

Range-wide habitat assessment of Loach minnow (*Tiaroga cobitis*) and Spikedace (*Meda fulgida*)

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Final Report

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INTRODUCTION

We conducted a range-wide, multi-scale habitat assessment of loach minnow (*Tiaroga cobitis*) and spikedace (*Meda fulgida*) to inform management decisions regarding the habitat requirements of these species and potential opportunities to repatriate extirpated populations. Field surveys were designed to quantify associated fish communities and habitats of these species at spatial scales from microhabitats to watersheds. In addition, we quantified the temporal dynamics of abundance and habitat use at our sample sites across two years. Sample sites include locations where these species have been extirpated and repatriated to test the role of habitat and fish community structure in the sustainability of populations in those areas. Quantitative estimates of population abundances were coordinated with a range-wide genetic analysis through the University of New Mexico to identify associations between habitat, population size and metrics of population genetic structure. Finally, a modification to this agreement added research to test the efficacy of using PIT tagging to track the survival of stocked fishes relative to PIT tagged wild fish in selected locations.

Specific objectives

- 1) Characterize and compare habitat available and entire fish community structure at ~ 20 sites that have either contemporary populations of loach minnow and spikedace or historically maintained populations of these species.
- 2) Quantify habitat for loach minnow and spikedace across multiple spatial and temporal scales.
- 3) Characterize relationships between genetic population structure determined in a companion study and habitat attributes across the currently occupied range of each species.
- 4) Validate the efficacy of implanting loach minnow and spikedace with 8mm PIT tags using hatchery fish.
- 5) Characterizing Dispersal and Survival of Stocked Loach Minnow and Spikedace

Results

Objectives 1&2: Characterize and compare habitat available and entire fish community structure at ~ 20 sites that have either contemporary populations of loach minnow and spikedace or historically maintained populations of these species; Quantify habitat for loach minnow and spikedace across multiple spatial and temporal scales

Thirty-one stream locations were visited (Table 1) and a synthesis of our findings can be found in a MS thesis by Crosby Hedden that was completed in fall 2020. See this link do access that thesis: <https://krex.k-state.edu/dspace/handle/2097/40946>. In short, this habitat assessment was conducted across multiple spatial scales, 1-km, 0.1-km, and the mesohabitat scale, to identify at what scale measured habitat variables most strongly predicted the presence of each species. We developed models to predict the presence of both of these species. At our two broadest scales, average velocity was the variable that best predicted the presence or absence of both species. At the finest scale, loach minnow associated with very specific habitats while spikedace occurred across a range of habitats. Data were used to rank streams based on habitat suitability and suggested that future habitat assessments should be conducted at larger spatial scales to evaluate if a location should be repatriated.

Table 1. Number of 1-km reaches that meet habitat requirements for loach minnow and spikedace. Areas where spikedace currently occur are denoted with the superscript SD, while areas where loach minnow currently occur are denoted with the superscript LM.

Location	Meets Loach Minnow Requirements	Meets Spikedace Requirements	Key in Reference to Site Map
Gila River - Bird Area ^{LM,SD}	Yes	Yes	1
Gila River – Grapevine ^{LM,SD}	Yes	Yes	2
Gila River - Middle Box ^{LM}	Yes	No	3
Gila River - Riverside	Yes	Yes	4
Gila River West Fork ^{LM,SD}	No	No	5
Gila River Middle Fork	Yes	No	6
Little Creek	No	No	7
Black River	No	No	8
Bear Creek ^{LM}	No	No	9
Blue River	No	No	10
Campbell Blue River ^{LM}	No	No	11
Eagle Creek ^{SD,}	Yes	Yes	12
Lower San Francisco River	Yes	Yes	13
Lower Tularosa River	Yes	No	14
San Francisco River - Glenwood ^{LM,SD}	Yes	Yes	15
Saliz Canyon	No	No	16
Tularosa River - Hell's Hole ^{LM}	Yes	No	17
Upper Blue River ^{LM}	Yes	No	18
Upper San Francisco River	No	No	19
Wet Beaver Creek	No	No	20
Bonita Creek	No	No	21
Fossil Creek ^{SD}	Yes	Yes	22
Oak Creek	No	No	23
Spring Creek ^{SD}	No	No	24
Sycamore Creek	No	No	25
Upper Verde River	No	No	26
West Fork Clear Creek	No	No	27
Aravaipa Creek ^{LM,SD}	Yes	Yes	28
Lower Aravaipa Creek ^{LM,SD}	Yes	Yes	29
Redfield Canyon	No	No	30

Objective 3: Characterize relationships between genetic population structure determined in a companion study and habitat attributes across the currently occupied range of each species

Habitat use data from our field surveys are available in a large database upon request. These data include depth, velocity and substrate associated with occurrences of native and nonnative species. Once completed, we can compare habitat use across populations that are genetically distinct.

Objective 4: Validate the efficacy of implanting loach minnow and spikedeace with 8mm PIT tags using hatchery fish.

Work for this objective has been completed. We completed a loach minnow and spikedeace survival study in which 50 individuals of each species were tagged with an 8-mm PIT tag on February 21, 2020. We observed high survival for both species (80% survival for loach minnow and 92% survival for spikedeace), which was comparable to typical survival levels for non-PIT tagged fish at Aquatic Research Conservation Center (ARCC). The completion of this study aided decisions to allow further PIT tagging of hatchery fish and allowing the use of PIT tags in wild fish in order to better understand and evaluate the successes and failures in repatriation across the basin. An interesting follow up observation from the staff at the ARCC, was the PIT tagged loach minnow spawned successfully, further indicating a limited impact of PIT tagging on these species.

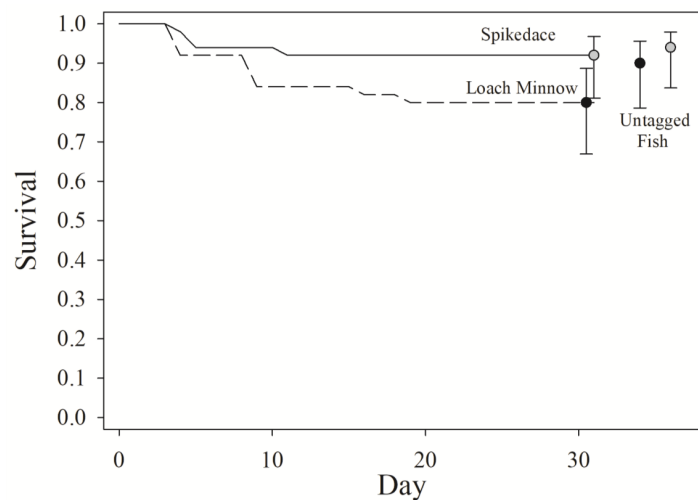


Figure 1. Survival of Spikedace and Loach Minnow PIT tagged at the Aquatic Research and Conservation Center, Cornville, AZ.

Objective 5: Characterizing Dispersal and Survival of Stocked Loach Minnow and Spikedace

We have conducted five survival studies associated with this project; West Fork of Gila River (2020 and 2021), Hot Springs (2020), Spring Creek (2020, 2021). A complete draft manuscript is available that describes the background, methods, results and findings from this study (see attached pdf file: Hedden_etal_draft_manuscript21dec2022). In summary, we quantified survival, emigration, movement directionality from stocking locations, and long term (24 months) trends in apparent survival of passive integrated transponder (PIT) tagged hatchery released fish and wild caught and tagged fish from three desert streams in Arizona and New Mexico. We PIT tagged and tracked 1,450 individuals from 2020 to 2022 and found that hatchery fish had high (>90%) estimates of survival that was similar to tagged wild fish. Hatchery fish had a five-fold higher emigration rate than wild fish shortly (<40 days) after being released. Emigrating hatchery fish also tended to move downstream, whereas wild fish were more likely to move upstream. Additionally, apparent survival estimates were ten times higher

for tagged wild fish than hatchery fish one year after release, where hatchery fish apparent survival neared zero after about 265 days after release but did not approach zero for wild fish until 625 days. Our results suggest emigration, rather than lowered survival might limit the success of stocking efforts. Thus, choosing appropriate stocking locations, such as upstream reaches, might improve retention of stocked fish. We suggest future work should examine the distances hatchery fish emigrate after they are released, explore different training and release strategies for hatchery-reared fish, and attempt to quantify hatchery fish reproductive output following translocations.

Survival and Movement of Imperiled Wild and Hatchery Fishes in Desert Streams

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Abstract

Monitoring vital population rates is needed to assess management actions such as stocking and translocation of rare and endangered fishes. Stocking streams with hatchery produced fishes has become necessary to restore native populations but these efforts might fail due to low survival or high emigration rates. We quantified survival, emigration, movement directionality from stocking locations, and long term (24 months) trends in apparent survival of passive integrated transponder (PIT) tagged hatchery released fish and wild caught and tagged fish from three desert streams in Arizona and New Mexico. We PIT tagged and tracked 1,450 individuals from 2020 to 2022 and found that hatchery fish had high (>90%) estimates of survival that was similar to tagged wild fish. Hatchery fish had a five-fold higher emigration rate than wild fish shortly (<40 days) after being released. Emigrating hatchery fish also tended to move downstream, whereas wild fish were more likely to move upstream. Additionally, apparent survival estimates were ten times higher for tagged wild fish than hatchery fish one year after release, where hatchery fish apparent survival neared zero after about 265 days after release but did not approach zero for wild fish until 625 days. Our results suggest emigration, rather than lowered survival might limit the success of stocking efforts. Thus, choosing appropriate stocking locations, such as upstream reaches, might improve retention of stocked fish. We suggest future work should examine the distances hatchery fish emigrate after they are released, explore different training and release strategies for hatchery-reared fish, and attempt to quantify hatchery fish reproductive output following translocations.

Introduction

Quantifying a population's survival rate is one of the most critical parameters to efficiently manage and conserve species, as it dictates current and future population sizes (Lebreton et al. 1992; Okamoto et al. 2016). Furthermore, survival of an organism is also linked other important parameters (i.e. growth, reproduction) and can be useful in understanding individual or population performance and fitness (Minto et al. 2008). Despite the critical importance of knowing how many individuals survive within a given season or year, reliable survival rates are unknown for many fish populations (Todd et al. 2017; Macaulay et al. 2021). This discrepancy is due to the difficulty in tracking individual fish through time because capture and recapture rates are low, detection of tagged individuals is not continuous, and constraints on our ability to track small (<100 mm) individuals (Lucas and Baras 2000; Pennock et al. 2018; Hedden and Gido 2020).

Quantification of population survival rates can examine the effectiveness of stocking hatchery-reared fishes (Miller 1954; Tennant et al. 2022). Hatchery-reared fish can differ in their behavior and survival when compared to wild-spawned fish, but often these results are variable among species and systems making it difficult to infer results to rare or understudied organisms (Araki and Schmid 2010). One common technique used to examine the success of stocking

hatchery-reared fish is to conduct post-stocking surveys to evaluate the establishment of the species. For example, the Gila River basin located in the southwestern U.S., has experienced severe native species declines, driven by numerous environmental, community, and stream alterations (Olden and Poff 2005). Hatchery-reared native fish have been stocked in numerous locations by state and federal agencies and their success determined by subsequent surveys to identify if the species has established (DFT 2006). But often, sampling streams in years following the stocking of thousands of hatchery fish results in small population estimates. In two Arizona streams, repeatedly stocking (>500 fish per year) of federally endangered Spikedace (*Meda fulgida*) and Loach Minnow (*Tiaroga cobitis*) has only resulted in modest population sizes (3-11 individuals per 100 m; Figure 1). In contrast, populations in nearby streams have shown positive population growth and expansion of translocated fish (Hickerson et al. 2021). Unfortunately, post-stocking surveys that quantify success cannot discern if high mortality rates or high emigration rates are leading to low population estimates.

Understanding if the stocking success at a location is driven by post stocking mortality or emigration can help refine stocking protocols for rare and endangered fish (Pennock et al. 2020). For example, alternative locations within the watershed, different release strategies such as soft releases, flow training in the hatchery, or altering the season or location of stocking might increase survival and retention of stocked fish (Brown and Day 2002; Franssen et al. 2021; Hickerson et al. 2022). Additionally, stocking of native fish in formerly occupied habitats often occurs upstream of natural or constructed fish barriers that prevent the establishment of nonnative fish (Fausch et al. 2006; Hickerson 2021). If stocked fish emigrate below those barriers they are not likely to persist due to negative interactions with nonnatives and will not contribute to the stream reach targeted for conservation.

Many mark-recapture studies do not distinguish between survival and movement, and only examine apparent survival (Leberon et al. 1992). Apparent survival is the probability an individual is in the population at a certain time, but true mortality and permanent emigration are confounded (Gilroy et al. 2012). Although apparent survival estimates are valuable for some ecological questions, the inability to separate survival from emigration can be problematic, especially when examining populations across broad spatial boundaries. But with the combination of well-designed studies and new technological improvements to marking and tracking fish of all sizes (i.e., PIT tags and submersible antennas), studies can now estimate both survival and emigration, answering questions regarding the success and limitations of stocking hatchery fish.

The objective of our study was to examine the post stocking survival and emigration of two hatchery-reared, federally endangered fish species, Spikedace *Meda fulgida* and Loach Minnow *Tiaroga cobitis*, and compare these estimates to their wild counterparts. We predicted that hatchery fish would have higher emigration rates, largely in a downstream direction due to their rearing in low flow raceways, that high flow events would exacerbate emigration rates, but survival rates would be similar to tagged wild fish given that hatchery fish have been seen to persist in low numbers following previous translocations into these streams (Figure 1). To test these predictions, we obtained estimates of survival, emigration, movement directionality from stocking locations, downstream movements over fish barriers, and long term (24 months) trends in apparent survival of hatchery and wild PIT tagged fish from three desert streams.

Methods

Study Area

The Gila River basin, which flows west across the southwestern portion of New Mexico and across the lower two thirds of Arizona, encompasses a broad range of elevation, temperature, and vegetative landscapes. Three streams were examined to assess native fish survival and movement and were located throughout the basin. The West Fork of the Gila River in New Mexico has an average stream width of about 8 m, an elevation around 1700 m above sea level, and is located on New Mexico Game and Fish property in the Gila National Forest. Hot Springs is a small tributary to the San Pedro River in southeast Arizona with an average stream width of about 4 m, an elevation around 1200 m above sea level, and is located on The Nature Conservancy property in the eastern part of the Sonoran Desert. Spring Creek is small tributary to Oak Creek, which joins the Verde River in central Arizona with an average stream width of about 2 m, an elevation around 1000 m above sea level, and is located on the Coconino National Forest (Figure 2). These three streams were chosen because they had few, if any, nonnative fish species present, robust native fish communities, and contained or are supplemented with stocked Loach Minnow and/or Spikedace (Propst et al. 2015; Gido et al. 2019; Robinson et al. 2019). Other native fish species present at these sites included wild populations of Longfin Dace (*Agosia chrysogaster*), Roundtail Chub (*Gila robusta*), Desert Sucker (*Catostomus clarkii*), Sonora Sucker (*Catostomus insignis*), and Speckled Dace (*Rhinichthys osculus*). Hot Springs was stocked with Loach Minnow and Spikedace from 2007-2011 and Spring Creek was stocked with Spikedace from 2015-2018; both of these streams have a downstream nonnative fish barrier that prevents upstream movement of fishes. The West Fork Gila River does not have a downstream nonnative fish barrier and contains wild populations of Loach Minnow and Spikedace as well as the native fishes listed above.

Fish Tagging and Tracking

Prior to field tagging we tagged 50 Spikedace and 50 Loach Minnow to identify the efficacy of tagging, survival, and reproductive output. Spikedace and Loach Minnow were reared at the Aquatic Research and Conservation Center (ARCC) operated by Arizona Game and Fish Department in Cornville, AZ. Fish were tagged with 8-mm PIT tags following the tagging procedures outlined by Pennock et al. (2016). Results indicated high and comparable survival rates (>85%) after 30 days and similar reproductive output (larvae produced per adult) to untagged hatchery fish after 120 days. Thus, we confirmed we could tag hatchery and wild fish (>45 mm) with 8-mm PIT tags with minimal effect.

For field experiments, hatchery fish were PIT tagged at the ARCC facility 1-3 days prior to transport and release into streams, while all wild fish were tagged after being collected with either a backpack electroshocker or seine. Each stream had a designated 600 m reach to assess emigration and survival within the reach. All reaches were bracketed by an upstream and downstream stationary antenna as well as a stationary antenna in the middle of the reach near the stocking/tagging location. Two stationary antennas were deployed in the middle of the reach, in each stream, during the first 3-6 days of PIT tagging fish to assess detection probability from stationary antennas. All stationary antennas were programmed to detect a unique tag every minute. Stationary antennas used were primarily 0.9 m circular submersible antennas (Biomark IS1001 readers) but occasionally submersible rectangular antennas 1.5 m x 0.7 m were also used (Biomark RM310 readers). In addition to stationary antennas, a mobile, floating antenna was used to detect fish throughout our 600 m reach and involved a person walking the stream while pulling the antenna. Mobile antennas were rectangular with dimensions of 1.0 m x 0.3 m (in 2020) or 1.5 m x 0.7 m (in 2021 and 2022) and recorded tags on either a handheld reader or a

reader board similar to stationary antennas (Biomark HPR plus or RM310 reader). Mobile antennas were used to scan the entire 600 m reach to detect any fish present and multiple passes (2-6 per day) were performed when tracking for fish.

