

**NATIVE FISH MONITORING ACTIVITIES IN THE COLORADO RIVER WITHIN  
GRAND CANYON DURING 2001**

**Final Report**

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## EXECUTIVE SUMMARY

The feasibility of and effort required to conduct a population estimate for humpback chub (*Gila cypha*) in the Colorado River in the Grand Canyon near the Little Colorado River (LCR) inflow were assessed in 2001. The largest aggregation of humpback chub (HBC) in the Colorado River mainstem occurs in the LCR area from River Mile (RM) 56.3, above the LCR, to RM 68.3 below the LCR. The LCR enters the Colorado River near RM 61.5. Population size was estimated using a two-pass mark-recapture design. Two sampling trips were conducted; one in July/August and the other in August/September using trammel nets and hoop nets to capture HBC. Sampling effort included a total of 264 person days, 605 hoop net sets comprising 13,524.5 sampling hours, and 1,102 trammel net sets comprising 2,151.9 sampling hours and resulted in 425 HBC captured. HBC greater than 100 mm TL were marked with PIT tags on the first trip and examined for marks on the second trip. We captured 104 HBC >100 mm TL on the first trip and 160 on the second trip. During the second trip no fish less than 200 mm TL were recaptured from the first trip. Thus, the population estimate was stratified to include only fish greater than 200 mm TL, resulting in 83 marked on the first trip and 111 examined on the second trip, with 8 recaptured fish, for a population estimate of 1,044 fish > 200 mm TL (95% confidence interval 559 to 2,137), with a coefficient of variation (CV) of 29%. Despite the large effort, the number of captures and recaptures was lower than expected, and the precision of the estimate was less than desired. Increased effort is recommended for future population estimates. However, the 2001 estimate was significantly different from similar estimates made in 1991-1993 (Valdez and Ryel 1995) and suggests a significant decline in the abundance of HBC in the mainstem Colorado River near the LCR.

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## 1.0 INTRODUCTION

The Grand Canyon Monitoring and Research Center (GCMRC) currently administers research and management actions for humpback chub (HBC) in the Grand Canyon. Recent stock assessment modeling and analysis using historical catch data for HBC in the Little Colorado (LCR) and Colorado Rivers in Grand Canyon have indicated a decline in population size since the early 1990s (GCMRC 2003). However, no rigorous population estimates had been made of HBC in the Colorado River in Grand Canyon since mainstem estimates reported by Valdez and Ryel (1995), and LCR estimates published by Douglas and Marsh (1996).

In 1980 and 1981 Kaeding and Zimmerman (1982) conducted the first population estimate that included the Colorado River at the inflow of the LCR. The study area included 32-km (RM 51.5-71.5) of the Colorado River around the mouth of the LCR and the 20-km reach of the LCR between the confluence with the mainstem and Blue Springs, the uppermost source of perennial flow in the LCR. While the authors suggest that many of the application criteria were not met in the study, they do provide a “ballpark” estimate using the Schnabel, modified Schnabel, and Schumacher/Eschmeyer methods. They estimated that the adult HBC population (>200 mm) was around 7,000–8,000. During 1990-1993 Valdez and Ryel (1995) used the program CAPTURE to estimate the Colorado River population of humpback chub to be 3,750 adults (>200 mm TL), with about 3,482 adults (95% CI = 2,682-4,281) in the aggregation near the LCR (RM 57-65.4). This was the only time the entire Colorado River population was estimated in the Grand Canyon. During the same period (1991-1992), Douglas and Marsh (1996), also using CAPTURE, estimated 4,508 humpback chub (>150 mm TL, no confidence intervals provided for this estimate) within the LCR. These two estimates appear to be sound and reliable (GCMRC 2003). Following these studies no further estimates were made until 2000. The U.S. Fish and Wildlife Service (FWS) estimated the LCR population in October of 2000 using Chapman-Petersen. The October 2000 estimate for HBC (>135 mm) was 1,590 fish (Coggins and Van Haverbeke 2001).

By 2001 there was a clear need for concurrent population estimates in the LCR and mainstem Colorado River to validate GCMRC’s HBC stock assessment modeling efforts. GCMRC determined that population estimates of HBC in the LCR and in the LCR-associated mainstem aggregation should be conducted to produce a total population estimate of HBC for the LCR/mainstem aggregation in 2001. A cooperative effort between GCMRC, SWCA, Inc., Environmental Consultants (SWCA), FWS, and Arizona Game and Fish Department (AGFD) was proposed to accomplish this and other complementary objectives.

Population estimate methodologies developed by the FWS during mark/recapture estimates in 2000 are promising for the LCR. However, accurate and precise population estimates have been difficult to make in the past, particularly in the mainstem, due to the large amount of time and effort necessary to produce an adequate number of fish captures (marks) and recaptures. Earlier population estimates made in both the LCR (Douglas and Marsh 1996) and the mainstem (Valdez and Ryel 1995) were produced from monthly sampling trips performed over several years.

Three-pass mark/recapture estimates in the mainstem were attempted in 2000 as part of a gear calibration study (Trammell and Valdez 2002). Population estimates were made for a portion of the LCR reach and for a portion of the Middle Granite Gorge (near Randy's Rock) but were imprecise because of insufficient captures and recaptures of fish and because the sample areas did not cover the entire range of the populations of interest. The low number of captures at these sites was the result of insufficient effort in time and possible gear saturation. However, sampling efforts in 2001 were confined to the largest aggregation (LCR), and the total effort was increased in an attempt to capture sufficient numbers of fish in this aggregation to perform a more precise population estimate.

SWCA proposed to perform a two-pass mark-recapture population estimate in the mainstem of the Colorado River from River Mile (RM) 56.3 to 68.3, to be conducted during late summer and early fall 2001, when fish movement between the rivers is at a minimum and catch rates are reasonably good (Figure 1). In the LCR, the FWS proposed to perform a two-pass mark/recapture population estimate in the spring (May/June), and another two-pass estimate in the fall (October/November). This schedule would result in three independent estimates. The spring LCR estimate would include resident LCR fish and resident mainstem fish spawning in the LCR in the spring. The fall LCR estimate would include only resident LCR fish. The summer mainstem estimate would include only resident mainstem fish. The fall LCR and summer mainstem estimates may be added together for an estimate of the total HBC population. This report addresses objectives 1, 2, and 4 (Section 1.1). Objective 3 will be addressed cooperatively with the FWS in a separate report.

## **1.1 Objectives of Mainstem Sampling**

1. Assess the potential and effort required of two-pass mark/recapture sampling to provide mainstem abundance estimates of HBC.
2. Capture HBC >100 mm TL to increase mark rates and collect mark/recapture data to support stock synthesis model to establish a baseline for future monitoring efforts. These data may be used to estimate relative proportion of the mainstem population to LCR population using proportional mark rates.
3. Assess changes in mark rates and population length frequency distributions over time using historical data to determine if recruitment is taking place, and attempt to estimate recruitment rates.
4. Assess predation by examining stomach contents of occasional rainbow trout (*Oncorhynchus mykiss*) > 300 mm TL, and all brown trout (*Salmo trutta*), channel catfish (*Ictalurus punctatus*), and black bullhead (*Ictalurus melas*) in the field.

## 2.0 METHODS

### 2.1 Study Area

The study area was the mainstem Colorado River in the Grand Canyon from RM 56.3 above the LCR (Kwagunt Rapid) to RM 68.3 below the LCR (Tanner Rapid). Kwagunt and Tanner rapids delineate the range of the LCR/mainstem aggregation (Valdez and Ryel 1995). The LCR enters the Colorado River at RM 61.5. River Mile designations from Lees Ferry (RM 0) to Diamond Creek (RM 226) were used to identify sampling areas (Stevens 1983).

### 2.2 Mark-Recapture Population Estimate

Two mainstem native fish sampling trips were conducted, one in late July/August (Jul 31-Aug 7) and one in late August/September (Aug 28-Sep 4). Each trip included 8 full days and nights of sampling and was a total of 12 days long including 1 day to rig the boats and equipment, 1 day to de-rig, and 2 days for travel. Trammel nets (TK; 75x6x10x100 and TL; 75x6x1.5x100) and baited hoop nets (HB; 2Ndia.) were used to sample the reach intensively to maximize captures of both adult and juvenile HBC. Hoop nets were baited with Aquamax trout chow suspended inside the net in a small mesh bag. Eleven personnel participated in each sampling trip.

