

# Monitoring Humpback Chub Aggregations in the Colorado River, Grand Canyon Fall 2019



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Cover photo: Pilar Wolters and Kenai “Ninja Fisherman” Van Haverbeke working up fish in the JCM West reach at upper Fall Creek camp eddy, 16 September 2019. Photo by D. Van Haverbeke.

## EXECUTIVE SUMMARY

During fall 2019, two river trips were conducted to monitor Humpback Chub (*Gila cypha*) in the mainstem Colorado River in Marble and Grand Canyons. The first trip (aggregation trip) occurred from 7-23 September. A second trip (Diamond down trip) occurred from 30 September to 8 October. The primary objectives of the aggregation trip were to continue long-term relative abundance (catch per unit effort, CPUE) monitoring of Humpback Chub in the historical “aggregation” sites (Valdez and Ryel 1995), and to mark fish with Passive Integrated Transponder (PIT) tags in three discrete reaches for mark-recapture studies. The objective of the second Diamond down trip was to assess chub catch per unit effort outside of aggregation areas, and served to function as a recapture trip at two of the mark-recapture locations.

During the aggregation trip, we sampled twelve river reaches, each approximately 1-2 miles in length using baited hoop nets. Submersible PIT tag antennas were also deployed within seven of the sample reaches to increase detections of tagged fish. Humpback Chub were captured in all sample reaches, with the highest number of chub captured in hoop nets near Bridge City (river mile [RM] 236.6-238.7,  $n = 1,020$  fish) during two nights of sampling. CPUEs for Humpback Chub generally increased going downriver, particularly below Havasu Creek, reaching a peak of 13 fish/net set at Bridge City. Flannemouth Sucker (*Catostomus latipinnis*) made up the majority of fish captures on the trip ( $n = 6,842$ ), with the highest CPUE (31 fish/net set) in the Havasu sample reach (including 1 net set in Havasu Creek itself).

A second objective of the 2019 aggregation trip was to function as a marking event to conduct closed Chapman Petersen abundance estimates of Humpback Chub and Flannemouth Sucker in three river reaches; these being 1) JCM West (RM 210.2-213.9), 2) Bridge City (236.6-238.7), and 3) 250-mile (249.7-252.5). The follow up Diamond down trip provided a recapture event for the Bridge City and 250-mile reaches. We utilized data from another independent river trip (fall JCM trip, USGS Grand Canyon Monitoring and Research Center) to function as a recapture event for the JCM West reach. Humpback Chub population ( $N$ ) and density (fish/mile) estimates were possible at all three locations, but only at two locations for Flannemouth Sucker. Estimated densities (fish/mile) of adult Humpback Chub ( $\geq 200$  mm TL) in the JCM West, Bridge City, and 250-mile reaches were 291 (95% CI: 204-378), 623 (95% CI: 519-727), and 158 (95% CI: 102-214), respectively. Flannemouth Sucker ( $\geq 200$  mm) estimated densities in the JCM West, and Bridge City reaches were 714 (95% CI: 604-825), and 290 (95% CI: 252-329), respectively.

## 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 endangered Humpback Chub, must be available to managers. Conducting mainstem 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 2018-2020 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 Flannemouth Sucker, Bluehead Sucker, and Speckled Dace populations in the Colorado River ecosystem.

## INTRODUCTION

Humpback Chub (*Gila cypha*) is a federally endangered cyprinid endemic to the Colorado River basin (USFWS 1967; U.S. Endangered Species Act [ESA 1973, as amended]). 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 Flannemouth 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.

The largest aggregation of Humpback Chub inhabits the Little Colorado River and nearby vicinity in the mainstem Colorado River (Douglas and Marsh 1996, USFWS 2002) and is referred to as the Little Colorado River (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, the Humpback Chub aggregations have been sampled using various gear types including baited and non-baited hoop nets, trammel nets, seining, and electrofishing (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).

Since Valdez and Ryel (1995) first described the aggregations of Humpback Chub in Marble and Grand Canyons, resource managers have been interested in estimating abundance of Humpback Chub within these aggregations and, more broadly, in assessing abundance of Humpback Chub at a larger spatial scale in Marble and Grand Canyons. Because of its size, and importance as a tributary associated population in the face of changing Glen Canyon Dam environmental conditions, most progress in estimating Humpback Chub population parameters has come from working on the LCR inflow aggregation (Valdez and Ryel 1995, Douglas and Marsh 1996, Coggins et al. 2006, Coggins and Walters 2009, Van Haverbeke et al. 2013, Dodrill et al. 2015). The LCR inflow aggregation was estimated at 11,000 (95% CI: 7,000-16,000) total adults  $\geq 200$  mm (Yackulic et al. 2014).

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 ( $\geq 200$  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-Hance, Bright Angel inflow, and Stephen Aisle aggregations. In July-September 2001, a closed mark-recapture effort obtained an estimate of 1,044 adult Humpback Chub residing in the mainstem for the LCR inflow aggregation, with  $p_1$  and  $p_2$  capture probabilities of 0.07 and 0.1, respectively (Trammel and Valdez 2003). 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  $p_1$  and  $p_2$  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. Juvenile Humpback Chub density in the LCR inflow of the mainstem has been successfully estimated using hoop nets and electrofishing (Dodrill et al. 2015).

With the exception of the cases described above, monitoring of Humpback Chub aggregations in the Colorado River in Marble and Grand Canyons outside of the LCR inflow aggregation 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 substantial focused and repetitive effort. Since 2017, aggregation trips have focused toward estimating absolute abundances and densities of Humpback Chub at select mainstem locations in Grand Canyon.

In 2017, we worked collaboratively with the USGS Grand Canyon Monitoring and Research Center (GCMRC) to obtain population estimates of Humpback Chub and Flannelmouth Sucker in the mainstem at two locations: the JCM East site below the confluence of the LCR (river mile [RM] 63.4-65.05) and the JCM West site (RM 210.19-213.76; Pillow et al. 2018). Our strategy was to use our late August/early September 2017 aggregation trip as a marking event at these sites and use the October JCM trip conducted by GCMRC biologists as a recapture event in order to conduct closed Chapman Petersen abundance estimates.

In 2018, we again made use of a multiple river trip strategy with the intent of obtaining abundance estimates of Humpback Chub and Flannelmouth Sucker in four discrete reaches of the Colorado River: 1) JCM East (RM 63.4-65.05), 2) JCM West (RM 210.19-213.79), 3) Bridge City (RM 236.65-238.67), and 4) Spencer (RM 245.8-247.9). Of these four reaches, we obtained Humpback Chub population estimates in two of these reaches, JCM East and Bridge City, and Flannelmouth Sucker population estimates in the Bridge City reach (Van Haverbeke et al. 2019).

In 2019, we again used a multiple river trip strategy to estimate abundances of Humpback Chub and Flannelmouth Sucker in the JCM West, Bridge City, and 250-mile (247.9-252.5) reaches. We used these abundance estimates, capture probability data, and CPUE data to formulate absolute abundance estimates for western Grand Canyon. Again, data from the GCMRC JCM trips was utilized to function as recapture events in the JCM reaches in 2017, 2018, and 2019.

## **OBJECTIVES**

1. Obtain August/September 2019 relative abundance (catch per unit effort, CPUE) estimates of Humpback Chub from aggregation sites in Grand Canyon, (e.g., 30-Mile, LCR inflow, Stephen Aisle, Middle Granite Gorge, Havasu Creek inflow, Pumpkin Spring) and compare these estimates to CPUE estimates since 2010.
2. Provide information related to Humpback Chub length frequency distributions, observed community composition, and sexual condition (e.g., ripe, not ripe).
3. Investigate the utility of passive antennae gear for detecting additional fish.

## **METHODS**

### Schedule, Sampling Sites, and Personnel

*Aggregation Trip* - During 7-23 September 2019, we sampled twelve reaches, seven of which were within the Humpback Chub aggregation reaches described by Valdez and Ryel (1995), or as modified by Persons et al. (2017). Four additional reaches outside of the defined aggregation reaches were also sampled (Table 2). Three locations were selected to conduct a marking event for closed mark-recapture population abundance efforts, including JCM West and two sites below Diamond Creek (Bridge City and 250-mile). Participants on the September 2019 aggregation trip were David Van Haverbeke (PI), Kirk Young (PI), Kristy Manuel, and Chase Ehlo (USFWS);

Cory Nielson and Pilar Wolters (AZGFD), Kenai Van Haverbeke, Robin Osterhoudt, and Kirsten Tinning (volunteers); and boatmen Marc Perkins (TL), Jeremy Swindlehurst, Brandon Green, and Derik Spice (Ceiba, Inc.).

*Diamond Down trip* - A separate trip was conducted below Diamond Creek from 30 September to 8 October 2019 to examine sites outside recognized aggregations and to function as a recapture trip for the Bridge City and the 250-mile reaches. Participants on the September/October Diamond down trip were David Van Haverbeke (PI), Kirk Young (PI), Olivia Williams, Tiffany Love-Chezm, and Ryan Green (USFWS); and boatmen Jeremy Swindlehurst (TL), Scott Perry, and Maggie Oliver (Ceiba, Inc.).

*GCMRC JCM trip* - An October 2019 JCM sampling trip conducted by GCMRC personnel was utilized to provide recaptured fish for the closed mark-recapture abundance estimation in the JCM West reach.

### Sampling Gear

*Aggregation and Diamond Down trips* - We sampled all locations with baited hoop nets set overnight. 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  $\frac{3}{4}$  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. We recorded set and pull times and net location (side, river mile, and habitat), and marked net locations on aerial photo maps provided by GCMRC. Hoop nets were deployed from two 4.9 m aluminum hulled Osprey fishing boats with 50-horsepower 4-stroke outboard motors. Hoop nets were tied to shore, and typically set at a depth of less than 3 m. With a few exceptions, hoop nets were set in the afternoon each day between 14:00 h and 19:00 h and pulled the next day between 07:00 h and 13:00 h. If possible, 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 Middle Granite Gorge with three small rapids in the sample reach).

On the aggregation trip, we also deployed 6-8 baited submersible Passive Integrated Transponder (PIT) tag antennas (Marsh & Associates, LLC) overnight within some sampling reaches. We did not employ antennas on the Diamond down trip. The antennas recorded PIT tags, dates, and times of detected PIT tagged fish. Antennas were generally deployed each evening between 16:30 and 19:00 h and retrieved between 06:00 and 13:30 h the following day. Within reaches, we set antennas on both sides of the river, and attempted to distribute the antennas evenly across each reach.

*GCMRC JCM trip* - Dimensions of hoop nets used were as above, but were not baited. Electrofishing was also used as capture gear, and antennas were used as detection gear.

### Data Collection

*Aggregation and Diamond Down trips* - 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. TL was recorded for all other species. All fish lengths herein refer to TL. Fish were scanned for presence of a PIT tag. Untagged

Flannemouth Sucker and Bluehead Sucker  $\geq 150$  mm and Humpback Chub  $\geq 80$  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 (Biomark). We entered data directly into tablet computers set up on each boat; PIT tags were uploaded to the tablet data files via Bluetooth connection. Because of time constraints, not all Flannemouth Sucker were measured for length at 250-mile reach. Water temperature was recorded on the aggregation trip with a Hobo Water Temp Pro V2 (Onset Computer Corp., Bourne, MA) deployed off the freighter boat and set at 15 minute intervals.

#### Catch per Unit Effort (CPUE)

*Aggregation and Diamond Down trips* - We calculated CPUE for hoop nets within each sampling reach as number of fish (Humpback Chub or Flannemouth Sucker) captured per overnight hoop net. Because not all Flannemouth Suckers were measured for TL at the 250-mile reach, we constructed a proportional relationship of total fish to fish  $\geq 200$  mm in western Grand Canyon to estimate our catch of Flannemouth Sucker  $\geq 200$  mm at the 250-mile site (Figure 2).

#### Abundance Estimation

During fall 2019, we conducted Humpback Chub mark-recapture studies in three discrete reaches of the mainstem Colorado River: 1) JCM West (RM 210.2-213.9), 2) Bridge City (236.6-238.7), and 3) 250-mile (249.7-252.5). Flannemouth Sucker mark-recapture studies were only conducted in the JCM West and Bridge City reaches. We used the September 2019 aggregation trip as a marking event in these reaches to conduct a marking event within each reach as part of two-pass Chapman Petersen mark-recapture efforts (Seber 1982). We used data collected on the October 2019 JCM monitoring trip conducted by GCMRC as a recapture event in the JCM West reach, and used data collected on our Sept/Oct Diamond down trip as a recapture event in the Bridge City and 250-mile reaches. 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 telemetry studies which show limited movement (Gerig et al. 2014). We also assumed that mixing of fish within the reaches occurred.

For Humpback Chub, we compare population estimates using 1) hoop net data alone during the marking events, and 2) using both hoop net and antennae data during the marking events. For clarification, antenna data were only used in the marking events to increase the number of marked fish, but were not used in the recapture events because they do not detect the unmarked portion of the population. In the JCM West reach, we utilized non-baited hoop net and electrofishing data in the recapture event.

