Monitoring Humpback Chub in the Colorado River, Grand Canyon during fall 2021

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Cover photos: Top row – Pilar Rinker, Kenai Van Haverbeke, Chase Ehlo. Middle row – Jeremy Swindlehurst and Kirsten Tinning, Daniel Young, Clay Nelson. Bottom row – Kirk Young, Daniel Young and Kirsten Tinning, crew watching kitchen boat stuck in Dubendorf Rapids, Chris McIntosh. Photos by D. Van Haverbeke.

EXECUTIVE SUMMARY

During fall of 2021, we conducted work on the mainstem Colorado River in Marble and Grand Canyons to monitor Humpback Chub (*Gila cypha*) and other fishes. The objectives of these trips were: 1) continue long-term relative abundance (catch per unit effort, CPUE) monitoring of Humpback Chub in historical "aggregation" sites described by Valdez and Ryel (1995), 2) apply capture probability to catch data to estimate abundances of Humpback Chub and Flannelmouth Sucker in western Grand Canyon, and 3) conduct opportunistic seining in backwaters of Marble and Grand Canyons.

To this end, from 3-18 September 2021, we conducted a river trip from Lees Ferry to Pearce Ferry (Aggregation trip) focusing on monitoring Humpback Chub aggregations and conducting sampling in reaches of river outside of the formal aggregations described by Valdez and Ryel (1995). We sampled thirteen river reaches, most approximately 1-2 miles in length, using baited hoop nets. Submersible Passive Integrated Transponder (PIT) tag antennas were deployed within seven reaches to increase detections of tagged fish.

CPUEs for Humpback Chub generally increased going downriver, particularly below Havasu Rapids, reaching a peak at the Travertine Falls sample reach (~river mile 230-231). Flannelmouth Sucker (*Catostomus latipinnis*) CPUEs were higher than Humpback Chub CPUEs at nearly all locations and were consistently highest in reaches between Fern Glen and Frogy Fault (~river mile 170-198).

Using capture probability data, and applying that to catch data, we estimate the abundance of adult Humpback Chub ≥200 mm between Havasu Rapids and Pearce Ferry in 2021 to be ~46,000 (CI: ~39,000-55,000), and the total abundance of Flannelmouth Sucker ≥200 between Havasu Rapids and Pearce Ferry to be ~105,000 (CI: ~91,000-123,000).

Finally, we seined 91 backwaters between river miles 35-270, capturing 3,517 total fish, of which 459 were Humpback Chub. Seven non-native species were captured, including five Green Sunfish (*Lepomis cyanella*).

JUSTIFICATION

Native fish populations in Grand Canyon are key resources of concern influencing decisions on operations of Glen Canyon Dam, including non-flow actions. To inform these decisions, accurate

and timely information on the status of fish populations, particularly the Humpback Chub (*Gila cypha*), must be available to managers. Conducting mainstem Humpback Chub aggregation monitoring trips is a conservation measure in the 2016 Biological Opinion (USFWS 2016), is a project element in the Glen Canyon Dam Adaptive Management Program 2021-2023 Triennial Work Plan, and helps to meet the following Glen Canyon Dam Adaptive Management Program Core Monitoring Information Needs (CMINs).

> CMIN 2.1.2. Determine and track recruitment of all life stages, abundance, and distribution of Humpback Chub in the Colorado River.
>
> CMIN 2.4.1. Determine and track the abundance and distribution of nonnative predatory fish species in the Colorado River.
>
> CMIN 2.6.1. Determine and track the abundance and distribution of Flannelmouth Sucker, Bluehead Sucker, and Speckled Dace populations in the Colorado River ecosystem.

INTRODUCTION

Humpback Chub 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 was recently downlisted to federally threatened (U.S. Office of the Federal Register 86:198 [2021]:57588-57610). Including the Razorback Sucker (*Xyrauchen texanus*), Humpback Chub is one of five remaining native fish species currently inhabiting the Colorado River and tributaries in Grand Canyon; the others being Flannelmouth Sucker (*Catostomus latipinnis*), Bluehead Sucker (*C. discobolus*), and Speckled Dace (*Rhinichthys osculus*). Humpback Chub currently exist as five populations: four upstream of Lake Powell (Black Rocks, Westwater Canyon, Desolation/Gray Canyons, and Cataract Canyon); and one downstream of Lake Powell (Marble and Grand Canyons).

During the early 1990s, Valdez and Ryel (1995) identified nine Humpback Chub "aggregations" in Marble and Grand Canyons (Figure 1). An aggregation of Humpback Chub was defined as a consistent and disjunct group of fish, with no significant exchange of individuals with other aggregations, as indicated by recapture of PIT tagged juveniles and adults and movement of radio tagged adults (Valdez and Ryel 1995). Generally, these aggregations were found in areas near springs or tributary inflows. A large aggregation of Humpback Chub inhabits the Little Colorado River (LCR) and nearby vicinity in the mainstem Colorado River (Douglas and Marsh 1996, USFWS 2002) and is referred to as the LCR inflow aggregation. Since 2014, however, a sizeable population of Humpback Chub has developed in western Grand Canyon (Van Haverbeke et al. 2017, Rogowski et al. 2018).

Since the early 1990s, Humpback Chub aggregations have been sampled using various gear types including baited and non-baited hoop nets, trammel nets, seines, and electrofishing equipment (Valdez and Ryel 1995, Gorman et al. 2005, Ackerman et al. 2008, Persons et al. 2017). Based on the results of those monitoring efforts, the original aggregation boundaries defined by Valdez and Ryel (1995) were slightly modified by Persons et al. (2017) to reflect a more recent distribution (Table 1). For example, the range of the original 30-Mile aggregation was expanded, the Lava-

Chuar to Hance aggregation was considered a continuation of the LCR inflow aggregation, and the Bright Angel Creek inflow aggregation was thought to be no longer present (Persons et al. 2017).

Resource managers are interested in estimating abundance of Humpback Chub within the described aggregations and, more broadly, in assessing abundance of Humpback Chub at a larger spatial scale in Marble and Grand Canyons. Most progress in estimating Humpback Chub population parameters has come from working on the LCR inflow aggregation, because of its size, and importance as a tributary associated population in the face of changing Glen Canyon Dam environmental conditions (Valdez and Ryel 1995, Douglas and Marsh 1996, Coggins et al. 2006, Coggins and Walters 2009, Van Haverbeke et al. 2013, Dzul et al. 2014, Dodrill et al. 2015). The LCR inflow aggregation was estimated at 11,000 (95% CI: 7,000-16,000) total adults ≥200 mm (Yackulic et al. 2014). Additionally, a primary reason that successful population parameters have been estimated for the LCR inflow aggregation is that this aggregation of Humpback Chub is potadromous, with a portion of the mainstem adults migrating into the LCR during spawning season. Because the LCR is a relatively small volume river system compared to the Colorado River, Humpback Chub can be more easily captured, marked, released, and then recaptured to estimate trends in abundance and survival using standard mark recapture methods.

Progress in estimating the abundance of Humpback Chub in the mainstem Colorado River outside of the LCR inflow aggregation has been more difficult and sporadic. Valdez and Ryel (1995) estimated abundances of adult Humpback Chub (≥200 mm TL) in six aggregations during the early 1990s (30-Mile, LCR inflow, Shinumo Creek inflow, Middle Granite Gorge, Havasu Creek inflow, and Pumpkin Spring). Except for the LCR inflow, all aggregations were small, ranging from 5-98 adult individuals. A lack of recaptures precluded obtaining reliable abundance estimates in the Lava Chuar-Hance, Bright Angel inflow, and Stephen Aisle aggregations. In July and September 2014, a closed mark-recapture effort yielded an estimate of 243 adult Humpback Chub (95% CI: 91-395) in a group of Humpback Chub found between 34-36 mile (within the 30-Mile aggregation as defined in Persons et al. 2017), with $p1$ and $p2$ capture probabilities of 0.15 and 0.12, respectively (Van Haverbeke, pers. com.). The aforementioned estimates were obtained primarily with the use of trammel nets, or with a combination of hoop nets and trammel nets. Because of potential stress to endangered fish (Hunt et al. 2012), trammel netting has largely been discontinued as a gear type in Grand Canyon.

Except for the cases described above, monitoring of Humpback Chub aggregations in the Colorado River in Marble and Grand Canyons has been largely restricted to obtaining relative abundance (catch per unit effort, CPUE) indices (Ackerman et al. 2008, Persons et al. 2017). This remains the case, because obtaining absolute abundance (N) estimates in the mainstem requires focused and repetitive effort. However, since 2017, opportunistic efforts associated with aggregation trips have focused on estimating absolute abundances and densities (fish/mile) of Humpback Chub at select mainstem locations. These have included 2017 abundance and density estimates of Humpback Chub and Flannelmouth Sucker at Juvenile Chub Monitoring (JCM) East and JCM West (Pillow et al. 2018), estimates in 2018 at JCM East and Bridge City (Van Haverbeke et al. 2019), 2019 estimates at JCM West, Bridge City, and 250-Mile (Van Haverbeke et al. 2020), and 2020 estimates at JCM West, Ghost camp, Separation, Island, and Columbine (Van Haverbeke et al. 2021). In 2021, we continued this pursuit, however with an emphasis on gathering catch data in

western Grand Canyon between Havasu rapids and Pearce Ferry to refine abundance estimates in western Grand Canyon.

OBJECTIVES

1. Obtain September 2021 relative abundance (catch per unit effort, CPUE) estimates of Humpback Chub from aggregation sites in Grand Canyon, (e.g., 30-Mile, Bright Angel, Stephen Aisle, Middle Granite Gorge).
2. Gather catch data at approximately 10-15 mile intervals between Havasu rapids and Pearce Ferry to estimate abundances of Humpback Chub and Flannelmouth Sucker in western Grand Canyon.
3. Provide information related to Humpback Chub length frequency distributions, observed community composition, and sexual condition (e.g., ripe, not ripe).
4. Investigate the utility of passive PIT antennae for detecting additional fish.
5. Investigate the utility of opportunistic seining on an aggregation trip.

METHODS

Schedule, Sampling Sites, and Personnel

During 3-18 September 2021, we conducted a river trip between Lees Ferry and Pearce Ferry, Arizona. Participants on the trip were David Van Haverbeke and Kirk Young (Principal Investigators, USFWS), Pilar Rinker, Chase Ehlo, and Kirsten Tinning (USFWS); Kenai Van Haverbeke and Daniel Young (volunteers); and boatmen Jeremy Swindlehurst (Trip Leader), Chris McIntosh, Tristan Slade, and Al Neil (Ceiba, Inc.).

Sampling Gear

During the trip, we sampled at fourteen sites, each approximately 1-2 miles in length (Table 2). We sampled thirteen of the reaches with baited hoop nets. Hoop nets were 0.5-0.6 m in diameter and 1.0 m long with 6 mm mesh and a single 10 cm throat (Memphis Net and Twine, Memphis, TN). All hoop nets were baited with approximately ¾ L Aquamax™ Grower 600 for Carnivorous Species (Purina Mills, Inc., Brentwood, MO) in 3 mm mesh bait bags that allowed fish to access and consume bait. Hoop nets were tied to shore, and typically set at a depth of less than 3 m. With few exceptions, hoop nets were set in the afternoon each day between 14:00 h and 18:00 h and pulled the next day between 07:00 h and 13:30 h. Hoop nets were set at a density of 1 net per 0.1 mile on each side of the river. In a few locations, rapids or fast shallow water prevented setting nets, and a few 0.1 mile sections were skipped (for example in Middle Granite Gorge, and other reaches containing small rapids).

In addition, we deployed 2-5 baited submersible Passive Integrated Transponder (PIT) antennas overnight within seven of the sampling reaches. The LCR inflow aggregation reach was sampled only with the antennas. The purpose of the antennas was to opportunistically augment fish detections. For both hoop nets and antennas, we recorded set and pull times and location (side, and river mile), and marked locations on aerial photo maps provided by GCMRC.

Finally, we conducted opportunistic seining on the 2021 aggregation trip. This was accomplished by utilizing a small crew of two or three biologists on the kitchen boat. Generally, while hoop

netting crews pulled nets in the morning, the seining crew departed downriver and seined backwaters until the hoop net crews caught up later in the day. Depending on backwater size, a small seine (~5 m x 1.5 m, 1/8" mesh) or a large seine (~8 m x 2 m, 1/8" mesh) was used. Seine widths were adjusted to conform to backwater widths. Seine haul lengths were measured, and a representative depth and width were taken in the midpoint of the seine haul. Because of traveling logistics, and because of few backwaters in some reaches, no seining was conducted from RM 71.7-93.7 (Hance to just above Granite Rapids), 93.8-115.7 (Granite Rapids to Stephen Aisle), 123.2-159.3 (Middle Granite Gorge to below Havasu), RM 225-240.1, 250-269.2, and 269.2-280.

Data Collection

Captured fish were identified to species. Total length (TL), fork length, sex (male/female), and sexual condition (ripe/not ripe) were recorded for Humpback Chub, Flannelmouth Sucker, and Bluehead Sucker. Fish captured in seines were generally tallied by species, but Humpback Chub were PIT tagged and measured. All fish lengths herein refer to TL. Fish were scanned for presence of a PIT tag. Untagged Humpback Chub ≥80 mm were implanted with PIT tags (134.2 kHz, 12.5 mm, Biomark, Boise, ID). PIT tags were detected using Biomark HPR Lite tag readers. We entered data directly into tablet computers on each boat; PIT tags were uploaded to the tablet data files via Bluetooth connection. Because of time constraints, not all Flannelmouth Sucker were measured for length or PIT tagged. However, to facilitate gathering catch data for population estimation by 50 mm size class, we either measured or counted all Flannelmouth Suckers in a net into length categories of <150 mm, 150-199 mm, and ≥200 mm. Table 3 shows specifics on Flannelmouth Sucker processing.

Water temperature was recorded with a Hobo Water Temp Pro V2 (Onset Computer Corp., Bourne, MA) deployed off a boat and set at 15 minute intervals. Turbidities (Nephelometric Turbidity Units, NTU) were recorded with a Hach 2100P Turbidimeter (Loveland, CO), and three or more readings were taken daily at a site and averaged.

Catch per Unit Effort (CPUE)

We calculated CPUE for hoop nets within each sampling reach as number of fish (Humpback Chub or Flannelmouth Sucker) captured per overnight hoop net set.

Abundance Estimation

Because there has been a significant expansion of Humpback Chub and Flannelmouth Sucker in western Grand Canyon since 2014, there is interest in quantifying the abundance (N) of these species. To this end, in 2017, 2018, 2019 and 2020 we conducted closed mark-recapture efforts within discrete river reaches in western Grand Canyon (Pillow et al. 2018, Van Haverbeke et al. 2019, 2020, 2021). Most of these reaches were ~2 miles in length. Because of logistics, all but one mark-recapture reach was conducted below Diamond Creek by USFWS, and one mark-recapture reach was conducted jointly by USFWS and GCMRC above Diamond Creek (JCM West). As a result of these mark-recapture efforts, a body of capture probability (*p*) data has been generated (Table 4). In 2021, we collected catch data at roughly 10-mile intervals in western Grand Canyon to apply this body of *p* data to catch data.