A minimum of two sampling passes is required for a mark/recapture population estimate. In mark/recapture estimates, as in any statistical exercise, precision increases with sample size. For a reasonably precise estimate, the target figure for the number of fish captured in the first pass was 10-20% of the total population. Because the population was not known until the estimate was made, estimating the number of captures necessary was speculative. However, using the Valdez and Ryel (1995) estimate of 3,482 adult HBC >200 mm in the mainstem LCR aggregation, we estimated a minimum of 350 fish captures would be required, with greater precision gained by increasing that number (Figure 2). The historic average catch rate of HBC >200 mm in August in the LCR inflow area (RM 60-69) is 41.2 fish/100 hours/100-ft net (based only on trammel net catch). This figure was derived from historical data, collected by the FWS, AGFD, and Bio/West from 1990 to 2000 and compiled by Lew Coggins (FWS). At 41.2 fish/100 hrs/100-ft, about 850 hours of effort would be necessary to produce 350 fish. The historic average does not include sampling above the LCR because very few samples were taken in this reach after 1993. Historic catch rates in this area were lower than those at or below the LCR inflow area. SWCA sampled above the LCR (from RM 56.3) as well as to RM 68.3 below the LCR. Our inclusion of sampling in this area was expected to reduce the overall catch rate, thus the sampling design provided for approximately 2,150 hours of trammel net effort using 75-ft nets, or 1,600 hours/100-ft nets, roughly twice the estimated effort needed. Trammel net sampling was also supplemented with about 13,000 hours of hoop net sampling to maximize the catch, particularly of juvenile fish.

The historical catch rate data (1990-1999) were also examined to determine if stratification of effort was needed. The 12-mile reach was divided into approximately one-mile sections, and catch and effort were evaluated for each section. The historical catch was correlated with effort ( $r^2 = 0.67$ ) below the LCR (Figure 3), and it was determined that stratification of effort was not appropriate below the LCR. However, effort above the LCR was not included in the data set

examined. Relatively little effort was spent historically above the LCR because catch was usually low; therefore, during this study, slightly less effort was expended per one-mile section above the LCR than was expended below. We strove to maintain equal effort for each one-mile section from the LCR to RM 65.5. Effort below this point was also reduced slightly due to logistical considerations. The proposal stipulated that hoop nets would not be deployed above the LCR, since hoop nets primarily target fish <200 mm in the mainstem, and few fish of this size class have been collected above the LCR confluence. However, in the field the decision was made to set hoop nets above the LCR as well as below to confirm this pattern and supplement the adult catch.

A general sampling schedule is shown in Table 1. Three netting boats were used. Each boat was assigned to an approximately one-mile section (limits were adjusted slightly due to occurrence of rapids and riffles), and each section was sampled for 2 days with hoop nets and trammel nets. Each boat deployed 5-7 trammel nets per set. There were three 2-hour sets each evening beginning at 1630 hours, and two 2-hour sets each morning, beginning at 0530 hours. Nets were checked every two hours, and all fish were removed and processed as defined below, and released or sacrificed as necessary. Each 2-hour set was considered one sample. Each netting boat also set or checked 18 hoop nets, which were checked every 24 hours and reset or moved and reset as necessary. Each 24-hour net set was considered one sample. Hoop nets were generally set beginning at 1300 hours, and it took approximately 2 hours to set, check, or move all hoop nets. All hoop nets were baited with commercial trout food (Aqua-max) by securing a handful of bait in the end of the net in a small mesh bag.

The abundance of HBC  $\geq 100$  mm in the LCR reach was estimated using the Chapman-Peterson estimation formula. This formula was chosen to be comparable with the same estimation formula used by the FWS for the LCR (Lew Coggins, personal communication). The Chapman-Peterson model is recommended for two-pass mark-recapture estimates (White et al. 1982). The estimation formulae from Ricker (1975) are:

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

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

where  $N^*$  is the estimated number of fish in the population,  $V[N^*]$  is the estimated variance of the number of fish in the population,  $M$  is the number of fish marked during the marking event,  $C$  is the number of fish captured during the recapture event, and  $R$  is the number of fish recaptured in the recapture event.

The Chapman-Peterson method assumes the population was closed (no emigration, immigration, mortality or recruitment) between the marking and recapture events. Additional requirements that must be met for unbiased estimates from two-event mark-recapture experiments on closed populations are that marking does not affect catchability of a fish and there is no ‘trap-happiness’ or ‘trap-shyness’. Additional assumptions, of which at least one must be fulfilled, are that all fish have an equal probability of being captured during the marking event, or all fish have an

equal probability of capture during the second event, or marked and unmarked fish mix completely between sampling events. These assumptions, and means to correct them if violated, were tested according to procedures outlined in Bernard and Hansen (1992).

### **2.3 Predation**

Occasional rainbow trout > 300 mm TL, and all brown trout, channel catfish, and black bullhead, and were sacrificed to examine the stomach contents. The stomach contents were examined in the field. If a sample appeared to contain fish, it was preserved in formalin for later identification. The stomach contents were examined in the laboratory to attempt to identify fish prey items to species using a ratio of standard length to snout-to-vent length to distinguish between cyprinids and catostomids. Brown trout > 125 mm have been PIT tagged by AGFD in recent years; therefore, all brown trout were scanned for a PIT tag prior to sacrifice.

### **2.4 Fish Processing/Data Collection**

Data were recorded on data sheets provided by GCMRC. Copies of the data sheets were delivered to GCMRC. Data were entered into a Microsoft ACCESS database designed by GCMRC and were delivered to GCMRC in electronic format. Data analysis was performed using Microsoft EXCEL.

All fish captured were processed according to the following list:

1. Total lengths were taken for all native fish, and fork lengths were taken on all native fishes >100 mm TL. Total lengths were taken for all non-native fishes.
2. Weights (g) on all native fishes (>100 mm TL) were taken.
3. All HBC > 100 mm TL and all native fish and brown trout > 125 mm TL were scanned for PIT tags. All native fish > 100 mm were PIT tagged, if not previously tagged. The tag numbers were recorded on data sheets and stored in the PIT tag readers for later download.
4. All native fish were examined for sex, sexual condition, and external parasites.
5. Global Positioning System (GPS) data were taken at the attachment point of each netting device as satellite acquisition allowed.
6. Genetic samples (fin clips) were taken as necessary, in coordination with concurrent genetic studies.

**Table 1.** General sampling schedule for humpback chub monitoring in the Colorado River in Grand Canyon, in July and September 2001.

Date	Am/Pm	Camp	Activities	RM	Sample Area
Day 1		Lees Ferry	rig/travel		
Day 2	AM		Travel		
	PM	Awatubi	set/check TR nets	RM 56.3-59.5	Kwagunt to 60 Mile
Day 3	AM/PM	Awatubi	set/check TR and hoop nets	RM 56.3-59.5	Kwagunt to 60 Mile
Day 4	AM		set/check TR and hoop nets	RM 56.3-59.5	
	PM	Crash	move camp to Crash	RM 59.5-63	60 Mile to 63
Day 5	AM/PM	Crash	set/check TR and hoop nets	RM 59.5-63	60 Mile to 63
Day 6	AM	Crash	set/check TR and hoop nets	RM 59.5-63	60 Mile to 63
	PM	Crash	set/check TR and hoop nets	RM 63-65.5	63 to Lava-Chuar
Day 7	AM/PM	Crash	set/check TR and hoop nets	RM 63-65.5	63 to Lava-Chuar
Day 8	AM		set/check TR and hoop nets	RM 63-65.5	63 to Lava-Chuar
	PM	Tanner	move camp to Tanner	RM 65.7-68.3	Lava-Chuar to Tanner
Day 9	AM/PM	Tanner	set/check TR and hoop nets	RM 65.7-68.3	Lava-Chuar to Tanner
Day 10	AM/PM	TBD	Travel	68.3-226 (164)	
Day 11	AM/PM	TBD	Travel	68.3-226 (225)	
Day 12		Home	travel/derig	RM 226	Diamond Creek

### 3.0 RESULTS

#### 3.1 Sampling Efforts and Fish Captures

Sampling effort was 264 person-days, with 605 hoop net sets for 13,524.5 sampling hours, and 1,102 trammel net sets for 2,151.9 sampling hours. A total of 859 samples was taken on Trip 1, and 852 samples were taken on Trip 2. Table 2 gives the total number of samples and total sampling hours for each gear type for each sampling trip. The total number of HBC captured by each gear type is also shown in Table 2. Ten species were captured on the first trip and 11 on the second trip. Total number of each species captured by gear type is shown in Tables 3 and 4. Humpback chub were captured by angling at one site on both trips (RM 62.5) as well as by the hoop nets and trammel nets, and these fish were included in the population estimate.

