Summer 7-12-2017

Evaluating the Relationship of Temperature and Growth of a Larval Colorado River Catostomid, C. latipinnis, Through Otolith Aging and Stable Isotopes (δ18O)

Adam Lawrence Barkalow
University of New Mexico

Adam Lawrence Barkalow

Candidate

Biology

Department

This thesis is approved, and it is acceptable in quality and form for publication:

Approved by the Thesis Committee:

Dr. Thomas F. Turner, Chairperson

Dr. Seth D. Newsome

Dr. Nicu-Viorel Atudorei

Dr. Mark C. McKinstry

EVALUATING THE RELATIONSHIP OF TEMPERATURE AND GROWTH OF A LARVAL COLORADO RIVER CATOSTOMID, *C. LATIPINNIS*, THROUGH OTOLITH AGING AND STABLE ISOTOPES ($\delta^{18}O$)

by

ADAM LAWRENCE BARKALOW

B.S., BIOLOGY, UNIVERSITY OF NEW MEXICO, 2008

THESIS

Submitted in Partial Fulfillment of the
Requirements for the Degree of

Master of Science

Biology

The University of New Mexico
Albuquerque, New Mexico

July 2017

ACKNOWLEDGEMENTS

I would like to thank many people who have had great influence on both my life and the work presented within this thesis. This list is however terribly insufficient and doesn't appropriately reflect the overwhelming gratitude that I have for those who have helped me complete this chapter of my life. First and foremost, I would like to thank my incredible wife, Stephani L. Clark Barkalow, for her emotional and intellectual support. Similarly, I would like to thank my friends and family for helping me make the journey through life's many adventures that have led me to this point.

This work would not have been possible if it weren't for the support from my fish family at American Southwest Ichthyological Researchers and the Museum of Southwestern Biology at the University of New Mexico; their financial, intellectual, and often emotional support were above and beyond what any researcher could hope for. I will forever be grateful the opportunities that you've provided and encouraged. Thank you Steven Platania for always encouraging me to do more with myself and always setting a high standard for research.

There were numerous people who assisted with various aspects of this work including fieldwork, sample acquisition, data analysis, and careful and thoughtful edits. Thank you for your contributions. Foremost I would like to thank the members of my committee Dr. Thomas F. Turner, Dr. Seth D. Newsome, Dr. Nicu-Viorel Atudorei, and Dr. Mark C. McKinstry. In addition I would like to thank contributing members from National Park Service, BIO-WEST, United States Geological Survey's Grand Canyon Monitoring and Research Center, CEIBA adventures, UNM Center for Stable Isotopes, and Near Shore Ecology Group (University of Florida).

EVALUATING THE RELATIONSHIP OF TEMPERATURE AND GROWTH OF LARVAL COLORADO RIVER CATOSTOMID, *C. LATIPINNIS*, THROUGH OTOLITH AGING AND STABLE ISOTOPES ($\delta^{18}O$)

by

Adam Lawrence Barkalow

B.S., Biology, University of New Mexico, 2008

M.S., Biology, University of New Mexico, 2017

ABSTRACT

Knowledge of early life history strategies and ecological dynamics of larval fish growth and development is invaluable for effectively managing and conserving common and endangered fish species. Isotopic analysis of otoliths (bony structures of the inner ear) from larval Flannelmouth Suckers *Catostomus latipinnis* obtained from the Colorado River in Grand Canyon could greatly facilitate understanding of thermally-regulated growth rates, thermal preferences, and ontogenetic habitat use by these fishes. Colorado River water temperatures in the Grand Canyon are highly modified from projected historic water temperatures present before closure Glen Canyon Dam. Cold water as result of Glen Canyon dam and hypolimnetic releases from Lake Powell are predicted to slow growth and development of ectothermic fish larvae unless young fish can occupy warmer aquatic microhabitats (i.e., channel margins or shallow backwaters) within the river. Isotope ratio mass spectrometry (IRMS) is a technique that reveals integral aspects of an individuals' life history that are often difficult to infer with traditional sampling