We calculated abundances within each mark recapture reach using the Chapman modified Petersen population estimator (Seber 1982). We first stratified the length data of the marked, captured, and

recaptured fish. The goal of this procedure was to minimize bias by defining length strata to optimize for more homogenous capture probability within length categories (Seber 1982). Optimal length strata bounds were determined using a computer program that conducted sequential contingency table calculations, each with a different length-strata bound, to find the bound that maximized the test statistic value (i.e., χ^2), with each strata containing 7 or more recaptures (as in Van Haverbeke et al. 2013). For example, this procedure in Island reach in 2020 resulted in two primary length divisions for Humpback Chub: <211 mm and >210 mm with $\chi^2 = 4.31$. We calculated abundances within these primary length divisions using the Chapman Petersen population estimator with standard formula presented in Seber (1982, p. 60). For estimation of fish within 50 mm size classes (e.g., 150-199 mm), we used the method of subcategories described by Seber (1982, pp. 100-101) within each χ^2 strata. The 95% confidence intervals of the Chapman Petersen estimates were approximated with a normal distribution following Seber (1982, p. 60). We conformed to requiring ≥7 recaptures to be 95% confident that bias in the abundance estimation is negligible (Robson and Reiger 1964, Seber 1982 p. 60). For both Humpback Chub and Flannelmouth Sucker, we determined either "pooled" or "summed" estimates of abundance and density ≥100 mm, ≥150 mm and ≥200 mm. Pooled estimates were used when not all relevant 50 mm size classes contained ≥7 recaptures. If each 50 mm size category contained ≥7 recaptures, these size specific abundance estimates (and variances) were summed to provide preferred summed abundance estimates of fish ≥150 mm and ≥200 mm. For most estimates, once fish were ≥300 mm, there were fewer than 7 recaptures, so that size class was always pooled.

Abundances (N) within each mark recapture reach were transformed into densities (fish/river mile). We computed the mean absolute maximum distance individual fish moved (MMDM) between the marking and recapture events within each reach and added ½ this distance onto each end of the sampling reach, then dividing abundance by the adjusted distance (Wilson and Anderson 1985, Karanth and Nichols 1988). This slightly expanded the reach to theoretically incorporate the "superpopulation" of fish that were potentially moving in or out of the reach boundaries, and slightly decreased density estimates. For Humpback Chub, MMDM was 0.263 miles in the Ghost camp reach (n = 117 fish), 0.154 miles in the Separation reach (n = 55 fish), 0.464 miles in the Island reach (n = 24 fish), and 0.403 miles in the Columbine reach (n = 96 fish). For example, 0.154 miles were added onto the reach distance of 2.0 miles in the Separation reach to adjust densities of Humpback Chub. In the JCM West reach, MMDM for Humpback Chub was 0.249 miles (n = 12 fish), but we used 0.747 (i.e., 3 x 0.249) because we had marked fish in 3 sub-reaches of the JCM reach, each separated by >0.249 miles. For Flannelmouth Sucker, MMDM was 0.08 in the Ghost camp reach (n = 222 fish), 0.1 miles in the Island reach (n = 52 fish) and 0.15 miles (n = 126 fish) in the Columbine reach.

To demonstrate the increase of total adult Humpback Chub abundance that has occurred since 2010 in western Grand Canyon (between the base of Havasu Rapid and Pearce Ferry), we applied p data to annual catch data via Monte Carlo simulation. Capture probability data was derived from mark-recapture efforts in western Grand Canyon during 2017, 2018, 2019, and 2020 (Pillow et al. 2018; Van Haverbeke et al. 2019, 2020, 2021). We calculated capture probability values for the mark trip ($p1$) as $p1 = R/C$, where C = number of unique Humpback Chub captured during the recapture trip, and R = number of Humpback Chub marked (PIT tagged) during the mark trip and subsequently recaptured during the recapture trip. Capture probabilities for the recapture trip ($p2$) were calculated as $p2 = R/M$, where M = number of Humpback Chub marked during the mark trip

(Table 4). We first bootstrapped the number of expected recaptures given the individual reach *p* estimates and the number of chub marked during the marking trip. We then simulated *p*1 and *p*2 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 (i.e., recapture) trip, respectively. We took the mean of the simulated reach *p* values and used this value as the mean capture probability. Then, using Monte Carlo analysis (10,000 replicates), annual sample reach catch was divided by the mean bootstrapped *p* value to obtain abundance estimates with 95% confidence intervals. We segregated the *p* values into low and high turbidity categories. This was performed because turbidity is a primary factor influencing catch of Humpback Chub (Stone 2010). Adult Humpback Chub *p* values during low turbidity conditions ranged from 0.085 - 0.238 (mean = 0.147, SE = 0.07, *n* = 11), and during high turbidity conditions from 0.06 – 0.072 (mean = 0.072, SE = 0.017, *n* = 2).

Abundances and densities within the mark-recapture reaches were calculated using the maximum number of nights during the mark and recapture events (usually 2 or 3 nights) in order to maximize recaptures, and to minimize the coefficients of variance. However, when applying *p* data to catch, we used *p* data based on one night of marking and one night of recapture using only hoop nets as gear type because catch data at sites outside of the mark recapture reaches were generally limited to one night of hoop net sampling. We also only used *p* data that contained ≥7 recaptures for Humpback Chub ≥200 mm. We assumed that the short time span between mark and recapture events (about a month) would serve to minimize fish movement in and out of the individual reaches between trips, helping to meet the closure assumption of the model. Additionally, we think that the closure assumption is justified over this short time frame in the mainstem based on Humpback Chub telemetry studies which show limited movement (Valdez and Hoffnagle 1999, Paukert et al. 2006, Gerig et al. 2014), and on evidence of little movement of Humpback Chub and Flannelmouth Sucker in between the mark and recapture events (generally <0.5 mile). We also assumed that mixing of fish within the reaches occurred. These same exercises were performed for Flannelmouth Sucker ≥200 mm.

Because site sampling was not always geographically evenly distributed each year, in 2021 we gathered catch data at roughly 10-15 mile intervals between Ledges and Columbine (~RM 151-276, Table 2). We supplemented this with 2019 catch data from three additional sites near 230 mile (Travertine), 236 mile (Bridge City), and 251-Mile. We believe this was appropriate because catch data in 2019 and 2021 were collected under low turbidity conditions. Also, we used 2019 catch data from the Havasu Reach since we did not sample at Havasu in 2021. We estimated abundances of Humpback Chub and Flannelmouth Sucker >=200 mm between Havasu Rapids and Pearce Ferry by connecting point density and confidence interval and summing the resulting extrapolated density estimates/mile.

RESULTS

Physical Parameters

Daily flows during September 2021 ranged from ~7,000-12,600, with weekend peaks ~11,400 cfs. There was one short (perhaps only a few hours in duration) water releases of ~14,000 cfs on September 15 to meet emergency power demands, but it was not noticeable during sampling some

220 miles downriver from Glen Canyon Dam. Daily turbidity ranged between 51-873 NTUs (Figure 2). The increase seen on 7 September at RM 115 was likely from a small spate coming out of the LCR on 4 September, while the short lived and red colored increase seen on 16 September at RM was likely because of small localized tributary input. Mean daily water temperature ranged between 16.6-21.4 ^{o}C, rising progressively between RM 93-280 (Figure 3).

PIT antenna detections –We queried antenna detected PIT tags in the GCMRC fish database to determine species, date, and location of the last capture, and compare the numbers of fish detected with antennas only, hoop nets only, and both gear types in reaches excluding the LCR inflow (where only antennas were deployed; Table 5). Of the 190 unique PIT tags detected with antennas, nearly all were Flannelmouth Sucker (n = 131) or Humpback Chub (n = 44). There were also three Common Carp (*Cyprinus carpio*), and 12 fish of unknown species not available in database records. In the LCR inflow reach, five antennas set between RM 60.19 and 61.53 detected 288 unique Humpback Chub, 60 unique Flannelmouth Sucker, one Brown Trout (*Salmo trutta*), one Common Carp, one Rainbow Trout (*Oncorhynchus mykiss*), and 13 of unknown species.

Seining – We seined 91 backwaters between RM 35.6 and 269.2 (Figure 4). Fish captures included 3,517 fish, of which 459 were Humpback Chub (Table 6). Most Humpback Chub captured with seines were age 0 fish ≤70 mm, with a much smaller juvenile proportion in the 100-150 mm size class (Figure 5). Seven non-native species were captured, including five Green Sunfish (*Lepomis cyanellus*) captured at RM 159.3, 165.2 (3 fish), and 199.5. Humpback Chub sampled from backwaters while seining indicated some areas of higher catch (Figure 6). These included from RM 35-40 (30-Mile aggregation area), and ~RM 117-124 (Stephen Aisle and Middle Granite Gorge area), likely because of a higher density of backwaters in those areas.

Hoop netting

We deployed 451 overnight hoop nets within discrete river reaches between RM 30.5-279.5 in the mainstem Colorado River (Table 2). The number of net sets per sample location varied based on length of the reach and travel logistics. Mean hoop net density per side of the river was 1.05 net/0.1 mile (SE = 0.04, n = 451 nets). The average net set time was 16.89 hours (SE = 0.92).

Hoop net fish captures - We captured a total of 10,317 fish in 451 hoop net sets (Table 7). Of those, 99.5% were native. Flannelmouth Sucker made up the majority of fish captured (79%, n = 8,193), followed by Humpback Chub (14%, n = 1,450), Speckled Dace (6%, n = 607), and Bluehead Sucker (<0.01%, n = 7). Seven Flannelmouth Sucker x Razorback Sucker hybrids were captured, one at RM 31.08 and the remainder in western Grand Canyon between RM 171-246. Non-native species captured were forty Rainbow Trout, nine Common Carp, two Fathead Minnow, and one Red Shiner. One crayfish was also captured at RM 274.

Hoop net catch per unit efforts (CPUE) - Humpback Chub CPUEs for all size classes, and for adults only were low (<1 fish/net) between the 30-Mile and Middle Granite Gorge reaches (between RM 30.5 and 129). Below that, numbers gradually increased from Ledges (above Havasu) to Travertine Falls reaches (RM 151-231), after which they slightly declined again (Figures 7-A&B).

Flannelmouth Sucker CPUEs for all size classes combined were significantly higher than Humpback Chub at most sites sampled and reached a high at Frogy Fault reach (~RM 196; Figure 7-A). CPUEs for Flannelmouth Sucker ≥200 mm were similar to all size classes combined, but reached a high at Chevron reach (~RM 183; Figure 7-B). Ordinarily, we see a spike of Humpback Chub and Flannelmouth Sucker CPUEs near the LCR, but in 2021 we did not deploy hoop nets in the LCR inflow aggregation.

Considering the historical data on aggregation trips since 2010, the number of overnight hoop nets per trip has remained relatively steady. However, catches of Humpback Chub and Flannelmouth Sucker have increased significantly during this period (Figure 8). CPUEs for Humpback Chub above Havasu reach (RM 29-156) have remained highest in the LCR inflow (Figure 9-A). CPUEs for Humpback Chub have increased significantly since 2014 in western Grand Canyon (RM 157-279; Figure 9-B), approaching those seen in the LCR inflow aggregation (Figure 9-A).

Length Frequencies (hoop nets only) - Length frequency distributions of Humpback Chub above and below RM 156 show that only adult Humpback Chub were captured above RM 156, while Humpback Chub of all size classes were captured below RM 156 (Figure 10-A). No chub smaller than 239 mm were captured above RM 156, albeit we did not hoop net in the LCR inflow aggregation. In stark contrast, we captured 43 Humpback Chub <100 mm and 343 between 100-199 mm below RM 156. All but one juvenile chub <100 mm were captured below Lava Falls; and of these, 38 were captured below Diamond Creek. This pattern also held true for Flannelmouth Sucker. Only one representatively measured Flannelmouth Sucker <150 mm was captured in hoop nets above RM 156, while 55 representatively measured Flannelmouth Sucker <150 mm were captured below RM 156 (Figure 10-B).

We show comparative annual length frequency distributions of Humpback Chub captured on aggregation trips in western Grand Canyon between 2010-2021 to illustrate the dramatic increase in catches of Humpback Chub during the post-2013 timeframe (Appendices 1-A, B, C). Of note is that in the 2014-2021 period, there has been increasingly more representation by all size classes of Humpback Chub in western Grand Canyon below Lava Falls (>RM 182). The first substantial signals of age 0 Humpback Chub in western Grand Canyon occurred in 2014, with these fish being captured near the Pumpkin Spring aggregation ~RM 212-216 and sampling below Diamond Creek ~RM 240-247. These early spikes of age 0 fish were followed by fish into larger size classes during the following years indicating a population level effect.

Sexual Condition and Parasites (Hoop nets)
Ripe fish encountered in 2021 included two female Humpback Chub (204 & 269 mm at RM 170.1 & RM 274.1), and one male (320 mm at RM 152.6). Ripe Flannelmouth Suckers included one female (445 mm, RM 92.3), and 18 males (357-495 mm, captured between RM 30.8-116.4). One female Humpback Chub (243 mm) was recorded with *Lernaea* sp. at RM 218.4.

Abundance Estimation and Density within Discrete Reaches (Hoop nets) –
Humpback Chub - We applied *p* data to annual catch data via Monte Carlo simulation to estimate abundances (N) of adult Humpback Chub (≥200 mm) since 2010 in western Grand Canyon. By doing so, we can see that there has been a significant increase of adult Humpback Chub in western

Grand Canyon post-2015 (Figure 11), two years after the initial spikes of age 0 chub, with the peak of the observed adult expansion occurring between ~RM 212-240 (JCM West to about Separation Canyon; Figure 12). This method estimates that there were ~46,000 (95% CIs: 39,000-55,000) adult Humpback Chub between Havasu Rapids and Pearce Ferry in 2021. Of these fish, it was estimated that ~24,000 (95% CI: ~20,000-29,000) were between Diamond Creek and Pearce Ferry in 2021.

Flannelmouth Sucker - Likewise, to demonstrate abundances (N) of Flannelmouth Sucker (≥200 mm) since 2010 in western Grand Canyon, we applied *p* data to annual catch data via Monte Carlo simulation. We can see that there seems to have been a relatively steady increase in the abundance of Flannelmouth Sucker in western Grand Canyon, as opposed to the seemingly more explosive post-2015 increase of Humpback Chub (Figure 13). For 2021, this method estimates that there were ~105,000 (CIs: ~91,000-123,000) Flannelmouth Sucker ≥200 mm between Havasu Rapids and Pearce Ferry. Also, in contrast to Humpback Chub, the abundance of Flannelmouth Sucker ≥200 mm declines moving downriver from Havasu, with an estimated abundance in 2021 of only ~15,000 (CI: ~13,000-18,000) below Diamond Creek to Pearce Ferry (Figure 14).

