)
Mean	3,574	0.84 (0.78–0.87)	0.92 (0.89–0.95)

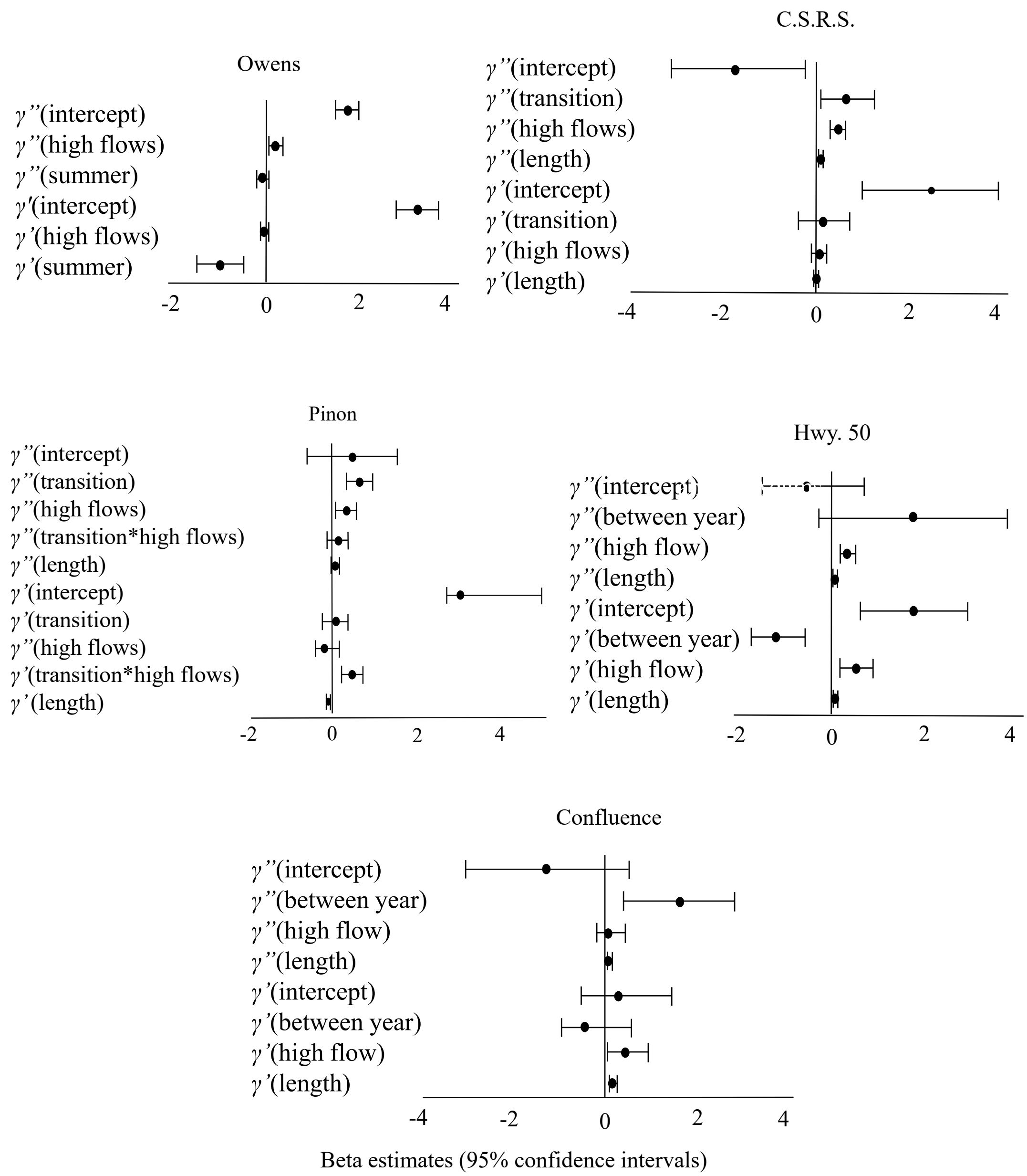

Figure 3. Beta estimates (95% confidence intervals), which display direction and magnitude of effects, for temporary emigration (γ) from the top model of robust design analyses for five sites. Note: C.S.R.S = Clear Springs Ranch South.

Objective 3. Provide gear and field protocol recommendations for future PIT tag studies.

Multistate modelling results provided inference on interesting site-specific interactions with seasons. For example, the overall beta for Owens was negative; however, the interaction with summer was strongly positive (Figure 4). This can be explained by fish moving back downstream and away from this site. Owens has the least complex habitat of all the sites, so when fish were not on their summer upstream spawning migration and trapped by the barrier, they completely evacuated this site. At times during summer sampling, there would be over 600 Flathead Chub collected in a relatively short amount of time. During winter, there were sampling events when no Flathead Chub were detected. This is shown in the Owens by winter interaction which is strongly negative and does not overlap zero. This overall trend in detection probability was corroborated by the robust design gear specific results as well. The 12-m array excelled at detecting fish trapped below the barrier at Owens. Robust design results showed the summer*12-m array beta as highly positive, while the winter*12-m array was highly negative (Figure 5).