We determined TL of Humpback Chub detected by antennas by 1) querying the GCMRC database for measured recaptured chub below 157 mile to determine daily growth increments within 50 mm size categories  $\geq 100$  mm FL, 2) using this data to build a predictive logistic growth curve, 3) calculating predicted length for time at large. Using this method, we found a high correlation ( $r^2 = 0.93$ , Figure 3) between predicted and measured lengths of chub.

We calculated abundances using the Chapman Petersen closed population estimator with standard formula presented in Seber (1982, p. 60). Because these mark-recapture efforts were inclusive of

Humpback Chub  $\geq 100$  mm and Flannemouth Sucker  $\geq 150$  mm, we used the method of subcategories described by Seber (1982, pp. 100-101) to apportion Chapman Peterson estimates of the entire sample to estimates of abundance for fish within select size classes (e.g., 100-149 mm, 150-199 mm,  $\geq 200$  mm, etc.). The 95% confidence intervals of the Chapman Petersen abundance estimates were approximated with a normal distribution, following Seber (1982, p. 60) for pooled size class estimates (e.g., Humpback Chub  $\geq 100$  mm and Flannemouth Sucker  $\geq 150$  mm), and Seber (1982, p. 101) for subcategory apportionments (e.g., 100-149 mm). We conformed to the general rule of requiring at least 7 recaptures in order to be 95% confident that bias in the abundance estimation is negligible (Robson and Reiger 1964, Seber 1982 p. 60), and applied this rule to both pooled estimates (e.g.,  $\geq 200$  mm) and to size specific 50 mm categories.

Absolute abundances (N) were transformed into fish densities (fish/river mile) by computing the mean absolute maximum distance individual fish moved (MMDM) between the 2019 marking and recapture events, adding  $\frac{1}{2}$  this distance onto each end of the sampling reach, and then dividing abundance by the adjusted distance (Wilson and Anderson 1985, Karanth and Nichols 1988). For Humpback Chub, MMDM was 0.285 miles in the JCM West reach ( $n = 62$  fish), and 0.225 miles in the Bridge City reach ( $n = 183$  fish), and 0.423 miles in the 250-mile reach ( $n = 41$  fish). For Flannemouth Sucker, MMDM was 0.193 miles in the JCM West reach ( $n = 178$  fish), and 0.01 miles ( $n = 309$  fish) in the Bridge City reach. For example, 0.225 miles were added onto the reach distance of 2.04 miles in the Bridge City reach to estimate densities of Humpback Chub.

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. The same methods were used for Flannemouth Sucker.

JCM West – During the 2019 aggregation trip, we sampled the JCM West between RM 210.2-213.8. Because of safety concerns, we divided the reach into two sections; sampling for one night above Little Bastard Rapid with 45 baited hoop net sets and eight baited antenna, and one night below Little Bastard Rapid with 25 hoop net sets and four baited antenna. During the follow up JCM monitoring trip, GCMRC sampled between RM 210.49-213.88 over the course of six nights (15-20 Oct) with 718 unbaited hoop net sets and 144 electrofishing efforts (2,724 shocking seconds). Data from the JCM West monitoring effort conducted by GCMRC personnel functioned as the recapture event.

Bridge City – During the 2019 aggregation trip, the Bridge City reach was defined as RM 236.6-238.7, and was fished for two nights for a total of 78 baited hoop net sets, and eight baited antenna. On the follow-up Diamond down recapture trip, we deployed 106 baited hoop net sets in the same reach over the course of three nights.

250-Mile – During the 2019 aggregation trip, the 250-mile reach was defined between RM 249.7-251.4, and was fished for two nights for a total of 62 baited hoop net sets, and six baited antenna. On the follow up Diamond down recapture trip, we deployed 121 baited hoop net sets in the reach over the course of three nights.



## RESULTS

### Sampling gear

*Hoop nets* – On the 2019 aggregation trip, we deployed 432 overnight hoop nets in the mainstem Colorado River, plus one in the mouth of Havasu Creek over the course of the 18-day trip (Table 2). The number of net sets per sample location varied based on length of the reach and travel logistics the following day. Mean hoop net density per side of the river was 1.01 net/0.1 mile (SE = 0.007,  $n = 432$  nets). The hoop net set in Havasu Creek was a few meters above the confluence.

On the Diamond down trip, we deployed 243 overnight hoop nets in the mainstem Colorado River over the course of the 8-day trip (Table 3). Mean net density per side of the river was 0.83 net/0.1 mile (SE = 0.02,  $n = 243$  nets). For both trips combined, mean net set time was 19.4 hrs. (SE = 3; Table 4), although in this report we consider CPUE as #fish/overnight net set.

*Antennas* – We also deployed submersible portable PIT tag reading antennas (Marsh and Assoc., Inc., Tempe, AZ) at select sampling locations. Because our primary focus for employing antennas was to detect (mark) additional fish within designated mark-recapture reaches (JCM West, Bridge City, and 250-mile), antennas were not employed at all sampling locations.

We queried antenna detected PIT tags in the GCMRC fish database to determine species, TL, date, and location of the last capture. Of the 727 total unique PIT tags detected only with antennas, most were Humpback Chub ( $n = 398$ , 42%) and Flannemouth Sucker ( $n = 310$ , 23%). There were also three Bluehead Suckers, two Common Carp, and 14 fish of unknown species not available in database records (Table 5).

*Water Temperature* – Mean daily water temperature on the aggregation trip ranged between 15.8-19.2 °C (Figure 4). The highest water temperatures were recorded at the JCM West and Bridge City sites. Water temperature was not taken on the Diamond down trip.

### Fish Captures

On the 2019 aggregation trip, we captured a total of 10,998 fish in 432 hoop nets (Table 6). Of those, 99.4% were native. Flannemouth Sucker made up the majority of fish captured (62%,  $n = 6,842$ ), followed by Speckled Dace (19%,  $n = 2,158$ ), Humpback Chub (18%,  $n = 1,925$ ), and Bluehead Sucker (<1%,  $n = 3$ ). Non-native species captured were one Common Carp (*Cyprinus carpio*), 37 Fathead Minnow (*Pimephales promelas*), one Green Sunfish (*Lepomis cyanellus*), six Rainbow Trout (*Oncorhynchus mykiss*), and 18 Red Shiner (*Cyprinella lutrensis*). Green Sunfish were captured at RM 211.4. In the mouth of Havasu Creek, we captured 47 Flannemouth Sucker (186-472 mm), and five Humpback Chub (220-360 mm) in one hoop net (included in Table 6).

On the Diamond down trip, we captured a total of 6,009 fish in 244 hoop net (Table 7). Of those, 99.7% were native. Flannemouth Sucker made up the majority of fish captured (80%,  $n = 4,816$ ), followed by Humpback Chub (15%,  $n = 912$ ), Speckled Dace (4%,  $n = 261$ ), and Bluehead Sucker (<1%,  $n = 1$ ). Non-native species captured were three Common Carp, 12 Fathead Minnow, and two Red Shiner (Table 7). One hundred and eleven Humpback Chub (157-361 mm) were captured in 12 hoop net sets in a very short reach (RM 229.92-230.83) at Travertine Falls, indicating chub are in dense numbers at this location, as well as lower down in the Bridge City and 250-mile

reaches. We also collected seven Northern Crayfish (*Faxonius virilis*) from the 250 mile reach and observed six newly hatched Spiny Softshell turtles (*Apalone spinifera*) emerging from a sandy bench in camp at RM 249.73, river left (Figure 5).

#### Catch per Unit Effort (CPUE)

CPUEs (# fish/overnight hoop net set) during the 2019 aggregation trip showed a peak for Flannemouth Sucker in the Havasu reach (near RM 158) and a peak for Humpback Chub further downriver at Bridge City (near RM 237). This was true for all size classes of fish combined, and for fish  $\geq 200$  mm (Figures 6-A, B). Ordinarily, there is a spike of Humpback Chub and Flannemouth Sucker CPUE near the confluence of the Little Colorado River, but in 2019 our sampling was conducted at the upper end of the LCR inflow aggregation (i.e., Awatubi, or above 60-mile Rapids) where densities are fewer than near the Confluence.

For all size classes, Flannemouth Sucker CPUE was highest at Havasu (31 fish/net including the Havasu Creek net set, slightly lower without) and Humpback Chub CPUE was highest at Bridge City (13 fish/net; Figure 6-A). For nets between 30-Mile and Middle Granite Gorge reaches, Flannemouth Sucker mean CPUE was 11 fish/net, and for nets between Chevron (~183 mile) and 250-mile reaches increased to a mean of 18 fish/net. Humpback Chub mean CPUE remained low (mean = 0.23 fish/net) until Havasu. From Chevron downriver, mean Humpback Chub CPUE was 7 fish/net. This trend was also true for Humpback Chub and Flannemouth Sucker  $\geq 200$  mm (Figure 6-B).

Considering the historical data, on aggregation trips since 2010 the number of overnight hoop nets per trip has remained relatively steady. However, CPUEs of Humpback Chub and Flannemouth Sucker have increased significantly during this time period (Figure 7). We attribute the decline of CPUEs in 2018 to turbid mainstem water conditions. Much of the elevated Humpback Chub CPUEs seen in the past few years is a result of higher Humpback Chub capture rates in the western Grand Canyon (from Havasu aggregation downriver), where there has been a dramatic increase of Humpback Chub (Van Haverbeke et al. 2017, Rogowski et al. 2018). For example, Pumpkin Spring, Bridge City, and Spencer had been extensively sampled using baited hoop nets prior to 2014, but capture rates for adult Humpback Chub were very low (Figure 7). Since 2014, capture rates indicate a significant increasing trend of Humpback Chub relative abundance in western Grand Canyon (Figure 8; Van Haverbeke et al. 2017). On the 2019 Diamond down trip, CPUEs for Humpback Chub and Flannemouth Sucker were lower at sampling sites below Diamond Creek than they were on the 2019 aggregation trip (Figures 9). We believe this was caused by higher turbidities on the Diamond down trip.

#### Length Frequencies

Length frequency distributions for Humpback Chub and Flannemouth Sucker between two river reaches (above and below RM 156) show that juvenile Humpback Chub size classes comprised a greater proportion of the catch in reaches below RM 156 than above RM 156 (Figure 10-A). We choose RM 156 as a cut-off in this instance because below RM 156 was inclusive of the entire Havasu sampling reach, although strictly speaking, we define “western Grand Canyon” as below Havasu Rapid, or below ~RM 157.5). This pattern was also true for Flannemouth Sucker, only to a greater degree (10-B) where nearly all juvenile and small adult Flannemouth Sucker  $< 300$  mm

were captured in western Grand Canyon and nearly all large adults >300 mm were captured above RM 156. This is also implied for Flannemouth Sucker <200 mm in Figure 2.

We show comparative length frequency distributions of Humpback Chub captured on aggregation sampling trips between two time periods (2010-2013 vs. 2014-2019) to illustrate the dramatic increase in catches of Humpback Chub in western Grand Canyon during the post-2013 timeframe (Figure 11). This change is particularly visible in reaches of river sampled below Lava Falls (~RM 182-253; Figure 11). Of note is that in the 2014-2019 time period, there is strong representation by all size classes of Humpback Chub in western Grand Canyon below Lava Falls. These patterns are further shown in annual western Grand Canyon subreach length frequency charts (Appendices 1-A, B, C), whereby the first substantial signals of age-0 Humpback Chub in western Grand Canyon occurred in 2014, with these fish being captured sampling near the Pumpkin Spring aggregation ~RM 212-216 and sampling below Diamond between ~RM 240-247. Importantly, these small spikes of age-0 fish are followed by fish in the age-1 cohort the next year, indicating a population level effect.

#### Sexual Condition and Parasites

Ripe fish (extruding gametes) encountered on the aggregation trip included one male Humpback Chub, 100 Flannemouth Suckers (all male), and one male presumed Razorback x Flannemouth Sucker hybrid (455 mm). The ripe male Humpback Chub (275 mm) was captured in the Bridge City reach. The ripe Flannemouth Sucker were captured sporadically in all sampled reaches except 250-mile, and the presumed hybrid was captured near Garnet Canyon at RM 115.51. One Humpback Chub (158 mm) was recorded with the anchorworm, *Lernaea* sp., at 250.02 mile. On the Diamond down trip, two ripe male Flannemouth Sucker were captured in the Bridge City reach. One Humpback Chub (263 mm) was recorded with *Lernaea* at 250.05 mile.

#### Abundance Estimation and Density

We provide comparative absolute abundance and density (fish per mile) estimates for Humpback Chub among the JCM West, Bridge City, and 250-mile reaches using two methods: 1) calculating utilizing hoop net data only in the marking events, and 2) calculating utilizing hoop net data plus antenna data in the marking events (Tables 8, 9, and 10 respectively). Further, in the Bridge City and 250-mile reaches we compare estimates and densities between marking fish during the marking event for one and two nights (Tables 9, 10). Note that some parameters improve by marking fish for two nights vs one, or with the addition of antennas in the marking events (e.g., numbers of marked and recaptured fish increase, coefficients of variance decrease, and ability to more confidently estimate abundances within 50 mm size classes increases by maintaining  $\geq 7$  recaptures).

In the JCM West reach, sufficient recaptures ( $\geq 7$ ) were obtained within 50 mm size classes between 100-250 mm to present size class abundance and density estimates (Tables 8-A, B). Similarly for Humpback Chub between 100-300 mm in the Bridge City reach (Tables 9-A, B, C). However, in the 250-mile reach, only the 150-199 mm size class had  $\geq 7$  recaptures (Tables 10-A, B, C), unless estimates were pooled (e.g.,  $\geq 200$  mm).