DISCUSSION

During September 2021, we sampled Humpback Chub aggregations in Marble and Grand Canyons to continue a long-term monitoring program that has historically focused on relative abundance (i.e., CPUE). In addition, we utilized additional mainstem monitoring in western Grand Canyon to estimate absolute abundance of Humpback Chub and Flannelmouth Sucker in western Grand Canyon. Finally, we conducted opportunistic seining in backwaters in Marble and Grand Canyons to monitor for presence/absence of juvenile Humpback Chub and non-native fishes.

Catch per Unit Effort

Since 1991, there have been four, 3-7 year periods in which CPUE population monitoring in the Grand Canyon Humpback Chub aggregations has occurred. These periods are: 1991-1993 (Valdez and Ryel 1995), 2002-2005 (Ackerman 2008), 2010-2013 and 2014-2021 (Persons et al. 2017, this study). Similar hoop net sampling methods were used across sampling periods, however, during the early period (1991-1993) hoop nets were not baited, and net-baiting techniques shifted from perforated PVC scent tubes to mesh bags in 2011. In addition, trammel netting was used much more extensively as a gear type during the 1990s period.

In general, except for at the LCR inflow aggregation, Humpback Chub mean capture rates were very low river-wide during the 1991-1993 and 2002-2006 periods (Valdez and Ryel 1995, Ackerman 2008), and have increased during the 2010-2021 period (Persons et al. 2017, this study). While most aggregations have increased in relative abundance during this later time period, dramatic and significant increases have occurred in western Grand Canyon (Van Haverbeke et al. 2017). On the September 2021 aggregation trip, the highest CPUEs for adult Humpback Chub ≥200 mm were in the 220-Mile and Travertine Falls reaches (between RM 218 and 231). CPUEs for Flannelmouth Sucker were higher than for Humpback Chub at nearly all locations.

Length Frequencies

Length frequency distributions for Humpback Chub show the presence of juvenile Humpback Chub in western Grand Canyon, especially below Lava Falls since 2014 (Appendices 1-A, B & C). In 2021, most juvenile Humpback Chub between 50 and 100 mm were captured below Diamond Creek (Appendix 1-C). These time series length frequencies and CPUEs, combined with the capture of ripe females below Diamond Creek (K. Young pers. com; D. Rogowski pers. com), and distribution and temporal patterns of larval Humpback Chub (Kegerries et al. 2016) strongly suggest mainstem spawning occurs in western Grand Canyon. Since 2014, catches of Humpback Chub in western Grand Canyon have dramatically increased in all size classes, particularly below Lava Falls. Annual length frequency distributions indicate sizeable numbers of age 0 Humpback Chub were detected in hoop nets in 2014 between Lava Falls and Pearce Ferry; with these fish being captured near the Pumpkin Spring aggregation ~RM 212-216 and sampling below Diamond between ~RM 240-247 (Appendices 1-B and 1-C). Age 0 Humpback Chub were noticeably absent in 2014 in the Havasu-Lava Falls reach (Appendix 1-A), suggesting that the age 0 production in 2014 was a result of mainstem spawning in reaches below Lava Falls and not outmigration from Havasu Creek. Further, 86% of Humpback Chub that have been translocated into Havasu Creek and then recaptured in the mainstem, were caught within 3 miles from the mouth of Havasu Creek (RM ~155-160), suggesting dispersion of adult translocated Humpback Chub from Havasu Creek is predominantly local (Figure 15). Evidence for *in situ* spawning of Humpback Chub below Lava Falls also comes from collection of ripe males and females at least as far down as Columbine reach (RM 273-276). Finally, we have seen much greater representation of juvenile Humpback Chub and Flannelmouth Sucker below RM 156 in 2019, 2020 and 2021 compared to upriver of RM 156 (Van Haverbeke et al, 2020; this report Figure 10-A&B).

Population Levels

Humpback Chub – The overall picture of Humpback Chub in Marble and Grand Canyon is one of decline sometime after the completion of Glen Canyon Dam, followed by more recent increases in abundance. Pre-dam records of Humpback Chub are very scarce, but one account suggests Humpback Chub were very abundant in the LCR during the early 1900s (Kolb and Kolb 1914), and at least one early photograph suggest they were likely plentiful in the mainstem Colorado River in Grand Canyon (Figure 16). After completion of Glen Canyon Dam, it is assumed that changes in the physical parameters of the Colorado River below the dam resulted in a decline of Humpback Chub, primarily attributed to cold hypolimnetic water releases from Glen Canyon Dam (Suttkus 1977, Kaeding and Zimmerman 1983, Minckley 1991). During the early 1990s, Humpback Chub were still abundant enough in the LCR to conduct successful monthly closed mark recapture population estimates (Douglas and Marsh 1996), but obtaining successful population estimates in the mainstem was more problematic because of very low densities (Valdez and Ryel 1995). It appears that Humpback Chub had found refuge in the warmer Little Colorado River where spawning could continue (Suttkus and Clemmer 1977), but that the mainstem portion of chub contracted into a meta population structure of small groups of chub known as "aggregations" (Valdez and Rye 1997). Sometime during the early to mid-1990s, even the Little Colorado River population suffered a decline for unknown causes related to lack of recruitment (Coggins et al. 2006, 2009).

By the early 2000s, even the Little Colorado River inflow population had declined to levels low enough to be of substantial concern. Annual abundance estimates of the adult spring spawning population in the LCR were <3,000 individuals (Van Haverbeke et al. 2013). This triggered a set

of conservation actions, including the removal of non-native rainbow trout in the mainstem near the vicinity of the LCR (Coggins et al. 2011, Yard et al. 2011), and translocation efforts within the LCR (Van Haverbeke 2010, Stone et al. 2020, Yackulic et al. 2021) and into other tributaries in Grand Canyon (Spurgeon et al. 2015, Healy et al. 2020).

The overall decline of Humpback Chub in Marble and Grand Canyon began to visibly reverse post-2006, when the LCR population experienced a significant increase, with adult fish reaching population levels of 4,000-7,000 fish during the spring spawning counts between 2007 and 2014 (Van Haverbeke et al. 2013). This reversal is thought to have begun as early as 2001 and was attributed to increases in juvenile production during the mid-late 1990s (Coggins 2007). Increases in water temperatures from Glen Canyon Dam beginning in ~2003 likely further increased survival of juvenile chub exported to the mainstem. Between 2010 and 2014, increases in the relative abundances (CPUEs) of adult Humpback Chub were detected in the mainstem Colorado River in most aggregations (Persons et al. 2017). These increases were not particularly remarkable but were detectable by pooling CPUE data into multi-year blocks.

After 2014, a substantial change occurred in western Grand Canyon, beginning with spikes of age 0 Humpback Chub that translated into significant increases in the adult population in the following years (Figure 11). Again, this increase was thought to be caused by increases in water temperature, which exceeded ~19 ^{o}C in 2014, easily within suitable spawning temperatures for Humpback Chub (Hamman 1982). The increase appears to have begun in the vicinity of the historical Pumpkin Spring aggregation, with the beginnings being visible as early as 2012 (Figure 12). By 2017, there was a clear signal of a substantial population of adult Humpback Chub present between Pumpkin Spring and Separation Canyon (~RM 210-240; Figure 12). Notably, much of this habitat is characterized by more canyon-bound shorelines with many small and large eddies thought to be conducive to Humpback Chub (Valdez and Ryel 1997). By 2021, our estimate of the adult population in western Grand Canyon (Havasu Rapids to Pearce Ferry) had reached ~46,000 (CI: ~39,000-55,000). Of these fish, we estimate ~24,000 (CI: ~20,000-29,000) adult chub between Diamond Creek and Pearce Ferry. Densities appear to diminish downriver from Separation (below ~RM 240) where shoreline habitat becomes increasingly dominated by the tall clay banks caused by former inundation of Lake Mead. Nevertheless, densities are still surprisingly high at some sites far downriver, such as at Columbine (~RM 273-275).

Humpback Chub spawning and production of age 0 chub may be highly localized in western Grand Canyon, such as on the large gravel debris fans exiting Separation Canyon or associated with springs in the Columbine reach. Although juvenile Humpback Chub were captured in all mark-recapture locations in 2020, we were only able to estimate density of Humpback Chub <100 mm in the Separation and Columbine reaches (Van Haverbeke et al. 2021). At all other mark-recapture reaches in 2020, lack of sufficient captures of chub <100 mm prevented population estimation.

Flannelmouth Sucker – As with Humpback Chub, we applied *p* data to annual catch data since 2010 to estimate abundances of Flannelmouth Sucker ≥200 mm from Havasu Rapids to Pearce Ferry. For 2021 this method estimated ~105,000 (CI: ~91,000-123,000). Of these fish, we estimate ~15,000 (13,000-18,000) between Diamond Creek and Pearce Ferry (Figures 13 and 14). Note, unlike adult Humpback Chub that show highest densities between ~RM 210 and 240, Flannelmouth Sucker ≥200 mm appear to steadily decline with distance downriver from Havasu

Creek (Figure 14). Although adult Humpback Chub have been captured on sampling trips in the mouth of Havasu Creek since the early 1990s (D. Van Haverbeke, pers. obs., USGS unpublished data), numbers have generally been very low, with only a few fish or less captured each sampling event. On the other hand, the mouth of Havasu Creek is recognized as a spawning ground for Flannelmouth Sucker (Douglas and Douglas 2000), and Bluehead Suckers. Nevertheless, given increasing densities of juvenile Humpback Chub and Flannelmouth Sucker downriver from Havasu Creek, it seems clear that both species are spawning in the mainstem in western Grand Canyon, especially from Lava Falls downriver.

Future considerations for mark-recapture efforts in western Grand Canyon-

Thus far, population estimates of Humpback Chub and Flannelmouth Sucker have been derived from applying capture probability (*p*) data to catch data, with capture probably data being obtained during closed mark-recapture efforts (Van Haverbeke et al. 2021). Recall, we used *p* data based on one night of marking and one night of recapture, and using only hoop nets as gear type, because sampling at most sites on aggregation trips is limited to one night of hoop net sampling. Although more precise (lower CVs) abundance and density (fish/mile) estimates are generally obtained from multiple nights of mark recapture, we have used $p1$ *and* $p2$ data based on one night of marking and one night of recapture. A potential problem with this is obtaining sufficient recaptures with only single night mark-recapture efforts, particularly in low density areas, or under high turbidity conditions when catch rates decline.

Second, it is preferred to conduct mark-recapture efforts that contain ≥7 recaptures within each 50 mm size class, and then summing the individual size class estimates for an overall estimate. This would contrast with pooling size classes to obtain ≥7 recaptures for an overall estimate. This philosophy of preferring ≥7 recaptures within 50 mm size classes stems from two considerations. First, capture probability can vary by size class. Second, by conforming to ≥7 recaptures within each 50 mm size class, Seber (1982) we should be 95% certain that bias in the abundance estimation is negligible (Robson and Reiger 1964, Seber 1982 p. 60). Despite this, both methods can be used (summed or pooled estimates), and there generally has not been significantly different estimates between the two methods, although summed estimates usually provide lower CVs. Regardless, when possible, mark recapture efforts should be carried out with intent to maximize recaptures.

Given the current densities of Humpback Chub and Flannelmouth Sucker in western Grand Canyon, it seems advisable to conduct a minimum of either 2 days of marking and 1 day of recapture, or 1 day of marking and 2 days of recapture in each reach, although we cannot provide a hard and fast rule. Estimates become more robust with two or more days of marking and two or more days of recapture as recaptures generally increase within size classes, and CVs generally decrease. For Humpback Chub, estimates of the smaller size classes may require two or more days of marking and recapture. This is because it appeared that the smaller size classes of chub (i.e., <150 mm) did not enter the nets until larger chub had become satiated on bait and vacated, as exhibited in our 3rd day of sampling at Columbine in 2020 (Van Haverbeke et al. 2021).

PIT antennas have marginal value in providing additional marks during closed mark recapture abundance efforts (Van Haverbeke et al. 2021). However, deployment of 3-5 PIT antennas in

addition to hoop netting can increase unique detections appreciably, and thus, are an efficient addition to detection, movement, and survival metrics.
It is possible that some of our abundance estimates could be biased high because of movement in and out of these "closed" mark-recapture reaches. However, average absolute movement between mark and recapture was low (0.31 miles for Humpback Chub and 0.15 miles for Flannelmouth Sucker), supporting use of a closed model. Site fidelity of Humpback Chub has consistently been noted by previous authors (Kaeding et al. 1990, Valdez and Hoffnagle 1999, Paukert et al. 2006, Gerig et al. 2014).

Seining

Seining in combination with the aggregation sample effort provided considerable data and better than expected geographic coverage. Species assemblage and relative abundances track fairly closely with more temporally extensive efforts (Keggeries et al. 2021). Number of backwaters, native and nonnative species sampled, and spatial coverage appear adequate to justify incorporating this effort into future aggregation sample trips when staffing allows.

Summary

We currently think there are many thousands of adult Humpback Chub in western Grand Canyon between Havasu Rapid and Pearce Ferry. We have a limited understanding of varying capture probabilities and densities of chub under varying environmental and habitat conditions, but are working to further refine estimates of abundance by considering habitat quality in the future. We hypothesize that the significant recent increases of Humpback Chub in western Grand Canyon are primarily the result of increased water temperatures being released from Glen Canyon Dam since about 2003, but especially in 2014, and the expansion of riverine habitat below Separation Canyon (from ~RM 240 to Lake Mead; Van Haverbeke et al. 2017). Higher water temperatures are assumedly contributing to faster growth rates, similar to or exceeding those found in the LCR (Figure 17). Little is known about food resources in western Grand Canyon, but captured Humpback Chub have been noted as having algae in them. Other contributing factors to population growth are believed to be available spawning habitat in the form of fresh tributary gravel debris fans, and a reduction in nonnative predators. It is possible that warmer mainstem waters in 2005 and the significant post-2006 expansion of the LCR inflow aggregation (Van Haverbeke et al. 2013) increased larval survival and drift from the LCR, increasing population levels of adult Humpback Chub in western Grand Canyon to critical spawning mass. This possibility, followed by unusually high mainstem water temperatures in 2014 apparently led to population expansions of both Humpback Chub and Flannelmouth Sucker in western Grand Canyon. Interestingly, we do not see this trend with Bluehead Sucker, probably a species more adapted to tributary spawning.

This project has demonstrated the ability to detect trends in CPUE at aggregation sites and benefitted our understanding of recruitment and distribution of Humpback Chub in the Colorado River. Particularly exciting are the findings of a downstream expansion of Humpback Chub in western Grand Canyon. This expansion is evident in the long-term CPUE monitoring data, the length frequency data, and with the mark-recapture efforts, all showing signs of recruitment and a well-represented range of size classes. The incorporation of passive PIT antenna data and the recent successes of closed mark-recapture abundance estimation (in areas outside the extensively

studied JCM reaches) shows the additional utility of the project in finding innovative monitoring strategies for native fish populations. These efforts provide accurate and timely information on the status of native fish populations in support of management decisions regarding key resources in Grand Canyon.