methods. I developed and evaluated IRMS of oxygen isotopes ($\delta^{18}O$) of larval fish otoliths as a means to reconstruct water temperatures experienced by fish during early developmental phases. Stable isotope analysis of larval fish otoliths allowed for confident determination of relative water temperatures experienced by individual larvae and the potential for larvae to influence the temperatures they experience through active transport. Water temperatures experienced by larval *C. latipinnis* closely mirrored the seasonal temperature variation and on average were slightly warmer ≅ 2°C than the temperatures present in the mainstem river. Ultimately, this technique, while not completely tested, may provide a better understanding of potential limiting factors for other catostomids. Further, this research has identified potential impacts to the interpretation of growth rates of wild caught catostomid larvae spawned in cold-water temperatures. This unforeseen result may indicate an adaptive response to colder temperature by changes in resource provisioning to eggs and changes in early developmental ontogeny.

TABLE OF CONTENTS

List of Tables

List of Figures

Introduction

The Colorado River basin stretches across 7 states and 2 countries in western North America. The basin drains 629,100 km^2 from Wyoming to its historical terminus in the Sea of Cortez. The Colorado River basin has a rich ichthyofaunal legacy. Over the last 100 years, construction of over a dozen major dams (Clarkson and Childs 2000) have negatively affected native fish species (Miller 1961; Holden and Stalnaker 1975; Dowling et al. 1996; Webb et al. 1999; Bezzerides and Bestgen 2002). Dams were constructed for a variety of reasons including flood control, irrigation, and hydroelectric power generation. Dams impact aquatic environments by moderating discharge, reducing sediment input, armoring river bottoms, altering the food web, and lowering and stabilizing water temperatures through hypolimnial water releases (Ligon et al. 1995; Clarkson and Childs 2000).

The Colorado River within the Grand Canyon provides an impressive example of downstream effects of hydroelectric dams and hydrologic alteration to the riverine system. Impacts include dramatically altered water temperatures, cessation of a natural flow regime, reduction in suspended sediment, and degradation of a the aquatic food web (Clarkson and Childs 2000; Cross et al. 2013; Kennedy et al. 2016).

Altered sediment budgets and dam operations reduce habitat for larval fishes and invertebrates (Korman et al. 2004; Yarnell et al. 2015; Kennedy et al. 2016). Because access to warm water habitats such as wetlands and backwaters is thought to be an important component in development of larval and juvenile fish it is important to assess what impacts the decreased temperatures of the modified Colorado River may have on

larval fish development (Modde et al. 2001; Hedrick et al. 2009; Bestgen et al. 2011). The combined effects of altered flow regime, habitat loss, and blockage of nutrient input from upstream have severely impacted aquatic food resources for native fishes which may be limited by the abundance of aquatic invertebrates (Cross et al. 2013).

The relationship between growth rate of larval fishes and temperature is generally well documented (McCormick 1977; Houde 1989). Decreased temperatures resulted in lower growth rates of Razorback Suckers in the laboratory (Bestgen 2008). Fishes are ectotherms, meaning that their body temperature and corresponding metabolic rates are influenced by environmental temperatures, thus, lower water temperatures yield decreased growth rates and extended larval period (O'Connor et al. 2007). Cooler water temperatures of the Colorado River below Glen Canyon Dam could limit larval sucker recruitment through increased mortality that results from increased time spent at small size and in vulnerable life stages. Access to food resources could also be reduced through reduced swimming and feeding performance of small and poorly developed larvae (Govoni et al. 1986; Ward et al. 2002).

The Colorado River within the Grand Canyon is bounded by Lake Powell upstream and Lake Mead downstream. Historical ichthyofaunal records in the lower Grand Canyon reach recorded numerous endemic species many with distinct physiological attributes (Minckley 1991). Three co-occurring catostomid species are among the eight fish species native to Grand Canyon National Park. Flannelmouth Sucker *Catostomus latipinnis,* Bluehead Sucker *Catostomus discobolus,* and Razorback Sucker *Xyrauchen texanus* appear in historic and contemporary records (Minckley 1991; Kegeries et al. 2016). All three catostomids have are thought to have similar life history

strategies, spawning ecologies, and were all thought to be historically abundant within the Colorado River Basin (Minckley 1991; Bezzerides and Bestgen 2002; USFWS 2002).