There was a general trend of summer*site multistate interactions having a positive beta. One explanation for this is that during summer, fish are more active due to increased feeding and spawning. Therefore, they are more available to gears and increasing detection probabilities.

Robust design analyses resulted in a negative beta for mean daily flow (MDF), which was expected as the higher the flows, the more space fish have available to avoid gears. The negative beta for fish length indicates that smaller fish are easier to detect than larger fish. This is likely due to improved swimming performance of larger fish, making them more capable of avoiding gears. Also, smaller fish tended to be more closely associated with cover rather than fleeing. There was a general trend of negative betas in the winter, though the betas did not overlap zero in two out of five sites.

Large wood intersecting the main channel seemed to concentrate Flathead Chub. Unfortunately, this transient habitat was noted but not quantified. Specifically, future studies should quantify large wood to determine the effect that this habitat type has on detection probability.

The management implications these results is that detection probability changes by site and by season. Therefore, if using future studies using mobile arrays to detect PIT tags it is important to use multiple gear types. For example, the 12-m array that was floated through sites had a higher detection probability in the summer when fish were in the main river channel to spawn or to move upstream on the spawning migration. However, the 2-m array had a higher detection probability in winter when fish were concentrated in shoreline cover and avoiding main channel current.

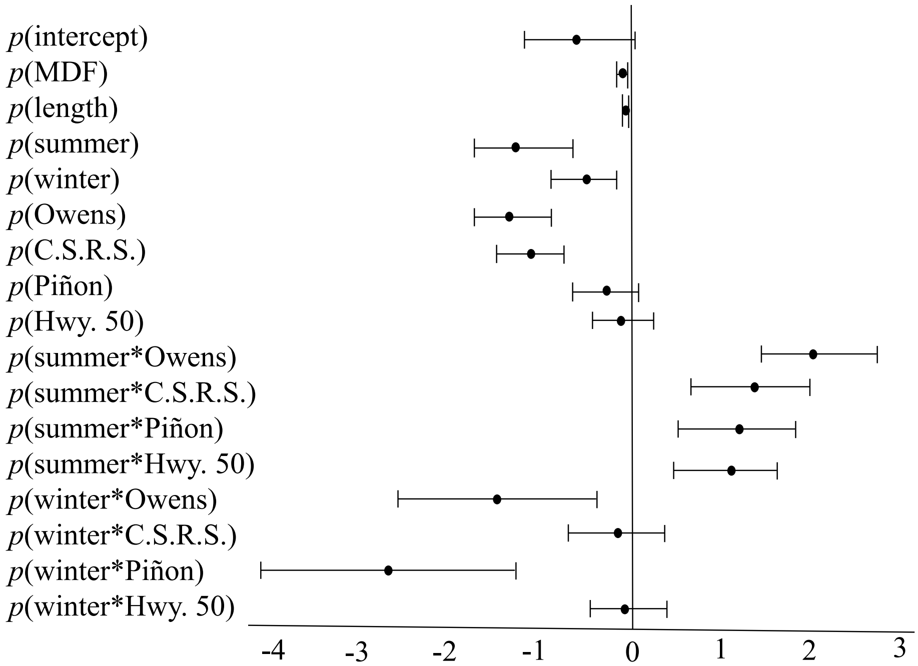

Figure 4. Multistate modeling results that provide beta estimates from the top model of for covariates affecting probability (*p*). Note: C.S.R.S = Clear Springs Ranch South.