We also provide abundance and density estimates of Flannemouth Sucker in the JCM West and Bridge City reaches using only hoop net data in the marking event (Table 11-A, B, C). Because of

time constraints in the field and not measuring all individuals, we do not provide a closed abundance estimates of Flannemouth Sucker in the 250-mile reach.

For visual purposes, we compare density estimates (fish/mile) of Humpback Chub and Flannemouth Sucker in the JCM, Bridge City, and 250-mile reaches (Figures 12 and 13). Note that despite differences in marking or detecting fish in the marking event (marking for 1 or 2 nights, or incorporating antenna data), the estimates of density are not significantly different. Recall that while we fished the JCM West reach for two nights, we are essentially marking fish for one night in the reach only because we fish one night above Little Bastard Rapid and one below. Additionally, we have not added antenna data into Flannemouth Sucker estimates because we have not yet built a growth/time relationship as we have for Humpback Chub (e.g., Figure 3).

## **Discussion**

During Sept 2019, 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 two additional mainstem monitoring trips to conduct closed mark-recapture experiments to estimate absolute abundance in three discrete reaches of the river (JCM West, Bridge City, and 250-mile). The latter two of these reaches are below Diamond Creek.

### **Catch per Unit Effort**

Since 1991, there have been four, 3-5 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 2014-2019 (Persons et al. 2017, this study). Similar hoop net sampling methods were used across sampling periods, however, net-baiting techniques shifted from perforated PVC scent tubes to mesh bags in 2011, and during the early period (1991-1993) hoop nets were not baited. 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 substantially during the 2010-2019 period (Persons et al. 2017, Van Haverbeke 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.

On the Sept 2019 aggregation trip, the highest CPUEs for Humpback Chub were in the Bridge City reach (~RM 237), and the highest CPUEs for Flannemouth Sucker were in the Havasu reach (~RM 157). On the 2019 Diamond down trip, CPUEs for Humpback Chub were lower at the Bridge City and 250-mile reach than on the previous aggregation trip, likely a result of increased turbidity on the Diamond down trip. We did not see the typical spike of Humpback Chub and Flannemouth Sucker CPUE near the LCR inflow during the 2019 aggregation trip, likely because we sampled above 60-Mile Rapids (Awatubi reach) that is the upper perimeter of the LCR inflow aggregation where densities are typically lower.

### Length Frequencies

Length frequency distributions for Humpback Chub and Flannemouth Sucker show the presence of high numbers of juvenile Humpback Chub and Flannemouth Sucker in western Grand Canyon below Lava Falls. The source of these recruits remains unknown, but distance from the LCR, 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 downriver. 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 illustrate that the first indication of sizeable numbers of age-0 Humpback Chub were detected in the Lava Falls to Diamond and the Diamond down reaches; with these fish being captured near the Pumpkin Spring aggregation ~RM 212-216 and sampling below Diamond between ~RM 240-247 (Appendices 1-A, B, C). Noticeably absent in 2014 were any age-0 Humpback Chub in the Havasu-Lava Falls reach, 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.

### Abundance Estimation

We provide absolute abundance (N) and density (fish/mile) estimates, and capture probabilities for Humpback Chub in the JCM West, Bridge City and 250-mile reaches, and of Flannemouth Sucker in the JCM West and Bridge City reaches during fall 2019. For Humpback Chub, we provide comparative abundance and density estimates using two methods: 1) calculating absolute abundance and density utilizing hoop net data only in the marking events, and 2) calculating utilizing hoop net data plus antenna data in the marking events. Antenna data was only used in the marking events to supplement the marked population, but were not used in the recapture events because they do not indicate the proportion of non-marked fish. In two reaches (Bridge City and 250-mile), we estimated abundance and density with hoop net data set for one, and for two nights during the marking event. Using the different approaches did not appear to significantly alter abundance or density estimates. However, marking fish for two consecutive nights as opposed to one night generally decreased coefficients of variance, increased capture probabilities in the marking event ( $p$ 1s), and increased our ability to more confidently estimate abundance within 50 mm size classes by increasing recaptures in some size classes. Adding antenna data to the marking events appeared to further augment the above.

We provide pooled estimates of abundance and density (e.g.,  $\geq 150$  mm,  $\geq 200$  mm), and when possible estimates within 50 mm size classes. We recognize that pooled estimates of abundance can be biased because capture probability can vary by size class. However, we do not view this as a large problem because summed size stratified estimates are not significantly different than the pooled estimates, here and in our multiple other estimates in the Little Colorado River. A similar case could be made for adding antenna data into the marking event, since capture probabilities are likely to be different for hoop nets vs antennas. However, our estimates of abundance and density were not significantly different, with or without inclusion of antenna data.

It is possible that some of our closed abundance estimates could be biased high because of movement in and out of these “closed” mark-recapture reaches. However, average absolute movement of both Humpback Chub and Flannemouth Sucker between mark and recapture was low (0.31 miles for Humpback Chub and 0.20 miles for Flannemouth 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).

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 conditions, but are working to refine an estimate of abundance. On the 2017 aggregation monitoring trip we captured 416 unique adult Humpback Chub in 11.9 miles of river sampled between Havasu Rapids and 250-mile (between RM 157.1-249.1). Using a  $p1$  of  $\sim 0.12$  estimated for adult chub in the JCM West reach equals an estimated abundance of 3,400 adult Humpback Chub for this 11.9 miles of river. In 2018, we captured 213 unique adult chub in 11.8 miles of river sampled between Havasu Rapids and Surprise Canyon (between RM 157.1-247.9). Using a  $p1$  of  $\sim 0.14$  for adults estimated in the Bridge City reach equals 1,500 fish in this 11.8 miles of river. In 2019 we captured 907 unique Humpback Chub in 11.5 miles of river sampled between Havasu Rapids and Horse Flat Canyon (between RM 157.1-252.5). Using a mean  $p1$  of 0.195 from the JCM West, Bridge City, and 250-mile reaches equals an estimate of 4,651 for this reach. There are about 121 miles of river between the base of Havasu Rapids and Pearce Ferry.

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, and the expansion of riverine habitat below Separation Canyon (from  $\sim$ RM 239 to Lake Mead; Van Haverbeke et al. 2017). Other contributing factors are believed to be available spawning habitat in the form of large tributary gravel debris fans, especially between Separation Canyon and 250-mile, and possibly drifting of larvae from tributaries such as Havasu Creek or LCR. It is possible that warmer mainstem waters in 2005 and the post-2006 expansion of the LCR inflow aggregation (Van Haverbeke et al. 2013) increased larval survival and drift from the LCR, increasing the very low population levels of adults in western Grand Canyon to critical spawning mass. This possibility, followed by unusually high mainstem water temperatures in 2014 apparently led to population explosions 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 and the length frequency data showing signs of recruitment, with a range of size classes well represented. The incorporation of passive antennae 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.

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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 of 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
Havasus inflow	155.8-156.7	Havasus 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 7-23 September 2019 aggregation monitoring trip.

Sample vicinity	Date	Nets (L)	RM (L)	Nets (R)	RM (R)	Total Nets
Little Redwall**	9/7/2019	12	32.8-34	12	34.5-35.7	24
Awatubi*	9/8/2019	15	58.6-60.1	15	58.6-60.1	30
Cremation*	9/9/2019	15	86.8-88.3	15	86.8-88.3	30
Schist Fist*	9/10/2019	15	91.5-93	15	91.5-93	30
Stephen Aisle*	9/11/2019	20	115.1-117.1	20	115.1-117.1	40
Middle Granite Gorge*	9/12/2019	13	(126.7-126.9)+(127.1-127.5)+(127.6-128.3)	13	(126.7-126.9)+(127.1-127.5)+(127.6-128.3)	26
Havasus (above rapid)*	9/13/2019	1	157.1-157.2	5	(156.7-157.1)+(157.2-157.3)	6
Havasus Creek*	9/13/2019	0		1		1
Havasus (below rapid)**	9/13/2019	6	(158-158.4)+(158.6-158.7)	6	(158-158.3)+(158.5-158.7)	12
Chevron	9/14/2019	11	182.5-183.6	13	182.5-183.7	24
JCM West (upper)	9/15/2019	23	210.2-212.5	22	210.2-212.5	45
JCM West (Lower)*	9/16/2019	13	212.5-213.8	12	212.6-213.7	25
Bridge City	9/17/2019	21	236.6-238.7	21	236.6-238.7	42
Bridge City	9/18/2019	18	236.6-238.3	18	236.6-238.4	36
Bridge City	9/19/2019	N/A		N/A		
250-mile	9/20/2019	16	250.8-252.5	15	250.6-252.2	31
250-mile	9/21/2019	16	249.7-251.4	15	249.8-251.1	31
Cowpie	9/22/2019	N/A		N/A		
Totals		215		218		433

Note: Sites with \* are within historical aggregation sites defined by Valdez and Ryel (1995). Sites with \*\* are within modified aggregation sites in Persons et al. 2017).

Table 3. Sampling vicinity, date, number of hoop nets deployed, side of the river (left [L], right [R]), and river miles (RM) during 30 Sept to 8 Oct 2019 Diamond Down monitoring trip.

<b>Sample vicinity</b>	<b>Set date</b>	<b>Nets (L)</b>	<b>RM (L)</b>	<b>Nets (R)</b>	<b>RM (R)</b>	<b>Total Nets</b>
Travertine Falls	9/30/2019	8	229.9-230.9	8	229.9-230.9	16
Bridge City	10/1/2019	21	236.6-238.6	21	236.6-238.7	42
Bridge City	10/2/2019	20	236.6-238.6	23	236.6-238.7	43
Bridge City	10/3/2019	10	236.7-238.6	11	236.7-238.7	21
250-mile	10/4/2019	23	249.7-252.2	23	249.7-252.2	46
250-mile	10/5/2019	22	249.7-252.2	24	249.7-252.2	46
250-mile	10/6/2019	15	249.7-252.2	14	249.8-252.2	29
Cowpie	10/7/2019	N/A		N/A		
<b>Totals</b>		<b>119</b>		<b>124</b>		<b>243</b>

Table 4. Total number of hoop nets set and mean ( $\pm$ SE) set times for each sampling location along the Colorado River on the 2019 aggregation and Diamond down trips combined. Asterisk designates sampling on the 2019 Diamond Down trip.

<b>Sampling Location</b>	<b>Hoop nets (n)</b>	<b>Mean set time (hrs)</b>	<b>SE</b>
30-mile	24	16.5	0.6
Awatubi	30	15.9	0.4
Cremation	30	16.3	1.0
Schist Fist	30	17.5	0.9
Stephen Aisle	40	17.9	1.4
Middle Granite Gorge	26	16.3	0.8
Havas	18	16.6	1.0
Havas Creek	1	14.6	
Chevron	24	16.3	1.1
JCM West (upper)	45	18.8	2.2
JCM West (lower)	25	16.8	1.2
Travertine Falls *	16	17.2	4.2
Bridge City	78	21.3	2.8
Bridge City *	106	22.0	1.9
250-mile	62	20.0	2.5
250-mile *	121	21.5	2.4
<b>Total</b>	<b>676</b>	<b>19.4</b>	<b>3.0</b>

Table 5. Numbers and percentages of unique fish captured in hoop nets, detected by antennas, and both captured in hoops and detected by antennas on 2019 aggregation trip, Colorado River.

<b>2019 Mainstem Aggregation: Hoop Net Captures vs. Antenna Detections</b>						
<b>Species</b>	<b>Hoop Net Only</b>		<b>Antenna Only</b>		<b>Both</b>	
	n	% Total	n	% Total	n	% Total
Bluehead Sucker	--	--	3	100%	--	--
Common Carp	--	--	2	100%	--	--
Flannelmouth Sucker	918	69%	310	23%	99	7%
Flannelmouth-Razorback Hybrid	2	100%	--	--	--	--
Humpback Chub	411	44%	398	42%	135	14%
Unknown Species	--	--	14	100%	--	--
<b>Total</b>	1,331	58%	727	32%	234	10%

Table 6. Total numbers of fish captured by sample location and species during the 2019 mainstem Colorado River Aggregation trip (i.e., within trip recaptures included). Sampling sites arranged upriver to downriver.

Reach	River Mile	BHS	CRP	FHM	FMS	FRH	GSF	HBC	RBT	RSH	SPD	Totals
Little Redwall	32.8-35.6				221			7	3			231
Awatubi	58.6-60.1				107			8	1			116
Cremation	86.8-88.3				317			6				323
Schist Fist	91.5-93.3				430	1		4				435
Stephen Aisle	115.1-117.2				618	1		3	1			623
Middle Granite Gorge	126.7-128.4				268			10				278
Havasus	156.7-158.7	1			541			56				598
Havasus Creek					47			5				52
Chevron	182.5-183.7				542			43			38	623
JCM West (upper)	210.2-212.5		1		1,009		1	271	1		742	2,025
JCM West (lower)	212.6-213.8			13	430			256			322	1,021
Bridge City	236.6-238.7			18	1,475	5		1,020		12	747	3,277
250-Mile	249.7-252.5	2		6	837			236		6	309	1,396
Totals		3	1	37	6,842	7	1	1,925	6	18	2,158	10,998

BHS = Bluehead Sucker (*Catostomus discobolus*), 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*), GSF = Green Sunfish (*Lepomis cyanellus*), RBT = Rainbow Trout (*Oncorhynchus mykiss*), RSH = Red Shiner (*Cyprinella lutrensis*), SPD = speckled dace (*Rhinichthys osculus*).