Both *X. texanus* and *C. latipinnis* share many similarities in life history and reproductive ecology. Both are known to migrate upstream prior to spawning (USFWS 2002; Carman 2007). Both species have similar spawning requirements and spawn in groups, typically over gravel beds in both riverine and lacustrine habitats (USFWS 2002; Carman 2007). Eggs are adhesive and demersal and settle into the gravel beds to incubate. *Xyrauchen texanus* and *C. latipinnnis* spawn in the spring in water temperatures as low as 6°C (Bozek et al. 1990; Carman 2007), although more often both species are reported to spawn in water temperatures exceeding 10°C, which is considered the lower thermal threshold for successful egg incubation (Bozek et al. 1990). Both species are omnivorous and their diet consists of algae, detritus, and benthic invertebrates (USFWS 2002; Carman 2007). Life-history similarities notwithstanding, subsequent to construction of mega dams along the Colorado River *X. texanus* became increasingly rare throughout the basin while *C. latipinnis* remained comparatively common. Despite detection of successful spawning in remaining populations of *X. texanus* there was little to no evidence of successful recruitment which eventually warranted the U.S. Fish and Wildlife Service to list *X. texanus* as federally endangered in 1991 under the Endangered Species Act.

There is no obvious single reason for low recruitment *of X. texanus* , but prevailing hypotheses include predation of eggs, larvae, and young fish (from non-native fish predators), contaminants, reduced water temperatures, limited food availability, and habitat loss (Marsh and Minckley 1989; Papoulias and Minckley 1990).

Managers and researchers have been attempting to determine reasons for the decline of Razorback Sucker while simultaneously trying to sustain and restore remaining populations. Conservation and management efforts include stocking programs, removal of exotic species, habitat restoration, seasonally timed and experimentally operated flows to mimic natural conditions, and implementation of water temperature control devices and fish passage. Conservation efforts are conducted on a basin-wide scale and implemented through the Upper Colorado River Basin Recovery Implementation Program, San Juan River Recovery Implementation Program, Glen Canyon Adaptive Management Program, and the Lower Colorado River Multi-Species Conservation Program that are mandated by the U.S. Congress. Despite these efforts, Razorback Sucker recruitment from larval to juvenile and adult life stages is limited and observed in only a few locations throughout the Colorado River basin (Papoulias and Minckley 1990; Albrecht et al. 2010). In rivers similar to the Colorado river, where successful recruitment of Razorback Suckers has been observed, off channel habitats are warm, food-rich environments, that appear to increase growth rates and survival of Razorback Sucker larvae (Bestgen et al. 2011; Sabo et al. 2012). Hypolimnetic releases from Lake Powell and Glen Canyon Dam have stabilized and significantly lowered mainstem maximum water temperatures (Stevens et al. 1997). Lower water temperatures reduce swimming performance, hatching success, and are likely a limiting factor for development, growth, and recruitment of Colorado River catostomid larvae (Marsh 1985; Ward et al. 2002).

Until recently (2014), *X. texanus* was absent from contemporary ichthyofaunal surveys and considered to be functionally extirpated from the Colorado River in the Grand Canyon (Bunch et al. 2012; Kegerries et al. 2016). Though *X. texanus* is present in

the river and there is some evidence of spawning, to date there is no evidence of recruitment to later life stages (e.g. sub-adult and adult). In contrast, *C. latipinnis* is prominent in the Colorado River within the Grand Canyon. Due to the similarities in life-history, prevalence in the river, and abundance of specimens available, *C. latipinnis* is a good proxy for *X. texanus* for elucidating potential impacts of temperature on larval fish growth in the Grand Canyon.

Thermal conditions experienced by wild catostomid larvae and the effects of environmental temperatures on growth rate and survival are difficult to study in nature. Although larval fishes are capable of short bursts of movement (active transport), passive transport (drift) is the main mode of movement for many big-river larval fishes (Carter et al. 1985; Dudley and Platania 2007) and largely determines habitats and water temperatures experienced by larvae. Active transport determines the time spent and position in a nursery habitat and also potentially affects the thermal environment a larval fish experiences. Traditional sampling methods for larval riverine fishes are only capable of substantiating that a larval fish collected anywhere in the system likely originated from that location or upstream of that position. Movement of larvae by drift in combination with larval use of thermally distinct shoreline associated microhabitats makes determining the thermal conditions experienced by larvae in a riverine system nearly impossible; however, oxygen isotope analysis of a larva's otoliths may be used to elucidate key insight into the thermal conditions experienced (Kitagawa et al. 2013).