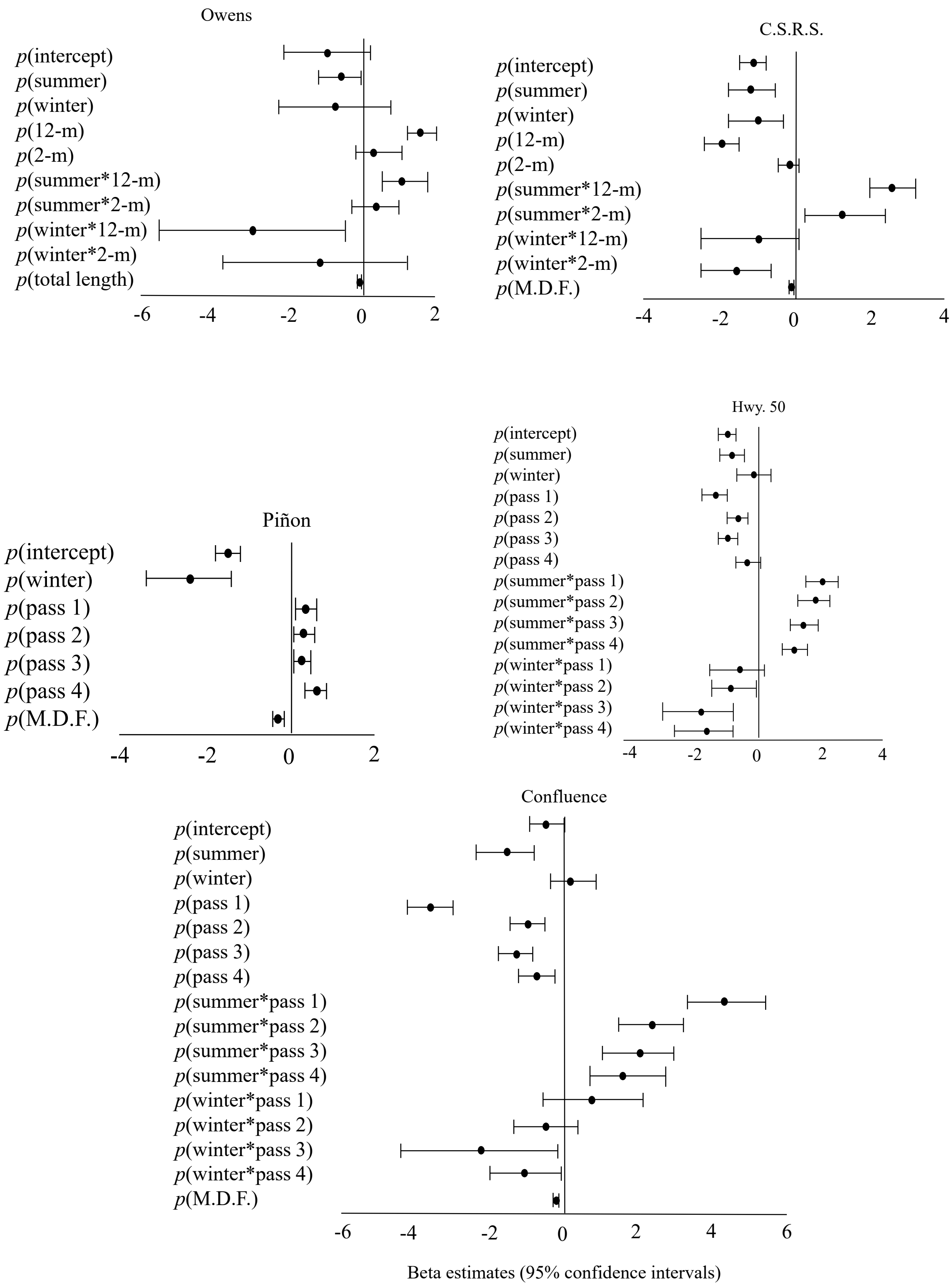

Figure 5. Robust design modeling results that provide beta estimates for detection probability (*p*; 95% confidence intervals) from the top model at each of the five sites. Note: C.S.R.S = Clear Springs Ranch South.

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.

RESEARCH PRIORITY:

Obtain quantifiable life history metrics for a Great Plains cyprinid.

OBJECTIVES:

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

See 2020 Progress Report for Introduction and Methods.

RESULTS AND DISCUSSION:

Age-0 fish were collected and their otoliths measured and averaged to provide the 50µm baseline. This validation provided a reference from which annuli beyond this point were counted to determine age (Figure 6).

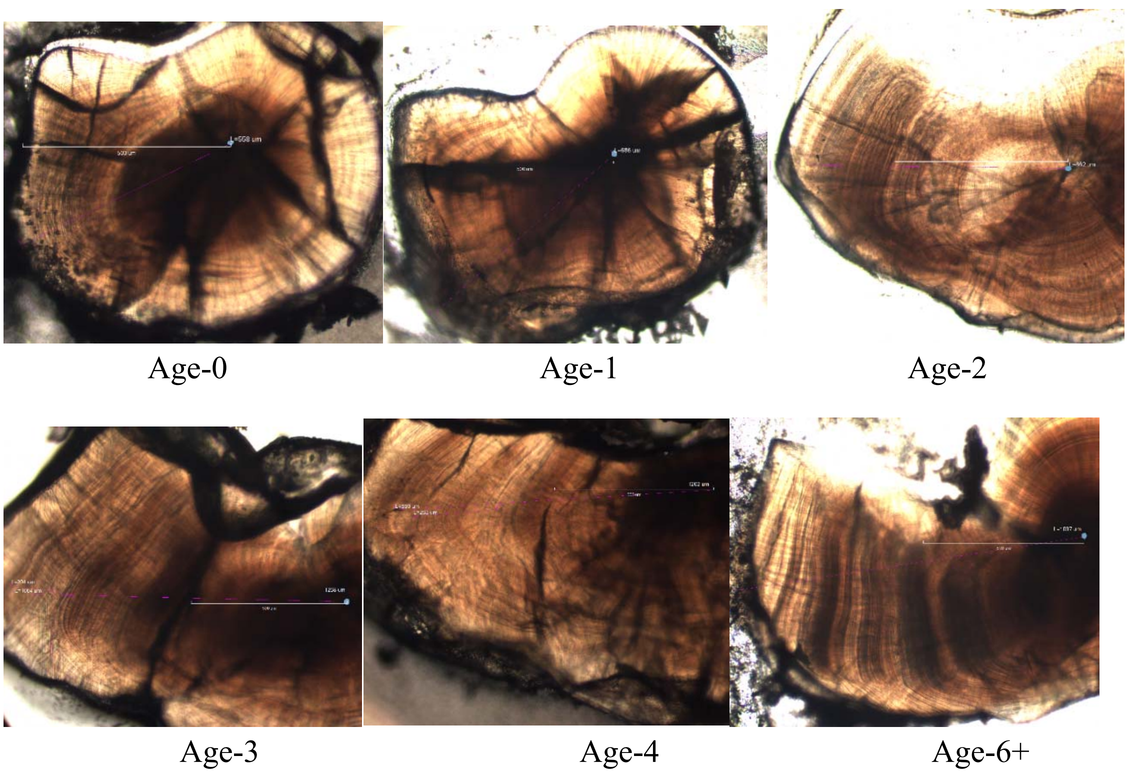

Figure 6. Otolith series showing differences in age of Flathead Chub, *Platygobio gracilis* collected from five locations in Fountain Creek, Colorado.