Table 7. Total numbers of fish captured by sample location and species during the 2019 mainstem Colorado River Diamond Down trip (i.e., within trip recaptures included). Sampling sites arranged upriver to downriver (see Table 3 for river miles of reaches).

Reach	River Mile	BHS	CRP	FHM	FMS	FRH	HBC	RSH	SPD	Totals
Travertine Falls	229.9-230.9		1		312		111		12	436
Bridge City	236.6-238.6		2	2	1,791	1	509		18	2,323
250-mile	249.7-252.2	1		10	2,713	1	292	2	231	3,250
Totals		1	3	12	4,816	2	912	2	261	6,009

BHS = Bluehead Sucker (*Catostomus discobolus*), 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*), RSH = Red Shiner (*Cyprinella lutrensis*), SPD = speckled dace (*Rhinichthys osculus*).

Table 8. Humpback Chub abundance estimates and densities in JCM West reach using A) only hoop net data in the marking event, and B) hoop net and antennae data in the marking event, Colorado River, 2019. Abundance estimates (N), 95% confidence intervals (95% CI), coefficient of variations (CV), and capture probabilities ( $p_1$  and  $p_2$ ) of size classes of Humpback Chub. M = number of marked fish, C = total number of fish captured in recapture event, and R = number of recaptured marked fish. P = proportion of chub  $\geq 100$  mm within a specific size category. Densities and 95% CI of chub are expressed as estimated fish/mile. Abundance estimates with fewer than 7 recaptures should be interpreted with caution (i.e., cannot be 95% confident that bias in N is negligible, Seber 1981, p. 60).

**A.) Hoops only (1 night marking) - JCM West**

Length (mm)	M	C	R	P	N	SE	95% CI		CV	$p_1$	$p_2$	Density	95% CI	
							Lower	Upper					Lower	Upper
$\geq 100$	513	279	49		2,877	347	2,197	3,558	0.12	0.176	0.096	741	565	916
$\geq 150$	447	214	37	0.84	2,417	355	1,722	3,112	0.15	0.173	0.083	622	443	801
$\geq 200$	230	77	15	0.39	1,131	172	793	1,469	0.15	0.195	0.065	291	204	378
100-149	65	65	12	0.16	457	76	307	607	0.17	0.185	0.185	118	79	156
150-199	217	137	22	0.45	1,286	194	905	1,667	0.15	0.161	0.101	331	233	429
200-249	154	47	12	0.25	732	116	505	959	0.16	0.255	0.078	188	130	247
250-299	52	22	2	0.10	279	51	179	378	0.18	0.091	0.038	72	46	97
$\geq 300$	24	8	1	0.04	120	27	67	173	0.22	0.125	0.042	31	17	44

**B.) Hoops and antennas (1 night marking) - JCM West**

Length (mm)	M	C	R	P	N	SE	95% CI		CV	$p_1$	$p_2$	Density	95% CI	
							Lower	Upper					Lower	Upper
$\geq 100$	645	279	62		2,870	300	2,282	3,458	0.10	0.222	0.096	739	587	890
$\geq 150$	566	214	49	0.85	2,434	316	1,814	3,054	0.13	0.229	0.087	626	467	786
$\geq 200$	312	77	20	0.43	1,229	165	904	1,553	0.13	0.260	0.064	316	233	400
$\geq 250$	119	30	7	0.16	473	70	335	611	0.15	0.233	0.059	122	86	157
100-149	77	65	13	0.15	430	65	302	557	0.15	0.200	0.169	111	78	143
150-199	253	137	29	0.42	1,202	162	884	1,520	0.13	0.212	0.115	309	228	391
200-249	193	47	13	0.26	756	106	548	964	0.14	0.277	0.067	195	141	248
250-299	82	21	4	0.11	330	52	227	432	0.16	0.190	0.049	85	59	111
$\geq 300$	37	8	3	0.05	140	27	87	193	0.19	0.375	0.081	36	22	50

Table 9. Humpback Chub abundance estimates and densities (fish/mile) in Bridge City reach using A) hoop nets only for 1 night in the marking event, and B) hoop nets only for 2 nights in the marking event, and C) and hoop nets and antennas for 2 nights in the marking event, Colorado River, 2019. See Table 5 above for heading descriptions (e.g., M,C, R, N, etc.). Note: Abundance estimates with fewer than 7 recaptures should be interpreted with caution (i.e., cannot be 95% confident that bias in N is negligible, Seber 1981, p. 60).

**A.) Hoops only (1 night marking) - Bridge City**

Length (mm)	M	C	R	P	N	SE	95% CI		CV	p1	p2	Density	95% CI	
							Lower	Upper					Lower	Upper
>=100	684	423	111		2,592	191	2,217	2,967	0.07	0.262	0.162	1,145	979	1,310
>= 150	593	384	103	0.88	2,275	219	1,845	2,705	0.10	0.268	0.174	1,004	815	1,194
>=200	368	222	54	0.54	1,395	139	1,122	1,668	0.10	0.243	0.147	616	496	736
100-149	91	39	8	0.12	318	40	240	395	0.13	0.205	0.088	140	106	175
150-199	225	162	49	0.34	880	92	699	1,060	0.10	0.302	0.218	388	309	468
200-249	194	110	34	0.27	703	76	554	851	0.11	0.309	0.175	310	245	376
250-299	101	70	15	0.16	406	48	312	500	0.12	0.214	0.149	179	138	221
>=300	73	42	5	0.11	286	37	214	358	0.13	0.119	0.068	126	95	158

**B.) Hoops (2 nights marking) - Bridge City**

Length (mm)	M	C	R	P	N	SE	95% CI		CV	p1	p2	Density	95% CI	
							Lower	Upper					Lower	Upper
>=100	934	423	153		2,573	151	2,278	2,869	0.06	0.362	0.164	1,136	1,006	1,267
>= 150	816	384	143	0.88	2,259	185	1,896	2,622	0.08	0.372	0.175	998	837	1,158
>=200	510	222	72	0.55	1,411	120	1,176	1,646	0.09	0.324	0.141	623	519	727
100-149	118	39	10	0.12	314	34	247	381	0.11	0.256	0.085	139	109	168
150-199	306	162	71	0.33	849	76	699	998	0.09	0.438	0.232	375	309	441
200-249	260	110	46	0.27	692	64	566	819	0.09	0.418	0.177	306	250	361
250-299	155	70	20	0.17	438	44	351	525	0.10	0.286	0.129	193	155	232
>=300	95	42	6	0.11	280	31	218	342	0.11	0.143	0.063	124	96	151

**C.) Hoops and antennas (2 nights marking) - Bridge City**

Length (mm)	M	C	R	P	N	SE	95% CI		CV	p1	p2	Density	95% CI	
							Lower	Upper					Lower	Upper
>=100	1,067	423	183		2,460	124	2,217	2,703	0.05	0.433	0.172	1,086	979	1,194
>= 150	946	384	172	0.89	2,180	163	1,860	2,500	0.07	0.448	0.182	962	821	1,104
>=200	625	222	99	0.57	1,408	109	1,194	1,622	0.08	0.446	0.158	622	527	716
100-149	121	39	11	0.11	280	29	223	337	0.10	0.282	0.091	124	99	149
150-199	321	162	73	0.31	772	65	645	898	0.08	0.451	0.227	341	285	397
200-249	300	110	58	0.27	663	57	551	774	0.09	0.527	0.193	293	243	342
250-299	191	70	28	0.18	439	41	359	518	0.09	0.400	0.147	194	158	229
>=300	130	42	13	0.12	299	30	240	359	0.10	0.310	0.100	132	106	159



Table 10. Humpback Chub abundance estimates and densities (fish/mile) in 250-mile reach using A) hoop nets only for 1 night in the marking event, and B) hoop nets only for 2 nights in the marking event, and C) and hoop nets and antennas for 2 nights in the marking event, Colorado River, 2019. See Table 5 above for heading descriptions (e.g., M,C, R, N, etc.). Note: Abundance estimates with fewer than 7 recaptures should be interpreted with caution (i.e., cannot be 95% confident that bias in N is negligible, Seber 1981, p. 60).

**A.) Hoops only (1 night marking) - 250 mile**

Length (mm)	M	C	R	P	N	SE	95% CI		CV	p1	p2	Density	95% CI	
							Lower	Upper					Lower	Upper
>=100	83	221	21		847	144	564	1,129	0.17	0.095	0.253	263	175	350
>= 150	76	189	20	0.87	733	169	401	1,065	0.23	0.106	0.263	227	124	330
>=200	34	84	4	0.40	341	82	181	502	0.24	0.048	0.118	106	56	156
100-149	7	32	1	0.13	114	31	53	174	0.27	0.031	0.143	35	16	54
150-199	42	105	16	0.46	392	93	209	575	0.24	0.152	0.381	122	65	178
200-249	24	39	3	0.21	179	46	90	269	0.25	0.077	0.125	56	28	84
250-299	9	24	1	0.11	96	27	43	148	0.28	0.042	0.111	30	13	46
>=300	1	21	0	0.08	66	20	27	105	0.30	0.000	0.000	20	8	33

**B.) Hoops (2 nights marking) - 250 mile**

Length (mm)	M	C	R	P	N	SE	95% CI		CV	p1	p2	Density	95% CI	
							Lower	Upper					Lower	Upper
>=100	200	221	36		1,205	161	889	1,521	0.13	0.163	0.180	374	276	472
>= 150	181	189	35	0.87	1,049	181	694	1,403	0.17	0.185	0.193	325	215	435
>=200	88	84	9	0.42	510	92	329	691	0.18	0.107	0.102	158	102	214
100-149	19	32	1	0.13	156	33	91	222	0.21	0.031	0.053	49	28	69
150-199	93	105	26	0.45	538	97	348	728	0.18	0.248	0.280	167	108	226
200-249	40	39	4	0.19	235	47	144	326	0.20	0.103	0.100	73	45	101
250-299	26	24	3	0.12	147	32	85	209	0.22	0.125	0.115	46	26	65
>=300	22	21	2	0.11	128	29	72	184	0.22	0.095	0.091	40	22	57

**C.) Hoops and antennas (2 nights marking) - 250 mile**

Length (mm)	M	C	R	P	N	SE	95% CI		CV	p1	p2	Density	95% CI	
							Lower	Upper					Lower	Upper
>=100	217	221	40		1,179	148	889	1,470	0.13	0.181	0.184	366	276	456
>= 150	198	189	39	0.87	1,031	168	701	1,361	0.16	0.206	0.197	320	218	422
>=200	103	84	13	0.50	590	100	394	785	0.17	0.155	0.126	183	122	244
100-149	19	32	1	0.13	148	30	89	208	0.21	0.031	0.053	46	27	64
150-199	95	105	26	0.44	516	88	343	689	0.17	0.248	0.274	160	106	214
200-249	47	39	5	0.20	240	45	152	328	0.19	0.128	0.106	74	47	102
250-299	32	24	5	0.13	151	31	91	212	0.20	0.208	0.156	47	28	66
>=300	24	21	3	0.11	124	27	72	177	0.21	0.143	0.125	39	22	55

Table 11. Flannemouth Sucker abundance estimates and densities (fish/mile) using hoop nets only in A) JCM West (1 night marking), and B) and C) Bridge City reaches (1 and 2 nights marking). Colorado River, 2019. See Table 5 above for heading descriptions (e.g., M,C, R, N, etc.). Note: no comparative abundance estimates made using both hoop net and antenna data are provided.

