ABSTRACT

Fish otolith and water chemistry were assessed in the Grand Canyon reach of the Colorado River and its tributaries. Aqueous strontium and selenium (in ratio to calcium) and carbon stable isotopic ratios were identified as markers with excellent potential to track the provenance and movements of the endangered humpback chub *Gila cypha*. Although otolith δ¹³C and Sr/Ca varied proportionately to water chemistry and provided a framework for detailed study of humpback chub movements, otolith Se/Ca showed ambiguous tracking of known water chemistries. As an application, we document the natal source and movement dynamics of n = 10 humpback chub and compare these findings from otolith microchemistry with the current paradigm of humpback chub spawning ecology. We found that seven of ten fish follow the current early life history paradigm and were spawned in the Little Colorado River and subsequently emigrated to the main stem Colorado River as juveniles. However, the otolith markers of three fish suggest an alternative early life trajectory with unknown provenance. Age and growth analyses demonstrate seasonally higher growth rates in the warmer Little Colorado River compared with the Colorado River. Combining natural markers with age and growth reconstructions provides a powerful tool for assessing the habitat use and success of humpback chub in the Grand Canyon. Copyright © 2012 John Wiley & Sons, Ltd.

KEY WORDS: otolith chemistry; humpback chub; Grand Canyon; fish growth/age; Colorado River

INTRODUCTION

The Grand Canyon reach of the Colorado River is an internationally recognized cultural and ecological treasure (UNESCO status 1979). Since 1963, this canyon-bound river reach has been highly influenced by operation of the Glen Canyon Dam. Completion of the dam created physical ecosystem changes, including attenuation of floods, reductions in sediment inputs, daily water level fluctuations due to hydropower production and relatively constant, cool water temperatures (Howard and Dolan, 1981; Stanford and Ward, 1991; Stevens *et al*., 1997; Schmidt *et al*., 1998; Topping *et al*., 2008). Concurrent biological effects include changes in food web dynamics across all trophic levels (Stevens *et al*., 1997; Shannon *et al*., 2001; Cross *et al*., 2011), including the introduction of several non-native fish species (Rinne and Janisch, 1995; Nico and Fuller, 1999; Olden and Poff, 2005), as well as a reduction or total loss of successful spawning and rearing conditions for native fishes in the Colorado River main stem (Robinson *et al*., 1998; Gorman and Stone, 1999).

Currently, only four of the endemic species historically found in Grand Canyon still exist there today. One of these, humpback chub *Gila cypha*, has received protection as an endangered species under the US Endangered Species Act (ESA) since the Act was enacted in 1973. The largest humpback chub population is located in Grand Canyon near the confluence of the Colorado and Little Colorado rivers (Valdez and Ryel, 1995; Coggins *et al*., 2006). In addition to the humpback chub population in Grand Canyon, five other humpback chub populations are known and all are found in the Colorado River basin. Management actions intended to enhance humpback chub populations include experimental flow regimes (Cross *et al*., 2011) and removal of non-native predators (Coggins *et al*., 2011). Although the numbers of adult humpback chub in the Grand Canyon population have increased in recent years, species persistence is thought to be dependent on a single spawning tributary (Little Colorado River). Research efforts continue to assess the mechanisms limiting humpback chub recovery (Coggins *et al*., 2006, 2011; Yard *et al*., 2011) and are used for assessment and development of management actions undertaken for species remediation.

Within Grand Canyon, the humpback chub population is structured as a series of nine discrete aggregations. The largest aggregation is located in the Colorado River near...
the confluence of the Little Colorado River (km ~100, as measured downstream of Lee’s Ferry, Arizona; Coggins et al., 2006). The Little Colorado River is a spring-fed tributary that is characterized by extensive travertine (i.e. calcium carbonate) riverbed deposition. Although perennial water flows in the Little Colorado River are supported by a large spring complex located approximately 21 km from the confluence with the Colorado River, only the lower ~14 km of the river are colonized by humpback chub owing to a natural barrier waterfall and naturally occurring high dissolved carbon dioxide levels (Robinson et al., 1998).

