# Effects of flow training on movement, survival, and habitat associations of age-1 Colorado pikeminnow stocked in the San Juan River, Utah, and New Mexico

by

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# Abstract

Stocking of hatchery-reared fish is an increasingly important management action to conserve and restore native fish populations. Colorado pikeminnow (*Ptychocheilus lucius*) was historically abundant throughout the Colorado River basin until the construction of dams and introduction of nonnative species caused a rapid decline and federal listing as endangered in 1973. Following the presumed extirpation of pikeminnow in the San Juan River in the late 1990s, a stocking program was developed in 2003. Due to an increasing adult abundance and potential for spawning, stocking protocols were changed from stocking young of year to age-1 pikeminnow with passive integrated transponders (PIT) tags to distinguish wild spawned and hatchery reared individuals. The addition of unique tags also allowed for hatchery experimentation such as flow training which was implemented in 2022 and 2023 to increase the survival of stocked age-1 pikeminnow.  I used radio telemetry to compare movement, survival, and habitat associations among flow-trained and different size classes of non-flow-trained pikeminnow. We also used PIT tags implanted in stocked pikeminnow to evaluate differences in detection rates across treatments. The mean rate of movement for radio-tagged pikeminnow was similar across treatments (-0.08 ± 0.48 km/hr). At the conclusion of 60 days, no control (0%), one flow-trained (3.3%) and 12 larger pikeminnow (34.3%) were detected and determined to be alive and within our study reach across both years. One individual from the radio-tagged flow-trained group was detected 255 river kilometers downstream approximately five days post stocking in 2022 (rate of -2.13 km/hr), demonstrating the ability to move long distances following stocking. Effects of terrestrial predation was observed in both years with 11 radio transmitters and 59 PIT tags found on dry land within the river corridor. Another radio transmitter was triangulated in a great blue heron (*Ardea herodias*). Overall, results suggest stocked pikeminnow dispersed quickly downstream; flow-trained pikeminnow prior to stocking did not increase retention of radio tagged individuals; and terrestrial predators may be a large source of mortality. Increasing the duration or velocities of flow training, prey training, or using alternative stocking sites may reduce losses due to predators and emigration.

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# Chapter 1 - Evaluating stocked age-1 Colorado pikeminnow movement, survival, and habitat associations.

## Introduction

In response to ongoing threats to freshwater ecosystems and their fisheries (Vorosmarty et al. 2010; Cooke et al. 2012; Dudgeon 2019), conservation strategies to mitigate these threats are being developed and include habitat restoration, prescriptions for environmental flows, and rearing of native fish in hatcheries (Roni et al. 2008; Hutson et al. 2012; Arthington 2015). Hatchery programs allow for the preservation of genetic diversity of extirpated populations (Meffe 1990; Osborne et al. 2012) and research on the behaviors and life history strategies of unique species (Platania and Altenbach 1998; Rakes et al. 1999; Ruble et al. 2019). Stocking fish is a critical component of many conservation and restoration efforts for endangered fish populations (Marsh et al. 2015; Archdeacon et al. 2022) and can be one of a few management actions available in highly modified systems (Schute et al. 2005; George et al. 2009). While negative effects and limitations of stocking have been documented (Araki et al. 2009; Araki and Schmid 2010; Christie et al. 2012), the successful reintroduction and restoration of native fish species is becoming increasingly more common (Cochran-Biedermann et al. 2015). Continual assessment of hatchery and stocking procedures and practices is essential to ensure their potential benefits will outweigh any negative effects (Archdeacon et al. 2023). Methods to evaluate stocking success include estimating apparent survival and emigration from stocking sites (Steffensen et al. 2010; Becker et al. 2023; Hedden et al. 2023). Telemetry can be used to evaluate stocking success and has become an increasingly important tool due to its application across a wide range of aquatic species and environments (Donaldson et al. 2014; Abecasis et al. 2018).

Approaches to improve success of stocking programs include the strategic selection of hatchery enrichment, size at stocking, or method of release (Brown and Day 2002; LeCheminant et al. 2021; Fonken et al. 2023). For example, experimentation from a typical “hard release” where fish are stocked directly into a given water body to a “soft release” where fish are provided an acclimation period has become more prevalent and accepted in conservation hatchery programs (Brown and Day 2002). During the rearing process, methods for enhancement can also be implemented to alter size at stocking, source populations, and complexity of the rearing environment (Hyvärinen and Rodewald 2013; Fonken et al. 2023). Fonken et al. (2023) experimented with size at stocking and found that larger fish had greater apparent survival and detection efficiency leading to a more cost-effective stocking strategy than stocking smaller fish. Flow training also has been used for a wide range of fishes and can increase swimming performance and fitness prior to stocking (Ward and Hilwig 2004; Boysen and Hoover 2009; Franssen et al. 2021). Flow training of razorback sucker (*Xyrauchen texanus),* an endangered fish endemic to the Colorado River basin, doubled the mean apparent survival for the first overwintering period following stocking (Franssen et al. 2021). Use of stocking enhancements such as flow training might play a critical role in the recovery of populations where establishing self-sustaining populations has been difficult.

The Colorado pikeminnow (*Ptychocheilus lucius*; hereafter “pikeminnow”) is a large, long-lived, highly migratory minnow endemic to the Colorado River basin. The pikeminnow, listed as endangered by the U.S. Fish and Wildlife Service in 1967 and protected under the Endangered Species Act in 1974 (Federal Register 39[3]:1175), has been the focus of intensive conservation efforts by the Upper Colorado River Endangered Fish Recovery Program and the San Juan Basin Recovery Implementation Program (SJRIP) due to declining populations (USFWS 2020). The most recent population estimate of adult pikeminnow in the upper Colorado River basin were approximately 50% below recovery goals, likely due to a combination of multiple stressors, including reductions to natural flow regimes, physical barriers to movement, and nonnative fish competition, present in the basin (USFWS 2020). In the San Juan River, efforts to stock pikeminnow have been ongoing since 2003 when a formal augmentation plan was adopted, resulting in the stocking of nearly 4.9 million young-of-year (YOY) pikeminnow (Zeigler et al. 2021). The adult population of pikeminnow increased following years of stocking YOY pikeminnow, thus the potential for natural spawning and the presence of wild spawned offspring also increased. Due to an inability to potentially determine future YOY as wild or hatchery raised, the SJRIP altered the pikeminnow augmentation plan from the annual stocking of approximately 400,000 age-0 pikeminnow to 12,000 age-1 pikeminnow in 2020. Each age-1 pikeminnow was implanted with a passive integrated transponder (PIT) tag which allowed researchers to distinguish wild from hatchery spawned pikeminnow, while also potentially decreasing predation by channel catfish (*Ictalurus punctatus*), allowing for hatchery experimentation and increasing recruitment to adulthood

To account for the lower total number of pikeminnow stocked each year, hatchery experimentation was implemented on the larger, PIT tagged age-1 pikeminnow to increase survival and recruitment to adulthood. In 2022 and 2023, a subset of age-1 pikeminnow were subjected to approximately seven weeks of flow training to test for differences in movement, survival, and habitat associations of flow-trained and non-flow-trained pikeminnow. Additionally, in 2023, a subset of the control pikeminnow were harvested from rearing ponds and stocked at a larger size than the remaining control and flow-trained pikeminnow of that year class. My objective was to use radio telemetry and PIT tag detections to evaluate stocking success among pikeminnow exposed to different hatchery conditions by quantifying 1) retention time at the stocking site, 2) movement and dispersal from the stocking site, 3) habitat associations, and 4) fate following stocking.

## Methods

### Study Site

Originating in southwest Colorado, the San Juan River drains an area of 99,200 $km^2$ in Colorado, New Mexico, Arizona, and Utah (Carlson and Carlson 1982). With the construction of Navajo Dam in 1962, the San Juan River now flows 365 km and is bounded by Navajo Reservoir (river kilometer, RKM 365) and Lake Powell (RKM 0). The operation of Navajo Dam modifies the natural flow regime (Propst and Gido 2004), intensifies the longitudinal thermal gradient, and has altered habitat downriver (Franssen et al. 2014). In addition to physical barriers, the introduction and establishment of invasive salt cedar (*Tamarix* spp.) and Russian olive (*Elaeagnus angustifolia*) has contributed to the straightening of the main river channel and loss of channel complexity (Franssen et al. 2014). Decreasing water levels in Lake Powell and the accompanying shift in the San Juan River channel has also resulted in the formation of the Piute

Farms Waterfall (PFW, Cathcart et al. 2018; Figure 1). This waterfall has only been passable for a two-week period in the summer of 2011 when the reservoir level exceeded the elevation of the waterfall; thus, it presents a barrier to fish movement (Durst and Francis 2016).

Age-1 Colorado pikeminnow were stocked at two sites, Hogback Canal and McElmo Creek (Figure 1). Hogback Canal diverts water for irrigation and includes three channels; a diversion, sluice, and main river (Figure 2). Hogback Canal was located 255.2 RKM upstream of PFW and is the most downstream managed diversion on the San Juan River. The diversion structure has a weir upstream of a bypass facility where water is sent to either an irrigation canal or a return channel that reconnects with the mainstem river (Figure 2). The function of the weir is to divert fish to the return channel however entrainment of pikeminnow and other native fishes has been previously documented (Brandenburg et al. 2017; Clark-Barklow et al. 2024). Due to its location and surrounding infrastructure, PIT tag antennas are operated at multiple locations that detect movement, passage, and entrainment (Figure 2). The McElmo Creek stocking site was located approximately 16 RKM upstream of its confluence with the San Juan River (RKM 161.7; Figure 1). Base flow in McElmo Creek is influenced by McPhee Reservoir through an irrigation system that maintains perennial flow throughout much of the year. McElmo Creek also has a combination of PIT antenna arrays though they are located either at or just downstream of its confluence to detect fish entry and exit into this tributary.

