



State of Utah  
DEPARTMENT OF NATURAL RESOURCES  
*Division of Wildlife Resources – Native Aquatic Species*

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## Assessment of Larval Colorado Pikeminnow Presence and Survival in Low Velocity Habitats in the Middle Green River: 2009–2012

Publication Number: 19-05  
Utah Division of Wildlife Resources  
1594 W. North Temple  
Salt Lake City, Utah  
Michal D. Fowlks, Director



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the Middle Green River: 2009–2012

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## **LIST OF KEY WORDS**

Age-0, larvae, Green River, Colorado pikeminnow, autumn recruitment, backwater, nursery habitat, seine, drift net, nonnative fish, block net

## EXECUTIVE SUMMARY

Despite consistent and high levels of Colorado pikeminnow (*Ptychocheilus lucius*; CPM) reproductive success, first year survival of larval fish remains poor. In order to better understand and perhaps remedy this situation, research was conducted to examine potential factors impeding survival in nursery backwater habitats. Based on results from a demonstration project conducted on the middle Green River (UT) in 2008 (*Backwater Restoration Ad Hoc Group* 2008), we conducted a study from 2009-2012 to investigate the influence of small-bodied nonnative fishes on age-0 CPM survival in backwater habitats. Our overall goal was to demonstrate experimentally that nonnative fishes have a negative impact on CPM autumn recruitment. Our main objectives were to (1) verify larval CPM arrival in the nursery reach, (2) reduce densities of nonnative fishes, particularly cyprinids, in backwater habitats before and after CPM arrival, (3) determine the efficacy of manipulating backwaters to increase CPM survival by removing and excluding nonnative fishes using various blocking techniques, and (4) document age-0 CPM abundance in backwaters as the summer progresses and assess small-bodied fish community composition resulting from the exclusion of nonnative fishes from backwater habitats. Examination of these objectives was originally slated for three consecutive years of study (2009–2011), but 2011 project implementation was postponed until 2012 due to extended high spring peak flows.

In each year of study, drift netting for larval CPM was conducted downstream of the Split Mountain boat ramp (river mile [RM] 310) following verification of larval CPM presence at Echo Park (RM 345) in Dinosaur National Monument. Following verification of drifting larvae

at Echo Park, arrival of larval fish in backwater habitats in the middle Green River was verified by seining backwaters downstream.

For objectives 2–4, significant changes to experimental design in 2009 and inadequate replication of experimental treatments in 2010 prohibited proper analysis and interpretation of data, so these years should be considered as pilot-level projects; only 2012 sampling design and analyses are thoroughly analyzed and discussed. More specifically, in 2012 we implemented a more complete study design with adequate replication (i.e., at least three replicates per treatment type). We scouted our study reach one week prior to sampling to select backwaters based on Interagency Standardized Monitoring Program selection criteria and feasibility of effectively depleting backwaters. Twelve backwaters were selected for a randomized block study design (RM 305–273.5) where four blocks (three backwaters/block) contained one of each treatment type by random selection (i.e., four replicates/treatment). All backwaters were blocked and initially depleted of nonnatives (90% depletion goal) on the first sampling trip with a beach seine. Blocking treatments included four Control backwaters that were not blocked after initial depletions, four backwaters blocked by 1/4" mesh block nets, and four backwaters blocked by 1/2" mesh block nets. Following backwater depletions, each experimental backwater was revisited every other week on six separate occasions (10 July–25 September 2012) to monitor fish community response. To analyze changes in fish community composition over time, we conducted a Repeated Measures ANOVA.

Environmental conditions (i.e., dry hydrology) and deteriorating habitat quality over time were not conducive to CPM larval drift, arrival in nursery areas, or age-0 recruitment in 2012. Thus, evaluation of CPM survival and recruitment in response to removal of nonnative fish in nursery habitats was not possible for this species. However, other age-0 native fishes collected in

2012 provided helpful insights on the effects of our experimental design. For example, 1/2" mesh block net treatments had a significant positive effect on survival in comparison to Control backwaters and 1/4" mesh blocking treatments throughout the experiment. Flannemouth sucker (*Catostomus latipinnis*) comprised the majority of the combined native fish metric, especially in 1/2" mesh blocking treatments. Substantial growth was observed throughout the study and by the final sample period (18–25 September 2012) flannemouth sucker achieved sizes >100 mm total length (TL). Red shiner (*Cyprinella lutrensis*) and other nonnative small-bodied fish species abundance increased over time following initial depletions in 2012, indicating that either movement between riverine and backwater environments or new production within backwaters was occurring. Abundance of nonnative fish was greatest in 1/2" mesh blocking treatments. Red shiner TL decreased over time, suggesting we were effective at capturing and removing nonnative fish as new cohorts became susceptible to our gear. Moreover, flannemouth sucker likely had the ability to move between habitats as needed for refuge from predation and to acquire necessary resources given that native fish were completely absent from Control backwaters before the fourth sample period (20–22 August 2012) and nearly eliminated from 1/4" mesh blocking treatments before the fifth sample period (4–5 September 2012).

Control backwaters contained the lowest abundance of all species, suggesting that predation by nonnative piscivores coming from riverine habitats may be a significant threat to small-bodied fishes and blocking treatments can positively influence survival in nursery areas (native and nonnative fishes). Moreover, we documented predation on an age-0 flannemouth sucker by an age-0 smallmouth bass [*Micropterus dolomieu*] in a control backwater. By blocking backwater nursery areas, we created predator free habitats that benefited most species of small-bodied fishes. Despite higher abundance of small-bodied nonnative fishes in 1/4" and 1/2" mesh

blocking treatments throughout our experiment, age-0 native fishes were also more abundant (1/2" mesh treatments containing the highest abundance). We do not discount that competition between age-0 native fishes and nonnative cyprinids can be intense in backwaters, but if we can control for predation by blocking backwaters, survival can be positively influenced despite ongoing competition for resources in nursery habitats.

Although largely unpredictable, we recommend continuing experimentation with backwater blocking and nonnative fish depletion treatments for an additional season when environmental factors (i.e., extreme drought or flooding) do not play such a large role in affecting experimental outcomes. Implementation of this study design under ideal flow conditions throughout the months of August and September (1,700–3,000 cfs for the middle Green River) would likely result in important management implications and recommended recovery actions for this endangered species. Lastly, it is crucial that such management activities occur as soon as possible and for several years to bolster current population declines, given that CPM require between 5-8 years to reach sexual maturity.

## **INTRODUCTION**

The Colorado River basin harbors a native fish community characterized by low diversity and high endemism. However, introduction and proliferation of nonnative fishes now make it one of the most altered fish assemblages in the United States of America (Fuller et al. 1999; Rahel 2000; Tyus and Saunders 2000). Introduction and establishment of nonnative fishes has mainly occurred via purposeful stocking events for angling opportunities and the deliberate illegal transfer of fish by members of the public (Rahel and Smith 2018). Furthermore, water manipulation in the southwestern United States has detrimentally impacted native fish habitats

(Minckley and Deacon 1968) and accelerated invasions by nonnative fishes (Tyus and Saunders 2000). For example, in the Green River sub-basin, Flaming Gorge Dam operations have greatly altered historic flow and temperature regimes, sediment transport dynamics, and several other key biotic and abiotic factors (Muth et al. 2000) allowing introduced species to flourish.

Competitive and predatory interactions from nonnative fishes negatively impacts native fish at all life stages (Haines and Tyus 1990; Karp and Tyus 1990; Tyus and Saunders 2000). Early life-stages of endangered Colorado River fishes are particularly vulnerable to negative interactions with nonnative fishes because they grow slowly and lack defense mechanisms to avoid predation (i.e., they evolved in isolation without these pressures). Reductions in survival of early life stages of native fishes has reduced recruitment to sexual maturity and subsequently resulted in the decline of many native fish populations. In fact, negative impacts from nonnative fishes, along with water development and withdrawal, are primary reasons for four native fishes' listing under the Endangered Species Act – Colorado pikeminnow (*Ptychocheilus lucius*; hereafter CPM), razorback sucker (*Xyrauchen texanus*), humpback chub (*Gila cypha*), and bonytail (*G. elegans*; USFWS 2002a; USFWS 2002b; USFWS 2002c; Jelks et al. 2008).

One of these endangered species, the Colorado pikeminnow, is the native apex predator of the warm water portions of the Colorado River ecosystem. The species is a large-bodied, long-lived, late maturing migratory spawner, using established spawning bars for egg deposition and fertilization (e.g., Bestgen et al. 2006; Bestgen and Hill 2016). Upon hatching, larval fish drift downstream into low gradient depositional reaches which provide low velocity, warm water backwater habitats for young fish. These warm-water backwater habitats provide higher food production, faster growth, and may harbor fewer predators than mainstem river habitats and are especially important for survival during the first summer of life (Muth et al. 2000; Grippo et al.

2017). One established CPM spawning location is Cleopatra's Couch in the Yampa River in Dinosaur National Monument, Colorado (Tyus 1990; Figure 1). Young fish (< 9 mm total length [TL]) produced here drift downstream into the Green River, through Whirlpool and Split Mountain canyons, and settle in productive backwater habitats throughout the alluvial nursery reach of the middle Green River between Jensen and Ouray, Utah (Figure 1).

The overwhelming abundance of nonnative cyprinids in essential backwater habitats have reduced habitat quality by reducing available food and increasing predation pressure. More specifically, fathead minnow (*Pimephales promelas*), red shiner (*Cyprinella lutrensis*), and sand shiner (*Notropis stramineus*) comprise up to 90–99% of the fish community in backwater habitats (Haines and Tyus 1990; McAda et al. 1994). Furthermore, red shiners pose a significant threat to early life-stage native fishes because of their wide distribution and high abundance in the Green River sub-basin (Tyus and Saunders 2000; Bestgen et al. 2006; Bestgen and Hill 2016). They both compete with (Karp and Tyus 1990; Seegert et al. 2014) and directly prey on native fishes (Ruppert et al. 1993; Bestgen et al. 2006), and their reproductive ecology affords them the ability to produce multiple successful cohorts within a single season (e.g., Herrington and DeVries 2008). With specific mention to young CPM, laboratory experiments demonstrated that both growth and survival are adversely affected by red shiners and fathead minnows due to similar activity schedules, space use patterns, shared habitat use, and aggressive behaviors (Karp and Tyus 1990; Bestgen et al. 2006).

Intensive investigations examining CPM early life history and recruitment in the middle Green River began in the late 1970's (e.g., Tyus and Haines 1991). A result of such studies, the Interagency Standardized Monitoring Program ([ISMP]; USFWS 1987) was developed to monitor autumn recruitment of age-0 CPM (defined as survival of age-0 CPM over the first

summer of life into the autumn months) and has been conducted annually since 1986. Every September–October, ISMP sampling occurs in the middle and lower Green River, as well as the lower Colorado River, and is conducted under Recovery Program Project #138 (e.g., Breen et al. 2017). Based on ISMP selection criteria, a suitable backwater habitat is defined as a low-velocity area that is isolated from main channel flows (i.e., no upstream connection), is  $\geq 25 \text{ m}^2$ , and is  $\geq 1$  ft in depth (USFWS 1987). The first two suitable backwaters encountered in each 5-mile sub-reach are sampled with a seine ( $\geq 25\%$  of each habitat) to determine abundance, catch rates, and distribution of age-0 CPM. Additionally, a variety of physical habitat measurements are collected at each site to relate autumn recruitment of age-0 CPM to environmental variables.

Beginning in 1994, drastic reductions in age-0 CPM abundance in the middle Green River were apparent, marking a substantial long-term decline in autumn recruitment (Breen et al. 2011) despite relatively stable upstream production and subsequent larval drift during those years (Bestgen et al. 1998; Bestgen and Hill 2016). Juvenile and adult CPM abundance is a function of age-0 recruitment (Bestgen et al. 2007a), thus continued population declines have been observed in recent years (Bestgen and Hill 2016; Bestgen et al. 2018).

Due to poor autumn recruitment of age-0 CPM despite continued production, several studies were conducted to examine potential factors impeding survival in nursery areas. Nonnative cyprinid control efforts in backwater habitats were attempted in the lower Colorado and Green rivers, but with little success in permanently shifting backwater species composition that would favor age-0 CPM survivorship (Trammell et al. 2002, 2004). Following the onset of intensive nonnative removal efforts (Tyus and Saunders 1996), two studies were initiated in the upper Colorado River basin in the early- to mid-2000's to investigate the response of early-life stage native fishes to nonnative predator removal (Bestgen et al. 2007b; Skorupski et al. 2012c).

However, this work was not very revealing for the middle Green River reach (Skorupski et al. 2012c), because there was not a true treatment (small-bodied nonnatives mechanically removed) and control reach (no mechanical removal) as was evaluated in the Yampa River (Bestgen et al. 2007b). Although ineffective elsewhere in the upper Colorado River basin, a demonstration project was conducted in the middle Green River in 2008 focused on nonnative cyprinid removal in backwaters prior to the arrival of drifting larval CPM (*Backwater Restoration Ad Hoc Group* 2008) with the hope of leading towards a more structured sampling design.

Previous attempts to enhance age-0 CPM survival in nursery habitats (Trammell et al. 2002, 2004; *Backwater Restoration Ad Hoc Group* 2008) prompted discussions at the 30<sup>th</sup> Annual Researcher's Meeting of the Upper Colorado River Endangered Fish Recovery Program and San Juan River Basin Recovery Implementation Program leading to the development of this project designed to fully investigate this matter in the middle Green River. This study seeks to address the influence that small-bodied nonnative fishes have on age-0 CPM in backwater habitats where these species overlap. Our overall goal was to demonstrate experimentally that nonnative fishes have a negative impact on CPM autumn recruitment. Our main objectives were to (1) verify larval CPM arrival in the nursery reach, (2) reduce densities of nonnative fishes, particularly cyprinids, in backwater habitats before and after CPM arrival, (3) determine the efficacy of manipulating backwaters to increase CPM survival by removing and excluding nonnative fishes using various blocking techniques, and (4) document age-0 CPM abundance in backwaters as the summer progresses and assess small-bodied fish community composition resulting from the exclusion of nonnative fishes from backwater habitats. Although the most recent version of the scope of work for this project contained five objectives, two of those were

combined for this report (objective four) to maintain a logical progression of tasks completed through time.

## **METHODS**

### *Study Area*

The Green River is the largest tributary of the Colorado River, and extends north to drain northwestern Colorado, eastern Utah, and southwestern Wyoming. Historically, the Colorado River basin was characterized by high spring flows from snowmelt runoff and low summer flows with warm water temperatures, punctuated by thunderstorm driven flash flood events. Water development and large dams have modified this pattern to one with less dramatic fluctuations, generally resulting in lower spring peaks and higher base flows (e.g., Muth et al. 2000; Bestgen and Hill 2016). Our study reach is located within the middle Green River (river mile [RM] 319-248), which lies downstream of higher gradient canyon sections of Dinosaur National Monument. Here, the Uinta Basin forms a broad, alluvial reach where the Green River largely consists of a sand bed substrate with backwater and side channel habitats used by several species of larval and small-bodied fishes (e.g., Breen et al. 2011). The middle Green River reach is influenced by the Flaming Gorge Dam on the Green River and the largely unregulated Yampa River. The Uinta Basin is located downstream from the confluence of these two rivers (Figure 1), and exhibits an intermediate hydrology driven by each river at different times of year. The Yampa River serves to maintain relatively high peak flows in spring and contributes large amounts of sediment to the alluvial nursery reach (Tyus and Saunders 2001). Managed releases from Flaming Gorge Dam can augment spring peak flows, thereby enhancing floodplain connections during the runoff period (Lagory et al. 2012), while maintaining higher base flows

than those found in pre-dam winter periods. Bestgen and Hill (2016) found, however, that despite higher flows over winter, mean monthly flows during June-August had decreased over the post-dam period.

