



United States Department of the Interior



FISH AND WILDLIFE SERVICE
Arizona Fish and Wildlife Conservation Office
Southwest Forest Science Complex, Bldg. 82 West
2500 South Pine Knoll Drive
Flagstaff, Arizona 86001-6381
(928) 556-2154

Mark-Recapture and Fish Monitoring Activities in the Little Colorado River in Grand Canyon from 2000 to 2022

David R. Van Haverbeke, Jess Newton, Kirk L. Young, Michael J. Pillow, and Pilar Rinker



U.S. Fish and Wildlife Document: USFWS-AZFWCO-FL-23-02

Submitted to USGS Grand Canyon Monitoring and Research Center 1 March 2023.

Photos: Arizona Fish and Wildlife staff members: Top row (L-R) Kerri Pedersen and Pilar Rinker, Ann Hall with Mike Pillow and Ryan Green, Tiffany Love-Chezm. Bottom row (L-R) Kirk Young, David Van Haverbeke and Kenai Van Haverbeke, Kirsten Tinning. Photos by D. Van Haverbeke, Mike Pillow, and Pilar Rinker.

Suggested report citation:

Van Haverbeke, D.R., J. Newton, K.L. Young, M.J. Pillow, and P. Rinker. 2023. Mark-Recapture and Fish Monitoring Activities in the Little Colorado River in Grand Canyon from 2000 to 2022. Submitted to USGS Grand Canyon Monitoring and Research Center, Flagstaff, Arizona. U.S. Fish and Wildlife Service, Flagstaff, Arizona. 53 pp.

Contents

Abstract	8
Introduction	9
Previous Investigations	10
Purpose and Objectives	11
Methods	12
Trips and Participating Personnel	12
Study Area	12
Gear and Effort	13
Fish Handling and Data Collection	14
Water Quality	15
Closed Mark-Recapture	15
Population Estimation using Capture Probability Data and Monte Carlo Simulation	17
Catch per Unit Effort	18
Results –rkm 0-13.57	19
Physical Parameters	19
Turbidity and Flow	19

Species Composition	19
Length Frequency Distributions.....	20
Humpback Chub.....	20
Bluehead Sucker	21
Flannelmouth Sucker.....	21
Parasites	21
Abundance Estimation (rkm 0-13.57).....	22
Adult and sub-adult Humpback Chub	22
Juvenile (age 1 and age 0) Humpback Chub	22
Bluehead and Flannelmouth Sucker.....	23
Results Chute Falls (rkm 13.57 to 17.7)	24
Physical Parameters	24
Turbidity and Temperature	24
Length Frequency Distributions and Catch	24
Sexual Condition and Parasites	24
Population Abundance Estimation	25
Discussion	25
Lower 13.57 rkm of the LCR	25
Chute Falls (rkm 13.57-17.7).....	28
Future Population Estimation Considerations	28
Acknowledgements.....	29
References Cited	29

Figure 1. Map of study areas, showing Boulders (river km [rkm] 0 to 5), Coyote (rkm 5 to 9.6) and Salt (rkm 9.6 to 13.57) reaches, and lower Chute Falls (rkm 13.57 to 14.1, Atomizer reach) and upper Chute Falls (rkm 14.1 to 17.7, Chute Falls reach), Little Colorado River. Camps are designated by triangles. 35

Figure 2. Hoop net sampling effort (hours) across all spring and fall monitoring trips 2000 to 2022 in lower 13.57 river km of the Little Colorado River..... 36

Figure 3. Hoop netting effort (hours) in the Atomizer and Chute Falls reaches during monitoring efforts between 2006 and 2022, Little Colorado River. Note: Atomizer reach extended from Lower Atomizer Falls (river km 13.57) to Chute Falls (river km 14.1). Chute Falls reach extended from top of Chute Falls (river km 14.1) to river km 17.7. Both reaches included a mark and recapture event from 2006-2009, but thereafter these reaches only included one trip per year..... 36

Figure 4. Average trip turbidities (nephelometric turbidity units, NTU) during spring and fall mark-recapture trips in the Little Colorado River, 2000-2022. Note, “missing” bars represent low trip turbidities (most < 54 NTUs), not missing data. Note: sampling was not conducted in spring 2020..... 37

Figure 5. Provisional mean daily discharge (cubic feet/second; cfs) from USGS gage station 0940200; Little Colorado River (LCR), Arizona, 1 January 2000 – 1 January 2023. 37

Figure 6. Observed spring and fall species composition ($\pm 95\%$ CI) of fish captured in hoop nets between fall 2000 and fall 2022; Little Colorado River (river km 0-13.57, n = 241,284 fish). 38

Figure 7. Catch per unit effort (CPUE, fish/net) of nonnative fish species in the lower 13.57 km of the Little Colorado River during A) spring and B) fall seasons. Note: dotted lines are linear trend lines. 39

Figure 8. Length frequency distributions of Humpback Chub captured in the Little Colorado River (river km 0-13.57) during (A) spring 2001-2021 and 2022 and (B) fall 2000-2021 and 2022. Note: sampling was not conducted in spring 2020. Note y-axis variations in the charts. 40

Figure 9. Length frequency distributions of Bluehead Sucker captured in the Little Colorado River (river km 0-13.57) during (A) spring 2001-2021 and 2022 and (B) fall 2000-2021 and 2022. Note: sampling was not conducted in spring 2020. Note y-axes variations in the charts. 41

Figure 10. Length frequency distributions of Flannelmouth Sucker captured in the Little Colorado River (river km 0-13.57) during (A) spring 2001-2021 and 2022 and (B) fall 2000-2021 and 2022. Note: sampling was not conducted in spring 2020. Note y-axes variations in the charts. 42

Figure 11. Percent occurrence of the external copepod parasite (*Lernaea cyprinacea*) on Humpback Chub in the Little Colorado River (river km 0-13.57), 2000-2022. 43

Figure 12. Chapman Petersen abundance estimates ($\pm 95\%$ CI) of Humpback Chub ≥ 150 mm and ≥ 200 mm in the Little Colorado River (0-13.57 river km) during (A) spring (2001-2022) and (B) fall seasons (2000-2022). Note: closed spring and fall abundance estimates of Humpback Chub > 150 mm in the Little Colorado River during 1991 and 1992 are from Douglas and Marsh (1996). Note: No sampling occurred in spring 2020. 44

Figure 13. Chapman Petersen spring abundance estimates ($\pm 95\%$ CI) of Humpback Chub in the 150-199 mm size category; Little Colorado River (0-13.57 river km), 2001-2022. Note: 2020 (red diamond) was estimated using multi-state model (Yackulic et al. 2014) vital rate parameters (see Methods). 45

Figure 14. (A) Spring season Monte Carlo simulated abundance estimates ($\pm 95\%$ CI), Chapman Petersen abundance estimates ($\pm 95\%$ CI), and catch per unit effort (CPUE, $\pm 95\%$ CI) of Humpback Chub (HBC) in the 91-161 mm size class (predominately age 1). Note: Age 1 Chapman Petersen abundance estimates were not performed in all years, and no estimates were made in 2020 because Covid-19 prevented sampling. (B) Fall season Monte Carlo simulated abundance estimates ($\pm 95\%$ CI), Chapman Petersen abundance estimates ($\pm 95\%$ CI), and CPUE ($\pm 95\%$ CI), of HBC in the 40-105 mm size class (age 0); Little Colorado River (0-13.57 river km). Note: Age 0 Chapman Petersen abundance estimates were not performed in all years, and Chapman Petersen estimates were not successful in 2018 and 2022 because of too few recaptures. 46

Figure 15. Annual Chapman Petersen abundances of Humpback Chub <100 mm (age 0 fish) during the fall, and abundances of Humpback Chub 100-149 mm (predominantly age 1 fish) the following spring, Little Colorado River (0-13.57 river km). Note, spring 2020 100-149 mm size class (2019 cohort) missing because spring 2020 sampling was not conducted..... 47

Figure 16. (A) Spring season Chapman Petersen abundance estimates ($\pm 95\%$ CI), Monte Carlo simulated abundance estimates ($\pm 95\%$ CI), and catch per unit effort (CPUE, $\pm 95\%$ CI), of Bluehead Sucker ≥ 150 mm, and (B) Flannelmouth Sucker ≥ 150 mm; Little Colorado River (0-13.57 river km). Note: Chapman Petersen abundance estimates were not performed in all years, and no spring sampling in 2020..... 48

Figure 17. Length frequency distributions of Humpback Chub captured during May 2022 above Chute Falls (river km 14.1-17.7, top graph) and in the Atomizer reach (river km 13.57-14.1, bottom graph), Little Colorado River. Colored stacked bars were captured chub that were translocated in years between 2013 to 2021, as evidenced by PIT tag recaptures. Grey bars were fish that were untagged upon capture in May 2022 (i.e., most likely fish that were spawned and grew up in these reaches, or upriver migrants)..... 49

Figure 18. (A) Numbers of juvenile Humpback Chub that have been translocated to the Chute Falls reach since 2003 (black bars); and abundances ($\pm 95\%$ CI) of adult chub (≥ 200 mm) in the Chute Falls reach (river km 14.1-17.7) calculated with Chapman Petersen method (red bars), and Monte Carlo simulation (light grey bars), and (B) abundances ($\pm 95\%$ CI) of adult chub in the Atomizer reach immediately downstream of Chute Falls (river km 13.57–14.1) calculated with Chapman Petersen method (red bars) and Monte Carlo simulation (light grey bars); Little Colorado River..... 50

Figure 19. Number of consecutive days with Little Colorado River (LCR) flows <30 cubic feet/second (cfs) at USGS Gage 0940200, near Cameron, AZ, 1947-2023. Note, flows <30 cfs at Cameron generally indicate base level flows below Blue Springs in LCR, unless there is input from local drainages downriver from Cameron gage. 51

Figure 20. Regression of LN10 summed mean daily flow (1 January-31 May, cfs) to LN10 fall abundance of age 0 Humpback Chub in Little Colorado River, 2000-2022..... 52

Figure 21. Regression of stratified Chapman-Petersen abundance estimates, and non-stratified Chapman-Petersen abundance estimates for A.) ≥ 150 mm, and B.) ≥ 200 mm size classes of Humpback Chub in Little Colorado River, 2000-2022..... 53

Tables

Table 1. List of reaches, subreaches, and river kilometers within each subreach; Little Colorado River..... 34

Mark-Recapture and Fish Monitoring Studies in the Little Colorado River in Grand Canyon from 2000 to 2022

By David R. Van Haverbeke, Jess Newton, Kirk Young, Michael J. Pillow, and Pilar Rinker

Abstract

Since 2000, monitoring using hoop nets has been conducted in the lower 13.57 river kilometers (rkm) of the Little Colorado River (LCR) to estimate abundance and track trends in abundance of the Humpback Chub (*Gila cypha*), and to monitor other fishes. These monitoring activities occurred during the spring and fall seasons. Native fish species continue to dominate the LCR, comprising 92.4 percent of fish captures since 2000. We used closed Chapman Petersen mark-recapture methods and catch per unit effort data to estimate either absolute or relative abundances of Humpback Chub of various size classes. Between 2000 and 2006, adult Humpback Chub (≥ 200 mm) maintained stable but low abundances of $< 3,000$ individuals during the spring and fall seasons. From 2007 to 2014, the spring abundance of adult Humpback Chub significantly increased, and remained at elevated levels ($\sim 4,000$ to $7,000$ individuals). Fall abundances of adult Humpback Chub were also generally elevated during 2007-2014, but to a lesser extent. In 2015 and 2016, the spring abundances of adult Humpback Chub in the LCR were depressed compared to the 2007-2014 timeframe. The reason why is uncertain, but it is thought that a large portion of the population remained in the mainstem during those years. From 2017 to 2019, the spring adult Humpback Chub abundance in the LCR returned to levels equal to or exceeding those during 2007-2014. During spring 2020, no sampling was conducted in the LCR because of COVID-19. In spring 2021, the spring abundance of adult Humpback Chub was again significantly depressed, thought to be a result of severe drought in the LCR, poor spawning substrate, and many adults remaining in the mainstem. Finally, in spring 2022 adult abundances rebounded again to the 2019 level.

Spring abundance estimates of sub-adult Humpback Chub (150-199 mm) were more variable than adults, but have also generally increased since 2006, with a rolling three-year average remaining above 1,250 fish. Smaller size classes of Humpback Chub (< 150 mm) also display significant annual variation in abundance and catch per unit effort, which is thought to be related to the LCR hydrograph; with 2019 and 2020 being represented by relatively high age 0 cohort production, but with an unexpected decline in age 1 Humpback Chub during spring

2021, again believed to be a result of prolonged drought in the LCR accompanied by severe accumulation of marl substrate during winter of 2020.

Bluehead Sucker (*Catostomus discobolus*) and Flannelmouth Sucker (*C. latipinnis*) were primarily monitored using catch per unit effort, but population estimates were also obtained using mark recapture and simulation. Relative catches of both native sucker species have generally increased in the post-2006 timeframe.

Reasons for the post-2006 increases of Humpback Chub, Bluehead Sucker, and Flannelmouth Sucker are thought to be related to several factors, including warmer mainstem Colorado River water temperatures since 2003, and possibly mechanical removal of nonnative fish in the mainstem Colorado River during 2003-2006.

Finally, annual monitoring has been conducted since 2006 to monitor Humpback Chub translocated to above Chute Falls in the LCR. Annual estimates of adult Humpback Chub above Chute Falls (>14.1 rkm) have ranged from 1-263, and annual estimates of adult chub in the Atomizer reach immediately below Chute Falls (13.57-14 rkm) have ranged from 16-435.

Introduction

With completion of the EIS and the Biological Opinion on Operations of Glen Canyon Dam (USBR 1995, USFWS 1994), the Glen Canyon Dam Adaptive Management Program (GCDAMP) was initiated. Within GCDAMP, the Adaptive Management Work Group (AMWG) is responsible for defining management objectives associated with resources downstream from Glen Canyon Dam and provides recommendations about development of long-term monitoring programs to assess those resources. The Long Term Experimental and Management Plan (LTEMP) EIS (USBR 2016) further identified important conservation measures for species including the Humpback Chub (*Gila cypha*). The U.S. Geological Survey Grand Canyon Monitoring and Research Center (GCMRC) is responsible for coordinating long-term monitoring programs in support of AMWG and the LTEMP. Assessing the status of the Humpback Chub is particularly important because it was listed as a federally endangered species (U.S. Office of the Federal Register 32:48 [1967]:4001; ESA; 16 U.S.C. 1531 et. seq.), and recently down listed to federally threatened (U.S. Office of the Federal Register 86:198 [2021]:57588-57610).

Because of the above needs, the U.S. Fish and Wildlife Service (USFWS) initiated a program in fall 2000 to conduct long-term monitoring of Humpback Chub in the Little Colorado River (LCR). Between 2000 and 2022, USFWS obtained closed mark-recapture population abundance estimates of Humpback Chub ≥ 150 mm in the lower 13.57 river kilometers (rkm) of the LCR (e.g., Van Haverbeke 2010, Van Haverbeke et al. 2013). Our spring mark-recapture efforts are aimed to coincide with Humpback Chub spawning in the LCR and provide an annual estimate of spring spawning abundance. Our fall mark-recapture efforts provide data about Humpback Chub

potentially overwintering in the LCR, particularly those in the juvenile and sub-adult life stages. Additionally, fall mark-recapture efforts temporally expand marks and recaptures of Humpback Chub in the LCR, thereby providing a foundation for other open or multi-state models (e.g., Coggins et al. 2006, Coggins and Walters 2009, Yackulic et al. 2014).

In addition to estimating abundance and population trends of sub-adult (150-199 mm) and adult Humpback Chub ≥ 200 mm, there is interest in tracking abundance of smaller size classes of Humpback Chub < 150 mm. This interest stems from a desire to further understand recruitment dynamics of Humpback Chub (e.g., Coggins and Walters 2009, Dzul et al. 2014). This information is also used to estimate the portion of age 0 Humpback Chub that are annually removed for translocations to other tributaries in Grand Canyon (i.e., Bright Angel, Shinumo, or Havasu creeks; NPS 2013, Trammel et al. 2012) and for maintenance of a refuge population of Humpback Chub at the Southwest Native Aquatic Research and Recovery Center (SNARRC).

Finally, Humpback Chub monitoring has also occurred upstream of rkm 13.57 in the LCR between 2006 and 2022 (e.g., Van Haverbeke 2010, Van Haverbeke et al. 2013). The purpose of this monitoring has been two-fold. First, this portion of the LCR (rkm 13.57 to ~ 17.7) became of interest after a series of translocations initiated in 2003 moved juvenile Humpback Chub from the lower reaches of the LCR (\sim rkm 1.15 to 9.85) to upstream of a natural travertine structure called Chute Falls located at rkm 14.1. Translocated chub were released at rkm 16.2. Monitoring efforts upstream of Chute Falls provide annual population estimates of these translocated chub as they grow into adulthood. Second, there is a small reach of river (Atomizer reach, rkm 13.57 to 14.1) that is not included during our mark-recapture efforts downstream of rkm 13.57. This is a section of river in which Humpback Chub naturally occur, and a section of river which many of the translocated chub occupy as they eventually disperse downstream. Monitoring in this short section of river helps to account for all Humpback Chub in the LCR system.

Previous Investigations

With emplacement of Glen Canyon Dam, the Colorado River throughout Grand Canyon has been predominately characterized by cold hypolimnetic release waters (Wright et al. 2009) that negatively affect Humpback Chub by lengthening the egg incubation period, decreasing egg and larval survival, and slowing growth at all life stages (Hamman 1982, Clarkson and Childs 2000, Robinson and Childs 2001, Coggins and Pine 2010). These factors were considered to have caused range contraction and decreases in abundance of Humpback Chub in Grand Canyon (Kaeding and Zimmerman 1983, Douglas and Marsh 1996, USFWS 2002) and limited the spatial distribution of Humpback Chub to near the LCR. Likewise, warmer mainstem waters that have occurred more often since about 2003 have likely led to the post 2006 LCR population expansion and post 2014 range expansion in

western Grand Canyon (Van Haverbeke et al. 2013, Van Haverbeke et al. 2017). Because of the need to conserve and recover this relict species, several studies have focused on estimating population abundance of the LCR population (Douglas and Marsh 1996, Coggins et al. 2006, Coggins and Walters 2009, Van Haverbeke et al. 2013, Yackulic et al. 2014).

Early studies on Humpback Chub in Grand Canyon began in the 1970s and focused on morphology, life history, and ecology (Suttkus and Clemmer 1977, Kaeding and Zimmerman 1983, Minckley 1996). In Grand Canyon, Humpback Chub are potadromous, with adults typically migrating from nearby areas in the Colorado River to the LCR to spawn during spring (Douglas and Marsh 1996, Gorman and Stone 1999). Young Humpback Chub rear in the LCR and some remain in the LCR until adulthood (Douglas and Marsh 1996, Gorman and Stone 1999) unless they emigrate or are transported out of the LCR by seasonal flood events (Valdez and Ryel 1995). Some portion of the LCR population is thought to be resident (Douglas and Marsh 1996, Yackulic et al. 2014). In addition to the LCR population, there are several smaller “aggregations” of Humpback Chub inhabiting the mainstem Colorado River in Grand Canyon (Valdez and Ryel 1995). With few exceptions in recent years, post-dam mainstem reproduction in other aggregations has been absent (but see Valdez and Masslich 1999, Andersen et al. 2010) and exchange of individuals from these aggregations to the LCR is limited (Paukert et al. 2006). However, since ~2014, evidence has accumulated that a significant population of Humpback Chub has developed in western Grand Canyon, likely sustained by mainstem spawning (Van Haverbeke et al. 2017, Rogowski et al. 2018).