Data Analysis

Detection Probability

To assess if any corrections were needed for stationary antenna detection probabilities across the three streams, we used logistic regression to predict the probability of a fish hitting both of the two stationary antennas that were placed in the middle of the reach for the first 3-6 days as a function of the total number of detections. If a fish was detected on only one antenna that individual was recorded as a zero but if a fish was detected on both middle antennas that individual was recorded as a one in the logistic regression analysis. These detections were plotted as a function of the total number of times the fish was detected on the antennas and was log transformed for the logistic regression. Detection probability from all 3 streams was high on stationary antennas and quickly approached 100% when fish remained in the area for more than 10 minutes (10 hits on the antenna; Supplemental Figure 1). Even fish that remained in an area for as short as 3 minutes had high detection probabilities (>60%). Due to these high and constant detection probabilities across all streams and that fish were typically (82.4% of fish) detected at least 2 times (i.e. remained in the area for >2 minutes) on an both the upstream and downstream stationary antennas that bracketed our 600 m reach, we made no correction for detection probability and plot raw values in terms of the number of individuals that emigrated from our study reach.

Emigration

To quantify emigration rates of hatchery and wild fish out of the 600 m reach we examined cumulative emigration in each stream for each of the stocking/tagging trips. Wild fish were pooled together to derive an overall emigration estimate for wild fishes, to be compared to each hatchery released species. In Hot Springs, estimates for emigration was calculated from 22 May-27 June 2020, in Spring Creek from 9 September-13 September 2020 and from 13 July-15 July 2021, and in the West Fork Gila River from 25 June-30 June 2020 and from 16 May-28 May 2021. Emigration was calculated as the proportion of stocked or tagged fish that were detected either at the upstream or downstream antenna and then was not detected again on any stationary or mobile antennas. To test for directional differences in emigration rates, we used Pearson's chi-square test to evaluate if hatchery fish had a stronger emigration directionality than wild fish.

To characterize the number of hatchery and wild fish lost from the system due to large downstream movements over nonnative fish barriers, stationary antennas were deployed below nonnative fish barriers on Spring Creek and Hot Springs. The barrier antenna in Hot Springs was deployed 3.8 river kilometers downstream of the stocking/tagging location from 22 May-3 August 2020 (74 days) while the barrier antenna in Spring Creek was deployed 2.5 river kilometers downstream of the stocking/tagging location from 9 September-5 October 2020 (27 days), 13 July-1 September 2021 (51 days), and 10 March-16 April 2022 (37 days).

Survival

To quantify survival rate, we used Cormack-Jolly-Seber (CJS) models in Program Mark. Given our ability to remove fish that emigrated from the reach, the CJS model provided a

measurement of true survival. Detections were binned into individual days for survival analysis and included both stationary and mobile antenna detections. A CJS model was ran for the same time periods described above for quantifying emigration, again for all species in each stream for each of the stocking/tagging trips, and additionally all wild fish were pooled together and ran to encompass an overall survival estimate for all wild fishes. Given the short time period and hence detections, survival estimates (ϕ) were held constant while detection probability (p) was allowed to be constant or vary through time. Models with the constant or time varying estimate of detection probability were ranked using Akaike Information Criterion corrected for small sample size (AICc) and the top model was selected to measure survival and associated 95% confidence interval.

Apparent Survival

Because our stationary antennas that bracketed our reach were not in place for the full duration of the study (2020-2022), we estimated apparent survival (combination of emigration and mortalities) using the mobile antenna detections to evaluate the long-term (2-24 months) persistence of fish in our study reaches. Detection probabilities for the mobile antennas from each stream and year were calculated by examining the average number of fish detected across all passes (10-24 passes) of the mobile antenna over the first 3-6 days after stocking/tagging. Given our ability to account for emigration out of our 600 m reach (bracketing the upstream and downstream ends with antennas), we were able to calculate the number of individuals detected from the total number of fish that remained within the 600 m study reach. Thus, a detection probability was calculated for each species within each stream by dividing the average number of fish detected across all passes by the total number of fish present in the 600 m reach (after accounting for migrates). The associated standard error was calculated and then used to estimate the 95% detection probability intervals. This detection probability was then used on subsequent trips where mobile antenna passes (3-16 total passes) encompassed the entire 600 m reach and species and stream specific population estimates could be calculated. We then divided these population estimates by the total number of fish stocked/tagged to calculate the apparent survival for that given time period. Mobile antenna passes were executed on at least 2 trips following the initial stocking/tagging but varied based on stream and tagging year. Additionally, given our 600 m study reach was replicated in West Fork Gila River from 2020 and 2021 and in Spring Creek from 2020-2022, fish stocked/tagged in 2020 had more apparent survival estimates than fish in 2021 and 2022. Given that program MARK accounts for detection probabilities as well as our analysis here for apparent survival, estimates all survival parameters have been corrected for variable detection rates among days that the stationary and mobile antennas were deployed.

To test for differences in hatchery and wild fish emigration and apparent survival rates, we ran an analysis of covariance on natural log transformed estimates of cumulative emigration and apparent survival. For emigration, we tested if cumulative emigration rates varied by origin (hatchery vs wild) while controlling for the number of days since fish were released. Apparent survival was tested similarly, but we also included a binary variable that represented if a high flow event (>3 times baseflow) occurred between apparent survival estimates. Models were run using the aov function in Program R (R Core Team 2020). Type III sum of squares were used to calculate P-values.

Results

Emigration

Overall, 612 hatchery fish and 838 wild fish were PIT tagged and released across the three streams from 2020-2022 (Appendix A). Cumulative emigration rates after stocking/tagging generally increased for the first 5 days before leveling off and remaining constant for the remaining 40 days (Figure 3). Hatchery fish on average had a five-fold higher emigration rate compared to wild fishes within the first 40 days. This was supported by a linear model that indicated higher cumulative emigration for hatchery fish (P-value = 0.053; Appendix B). Hatchery fish also had more of a downstream emigration bias compared to wild fish (chi-square = 16.6, P-value<0.001) with 78% (40 of 51) of emigrating hatchery fish moving downstream compared to 36% (15 of 41) of emigrating wild fish moving downstream.

Long distance downstream movements towards nonnative fish barrier antennas were rarely observed (4.1% of hatchery fish; 0% of wild fish) over the 27-74 days barrier antennas were deployed in each stream. In Hot Springs, five hatchery Spikedace (4.6%) were detected shortly (<4 days) after stocking with one more individual (0.9%) detected 60 days after stocking. In Spring Creek, no fish were detected in 2020 but two hatchery Spikedace were detected in 2021, one individual (1.0%) tagged in 2020 and one individual (0.9%) in 2021. In 2022, 17 hatchery Spikedace, three individuals (2.7%) tagged in 2021 and 14 individuals (7.3%) tagged in 2022 were detected below the barrier.

Survival

Survival estimates from Program Mark revealed high (>90%) and almost identical estimates for hatchery and wild fish over the first 40 days after release (Table 1) and these estimates were largely uniform across all species, sites, and years (Appendix C).

Apparent Survival

Apparent survival estimates declined exponentially with time since release for both hatchery and wild fish but hatchery fish had lower apparent survivals than wild fish (Figure 4). ANCOVA results suggested lower apparent survival for hatchery fish (P-value<0.05) but there was no effect (p-value=0.854) on the binary response of high flow events (Appendix B). The magnitude difference of apparent survival between hatchery and wild fish was most similar within the first 5 days (4.5% difference) and after 600 days (4.0% difference) since release, but substantial differences (>25% difference) were observed between 55 and 245 days after release and tagging. Hatchery fish apparent survival asymptotes below 5% after 265 days while wild fish did not asymptote below 5% until almost a year later at day 625. After one year, hatchery fish apparent survival was ten times lower than wild fish (1.6% and 17.7%). Given that different size mobile antennas were used across year, direct comparisons of detection probability across streams and years was not feasible. Overall detection probability of the mobile antennas averaged 14.6% for hatchery fish and 5.6% for wild fish (Appendix D).

Discussion

Hatchery fish had high and similar estimates of survival to wild fish across all streams and years. Although our 'true' survival estimates only encompassed a relatively short time period (<40 days), we suspect survival estimates are likely to continue to remain high and similar to wild fish for longer time periods, given hatchery fish mortality after stocking is typically acute. For example, almost all mortalities from endangered hatchery fish stockings in Australia occurred within the first month, with the rate of mortality of those surviving being similar to wild fish thereafter (Ebner and Thiem 2009). Similarly, in the heavily studied salmonid literature, if

hatchery fish are able to persist after the initial stocking, their mortality rate is often low or similar to wild fish (Thorstad et al. 2007; Losee et al. 2019). The high survival of the endangered hatchery fish in our study was potentially attributed to their genetic similarity to wild fishes, as wild fish are regularly being added to the hatchery brood stock, with the hopes of maintaining similar genetic diversity and fitness as their wild counterparts (CITE). But despite this regular addition of wild genetics into the hatchery brood stock, differences in movements were observed between wild and hatchery fish suggest the rearing environment is also likely to influence survival of stocked fish.

As predicted, hatchery fish exhibited higher emigration rates, with an about five-fold increase in their emigration out of our 600 m study reach within the first few weeks after being released, relative to wild fish. Higher emigration rates were expected because hatchery Spikedace and Loach Minnow were spawned in low flow (XXX cfs) raceways and reared in circular, no flow, holding tanks until adulthood. The lack of exposure and conditioning to flows might explain the higher proportion of hatchery fish moving downstream, a result also observed in many other hatchery-released species (Cresswell 1981; Horka et al. 2015; Franssen et al. 2021; Tennant et al. 2022). This relationship of higher emigration rates from the stocking location was also observed in our longer-term analysis examining apparent survival, which we attribute to the continued high emigration rates of hatchery fish. Surprisingly, and contrary to our predictions, high flow events did not have an observed effect on estimates of apparent survival for either wild or hatchery fish, a result that contrasts other studies, especially in small streams (Vincenzi et al. 2012; Brignone et al. 2022). One potential explanation are these species ability to resist displacement by floods, as evidenced by a lack of long-term population declines following large floods (Minckley and Meffe 1987; Ward et al. 2003; Gido et al. 2019).

Differences in emigration observed between wild and hatchery fish can have important management implications for future stockings. Hatchery fish have historically been stocked in easy to access stream locations (Stickney 1994), but our results suggest that fish may need to be stocked further upstream in order to establish a population within a specific area, especially if impassible fish barriers are present downstream of stocking locations. Although antennas that were placed near the barrier location in our study were not run continuously, they suggested that only a small fraction of the stocked fish (~4%) dispersed below these barriers, and the only species observed at the barrier antennas was hatchery released Spikedace. However, longer-term monitoring might indicate higher rates of emigration. Indeed, apparent survival estimates within our 600 m after 1 year was 1.6% for hatchery fish (17.6% for wild fish), thus if it is desired to have hatchery fish remain in a stocking and to reproduce in that specific area, the number of fish released could be adjusted to account for these high emigration rates. Alternatively, understanding that almost half of hatchery fish disperse in a relatively short amount of time (first 60 days), this could inform the time of year fish are stocked. If the sole purpose of introducing hatchery fish is for these individuals to spawn, stocking may be more successful if fish are stocked out just before their reproductive season.

Although we were able to detect some individuals moving large distances over fish barriers, we were unable to detect how hatchery fish redistribute after being released, despite our ability to show their affinity for downstream movement. Management decisions would be more informed if future studies examined how far and what proportion of released individuals move and redistribute, quantifying this redistribution may help reduce any potential ecological risks and unnecessary loss of hatchery fish (Ham and Pearsons 2011). Additionally, examining the utility of different training and release strategies may improve the retention or the lopsided

directionality of movement of hatchery fish. For example, federally listed species in the Colorado River basin have seen apparent survival estimates double after flow conditioning, largely attributed to the lower immediate emigration of conditioned hatchery fish (Franssen et al. 2021). Also, only minor shifts in hatchery activities can regularly improve hatchery fish survival and movement parameters to better mimic wild fish (Harbicht et al. 2020). Lastly, the looming question of how many individuals contribute to the establishment of a population still exists, as no information exists on the reproductive output of these hatchery fish in relation to their wild counterparts, despite evidence suggesting hatchery fish on average can have much lower (50%) of the output as wild fish (Araki et al. 2008; Christie et al. 2014).

Our study was able to obtain estimates of survival, emigration, movement directionality, and long-term persistence of hatchery and wild imperiled fishes. By quantifying survival, we showed hatchery released fish in desert streams is high but these fish are more prone to downstream movements, resulting in almost all fish either dying or leaving the stocking area in less than one year. We also showed these hatchery fish are capable of large distance movements below fish barriers where they can longer contribute to the populations. This information will better inform managers on deciding optimal placement of future stockings, the number of fish to release, adjusting the timing of stocking, and factors that may lead to site specific successes and failures in stockings to help improve conservation management activities and increase our chances at recovering these species.

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Table 1: Estimates of survival from wild and hatchery released tagged in three streams across Arizona and New Mexico in 2020 and 2021.

Stream	Year	Origin	Species	Survival	Lower 95% CI	Upper 95% CI	Duration (Days)
Spring Creek	2021	Hatchery	Spikedace	1	1	1	3
Spring Creek	2021	Wild	All Fishes	0.916	0.666	0.983	3
West Fork Gila River	2021	Wild	All Fishes	1	1	1	3
Spring Creek	2020	Hatchery	Spikedace	1	1	1	5
Spring Creek	2020	Wild	All Fishes	1	1	1	5
Hot Springs	2020	Hatchery	Spikedace	0.961	0.925	0.981	6
Hot Springs	2020	Hatchery	Loach Minnow	1	1	1	6
Hot Springs	2020	Wild	All Fishes	1	1	1	6
West Fork Gila River	2020	Wild	All Fishes	0.915	0.851	0.953	6
West Fork Gila River	2020	Wild	All Fishes	0.939	0.921	0.953	13
Hot Springs	2020	Hatchery	Loach Minnow	0.957	0.945	0.966	37
Hot Springs	2020	Hatchery	Spikedace	0.975	0.966	0.981	37
Hot Springs	2020	Wild	All Fishes	0.968	0.961	0.974	37

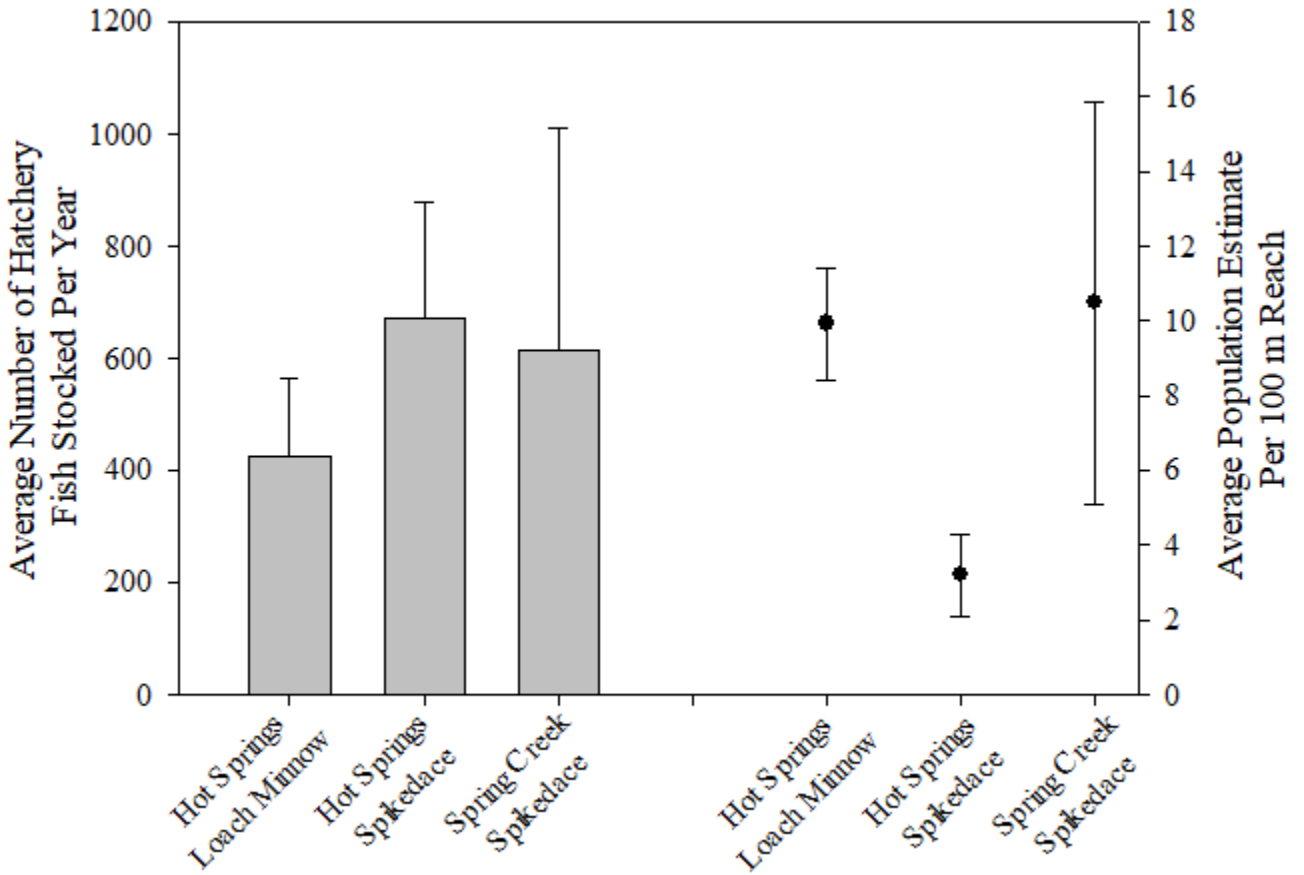


Figure 1: Average yearly number of hatchery fish stocked (left axis) and population estimate per 100 m reach (right axis) for loach minnow and spikedace in two Arizona streams. Stockings occurred from 2007-2011 in Hot Springs and from 2015-2018 in Spring Creek while population estimates encompass 2009-2019 in Hot Springs and 2015-2019 in Spring Creek. Error bars represent standard error.