Fin rays were not a reliable method to age Flathead Chub. Most Flathead Chub were only aged to be two or three years old according to this method, even though there were fish that were PIT tagged, recaptured, and known to be older than these estimates. The estimates between right and left fin ray, as well as compared to otoliths, were not reliable and greatly underestimated the age of the fish. Although fin rays were not reliable in this study, they have been shown to be useful in aging other Great Plains fish taxa. For example, Sweet et al. (2009) successfully aged Bluehead Suckers (*Catostomus discobolus*) and Flannelmouth Suckers (*Catostomus latipinnis*) in the Upper Colorado River Basin, Wyoming. However, this species is much larger bodied than Flathead Chub. The larger body makes it easier to successfully cut into the core of the fin ray to obtain an accurate reading. I do not recommend fin ray ageing for other small-bodied Great Plains cyprinids.

Individual growth rates were obtained from 285 fish that were part of a mark-recapture study. The overall Flathead Chub monthly growth rate was 0.74 mm/month (range -7.14–7.71; n=576). There were seasonal differences in growth rates (Table 3). As expected, summer had the highest growth with 0.83 mm/day (range -5.17–4.39; n=31; Table 3). Fall had a very low rate at 0.22 mm/day (-7.14–4.05; n=46). The winter growth rate was negative, which is likely a result of measurement error. These are very small fish that grow slowly, and by multiplying the daily growth rate by 30, any small errors in measurement were multiplied when calculating the monthly rate.

Table 3. Flathead Chub seasonal growth rates (mm/month). The monthly rates were taken by calculating the daily growth rate, then multiplying by 30. To reduce measuring error bias, only fish collected more than four weeks were included in this analysis. No spring recaptures met this criteria.

	Average	Minimum	Maximum	n
Summer	0.83	-5.17	4.39	31
Fall	0.22	-7.14	4.05	46
Winter	-0.48	-2.50	0.66	28

Growth rates were very low for adult Flathead Chub. Larger fish had lower growth rates than smaller fish, in particular indicated by large fish that had negative growth rates (Figure 7). The winter growth curve was very flat and concentrate near the zero growth line (Figure 7). Flathead Chub in Fountain Creek are slower growing and older than previously thought.