## Hatchery Enrichment

Age-1 pikeminnow for the 2022 and 2023 stocking were spawned from a broodstock held at the Southwestern Native Aquatic Resources and Recovery Center (SNARRC) in Dexter, New Mexico. Pikeminnow were reared in outdoor ponds until they were collected, implanted with a PIT tag, and transferred to one of two outdoor raceways that were 12.1 m x 1.8 m wide and consisted of an inflow and outflow on opposite ends with constant flow through water (Figure 3). Due to the gradual slope in the raceways, water depth ranged from ~0.6 – 0.7 m. The flow training raceway consisted of a central cinderblock wall, cinderblock baffles approximately every 3 m and a pump on the upstream end of the flowing raceway (Franssen et al. 2021). This design provided constant flowing water and heterogenous habitat. The raceway used for control pikeminnow did not contain any additional features. Once pikeminnow were separated between the two raceways, water velocities in the flow treatment were incrementally increased over two-week intervals for approximately seven weeks prior to stocking. In 2022, the average velocity recorded in the raceway was 0.05 m/s ± 0.03 during the first week of flow training increasing to 0.10 m/s ± 0.03 by weeks. In 2023, average velocities were similar in the first week (0.06 m/s ± 0.03) and last week (0.08 m/s ± 0.03; Figure 4). In 2023, pikeminnow available for stocking grew into two distinct size classes (mean length ± 1 SD of radio tagged pikeminnow; 184 ± 7.5 mm and 255 ± 10.9 mm TL). Due to concerns of stress, cannibalism and demands for limited space within the hatchery, larger pikeminnow were graded, held separately from the control and flow-trained pikeminnow in a large indoor holding tank and stocked approximately one month earlier.

## Tagging

Prior to stocking, all pikeminnow were collected and scanned for a PIT tag with any pikeminnow that had shed its tag given a new tag. One week prior to stocking, radio-tagged pikeminnow were collected, tagged with a surgically implanted radio transmitter, and held in an indoor holding tank before stocking. Fifteen flow-trained and control pikeminnow were tagged in 2022 and 2023, and an additional 37 larger pikeminnow tagged in 2023 (Table 1). Each pikeminnow was sedated in a bath of tricaine methanesulfonate (MS-222; Syndel Syncaine®) until they were immobilized. After measuring total length, an incision was made anterior to the pelvic girdle and offset of the linea alba where a unique, noncoded, external whiptail radio transmitter (2022 and 2023 control and flow-trained pikeminnow = Advanced Telemetry Systems, Isanti, Minnesota, F1545, 1.0 g, ~55-day battery life; 2023 larger pikeminnow = F1560, 2.8 g, ~198-day battery life) was surgically implanted. Radio transmitters did not exceed 3.5% of the pikeminnow mass (estimated from a length-mass regression) with an estimated tag burden ranging from 1.7% to 3.5% for control and flow-trained pikeminnow and 1.9% to 3.3% for the larger pikeminnow. In 2022, the external whiptail antenna was sutured in place at the posterior end of the incision. Whereas in 2023, a hollow needle was used to create a conduit from an opening posterior of the pelvic girdle to the main incision site and the external whiptail antenna was fed through the needle. During surgeries, the gills were irrigated with MS-222 dosed water. Following surgery, pikeminnow were transferred to an indoor holding tank where they received an 8 h salt and antibiotic treatment to assist with wound healing and held for approximately one week before they were transported to the stocking site. During the one-week period following surgeries, one mortality was observed in the larger pikeminnow treatment, and no impaired swimming ability was observed leading up to their transport to the stocking site. During stocking of the larger pikeminnow, one radio transmitter was shed during the stocking process with the individual removed from further analysis.

## Stocking Site Retention

I evaluated retention to the stocking site between the Hogback Canal and confluence of the Hogback return channel, and San Juan River main channel (Hogback Confluence) within the first three days post-stocking using a combination of mobile telemetry surveys and stationary antenna detections (Figure 2). In 2022, the Hogback Confluence antenna consisted of one, 4-element yagi antenna pointed downstream. In 2023, an additional 4-element yagi antenna pointed upstream was added to the Hogback Confluence antenna. In both years, mobile telemetry surveys consisted of walking or rafting from the Hogback Canal to the Hogback Confluence using a 3-element foldable yagi antenna pointed downriver (Figure 2). Any radio transmitter heard within this reach by either stationary antenna or mobile telemetry was designated as retained within the stocking site for that given day.

A stationary antenna was deployed at the PFW throughout the study period (i.e., 60 days after each stocking event) to estimate pikeminnow movement over the waterfall. Pikeminnow detected at this antenna were assumed to have passed over the waterfall into Lake Powell and unable to move back upstream into the San Juan River. This stationary antenna consisted of two, 4-element yagi antennas pointed upstream and downstream of the waterfall with frequencies of each radio-tagged pikeminnow scanned on both antennas separately. Prior to the 2023 October 24 stocking, the scanning of radio frequencies was modified from each antenna scanning a frequency table separately to scanning each frequency table with both antennas in tandem. This change was made due to the large number of tagged pikeminnow in the river between the two stockings in 2023, and the duration it would take to scan through each frequency table. The maximum duration spent scanning each frequency table with both antennas in tandem was approximately

30 s (30 min/cycle maximum) in contrast to an approximately 90 s maximum duration when antennas scanned frequencies separately (97 min/cycle maximum). Whereas pikeminnow orientation in relation to PFW would not be able to be determined with antennas scanning in tandem, the maximum duration needed to scan each frequency table would be similar to when only one cohort of radio-tagged pikeminnow was present in the river at a given time and frequency tables were scanned on each antenna separately.

## Movement and Dispersal

Stationary antennas were installed approximately 85.0 river RKM apart to detect pikeminnow moving downstream of the stocking site (Figure 5). Including antennas used for stocking site retention at Hogback Canal and PFW, stationary antennas were also deployed downstream of the confluence of McElmo Creek and the San Juan River (RKM 161.7) and upstream of the Mexican Hat boat launch (RKM 85.1). While the Hogback Canal stationary antenna was removed 3 days post stocking, the remaining stationary antennas were active for 60 days following stocking. Similar to the PFW antenna, the Mexican Hat and McElmo Creek antennas consisted of two, 4-element yagi antennas oriented upstream and downstream and scanned frequency tables separately on each antenna in 2022 and 2023. Prior to the 2023 October 24 stocking, the scanning of radio frequencies was modified from each antenna scanning a frequency table separately to scanning each frequency table with both antennas in tandem due to similar concerns given for the PFW stationary antenna modification.

Mobile telemetry surveys were conducted the first week and approximately every 2-, 4-, and 7-weeks post-stocking between Hogback Canal and the Sand Island boat launch (RKM 123.0). Within the first week after stocking, mobile telemetry surveys were conducted the day of stocking and three days following stocking, focusing on a short reach above and below the Hogback Canal. Tracking efforts after this first week involved rafting trips between Hogback Canal to the Sand Island boat launch. During mobile telemetry surveys, the main channel was typically followed; however, if conditions allowed, side channels were floated opportunistically. When a pikeminnow was detected, the gain of the receiver was reduced and the pikeminnow was approached from different directions until the pikeminnow's position could be estimated to within approximately a 1 m x 1 m area.

Stocking of PIT-tagged age-1 pikeminnow occurred in 2021, 2022, and 2023, with variable numbers stocked across treatments and locations (Table 1). Five stationary PIT antennas downstream of stocking sites were used to monitor downstream dispersal (Figure 5). These PIT antennas were operated continuously and consisted of their own, self-sustaining, solar charging system. These antennas span a portion of the river channel, thus only detecting a proportion of pikeminnow that pass by each antenna at a given time.

I used a mixed-effects general linear model to test for differences in movement rates of radio-tagged pikeminnow between treatments. The model included rates of movement as the response and days since stocking, year, and treatment as fixed, additive effects. Individuals were included as a random effect due to subsequent detections of the same individual not being independent. Testing the fixed effect of year represents both the change in the stocking site from the Hogback diversion canal in 2022 to the Hogback sluice channel in 2023 as well as variation in environmental conditions (e.g., flow, temperature) between the stockings. Using individuals (i.e., tag ID) as a random effect was necessary because relocations of the same pikeminnow was not independent. I also used an analysis of variance (ANOVA) to test for differences in treatment and distance moved from the stocking site to the RKM of each radio-tagged pikeminnow's last detection.

## Habitat

Differences in habitat associations for each treatment was based on point locations of radio-tagged pikeminnow collected during mobile telemetry surveys following stocking. Once pikeminnow were triangulated to a 1 m x 1 m area, I recorded a GPS point and habitat data, including depth (m), velocity (m/s), substrate, and mesohabitat type. Depth was measured with a topset rod, velocity was collected by a Hach flow meter (FH950), and substrate was visually classified into either silt, sand, gravel, rubble, cobble, boulder, or bedrock from a modified Wentworth scale (Wentworth 1922). Classification of mesohabitat types was based on Bliesner et al. (2009). Only the first detection was included for pikeminnow triangulated multiple times in the same mobile survey. For data analysis, habitat associations were standardized to proportions due to the varying number of detections between groups. I used an ANOVA to test for differences between depth and velocity habitat associations between treatments. I also used a t-test to test for differences between depth and velocity habitat associations between years.