Purpose and Objectives

The purpose of this report is to summarize Humpback Chub mark-recapture studies in the LCR from 2000 through 2022 and evaluate the status and trends of Humpback Chub during those years. Population variables evaluated in the study include closed population estimates, length frequency distributions, and external parasites of Humpback Chub during the spring and fall seasons. In addition, data are presented on relative abundance (catch per unit effort) of smaller size classes of Humpback Chub (<150 mm), as well as Flannelmouth Sucker (*Catostomus latipinnis*) and Bluehead Sucker (*C. discobolus*) ≥ 150 mm. The specific objectives of this report are:

1. Present closed Chapman-modified Peterson (Chapman Petersen) abundance estimates of Humpback Chub ≥ 150 mm and ≥ 200 mm in the lower 13.57 rkm of the LCR during the spring and fall season.
2. Present Chapman Petersen abundance estimates of age 0 Humpback Chub (40-99 mm) in the lower 13.57 rkm of the LCR during the fall season.

3. Document translocation efforts and present Chapman Peterson abundance estimates of Humpback Chub in the LCR between rkm 13.57 and 14.1 and between rkm 14.1 to 17.7 (Atomizer and Chute Falls reaches).
4. Present additional information related to physical parameters of the LCR, length frequency distributions of native fishes, species composition, and parasites.
5. Present 2022 annual data alongside previous data to provide data continuity.

Methods

Trips and Participating Personnel

Between September 2000 and October 2022, 87 field trips were conducted to perform 43 mark-recapture efforts to estimate abundance of Humpback Chub ≥ 150 mm and ≥ 200 mm in the lower 13.57 rkm of the LCR. A mark-recapture event occurred each spring (generally during April and May, except for 2020 because of COVID-19), and each fall (generally during September and October). In addition, between 2006 and 2022, twenty-one trips were conducted to estimate abundances of Humpback Chub upstream of rkm 13.57 in the LCR (Atomizer and Chute Falls reaches). These trips occurred during May or June, except the 2020 trip occurred in October. Personnel on the above-mentioned trips included USFWS staff from the Arizona Fish and Wildlife Conservation Office, USFWS volunteers, collaborative staff from the Arizona Game and Fish Department, Navajo Nation Fish and Wildlife Department, Grand Canyon National Park, and GCMRC. During 2022, four trips were conducted in the lower 13.57 rkm of the LCR: 19-29 April, 17-27 May, 13-23 September, and 18-28 October. Additional details of the spring and fall 2022 monitoring trips can be found in Pillow (2022) and Pillow (2023).

Study Area

Work during the four trips in the lower 13.57 rkm of the LCR was conducted downstream of a large travertine structure called Lower Atomizer Falls, with the confluence of the LCR and the mainstem Colorado River designated as rkm 0 (Figure 1). During these trips, the LCR was divided into three contiguous reaches (Boulders, Coyote, and Salt). Each reach was divided into three sub reaches (Table 1).

In addition, during work in the May trip, the LCR upstream of rkm 13.57 was divided into Atomizer reach from the top of Lower Atomizer Falls (rkm 13.57) to the base of Chute Falls (rkm 14.1), and Chute Falls reach from the top of Chute Falls (rkm 14.1) to ~rkm 17.7 (Figure 1, Table 1).

Gear and Effort

During the trips in the lower 13.57 rkm of the LCR, unbaited hoop nets (0.5 - 0.6 m diameter, 1.0 m length, 6 mm [1/4"] mesh, with a single 0.1 m throat) were used to sample fishes. During 2001 and 2002, nets were baited with AquaMax Grower 600 for Carnivorous Species (Purina Mills, Inc., Brentwood, MO). Baiting in the lower 13.57 rkm was then discontinued because of uncertainty about PIT tagging mortality in fish with full stomachs. With few exceptions, ~540 net sets were deployed during each monitoring trip. Each "net set" consisted of a hoop net being deployed in the river for ~24 hours, after which it was checked for fish. This resulted in ~180 net sets being deployed per reach (i.e., Boulders, Coyote, and Salt reaches). Each reach was divided into three sub reaches, and within each subreach, ~20 nets were set for three 24-hour periods. This design resulted in a spatially and temporally stratified netting effort among sampling trips across years (Figures 1 and 2). Exceptions to the above were five trips during 2001 and 2002, when nets were set for four 24-hour periods rather than three. Minor variation also occurred because helicopter logistics occasionally resulted in slightly shortened trips (e.g., spring 2006, and fall 2011, 2013, 2014 and 2017 mark trips). In addition, one trip was cancelled in fall 2018 because of inclement weather and flooding, and both spring trips were cancelled in 2020 because of COVID-19. With exclusion of the five higher effort trips in 2001 and 2002, average hoop netting effort among spring trips was 12,450 hours per trip (SE = 853), and among fall trips was 12,268 hours per trip (SE = 997). During spring 2022, 543 hoop net sets were deployed in April yielding 12,636 hours of effort, and in May 540 hoop net sets were deployed yielding 12,452 hours of effort. In fall 2022, 456 hoop net sets were deployed during the September trip yielding 11,292 hours of effort, and 504 hoop net sets were deployed during the October trip yielding 11,830 hours of effort. The slightly decreased effort during the fall was because of flooding and some nets being pulled.

During the Chute Falls monitoring trips, hoop nets were baited near their cod ends by attaching nylon mesh bags (30 x 30 cm, 6 mm mesh) filled with ~160 g AquaMax Grower 600 for Carnivorous Species (Purina Mills Inc., Brentwood, MO). This was done to increase fish captures to track translocated fish (Stone 2005) more closely. In addition, because our population estimation technique for these reaches involves using capture probability data collected while using baited nets between 2006 and 2009, we have continued to bait nets in the Atomizer and Chute Falls reaches. Typically, the Atomizer reach (rkm 13.57-14.1) was sampled with 17 hoop nets, and the Chute Falls reach (rkm 14.1 to ~rkm 17.7) was sampled with 33 hoop nets, all of which were run for three 24-hr periods. During 2006-2009, monitoring in the Atomizer and Chute Falls reaches entailed two trips per year (a marking trip and a recapture trip), but years 2010-2022 included only annual marking trips. Sampling effort (hours of hoop netting) was uniform across trips, both in the Atomizer reach and in the Chute Falls reach, respectively (Figure 3). More effort than usual was spent in the Chute Falls reach during 2016 because of a small flood (i.e., three high

turbidity sampling days were repeated with sampling during three ensuing low turbidity days). Between 2006-2022, average trip hoop netting effort in the Atomizer reach was 1,150 hours (SE = 35), and average hoop netting effort in the Chute Falls reach was 2,450 hours (SE = 406). During 2022, 51 net sets were deployed in the Atomizer reach yielding 1,213 hours of effort, and 99 net sets were deployed in the Chute Falls reach yielding 2,410 hours of effort.

On all LCR monitoring trips, nets were positioned in habitat suspected to catch Humpback Chub and were frequently repositioned or moved if the catch was poor and if an alternative site was available. Nets were spread throughout each subreach within constraints of river hydrology and depth. Most nets were set near shore, but some were set further midstream if access allowed. Each net set was checked and emptied of fish daily. Net locations on all sampling trips above were recorded as distance (rkm) upstream of the confluence.

Fish Handling and Data Collection

Data collected from fish included species, total and fork length (mm), sex, sexual condition (ripe, not ripe, gravid), and sexual characteristics (tuberculate, spawning colors). Speckled Dace and nonnative fish were generally measured only for total length. Humpback Chub and other fish were visually checked for the presence of the external copepod parasite (*Lernaea cyprinacea*), but the internal Asian fish tapeworm (*Bothriocephalus acheilognathi*) was not monitored. All fish lengths reported refer to total length.

Humpback Chub, Flannelmouth Sucker, and Bluehead Sucker ≥ 150 mm were scanned for a Passive Integrated Transponder (PIT) tag (Biomark Inc., Boise, ID), and if lacking a tag were PIT tagged. From 2000 through 2002, 400 kHz PIT tags were used. Thereafter, 134 kHz PIT tags were used and fish containing a 400 kHz tag were retagged with a 134 kHz tag. From 2000 to 2003 and from 2009 onward Humpback Chub ≥ 100 mm were PIT tagged in the lower 13.6 km of the LCR. From 2012 through 2022 some Humpback Chub as small as 65 mm TL were PIT tagged, but generally chub below 80 mm were not PIT tagged. From 2000 through spring 2003, most fish were weighed (g). Methods for collection of fish data followed the Standardized Methods for Grand Canyon Fisheries Research 2012 (Persons et al. 2013) with the following additions:

1. Humpback Chub ≥ 100 mm in the Chute Falls monitoring efforts were PIT tagged from 2006 to 2012, and ≥ 80 mm from 2012 onward.
2. From 2010 onward, Visible Implant Elastomer (VIE, Northwest Marine Technology, Shaw Island, WA) tags were used during the fall mark-recapture trips in the lower 13.57 rkm to mark Humpback Chub that were too small to PIT tag effectively. If they were deemed too small to PIT tag, during the September marking trips chub 40-99 mm received a specific colored VIE tag (usually red or orange depending on the year) VIE tag, and chub 40-99 mm during the October recapture trips received a different specific colored VIE tag (generally blue or green depending on the year). Tag placement was trip specific (e.g., below, anterior, or posterior to dorsal fins, and left or right side of

dorsal fins). Secondary VIE tags were inserted (generally on opercles or on top of head) for size strata marks (e.g., 40-59 mm, 60-80 mm).

Water Quality

Measured water quality parameters for the spring and fall trips included turbidity readings (nephelometric turbidity units, NTUs) collected near Salt Camp (rkm 10.4), or Coyote Camp (rkm 9.0). Generally, three or more turbidity daily readings were taken with a Hach 2100P or 2100Q Turbidimeter (Loveland, CO) and averaged. During Chute Falls trips, these measurements were also taken at Translocation Camp (rkm 16.2). Provisional data (mean daily discharges in cubic feet per second; cfs) were downloaded (http://waterdata.usgs.gov/az/nwis/uv?site_no=09402000) for USGS gage station 0940200 located on the LCR near Cameron, Arizona.

Closed Mark-Recapture

Closed Chapman Petersen mark-recapture efforts were conducted during the spring (2001-2019, and 2021-2022) and fall (2000-2022) to estimate abundance of Humpback Chub ≥ 150 mm in the lower 13.57 rkm of the LCR. Like Douglas and Marsh (1996), our approach was to obtain closed abundance estimates of Humpback Chub ≥ 150 mm via fishing the lower 13.57 rkm of the LCR with hoop nets. However, our efforts only provide abundance estimates during the spring and fall seasons of each year rather than monthly as in Douglas and Marsh (1996). This is because Douglas and Marsh (1996) conducted monthly sampling from 1991-1992 whereas this study only collected data four times per year. Nevertheless, within a given set of months, and within a given size class of fish (≥ 150 mm), our abundance estimates are comparable to Douglas and Marsh (1996).

Abundance estimates were obtained for all Humpback Chub ≥ 150 mm and ≥ 200 mm, and in some years was estimated for smaller size classes (e.g., Humpback Chub ≥ 100 -149 mm from 2010 onward, and ≥ 40 -99 mm during fall trips 2010 onward). We first examined our data to define the sampled population. Bernard and Hansen (1992) suggest setting the lower boundary of the sampled population equal to the length of the smallest fish recaptured. However, we allowed for growth and measurement error that could have occurred between the marking and recapture events (generally ~ 10 mm). We did not truncate the upper length end of our abundance estimates, because the types of hoop nets used in our study have been shown to capture large Humpback Chub (Gorman and Stone 1999, Stone and Gorman 2006).

The closed Chapman modified Petersen two-sample mark-recapture model (Seber 2002) was used to estimate abundance, where:

$$N^* = \frac{(M+1)(C+1)}{R+1} - 1 \quad (1)$$

$$V[N^*] = \frac{(M+1)(C+1)(M-R)(C-R)}{(R+1)^2(R+2)} \quad (2)$$

Where:

N^* = the estimated number of fish in the population,

$V[N^*]$ = the estimated variance of the number of fish in the population,

M = the number of fish marked during the marking event,

C = the number of fish captured during the recapture event,

R = the number of fish recaptured from the marked population during the recapture event.

To estimate the abundance of Humpback Chub within discrete size classes (e.g., ≥ 200 mm, 150-199 mm, 100-149 mm), the Chapman Petersen estimates of Humpback Chub ≥ 100 mm were multiplied by the proportion of Humpback Chub ≥ 200 mm (for example) with the formulae presented in Seber (2002) as:

$$N_x^* = \frac{M_x + C_x - R_x}{M + C - R} N^* = P_x(N^*) \quad (3)$$

$$V[N_x^*] = N_x^{*2} \left[\frac{1}{R} + \frac{2}{R^2} + \frac{6}{R^3} \right] + \frac{N_x^*(N^* - N_x^*)}{(M + C + 1)} \quad (4)$$

Where: P_x indicates the proportion of fish within a particular size class and the subscript x indicates fish that belong to a particular size class (e.g., ≥ 200 mm). The 95% confidence limits on our abundance estimates assume a normal distribution and are appropriate given the ratios of R/C and R/M observed in the experiments (Seber 2002).

Using the Chapman Peterson model requires only two trips (i.e., a marking trip and a recapture trip) to generate abundance estimates, and therefore requires less handling of fish. In addition, data gathered to produce the Chapman Petersen estimates can be incorporated into open and multi-state models (e.g., Coggins et al. 2006, Coggins and Walters 2009, Yackulic et al. 2014, Dzul et al. 2014, Van Haverbeke et al. 2015). Assumptions necessary for unbiased estimates of abundance using the Chapman Petersen estimator are:

1. The population is closed with no additions (i.e., recruitment, immigration) or losses (i.e., mortality, emigration) between marking and recapture events
2. All individuals in the target population have an equal probability of capture. Specifically:
 - a. Marked individuals mix completely with unmarked individuals prior to the recapture event.

- b. Marking does not affect capture probability (p) during the recapture event (i.e., animals are not 'trap-happy' or 'trap-shy').
3. Marks (tags) are not lost between the mark and recapture events.
4. All marked individuals captured can be recognized from unmarked individuals.

The first assumption, addressing population closure, could potentially be violated in this system because Humpback Chub in the LCR have access to the mainstem Colorado River. This assumption has a higher probability of being violated during the spring than during the fall mark-recapture events. Humpback Chub movement and migration is known to occur during the springtime (Kaeding and Zimmerman 1983, Douglas and Marsh 1996), but is thought to be much lower during the fall and winter months (Douglas and Marsh 1996). We minimized potential for violating this assumption by allowing less than a month to elapse between mark and recapture events. It was also assumed that growth-related recruitment was minimal because of the short time span between the mark and recapture events. Finally, all fish captured during the mark-recapture efforts were handled with care according to protocols (Persons et al. 2013) to minimize injury or stress to fish.

If the Humpback Chub population experiences only losses between mark and recapture events, the Chapman Petersen estimator will be unbiased and pertain to population abundance during the marking event. Conversely, if the Humpback Chub population experiences only additions, population estimates will be unbiased and pertain to abundance during the recapture event. However, if both additions and losses occur, there is no possible correction and the estimator will overestimate abundance (Otis et al. 1978, Seber 1982). For further explanation about population estimation, and measures taken to minimize assumption violation during these studies, see Van Haverbeke et al. (2013).

Population Estimation using Capture Probability Data and Monte Carlo Simulation

In addition, we used capture probability (p) data and Monte Carlo simulation in some instances to estimate abundances of Humpback Chub. From 2010 onward, p data and Monte Carlo simulation rather than the Chapman Petersen estimator was used to estimate abundance of adult Humpback Chub in the Atomizer and Chute Falls reaches. This was done because closed Chapman Petersen efforts were not performed in the Atomizer and Chute Falls reaches after 2010 (i.e., only one annual marking trip was conducted each year, but no recapture trip). Annual catch of adult Humpback Chub in each reach from 2010–2022 was divided by annual p data derived from Chapman Petersen mark-recapture efforts conducted from 2006–2009. Capture probabilities were calculated as:

$p1 = R/C$, where C = total number of unique adult chub captured during a recapture trip, and R = number of adult chub marked (tagged) during a mark trip and subsequently recaptured during the recapture trip; and $p2 = R/M$, where M = the number of unique adult chub marked. Adult Humpback Chub p values in the Chute Falls reach between 2006 and 2009 ranged from 0.35 - 0.91 (mean = 0.67, SE = 0.17, $n = 8$), while adult Humpback Chub p values in the Atomizer reach between 2006 and 2009 ranged from 0.54 - 0.74 (mean = 0.67, SE = 0.15, $n = 8$). To estimate abundance using the 2006-2009 p data, we first bootstrapped the number of expected recaptures given the individual trip p estimates and the number of chub marked during the marking trip. We then simulated $p1$ and $p2$ capture probabilities by dividing the bootstrapped number of recaptures by the number of marked fish, and by the number of fish captured during the second trip, respectively. We took the mean of the simulated trip p values and used this value as the trip capture probability. Then, using Monte Carlo analysis (10,000 replicates), trip catch was divided by the mean bootstrapped p value to obtain abundance estimates with 95% confidence intervals. Because hoop netting effort and turbidity conditions in the Atomizer and Chute Falls reaches during 2010-2022 were nearly identical to those of 2006–2009, we considered it reasonable to assume that detection values were similar between the two time periods and unnecessary to correct for turbidity (see below).

Monte Carlo simulation was also performed to fill in abundance estimates for the fall age 0 (<100 mm) and spring age 1 (100-149 mm) cohorts during years in which the Chapman Petersen estimator was not used (i.e., those years between 2000 and 2022 when those size classes were not PIT tagged), and as a comparative index to available Chapman Petersen estimates. We used the method described above, using size specific p values, and segregating the p values into low, intermediate, and high turbidity categories based on Stone (2010). This was performed because turbidity is a primary factor influencing catch of Humpback Chub (Stone 2010). Finally, we used Monte Carlo simulation to estimate abundances of Bluehead Sucker and Flannelmouth Sucker using available species and size specific (≥ 150 mm) p values segregated by turbidity.

