Nearshore Ecology (NSE) of Grand Canyon Fish 2009 Progress Report

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Note: All information presented should be considered draft and subject to revision based on corrections and updates to field data and analytical approaches. This document is prepared as a courtesy to our cooperators.

Nearshore Ecology Project 2009 Research Progress Report

The NSE project is designed to assess whether juvenile native fish survival and recruitment are influenced by planned flow experiments from Glen Canyon Dam that will occur during September and October 2009-2011. To make this assessment, our research is defined by three domains each informed by 2009 sampling and detailed in our original full proposal. Three key areas where this project seeks to fill existing information gaps are:

> *(1) Evaluating how steady flows influence juvenile native fish growth and survival,*
> *(2) Assess habitat use and movement of juvenile fish in response to steady flows, and*
> *(3) Identify the spatial source of juvenile native fish found in the mainstem.*

This report serves as a preliminary presentation of research results from 2009. These results focus on sampling and analyses methodologies to inform 2010 field efforts and primarily include catch-rate, movement, capture probability, and abundance estimates for juvenile humpback chub (HBC) as well as microchemical and isotopic water atlas development and assessment of otolith microchemistry techniques. All information presented should be considered draft and subject to revision based on corrections and updates to field data and analytical approaches through collaboration revisions between NSE core research team and GCMRC cooperators.

Field Sampling Overview

Field efforts in 2009 included a total of four sampling trips, two trips prior to the steady flow experiment (launch dates of mid-July and mid-August) and two trips following the start of the steady flow experiment (experiment began September 1, trip launch dates early September and mid-October). Our sampling universe covered an area from Heart Island (just downstream of the Little Colorado River confluence) to an area just upstream of Lava Chuar rapid (about RM 65.5). Within this sampling universe we established three sampling sites (Sites 1, 2, and 3) of approximately equal length (about 1500-m) depending on hydrologic features. Each Site was then subdivided further into individual spatially referenced 50-m segments ("habitat sub-unit", HSU). We used slow-speed boat electrofishing during night time to sample each HSU. Fish captured in each

HSU were placed in a numbered bucket corresponding to the HSU to track catch spatially by HSU. All fish collected in each HSU were identified, measured (TL and FL) and given one of two tag types following established fish handling protocols in Grand Canyon. We examined all native fish for PIT tags and tagged HBC greater than 100-mm TL and all other natives greater than 150-mm TL with a PIT tag. All native fish less than 100-mm TL and fathead minnows received a Visual Implant Elastomer (VIE) mark that identified gear and Site (1, 2, or 3) the fish was captured (the marks are not unique to individual fish). The use of VIE marks was required because the smaller size fish are simply too small to mark with PIT tags. We sampled each site over multiple nights and kept track of the cumulative numbers of captures and recaptures of fish (all species and tag types). We then used this information to estimate abundance for each site. Because different sampling gears have different sampling selectivities for a given species, or fish size, or habitat, in Site 1 we also employed hoopnets (standardized mini-hoopnets used by cooperating agencies for mainstem fish sampling, approximately 0.5-m in diameter, 1.0-m length, 6-mm mesh, and single 10-cm throat; n = 47 nets trip 1, n = 60 nets trips 2, 3, and 4) as an additional technique to sample juvenile fish. Hoopnets (0.5-0.6 m diameter, 1.0 m length, 6 mm nylon mesh, single 0.1 m throat) were checked every 24-hours and were fished for 12-14 nights for each trip. All collected fish were processed similarly to the fish captured via electrofishing.

Water chemistry and isotope sampling

Water samples were collected in May, July, August, September, and October 2009. Acidified (1% HNO_3) samples were analyzed for trace elements with inductively coupled plasma optical emission spectrometry (ICP-OES) or inductively coupled plasma mass spectrometry (ICP-MS). Our limit of detection for most elements was in the low parts per billion. Following completion of quality control samples, results were only accepted if relative standard deviation (standard deviation / mean * 100) < 10%. Unacidified samples were analyzed for stable isotopic ratios of Sr, O, H, and C. $^{87:86}Sr$ was analyzed in the Department of Earth Sciences, Syracuse University, using thermal ionization mass spectrometry (TIMS). Oxygen ($\delta^{18}O$) and hydrogen:deuterium (H:D) ratios were analyzed by isotope ratio mass spectrometry at the Center for Stable

Isotope Biogeochemistry at the University of California at Berkeley. Carbon ($\delta^{13}C$) ratios were analyzed at the Stable Isotope Facility at the University of California at Davis.

The use of otolith chemistry as a natural marker is based on a predictable relationship between water and otolith chemistry such that fish movements between water masses with differing trace elemental chemistry is reflected in otolith composition. Our efforts have focused on identifying unique otolith chemical markers that discriminate native fish residency in the Little Colorado River and mainstem Colorado River. To this point, all otolith chemistry analyses have been conducted on a very limited number of native fish that were either incidental mortalities during sampling by the NSE project or other cooperators (i.e. HBC, n = 4) or a very small number of flannelmouth sucker (FMS) and bluehead sucker (BHS) that were kept for otolith microchemistry analyses (n < 30). With all fish, the lapillus otoliths were removed and polished to the core in order to permit retrospective otolith chemistry analysis over the life of the fish. In addition, we have also investigated age and growth of these same young-of-year native fishes using otolith daily increment analyses.

A single analytical technique cannot be used to quantify all potential otolith chemistry markers. Therefore, we have used three different analytical methodologies to capitalize on the analytical strengths each technique offers. Laser-ablation inductively coupled plasma mass spectrometry (LA-ICPMS) is routinely used to quantify heavier trace elements in otoliths such as Sr, Ba, Mg, Mn, Ca, and Pb. This approach uses a high energy laser to remove otolith material that is swept into a mass spectrometer using a carrier gas flow. This technique assays specific sample locations on the otolith and permits the simultaneous analysis of multiple elements. In this study, we have used LA-ICPMS to analyze FMS otoliths for a suite of fifty-four trace elements. Analyses consisted of continuous transects beginning at the otolith edge, extending through the otolith core and ending at the opposite edge. Detection limits ranged from high parts-per-billion (ppb) to low parts-per-million (ppm). Otoliths were obtained from individuals

collected from the Colorado River (upstream and downstream of the LCR-COR confluence) in addition to the Little Colorado River. All fish ranged from ~50-200mm TL.

In addition to LA-ICPMS analyses, we have analyzed several otoliths using synchrotron-based scanning x-ray fluorescence microscopy (synchrotron SXFM) at the Cornell High Energy Synchrotron Source (CHESS) facility. This method uses focused high-energy x-ray radiation to elicit a characteristic, element-specific fluorescence from the otolith used to determine trace elemental concentrations. Although many of the elements that can be quantified using SXFM overlap with LA-ICPMS techniques, SXFM has the advantage of being non-destructive (i.e., material is not removed from otolith) and can produce fine scale analyses of the otolith (beam size ~15-25μm). Additionally, this technique lends itself to creating 2D surface maps of otolith chemistry. A suite of HBC, SPD, and FMS fish otoliths were analyzed using this technique. As with the otoliths used for LA-ICPMS analyses, fish were collected from the Little Colorado River and upstream/downstream of the LCR confluence.

Geologic and biological processes often result in shifts in natural isotopic abundances for some elements in the water. Given that these shifts are often recorded in otolith chemistry, we used secondary ion mass spectrometry (SIMS) to quantify oxygen ($\delta^{18}O$) and carbon ($\delta^{13}C$) stable isotope ratios in otoliths. This technique bombards the surface of the otolith sample using a cesium ion beam to create oxygen and carbon ions. These ions are then separated by isotopic mass, and abundances are quantified using a very sensitive mass spectrometer. Given the high demand and high cost of SIMS instrument time (only 3 SIMS instruments exist in US), we were only able to analyze otoliths from one HBC that was collected in the Colorado River (33mm TL), one FMS that was collected in the Little Colorado River at Boulder Camp (64mm TL), and one BHS larva also collected in the Little Colorado River at Boulder Camp (14mm TL).

Finally, a combination of light (brightfield, phase contrast, differential light microscopy) and electron microscopy (SEM) were used to enumerate daily growth increments

recorded in the otoliths. Samples were prepared as described above using otoliths from the limited number of native fish collected and described above.

Results and Analyses to date

Catch and size frequency analyses

Across all four trips, in all sites, using both gears, we collected nearly 12,000 fish from 14 identifiable species, a group of unidentifiable suckers (mostly larvae) and a few species that were unidentified (Table 1). Unidentified samples were unidentified suckers and were not retained due to concern that these may be HBC. The top three species caught (by number) were generally native HBC and FMS and nonnative fathead minnows. We focus the results in this report on juvenile HBC <100-mm TL as they are the endangered species of management interest in this system.

Humpback chub generally represented between 3 and 22% of the total catch in electrofishing and between 52 and 81% of total hoop net catch (Table 1). Size frequency analyses show that both gears captured a wide size range of fish but electrofishing generally captured smaller fish than hoopnets (Figure 2). Total catch of all sizes of HBC was higher in hoopnets than from electrofishing (Figure 3), but the catch of small HBC (<100 mm TL) was much higher with electrofishing than in hoopnets. Overall, hoopnets appeared to be more effective at capturing total numbers of HBC, but our slow speed electrofishing technique appeared to be more effective at capturing small bodied fish such as juvenile HBC and fathead minnows than hoopnets, although the total catch of these species is lower (Table 1, Figures 1-3).

We assessed cumulative catches of HBC in three size categories < 100-mm TL (Figures 4 and 5), 100-150-mm TL (Figure 6), and 150-mm+ TL (Figure 7) for both gear types to assess whether catches declined through repeated sampling. We found that cumulative hoopnet catch of HBC for all sizes was positive and generally linear showing a consistent pattern in the HBC catch (Figure 4). We also plotted mainstem turbidity simultaneously with HBC cumulative catches to assess whether catch and turbidity showed any similar pattern. We did not find any apparent relationship between turbidity

and cumulative catch (Figures 4-7). We were also able to use the cumulative catch plots to assess two choices related to effort allocation of electrofishing. Electrofishing passes in Site 1 and 2 were conducted with 48 hours between passes while electrofishing passes in Site 3 were 24 hours apart. From the plot of cumulative catches it does not appear that there were differences in catch-rates with 24 or 48-hour time periods between electrofishing passes (Figures 5-7). We also assessed size-frequency per pass of HBC, and it did not appear that size distributions changed with each pass (Figure 8). Thus, it appeared that the frequency of our sampling did not result in diminishing returns of fish collected, suggesting that our sampling design is adequate for our objectives.

Spatial distribution of catch

To examine the spatial distribution of HBC catch, we created a plot of HBC catch by size class on habitat sub-unit (HSU; Figure 9). The HSU represents the spatial grid cell of each electrofishing transect sample. We structured this plot such that the HSUs for river right (sites 140-300) are found on the primary x-axis and the HSUs for river left (HSU 450-650) are found on the secondary x-axis (Figure 9). The catch in each of these grid cells (y-axis) then correspond to each x-axis such that catches close to zero for a given HSU are near the axis corresponding to that HSU (either primary or secondary x-axis) and non-zero catches are a greater distance away from the corresponding x-axis. Catches of HBC of all sizes by gear and trip were widely distributed throughout each site from electrofishing and a similar pattern is apparent for HBC in hoopnet samples in Site 1. We are currently working with GCMRC cooperators to link spatial catch information with habitat information available for each HSU to assess habitat use vs. availability. We will also use this same habitat information, linked with our catch information and site based capture probabilities (discussed below) to develop density estimates of HBC and other fish species by habitat type.

Movement of tagged fish

Tables 2 and 3 detail movement of VIE tagged HBC by trip and site. As a reminder, VIE tagged HBC are < 100-mm TL. Movement patterns of HBC within a trip were generally

restricted to the site of tagging with the majority of recaptures occurring in the same site in which the fish was tagged. Recaptures of fish outside of the site they were originally tagged occurred both downstream and upstream (i.e., Trip 3, Site 1, 2 fish were recaptured in Site 3; Table 2). Highest marking rates were in Site 1 because of the additional sampling effort associated with fishing hoopnets in this site.

Capture probability

We assessed capture probability of juvenile HBC in three different size classes, sample, and gear (Table 4) and found that across these attributes capture probabilities were generally low (about 4-13%) with limited recaptures. Graphs of the distributions of capture probability for each trip, site, and gear show that capture probabilities for hoopnets were generally lower, but more precise than capture probabilities for electrofishing (Figure 10). These differences in precision are likely due to the higher number of hoopnet samples taken at Site 1 (12 nights of sampling) than the three or four samples taken at each electrofishing site. Graphs of capture probability by size class for HBC show similar patterns between size classes. Recaptures of HBC > 150-mm were rare hence the reason most estimates of capture probability for this size class of fish failed (i.e., flat blue lines in figure Figure 10).

Abundance

We estimated abundance of juvenile HBC using closed population models described by Gazey and Staley (1986). Abundance of HBC < 100-mm TL maximum likelihood estimates (MLEs) and uncertainty profiles for each site and trip (Figures 11-13) show that abundance in Sites 1 and 2 were fairly similar (approximately 500 -1000 fish). Estimates for Site 3 were slightly lower with MLE estimates < 500 fish. Estimates with very high uncertainty (i.e. Trip 1, Site 2 or Trip 4, Site 2; Figure 13) where convergence was not met and estimates are not possible was likely due to extremely low (if any) recaptures of marked fish. We developed estimates for each gear type separately in Site 1 (hoopnets blue line, electrofishing black line) and found generally similar patterns and overlapping likelihood profiles with each gear type (Figure 13). We also assessed how many samples were necessary to generate parameter estimates for each gear type

(Figures 11 and 12). Figure 11 provides an example of this type of inference where we have plotted the abundance estimates for HBC in Site 1 for Trip 1 from electrofishing after each nightly pass. Each line on the graph represents an abundance estimate following a night of sampling where the flat line represents the abundance estimate after sampling 1 night (no estimate, as no recaptures were made) and then the subsequent night (night 2) of sampling all yields a poorly defined likelihood estimate (not a well defined dome). After the third night of sampling the estimate is much better defined and the MLE estimate (thick black line) of abundance is plotted. Figure 12 shows the same type of graph for hoopnetting where generally sigmoidal (logistic) curves are plotted for each night of sampling, until sufficient recaptures are made (after approximately seven nights of sampling) resulting in a defined likelihood estimate (dome shaped curve) that with subsequent samples (and recaptures) becomes better defined (until MLE is reached, thick black line). These plots demonstrate that at least three nights of sampling are needed to generate estimates of abundance using electrofishing and seven nights of sampling are needed for hoopnetting (Figure 12).

Trace element and isotopic water chemistry

Water analyses revealed strong differences in several trace elemental and isotopic composition, with some tributaries (notably Paria, Little Colorado and Nankoweep) showing periodically high levels of trace elements (e.g., cobalt, copper, lead, rubidium, and selenium). Some of these appeared to become elevated after rain events (T. Hayden, SUNY-ESF, Unpublished). Perhaps the most interesting data were the stable isotopic ratios of carbon, hydrogen, and oxygen, which exhibited tributary-specific signatures well (Figures 14-16). In particular, the Little Colorado River is separated extraordinarily well by $\delta^{13}C$ from most of the other sites, although Havasu Creek has values somewhat close. Principal components analysis, along with visual inspection of the data, emphasizes the fact that the mainstem “chemical fingerprint” is highly consistent from Lee’s Ferry down past Diamond Creek.

LA-ICPMS otolith chemistry

Of the fifty-four trace elements quantified using LA-ICPMS techniques, only seven elements were consistently above detection limits (Figure 18). For the purpose of this report, we have focused on a subset of three elements measured in three representative FMS. Chemistry transects exhibited multiple distinct peaks in Sr:Ca and Ba:Ca ratios in the interior portion of the otolith, regardless of fish capture location (Figure 19). These distinct peaks suggest that fish were undertaking directed movements between locations with different water chemistry or alternatively, or the fish were not moving and water chemistry was changing in the system. Elemental chemistry at the otolith edge (i.e., otolith chemistry at time of capture) was similar for all fish collection locations. Mn:Ca exhibited a slightly different pattern of a single distinct peak in the otolith core and low ratio values outside of the otolith core (Figure 19).

CHESS- XRF

Results of the synchrotron x-ray fluorescence analyses confirmed patterns observed in LA-ICPMS analyses for elements quantified using both analytical techniques. Two-dimensional surface maps of otolith Sr:Ca ratios displayed distinct bands of high and low Sr:Ca (Figure 20). Except for two fish collected in the Little Colorado River that had distinct regions of higher Cu:Ca concentration near the otolith core, most otoliths displayed uniform elemental ratios and little patterning across the otolith surface (Figure 21). As with Cu:Ca, Se:Ca ratios were consistent and showed little patterning for most otoliths except for two fish, collected in the mainstem Colorado River, that exhibited a distinct band of elevated Se:Ca ratios (Figure 22).