The amount of effort by one-mile sections (in number of hoop net and trammel net sets), with associated captures of HBC, is shown in Figure 4. Effort was commensurate between trips in number and hours of samples. The majority of effort was expended between RM 60 and 65.5, and less effort expended above RM 60 (above the LCR) and below RM 65.5 (Lava-Chuar Rapid). The reduced effort below RM 65.5 was primarily a result of the character of the river channel in this section. This area has relatively fast water, with shallow, rocky shorelines that limited the areas where nets could be set, as well as shallow, rocky rapids that are dangerous to up-run in the sampling boats.

### **3.2 Mainstem HBC Population Estimate**

During the first sampling trip (Trip 1), a total of 121 humpback chub were captured. Of those, 1 was not measured, 16 were less than or equal to 100 mm and 104 were greater than 100 mm. These 104 fish were PIT tagged if not already tagged and all were counted as marks. On the second trip (Trip 2), 304 HBC were captured. Two fish escaped before measurement or tagging. Five were captured twice, for a total of 297 individuals. Of these, 137 were less than or equal to 100 mm, and 160 were greater than 100 mm. A total of eight fish were recaptured from the first trip. No fish were recaptured less than 200 mm (Figure 5), thus the estimate was stratified for size as discussed in the next section, resulting in estimates for fish greater than 200 and 250 mm TL (Table 5).

### **3.3 Marks**

The percentage of HBC marked with PIT tags was estimated in two ways. We calculated the percentage of fish marked by the FWS and recaptured by SWCA during this study. In the mainstem, 18 LCR-tagged fish were recaptured out of 210 fish greater than \$150 mm, for a tagged percentage of 8.6%. We also calculated the historical marks, which included all fish that were tagged prior to and including the 2001 study year. Adult fish in this population have been captured and tagged by researchers since PIT tags began to be used extensively in the Grand Canyon in 1990 and many of these marked fish continue to be captured. Of previously tagged fish captured during this study, 92.6% had been tagged prior to 2001. A total of 258 unique fish \$100 mm TL were captured during the two mainstem trips. The percentage of marked fish in the mainstem for fish \$100 mm and #200 mm was 1.7% (1/59). The percentage of marked fish \$200 mm and #300 mm was 23.7% (9/38), for fish \$300 mm and #400 the percentage was 86.6% (97/112), and for fish \$400 mm, the percentage was 95.9% (47/49). Thus the percentage of tagged fish increased with the size of the fish.

### **3.4 Large Predator Stomach Samples**

Some large-bodied predators were sacrificed to evaluate predation on humpback chub. On Trip 1, 11 brown trout (BNT) were captured ranging from 336 to 622 mm TL. All were sacrificed for stomach samples, including one recaptured fish with a clipped adipose fin and a PIT tag (532376585C). Three fish (two captured in trammel nets and one in a hoop net) contained fish or fish bones. On Trip 2, 27 BNT were captured. Of these, 23 were examined: 17 were empty, and 6 contained fish (3 from trammel nets and 3 from hoop nets). One (from a hoop net) contained a HBC (121 mm) that had been PIT tagged the day before at the same site.

This direct evidence of predation by BNT upon HBC would be more meaningful if the BNT had not been captured in a hoop net, where the juvenile HBC are more vulnerable to predation because they cannot escape and there is no cover. In addition, of 127 rainbow trout examined, 3 (307, 353, and 412 mm) appeared to contain fish. All three were captured in trammel nets. The preserved stomach samples were examined to identify the species of the fish remains.

Most of the specimens were too far digested to determine species. In many cases, all that remained were a few small bones. Skulls were frequently missing in otherwise intact specimens. The diagnostic pharyngeal teeth are located in the head so specimens without skulls could not be identified. The specimens with intact skulls were gently boiled to remove the remaining flesh; however, this method was unfortunately too severe to allow subsequent identification of pharyngeal bones. The only specimen out of 10 fish items that was definitely identified as a HBC was the PIT tagged fish. Other stomach items that were not fish were not identified, except for one small bat in the 307-mm RBT (Table 6).

**Table 2.** Total number of samples and hours of effort for each gear type, number of humpback chub captured, and mean CPE by each gear type on two mainstem sampling trips, 2001.

Gear	Trip 1				Trip 2			
	Samples	Hours	HBC	HBC cpe/100hrs/100'	Samples	Hours	HBC	HBC cpe/100hrs/100'
AN	3	NA	3	NA	1	NA	2	NA
HB	302	6,930.8	39	0.6	303	6,593.7	190	2.8
TN	554	1,090.3	79	9.8	548	1,061.6	112	13.64
<b>TOTAL</b>	<b>859</b>	<b>8,021.1</b>	<b>121</b>		<b>852</b>	<b>7,655.3</b>	<b>304</b>	

AN=angling, HB=hoop nets (2-ft dia.), TN=trammel nets: either TK = (75-ft x 6-ft x 1.0 in mesh), or TL= (75-ft x 6-ft x 1.5 in mesh).

**Table 3.** Number of each fish species captured in the mainstem Colorado River, by each gear type during Trip 1, 2001.

Gear	BBH	BHS	BNT	CCF	CRP	FHM	FMS	HBC	RBT	RSH	SPD
AN								3			
HB		1	4	1		3	5	39	62	1	1
TK		5	6		7		180	56	172		
TL		1	1				16	23	7		
<b>Total</b>	<b>0</b>	<b>7</b>	<b>11</b>	<b>1</b>	<b>7</b>	<b>3</b>	<b>201</b>	<b>121</b>	<b>241</b>	<b>1</b>	<b>1</b>

BBH= black bullhead, BHS=bluehead sucker, BNT=brown trout, CCF=channel catfish, CRP=common carp, FHM=fathead minnow, FMS=flannelmouth sucker, HBC=humpback chub, RBT=rainbow trout, RSH=red shiner, SPD=speckled dace.

**Table 4.** Number of each fish species captured in the mainstem Colorado River, by each gear type during Trip 2, 2001.

Gear	BBH	BHS	BNT	CCF	CRP	FHM	FMS	HBC	RBT	RSH	SPD
AN								2			
HB	1	1	3	1	1	11	12	190	80	2	2
TK	1	42	17	2			177	83	335		
TL		3	7		2		77	29	45		
<b>Total</b>	<b>2</b>	<b>46</b>	<b>27</b>	<b>3</b>	<b>3</b>	<b>11</b>	<b>266</b>	<b>304</b>	<b>460</b>	<b>2</b>	<b>2</b>

BBH= black bullhead, BHS=bluehead sucker, BNT=brown trout, CCF=channel catfish, CRP=common carp, FHM=fathead minnow, FMS=flannemouth sucker, HBC=humpback chub, RBT=rainbow trout, RSH=red shiner, SPD =speckled dace.

**Table 5.** Humpback chub population estimate for fish >200 mm TL, in the mainstem Colorado River, 2001.

Size (TL )	Marks (Trip 1)	Examined (Trip 2)	Recaptured fish	N estimate	SE	CV	95% Poisson confidence intervals	
>100 mm	104	160	8	NA	--	--	--	--
>200 mm	83	111	8	1,044	300	.29	559	2,137
>250 mm	75	93	5	1,190	418	.35	561	2,747

**Table 6.** Fish specimens in trout stomachs. Specimens lacking skull bones could not be identified. TLest = Total Length estimated from measured standard length (SL) to account for digested caudal fin. V= measured snout to Vent length. AR = Anal rays.