Tagging studies on humpback chub have been ongoing since the mid-1980s and have provided a general framework for tracking adult fish movements between the Little Colorado River and the Colorado River. The resulting data suggest that the Little Colorado River and Colorado River habitats are linked by seasonal potamodromous spawning migrations of adult humpback chub. Adult Individuals move from the Colorado River to the Little Colorado River in March to June for spawning, returning to the main stem after spawning (Gorman and Stone, 1999). As part of this tagging program, the minimum size for tagging humpback chub has been 150 mm total length (TL) or approximately age 4 years (Coggins et al., 2006), and in recent years, the minimum size at tagging has shifted to 100 mm TL (ages 2 or 4 years). However, very little information is available on the movement dynamics of smaller, younger humpback chub because they are below the minimum size for Passive Integrated Transponder (PIT) tagging. The current paradigm of early life humpback chub ecology suggests that after hatching in the Little Colorado River, larval and juvenile humpback chub emigrate to the Colorado River at different life stages and times (Gorman and Stone, 1999). Hypotheses for the decline of humpback chub populations in Grand Canyon include (1) changes in habitat, including reductions in water temperature and sediment (Converse et al., 1998; Clarkson and Childs, 2000); (2) non-native fish interactions (Yard et al., 2011); and (3) non-native parasites (Hoffnagle et al., 2006). Given the importance of early life ecology on population persistence and that the survival of emigrating humpback chub is thought to differ with fish size and age, juvenile humpback chub are increasingly the focus of research efforts and management actions, including the removal of rainbow trout (Coggins et al., 2011; Yard et al., 2011), the experimental flow releases from Glen Canyon Dam and the translocations of juvenile humpback chub from the Little Colorado River to other tributaries within Grand Canyon (B. Healey, Grand Canyon National Park, Flagstaff, Arizona, personal communication). The goals of these management actions include establishing humpback chub populations within the Colorado River and establishing refuge populations in other tributaries. As it contains approximately 95% of the Grand Canyon population of humpback chub (Kaeding and Zimmerman, 1983; Coggins et al., 2006), the Little Colorado–Colorado River confluence region is of critical conservation importance for the persistence of humpback chub and the recovery of this unique species.

The other Grand Canyon humpback chub aggregations consist of small pockets of fish that are not known to spawn successfully (but see Andersen et al., 2010 and references therein), although the aggregations persist and are consistent in their presence and relative abundance across years and sampling events (Paukert et al., 2006). Of these aggregations, only one is found upstream of the Little Colorado River near a spring complex that flows directly into the Colorado River (km 48), whereas the remaining aggregations are located downstream of the Little Colorado confluence.

A key uncertainty in our knowledge of humpback chub ecology is related to the source and fates of juvenile humpback chub. Given the cold water and non-native predator densities in the main stem, the survival of larval and small juvenile humpback chub emigrating from the warmer, unregulated Little Colorado River is thought to be lower than later emigrants due to reduced growth and increased predation risk (Valdez and Ryel, 1997; Robinson et al., 1998; Robinson and Childs, 2001). However, recent assessments have shown an increasing trend in the Grand Canyon humpback chub population (Coggins et al., 2006; Coggins and Walters, 2009) and these increases could be from the increased production of humpback chub and or the increased survival of juvenile humpback chub. If so, these increases are solely coming from Little Colorado River–spawned fish or do other unknown spawning areas exist? Is the Grand Canyon population of humpback chub supported only by fish whose provenance is the Little Colorado River? If so, this would further emphasize the need for strong conservation of the Little Colorado River to reduce the risk of extinction due to catastrophic events within the Little Colorado River watershed.

In this study, we begin to address this uncertainty by characterizing and identifying site-specific water and humpback chub otolith chemistry markers in the Grand Canyon. We then use these otolith chemistry markers to identify humpback chub fish provenance and migration trajectories to inform our knowledge of the spatial ecology of juvenile and adult humpback chub. Multiple trace elements and carbon stable isotopes were evaluated as potential natural markers. These naturally occurring otolith “tags” (suites of incorporated trace elements and isotopes) are increasingly used to study fish migrations and provenance (Elsdon et al., 2008). Given that some trace metals and isotopes become incorporated in the calcium carbonate structure of the otolith in relation to their presence in ambient water, otoliths eliminate the need to artificially mark fish for future recaptures. Otolith tags also permit the analysis of larval and juvenile fish that are too small to bear artificial tags. Furthermore, fish otoliths are chronometric structures that are present before egg hatching and grow throughout the life of the fish.
such that analyses of otolith chemistry can provide lifetime trajectories of an individual fish’s movements, provided that these migrations include waters with different chemistries (Campana and Thorrold, 2001; Weidel et al., 2007). When chemistries are combined with traditional age and growth analysis, the two approaches may yield great insights into how specific habitats affect fish growth and ultimately survival.

METHODS

Humpback chub (n = 10) were obtained from collections maintained by the US Geological Survey (USGS) Grand Canyon Monitoring and Research Center (GCMRC) in Flagstaff, Arizona for otolith chemistry investigations. Given the endangered species status of humpback chub, specimen collection is highly regulated and all fish used in this study were incidental mortalities during research and monitoring activities of different cooperating agencies in Grand Canyon. Fish used in this study represent individuals collected from three of the known humpback chub aggregations in Grand Canyon. We chose these aggregations to represent a range of possible provenance and life history trajectories. In the Colorado River, fish were available from the 30-Mile Spring aggregation (~48 rkm), the Colorado River–Little Colorado River confluence aggregation (~100 rkm) and the final 12 km of the Little Colorado River aggregation (Table I; Figure 1). Of the ten fish included in this study, eight individuals in the collection were small and were presumed to be juveniles, whereas two individuals were larger adults or subadults (Kaeding and Zimmerman, 1983; Table I).