Recommendations

We recommend continued reach specific abundance estimates, and refinement of western Grand Canyon population abundance estimates. In pursuit of this we suggest an annual Diamond down trip paired with the aggregation trip over the next three years and consideration of a second aggregation trip to facilitate abundance estimates of at least three sites above Diamond Creek. We recommend, when possible, to conduct mark recapture efforts with intent of recapturing ≥7 fish per 50 mm size classes. We recommend using antennas to supplement marks (detected fish) during marking events of mark recapture efforts when it is expected that hoop net captures alone will likely not result in ≥7 recaptures. Last, as staffing allows, we recommend incorporating seining into the aggregation effort to monitor Humpback Chub reproduction and aquatic invasive species.

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Table 1. Grand Canyon Humpback Chub aggregation locations based on the aggregations identified by Valdez and Ryel (1995), and on aggregations as modified in Persons et al. (2017). Note, Valdez and Ryel (1995) based river miles (RM) off Belknap and Evans (1989), while Persons et al. (2017) based RM off of Martin and Whitis (2007).

Valdez & Ryel (1995)		Persons et al. (2017)	
Aggregation	RM	Aggregation	RM
30-Mile	29.8-31.3	30-Mile	29.8-36.3
LCR inflow	57-65.4	LCR inflow	57-77.2
Lava Chuar-Hance	65.7-76.3		
Bright Angel	83.8-92.2		
Shinumo inflow	108.1-108.6	Shinumo inflow	107.8-110
Stephen Aisle	114.9-120.1		
Middle Granite Gorge	126.1-129	Middle Granite Gorge	125-129.7
Havasu inflow	155.8-156.7	Havasu inflow	155.8-159.2
Pumpkin Spring	212.5-213.2	Pumpkin Spring	212.5-216

Table 2. Sampling vicinity, date, number of hoop nets deployed, side of the river (left [L], right [R]), and river miles (RM) during 1-17 September 2021 aggregation monitoring trip.

Sample vicinity	**Date**	Nets (L)	RM (L)	Nets (R)	RM (R)	Total Nets
Lees Ferry	9/3/2021	N/A		N/A		
30-Mile	9/4/2021	15	30.5-32	15	30.5-32	30
LCR**	9/5/2021	0	60.2-60.6	0	60.2-60.6	0
Schist Fist*	9/6/2021	15	91.6-93.1	15	91.6-93	30
Lower Garnet*	9/7/2021	20	115.1-117.1	20	115.1-117.1	40
Middle Granite Gorge*	9/8/2021	16	126.7-128.3	16	126.7-128.4	32
Ledges	9/9/2021	15	151.2-152.7	15	151.3-152.8	30
Above Stairway	9/10/2021	20	169.8-171.8	20	169.8-171.8	40
Chevron	9/11/2021	15	182.2-183.7	15	182.2-183.7	30
190 Mile	9/12/2021	15	189-190.5	15	189-190.5	30
Froggy Fault	9/13/2021	15	196.3-197.8	16	196.3-197.8	31
Big Bar	9/14/2021	14	207.7-209.1	14	207.7-209.1	28
Upper 220	9/15/2021	20	218.4-220.4	19	218.4-220.3	39
Travertine Falls	9/16/2021	10	229.9-230.9	11	229.8-230.9	21
244 Mile-Spencer	9/17/2021	15	244.7-246.2	15	244.7-246.2	30
Columbine	9/18/2021	20	273.9-275.9	20	273.9-275.9	40
Totals		225		226		451

Note: Sites with * are within historical aggregation sites defined by Valdez and Ryel (1995). Site with ** (mainstem near Little Colorado River) was only sampled with antennas.

Table 3. Measuring and counting tactics for Flannelmouth Sucker on 2021 Aggregation trip. Flannelmouth Sucker were either measured or counted by three size classes (<150 mm, 150-199 mm, and ≥200 mm). All recaptured PIT tagged individuals were measured, except three that escaped. To increase PIT tags in the general population, an effort was made to PIT tag every 5th Flannelmouth Sucker ≥150 mm that did not already have a PIT tag.

	<150 meas.	<150 count	150-199 meas.	150-199 count	≥200 meas.	≥200 count
30-Mile	0	0	0	0	107	163
Schist Fist	0	0	0	0	115	234
Stephen Aisle	0	0	1	1	172	587
Middle Granite Gorge	1	0	0	4	126	364
Ledges	1	0	4	2	60	232
Above Stairway	2	0	6	9	118	672
Chevron	1	4	20	100	104	511
Frogy Fault	11	26	27	157	93	502
Big Bar	3	43	15	234	63	277
220-Mile	1	69	29	408	39	420
Travertine	6	2	33	81	34	76
244-Mile	6	30	9	132	80	459
Columbine	9	83	19	146	42	62
Totals	41	257	163	1,274	1,153	4,559

Table 4. Adult Humpback Chub (≥200 mm) capture probabilities (*p*1 and *p*2) obtained in sample reaches on aggregation trips 2017-2020. Note: these capture probabilities are for a sampling regime of 1 night of marking and 1 night of recapture using hoop nets only as gear (i.e., in order to be relevant to apply to one night of catch in areas outside of the mark recapture reaches). Hence, only *p*1 is shown for JCM West because *p*2 is reflective of multiple nights of effort using hoop nets and electrofishing.

	JCM West	Ghost	Bridge City	Separation	Spencer-Surprise	250-Mile	Island	Columbine
	~RM 210.7-213.8	RM 227.2-229.2	RM 236.6-238.7	RM 240-242	RM 245.8-247.9	RM 249.7-252.5	RM 265-267	RM 273.9-275.9
2017	0.117							
2018	N/A (only 1 recap)		0.06, 0.179		Insufficient marked			
2019	0.195		0.239, 0.084			N/A (only 4 recaps)		
2020	N/A (only 3 recaps)	0.133, 0.117		0.123, 0.085			0.15, 0.116	0.161, 0.176

Table 5. Numbers of unique fish detected by antennas only, hoop nets only, or in both on 2021 Aggregation trip, where both gears were set concurrently, Colorado River. Note this table does not include antenna detections in the LCR Inflow since hoop nets were not concurrently set in that sample reach, or hoop net data from sites where antennas were not concurrently set with hoop nets. Also note that to make antenna and hoop net numbers comparable, hoop net numbers represent only fish that were recaptured (not those marked [tagged] during the trip).

Species	Antenna Only		Hoop Net Only		Both		Total
	n	% of total	n	% of total	n	% of total	
Common Carp	3	*100.0%*	--	--	--	--	**3**
Flannelmouth Sucker	131	*24.7%*	396	*74.6%*	4	*0.8%*	**531**
Humpback Chub	44	*63.8%*	22	*31.9%*	3	*4.3%*	**69**
Unknown Species	12	*100%*	--	--	--	--	**12**
Total	**190**	***30.9%***	**418**	***68.0%***	**7**	***1.1%***	**615**

Table 6. Total numbers of fish species captured with seine nets by 20-mile river increments on 2021 mainstem Colorado River Aggregation trip.

	Species											
River Mile	**BHS**	**CRP**	**FHM**	**FMS**	**GSF**	**HBC**	**MOS**	**PKF**	**RBT**	**RSH**	**SPD**	**Grand Total**
30-50				107		140			9			256
50-70			31	309		19		116	3			478
70-90		1	36	29		1		90				157
90-110			11	28		4		32			1	76
110-130			24	379		121		121			21	666
150-170			69	131	4	61		34	2		91	392
170-190			22	168		45		98			112	445
190-210	1	1	15	188	1	34		89			501	830
210-230			3	19		19		1			60	102
230-250				12		12		2		2	32	60
250-270			5	23		3	10	5			9	55
Grand Total	**1**	**2**	**216**	**1393**	**5**	**459**	**10**	**588**	**14**	**2**	**827**	3517

BHS = Bluehead Sucker (*Catostomus discobolus*), CRP = Common Carp (*Cyprinus carpio*), FHM = Fathead Minnow (*Pimephales promelas*), FMS = Flannelmouth sucker (*C. latipinnis)*, GSF = Green Sunfish (*Lepomis cyanellus*), HBC = Humpback Chub (*Gila cypha*), MOS = Mosquitofish (*Gambusia affinis*), PKF = Plains Killifish (*Fundulus zebrinus*), RBT = Rainbow Trout (*Oncorhynchus mykiss*), RSH = Red Shiner (*Cyprinella lutrensis*), SPD = Speckled Dace (*Rhinichthys osculus*).

Table 7. Total numbers of fish captured in hoop nets by sample location and species during the 2021 mainstem Colorado River Aggregation trip (i.e., within trip recaptures included). Sampling sites arranged upriver to downriver. Note, no hoop netting was conducted near LCR, only PIT antennas deployed.

	BHS	CRA	CRP	FHM	FMS	FRH	HBC	RBT	RSH	SPD	Grand Total
30-Mile					273	1	2	3			279
Schist Fist					350		2	2			354
Stephens Aisle	1				761		2	1			765
Middle Granite Gorge	1		1		495		1	3		1	502
Ledges					300		18	1		2	321
Above Stairway			2		807	2	45	4		4	864
Chevrons	1				740		44	12		28	825
190-Mile					724		105	6		30	865
Froggy Fault			1	1	816		170	4		83	1,075
Big Bar			2		636		122	2		154	916
220	1		3		966	1	282			32	1,285
Travertine					232		181			73	486
243-Spencer				1	716	3	181	1	1	109	1,012
Columbine	3	1			377		295	1		91	768
Totals	7	1	9	2	8,193	7	1,450	40	1	607	10,317

BHS = Bluehead Sucker (*Catostomus discobolus*), CRA = crawdad, CRP = Common Carp (*Cyprinus carpio*), FHM = Fathead Minnow (*Pimephales promelas*), FMS = Flannelmouth sucker (*C. latipinnis)*, FRH = Flannelmouth Sucker x Razorback Sucker hybrid (*C. latipinnis* x *Xyrauchen texanus*), HBC = Humpback Chub (*Gila cypha*), RBT = Rainbow Trout (*Oncorhynchus mykiss*), RSH = Red Shiner (*Cyprinella lutrensis*), SPD = Speckled Dace (*Rhinichthys osculus*).

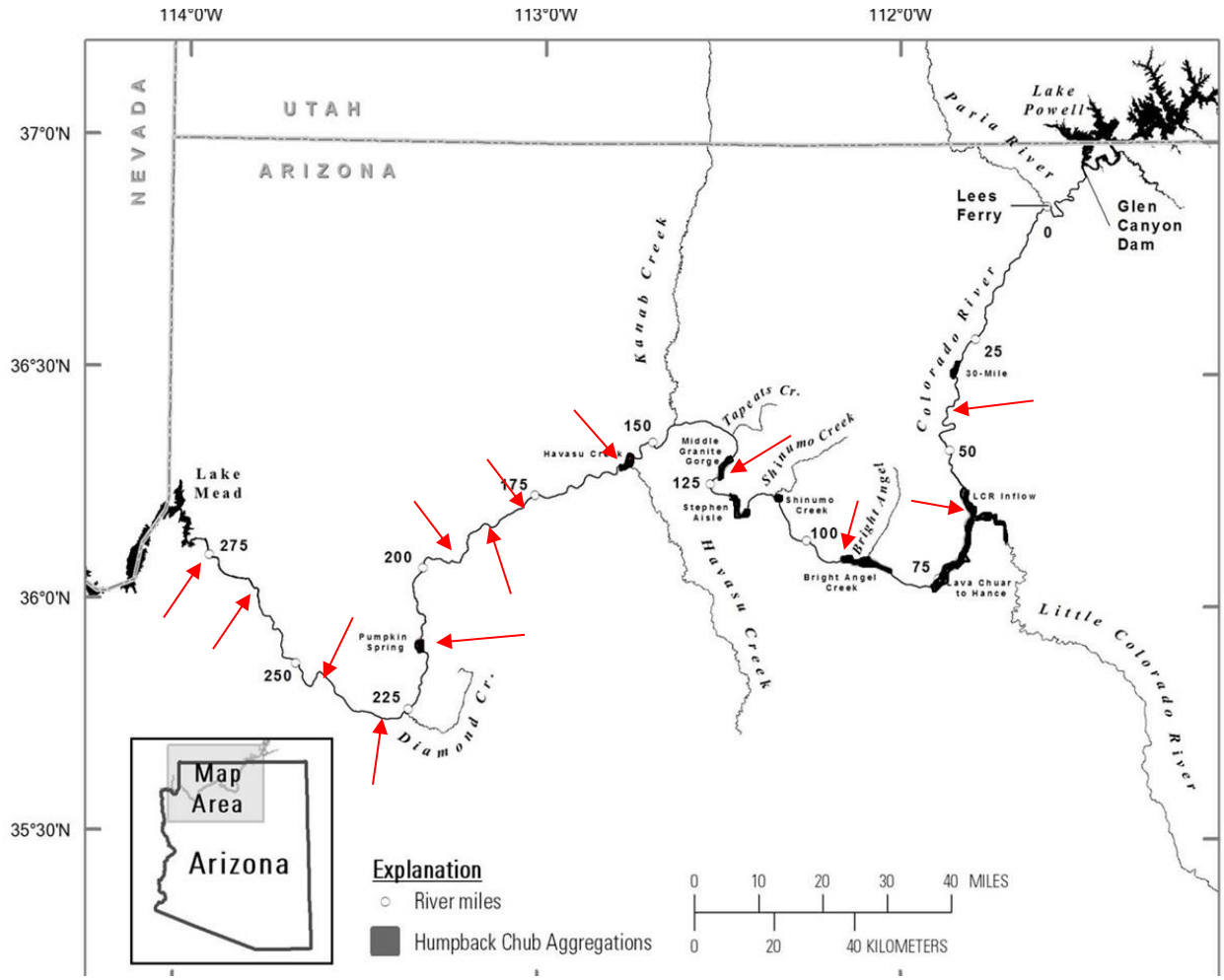


Figure 1. Map of the Colorado River from Lake Powell to Lake Mead showing the nine Humpback Chub aggregations (black), as defined by Valdez and Ryel (1995): 30-Mile, Little Colorado River inflow, Lava Chuar–Hance Rapid, Bright Angel inflow, Shinumo Creek inflow, Stephen Aisle, Middle Granite Gorge, Havasu Creek inflow, Pumpkin Spring. Red arrows indicate locations sampled in 2020. Map: Tom Gushue, GCMRC. Note: distance points shown along the river are in miles.