Trip	RM	Gear	Predator		Prey					
			Species	TL	TLest.	SL	V	AR	Species	Comments
1	63.0	TN	BNT	336	~175	154	109	7	Catostomid	
	63.2	HB	BNT	345	<150 <150	--	--	--	?	two fish, no skulls
	63.2	HB	RBT	412	<150	--	--	--	?	no skull
2	63.2	TN	BNT	337	<150	--	--	--	?	few small bones
	63.04	TN	BNT	492						empty
	63.55	TN	BNT	310	<150	--	--	--	?	few small bones
	61.9	TN	BNT	348	~142	115	78	8	HBC?	Intact
	61.9	TN	BNT	366	~156	127	90	8	HBC?	Intact
	63.1	HB	BNT	306	~102	83	59	10	HBC	PIT# 423F0D6A16, live TL 121 mm.
	58.1	TN	RBT	353	<150	--	--	--	?	few small bones
68.3	TN	RBT	307					--	small bat	

## **4.0 DISCUSSION**

### **4.1 Population Estimate**

#### **4.1.1 Assessment of Bias**

The assumptions that must be met for unbiased estimates from two-event mark-recapture experiments on closed populations were tested according to procedures outlined in Bernard and Hansen (1992). We neither detected nor suspected handling-induced mortality, trap-happiness, or trap-shyness. No immediate mortality was observed, and many large adult fish had been tagged during prior studies up to 10 years ago, so no mortality was suspected for fish  $\geq 200$  mm TL. Trap-happiness was not evident, since recaptured fish were recaptured once only during this study. Trap-shyness cannot be ruled out since few fish were recaptured, but it is thought to be minimal, as about 71.5% of adult fish ( $\geq 200$  mm) had been captured and tagged during this or some previous study.

The assumption of closure was evaluated. First, we assumed the population was closed to mortality, growth-recruitment, and emigration because the capture and recapture sampling events were separated by only 3 weeks. There may have been some mortality due to predation; however, because all of the fish found in predator stomachs in this study were  $< 200$  mm and the estimate was made for fish  $\geq 200$  mm, the effect of predation mortality on the estimate is thought to be minimal. Valdez and Ryel (1995) reported that adult HBC exhibit little movement and remained within the study area when not spawning; emigration is thus thought to be negligible.

The length and geographical distributions of marked, examined, and recaptured fish were tested for structural bias (Bernard and Hansen 1992). The catch of HBC  $< 200$  mm in the hoop nets increased during Trip 2 (Figure 5), likely as a result of monsoonal floods which occurred in the LCR during the second sampling event and flushed smaller fish out of the LCR. Valdez and Ryel (1995) also observed the phenomenon of monsoonal flushing of small fish from the LCR. This immigration of fish likely did not violate the assumption of closure for this estimation because the estimate was made only for fish  $\geq 200$  mm. There was also a small increase in the catch of fish between 200 and 250 mm. The result of an immigration bias would be to inflate the estimate of fish  $\geq 200$  mm due to dilution of marked fish in this size class; however, the estimation of fish  $\geq 250$  mm is actually larger than the estimation of all fish  $\geq 200$  mm, with less precision (Table 5). Thus, the more conservative estimation for all fish  $\geq 200$  mm is preferred.

Next, we examined the length distributions of HBC on the two sampling events to determine if the distributions were similar or if the population estimate should be stratified by length class. A cumulative distribution frequency was plotted (Figure 6). The length distributions of fish captured during the first and second events and of recaptured fish were all dissimilar. A larger proportion of HBC  $\geq 250$  mm were captured during the second event, all the recaptured fish were  $\geq 200$  mm, and the smallest recaptured fish was 205 mm (Figure 5); therefore, we stratified the estimate to include only fish  $\geq 200$  mm to partially correct for this bias. The other correction for this bias is that the estimate applies only to the second occasion. Because the occasions were only three weeks apart, this difference is considered unimportant.

Finally, we examined if the fish were adequately mixed between sampling events, or if the estimate should be geographically stratified. All eight recaptured fish moved at least 0.1 mile, and up to 3.1 miles, with an average of 0.75 mile between captures, and at least one fish was recaptured in all but one section (Figure 8). We concluded that the fish were likely adequately mixed between the first and second events; thus, the estimate is germane to the entire reach. Table 5 shows the number of fish marked on the first trip, the number examined for marks on the second trip, number of recaptures, and the population estimates for fish \$200 and \$250 mm TL.

#### **4.1.2 Assessment of Precision, Feasibility, and Effort**

Despite the large amount of effort invested in this population estimate, the number of captures and recaptures was lower than expected. The average catch rate for trammel nets was only 9.8 (HBC/100h/100ft) for the first sampling event and 13.64 for the second event, compared to the historical average of 41.2. Although the estimated trammel net effort needed to produce 350 HBC was doubled and effort was supplemented with > 13,000 hours of hoop net sampling, only 104 HBC were captured on the first occasion. Only 83 of those were \$200 mm and could be included in the stratified population estimate for fish \$200 mm. These represent 8% of the estimated population of 1,044 fish \$200 mm. The desired coefficient of variation ( $CV = SE/N$ ) was 10-15%, while the actual CV was 29%. Increasing the initial effort or completing a third sampling pass would increase the precision of future estimates (White et al. 1982). Although the precision was less than desired, we feel this is a useful estimate and can be effectively compared to past and future estimates.

Past estimates of HBC in the mainstem LCR inflow area are available for 1991, 1992, and 1993 (Valdez and Ryel 1995). They presented population estimates made with 11 different models. All estimates made by Valdez and Ryel (1995) included multiple capture events ( $t > 2$ ). They selected the Chao (Mh) estimate as the most appropriate model because it is sensitive to low numbers of recaptures. However the Chao (Mh) estimates were not significantly different from most of the other estimates used including the Schnabel estimate, which is similar to the Chapman-Peterson estimate adapted to more than two capture occasions (White et al. 1982). The Chapman-Peterson estimate used in this study is most suited for use with only two capture events (mark and recapture).

We compared our Chapman-Peterson estimate to the Schnabel estimate reported in Valdez and Ryel (1995) and also conducted a Chao (Mh) estimate on our data to compare directly with the Valdez and Ryel estimate of Chao (Mh) (Figure 9). The comparison of the Schnabel and Chapman-Peterson estimates indicated a significant decline in the estimated abundance between the earlier estimates (~3,000) and our 2001 estimate (1,044). However, the comparison of the Chao (Mh) estimates showed a non-significant decline from ~3,500 to 2,166. However, we note that the abundance of HBC must decline by at least 50% for the Chao (Mh) estimates to be statistically different given the confidence intervals of 50% of the mean. We feel that the decline is likely biologically significant although not statistically significant. This decline supports the FWS model and analysis suggesting a significant decline in recruitment and abundance in the LCR over this time period (Lew Coggins, USGS, personal communication).

### 4.1.3 Link to LCR Estimates

To obtain an estimate of the total HBC population in the LCR and the mainstem LCR inflow area, in addition to SWCA's mainstem estimate, the FWS performed independent two-pass mark/recapture population estimates in the LCR, one in the spring (May/June) and another in the fall (October/November). To be additive, the independent estimates must assume that during the time of sampling a portion of the total population was resident in the LCR and a portion in the mainstem, and there was no exchange of individuals between the groups. For part of each year, this assumption is met, with the majority of the total population residing in the mainstem and a smaller proportion residing in the LCR. However, this assumption is violated primarily during spring spawning runs, when a portion (probably a large portion) of the adult mainstem population moves into the LCR to spawn and then returns to the mainstem (Valdez and Ryel 1995). Valdez and Ryel (1995) found that adult HBC congregated at the LCR inflow and then ascended in March – May, and dispersed back to the mainstem in late June – July. Because the earlier estimates were performed over a period of years, exchanges and overlap in the populations were certainly reflected in the estimate, but the extent of the overlap is not known. To ensure that independent estimates in the LCR and the mainstem were additive, the mainstem effort was conducted during July/August and August/September, when exchange between the populations was at a minimum and historic catch rates were reasonably good (Figure 2). This schedule resulted in three independent estimates. The spring LCR estimate included resident LCR fish and mainstem spawners. The fall LCR estimate included only LCR resident fish. The mainstem estimate included only resident mainstem fish and a few recent juvenile immigrants. All the estimates can be evaluated and cross-checked in several ways.

Ideally, if all mark/recapture estimates were precise and accurate, the fall LCR estimate and the mainstem estimate could be added to give a total population estimate for the LCR/mainstem aggregate. The fall LCR estimate subtracted from the spring LCR estimate would give an estimate of the number of mainstem spawners present in the LCR in the spring. That number of mainstem spawners subtracted from the mainstem estimate gives the number of fish remaining in the mainstem during spring spawning. The abundance estimate for fish > 150 mm in fall in the LCR was 1,106 (Van Haverbeke and Coggins 2002), which added to our mainstem estimate of 1,044 fish > 200 mm yields 2,150 fish which is quite similar to the spring LCR estimate of 2,090 fish >150 mm. The agreement between the estimates is encouraging; however, the lack of precision of the estimates, particularly of the mainstem estimate (CV=29%), suggests caution in interpretation of these results. The relationship between the independent mainstem and LCR estimates will be further examined cooperatively with the FWS in a separate report.