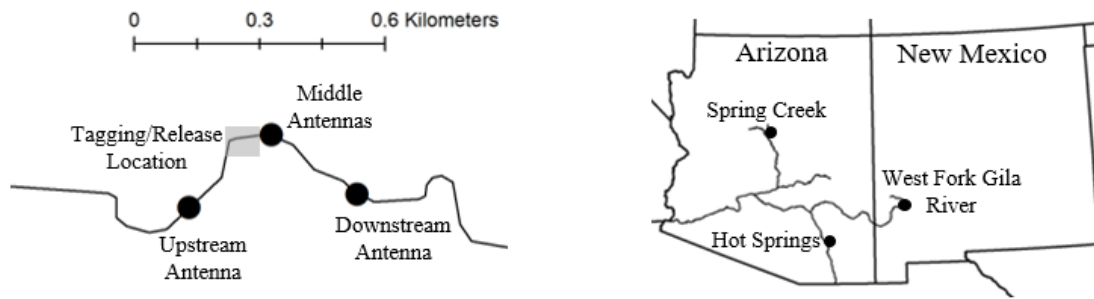


Figure 2: Locations of three streams where fish were tracked following tagging of wild and release of hatchery fish from 2020-2022. At each site, three antennas were evenly placed across 600 m of the stream with fish being released or tagged just upstream of the middle antenna

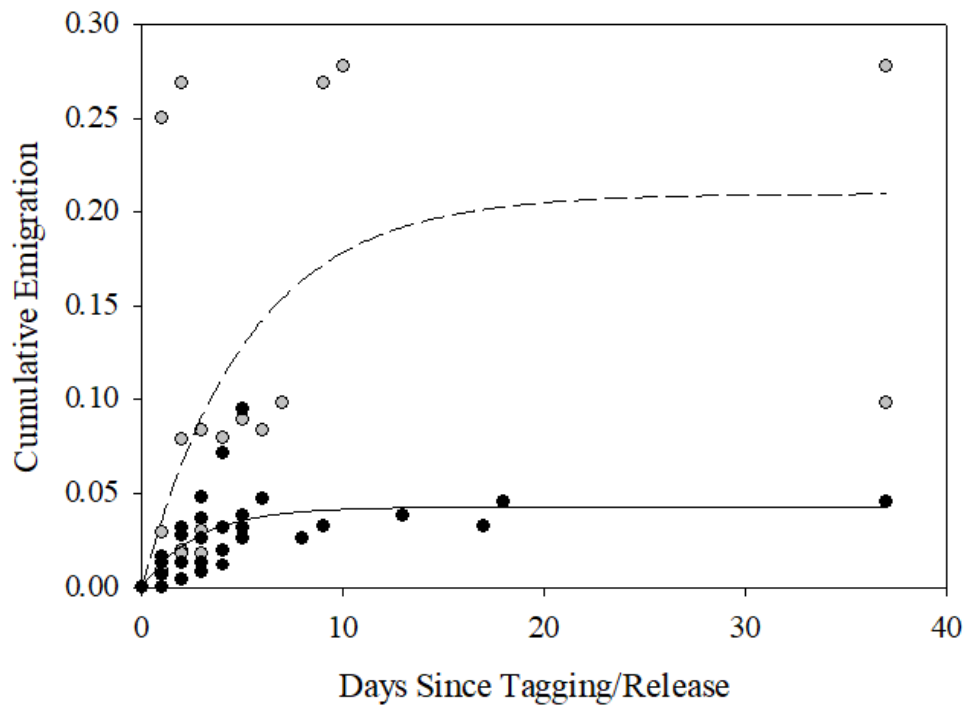


Figure 3: Cumulative proportion of fish emigrating out of a 600 m reach of stream in 2020 and 2021 from wild (black dots, solid line) and hatchery released (grey dots, dashed line) tagged fish in three streams in Arizona and New Mexico.

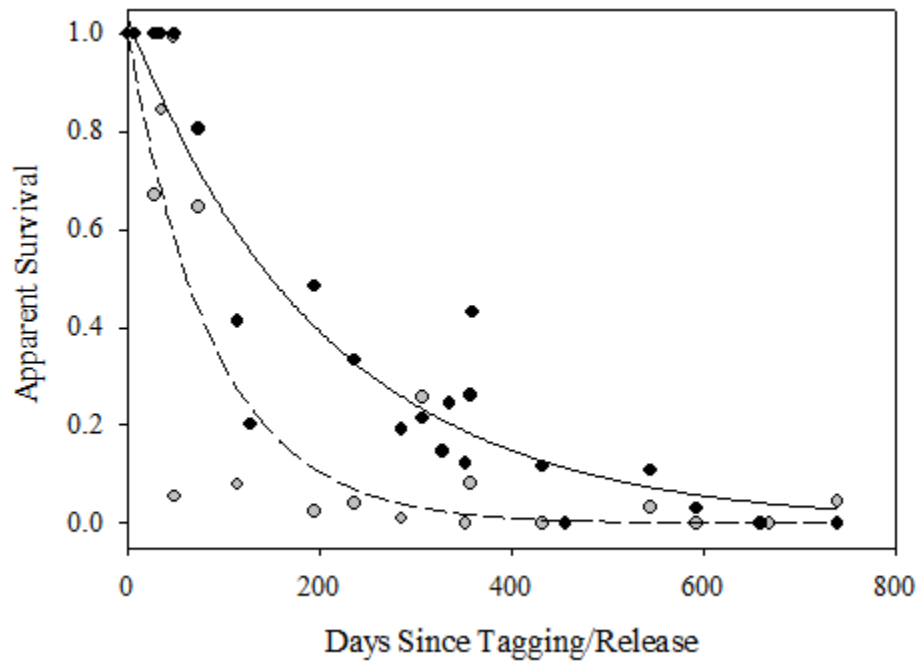


Figure 4: Apparent survival estimates within a 600 m reach of stream from 2020-2022 from wild (black dots, solid line) and hatchery released (grey dots, dashed line) tagged fish in three streams in Arizona and New Mexico.

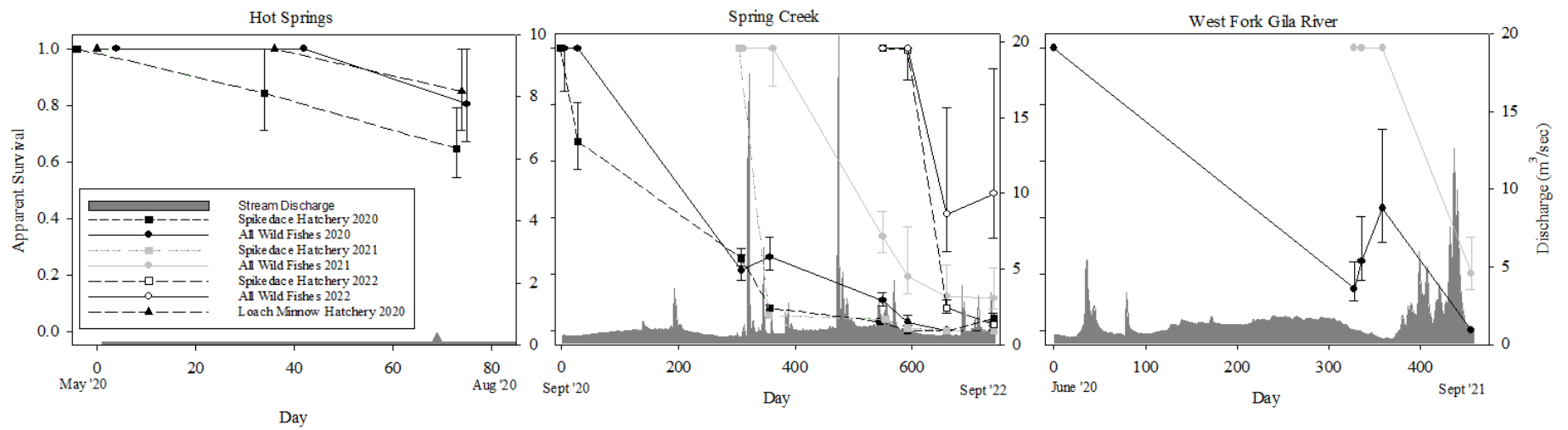
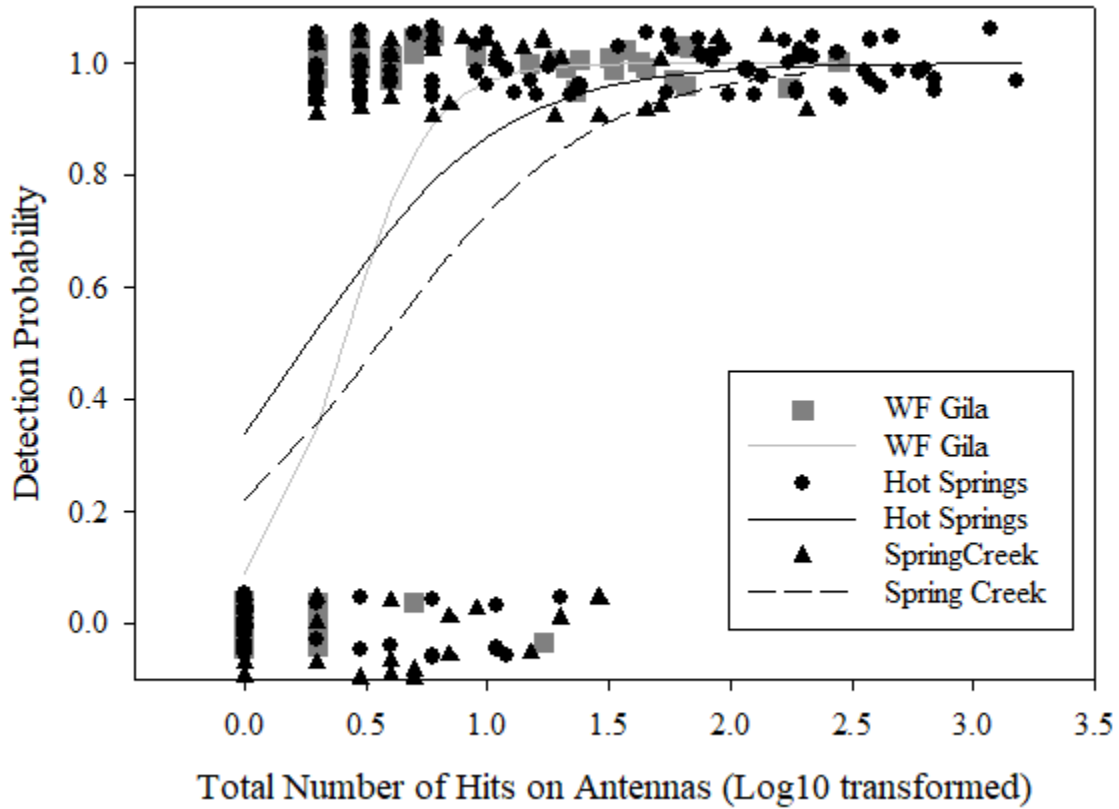


Figure X: Apparent survival estimates of wild (solid) and hatchery (dashed) released tagged fish from three different streams in 2020 (black), 2021 (gray), and 2022 (white). Error bars represent 95% confidence intervals. Dark gray shading represents stream discharge from the nearest USGS gage.



Supplemental Figure 1: Detection probability predicted as a function of number hits from two submersible antennas placed in the same pool within three different streams in 2020. Values are jittered around zero (hit only one antenna) and one (hit both antennas). Each dot represents one individual fish.

Appendix A: Number of individuals tagged and mean total length of wild and hatchery released fish in three streams across Arizona and New Mexico from 2020-2022.

Stream	Year	Origin	Species	Individuals Tagged	Total Length (mm)
Hot Springs	2020	Hatchery	Loach Minnow	102	49
Hot Springs	2020	Hatchery	Spikedace	108	53
Hot Springs	2020	Wild	Longfin Dace	63	72
Hot Springs	2020	Wild	Roundtail Chub	10	110
Hot Springs	2020	Wild	Desert Sucker	20	126
Hot Springs	2020	Wild	Speckled Dace	62	66
Spring Creek	2020	Hatchery	Spikedace	101	56
Spring Creek	2020	Wild	Longfin Dace	25	80
Spring Creek	2020	Wild	Roundtail Chub	26	79
Spring Creek	2020	Wild	Spikedace	22	67
Spring Creek	2020	Wild	Desert Sucker	22	113
Spring Creek	2020	Wild	Speckled Dace	31	68
Spring Creek	2021	Hatchery	Spikedace	110	54
Spring Creek	2021	Wild	Longfin Dace	25	65
Spring Creek	2021	Wild	Roundtail Chub	31	84
Spring Creek	2021	Wild	Desert Sucker	19	123
Spring Creek	2021	Wild	Speckled Dace	31	59
Spring Creek	2022	Hatchery	Spikedace	191	57
Spring Creek	2022	Wild	Longfin Dace	17	65
Spring Creek	2022	Wild	Speckled Dace	44	63
West Fork Gila River	2020	Wild	Longfin Dace	52	58
West Fork Gila River	2020	Wild	Spikedace	63	51
West Fork Gila River	2020	Wild	Desert Sucker	50	73
West Fork Gila River	2020	Wild	Speckled Dace	17	55
West Fork Gila River	2020	Wild	Loach Minnow	52	54
West Fork Gila River	2021	Wild	Longfin Dace	32	56
West Fork Gila River	2021	Wild	Sonora Sucker	31	253
West Fork Gila River	2021	Wild	Spikedace	49	49
West Fork Gila River	2021	Wild	Desert Sucker	4	115
West Fork Gila River	2021	Wild	Loach Minnow	40	53

Appendix B: Model output from natural log transformed cumulative emigration and apparent survival of fish stocked/tagged in three streams across Arizona and New Mexico from 2020 and 2022.

Model	Sum of Squares	Df	F-Value	P-values
Cumulative Emigration ~ Origin + Days Since Tagging				
Intercept	300.32	1	132.89	<0.001
Origin	8.82	1	3.9	0.053
Days Since Tagging	30.68	1	13.58	<0.001
Residuals	122.04	54		
Apparent Survival ~ Origin + Days Since Tagging + High Flows				
Intercept	5.24	1	2.93	0.093
Origin	11.65	1	6.52	0.014
Days Since Tagging	140.57	1	78.68	<0.001
High Flows	0.06	1	0.03	0.854
Residuals	89.33	50		

Appendix C: Species specific estimates of true survival of fish stocked/tagged in three streams across Arizona and New Mexico in 2020 and 2021.

Stream	Year	Origin	Species	Survival	Lower 95% CI	Upper 95% CI	Duration (Days)
Hot Springs	2020	Hatchery	Spikedace	0.961	0.925	0.981	6
Hot Springs	2020	Hatchery	Loach Minnow	1	1	1	6
Hot Springs	2020	Wild	Longfin Dace	1	1	1	6
Hot Springs	2020	Wild	Roundtail Chub	0.974	0.839	0.996	6
Hot Springs	2020	Wild	Desert Sucker	1	1	1	6
Hot Springs	2020	Wild	Speckled Dace	1	1	1	6
Hot Springs	2020	Wild	All Fishes	1	1	1	6
Spring Creek	2020	Hatchery	Spikedace	1	1	1	5
Spring Creek	2020	Wild	Spikedace	1	1	1	5
Spring Creek	2020	Wild	Longfin Dace	1	1	1	5
Spring Creek	2020	Wild	Roundtail Chub	1	1	1	5
Spring Creek	2020	Wild	Desert Sucker	0.982	0.765	0.999	5
Spring Creek	2020	Wild	Speckled Dace	1	1	1	5
Spring Creek	2020	Wild	All Fishes	1	1	1	5
Spring Creek	2021	Hatchery	Spikedace	1	1	1	3
Spring Creek	2021	Wild	Longfin Dace	1	1	1	3
Spring Creek	2021	Wild	Roundtail Chub	0.814	0.272	0.981	3
Spring Creek	2021	Wild	Desert Sucker	0.793	0.529	0.929	3
Spring Creek	2021	Wild	Speckled Dace	0.831	0.579	0.946	3
Spring Creek	2021	Wild	All Fishes	0.916	0.666	0.983	3
West Fork Gila River	2020	Wild	Spikedace	1	1	1	6
West Fork Gila River	2020	Wild	Loach Minnow	1	1	1	6
West Fork Gila River	2020	Wild	Longfin Dace	0.729	0.498	0.88	6
West Fork Gila River	2020	Wild	Desert Sucker	0.876	0.807	0.922	6
West Fork Gila River	2020	Wild	Speckled Dace	0.886	0.565	0.979	6

West Fork Gila River	2020	Wild	All Fishes	0.915	0.851	0.953	6
West Fork Gila River	2021	Wild	Spikedace	1	1	1	3
West Fork Gila River	2021	Wild	Loach Minnow	1	1	1	3
West Fork Gila River	2021	Wild	Longfin Dace	1	1	1	3
West Fork Gila River	2021	Wild	Desert Sucker	1	1	1	3
West Fork Gila River	2021	Wild	Sonora Sucker	0.909	0.781	0.966	3
West Fork Gila River	2021	Wild	All Fishes	1	1	1	3
West Fork Gila River	2021	Wild	Spikedace	0.976	0.898	0.994	13
West Fork Gila River	2021	Wild	Loach Minnow	0.951	0.908	0.975	13
West Fork Gila River	2021	Wild	Longfin Dace	0.958	0.857	0.989	13
West Fork Gila River	2021	Wild	Desert Sucker	0.938	0.784	0.984	13
West Fork Gila River	2021	Wild	Sonora Sucker	0.894	0.842	0.931	13
West Fork Gila River	2021	Wild	All Fishes	0.939	0.921	0.953	13
Hot Springs	2020	Hatchery	Spikedace	0.975	0.966	0.981	37
Hot Springs	2020	Hatchery	Loach Minnow	0.957	0.945	0.966	37
Hot Springs	2020	Wild	Longfin Dace	0.98	0.971	0.987	37
Hot Springs	2020	Wild	Roundtail Chub	0.987	0.964	0.995	37
Hot Springs	2020	Wild	Desert Sucker	0.989	0.976	0.995	37
Hot Springs	2020	Wild	Speckled Dace	0.947	0.928	0.961	37
Hot Springs	2020	Wild	All Fishes	0.968	0.961	0.974	37

Appendix D: Detection probability and associated 95% confidence intervals (CI) from pulling a mobile antenna across 600 m of stream containing PIT tagged wild and hatchery fish in Arizona and New Mexico from 2020 and 2022.

