Monitoring Humpback Chub Aggregations in the Colorado River, Grand Canyon during fall 2018

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Cover photo: Crew fishing in Osprey boat near 181 mile camp. Photo by D. Van Haverbeke.

Executive Summary

During fall 2018, 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 21 August to 7 September. A second trip (Diamond down trip) occurred from 28 September to 3 October.

The primary objective of the aggregation trip was to continue long term relative abundance (catch per unit effort) monitoring of Humpback Chub in the known historical "aggregation" sites (Valdez and Ryel 1995). Relative abundances of Humpback Chub in Marble and Grand Canyon have increased in recent years, and since 2010, we have documented a general increase in Humpback Chub capture rates river-wide with greatest increases occurring in western Grand Canyon. Further, at the three most downstream sample sites, capture rates have increased since 2014. Two of these sites are below Diamond Creek. Length frequencies illustrate that since 2014, western Grand Canyon has been populated by Humpback Chub represented by all size classes.

During the aggregation trip, we sampled twelve river reaches, each approximately 1.8 miles in length (mean = 1.78 miles, SE = 0.35) 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 sampling reaches, with the highest number of Humpback Chub captured with hoop nets below the Little Colorado River between river mile (RM) 62.86-65.05 (n = 301) after two nights of sampling. Outside of this reach (Little Colorado River inflow aggregation), per-site Humpback Chub captures generally increased downriver. Flannelmouth Sucker (*Catostomus latipinnis*) made up the majority of fish captures on the trip (n = 3,990), and were captured in highest numbers in the Juvenile Chub Monitoring (JCM) West reach (n = 960, RM 210.2-214.1).

A second objective of the 2018 aggregation trip was to function as a marking event to conduct closed Chapman Petersen abundance estimates of Humpback Chub and Flannelmouth Sucker in four river reaches; these being 1) the JCM East reach, 2) the JCM West reach, 3) Bridge City reach, and 4) Spencer reach. The follow up Diamond down trip was to function as the Chapman Petersen recapture event for the Bridge City and Spencer reaches. Additionally, 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 East and JCM West reaches. Humpback Chub population (N) and density (fish/mile) estimates were possible at two locations, JCM East and Bridge City, with a density of 1,449 (95% CI: 753-2,145) and 308 (95% CI: 236-380) adult fish (pooled ≥200 mm) per mile in each reach, respectively. Population and density estimates of Humpback Chub at the other locations were not presented, because of an insufficient number of marked or recaptured fish. In addition, Flannelmouth Sucker (pooled ≥200 mm) abundance and density was estimated in the Bridge City reach, with a density of 167 (95% CI: 126-209) fish per river mile.

Introduction

Humpback Chub (*Gila cypha*) is a large-bodied, federally endangered Cyprinid endemic to the Colorado River basin (USFWS 1967; U.S. Endangered Species Act [ESA 1973, as amended]). The species currently exists 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). The largest of these populations 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. Including the endangered Razorback Sucker (*Xyrauchen texanus*), the 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 (*Catostomus discobolus*), and Speckled Dace (*Rhinichthys osculus*).

In addition to the LCR inflow aggregation, Valdez and Ryel (1995) identified eight additional Humpback Chub aggregations during the early 1990s in Marble and Grand Canyons generally in areas near springs or tributary inflows (Figure 1). An aggregation of Humpback Chub was defined by Valdez and Ryel (1995) 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. In subsequent years, these 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 modified by Persons et al. (2017) to reflect a more recent distribution (Table 1). For example, the original 30-Mile aggregation was expanded in range, 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 comparing the abundance of

Humpback Chub among these aggregations and, more broadly, in assessing abundance of Humpback Chub at a larger spatial scale in Marble and Grand Canyons. Because the largest population of Humpback Chub in the Colorado River ecosystem resides in the LCR inflow, most progress in estimating population parameters of Humpback Chub has come from working on this specific 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); with a recent estimate of 11,000 (95% CI: 7,000-16,000) total adults in this aggregation (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 adult Humpback Chub in the mainstem Colorado River outside of the LCR inflow has been made more sporadically. Valdez and Ryel (1995) estimated abundances of adult Humpback Chub 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 $p1$ and $p2$ capture probabilities of 0.07 and 0.1 (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 discovered between 34-36 mile, with $p1$ and $p2$ capture probabilities of 0.15 and 0.12 (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 intense monthly sampling efforts during the early 1990s by Valdez and Ryel (1995), and 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) indices (Ackerman et al. 2008, Persons et al. 2017). This remains the case, because obtaining absolute abundance estimates in the mainstem requires substantial focused and repetitive effort.

In 2017, we worked collaboratively with biologists at the USGS Grand Canyon Monitoring and Research Center (GCMRC) to successfully obtain population estimates of Humpback Chub and Flannelmouth Sucker in the mainstem Colorado River 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 to 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 discreet 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.

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 Flannelmouth Sucker, Bluehead Sucker, and Speckled Dace populations in the Colorado River ecosystem.

Objectives:

1. Obtain August/September 2018 relative abundance (catch per unit effort, CPUE) estimates of Humpback Chub and other species 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 2002.
2. Provide information related to Humpback Chub length frequency distributions, observed community composition, and sexual condition (ripe, not ripe).
3. Investigate the utility of passive antennae gear for detecting additional fish.

Methods

Schedule, Sampling Sites, and Personnel

Between 21 August and 7 September 2018, 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). Five additional reaches outside of the defined aggregation reaches were also sampled (Table 2). Four reaches were selected to perform a marking effort for closed mark-recapture population abundance efforts, including two sites above Diamond Creek (JCM East, RM 63.4-65.05 and JCM West, 210.19-214.1), and two sites below Diamond Creek (Bridge City, 236.65-238.67 and Spencer, 245.8-247.9; Table 2).

A separate trip conducted downriver from Diamond Creek by USFWS from 28 September to 3 October 2018 was to function as a recapture trip for both the Bridge City and the Spencer reaches. However, a Spencer reach recapture effort was not conducted because not enough fish were marked in this reach during the 2018 aggregation trip. Finally, an October 2018 JCM sampling trip conducted by GCMRC personnel was utilized to provide recaptured fish for closed mark-recapture abundance estimation in the JCM East and JCM West reaches.

Personnel participating on the August/September 2018 aggregation trip were: David Van Haverbeke, Kirk Young, Michael Pillow, and Kristy Manuel (USFWS), Mike Dodrill and Laura Tennant (GCMRC), Cory Nelson (AZGFD), and boatmen Nate Jordan, Jeremy Swindlehurst, Sam Jones, and Brandon Green (St. Jude, Inc.). Participants on the September/October Diamond down trip included Kirk Young, David Van Haverbeke, Kristy Manuel, and Chase Ehlo (USFWS), David Ward (GCMRC), and boatmen Jeremy Swindlehurst, and Jeremy Draper (St. Jude, Inc.).

Sampling Gear

We sampled each location 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 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. At each sample location, we deployed hoop nets 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, 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. As a result, a few 0.1 mile sections were set with 2 nets per 0.1 mile (e.g., either below or above the rapid) to keep the overall net density on each side of the river and within the sampling reach at 1 net per 0.1 mile. Mean net density per each side of the river was 1.0 net/0.1 mile (SE = 0.006, n = 537 nets). One additional hoop net was set in Havasu Creek within a few meters of the confluence.

We also deployed between one and six baited submersible Passive Integrated Transponder (PIT) tag antennas (Marsh & Associates, LLC) overnight within most sampling reaches. These battery powered antennas detect and record PIT tags along with date and detection time of PIT tagged fish. Antennas were deployed each evening between 16:30 and 19:00 h and were retrieved between 06:00 and 13:30 h the following day. We set antennas throughout each reach on both sides of the river, each antennae adjacent (within ~5 m) to a hoop net set. An attempt to distribute the antennas evenly across each reach was made by pairing them with hoop nets at either end and near the middle of the sampled reach. We exchanged batteries in all antennas halfway through the trip and downloaded detection data periodically. During the course of the trip, some antennas malfunctioned prior to deployment or had batteries die overnight, while other antennas became unusable and were discontinued.