Lapillar otoliths were selected for otolith chemistry analyses and were removed via dissection and cleaned of any adhering organic matter by immersion in a dilute (10% v/v) bleach–water solution. Preparation of otoliths for microchemical analyses was adapted from Secor et al. (1991). A single otolith was randomly chosen for elemental analyses and was cast into rectangular molds using EpoFix (Struers) cold-set epoxy. Epoxy blocks were sectioned in the frontal plane through the core with a low-speed diamond saw (Buehler, IsoMet) and then polished using progressively finer grades of aluminium oxide lapping film until the otolith core was exposed, as determined by bright-field light microscopy. Polished otoliths were subsequently mounted on fused-quartz glass slides using cyanoacrylate adhesive (Loctite). Immediately before elemental analyses, all samples were ultrasonically cleaned in deionized water. Otoliths were photographed at magnifications of 200× to 630× and the daily rings were counted without prior knowledge of the identity (i.e. the ID code or collection location) of the fish. For the two larger fish, ages in years were determined, and daily rings deposited from hatching during the juvenile phase were enumerated until growth slowed so much that daily rings could no longer be discerned.

Table I. Collection dates, locations and lengths and ages at capture for humpback chub used in this study

<table>
<thead>
<tr>
<th>Fish no.</th>
<th>Collection date</th>
<th>Collection location</th>
<th>rkm</th>
<th>TL (mm)</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20 August 2009</td>
<td>COR</td>
<td>101</td>
<td>33</td>
<td>140 days</td>
</tr>
<tr>
<td>2</td>
<td>May 2003</td>
<td>LCR</td>
<td>1.1</td>
<td>37</td>
<td>1+ year</td>
</tr>
<tr>
<td>3</td>
<td>24 September 2006</td>
<td>COR</td>
<td>64.6</td>
<td>19</td>
<td>83 days</td>
</tr>
<tr>
<td>4</td>
<td>24 September 2006</td>
<td>COR</td>
<td>64.6</td>
<td>18</td>
<td>na</td>
</tr>
<tr>
<td>5</td>
<td>25 September 2006</td>
<td>COR</td>
<td>78.1</td>
<td>21</td>
<td>75</td>
</tr>
<tr>
<td>6</td>
<td>June 2010</td>
<td>LCR</td>
<td>1.6</td>
<td>27</td>
<td>33 days</td>
</tr>
<tr>
<td>7</td>
<td>June 2010</td>
<td>LCR</td>
<td>3</td>
<td>28</td>
<td>39 days</td>
</tr>
<tr>
<td>8</td>
<td>June 2010</td>
<td>LCR</td>
<td>12</td>
<td>25</td>
<td>40 days</td>
</tr>
<tr>
<td>9</td>
<td>20 July 2009</td>
<td>LCR</td>
<td>1.6</td>
<td>112</td>
<td>1+ year</td>
</tr>
<tr>
<td>10</td>
<td>7 October 2009</td>
<td>LCR</td>
<td>9</td>
<td>255</td>
<td>5+ years</td>
</tr>
</tbody>
</table>

COR, Colorado River; LCR, Little Colorado River; rkm, river kilometre (for COR, downstream of Lee’s Ferry, Arizona; for LCR, upstream of LCR–COR confluence); TL, total length.
Multiple otolith trace elemental concentrations were quantified using scanning X-ray fluorescence microscopy at the F3 Beamline Station at the Cornell High Energy Synchrotron Source. Scanning X-ray fluorescence is a spectral technique that uses high-energy X-rays to produce an elemental fluorescence spectrum. A double-bounce multilayer monochromator provides a 16.1-keV incident beam with 0.6% bandpass. A single-bounce glass capillary was used to focus the incident beam to a 20-μm (horizontal) by 10-μm (vertical) spot at the sample with a photon flux of approximately 10^{11} counts per second (Bilderback et al., 2003; Cornaby, 2008). Two-dimensional surface maps of elemental concentrations were created by stepping the beam across the entire surface of the sample in a sequential, non-overlapping grid pattern. At each step, the fluorescence spectrum was integrated for 3 s before moving to the adjacent sample location. Fluorescence X-rays were detected with a Vortex energy-dispersive silicon drift detector fitted with an aluminium foil attenuator to reduce high-intensity calcium fluorescence and increase sensitivity to trace elements. Initial spectral processing consisted of screening for a suite of 25 trace elements. However, only Se, Sr and Ca concentrations exhibited consistent variation between and within fish. Samples are reported as molar ratios to Ca (millimole element per mole Ca; Campana, 1999). Instrumental calibration was achieved using an in-house standard reference material consisting of ground otolith material of known trace elemental concentrations (Limburg et al., 2011). Data reduction and processing were completed using PyMCA (Solé et al., 2007) and in-house software developed at Cornell High Energy Synchrotron Source to produce two-dimensional elemental maps and spatially explicit numerical output. Numerical data were imported to a geographic information system to extract sequences (i.e. transects) of elemental concentrations from the two-dimensional maps (Quantum GIS Development Team, 2011). All elemental sequences extended from the otolith core to the otolith edge, parallel to the longest growth axis of the otolith.