**A.) Hoops only (1 night marking) - JCM West**

Length (mm)	M	C	R	P	N	SE	95% CI		CV	p1	p2	Density	95% CI	
							Lower	Upper					Lower	Upper
>=150	779	1,321	178		5,760	350	5,073	6,447	0.06	0.135	0.228	1,518	1,337	1,700
>= 200	399	609	104	0.47	2,709	214	2,290	3,128	0.08	0.171	0.261	714	604	825
>=300	84	52	9	0.07	381	42	297	464	0.11	0.173	0.107	100	78	122
150-199	380	712	74	0.53	3,051	238	2,583	3,518	0.08	0.104	0.195	804	681	927
200-249	260	472	83	0.34	1,945	158	1,635	2,255	0.08	0.176	0.319	513	431	594
250-299	55	85	12	0.07	384	43	300	467	0.11	0.141	0.218	101	79	123
300-349	20	21	2	0.02	117	20	78	156	0.17	0.095	0.100	31	21	41
350-399	38	18	4	0.03	156	24	110	202	0.15	0.222	0.105	41	29	53

**B.) Hoops only (1 night marking) - Bridge City**

Length (mm)	M	C	R	P	N	SE	95% CI		CV	p1	p2	Density	95% CI	
							Lower	Upper					Lower	Upper
>=150	463	794	195		1,881	88	1,708	2,054	0.05	0.246	0.421	879	798	960
>= 200	193	257	81	0.35	654	53	549	758	0.08	0.315	0.420	305	257	354
>=300	48	29	12	0.06	115	15	85	145	0.13	0.414	0.250	54	40	68
150-199	270	537	114	0.65	1,227	92	1,047	1,408	0.07	0.212	0.422	574	489	658
200-249	102	197	54	0.23	434	38	359	509	0.09	0.274	0.529	203	168	238
250-299	43	31	15	0.06	105	14	76	133	0.14	0.484	0.349	49	36	62
300-349	31	20	8	0.04	76	12	53	99	0.15	0.400	0.258	36	25	46
350-399	17	8	4	0.02	37	8	22	53	0.21	0.500	0.235	17	10	25

**C.) Hoops (2 nights marking) - Bridge City**

Length (mm)	M	C	R	P	N	SE	95% CI		CV	p1	p2	Density	95% CI	
							Lower	Upper					Lower	Upper
>=150	712	794	309		1,828	61	1,708	1,947	0.03	0.389	0.434	854	798	910
>= 200	271	257	121	0.34	621	42	539	703	0.07	0.471	0.446	290	252	329
>=300	62	29	13	0.07	119	13	93	145	0.11	0.448	0.210	56	43	68
150-199	441	537	188	0.66	1,206	72	1,064	1,348	0.06	0.350	0.426	564	497	630
200-249	153	197	90	0.22	397	30	338	455	0.08	0.457	0.588	185	158	213
250-299	56	31	18	0.06	105	13	81	130	0.12	0.581	0.321	49	38	61
300-349	43	20	9	0.05	82	11	61	104	0.13	0.450	0.209	39	29	48
350-399	19	8	4	0.02	35	7	22	48	0.19	0.500	0.211	16	10	23

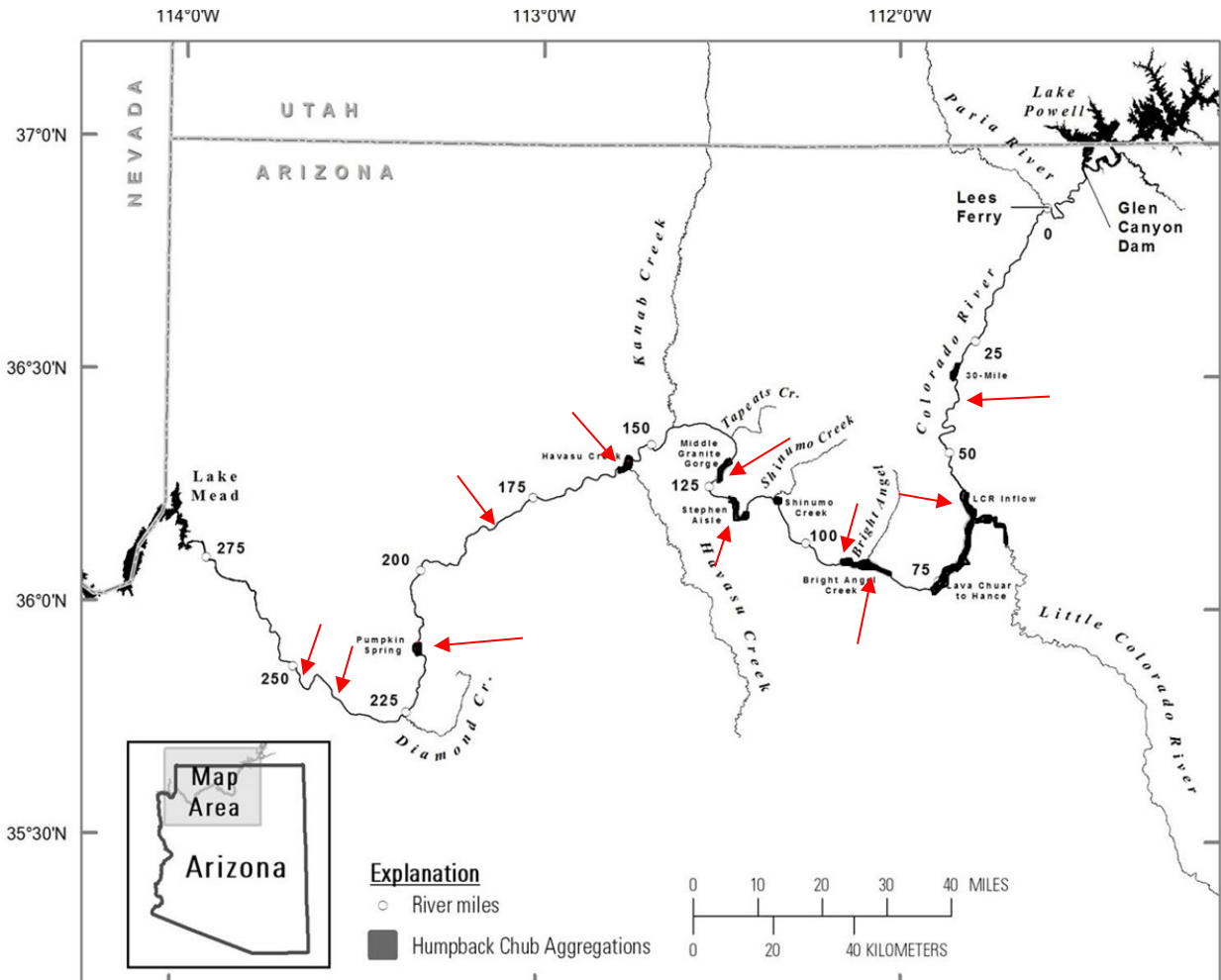


Figure 1. Map of the Colorado River from Lake Powell to Lake Mead showing the nine Humpback Chub aggregations (black), as defined by Valdez and Ryel (1995): 30-Mile, Little Colorado River inflow, Lava Chuar–Hance Rapid, Bright Angel inflow, Shinumo Creek inflow, Stephen Aisle, Middle Granite Gorge, Havasu Creek inflow, Pumpkin Spring. Red arrows indicate locations sampled in 2019. Map: Tom Gushue, GCMRC. Note: distance points shown along the river are in miles.

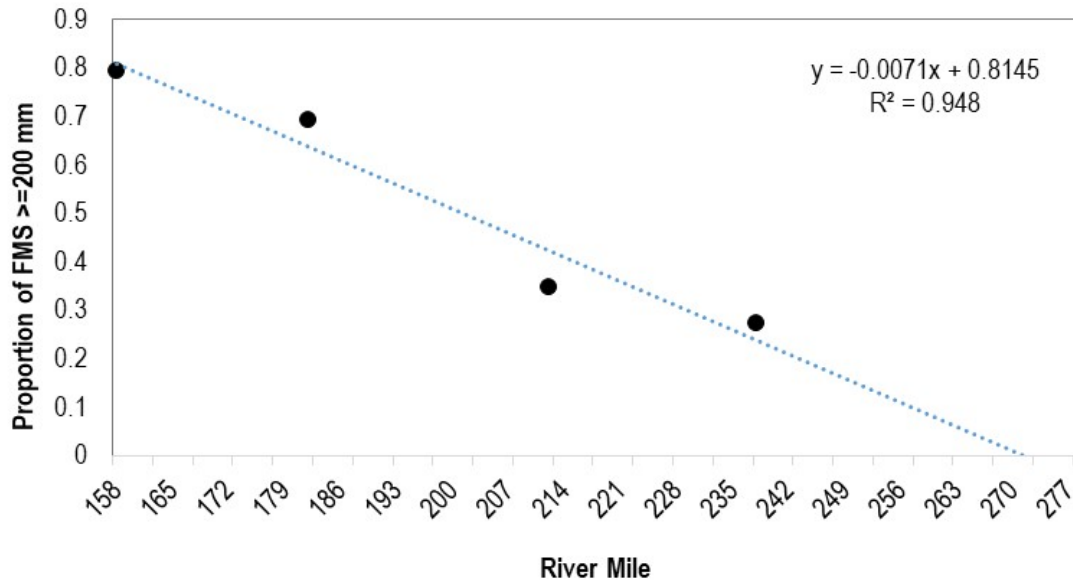


Figure 2. Proportion of total Flannemouth Sucker  $\geq 200$  mm at 4 sample reaches (below Havasu Rapids, Chevron, JCM West, and Bridge City reaches), during 2019 aggregation trip.

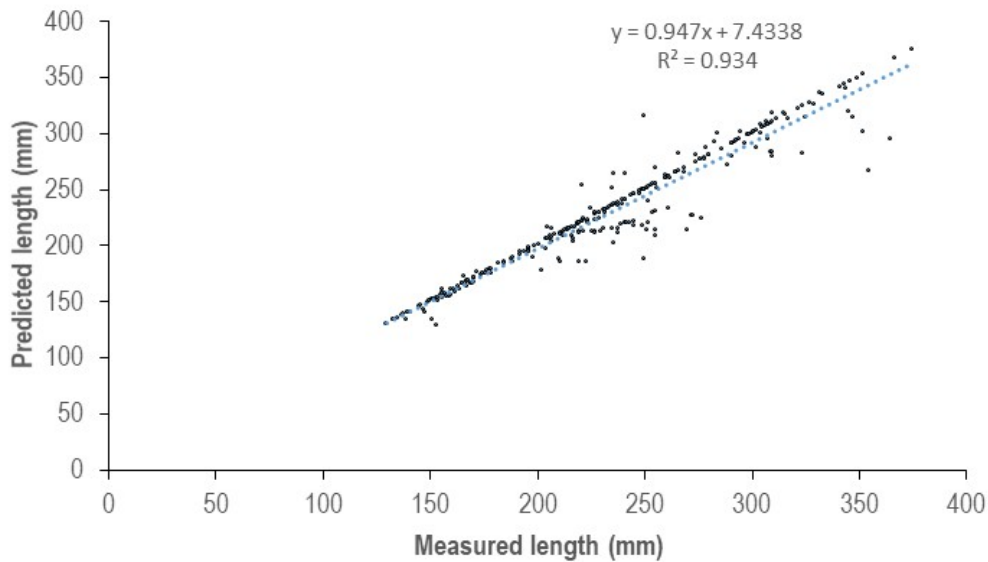


Figure 3. Predicted vs. measured length (mm TL) of Humpback Chub ( $n = 298$ ) that were detected on antennas, and captured with hoopnets or electrofishing in western Grand Canyon. Antenna detections came from the September 2019 Aggregation trip in the JCM West, Bridge City, and 250-mile reaches. Measured chub came from the Sept 2019 Aggregation trip from above three reaches, or from the Oct 2019 JCM West reach trip conducted by GCMRC.

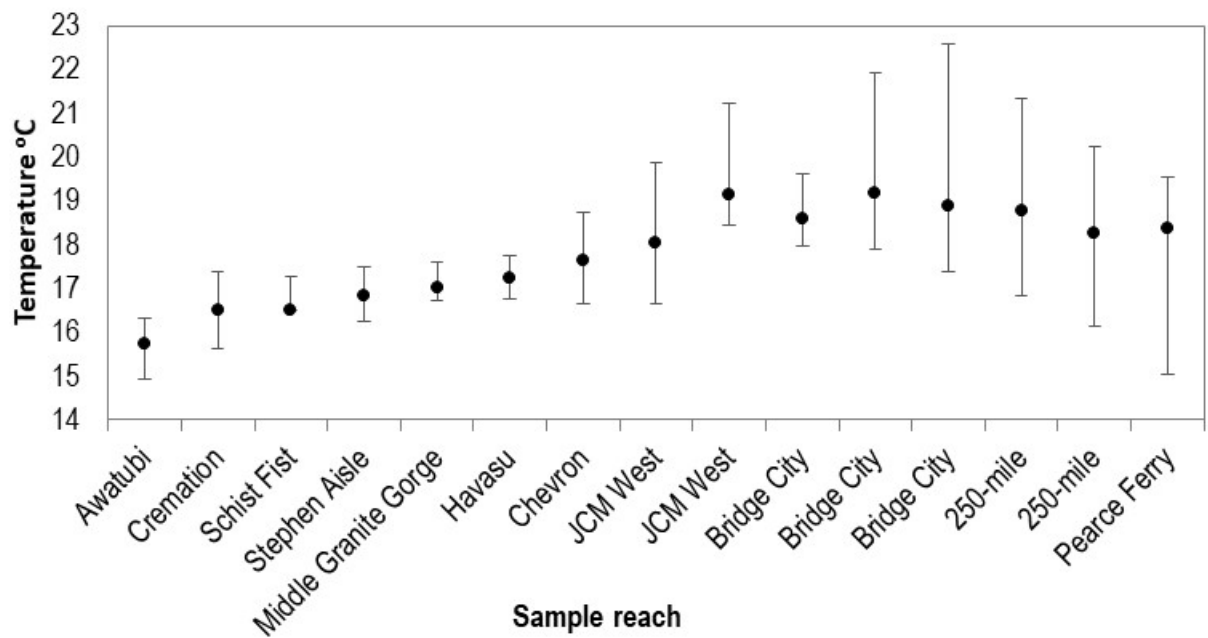
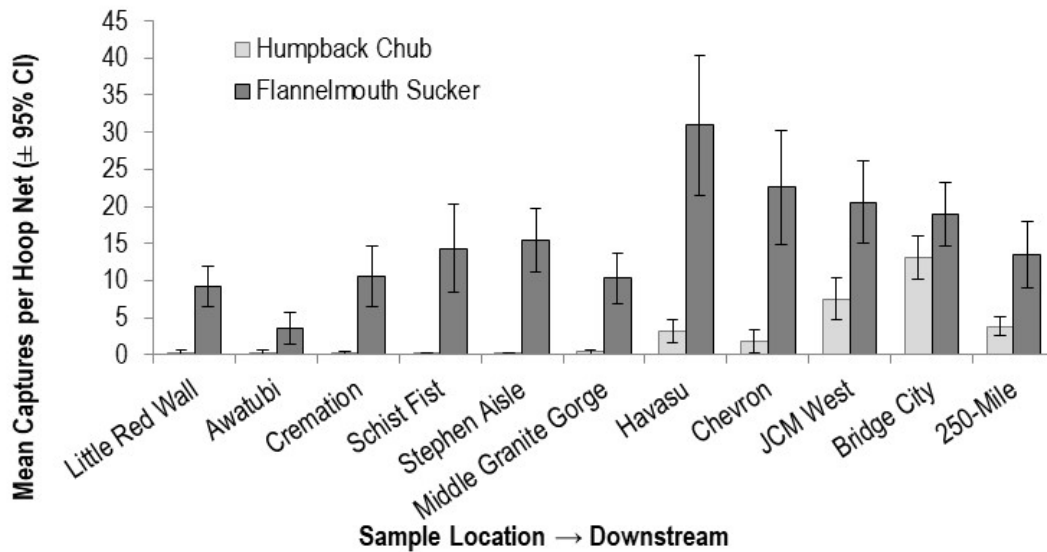


Figure 4. Mean daily water temperature (°C) with daily minimum and maximum temperature bars at sample reaches on the 2019 aggregation trip, Colorado River.