## Fate of Tagged Pikeminnow

The fate of radio-tagged pikeminnow was assessed based on the recovery of tags that were determined to be mortalities in addition to detections of pikeminnow during mobile telemetry surveys and stationary antennas (Figure 6). Probability of detection during mobile telemetry surveys was also calculated for a range of tracking parameters to evaluate the likelihood of detecting or missing radio-tagged pikeminnow. This entailed assigning a random distribution of pikeminnow locations along a linear path and assuming constant values for the parameters of radio-tag frequencies (30) and scan rate (3 seconds/frequency). A range of values were used for different combinations of the parameters boat speed (0-4 mi/hr) and antenna read range (10-150m) with 10 simulations run for each combination. Using the random distribution of each frequency along the linear path, at which stage of the frequency table that given frequency was in the cycle when being passed, the boats speed, and antennas read range, an average estimate of the percent of pikeminnow that would be detected was then generated from these simulations.

The fate of PIT-tagged pikeminnow from the 2021 and 2022 stocking was assessed as either detected (i.e., alive) or unknown using data available in the Species Tagging Research and Monitoring System (STReaMS) database (STReaMS 2024). STReaMS data includes a combination of passive detections from permanent and temporary PIT antenna arrays, active captures from river-wide systematic sampling for long-term monitoring, and other research projects occurring throughout the river. The fate of PIT-tagged pikeminnow was assessed following the first overwintering period that began on January 1 of the following year. Due to the inconsistent nature of PIT antenna detection probabilities and seasonal nature of active sampling activities, the fate of pikeminnow stocking cohorts were assessed on a yearly basis with only the pikeminnow detected considered known and alive. Data from pikeminnow stocked in 2023 and

2024 detections were included in this assessment; however, due to an entire calendar year having not passed since the time of stocking these data are preliminary.

# Results

## Stocking Site Retention

The percent of pikeminnow detected within the stocking site the first three days following stocking did not differ between treatments with 21.9% of pikeminnow detected (z-test; $P = 0.76$). There was a significantly greater percent of pikeminnow detected that were stocked in the Hogback diversion canal (38.3%) than pikeminnow stocked in the Hogback sluice channel (14.2%) following the changes made to the stocking site location in 2023 (z-test; $P < 0.05$; Figure 7). Of the 95 radio-tagged pikeminnow stocked, only one flow-trained pikeminnow was detected on the PFW stationary antenna five days post-stocking in 2022. The PFW PIT antenna detected an additional six pikeminnow (without radio transmitters) within the first 60 days following stocking at Hogback Canal. Of these detections, two were control pikeminnow from 2021 (3- and 32-days post stocking), and 1 flow-trained (22 days post stocking) and 3 larger pikeminnow (3-, 4-, and 4-days post stocking) were from 2023. No pikeminnow from the McElmo stocking were detected at the waterfall within 60 days following stocking.

## Movement and Dispersal

The results of the mixed-effects model suggested there was not a difference in movement rates between treatments of radio-tagged pikeminnow following stocking with a mean rate of movement for all treatments of $-0.08 \pm 0.48$ km/hr (i.e., downstream). There was a difference in estimated rate of movement between years with pikeminnow movement rates 5.46 km/hr slower in 2023 than 2022. No pikeminnow were detected during mobile surveys upstream of the stocking site at Hogback Canal (Figure 8). There was no difference for mean distance of each pikeminnow’s last detection from the stocking site among treatments (ANOVA; $F(2)=1.8$; $P = 0.17$; Figure 9). Dispersal downstream of the stocking site by PIT-tagged pikeminnow occurred in two “waves”, with increased unique daily detections of pikeminnow on multiple PIT antennas downstream of the stocking site after approximately 5 and 30 days (Figure 10).

## Habitat

Habitat association data were collected at 23 points in 2022 and 60 points in 2023 with all treatments associating with similar depths (ANOVA; $F(2)=2.51$; $P = 0.09$) and velocities (ANOVA; $F(2)=0.78$; $P = 0.46$; Table 2). Habitat associations for all treatments were also similar between years for depth (t-test; $F(20.87)=-1.55$; $P = 0.22$) and velocity (t-test; $F(23.31)=1.22$; $P = 0.38$). Of the seven substrate classifications, pikeminnow only associated with silt, sand, and cobbles. Sand constituted the largest percent of observed pikeminnow habitat associations (cobble = 28.2%; sand = 62.1%; silt = 9.7%; z-test; P-value $\leq 0.05$). Pikeminnow most often associated with slackwater mesohabitats (55.1%) followed by run (32.0%), riffle (5.0%), eddy (6.6%) and backwater (1.4%; z-test; $P < 0.05$). Control pikeminnow more frequently occurred in runs (40.5%) than flow-trained pikeminnow (z-test; $P < 0.05$), whereas flow-trained pikeminnow associated more with eddy mesohabitat (11.8%) than control pikeminnow (1.4%; z-test; $P < 0.05$).

## Fate of Tagged Pikeminnow

Only 13 of the 95 radio-tagged pikeminnow stocked between 2022 and 2023 were detected on the final mobile tracking trip and assumed to have survived the first 60-days post-stocking. One additional pikeminnow was detected moving downstream at the Mexican Hat stationary antenna and out of our study reach. Twelve pikeminnow mortalities were attributed to terrestrial predation. In 2022, two radio transmitters were triangulated out of the river with one on a tree branch and another in a scat pile along with 46 additional PIT tags from the same stocking (Figure 11). In 2023, nine radio transmitters and three PIT tags were found from the larger pikeminnow stocking and one radio transmitter was found from the control and flow-trained pikeminnow stocking with 10 PIT tags (2022: flow-trained = 1; 2023: control = 4, flow-trained = 2, larger = 3). One of the nine radio transmitters from the larger pikeminnow stocking was also triangulated multiple times in a great blue heron as it flew downriver during a mobile telemetry survey. An additional radio-tagged pikeminnow was found dead along the riverbank with a juvenile channel catfish lodged in its mouth. Five more radio tags were recovered within the wetted area of the river (presumed from tag shedding) and removed from further evaluation leaving 63 pikeminnow at large. Six of the 63 pikeminnow remaining pikeminnow were considered lost to the system by way of either entrainment (n = 5) or passing over PFW (n = 1) leaving 57 pikeminnow (60%) remaining undetected and their fate unknown (Table 3).

The percent of PIT-tagged pikeminnow from each stocking cohort decreased at least 88% following the first overwintering period (Table 4). Additionally, less than 2% of uniquely tagged pikeminnow were detected two years following stocking. The percent of pikeminnow detected following the first overwintering period was more than five times greater for the 2021 cohort than the 2022 cohort. Following the 2021 stocking, the percent of detected pikeminnow stocked at McElmo Creek was more than double than those stocked at Hogback Canal with an inverse pattern observed after the 2022 stocking. Following the first overwintering period, an additional 23 PIT-tagged pikeminnow were detected below the PFW waterfall, all of which were from the control treatment. Of these 23, nine were stocked at the Hogback Canal and 14 were stocked at McElmo Creek.

## Discussion

My findings suggest that the low retention of stocked pikeminnow in our study reach was due to rapid dispersal downstream and loss to terrestrial predation, with limited effects of flow training stocked pikeminnow. Following the final mobile survey of the 60-day study period, few (<5%) of the smaller control and flow-trained pikeminnow were detected in our study reach. While a greater percentage of the larger control pikeminnow were detected (34.3%), two thirds of the pikeminnow were either undetected or confirmed mortalities from terrestrial predation. In addition to predation, pikeminnow were also lost to the system by entrainment into the Hogback irrigation canal or passing over the PFW. A concerning outcome was the proportion of pikeminnow that were not detected with an unknown fate at the end of the study period. My post hoc simulations to predict detection probabilities based on frequency scan rate, number of tagged pikeminnow, and a range of values for mobile telemetry parameters that included boat speed and read range suggest that even using conservative estimates for read range and boat speed, it is highly unlikely that I would not detect radio-tagged pikeminnow if they were in the study reach (Figure 12). I also eliminated the possibility of drained radio transmitter batteries because recovered transmitters were functional past the 60-day study period. Thus, the evidence suggests that avian predators that have the capability of carrying pikeminnow outside the river corridor may be the most likely explanation for the absence of radio-tagged pikeminnow.

Avian predation on fish can be intense (Jepsen et al. 2018; Schultz et al. 2022) with Pitt et al. (2008) estimating that one great blue heron can consume between 127 to 451g of fish per day. During our study, I observed evidence of terrestrial predators consuming pikeminnow on several occasions. For example, I found 46 PIT tags from stocked pikeminnow along with a radio transmitter in what appeared to be the scat from one bird (Figure 11). Additional evidence included groups of one, two, or three radio transmitters on exposed cobble bars, in leaf litter under large riparian trees or on top of tree limbs that also appeared to be defecated by an avian predator. While it is difficult to assign every predation event to a specific species, one radio transmitter was detected in a great blue heron multiple times in addition to observations in the field of great blue herons and common mergansers (*Mergus merganser*) actively searching and preying upon fish downstream of the stocking site. Many of our observations of terrestrial predation were opportunistic in nature due to the presence of a radio transmitter in a predator, the transmitter being defecated adjacent to the river, or transmitters found with PIT tags. Thus, it is likely that the 12 pikeminnow (11.6%) from this study that were lost to terrestrial predation represents an underestimate of the number of pikeminnow that are removed from the system. Other studies have found that terrestrial predation by white pelicans (*Pelecanus erythorhynchos*) and double-crested cormorants (*Phalacrocorax auratus*) on stocked rainbow trout (*Oncorhynchus mykiss*) can also be quite intense, with predation rates ranging from 0-48% of PIT tagged fish (Meyer et al. 2016). If half of the undetected pikeminnow were due to predation, it is possible that approximately an additional third of the stocked pikeminnow may have been lost to avian predation within the first weeks following stocking.