Data Collection

For each hoop net, we recorded set and pull times, and net location (side, river mile, and habitat) along with fish captures. We marked net locations and set times on aerial photo maps provided by GCMRC. All captured fish were identified to species. Total length (TL), fork length, sex (male/female), and sexual condition (ripe/not ripe) were recorded for all Humpback Chub, Flannelmouth Sucker, and Bluehead Sucker. TL was recorded for all other species. All fish lengths herein refer to TL. At all sampling locations, a subsample of native fish were weighed using a digital laboratory scale. All large-bodied fish were scanned for the presence of a PIT tag. Untagged Flannelmouth Sucker and Bluehead Sucker ≥150 mm and Humpback Chub ≥80 mm were implanted with PIT tags (134.2 kHz, 12.5 mm; Biomark, Boise, ID).

We entered all hoop net and fish data directly into data files on tablet computers set up on each boat. PIT tags were detected using Biomark HPR Plus tag readers powered by a 12V external battery and were uploaded to the tablet data files via Bluetooth connection.

For each antenna deployed, we recorded set time, pull time, river side, and river mile. Typically, we programmed antennas to turn on at 17:00 h and off at 08:00 h the next day, however, they were often deployed after and retrieved before those times. As such, we used set and pull times along with power up and power down times to determine actual set duration.

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. We also used a generalized linear modeling approach to predict catch at aggregation and non-aggregation sites among four discreet sampling periods between 1991 and 2018. For detailed methods on the generalized linear modeling approach that we used see Persons et al. (2017).

Abundance Estimation

During fall 2018, we attempted to conduct Humpback Chub mark-recapture studies in four discreet reaches of the mainstem Colorado River: 1) JCM East, 2) JCM West, 3) Bridge City, and 4) Spencer. However, we abandoned the recapture event in the Spencer reach because of concern about an insufficient number of Humpback Chub marked in this reach during the 2018 aggregation trip. In addition, an insufficient number of Humpback Chub recaptures (only 3 total) in the JCM West reach precluded obtaining an accurate population estimate.

We used the Sept/Oct 2018 aggregation trip as a marking event within each discreet reach to conduct a two-pass Chapman Petersen mark-recapture effort (Seber 1982). We used data collected on the October 2018 JCM monitoring trip conducted by GCMRC as a recapture event in the JCM East and JCM West reaches. Finally, we used data collected on the Sept/Oct Diamond down trip as a recapture event in the Bridge City reach. We assumed that the short time span between mark and recapture events (about a month) would help ensure that fish moving in and out of the individual reaches would be limited between trips, meeting the closure assumption of the model. Additionally, we feel that the closure assumption is justified over this short time frame in the mainstem Colorado based on telemetry studies which show very limited movement (Gerig et al. 2014). However, we also assumed that mixing of fish within the reaches would occur.

For Humpback Chub, we compare population estimates using hoop net data alone during the marking events, and 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. We adjusted total lengths of Humpback Chub detected by antennas by adding 1.2 mm/30 days to total length at the last day of capture. Valdez and Ryel (1995) found that Humpback Chub older than about 3 years of age grew faster in the mainstem (0.79–2.79 mm/30 days) than in the LCR (<1–1.4 mm/30 days). Adult chub >300 mm in the mainstem

grew about 1.2 mm/30 days (Valdez and Ryel 1995). We chose this simplistic method to approximate length at detection because it represents a minimum growth rate. Though it may result in an overestimation of abundance in subadult size classes (100-200 mm), it should not overestimate fish in the adult size classes. Average size of unique Humpback Chub and detected only by antennas in the JCM East reach during the 2018 aggregation trip was 242 mm (SE = 65, n = 218), and in the Bridge City reach was 218 mm (SE = 60, n = 20).

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 all Humpback Chub ≥80 mm and all Flannelmouth Sucker ≥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, ≥200 mm, etc.). We apply the general rule of needing at least 7 recaptures in order to be 95% confident that abundance estimation is negligible (Robson and Reiger 1964, Seber 1982 p. 60). We apply this rule to both pooled estimates (e.g., ≥200 mm) and to size specific 50 mm categories. Of course, we recognize that this is not the only factor that can cause bias, but it is a general rule that we follow. The 95% confidence intervals of the Chapman Petersen abundance estimates were approximated with a normal distribution, following Seber (1982, p. 60) for Humpback Chub ≥100 mm and Flannelmouth Sucker ≥150 mm, and Seber (1982, p. 101) for subcategory apportionments.

Abundances (N) were transformed into fish densities (fish/river mile) by first computing the mean absolute maximum distance fish moved (MMDM) between the marking and recapture events, adding ½ this distance onto each end of the sampling reach, and then dividing abundance by the MMDM adjusted distance (Wilson and Anderson 1985, Karanth and Nichols 1988). For Humpback Chub, MMDM was 0.1 miles in the JCM East reach (n = 19 fish), and 0.2 miles in the Bridge City reach (n = 80 fish). For Flannelmouth Sucker in Bridge City reach MMDM was 0.12 miles (n = 93 fish). For example, 0.2 miles were added onto the reach distance of 2.02 miles for the Bridge City reach.

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 for Flannelmouth Sucker.

<u>JCM East</u> – During the 2018 aggregation trip, the JCM East reach was defined as RM 63.4-65.1 (top of New Fish Camp eddy to middle of Carbon delta). This was done to conform to the original JCM East reach (RM 63.4-65.05). It was fished with baited hoop nets spread throughout the reach for two nights (total 69 hoop net sets), and with five baited antenna set for two nights.

During the follow up JCM monitoring trip, GCMRC personnel sampled between RM 63.4-65.1 over the course of five nights (9-14 October) with 455 unbaited hoop net sets and 75 electrofishing efforts (2,251 shocking seconds). Again, we used data from GCMRC's JCM East monitoring trip as the recapture event for a two-pass Chapman Petersen effort.
JCM West – During the 2018 aggregation trip, the JCM West reach was defined as RM 210.19-214.1. 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 three baited antenna, and one night below Little Bastard Rapid with 31 hoop net sets and three baited antenna. During the follow up JCM monitoring trip, GCMRC sampled between RM 210.52-213.97 over the course of six nights (18-24 Oct) with 702 unbaited hoop net sets and 120 electrofishing efforts (3,332 shocking seconds). As with the JCM East, data from the JCM West monitoring effort conducted by GCMRC personnel functioned as a recapture event. Unfortunately, there were an insufficient number of recaptured Humpback Chub to perform a closed mark-recapture estimate in JCM West.

Bridge City – During the 2018 aggregation trip, the Bridge City reach was defined between RM 236.65-238.67, and was fished for two nights for a total of 84 baited hoop net sets, and six baited antenna. On the follow-up Diamond down recapture trip, we deployed 126 baited hoop net sets (42 net sets/night) over the course of three nights (28 Sept–1 Oct).

Results

Hoop Nets
We deployed a total of 537 overnight hoop nets in the mainstem over the course of the 18-day aggregation monitoring trip (Table 3). The number of nets set per sample location varied based on the length of the reach and travel logistics the following day. We also set one hoop net in the mouth of Havasu Creek, where we captured seven adult Humpback Chub (244-415 mm) and 76 Flannelmouth Sucker (298-491 mm). This net is included in the total captures, CPUEs, and length frequency analyses.

Fish Captures
We captured a total of 5,004 fish using hoop nets during the 2018 aggregation monitoring trip (Table 4). Of those, 99.8% (n = 4,995) were native to the Colorado River in Grand Canyon. Flannelmouth Sucker made up the majority of fish captured (80%, n = 3,990), followed by Humpback Chub (15%, n = 762), Speckled Dace (5%, n = 237), and Bluehead Sucker (<1%, n = 6). Non-native species captured were three Rainbow Trout (*Oncorhynchus mykiss*), three Fathead Minnows (*Pimephales promelas*), and three Common Carp (*Cyprinus carpio*) (Table 4).

Although the number of overnight hoop nets has remained relatively steady over the years among trips, the numbers of Flannelmouth Sucker and Humpback Chub captures have shown

notable increases (Figure 2). Further, although catches of Humpback Chub and Flannelmouth Sucker have increased consistently since 2010, they were lower than 2017, likely because of turbid water conditions in the mainstem during 2018 (Figure 2).