Stream	Year	Origin	Species	Detection Probability	Lower 95% CI	Upper 95% CI
Hot Springs	2020	Hatchery	Spikedace	0.110	0.090	0.130
Hot Springs	2020	Hatchery	Loach Minnow	0.054	0.044	0.064
Hot Springs	2020	Wild	Longfin Dace	0.029	0.019	0.039
Hot Springs	2020	Wild	Roundtail Chub	0.020	0.000	0.040
Hot Springs	2020	Wild	Desert Sucker	0.000	0.000	0.000
Hot Springs	2020	Wild	Speckled Dace	0.023	0.016	0.030
Hot Springs	2020	Wild	All Fishes	0.023	0.018	0.027
Spring Creek	2020	Hatchery	Spikedace	0.118	0.098	0.138
Spring Creek	2020	Wild	Spikedace	0.049	0.031	0.067
Spring Creek	2020	Wild	Longfin Dace	0.007	0.001	0.014
Spring Creek	2020	Wild	Roundtail Chub	0.004	0.000	0.009
Spring Creek	2020	Wild	Desert Sucker	0.000	0.000	0.000
Spring Creek	2020	Wild	Speckled Dace	0.012	0.005	0.019
Spring Creek	2020	Wild	All Fishes	0.015	0.010	0.019
Spring Creek	2021	Hatchery	Spikedace	0.224	0.197	0.251
Spring Creek	2021	Wild	Longfin Dace	0.075	0.050	0.100
Spring Creek	2021	Wild	Roundtail Chub	0.031	0.018	0.045
Spring Creek	2021	Wild	Desert Sucker	0.033	0.008	0.058
Spring Creek	2021	Wild	Speckled Dace	0.075	0.054	0.096
Spring Creek	2021	Wild	All Fishes	0.056	0.044	0.067
Spring Creek	2022	Hatchery	Spikedace	0.223	0.163	0.282
Spring Creek	2022	Wild	Longfin Dace	0.221	0.110	0.331
Spring Creek	2022	Wild	Speckled Dace	0.102	0.042	0.162
Spring Creek	2022	Wild	All Fishes	0.135	0.071	0.200
West Fork Gila River	2021	Wild	Spikedace	0.055	0.025	0.086

West Fork Gila River	2021	Wild	Loach Minnow	0.111	0.056	0.165
West Fork Gila River	2021	Wild	Longfin Dace	0.022	0.009	0.034
West Fork Gila River	2021	Wild	Desert Sucker	0.025	0.000	0.071
West Fork Gila River	2021	Wild	Sonora Sucker	0.010	0.000	0.029
West Fork Gila River	2021	Wild	All Fishes	0.052	0.032	0.073

Habitat evaluation of native fishes in the Gila River basin

by

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B.S., Colorado State University, 2018

A THESIS

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Abstract

Habitat loss and alteration is a major cause of declining native fish diversity in streams across the southwestern United States. Much of this habitat alteration is a consequence of human's actively extracting or diverting water from lotic systems. The Gila River and its tributaries are home to multiple dams, diversions and also supply water to agricultural and industrial municipalities throughout the region, leading to continued decline of native fishes. We conducted a range-wide habitat assessment in an effort to identify critical habitat needs of two endangered minnow species, spikedace (*Meda fulgida*) and loach minnow (*Tiaroga cobitis*). This habitat assessment was conducted across multiple spatial scales in an effort to identify at what scale habitat variables most strongly influence the presence of these species. Further, we conducted an enclosure experiment using these two endangered fishes, as well as two other native cyprinids, speckled dace (*Rhinichthys osculus*) and longfin dace (*Agosia chrysogaster*), to test mechanisms influencing resource availability and growth rates. Our results point towards water velocity as the driving variable associated with presence and performance of endangered species in this system. We provide guidance on priority habitats and potential repatriation efforts, to ensure that these native species are not lost from this basin.

Table of Contents

List of Figures	vi
List of Tables	viii
Acknowledgements	ix
Chapter 1 - Multi-scale Habitat Assessment of Loach Minnow and Spikedace Across the Gila	
River Basin	1
Abstract	1
Introduction.....	2
Methods	5
Study area.....	5
Fish Sampling	6
Habitat Measurements	6
Data Analysis	7
Results.....	8
Discussion.....	9
Acknowledgements.....	13
Figures	14
Tables.....	18
Chapter 2 - How Fast it too fast? Water velocity differentially effects growth of four Gila River,	
native cyprinids.....	23
Abstract.....	23
Introduction.....	24
Methods	26
Study Site	26
Enclosure Experiment.....	27
Data analysis	28
Results.....	29
Discussion.....	30
Acknowledgements.....	34
Figures	35

References..... 42

List of Figures

Figure 1.1. Visual representation of the three differing spatial scales sampled at each reach throughout the Gila River basin.	14
Figure 1.2 Map of reaches sampled throughout the course of this study in 2019 and 2020 in Arizona and New Mexico. Numbers correspond to locations reported on Table 1.3.	15
Figure 1.3 Classification tree for the loach minnow describing variables that predict species presence-absence at three differing spatial scales: 1-km reach (top left), 0.1-km reach (top right), and mesohabitat (bottom). Numbers within each box denote the number of observations where this species is truly present or absent (numerator), and the number of habitats that meet the requirements being described with no reference to fish presence absence (denominator).	16
Figure 1.4 Classification tree for the spikedace describing variables that predict species presence-absence at three differing spatial scales: 1-km reach (top left), 0.1-km reach (top right), and mesohabitat (bottom). Numbers within each box denote the number of observations where this species is truly present or absent (numerator), and the number of habitats that meet the requirements being described with no reference to fish presence absence (denominator).	17
Figure 2.1 The four native species used in the enclosures experiment (A) and the enclosures within the West Fork of the Gila River (B). The four species used in the experiment are: Speckled Dace (top left), longfin dace (top right), loach minnow (bottom left), and spikedace (bottom right).	35
Figure 2.2 A graphical depiction of our predictions in regard to the response of food availability and growth rate of drift and benthic feeders.	36
Figure 2.3 Variation in velocity within each enclosure over the course of the study in 2019 (top) and 2020 (bottom). Cage rank (1 = lowest mean velocity enclosure, 18 = highest mean velocity enclosure) is on the x-axis and velocity (m/s) is on the y-axis.	37
Figure 2.4 Relationship between enclosure velocity and individual growth rate, measured as change in total length (mm/d) between the beginning and end of the experiment for speckled dace, longfin dace, loach minnow, and spikedace. Each dot represents an individual fish and trend-lines are only included for significant relationships.	38

Figure 2.5 Relationship between enclosure velocity and individual growth rate, measured as change in mass (g/d) between the beginning and end of the experiment for speckled dace, longfin dace, loach minnow, and spikedace. Each dot represents an individual fish and trend-lines are only included for significant relationships..... 39

Figure 2.6 Biomass of drifting (left) and benthic (right) macroinvertebrates < 6 mm in enclosures (circles, black) and outside of enclosures (triangles, grey) across a velocity gradient in 2019. Both Y-axes are log transformed. 40

Figure 2.7 Biomass (< 6 mm) of drifting (triangles) and benthic (circles) macroinvertebrates in enclosures across a velocity gradient in 2020. Y-axis is log transformed. Each dot represents the average biomass of invertebrates in each enclosure. 41

List of Tables

Table 1.1	List of habitats sampled over the course of this study throughout the Gila River basin. Means and standard deviations are reported for depth, velocity, and substrate sizes.....	18
Table 1.2	Values for Mean Gini Decrease for loach minnow and spikedace at all spatial scales in which Classification Trees generated from Random Forest with 500 iterations that were able to describe the presence of these species. Values indicate node purity identifying which variables were able to most accurately predict the presence of these species.	19
Table 1.3	Number of 1-km reaches that meet habitat requirements for loach minnow and spikedace. Areas where spikedace currently occur are denoted with the superscript SD, while areas where loach minnow currently occur are denoted with the superscript LM.....	20
Table 1.4	Number of 0.1-km reaches that meet habitat requirements for loach minnow and spikedace. Areas where spikedace currently occur are denoted with the superscript SD, while areas where loach minnow currently occur are denoted with the superscript LM.....	21
Table 1.5	Number of habitat points and percentage of suitable habitat from transect data that meets the fine scale habitat requirements for loach minnow. Streams that currently have loach minnow present are denoted with the superscript LM.	22

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Chapter 1 - Multi-scale Habitat Assessment of Loach Minnow and Spikedace Across the Gila River Basin

Abstract

Habitat loss and alteration is a major cause of declining native fish diversity and abundance in streams across the southwestern United States. Much of this habitat alteration is a consequence of humans extracting or diverting water from lotic systems. The Gila River and its tributaries have multiple dams and diversions that supply water to agriculture, industry, and municipalities throughout much of Arizona, contributing to the decline of native fishes. To counter these declines, repatriation efforts have been implemented throughout the basin. While repatriation efforts have successfully re-established some populations, others have been unsuccessful. To aid in future management of imperiled Gila basin fishes, we conducted a range-wide habitat assessment in an effort to identify critical habitat needs of two federally-protected species, spikedace (*Meda fulgida*) and loach minnow (*Tiaroga cobitis*). This habitat assessment was conducted across multiple spatial scales, 1-km, 0.1-km, and the mesohabitat scale, to identify at what scale measured habitat variables most strongly predicted the presence of each species. We included minimum, maximum, and average depth, velocity, and substrate sizes within our model to predict the presence of both of these species. At our two broadest scales, average velocity was the variable that best predicted the presence or absence of both species. At the finest scale, loach minnow associated with very specific habitats while spikedace occurred across a range of habitats. Data were used to rank streams based on habitat suitability and suggested that future habitat assessments should be conducted at larger spatial scales to evaluate if a location should be repatriated.

Introduction

Repatriation, the placement of native animals in areas they have since been extirpated, is an increasingly common conservation strategy for restoring extirpated or enhancing diminished populations of terrestrial and aquatic species globally (Ripple & Beschta, 2003; King, Berg & Hay, 2004; Lamothe & Drake, 2019). Although there is a long history of fish stocking aimed at enhancing recreational or commercial fisheries, there is only recent interest for species that do not possess economic value (Lamothe & Drake, 2019; Desert Fishes Team, 2003; Marsh, Kesner & Pacey, 2005). Typically, repatriation of imperiled species and populations takes place in historically occupied habitat, presumably following the amelioration of factors that influenced the initial extirpation (Marsh, Kesner, & Pacey, 2005). Management actions to restore natural environmental characteristics include mimicking the natural flow regime, removal of nonnatives, and habitat improvements (Knopf et al. 1988; Tyus, 1992; Dodrill et al. 2015). Presumably, some combination of habitat restoration and eradication of nonnatives should set the stage for successful reintroduction of native fishes.

Our focus in this paper is to characterize habitat requirements of native Gila basin fishes, which can be challenging because habitat needs observed at one spatial scale may not be representative of what is needed at other scales (Winemiller, Flecker, & Hoeninghaus, 2010; Logue et al. 2011). With this in mind, studies evaluating habitat requirements for a species, as well as evaluating habitat for repatriation requirements, should be conducted at multiple spatial scales. Our goal with this approach was to provide additional information to inform future management in regard to the repatriation of imperiled species, to consider what spatial scale measurements should be taken, and what processes affected our observations. Until recently, community ecology focused on fine scale interactions, such as the mesohabitat scale, with the

assumption that broad scale approaches, such as those occurring over multiple kilometers, were less important (Fausch et al. 2002). In the past, research was conducted only within short reaches of rivers due to the assumption that individuals complete their life cycle within this confined area (Gatz & Adams, 1994; Fausch et al. 2002). By assessing habitats at multiple scales, we hope to gain a better understanding of what scale is most appropriate to characterize habitats amenable to repatriation of native fishes.

Native fish declines in the American Southwest have been ongoing for decades and management efforts have been aimed at combating these declines (Propst, Williams, Bestgen, & Hoagstrom, 2020). The Gila River basin is home to multiple imperiled native fishes, all of which have been negatively affected by a combination of habitat loss and introduction of nonnative species (Propst, Gido, & Stefferud, 2008; Ruhi, Olden, & Sabo, 2016). In response to these declines, multiple management approaches have been implemented within the basin. Specifically, there have been extensive efforts to isolate reaches above natural or artificial barriers after eradication of nonnatives and establishment of native populations. However, due to continued declines, extensive evaluation of factors affecting successes or failures in establishing naturally reproducing populations in these renovated waters or elsewhere are needed.

We conducted a multi-scale habitat assessment in streams across the Gila River basin in an effort to identify variables associated with the presence-absence of loach minnow (*Tiaroga cobitis*) and spikedace (*Meda fulgida*), two federally-protected cyprinids that have been extirpated from > 80% of their native ranges (U.S. Fish and Wildlife Service, 2012). Whereas large-scale repatriation efforts have attempted to protect these populations from extinction (Desert Fishes Team, 2003), knowledge of the appropriate scale to conduct habitat measurements for the successful establishment of populations is critical (Lamothe & Drake, 2019). Repatriation

of spikédace and loach minnow has been taking place for many years, but results have been mixed in regard to establishing reproductive populations (Desert Fishes Team, 2003; Clarkson & Marsh, 2010).

To gain an understanding of the habitat requirements of loach minnow and spikédace that might enhance success of repatriation efforts, we selected reaches throughout the Gila River basin where spikédace and loach minnow either have natural populations, extirpated populations, successfully repatriated populations, or unsuccessfully repatriated populations to test if habitat variables are driving population persistence. The main objectives of this study were to 1) identify abiotic variables associated with successfully establishing naturally sustaining populations of loach minnow and spikédace and 2) identify the spatial scales at which to measure habitat variables to best predict the presence-absence of these species. We used a nested design where habitat data from our mesohabitat spatial scale were grouped together to represent our 0.1-km spatial scale, but habitat at our 1-km scale was defined by transects taken throughout the reach (Figure 1.1). Fish were collected at the mesohabitat spatial scale and grouped together to represent both the 0.1-km and 1-km spatial scales.

Previous research on these species has provided insight into their habitat associations. Loach minnow are a riffle obligate species completing most essential life-history processes in high velocity habitats (Rinne, 1989; Propst & Bestgen, 1991). For spikédace, increased water velocity is necessary for reproduction and feeding; although they do not solely occupy high velocity habitats for extended periods of time (Barber & Minckley, 1983; Propst & Bestgen, 1986). Assuming loach minnow are riffle obligates, we predicted flow velocity and depth will predict occurrences at fine scales due to their direct influence on species well-being and habitat occupation. At broader scales, increasing mean velocity should also provide an index of the total

amount of suitable habitat, and over some threshold value, should predict the occurrence of this species. For spikédace, we predicted that at fine scales they will require moderate flow velocity that sustains drifting invertebrates while not imposing excess energy expenditure. At the broader scales, we also predicted that intermediate mean flow velocity would associate with sites with a mosaic of intermediate and fast velocity habitats that might optimize feeding efficiency and energy use of this species. While we predicted velocity would predict occurrences of both species across all scales, we predict loach minnow would occur in higher velocities than spikédace.

Methods

Study area

The Gila River and its tributaries are part of the lower Colorado River basin and flows southwesterly through southwestern New Mexico, then in a westerly direction once it reaches Arizona, where it eventually drains into the Colorado River in southwest Arizona, near the town of Yuma, AZ. The upper Gila River basin remains free flowing with relatively low human impact due to its remoteness and presence of federally-administered lands. However, the other sub-basins of the catchment have moderate to heavy human impacts, running through populated areas as well as having multiple dams and diversions. We sampled 31 1-km stream reaches between October 2018 and October 2019 (Figure 1.2). Eighteen reaches were sampled in October 2018 and June 2019. During October 2019, 11 reaches previously sampled were replaced with new reaches. Six reaches were sampled throughout the study, because they either were highly dynamic or had variable occurrences of loach minnow or spikédace. Dropping and adding sites was done to increase overall sample size to provide a more comprehensive view of the range of habitats currently and potentially occupied by each species. The elevation of reaches

sampled over the course of the study ranged from 953 to 2185 m above sea level. Individual reaches were selected based on accessibility to each location and in consultation with various state agencies.