***Verify larval Colorado pikeminnow drift and arrival in backwater nursery habitats (Objective 1)***

To accomplish the first objective, larvae were sampled downstream of the Split Mountain boat ramp in Dinosaur National Monument (Figures 1-3). Drift net sampling began when the Colorado State University Larval Fish Laboratory (CSU-LFL) indicated larval CPM were captured at the Echo Park site (Bestgen et al. 2010, 2011, 2012) ca. 25 river miles upstream. Drifting larvae were collected with three conical drift nets, each measuring 50 x 30 cm at the mouth and 4 m long, with 560  $\mu$ m mesh. Drift nets were deployed at 0600 and sampled for approximately two hours, unless high debris loads necessitated a shorter sampling duration. Nets were deployed near shore with the inshore net fully submerged and subsequent nets extending away from shore. Flow meters (General Oceanics model 2030) were placed in the mouth of each net to estimate stream velocity, which was later used to estimate river volume sampled. Entire drift net sample contents were preserved in 100% ethanol and sorted in the laboratory immediately upon return from the field (within 2 hrs). Larvae were then preserved in fresh 100% ethanol after separation from debris. All larvae collected were sent to CSU-LFL for identification, measurement, and curation.

To verify drifting larvae had arrived in the nursery habitats, low velocity habitats were seined for larval CPM. For this objective, we defined backwaters as habitats with no flow velocity that were connected but defined from the main channel—usually by a sand bar oriented roughly parallel to the river channel. For this objective, we did not use pre-established criteria

(i.e., ISMP standards) to select backwaters, but instead attempted to detect larvae wherever possible. Backwaters were seined after drift net sampling at Echo Park verified larval CPM presence, typically late-July through mid-August, except in 2012 when most sampling occurred in mid- to late-July. Seining was conducted between Red Wash (RM 298.5) and Ouray, UT (RM 248), but focused intensively on the reach in and below Ouray National Wildlife Refuge (RM 265-250) with the exception of backwaters selected for experimental manipulations (see below). Backwaters were sampled with a 4.6 m x 1.2 m seine (1/32" mesh). In larger backwaters, seines were typically pulled parallel to shore to avoid seining deeper water where sampling becomes less effective.

Larval and juvenile fish were picked from seines and preserved immediately in 100% ethanol. Samples were later transferred into fresh ethanol and new storage containers upon returning to the lab each day. These samples were sent to CSU-LFL for positive identification, enumeration, and measurement (TL). When age-0 native fishes were large enough to positively identify, they were enumerated, measured, and returned to the backwater. Backwaters were also measured for overall length, width, maximum depth, and temperature, similar to ISMP methods (USFWS 1987; McAda et al. 1994).

***Nonnative depletions (Objective 2), experimental backwater treatments (Objective 3), and fish community response (Objective 4)***

Given the evolving nature of this study, depletion protocols (objective two) and experimental blocking scenarios (objective three) evolved each year (Table 1); 2009 and 2010 should only be considered as pilot-level projects. Additionally, primary investigators have changed during each year of implementation (Hedrick et al. 2009, 2010; Skorupski et al. 2012a), bringing new ideas and project improvements. Examination of all objectives was

originally slated for three consecutive years of study (2009–2011). However, 2011 project implementation was postponed until 2012 due to extended high peak flows in 2011, which proved to be valuable as age-0 CPM were absent from autumn sampling in the middle Green River (Skorupski et al. 2011).

In 2009, nine experimental backwaters (three of each treatment type; Table 1) were selected (random treatment assignment) within the study area (Figure 1). Treatment A backwaters were blocked with a block net (1/2" mesh; 2 m tall x 50–100 m long depending on backwater width) at the mouth of the backwater, depleted of small-bodied nonnative fish, and then opened to restore river connection. Treatment B backwaters were blocked, depleted of nonnatives, and the block net (1/2" mesh) remained in place for the duration of the study. Control backwaters were depleted of nonnatives and left unblocked; they were not blocked during depletions. Backwaters two and five (Figure 1) were specifically selected because they were sampled during preliminary surveys conducted in 2008 (*Backwater Restoration Ad Hoc Group* 2008). Nonnative fish depletion efforts (90% depletion goal) began on 20 July 2009 and were conducted every other week for the first three sampling trips using a beach seine (4.6 m x 1.2 m; 1/4" mesh) and as many seine hauls as needed to achieve desired levels of depletion. Nonnative fish depletions were calculated as the percent nonnatives depleted from a backwater:  $100 - (\text{SUM of nonnatives collected in the final seine haul} / \text{SUM of nonnatives collected in all subsequent seine hauls} * 100)$ . When age-0 CPM were identified in a given backwater during depletion efforts, seining was terminated to avoid additional stress. During the third sampling trip (17–20 August 2009), an abundance of CPM were collected from backwater three (Control) and a block net was established for the remainder of the study (i.e., no longer a control backwater). Following the third sampling trip, backwater sampling and collection of habitat

information was conducted in accordance with ISMP protocols (USFWS 1987; McAda et al. 1994) to reduce CPM mortality (i.e., 1-2 seine hauls/backwater instead of many; see Results). During the fourth sampling trip (31 August–2 September 2009), it was discovered that the block net in backwater five (Treatment B) was stolen, and it remained unblocked for the remainder of the study. Fish community response to nonnative depletions (objective four) was determined by seining (1/4" and 1/8" mesh nets) study backwaters every other week from 20 July 2009 to 29 September 2009 (six sample periods total).

Fewer backwaters were sampled in 2010, as suggested by the Recovery Program, to increase project feasibility (i.e., depletions proved time consuming in 2009; see Results), and experimental treatments were altered to improve sampling design by focusing more on blocking techniques than open backwaters (Table 1). Six experimental backwaters were selected (random treatment assignment) within the study area (Figure 2). Two backwaters were treated as Controls, two backwaters were blocked with 1/4" mesh block nets, and two backwaters were blocked with 1/2" mesh block nets. For initial depletions, seine hauls were repeated until depletion goals were met; when CPM were captured in a backwater, depletion efforts were terminated to avoid additional stress. Block net treatments were blocked at the mouth of the backwater, depleted of nonnatives, and the block net width remained throughout the study period. For Control backwaters, a temporary block net was positioned during the initial depletion and then removed for the duration of the study. Following the initial depletion (first sampling trip only, 27–29 July 2010), all study backwaters were sampled every other week (five sample periods total) following ISMP protocols (USFWS 1987; McAda et al. 1994; 1-2 seine hauls/backwater, 1/8" mesh seine). On the third sampling trip (23–24 August 2010), it was discovered that a 1/2" block net had been stolen (backwater three; Figure 2), and that treatment

had to be excluded from the experiment. On the final sampling trip (21–23 September 2010), backwater two (Control) was disconnected from the river, and backwater six (1/2" mesh block net) was discovered to have a large hole in the block net, resulting in exclusion from the experiment in both instances.

Experimental treatments were altered again in 2012 to improve treatment replication for a more robust analysis (Table 1). Twelve backwaters were selected within RM 305–273.5 for a randomized block study design (Figure 3). Four blocks (three backwaters/block) contained one of each treatment type by random selection (i.e., four replicates/treatment), all of which were blocked when initially depleted of nonnatives (objective two) on the first sampling trip (25–28 June 2012). Specific blocking treatments (objective three) included four Control backwaters that were not blocked after initial depletions, four backwaters blocked by 1/4" mesh block nets, and four backwaters blocked by 1/2" mesh block nets. The study reach was scouted one week prior to sampling to select backwaters based on ISMP selection criteria (USFWS 1987; McAda et al. 1994) and feasibility of effectively depleting backwaters. Only 1/8" mesh seines were utilized in 2012 (enlisting ISMP seining protocols following initial depletions); two seines were woven together to create one 9.1 m x 1.2 m seine for better depletion capabilities (Figure 4). After establishing block nets (2 m tall x 50–100 m long depending on backwater width) and depleting backwaters (Figure 4), they were revisited every other week on six separate occasions (seven sample periods total) to monitor fish community response (objective four), with the last sampling event occurring from 18–25 September 2012. As water levels consistently dropped throughout the summer (see Results), river connection with backwater four (Control) was receding by the fourth sample period (7–9 August 2012); this backwater was completely isolated prior to the seventh sampling event. During the fifth sampling period (20–22 August

2012) we discovered that the block net was stolen from backwater six (1/2" mesh). Both backwaters were excluded from the experiment as a result; however, a minimum of three of each treatment type remained for a more robust analysis than previous years.

Beyond backwater blocking scenarios and depletion protocols, several similarities in sampling techniques existed among years. All block nets were erected with t-posts and reinforced by chicken wire, then tied together with zip ties (Figure 4) to limit damage by beavers (*Castor canadensis*), muskrats (*Ondatra zibethicus*), and river otters (*Lontra canadensis*). However, previous observations of fish escapement/entrance in blocked backwaters (see Results) prompted the addition of spiral anchors (30.5 cm in length) to ensure a good seal of nets along substrata in 2012. Temperature loggers (HOBO® Pendant) were placed in each backwater, and their location was adjusted throughout the study as habitat size decreased. Habitat measurements of backwaters (in addition to standard ISMP habitat data) were taken at every sampling event and included date and time of sampling, temperature logger depth, maximum and average backwater depth (2012 only), and the width and length of backwaters to monitor habitat change over time (2012 only). Identifiable age-0 CPM and other native fishes were measured (TL) and released alive in their respective backwater. However, in 2009 thousands of age-0 CPM were collected from backwater number nine (Treatment B; L. Monroe field notes), and fish were not measured or enumerated to reduce the potential for desiccation; other exceptions will be discussed in the Results. With the exception of 2012, where 30 individuals of each nonnative species were sub-sampled for measurement, nonnative fish were only enumerated. In 2012, when seine hauls were overwhelming (i.e., thousands of fish), all fish collected from a seine haul were placed in an in-stream live well to limit mortalities, while we sorted and measured all native fishes and rare nonnative species (Figure

4), then we sub-sampled to enumerate other nonnatives. Unidentifiable specimens were preserved in 100% ethanol and sent to CSU-LFL for later identification.

### ***Data Analysis***

To analyze data collected for the first objective, transport indices were calculated for CPM larvae sampled at Split Mountain after Bestgen and Hill (2016). The transport abundance index was calculated by dividing the number of larvae collected per hour by the proportion of total river discharge sampled. Average daily discharge was obtained from the USGS Green River gauge at Jensen, UT (gauge #09261000), approximately three RM downstream from the drift net site. Additionally for the first objective, we calculated mean maximum depth and mean temperature difference between main channel and backwater habitats with and without CPM.

Analysis of objective four was limited for 2009 based on available data. For reasons described previously, backwater number three (Control) and five (Treatment B) were excluded from all 2009 analyses. Therefore, statistical comparisons of age-0 CPM abundance between treatment types were inappropriate due to lack of replication; only two Controls, three Treatment A, and two Treatment B backwaters were available for comparison (Table 1). As a result, we only report mean CPM abundance by treatment type ( $\pm 1$  standard error), incorporating all backwater data that was not excluded due to violation of experimental assumptions. Given that CPM were abundant, we evaluated differences in CPM growth between treatment types using the non-parametric Mann-Whitney Rank Sum Test because raw data and log-transformed data were not normally distributed. However, with deficiencies in sample size we were only able to compare Treatment A and Treatment B backwaters from the fifth sample period. To assess community composition following nonnative fish depletions, data from sample periods 4-6 were

compiled for each species and the percent species composition was calculated for each backwater treatment type (sample periods combined).

Objective four analyses were limited for 2010 based on available data. Abundance of CPM was plotted for each treatment type and sample period (similar treatments combined within sample periods). However, statistical comparisons of CPM abundance between treatment types were inappropriate due to lack of replication by the conclusion of the experiment (i.e., only one Control and two 1/4" mesh treatments lasted the duration of the experiment; Table 1). Therefore, we only report mean CPM abundance by treatment type ( $\pm 1$  standard error), incorporating all backwater data that was not excluded due to violation of experimental assumptions. In addition, three age-1 CPM collected during the first sample period (87, 96, and 120 mm TL) were excluded from all analyses given that age-0 CPM survival is the focus. To assess community composition following nonnative fish depletions, data from sample periods 2-4 were compiled for each species and percent species composition was calculated for each backwater treatment type (sample periods combined). Note that this only includes one 1/2" mesh treatment as described previously; the fifth sample period is ignored for a more complete comparison.

Given a much more robust data set with increased replication (i.e., no longer a pilot study; Table 1), additional analyses were conducted in 2012 to evaluate experimental conditions (objective three), linking objective four results more clearly to biotic and abiotic factors. Based on fundamental findings of Bestgen and Hill (2016), we plotted mean daily discharge data collected at Jensen, UT (USGS gauge #09261000) for the months of August and September to illustrate whether ideal flow targets to benefit age-0 CPM recruitment were achieved during the experimental portion of each study year. We also plotted backwater temperature throughout the

experiment and analyzed backwater habitat change through time (i.e., loss of depth and area of backwaters).

Given that flannelmouth sucker was the most abundant native fish, we summarized their growth by treatment type. To assess community composition following nonnative fish depletions, data from sample periods 2-7 were compiled for each species and the percent species composition was calculated for each backwater treatment type (sample periods combined).

To analyze changes in fish community composition over time (objective four), we conducted a Repeated Measures ANOVA for 2012 data. This analysis is appropriate given the nature of the study (i.e., measurements on the same experimental unit through time; n=6 post-depletion sample periods for comparison) and was determined a priori. Given that only one age-0 CPM was collected post-depletion, we combined all native fish as a surrogate response variable to characterize the response of CPM to removal of nonnative fishes. Total abundance was used as the dependent variable to evaluate survival. We also analyzed red shiner total abundance and TL given they were one of the most abundant species collected post-depletion (see Results) and a significant threat to CPM survival (e.g., Bestgen and Hill 2016). Log-transformed data was used for native fish total abundance, whereas normality assumptions were met for red shiner total abundance and TL, thus raw data was examined. Generalized least squares were fit for each data set; mean values for each treatment type and sample period were used for all analyses. Covariates evaluated to determine abiotic effects on survival included backwater area, maximum depth of backwaters, backwater temperature, and main channel temperature at time of sampling. Excluded backwaters (see Methods above and Table 1) both occurred in the second block (Figure 3); therefore, we also excluded the 1/4" mesh treatment in the second block from this analysis to maintain a balanced design for our analysis (i.e., three replicates/treatment type).

Backwater (the experimental unit) was treated as the random effect and all other variables were fixed factors. Covariance structure was evaluated in our analysis (Littell et al. 2000) and models were fit using the R statistics software (R Core Development Team 2014). An information-theoretic approach was used to select among competing models explaining variability in total abundance and TL (Anderson and Burnham 2002; Burnham and Anderson 2002). Six candidate models were developed for each response variable and Akaike's information criterion adjusted for small sample size ( $AIC_c$ ) was used to compare candidate models and select the best model (i.e., lowest  $AIC_c$  value; Burnham and Anderson 2002). Once the best model was selected we conducted a 2-way ANOVA to determine significant differences between treatments. A Tukey Contrasts test for multiple comparisons of means was used for additional post-hoc analyses to determine differences between sample periods.

## RESULTS

### *Verify larval Colorado pikeminnow drift and arrival in backwater nursery habitats (Objective 1)*

#### **2009 Results**

For our first objective, drift net sampling occurred for 25 days between July 16 and 20 August (red data presented in Figure 5a). During this time 18 larval CPM were captured, with 14 captures occurring 21-29 July. The transport index for days when CPM were captured ranged from 289-773 larvae/hr.