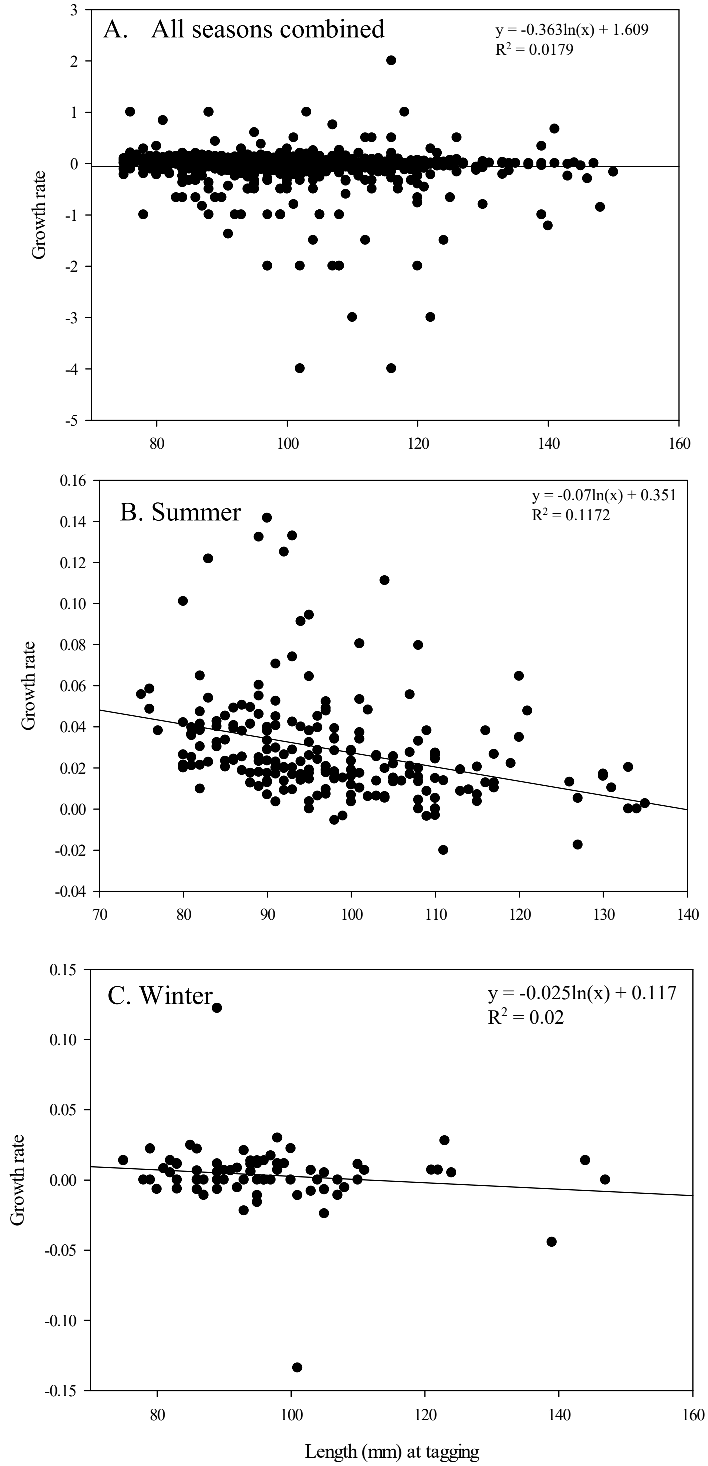

Figure 7. Flathead Chub growth rate (mm/day) at length for (A) all seasons combined, (B) summer, and (C) winter.

ACKNOWLEDGEMENTS:

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

REFERENCES:

Sweet, D. E., R. I. Compton, and W. A. Hubert. 2009. Age and growth of bluehead suckers and flannelmouth suckers in headwater tributaries, Wyoming. Western North American Naturalist 69:35–41.

RESEARCH PRIORITY:

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

OBJECTIVES:

Determine the amount and timing of native fish movement through the Owens-Hall fish passage structure (Figure 8).

See 2020 Progress Report for Introduction and Methods.

Figure 8. Fish passage structure on the Owens-Hall Diversion, Fountain Creek, Colorado. Note the technician at bottom of the passage structure for scale.

RESULTS AND DISCUSSION:

Evaluation of the fish passage structure is ongoing. During 2020, weekly sampling efforts resulted in a total of 548 fish from seven species were implanted with PIT tags (Table 4). The vast majority of these (n=494) were Flathead Chub. To date, four species of plains fish have been documented successfully passing the structure (Table 4). This is encouraging as one species, the Creek Chub, only had one individual tagged, but it successfully navigated the fish passage structure.

Table 4. Summary of the number of fish PIT tagged per species below the Owens Hall fish passage structure on Fountain Creek, Colorado.

Species	Number tagged	Number detected in structure	Successful passage if detected in passage structure
Flathead Chub, *Platygobio gracilis*	494	15	90%
Creek Chub, *Semotilus atromaculatus*	1	1	100%
Stoneroller, *Campostoma anomalum*	10	1	100%
White Sucker, *Catostomus comersonii*	35	1	100%
Longnose Sucker, *Catostomus catostomus*	5	0	-
Sand Shiner, *Notropis stramineus*	1	0	-
Fathead Minnow, *Pimepheles promelas*	2	0	-
	548	18	95%

The top model from a Cormac Jolly Seber analysis indicates differences in passage based on arrays, but little difference in detection probability among arrays (Table 5). The differences in passage is because a released fish needs to find the entrance to the fish passage structure. Therefore, you would expect that passage success to be low. However, once fish are in the structure, there were high rates of successfully navigating it. Specifically, there is a 3% probability of a released fish encountering the first array, but once in the structure, there is a high probability that the fish will successfully pass it (transition probability from array one to array three was 82%).

Table 5. Models with weight from a Cormac Jolly Seber analysis examining probability of entering and successfully passing the fish passage structure. Covariates for both passage and detection were the three arrays as well as fish length as an individual covariate.

Model	AICc	ΔAICc	AICc weight	Model Likelihood	# Parms.	-2Log(L)
ϕ(array + length) p(.)	261.4710	0.0000	0.3752	1.0000	5	251.4010
ϕ(array + length) p(length)	262.6701	1.1991	0.2060	0.5490	6	250.5720
ϕ(array + length) p(array)	263.3189	1.8479	0.1489	0.3970	7	249.1881
ϕ(array) p(.)	263.9791	2.5081	0.1071	0.2854	4	255.9325
ϕ(array + length) p(array + length)	265.1700	3.6990	0.0590	0.1573	8	249.0015
ϕ(array) p(length)	265.7579	4.2869	0.0440	0.1173	5	255.6880
ϕ(array) p(array)	265.8116	4.3406	0.0428	0.1141	6	253.7136
ϕ(array) p(array + length)	267.6579	6.1869	0.0170	0.0453	7	253.5270

One interesting initial result is that most movement in the passage structure occurred at night. There was concern with this design over avian predation, but this form of predation should be minimized with most movements occurring in the dark.

The Owens Fish Passage Structure was designed to be a relatively inexpensive structure that can be deployed quickly throughout the Front Range. It appears to be successful in allowing fish passage of multiple species, and should be viewed as a viable option especially when there is a large vertical drop and not a lot of horizontal distance to install other structures (such as a rock ramp).

ACKNOWLEDGEMENTS:

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RESEARCH PRIORITY:

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

OBJECTIVES:

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

See 2020 Progress Report for Introduction and Methods.