Catch per Unit Effort

In addition to mark-recapture and simulation, catch per unit effort (CPUE) was used to monitor relative trends of smaller size classes of Humpback Chub <150 mm, and for Flannelmouth and Bluehead Suckers ≥ 150 mm. This was because Humpback Chub <150 mm were not PIT tagged during all years of this study, and because in several years numbers of recaptured suckers were insufficient for population estimation. In the LCR, catch rates of these fish in hoop nets can be significantly affected by high turbidities resulting from flood events (Stone 2010). We minimized for the effect of turbidity by calculating CPUEs from data gathered during May of each year, and from October during the fall, although some exceptions were made. For example, in 2015 and 2016 we used

CPUEs from September to estimate the relative abundance of the Humpback Chub age 0 cohort (<100 mm) because September had lower turbidities than October during those years. If necessary, we obtained CPUEs from select days within a trip when turbidity was low (e.g., fall 2016 CPUEs from 6 days in September trip when the LCR was experiencing low turbidities [mean = 36 NTUs], spring 2015 CPUEs from five days in May when daily NTUs were <54, spring 2016 CPUEs from 6 days in May when turbidity ranged 58-83 NTUs). Because net sets were set very close to 24 hours each, all CPUEs in this report are expressed as number of fish captured per net set (Stone 2010).

Results –rkm 0-13.57

Physical Parameters

Turbidity and Flow

In general, turbidities encountered during fall 2000-2022 mark-recapture events were much higher than those of spring 2001-2022 mark-recapture events (Figure 4). This is because monsoonal flooding events during September tend to cause far higher turbidities than spring runoff flows of April. More specifically, turbidity tended to be higher during spring mark trips because of snow run-off, and lower during the spring recapture trips when the LCR generally returned to base flows; whereas turbidity tended to be higher during the fall mark trips because of monsoonal flooding, and lower during the fall recapture trips when the LCR generally returned to base flows (Figure 5). Average trip turbidities encountered during the spring (2001-2021) ranged from 4 to 6,343 NTUs (grand mean = 461 NTUs, SE = 1,076, n = 40 trips). Average trip turbidities during the fall (2001-2021) ranged from 5 to 75,956 NTUs (grand mean = 11,228 NTUs, SE = 19,977, n = 43 trips). During the spring 2022 trips, average daily turbidity was 80 NTUs (SE = 29). During the fall 2022 trips, average daily turbidity was 34,838 NTUs (SE = 24,297).

Species Composition

Humpback Chub have generally dominated hoop net catches in the lower 13.57 rkm of the LCR since 2000 (Figure 6). Native fish have comprised 92.4% of all fish captured since 2000. Fathead Minnow (*Pimephales promelas*) were generally the dominant nonnative fish captured, comprising 6% of catch since 2000. Catches of Fathead Minnow were variable and thought to result either from immigration of fish caused by floods in the mid-upper portions of the LCR watershed (Stone et al. 2007, 2018), or from extended periods of base flows during

which local populations may have expanded. Other nonnative fish captured since 2000, listed in order of decreasing catch, included Common Carp (*Cyprinus carpio*), Black Bullhead (*Ameiurus melas*), Channel Catfish (*Ictalurus punctatus*), Red Shiner (*Cyprinella lutrensis*), Plains Killifish (*Fundulus zebrinus*), Rainbow Trout (*Oncorhynchus mykiss*), Green Sunfish (*Lepomis cyanellus*), and Brown Trout (*Salmo trutta*). A few fish thought to be Razorback Sucker (*Xyrauchen texanus*) and Flannelmouth Sucker hybrids have been captured since 2000, and these fish are treated as Flannelmouth Sucker for this report. Such hybrids were infrequently captured during monitoring efforts in the early 1990s (Douglas and Marsh 1998). Presumably under-represented in hoop net catches were adult Channel Catfish and adult Common Carp, which seldom enter our nets but are seen by field crews and captured by angling, and Plains Killifish which tend to inhabit shallower waters than our hoop nets fish. In 2022, Humpback Chub dominated trip catches (57% of captures), followed by Bluehead Sucker and Speckled Dace (each 15%), Flannelmouth Sucker (6%), Fathead Minnow (6%), and Carp, Red Shiner, Rainbow Trout, and Black Bullhead (<1%). Native fish comprised 93% of fish captures in 2022.

Overall, both the spring and fall CPUEs of nonnative fishes appear to be declining since 2000 for reasons unknown (Figures 7-A and 7-B). Two obvious exceptions were spring of 2006 and fall 2009, both spikes caused by high captures of Fathead Minnows that comprised 98% and 94% of the nonnative catch in those instances, respectively. These downward trends hold even if deleting 2006 spring data (with exceptionally high captures of Fathead Minnow), and 2020 spring data (trip cancelled in 2020 because of COVID-19) and deleting 2009 fall data (again with exceptionally high captures of Fathead Minnow).

Length Frequency Distributions

Humpback Chub

Humpback Chub in the lower 13.57 rkm of the LCR show a widely distributed size structure, with all size classes represented and recruitment of young fish apparent (Figure 8-A). Cumulatively from 2001-2021, the spring season shows sizeable presence of age 1 fish in the roughly 80-130 mm size class. This peak was also seen in spring 2022, but the mode was slightly larger than in previous years.

During fall 2022 in the lower 13.57 rkm of the LCR, there is also indication of a healthy population structure for Humpback Chub, with multiple size class representation (Figure 8-B). However, there was a relative lack of fish in size classes 100-200 mm, suggesting a lack of recent recruitment. For example, cumulatively since 2000, there

has been sizeable production of age 0 Humpback Chub, but comparatively less so in fall 2022. These fish hatch during the spring seasons, but most are still <100 mm by fall (Figure 8-B).

Noticeably fewer fish are captured in the 200-300 mm size class during the fall (12,545 between 2000-2022) compared to spring (22,834 between 2001-2022), because many of these fish presumably vacate the LCR after the spring spawning season. Finally, captures of large adult Humpback Chub >300 mm were fewer during the fall season (1,304 captured since 2000), compared to the spring (5,875 captured since 2001).

Bluehead Sucker

Bluehead Sucker also show signs of a healthy population structure in the lower 13.57 rkm of the LCR, with adult fish and recruiting young fish present (Figure 9-A). Cumulatively from 2001-2021, the spring season displays a sizeable group of presumed age 0 fish (mode at 65-70 mm), and another group of sub-adult/adult fish (mode ~240 mm). This pattern was distinctly different in spring 2022, with three rather than two modes, and with the sub-adult size classes being more abundant in 2022 (Figure 9-A). Fall 2022 showed only 1 age 0 Bluehead Sucker, again noticeably different from previous years (Figure 9-B).

Flannelmouth Sucker

Cumulatively, Flannelmouth Sucker show multiple size classes during the spring (2001-2021, Figure 10-A), with representatives from the age 0 (<80 mm) and age 1 cohorts (~80-150 mm) being most distinguishable (i.e., older cohorts tend to blur). Both spring and fall of 2022 showed a comparative lack of Flannelmouth Sucker in size classes <300 mm (Figures 10 A&B), possibly indicating that the lack of flooding in the LCR during 2021 was not conducive for their production.

Parasites

Percent occurrence of the external parasite (*Lernaea cyprinacea*) on Humpback Chub during spring trips was generally lower compared to fall trips, with infestation rate representing the percent of chub captured observed carrying the parasite (Figure 11). Infestation rates during spring trips averaged 1.3% (range 0-8%); while those on fall trips averaged 6.9% (range 0.07% to 35%). The highest infestation rate was during fall 2009 at 35%. Typically, only one or two parasites are seen on individual Humpback Chub in the LCR, although numbers are sometimes higher. For spring 2022 trips, the infestation rate on Humpback Chub was 0.9%, and for fall 2022 trips, the infestation rate was 0.24%. Very infrequently, this parasite is seen on other species of native and nonnative fish

(e.g., Flannelmouth, Bluehead, Dace, Fathead Minnows), again typically at low frequencies with only one or two parasites per fish.

Abundance Estimation (rkm 0-13.57)

Adult and sub-adult Humpback Chub

Following the decline in sub-adult (150-199) and adult (≥ 200 mm) Humpback Chub abundance documented in the early-mid 90s (Coggins et al. 2006), mark-recapture abundance estimates in the LCR remained at reduced but stable levels during the early-mid 2000s (Figure 12). After 2006, abundance of Humpback Chub ≥ 150 mm and ≥ 200 mm in the LCR during the spring and fall seasons significantly increased and remained at elevated levels until 2014. In 2015 and 2016, the spring abundance of Humpback Chub ≥ 150 mm and ≥ 200 mm significantly declined, thought to be caused by a large portion of the population remaining in the mainstem Colorado River during spawning season. Post-2016, numbers rebounded again, and in 2017-2019 equaled or surpassed the abundance estimates during the 2007-2014 period (Figure 12-A). There was no estimate for spring 2020 because of no sampling. In spring 2021, adult chub abundance was again significantly depressed, thought to be a result of drought in the LCR, poor spawning substrate, and many adults remaining in the mainstem. In spring 2022 adult abundances rebounded again to the 2019 level. For 2022, the Chapman Petersen abundance estimate of Humpback Chub ≥ 150 mm was 10,563 (SE = 727), of which an estimated 8,525 (SE = 665) were ≥ 200 mm. For fall 2022, the abundance estimate of Humpback Chub ≥ 150 mm was 2,320 (SE = 367), of which an estimated 1,888 (SE = 321) were ≥ 200 mm (Figure 12-B).

For subadults only (150-199 mm), ten spring abundance estimates during the post-2006 increase period have been $>1,250$ fish, whereas six have been below 1,250 (Figure 13). Recall that the number 1,250 functions as a desired three-year rolling average for subadult Humpback Chub abundance during spring in the LCR. For spring 2022, the abundance estimate for Humpback Chub in the 150-199 mm size class was 2,056 (SE = 149).

Juvenile (age 1 and age 0) Humpback Chub

Mark-recapture estimates of Humpback Chub in the age 1 size class (91-161 mm) during the spring season were conducted in 2001, 2002, 2009-2019, 2021, and 2022 and ranged between 818 and 11,727 fish (mean = 3,675, SE = 2,959; Figure 14-A). For spring 2022, the abundance estimate of Humpback Chub in the age 1 size class was 1,766 (SE = 357). CPUEs of age 1 Humpback Chub during the spring recapture trips between years 2001-2021 ranged between 0.14 and 3.52 fish per 24-hour net set (mean = 1.4; Figure 14-A). For spring

2022, CPUE for age 1 Humpback Chub was 0.43 fish per net set during May. Several years (2001, 2003, 2005, 2007, 2010, and 2019) can be characterized as years with low relative age 1 abundance. Six of these years (e.g., 2001, 2003, 2007, 2010, 2019, 2022) correspond to low age 0 CPUEs of the previous fall (Figure 14-B). The age 1 mark-recapture estimates show good correlation with the age 1 CPUE calculations ($r^2 = 0.84$, $n = 15$ years).

Annual Chapman Petersen efforts using VIE and PIT tagging to estimate abundance of Humpback Chub in the age 0 size class (40-105 mm) in the LCR began in fall 2010 and continued through fall 2022 (Figures 14-B and Figure 15). Between fall 2010 and fall 2022, the average of Chapman Petersen abundance estimates of the fall age 0 cohorts is 5,609 (SE = 4,285). A high of 13,896 was seen in 2020, and a low of only 637 was obtained via Monte Carlo simulation in 2018 because a Chapman Petersen estimate failed. For fall 2022, a Monte Carlo estimate of 2,757 (SE = 281) age 0 chub was obtained because a Chapman Petersen estimate failed (only 3 recaptures). CPUE in years 2000-2022 has ranged between 0.05 and 3.27 (mean = 1.4) fish per 24-hour net set (Figure 14-B). For October 2022, CPUE for age 0 Humpback Chub was 0.1 fish per net set.

Bluehead and Flannemouth Sucker

Bluehead Sucker and Flannemouth Sucker (≥ 150 mm) abundance and trends in the LCR were estimated using Chapman Petersen mark-recapture, Monte Carlo simulation, and CPUE data from spring recapture trips when turbidities were low (Figure 16 A&B). Like Humpback Chub, Bluehead and Flannemouth Sucker underwent a post-2006 period of significant increase, although this increase was of a lesser magnitude for Flannemouth Sucker. Bluehead Sucker abundance and CPUE reached a peak in 2008, and since then have declined (16-A). Flannemouth Sucker appeared to reach peaks in 2010 and again in 2018 (Figure 16-B). For May 2022, Bluehead Sucker CPUE was 1.74 (SE = 0.36) fish per net set and Flannemouth Sucker CPUE was 0.48 (SE = 0.05). There were sufficient recaptures of Bluehead and Flannemouth suckers in spring 2022 for Chapman-Petersen estimates (19 for Bluehead Sucker and 13 for Flannemouth Sucker). Hence, we obtained Chapman Petersen estimates of 24,452 (SE = 5,178) Bluehead Sucker ≥ 150 mm, and 3,894 (SE = 945) Flannemouth Sucker ≥ 150 mm (Figures 16-A and 16-B) in spring 2022.

Results Chute Falls (rkm 13.57 to 17.7)

Physical Parameters

Turbidity and Temperature

Excluding two sampling days during 2015 and three sampling days during 2016 when some flooding occurred, average trip turbidities during the 2006-2021 Chute Falls monitoring trips were consistently low, ranging from 0.9-6.9 NTUs (mean = 4.2 NTUs, N = 20 trips), and were accompanied by base flows. For the May 2022 trip, average daily turbidity in the Chute Falls reach was 1.3 NTUs (SE = 0.2). Average daily afternoon water temperature was 22 °C (SE = 0.7).

Length Frequency Distributions and Catch

Length frequencies demonstrate that Humpback Chub translocated to upstream of Chute Falls can remain upstream of Chute Falls and grow to adulthood. Also seen are a smaller group of Humpback Chub with a mode of ~135 mm. These are chub that were captured but had no PIT tag and were presumably spawned above Chute Falls (Figure 17, upper chart).

Immediately downstream of Chute Falls in the Atomizer reach shows Humpback Chub composed mostly of adults ≥ 200 mm, and a smaller group with a mode of ~125 mm. Again, these represent Humpback Chub that had no PIT tag upon capture and were likely born in the Atomizer or possibly the Chute Falls reaches (Figure 17, lower chart).

Sexual Condition and Parasites

During May 2022, we captured 12 ripe female Humpback Chub (172-337 mm) above Chute Falls (between rkm 15.66 and 16.96 km). Another 223 ripe male chub (168-354 mm) were captured above Chute Falls (between rkm 14.68 and 17). In the Atomizer reach, we captured 94 ripe males (174-451 mm), but no ripe females.

Like spring trips in the lower 13.57 rkm, infestation rates of *Lernaea cyprinacea* on Humpback Chub in the Atomizer and Chute Falls reaches from 2006-2022 tended to be low, ranging from 0% to 6% (mean = 0.7%). The highest infestation rate was 6% in 2009. During May 2022, infestation rate was 0%, with no infested fish detected.

Population Abundance Estimation

Translocations of Humpback Chub to above Chute Falls began in 2003 (Figure 18-A). From 2003-2005, 1,149 juvenile chub were collected from the lower reaches of the LCR (~ rkm 1.15-3.16), and transported upriver to rkm 16.2, where they began to show up as adult fish within a year or two. Following no translocations in 2006 and 2007, adult chub abundance in the Chute Falls reach then experienced a period of decline (2007-2008; Figure 18-A). Translocations resumed in 2008, followed by higher abundances of adult chub by 2009. In March 2010, a prolonged steady spring flood accompanied by heavy sediment deposition and elimination of deep pools is thought to have resulted in a near absence of adult chub in the Chute Falls and Atomizer reaches during June 2010 (Figures 18-A and 18-B). Continued annual translocations from 2010-2021, and likely the returning of deep pool riverine habitat, have again resulted in increases in the abundance of adult chub upstream of Chute Falls. In May 2021, another 565 juvenile chub were translocated above Chute Falls, and on October 28, 2022 we translocated 196 juvenile Humpback Chub (TL range 60-110 mm) via helicopter from Coyote camp to Chute Falls Camp (Pillow 2023).

In May 2022, it was estimated that there were 623 (SE = 79) Humpback Chub ≥ 100 mm in the Chute Falls reach, of which 379 (SE = 48) were ≥ 200 mm. For May 2022 in the Atomizer reach, it was estimated that there were 572 Humpback Chub ≥ 100 mm (SE = 25), of which 307 (SE = 15) were ≥ 200 mm. For the Atomizer and Chute Falls reaches combined, there was high correlation between the annual Chapman Petersen abundance estimates and the annual Monte Carlo simulation abundance estimates (2006-2009), both for Humpback Chub ≥ 100 mm ($r^2 = 0.90$, $n = 8$) and for Humpback Chub ≥ 200 mm ($r^2 = 0.93$, $n = 8$).

Discussion

Lower 13.57 rkm of the LCR

Population estimates of Humpback Chub indicate that sometime between the early 1990s and 2000 the abundance of adult Humpback Chub (≥ 200 mm) underwent a significant decline in the LCR (Coggins et al. 2006). This was followed by a period of relatively low but stable abundance between 2000 and 2006, and by a 2007-2014 period when abundance levels significantly increased (Van Haverbeke et al. 2013). The post-2006 increases of Humpback Chub ≥ 150 mm and ≥ 200 mm are visible during both spring and fall seasons but are more apparent during spring (Figures 12-A and 12-B). The decline in the 2011 spring abundance of Humpback Chub ≥ 150 mm (Figure 12-A) may be a result of relative low production of age 0 fish during 2009 as suggested by Figure 14-B.

Spring 2015 and 2016 saw another apparent decline of abundance of Humpback Chub ≥ 150 mm and ≥ 200 mm (Figures 12-A and 12-B). This decline was particularly visible during the spring seasons. The cause of this decline remains uncertain, but high CPUEs in the mainstem Colorado River near the LCR in fall 2015 and 2016 (M. Dodrill, pers. com.) suggested that a high proportion of chub may have emigrated into the mainstem and may not have returned to the LCR during the spring spawn in those years. This may have been caused by poor condition factor, possibly caused by depressed food resources (C. Yackulic, pers. com.). Alternatively, the lowered abundance in 2015 and 2016 could have been caused by relatively low age 0 production in 2013 and 2014 (Figure 14-B). Regardless, the spring seasons of 2017-2019 witnessed another increase in the abundance of Humpback Chub ≥ 150 mm and ≥ 200 mm, approaching or exceeding levels seen during the 2007-2014 period. No sampling was conducted in spring 2020 because of COVID-19. In spring 2021 there was a significant decline in the abundance of both adult and sub-adult chub (Figures 12-A and 13), thought to be related to an extended lack of flooding in the LCR from May 2020 to July 2021 (Figures 5 and 19), and possibly many chub remaining in the mainstem. Finally, in spring 2022, abundances of subadult and adult Humpback Chub rebounded again to 2019 levels (Figure 12-A).