## 4.2 Marks

Theoretically, a population estimation of HBC in the mainstem is possible using a proportional mark rate or a two-pass mark/recapture estimate, with the LCR sampling as the first capture (marking) occasion, and the mainstem sampling as the second (recapture) occasion. To be successful, this estimate assumes that most mainstem HBC are in the LCR during the LCR estimate, and that most of the fish disperse back into the mainstem during the mainstem sampling, so that the entire population is available for capture during both sampling events. It is critical that the capture probability in the LCR is equal between resident and non-resident fish,

and the proportion of marked individuals in the LCR is equal between the resident LCR population and the non-resident mainstem spawners. If these assumptions were met, then the mark rate (proportion of the population that is marked) would be the same in the LCR and in the mainstem.

We observed a large difference in the percentage of marked fish between the LCR and the mainstem. The FWS marked 819 unique fish greater than 150 mm, and calculated that the population of fish greater than 150 mm was 2,090; therefore, they estimated a total mark of ~39% (Randy Van Haverbeke, FWS, personal communication). The estimated mark rate of fish in the LCR (39%) and the estimated mark rate of LCR fish in the mainstem (8.6%) were substantially different. The amount that the mark rate differs is a function of two unknown variables: the total population and the proportion that remain in the LCR. The independent mark/recapture estimate in the mainstem may allow quantification of these variables. The discrepancy may be a result of high tag loss in the LCR after the FWS estimated the mark rate, or insufficient mixing in the LCR population resulting in an underestimate of the population and overestimation of the mark rate, or it may be a result of a larger number of juvenile fish in the mainstem than previously thought or a large percentage of adult fish remaining in the LCR during the mainstem estimate. The discrepancy and the source of the bias will be examined in cooperation with the FWS in a separate report.

If the proportional mark rate method were successful, it would be an efficient and cost-effective way to monitor the population of HBC in the future without continuing to conduct two-pass mark recapture efforts on the mainstem. However the extent of bias likely will make this method unreliable, suggesting the need for further independent mainstem estimates.

One of the goals of this study was an estimate of recruitment, or abundance of fish  $\geq 200$  mm. Because no valid estimate was possible for fish  $\geq 200$  mm in the mainstem due to lack of recaptured fish  $\geq 200$  mm, recent mainstem recruitment could not be assessed. Multiple censuses performed over several years will give estimates of long-term recruitment if the annual estimates are stratified by size or age and changes can be detected in size classes over years. However growth is slow in HBC after age 4 or 5 and shifts in age proportion are very difficult to detect (Valdez and Ryel 1995). It is useful to detect success or failure of recruitment in the first 2 or 3 years, on short time scales. Thus a means must be found to estimate the abundance of the smaller sizes. Mortality due to handling (insertion of PIT tag) or tag loss in fish less than 150 mm was suspected in the LCR (Lew Coggins, personal communication). Few fish this size were captured in the mainstem, so no tag loss was detectable. However, consensus among researchers was that PIT tagging of fish less than 150 mm should be discontinued, and an alternative marking method should be used.

## 5.0 CONCLUSIONS AND RECOMMENDATIONS

### 5.1 Conclusions

1. *Assess the potential and effort required of two-pass mark/recapture sampling to provide mainstem abundance estimates of HBC.*

Although the effort expended was considerable, the estimate did not reach the desired level of precision. The target level of precision was to achieve a CV of 10-15%; however the actual CV was 29%. Increasing the level of effort on each of two passes, or completing a third pass would increase precision; however, the impact of increased handling on the HBC should be considered.

2. *Capture HBC >100 mm TL to increase mark rates and collect mark/recapture data to support stock synthesis model to establish a baseline for future monitoring efforts. These data may be used to estimate relative proportion of the mainstem population to LCR population using proportional mark rates.*

HBC > 100 mm TL were captured and tagged; however, no fish less than 200 mm were recaptured. Thus no additional recapture data on fish < 200 mm could be provided to support the stock synthesis model. Capture and recapture data on fish > 200 mm were provided.

The estimated percentage of marked fish in the LCR (39%) and the estimated percentage of marked LCR fish in the mainstem (8.6%) were substantially different. The amount that the mark rate differs is a function of two unknown variables: the total population and the proportion that remain in the LCR. The discrepancy may be a result of high tag loss or insufficient mixing in the LCR population resulting in an underestimate of the population and overestimation of the mark rate. It may be a result of a larger number of juvenile fish in the mainstem than previously thought, or a large percentage of adult fish either remaining in the LCR during the mainstem estimate or remaining in the mainstem during the LCR estimate. The discrepancy and the source of the bias will be examined in cooperation with the FWS in a separate report.

If the proportional mark rate method were successful, it would be an efficient and cost-effective way to monitor the population of HBC in the future without continuing to conduct two-pass mark recapture efforts on the mainstem. However the extent of bias likely will make this method unreliable, suggesting the need for further independent mainstem estimates.

3. *Assess changes in mark rates and population length frequency distributions over time using historical data to determine if recruitment is taking place, and attempt to estimate recruitment rates.*

Because no valid estimate was possible for fish #200 mm in the mainstem due to lack of recaptured fish #200 mm, recent mainstem recruitment could not be assessed.

Multiple censuses performed over several years will give estimates of long-term recruitment if the annual estimates are stratified by size or age, and changes can be detected in size classes over years.

4. *Assess predation by examining stomach contents of large-bodied non-native fish (brown trout BNT, rainbow trout RBT, channel catfish CCF, and black bullhead BBH) in the field.*

Of 38 BNT examined, 9 contained fish remains. One RBT also contained fish remains. Three CCF and two BBH were examined but contained no fish. The only specimen out of 10 fish items that was definitely identified to species was a HBC that had been PIT tagged the previous night in the same location. This specimen was recovered from a BNT captured in a hoop net. This direct evidence of predation by BNT upon HBC would be more meaningful if the BNT had not been captured in a hoop net, where the juvenile HBC are more vulnerable to predation because they cannot escape and there is no cover.

## **5.2 Recommendations**

1. Continue to conduct mark-recapture population estimates in the mainstem Colorado River in Grand Canyon near the LCR inflow and increase the level of effort to increase the precision of the estimates. The suggestion of a decline in abundance since the early 1990s is a great cause for concern for this endangered species, and the significance of this apparent decline should be validated.
2. Continue to research alternative methods for marking HBC < 150 mm TL to support estimates of recruitment.
3. Conduct studies to determine the cause of the recent decline in HBC in the Colorado River in Grand Canyon.