Figure 5. Spiny Softshell turtles *Apalone spinifera* emerging from a sandy bench in camp at RM 249.73, river left, Colorado River in western Grand Canyon.

A)



B)

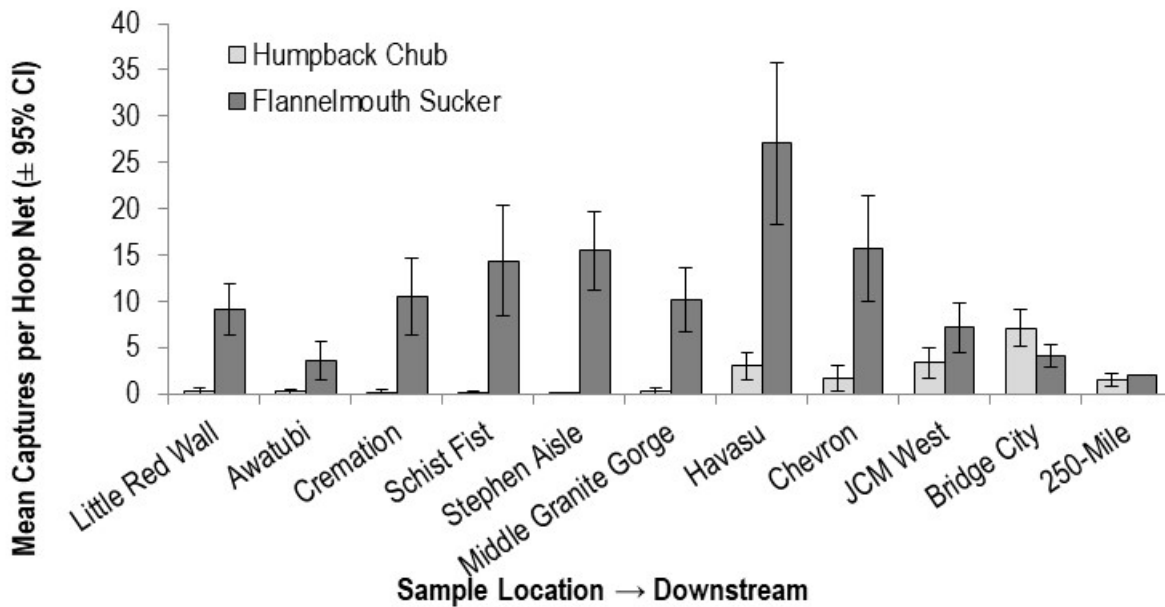


Figure 6. Mean catch per unit effort (CPUE  $\pm$  95% CI, captures per overnight hoop net) for Humpback Chub and Flannemouth Sucker A) all size classes, and B) fish  $\geq 200$  mm only at each sample location on the 2019 Aggregation monitoring trip. Note: CPUE for Flannemouth Sucker  $\geq 200$  mm at 250-Mile estimated by using equation in Figure 2 to calculate proportion of catch  $\geq 200$  mm (hence no error bar).



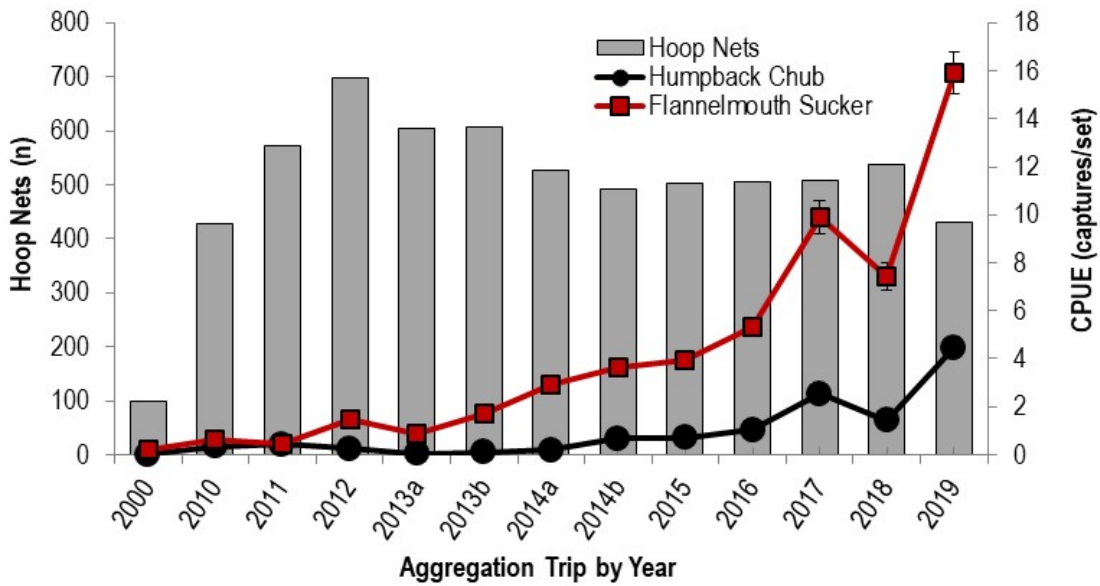


Figure 7. CPUEs of Humpback Chub and Flannemouth Sucker (all size classes) paired with total hoop nets set for each Grand Canyon aggregation trip 2010-2019. Note in 2013 and 2014, two hoop netting aggregation trips (July, September) were conducted.

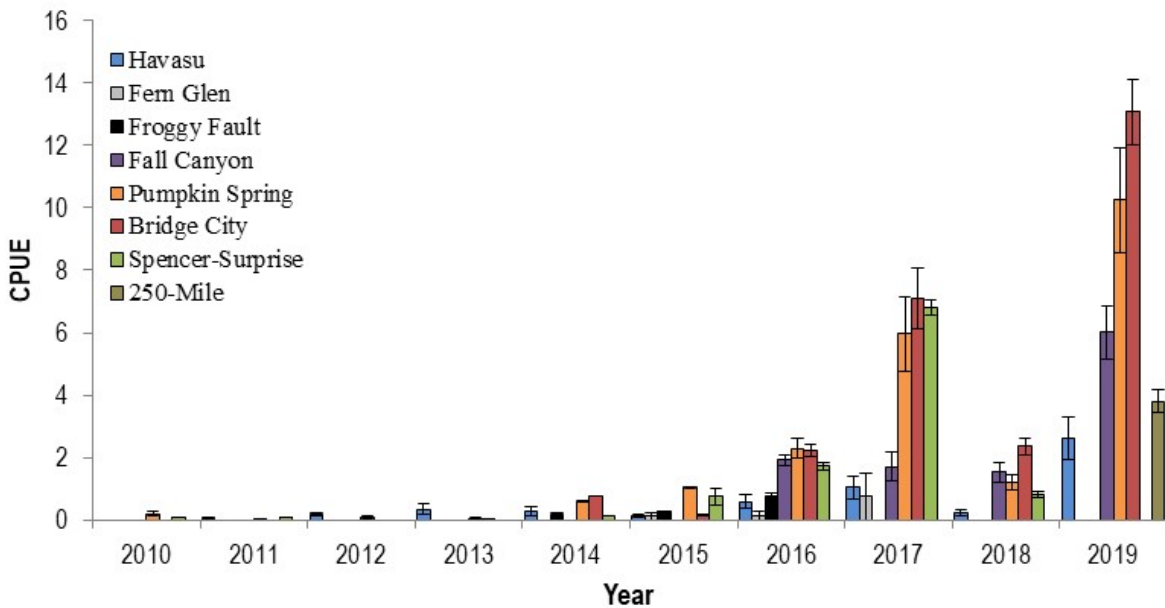


Figure 8. Mean CPUE  $\pm$  95% CI, captures per overnight hoop net) of adult Humpback Chub ( $\geq 200$  mm TL) for sampling reaches from Havasu downriver 2010-2019. The 250-mile reach was not sampled until 2019.



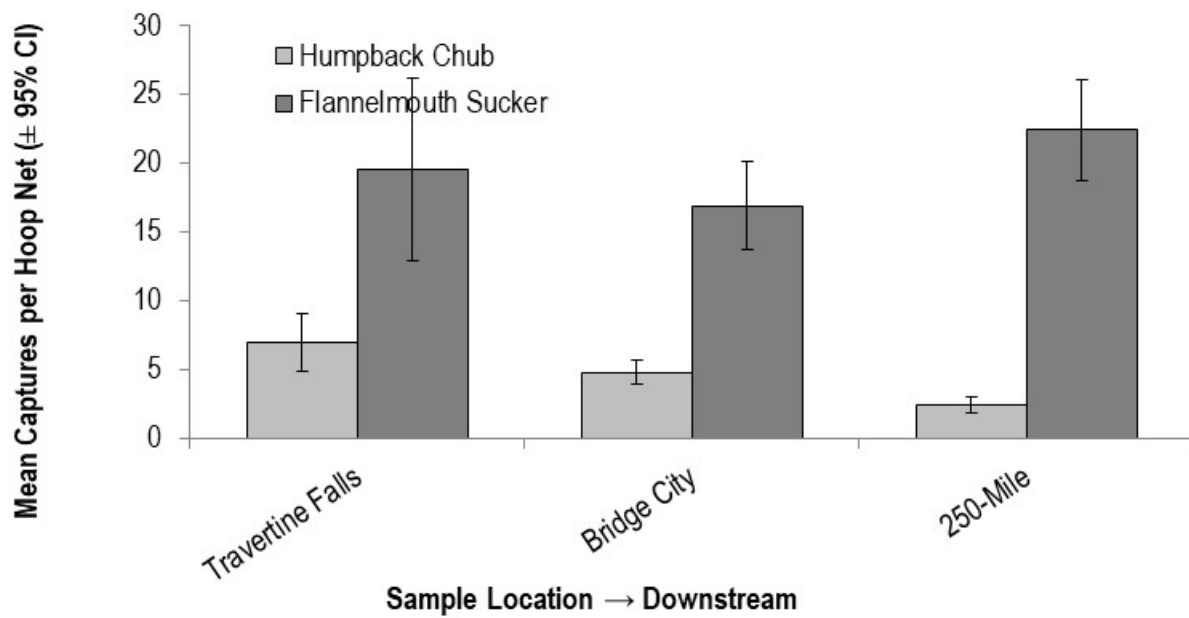
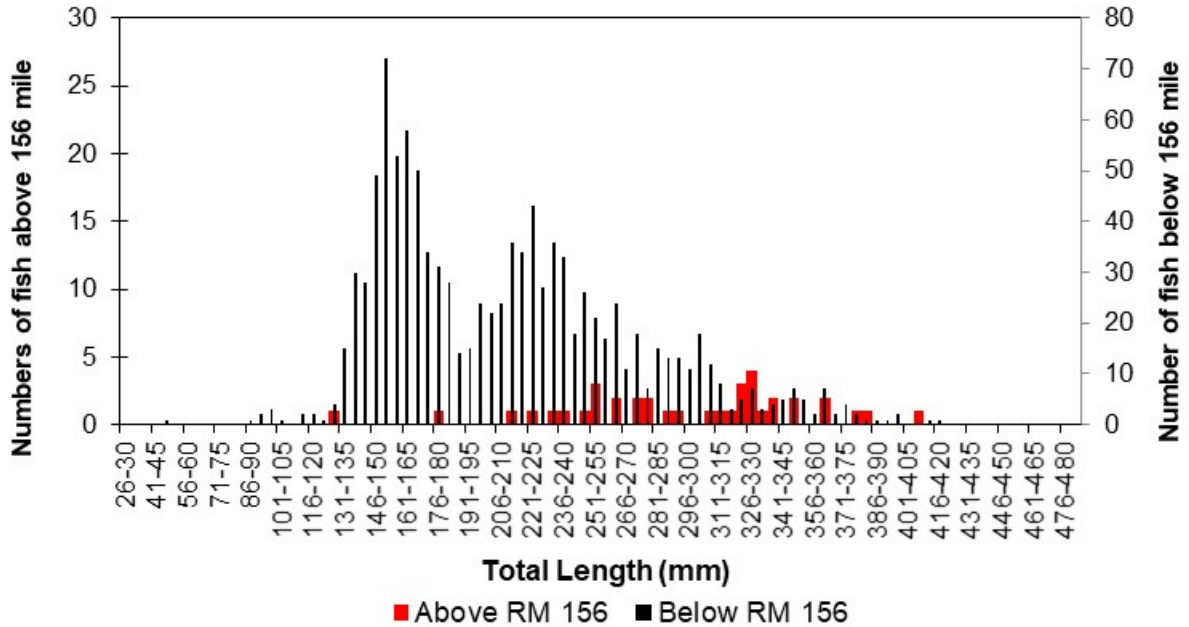


Figure 9. Catch per unit effort (fish/overnight net set, CPUE) of Humpback Chub and Flannemouth Sucker (all size classes) in sampling reaches on the Sept/Oct 2019 Diamond down trip.