Ripe fish (extruding gametes) on the trip included three male Humpback Chub, 32 Flannelmouth Suckers (30 male, 2 female), and one male Bluehead Sucker. One ripe Humpback Chub was captured in the JCM East reach and two in the Bridge City reach. The ripe male Flannelmouth Suckers were captured sporadically in all sampled reaches except Bridge City, and the two ripe females were captured at RM 34.6 and RM 126.8.

Catch per Unit Effort (CPUE)

We calculated mean CPUE (fish captured per overnight hoop net) for Humpback Chub and Flannelmouth Sucker at each sample location for the 2018 aggregation trip (Figure 3). Flannelmouth Sucker CPUE was higher than Humpback Chub CPUE at all locations except the LCR inflow aggregation. Flannelmouth Sucker saw a noticeable increase in mean CPUEs down river starting at Havasu reach (Figure 3). For nets between 30 Mile and Middle Granite Gorge, Flannelmouth Sucker mean CPUE was 4.1 (SE = 1.8), and from Havasu reach to Spencer reach it increased to 11.4 (SE = 6.4). Flannelmouth mean CPUE was highest in the 220-mile reach (22.4, SE = 33.9) and lowest at the LCR inflow aggregation (1.8, SE = 4.2).

Also on the 2018 aggregation trip, Humpback Chub mean CPUE peaked in the LCR inflow aggregation (3.9, SE = 5.1), and then dropped to less than one capture per net until the JCM West reach in western Grand Canyon (Figure 3). After the LCR inflow aggregation, Bridge City had the next-highest Humpback Chub CPUE at 2.4 (SE = 3.8), but JCM West (1.4, SE = 2.4) and 220-mile (1.0, SE = 2.6) were also within the margin of error.

Elevated Humpback Chub capture rates in the western Grand Canyon (i.e., from Havasu aggregation downriver) are a relatively recent occurrence (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 Humpback Chub were low (Figure 4). Since 2014, we have sampled most of these localities annually, and capture rates indicate a significant increasing trend of Humpback Chub relative abundance in western Grand Canyon (Figure 4, Van Haverbeke et al. 2017).

We also show comparative CPUE points for aggregation and non-aggregation sites among four distinct sampling periods (Figure 5). Because of variability of annual CPUE estimates, pooled sampling periods (4-5 years per period) were used to better illustrate changes over time. All sites since 2010 have shown a significant increase in Humpback Chub CPUEs compared to earlier periods between 1991 and 2006.

Length Frequencies

Length frequency distributions for Humpback Chub and for Flannelmouth Sucker between two river reaches (above and below RM 156) show two patterns. First, juvenile Humpback Chub size classes comprised a greater proportion of the catch in reaches below RM 156 (western Grand Canyon) than above RM 156 (Figure 6-A). This pattern was also true for Flannelmouth Sucker, only to a greater degree (6-B) where nearly all juvenile and small adult Flannelmouth Sucker <300 mm were captured in western Grand Canyon and nearly all large adults >300 mm were captured above RM 156.

We also show comparative length frequency distributions of Humpback Chub captured on aggregation sampling trips between two time periods (2010-2013 vs. 2014-2018) to illustrate the dramatic increase in catches of Humpback Chub in western Grand Canyon during the post-2013 timeframe (Figure 7). This change is particularly visible in reaches of the reaches of river sampled below Lava Falls (~ RM 182-250; Figure 7). Of note is that in the 2014-2018 time period, there is strong representation by all size classes of Humpback Chub in western Grand Canyon below Lava Falls.

Condition

We used relative weight (measured/expected weight by TL) as a metric to evaluate the condition of Humpback Chub and Flannelmouth Sucker at sample locations (Figure 8). In general, condition remained the same down-river for both species.

Antennas

We queried all detected PIT tags in the GCMRC fish database to determine species, total length, date, and location of the last capture. Of the 613 total unique PIT tags detected with antennas, most were Humpback Chub (n = 392, 64%) and Flannelmouth Sucker (n = 190, 31%). There were also nine Bluehead Suckers, one Common Carp, and one Rainbow Trout detected comprising 2% of detections. One razorback sucker x flannelmouth hybrid (3DD.003BFB07B1) was captured at RM 247.8. There were 19 (3%) PIT tags detected over the course of the trip that were not yet found in the database.

Abundance Estimation and Density

We provide comparative abundance and density (fish per mile) estimates for Humpback Chub in the JCM East and Bridge City reaches using only hoop net data in the marking events, and using hoop net and antenna data in the marking events (Tables 5 and 6). In the Bridge City reach, sufficient recaptures (≥7) were obtained within most 50 mm size classes to present size class abundance and density estimates. For example, we estimated that there were ≥59 Humpback Chub per mile within all 50 mm size classes in the Bridge City reach (Tables 6A and 6B). In the JCM East reach, we provide pooled abundance estimates for the adult size class (≥200 mm, Tables 5 A&B), and for the 200-249 mm size class using hoop nets and antennae data (Table

5B). Except for the 200-249 mm size class, there were insufficient recaptures (<7) within 50 mm size classes in the JCM East reach, and hence they are not shown.

We also provide abundance and density estimates of Flannelmouth Sucker in the Bridge City reach using only hoop net data in the marking event (Table 7). Unfortunately, because of uncertainties about tagging protocols in the recapture trips for Flannelmouth Sucker in the JCM East and West reaches, we only estimated abundances and densities of Flannelmouth Sucker in the Bridge City reach.

Discussion

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 2014-2018 (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 earliest period, although the CPUEs shown in Figure 5 reflect only hoop net captures. In general, Humpback Chub mean capture rates were lowest river-wide during the 1991-1993 and 2002-2005 periods (Figure 5). An increase in catch rates for most aggregations was documented when sampling resumed from 2010 to 2013, and then further increased at several aggregations between 2014 and 2018. This later period is also when we noticed the significant increases in Humpback Chub capture rates at non-aggregation sites, most of which are in the western, downstream part of Grand Canyon (Figures 2-5).

We also saw a very marked increase in Humpback Chub CPUE in the LCR inflow aggregation during the 2014-2018 period (Figure 5). We think this may be partly a result of a large portion of the subadult and adult population remaining outside of the LCR and in the nearby mainstem during 2015 and 2016.

Length Frequencies

Length frequency distributions for Humpback Chub and Flannelmouth Sucker show the presence of considerable numbers of juvenile Humpback Chub and Flannelmouth Sucker in western Grand Canyon (Figures 6A and 6B). This suggests the potential for mainstem spawning and recruitment of these species. While the source of these recruits is unknown, because of the distance from the LCR this suggests that an alternative spawning source exists downriver, likely within the mainstem that is contributing to the population increases in western Grand Canyon

(Van Haverbeke et al. 2017). Since 2014, catches of Humpback Chub in western Grand Canyon have dramatically increased in all size classes, particularly below Lava Falls (Figure 7).

Abundance Estimation

We provide abundance and density estimates, and capture probabilities for Humpback Chub in the JCM East and Bridge City reaches (Tables 5 and 6), and of Flannelmouth Sucker in the Bridge City reach (Table 7) during fall 2018. For Humpback Chub, we provide comparative estimates using hoop net data only in the marking event, and including hoop net and antenna data in the marking event.

Unfortunately, population estimation failed in two of our planned mark recapture reaches during 2018 (JCM West and Spencer reaches). One factor likely causing this was that the river during September 2018 was highly turbid, possibly leading to poor catch with resulting low marking and recapture rates. For instance, in the JCM West reach we did not obtain a sufficient number of recaptured Humpback Chub (only 3 recaptures total including antenna data) to conduct population estimation. This was not the case in 2017, when catches were higher and population estimates were made for both Humpback Chub and Flannelmouth Sucker in the JCM East and JCM West reaches (Pillow et al. 2018). Because of time constraints and the limited number of fish marked in the Spencer reach, we chose to focus our Diamond down recapture trip solely on the Bridge City reach where we had marked more chub during the previous aggregation trip (thus increasing the number of known individuals). This improved estimation in the Bridge City reach, but sacrificed a mark recapture effort in the Spencer reach.