During the 2009 seining effort (also objective one), we were able to sample 32 individual backwaters from 28 July to 18 August. Of these 32 sites, CPM larvae were present in 22 backwaters (69%) at least once. We captured 390 larvae during this period, ranging between 9-

33 mm TL (mean=21.5 mm). The catch-per-unit-effort (CPUE) for 2009 was 4.91 fish/100 m<sup>2</sup>. There was not a statistically significant difference in mean maximum depth for occupied and unoccupied habitats.

### **2010 Results**

Drift net sampling for 2010 occurred from 13 July until 13 August, totaling 23 days (Figure 5b). A total of seven larvae were captured in the 2010 sampling, with the majority of captures (n=6) again between 21-29 July. Transport indices in 2010 were between 149-534 larvae/hr.

Seine sampling took place 27 July through 10 August, and we were able to sample 27 backwaters. Eleven of 27 backwaters had CPM at least once (40.7%). Only two CPM larvae (17.4 and 18 mm TL) were caught in the early sampling (27 July), and the rest of the fish (n=68) were collected in August. By August 9, CPM were large enough to identify and return to the water. The catch rate for the entire seining period was 0.53 fish/100 m<sup>2</sup>, and for August it was 0.87 fish/100 m<sup>2</sup>. There was not a statistically significant difference in mean maximum depth for occupied and unoccupied habitats.

### **2012 Results**

The 2012 drift netting occurred 26 June through 27 July, representing 23 days of effort (Figure 5c). Earlier sampling was due to below average runoff and an early peak flow (USGS gauge #09261000). Flows during the sampling period were also lower than previous years, so drift nets were able to sample a larger percentage of river volume. As a result, a total of 16 CPM larvae were captured in 2012, but transport indices were generally lower, with the exception of a pulse of fish captured 28-29 June. Despite this pulse, transport indices were still relatively low and comparable to previous years (89-761 larvae/hr).

We sampled 18 backwaters between 10 July and 3 August. We were unable to collect any CPM during this time, although other species of larval and juvenile fish were collected, including catostomids, nonnative cyprinids, and gizzard shad (*Dorosoma cepedianum*). There was not a statistically significant difference in mean maximum depth for occupied and unoccupied habitats.

Drift net results for Split Mountain were compared to those from Echo Park (Bestgen and Hill 2016) across the three years of sampling, since larvae drifting from the spawning bar should pass Echo Park first before passing Split Mountain and entering the nursery reach. Bestgen et al. (1998) found that CPM larvae generally exhibited sporadic peaks of abundance at the Echo Park site, and that these peaks were often detected the same day or one day later at a site near the Split Mountain location. Increased catch of larvae at Split Mountain typically followed a peak of larvae at the Echo Park site, although the Split Mountain transport index peaks were consistently lower than those observed at Echo Park a few days prior. Since the Split Mountain site was not sampled continuously, there were also gaps in the data set where the transport indices cannot be compared between sites. Despite lower transport indices and fewer larval captures, general trends in larval transport were similar between the two sites, and pulses of larvae could be detected downstream a few days after being seen at Echo Park.

***Nonnative depletions (Objective 2), experimental backwater treatments (Objective 3), and fish community response (Objective 4)***

### **2009 Results**

For objective two, nonnative fish depletions occurred on the first three sampling trips; 20–24 July 2009, 3–6 August 2009, and 17–20 August 2009. Combining trips, a total of 103, 127, and 207 seine hauls were completed in Control, Treatment A, and Treatment B backwaters,

respectively (see Table 1). During depletion passes a total of 28,366 nonnatives were removed: 11,348, 10,546, and 6,472 on trips 1-3, respectively (Table 2).

For the backwater manipulation objective, three Treatment A backwaters, two Controls, and two Treatment B backwaters lasted for the duration of the experiment (Table 1). Mean daily discharge from August 1 to September 30 was  $2,478.5 \pm 13.8$  cfs (Figure 6).

A total of 108 age-0 CPM were collected from excluded backwaters (89 from the Control that was later blocked, 19 from the Treatment B backwater with the stolen net); these fish were excluded from all analyses as mentioned previously, and TL measurements were not incorporated either. It is important to note that even without the exclusion of two experimental backwaters in 2009, CPM abundance data is biased for the third sample period given that an abundance of CPM were captured during the first seine haul from backwater nine (Treatment B). On that sampling occasion all fish were returned without identification or enumeration, and no further seine hauls were attempted in that backwater on that date (i.e., no data other than L. Monroe qualitative notes on “thousands” of CPM captured). Including all sample periods, 263 age-0 CPM were collected; 12, 22, and 229 from Control, Treatment A, and Treatment B backwaters, respectively (Figure 7). Although there appears to be a significant difference in CPM abundance between Treatment B backwaters and other treatments (Figure 8), this information should be interpreted cautiously because only two backwaters were analyzed for Control and Treatment B backwaters. Mean CPM TL for all captures was  $43.5 \pm 0.5$  mm. There was a significant difference in CPM size between Treatment A ( $n=12$ ; median=31.5 mm TL) and Treatment B ( $n=42$ ; median=41 mm TL) backwaters ( $U=103.5$ ;  $P=0.002$ ). Age-0 CPM were not captured until the third sample period and captures were highest during the fourth sample period, with Treatment B backwaters consistently containing more CPM for sample periods 4-6 than

other treatment types (Figure 9). Again, these results should be interpreted cautiously for reasons described above.

With regards to fish community composition, a total of 1,205 (99.5% nonnatives), 1,604 (99.2% nonnatives), and 2,733 (91% nonnatives) fishes were collected from Control, Treatment A, and Treatment B backwaters, respectively, during post-depletion sample periods 4-6 (31 August–2 September, 14–15 September, and 23–29 September 2009). Species composition mainly consisted of nonnative cyprinids (70.2%, 93.9%, and 81.1% for Control, Treatment A, and Treatment B backwaters, respectively) with red shiners dominating the assemblage in all treatment types (Figure 10). Additionally, black crappie (*Pomoxis nigromaculatus*; n=1; 100% of the post-depletion total for this species), bluehead sucker (*C. discobolus*; n=1; 100%), brook stickleback (*Culaea inconstans*; n=2; 100%), flannelmouth sucker (*C. latipinnis*; n=4; 67%), green sunfish (*Lepomis cyanellus*; n=147; 66%), smallmouth bass (*Micropterus dolomieu*; n=5; 83%), and white sucker (*C. commersonii*; n=3; 75%) were only collected from or were more abundant in Control backwaters (Figure 10). Additional native fishes collected during depletion sampling from backwater five (Treatment B) that were excluded from analyses included one bonytail (209 mm TL; 1<sup>st</sup> trip), two flannelmouth sucker (110 and 129 mm TL; 2<sup>nd</sup> trip), and one bluehead sucker (no measurement; 3<sup>rd</sup> trip). Other than CPM, the only other measurements taken on fish during post-depletion sampling included one smallmouth bass (46 mm TL) and two flannelmouth sucker (52 and 68 mm TL) captured during the 6<sup>th</sup> sample period. Note that community composition data is not completely accurate for all sample periods given that smaller unknown species (especially unknown catostomids) are not accounted for, many of which were released without measurement or preservation. Additionally, it is important to note that community data is biased for the third depletion period because backwater nine (Treatment B)

was essentially never sampled (see above). Finally, field observations suggest that there was a poor seal for blocked backwaters because fish got through and/or around nets in some instances (Hedrick et al. 2009), possibly affecting species composition over time.

### **2010 Results**

For objective two, nonnative fish depletions occurred on the first sampling trip from 27–29 July 2010. Considering all treatment types, an average of 26 seine hauls (range=12–48) was necessary to achieve 90% depletion, a goal that was reached for all but one 1/4" mesh treatment (Hedrick et al. 2010). During depletion passes a total of 726, 781, and 738 fishes were sampled from Controls, 1/4" treatments, and 1/2" treatments, respectively (Table 2).

For the backwater manipulation objective, only one Control backwater and two 1/4" mesh block net treatments lasted for the duration of the experiment (Table 1). However, all backwater treatments (less one 1/2" block net stolen early on) are comparable through the fourth sample period. Mean daily discharge from August 1 to September 30 was  $2,165.4 \pm 13.8$  cfs (Figure 6).

For the fish community response objective, including all sample periods, two, seven, and 49 age-0 CPM were collected from Control, 1/2", and 1/4" backwater treatments, respectively. Although there appears to be a significant difference in CPM abundance between 1/4" treatments and Controls (Figure 11), this information should be interpreted cautiously because sample sizes only consisted of one or two backwaters and statistical analyses comparing treatments are not possible. With the exception of 22 CPM that were not measured on 23 August, mean TL was  $36 \pm 1.05$  mm (n=36). Given that 38% of age-0 CPM were not measured in the field, an accurate comparison of treatment-specific growth through time is not possible. Age-0 CPM were not captured until the third sample period and captures were highest during the fourth sample period,

with 1/4" treatments consistently containing more CPM than 1/2" and Control backwaters (Figure 12). This information should be interpreted cautiously for reasons described above.

To assess fish community composition, a total of 957 (99.9% nonnatives), 1,436 (99.5% nonnatives), and 4,374 (99.3% nonnatives) fishes were collected from Control, 1/2", and 1/4" backwaters, respectively, from sample periods 2-4. Species composition mainly consisted of nonnative cyprinids (88.9%, 97.9%, and 98.2% for Control, 1/2", and 1/4" backwaters, respectively) with red shiners dominating the assemblage in all treatment types (Figure 13). Additionally, smallmouth bass (n=11) and white sucker (n=3) were only collected from Control backwaters, and common carp (*Cyprinus carpio*) were more abundant in Controls (Figure 13). Although only qualitative based on visual observations, the size of nonnative fish decreased with an increasing level of exclusion (i.e., smallest fish observed in 1/4" mesh treatments; Hedrick et al. 2010). Despite excellent depletion in most backwaters, observations during following sample periods suggest a poor seal for blocked backwaters (Hedrick et al. 2010), possibly affecting species composition over time.

### **2012 Results**

For objective two, nonnative fish depletions occurred on the first sampling trip from 25–28 June. Considering all treatment types, an average of 4.3 seine hauls (range=3–8) was necessary to achieve 90% depletion. Nonnative depletions were 94.8% effective on average (range=91.0-100%) when considering all 12 experimental backwaters. During depletion passes a total of 18,595, 4,909, and 7,628 fishes were sampled from Controls, 1/4" treatments, and 1/2" treatments, respectively (Table 2). Note that Control backwater totals are biased from a single outlier backwater that produced 13,530 fish; 1,471 black bullhead (100%), six common carp (100%), 10,158 fathead minnows (82%), 123 green sunfish (95%), 1,700 red shiner (39%), 52

sand shiners (22%), and 20 white suckers (100%). However, there is no effect on the remainder of the experiment as this backwater was excluded (see below). Additional information on mean TL, TL range, and sample or subsample size for all fish removed during depletions are included in Table 3. Additionally, 231 unknown catostomids were collected; not all were removed because of the uncertainty of whether they were native fishes. Known native fishes collected and released during depletion efforts are included on Table 4. Based on size and time of collection (see objective one results), CPM were all age-1 or age-2 fish, thus excluded from the experiment given that age-0 survival was the focus; if collected in a backwater assigned a blocking treatment, CPM were returned to the river to reduce stress in their early life stages.

For our backwater manipulation objective, all but one Control backwater and one 1/2” mesh block net treatment lasted for the duration of the experiment, resulting in adequate replication (i.e., at least three replicates/treatment type). Mean daily discharge from August 1 to September 30 was  $1,402.8 \pm 12.3$  cfs (Figure 6). Resulting from drought conditions in 2012 (i.e., prolonged low flows [Figure 6] and higher than normal air temperatures), backwater habitats experienced elevated temperature regimes throughout our experiment (Figure 14). Our habitat analysis revealed drastic changes in availability and quality over the course of the study period. Specifically, lack of flows led to the eventual disconnection of one of our Control backwaters (Figure 15). Moreover, substantial loss in average depth and area was observed (Table 5).

Only one age-0 CPM was collected from treatment backwaters during the entire study (post-depletion). A 39 mm TL CPM was collected on August 20 from backwater number one (1/4” mesh blocking treatment), the farthest upstream backwater habitat in our experiment (Figure 3; RM 291.2). Flannelmouth sucker were the most abundant native fish (n=406) and were collected on each post-depletion sampling event for the remainder of the study (Table 6).

With the exception of the July 10-12 sample period, backwaters blocked with 1/2" mesh nets consistently had the most flannelmouth sucker captures, followed by 1/4" mesh treatments, and then Controls, which did not contain flannelmouth suckers after the August 20-22 sampling event (Table 6). Additionally, substantial growth was observed for flannelmouth sucker, achieving sizes greater than 100 mm TL (maximum=111 mm TL) by the final sample period (Table 6). Additional native fishes collected post-depletion (Figure 16) included 12 (mean=29.3±1.6; range=18–39), seven (mean=34.3±5.3; range=20–55), and 52 bluehead suckers (mean=47.2±1.7; range=23–67) from Control backwaters (absent after August 7-9), 1/4" mesh (absent after August 20-22) and 1/2" mesh treatments (14 individuals collected September 18-25), respectively, as well as two speckled dace (*Rhinichthys osculus*; 26 and 58 mm TL).

To assess fish community composition, a total of 8,182 (99.2% nonnatives), 16,532 (99.2% nonnatives), and 17,036 (98.4% nonnatives) fishes were collected from Control backwaters, 1/4", and 1/2" treatments, respectively, from sample periods 2-7. Species composition mainly consisted of nonnative cyprinids; 99.1%, 98.4%, and 98.0% for Control backwaters, 1/4", and 1/2" treatments, respectively (Figure 16). Interestingly, fathead minnow thrived in blocking treatments (29.8% in 1/4" treatments and 31.8% in 1/2" treatments vs. 6.1% in Control backwaters), sand shiners were more dominant in Control backwaters and 1/2" treatments (44.1% and 42.1%, respectively) and less of a component of the assemblage in 1/4" treatments (30.0%), and red shiners were by far most dominant in Control backwaters (48.9%; Figure 16). Unlike 2009 and 2010, smallmouth bass were rare in 2012. We collected one each on 10-12 July (40 mm; 1/4" treatment), 23-25 July (51 mm; 1/2" treatment), 7-9 August (88 mm; Control), and 20-22 August (62 mm; Control). Note that the 88 mm TL smallmouth bass had

swallowed a 54 mm flannelmouth sucker (TL estimated, tail was already digested). Additional fish collected from excluded backwaters are included on Table 7.

Native fish captures decreased through time for all treatments (Figure 17) and more fish were captured on average in blocked backwaters than Control backwaters, with 1/2" blocking treatments containing the most fish (Figure 18). Despite decreasing abundance over time, blocked backwaters contained more fish in all sample periods (Figure 17), with the exception of the first event, suggesting increased survival over Control backwaters. Moreover, native fish were completely absent from Control backwaters before the fourth sample period and nearly absent from 1/4" treatments before the fifth sample period (one and two fish collected during the fifth and sixth sample period, respectively; Figure 17).