Current hatchery protocols for raising age-1 pikeminnow may be selecting for disadvantageous behavioral traits that reduce the overall survival of stocked pikeminnow. Feeding pikeminnow at the surface with floating feed, where they learn to orient to the surface, may be inadvertently leading to increasing rates of terrestrial predation. The multiple piles of PIT tags and radio-transmitters found during the study may be evidence of how vulnerable recently stocked pikeminnow are to terrestrial and visual predators. The time of stocking and the associated turbidity of the river may also affect the ability of terrestrial predators to locate and prey upon pikeminnow. Additionally, >50% of pikeminnow habitat associations were in slackwater habitat, which may provide avian predators an advantage where they can better stalk their prey. For example, a radio transmitter was found on an exposed sand bar that was adjacent to a slackwater habitat with a PIT tag from a pikeminnow stocked the previous year. This evidence suggests that even after a full year in the river, overlap between pikeminnow habitat associations and terrestrial predation may present a continued source of mortality. Altered hatchery rearing and stocking protocols such as live prey training, where pikeminnow are accustomed to foraging for wild prey and are not conditioned to feeding at the surface, or stocking pikeminnow when river conditions are more turbid may help increase the overall immediate survival of stocked pikeminnow and reduce interactions with terrestrial predators following stocking.

The number of PIT-tagged pikeminnow detected after the first overwintering periods followed a similar pattern of decline observed in radio-tagged pikeminnow. While raw number of detections does not account for detection efficiency, the low number of pikeminnow detected may be cause for concern given the evidence of terrestrial predation on radio-tagged pikeminnow. Prior to the first overwintering period, a similar short-term pattern was observed by Golden et al. (2006) where the majority of stocked pikeminnow typically were lost within the first few weeks following stockings. Other studies also documented a similar long-term pattern with infrequent encounters of pikeminnow three years post stocking (Durst and Franssen 2014). A similar pattern has been observed following the change to stocking age-1 pikeminnow in 2021 with no detections three years post-stocking through the first third of 2024; however, these data are still preliminary and do not encompass the entirety of the third year. With the addition of the larger pikeminnow treatment in 2023, I have observed increased retention and survival of larger pikeminnow stocked in September 2023 thus far. This might suggest that size at stocking may lead to better short-term survival (Clark et al. 2018) and possibly increase long-term survival though I recommend that additional data are collected to more accurately evaluate this observation over a longer temporal scale.

Entrainment of pikeminnow in the Hogback irrigation canal in 2022 was an unanticipated source of mortality following stocking. Entrainment was estimated using PIT antenna detections on the Hogback irrigation canal antenna downstream of the stocking site, which included 16.7% of radio-tagged pikeminnow (n = 5) and 14.9% of PIT-tagged pikeminnow (n = 709). Research has shown the weir wall to be successful in reducing the entrainment of sub adult and adult species of flannelmouth sucker (*Catastomus latipinis*) and bluehead sucker (*C. discobolus*; McKinstry 2016); however, these species tend to orient along the river bottom and may be less susceptible to passing over the weir. In contrast, stocked age-1 pikeminnow are fed at the surface in the hatchery and may tend to orient higher in the water column and thus may be more susceptible to passing over the weir. While the proportion of radio-tagged pikeminnow retaining within the Hogback diversion canal stocking reach during the first three days post stocking was greater than the Hogback sluice channel, the risk of entrainment may outweigh any benefit of an acclimation period that this site may provide.

The lack of an extended acclimation period may intensify terrestrial predation, downstream dispersal, and the overall survival of stocked pikeminnow. Control and flow-trained pikeminnow stocked at the Hogback sluice channel, which contains higher velocities and lower depths than the Hogback diversion canal, were detected exiting the stocking site 30 minutes following stocking. Radio-tagged pikeminnow from this stocking cohort also had the fewest number of detections during the study period, which may have been have a result of pikeminnow rapidly dispersing downstream and an increased rate in predator interactions. In contrast, larger radio-tagged pikeminnow stocked at the same site only one month earlier were found within the stocking reach three days post-stocking and had the most detections during the study period, suggesting pikeminnow size or environmental factors may increase stocking site retention. Pikeminnow stocked at the Hogback diversion canal had increased retention to the stocking site, which may have been from a prolonged acclimation period; however, the risk of entrainment at this site was elevated. A stocking site that limits the risk of entrainment and provides some form of an acclimation period such as lower velocities or a softer release may result in a greater proportion of pikeminnow retaining to the stocking site before dispersing downstream.

The ability of stocked pikeminnow to disperse rapidly downstream and pass over the PFW may greatly reduce the overall success and survival of current and future stockings. During the 60-day study period, one radio-tagged and six PIT-tagged pikeminnow stocked at Hogback Canal were detected at PFW, with two individuals traveling downstream 255 RKM in three days. Following the first overwintering period, an additional 23 pikeminnow were detected at PFW, 14 of which were stocked at McElmo Creek and the remaining nine at Hogback Canal. These data suggest that regardless of stocking site, and even after pikeminnow have established in the San Juan River following stocking, the threat of emigrating over the PFW still exists and could contribute to the infrequent encounters of pikeminnow three years following stocking (Clark et al. 2018). Additionally, due to the orientation of the PFW PIT antenna below PFW (Figure 13), these detections likely represent an underestimate of the number of pikeminnow moving downstream over the waterfall (Bogaard et al. 2023); thus, the number of pikeminnow lost to the system. While there have been efforts in place that translocate imperiled fish upstream of the waterfall (Pennock et al. 2020), this management action is relatively costly and requires continued effort to return fish to the system.

The lack of a difference between the rate of movement of control and flow-trained pikeminnow was unexpected and suggests that modifications to flow training protocols may be necessary. Additionally, I did not detect a difference between the average depth or velocity of habitat associations between the two groups. Velocities recorded during flow training showed that flows can vary greatly within each raceway as well as between years (Figure 4). While a steady increase in raceway velocities during flow training may be desired, providing pikeminnow with variable flows may better replicate the more heterogenous habitats that pikeminnow encounter once they are stocked. Future work might include swimming performance experiments during the flow training process to better detect when significant differences in swimming ability have been achieved (Ward and Hilwig 2004). Quantifying the threshold in which there is a difference in swimming performance may relate to detectable differences in rate of movements or associated habitat features following stockings.

Stocking endangered fish for conservation efforts requires continued evaluation to improve overall success and reach recovery goals. Our immediate evaluation of the stocking of age-1 pikeminnow in the San Juan River found a combination of unanticipated negative outcomes. I found evidence of terrestrial predation, with one individual predator consuming 46 pikeminnow, which may have the potential to drastically reduce the overall survival of stocked pikeminnow. Pikeminnow also showed the ability to rapidly disperse downstream, emigrating over the PFW where they were lost to the system, highlighting the importance of stocking site distance from major barriers as well as habitat connectivity. Entrainment of pikeminnow in the Hogback irrigation canal in 2022, where nearly 15% of PIT-tagged pikeminnow were entrained, emphasized the importance of stocking site selection. In response to this, our immediate evaluation allowed managers to act quickly and alter the stocking site in 2023 where entrainment in the Hogback irrigation canal was no longer a risk. Finally, I found that there was no difference in the rate of movement between flow-trained and control pikeminnow under current hatchery protocols. With the information gained from our immediate evaluation, managers can adjust hatchery and stocking methods, including increasing the duration or velocities during flow training, prey training, and experimenting with stocking site location and timing. These changes may lead to increased success of future stockings and improved survival of pikeminnow, which may lead to the recovery of this species in the San Juan River.