Including antennae data in the marking event appeared to improve population parameters. For instance, it resulted in increased numbers of recaptures which increased our ability to obtain estimates with 50 mm size classes. It also decreased coefficients of variation, and increased $p1$ capture probability values. A problem in using antenna data, however, is calculating growth since previous detection in a riverine system that is highly variable in temperature. We took a minimalist approach to this problem (i.e., applying a uniform minimal growth rate to all Humpback Chub), since we were interested in the minimum number of Humpback Chub growing into adulthood. This approach seemed to be acceptable, because population estimates of all 50 mm size classes of chub in the Bridge City reach were not significantly different, whether we used hoop net data only (where length of all marked fish were measured), or included antenna data where lengths were estimated (Tables 5A and 5B).

Despite the above difficulties, our efforts in 2017 (Pillow et al. 2018) and 2018 appear to show that densities of adult Humpback Chub (≥200 mm) in the JCM East reach are roughly 4-6 fold higher than in the JCM West or Bridge City reaches. Similarly with Flannelmouth Sucker ≥200 mm (see Pillow et al. 2018).

Abundance and density estimates of adult Humpback Chub in the JCM East site suggest a population size similar to estimates provided by Yackulic et al. (2014) for the LCR inflow aggregation. Assuming that most Humpback Chub in the LCR inflow aggregation during the fall reside in the mainstem between RM 60-66 (60-mile Rapids to Lava/Chuar Rapids), then a density estimate of ~1,400-2,500 adult Humpback Chub per mile in the JCM East reach (1,400 from Table 5 in Pillow et al. 2018 and 2,500 from Table 5 this report) extrapolates to 8,400-15,000 individuals in the mainstem between RM 60-66. Using an open model, Yackulic et al. (2014) estimated a total of 7,000-16,000 adults for the entire LCR inflow aggregation in 2011 (i.e., inclusive of those in the LCR). For fall 2017, it was estimated there were another 1,921 adult Humpback Chub in the LCR (Van Haverbeke et al. 2018), and for fall 2018 it was estimated there were another 2,779 adult Humpback Chub in the LCR (Van Haverbeke et al. 2019).

Humpback Chub abundance estimates in the Bridge City reach showed multiple size classes (Table 6), indicating recruitment and growth into adulthood in western Grand Canyon, much like abundance estimates showed in the JCM West reach during 2017 (Pillow et al. 2018). At present, we have a limited understanding of mainstem capture probability using different gear types and a limited understanding of spatial patterns in density of Humpback Chub along the river corridor. Based on the observed Humpback Chub catch in recent years and the range of observed capture probabilities there is likely a population of several thousand adult Humpback Chub residing in western Grand Canyon. For instance, on the 2017 aggregation monitoring trip we captured 416 unique adult Humpback Chub in 11.9 miles of river sampled below Havasu Creek, with a capture probability of ~0.12 for adult chub in the JCM West reach. A simple abundance estimate derived from catch/capture probability would equal an abundance of about 3,400 fish for this 11.9 miles of river. In 2018, we captured 213 unique adult chub in 11.8 miles of river with a capture probability of ~0.14 for adults in the Bridge City reach for an approximate estimate of about 1,500 fish in this 11.8 mile reach.

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 of both Humpback Chub and Flannelmouth Sucker between mark and recapture was low (≤0.2 miles), supporting use of a closed model. Site fidelity of Humpback Chub has been noted by previous authors (Kaeding et al. 1990, Valdez and Hoffnagle 1999, Paukert et al. 2006, Gerig et al. 2014).

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.

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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 Whittis (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 nets deployed, side of the river (left [L] right [R]), and river miles (RM) during 21 August-7 September 2018 aggregation monitoring trip.

Sample vicinity	**Date**	Nets (L)	RM (L)	Nets (R)	RM (R)	Total Nets
Little Redwall	8/22/2018	12	32.8-34	12	34.5-35.7	24
Crash	8/23/2018	4	62.9-63.4	5	62.9-63.4	9
JCM East (day 1)	8/23/2018	18	63.4-65.1	17	63.4-65.1	35
JCM East (day 2)	8/24/2018	17	63.4-65.1	17	63.4-65.1	34
Cremation	8/25/2018	15	86.8-88.3	15	86.8-88.3	30
Stephen Aisle	8/26/2018	20	118.5-120.5	20	118.5-120.5	40
Middle Granite Gorge	8/27/2018	16	126.7-128.3	16	126.7-128.3	32
Havasu (above rapid)	8/28/2018	5	156.7-157.2	7	156.7-157.3	12
Havasu Creek	8/28/2018	1				1
Havasu (below rapid)	8/28/2018	9	158-158.9	7	158-158.7	16
Chevron	8/29/2018	15	182.8-184.2	15	182.7-184.2	30
JCM West (Upper)	8/30/2018	23	210.2-212.5	23	210.2-212.5	46
JCM West (Lower)	8/31/2018	15	212.5-214.1	16	212.5-214.1	31
220-mile	9/1/2018	15	218.4-219.9	15	218.4-219.9	30
Bridge City (Day 1)	9/2/2018	21	236.7-238.7	21	236.6-238.6	42
Bridge City (Day 2)	9/3/2018	21	236.7-238.7	21	236.6-238.6	42
Spencer (Day 1)	9/4/2018	21	245.8-247.9	21	245.8-247.9	42
Spencer (Day 2)	9/5/2018	21	245.8-247.9	21	245.8-247.9	42

Table 3. Total number of hoop nets set and mean (±SD) set times for each sampling location along the Colorado River on the 2018 aggregation trip. The LCR, JCM West, Bridge City and Spencer locations were sampled for two consecutive nights.

Sampling Location	Hoop Nets (n)	Mean Set Time (hrs)	Set Time (SD)
Little Redwall	24	17.45	0.78
LCR	78	20.00	2.09
Cremation	30	16.90	0.36
Stephen Aisle	40	17.07	0.72
Middle Granite Gorge	32	16.34	0.42
Havasu *	29	17.33	0.50
Cheveron	30	16.14	0.97
JCM West	77	18.13	1.48
220-mile	30	17.55	0.74
Bridge City	84	19.92	3.31
Spencer	84	20.58	3.83
Grand Total	**538**	**18.59**	**2.73**

*Includes 1 hoop net set in Havasu Creek.

Table 4. Numbers of fish captured by sample location and species during the 2018 mainstem Colorado River aggregation trip. Sampling sites are arranged from upriver to downriver. See Table 4 for river miles of sampling reaches.

Sample reach	Aggregation	BHS	CRP	FHM	FMS	HBC	RBT	SPD	Totals
Little Red Wall	30-mile				125	5	1		131
JCM East	LCR inflow	2		1	142	301			446
Cremation	Bright Angel			1	81	3		1	86
Stephen Aisle	Stephen Aisle	1			250	6	1		258
Middle Granite Gorge	Middle Granite Gorge		1		145	2	1		149
Havasu Creek	Havasu Inflow				76	7			83
Havasu (in mainstem)	Havasu inflow		1		181	8		4	194
Chevron		2			384	25		1	412
JCM West	Pumpkin Spring	1	1		960	106		24	1092
220-mile					672	31		5	708
Bridge City					290	198		34	522
Spencer				1	684	70		168	923
Totals		6	3	3	3990	762	3	237	5004

BHS = Bluehead Sucker (*Catostomus discobolus*), CRP = Common Carp (*Cyprinus carpio*), FHM = Fathead Minnow (*Pimephales promelas*), FMS = Flannelmouth sucker (*Catostomus latipinnis*), HBC = Humpback Chub (*Gila cypha*), RBT = Rainbow Trout (*Oncorhynchus mykiss*), SPD = speckled dace (*Rhinichthys osculus*).

Table 5. Humpback Chub abundance estimates and densities in JCM East reach using A) only hoop net data in the marking event, and B) hoop net and antennae data in the marking event, Colorado River, 2018. 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. Marked = number of marked fish, Captured = total number of fish captured in recapture event, and Recaptured = number of recaptured marked fish. Densities of fish are shown as estimated fish/mile.