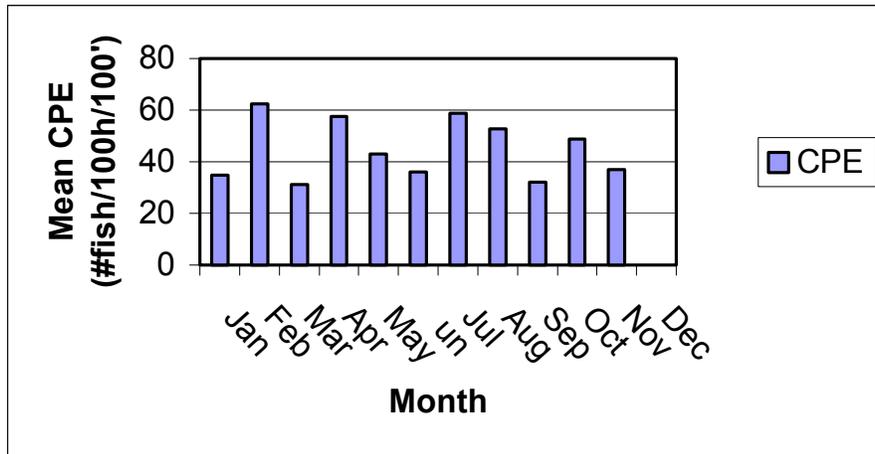
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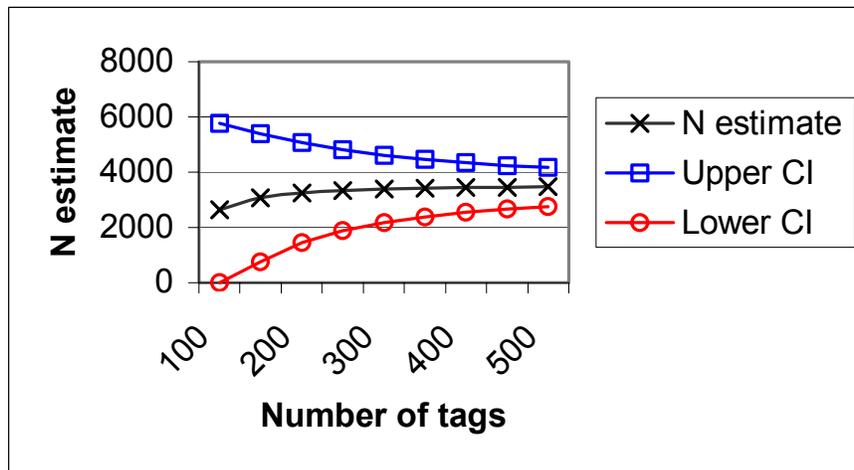
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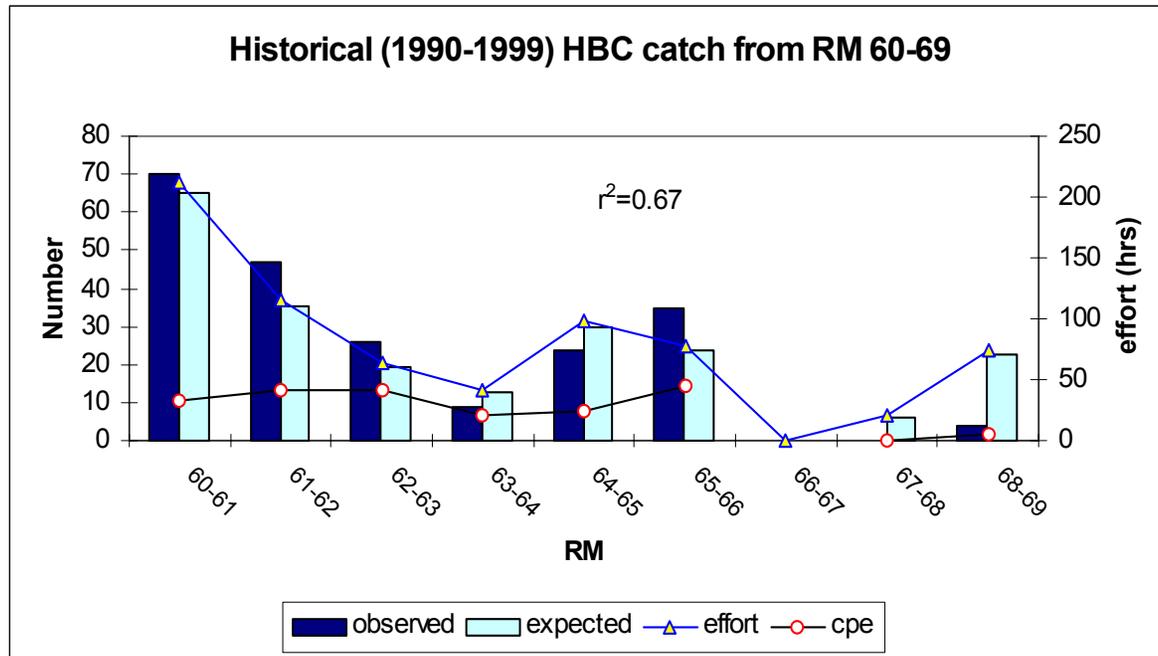
## 7.0 FIGURES



**Figure 1.** Mean monthly trammel net CPE (#fish/100h/100') for data collected 1990-2000 by Bio/West, AGFD, and FWS and compiled by Lew Coggins (FWS).



**Figure 2.** Example of increasing precision of N estimate as number of initial marks (tags) increases, for an actual population (N) of 3,500 and an equal number of captures on first and second passes.



**Figure 3.** Observed catch of humpback chub (HBC) and expected catch based on effort (hours of trammel net sets) in each one-mile section of the Colorado River mainstem between RM 60 and 69, from 1990 to 1999.