SIMS

As with LA-ICPMS and XRD, SIMS is a microbeam-based technique that permits analyses of discrete locations on an otolith. Using this technique, we quantified carbon and oxygen stable isotopes at two locations on the otolith from the 33-mm Colorado River HBC, 11 locations on the 64-mm LCR Boulder Camp FMS otolith, and at and one location (core) on the 14-mm BHS. Observed $\delta^{18}O$ values were similar for the BHS and core analysis of the HBC (Figure 23). Furthermore, estimates of $\delta^{18}O$ of the same HBC at the edge were substantially lower than the BHS analysis (Figure 23). A similar

pattern was observed in the $\delta^{13}C$ data. The HBC otolith core $\delta^{13}C$ values and bluehead $\delta^{13}C$ stable isotope ratios were similar and both were substantially higher than $\delta^{13}C$ values measured at the edge of the HBC otolith (Figure 24). Given the small size at capture of the BHS, it is unlikely that this fish had migrated a large distance from the spawning site and it is likely that the measured otolith $\delta^{18}O$ and $\delta^{13}C$ represent the LCR chemistry. The similarity between the BHS chemistry and HBC core chemistry suggest that the HBC was spawned in the LCR and subsequently migrated to the MS where it incorporated a lower $\delta^{13}C$ and $\delta^{18}O$ otolith chemistry. These data are congruent with observed patterns in $\delta^{18}O$ and $\delta^{13}C$ in the water.

Analysis of the FMS otolith included 11 spot analyses extending from otolith edge through the core region to the opposite edge (Figure 25). Observed $\delta^{13}C$ values for sample spots 1-5 and 8-11 were similar to the purported Little Colorado River otolith chemistry signature (compare Figures 15 and 24). Sample locations 6 and 7 had very high $\delta^{13}C$ values and were different from all other sample spots (Figure 25). These values may have been the result of instrument instability and a resulting restart procedure, or may truly represent fish movement to a location with very different chemistry.

Regardless of the analytical method used to decode the otolith chemistry, interpreting and identifying site-specific otolith chemistry markers by analyzing otolith edge chemistry in mobile, wild caught fish is problematic as recent immigrants to the collection location may not reflect otolith chemistry at the collection location but rather represent the chemistry of previous habitats. This problem may be overcome by validating site-specific otolith chemistry using larval or juvenile fish that are unlikely to have moved far from the hatch site or by conducting an experimental enclosure study to prevent movement from a particular site. This will hopefully be addressed through experiments or targeted collections during 2010 field efforts.

Fish age/growth

Fish age was determined by enumerating all daily growth increments, starting at the otolith core and extending to the otolith edge using standard light microscopy techniques. A consistent pattern of approximately 35-70 daily growth increments followed by a large growth check and rapid transition to closely spaced increments was observed in most fish (Figure 26). Often, the region of closely spaced increments continued to the edge of the otolith but in some cases, growth increments increased in width towards the otolith edge. In most fish, the closely spaced rings were quite difficult to resolve using standard bright-field light microscopy techniques and high contrast light microscopy techniques (phase contrast and differential interference contrast) improved optical resolution (Figure 26). To further validate the presence of growth increments, electron microscopy (SEM) was used to confirm the presence of growth increments. Given that the widths of daily growth increments are directly proportional to fish growth rate and temperature strongly influences growth, the rapid shift from widely spaced growth increments to tightly spaced growth increments is consistent with the fish experiencing a rapid shift from warm water (fast growth) to cold water (slow growth).

2010 Work plan

Field efforts for mark-recapture

As in 2009, we plan four sampling trips during 2010, two trips prior to the steady flow experiment (launch dates of mid-July and mid-August) and two trips following the start of the steady flow experiment (experiment begins September 1, trip launch dates early September and mid-October). Also as in 2009 our sampling universe will cover an area from Heart Island (just downstream of the Little Colorado River confluence) to an area just upstream of Lava Chuar rapid (about RM 65.5). As defined in 2009 our sampling universe is divided into three sampling reaches of approximately equal length (about 1500-m) depending on hydrologic features. Within each reach we subdivide the area further into individual 50-m segments.

We will use slow-speed boat electrofishing during night time to sample each sub-reach and use a bucket based accounting system to track catch in each sub-reach. We then process each bucket of fish identifying and measuring each fish caught and applying

one of two mark types to each fish. Following established native fish monitoring protocols, we will examine all native fish for PIT tags and tag HBC greater than 100-mm TL and all other natives greater than 150-mm TL with a PIT tag. All native fish less than 100-mm TL and selected non-native species (fathead minnows) receive a Visual Implant Elastomer (VIE) mark that uniquely identifies only the gear and sample reach where the fish was captured (the marks are not unique for individual fish). The use of VIE marks is required because the smaller size fish are simply too small to mark with PIT tags. We will then sample each reach over multiple nights and keep track of the cumulative numbers of captures and recaptures of fish (all species and tag types). We will then use this information to estimate abundance for each reach. Because different sampling gears have different sampling selectivity for a given species, or fish size, or habitat, in Reach 1 we will also employ hoopnets (n = 55 nets) as an additional technique to sample juvenile fish. Hoopnets will be checked every 24-hours and generally fished for 12-14 nights each trip. All fish collect with hoopnets will be processed similarly to the fish captured via electrofishing.

We will use the same analyses for fish collected in hoopnets and by electrofishing. Growth will be assessed by examining modal progression of fish lengths collected across trips with each gear. Additionally, a sub-set of fish collected in 2010 will have their growth rates estimated by assessing growth increments from otoliths.

Field efforts for habitat use assessment

Habitat use will be assessed using two approaches for two different sizes of native fish. First, catch-rate information is available at the 50-m spatial unit (from the slow speed electrofishing samples and hoopnets described above) such that catch-rate indices can be assessed at a spatial resolution less than the reach level (< 1500-m). Fish densities are estimated both by using reach specific abundance estimates divided by reach length and by catch-rate in each 50-m spatial unit divided by the length of the habitat unit. It should be noted that inferences based on catch-rate do not adjust catches to account for capture probabilities < 1. We will work with cooperators to potentially intensively sample specific habitat types to develop species and habitat specific capture

probabilities depending on reach specific estimates collected in 2009 and currently being analyzed. Our second approach to estimate habitat use is through the use of a small number of telemetered fish. During 2009 we successfully deployed a small autonomous receiver array and were able to collect, tag, release, and track within the array and via boat a small number of native fish between 180-200 mm TL. Although these fish are larger in size than the VIE fish, they provide key inference in a couple of areas.

- One, the "virtual" capture probability of a telemetered fish is the probability of detecting a fish on the boat receiver or on the array given that the fish is present and available to be detected. The array is deployed such that if fish exit our sampling domain they cross a detection gate. This is useful in informing us on the probability of a fish emigrating from each sampling reach. Since the telemetered fish does not have to be handled to detect its movement beyond the array, the virtual capture probability is much higher than the capture probability estimated from marking and recapturing fish.
- Two, we are able to estimate directly capture probabilities from our telemetered fish in the electrofishing and hoop net samples. We know from multiple daily relocations of the telemetered fish (usually every 6 hours) what their locations are. We can then compare these locations, and any captures of these animals, with the hoopnetting and electrofishing samples to provide an "empirical" capture probability estimate (simply put, the number of telemetered animals captured per unit of electrofishing or hoopnetting divided by the number of telemetered animals present to be captured with those gears). This empirical capture probability, although for larger size fish, provides a useful Bayesian prior for use in estimating abundance for all juvenile fish.
- Three, we can estimate habitat use and possibly home range directly from the relocations of telemetered fish.

<u>Assessment of natal spawning and rearing areas</u>

Any change in abundance of juvenile fish that occurs during the July-October NSE sampling period could be a result of a variety of factors including changes in habitat

use, capture probability, or simply immigration of fish from the LCR into the NSE study reach. Because the planned flow experiments occur simultaneously with the summer monsoon rains and associated freshets from the LCR (which can transport fish into the mainstem) we have sought to be able to understand the timing of outmigrations from the LCR to the mainstem through the use of elemental microchemistry or isotopes. This understanding would both help with interpreting any change in abundance or density that occurred concurrently with the flow experiment (as described in our original proposal in detail) and also provide a base from which a variety of questions associated with mainstem spawning, migratory patterns, and natal sources of non-native fish could be addressed. Following preliminary development of a geochemical atlas of trace elements and stable isotopes in waters throughout the Grand Canyon and successful screening of useful chemistries within otoliths in 2009, we have determined that two of the clearest "fingerprint" parameters to distinguish Little Colorado from mainstem Colorado are the stable isotope ratios of carbon and oxygen, respectively. However, we need to book instrument time at the University of Wisconsin Secondary Isotope Mass Spectrometry (SIMS) facility and if possible analyze a suite of otoliths of fish whose capture histories are known, and then correlate the carbon and oxygen isotope data with other chemistries as well as with daily growth increments in the otoliths. We need to do this despite the high expense and difficulty of obtaining time on the SIMS as this is the best approach to help us calibrate other chemistries and growth increments. Furthermore, in order to successfully link natural fluctuations in water chemistry with otolith chemistry and migratory behaviors, we would like to conduct a reciprocal transplant experiment using small enclosures. Ideally we would transfer fish between multiple locations in the mainstem and LCR. This work would allow us to estimate the amount of time a fish needs to spend in a particular water mass to obtain a habitat specific otolith signature as well as provide insight on the affects of short duration flood events on otolith chemistry signatures. These transplants would be conducted using FMS.

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References

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Table 1. Total catch and catch fraction (in parenthesis) of species collected from hoops and electrofishing during the Nearshore Ecology (NSE) sampling program in the Grand Canyon reach of the Colorado River during 2009. Note we have adopted the standard naming nomenclature of other researchers delineating trips by their launch date such that GC20090709 is our first trip which launched on July 9, 2009.

	GC20090709		GC20090812		GC20090905		GC20091012	
SPECIES	Electrofishing	Hoop Nets	Electrofishing	Hoop Nets	Electrofishing	Hoop Nets	Electrofishing	Hoop Nets
Brown bullhead	0 (0)	2 (0)	0 (0)	1 (0)	5 (0)	9 (0.01)	2 (0)	1 (0)
Bluehead sucker	47 (0.03)	22 (0.04)	139 (0.08)	47 (0.08)	137 (0.06)	17 (0.02)	56 (0.01)	10 (0.03)
Black crappie	0 (0)	0 (0)	0 (0)	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)
Brown trout	1 (0)	5 (0.01)	3 (0)	0 (0)	4 (0)	0 (0)	3 (0)	1 (0)
Channel catfish	1 (0)	2 (0)	0 (0)	4 (0.01)	1 (0)	1 (0)	0 (0)	2 (0.01)
Common carp	2 (0)	0 (0)	10 (0.01)	0 (0)	2 (0)	0 (0)	0 (0)	0 (0)
Fathead minnow	439 (0.31)	24 (0.04)	672 (0.4)	11 (0.02)	1460 (0.67)	77 (0.09)	3939 (0.91)	74 (0.25)
Flannelmouth sucker	83 (0.06)	28 (0.05)	182 (0.11)	42 (0.07)	79 (0.04)	54 (0.06)	46 (0.01)	51 (0.17)
Humpback chub	312 (0.22)	468 (0.78)	355 (0.21)	505 (0.81)	302 (0.14)	675 (0.8)	135 (0.03)	158 (0.52)
Plains killifish	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0)	3 (0)	0 (0)
Rainbow trout	72 (0.05)	6 (0.01)	90 (0.05)	4 (0.01)	55 (0.03)	7 (0.01)	46 (0.01)	2 (0.01)
Red shiner	256 (0.18)	1 (0)	18 (0.01)	1 (0)	7 (0)	0 (0)	9 (0)	0 (0)
Speckled dace	106 (0.08)	44 (0.07)	130 (0.08)	4 (0.01)	102 (0.05)	0 (0)	81 (0.02)	2 (0.01)
Unidentified sucker	66 (0.05)	0 (0)	93 (0.05)	0 (0)	29 (0.01)	0 (0)	6 (0)	0 (0)
Unidentified fish	2 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0)	0 (0)
Yellow bullhead	16 (0.01)	0 (0)	4 (0)	4 (0.01)	0 (0)	0 (0)	0 (0)	0 (0)
Total fish captured	1403	602	1696	623	2184	841	4327	301

Table 2. Total marks (M) and recaptures (R) of all VIE tagged fish from each site and trip combination for the 2009 NSE sampling. This matrix can be read across the first diagonal to interpret catch and movement within a trip and horizontally to assess movement across trips from each site.

Total Fish			Trip1			Trip2			Trip3			Trip4		
			Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
		M	R	R	R	R	R	R	R	R	R	R	R	R
Trip 1	Site 1	339	41	1	1	58	19	6	47	13	5	13	7	2
	Site 2	248	0	21	9	0	13	23	4	13	8	0	5	21
	Site 3	418	2	0	62	0	6	60	0	7	5	0	0	24
Trip 2	Site 1	358	-	-	-	39	2	3	79	13	1	18	1	2
	Site 2	246	-	-	-	0	28	15	0	39	10	0	8	31
	Site 3	451	-	-	-	0	1	67	0	4	18	0	2	36
Trip 3	Site 1	801	-	-	-	-	-	-	90	30	10	15	11	20
	Site 2	513	-	-	-	-	-	-	0	32	7	1	12	18
	Site 3	370	-	-	-	-	-	-	3	1	23	1	2	26
Trip 4	Site 1	797	-	-	-	-	-	-	-	-	-	72	25	31
	Site 2	774	-	-	-	-	-	-	-	-	-	0	58	75
	Site 3	1586	-	-	-	-	-	-	-	-	-	0	0	375

Table 3. Total marks (M) and recaptures (R) of all humpback chub tagged with VIE marks from each site and trip combination for the 2009 NSE sampling. This matrix can be read across the first diagonal to interpret catch and movement within a trip and horizontally to assess movement between sites across trips.

Humpback chub			Trip1			Trip2			Trip3			Trip4		
			Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
		M	R	R	R	R	R	R	R	R	R	R	R	R
Trip 1	Site 1	190	24	0	0	40	0	0	42	2	0	13	0	0
	Site 2	48	0	1	0	0	0	2	3	1	0	0	2	1
	Site 3	40	2	0	1	0	0	8	0	3	0	0	0	6
Trip 2	Site 1	220	-	-	-	24	0	0	49	4	0	12	0	0
	Site 2	34	-	-	-	0	3	2	0	5	0	0	1	4
	Site 3	53	-	-	-	0	0	8	0	1	1	0	0	7
Trip 3	Site 1	252	-	-	-	-	-	-	40	1	1	13	2	1
	Site 2	52	-	-	-	-	-	-	0	2	0	0	1	0
	Site 3	24	-	-	-	-	-	-	3	0	1	0	1	2
Trip 4	Site 1	95	-	-	-	-	-	-	-	-	-	5	0	0
	Site 2	9	-	-	-	-	-	-	-	-	-	0	0	0
	Site 3	31	-	-	-	-	-	-	-	-	-	0	0	2

Table 4. Number of marks (M), recaptures (R), and capture probability (pcap) for humpback chubs of different size classes from NSE trip 1 (20090709, July 2009).