There is considerable annual variation in the spring abundance estimates of sub-adult Humpback Chub (150-199 mm; Figure 13). Since 2016 there have been two years (2020 and 2021) in which the spring abundance estimate for this size class has dropped below 1,250 fish (Figure 13). This is important because the Biological Assessment (USBR 2016) and the Biological Opinion (USFWS 2016) for the Glen Canyon Dam Long-Term Experimental and Management Plan set forth triggers under which additional conservation measures would occur to minimize the likelihood for mechanical removal of predators, such as rainbow trout. One of the trigger criteria is maintaining a running three-year average of 1,250 sub-adults in the LCR during the spring spawning season (USBR 2016). The mean spring estimate for Humpback Chub in the sub-adult category (150-199 mm) during spring in the LCR for the past 3 years (2020-2022) was 1,248 fish. As a result, we plan on conducting an additional translocation of juvenile chub to above Chute Falls in May 2023 as a conservation action.

Concerning juvenile Humpback Chub < 150 mm, both the mark-recapture estimates and CPUE indices suggest high annual variability in abundance of the fall age 0 and the spring age 1 size class of Humpback Chub (Figures 14-B and 14-A, respectively). Despite this variability, during most years the abundance of age 1 chub in the spring appears to closely track the abundance of age 0 chub during the previous fall, suggesting good overwinter survival during most years, and that much of the age 0 outmigration/displacement to the mainstem may occur during the summer monsoon period prior to our fall sampling efforts (as opposed to overwinter outmigration). This relationship did not hold true for the large 2020 cohort of age 0 chub, as can be seen by an anomalous low

abundance of age 1 chub in spring 2021 (Figure 15). This breakdown may have been caused by a drought in the LCR, whereby there was essentially a complete lack of above base flows in the LCR between the beginning of May 2020 through mid-July 2021. The drought lasted approximately 445 days and is the longest drought on record since hydrograph measurements began at the Cameron gage station in 1947 (Figure 19). Further, it resulted in an extreme buildup of travertine and marl deposition in the LCR and filled in near shore habitat that juvenile chub utilize, and possibly reducing adult spawning habitat.

Annual variation in the production of the fall age 0 cohort is thought to be related to springtime hydrology of the LCR and cleansing of spawning substrate materials (Van Haverbeke et al. 2013). However, this relationship is not clear, and other factors are surely in play. Nevertheless, it appears that age 0 production has been consistently very low when there is essentially no spring runoff, but higher and more variable when there is some degree of spring runoff ($r^2 = 0.58$, $n = 23$ years, Figure 20). For example, during February and March in 2019 and 2020 the LCR experienced flooding above 1,500 cfs peak mean daily flows (not particularly high flooding), and there was high production of age 0 Humpback Chub. In contrast, there was no LCR spring runoff in 2018 and 2021, accompanied by very low age 0 chub production (Figure 14-B). Spring of 2022 was also relatively dry with a peak mean daily flow of 421 cfs occurring on April 4, 2022. This, combined with two events of sustained mean daily flow flooding >2100 cfs in July and August (that may have transported age 0 fish into the mainstem) appears to have resulted in relatively poor abundance of age 0 chub during Sept/Oct 2022 (Figure 14-B).

One reason for estimating the abundances of the juvenile cohorts is to gain insight into what percentages of these fish are being harvested (cropped) each year for various translocation projects, and for maintaining a refuge at SNARRC. However, translocations above Chute Falls have been shown to provide a positive population benefit to the LCR aggregation (Yackulic et al. 2021). Beginning in 2014, Humpback Chub cropped for translocation activities outside of the LCR have been harvested as larval fish (<30 mm), thus alleviating concerns about potential overharvesting of age 0 chub (Pine et al. 2013).

Both abundance and relative abundance (CPUE) of Bluehead Sucker and Flannelmouth Sucker ≥ 150 mm significantly increased in the post-2006 timeframe during the spring spawning season (Figures 16 A&B), with Bluehead Sucker reaching peak abundance in 2008 and Flannelmouth Sucker reaching peak abundances in 2010 and 2018. These data suggest that Bluehead Sucker can become very abundant in the LCR during the spring spawning season, reaching peak abundances into the tens of thousands, whereas Flannelmouth Sucker are less numerous, reaching peak abundances of only several thousand individuals. In May 2010 we saw an outbreak of fungus on many Bluehead Sucker, with many dead suckers seen in nets and along the shorelines (Van Haverbeke, pers. obs.). Bluehead Sucker catches have been about 12-fold lower during the fall season, suggesting mass

annual emigration for this species from the LCR to the mainstem. Finally, because few age 0 suckers are captured in the fall season, this may indicate that both sucker species tend to drift as larvae and emigrate from the LCR before the onset of the fall season (see Robinson et al. 1998).

Chute Falls (rkm 13.57-17.7)

Since 2003, ~4,874 juvenile Humpback Chub have been translocated from the lower reaches of the LCR to upstream of Chute Falls and have been found to grow to adulthood, although not all translocated chub have remained upstream of Chute Falls. Most have either eventually moved into the short Atomizer reach immediately below Chute Falls or moved further downriver (below Lower Atomizer Falls). Some have been recaptured in the mainstem Colorado River. Humpback Chub translocated to above Chute Falls generally experience increased growth and survival rates (Stone et al. 2020, Yackulic et al. 2021), providing a net benefit during most years to the LCR Humpback Chub population, a monetary savings to the program, and reducing the need for mainstem mechanical removal efforts (Yackulic et al. 2021).

Exceptions to the above pattern can occur if high mortality or early emigration of translocated Humpback Chub out of the Atomizer/Chute Falls reaches occurs. For example, between summer of 2009 and summer 2010, nearly all Humpback Chub vacated the Chute Falls and Atomizer reaches (Figure 18), as well as the upper portion of Salt reach (12.3 to 13.57 km). It is thought that this was a result of a prolonged spring flood during early 2010 that deposited a heavy sand load in the upper reaches of the LCR, filling in much of the available deep pool habitat. This phenomenon may explain why there were no Humpback Chub upstream of Chute Falls prior to the translocation efforts. High environmental stochasticity causing emigration in this section of river may override lower natural colonization rates. With the re-commencement of translocation activities in 2010, Humpback Chub have again been found to remain and grow to adulthood in the upper and lower reaches of Chute Falls. The capture of ripe adult Humpback Chub and occasionally unmarked juvenile chub above Chute Falls suggests spawning occurs above Chute Falls.

Future Population Estimation Considerations

Population estimation procedures were described in the Methods section above, and more fully in Van Haverbeke et al. (2013). Among the procedures described is stratification of the marked, captured, and recaptured population of fish by geographic reaches (i.e., Salt, Coyote, and Boulders), and/or by length (TL). Typically, a series of investigative tests were performed to search for significant differences between the captured and recaptured populations (mark rate, p_1) and the marked and recaptured populations (recap rate, p_2). For geographic

stratification, this entails chi-square tests that search for significant differences in the mark or recapture rates among the reaches. For length stratification, we used a computer program that conducted sequential contingency table calculations, each with a different length stratum bound, to find the bound that maximized the test statistic value (i.e., χ^2). The goal of this procedure was to minimize abundance estimate bias by defining length strata with homogenous capture probability (Seber 1982). We pooled data (reaches and/or length strata) if needed to ensure seven or more recaptures were maintained within any given geographic or length-based stratum (Seber 1982). The purpose of all the exercises was to minimize the potential for bias with differing capture probabilities among reaches or among 50 mm size classes.

Carrying out the above methodologies, we have conducted 43 mark recapture efforts in the LCR between fall 2000 and spring 2022. We have compared our stratified estimates with non-stratified estimates with regression analyses (Figure 21-A&B) and found high correlations between stratified and non-stratified estimates for Humpback Chub ≥ 150 mm ($r^2 = 0.98$, $n = 43$), and ≥ 200 mm ($r^2 = 0.97$, $n = 43$). Z-tests indicate no significant difference between stratified and non-stratified estimates for chub ≥ 150 mm ($Z = 0.38$, $n = 43$), and for chub ≥ 200 mm ($Z = 0.29$, $n = 43$).

Acknowledgements

We thank the Navajo Nation for permitting this work. We especially thank Ann-Marie Bringhurst, Seth Felder, and Lucien Bucci (GCMRC) for providing logistical support, and our helicopter pilots. Thanks to David Ward (GCMRC) for a review and editorial suggestions. We also especially thank the many volunteers who have assisted on trips, and the Navajo Nation Fish and Wildlife Department for their support. Funding was provided by the U.S. Geological Survey, Grand Canyon Monitoring and Research Center.

References Cited

- Andersen, M.A., M.W. Ackerman, K.D. Hilwig, A.E. Fuller, and P.D. Alley. 2010. Evidence of young humpback chub overwintering in the mainstem Colorado River, Marble Canyon, Arizona, USA. *The Open Fish Science Journal*, 2010: 3, 42-50.
- Bernard, D.R. and P.A. Hansen. 1992. Mark-recapture experiments to estimate the abundance of fish. Alaska Department of Fish and Game, Special Publication No. 92-4. Anchorage, Alaska.
- Clarkson, R.W. and M.R. Childs. 2000. Temperature effects of hypolimnial-release dams on early life stages of Colorado River basin big-river fishes. *Copeia* 2000:402-412.

- Coggins, L.G. Jr. and W.E. Pine III. 2010. Development of a temperature-dependent growth model for the endangered humpback chub using capture–recapture data. *The Open Fish Science Journal* 3:122-131.
- Coggins, L.G. Jr. and C.J. Walters. 2009. Abundance trends and status of the Little Colorado River population of humpback chub; an update considering data from 1989-2008: U.S. Geological Survey Open-File Report 2009-1075, 18 p.
- Coggins, L.G., Jr., W.E. Pine, III, C.J. Walters, D.R. Van Haverbeke, D. Ward, and H. Johnstone. 2006. Abundance Trends and Status of the Little Colorado River Population of Humpback Chub. *North American Journal of Fisheries Management* 26:233-245.
- Cooley, M. E. 1976. Spring flow from pre-Pennsylvanian rocks in the southwestern part of the Navajo Indian Reservation, Arizona. U.S. Geological Survey Professional Paper 521-F.
- Douglas, M.E. and P.C. Marsh. 1998. Population and survival estimates of *Catostomus latipinnis* in northern Grand Canyon, with distribution and abundance of hybrids with *Xyrauchen texanus*. *Copeia* 4:915-925.
- Douglas, M.E. and P.C. Marsh. 1996. Population estimates/population movements of *Gila cypha*, an endangered Cyprinid fish in the Grand Canyon region of Arizona. *Copeia* 1996(1): 15-28.
- Dzul, M.C., C.B. Yackulic, D.M. Stone, and D.R. Van Haverbeke. 2014. Survival, growth, and movement of subadult humpback chub, *Gila cypha*, in the Little Colorado River, Arizona. *River Research and Applications*. Available: <http://onlinelibrary.wiley.com/doi/10.1002/rra.2864/pdf>
- Gorman, O.T. and D.M. Stone. 1999. Ecology of spawning humpback chub (*Gila cypha*), in the Little Colorado River near Grand Canyon, Arizona. *Environmental Biology of Fishes* 55: 115-133.
- Hamman, R.L. 1982. Spawning and culture of humpback chub. *Progressive Fish Culturist* 44:213-216.
- Kaeding, L.R. and M.A. Zimmerman. 1983. Life history and ecology of the humpback chub in the Little Colorado and Colorado Rivers of the Grand Canyon. *Transactions of the American Fisheries Society* 112:577-594.
- Minckley, C.O. 1996. Observations on the biology of the humpback chub in the Colorado River basin 1908-1990. Doctoral dissertation. Flagstaff: Northern Arizona University. Available: <http://www.nativefishlab.net/library/textpdf/21260.pdf> (February 2013).
- NPS (National Park Service). 2013. Comprehensive Fisheries Management Plan: Environmental Assessment. Grand Canyon National Park and Glen Canyon National Recreation Area. 151 pp. Available: <http://parkplanning.nps.gov/documentsList.cfm?parkID=65&projectID=35150>
- Otis, D.L, K.P. Burnham, G.C. White, and D.R. Anderson. 1978. Statistical Inference from Capture Data on Closed Animal Populations. *Wildlife Monographs* 62:3-135.
- Paukert, C.R., L.G. Jr. Coggins, and C.E. Flaccus. 2006. Distribution and movement of humpback chub in the Colorado River, Grand Canyon, based on recaptures. *Transactions of the American Fisheries Society* 135:539-544.

- Persons, W.R., D.L. Ward, L.A. Avery. 2013. Standardized methods for Grand Canyon fisheries research 2012. U.S. Geological Survey, Grand Canyon Monitoring and Research Center, Flagstaff, Arizona. 19 pp. Available: <http://pubs.usgs.gov/tm/tm2a12/tm2a12.pdf>
- Pillow, M.J. 2022. Spring 2022 monitoring of Humpback Chub (*Gila cypha*) and other fishes in the lower 13.57 km of the Little Colorado River, Arizona: Trip Report 19-29 April and 17-27 May 2022. USFWS Document No. USFWS-AZFWCO-22-05. 15 pp.
- Pillow, M.J. 2023. Fall 2022 Monitoring and Translocation of Humpback Chub (*Gila cypha*) and other Fishes in the Lower 13.57 km of the Little Colorado River, Arizona. Trip Report 13-23 September and 18-28 October 2022. U.S. Fish and Wildlife Service, Flagstaff, AZ. Interagency Acquisition No. G17PG00059. USFWS Doc. # USFWS-AZFWCO-23-01. 13 pp.
- Pine, W.E. III, B. Healy, E.O. Smith, M. Trammel, D. Speas, R. Valdez, M. Yard, C. Walters, R. Ahrens, R. Van Haverbeke, D. Stone, W. Wilson. 2013. An individual based model for population viability analysis of humpback chub in Grand Canyon. *North American Journal of Fisheries Management* 33:3, 626-641.
- Robinson A.T. and M.R. Childs. 2001. Juvenile growth of native fishes in the Little Colorado River and a thermally modified portion of the Colorado River. *North American Journal of Fisheries Management* 21:809-815.
- Robinson, T.A., R.W. Clarkson, and R.E. Forrest. 1998. Dispersal of larval fishes in a regulated river tributary. *Transactions of the American Fisheries Society* 127:772-786.
- Rogowski, D.L., R.J. Osterhoudt, H.E. Mohn, and J.K. Boyer. 2018. Humpback chub (*Gila cypha*) range expansion in the western Grand Canyon. *Western N. American Naturalist* 78(1):26-38.
- Seber, G.A.F. 2002. *The Estimation of Animal Abundance*, 2nd edition. Blackburn Press, New Jersey. 654 pp.
- Stone, D.M. 2010. Overriding effects of species-specific turbidity thresholds on hoop-net catch rates of native fishes in the Little Colorado River, Arizona. *Trans. Am. Fish. Soc.* 139:1150-1170.
- Stone, D. M. 2005. Effect of baiting on hoop net catch rates of endangered humpback chub. *North American Journal of Fisheries Management* 25:640-645.
- Stone, D.M. and O.T. Gorman. 2006. Ontogenesis of endangered humpback chub (*Gila cypha*) in the Little Colorado River, Arizona. *American Midland Naturalist* 155:123-135.
- Stone, D.M, D.R. Van Haverbeke, D.L. Ward, and T.A. Hunt 2007. Dispersal of nonnative fishes and parasites in the intermittent Little Colorado River. *The Southwestern Naturalist* 52(1):132-138.
- Stone, D.M., K.L. Young, W.P. Mattes, and M.A. Cantrell. 2018. Abiotic controls of nonnative fishes in the Little Colorado River, Arizona. *Am. Midl. Nat.* 180:119-142.
- Stone, D.M. M.J. Pillow, K.L. Young, D.R. Van Haverbeke, and J.D. Walters. 2020. Effects of Disparate Water Temperatures and food bases on Humpback Chub growth rates within the Little Colorado River, Arizona. *N. Amer. J. Fish. Management* 40: 475-487.

- Suttkus, R.D. and G.H. Clemmer. 1977. The humpback chub, *Gila cypha*, in the Grand Canyon area of the Colorado River. Occasional Papers of the Tulane University Museum of Natural History 1:1-30.
- Trammel, M., B. Healy, E. Omana, and P. Sponholtz. 2012. Humpback chub translocation to Havasu Creek: Implementation and Monitoring Plan. Natural Resource Report NPS/GCRA/NRR-2012/586. National Park Service, Fort Collins, Colorado. Available: <https://irma.nps.gov/App/Reference/Profile?Code=2190488>
- USBR [U.S. Bureau of Reclamation]. 1995. Operation of Glen Canyon Dam: Final Environmental Impact Statement. U.S. Bureau of Reclamation. 337 pp. plus attachments.
- USBR. 2016. Biological assessment for the Glen Canyon long-term experimental and management plan (LTEMP). U.S. Bureau of Reclamation. Sept 27, 2016. 184 pp.
- USFWS (U.S. Fish and Wildlife Service). 2016. Biological Opinion for the Glen Canyon Dam Long-Term Experimental and Management Plan. Phoenix, Arizona. USFWS. AESO/SE 02EAAZ00-2012-F-0059 and AESO/SE 02EAAZ00-2014-CPA-0029. Available: https://www.fws.gov/southwest/es/arizona/Documents/Biol_Opin/120059_LTEMP%20BiOp_11-25-16.pdf (January 2016).
- USFWS. 2002. Humpback Chub (*Gila cypha*) Recovery Goals: amendment and supplement to the Humpback Chub Recovery Plan. Denver, CO: U.S. Fish and Wildlife Service, Mountain-Prairie Region (6), Denver, Colorado.
- USFWS. 1994. Final Biological Opinion: Operation of Glen Canyon Dam as the modified low fluctuating flow alternative of the final environmental impact statement. Ecological Services Arizona State Office, Phoenix. 56 pp.
- Valdez R.A. and W.J. Masslich. 1999. Evidence of reproduction by humpback chub in a warm spring of the Colorado River in Grand Canyon. The Southwestern Naturalist 44:384-387.
- Valdez R.A. and R.J. Ryel. 1995. Life history and ecology of the humpback chub (*Gila cypha*) in the Colorado River, Grand Canyon, Arizona. Final Report to Bureau of Reclamation, Salt Lake City, Utah. Contract No. 0-CS-40-09110. Salt Lake City, Utah. Available: http://www.gcmrc.gov/library/reports/biological/Fish_studies/Biowest/Valdez1995f.pdf
- Van Haverbeke, D.R. 2010. The humpback chub of Grand Canyon. pp. 261-268 in T.S. Melis et al. (eds.). Proceedings of the Colorado River Basin Science and Resource Management Symposium, November 18-20, 2008, Scottsdale, AZ. U.S. Geological Survey Investigations Report 2010-5135, 372 p.
- Van Haverbeke, D.R., L.G. Coggins Jr., D.M. Stone and M.J. Pillow. 2013. Long term monitoring of an endangered desert fish and factors influencing population dynamics. Journal of Fish and Wildlife Management 4(1):163-177. Available: <http://www.fwspubs.org/doi/pdf/10.3996/082012-JFWM-071>
- Van Haverbeke, D.R., K. Young, D.M. Stone, and M.J. Pillow. 2015. Mark-Recapture and Fish Monitoring Activities in the Little Colorado River in Grand Canyon from 2000 to 2014. Submitted to USGS Grand Canyon

- Monitoring and Research Center, Flagstaff, Arizona. U.S. Fish and Wildlife Service, Flagstaff, Arizona. 55 pp.
- Van Haverbeke, D.M. Stone, D.R., M.J. Dodrill, K.L. Young, and M.J. Pillow. 2017. Population expansion of humpback chub in western Grand Canyon and hypothesized mechanisms. *Southwestern Naturalist* 62(4):285-292.
- Van Haverbeke, D.R., K.L. Young, M.J. Pillow, and Pilar Wolters. 2021. Mark-Recapture and Fish Monitoring Activities in the Little Colorado River in Grand Canyon from 2000 to 2020. Submitted to USGS Grand Canyon Monitoring and Research Center, Flagstaff, Arizona. U.S. Fish and Wildlife Service, Flagstaff, Arizona. 48 pp.
- Wright, S.A., C.R. Anderson, N. Voichick. 2009. A simplified water temperature model for the Colorado River below Glen Canyon Dam. *River Research and Applications* 25:675-686.
- Yackulic, C.B., M.D. Yard, J. Korman, and D.R. Van Haverbeke. 2014. A quantitative life history of endangered humpback chub that spawn in the Little Colorado River: variation in movement, growth, and survival. *Ecology and Evolution* 4(7): 1006-1018. Available: <http://onlinelibrary.wiley.com/doi/10.1002/ece3.990/pdf>
- Yackulic, C.B., D. Van Haverbeke, M. Dzul, K.Y. Young, L. Bair. 2021. Assessing the population impacts and cost effectiveness of a conservation translocation. *J. of Applied Ecology* 58 (8): 1602-1612.