Fish Sampling

Fish communities were sampled using a combination of backpack electro-fishing (Smith-Root LR 20B, 200-250 V, Freq: 30 Hz, Duty Cycle: 30%) and seining (4.6 x 1.8 m, 3.2-mm mesh). Every stream was evaluated at three different spatial scales: 1-km reach, 0.1-km reach, and mesohabitat (0.5-72 m²). Mesohabitats were identified and selected on site by individuals conducting the sampling based upon their habitat characteristics (Table 1). All available habitat types present within each 1-km reach were sampled. Sampling was conducted by capturing fish in each mesohabitat independently of other samples. Fish captured within each mesohabitat were identified and measured to total length prior to release. Fish were released downstream of the sampling area in an effort to not capture the same individual more than once. All fish data were pooled across mesohabitats to represent our 0.1-km reach. Finally, fish data from all 0.1-km reaches were pooled to represent the fish community at the 1-km reach.

Habitat Measurements

Within each mesohabitat, three point measurements were taken across three transects. Each point measurement included depth, velocity, substrate (clay, silt, sand, gravel, pebble, cobble, boulder, bedrock), based on the scale set by Wentworth, 1922, and presence-absence of cover (i.e., overhanging vegetation or macrophyte presence-absence). All habitat data collected from the 8-10 mesohabitats within the downstream, midstream, or upstream 0.1-km reach were pooled to represent the habitat variables at the intermediate spatial scale. To capture the habitat variables at our 1-km scale, habitat transects were taken along the entire 1-km reach, providing

an independent habitat assessment of habitat availability at this broad spatial scale. These transects were taken perpendicular to the thalweg every 25 or 50 m throughout the 1-km reach and habitat was measured at points 0.5, 1.0, or 2.0 m increments across the entirety of the stream. Transect intervals were chosen to yield approximately 200 individual habitat measurements within each 1-km reach.

Data Analysis

Classification trees were used to identify variables associated with the presence of spinedace and loach minnow across three spatial scales. Variables included in the models were the mean, maximum, and minimum of velocity, depth and substrate size. Classification trees use a recursive partitioning method to identify which variables most strongly affect the differentiation in the presence-absence of these species across the basin and across spatial scales (Strobl, Malley, & Tutz, 2009). Trees are initially overfitted using as many variables as possible to predict presence-absence and are then pruned to remove nodes in an effort to minimize error within the model (Khoshgoftaar & Allen, 2001). Random forest was conducted, with 500 iterations (Archer & Kimes, 2008), to identify variable importance within each classification tree by identifying the degree to which each variable affects the Mean Decrease Gini (MDG). MDG is a metric that shows the purity of a particular node split within classification trees, where larger values indicate increased variable importance within the model (Han, Guo, & Yu, 2016). By conducting this analysis, we were able to identify habitat variables that were important drivers of presence or absence of loach minnow and at which scale habitat measurements should be conducted to assess a location's suitability for either species. If a significant model was derived at the mesohabitat scale, we calculated the percent of suitable habitat for a species by using each individual points within the habitat transects throughout the 1-km reach. The percent suitable

habitat was used to predict fish density using linear regression. Classification trees were generated in program R version 3.5.2 using the packages *rpart* and *randomForest*, while linear models were made using package *glm* (R Core Team, 2018). Sites were ranked based on their suitability for repatriation based on our ability to classify occurrences at the different spatial scales and the percent of adequate habitat throughout the reach.

Results

Classification trees indicated that mean velocity was the strongest predictor of presence-absence of both loach minnow and spikedace across all spatial scales, with the exception that no variable was able to predict presence-absence of spikedace at the mesohabitat scale (Figures 1.3 & 1.4). Further, random forest analysis suggested that for both species and at all scales, mean velocity was always the most important variable for predicting the presence-absence of these species (Table 1.2). At the 1-km reach scale, loach minnow presence was more likely in reaches with mean velocity > 0.19 m/s, and spikedace presence was more likely when average velocity was > 0.21 m/s. At the 0.1-km reach scale, mean velocity greater than 0.38 and 0.39 m/s were associated with the presence of loach minnow and spikedace, respectively. At the mesohabitat scale, mean velocity, depth, and substrate size predicted loach minnow presence, identifying two different habitat types that can describe this species presence. The first included deeper water (≥ 0.21 m) with very high velocities (≥ 0.91 m/s). The second was characterized by shallow, high velocity mesohabitats with coarser substrate sizes (< 0.21 m depth, ≥ 0.57 m/s velocity, < 4.6 substrate size [indicating gravel/pebble substrate]).

Of the 51 1-km reaches sampled, 25 had average velocities that were predicted to be occupied by loach minnow. However, loach minnow only occupied 16 (64%) of the 25 1-km reaches, as indicated by the numbers within the classification trees (16/25 reaches with velocity

that met threshold had loach minnow occupying them). Of the 153 0.1-km reaches sampled, loach minnow occupied 20 of the 22 (91%) reaches where they were predicted to occur.

Locations with adequate habitat without loach minnow were Eagle Creek and the lower San Francisco River. Of the 1246 mesohabitats sampled, loach minnow occupied 34 of the 47 (72%) classified as suitable habitat. Nine 1-km reaches sampled possessed at least one adequate mesohabitat without loach minnow present (Table 1.5). To further illustrate the importance of adequate fine-scale habitat, we found the percent of suitable mesohabitats was positively correlated with loach minnow density across the basin (p -value = 0.005).

Spikedace occupied 14 of 19 1-km reaches (74%) classified as suitable habitat. Five of the locations with suitable broad scale habitat locations were not currently occupied: Tularosa River at Hell's Hole, Eagle Creek, Lower Tularosa River, San Francisco River (not present at the time, have since inhabited this location), and the lower San Francisco River. Fifteen of the 20 (75%) 0.1-km reaches classified as possessing suitable habitat were occupied by spikedace. Of these 0.1 km reaches, Campbell Blue River, Gila River at Grapevine campsite (x2; was present in one of the three 0.1-km reaches), and the lower San Francisco River (x2) did not have spikedace. We were not able to classify occurrences of spikedace at the scale of mesohabitats, thus we were not able to compare densities to suitable habitat for this species.

Discussion

Classification trees described loach minnow and spikedace presence-absence using the same habitat variables across the two broadest spatial scales. As predicted, mean velocity was the environmental factor driving the model for both species. Water velocity influences multiple processes within streams and mediates many essential activities in the life history and well-being of fishes, such as increased food availability and required habitat (Barber & Minckley, 1983;

Facey & Grossman, 1992), and both species exhibit increased growth in high velocity habitats (CH, unpublished data). At the mesohabitat scale, loach minnows appear to require either shallow or very deep habitats with elevated velocity and coarse substrate sizes, aligning with our initial hypothesis. While loach minnow occurrences are very well described at the mesohabitat scale, no variables were able to predict spikedeace presence-absence at this scale, contradicting our hypothesis. This is likely because spikedeace is a vagile species, moving between a variety of high and low velocity mesohabitats and thus leading to our observation of no variable predicting their presence at this fine scale. Further, we believe higher flow velocities at the broader scales are indicative of higher habitat heterogeneity, potentially suggesting the importance of a variety of mesohabitats at the 0.1 km scale. Loach minnow seemingly require very specific fine scale requirements to be able to persist in a stream, while spikedeace might require several mesohabitats at fine scales.

While some studies have highlighted the need to control for spatial scale in fisheries research (Fausch et al. 2002; Hale et al. 2019), others highlight the importance of incorporating multiple scales (Rowe, Pierce, & Wilton, 2009; Cheek et al. 2016; Bruckerhoff, Pennock, & Gido, 2020). The differentiation of velocity required across scales highlights the discrepancy of what these values indicate. Habitat measured at the fine spatial scale predicts specific requirements for an individual at a point in time, while at broader scales, habitat might represent requirements of the population or individuals over larger time scales. Taking a layered approach to characterizing habitat needs for both species, enabled the incorporation of spatial considerations in regard to habitat and expands our current knowledge on species habitat requirements.

Spikedace and loach minnow have been repatriated to locations throughout the Gila River basin with varying degrees of success (Desert Fishes Team, 2003; Robinson, et al. 2009). Our data suggests that some areas where they have been repatriated, but failed, should not be considered in the future. In addition, our examination identified several potentially suitable locations for stocking these species (Tables 1.3-1.5). These determinations were made by considering suitable habitat at all scales, as well as using transect data to identify the percent of habitat that meets the mesohabitat criteria for loach minnow. We found a positive correlation with percentage of suitable habitat in relation to the density of loach minnow across the basin. With this analysis, we determined that Eagle Creek in Arizona (average velocity: 1-km = 0.21 m/s, one 0.1-km reach meeting criteria, 2.39% of stream possessed suitable fine scale habitat) is a potential location to repatriate loach minnow, as this species is not currently found in this stream, although historically it occupied it (Marsh et al. 2003), and it meets the requirements at all spatial scales. We also identified locations that should be avoided in regard to repatriating these species in the future as they do not appear to have suitable habitat. Specifically, Bonita Creek (average velocity: 1-km = 0.02 m/s, no 0.1-km reaches meet criteria), Oak Creek (average velocity: 1-km = 0.14 m/s, no 0.1-km reaches meet criteria), the San Pedro River (average velocity: 1-km = 0.15 m/s, no 0.1-km reaches meet criteria), Clear Creek (average velocity: 1-km = 0.12 m/s, no 0.1-km reaches meet criteria), Saliz Canyon (average velocity: 1-km = 0.12 m/s, no 0.1-km reaches meet criteria), and Redfield Canyon (average velocity: 1-km = 0.02 m/s, no 0.1-km reaches meet criteria) do not have an adequate amount of elevated velocity habitats at broad scales for either species, as well as having no suitable habitat for loach minnow at the fine scale.

With continued alteration of stream habitats coupled with ongoing stream dewatering in the American Southwest, elevated velocity habitats are declining and, in turn, diminishing suitable habitats for native species throughout the basin (Davey, Kelly, & Biggs, 2006; Stradmeyer et al. 2008). With continued loss and alteration of aquatic habitats, identifying the needs of imperiled species, especially at differing scales, is essential for sustaining these populations. For both species evaluated in this study, we found that transects spaced out at equal intervals are the most objective approach to depicting habitats available within a reach. For loach minnow, transect data can be used to calculate the percentage of habitat available for it using the mesohabitat scale model. For spikedace, transects provides a more general measure of habitat suitability that might reflect habitat heterogeneity. Because habitat requirements for both species are very similar at intermediate and broad spatial scales, suitable areas could have the potential for the management of both of these species.

This study presents key variables that need to be considered prior to repatriation of loach minnow and spikedace and how spatial scale affected the variables driving these patterns. The three tier approach, in regard to spatial scale, used during this study provided a robust assessment of a streams ability to support the two species in question. Of the variables included within our models, water velocity had the greatest predictive power. While other factors must be considered in regard to repatriating these species, such as the presence of nonnative fishes, we provide a baseline for the abiotic requirements for these species. We recommend a widespread habitat assessment, using habitat transects, to identify locations throughout the basin where we can expand our current repatriation program and establish more self-sustaining populations of loach minnow and spikedace.

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Figures

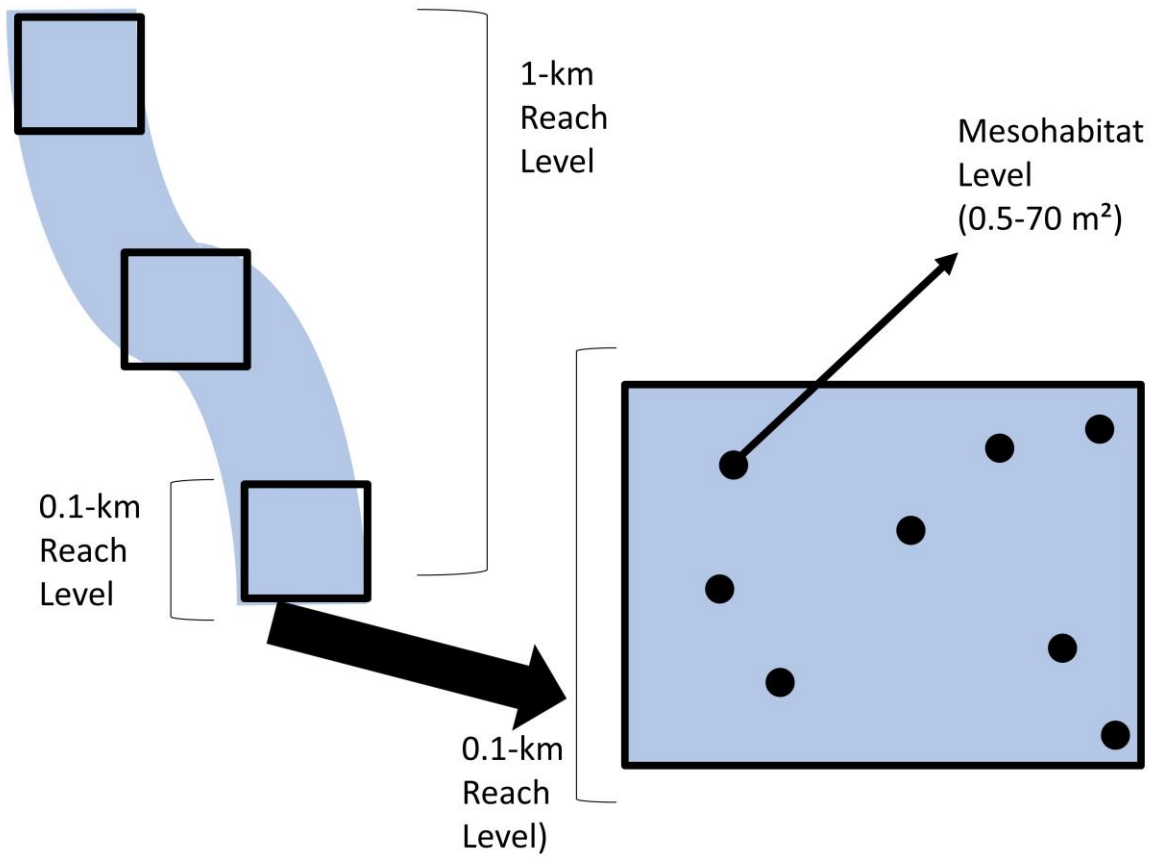


Figure 1.1. Visual representation of the three differing spatial scales sampled at each reach throughout the Gila River basin.

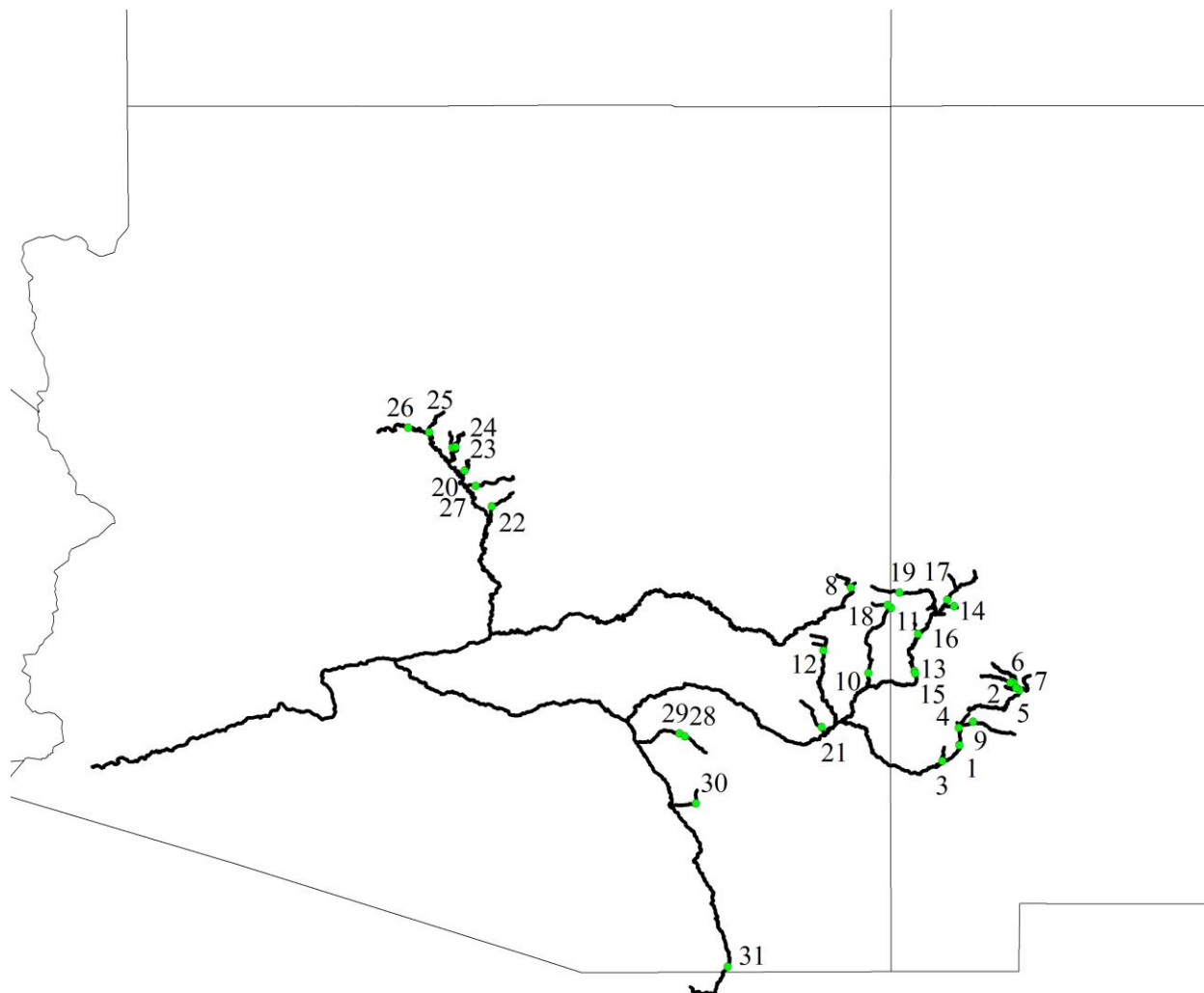


Figure 1.2 Map of reaches sampled throughout the course of this study in 2019 and 2020 in Arizona and New Mexico. Numbers correspond to locations reported on Table 1.3.