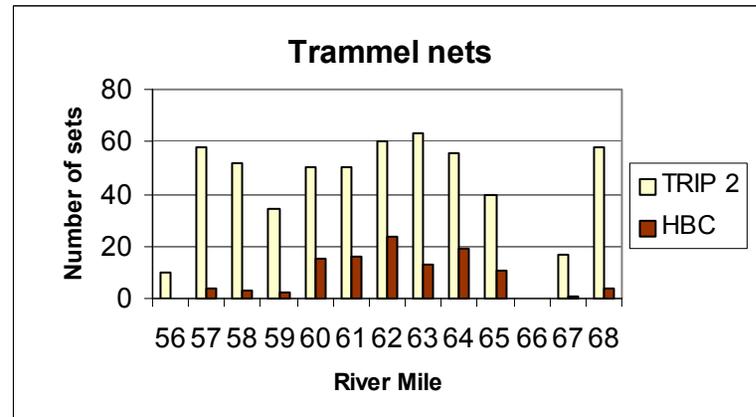
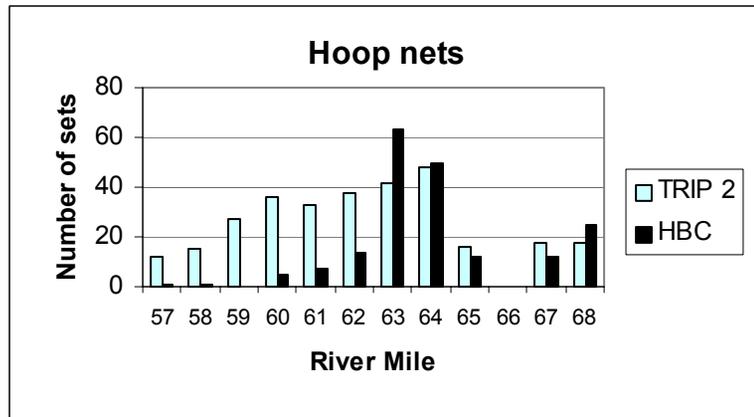
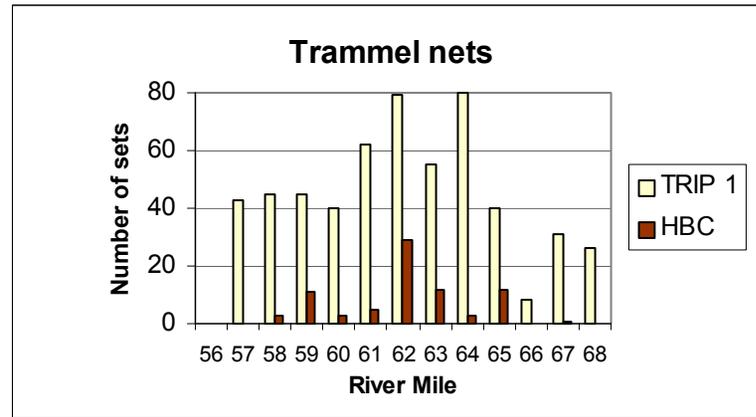
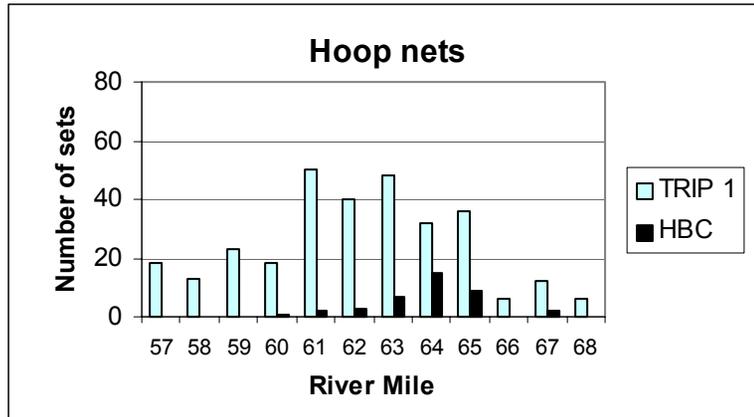
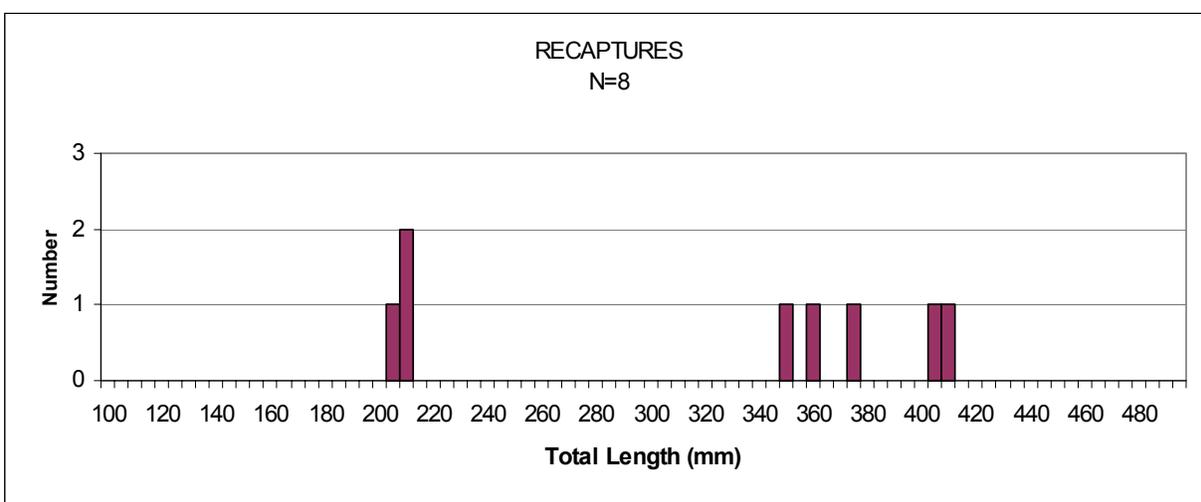
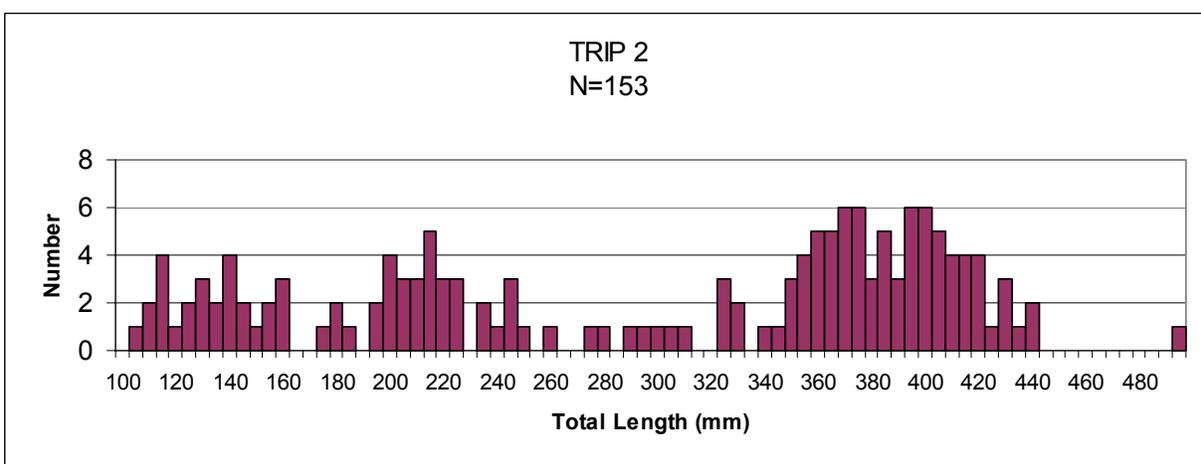
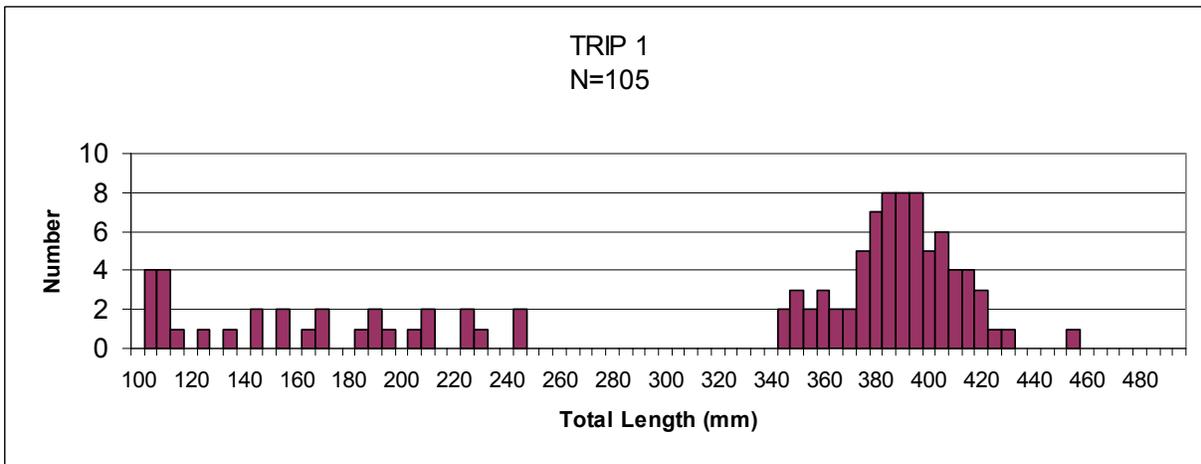
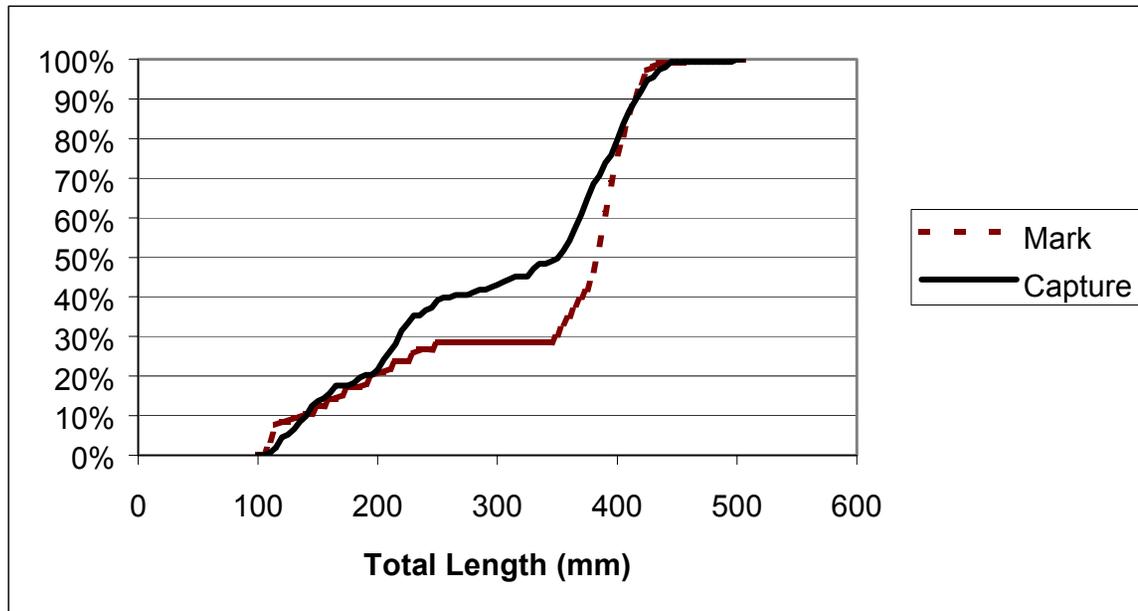