# Figures

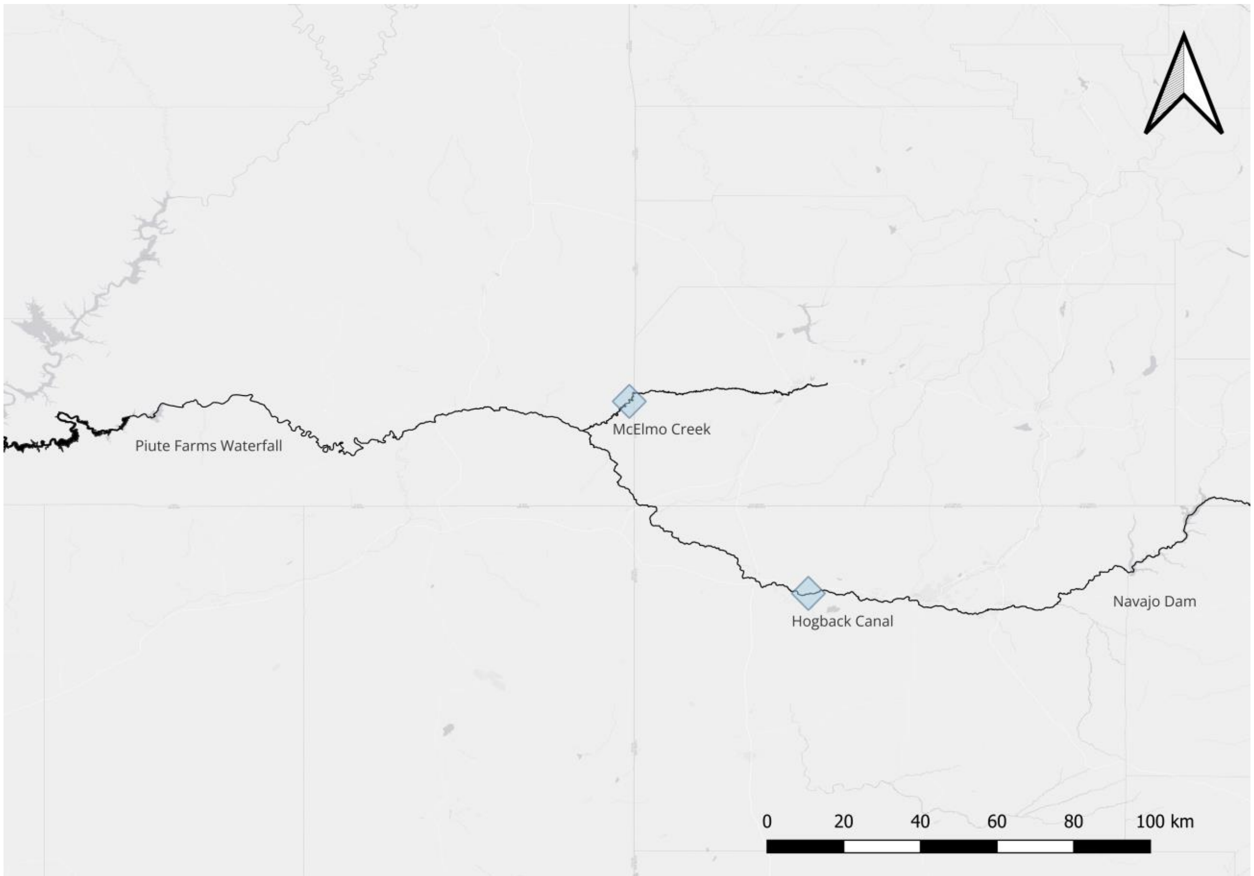


**Figure 1: Colorado pikeminnow stocking locations indicated by blue diamonds and their relative position to major barriers to fish passage (Piute Farms Waterfall and Navajo Dam) on the San Juan River.**

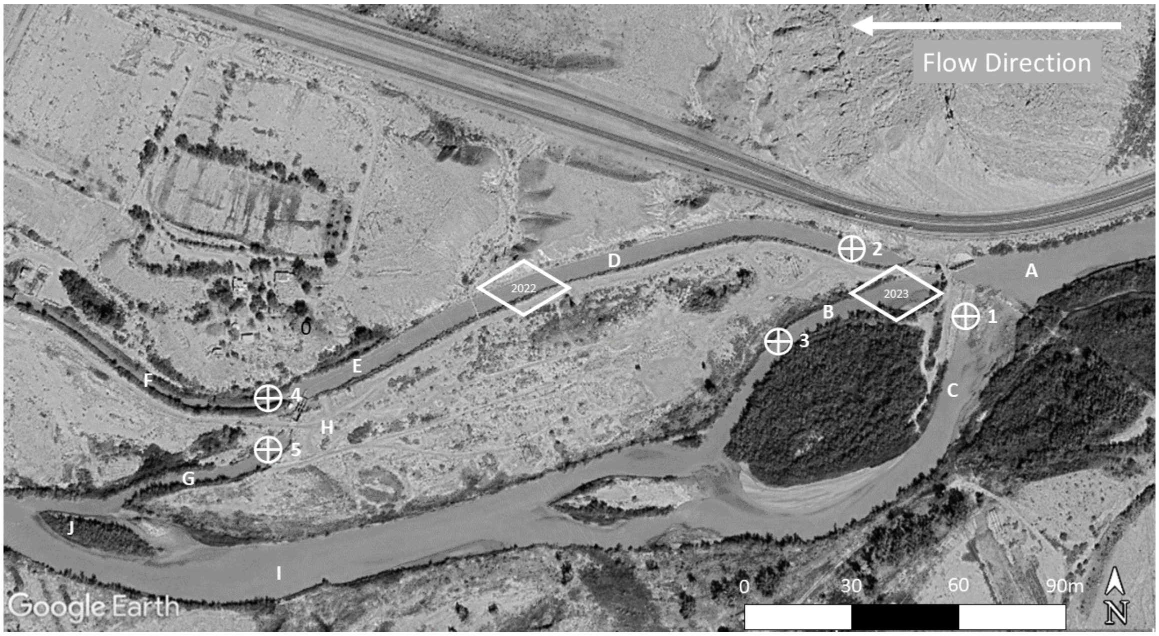


**Figure 2: Aerial image of Hogback Canal, where Colorado pikeminnow have been previously stocked, with key features identified. (A) San Juan River upstream of the headgate and trash rack; (B) sluice channel; (C) main channel with a constructed fish passage; (D) Hogback diversion canal; (E) weir where water that passes over is directed to the irrigation canal; (F) irrigation canal; (G) return channel; (H) by-pass facility below the weir; (I) San Juan River main channel; and (J) stationary antenna downstream of the stocking site. PIT antenna arrays are indicated for the following sites: (1) fish passage array; (2); Hogback diversion canal; (3) sluice channel; (4) irrigation canal; and (5) return channel. Stocking sites are indicated by open diamonds with the year Colorado pikeminnow were stocked at that location.**

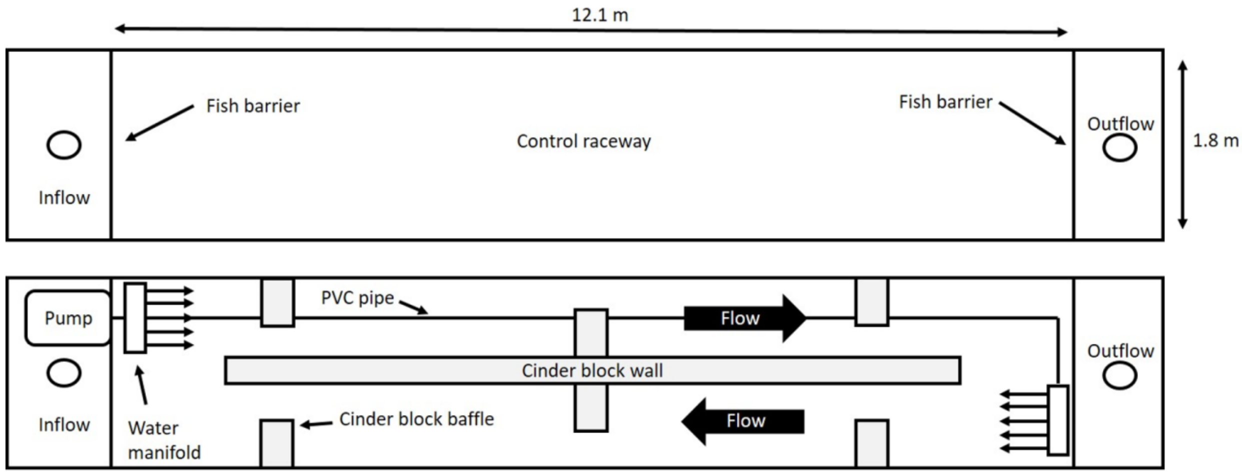


**Figure 3: Design of flow training and control raceways used for hatchery enrichment of age-1 Colorado pikeminnow (adapted from Franssen et al 2021).**