Given that otolith and water chemistry are correlated for many trace elements and isotopic ratios, Sr/Ca, Se/Ca and carbon stable isotopic ratios (δ^{13}C)\textsuperscript{1} in both dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC) were quantified at multiple locations within the Grand Canyon during a 21-day research expedition in October 2009. In addition to water samples collected in the Colorado River, major tributaries were sampled to quantify the influence of tributary chemistry on the Colorado River chemistry and identify tributaries with unique water chemistries for the assessment of potential humpback chub spawning and rearing sites. Fifteen locations were sampled within the Colorado River at sites located immediately upstream and downstream of major tributary confluences. Additionally, samples were collected from eight major tributaries (Paria River, Little Colorado River, Nankoweap, Bright Angel, Shinumo, Tapeats, Havasu and Diamond creeks) near their confluence with the Colorado River and one spring that flows directly into the Colorado River was sampled (30-Mile Spring). At all sample locations, two separate samples were collected for quantifying trace elemental concentrations and δ^{13}C stable isotope ratios. For trace elemental analyses, 500 mL polyethylene bottles containing 3 mL of high-purity nitric acid (OPTIMA) were filled by submerging the bottle in the water (Eaton and Franson, 2005). Samples for dissolved inorganic and organic δ^{13}C isotopic ratios were filtered in the field using a syringe fitted with an in-line filter enclosure containing a glass microfiber filter (filter enclosure, Pall; filter, Whatman GF/F, 25 mm diameter). Filtered water was collected in 50 mL screw top centrifuge vials such that no bubbles or headspace were present or introduced when collecting sample. All samples were stored in darkness and on ice until completion of the sampling trip. Immediately following the conclusion of the research expedition, samples for DIC and DOC δ^{13}C isotopic ratio analyses were shipped to the University of California-Davis Stable Isotope facility for analysis using isotope ratio mass spectroscopy. Concentrations of trace elements were determined at State University of New York College of Environmental Science and Forestry using inductively coupled plasma optical emission spectrometry (ICP-OES) (Perkin-Elmer Optima 3300DV) and inductively coupled plasma mass spectrometry (ICP-MS) (Perkin Elmer Elan DRC-e 6100). Strontium and calcium were quantified using ICP-OES and Se was quantified using the ICP-MS outfitted with a dynamic reaction cell set to monitor 80Se. Instruments were calibrated using external standards and monitored for drift by analyzing quality control samples after every 10 unknown samples. For a run to pass quality controls, all quality control samples had to be within ±10% of known values. All water samples were above detection limits for all reported analytes. Multivariate trends in water chemistry were visualized using a principal component analysis of the correlation matrix.

In addition to analyses of trace elements in water and otoliths, in situ carbon stable isotope measurements of one juvenile humpback chub otolith were conducted using the CAMECA IMS 1280 secondary ion mass spectrometer instrument at the Northeast National Ion Microprobe Facility (Woods Hole, Massachusetts). This instrument is a large radius, double-focussing mass spectrometer fitted with an ion detection system consisting of two Faraday cups and a single electron multiplier. The secondary ion extraction system consisted of a Cs\textsuperscript{+} ion beam combined with a high-energy normal-incidence electron gun for charge compensation. Ions were extracted by rastering the beam over the

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1. The δ\textsuperscript{13}C denotes the ratio of rare and heavy (\textsuperscript{13}C) to abundant, light (\textsuperscript{12}C) isotopes, relative to the same ratio in a standard, in this case Pee Dee Belemnite (PDB). By convention the PDB δ\textsuperscript{13}C = 0.
RESULTS AND DISCUSSION