RESULTS AND DISCUSSION:

Experiments have concluded and data analysis is ongoing. Preliminary results of this study include the 60-day duration having the highest egg predation among treatments. Egg and larval production were similar among treatments (Figure 9). However, the timing of spawning initiation was significantly different among treatments where all three of the 12°C treatments began spawning earlier in the winter before the three 4°C treatments. These results will be compiled in a thesis that will be incorporated in the next reporting cycle.

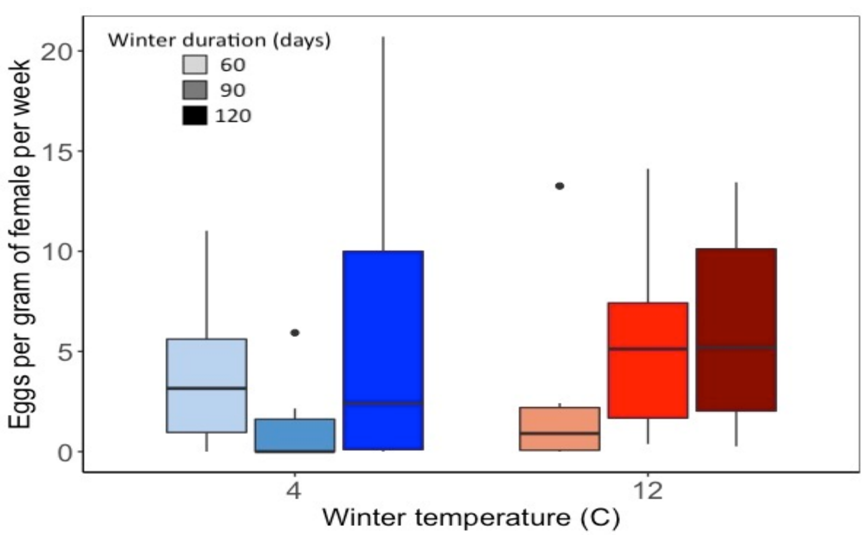

Figure 9. Egg per gram of Johnny Darter female per week produced per tank at two winter temperatures (4°C and 12°C) and three durations (60, 90, and 120 days). The color

refers to the winter temperature treatment and the shade of the color refers to the winter duration treatment.

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

RESEARCH PRIORITY:

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

OBJECTIVES:

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

INTRODUCTION:

Long-term trends of increasing water temperatures have been observed in many streams and rivers on a global scale, particularly in urban environments. While stream temperatures are correlated with air temperatures and have increased due to climate change, there is evidence that urbanization can exacerbate these increases. Particular causes of stream warming due to urbanization include decreased shading due to deforestation, runoff from impervious surfaces, warm water releases from water storage and diversions, and discharges of warm wastewater treatment plant (WWTP) effluent. WWTP effluent temperatures are increased by rapid residential and commercial energy consumption, and tend to have the greatest influence on stream temperature in winter months (Graham et al. 2014). Additionally, long-term increasing trends in urban WWTP effluent temperature have been shown (Knouchi et al. 2007) and will likely continue as worldwide projections demonstrate substantial increases in urbanization and urban population growth by 2050. Impacts of WWTP effluent on stream temperature and its potential to impact stream ecology deem further investigation to mitigate long term deterioration of native biota and stream function.

The South Platte River Basin along the Colorado Front Range supported 68% of the state's total population in 2002, with its growth and water consumption expected to continue to rise. Here, WWTP effluent comprises 69% of the annual streamflow, making up a majority of the downstream South Platte River section for two thirds of the year and can increase temperatures downstream of WWTPs by over 10ºC for up to 27 km (Lewis and McCutchan 2012). The current state standard for winter weekly average maximum effluent temperature is 12ºC for Warm Stream Tier I (WS-I) waters, classified by presence of Johnny Darter. Despite this standard allowing WWTP effluent temperatures to be 7ºC greater than normal instream winter temperatures, some South Platte WWTPs still exceed it. Understanding how effluent-impacted flows influence stream temperature, particularly in winter months when native fishes would be forced to endure unnaturally warm environments, is crucial towards successful population management solutions in urbanized environments.

Johnny Darter (*Etheostoma nigrum*) are a native percid in the South Platte River basin and one of the most common darters in eastern Canada and the Midwestern United States. In the South Platte River, Johnny Darters appear to spawn later in the year compared to those in its central range, suggesting this species is reliant on temperature for reproductive development and initiation of spawning They also have unique reproductive requirements and timing in relation to other South Platte native fishes (Propst 1982). Recent studies have shown that familial Yellow Perch (*Perca flavescens*) produced smaller eggs and reduced hatching success after exposer to shorter warmer winters believed to be the cause of subsequent low recruitment (Farmer et al. 2015). Also, Firkus et al. (2018) found that in the laboratory Johnny Darter exposed to elevated overwintering temperatures resulted in early out of season spawning, which additionally had the effect of lowering overall fecundity. Because of its potential sensitivity to unseasonably high winter stream temperatures, Johnny Darter presence is used by Colorado Department of Public Health and the Environment (CDPHE) and Colorado Parks and Wildlife (CPW) to establish and update thermal regulations for streams classified as WS-I. This, along with its widespread native range, is why we chose it as our focal species to study influences of increased winter temperatures from WWTP effluent on fish reproductive development.