GC20090709	HBC 40-59mm			HBC 60-79mm			HBC 80-99mm			ALL HBC<100mm		
	M	R	pcap	M	R	pcap	M	R	pcap	M	R	pcap
Site1_Hoop_Pass1	0	0	NA	0	0	NA	0	0	NA	0	0	NA
Site1_Hoop_Pass2	1	0	0	4	0	0	7	0	0	12	0	0.00
Site1_Hoop_Pass3	2	0	0	6	0	0	11	0	0	19	0	0.00
Site1_Hoop_Pass4	3	0	0	6	0	0	14	0	0	23	0	0.00
Site1_Hoop_Pass5	3	0	0	11	0	0	15	0	0	29	0	0.00
Site1_Hoop_Pass6	3	0	0	13	0	0	20	1	0.05	36	1	0.03
Site1_Hoop_Pass7	4	0	0	16	0	0	26	0	0	46	0	0.00
Site1_Hoop_Pass8	5	0	0	18	0	0	29	2	0.07	52	2	0.04
Site1_Hoop_Pass9	7	0	0	22	0	0	31	1	0.03	60	1	0.02
Site1_Hoop_Pass10	7	0	0	23	0	0	36	0	0	66	0	0.00
Site1_Hoop_Pass11	7	0	0	25	0	0	41	2	0.05	73	2	0.03
Site1_Hoop_Pass12	7	0	0	37	0	0	51	1	0.02	95	1	0.01
Site1_EF_Pass1	0	0	NA	0	0	NA	0	0	NA	0	0	NA
Site1_EF_Pass2	4	0	0	13	2	0.15	9	0	0	26	2	0.08
Site1_EF_Pass3	12	0	0	31	1	0.03	18	3	0.17	61	4	0.07
Site2_EF_Pass1	0	0	NA	0	0	NA	0	0	NA	0	0	NA
Site2_EF_Pass2	3	1	0.33	5	0	0	4	0	0	12	1	0.08
Site2_EF_Pass3	5	0	0	11	0	0	7	0	0	23	0	0.00
Site3_EF_Pass1	0	0	NA	0	0	NA	0	0	NA	0	0	NA
Site3_EF_Pass2	7	0	0	11	0	0	3	0	0	21	0	0.00
Site3_EF_Pass3	8	1	0.13	14	0	0	4	0	0	26	1	0.04
Site3_EF_Pass4	9	0	0	20	0	0	6	0	0	35	0	0.00

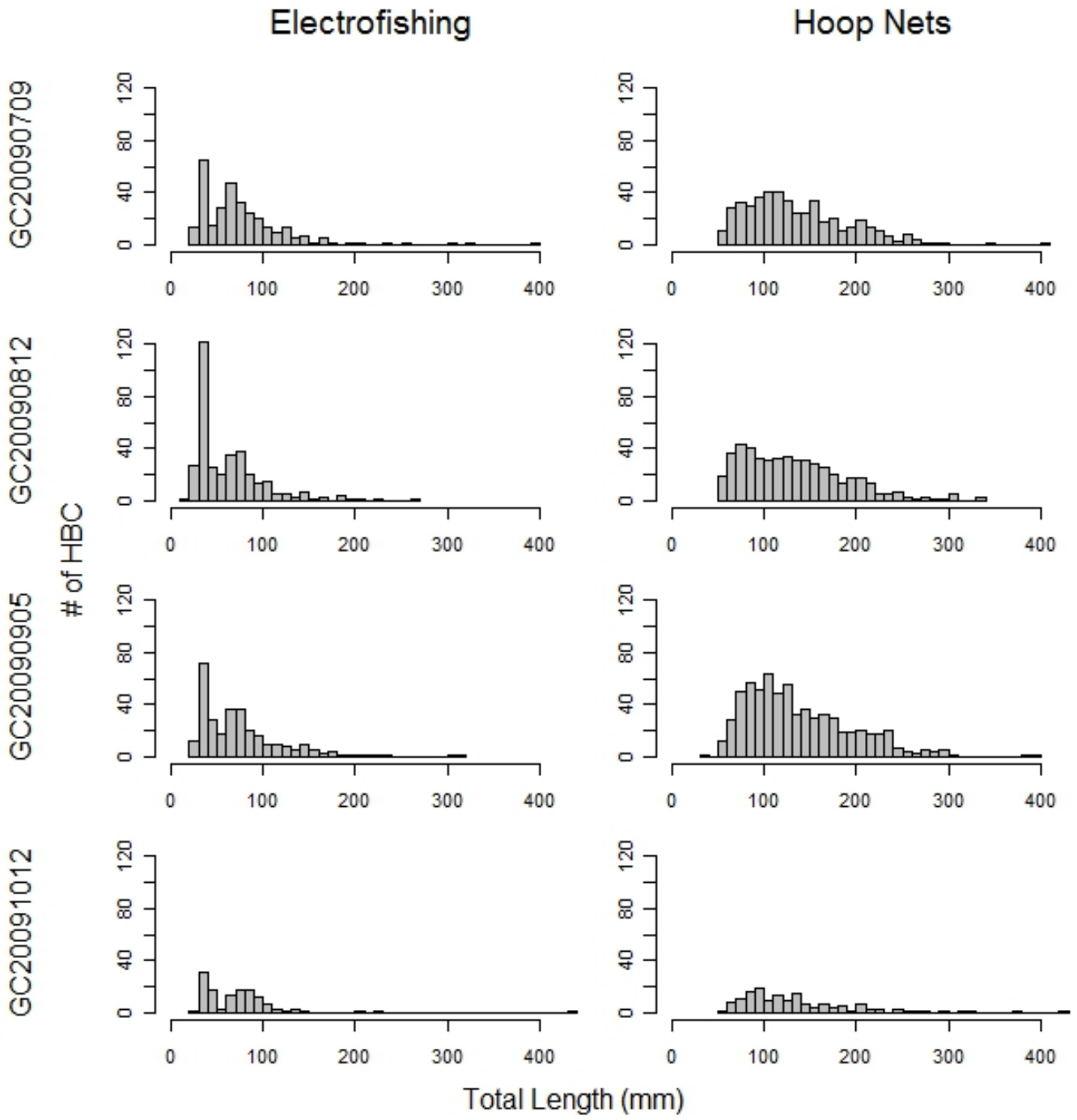


Figure 1. Length frequency plots for HUMPBACK CHUB collected from each 2009 NSE trip (rows) by electrofishing (left column) and hoopnetting (right column). Bin interval is 10-mm.

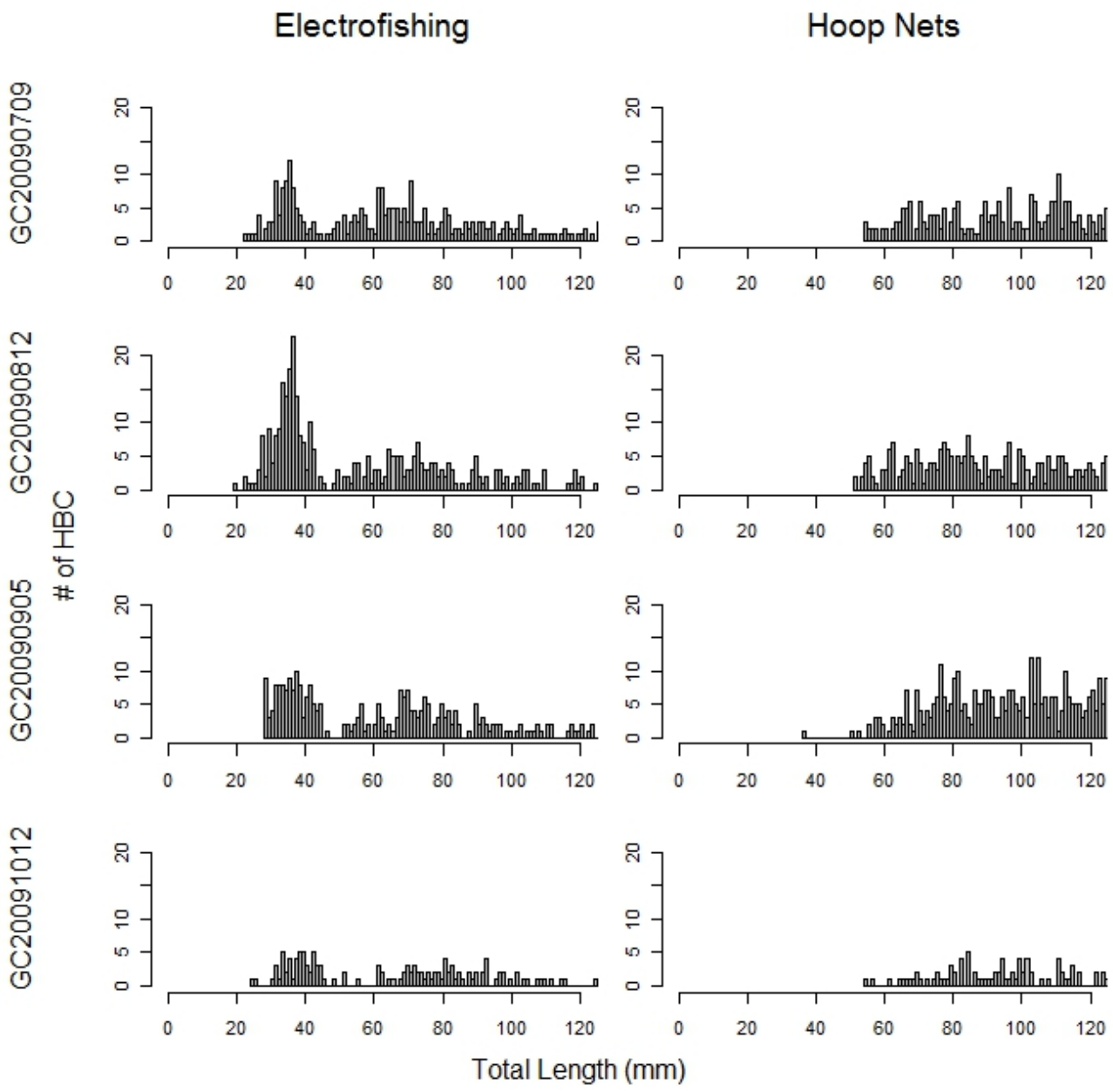


Figure 2. Length frequency plots for small HUMPBACK CHUB collected from each 2009 NSE trip (rows) by electrofishing (left column) and hoopnetting (right column). Bin interval is 1-mm.

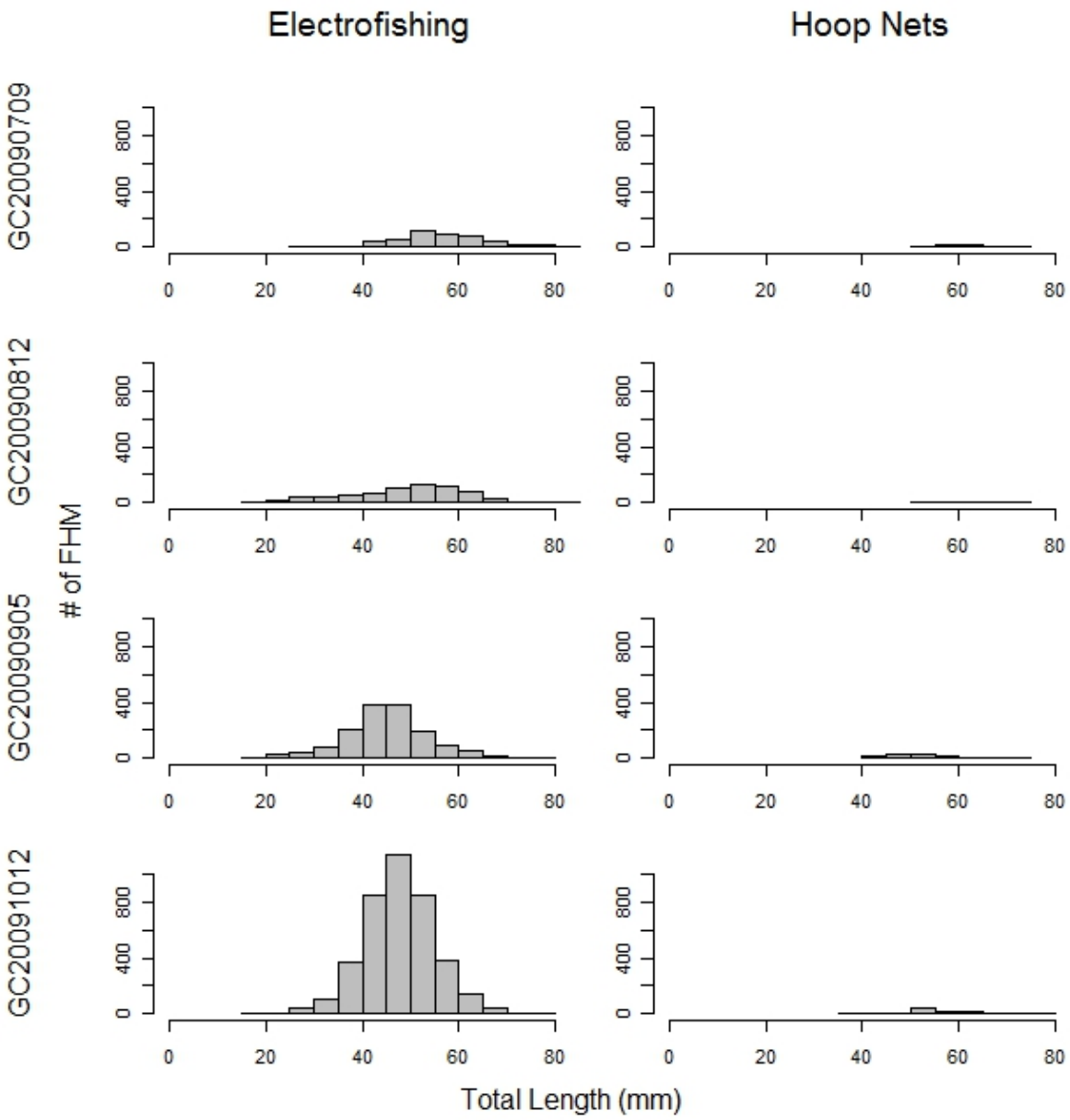


Figure 3. Length frequency plots for fathead minnows (FHM) collected from each 2009 NSE trip (rows) by electrofishing (left column) and hoopnetting (right column). Bin interval is 5-mm. We have included this plot to demonstrate the large difference in gear selectivity for this small bodied species between hoopnets and electrofishing.

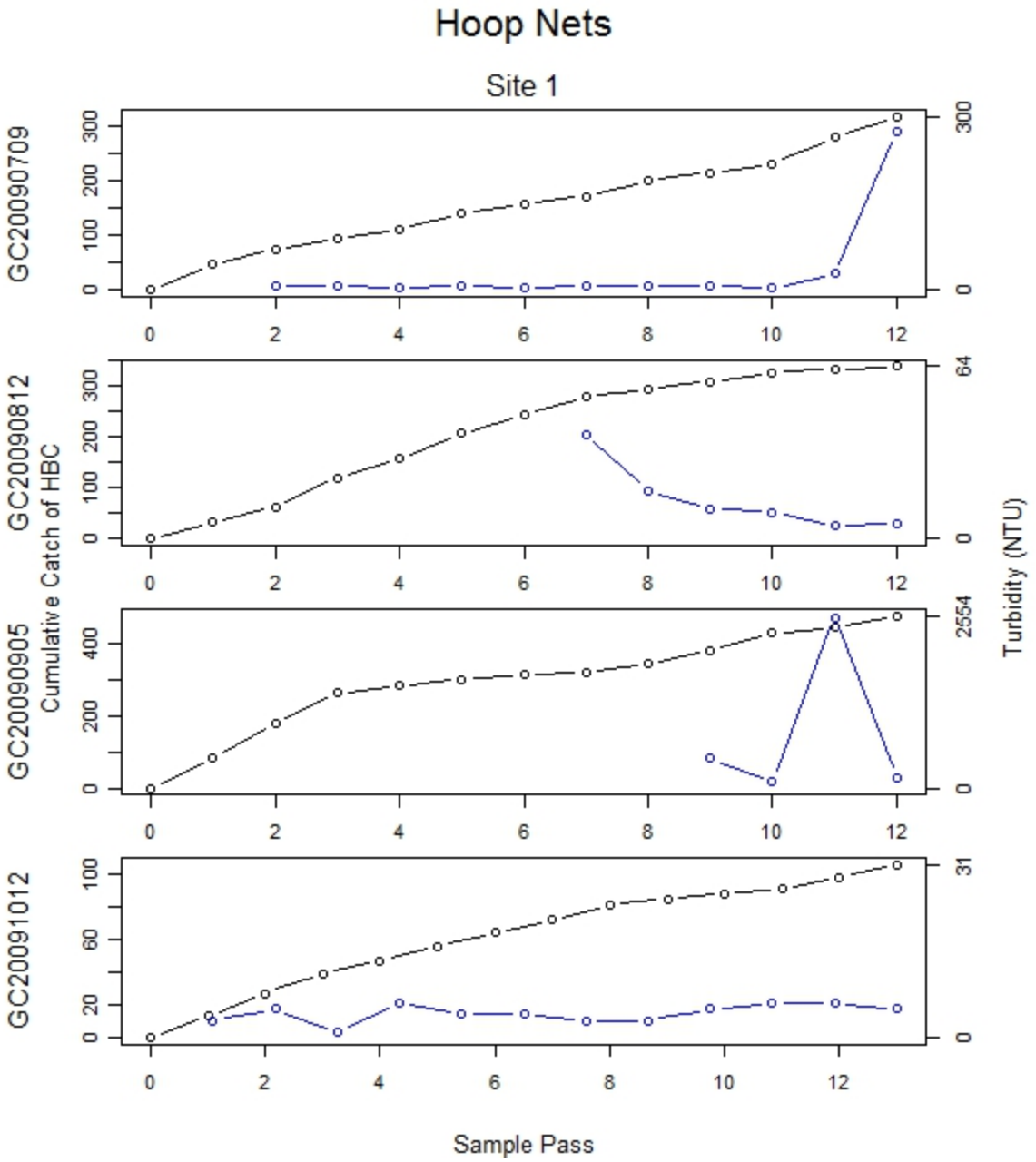


Figure 4. Cumulative catch of HUMPBACK CHUB < 100-mm TL from hoopnets in Site 1 for each NSE trip (rows) during 2009. Blue line represents turbidity measures (measured on second y-axis) measured in camp within the mainstem each trip. Note each axis differs in each plot.