Table 1. List of reaches, subreaches, and river kilometers within each subreach; Little Colorado River.

Sub-reach	Reach	River km (range)
Boulders		
Confluence-Jump Off Rock		0.0-1.8
Jump Off Rock-Powell Pool		1.8-3.0
Powell Pool-5.0 rkm		3.0-5.0
Coyote		
5.0 rkm - White Spot (Kachina Falls)		5.0-6.5
White Spot-Redbud Canyon		6.5-8.0
Redbud Canyon-House Rock		8.0-9.6
Salt		
House Rock-Hell Hole		9.6-11.2
Hell Hole-Triple Drop		11.2-12.3
Triple Drop-Lower Atomizer Falls		12.3-13.57
Chute Falls		
Lower Atomizer Falls-Chute Falls		13.57-14.1
Chute Falls-17.7 rkm		14.1-17.7

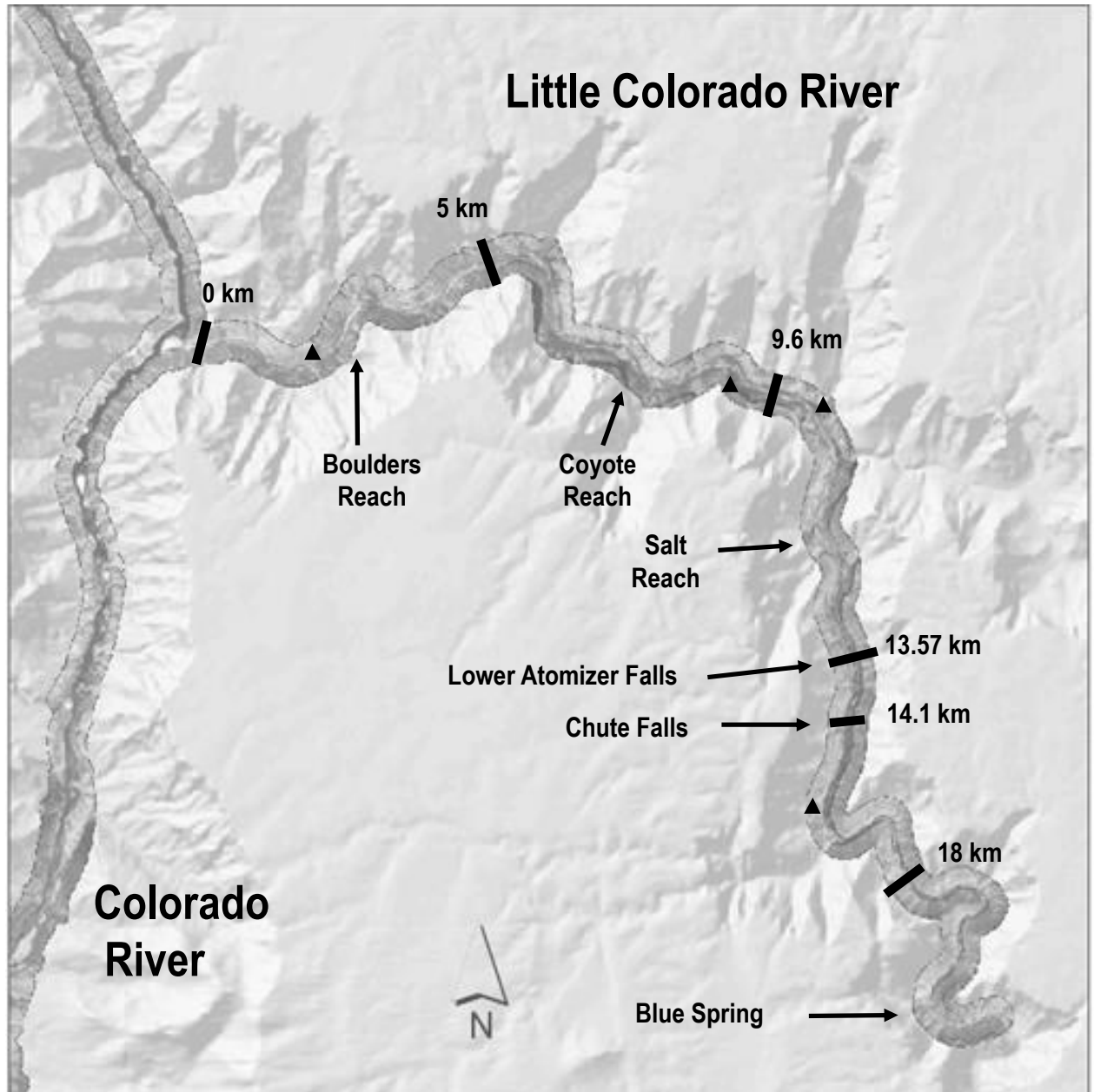


Figure 1. Map of study areas, showing Boulders (river km [rkm] 0 to 5), Coyote (rkm 5 to 9.6) and Salt (rkm 9.6 to 13.57) reaches, and lower Chute Falls (rkm 13.57 to 14.1, Atomizer reach) and upper Chute Falls (rkm 14.1 to 17.7, Chute Falls reach), Little Colorado River. Camps are designated by triangles.

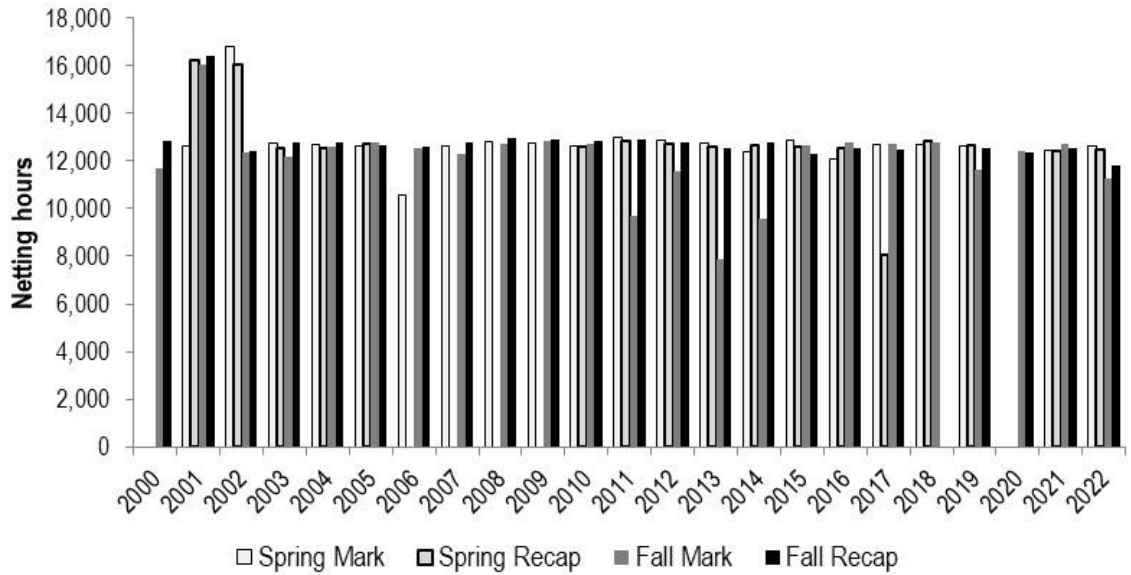


Figure 2. Hoop net sampling effort (hours) across all spring and fall monitoring trips 2000 to 2022 in lower 13.57 river km of the Little Colorado River.

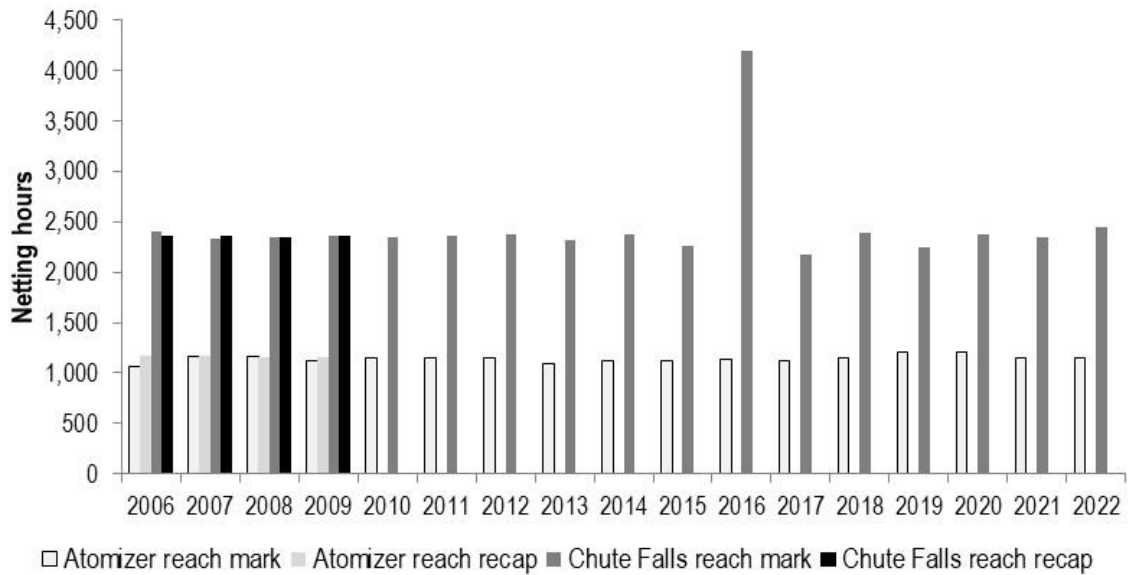


Figure 3. Hoop netting effort (hours) in the Atomizer and Chute Falls reaches during monitoring efforts between 2006 and 2022, Little Colorado River. Note: Atomizer reach extended from Lower Atomizer Falls (river km 13.57) to Chute Falls (river km 14.1). Chute Falls reach extended from top of Chute Falls (river km 14.1) to river km 17.7. Both reaches included a mark and recapture event from 2006-2009, but thereafter these reaches only included one trip per year.

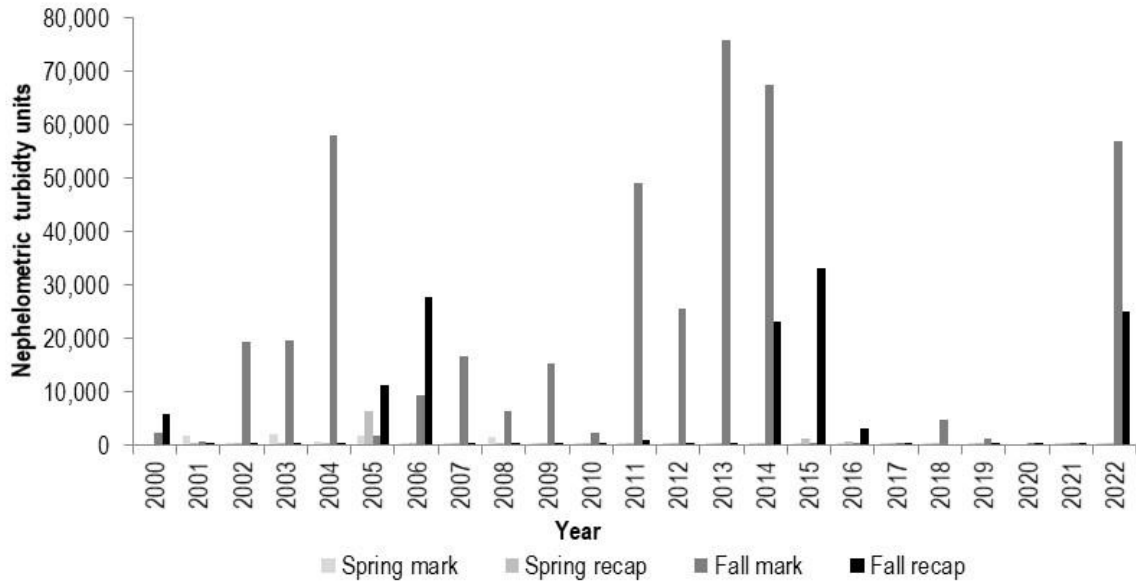


Figure 4. Average trip turbidities (nephelometric turbidity units, NTU) during spring and fall mark-recapture trips in the Little Colorado River, 2000-2022. Note, “missing” bars represent low trip turbidities (most < 54 NTUs), not missing data. Note: sampling was not conducted in spring 2020.

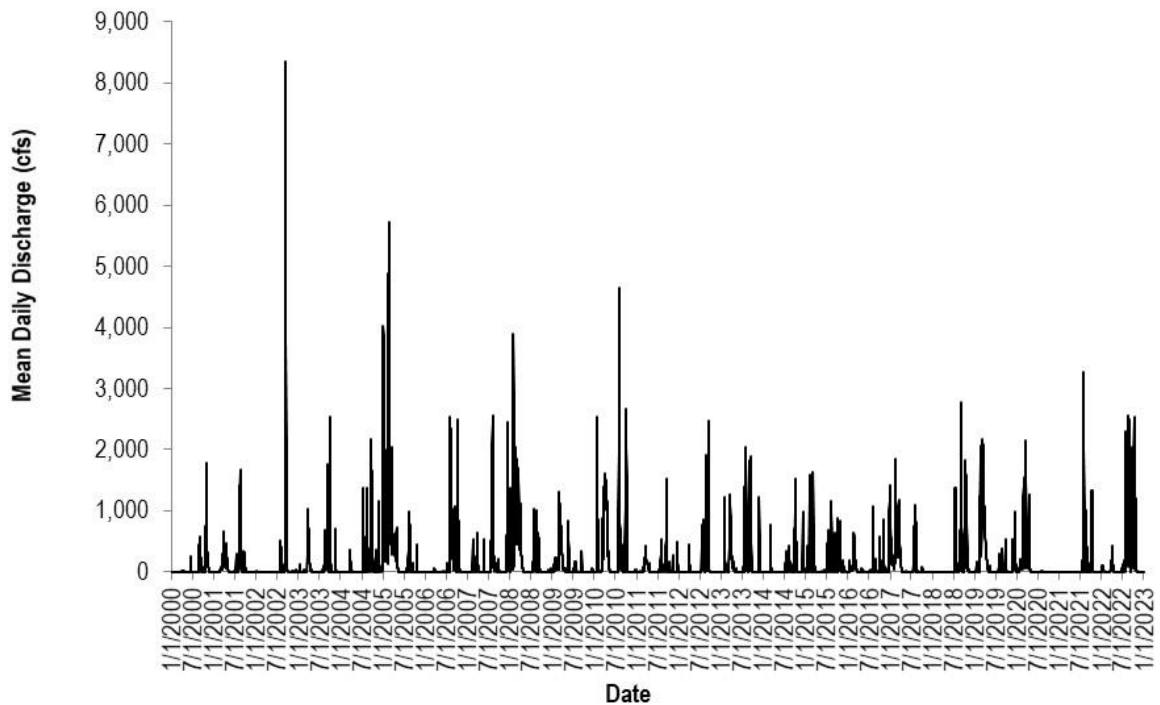


Figure 5. Provisional mean daily discharge (cubic feet/second; cfs) from USGS gage station 0940200; Little Colorado River (LCR), Arizona, 1 January 2000 – 1 January 2023.

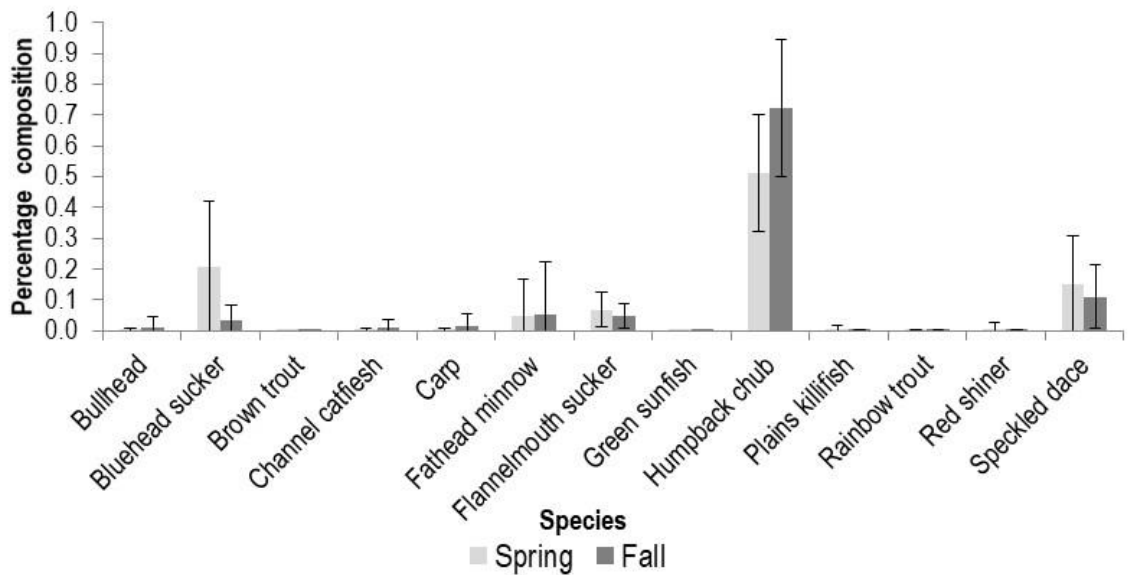
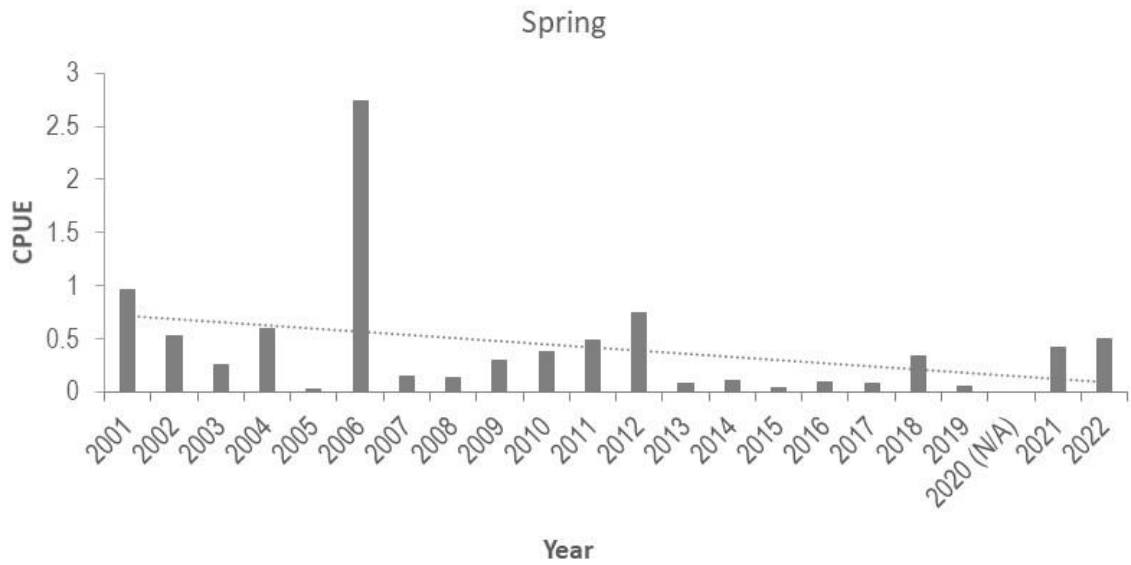


Figure 6. Observed spring and fall species composition ($\pm 95\%$ CI) of fish captured in hoop nets between fall 2000 and fall 2022; Little Colorado River (river km 0-13.57, n = 241,284 fish).