Spatial trends in water chemistries

Water chemistries of the Colorado River and tributaries in Grand Canyon were spatially and temporally heterogeneous for Sr/Ca, Se/Ca, DOC $\delta^{13}$C and DIC $\delta^{13}$C across sites and sample dates (Table II). Compared with the Colorado River, tributary water chemistry exhibited substantially more variability (Table II). Similarly, for all elemental and isotope ratios quantified, the range of values measured was substantially greater in the tributaries than those measured in the Colorado River (Table II). Inspection of vector loading plots from principal component analysis revealed that dimension 1 is positively correlated with DIC and DOC $\delta^{13}$C and dimension 2 is correlated with Sr/Ca and Se/Ca (Figure 2a). Overall, the first two principal components accounted for 84% of the variance in the data set (dimension 1 = 59%, dimension 2 = 25%; Figure 2a). The water chemistry of the Colorado River was similar throughout the entire Grand Canyon and tightly grouped in principal component analysis (PCA) biplots (Figures 1 and 2b). Lake Powell is a large reservoir and serves to homogenize the water chemistry at the beginning of the Grand Canyon reach of the Colorado River. The consistency of water chemistry throughout the Grand Canyon suggests that tributary inputs to the Colorado River are small and quickly diluted. With the possible exception of Paria River and Nankoweap Creek, all tributary multivariate water chemistry signatures were readily distinguished from the main stem Colorado River (Figures 1 and 2b). Comparing tributary water chemistry, Bright Angel, Tapeats and Shinumo creeks were similar but had lower trace element and isotopic elemental ratios compared with the Colorado River (Figures 1 and 2b). The Little Colorado River, Havasu Creek and 30-Mile Spring had water chemistries that were characterized by high $\delta^{13}$C values and low element to calcium ratios compared with the Colorado River chemistry (Figures 1 and 2b).

Table II. Water elemental and isotopic ratios for the Colorado River and tributary streams, October 2009

<table>
<thead>
<tr>
<th></th>
<th>Sr/Ca (mmol·mol⁻¹)</th>
<th>Se/Ca (mmol·mol⁻¹)</th>
<th>$\delta^{13}$DOC, ‰ (PDB)</th>
<th>$\delta^{13}$DIC, ‰ (PDB)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>COR</td>
<td>Trib</td>
<td>COR</td>
<td>Trib</td>
</tr>
<tr>
<td>Mean</td>
<td>4.7</td>
<td>2.4</td>
<td>22.7</td>
<td>16.4</td>
</tr>
<tr>
<td>SD</td>
<td>0.12</td>
<td>2.4</td>
<td>1.7</td>
<td>10.7</td>
</tr>
<tr>
<td>N</td>
<td>15</td>
<td>8</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>Min</td>
<td>4.3</td>
<td>0.5</td>
<td>19.7</td>
<td>6.7</td>
</tr>
<tr>
<td>Max</td>
<td>4.8</td>
<td>7.4</td>
<td>25.6</td>
<td>33.7</td>
</tr>
</tbody>
</table>

Tributaries include Paria and Little Colorado rivers and Nankoweap, Bright Angel, Shinumo, Tapeats, Havasu and Diamond creeks. COR, Colorado River; Trib, tributaries; Mean, mean water chemistry; SD, standard deviation of water chemistry; N, number of samples; Min, minimum value; Max, maximum value.

residency in these habitats. In this way, fish with markers that do not match the Little Colorado River can be identified as fish that were spawned or reared in a previously unknown location. This is of significant conservation interest because, if observed, it would suggest that spawning takes place outside of the Little Colorado River.

To validate our assumptions related to the incorporation of identifying water characteristics into the fish otoliths, the otolith trace elemental chemistry of three resident Little Colorado River humpback chub was quantified. Fishes 6 to 8 (Table I) were collected in the Little Colorado River as juveniles (~25 mm TL) upstream from the Little Colorado–Colorado River confluence (Figure 1). Given their small size and capture locations, it is likely that these fish originated in the Little Colorado River and are not recent immigrants. Trace element otolith chemistry transects between the otolith core and edge were characterized by constant Sr/Ca ratios, suggesting that water chemistry was temporally stable during the time period in which these fish inhabited the system (Figure 3, fishes 6–8). These otoliths provide a ‘known’ Sr/Ca signature for the Little Colorado River. Daily growth rings in these fish were very clear and large (Figure 4, top panels).

In addition to analysis of resident Little Colorado River humpback chub otoliths, we quantified Sr/Ca ratios in a 33-mm humpback chub collected in the Colorado River near the Little Colorado River confluence (Table I, fish 1). The otolith core to edge transects revealed that Sr/Ca ratios doubled between the otolith core (Sr/Ca = 0.5 mmol·mol⁻¹) and edge (Sr/Ca = 1 mmol·mol⁻¹; Figure 3, fish 1). Core Sr/Ca ratios in fish 1 were similar to the Sr/Ca ratios measured in the resident Little Colorado River fish (fishes 6–8), suggesting that this fish originated in the Little Colorado River. Given that water Se/Ca and Sr/Ca ratios are lower in the Little Colorado River than that in the Colorado River and that this fish was collected in the Colorado River, the higher Sr/Ca ratios at the otolith edge (i.e. portion of otolith formed immediately before capture) is consistent with a migration to the Colorado River (Figure 3, fish 1).