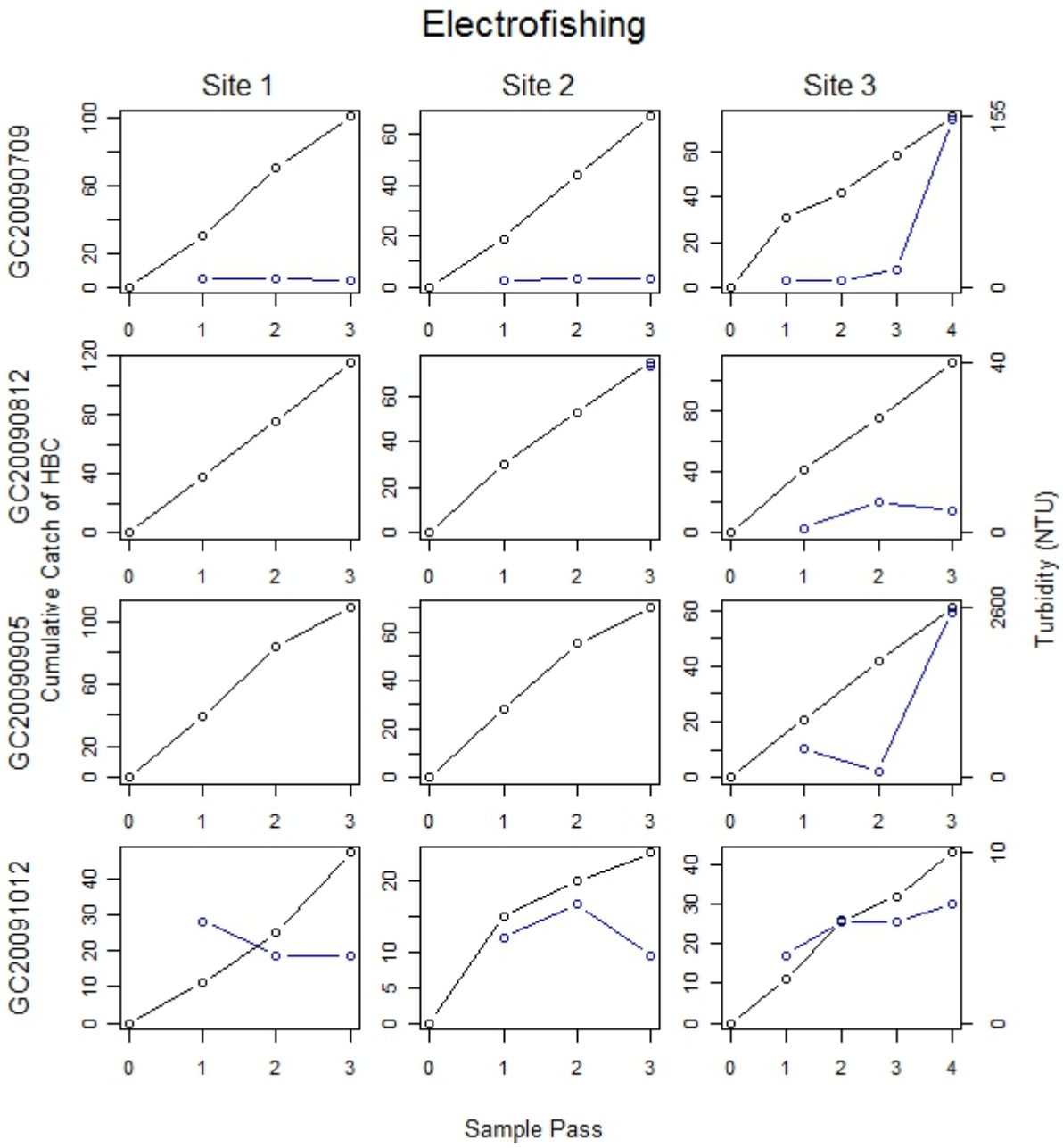


Figure 5. Cumulative catch of HUMPBACK CHUB < 100-mm TL from electrofishing in each site, in each trip. Blue line represents turbidity measures (on second y-axis) measured in camp within the mainstem each trip. Note each axis differs in each plot.

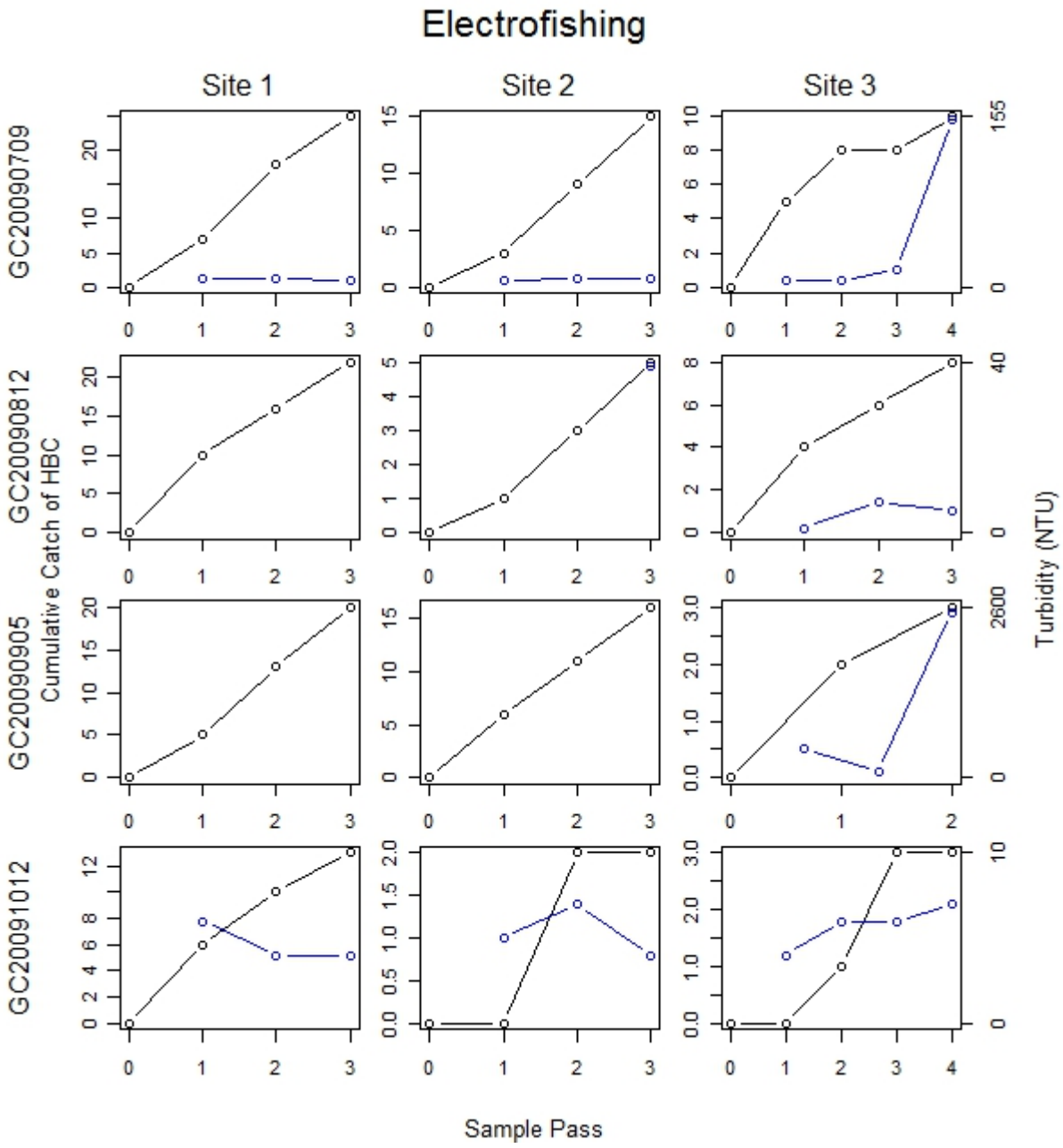


Figure 6. Cumulative catch of HUMPBACK CHUB 100-150-mm TL from electrofishing in each site, in each trip. Blue line represents turbidity measures (on second y-axis) measured in camp within the mainstem each trip. Turbidity measures (blue line) are along the secondary y-axis. Note each axis differs in each plot.

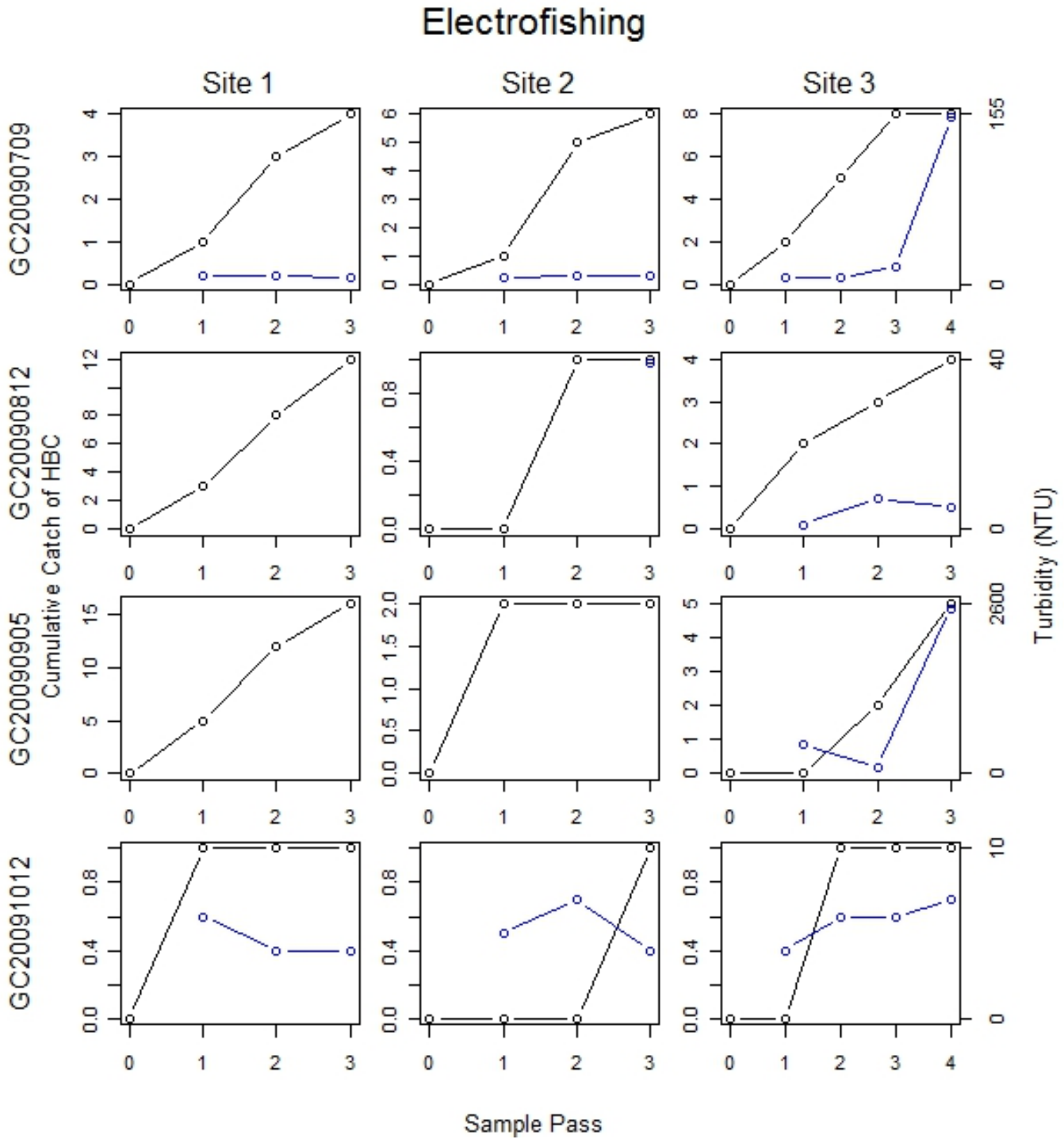


Figure 7. Cumulative catch of HUMPBACK CHUB >150-mm TL from electrofishing in each site, in each trip. Blue line represents turbidity measures (on second y-axis) measured in camp within the mainstem each trip. Turbidity measures (blue line) are along the secondary y-axis. Note each axis differs in each plot.

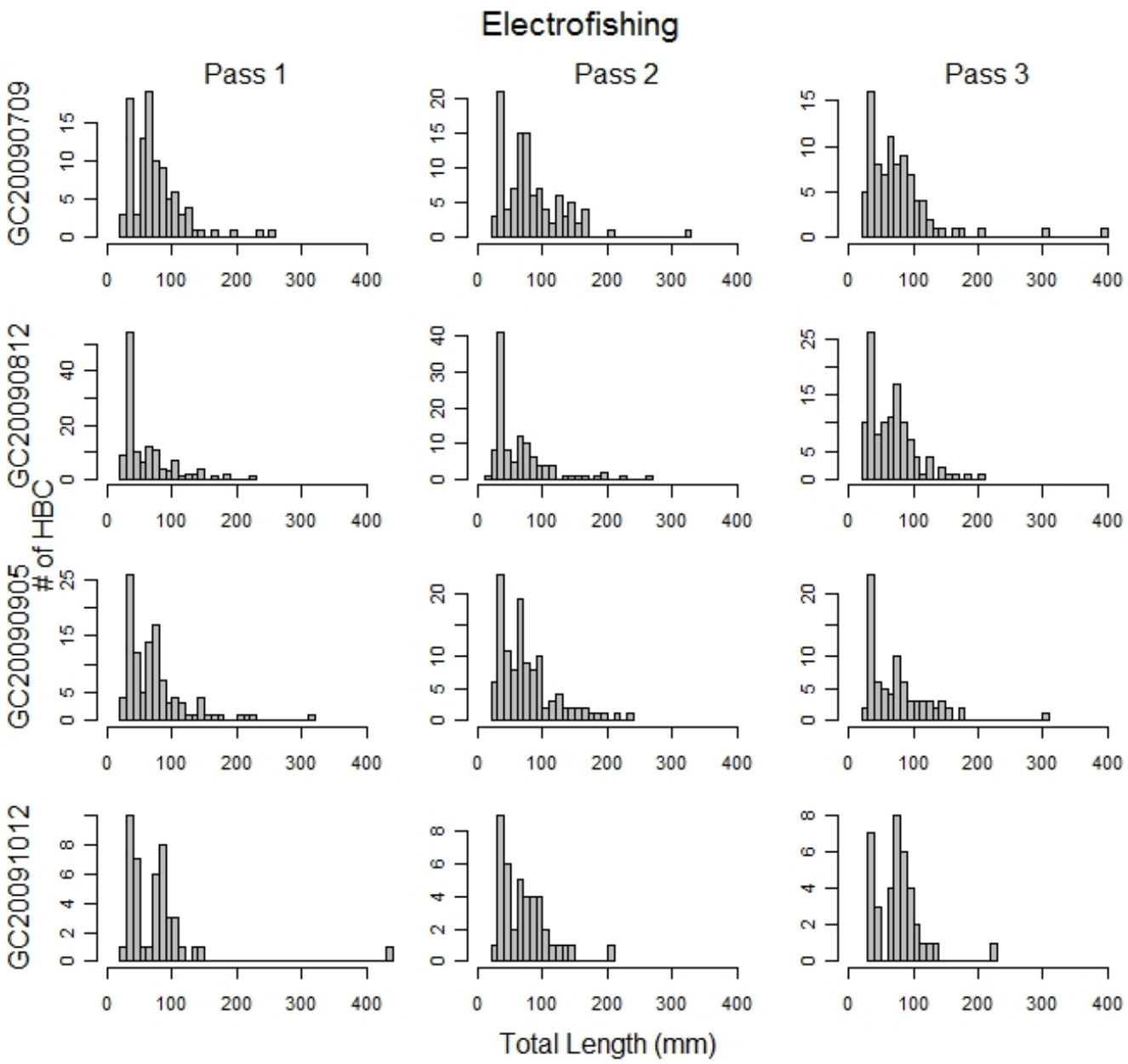


Figure 8. Size distribution (TL-mm) of humpback chub (HBC) collected via electrofishing in each site (columns) across all NSE trips (rows) during 2009. Note each axis differs in each plot.