METHODS:

Johnny Darter will be collected in reference reaches representing normal winter thermal regimes within the South Platte drainage, and their reproductive condition will be compared to those collected both upstream and downstream of thermal stream altering WWTPs. Reproductive condition will be determined through histological analysis of gonadal tissue. Histological analysis can determine the current state of gonadal development and used to assess the fish's reproductive developmental progress (Figures 10 and 11). Water quality metrics including temperature will be collected at each site, compared, and correlated with histological analyses. These analyses will allow for the determination of any differences in reproductive development due to differences in stream temperature and guide Colorado thermal water quality regulations.

RESULTS AND DISCUSSION:

From March to October 2020, exploratory collections were made on three streams (Big Thompson River, St. Vrain Creek, and the Cache la Poudre River) for a total of 50 sampling occasions. A total of 294 Johnny Darter were collected, of which 172 were female. Temperature loggers were deployed at each site. Histological analyses of gonads is ongoing (Figure 11).

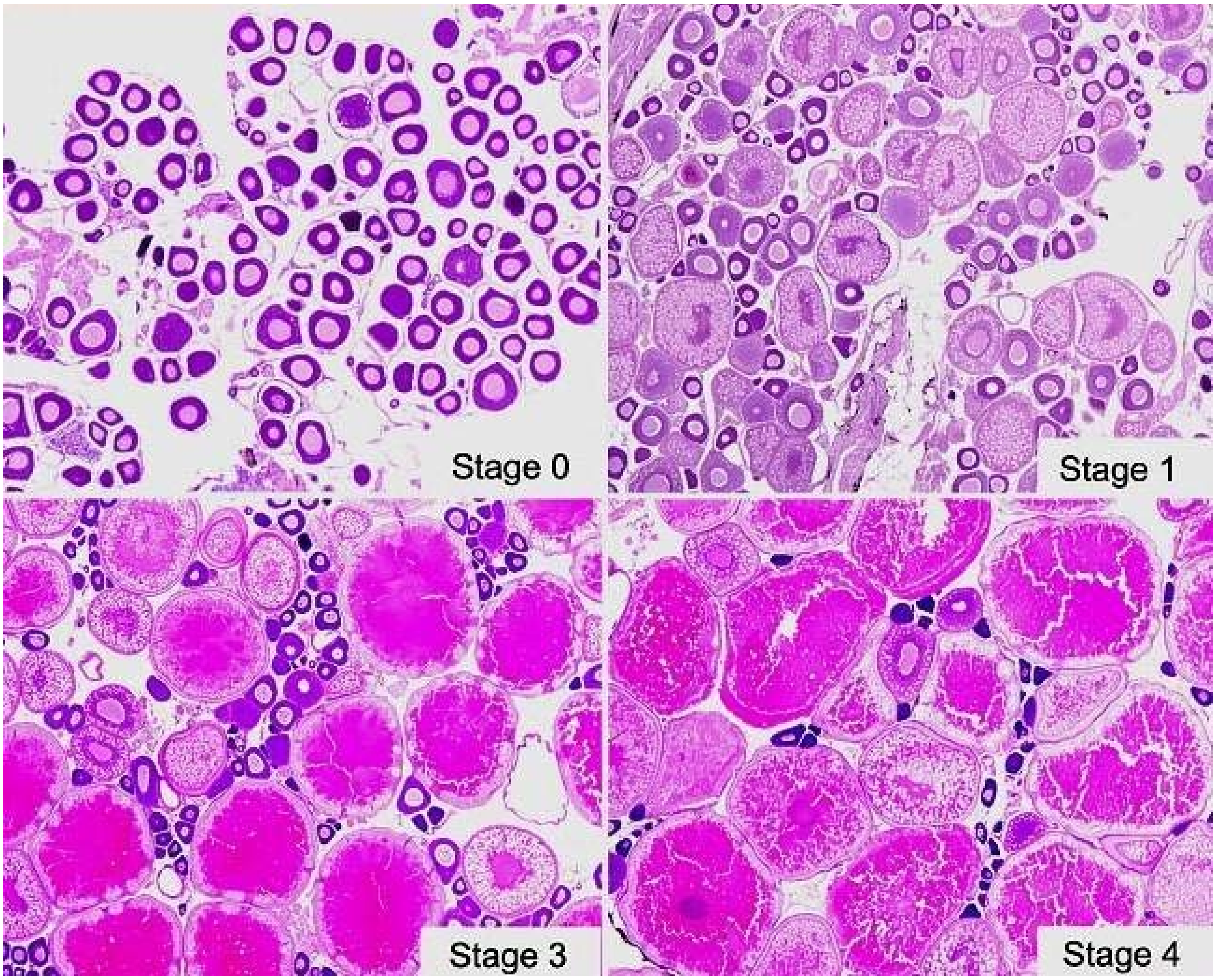

Figure 10. Examples of the staging system described by Johnson et al. 2009 applied to the ovaries of Fathead Minnow *Pimephales pimales* (adapted from Johnson et al. 2009).