In addition to quantifying otolith trace element chemistries, fish 1 was also analyzed for carbon stable isotope ratios at its core and outer edge (Table I; Figure 3). In this case, Se/Ca and Sr/Ca transects measured in juvenile humpback chub otoliths using scanning X-ray fluorescence. Transects started at the otolith core and extended to otolith edge, parallel to the growth axes of otoliths. Arrows in pane 1 denote the locations of secondary ion mass spectrometer analyses depicted in Figure 5. See Table I for biological characteristics of fish.
fish, the $\delta^{13}C$ value measured at the otolith core and edge was approximately $-1\%$ (PDB) and $-13\%$ (PDB), respectively (Figure 5). The otolith core $\delta^{13}C$ is a close match for values measured in the Little Colorado River water, whereas the $\delta^{13}C$ values measured at the outer edge of the otolith are a close match for Colorado River water. The difference between otolith core and edge $\delta^{13}C$ in fish 1 is substantial ($-13\%$) and as such, we confirm the results from the trace elemental chemistry that this individual originated in the Little Colorado River and moved to the Colorado River where it was collected at an early age. Furthermore, outgassing of mantle-derived carbon dioxide with a high $\delta^{13}C$ value ($-5\%$) in the Blue Spring (Figure 1, 21 km upstream of the confluence of the Little Colorado River and the main stem) is well documented and supports the observed differences in otolith chemistry between otolith core and edge in fish 1 (Crossey et al., 2006, 2009). As such, this individual provides information concerning the characteristic otolith Sr/Ca signature of the Colorado River.

The age and growth history of fish 1 corresponded well with otolith chemistry. From hatching (estimated to be at the beginning of April), there were 37 large, distinct daily growth increments (through early May), followed by 59 very small increments (i.e. into early July), followed by a period of such slow growth that we could only estimate the number of growth rings as 40 to 45. This last period...
corresponds to the increase in Se/Ca and Sr/Ca and decline in δ
13C, confirming that summertime growth rate slows dramatically in the cold waters of the main stem Colorado River.

Fish 2 exhibited a complicated migration history based on otolith Sr/Ca ratios. The otolith Sr/Ca transect of fish 2 was similar to fish 1, suggesting that this fish originated in the Little Colorado River and migrated to the Colorado River (Figure 3). However, this individual (fish 2) was collected in the Little Colorado River approximately 1.1 km above the Little Colorado–Colorado River confluence, despite the Colorado River Sr/Ca ratio at the otolith edge. The mismatch between collection location and edge otolith chemistry may be explained by recent back-migration to the Little Colorado River such that its otolith chemistry had not equilibrated with the water at the collection location. This fish had more than 140 visible daily growth increments, and apparently overwintered in the Colorado main stem, and thus should be classified as a +1-year-old fish rather than a juvenile. We note its very small size (37 mm; Table I).

Three juvenile humpback chub were available that were collected in the Colorado River upstream of the Little Colorado River. Two of these fish (fishes 3 and 4) had core Sr/Ca chemistries that was similar to the resident Little Colorado River specimens (fishes 6, 7 and 8) but also had increasing Sr/Ca ratios between the otolith core and edge, similar to fish 1, which migrated from the Little Colorado River to the Colorado River. Taken at face value, this suggests that fishes 3 and 4 originated from the Little Colorado River and moved to the Colorado River (Figure 3). However, fishes 3 and 4 were collected in the Colorado River at rkm 64.6 (~38 km upstream of the Little Colorado River–Colorado River confluence) and were very small (19 and 18 mm TL, respectively; Table I). Although it cannot be ruled out, it is unlikely that fishes 3 and 4 originated in the Little Colorado River as the Sr/Ca chemistry suggests and moved more than 35 km upstream to the collection location as approximately 20 mm TL individuals. The 30-Mile Spring region (rkm ~48) of the Colorado River contains a number of warm springs associated with Fence Fault where larval humpback chubs have been observed and thought to have been spawned (Valdez and Masslich, 1999; Andersen et al., 2010). Mantle-derived groundwater feeds both the 30-Mile Spring complex and the Blue Spring in the Little Colorado River and as such, the water chemistry of 30-Mile Spring is similar to the Blue Spring and both spring complexes are outlets for warm water with high carbon dioxide loads (Crossey et al., 2006, 2009). Although our analyses of Little Colorado River and 30-Mile Spring water chemistry suggest that fish residing in these locations should result in unique otolith chemistry signatures, we sampled only one spring in the area. It is possible that an unknown spring (possibly subaqueous within the Colorado River) in the area may have indistinguishable water chemistry from the Little Colorado River and support limited humpback chub spawning. Additional analyses of fish and water samples collected from 30-Mile Spring are needed to differentiate these locations with confidence (Figure 2). Therefore, it is plausible that fishes 3 and 4 may have originated in the 30-Mile Spring area and subsequently drifted downstream before capture (Figure 1).