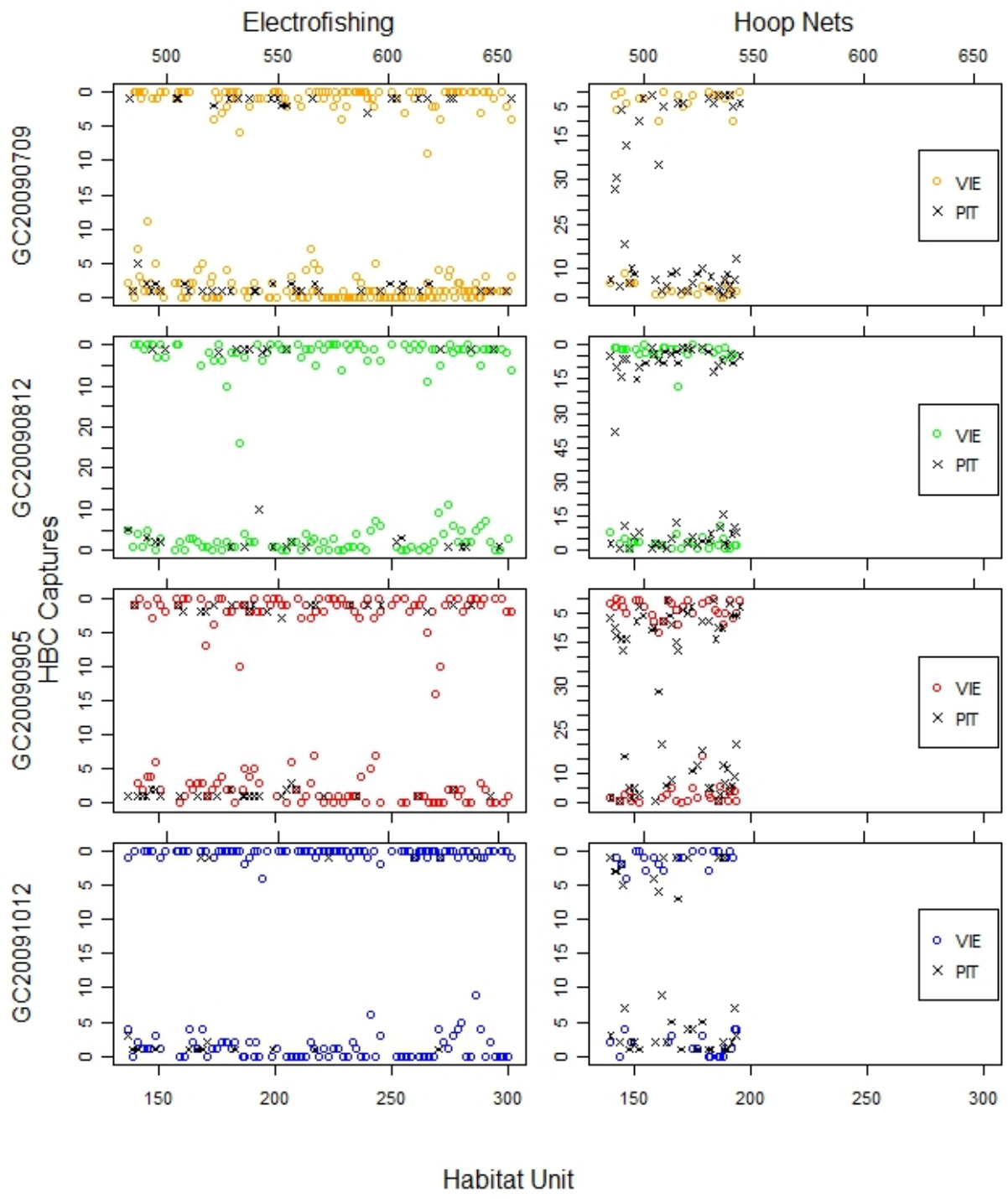


Figure 9. Spatial distribution of HUMPBACK CHUB caught during each trip (rows) by electrofishing (left column) and hoopnets (right column). Circles indicate fish caught and marked with VIE (< 100-mm TL) and X indicate fish tagged with PIT tags (≥100-mm TL). Habitat Unit (primary and secondary x-axis) represents spatially referenced 50-m shoreline sub-reaches. Primary x-axis represents river right HSU sites (facing downstream) and secondary x-axis represents river left HSU sites.

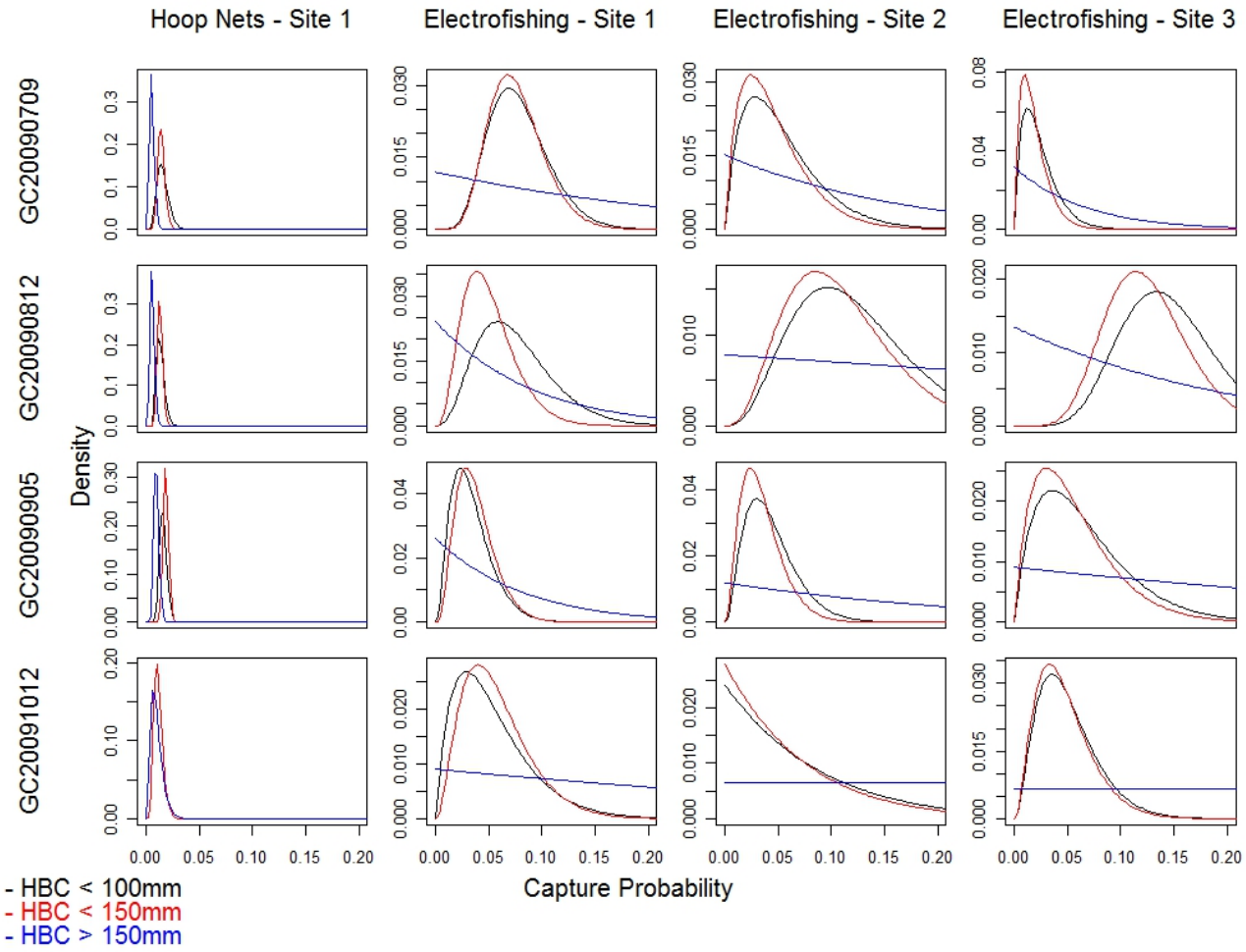


Figure 10. Maximum likelihood estimates of humpback chub capture probability by size class (colored lines) for humpback chub collected during each NSE sampling trip (rows) by sampling site and collection gear (columns) in 2009.

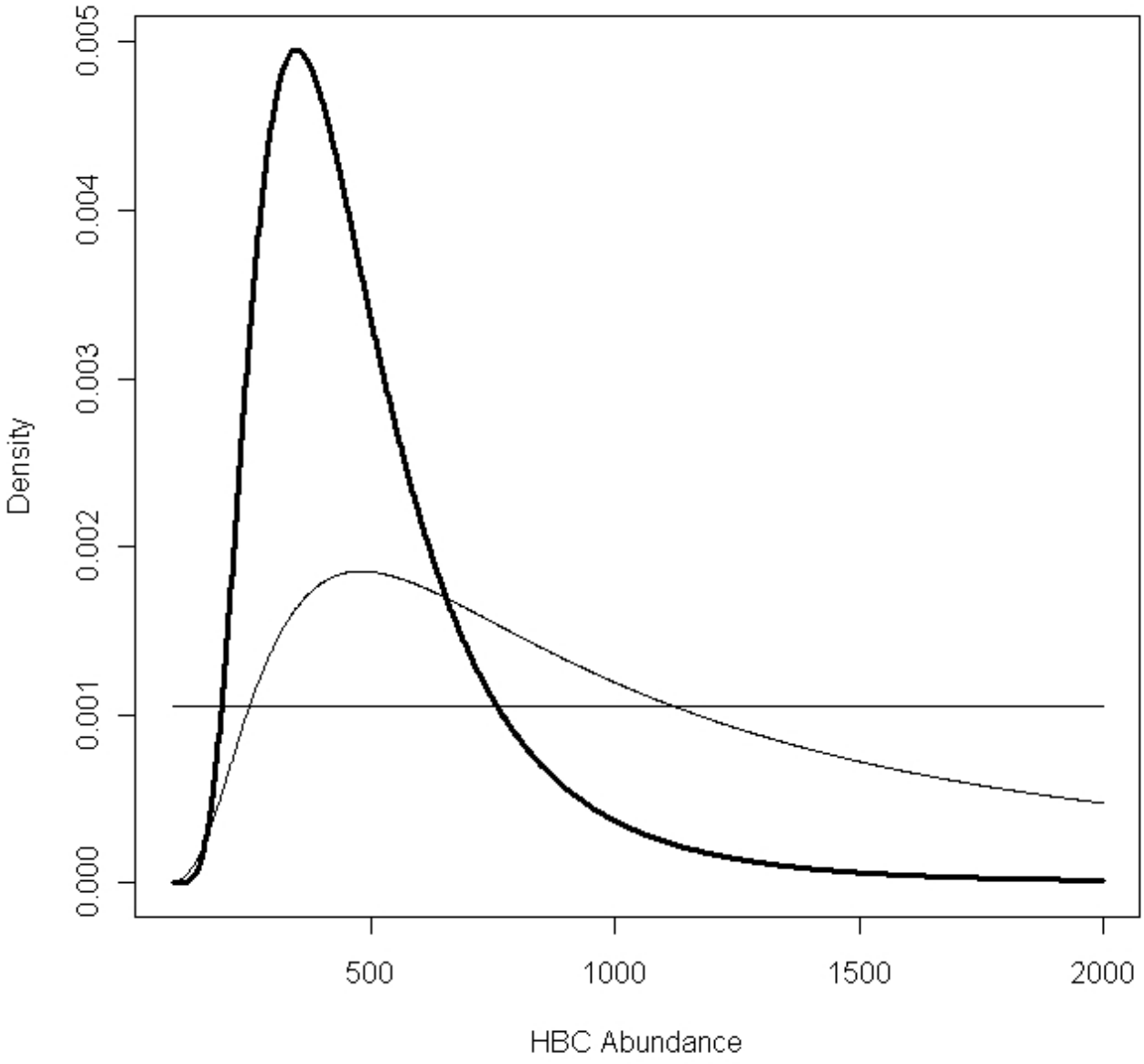


Figure 11. Likelihood estimates of humpback chub <100-mm TL abundance from electrofishing data collected for Site 1, Trip 1 (20090709). Thick black line represents the maximum likelihood estimate (MLE) of abundance after three electrofishing passes while the thin horizontal line represents the (unconverged) likelihood estimate of abundance after 1 pass while the thin dome shaped line with the long tail represents the likelihood estimate after 2 electrofishing passes.

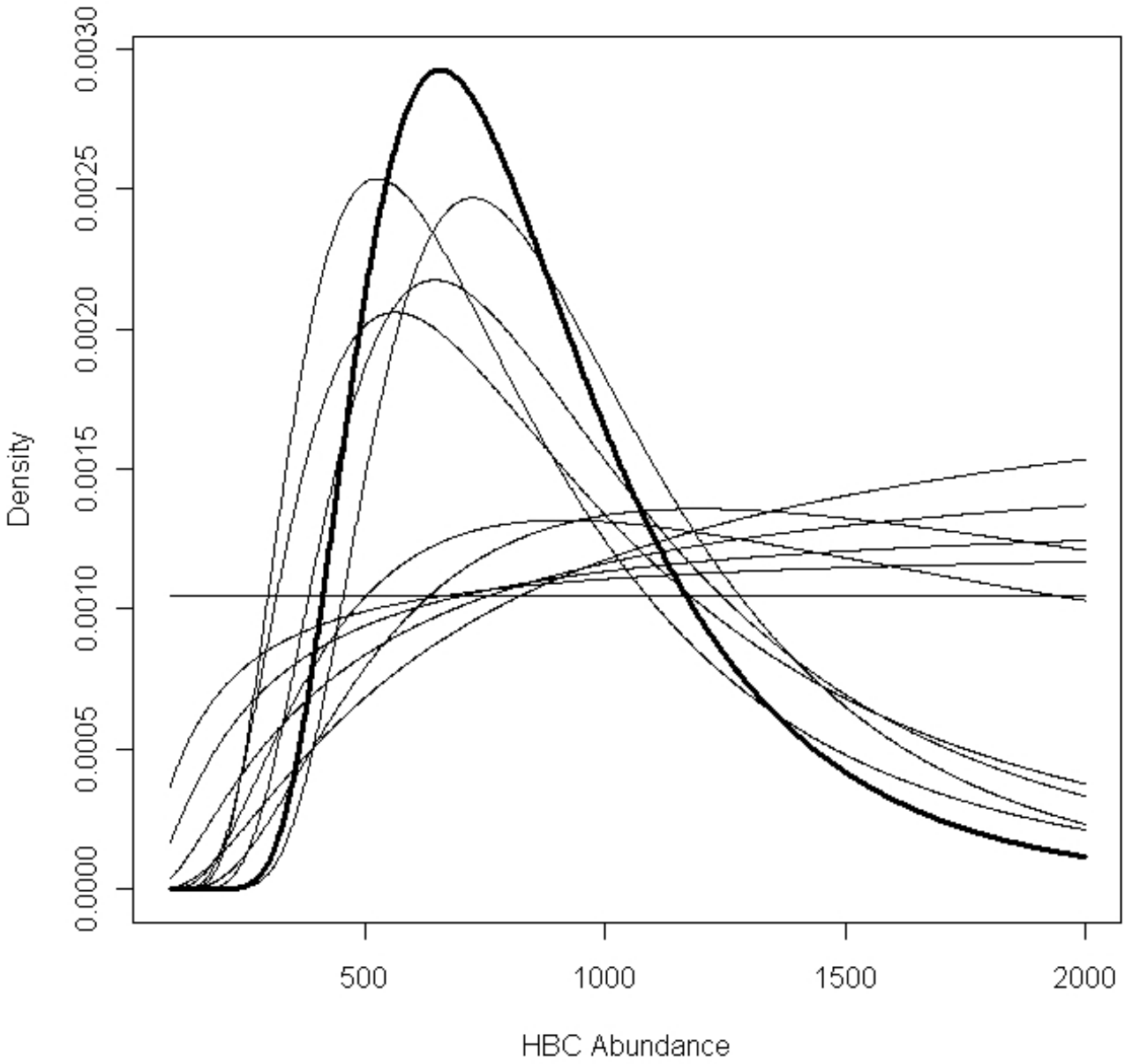


Figure 12. Likelihood estimates of humpback chub <100-mm TL abundance from hoopnetting data collected for Site 1, Trip 1 (20090709). Thick black line represents the maximum likelihood estimate (MLE) of abundance after 12 nights of hoopnet sampling while the thin horizontal line represents the (unconverged) likelihood estimate of abundance after 1 night of sampling. Other thin lines represent likelihood estimates after sequential nights of electrofishing showing that a minimum of 7 nights of electrofishing were required before a credible (i.e., dome shaped) estimate of abundance were made. With increasing samples (nights of fishing) likelihood estimate becomes better defined and resulting MLE is plotted as the think black line.