A)



B)

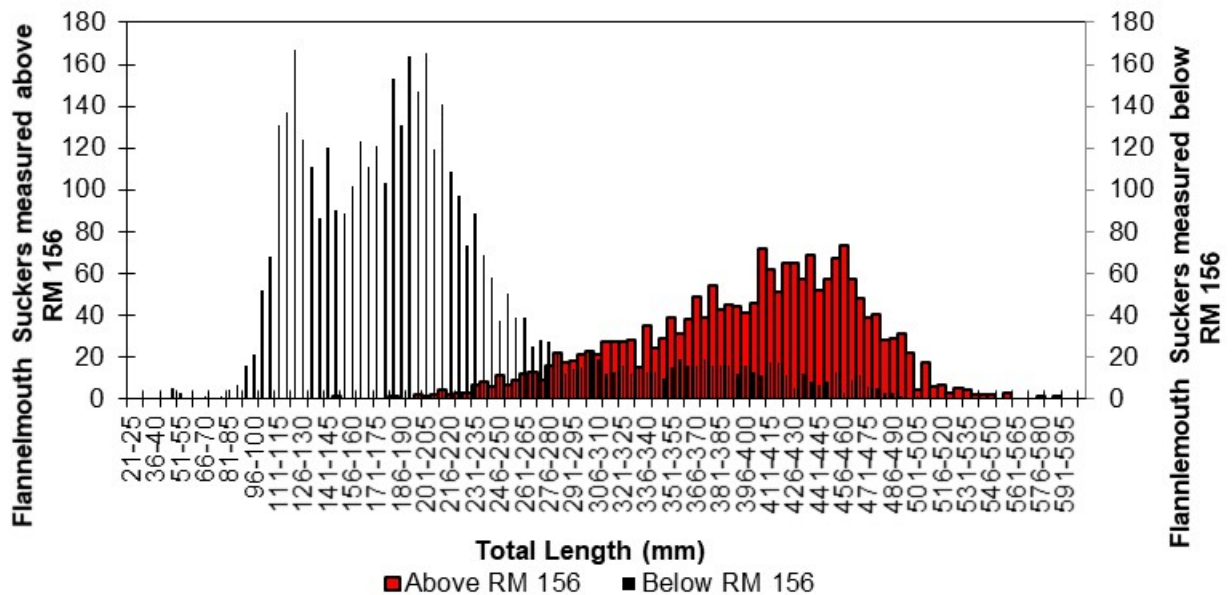


Figure 10. Length frequency distributions for A) Humpback Chub and B) Flannemouth Sucker captured during the 2019 Aggregation trip above and below river mile RM 156. Note: For Flannemouth Sucker below RM 210, representative samples of length measurements were sometimes taken. Thus numbers of Flannemouth sucker below RM 156 is underrepresented by 796 fish.

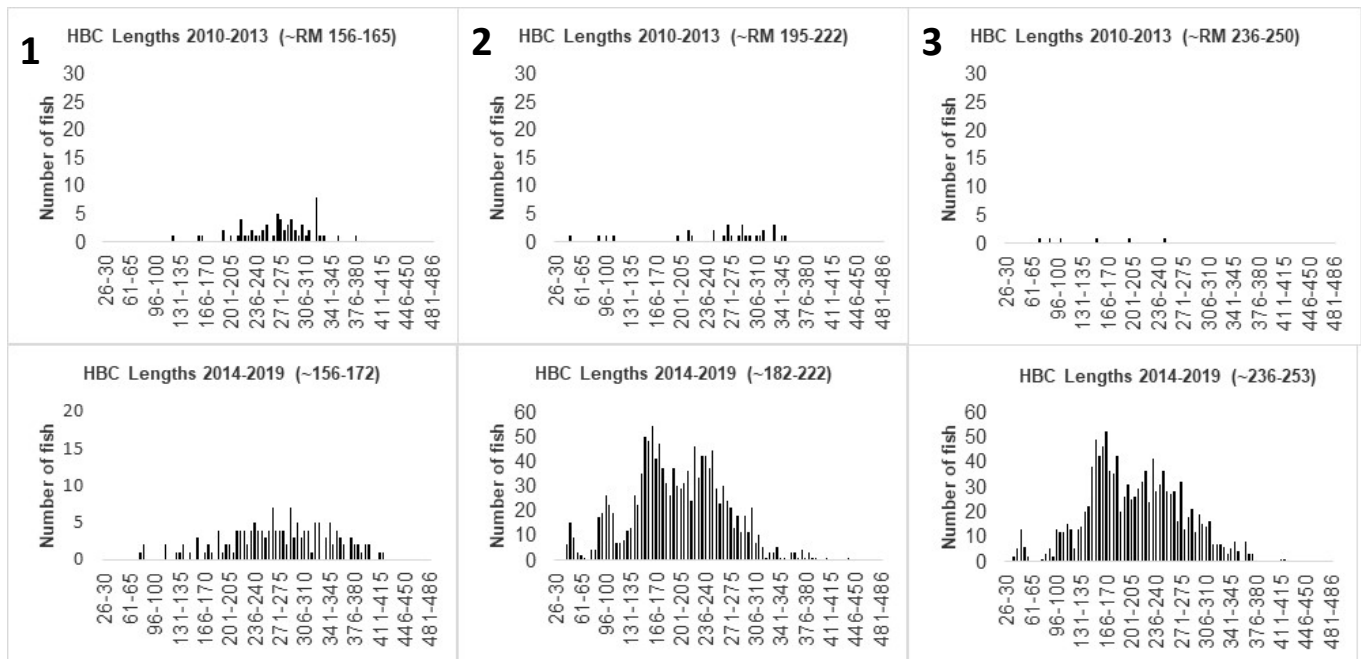
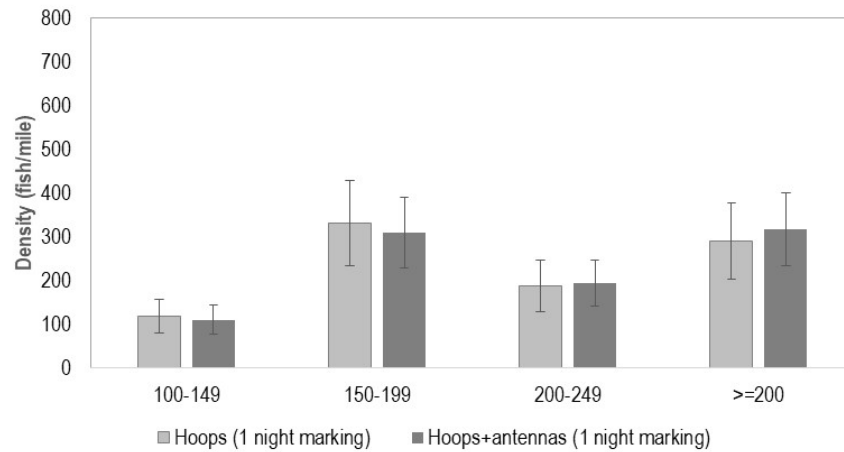
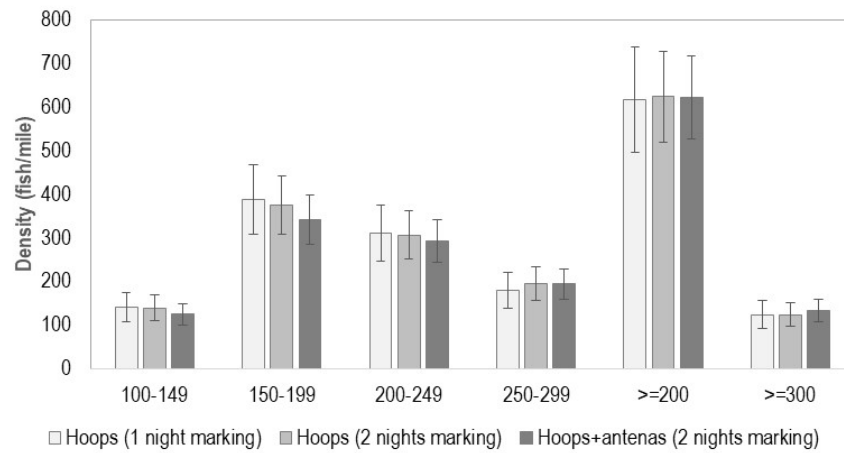


Figure 11. Comparative length frequency distributions of Humpback Chub (HBC) captured on aggregation sampling trips during two time periods (2010-2013 [upper row] vs. 2014-2019 [lower row]) in three reaches of western Grand Canyon: 1) from just above Havasu Creek to above Lava Falls (~river mile [RM] 156-172), 2) from below Lava Falls to above Diamond Creek (~ RM 182-222), and 3) from below Diamond Creek to ~RM 253. Note: 2010-2013 represented by 5 sampling trips and 2014-2019 represented by 7 sampling trips.

A) JCM West reach



B) Bridge City reach



C) 250-mile reach

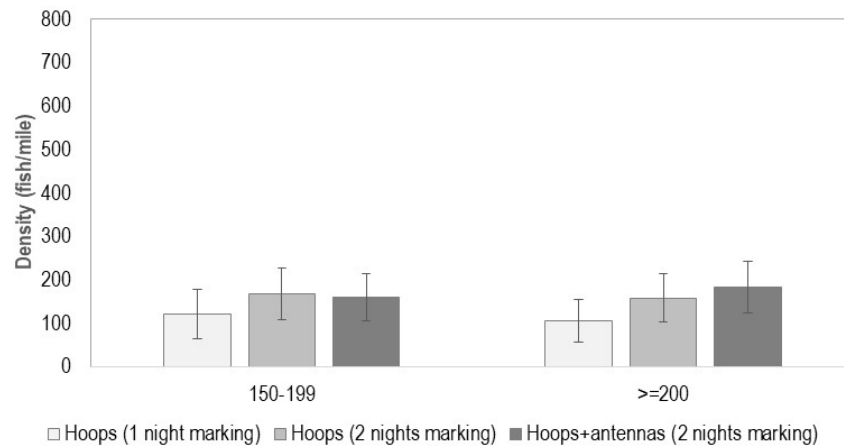
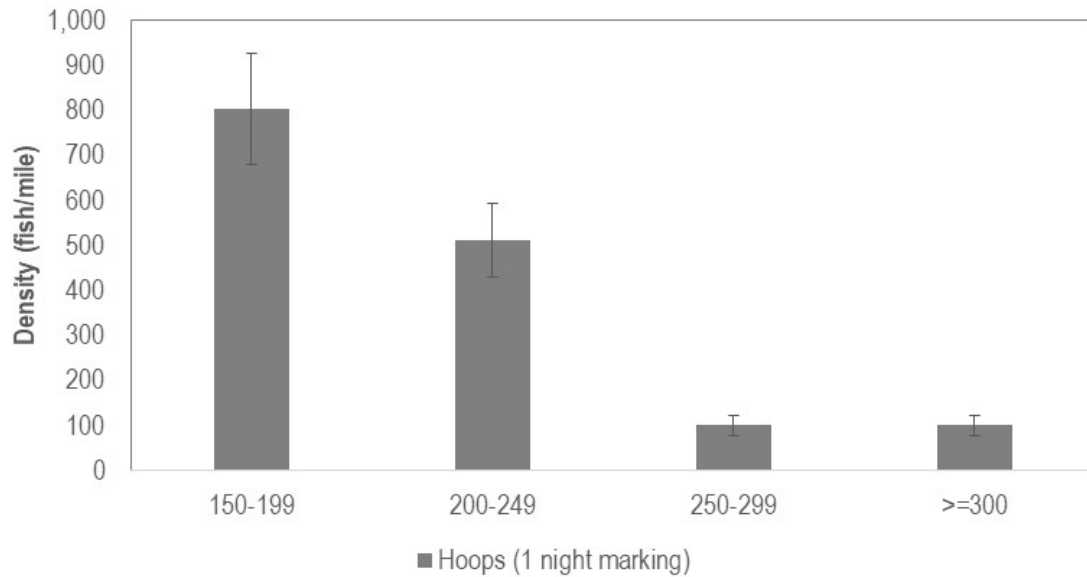


Figure 12. Densities (fish/mile) of Humpback Chub in size classes (mm) in A) JCM West, B) Bridge City, and C) 250-mile reaches, Colorado River, September 2019.