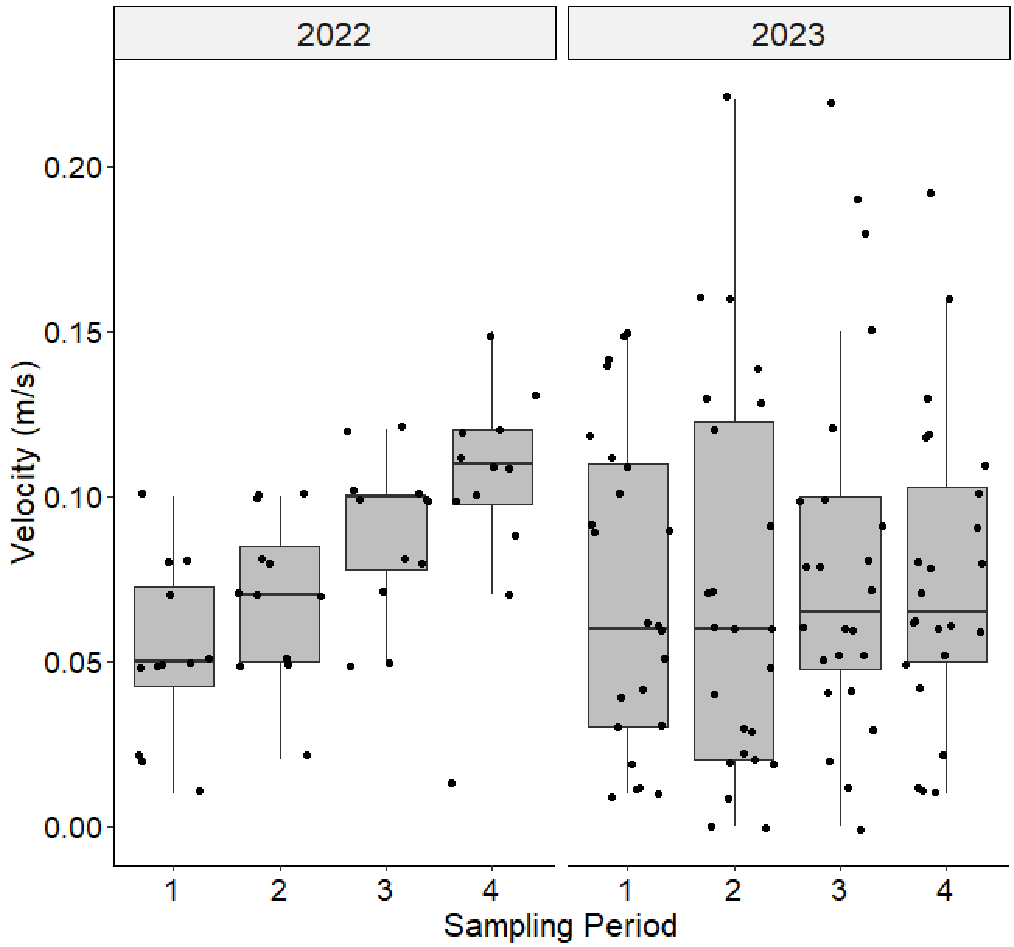


**Figure 4: Absolute value of velocities measured throughout the raceway at set locations during hatchery enrichment flow-training of age-1 Colorado pikeminnow in 2022 and 2023 at the Southwest Native Aquatic Resource and Recovery Center.**

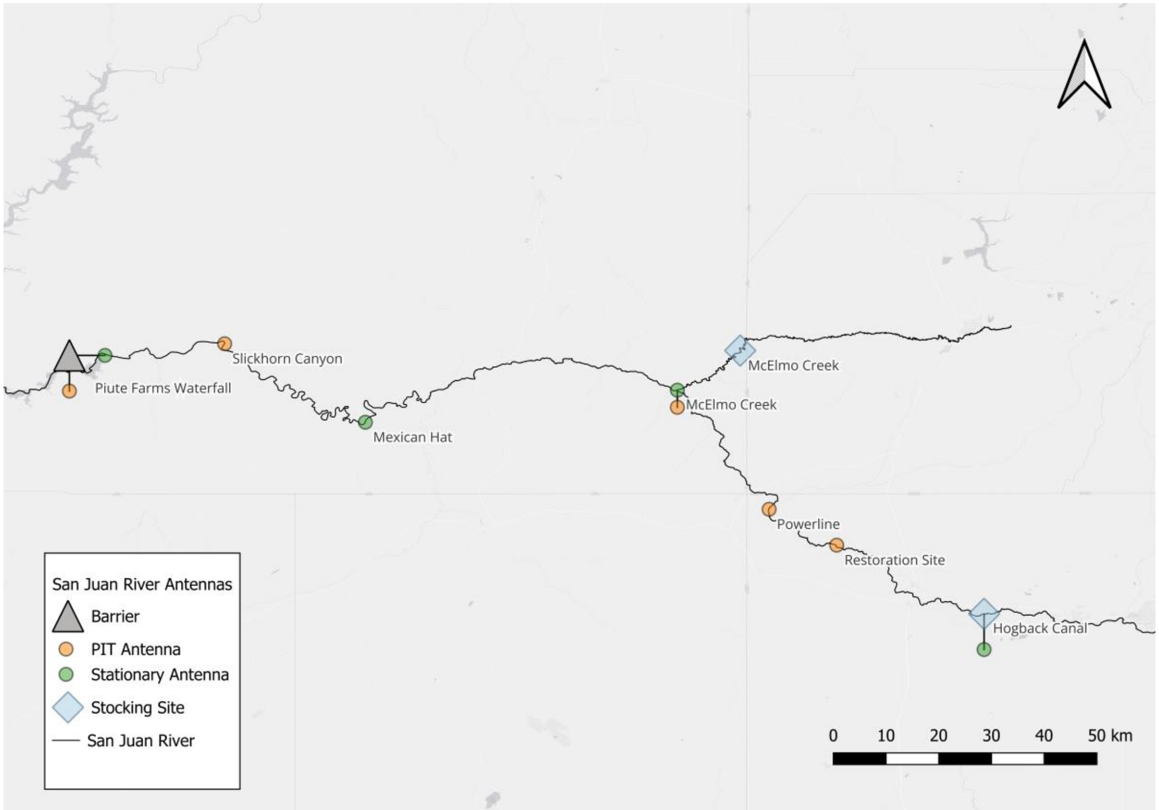


**Figure 5: Location of stationary and PIT antennas used to evaluate movement and survival of stocked age-1 Colorado pikeminnow on the San Juan River. Stationary antennas were located at RKM 254.9 (Hogback Canal), 161.7 (McElmo Creek), 85.3 (Mexican Hat), and 0.0 (Piute Farms Waterfall). PIT antennas were located at RKM 216.5 (Restoration Site), 200 (Powerline), 161.7 (McElmo Creek), 28.5 (Slickhorn Canyon) and 0.0 (Piute Farms Waterfall).**

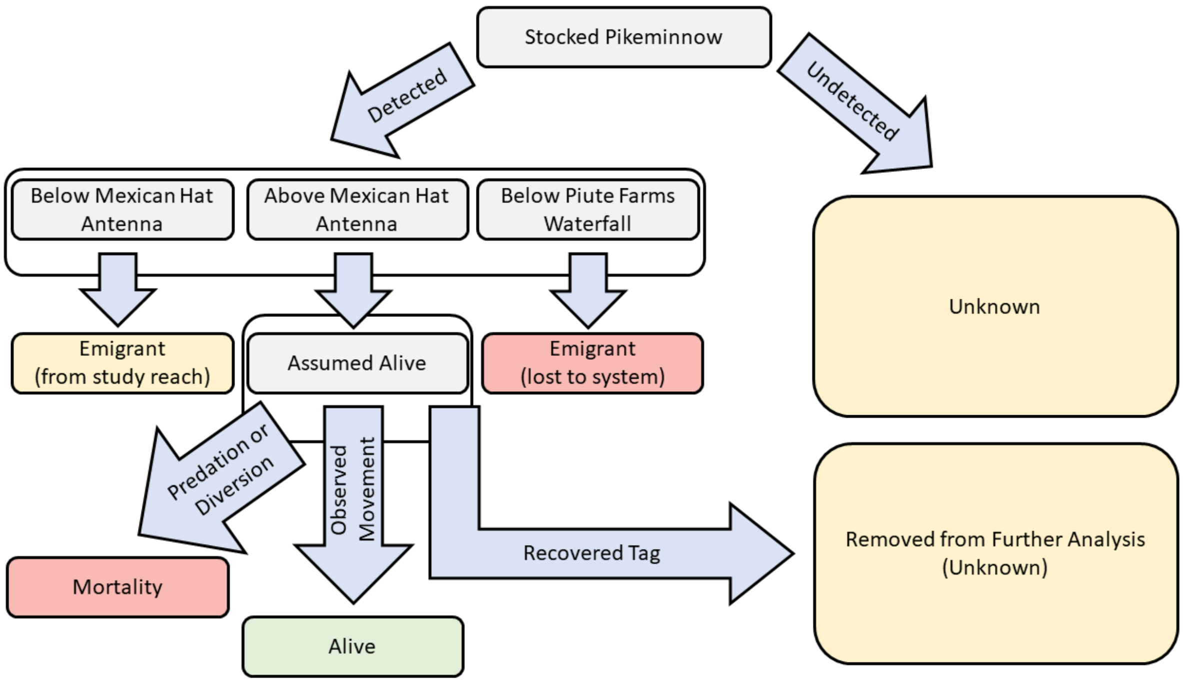


**Figure 6: Flow chart of the stepwise assessment of radio-tagged age-1 Colorado pikeminnow used to determine the fate of individuals following the 60-day study period.**