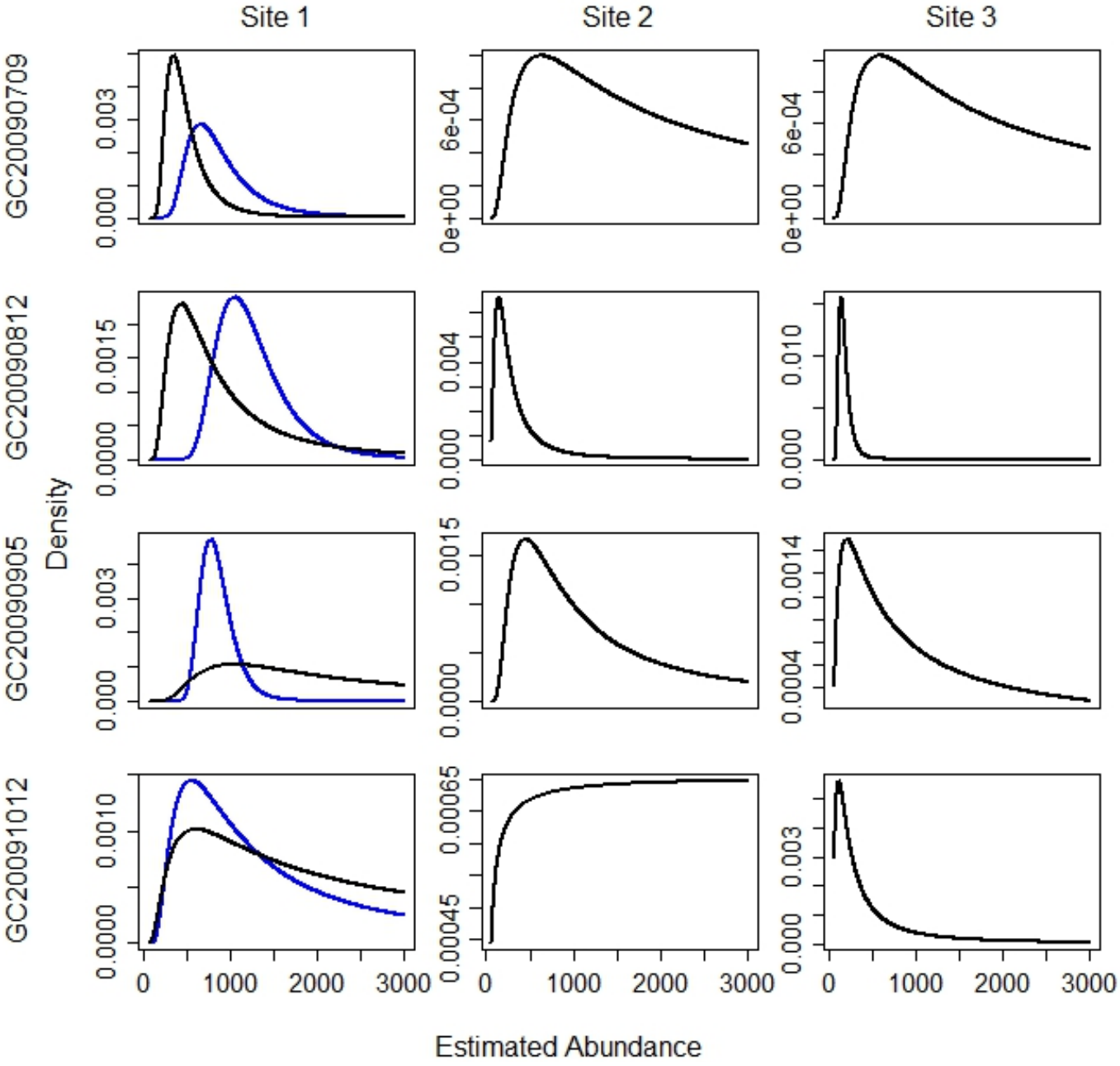


Figure 13. Estimates of humpback chub <100-mm TL abundance in each NSE sampling site (columns) for each trip (rows) during 2009 using closed population abundance methods from Gazey and Staley (1986). Estimates in Site 1 (left most column) were made using both electrofishing (black line in all plots) and hoopnets (blue line) while only electrofishing was used in other sites.

Figure 14. Example of varying trace elemental water chemistry, showing dissolved lead concentrations in July (top panel) and September (bottom panel), 2009. Note how lead is elevated upstream of the Little Colorado (LCR) mouth; it is likely that upstream sampling occurred in an eddy associated with a freshet discharge from the LCR.

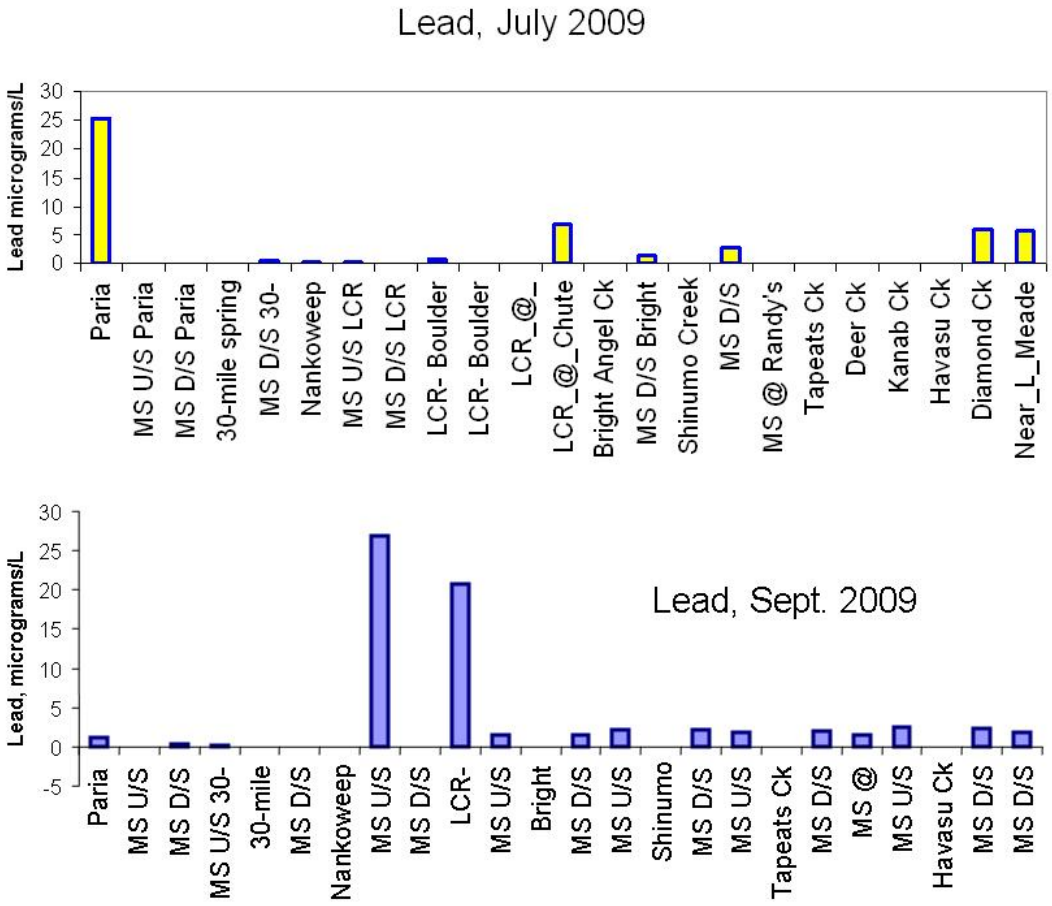
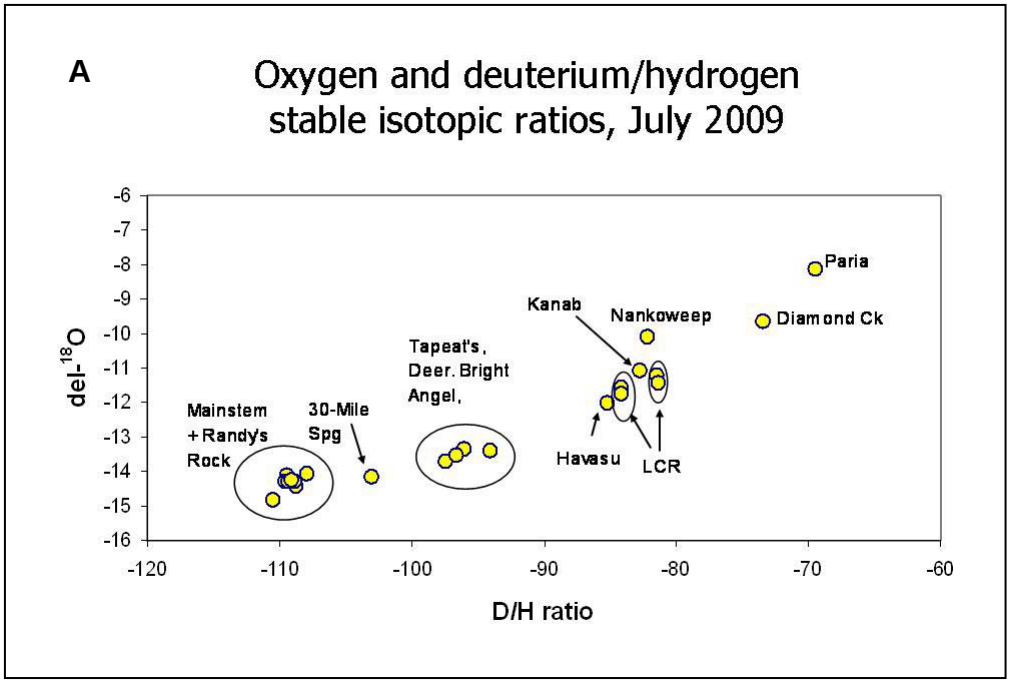


Figure 15. Stable oxygen ($\delta^{18}O$) and deuterium:hydrogen (D/H) ratios in Colorado River water and tributaries in the Grand Canyon. (a) July; (b) October.



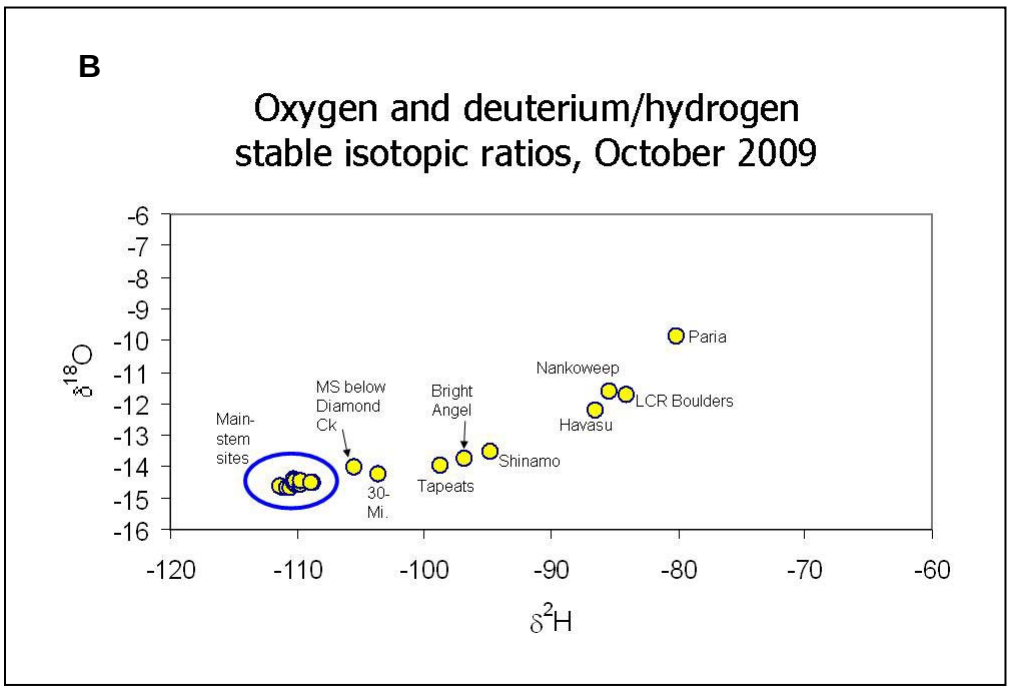


Figure 16. Carbon ($\delta^{13}C$) stable isotopic ratios in dissolved inorganic and dissolved organic C in Colorado River and tributary water, October 2009.

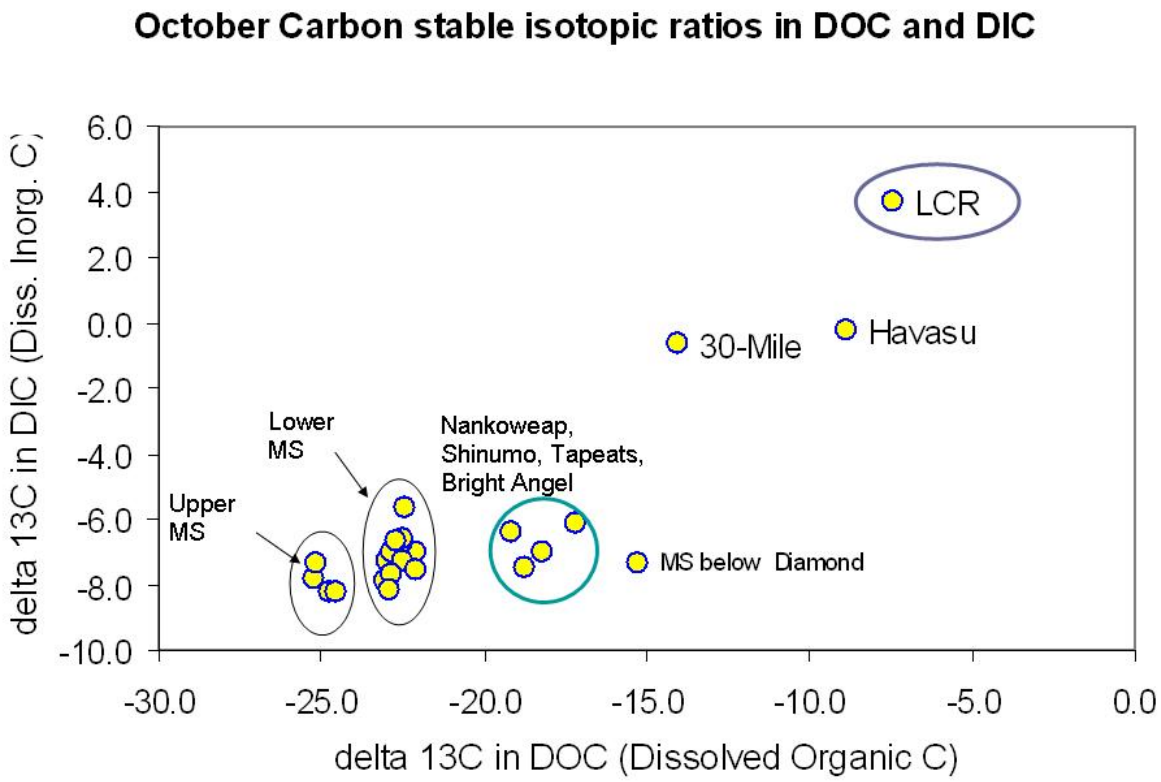


Figure 17. Principal components analysis of $\delta^{13}C$, D/H, and $\delta^{18}O$ in water showing sites arrayed along the first two factors. PC-1 is magnitude of stable isotopic ratios; PC-2 is a gradient of $\delta^{13}C$ (higher values of PC-2) vs. D/H, and $\delta^{18}O$ (lower values of PC-2). Analysis was limited to sites represented in May and October samples.