A) JCM West



B) Bridge City

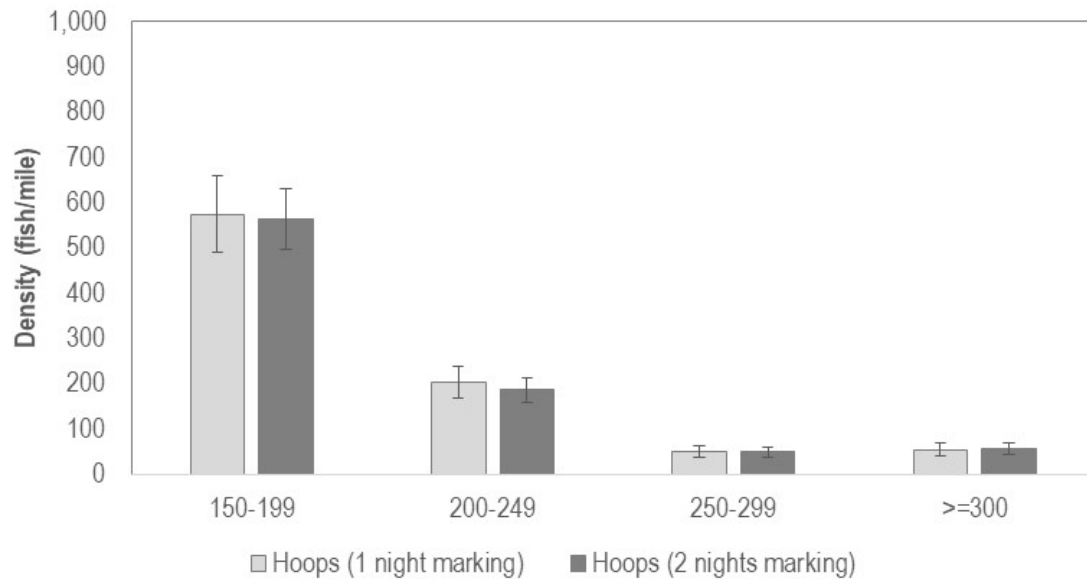
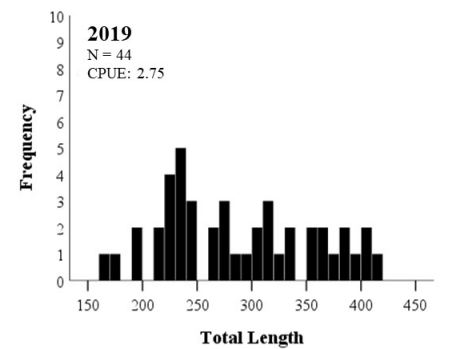
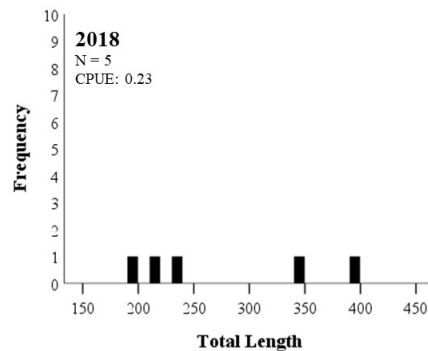
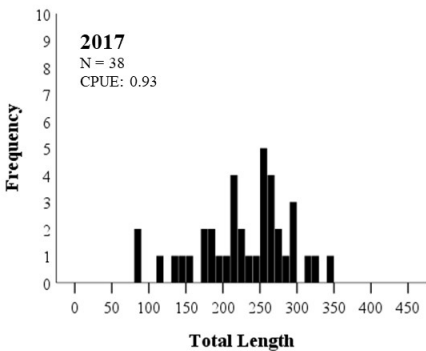
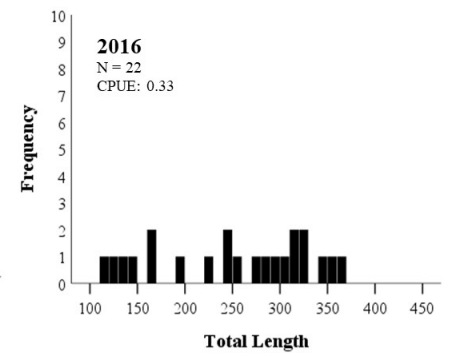
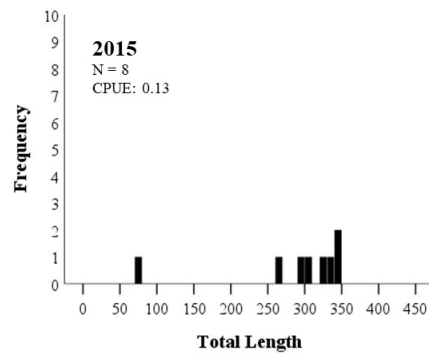
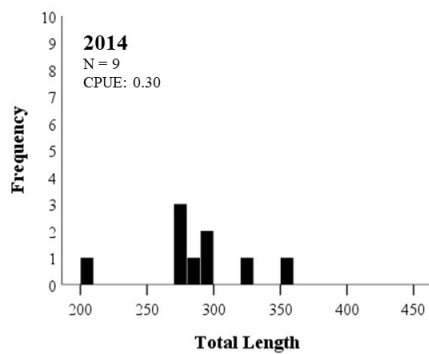
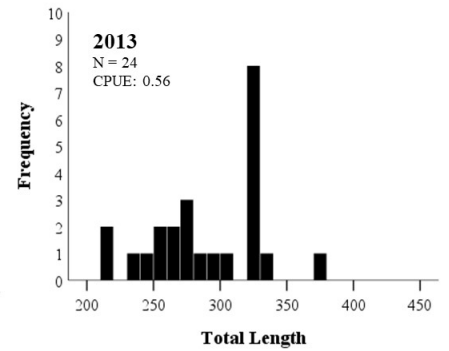
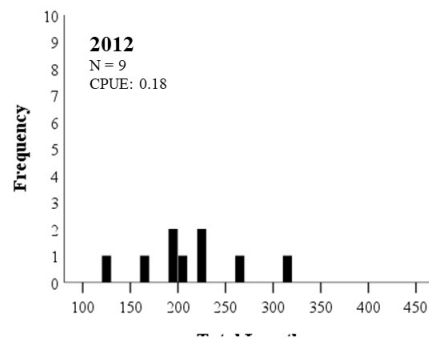
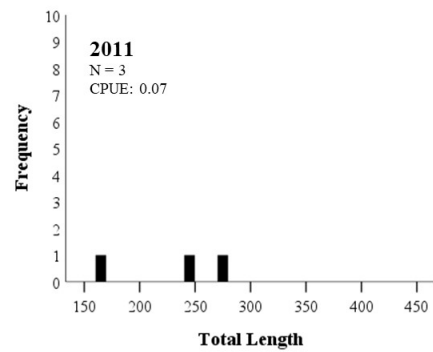
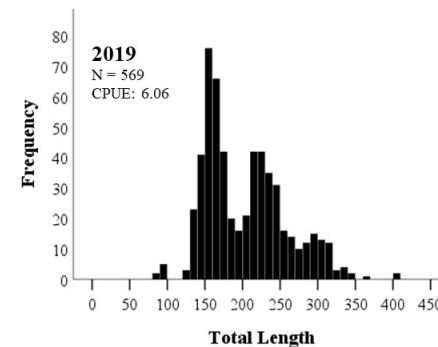
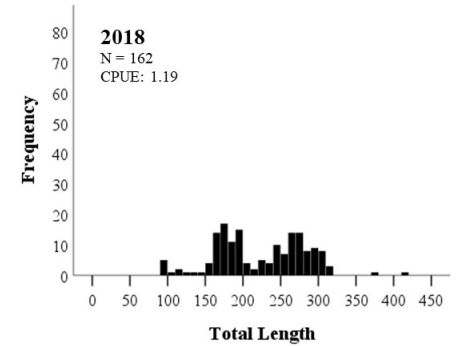
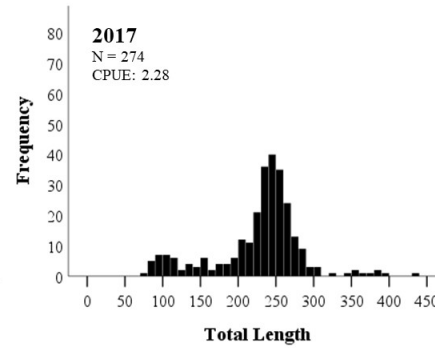
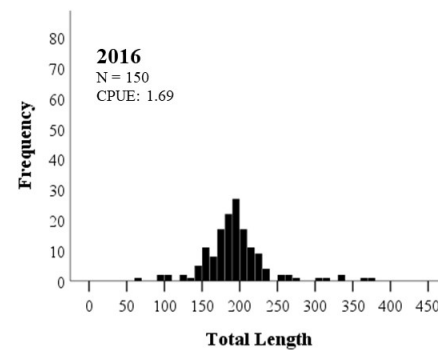
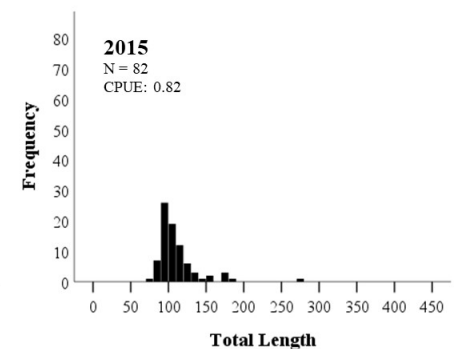
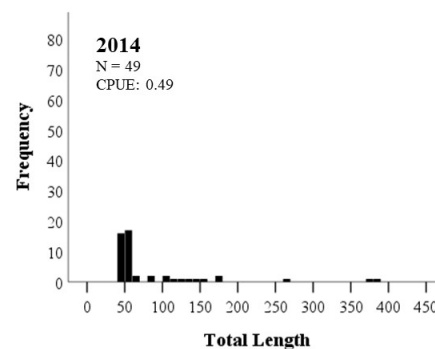
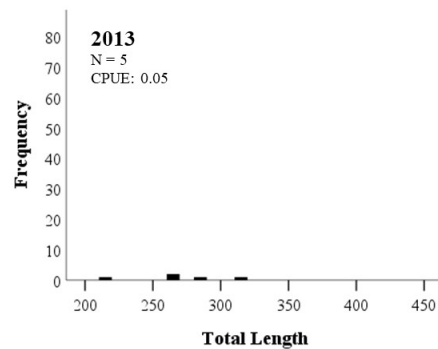
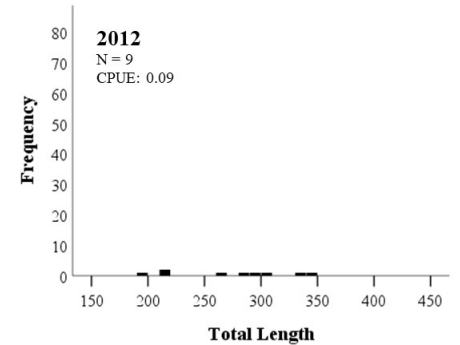
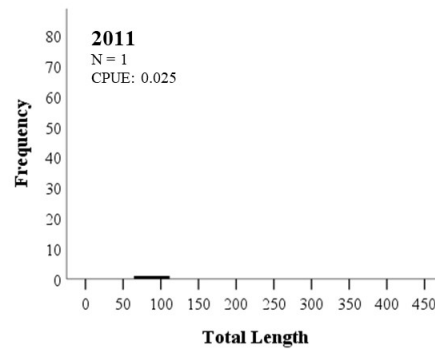
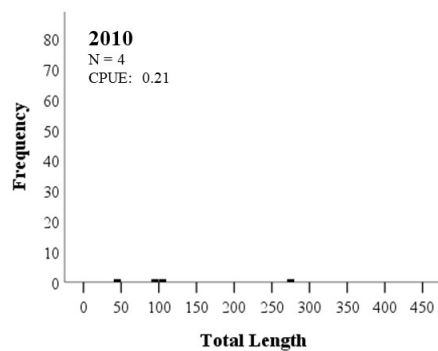


Figure 13. Densities (fish/mile) of Flannelmouth Sucker in size classes (mm) in A) JCM West, and B) Bridge City, Colorado River, September 2019.

Appendix 1-A. Annual length frequency distributions of Humpback Chub from just above Havasu Creek mouth to Lava Falls reach (river mile 157.04-180). Note: No Humpback Chub were captured in hoop nets in this reach in 2010.



Appendix 1-B. Annual length frequency distributions of Humpback Chub in the Lava Falls to Diamond Creek reach of western Grand Canyon (river mile 181-225).



Appendix 1-C. Annual length frequency distributions of Humpback Chub in the Diamond Creek to Horse Flat Canyon reach of western Grand Canyon (river mile 225-253). Note: No Humpback Chub were captured while sampling below Diamond Creek in 2012.