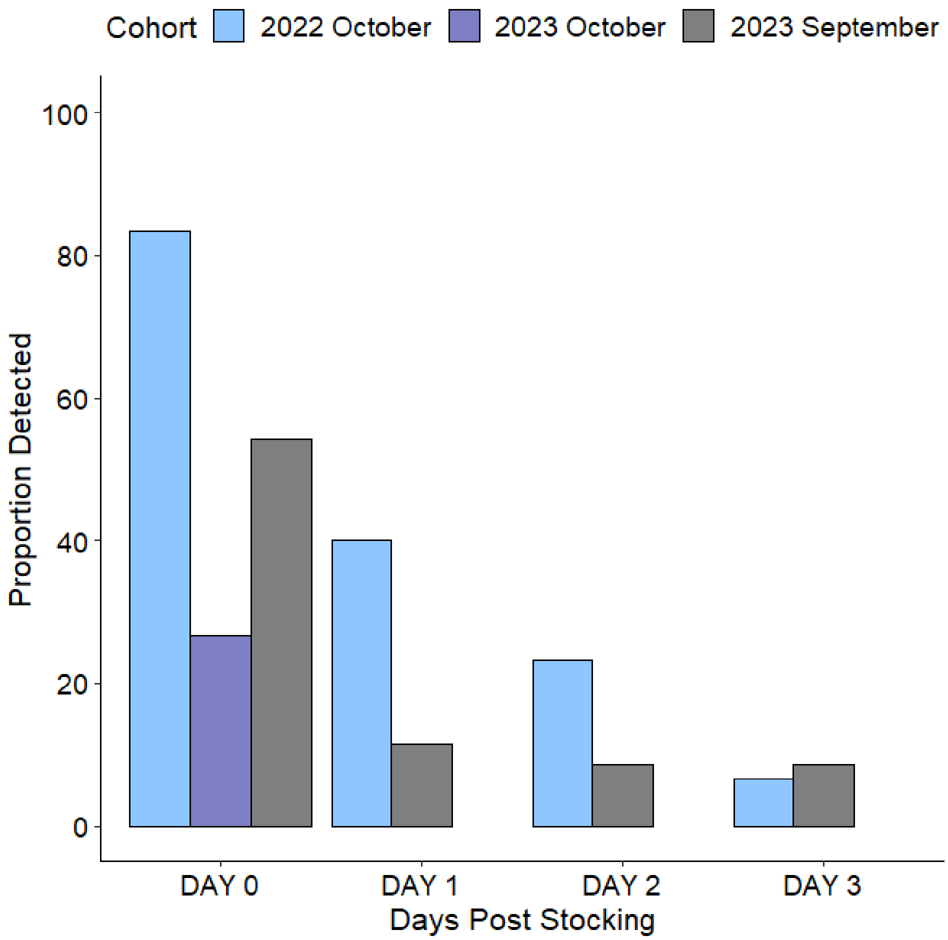


**Figure 7: The percent of Hogback diversion canal control and flow trained Colorado pikeminnow (2022 October), Hogback sluice channel control and flow trained Colorado pikeminnow (2023 October) and Hogback sluice channel larger Colorado pikeminnow (2023 September) detected by mobile surveys and stationary antenna within the stocking reach the first three days following stocking.**

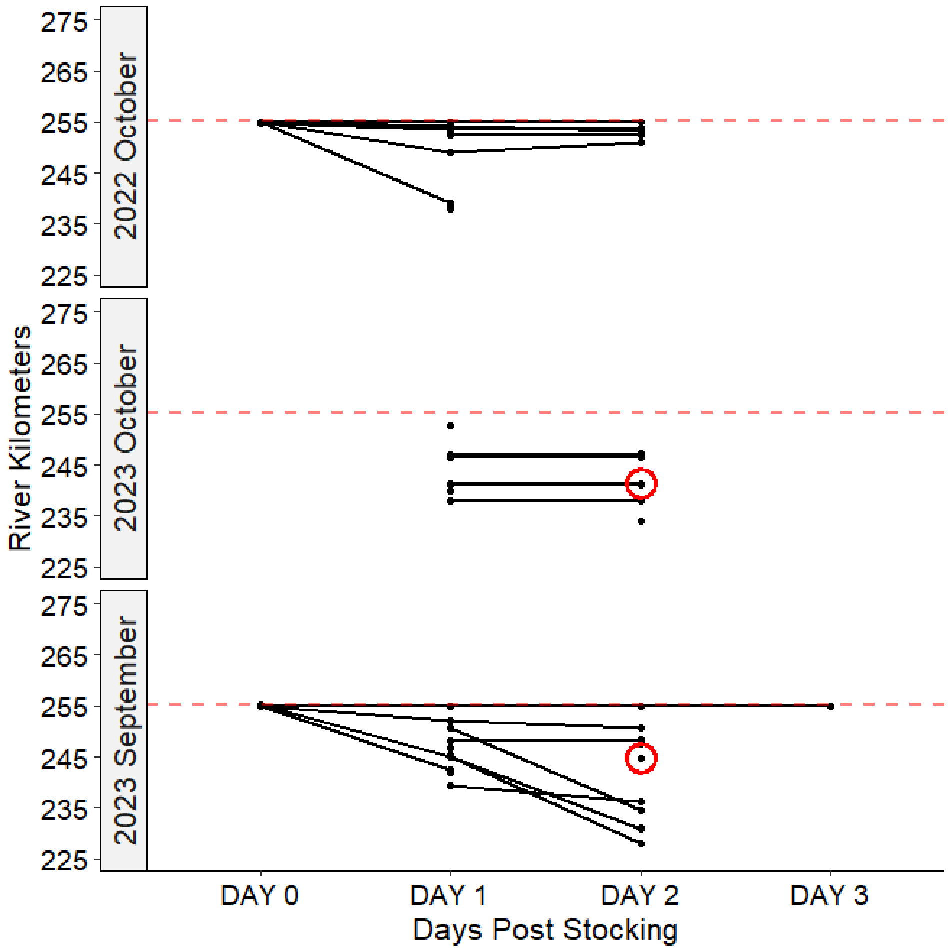


**Figure 8: Longitudinal position of Colorado pikeminnow detected during mobile surveys in relation to the stocking site the first 3 days post-stocking for each stocking cohort. Colorado pikeminnow stocked in October 2022 and 2023 were from the control and flow-trained cohort. Colorado pikeminnow stocked in September 2023 were from the larger control cohort. The red dashed line represents the stocking site river kilometer. The open red circles indicate Colorado pikeminnow that were mortalities due to terrestrial predation.**

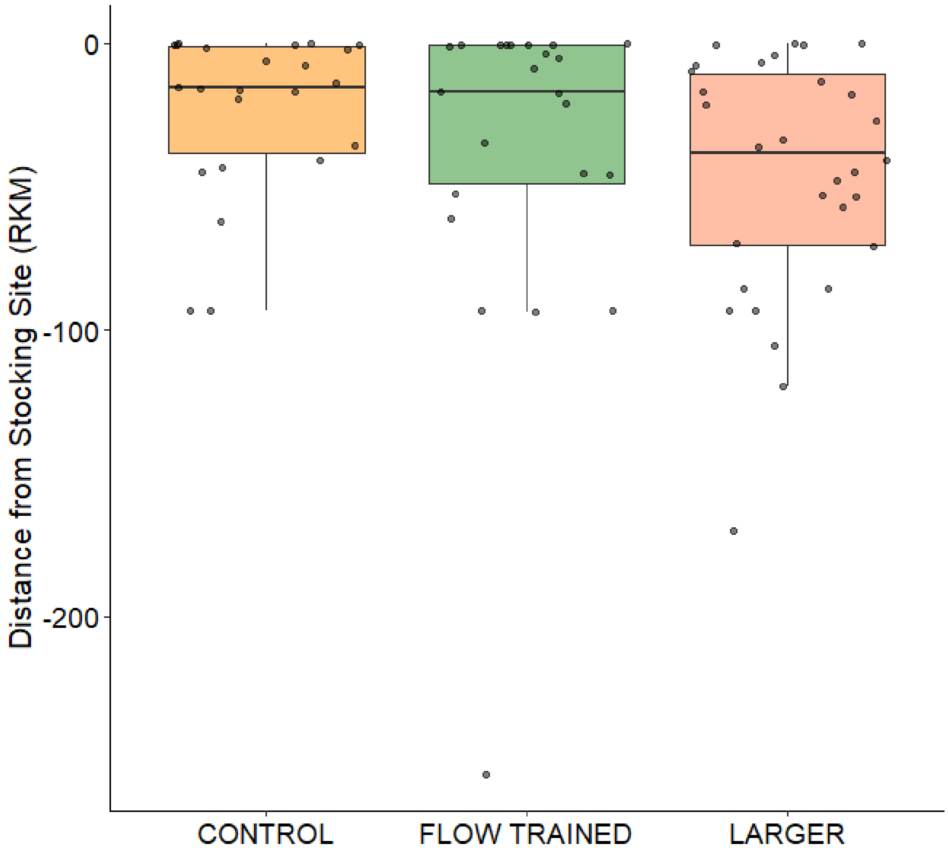


**Figure 9: Boxplots of the distance each radio-tagged Colorado pikeminnow travelled from the stocking site (RKM 0.0) to the river kilometer of their final detection at the end of the 2022 and 2023 study period. Individual Colorado pikeminnow are grouped by the treatments of control (raceway with no flow), flow-trained (raceway with increasing flows), and larger (larger total length in raceway with no flow).**