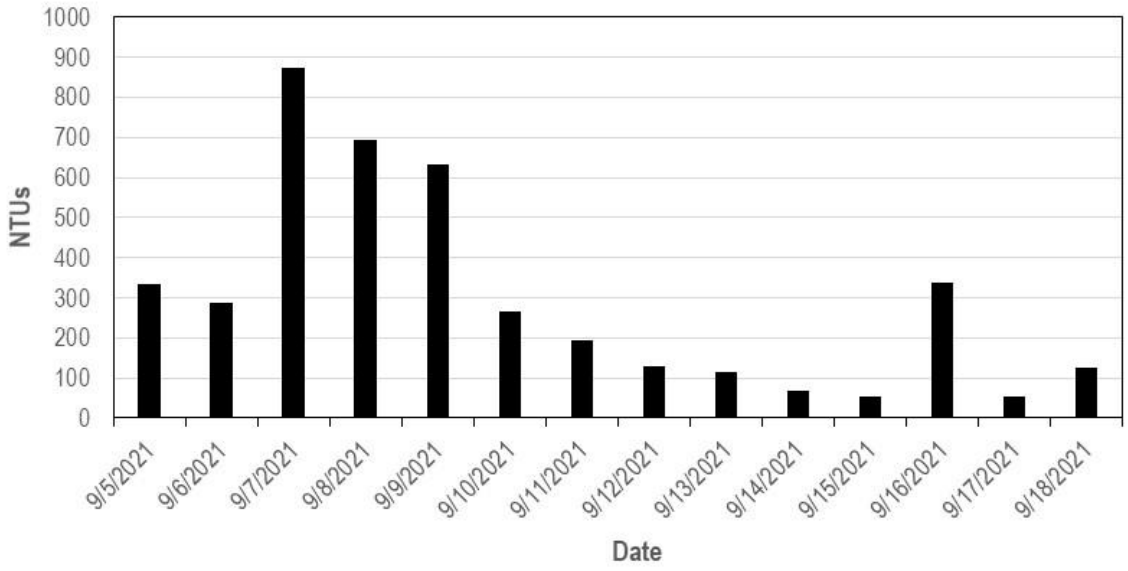


Figure 2. Average daily turbidity (Nephelometric Turbidity Units, NTUs) during September 2021 Humpback chub aggregation monitoring trip.

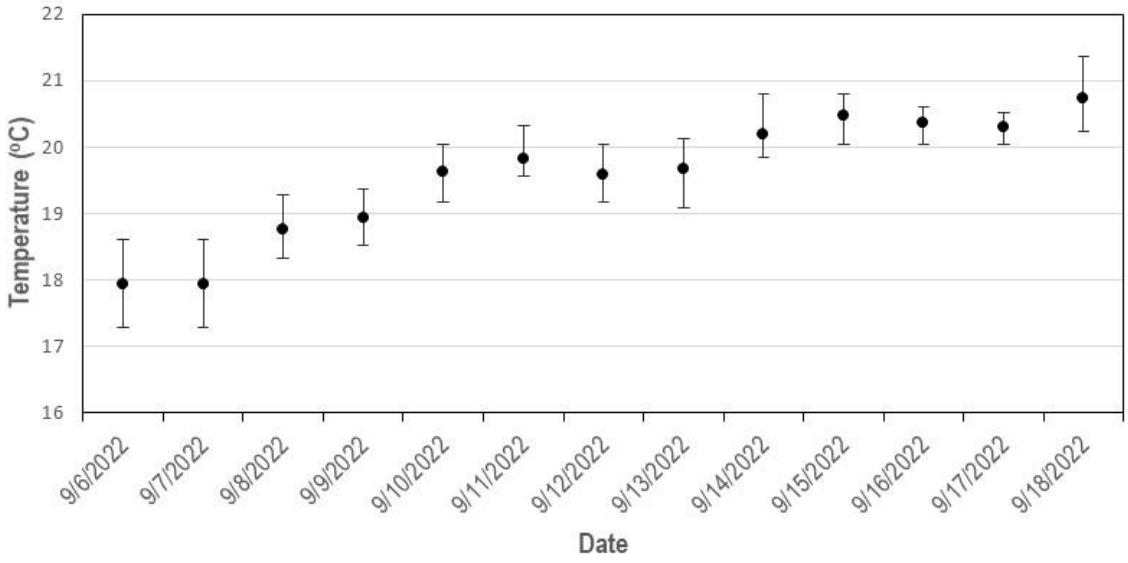


Figure 3. Mean (with minimum and maximum) daily water temperatures in mainstem Colorado River.

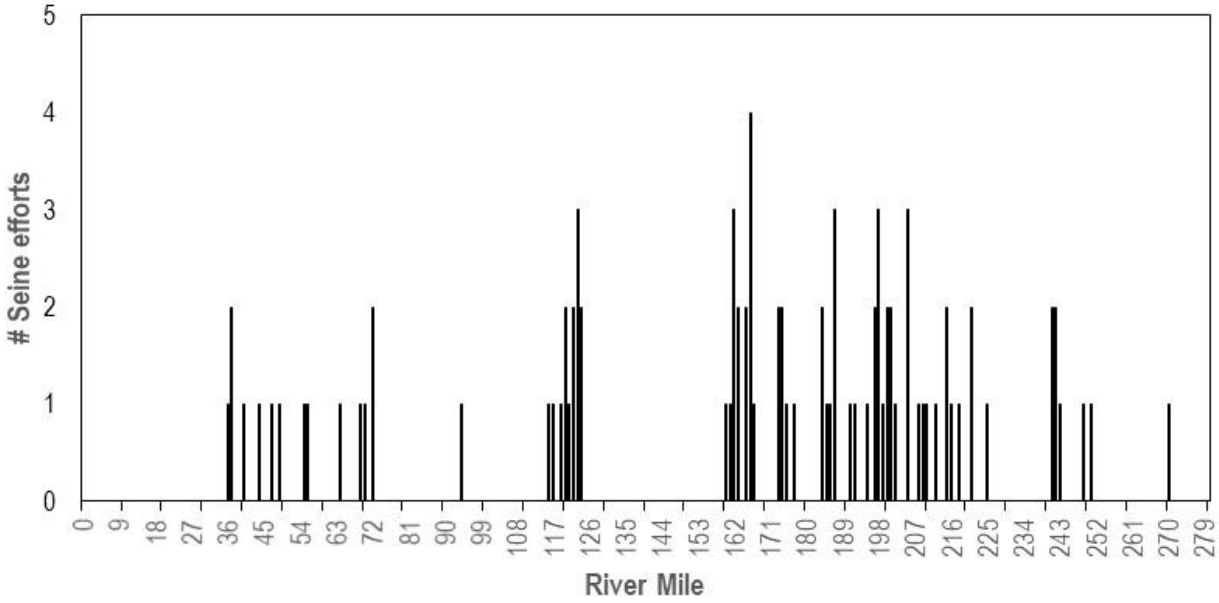


Figure 4. Number of seining efforts by river mile on September 2021 Humpback Chub monitoring aggregation trip.

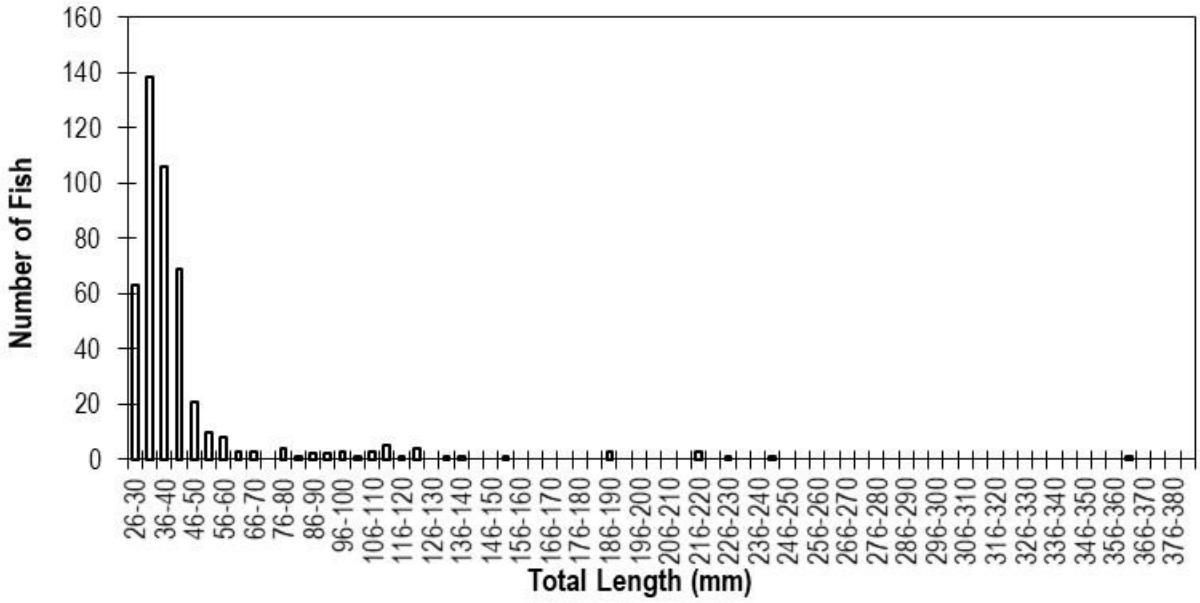


Figure 5. Length frequencies of Humpback Chub captured with seines on the 2021 Humpback Chub aggregation monitoring trip.

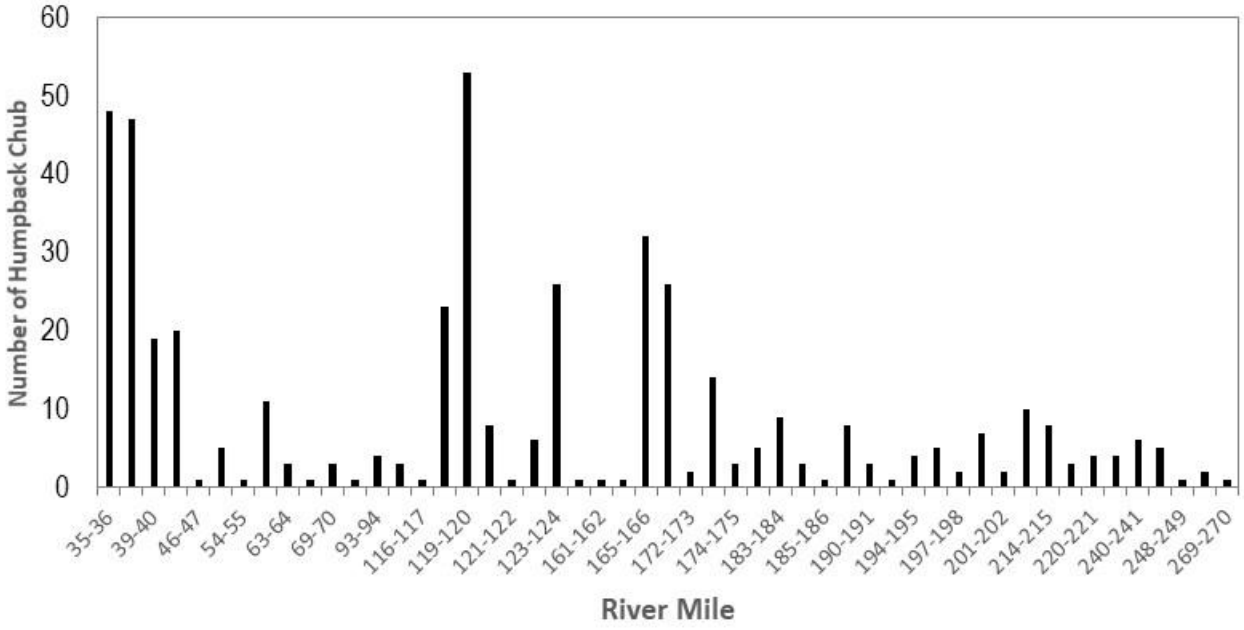
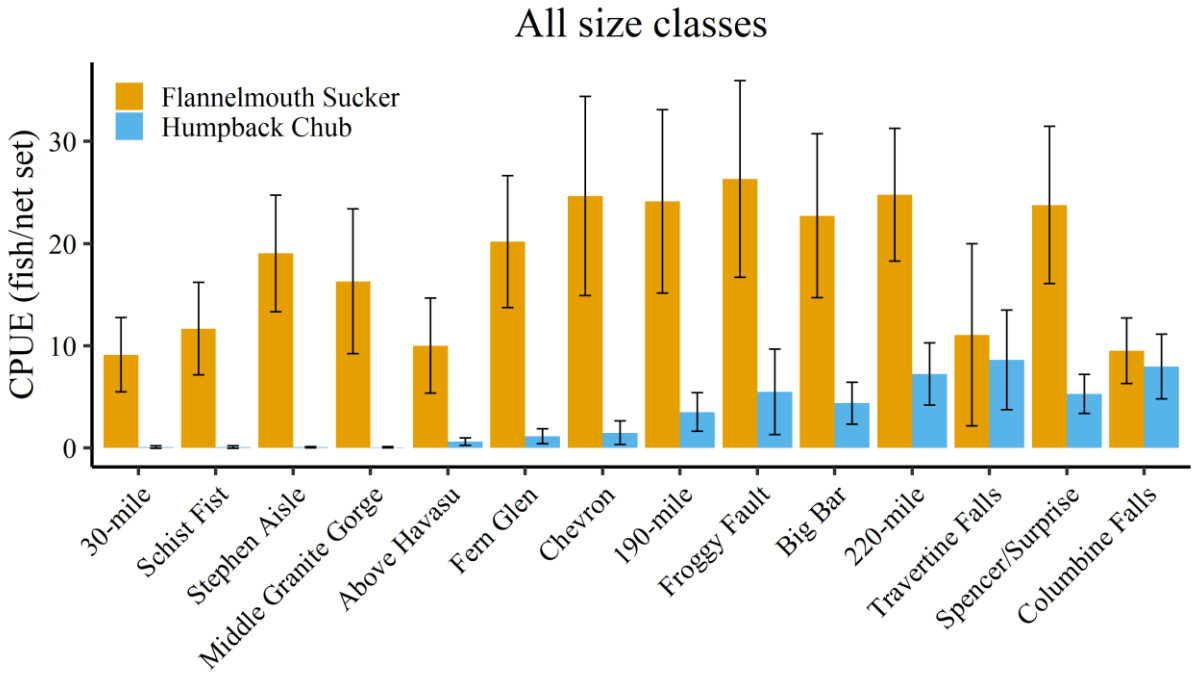


Figure 6. Number of Humpback Chub captured in seines by river mile, September 2022, Colorado River. Note, 432 were <100 mm, 17 were 100-149 mm, 4 were 150-199 mm, and 6 were ≥200 mm.