A)

Humpback Chub abundance estimates JCM East (river mile 63.4-65.05) fall 2018 - Hoop nets only

						95% CI					
Length (mm)	Marked	Captured	Recaptured	N	SE	Lower	Upper	CV	*p* 1	*p* 2	fish/mile
>=200	205	62	8	2,459	878	739	4,180	0.36	0.13	0.04	1,408

B)

Humpback Chub abundance estimates JCM East (river mile 63.4-65.05) fall 2018 - Hoop nets and antennas

						95% CI					
Length (mm)	Marked	Captured	Recaptured	N	SE	Lower	Upper	CV	*p* 1	*p* 2	fish/mile
>=200	347	62	13	2,531	620	1,317	3,746	0.24	0.21	0.04	1,449
200-249	134	44	7	1,093	275	555	1,631	0.25	0.16	0.05	626

Table 6. Humpback Chub abundance estimates and densities (fish/mile) in Bridge City reach using A) hoop nets only in the marking event, and B) hoop nets and antennas in the marking event, Colorado River, 2018. 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. Marked = number of marked fish, Captured = total number of fish captured in recapture event, and Recaptured = number of recaptured marked fish. Densities are shown as estimated fish/mile. 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)

Humpback Chub abundance estimates Bridge City reach (river mile 236.65-238.7) fall 2018 - Hoop nets

						95% CI					
Length (mm)	Marked	Captured	Recaptured	N	SE	Lower	Upper	CV	*p* 1	*p* 2	fish/mile
100-149	10	67	3	140	24	94	186	0.17	0.04	0.30	63
150-199	81	149	27	385	54	280	490	0.14	0.18	0.33	173
200-249	28	95	18	199	31	139	260	0.15	0.19	0.64	90
250-299	41	143	13	324	46	234	415	0.14	0.09	0.32	146
>=300	20	100	7	214	33	150	279	0.15	0.07	0.35	97
Sum				1,263							569

B)

Humpback Chub abundance estimates Bridge City reach (river mile 236.65-238.7) fall 2018 - Hoop nets and antennas

						95% CI					
Length (mm)	Marked	Captured	Recaptured	N	SE	Lower	Upper	CV	*p* 1	*p* 2	fish/mile
100-149	12	67	4	130	21	90	170	0.16	0.06	0.33	59
150-199	82	149	28	352	45	263	441	0.13	0.19	0.34	159
200-249	33	95	20	187	27	134	241	0.14	0.21	0.61	84
250-299	49	143	20	299	39	221	376	0.13	0.14	0.41	134
>=300	23	100	10	196	28	141	251	0.14	0.10	0.43	88
Sum				1,165							525

Table 7. Flannelmouth Sucker abundance estimates and densities (fish/mile) using hoop nets only in Bridge City reach. Colorado River, 2018. Estimates of abundance (N), 95% confidence intervals, coefficient of variations (CV) and capture probabilities (*p*1 and *p*2) of size classes of Flannelmouth Sucker. Marked = number of marked fish, Captured = number of fish captured in recapture event, and Recaptured = number of recaptured marked fish. Densities of fish are shown as estimated fish/mile. Note: no comparative abundance estimates made using both hoop net and antenna data are provided.

Flannelmouth Sucker abundance estimates Bridge City reach (river mile 236.65-238.67) fall 2018

						95% CI					
Length (mm)	Marked	Captured	Recaptured	N	SE	Lower	Upper	CV	*p* 1	*p* 2	fish/mile
150-199	57	121	21	261	34	194	329	0.13	0.17	0.37	122
200-249	38	66	20	140	21	99	180	0.15	0.30	0.53	65
250-299	26	55	17	106	17	73	140	0.16	0.31	0.65	50
>=300	34	47	14	111	17	77	146	0.16	0.30	0.41	22
Sum				619							260

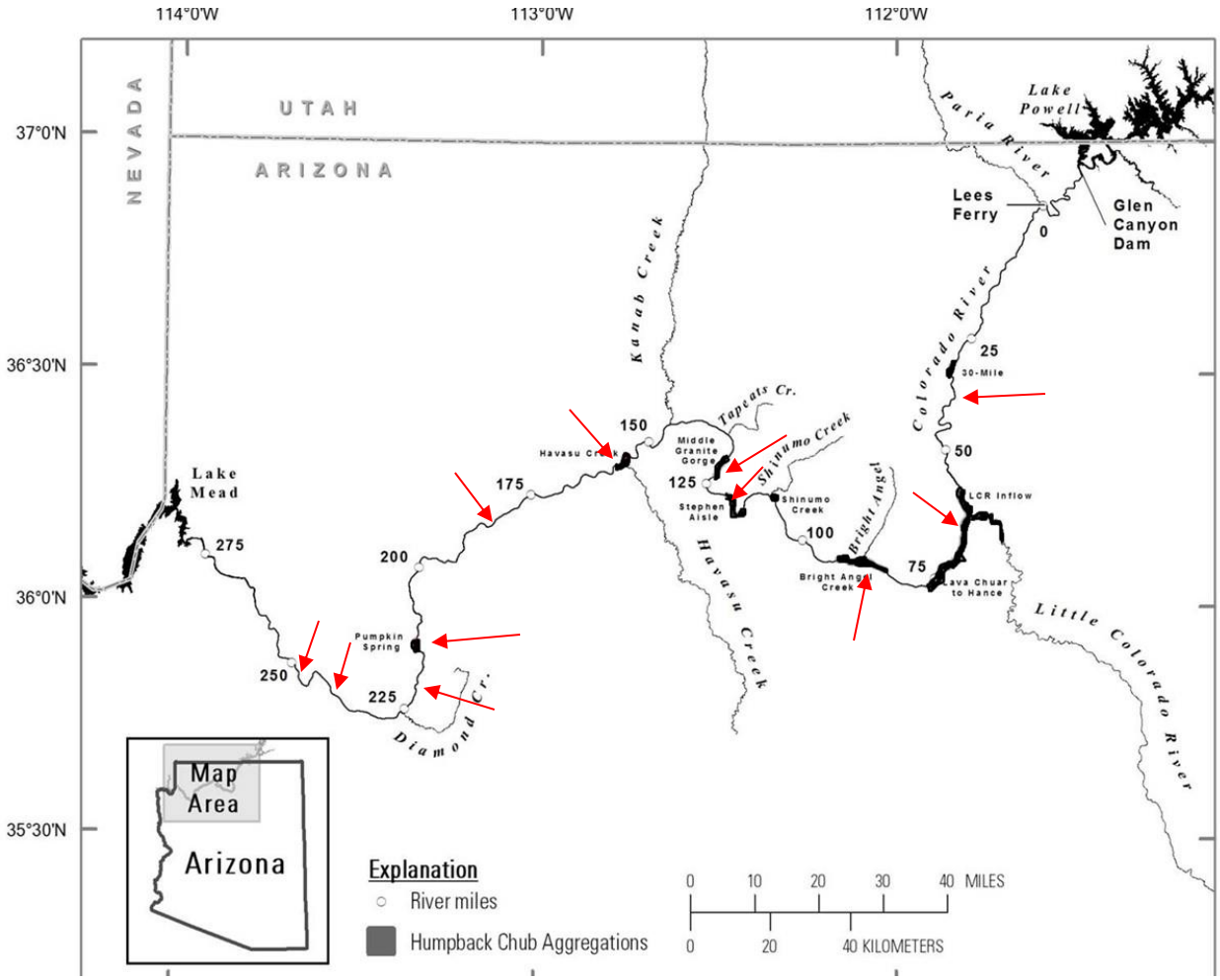


Figure 1. Map of the Colorado River from Lake Powell to Lake Mead showing the nine Humpback Chub aggregations (black), as defined by Valdez and Ryel (1995): 30-Mile, Little Colorado River inflow, Lava Chuar–Hance Rapid, Bright Angel inflow, Shinumo Creek inflow, Stephen Aisle, Middle Granite Gorge, Havasu Creek inflow, Pumpkin Spring. Red arrows indicate locations sampled in 2018. Map: Tom Gushue, GCMRC. Note: distance points shown along the river are in miles.