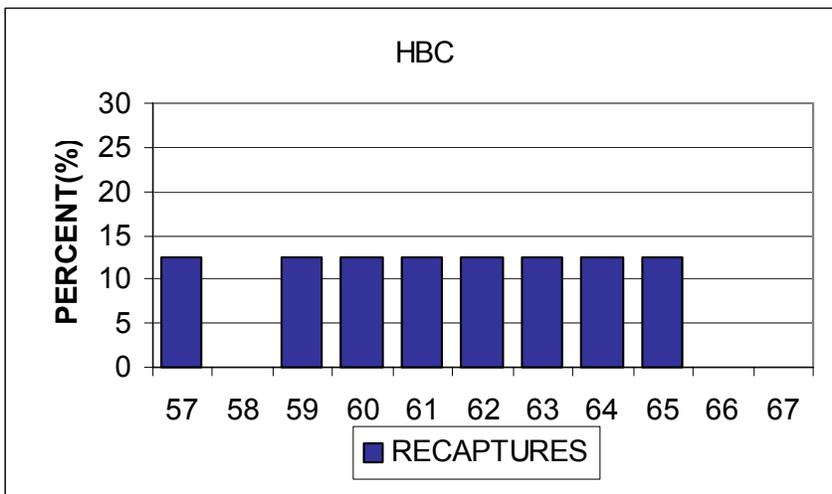
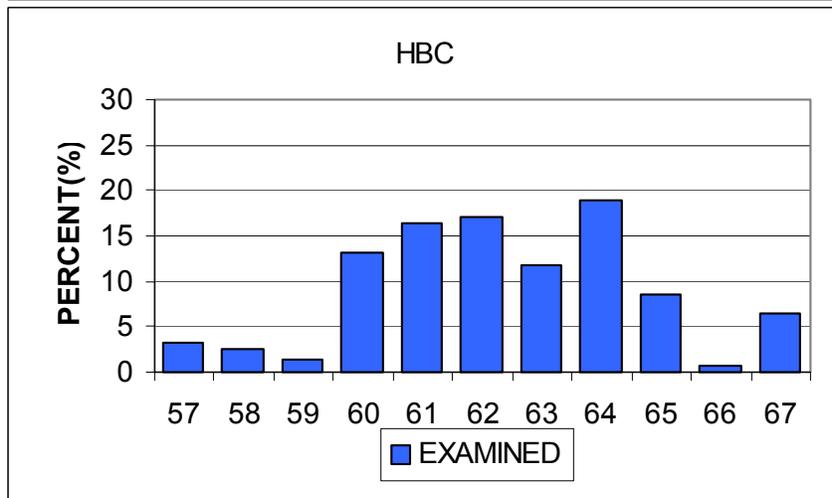
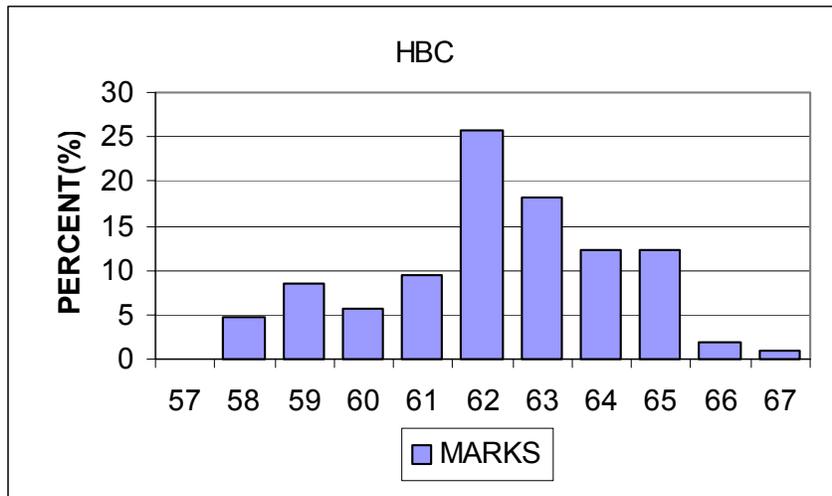
Figure 4. Effort in number of hoop and trammel net sets, and catch of humpback chub (HBC) for RM 56-69, Colorado River mainstem, 2001.



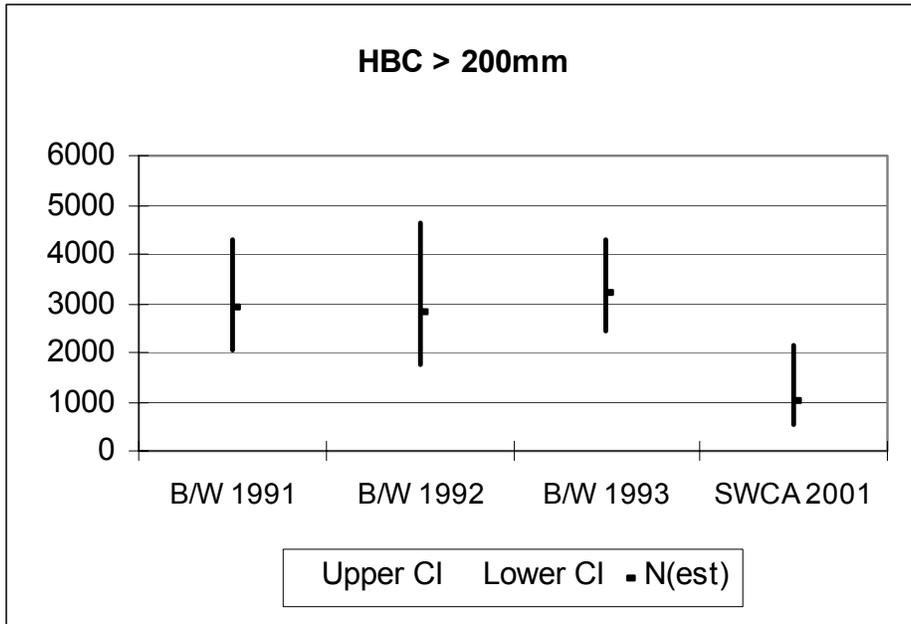
**Figure 5.** Length frequency of humpback chub (HBC) captured and recaptured during Colorado River mainstem sampling trips in 2001.



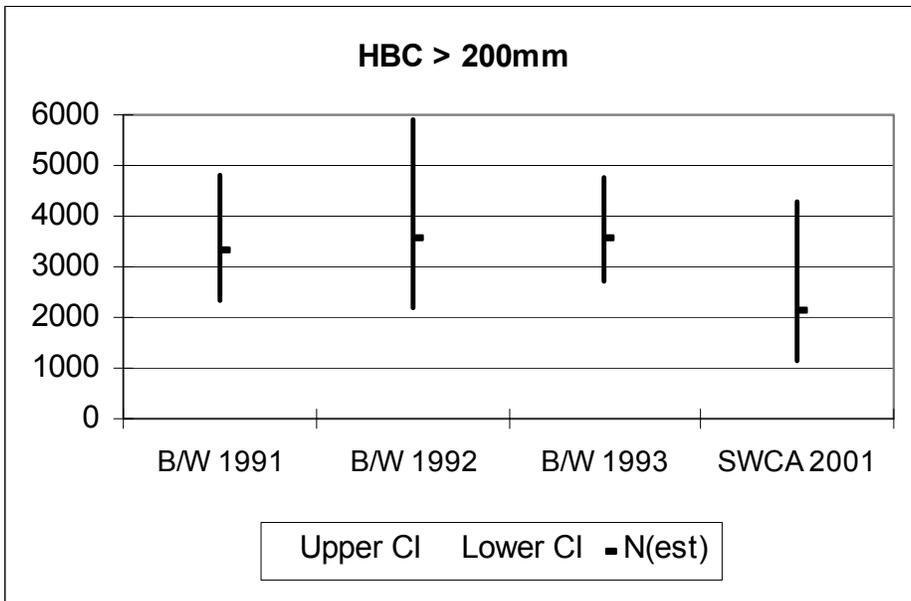
**Figure 6.** Cumulative length frequency distribution of humpback chub (HBC) marked during Trip 1 and examined (Capture) during Trip 2 on the Colorado River mainstem sampling trips in 2001.



**Figure 7.** Percent distribution by river mile of captures and recaptures of humpback chub (HBC) during sampling in the mainstem Colorado River 2001



**Figure 8.** Population estimates with 95% confidence intervals of humpback chub (HBC) in the Colorado River mainstem from RM 56-69 made by Bio/West (B/W) in 1991-1993 using the Schnabel estimator (Valdez and Ryel 1995), and by SWCA in 2001 using the Chapman-Petersen estimator.



**Figure 9.** Population estimates with 95% confidence intervals of humpback chub (HBC) in the Colorado River mainstem from RM 56-69 made by Bio/West (B/W) in 1991-1993 using the Chao (Mh) model in CAPTURE (Valdez and Ryel 1995), and by SWCA in 2001 using the Chao (Mh) model in CAPTURE.