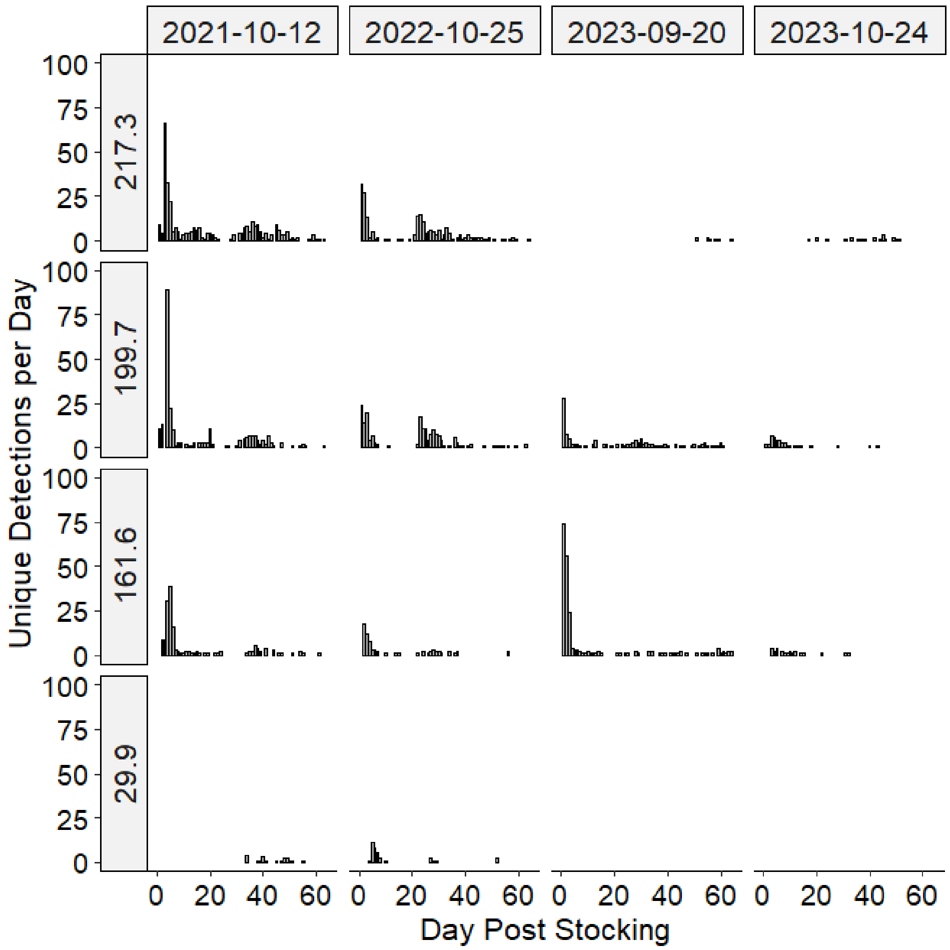


**Figure 10: Number of unique detections of Colorado pikeminnow stocked at Hogback Canal per day on PIT antennas located downstream of the stocking site. PIT antennas were labeled by their associated RKM (i.e. how far upstream of Piute Farms Waterfall, an impassable fish barrier). Data from the PIT antenna located at RKM 29.9 were not yet available for Colorado pikeminnow stocked in 2023.**

**Figure 11: Evidence of the terrestrial predation on stocked Colorado pikeminnow found during a mobile telemetry survey downstream of the stocking site in 2022. This pile of 46 PIT tags from Colorado pikeminnow was found adjacent to a recovered radio tag (not pictured).**

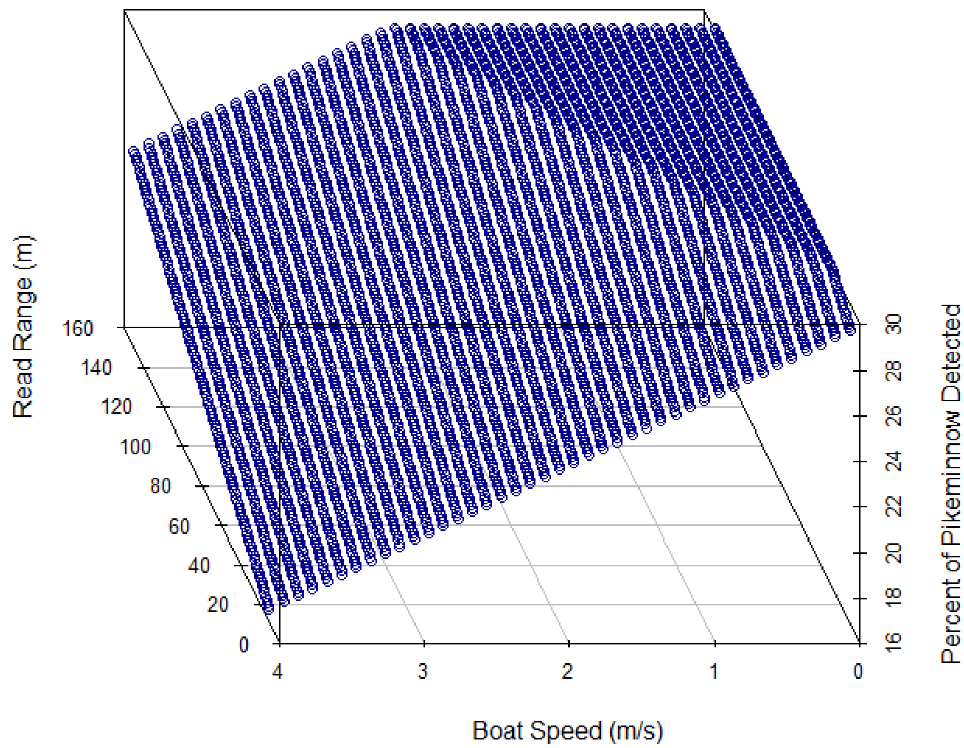


**Figure 12: Predicted number of Colorado pikeminnow detected using probabilities generated from simulations using the mobile survey parameters including antenna read range, scan time on each frequency, number of radio-tagged Colorado pikeminnow and boat speed. The parameters of scan time (3s) and number of tagged Colorado pikeminnow (30) remained constant for all simulations. A range of values was used for boat speed (0 – 4 m/s) and read range (10 – 150 m) to account for scenarios that may have been encountered in the field.**

**Figure 13: Location and orientation of the PIT antenna located downstream of the Piute Farms Waterfall.**

# Tables

**Table 1: Count of radio-tagged and PIT-tagged age-1 Colorado pikeminnow for each hatchery enrichment treatment and the stocking site where Colorado pikeminnow were released.**

| Date | Site | Treatment | # Radio Tagged | # PIT Tagged |
|---|---|---|---|---|
| 10/12/2021 | Hogback Canal | Control | 0 | 6,107 |
| 10/12/2021 | McElmo Creek | Control | 0 | 5,971 |
| 10/25/2022 | Hogback Diversion | Control | 15 | 2,324 |
| 10/25/2022 | Hogback Diversion | Flow-trained | 15 | 2,436 |
| 10/25/2022 | McElmo Creek | Control | 0 | 7,421 |
| 9/20/2023 | Hogback Sluice Channel | Large | 37 | 3,737 |
| 10/24/2023 | Hogback Sluice Channel | Control | 15 | 1,190 |
| 10/24/2023 | Hogback Sluice Channel | Flow-trained | 15 | 1,195 |

**Table 2: Mean, one standard deviation, and range of depth and velocity habitat associations of age-1 stocked Colorado pikeminnow in 2022 and 2023.**

| Treatment | Mean Depth (m) | Depth Range | Depth SD | Mean Velocity (m/s) | Velocity Range (m) | Velocity SD |
|---|---|---|---|---|---|---|
| Control | 0.54 | 0.20 – 1.10 | ± 0.24 | 0.35 | 0.12 – 0.64 | ± 0.14 |
| Flow-trained | 0.58 | 0.14 – 1.25 | ± 0.29 | 0.30 | 0.00 – 0.58 | ± 0.17 |
| Large | 0.46 | 0.10 – 1.20 | ± 0.22 | 0.35 | 0.00 – 0.71 | ± 0.17 |

**Table 3: Fate of radio-tagged Colorado pikeminnow at the conclusion of the 60-day study period. Colorado pikeminnow that shed their tags were removed. Colorado pikeminnow detected below the stationary antenna at Mexican Hat were considered emigrated from the study site. Colorado pikeminnow detected at PFW were considered to have emigrated from the system and thus, a mortality. Colorado pikeminnow detected at the diversion were entrained and considered a mortality.**

| | 2022 Control and Flow-trained | 2023 Larger Pikeminnow | 2023 Control and Flow-trained |
|---|---|---|---|
| Number Tagged | 30 | 35 | 30 |
| Detected | 0 | 12 | 1 |
| Below Mexican Hat | 0 | 1 | 0 |
| Piute Farms Waterfall | 1 | 0 | 0 |
| Shed Tag | 2 | 3 | 0 |
| Diversion | 5 | 0 | 0 |
| Mortality | 2 | 10 | 1 |
| Unknown | 20 | 9 | 28 |

**Table 4: Number of uniquely PIT-tagged Colorado pikeminnow and the cumulative number and percent detected following the first overwintering period (beginning on January 1 following stocking) for each subsequent year. Bolded rows represent unique yearly detections of the entire stocking cohort. Counts of Colorado pikeminnow in italics are preliminary and do not encompass totals for the entire 2024 calendar year.**

| Cohort | Total Stocked | 2022 Detections | 2023 Detections | 2024 Detections* |
|---|---|---|---|---|
| 2021 Hogback Control | 6,107 | 384 (6.3%) | 19 (0.3%) | *0* |
| 2021 McElmo Control | 5,971 | 947 (15.8%) | 16 (0.3%) | *0* |
| **2021 Cohort** | **12,078** | **1,331 (11.0%)** | **35 (0.3%)** | ***0*** |
| 2022 Hogback Control | 2,324 | | 59 (2.5%) | *0* |
| 2022 Hogback Flow-trained | 2,436 | | 62 (2.5%) | *0* |
| 2022 McElmo Control | 7,421 | | 86 (1.2%) | *0* |
| **2022 Cohort** | **12,181** | | **207 (1.7%)** | ***2 (< 0.1%)*** |
| 2023 Hogback Larger | 3,737 | | | *10 (0.3%)* |
| 2023 Hogback Control | 1,190 | | | *5 (0.4%)* |
| 2023 Hogback Flow-trained | 1,195 | | | *0* |
| **2023 Cohort** | **6,122** | | | ***15 (0.2%)*** |

* counts of uniquely PIT-tagged pikeminnow detected in 2024 as of 2/2/2024

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