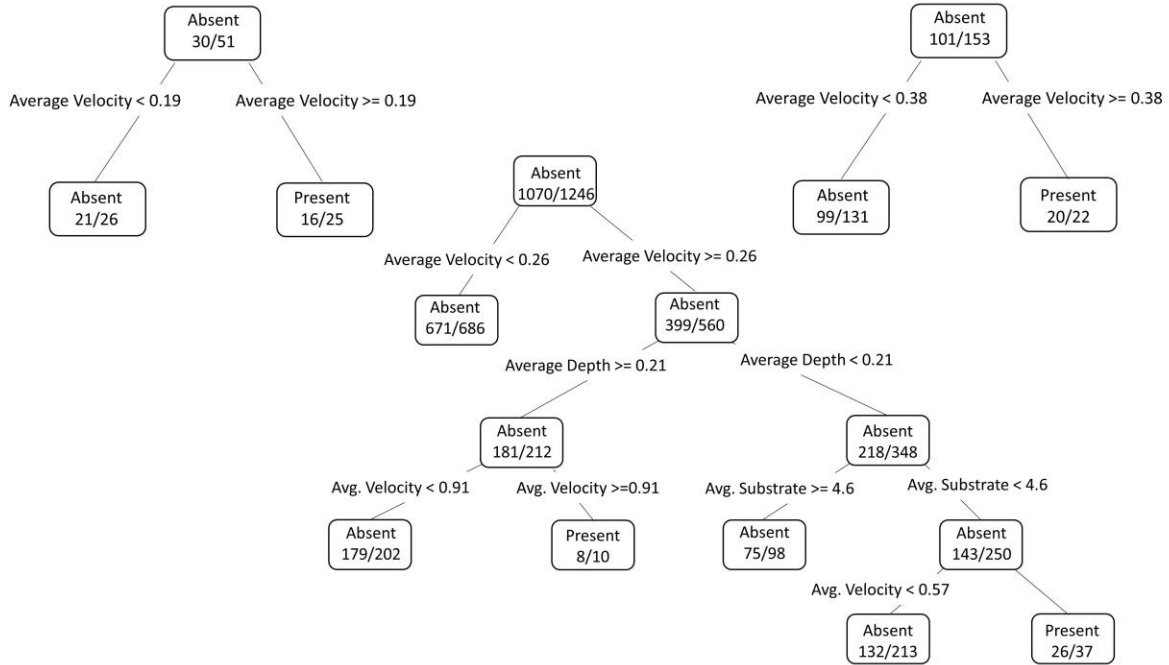


Figure 1.3 Classification tree for the loach minnow describing variables that predict species presence-absence at three differing spatial scales: 1-km reach (top left), 0.1-km reach (top right), and mesohabitat (bottom). Numbers within each box denote the number of observations where this species is truly present or absent (numerator), and the number of habitats that meet the requirements being described with no reference to fish presence absence (denominator).

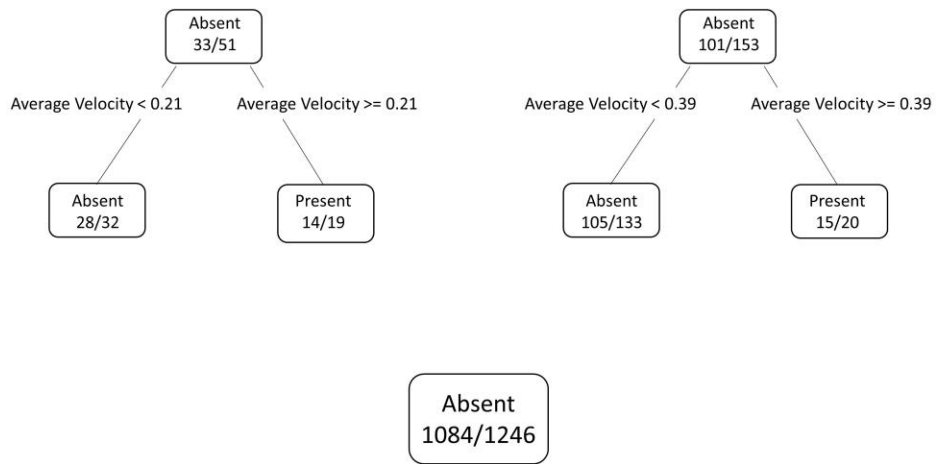


Figure 1.4 Classification tree for the spikedeace describing variables that predict species presence-absence at three differing spatial scales: 1-km reach (top left), 0.1-km reach (top right), and mesohabitat (bottom). Numbers within each box denote the number of observations where this species is truly present or absent (numerator), and the number of habitats that meet the requirements being described with no reference to fish presence absence (denominator).

Tables

Table 1.1 List of habitats sampled over the course of this study throughout the Gila River basin.

Means and standard deviations are reported for depth, velocity, and substrate sizes.

Habitat	Average Depth (m)	Average Velocity (m/s)	Average Substrate Size	Standard Deviation of Depth	Standard Deviation of Velocity	Standard Deviation of Substrate Size
Backwater	0.12	0.02	2.67	0.17	0.03	1.57
Chute	0.27	0.50	4.04	0.20	0.32	1.66
Eddie	0.19	0.04	3.39	0.14	0.07	1.68
Embayment	0.15	0.02	2.56	0.07	0.04	1.86
Plunge Pool	0.23	0.09	3.33	0.14	0.06	2.89
Pool	0.47	0.05	2.96	0.33	0.08	1.65
Pool-Run	0.28	0.20	3.39	0.18	0.14	1.40
Pool-Shore	0.22	0.04	2.83	0.18	0.09	1.57
Riffle	0.12	0.32	4.14	0.10	0.23	1.06
Riffle-Run	0.15	0.30	4.15	0.11	0.21	1.06
Run	0.19	0.25	3.65	0.15	0.17	1.31
Run-Shore	0.13	0.11	3.32	0.10	0.13	1.46
Shoal	0.12	0.07	3.08	0.12	0.09	1.33

Table 1.2 Values for Mean Gini Decrease for loach minnow and spikedace at all spatial scales in which Classification Trees generated from Random Forest with 500 iterations that were able to describe the presence of these species. Values indicate node purity identifying which variables were able to most accurately predict the presence of these species.

Loach Minnow	Variable	Mean Decrease Gini		Variable	Mean Decrease Gini	Spikedace
	Mean Depth	6447	0.1-km Reach	Mean Depth	8719	
	Mean Velocity	21577		Mean Velocity	35366	
	Mean Substrate Size	3784		Mean Substrate Size	11876	
	Variable	Mean Decrease Gini	1-km Reach	Variable	Mean Decrease Gini	
	Mean Depth	24907		Mean Depth	42263	
	Mean Velocity	31721		Mean Velocity	45898	
	Mean Substrate Size	6343		Mean Substrate Size	21163	
	Variable	Mean Decrease Gini	Mesohabitat			
	Mean Depth	4018				
	Mean Velocity	4534				
	Mean Substrate Size	1672				

Table 1.3 Number of 1-km reaches that meet habitat requirements for loach minnow and spikédace. Areas where spikédace currently occur are denoted with the superscript SD, while areas where loach minnow currently occur are denoted with the superscript LM.

Location	Meets Loach Minnow Requirements	Meets Spikédace Requirements	Key in Reference to Site Map
Gila River - Bird Area ^{LM,SD}	Yes	Yes	1
Gila River – Grapevine ^{LM,SD}	Yes	Yes	2
Gila River - Middle Box ^{LM}	Yes	No	3
Gila River - Riverside	Yes	Yes	4
Gila River West Fork ^{LM,SD}	No	No	5
Gila River Middle Fork	Yes	No	6
Little Creek	No	No	7
Black River	No	No	8
Bear Creek ^{LM}	No	No	9
Blue River	No	No	10
Campbell Blue River ^{LM}	No	No	11
Eagle Creek ^{SD}	Yes	Yes	12
Lower San Francisco River	Yes	Yes	13
Lower Tularosa River	Yes	No	14
San Francisco River - Glenwood ^{LM,SD}	Yes	Yes	15
Saliz Canyon	No	No	16
Tularosa River - Hell's Hole ^{LM}	Yes	No	17
Upper Blue River ^{LM}	Yes	No	18
Upper San Francisco River	No	No	19
Wet Beaver Creek	No	No	20
Bonita Creek	No	No	21
Fossil Creek ^{SD}	Yes	Yes	22
Oak Creek	No	No	23
Spring Creek ^{SD}	No	No	24
Sycamore Creek	No	No	25
Upper Verde River	No	No	26
West Fork Clear Creek	No	No	27
Aravaipa Creek ^{LM,SD}	Yes	Yes	28
Lower Aravaipa Creek ^{LM,SD}	Yes	Yes	29
Redfield Canyon	No	No	30
San Pedro River	No	No	31

Table 1.4 Number of 0.1-km reaches that meet habitat requirements for loach minnow and spikedace. Areas where spikedace currently occur are denoted with the superscript SD, while areas where loach minnow currently occur are denoted with the superscript LM.

Location	0.1 km Reaches that Meet Loach Minnow Requirements	0.1 km Reaches that Meet Spikedace Requirements	Total 0.1 km Reaches Sampled
Gila River - Bird Area ^{LM,SD}	0	0	3
Gila River – Grapevine ^{LM,SD}	2	2	3
Gila River - Middle Box ^{LM}	0	0	3
Gila River - Riverside	4	4	6
Gila River West Fork ^{LM,SD}	2	2	6
Gila River Middle Fork	0	0	3
Little Creek	0	0	6
Black River	0	0	6
Bear Creek ^{LM}	0	0	3
Blue River	0	0	6
Campbell Blue River ^{LM}	1	1	6
Eagle Creek ^{SD,}	1	0	6
Lower San Francisco River	2	2	3
Lower Tularosa River	0	0	6
San Francisco River - Glenwood ^{LM,SD}	2	2	6
Saliz Canyon	0	0	6
Tularosa River - Hell's Hole ^{LM}	1	0	3
Upper Blue River ^{LM}	0	0	3
Upper San Francisco River	0	0	3
Wet Beaver Creek	0	0	6
Bonita Creek	0	0	6
Fossil Creek ^{SD}	0	0	9
Oak Creek	0	0	3
Spring Creek ^{SD}	0	0	9
Sycamore Creek	0	0	3
Upper Verde River	0	0	6
West Fork Clear Creek	0	0	3
Aravaipa Creek ^{LM,SD}	5	5	6
Lower Aravaipa Creek ^{LM,SD}	3	3	3
Redfield Canyon	0	0	3
San Pedro River	0	0	6

Table 1.5 Number of habitat points and percentage of suitable habitat from transect data that meets the fine scale habitat requirements for loach minnow. Streams that currently have loach minnow present are denoted with the superscript LM.

Location	Points That Meet Loach Minnow Requirements	Total Points Taken	Percent of Suitable Habitat
Aravaipa Creek ^{LM}	82	666	12.31
Lower Aravaipa Creek ^{LM}	32	288	11.11
Fossil Creek	20	276	7.25
Gila River - Riverside ^{LM}	20	342	5.85
Lower San Francisco River*	7	217	3.23
Eagle Creek	10	418	2.39
Spring Creek	6	395	1.52
San Francisco River - Glenwood ^{LM}	5	344	1.45
Gila River - Bird Area ^{LM}	3	209	1.44
Lower Tularosa River	7	499	1.40
Gila River - Grapevine ^{LM}	2	247	1.21
Gila River West Fork ^{LM}	6	544	1.10
Little Creek	3	273	1.10
Upper Verde River	4	565	0.71
Campbell Blue River	3	587	0.51
Gila River - Middle Box ^{LM}	1	196	0.51
Upper San Francisco River	1	282	0.35
Beaver Creek	1	301	0.33
Blue River ^{LM}	1	305	0.33
Upper Blue River ^{LM}	1	333	0.30
Bonita Creek	1	353	0.28
Bear Creek ^{LM}	0	225	0
Black River	0	542	0
Tularos River - Hell's Hole ^{LM}	0	99	0
Middle Fork Gila River	0	266	0
Oak Creek	0	163	0
Redfield Canyon	0	216	0
Saliz Canyon	0	411	0
San Pedro River	0	415	0
Sycamore River	0	264	0
West Fork Clear Creek	0	172	0

Chapter 2 - How Fast is too fast? Water velocity differentially effects growth of four Gila River, native cyprinids

Abstract

Lotic systems are highly heterogeneous, providing a range of habitats for stream fishes. Understanding trade-offs associated with occupying various habitats provides a mechanistic understanding of habitat needs that can be used to evaluate the consequences of habitat loss or alteration. The objective of this study was to identify how velocity affects the growth rates of four native species in the upper Gila River basin, USA: spikedace (*Meda fulgida*), loach minnow (*Tiaroga cobitis*), longfin dace (*Agosia chrysogaster*), and speckled dace (*Rhinichthys osculus*). Elevated velocity was predicted to increase food delivery through drift or through stimulating benthic primary production. Energetic costs of high velocity habitat were predicted to vary with morphology and behavior and would be lowest for speckled dace and loach minnow, because they are adapted to living in interstitial spaces of the substrate in riffles. Spikedace and longfin dace should perform best in moderate velocities, where there is a trade-off between exposure to drifting macroinvertebrates and the energetic costs of maintaining position in the water column. As predicted, growth rates of loach minnow and speckled dace increased in higher velocities but, contrary to our initial predictions, spikedace growth rates also increased in high velocity habitats while longfin dace grew fastest in low velocity habitats. These results indicate that for spikedace, the increased abundance of drifting macroinvertebrates in high velocity habitats out-weighs the energy expenditure, but for longfin dace the energetic costs of occupying high velocity habitats out-weighs the benefit to increased food availability. Our experimental manipulations provide a mechanistic understanding of habitat requirements across species and might inform predictions on how modifications or restoration of riverine ecosystems influence native fish diversity.

Introduction

Habitat use by stream fishes can be influenced by resource availability, energetic costs, predator avoidance, and competition (Werner et al. 1983; Hill & Grossman, 1993; Thompson, Petty & Grossman, 2001). Partitioning of resources along habitat gradients (i.e. depth, velocity, substrate, cover) can facilitate species coexistence (Fausch et al. 2002) and influences the composition of fish assemblages (Ross, 2013). As specific habitats are reduced or eliminated from aquatic systems, species either cannot survive because those habitats are critical for their survival or they are forced into habitats with other species where coexistence may be compromised. The loss or reduction of essential habitats facilitates the biotic homogenization of fish assemblages by reducing native species and potentially favoring the invasion of new species (Walters, Leigh, & Bearden, 2003). Therefore, gaining a mechanistic understanding of species habitat use can aid conservation efforts by providing information to focus habit preservation and restoration activities on biologically relevant attributes.

Water velocity is tied to both abiotic and biotic characteristics of habitats in lotic systems (Frissell et al. 1986). Elevated velocity habitats typically have a higher biomass of benthic macroinvertebrates, due to increased exchange of nutrients that increase algal and insect production (Buffagni & Comin, 2000). While food availability is elevated in these high velocity habitats, energetic costs associated with increased swimming exertion can also be higher, but is not always the case due to body morphology and behavior (Hill & Grossman, 1993). The trade-off between increased food availability and increased energetic costs of high velocity habitats contributes to shaping a species niche in a way that promotes the maximum amount of energy intake (feeding) while minimizing the amount of energy expenditure, although other factors are in play. Thus, the velocity gradients that occur naturally within lotic systems should include

habitats to which their life history traits optimize energy intake to expenditure trade-offs.

Conversely, a species that are forced to occupy habitat that does not align with its autecology should suffer from negative fitness consequences.