Fishes 3 and 4 were collected as approximately 20 mm individuals in September 2006 in the main stem Colorado River. Such small sizes in the Little Colorado would correspond to fish of approximately 1 month in age; however, fish 3 had 83 daily growth increments (Figure 4, lower panels; fish 4 was slightly overpolished and could not be aged). Fish 3 was therefore hatched in mid-June 2006, later than the primary humpback chub spawning period of March to May. Fish 3 had approximately 2 weeks of initially high growth, followed by a rapid transition to very slow, even growth (Figure 4, lower panel inset). Thus, we interpret fish 3 (and fish 4) as having been reared in a nursery area in the 30-Mile Spring complex, after which they were advected into the main stem and survived, albeit with very slow rates of growth.

Although the otolith core Sr/Ca chemistry and capture location of fishes 3 and 4 suggest a complex migration trajectory, the edge Sr/Ca ratios of these otoliths were similar to that on the otolith edge of fish 1, suggesting that these individuals spent sufficient time in the Colorado River to obtain a Colorado River otolith signature, where they were collected (Figure 3). Again, this corresponds well with the growth history (Figure 4).

The otolith chemistry of fish 5, collected in the Colorado River upstream of the Little Colorado River, had a unique Sr/Ca chemistry compared with all other fish in this study. As with fishes 3 and 4, this individual was collected downstream (rkm ~78) of the 30-Mile Spring as a 21-mm TL individual in September 2006 and was aged at 75 days (Table I). The Sr/Ca transect between the otolith core and edge of this fish was characterized by a constant, low Sr/Ca ratio (Figure 3). In fact, the constant chemistry between the otolith core and edge and the fact that its chemistry was lower than any other fish in the study suggest that this fish was not spawned in the Little Colorado River. The low otolith chemistry of this fish does not match the typical main stem Colorado River chemistry signature that all other fish analyzed to date have incorporated into their otoliths. Given our knowledge of water chemistry in the system, this fish likely spawned in a previously unknown site within the Colorado River and exhibited high site fidelity to this location. However, fish 5’s daily growth increments were very narrow, suggesting cooler water conditions in the unknown nursery habitat as compared with the Little Colorado or 30-Mile Spring nurseries.

In contrast to the consistent patterns in Sr/Ca observed in the otolith core–edge transects, Se/Ca transects were
inconsistent for seven of eight juvenile humpback chub examined (Figure 3). Otolith Se/Ca ratios in fish 1 increased between the otolith core and edge from approximately 0.002 to 0.0035 mmol-mol$^{-1}$. On the basis of water chemistry, Sr/Ca ratios and $\delta^{13}$C, the observed increase of Se/Ca ratios between the otolith core and edge in fish 1 is consistent with a migration between the Little Colorado River and the Colorado River. Within an individual, the Se/Ca ratios of fishes 2 to 8 varied between otolith core and edge but transect trends were not consistent across individuals with similar collection locations and migratory histories as identified using otolith Sr/Ca, otolith $\delta^{13}$C or water chemistry. We have observed more variability in aqueous Se/Ca during flooding events (Hayden et al., in prep.), which may partly explain the variability. Additionally, physiological interactions of Se with metals such as mercury could potentially affect Se uptake (Lochet et al., 2010). Mercury concentrations were not quantified in this study and as such, the role of mercury remains unknown in terms of affecting Se/Ca in these juvenile otoliths.

**Larger humpback chub otolith chemistry**

Fishes 9 and 10 were larger humpback chub and their otolith chemistry transects exhibited multiple Sr/Ca and Se/Ca peaks between the otolith core and edge (Figure 6). In both fish, the maximum Sr/Ca ratio observed in the transect was located approximately 40% to 60% of the distance between the core and edge with subsequent Sr/Ca peaks decreasing toward the otolith edge (Figure 6). Although the maximum Sr/Ca ratios in fishes 9 and 10 are higher than observed in fishes 1 to 8, this may represent interannual variation in site-specific otolith chemistry owing to basin-wide processes such as Glen Canyon Dam releases or discharge within the Little Colorado River. Temporal shifts in otolith chemistry are well documented when comparing site-specific otolith chemistries across multiple years (Gillanders, 2002). Given our observations of high Sr/Ca ratios with Colorado River residency and low Sr/Ca with Little Colorado River residency, the multiple peaks in the adult humpback chub chemistry between otolith core and edge likely represent multiple fish movements between the Little Colorado River and the Colorado River (Figure 6).