### **2012 Modeling Summary**

The smallest AIC values (most parsimonious) were used to select the best candidate models to analyze changes in fish community composition over time. This included a Symmetric covariance structure for native fish total abundance, an Unstructured covariance structure for red shiner total abundance, and a Simple covariance structure for red shiner TL (Table 8). For native fish total abundance there was a significant difference between treatment type ( $df=2$ ,  $F=19.24$ ,  $P<0.0001$ ), sample period ( $df=5$ ,  $F=66.33$ ,  $P<0.0001$ ), and the interaction term ( $df=10$ ,  $F=11.38$ ,  $P<0.0001$ ). With the exception of red shiner for the last sample period, 1/2" treatments consistently contained more nonnative cyprinids, and there was an increasing trend for all three nonnative cyprinids over the course of the experiment (Figure 19). This result may suggest increased survival (i.e., exclusion from predation) over Control backwaters and 1/4" treatments and/or immigration into backwaters or within backwater reproduction. For red shiner total abundance there was a significant difference between treatment type ( $df=2$ ,  $F=204.14$ ,

$P < 0.0001$ ), sample period ( $df=5$ ,  $F=70.33$ ,  $P < 0.0001$ ), and the interaction term ( $df=10$ ,  $F=4.15$ ,  $P < 0.0001$ ). There was also a significant difference for red shiner TL between treatment type ( $df=2$ ,  $F=7.22$ ,  $P=0.0023$ ) and sample period ( $df=5$ ,  $F=2.84$ ,  $P=0.0294$ ), but not the interaction term ( $df=10$ ,  $F=1.29$ ,  $P=0.2709$ ). By the last sample period red shiner TL was similar among treatments (~30 mm), but size in controls did not decline initially as in blocked backwaters (Figure 19). After an initial decline in red shiner TL in blocked backwaters there was a rebound in size until it appears an equilibrium was reached (Figure 19). Sand shiner and fathead minnow TL also appear to reach an equilibrium for all treatments by the last sample period (~30-40 mm); however, sand shiner TL increases over time for blocked backwaters whereas fathead minnow demonstrate a similar pattern to red shiner.

Post-hoc analyses were not helpful for determining paired differences (i.e., no significant differences detected) in any of the three analyses. Covariates were removed from analyses because they provided too many parameters (i.e., 3 treatments x 3 backwaters/treatment x 6 sample periods=36) and AIC penalizes for additional parameters (Anderson and Burham 2002). Furthermore, the covariates were collected per our study design, but excluded from analyses because our data did not justify including these additional parameters; we had to simplify by excluding covariates in order to analyze our main objective to answer our study question regarding CPM survival in backwater nursery habitats.

## DISCUSSION

This study was originally intended to expand on work conducted by the *Backwater Restoration Ad Hoc Group* (2008). As such, the goal was to gather preliminary data (2009 and 2010 were truly pilot-level projects) and refine techniques that could be used to investigate

potential causes of low CPM autumn recruitment (Breen et al. 2011) and implement management actions to increase survival of age-0 CPM. The drift netting component and larval backwater seining for CPM abundance (first objective) were designed to verify that larval CPM were arriving in the nursery reach and to determine when mortality might reduce their abundance. Results from 2009 and 2010 indicated relatively good larval CPM production at the Yampa River spawning bar (Bestgen and Hill 2016), and larvae were also captured downstream at the Split Mountain drift net site (Figure 5a-b). In 2012, the number of larvae captured was low at both sites, suggesting few CPM larvae were arriving in the nursery reach where seining efforts occurred (Figure 5c).

Patterns of larval transport from drift net sampling were consistent with those of seine sampling in nursery reach backwaters. Higher transport rates of larvae in 2009 and 2010 translated into a higher proportion of backwaters occupied by age-0 CPM. In contrast, 2012 had low transport abundance, and no CPM were captured in seine samples that year, likely due to few fish being available for capture. Results from ISMP sampling were also consistent with those from this study, with high total captures and catch rates of CPM in 2009–2010 and low numbers in 2012 (Badame et al. 2009, 2010; Skorupski et al. 2012a; Bestgen and Hill 2016). Bestgen and Hill (2016) found a relationship between mean August–September base flows in the middle Green River and the abundance of age-0 CPM in that reach, where base flows in the 1,700–3,000 cfs range produced large year-classes of CPM. Base flows in 2009 and 2010 were within this range (Figure 6), and produced high numbers of age-0 CPM when compared to the 1994–2012 period. However, mean August–September flows that remained below this range in 2012 (Figure 6) was consistent with a low abundance of age-0 CPM in this study and ISMP sampling (Skorupski et al. 2012b).

There was one apparent discrepancy in the results of this study when comparing them to both the Echo Park drift net sampling and ISMP sampling. Larval transport abundance in 2010 from Echo Park and high numbers of CPM from the ISMP sampling would suggest that 2009 and 2010 were similar years in terms of CPM production and recruitment. Our results from the backwater larval seine surveys showed much higher numbers of CPM in 2009 than 2010. One possible explanation is that sampling occurred about a week later in 2009, and many of the CPM captured were recently transformed juveniles that could be identified in the field. In 2010, fish of this size were present only at the very end of sampling. For 2009, large numbers of age-0 fish were present in samples August 13–17, which was 23–27 days after the last large pulse of larvae at Echo Park. In 2010, sampling ended August 10, which was only 19 days after the last large group of larvae was detected at Echo Park, and there was a later, smaller pulse detected at the end of July. It is possible that backwater sampling had ceased before larval drift was finished and larvae had enough time to grow into more easily identifiable juveniles.

One shortcoming of this study for objective one was that drift net sampling did not commence at Split Mountain until after larvae were confirmed at Echo Park. Transport rates from Echo Park to Split Mountain have been estimated at about one day (Bestgen et al. 1998), so delaying sampling at the downstream location likely would miss the first pulse of larvae detected. Also, the drift nets were not deployed every day at the Split Mountain site, which would increase the probability of missing peaks of larval drift. Previous studies (Bestgen et al. 1998; Bestgen and Hill 2016) found that large peaks of larval transport often lasted only a single day, and these pulses could represent a large proportion of the annual transport abundance. The Split Mountain site also exhibited lower transport indices each year and for pulses detected during similar time periods. It is possible that lower transport indices and less dramatic peaks in

transport observed at Split Mountain could reflect pulses of larvae being spread out as they continue drifting downstream from Echo Park. This could result in larvae being spread out over longer time periods at lower densities. These lower values could also result from larvae experiencing some rate of mortality as they drift between the two stations, however, our data do show that at least some larvae made it to the beginning of the nursery reach in 2009 and 2010. Additionally, this may be a result of sampling a small volume of water from a larger river flow compared to the Yampa River site. The Split Mountain station receives input from both the Yampa and Green rivers, but larvae are almost exclusively produced from the Yampa River spawning site. Thus, larvae are diluted at the confluence and distributed within the combined flow of the two rivers. In low flow years, the total number of larvae captured can be high, since a larger proportion of the river is sampled (Bestgen et al. 1998; Bestgen and Hill 2016). However, these captures are extrapolated to the entire river flow, thus transport index is low. The opposite pattern is observed when few larvae are captured during higher flow conditions, since a small proportion of the river is sampled.

Additional evidence to support limited larval drift in 2012 is obvious from the results of our backwater blocking experiment. More specifically, we only captured a single age-0 CPM (not collected until August 20, after larval seining was completed in the Ouray reach [RM 265–250]), and that fish was collected at the farthest upstream backwater (RM 291.2). Catch rates were similarly low during ISMP sampling in 2012 (Skorupski et al. 2012a), when a single age-0 CPM (39 mm TL) was collected farther downstream in a backwater at RM 243.1 and another (68 mm TL) at RM 233.4 (2012 CPUE=0.03 fish/100 m<sup>2</sup>). This represented the second lowest catch rate since 1990, the 2011 high flow year being the lowest (CPUE=0 in 2011, Skorupski et al. 2011; Skorupski et al. 2012a). It is possible that these fish drifted downstream from the White

River (confluence at RM 246.1) which was documented in 2011, (Webber et al. 2013), but conditions that year contrast sharply with reduced flows observed in 2012 (Figure 6).

Alternatively, limited habitat availability in the Green River (Table 5; Figure 15) could also explain the lack of captures in our study reach with additional captures occurring downstream (i.e., fish had difficulties finding a location to settle and continued drifting farther downstream). Regardless, with such small sample sizes in both instances, interpretations are difficult to make and trends are not supported; two age-0 CPM is a 100% increase over one capture, but we are still only discussing three individuals.

Depletion of nonnative fish from experimental backwaters (objective two; Table 2) occurred prior to sampling experimental blocking treatments on a temporal basis in 2010 and 2012, and throughout the study period in 2009. It is unclear whether depletion goals were met in 2009 (Hedrick et al. 2009), a year in which the experimental design was inconsistent (i.e., truly a pilot year), and hundreds of seine hauls on average were necessary to exploit nonnative fishes. Nonnative fish depletion goals were met in 2010 (Hedrick et al. 2010), but an average of 26 seine hauls for each experimental backwater was necessary to accomplish this task. Prior to 2012 sampling, we considered hardships with this task experienced in 2009–2010 and reverted to previous experience using a larger seine to proficiently collect large numbers of fish in a timely manner (Breen and Ruetz 2006). By combining two smaller seines to create one 9.1 m seine, we achieved an average of 94.8% nonnative depletion (considering all 12 experimental backwaters) with an average of 4.3 seine hauls per backwater. Our goal was to increase the feasibility of this task to stay within budget, and we clearly demonstrated our ability to do so; less time was necessary (four days total) and we were more effective (Table 2). Additionally, initial depletions in 2012 were more closely matched with initial arrival of drifting larvae in nursery habitats (i.e.,

first larval CPM detection at Echo Park on 20–22 June 2012; Bestgen and Hill 2016), whereas 2009 and 2010 depletions generally occurred on the tail end of higher larval densities (Hedrick et al. 2009, 2010; Bestgen and Hill 2016).

Trammell et al. (2002, 2004) conducted similar depletion studies in the lower Green and Colorado rivers, which led to consideration for implementation in the middle Green River (*Backwater Restoration Ad Hoc Group* 2008). In addition to removal of small-bodied nonnative fishes from backwater habitats, Trammell et al. (2004) also included removal from shoreline areas adjacent to backwaters, and the *Backwater Restoration Ad Hoc Group* followed suit for removal trials in the middle Green River (2008). Considering only the lower Green River reach (RM 102.0–52.0), a recognized shortcoming of Trammell et al. (2004) was that sampling occurred during high spring flows (last depletion occurring on 17 June 1998) prior to backwater formation. This led to the recommendation that such studies should occur after peak flows, therefore this project element was incorporated into initial trials in the middle Green River (*Backwater Restoration Ad Hoc Group* 2008). Although block-netting could not be properly assessed in the lower Green River, due to breaches and damages from animals and flash floods, Trammell et al. (2004), also recommended the use of block nets to exclude nonnative cyprinids from off-channel habitats. Our study stemmed from lessons learned from this previous research for an improved sampling design; however, we excluded shoreline removals to improve project feasibility, focusing on the main nursery habitats only.

The study design to accomplish objective three evolved each year of this project until 2012 when adequate replication was achieved for a more complete analysis. It is unfortunate that meaningful analyses were not possible for 2009 and 2010 data when CPM were more abundant (Hedrick et al. 2009, 2010; Bestgen and Hill 2016). However, 2012 analyses were quite

revealing for the outcome of backwater experimental treatments and the response of native fishes other than CPM to removal of nonnative fishes. Experimentation in 2009 was hindered by inadequate replication, with three Treatment A, two Treatment B, and two Control backwaters (most of which did not maintain consistency through time; Table 1), thus appropriate statistical inference on whether we affected CPM survival through removal of nonnative fish in backwater nursery habitats was not warranted. Although a more streamlined approach was adopted in 2010, experimentation ended with only one Control and two 1/4" treatment backwaters justifiable for analysis (Table 1), again voiding any statistically meaningful inferences and limiting biological interpretations. In 2012 the study design included suitable replication (i.e., three replicates/treatment; Table 1) for a robust Repeated Measured ANOVA.

Although the appropriate design was in place to detect differences in survival between backwater treatments in 2012, environmental and biological variables played a large role in influencing the experimental results (Table 5; Figures 6 and 15), leading to a decrease in habitat availability and quality (i.e., loss of backwater depth, area, and eventual disconnection in some cases). Moreover, drought conditions led to poor larval transport to important nursery habitats (Figure 5c) and to poor survival through time after arrival resulting from alterations in fish community composition derived from warmer temperature regimes, specifically nonnative cyprinid reproductive capacity (Lentsch et al. 1996; Herrington and DeVries 2008; Bestgen and Hill 2016). Additionally, there were substantial diel temperature fluctuations within backwaters (i.e., temperatures exceeded 32.2° C during daytime hours) and there was no real change through time until later in September (Skorupski et al. 2012a; Figure 14). Furthermore, tolerance to temperature extremes favor nonnative cyprinids, aiding their ability to persist and recolonize low-velocity riverine habitats (Trammell et al. 2004).

By analyzing impacts in 2012 to native fishes as a surrogate for Colorado pikeminnow, we determined that backwater blocking treatments and removal of nonnative fish through time (objective four) positively affects the native fish community, especially for flannemouth sucker in 1/2" mesh blocking treatments. Therefore we hypothesize that blocking treatments would have a positive effect on CPM survival in nursery habitats. There was a significant difference for all factors analyzed by Repeated Measures ANOVA over the course of our study: native fish total abundance, red shiner total abundance, and red shiner total length. Analysis of all age-0 native fishes (combined as a single factor) in 2012 provided important insights on the effects of our experimental design; 1/2" mesh block net treatments (n=277 native fish collected over the entire experiment) had a significant positive effect on survival compared to 1/4" mesh blocking treatments (n=138) and Control backwaters (n=66) throughout the summer growth period (Figure 18). Flannemouth sucker, which comprised the majority of the combined native fish metric, especially thrived in 1/2" mesh blocking treatments throughout the experiment (Table 6). Although not all treatments contained flannemouth sucker by the final sample period for direct comparison (i.e., August 7-9 was the last sample period where all treatments contained adequate numbers; Table 6), substantial growth was observed in blocked backwaters throughout the study with flannemouth achieving sizes >100 mm TL. This could be an artifact of elevated temperature regimes optimal for growth (e.g., Bestgen 2006; Bestgen et al. 2006; Breen et al. 2011).

Red shiner and other nonnative small-bodied fish species abundance increased over time following initial depletions in 2012 (Figure 19) with the greatest abundance occurring in 1/2" mesh blocking treatments. Trammell et al. (2004) observed a similar pattern in the lower Green River where nonnative captures increased following depletions (re-invasion within 2-3 days),

and hypothesized that this was derived from immigration, reproduction, and growth of larval nonnative fish. In that study, a positive effect was not detected following habitat manipulation as observed from fall ISMP monitoring (Breen et al. 2017, Tables 5 and 7).

Despite increases in abundance of nonnatives over time in our study, especially for cyprinids, red shiner TL, as well as fathead minnows and sand shiners (Figure 19) reached an equilibrium over time. This result suggests that we were effective at cropping out nonnative fish as new cohorts became susceptible to our gear, also indicating that movement between riverine and backwater environments was possible for small, fusiform fish but perhaps blocking larger predatory fish (1/2" mesh blocking treatments; 1/4" mesh blocking treatments to a lesser extent). The larger, deep-bodied and more predaceous red shiner and age-0 smallmouth bass (see below) may have been excluded from the 1/2" mesh treatments making predation a limited factor and likely increasing native fish survival (Tyus and Karp 1990; Bestgen and Hill 2016). Moreover, the more streamlined flannelmouth sucker (mean=74.8 mm TL by last sample period) likely had the ability to move between habitats as needed for refuge from predation, to alleviate stress from competition with nonnative cyprinids in blocked backwaters, acquisition of necessary resources, to escape thermal extremes as necessary, and possibly other factors as well. For example, native fish were completely absent from Control backwaters before the fourth sample period, likely due to riverine piscivores foraging at will, and were nearly eliminated from 1/4" mesh blocking treatments before the fifth sample period (Figure 18). More specifically, three 1/2" treatments and four 1/4" treatments were available and natives were still much more abundant in 1/2" treatments and persisted in numbers through the entire experiment. Although not statistically proven in 2009 or 2010, backwater blocking has shown signs of positively influencing native fish survival in every year of implementation, including a higher abundance of CPM in 1/4" mesh

treatments in 2010. Backwaters blocked by ½” mesh nets had higher CPM abundance in 2009, and most importantly, there was a significant difference in native fish abundance in 2012 where ½” mesh treatments provided greater survival than ¼” mesh blocking treatments.