A)



B)

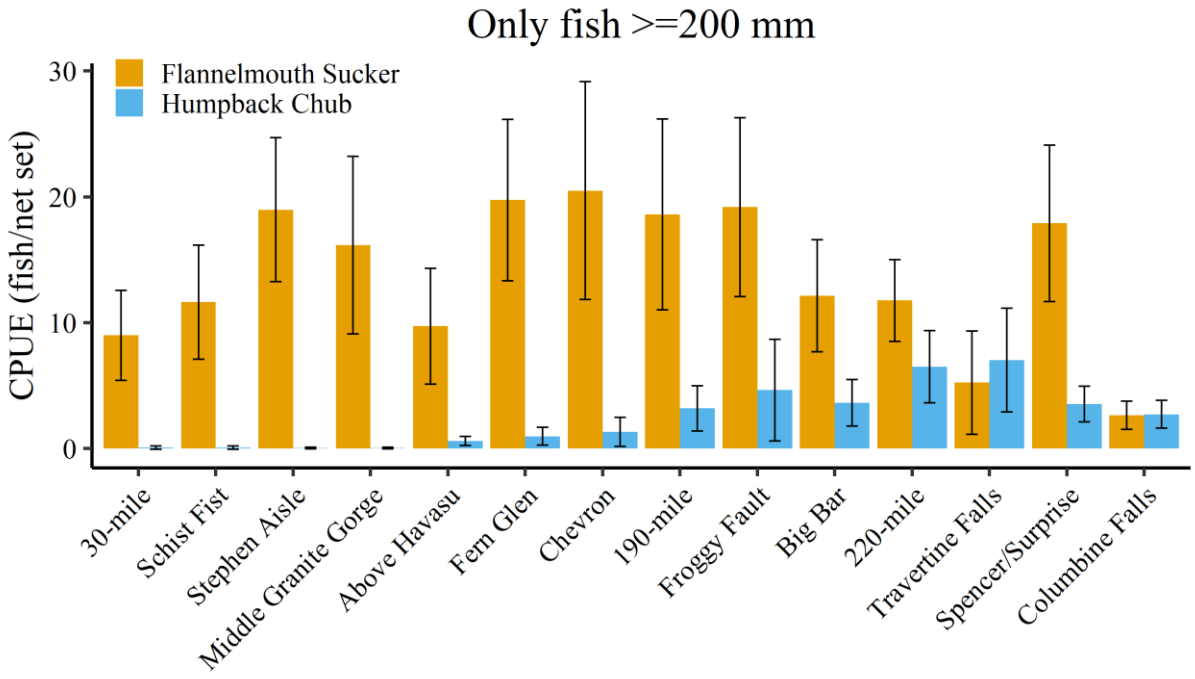


Figure 7. Mean catch per unit effort (CPUE ± 95% CI, captures per overnight hoop net) for Humpback Chub and Flannelmouth Sucker; A) all size classes, and B) fish ≥200 mm at sample locations on the September 2021 Aggregation monitoring trip.

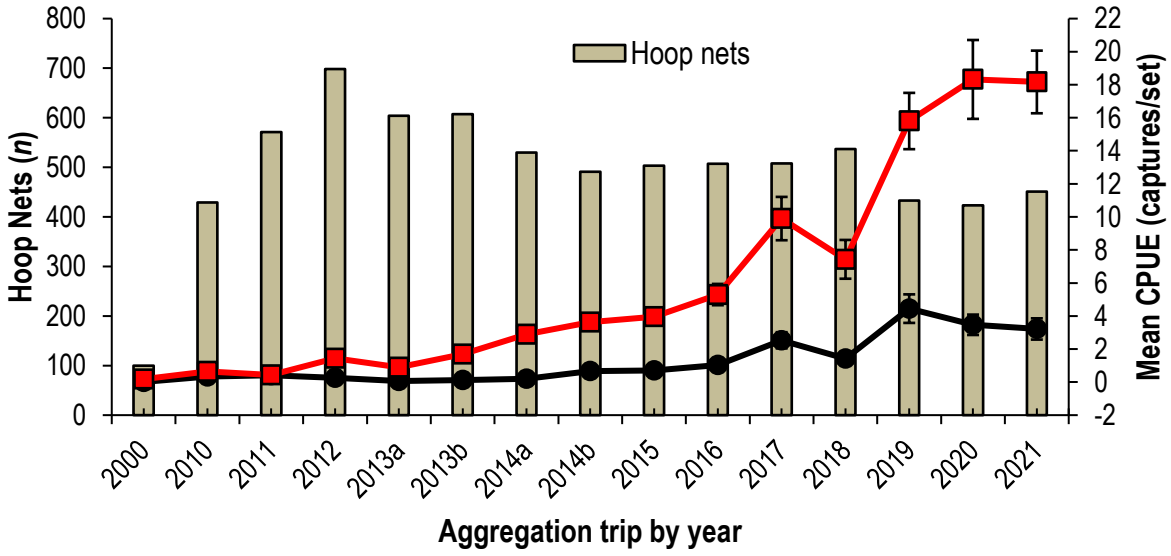
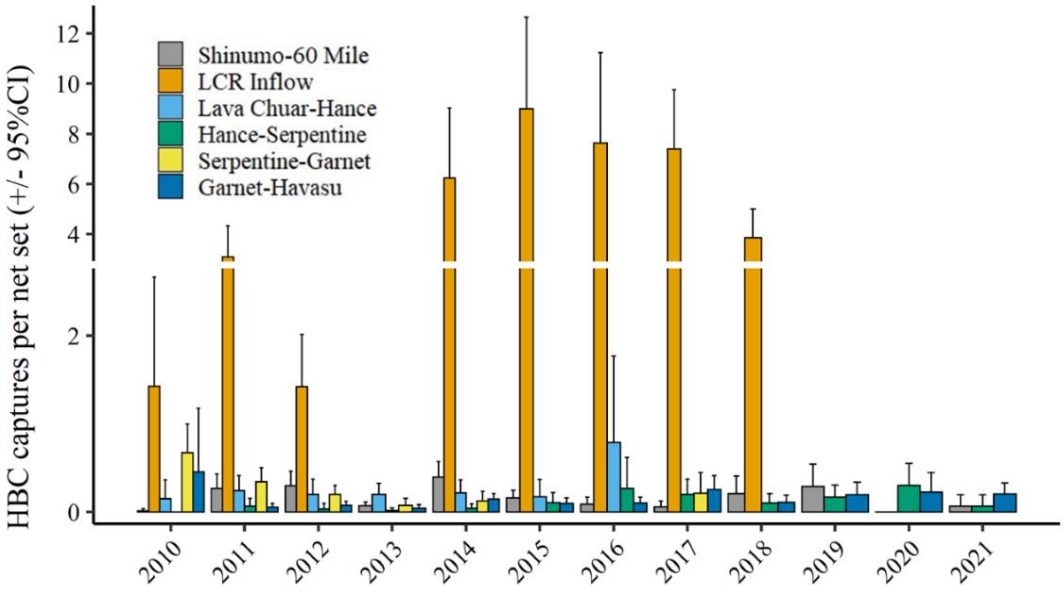


Figure 8. Catch per Unit Effort (CPUE) of Humpback Chub and Flannelmouth Sucker (all size classes) paired with total hoop nets set for each Grand Canyon aggregation trip 2010-2021. Note in 2013 and 2014, two hoop netting aggregation trips (July, September) were conducted. Note: No hoop netting was conducted at the LCR Inflow during 2019, 2020 and 2021, and in 2013 was conducted in LCR Inflow reach above 60-mile rapid where Humpback Chub densities are lower.

A) Above Havasu



B) Below Havasu

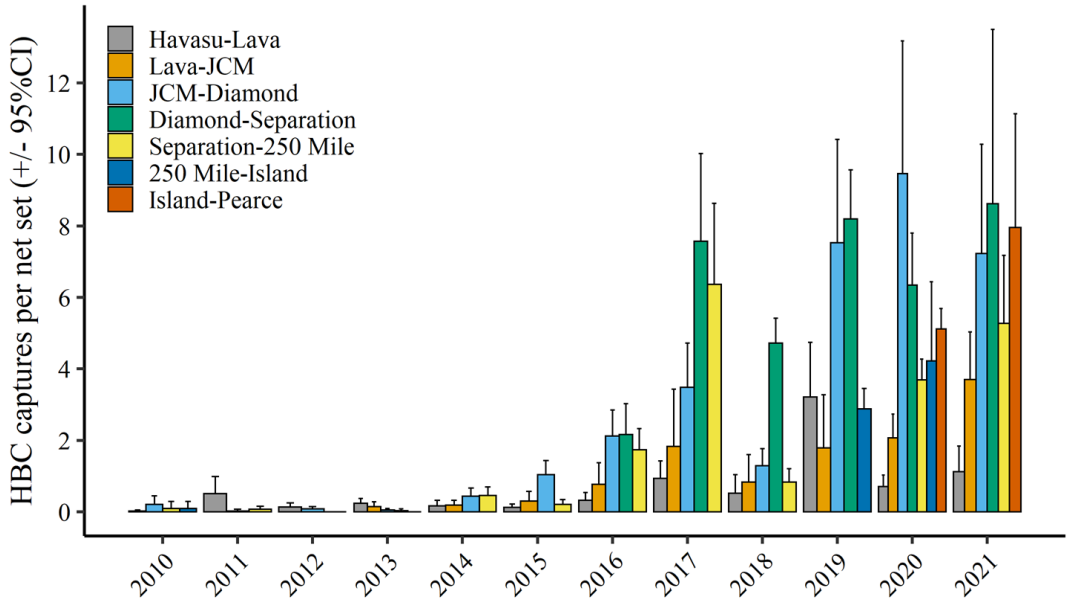
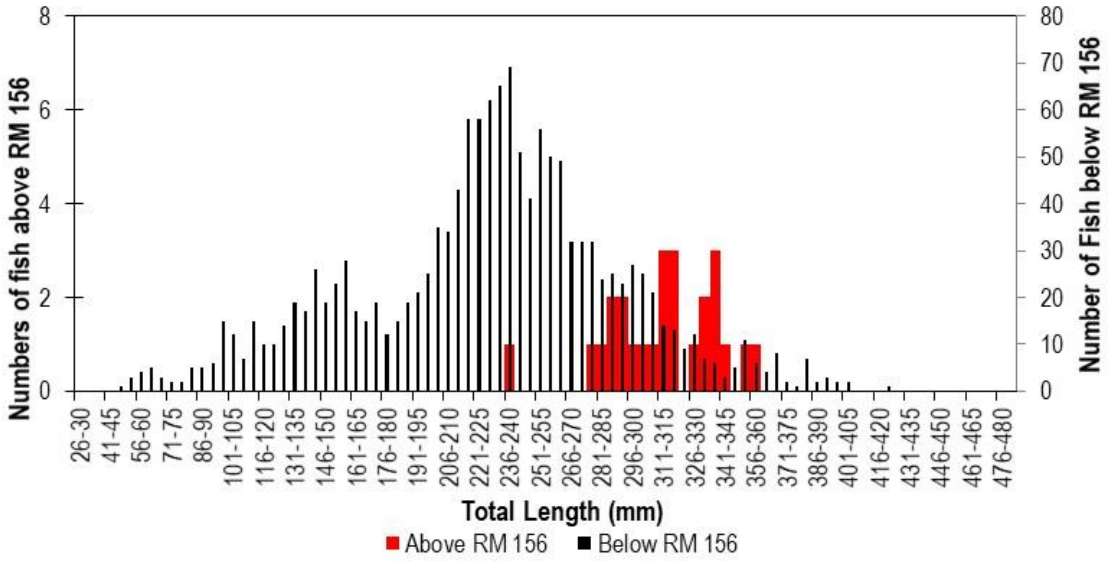


Figure 9. Mean CPUE ± 95% CI, captures per overnight hoop net) of all Humpback Chub for sampling reaches A) from Shinumo Wash to top of Havasu reach (RM 29-156) and B) from Havasu reach to bottom of Columbine reach (RM 157-279), Colorado River, 2010-2021. Note: In Figure 9-A no hoop netting was conducted at the LCR Inflow during 2019, 2020 and 2021, and in 2013 was conducted in LCR Inflow reach above 60-mile rapid where Humpback Chub densities are lower.

A) Humpback Chub



B) Flannelmouth Sucker

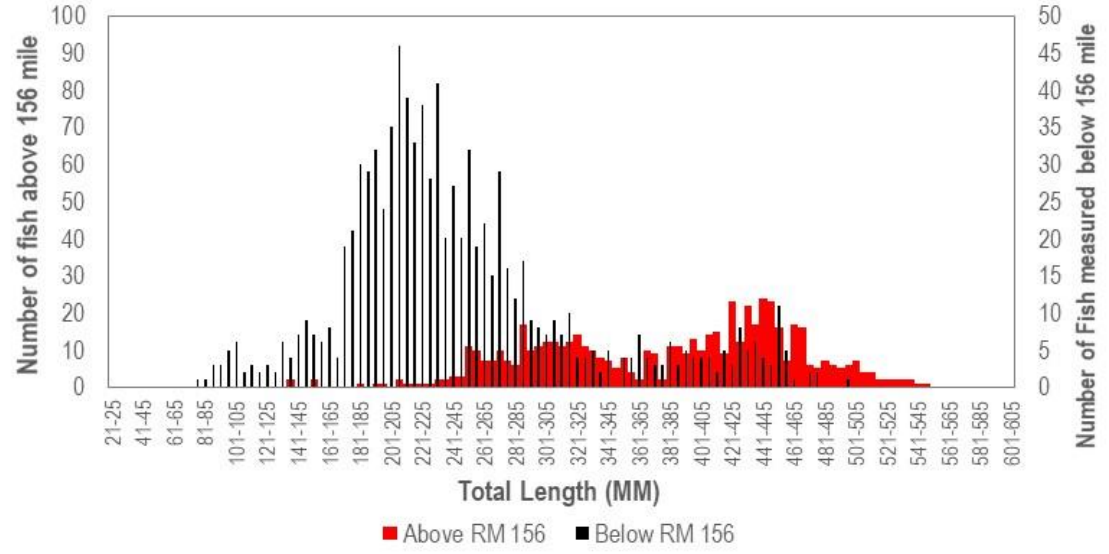


Figure 10. Length frequency distributions for A) Humpback Chub and B) Flannelmouth Sucker captured during the 2021 Aggregation trip above and below RM 156. Note: No fish were sampled from LCR inflow; For Flannelmouth Sucker, representative samples of length measurements were used.

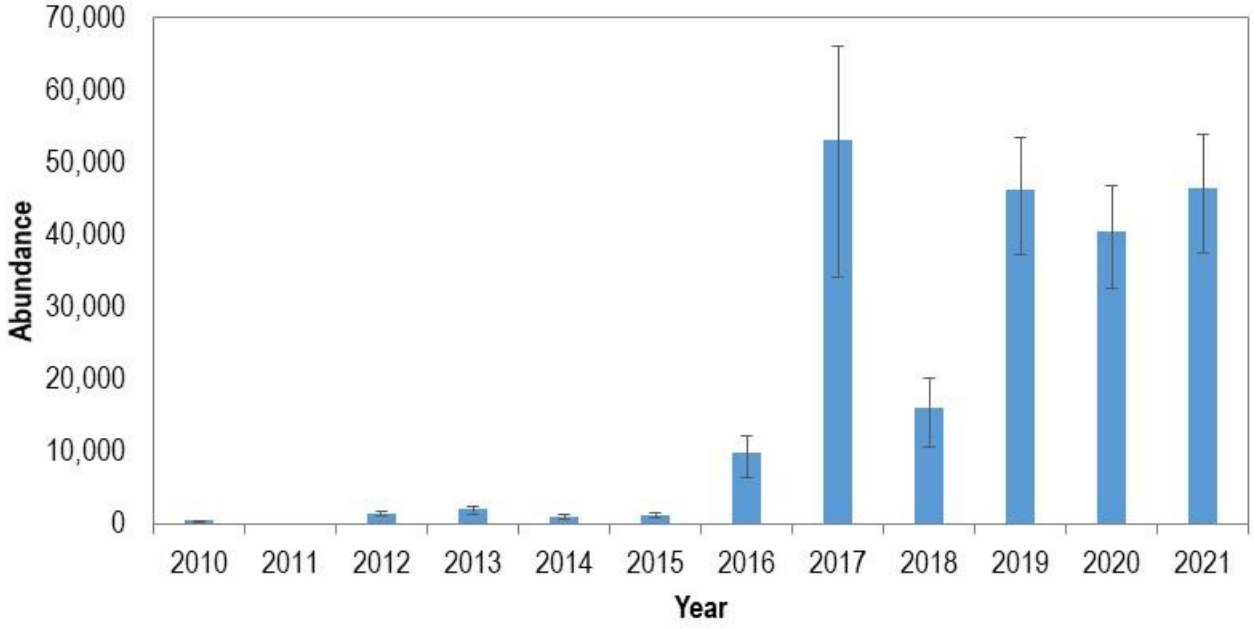


Figure 11. Estimated annual abundances of adult Humpback Chub (≥200 mm) between Havasu Rapids and Pearce Ferry (~river mile 157-280) from 2010 to 2021.