Otolith Se/Ca ratios also increased between otolith core and edge in the adult humpback chub. In the largest adult fish included in the study (fish 10, TL = 255 mm), Se/Ca peaks were positively correlated with Sr/Ca, as expected by water chemistry analyses (Figure 6). In fish 9, the correlation between otolith Sr/Ca and Se/Ca was observed but was not as strong.

Fish 9 was more than 1 year old and fish 10 was more than 5 years old (Figure 7). Otolith daily increments deposited

![Figure 6. Otolith Se/Ca and Sr/Ca transects of larger humpback chub using scanning X-ray fluorescence. Transects started at the otolith core and extended to the otolith edge, parallel to the longest growth axis of the otolith. See Table I for biological characteristics.](image-url)
during the first growing season were visible out to 138 and 176 days, respectively. We note the stark contrast between fish 9 (112 mm) and fish 2. The latter fish, originally thought to be a 37-mm juvenile but with more than 140 visible daily increments and a capture date of May, is likely either a more than 1-year-old fish spawned in the spring or a fall-spawned fish. Further analysis will reveal whether or not such small fish actually recruit to the adult population.

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, strong discrimination of the Little Colorado River from the Colorado River by carbon stable isotopes measured in water was observed in otolith chemistry and provides a distinct marker for fish movements between these two systems. Furthermore, results of water sampling efforts throughout the Grand Canyon suggest that the carbon stable isotope ratios in the Little Colorado are unique, although several tributaries (e.g. Havasu Creek) and 30-Mile Spring show some similarity (Limburg et al., in prep.). Although more subtle, differences in otolith trace elements, specifically Sr/Ca ratios, can also resolve fish movements between the Colorado River and the Little Colorado River. Our results suggest that incorporation of Se/Ca in otoliths may be influenced by hydrological events (and perhaps by concentrations of other elements such as mercury) and thus may not be a reliable natural marker, although further study is needed.

In this system, otolith chemistry may be an effective tool for identifying fish migration trajectories, source-sink dynamics, provenance or dispersal. We have shown that linking otolith chemistry and microstructure provides important information concerning the timing of fish movements and resulting growth patterns that may be important for future management of the Grand Canyon humpback chub population.

On the basis of our results, are we able to resolve any of the uncertainties related to movement dynamics of juvenile native fish? The fish used in this study are not random samples from the population but instead, as incidental mortalities from ongoing monitoring, represent available samples of juvenile humpback chub from three of the known
aggregations of humpback chub within the Grand Canyon population. Thus, absolute estimates of spawning contributions from different areas in the Grand Canyon are not possible at this time. However, our results do confirm the current spawning ecology paradigm for this species by highlighting the importance of the Little Colorado River as the primary spawning location. Our results also suggest that other spawning may be occurring in the main stem in the 30-Mile Spring region and that some larvae that spawn in this region may persist in the main stem Colorado River beyond the immediate 30-Mile Spring area. A larger random sample of humpback chub juveniles across multiple sizes classes and different spatial locations would be required to further assign the probabilities of these different natal origin and rearing life history types and, ultimately, what proportion of the adult recruits use, and especially spawn, in this area outside of the known Little Colorado River spawning aggregation.

Our most significant result is the documentation of otolith microchemistry methods to delineate the natal origins of juvenile humpback chub in Grand Canyon. This is a key result going forward; humpback chub populations increased during the first decade of the 2000s (Coggins et al., 2006; Coggins and Walters, 2009), but the mechanisms for this increase are unknown. Increases in population size can result from either increase in birth rate or decreases in death rate. Understanding which of these is occurring in Grand Canyon is critical for determining what management actions are necessary to aid in the recovery of the species. Recent management actions have focused on the removal of non-native predators (primarily rainbow trout Oncorhynchus mykiss) in an effort to reduce mortality on juvenile humpback chub (Coggins et al., 2011; Yard et al., 2011). Although humpback chub populations have increased during this period of reduced predator abundance, apparently due to increases in recruitment of juveniles (Coggins and Walters, 2009), whether this was related to the removal of non-native fish or other factors influencing recruitment is still uncertain (Coggins et al., 2011; Yard et al., 2011). Because of the recognized need to conserve and enhance native fish populations in the main stem Colorado River, understanding the source or sources of these recruits is important to understanding the overall population dynamics of humpback chub in Grand Canyon. If the Little Colorado River continues to be the only suitable spawning location (in terms of recruitment success), then the recovery of humpback chub in Grand Canyon will be limited to the spawning and rearing carrying capacity of the Little Colorado River—further highlighting the critical need for conservation to protect the Little Colorado River as a critical humpback chub habitat to ensure long-term viability of this species in Grand Canyon. With a larger sample of juvenile humpback chub that reflects spatial and temporal considerations, the increased statistical power should make these questions amenable to answers.

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