Unlike 2009 and 2010 (Hedrick et al. 2009, 2010), smallmouth bass (n=4) were rare in collections from backwaters in 2012 during the experiment. Although abundant in overall catch during mechanical removal efforts in 2012 (i.e., highest CPUE on record), substantial growth occurred for this species (i.e., smallmouth bass exceeded 150 mm by October in many cases) as well due to an earlier spawn and warmer summer temperatures, which likely led to an earlier transition from low-velocity habitats to riverine environments (Skorupski and Breen 2012). This was also confirmed by only one capture in backwaters during fall ISMP sampling in 2012 (Skorupski et a. 2012a), and the last capture in this study occurred on 22 August 2012. In years with wetter hydrology, growth can be much slower for bass and they likely spend more time in backwater habitats before transitioning to riverine habitats. For example, in 2016, a much better water year than 2012, smallmouth bass (mean TL = 53.1) were collected from 34% of backwaters sampled during autumn ISMP sampling (Breen et al. 2016). Furthermore, only two bass captures occurred in blocked treatments and were absent after 25 July 2012, suggesting that they were effectively cropped out by that time. The other two bass captures occurred in a Control backwater, one of which (88 mm TL) had consumed a 54 mm TL flannelmouth sucker, possibly suggesting that movement into backwater nursery habitat to forage is commonplace and perhaps occurs at night, given that daytime ISMP captures were limited.

In conclusion, we have provided several lines of evidence suggesting that blocking treatments benefit native fish survival when compared to controls (same pattern observed for nonnative cyprinids also). Although we could not test for significant differences between

blocking treatments in 2009 and 2010, blocked backwaters contained more CPM than unblocked backwaters in both years (Figure 8 and 11). Likewise, 2012 Control backwaters contained the lowest abundance of all species, suggesting that predation by nonnative piscivores coming from riverine habitats (e.g., smallmouth bass example above) is a significant threat to small-bodied fishes and blocking treatments positively affect survival in nursery areas (native and nonnative fish). By blocking backwater nursery areas, we essentially created more desirable habitat by removing predation as a factor. Furthermore, good habitat for one species apparently benefits most or all species of small-bodied fishes, explaining higher nonnative abundance in blocking treatments because predation is likely removing nonnative small-bodied fish in Control backwaters over time in addition to our sampling. Competition between age-0 native fish and nonnative cyprinids in backwater nursery habitats can strongly influence native fish survival (Karp and Tyus 1990; Seegert et al. 2014; Bestgen and Hill 2016), but our results demonstrate that negative effects of predation may outweigh competitive factors. More specifically, despite higher abundance of small-bodied nonnative fishes in 1/4" and 1/2" mesh blocking treatments throughout our experiment in 2012, age-0 native fishes were also more abundant (1/2" mesh treatments containing the highest abundance; Figure 18), suggesting that competition for resources in nursery habitats is less of an important factor than predation, thus improving probability of survival.

This study should be repeated to monitor the effects and success of experimental base flows aimed at increasing age-0 CPM recruitment (Bestgen and Hill 2016). Objectives would be similar in monitoring abundance and distribution of larvae reaching the nursery reach. By blocking and depleting backwaters of nonnatives, we would be better equipped to tease apart the relative impacts of flows and nonnative fishes on CPM survival. Current monitoring consists of

collecting larvae at the Echo Park drift net site and ISMP monitoring in the fall with no sampling during the summer. In years where few CPM are collected, little information is available to determine why survival of age-0 fish is low (i.e., ISMP is only a snapshot in time following months of predation). Repeating and improving this study could assist in determining the fate of larval CPM when they first arrive in the nursery reach, and the backwater depletions can determine whether nonnative fishes affect CPM survival as was determined for other native fishes in 2012. As discussed, lower base flows during the months of August–September in 2012 (Figure 6) resulted in poor CPM recruitment (Skorupski et al 2012a), whereas flows in 2009 and 2010 were between 1,700–3,000 cfs (Figure 6) and CPM recruitment in the middle Green River was highest since the pre-crash era (Badame et al. 2009, 2010; Breen et al. 2011). Due in part to elevated releases from Flaming Gorge Dam in an attempt to improve nursery habitat conditions, 2009 and 2010 closely matched flow targets identified at a later date (Bestgen and Hill 2016) and CPM recruitment success appeared to be reflected in the results. In 2015, we observed the highest level of CPM autumn recruitment since 2010 and this would have been an excellent year to repeat the 2012 experiment to provide further insights specifically geared towards CPM. In contrast, August–September 2017 discharge exceeded recommended targets (i.e., >3,000 cfs during this timeframe) and again, CPM recruitment was limited to only a single age-0 CPM (Breen et al. 2017), despite the fact that production levels of CPM determined in drift from upstream spawning locations was near average. Furthermore, at moderate flow levels within the above ranges, backwater abundance and area may be optimized, thus providing sufficient habitat to maximize survival of age-0 CPM following larval transport to nursery areas (Bestgen and Hill 2016).

## CONCLUSIONS

- We were able to determine that CPM larvae were arriving at the upstream extent of the nursery reach (Split Mountain), although the timing of drift net sampling likely missed pulses of larvae observed at Echo Park.
- We confirmed that larvae were present in nursery reach backwaters in July and August for 2009 and 2010.
- Few larvae were captured via drift nets, and none were collected in backwaters in 2012, indicating larval abundance and transport were low.
- Despite excellent age-0 CPM recruitment in 2009 and 2010, backwater blocking techniques were only in a pilot-level phase, limiting our ability to determine whether treatments had an effect on CPM survival.
- We implemented an improved study design in 2012 (i.e., adequate replication for a more robust analysis), but environmental conditions did not benefit CPM larval drift, arrival in nursery areas, or age-0 recruitment, thus we were unable to assess CPM survival in nursery habitats so we switched our focus to other native fishes as a surrogate.
- We successfully depleted randomly selected backwaters (25–28 June 2012) of nonnative fish prior to applying three experimental treatments to improve CPM survival and monitor fish community response every other week for six sample periods from 10 July–28 September 2012.
- Other age-0 native fishes in 2012 provided insights on the effects of our experimental design; 1/2" mesh block net treatments had a significant positive effect on survival in comparison to Control backwaters and 1/4" mesh treatments throughout summer.

- Flannemouth sucker, which comprised the majority of the combined native fish metric, especially thrived in 1/2" mesh blocking treatments throughout the experiment. Substantial growth was observed throughout the study and by the final sample period (18–25 September 2012) flannemouth sucker achieved sizes >100 mm TL.
- Abundance of red shiner and other nonnative cyprinids increased over time following initial depletions in 2012; greatest abundance occurring in 1/2" mesh blocking treatments.
- Red shiner TL decreased and then increased slightly until an equilibrium was reached. Lower average TL on our final sampling period may suggest that we were effective at cropping out nonnative fish as new cohorts became susceptible to our gear, but this metric was also likely affected by reproduction in blocked backwaters, as well as movement of new fish into backwaters from the river (1/2" mesh blocking treatments; 1/4" mesh blocking treatments to a lesser extent).
- Flannemouth sucker, and other native fishes, likely had the ability to move between backwater and riverine habitats as needed for refuge from predation and necessary resources given that native fish were completely absent from Control backwaters before the fourth sample period (20–22 August 2012) and nearly eliminated from 1/4" mesh blocking treatments before the fifth sample period (4–5 September 2012).
- Control backwaters contained the lowest abundance of all species, suggesting that predation by nonnative piscivores coming from riverine habitats is a significant threat to small-bodied fishes and blocking treatments positively affect survival in nursery areas (native and nonnative fish). By blocking backwater nursery areas, we essentially created more desirable habitat for all small-bodied fishes present in each treatment.

- Despite higher abundance of small-bodied nonnative fishes in 1/4” and 1/2” mesh blocking treatments throughout our experiment in 2012, age-0 native fishes, including bluehead sucker, were also more abundant (1/2” mesh treatments containing the highest abundance; Figure 18), suggesting that competition for resources in nursery habitats is less of an important factor than predation, thus improving probability of survival.

## **RECOMMENDATIONS**

- We recommend continuing experimentation with fish depletion and backwater blocking treatments (objectives 2–4) for an additional season when environmental factors do not play such a large role in affecting experimental outcomes (i.e., flow manipulation can buffer extremes observed in 2012).
- Based on 2012 results, we recommend that only Control backwaters and 1/2" mesh block net treatments will be necessary for future experimentation. This change would allow for increased replication and project feasibility.
- If future studies can verify a positive influence on CPM survival (as was observed for other native fishes in 2012), we recommend moving towards widespread implementation where Control backwaters would not be necessary, again allowing for increased replication and project feasibility.
- Prior to implementation on a larger scale, and with verification that backwater blocking can positively influence CPM survival as observed for other native fishes in 2012, we recommend a comprehensive analysis to extrapolate results to the entire middle Green River nursery reach. This analysis would allow us to determine if implementation with only blocked backwaters could have a population level effect and should be considered as

an ongoing management action.

- We recommend continuing larval drift and larval seine sampling, which we believe will be valuable in evaluating flow targets identified in Bestgen and Hill (2016). Such sampling would help monitor the effects of ideal flow implementation on larval CPM transport to and survival in nursery habitats.
- We recommend conducting the drift net sampling continuously (7 days/week), as was done by Bestgen and Hill (2016). There were gaps in the current data from this project, and based on their timing, we likely missed pulses of larvae that were detected at Echo Park. These pulses were sporadic and short-lived, making them difficult to detect without continuous monitoring.
- We recommend sampling middle Green River backwaters over the course of the summer following arrival of larval native fishes (next bullet for details). Results from objectives 3-4 provided insights on the importance of a temporal sampling design to detect changes in the fish community over the summer, rather than relying on a snapshot in time from ISMP sampling (i.e., the fish assemblage is already altered at that point). Given that we cannot truly measure a native fish response to nonnative removal in the middle Green River (Skorupski et al. 2012c), which is essential to understand effects of our control efforts, we recommend revising the Project #138 scope of work. We suggest replacing sampling of a third backwater in each sub-reach of the middle Green River with a summer sampling regime (tasks to be determined upon consideration).
- We recommend evaluation of diel movement in and out of backwaters by deploying fine mesh directional fyke nets at the mouth of select backwaters every other week following larval drift of native fishes. Besides determining changes in the fish community over

time, this will allow us to better understand the dynamics and extent of nonnative fishes moving between riverine and backwater habitats to forage on native fishes. As with our first recommendation, this knowledge may lead to additional recovery actions for endangered and state threatened species.

- We recommend that the Recovery Program and respective partners strive to achieve middle Green River base flow targets suggested by Bestgen and Hill (2016) so that we can accumulate several years of comparable data for a better understanding of what it takes for successful CPM recruitment. Furthermore, it is crucial that elevated base flows occur as soon as possible and for several years to bolster current population declines (Bestgen et al. 2018), given that CPM require between 5-8 years to reach sexual maturity.

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Table 1. Backwater experimental treatments applied during each year of study to determine effects of nonnative fish depletions on age-0 Colorado pikeminnow survival and the overall fish community.

<b>Year</b>	<b>Backwaters Studied</b>	<b>Study Dates</b>	<b>Experimental Treatments</b>	<b>Backwaters Excluded from the Experiment &amp; Subsequent Analyses</b>
2009	9	20-Jul to 29-Sep	3 Controls; unblocked for initial depletions & entire experiment 3 Treatment A; blocked for initial depletions, river connection was then restored 3 Treatment B; blocked for initial depletions & remained blocked with 1/2" mesh nets	1 Control; blocked to protect abundant CPM 1 Treatment B; net stolen
2010	6	27-Jul to 23-Sep	2 Controls; maintained river connection following initial depletions 2 backwaters blocked with 1/4" mesh nets for duration of experiment 2 backwaters blocked with 1/2" mesh nets for duration of experiment All backwaters were blocked for initial depletions, then treatments applied	1 Control; disconnected from the river 1 - 1/2" mesh treatment; net stolen 1 - 1/2" mesh treatment; hole in net
2012	12	25-Jun to 25-Sep	Randomized block study design; 4 blocks, each with one of each treatment type 4 Controls; maintained river connection following initial depletions 4 backwaters blocked with 1/4" mesh nets for duration of experiment 4 backwaters blocked with 1/2" mesh nets for duration of experiment All backwaters were blocked for initial depletions, then treatments applied	1 Control; disconnected from the river 1 - 1/2" mesh treatment; net stolen

Table 2. Nonnative fish removed from experimental backwaters during depletion efforts in 2009 (three sampling trips; 20–24 July, 3–6 August, and 17–20 August), 2010 (one sampling trip; 27–29 July), and 2012 (one sampling trip; 25–28 June). Note that Control backwater totals are biased from a single outlier backwater that produced 13,530 fish; 1,471 black bullhead (100%), six common carp (100%), 10,158 fathead minnows (82%), 123 green sunfish (95%), 1,700 red shiner (39%), 52 sand shiners (22%), and 20 white suckers (100%).

Species	2009			2010			2012		
	Control	A	B	Control	1/4"	1/2"	Control	1/4"	1/2"
red shiner	7,877	7,126	4,219	275	597	492	4,350	1,609	5,572
sand shiner	3,129	868	605	291	104	116	241	298	199
fathead minnow	379	1,299	315	85	18	25	12,378	2,915	1,790
common carp	720	513	219	58	55	85	6	4	3
green sunfish	331	356	270	1	6	1	129	37	18
white sucker	7	20	4	3	—	2	20	13	13
smallmouth bass	4	4	—	11	—	17	—	—	—
black bullhead	29	49	10	—	—	—	1,471	33	33
channel catfish	—	—	3	—	—	—	—	—	—
brook stickleback	1	—	9	—	—	—	—	—	—

Table 3. Nonnative fish removed from Control (C) and blocked backwaters (1/2" and 1/4" mesh nets) during depletion efforts from 25–28 June 2012. Mean total length (TL) and range are reported for each treatment; subsampling procedures applied to some calculations.

Species	Mean TL ± Standard Error			Sample Size / TL Range		
	C	1/2"	1/4"	C	1/2"	1/4"
black bullhead	60.2±1.2	67.7±1.9	59.2±1.2	36/44-75	33/52-101	33/47-74
common carp	134.5±7.4	120.3±7.4	116.3±4.6	6/112-164	3/111-135	4/108-127
fathead minnow	46.6±0.5	48.1±0.7	50.0±0.5	121/30-63	120/27-65	198/33-67
green sunfish	47.0±2.0	60.1±2.1	52.5±1.6	37/27-70	18/42-72	35/36-76
red shiner	37.0±1.0	37.5±0.8	48.3±0.9	131/21-76	127/24-62	134/27-77
sand shiner	43.2±0.8	43.0±0.8	45.4±0.9	110/30-66	95/26-66	125/14-66
white sucker	93.2±2.3	92.3±3.8	89.8±3.1	20/71-109	13/66-115	5/80-97

Table 4. Native fishes collected and released during depletion efforts from 25–28 June 2012.

Species	Count	Mean TL	Range
bluehead sucker	5	72.2±2.9	67-82
Colorado pikeminnow	6	98.7±21.3	52-165
flannelmouth sucker	28	47.6±3.2	34-86
Gila spp.	3	67.7±2.3	63-70
speckled dace	2	—	50, 57
unknown native catostomid	237	29.8±0.3	20-40

Table 5. Average percent loss of depth and area for experimental backwater treatments in 2012.