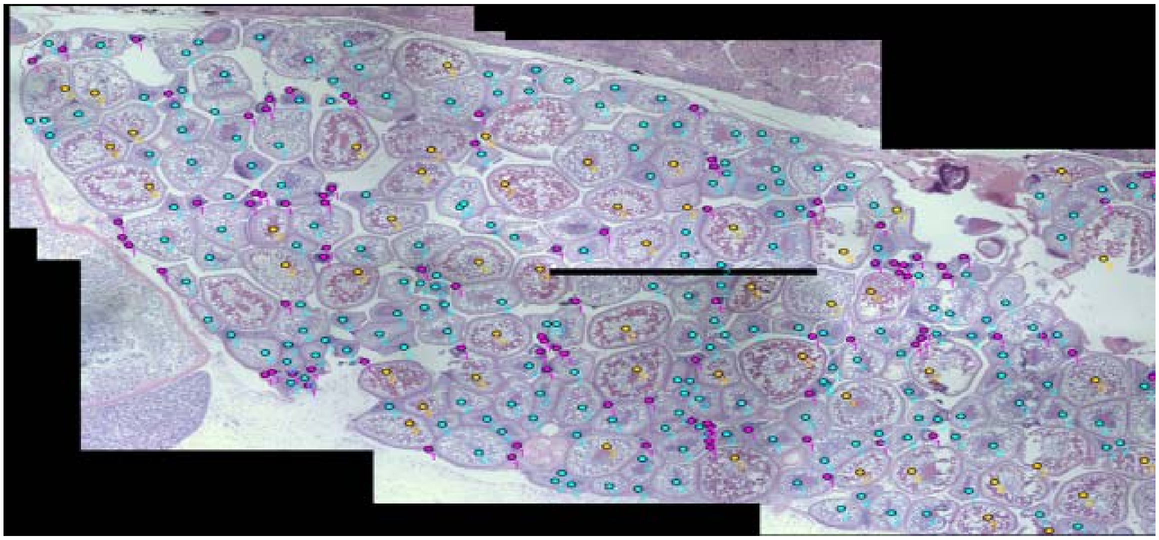

Figure 11. Johnny Darter ovary, with dots indicating eggs at various stages of development.

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REFERENCES:

Farmer, T. M., E. A. Marschall, K. Dabrowksi, and S. A. Ludsin. 2015. Short winters threaten temperate fish populations. Nature Communications 6:7724. DOI:10.1038/ncomms8724.

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.

Graham, J.L., Stone, M.L., Rasmussen, T.J., Foster, G.M., Poulton, B.C., Paxson, C.R., and Harris, T.D., 2014, Effects of wastewater effluent discharge and treatment facility upgrades on environmental and biological conditions of Indian Creek, Johnson County, Kansas, June 2004 through June 2013: U.S. Geological Survey Scientific Investigations Report 2014–5187, 78 p., http://dx.doi.org/10.3133/sir20145187.

Johnson, R., J. Wolf, and T. Braunbeck. 2009. OECD Guidance document for the diagnosis of endocrine-related histopathology of fish gonads. Organization for Economic Co-operation and Development.

Kinouchi, T., H. Yagi, and M. Miyamoto. 2007. Increase in stream temperature related to anthropogenic heat input from urban wastewater. Journal of Hydrology. 335:78-88.

Lewis, W. M., and J. H. McCutchan. 2012. Regulatory temperature compliance for the South Platte River downstream of the Metro District R. W. Hite Treatment Facility (RWHTF). Metro Wastewater Reclamation District Report 326.

Propst, D. L. 1982. Warm water fishes of the Platte River Basin, Colorado; distribution, ecology, and community dynamics. Doctoral dissertation. Colorado State University, Fort Collins, Colorado.

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 2020 Progress Report for Introduction and Methods. This project is scheduled to be an ongoing, annual site selection tool.

RESULTS AND DISCUSSION:

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

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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. Colorado Parks and Wildlife. Fort Collins, Colorado.

RESEARCH PRIORITY:

Incorporating environmental DNA metabarcoding into the plains fish monitoring protocol.

OBJECTIVES:

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

See 2020 Progress Report for Introduction and Methods.

RESULTS AND DISCUSSION:

Initial fish tissue sample collection took place in 2020 and will continue in 2021. A total of 171 samples from 36 species had tissues collected (Table 6). Of these, 26 are native and 10 are nonnative. The only east slope native plains fish species that we still need to collect is the Lake Chub, *Couesius plumbeus* which we plan to collect soon. High priority specimen collection included species that are listed as special status by the State of Colorado. These included four Species of Concern, three Threatened species, and four Endangered species (Table 6). We are currently working with other state agencies to obtain tissue samples of additional invasive fish species that have not been documented in Colorado.

A contract has been established with the USDA APHIS National Wildlife Research Center to conduct genetic analyses. DNA extraction of tissues has begun and sequencing will commence soon. Preliminary field analysis of the ANDe collection system will occur during 2021.

ACKNOWLEDGEMENTS:

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Table 6. Tissue samples that have been collected for the plains fish eDNA study. Note: SOC refers to Species of Concern.

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