A.



B.

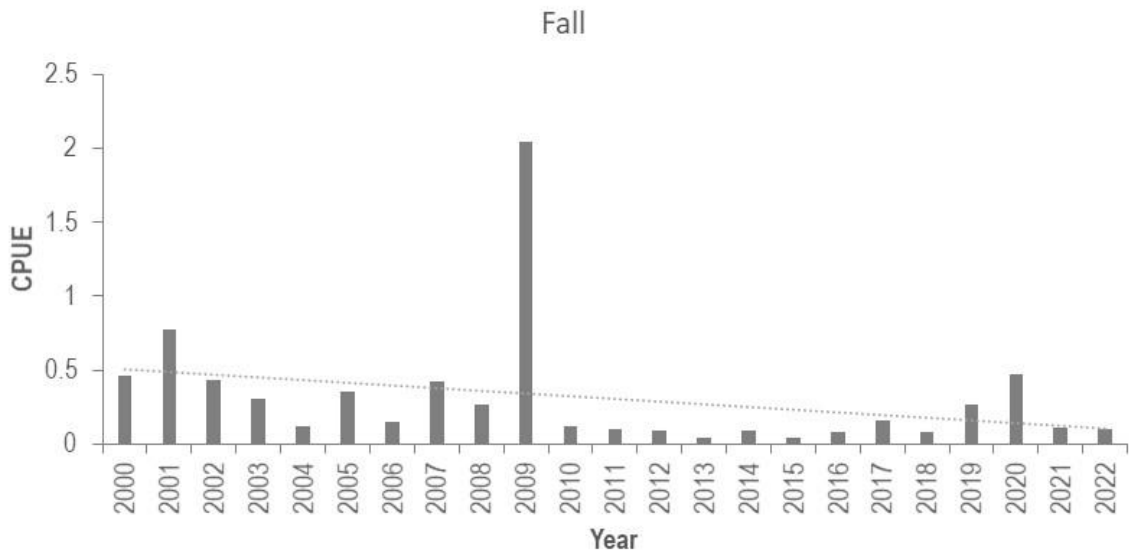
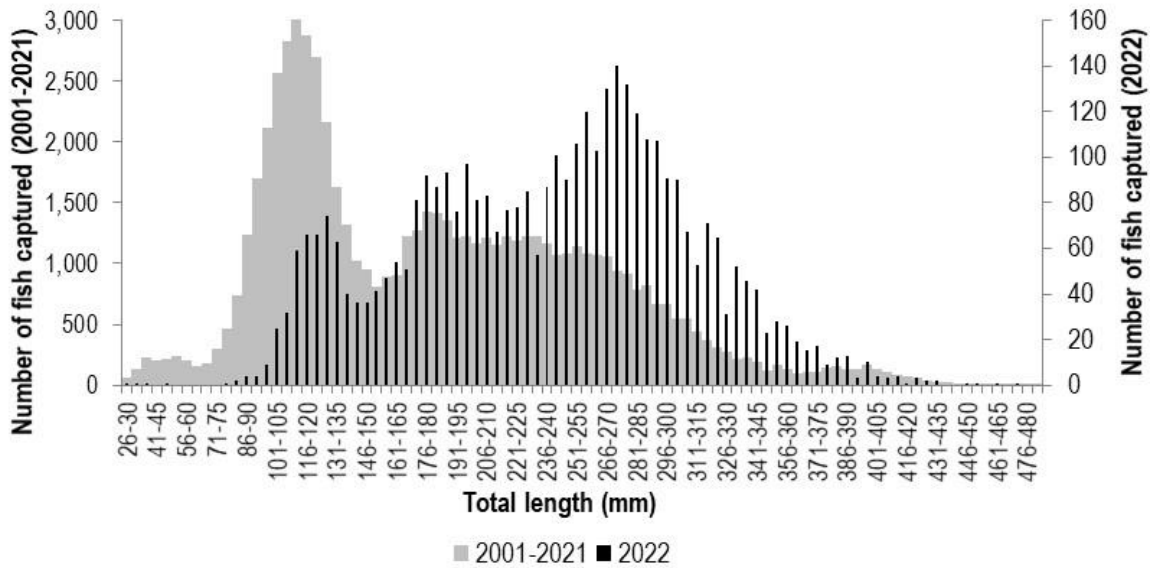


Figure 7. Catch per unit effort (CPUE, fish/net) of nonnative fish species in the lower 13.57 km of the Little Colorado River during A) spring and B) fall seasons. Note: dotted lines are linear trend lines.

A.



B.

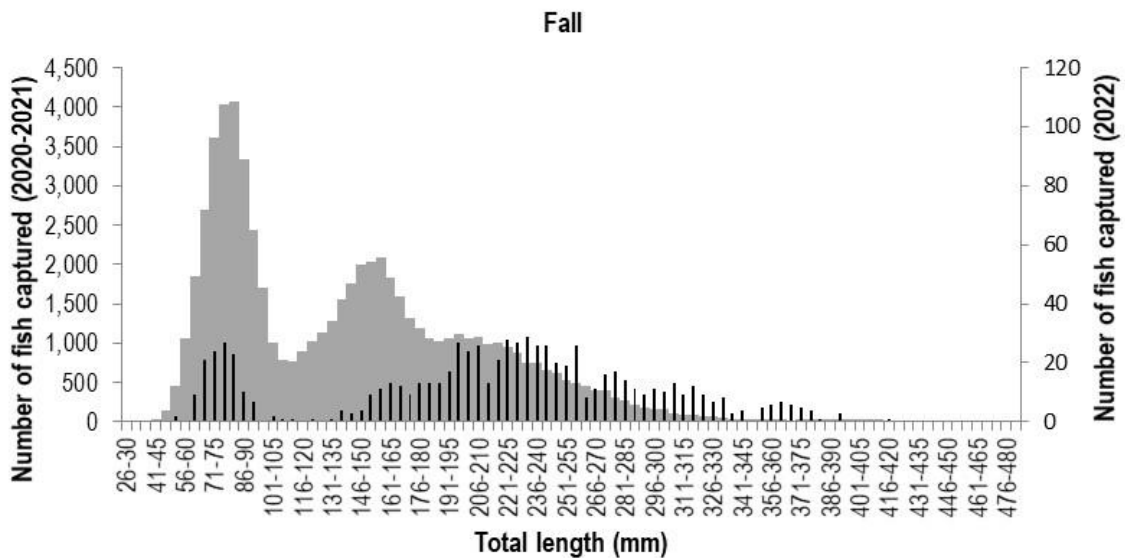
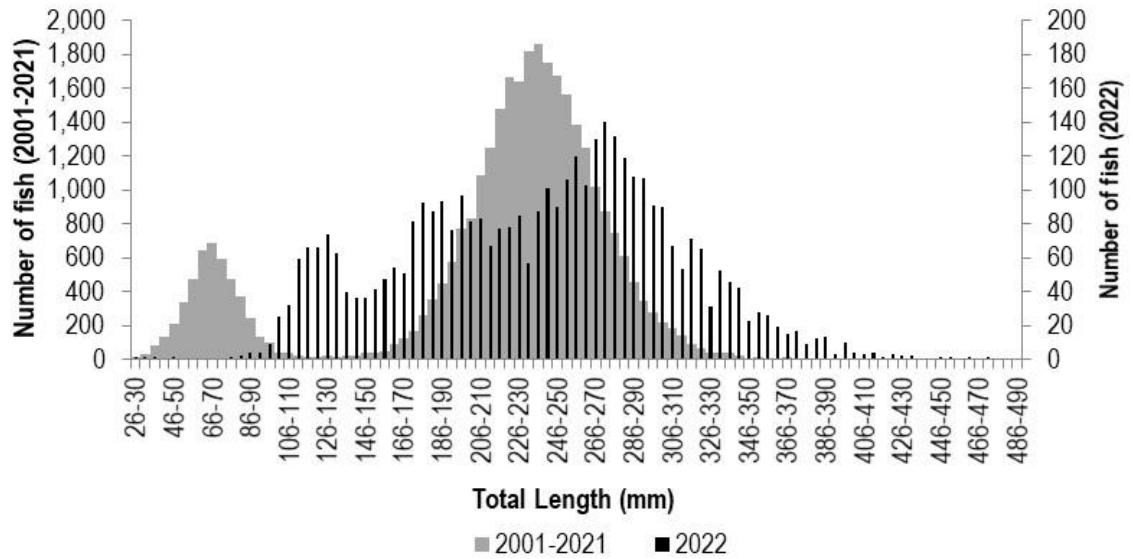


Figure 8. Length frequency distributions of Humpback Chub captured in the Little Colorado River (river km 0-13.57) during (A) spring 2001-2021 and 2022 and (B) fall 2000-2021 and 2022. Note: sampling was not conducted in spring 2020. Note y-axes variations in the charts.

A.



B.

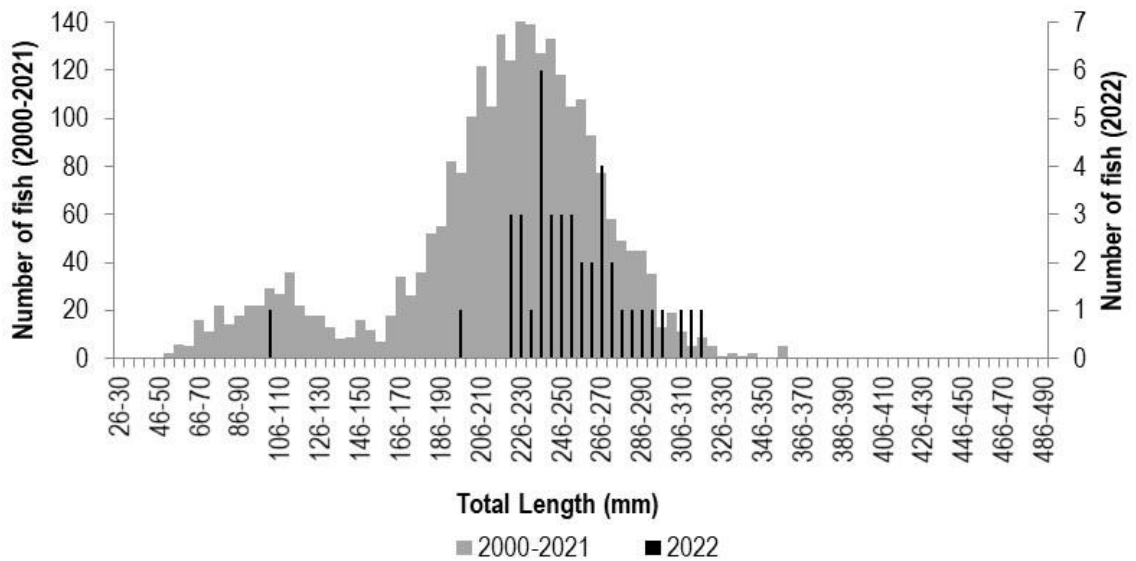
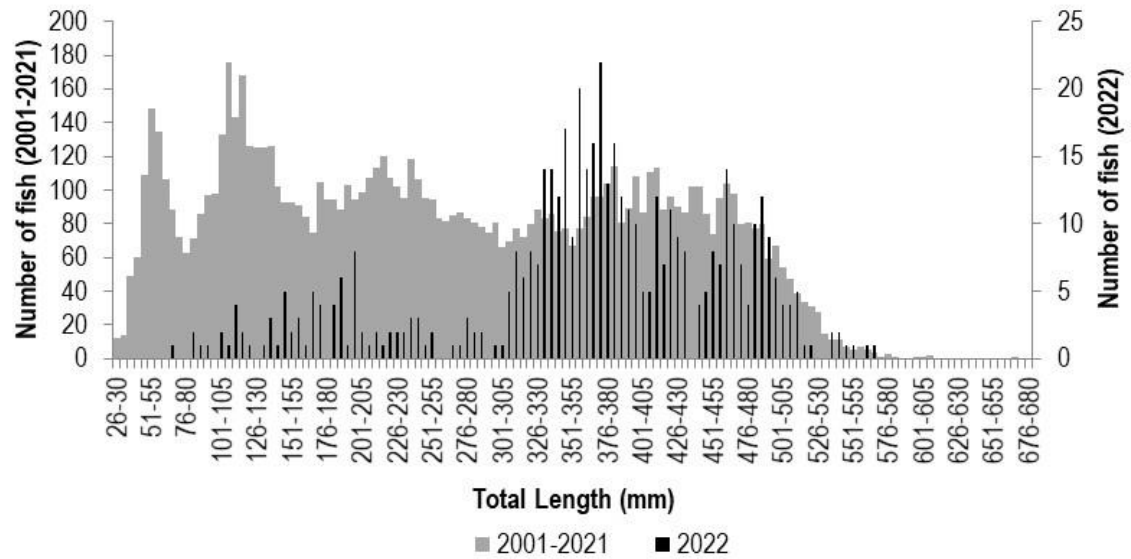


Figure 9. Length frequency distributions of Bluehead Sucker captured in the Little Colorado River (river km 0-13.57) during (A) spring 2001-2021 and 2022 and (B) fall 2000-2021 and 2022. Note: sampling was not conducted in spring 2020. Note y-axes variations in the charts.

A.



B.

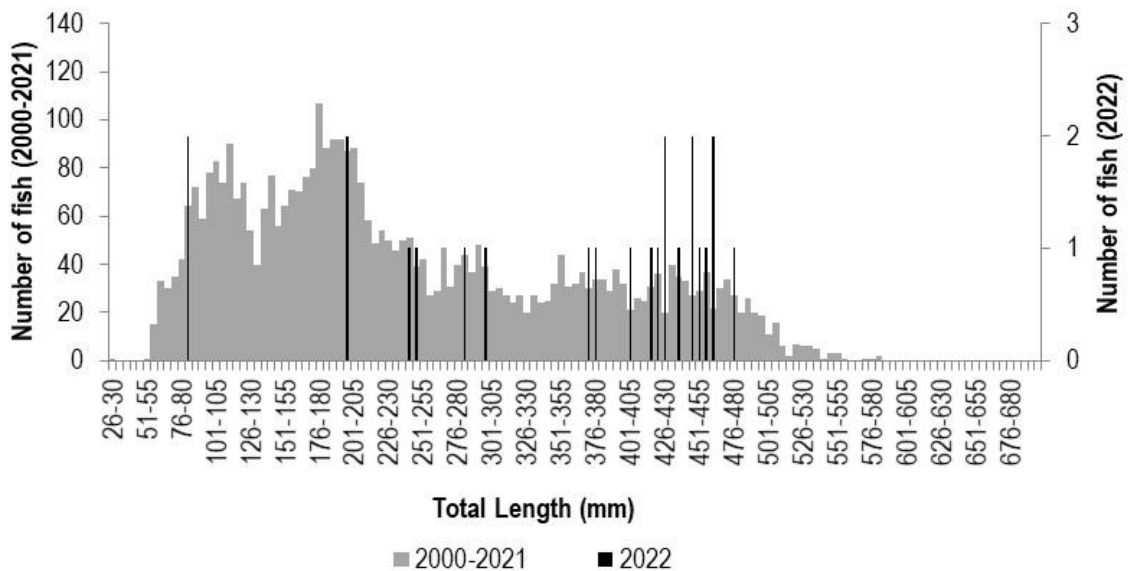


Figure 10. Length frequency distributions of Flannelmouth Sucker captured in the Little Colorado River (river km 0-13.57) during (A) spring 2001-2021 and 2022 and (B) fall 2000-2021 and 2022. Note: sampling was not conducted in spring 2020. Note y-axes variations in the charts.