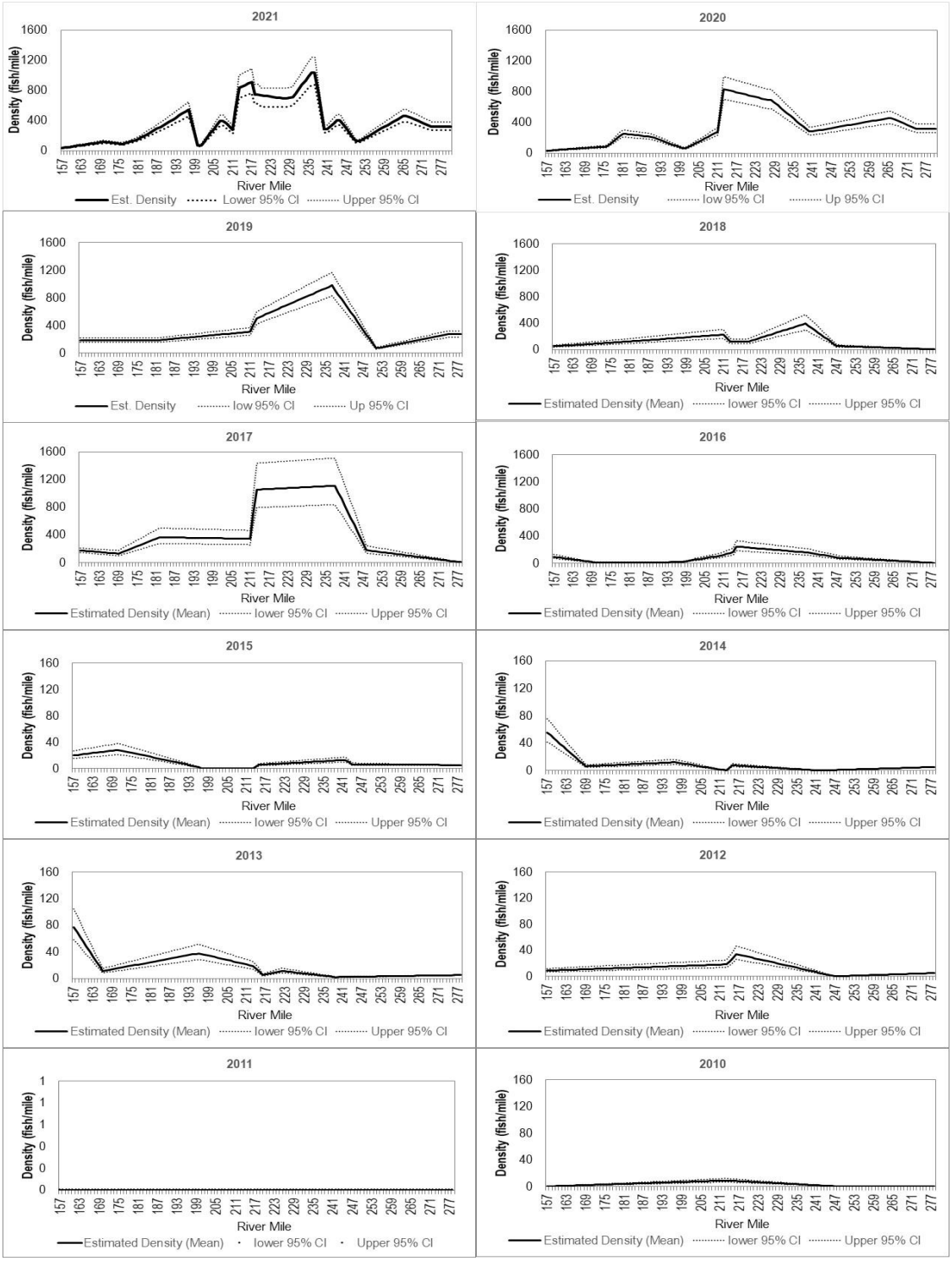


Figure 12. Annual estimated abundances of Humpback Chub ≥200 mm between Havasu rapids and Pearce Ferry (RM 158-280), 2010-2021, as estimated by applying capture probability data to catch data. Note: For 2021, we included density points from three 2019 sites (Travertine, Bridge, and 250-Mile) because turbidity was low during 2019 and 2021. Note 10-fold change in y-axis during 2016-2021.

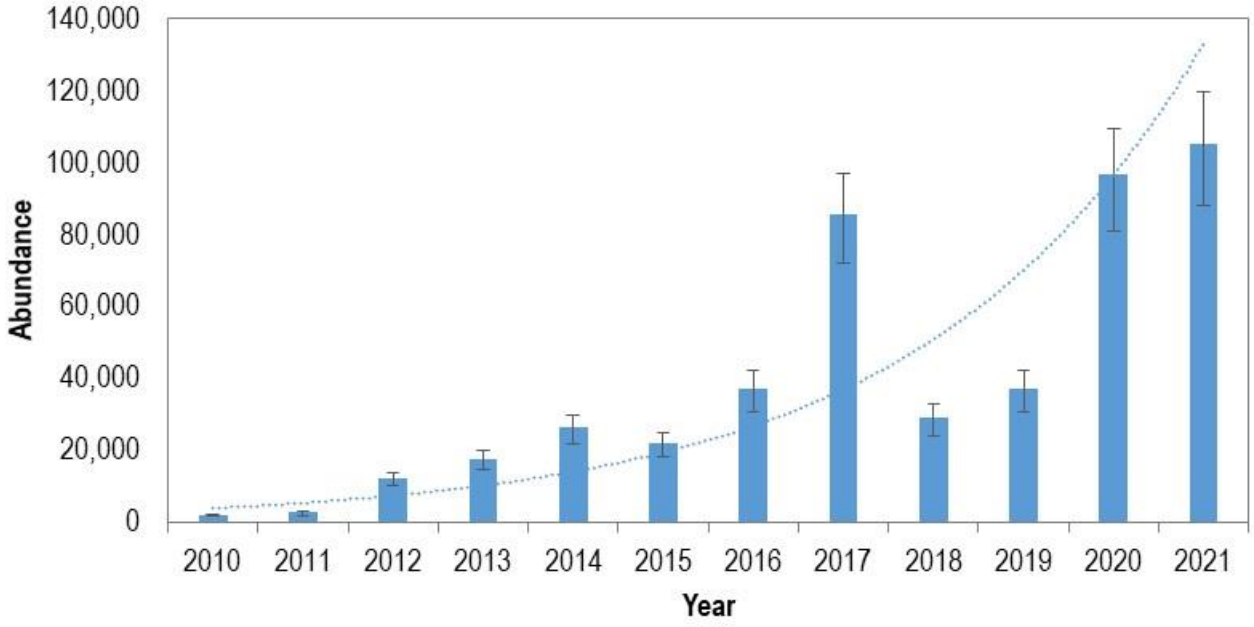


Figure 13. Estimated annual abundances of Flannelmouth Sucker (≥200 mm) between Havasu Rapids and Pearce Ferry (~river mile 157-280) from 2010 to 2021.

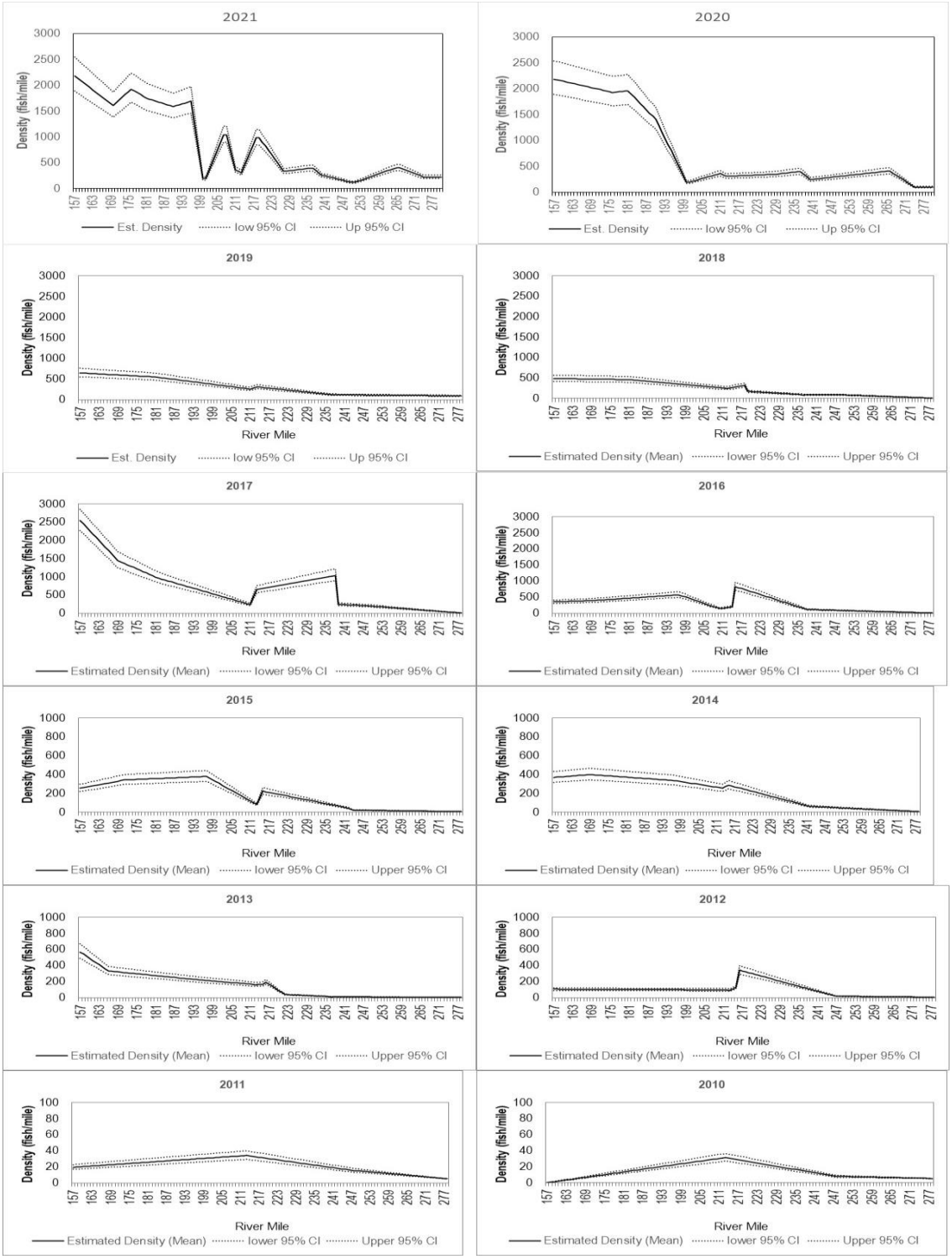


Figure 14. Annual abundances of Flannelmouth Sucker ≥200 mm between Havasu rapids and Pearce Ferry (RM 158-280), 2010-2021, as estimated by applying capture probability data to catch data. Note: For 2021, we included density points from three 2019 sites (Travertine, Bridge, and 250-Mile) because turbidity was low during 2019 and 2021. Note 3-fold change in y-axis post-2015.

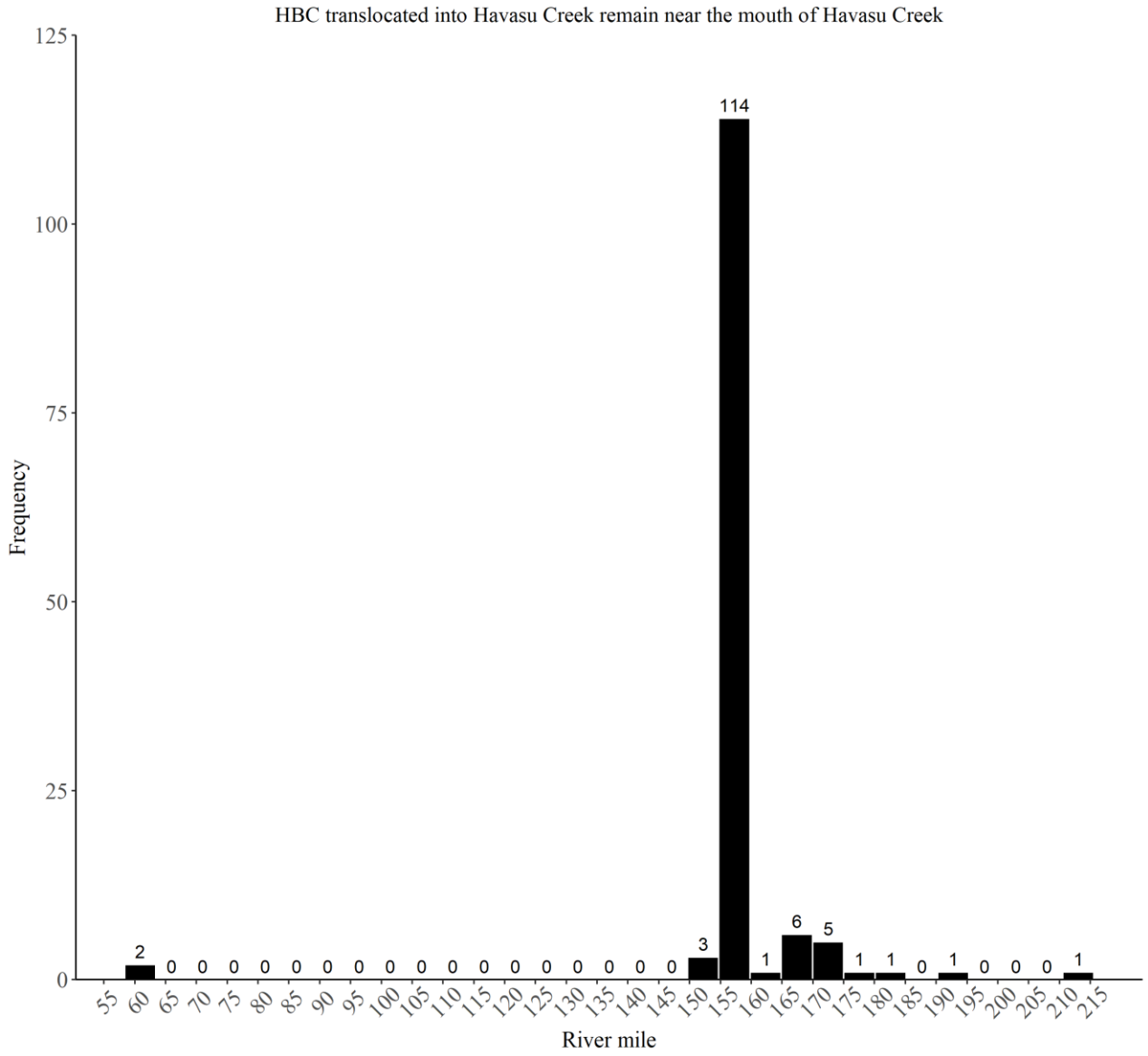


Figure 15. Mainstem recaptures of Humpback Chub that have been translocated into Havasu Creek, as through September 2021. Note: From 2011-2016, National Park Service translocated 1,956 Humpback Chub into Havasu Creek below Beaver Falls. Havasu Creek is 157.26).