In this study, we used a stream enclosure experiment to investigate how velocity influenced adult growth of four small-bodied cyprinids native to the Gila River basin: longfin dace (*Agosia chrysogaster*), speckled dace (*Rhinichthys osculus*), spikedace (*Meda fulgida*), and loach minnow (*Tiaroga cobitis*) (Figure 2.1). In 2019, this experiment was conducted with speckled dace and longfin dace, while in 2020 it was conducted with the federally-endangered loach minnow and spikedace (U.S. Fish and Wildlife Service, 2012). To visualize our predictions for this study, we modified a conceptual figure that builds upon the work of Hill & Grossman (1993), depicting the differences in energy expenditure, food availability and feeding efficiency in response to a velocity gradient for both drift and benthic feeding fishes (Figure 2.2). Of the focal species, speckled dace and loach minnow are benthic, feeding primarily on benthic macroinvertebrates and are typically found in elevated velocity habitats (Figure 2.2B; Schreiber & Minckley, 1981; Moyle & Baltz, 1985; Bonar, Mercado-Silva & Rogowski, 2010; Pilger, Gido & Propst, 2010). While both of these species are typically found in elevated velocity water, loach minnow is a riffle obligate, very rarely making forays out of these habitats, while speckled dace are not obligate and can be found in a variety of habitats. Longfin dace and spikedace feed higher in the water column and occupy an array of habitats including pools, runs, glides, and riffles (Figure 2.2A; Stefferud, Gido & Propst, 2011). Longfin dace are omnivores feeding on drifting invertebrates, benthic macroinvertebrates, algae, and terrestrial inputs (Bonar, Mercado-Silva & Rogowski, 2010; Pilger, Gido & Propst, 2010). Spikedace are less omnivorous than longfin dace and rely more strongly on drifting invertebrates and terrestrial inputs. We predicted

that adult speckled dace and loach minnow would have increased growth in high velocity habitats and the lowest growth in moderate to low velocity habitats (Figure 2.2B). This is due to their morphology and behavior allowing them to remain on the substrate while minimizing energy expenditure. Furthermore, elevated velocity habitats typically have increased production and increased aquatic macroinvertebrate biomass, enhancing fish growth (Hintz & Wellnitz, 2013). We further predicted that longfin dace and spikédace would have the highest growth in moderate velocities and the lowest growth in low and high velocity habitats (Figure 2.2A). This is due to increased energetic cost associated with elevated velocities for a water column species (Hill & Grossman, 1993), as well as the lower amount of resource availability in low velocity habitats (Buffagni & Comin, 2000). While we predicted these species would have similar responses in growth to velocity; spikédace should perform better at marginally higher velocities than longfin dace due to their fusiform body and embedded scales, presumably adaptations to elevated velocity.

Methods

Study Site

This experiment took place in the West Fork Gila River in southwestern New Mexico, USA. The experiment was conducted approximately 0.65 km downstream of the confluence of the West and Middle forks Gila River. The Gila River upstream of our study site has a natural flow regime and the watershed is almost entirely within US Forest Service managed lands. The stream in this area has an average width of 6.9 m and an average discharge of 0.23 m³/s in the months this experiment was conducted. The upper Gila River possesses a cottonwood-boxelder-willow riparian area in a narrow valley at an elevation of approximately 1700 m.

Enclosure Experiment

In June 2019, three speckled dace (mean total length [TL] = 57.5 mm, SD = 3.4; mean weight = 2.04 g, SD = 0.40) and three longfin dace (mean TL = 55.3 mm, SD = 4.1; mean weight = 1.98 g, SD = 0.43) were placed together (6 individuals per cage) in each of 18 enclosures (0.914 x 0.914 m, 6.35 mm mesh) across a velocity gradient (range: 0.00 – 0.57 m/sec) to test how velocity and aquatic invertebrate abundances affected the growth of these species.

Enclosures were maintained for 30 days. Fishes for the experiment were collected using a combination of backpack electrofishing (Smith-Root LR 20B, 200-250 V, Freq: 30, Duty Cycle: 30) and seining (4.6 x 1.8 m, 3.2 mm mesh) from the experimental reach. In July 2020 this experiment was repeated with spikedace (mean TL = 58.8 mm, SD = 3.4; mean weight = 1.8 g, SD = 0.37) and loach minnow (mean TL = 57.5 mm, SD = 3.5; mean weight = 2.00 g, SD = 0.37) across a velocity range of 0.00 – 0.97 m/sec. Enclosures were maintained for 46 days. For all four species, only age-1 individuals over 50 mm were used during this experiment so that fish would not be able to escape through the mesh of the enclosures. Because this study took place during the breeding season for each species, each fish was carefully evaluated to ensure that no gravid individuals were placed in enclosures. Prior to the beginning of the experiment, each individual was anesthetized with MS-222 while being measured, weighed, and uniquely marked (to enable individual identification) with a distinguishable Visible Implant Elastomer (VIE) Tag, and assigned cage placement. To minimize handling, fish were only measured prior to placement in enclosures and following extraction at the conclusion of the experiment. Following measurement, fish recovered in a cooler for a minimum of 10 minutes before being released into enclosures. When mortality occurred in an enclosure, the individual was replaced as long as there was at least 2 weeks remaining in the experiment. All analyses were converted to growth per-day

because individuals were held for different lengths of time. Depth, velocity, and substrate composition within each cage were measured every 10 days during the experiment. We filled enclosures with substrate that was representative of the habitat they were placed in (e.g., silt/sand in low velocity habitats and pebble/cobble in high velocity habitats). All cages were given a few large cobbles to provide cover for fish. Cage mesh was cleaned of algae two to five times daily to minimize the effect of algae and debris accumulation on velocity within each enclosure. To estimate drifting invertebrate biomass, drift net samples (0.22 x 0.23 m opening, 1 mm mesh) were taken over thirty minutes in the evening (between 1700 and 1900 hrs) for three to seven nights a week in randomly selected enclosures. Two to three samples were taken in each enclosure over the course of the experiment. In 2019, nets were placed inside enclosures and directly adjacent to enclosures to determine if enclosure mesh reduced the biomass of drifting aquatic invertebrates available to fish. To test if biomass of benthic macroinvertebrates were affected by velocity, core samples were taken every two weeks. Only invertebrates ≤ 6 mm in length were analyzed, because we assumed larger invertebrates would not pass through the mesh. All macroinvertebrates were identified to order, with the exception of common dipterans and hemipterans that were identified to family.

Data analysis

We used mixed-effects models to test for differences in daily growth rates (change in length and change in weight per day) as a function of water velocity. Models were fit using the *lmer* function in the *lme4* package implemented in R, version 3.5.2 (Bates et al., 2014; R Core Team, 2018). We included cage number as a random effect to account for differences in potentially confounding factors such as depth and substrate composition across cages and focused on the fixed effect of velocity. We evaluated significance by comparing an intercept

only model to a model with additive fixed effects of velocity using a Wald chi-square test. Further, we used the *lm* function in the *lme4* package to test if drifting and benthic macroinvertebrate biomass increased with velocity. To evaluate potential cage effects on invertebrate biomass, paired t-tests were used to test for differences between the average biomass of samples taken in the enclosures versus those collected in the river for both benthic and drifting invertebrates.

Results

Each species in this experiment experienced significant changes in total length or mass, or both, in response to the velocity gradient, with three species responding positively to increased velocity and one responding negatively. Further, in both years of this study the average velocity within each cage decreased throughout the study (Figure 2.3). In 2019, longfin dace in low velocity enclosures gained more weight compared to individuals in high velocity enclosures, increasing by as much as 0.015 g/d at low velocities relative to high velocities ($p = 0.02$; Figures 2.4 & 2.5). However, no significant differences were observed in length ($p = 0.30$; Figure 2.4). In contrast, speckled dace grew faster as measured in total length ($p < 0.001$; Figure 2.4), increasing by as much as 0.30 mm/d, and in weight ($p = 0.03$; Figure 2.4) increasing by as much as 0.05 g/d in high velocity habitats.

Both spikédace and loach minnow performed better in high velocity habitats in 2020. Spikédace gained more weight in elevated velocities ($p = 0.03$) growing by as much as 0.26 g/d more than in lower velocity enclosures (Figure 2.5). While spikédace weight increased in response to elevated velocity, change in length did not respond to velocity ($p = 0.48$; Figure 2.4). In one enclosure, with an average velocity of 0.57 m/s, spikédace were not able to maintain themselves for more than 24 hours at the beginning of the experiment, potentially indicating a

physiological threshold for what this species can tolerate for prolonged periods of time. Lastly, total length ($p = 0.01$) and mass ($p < 0.001$) for loach minnow were positively correlated with velocity (Figures 2.4 & 2.5).

In 2019, biomass of both drifting and benthic invertebrates did not differ between inside and adjacent to the cages. Drifting invertebrates had increased biomass in higher velocity in both the enclosures and the river ($p = 0.008$ & 0.04 respectively), while benthic invertebrates did not respond to velocity in either the enclosures or river ($p > 0.21$; Figure 2.6). In 2020, drifting aquatic invertebrate biomass was 3.6 times greater in high velocity rather than in lower velocity habitats, although a significant relationship was not observed ($p = 0.056$). Benthic invertebrate biomass increased with velocity in 2020 ($p = 0.04$; Figure 2.7). There was a shift in invertebrate community composition with velocity; chironomidae and trichopteran larvae were collected in highest abundance in elevated velocity, while corixid adults were the most abundant species collected in slower velocity habitats. Lastly, t-tests showed that there was no difference in invertebrate biomass of either benthic ($p = 0.29$) or drifting invertebrates ($p = 0.38$) between samples within enclosures and those adjacent to enclosures in 2019.

Discussion

Three of the four species tested in the enclosures experienced increased growth with increasing velocity, confirming our initial hypotheses for loach minnow and speckled dace, but not spikedace. In field studies conducted to evaluate the habitat use of native fish in the Gila River basin, loach minnow occupied habitats had an average velocity of 0.59 m/s (± 0.93 SD) and speckled dace occupied habitats with an average velocity of 0.30 m/s (± 0.21 SD; CH, unpublished data), which is similar to the maximum velocities in our field enclosures for loach minnow in 2020 (maximum velocity 0.57 m/s) but not for speckled dace in 2019 (maximum

velocity 0.44 m/s). Further, the slope of the relationship between velocity and growth for loach minnow was greater than that for speckled dace or spikedace, indicating loach minnow might gain a larger benefit from high velocity habitats. Whereas both loach minnow and speckled dace are benthic species with a hydrodynamic morphology (e.g. down turned snout, large pectoral fins, and flat ventral), speckled dace can be found in habitats with relatively low velocity while loach minnow are a riffle obligate. Moreover, loach minnow has a reduced swim bladder, a trait not shared with speckled dace (Propst & Bestgen, 1991), providing it an enhanced ability to maintain position and navigate in elevated velocities with minimal energy expenditure.

Linear increase in growth of spikedace with velocity contradicted our expectation of a unimodal distribution in response to a velocity gradient. Spikedace have an extremely streamlined body with deeply embedded scales, adaptations for occupying habitat with elevated velocity. Our results suggest this morphology allows spikedace to occupy elevated velocity habitats, with increased food supply, without exacerbating energetic costs. However, we did have one enclosure, with an average velocity of 0.57 m/s, where spikedace could not sustain themselves for more than 24 hours, and were unable to include this in our analyses. Although we were not able to identify a threshold where energy expenditure outweighed the costs of increased food supply, this observation, albeit only a single enclosure, suggests a threshold response at where this species can no longer maintain itself for extended periods of time. Douglas et al. (1994) observed that in the presence of nonnative red shiner (*Cyprinella lutrensis*), spikedace occurred mainly in higher velocity habitats (mean velocity 0.40 m/s) than when red shiner is not present (mean velocity 0.36 m/s), a marginal difference. Interestingly, the shift to higher velocity was close to the optimal velocities for growth found in our experiment questioning if the shift in habitat was detrimental to the species. While this displacement affects multiple factors in a

species life, combined field observations and our results generally suggest a performance benefit associated with elevated velocity habitats for spikedace.

Longfin dace fared best in low velocity habitats, contradicting our hypothesis that they would perform best in moderate velocities and indicating that its well-being is not linked to availability of drifting invertebrates. Field observations suggest longfin dace occupy a range of habitat types with an average overall velocity of 0.30 m/s (SD 0.18; CH, unpublished data), which is only slightly less than for spikedace and contradicts results from our experiment. While field data shows this species can be found in moderate velocity habitats, the energetic costs of occupying these habitats for prolonged periods of time might offset the benefits for longfin dace. Further, longfin dace is an omnivore (Minckley 1973; Grimm, 1988; Pilger, Gido & Propst, 2010), thus growth rates might not be dependent on biomass of invertebrate prey. We also noted a slight increase in temperatures in lower velocity habitats; lowest velocity enclosure was 2° C warmer than highest velocity enclosure, which serve as a potential benefit for this species if this pushes them closer to their optimal thermal requirements (Bonar, Mercado-Silva & Rogowski, 2010). In contrast to the other species evaluated, the trade-off between energetic costs and resource availability along a velocity gradient are different for longfin dace.

In both 2019 and 2020, the biomass of drifting invertebrates was higher in enclosures with higher velocity, which was not surprising with an overall increase in water volume moving through these cages. In 2019, the biomass of benthic invertebrates did not change with velocity but in 2020 significantly increased with velocity, as reported in other studies (Gibbins, Vericat & Batalla, 2010). It is possible that any increases in benthic invertebrates associated with velocity in 2019 were offset by consumption by fish, which may not have occurred in 2020 because of higher overall production. Thus, higher abundance of invertebrates in high velocity treatments is

likely responsible for increase growth rates. Indeed, shifts in assemblage composition towards more chironomidae larvae in high velocity habitats, is also consistent with findings in Pilger et al. (2010), where chironomid larvae are a frequent item in the diet of loach minnow, speckled dace, and spikedace while contributing less to the diet of longfin dace.

This study suggests mechanisms that drive habitat use of different species, highlighting that the two endangered species in the Gila River basin prefer elevated water velocity. Repatriation of spikedace and loach minnow is ongoing and has had varying degrees of success across the basin. We found a comparatively narrow range of velocities influenced growth of native cyprinids; nonetheless there are other factors limiting repatriation success. Increased growth rates of endangered species in higher velocity habitats also might suggest consequences of habitat alteration. Dewatering is known to degrade and cause the loss of certain habitats, particularly those of elevated velocity (Davey, Kelly, & Biggs, 2006; Stradmeyer et al. 2008; Perkin et al. 2015), which might disproportionately affect speckled dace, loach minnow, and spikedace. It has been observed that during dewatering events, fishes that typically occupy riffles are forced to relocate to pool habitats due to the loss of these high velocity areas (Stradmeyer et al. 2008). As velocity changes from what a species prefers the muscle composition, growth, and body condition of an individual can also change over time, and, thus, loss of habitat will negatively affect the individual being displaced (Hill & Grossman, 1993; Young, Cech Jr., & Thompson, 2011). Moreover, as drought is projected to become more common across the American Southwest in the coming decades, which likely will cause diminished water levels in rivers, knowledge of how this affects the habitat and well-being of a variety of species within the region (Morehouse, 2000; Cook, Ault, & Smerdon, 2015) will be critical to their conservation.

Acknowledgements

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Figures

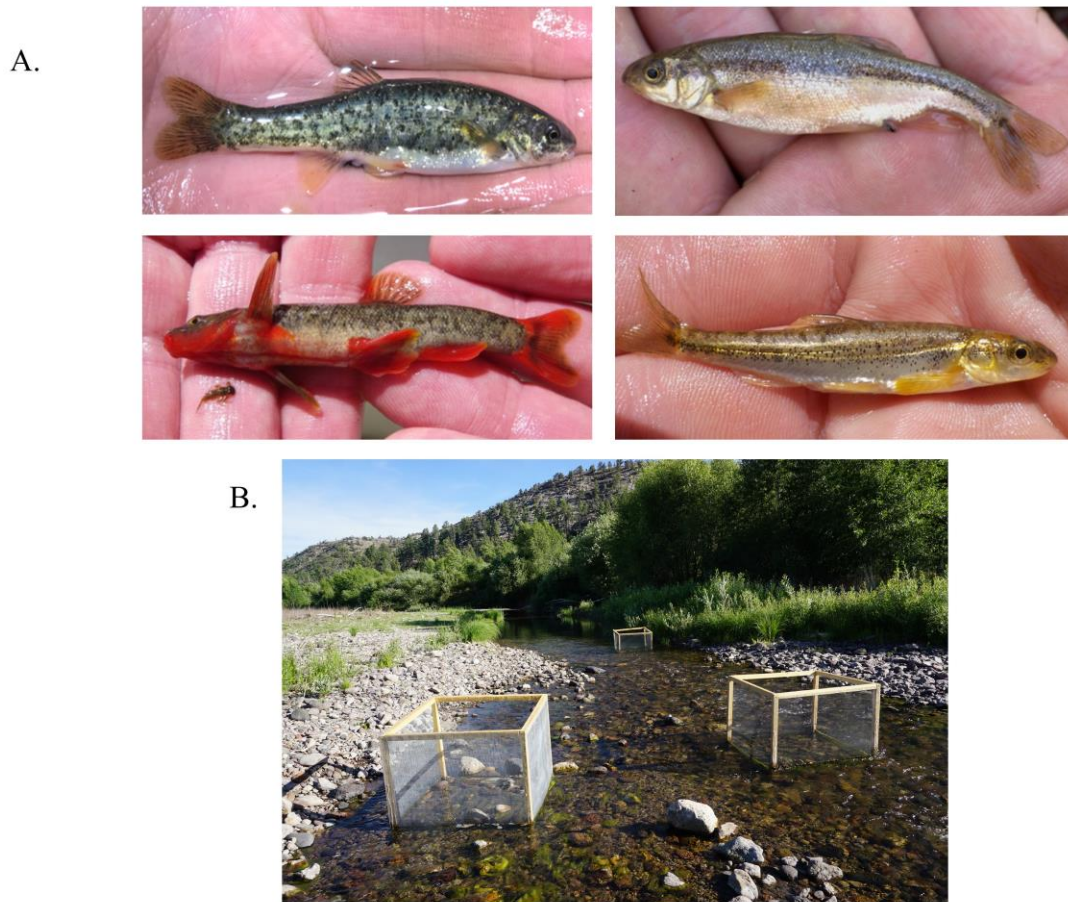


Figure 2.1 The four native species used in the enclosures experiment (A) and the enclosures within the West Fork of the Gila River (B). The four species used in the experiment are: Speckled Dace (top left), longfin dace (top right), loach minnow (bottom left), and spikedace (bottom right).