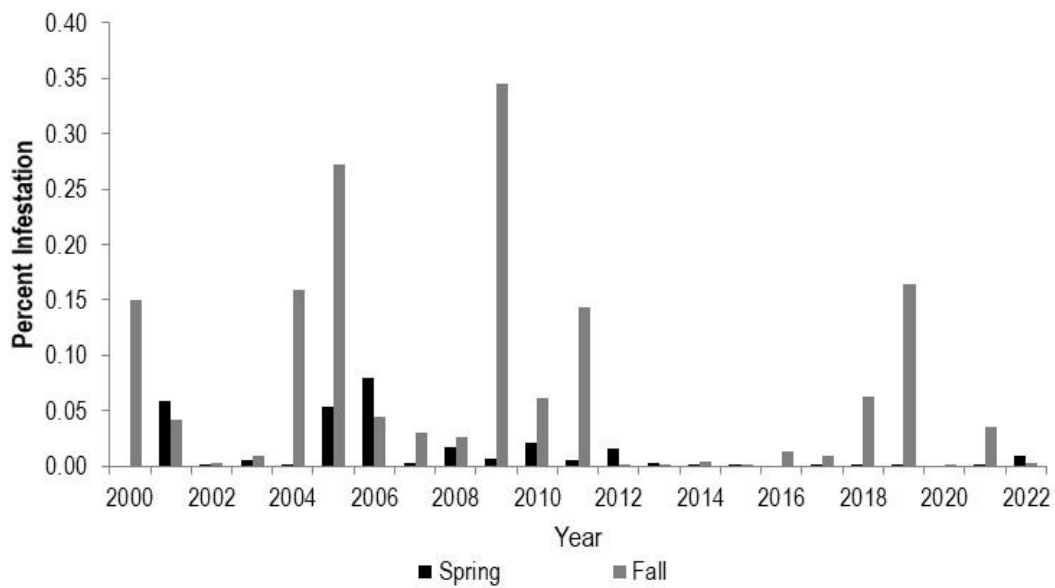
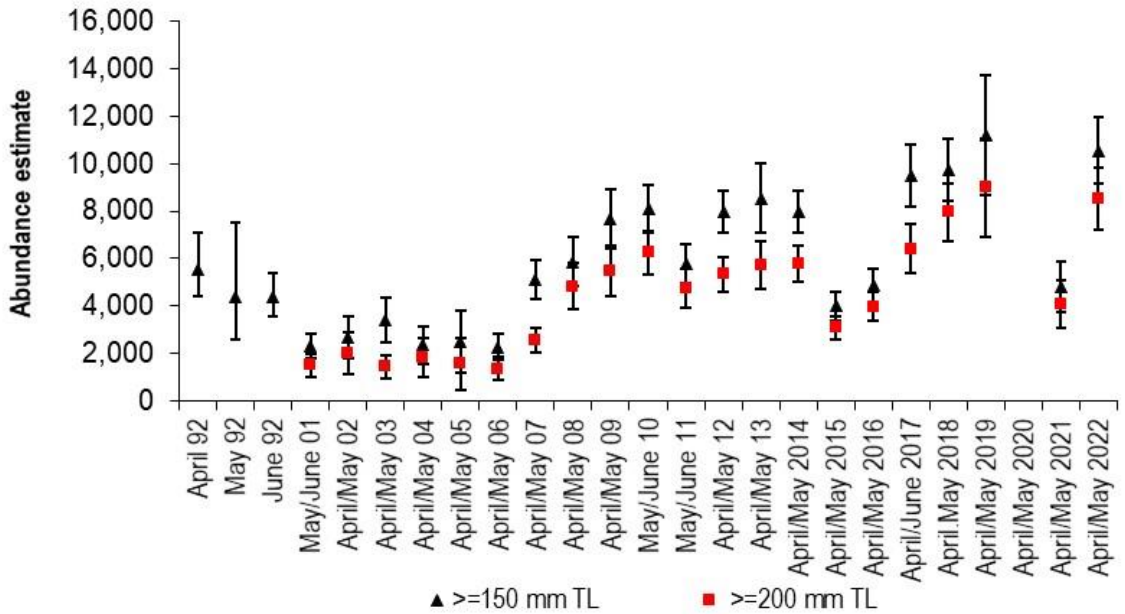


Figure 11. Percent occurrence of the external copepod parasite (*Lernaean cyprinacea*) on Humpback Chub in the Little Colorado River (river km 0-13.57), 2000-2022.

A.



B.

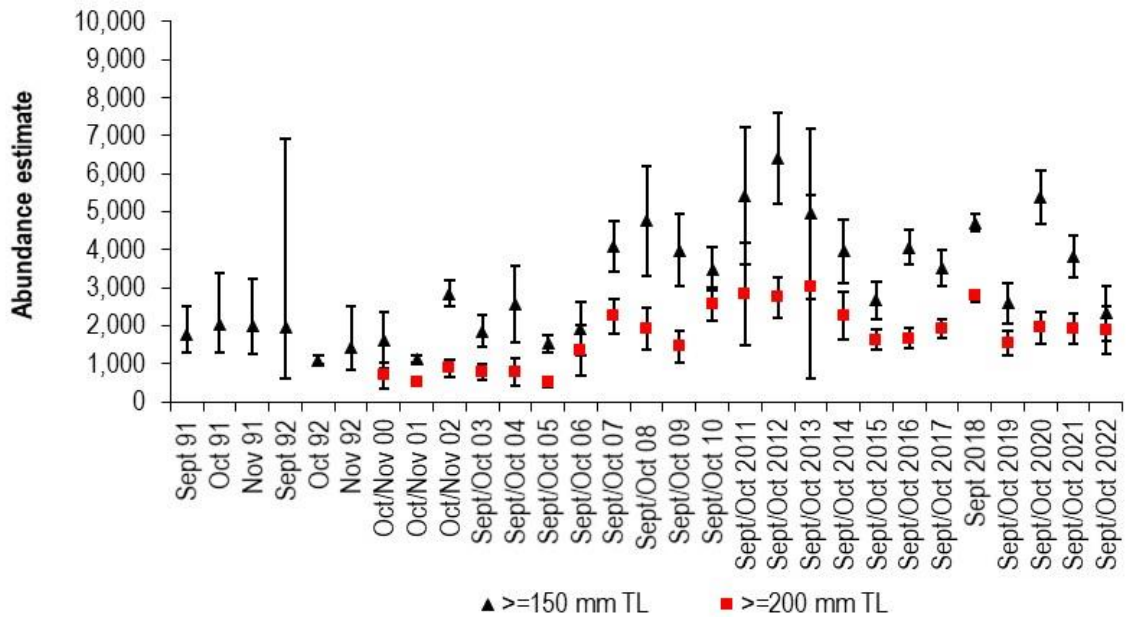


Figure 12. Chapman Petersen abundance estimates ($\pm 95\%$ CI) of Humpback Chub ≥ 150 mm and ≥ 200 mm in the Little Colorado River (0-13.57 river km) during (A) spring (2001-2022) and (B) fall seasons (2000-2022). Note: closed spring and fall abundance estimates of Humpback Chub > 150 mm in the Little Colorado River during 1991 and 1992 are from Douglas and Marsh (1996). Note: No sampling occurred in spring 2020.

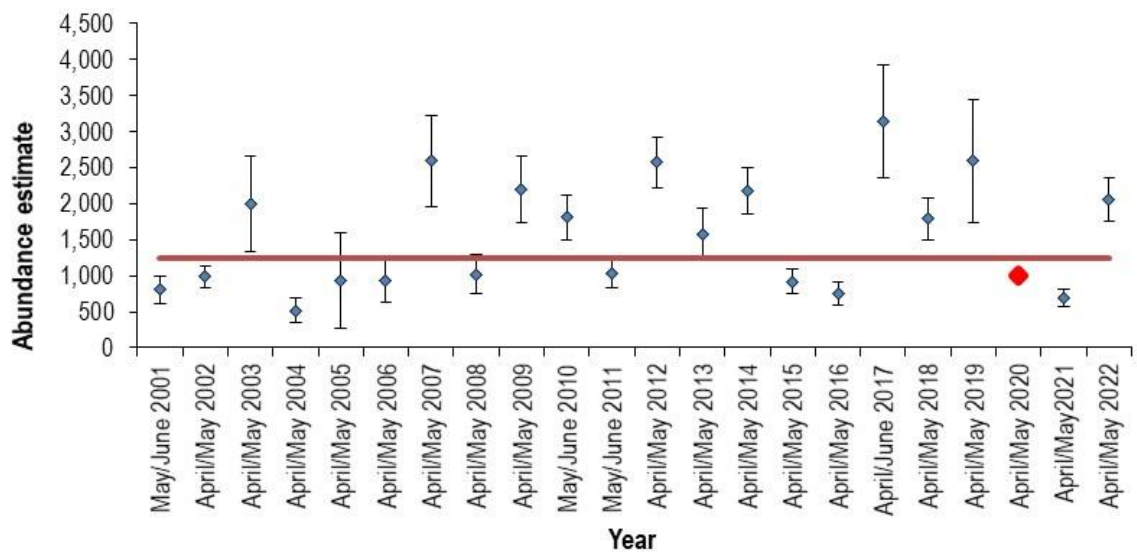
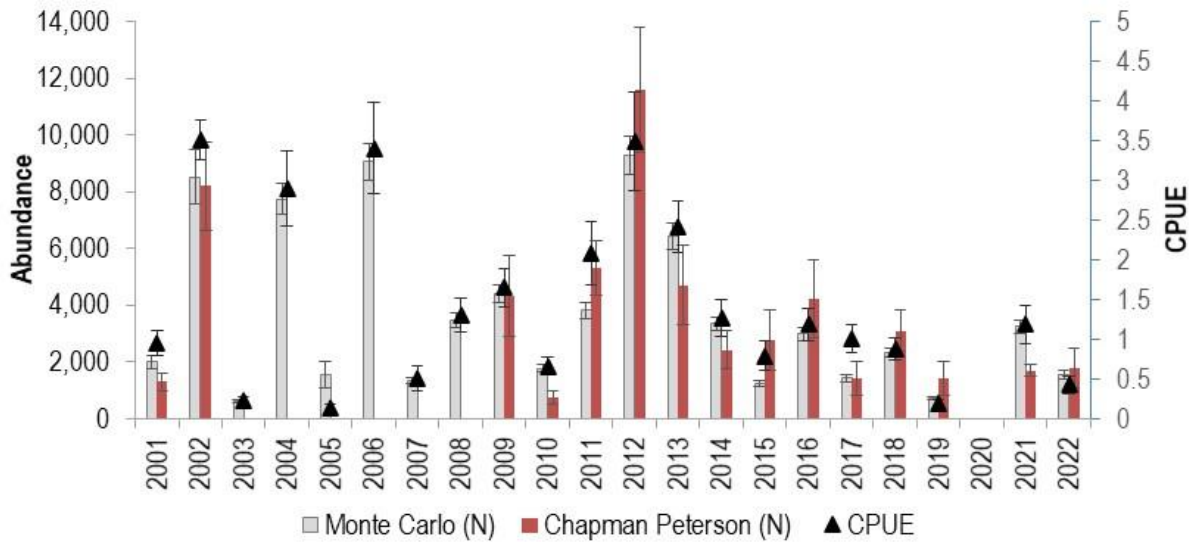


Figure 13. Chapman Petersen spring abundance estimates ($\pm 95\%$ CI) of Humpback Chub in the 150-199 mm size category; Little Colorado River (0-13.57 river km), 2001-2022. Note: 2020 (red diamond) was estimated using multi-state model (Yackulic et al. 2014) vital rate parameters (see Methods).

A. Spring age 1



B. Fall age 0

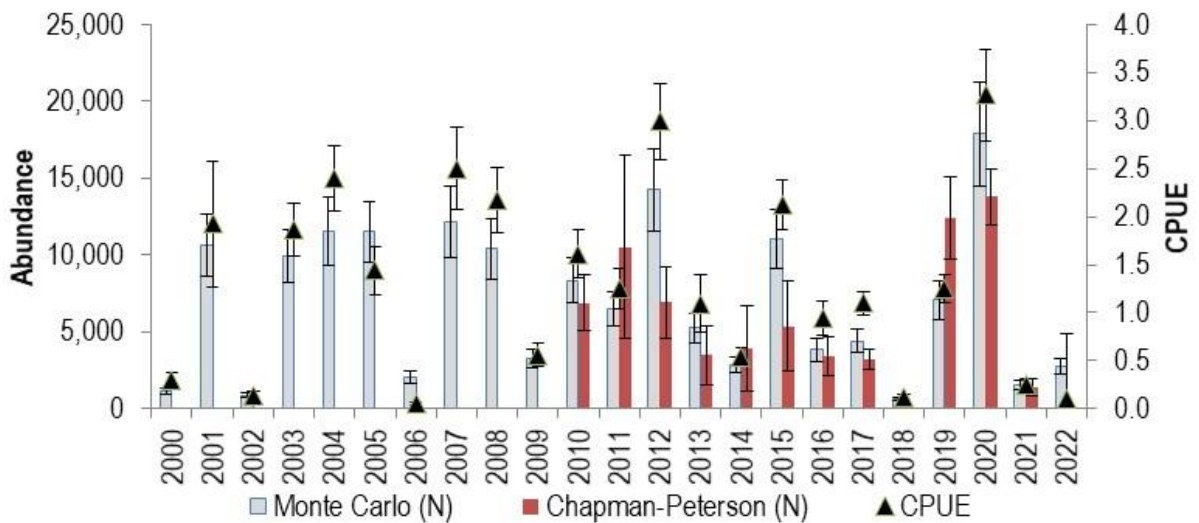


Figure 14. (A) Spring season Monte Carlo simulated abundance estimates ($\pm 95\%$ CI), Chapman Petersen abundance estimates ($\pm 95\%$ CI), and catch per unit effort (CPUE, $\pm 95\%$ CI) of Humpback Chub (HBC) in the 91-161 mm size class (predominately age 1). Note: Age 1 Chapman Petersen abundance estimates were not performed in all years, and no estimates were made in 2020 because Covid-19 prevented sampling. (B) Fall season Monte Carlo simulated abundance estimates ($\pm 95\%$ CI), Chapman Petersen abundance estimates ($\pm 95\%$ CI), and CPUE ($\pm 95\%$ CI), of HBC in the 40-105 mm size class (age 0); Little Colorado River (0-13.57 river km). Note: Age 0 Chapman Petersen abundance estimates were not performed in all years, and Chapman Petersen estimates were not successful in 2018 and 2022 because of too few recaptures.

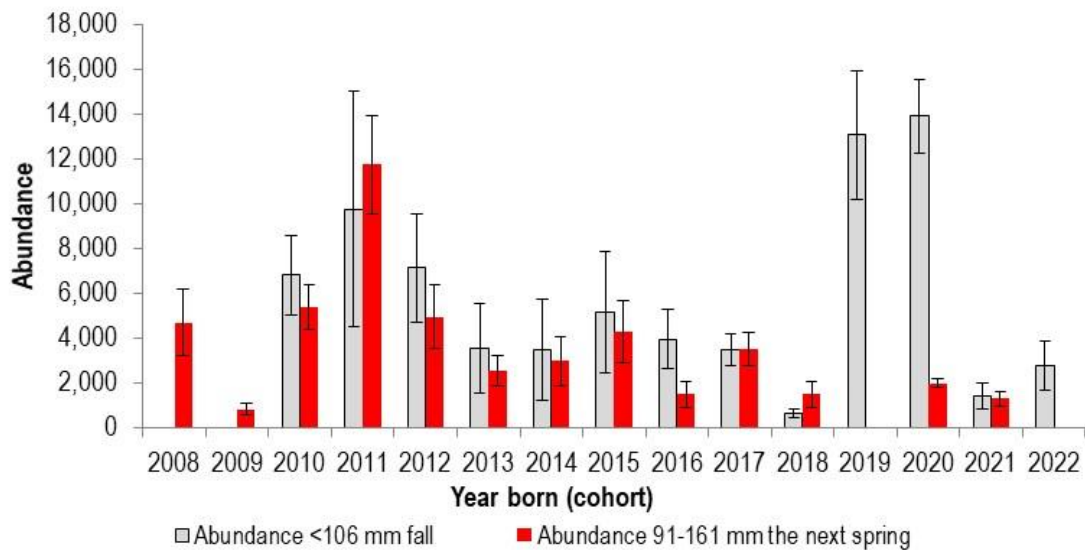
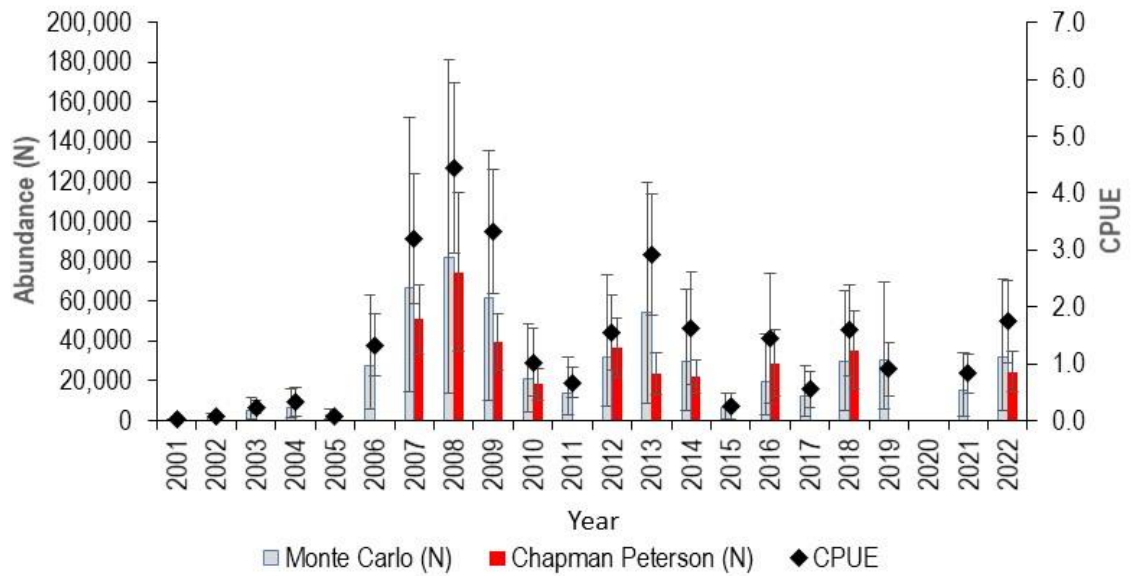


Figure 15. Annual Chapman Petersen abundances of Humpback Chub <100 mm (age 0 fish) during the fall, and abundances of Humpback Chub 100-149 mm (predominantly age 1 fish) the following spring, Little Colorado River (0-13.57 river km). Note, spring 2020 100-149 mm size class (2019 cohort) missing because spring 2020 sampling was not conducted.

A.



B.

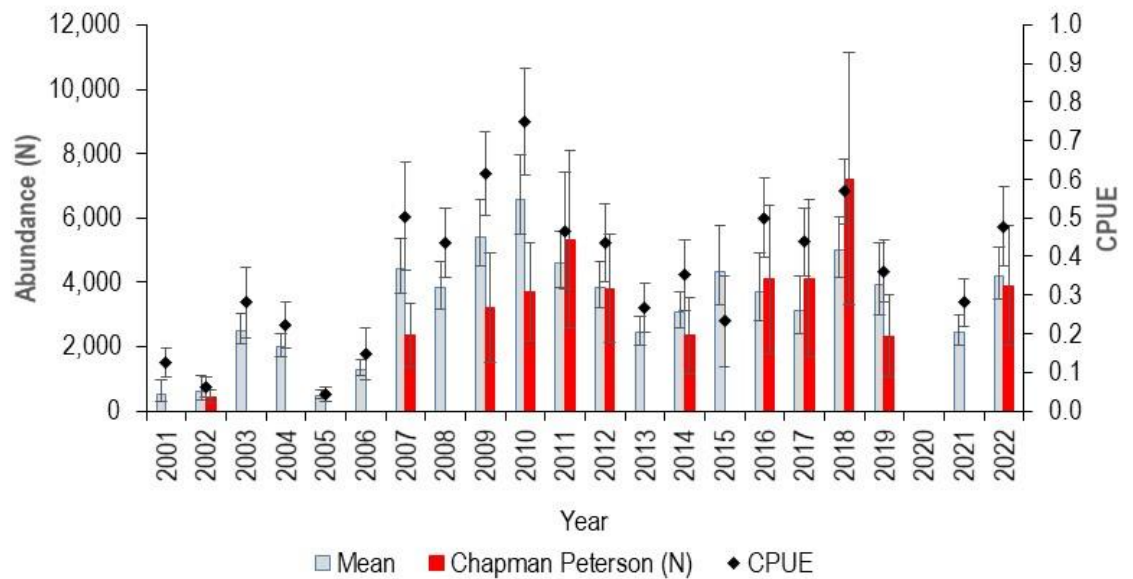


Figure 16. (A) Spring season Chapman Petersen abundance estimates ($\pm 95\%$ CI), Monte Carlo simulated abundance estimates ($\pm 95\%$ CI), and catch per unit effort (CPUE, $\pm 95\%$ CI), of Bluehead Sucker ≥ 150 mm, and (B) Flannelmouth Sucker ≥ 150 mm; Little Colorado River (0-13.57 river km). Note: Chapman Petersen abundance estimates were not performed in all years, and no spring sampling in 2020.