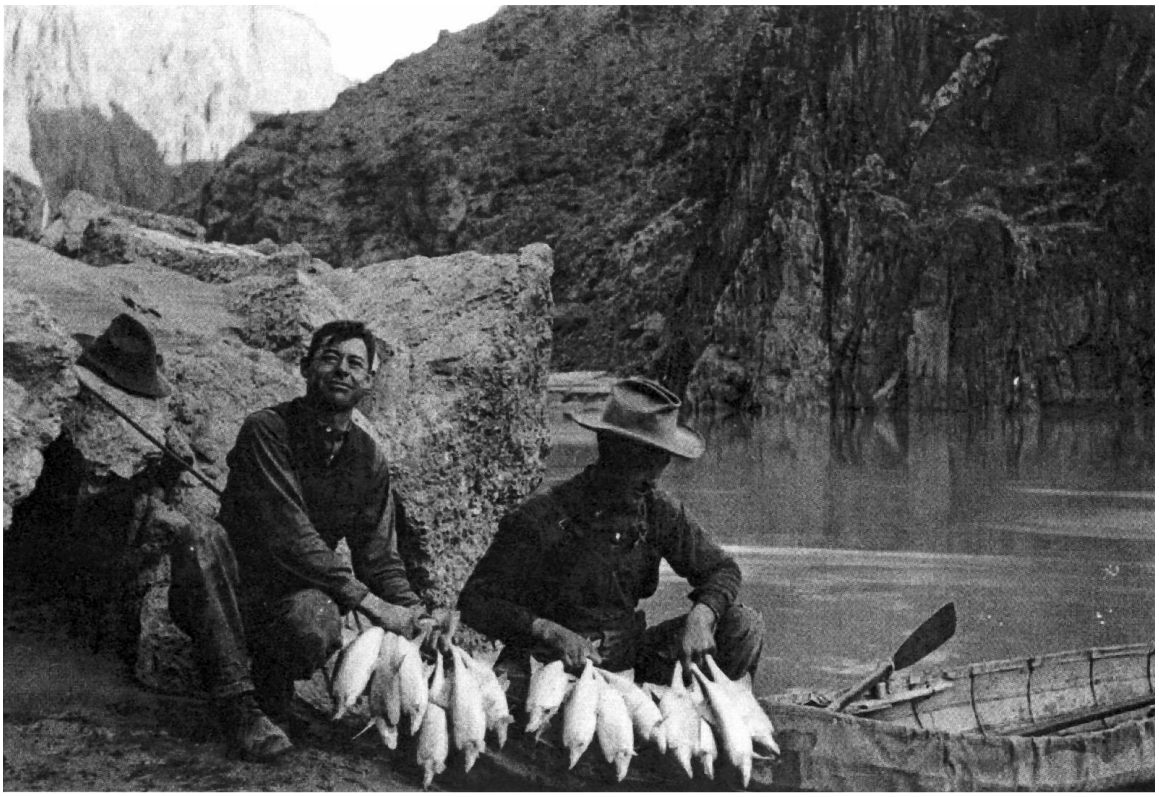


Figure 16. Early photograph of humpback chub taken on the mainstem Colorado River a short distance upriver from Bright Angel Creek. Photograph from Grand Canyon Archive.

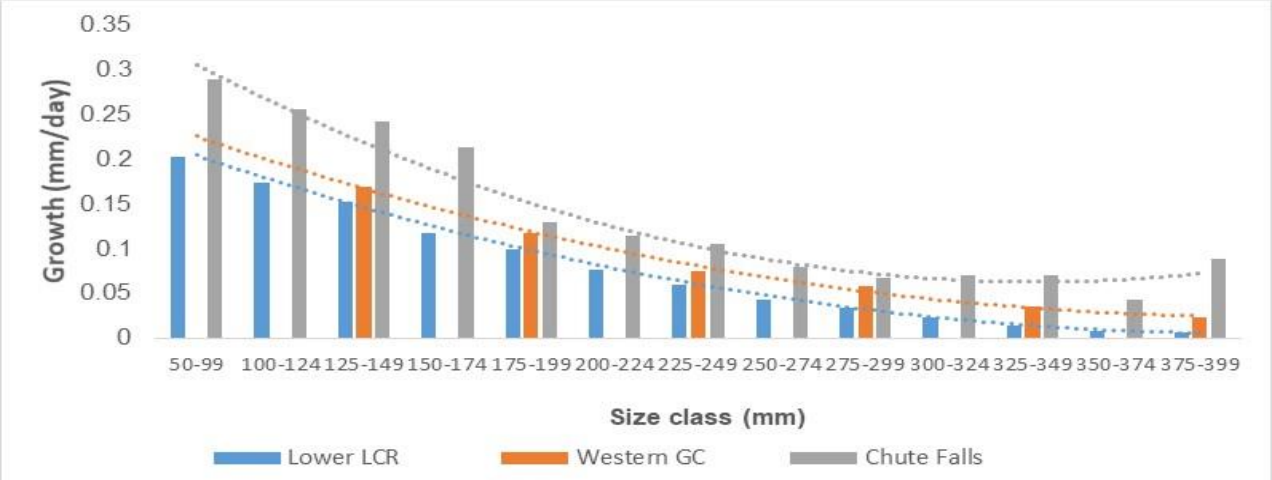
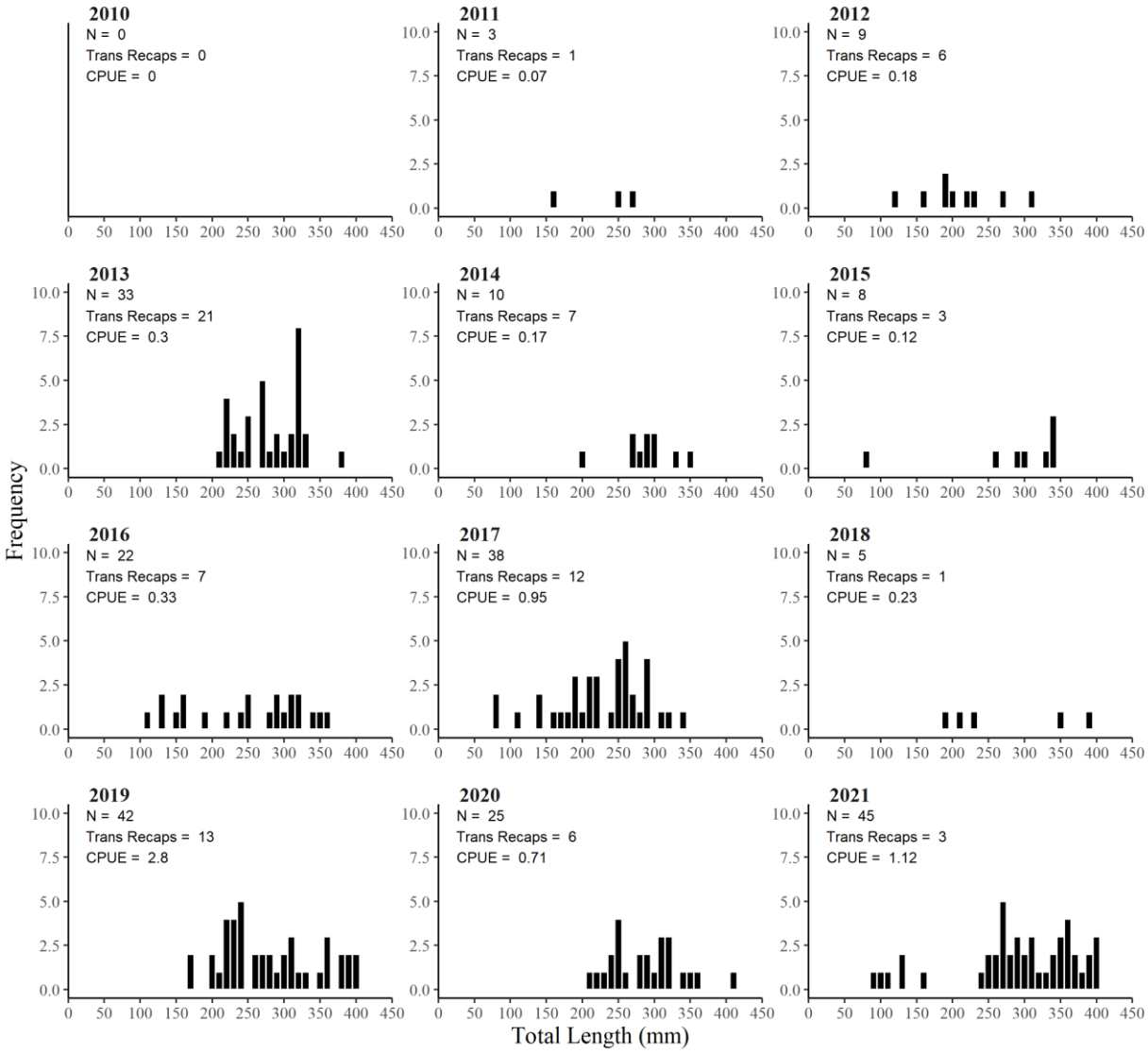
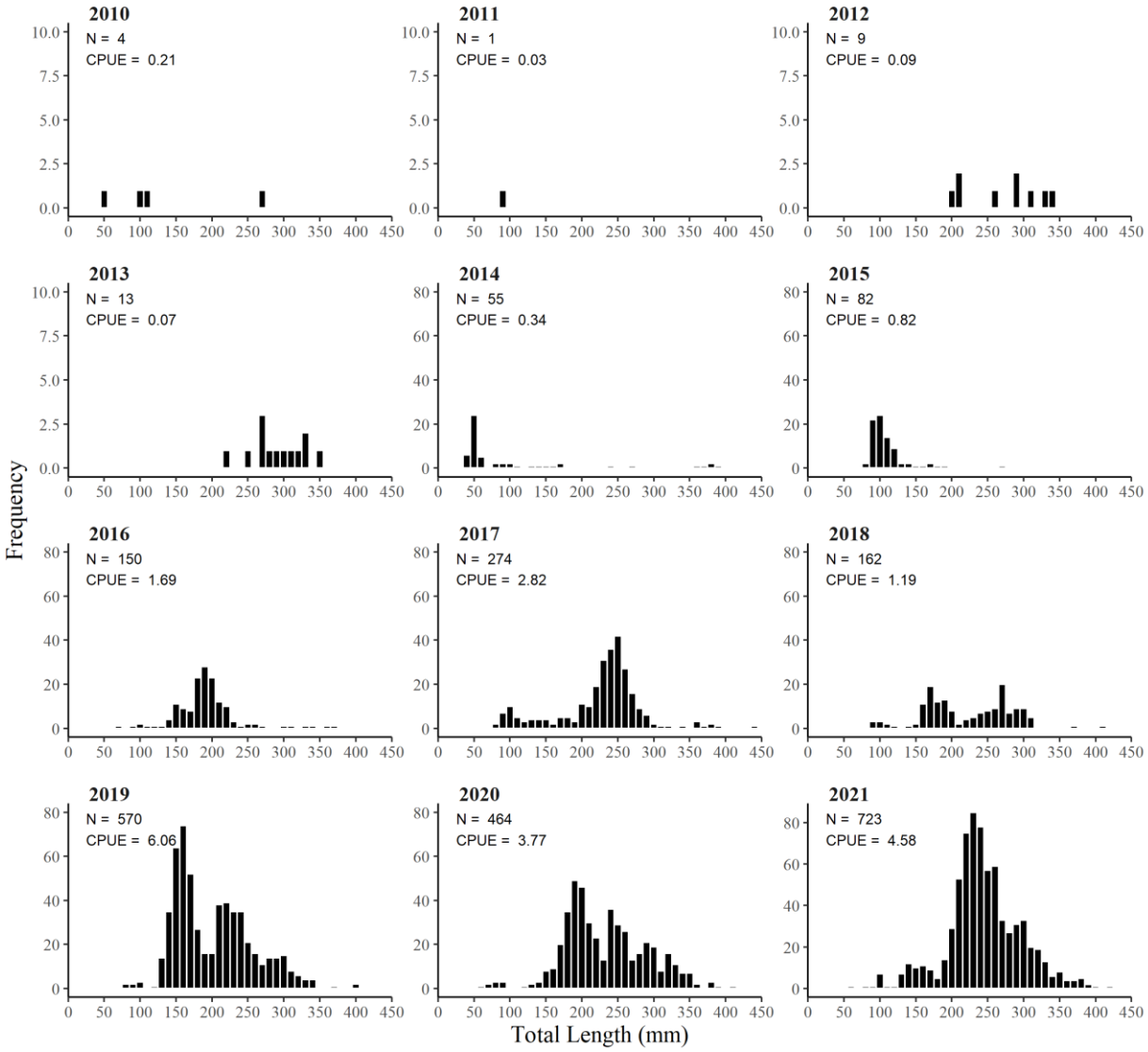


Figure 17. Growth rates (mm/day) of 25 mm size classes of Humpback Chub Density in the lower Little Colorado River (estimated from recaptured fish between river km 0-13.56), western Grand Canyon (estimated from recaptures between river miles 156-253), and Chute Falls (estimated from recaptures between river km 13.57-17.6).

Appendix 1-A. Annual length frequency distributions of Humpback Chub at sites sampled from just above Havasu Creek mouth to Lava Falls (river mile 157.04-180). Note: No Humpback Chub were captured in hoop nets in this reach in 2010. Also, low catches in 2018 was likely negatively affected by high turbidity. Trans recaps = number of chub captured that had been previously translocated into Havasu Creek and recaptured in the mainstem in the Havasu to Lava reach.



Appendix 1-B. Annual length frequency distributions of Humpback Chub at sites sampled between Lava Falls to Diamond Creek in western Grand Canyon (river mile 181-225). Note y-axis change after 2013.



Appendix 1-C. Annual length frequency distributions of Humpback Chub at sites sampled between Diamond Creek to Pearce Ferry in western Grand Canyon (river mile 225-280). Note: No Humpback Chub were captured while sampling below Diamond Creek in 2012. Note y-axis change after 2015.