Backwater Treatment	% Loss in Depth	% Loss in Area
1/2" block net	16.9	18.4
1/4" block net	22.8	25.4
Control	29.6	20.4

Table 6. Average total length (TL) and TL range for flannelmouth sucker (n=406) collected during each sampling event in 2012 from experimental backwater treatments following initial nonnative fish depletions.

Sample Dates	Treatment Type								
	Control			1/4" Mesh			1/2" Mesh		
Sample Dates	Sample Size	Mean	Range	Sample Size	Mean	Range	Sample Size	Mean	Range
July 10-12	27	34	34-51	46	29	29-51	3	39	39-44
July 23-25	10	35	35-60	42	31	31-65	71	30.5	30.5-65
August 7-9	15	61.4	40-75	16	67	40-88	49	51.6	40-72
August 20-22	1	46	—	22	62.8	50-74	30	68.5	54-91
September 4-6	0	—	—	1	64	—	53	71.1	51-93
September 18-25	0	—	—	2	101.5	92-111	18	74.8	60-101

Table 7. Fish collected post-depletion from one Control backwater and one 1/2" mesh treatment prior to exclusion from the 2012 experiment; WS x BH = white sucker x bluehead sucker hybrid.

<b>Species</b>	<b>Control</b>	<b>1/2" Mesh</b>
black bullhead	41	2
bluehead sucker	2	—
unknown cyprinid	718	1
fathead minnow	1,017	520
flannelmouth sucker	10	24
green sunfish	20	5
red shiner	3,331	963
speckled dace	—	1
sand shiner	773	522
unknown	13	3
unknown sucker	—	6
white sucker	3	—
WS x BH	2	—

Table 8. Models predicting trends in native fish and red shiner total abundance, and red shiner total length (TL). Akaike’s information criterion corrected for small sample size (AICc), the change in Akaike’s information criterion ( $\Delta AIC_c$ ), and Akaike’s weight ( $w_i$ ) were used to evaluate candidate models.

Model	<i>K</i>	AIC <sub>c</sub>	$\Delta AIC_c$	$w_i$
<b>Native fish total abundance</b>				
Symmetric	34	102.6984	0	0.929191
Unstructured	39	108.2839	108.2839	1
Compound Symmetric	20	112.3405	9.6421	0.007488
Autoregressive, Order 1	20	113.0710	10.3726	0.005197
Simple	19	116.1351	13.4367	0.001123
Autoregressive Heterogeneous Variances	25	121.2665	121.2665	8.63E-05
<b>Red shiner total abundance</b>				
Unstructured	39	84.16005	0	0.526582
Symmetric	34	84.37844	0.21839	0.47211
Autoregressive, Order 1	20	97.58951	13.42946	0.000639
Autoregressive Heterogeneous Variances	25	99.11814	14.95809	0.000297
Simple	19	99.69141	15.53136	0.000223
Compound Symmetric	20	100.4942	16.33414	0.000149
<b>Red shiner total length</b>				
Simple	19	301.2763	0	0.405127
Compound Symmetric	20	301.9523	0.676	0.288935
Autoregressive, Order 1	20	303.2580	1.9817	0.150408
Symmetric	34	303.3096	2.0333	0.146577
Unstructured	39	309.8457	8.5694	0.005582
Autoregressive Heterogeneous Variances	25	310.8545	9.5782	0.003371

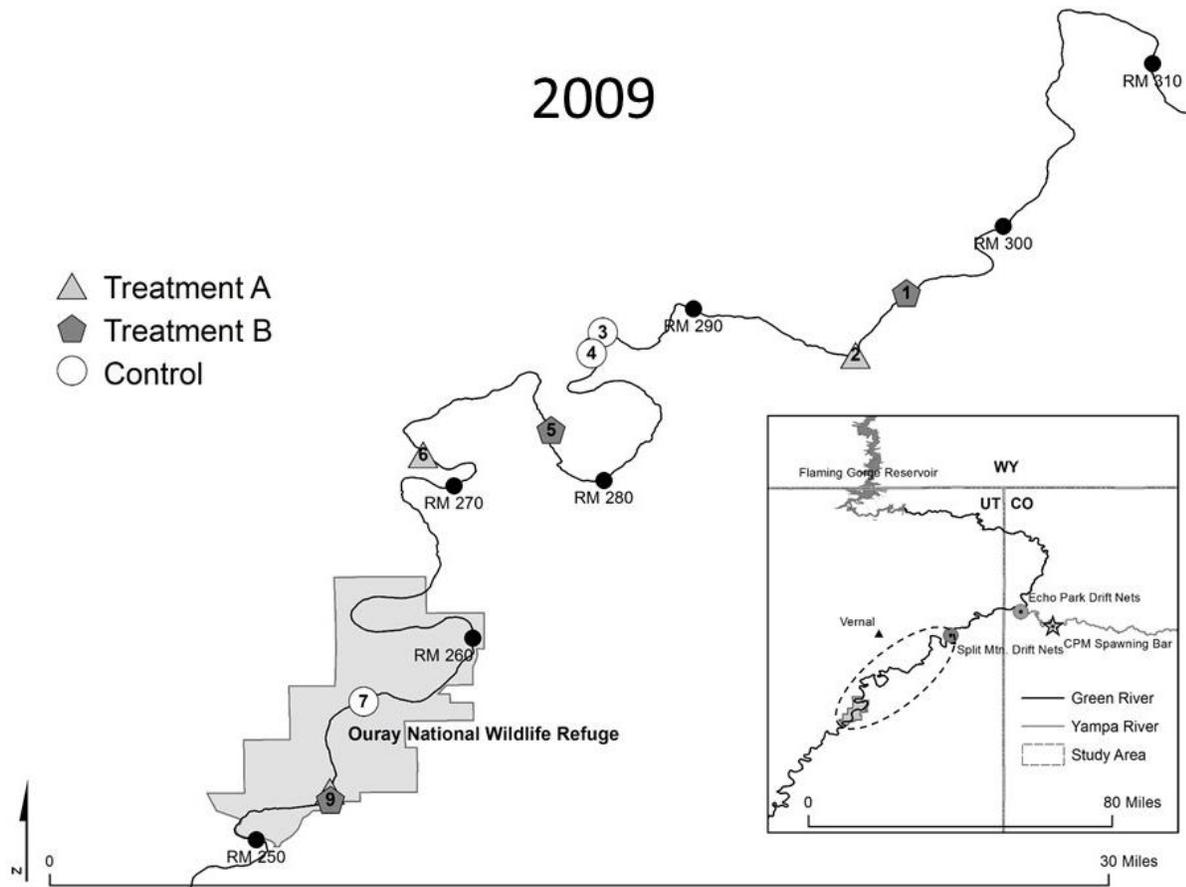


Figure 1. Study reach used to assess Colorado pikeminnow (CPM) presence and survival in the middle Green River in 2009. The inset depicts the CPM spawning bar located in Yampa Canyon (Cleopatra’s Couch; star) and the placement of drift nets at Echo Park and the Split Mountain boat ramp (circles) in Dinosaur National Monument to verify larval drift (16 July–20 August). Seining for larval CPM was conducted in various backwater habitats within Ouray National Wildlife Refuge to verify larval CPM arrival in nursery areas (28 July–13 August) and symbols represent backwaters selected for experimental treatments using a variety of blocking scenarios and nonnative fish depletion protocols (July–September).

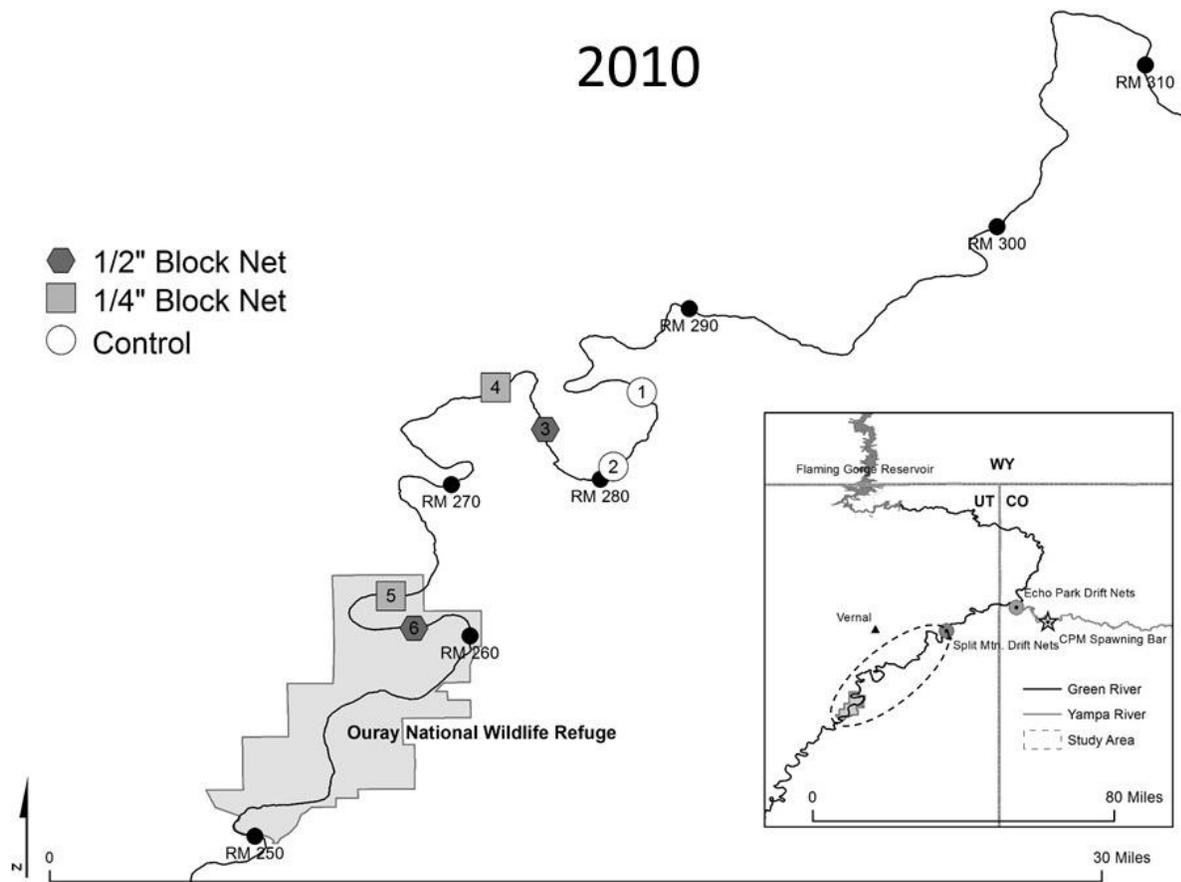


Figure 2. Study reach used to assess Colorado pikeminnow (CPM) presence and survival in the middle Green River in 2010. The inset depicts the CPM spawning bar located in Yampa Canyon (Cleopatra’s Couch; star) and the placement of drift nets at Echo Park and the Split Mountain boat ramp (circles) in Dinosaur National Monument to verify larval drift (13 July–13 August). Seining for larval CPM was conducted in various backwater habitats within Ouray National Wildlife Refuge to verify larval CPM arrival in nursery areas (27 July–10 August) and symbols represent backwaters selected for experimental treatments using a variety of blocking scenarios and nonnative fish depletion protocols (July–September).

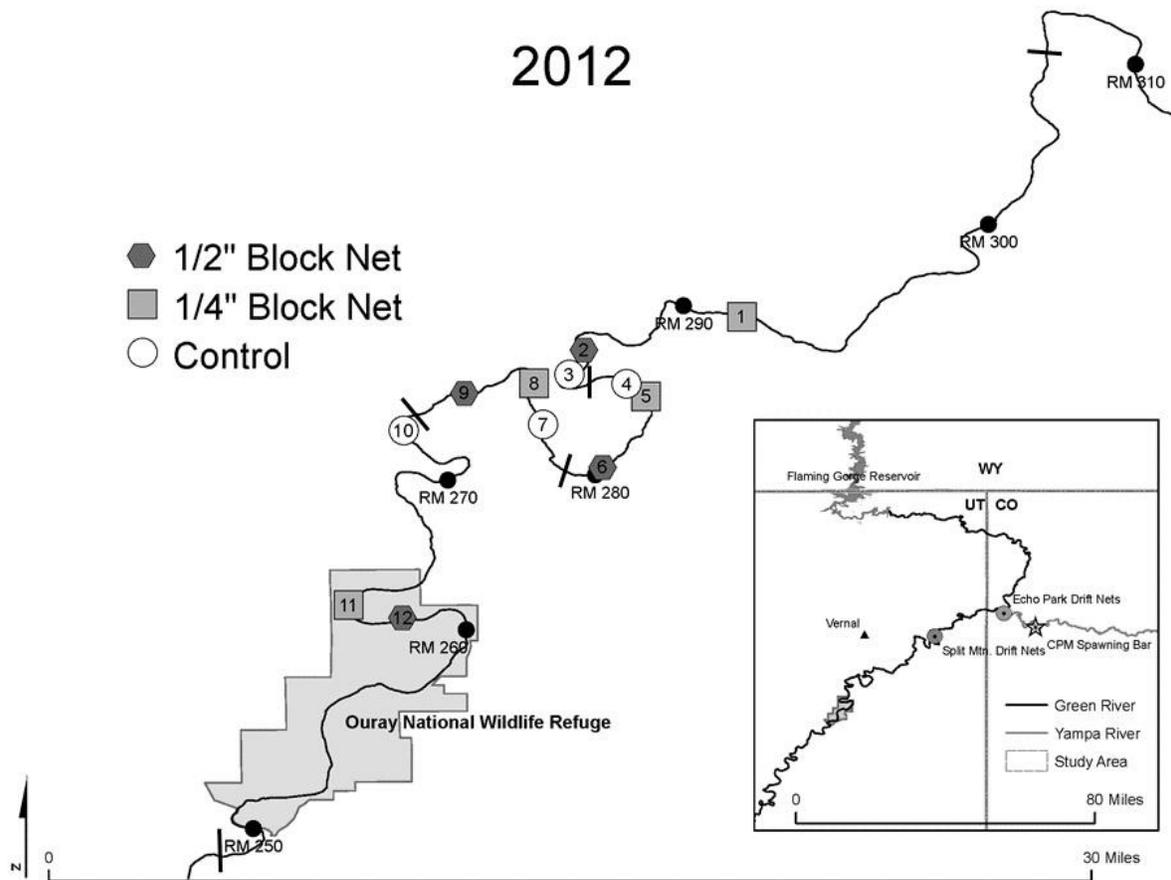


Figure 3. Study reach used to assess Colorado pikeminnow (CPM) presence and survival in the middle Green River in 2012. The inset depicts the CPM spawning bar located in Yampa Canyon (Cleopatra’s Couch; star) and the placement of drift nets at Echo Park and the Split Mountain boat ramp (circles) in Dinosaur National Monument to verify larval drift (26 June–27 July). Seining for larval CPM was conducted in various backwater habitats within Ouray National Wildlife Refuge to verify larval CPM arrival in nursery areas (10 July–3 August) and symbols represent backwaters selected for experimental treatments using a variety of blocking scenarios and nonnative fish depletion protocols (June–September). Solid black lines indicate boundaries for a stratified random block design (four blocks, one of each treatment randomly assigned to each block = four replicates/treatment, 12 experimental units total).



Figure 4. Experimental backwater (1/2" mesh block net; top left) set up prior to nonnative fish depletion passes (top right) in 2012. Bottom picture depicts the live cage used to process overwhelming seine hauls to remove native fishes before subsampling nonnatives.