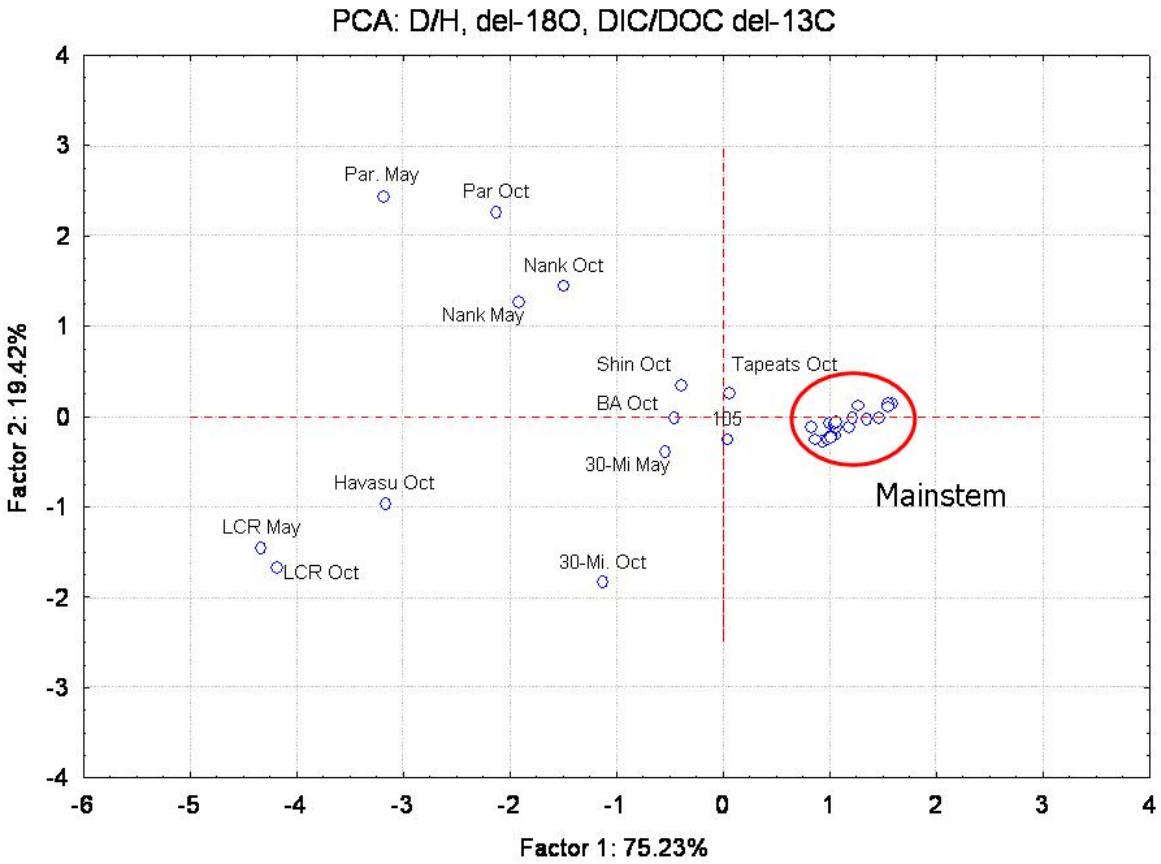


Figure 18. Periodic table of elements quantified in flannelmouth sucker otoliths using LA-ICPMS. (a) Elements quantified (outlined in red) (b) Elements above instrument detection limits in otoliths (outlined in Red)

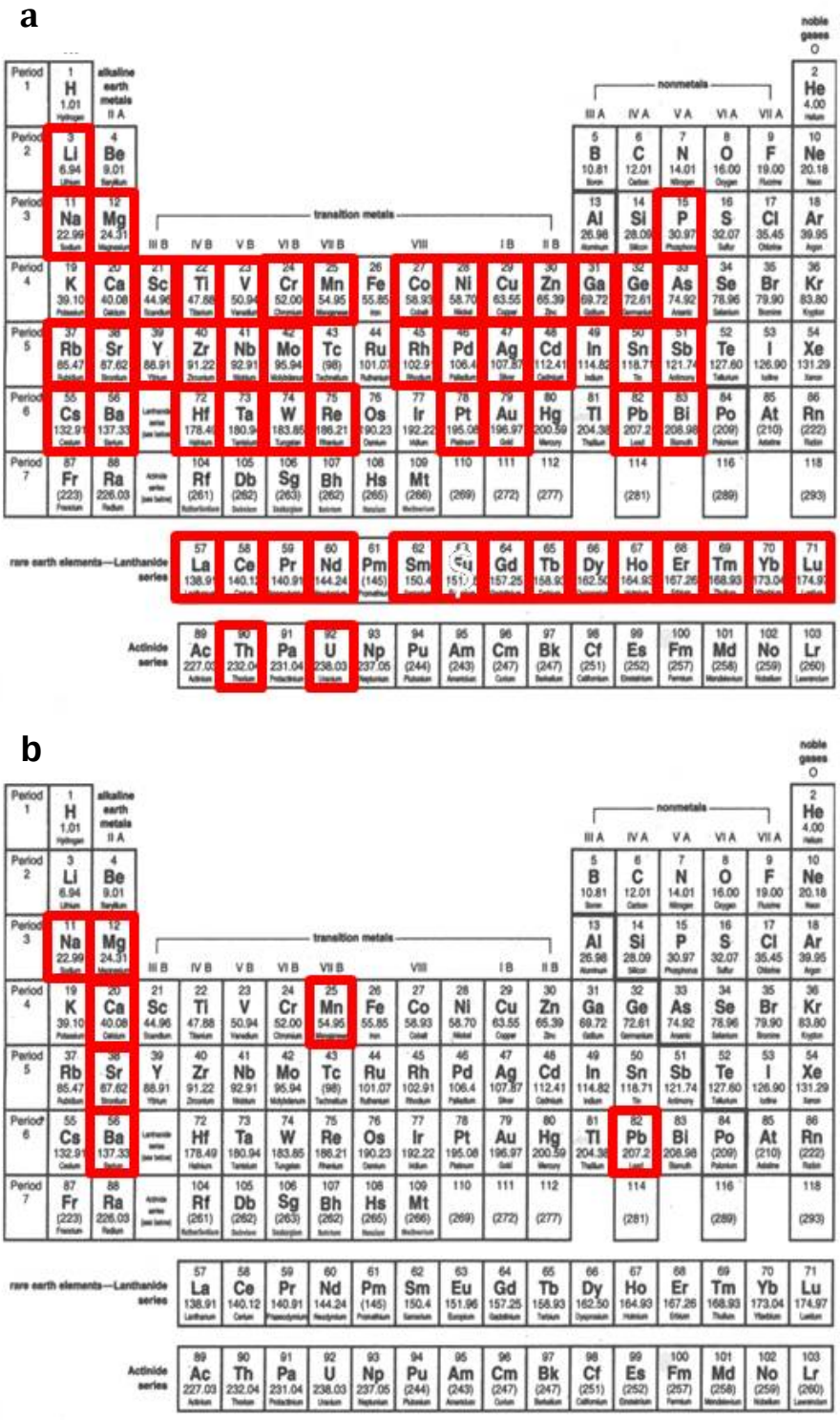


Figure 19. Representative LA-ICPMS Sr:Ca, Ba:Ca, Mn:Ca transects from three fish collected in the Grand Canyon. Top pane is from a fish collected in Colorado River above LCR confluence, middle pane is a fish collected in LCR, and bottom pane is a fish collected from Colorado River below LCR confluence. Long arrows in optical images of otoliths denote direction of laser transect. Short red arrows in optical images and graphs denote otolith core (notice Mn:Ca peak).

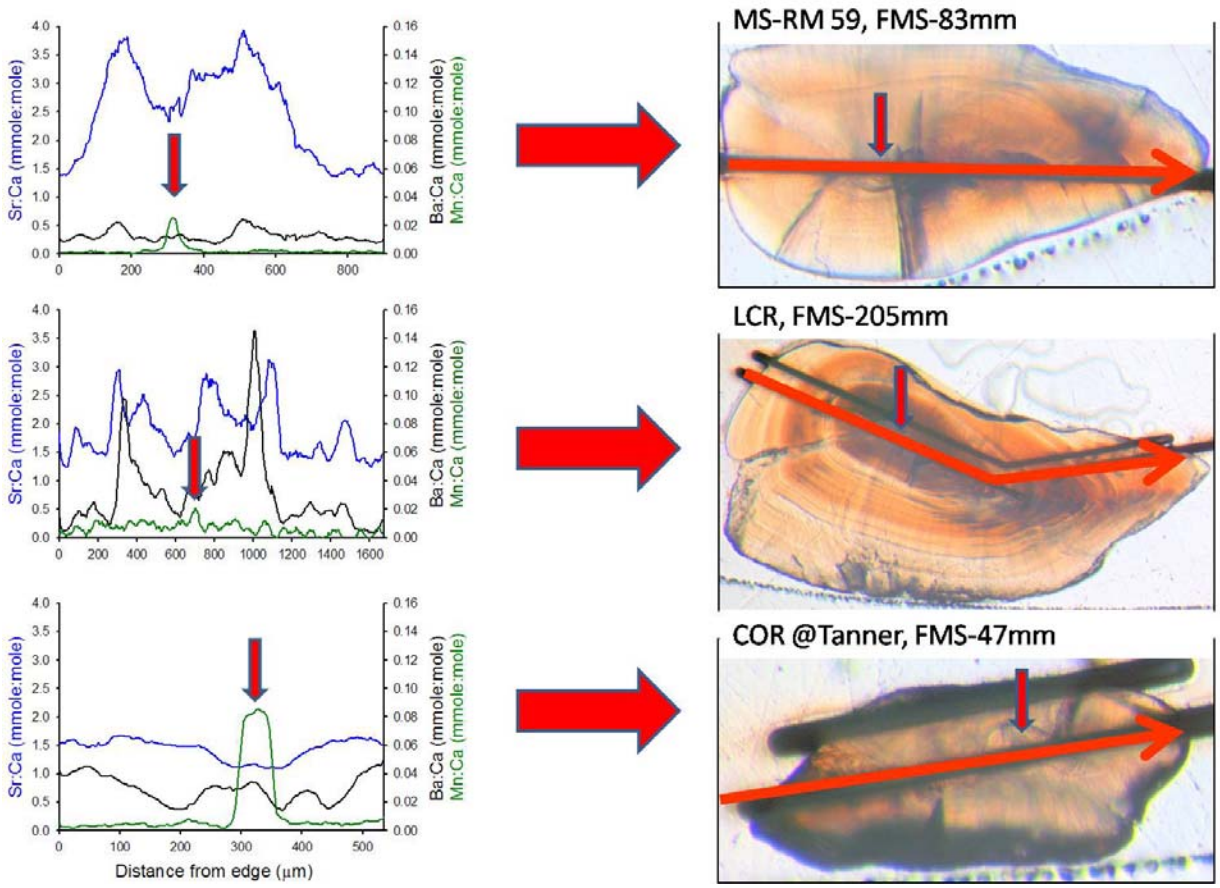


Figure 20. False-color 2-D surface maps of otolith Sr:Ca images from CHESS-XRF analyses. Column headings denote collection location. Purple is high Sr:Ca, blue is low Sr:Ca. Surface map color scale vary for each fish. High concentrations at immediate otolith edges are artifacts of analytical technique.

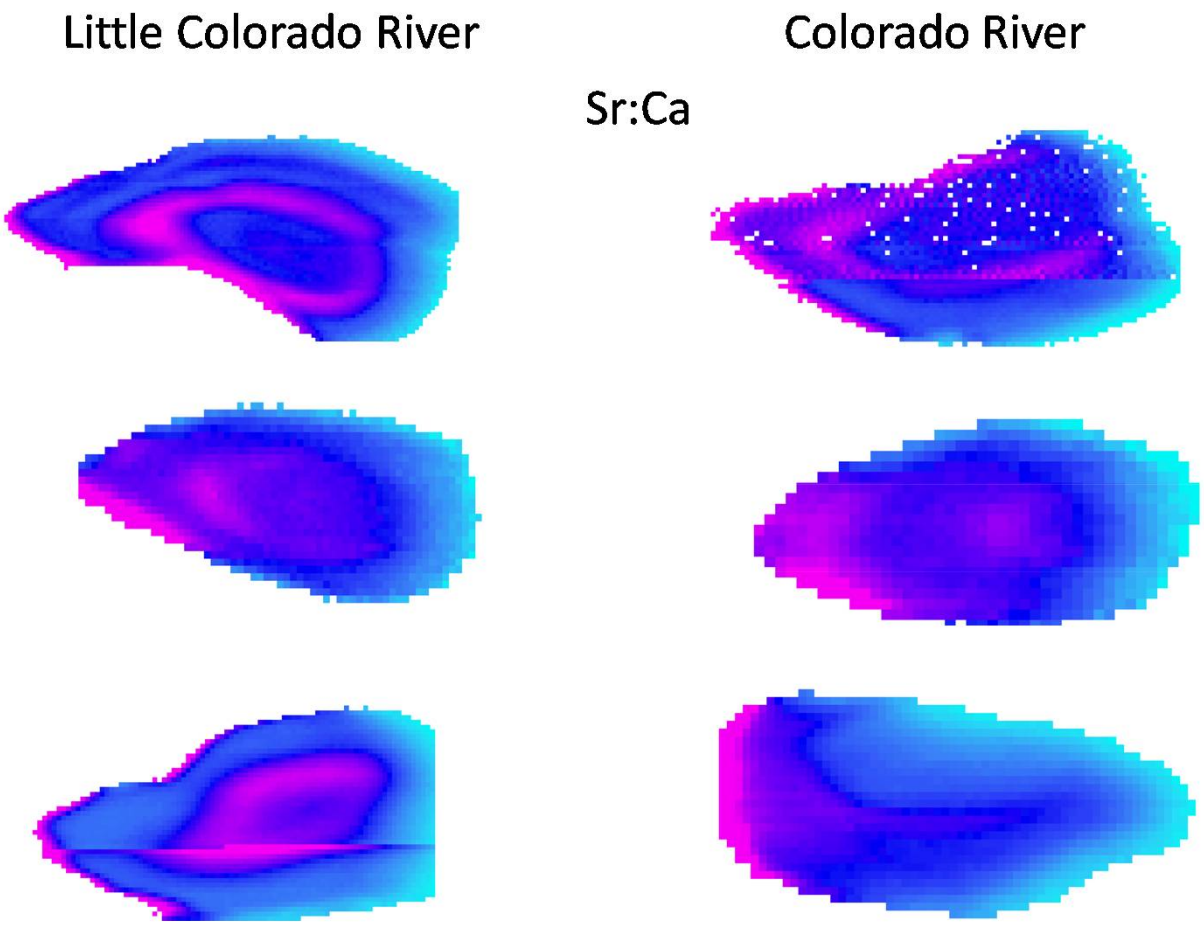


Figure 21. False-color 2-D surface maps of otolith Cu:Ca ratios from CHESS-XRF analyses. Purple is high Cu:Ca, blue is low Cu:Ca. Surface map color scale is the same for each image. High concentrations at immediate otolith edges are artifacts of analytical technique.

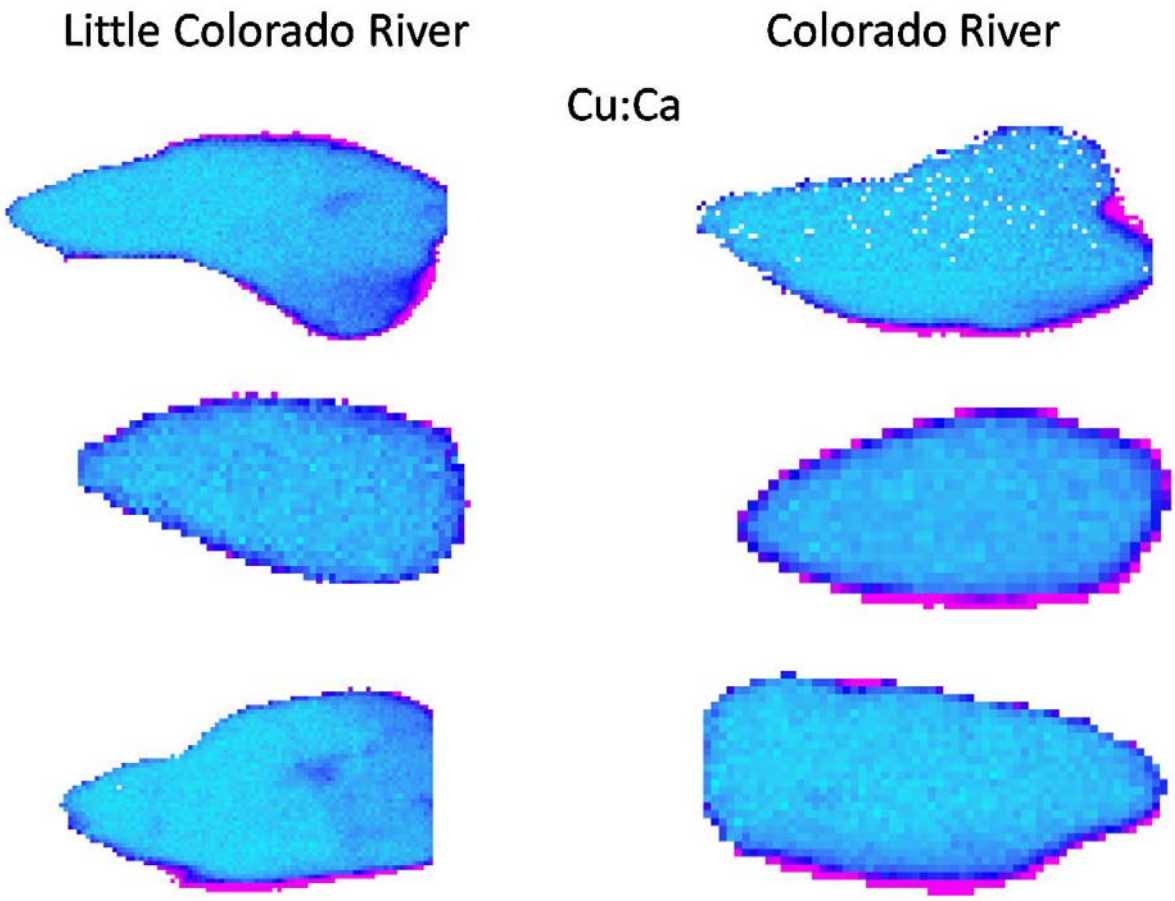


Figure 22. False-color 2-D surface maps of otolith Se:Ca ratios from CHESS-XRF analyses. Purple is high Se:Ca, blue is low Se:Ca. Otoliths are scaled the same for each fish. High concentrations at immediate otolith edges are artifacts of analytical technique.