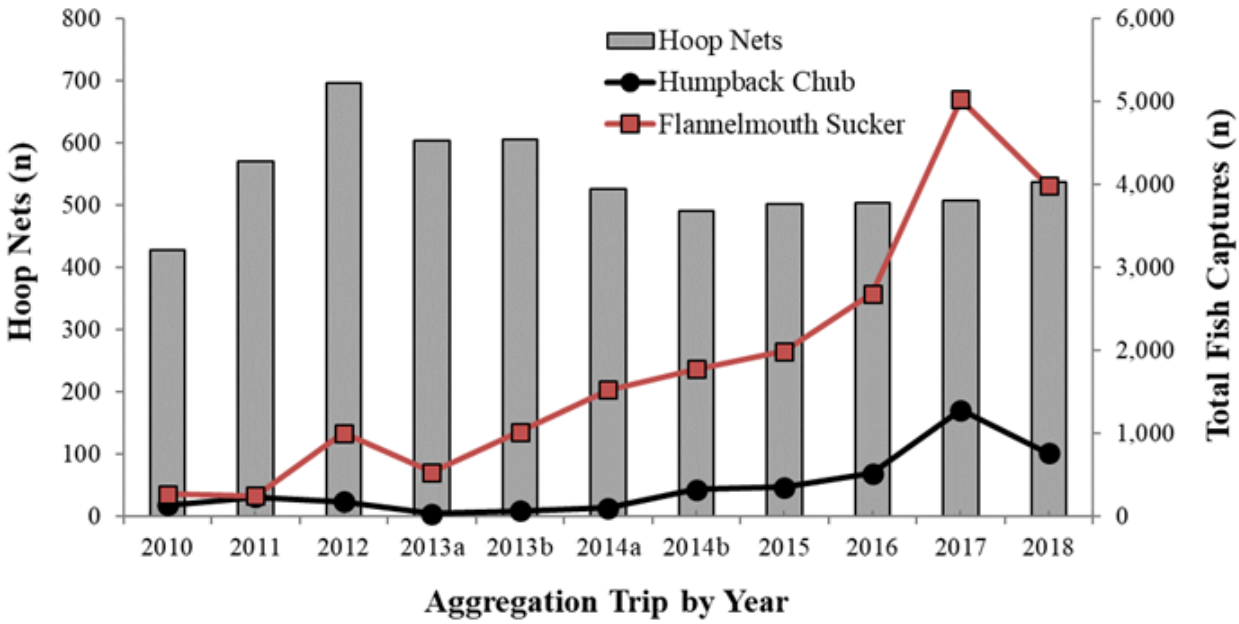


Figure 2. Total fish captures for Humpback Chub and Flannelmouth Sucker paired with total hoop nets set for each Grand Canyon aggregation trip 2010-2018. In 2013 and 2014, two hoop netting aggregation trips (July, September) were undertaken.

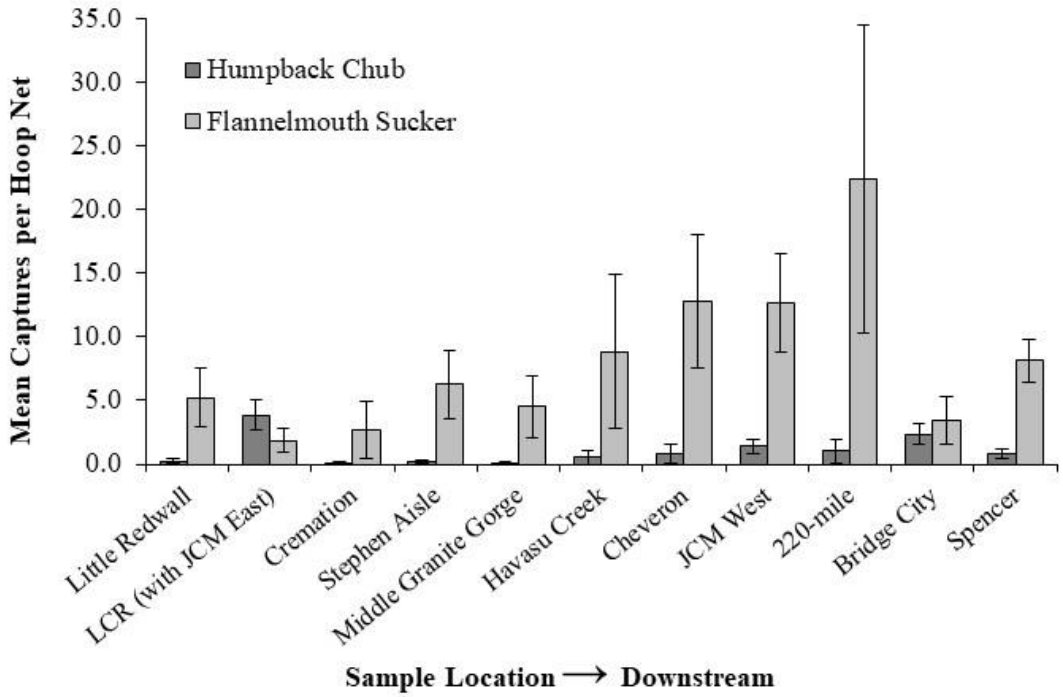


Figure 3. Mean catch per unit effort (CPUE ± 95% CI, captures per overnight hoop net) for Humpback Chub and Flannelmouth Sucker at each sample location on the 2018 aggregation monitoring trip.

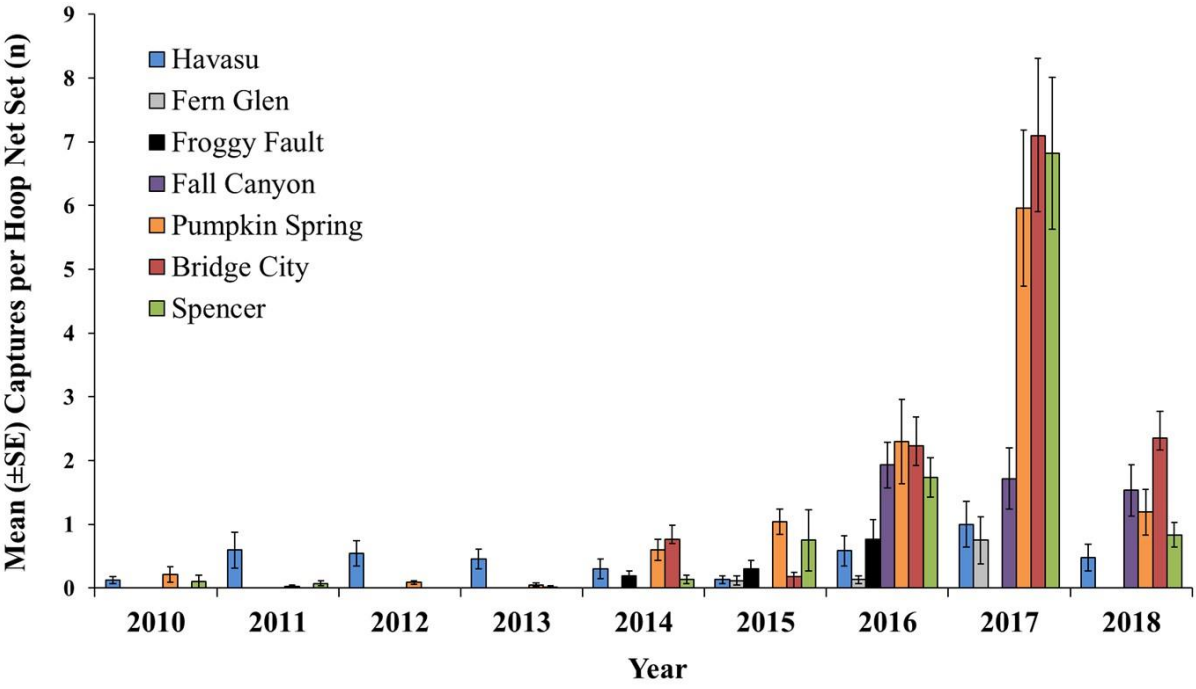


Figure 4. Mean Humpback Chub catch per unit effort (CPUE ± 95% CI, captures per overnight hoop net) for sampling reaches from Havasu downriver 2010-2018. Bridge City was not sampled in 2010, 2011 or 2012 and Spencer was not sampled in 2012 or 2013.