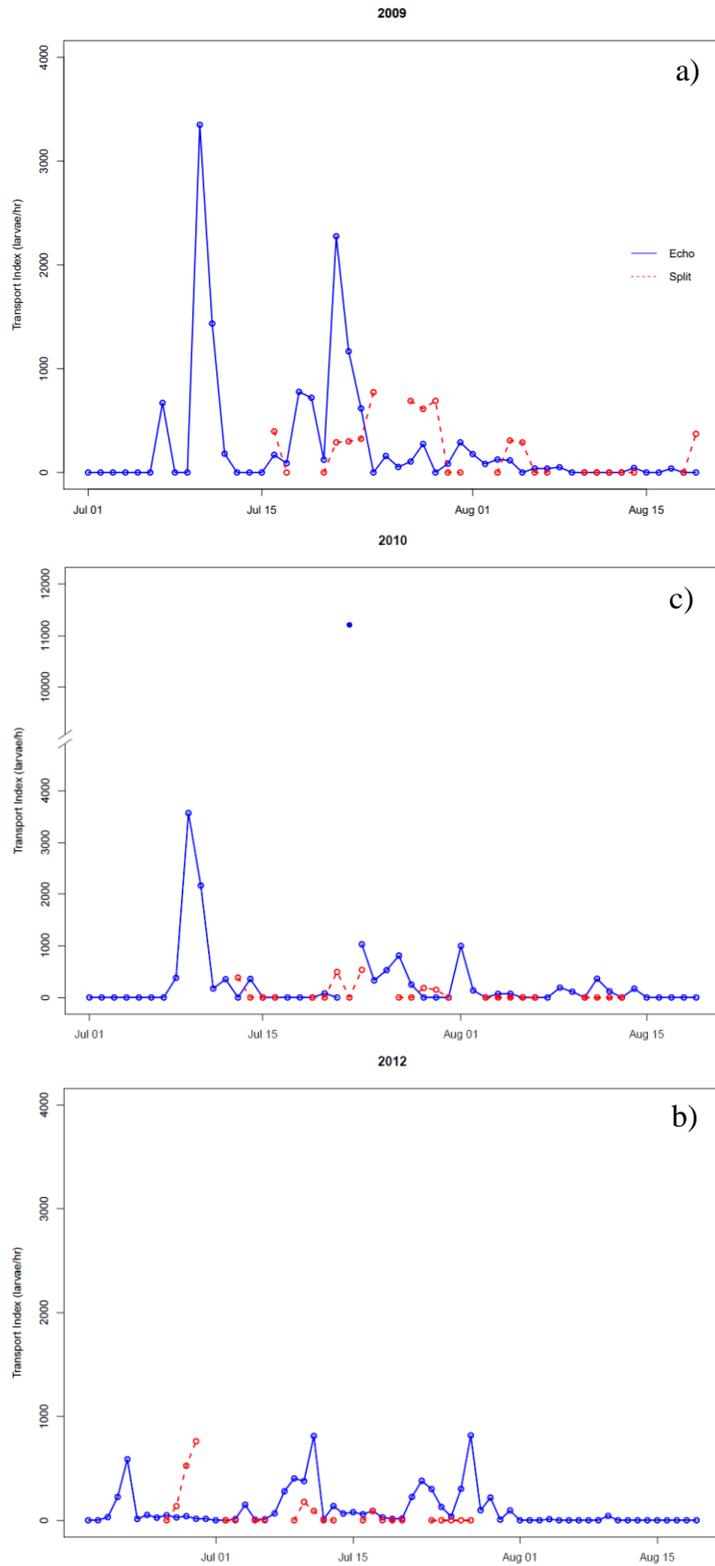


Figure 5. Transport indices of Colorado pikeminnow larvae drifting past drift net sites at Split Mountain and Echo Park, 2009-2010 and 2012. Echo Park data from Bestgen and Hill (2016).

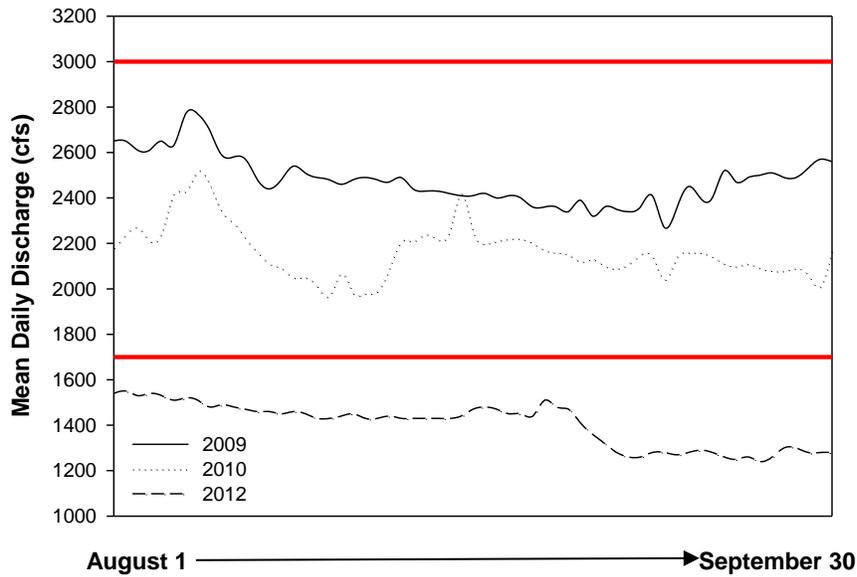


Figure 6. Middle Green River mean daily discharge (cubic ft/sec; cfs) for 2009, 2010, and 2012 measured at Jensen, UT (USGS gauge #09261000). Areas in-between the red lines indicate the ideal discharge for the months of August and September to benefit age-0 Colorado pikeminnow recruitment based on Bestgen and Hill (2016).

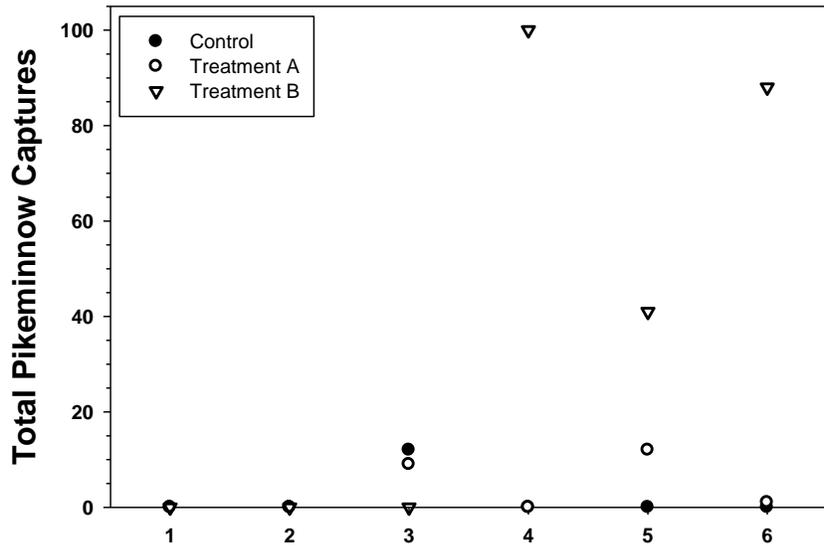


Figure 7. Age-0 Colorado pikeminnow (CPM) captures for experimental backwaters in 2009. Sample periods 1-6 occurred from 20–24 July, 3–6 August, 17–20 August, 31 August–2 September, 14–15 September, and 23–29 September, respectively. Note that data from one Control and one Treatment B backwater were excluded from the entire experiment, and data is not adequately represented for one Treatment B backwater on the third sample period when age-0 CPM were released immediately without being enumerated due to an abundance of captures.

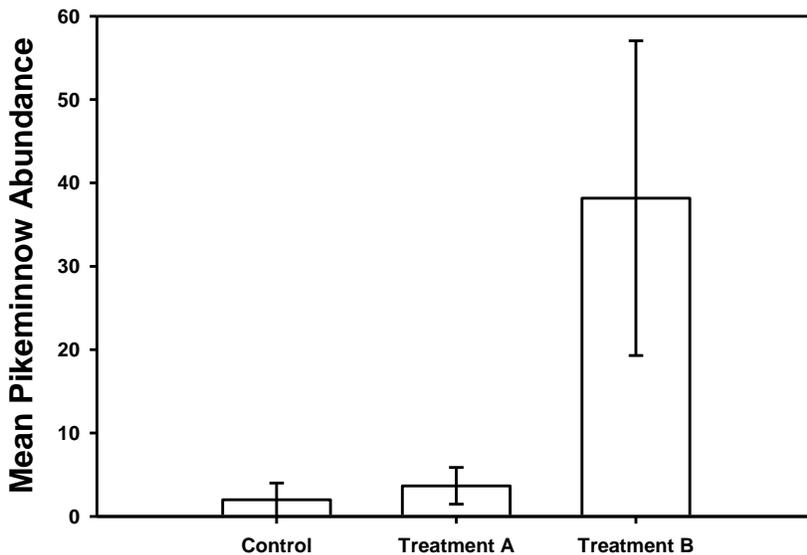


Figure 8. Mean ( $\pm$  SE) Colorado pikeminnow abundance by treatment type from six sampling occasions; 20 July–29 September 2009. Note that data from one Control and one Treatment B backwater were excluded from the entire experiment, and data is not adequately represented for one Treatment B backwater on the third sample period when age-0 pikeminnow were released immediately without being enumerated due to an abundance of captures.

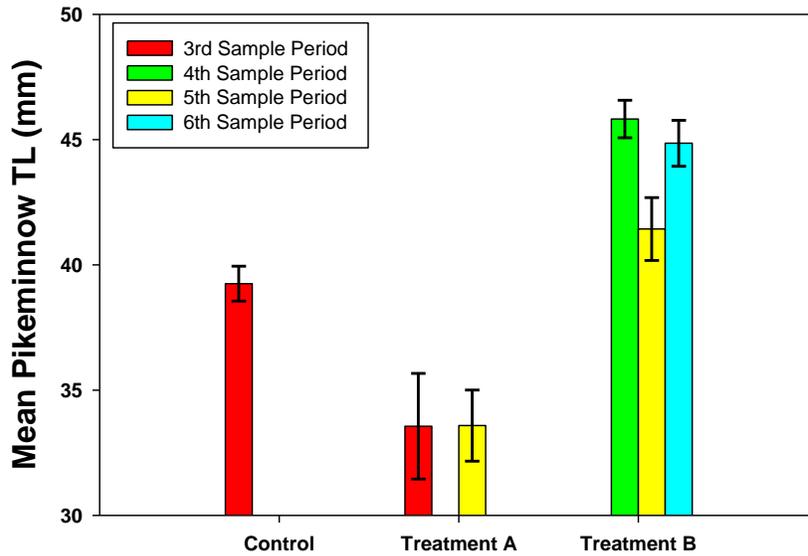


Figure 9. Mean ( $\pm$  SE) Colorado pikeminnow total length (TL) by treatment type for experimental backwaters in 2009. The 3-5<sup>th</sup> sample periods occurred on 17–20 August, 31 August–2 September, 14–15 September, and 23–29 September, respectively.

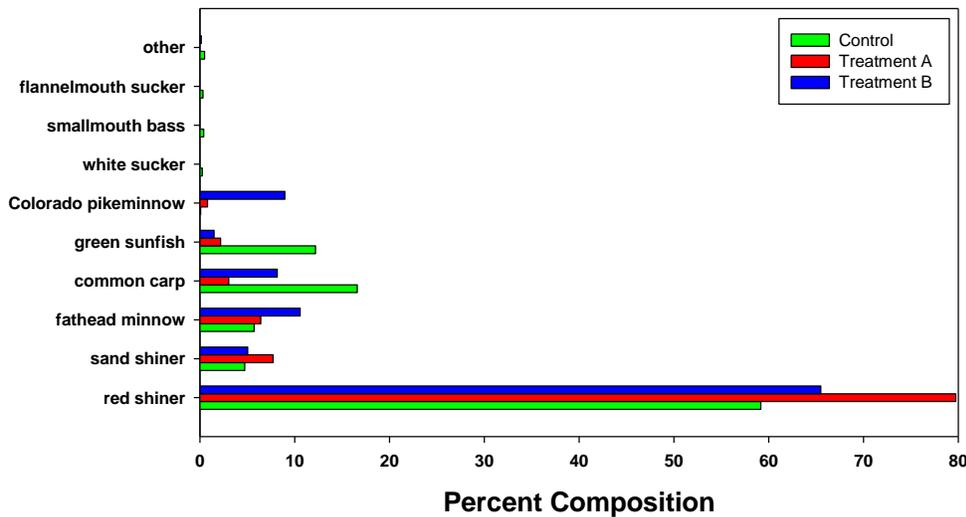


Figure 10. Percent species composition sampled from experimental backwaters in 2009 during sample periods 4-6 (31 August–2 September, 14–15 September, and 23–29 September respectively). Data is excluded from one Control and one Treatment B backwater that were removed from the experiment. The “other” category includes one black bullhead, one black crappie, one bluehead sucker, one channel catfish, and two brook sticklebacks from Controls, along with one black bullhead and three channel catfish captured from Treatment B backwaters.

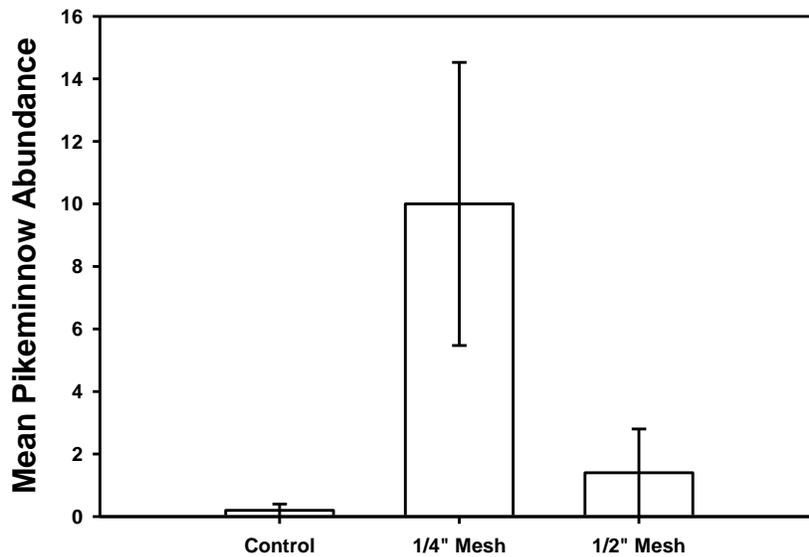


Figure 11. Mean ( $\pm$  SE) Colorado pikeminnow abundance by treatment type from five sampling occasions; 27 July–23 September 2010. Note that data from one 1/2" mesh treatment was excluded from the entire experiment, and data from one Control backwater and the remaining 1/2" mesh treatment were excluded from the fifth sample period (i.e., only one Control backwater and two 1/4" treatments were examined for the entire experiment).