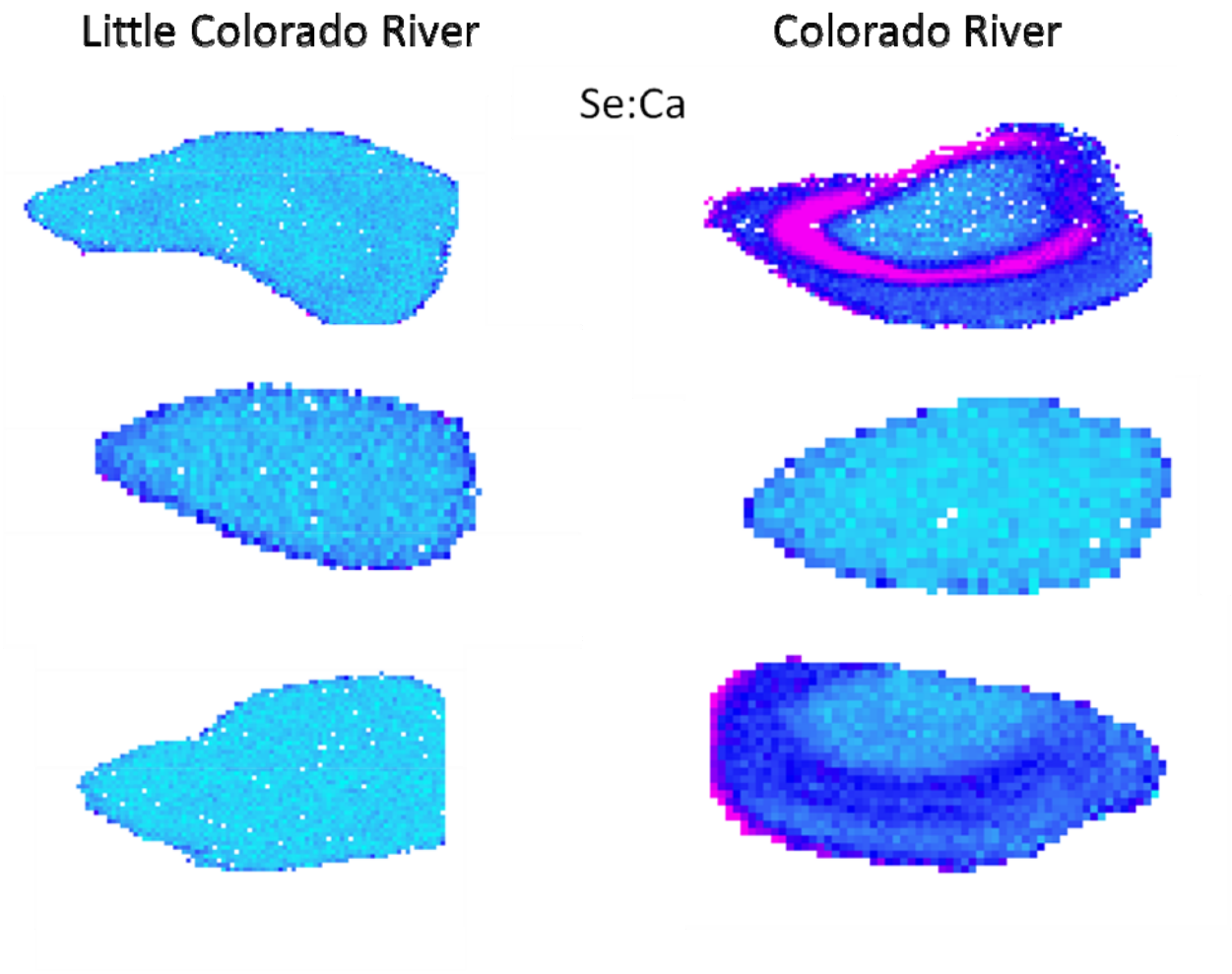


Figure 23. $\delta^{18}O$ otolith measured using SIMS. Optical image is otolith from 33mm HBC. Red circles in optical image (core= right circle, edge=left circle) represent location of SIMS analysis. Error bars denote internal precision of instrument. Optical image of bluehead sucker otolith is not shown.

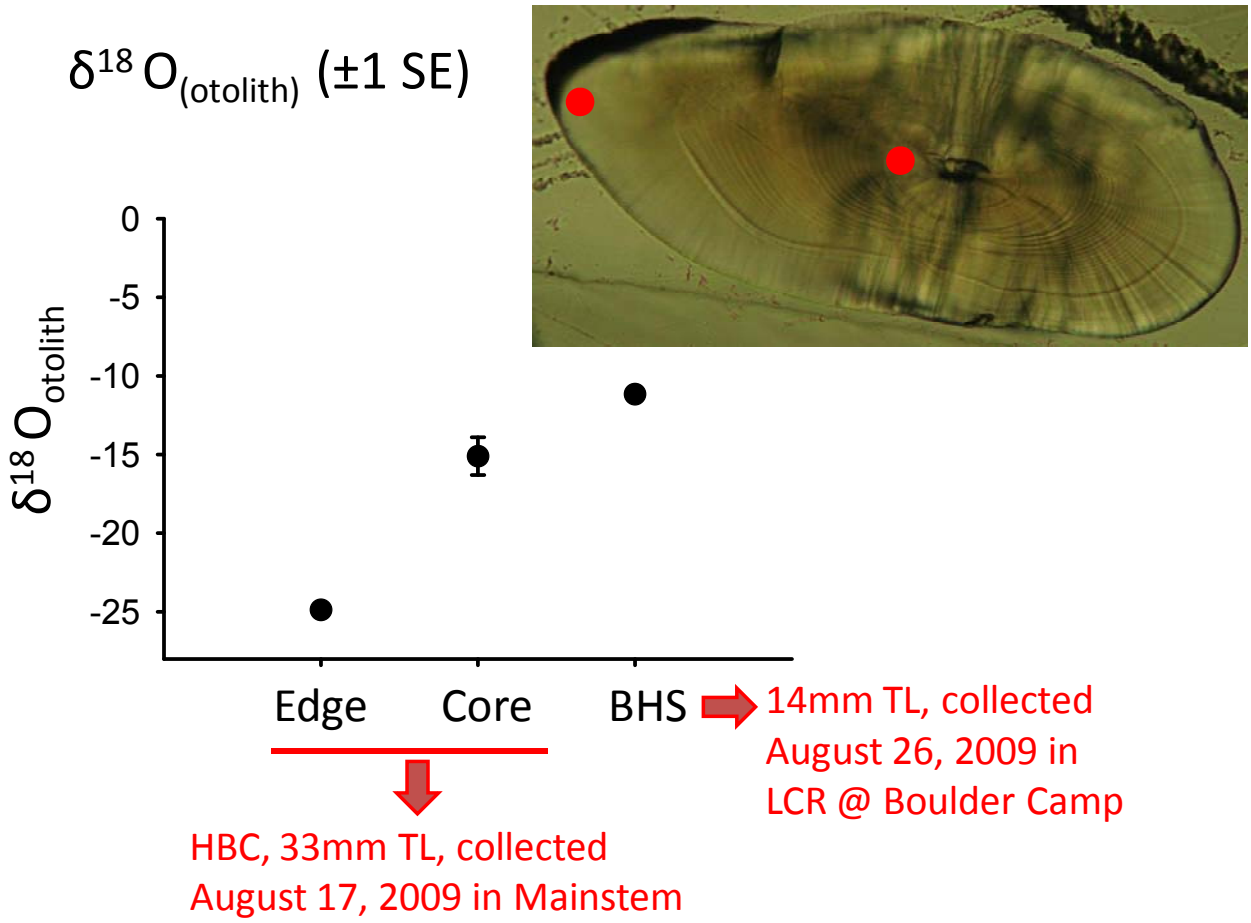


Figure 24. $\delta^{13}C$ of an otolith measured using SIMS. Optical image is otolith from a 33-mm humpback chub. Red circles in optical image (core= right circle, edge=left circle) represent location of SIMS analysis. Error bars denote internal precision of instrument. Optical image of bluehead sucker otolith is not shown.

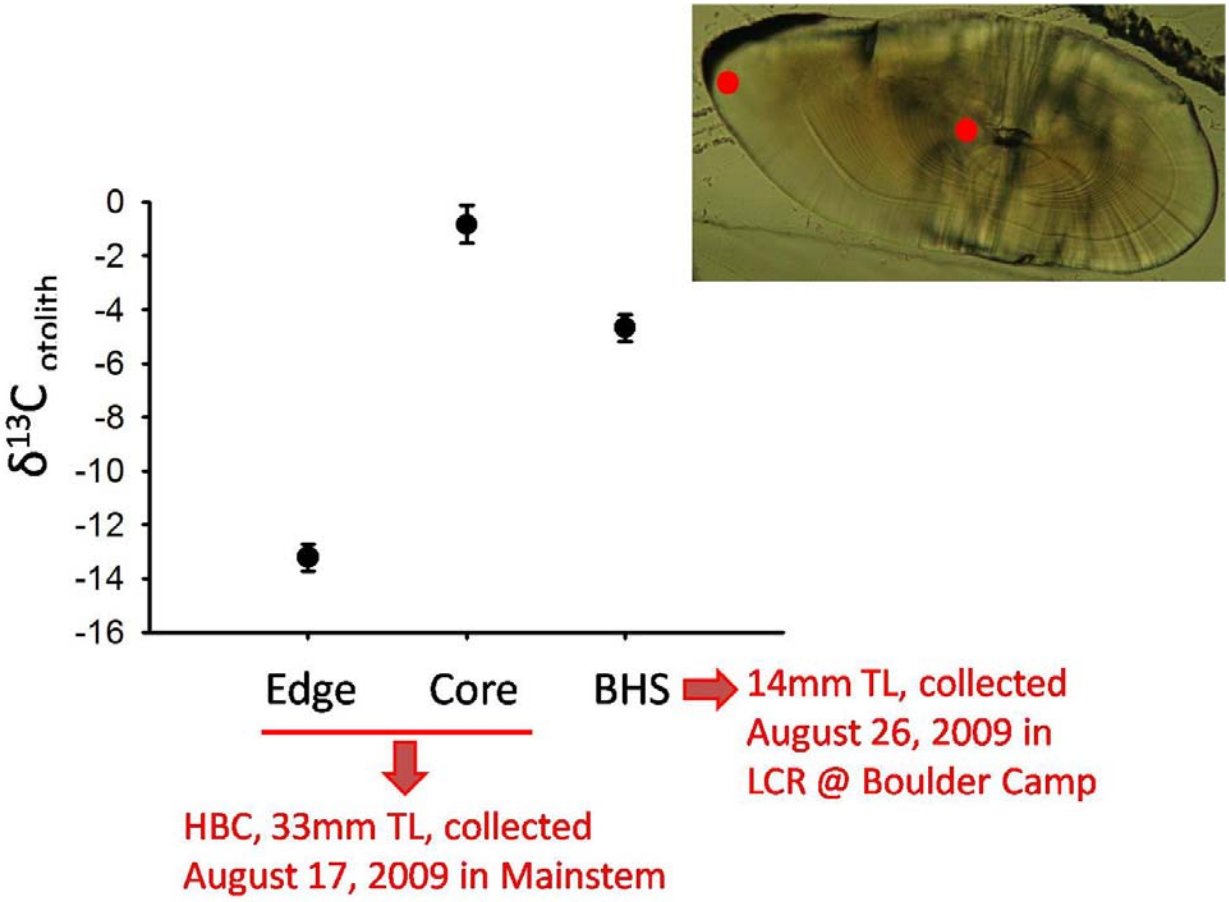


Figure 25. $\delta^{13}C$ of an otolith measured using SIMS. Otolith is from a 64-mm flannelmouth sucker. Red circles in optical image correspond to sample spots displayed on x-axis.

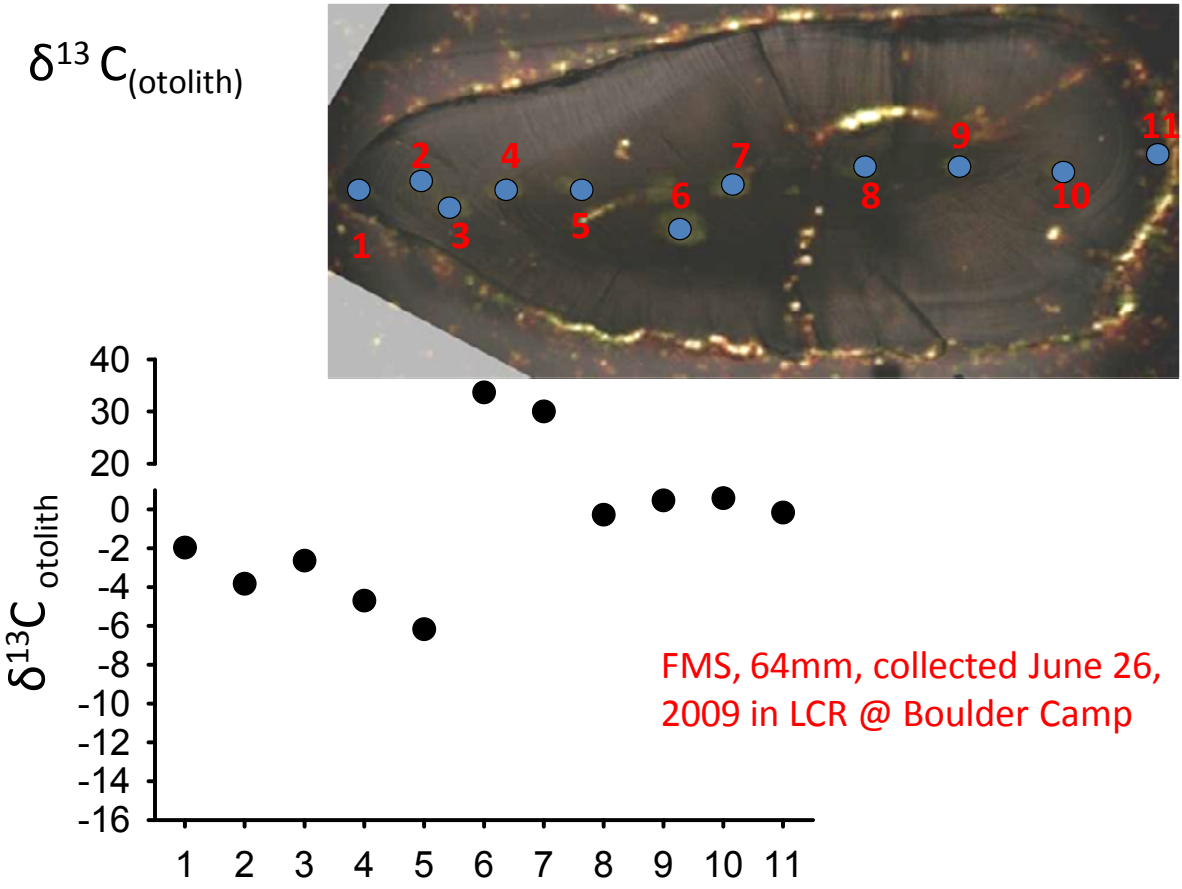


Figure 26. Light micrographs of HBC otoliths collected in LCR. (a) Bright-field light micrograph showing distinct growth check occurring 27 days post hatch (arrow). (b) Differential interference contrast micrograph showing growth check region and transition to closely spaced growth increments. Arrow points to distinct growth check between wide growth increments (fast growth) and narrow growth increments (slow growth). (c) SEM micrograph of widely spaced growth increments transitioning to closely spaced increments and back to wider spaced growth increments. Red vertical marks denote daily growth increments. Core is to the left in micrograph, otolith edge is to right.