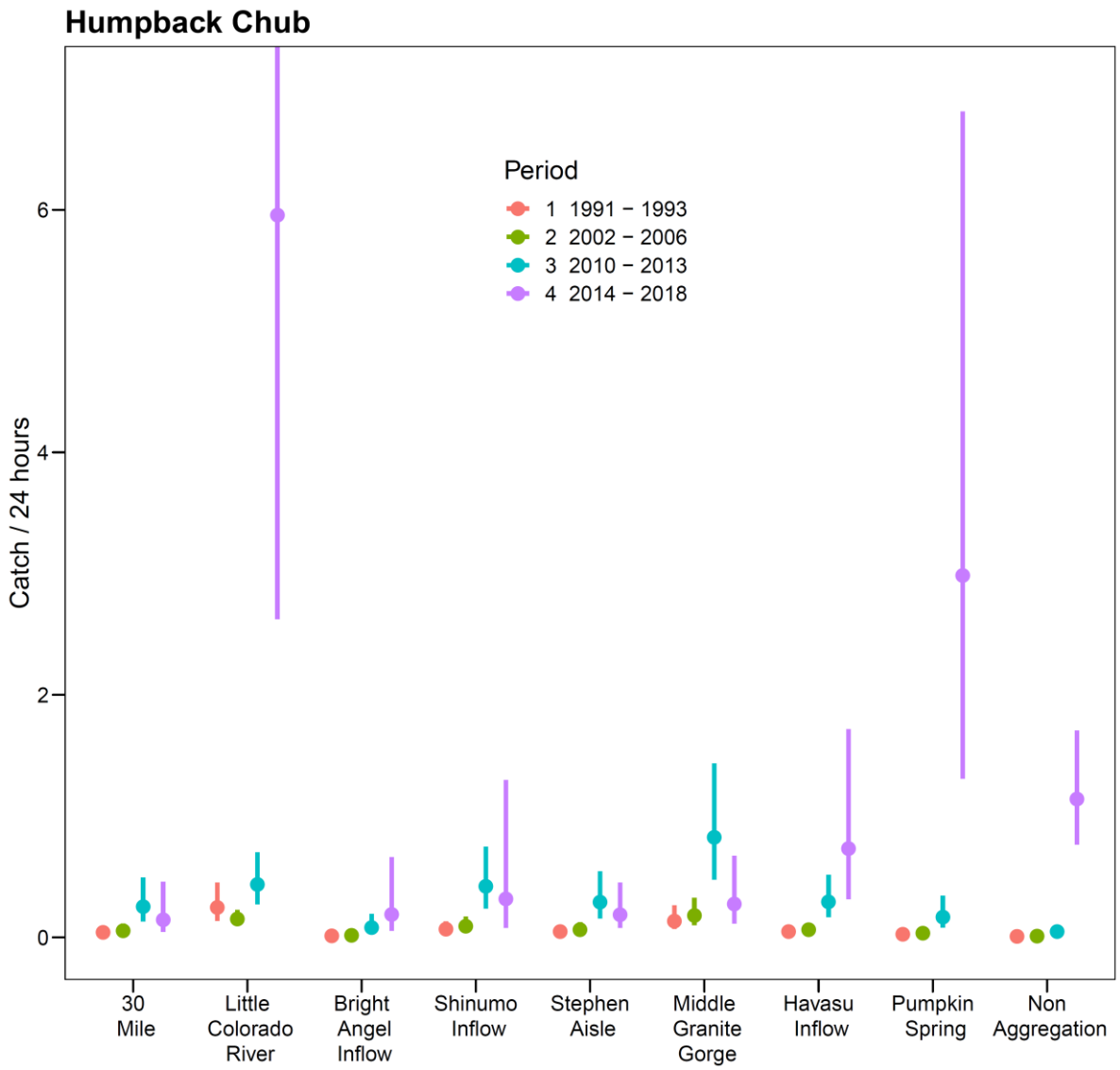
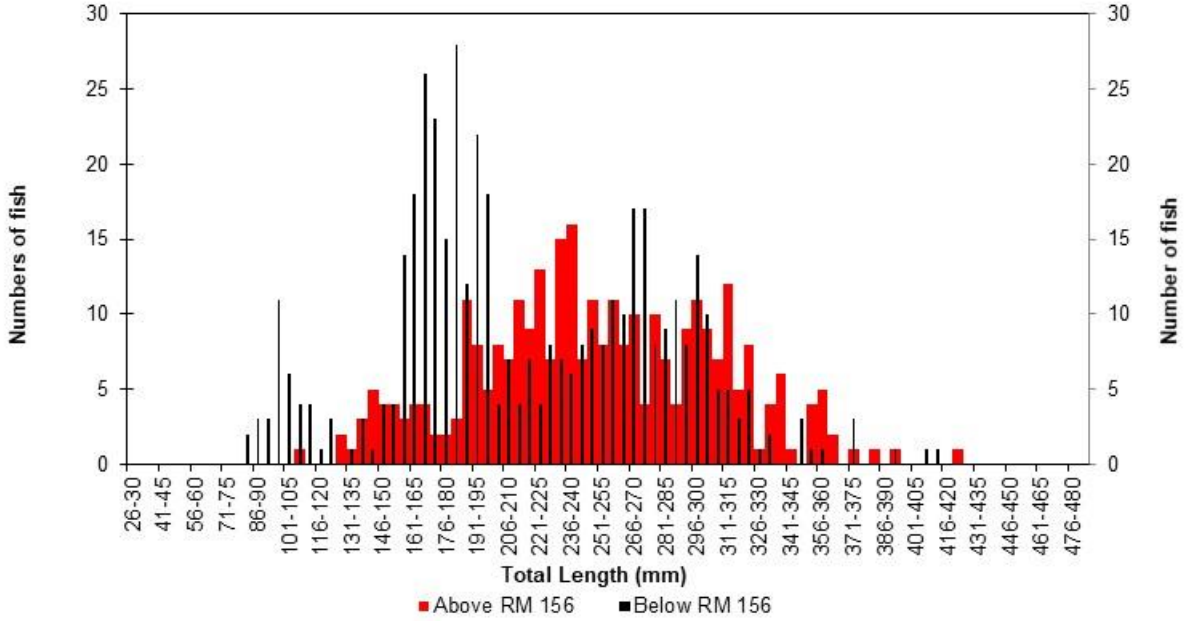


Figure 5. Predicted catch of adult Humpback Chub per 24 hours for hoop nets at each Grand Canyon aggregation during four sampling periods between 1991 and 2018. Orange points (furthest left point within each period) indicate sampling events conducted between 1991 and 1993 (Valdez and Ryel 1995). Green points (second from left) indicate sampling events conducted between 2002 and 2006 (Ackerman 2008). Blue points (third from left) indicate sampling events conducted between 2010 and 2013 (Persons et al. 2017). Purple points (furthest right point within each period) represent sampling events conducted between 2014 and 2018. Error bars represent 95 percent confidence intervals. "Non-Aggregation" includes all locations not previously defined as an aggregation. Figure supplied by M. Dodrill (USGS Grand Canyon Monitoring and Reasearch Center).

A.



B.

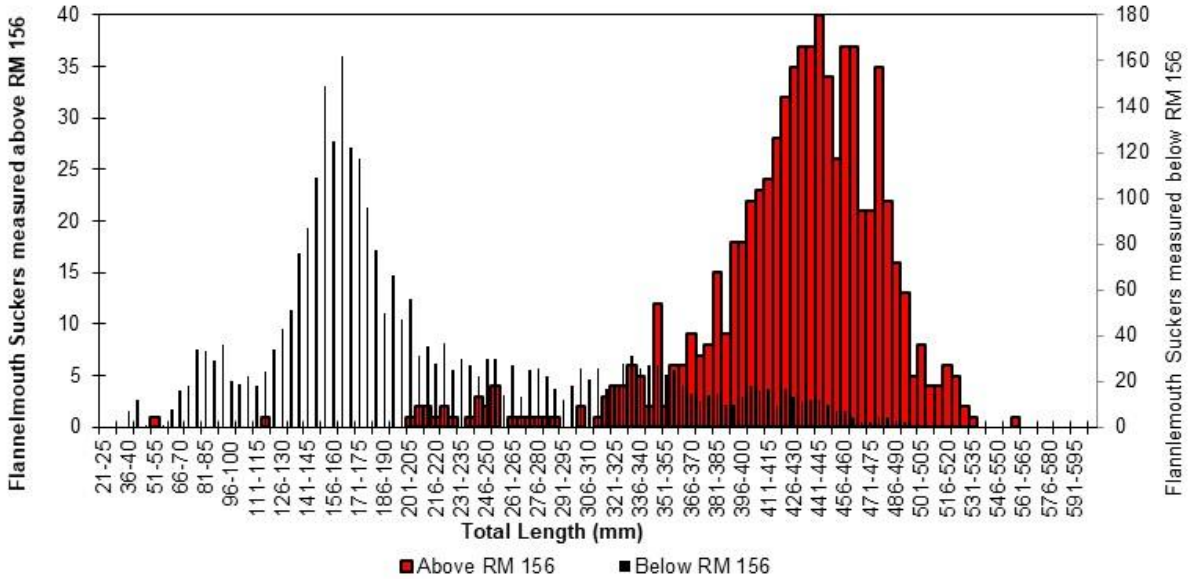


Figure 6. Length frequency distributions for A) Humpback Chub and B) Flannelmouth Sucker captured during the 2018 aggregation trip above and below river mile RM 156. Note: For Flannelmouth Sucker below RM 156, representative samples of length measurements were sometimes taken. Thus, the y-axis for Flannelmouth Suckers measured below RM 156 is considerably smaller than if all fish had been measured.