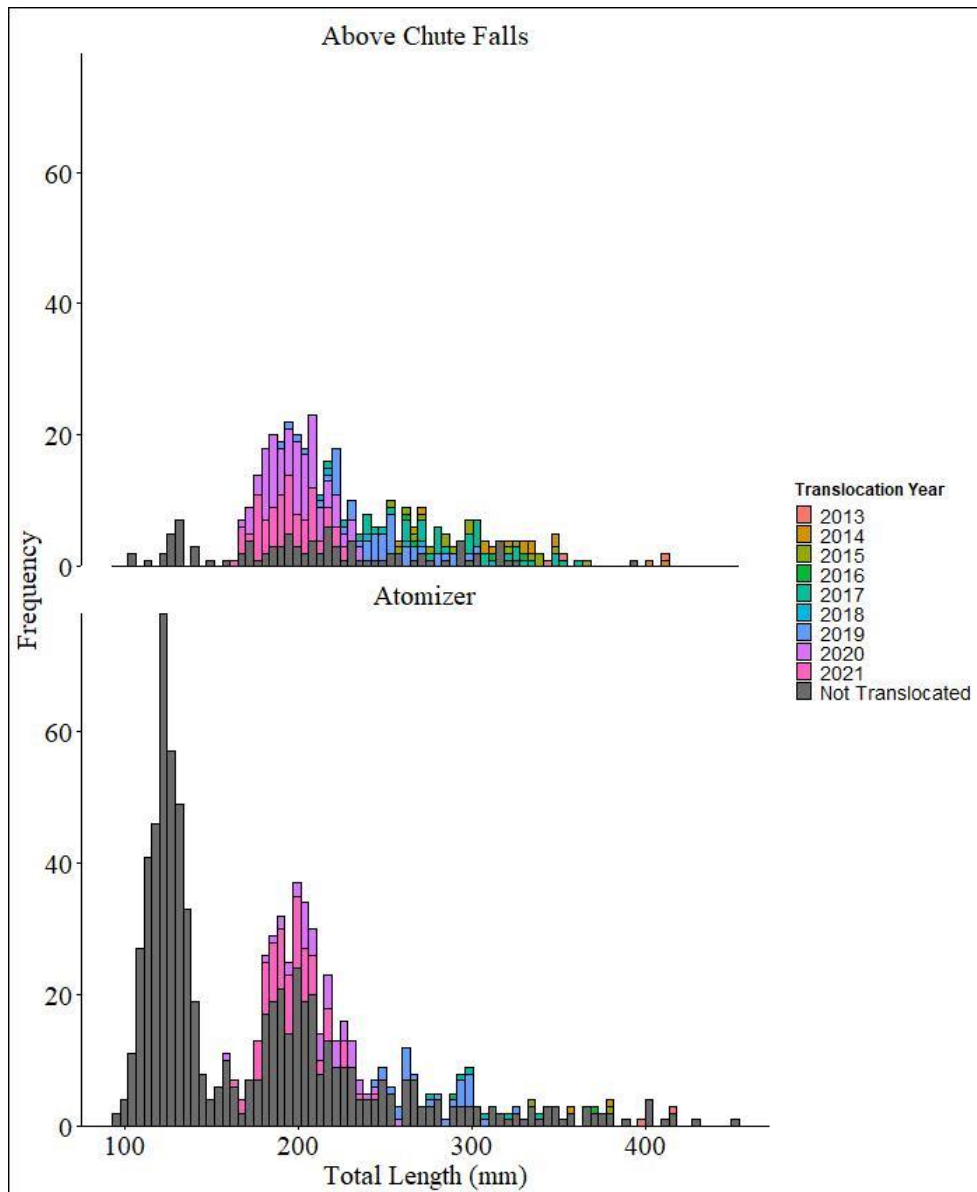
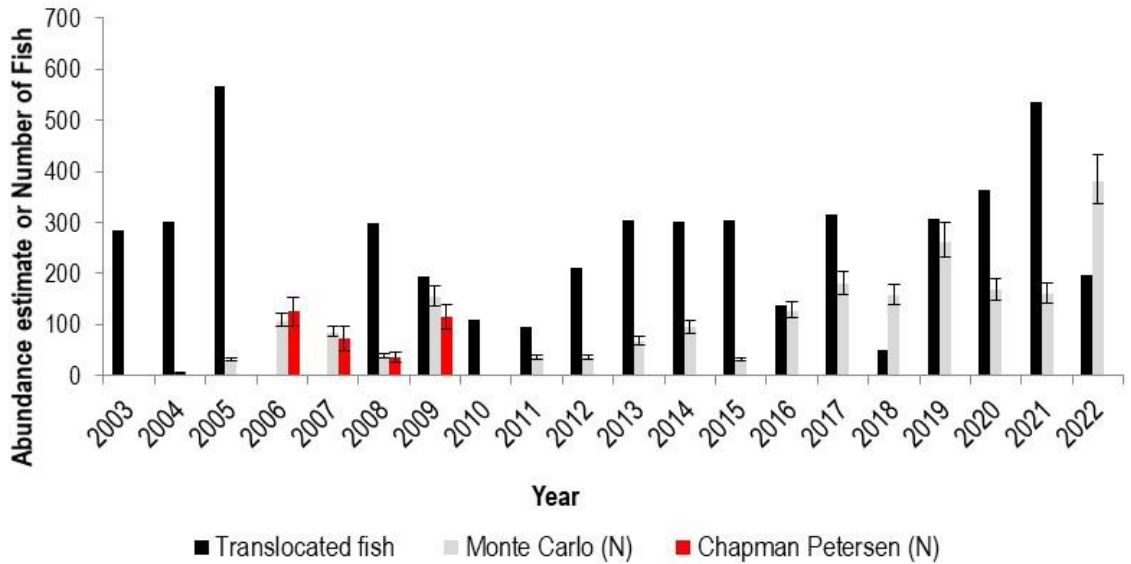


Figure 17. Length frequency distributions of Humpback Chub captured during May 2022 above Chute Falls (river km 14.1-17.7, top graph) and in the Atomizer reach (river km 13.57-14.1, bottom graph), Little Colorado River. Colored stacked bars were captured chub that were translocated in years between 2013 to 2021, as evidenced by PIT tag recaptures. Grey bars were fish that were untagged upon capture in May 2022 (i.e., most likely fish that were spawned and grew up in these reaches, or upriver migrants).

A.



B.

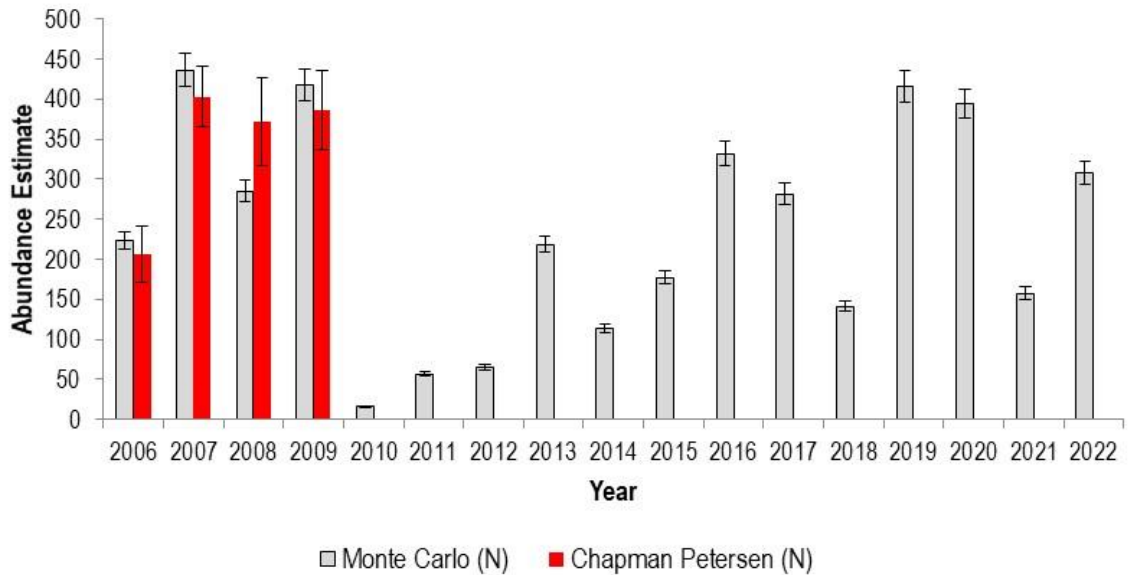


Figure 18. (A) Numbers of juvenile Humpback Chub that have been translocated to the Chute Falls reach since 2003 (black bars); and abundances ($\pm 95\%$ CI) of adult chub (≥ 200 mm) in the Chute Falls reach (river km 14.1-17.7) calculated with Chapman Petersen method (red bars), and Monte Carlo simulation (light grey bars), and (B) abundances ($\pm 95\%$ CI) of adult chub in the Atomizer reach immediately downstream of Chute Falls (river km 13.57–14.1) calculated with Chapman Petersen method (red bars) and Monte Carlo simulation (light grey bars); Little Colorado River.

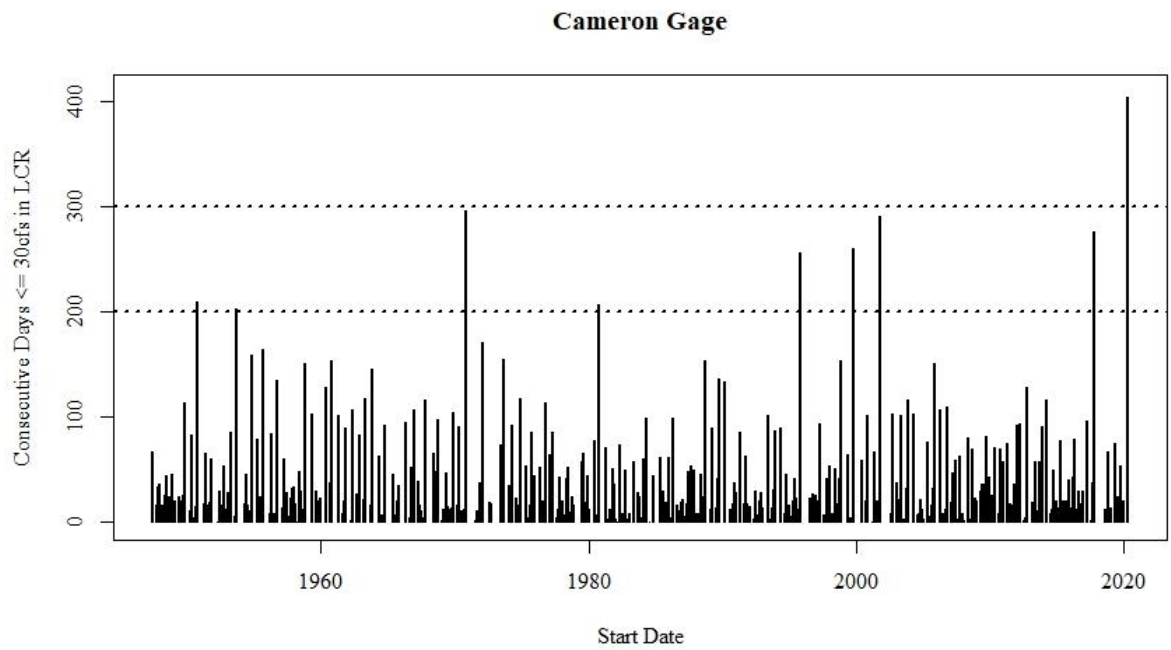


Figure 19. Number of consecutive days with Little Colorado River (LCR) flows <30 cubic feet/second (cfs) at USGS Gage 0940200, near Cameron, AZ, 1947-2023. Note, flows <30 cfs at Cameron generally indicate base level flows below Blue Springs in LCR, unless there is input from local drainages downriver from Cameron gage.

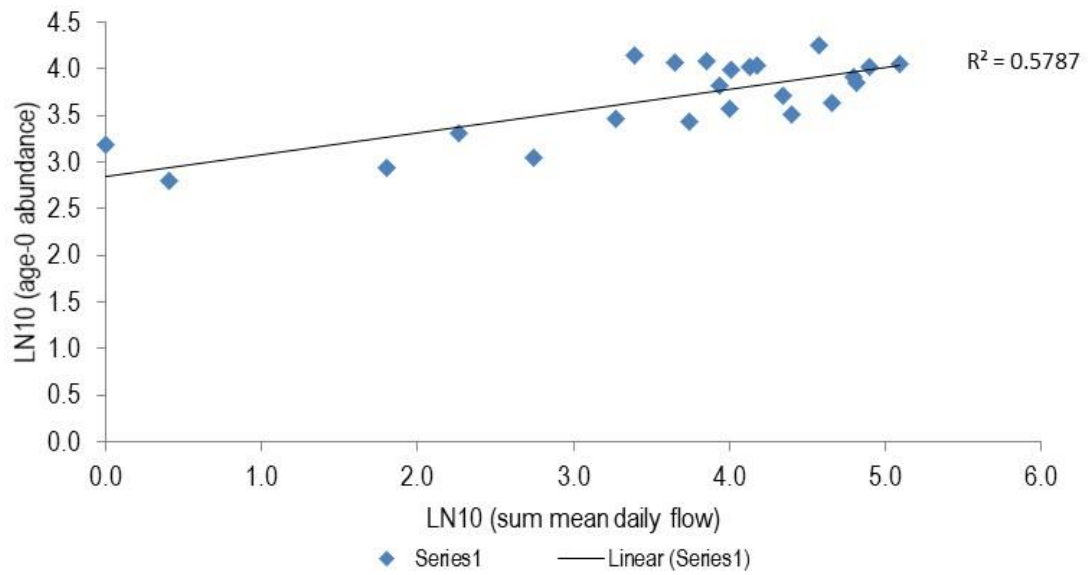
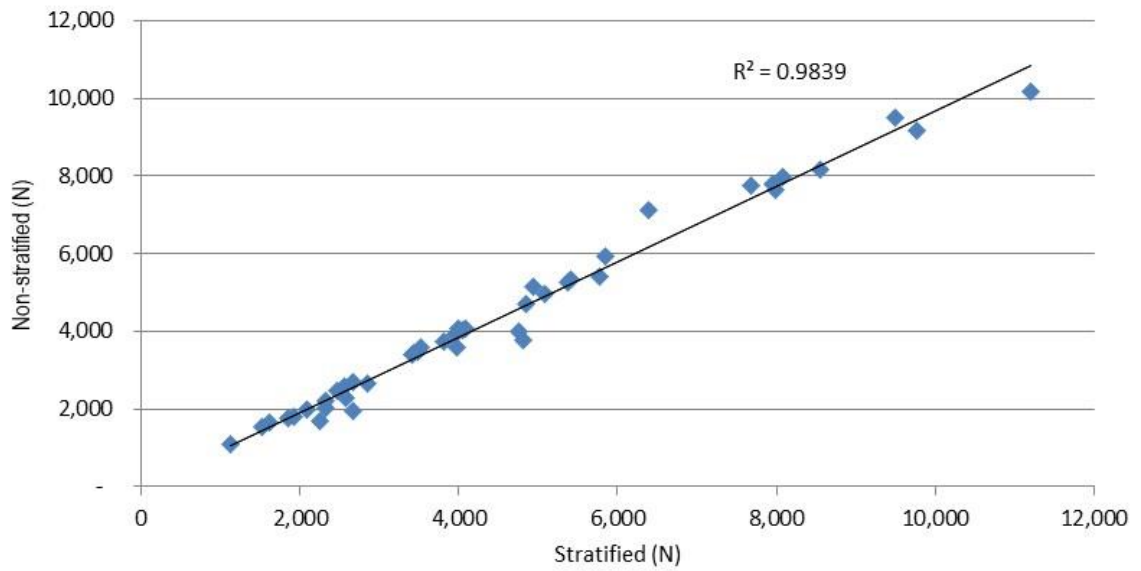


Figure 20. Regression of LN10 summed mean daily flow (1 January-31 May, cfs) to LN10 fall abundance of age 0 Humpback Chub in Little Colorado River, 2000-2022.

A.)



B.)

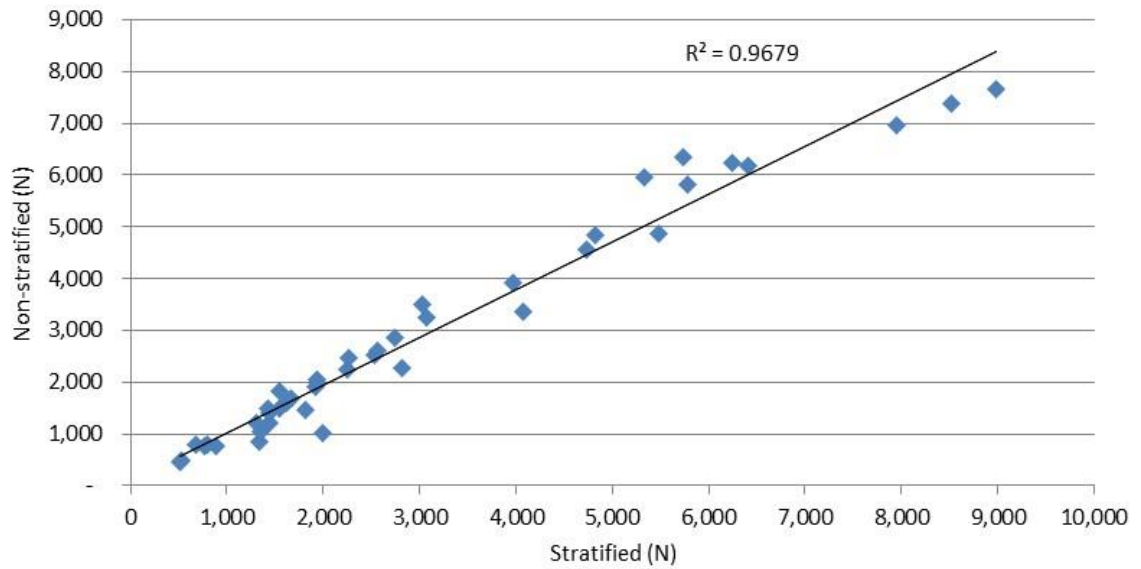


Figure 21. Regression of stratified Chapman-Petersen abundance estimates, and non-stratified Chapman-Petersen abundance estimates for A.) ≥ 150 mm, and B.) ≥ 200 mm size classes of Humpback Chub in Little Colorado River, 2000-2022.