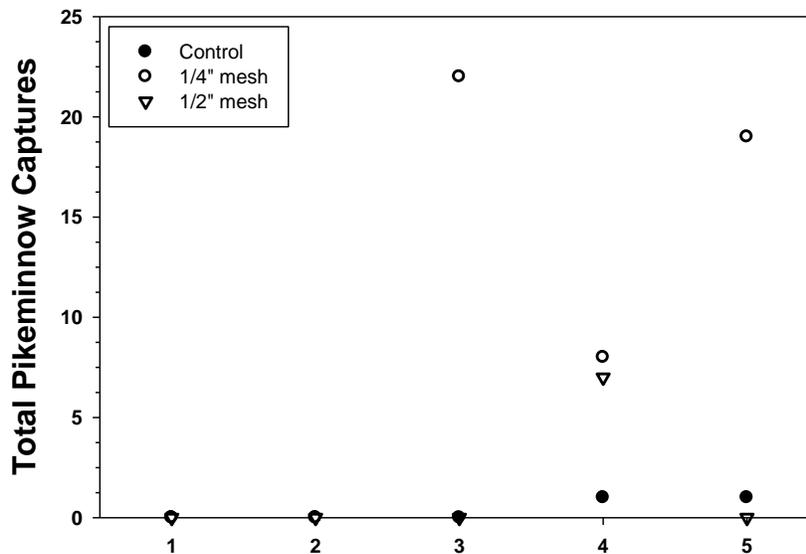


Figure 12. Age-0 Colorado pikeminnow captures for experimental backwaters in 2010. Sample periods 1-5 occurred from 27–29 July, 9–10 August, 23–24 August, 9 September, and 21–23 September, respectively. Note that data from one 1/2" mesh treatment was excluded from the entire experiment, and data from one Control backwater and the remaining 1/2" mesh treatment were excluded from the fifth sample period (i.e., only one Control backwater and two 1/4" treatments were examined for the entire experiment).

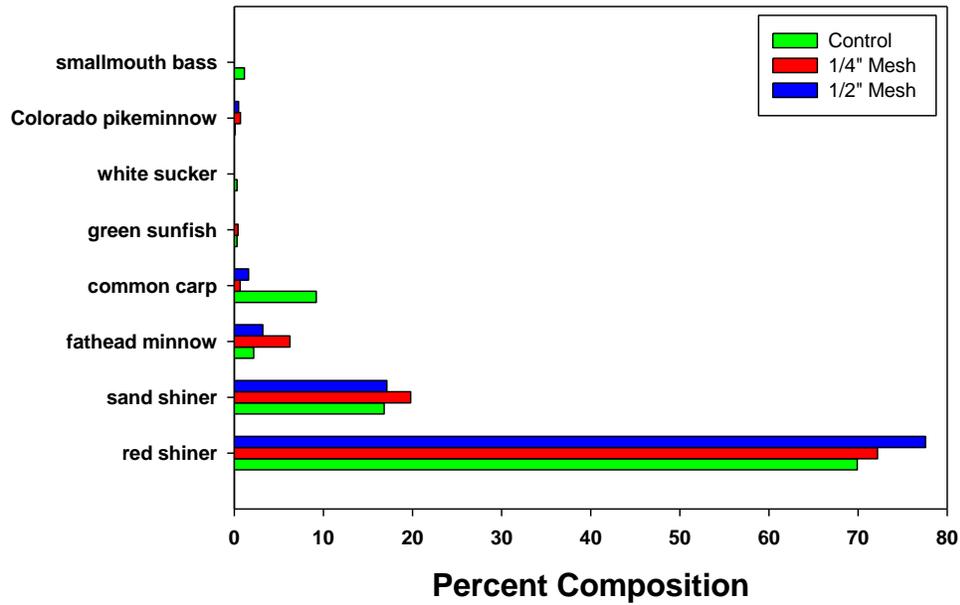


Figure 13. Percent species composition sampled from experimental backwaters in 2010 during sample periods 2-4 (9–10 August, 23–24 August, and 8 September, respectively). Data is excluded from one 1/2" mesh block net treatment that was vandalized early in the experiment.

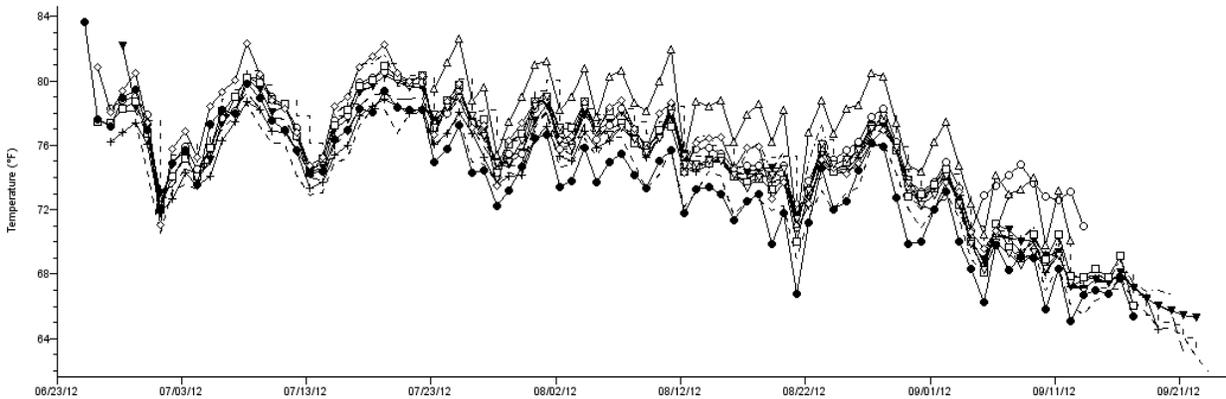


Figure 14. Mean daily water temperatures for all experimental backwaters in 2012 for the duration of the experiment.



Figure 15. Time series depicting backwater habitat loss during drought conditions observed during experimentation conducted in 2012. Photographs of the same Control backwater that was eventually excluded from the experiment were taken on 25 June 2012 (top left), 10 July 2012 (top right), and 18 September 2012 (bottom).

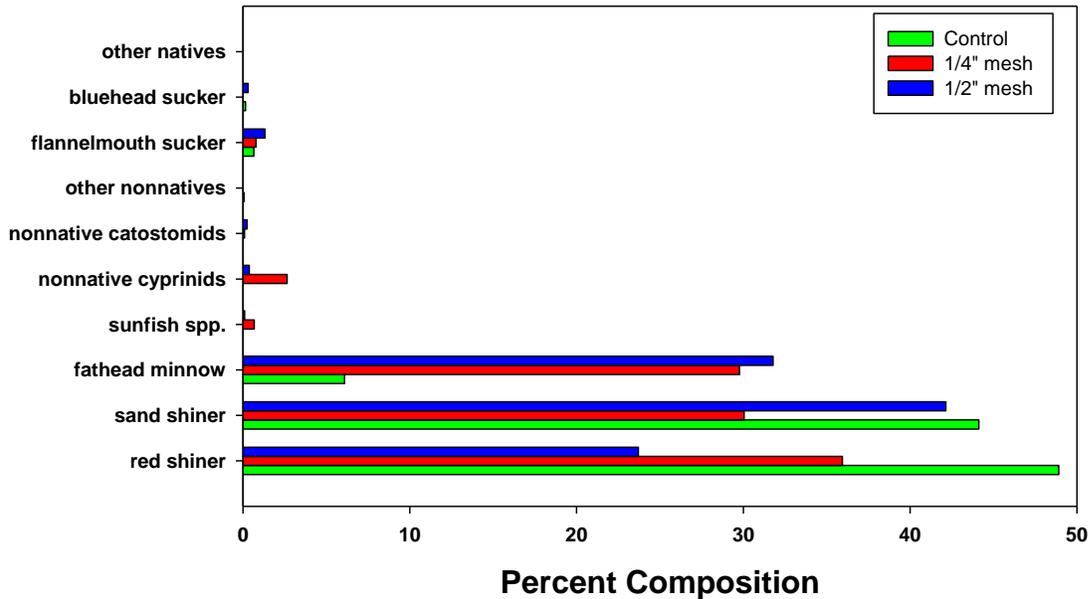


Figure 16. Percent species composition sampled from experimental backwaters in 2012 during sample periods 2-7 (10-12 July, 23-25 July, 7-9 August, 20-22 August, 4-6 September, and 18-25 September, respectively). Data is excluded from one Control backwater due to disconnection from the Green River and one 1/2" mesh block net that was stolen. Combined categories include: other natives (Colorado pikeminnow and speckled dace), other nonnatives (black bullhead, channel catfish, Iowa darter, and smallmouth bass), nonnative catostomids (white sucker and white sucker x flannelmouth sucker hybrids), nonnative cyprinids (too small to accurately identify in the field), and sunfish spp. (green sunfish and unknown *Lepomis* spp.).

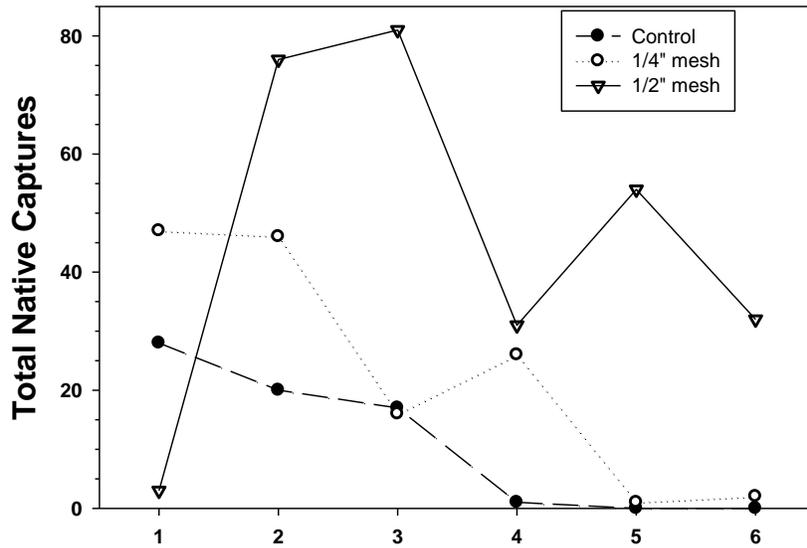


Figure 17. Post-depletion age-0 native fish captures (species combined) from experimental backwaters in 2012; data includes 71 bluehead sucker, one Colorado pikeminnow, 406 flannemouth sucker, and two speckled dace. Sample periods occurred from 10 July–25 September. Note that data from one Control and one 1/4" mesh backwater were excluded from the entire experiment.

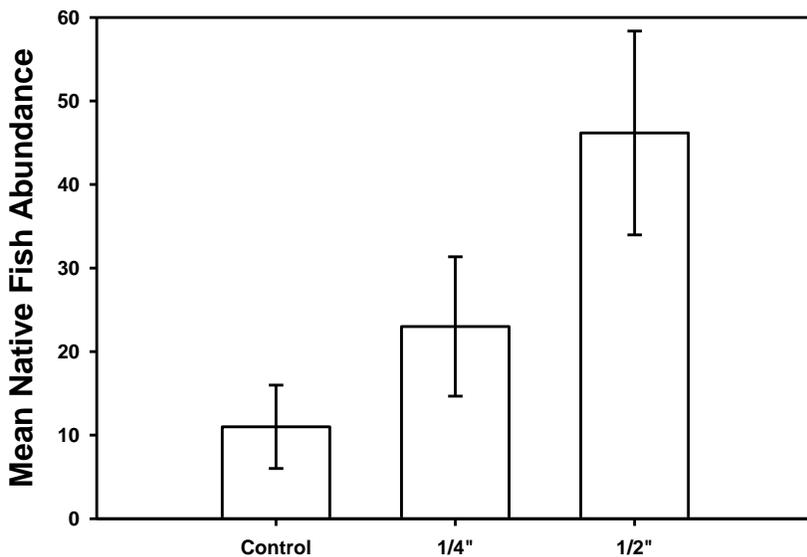


Figure 18. Mean ( $\pm$  SE) age-0 native fish abundance by treatment type from six post-depletion sampling occasions; 10 July–25 September 2012. Combined data includes 71 bluehead sucker, one Colorado pikeminnow, 406 flannemouth sucker, and two speckled dace. Note that data from one Control and one 1/4" mesh backwater were excluded from the entire experiment.

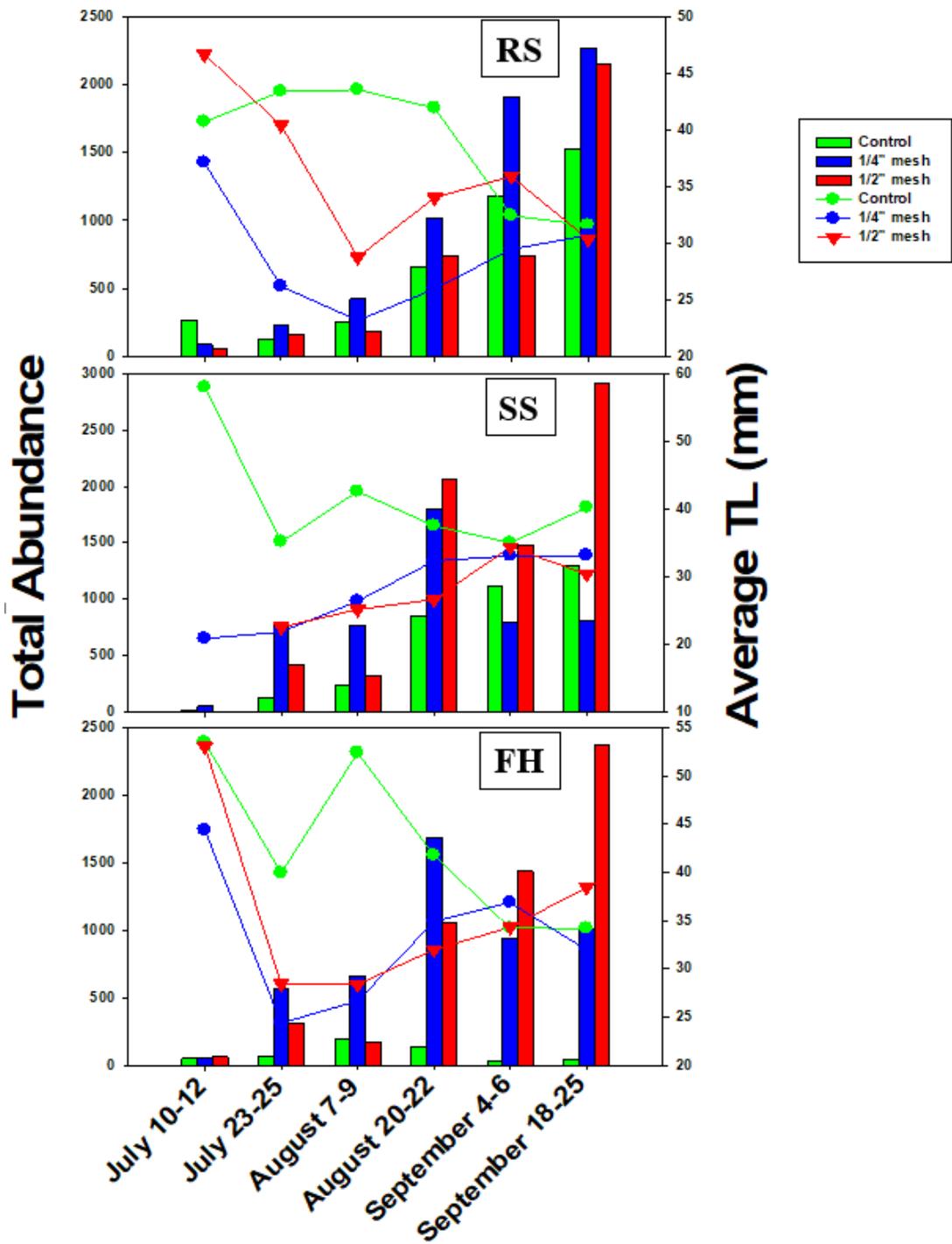


Figure 19. Total abundance (left Y-axis; bars) of red shiner (RS), sand shiner (SS), and fathead minnow (FH) captured from experimental backwaters during each post-depletion sample period. The right Y-axis displays average total length (TL; lines) for each species during each sample period. Length averages are derived from measurement of 1,848, 1,636, and 1,645 red shiner, sand shiner, and fathead minnow, respectively; only one 54 mm sand shiner was measured for the 1/2" mesh treatment during the July 10-12 sample period. Note that data from one Control and one 1/4" mesh backwater were excluded from the entire experiment. Also note the differences in the Y-axis scales for each panel.