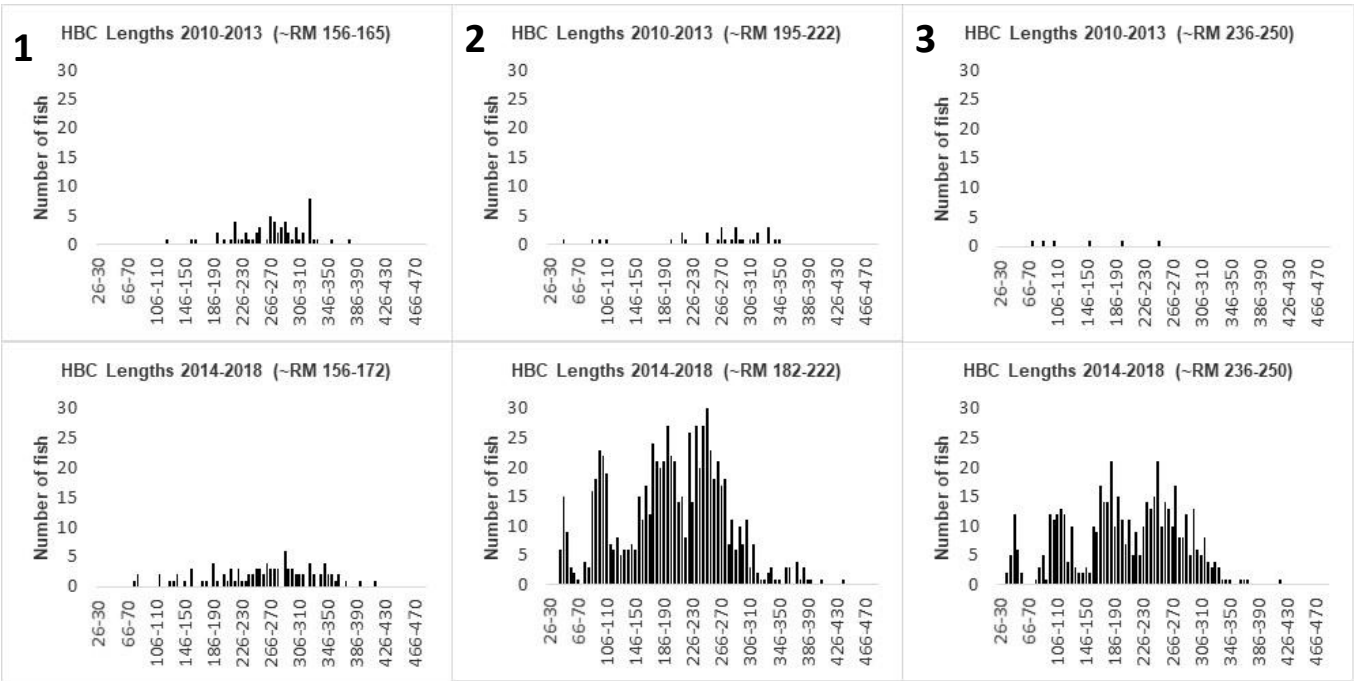


Figure 7. Comparative length frequency distributions of Humpback Chub (HBC) captured on aggregation sampling trips during two time periods (2010-2013 [upper row] vs. 2014-2018 [lower row]) in three reaches of western Grand Canyon: 1) from just above Havasu Creek to above Lava Falls (~river mile [RM] 156-172), 2) From below Lava Falls to above Diamond Creek (~ RM 182-222), 3) From below Diamond Creek to Red Clay Tank (~RM 236-250). Note: 2010-2013 represented by 5 sampling trips and 2014-2018 represented by 6 sampling trips.

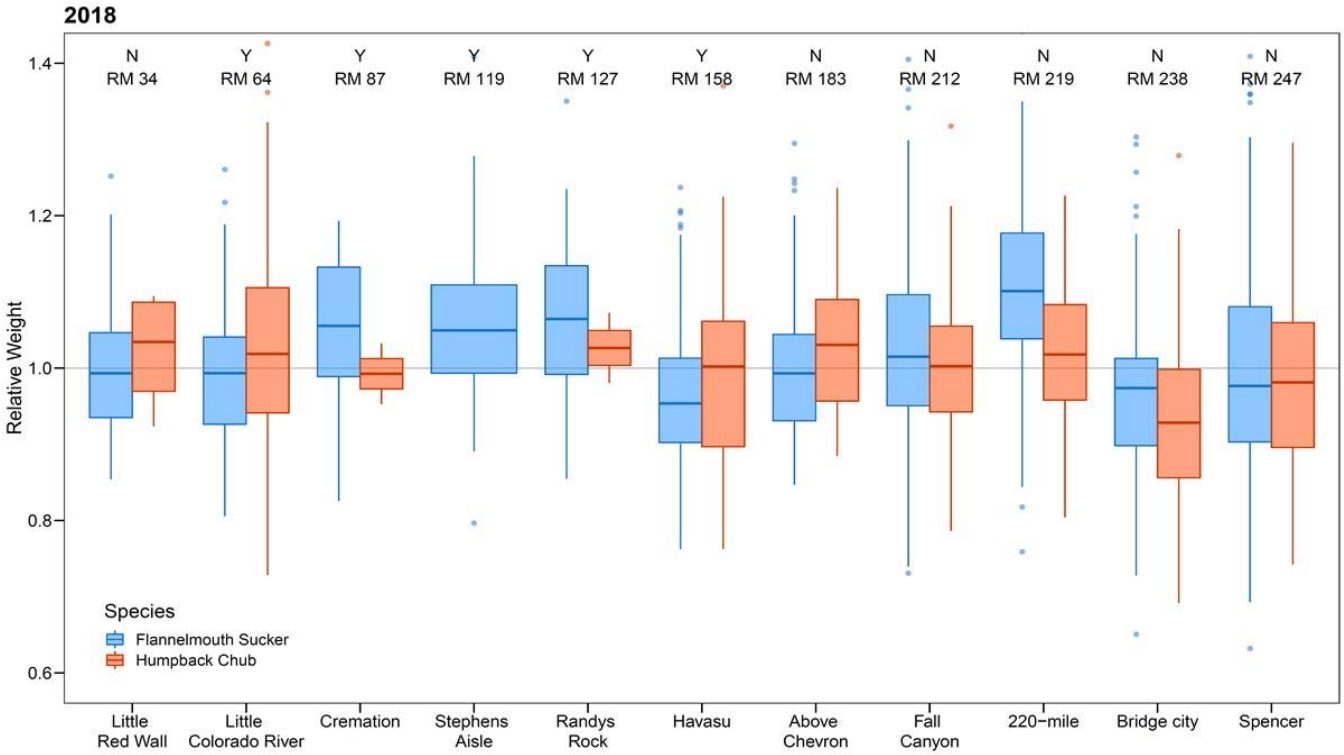


Figure 8. Relative weight (% of expected weight by total length) for Flannelmouth Suckers (blue) and Humpback Chub (Orange) collected in 2018 at sample locations. Figure supplied by M. Dodrill (GCMRC).