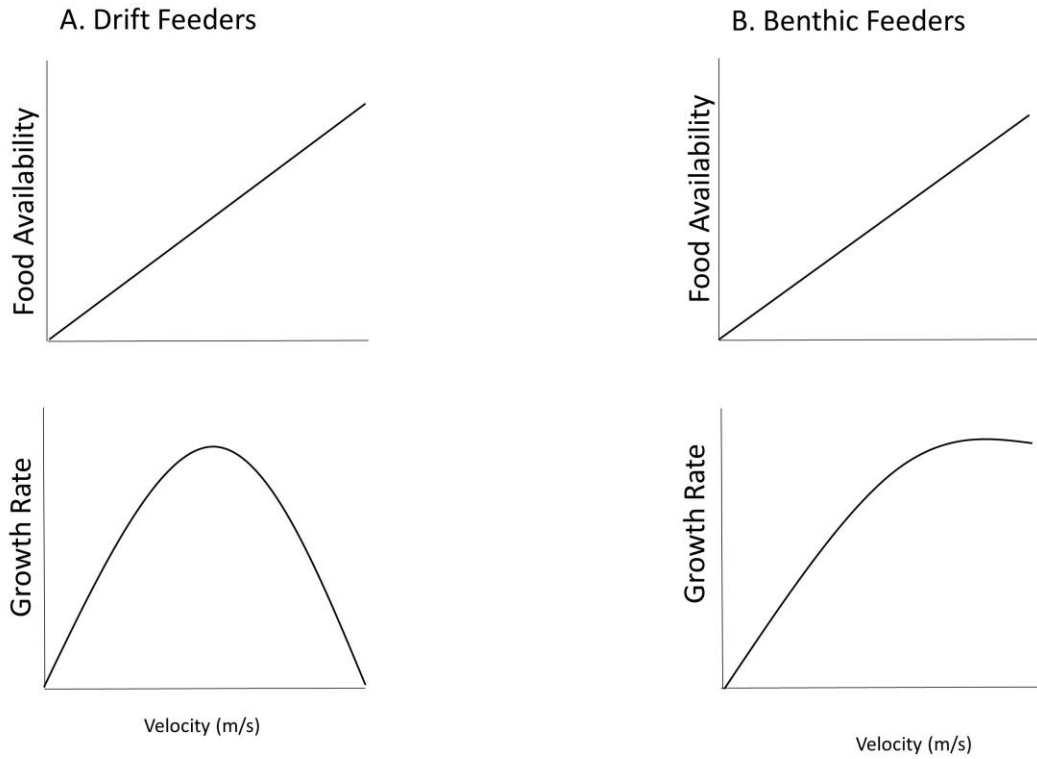


Figure 2.2 A graphical depiction of our predictions in regard to the response of food availability and growth rate of drift and benthic feeders.

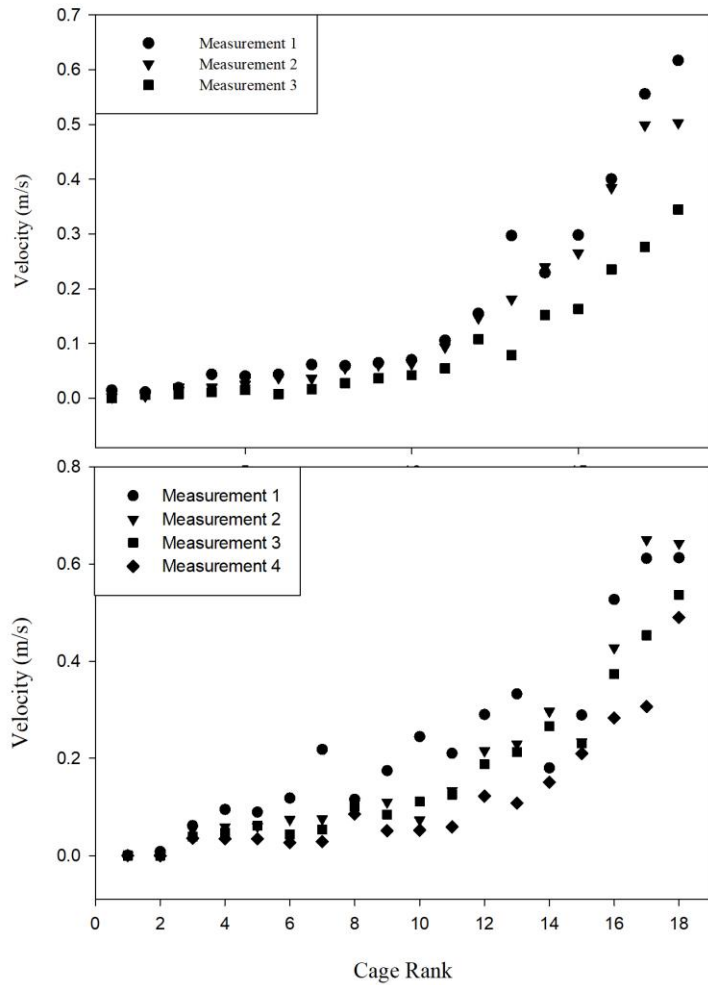


Figure 2.3 Variation in velocity within each enclosure over the course of the study in 2019 (top) and 2020 (bottom). Cage rank (1 = lowest mean velocity enclosure, 18 = highest mean velocity enclosure) is on the x-axis and velocity (m/s) is on the y-axis.

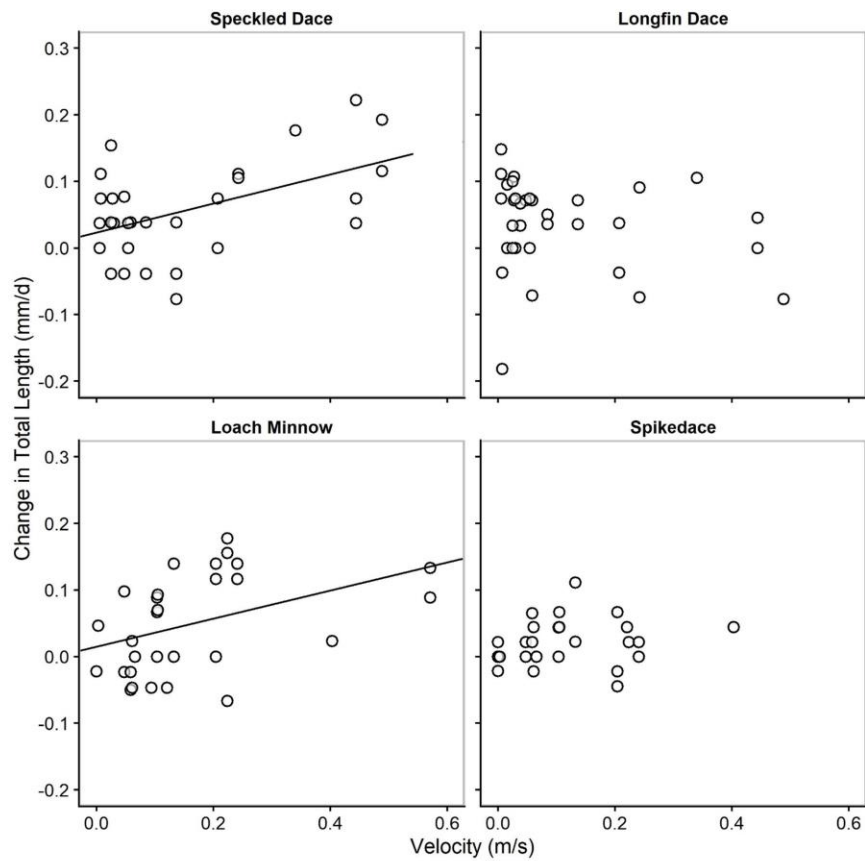


Figure 2.4 Relationship between enclosure velocity and individual growth rate, measured as change in total length (mm/d) between the beginning and end of the experiment for speckled dace, longfin dace, loach minnow, and spikedace. Each dot represents an individual fish and trend-lines are only included for significant relationships.

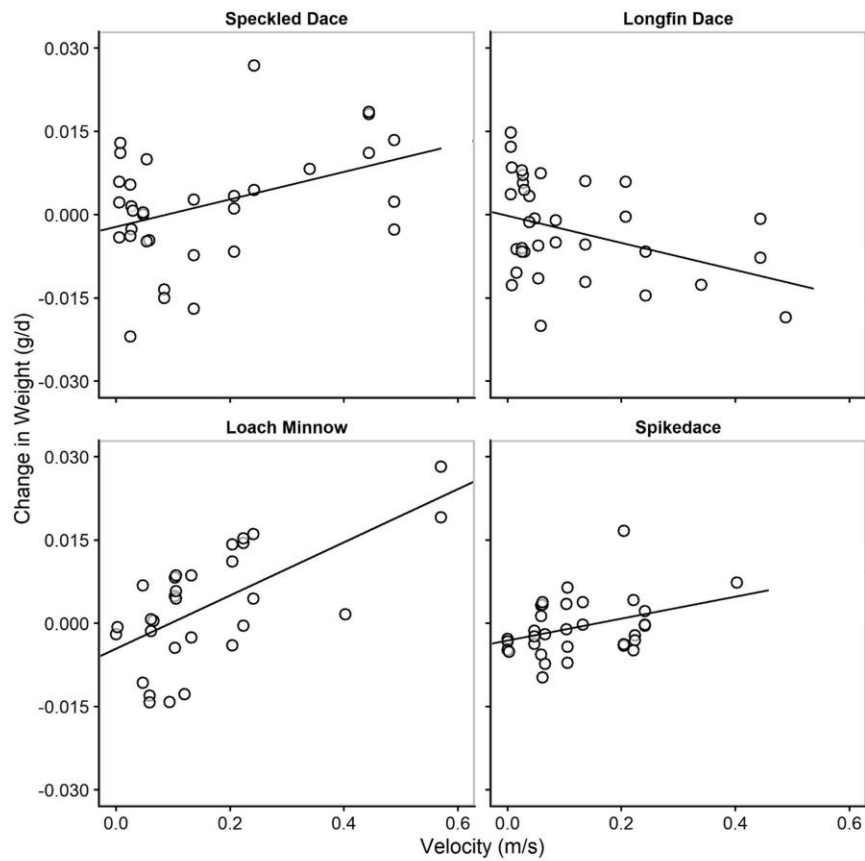


Figure 2.5 Relationship between enclosure velocity and individual growth rate, measured as change in mass (g/d) between the beginning and end of the experiment for speckled dace, longfin dace, loach minnow, and spikedace. Each dot represents an individual fish and trend-lines are only included for significant relationships.

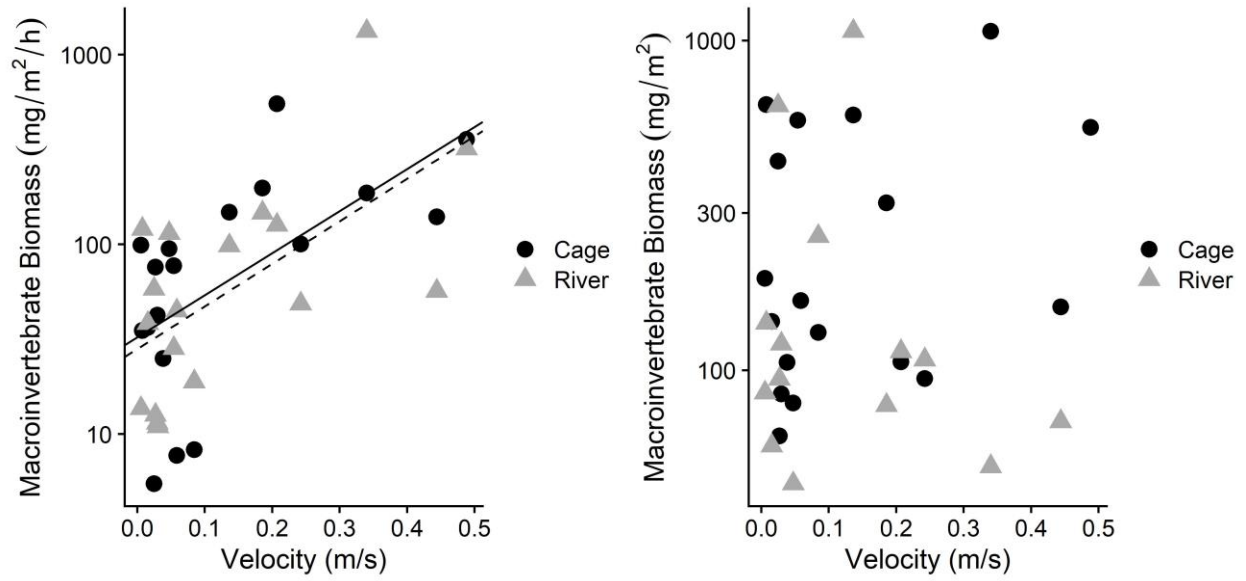


Figure 2.6 Biomass of drifting (left) and benthic (right) macroinvertebrates < 6 mm in enclosures (circles, black) and outside of enclosures (triangles, grey) across a velocity gradient in 2019. Both Y-axes are log transformed.

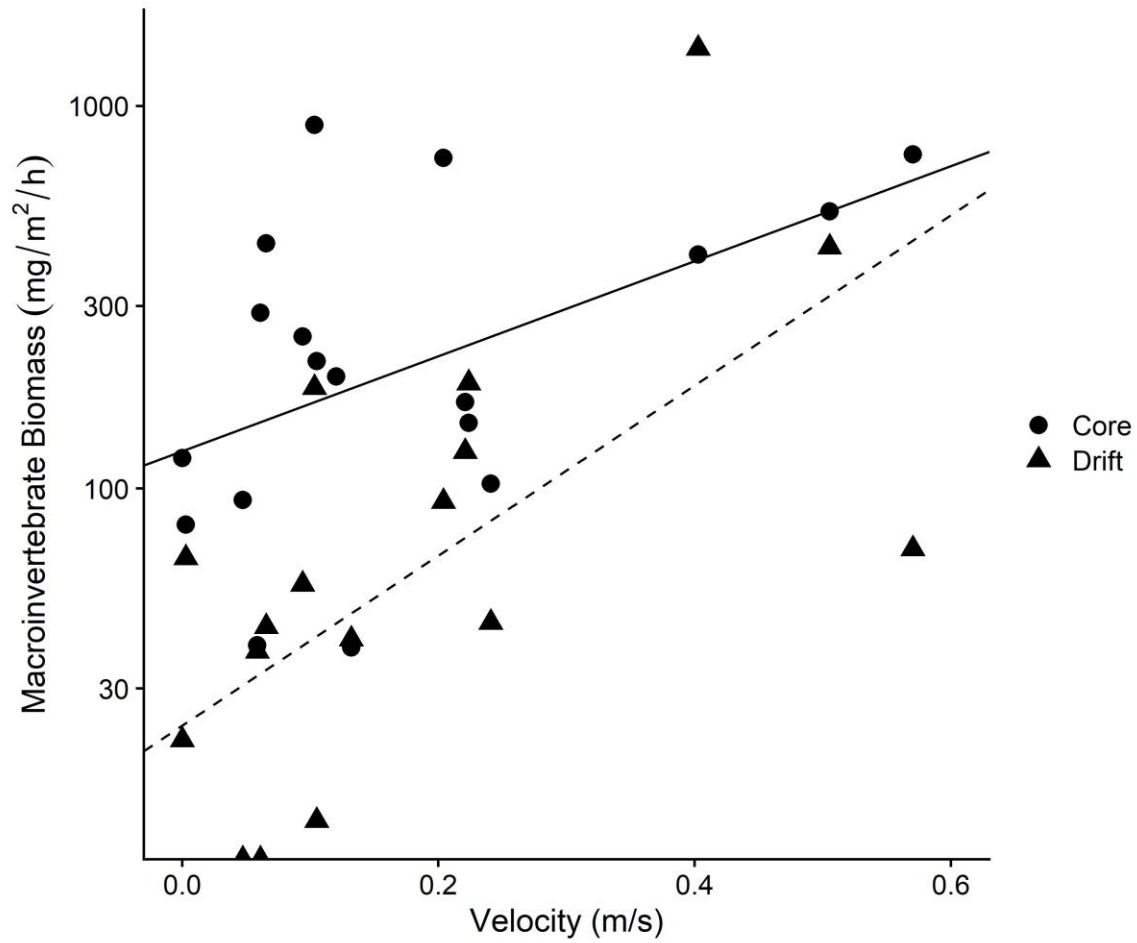


Figure 2.7 Biomass (< 6 mm) of drifting (triangles) and benthic (circles) macroinvertebrates in enclosures across a velocity gradient in 2020. Y-axis is log transformed. Each dot represents the average biomass of invertebrates in each enclosure.

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