Dendrochronological and micro-chemical analyses of otoliths have been used to determine growth rates and temperatures experienced by fishes over their lives (Edmonds et al. 1991; Campana 1999; Guiguer et al. 2003; Zeigler and Whitledge 2011; Matta et al.

2013). Otoliths are accretionary hard structures comprised of biogenic calcium carbonate within the inner ear of a fish formed at the earliest stages of larval development. Otoliths grow continuously throughout the life of the fish and, during growth, otoliths form daily and annual growth rings called annuli that can be used to determine age in days or years (Panella 1971; Campana 2005). Otoliths are present when a catostomid larvae hatches, and annuli are accreted at a rate of one per day, allowing for reliable age estimates of even the earliest stages (Bundy and Bestgen 2001). In addition to a temporal record, otoliths accrete elements from the environment in which the fish was living when the otolith was formed, so otoliths also serve as a geochemical record (Elsdon et al. 2008). Otoliths are acellular, thus material and isotopes that are accreted in the annuli remain permanent fixtures in the structures. Recent advances in isotopic and elemental analysis have led to increased awareness and use of microchemical analysis of otolith material to study fish life history (Campana 2005; Elsdon et al. 2008).

As an otolith grows, some elements and their isotopes from the environment are incorporated into the $CaCO_3$ matrix of the otolith, thus creating a permanent record of the fish's life history (Campana 1999). Analysis of elements and isotopic ratios of otolith material have been used to elucidate natal origin, stock differentiation, migration, diet, and thermal history (Edmonds et al. 1991; Campana 1999; Guiguer et al. 2003; Zeigler and Whitledge 2011; Matta et al. 2013). Thermal history of environments can be determined through analysis of oxygen isotopes in the calcium carbonate structure of materials such as ice cores, carbonate rocks, shells, and otoliths, and is often referred to as paleothermometry (Patterson et al. 1993; Fry 2006). Because the record of a larva's thermal history is difficult to infer with traditional sampling methods, otolith stable

isotope analysis (SIA) may be used and enables determination of average water temperature experienced by catostomid larvae within the Grand Canyon. Reconstruction of water temperatures experienced by freshwater riverine fishes by otolith oxygen isotope analysis is uncommon and is unknown for larval riverine fishes.

There are three stable isotopes of oxygen, O^{16}, the most abundant form of oxygen on earth (99.76%); O^{18}, far less abundant (0.20%); and O^{17}, which is extremely rare (< 0.04%, Fry 2006). The relative abundance of isotopes in a sample is calculated as a ratio of the rare isotope to the most abundant form, divided by a standard. This is denoted with the Greek letter δ and reported as per mil (i.e., ‰). Determination of temperatures experienced by a fish is possible because oxygen isotopes are incorporated into otoliths at near equilibrium with the isotopic composition of the surrounding water; (Degens et al. 1969; Campana 1999; Guiguer et al. 2003). However, values of $\delta^{18}O$ measured from otolith material differ from $\delta^{18}O$ of environmental water due to temperature-dependent fractionation of oxygen isotopes in the formation of otoliths (composed of the biogenic calcium carbonate crystalline material known as aragonite). At cooler temperatures more O^{18} is incorporated in the crystalline structure due to its greater bonding affinity in colder temperatures. (Epstein et al. 1953; Grossman and Ku 1986; Guiguer et al. 2003).

The use of oxygen isotope ratios from otoliths to interpret water temperature was developed in marine and estuarine environments (Devereux 1967), and has more recently been applied in freshwater systems (Guiguer et al. 2003). In a large volume well-mixed system (e.g., oceans) the $\delta^{18}O$ of seawater (H_2O^{18}) is well mixed and consistent over time. However, freshwater systems are subject to periodic fluctuations in isotope abundance as a result of inputs from precipitation, ground water, and snowmelt (Kendall

and Coplen 2001). Moreover, the baseline amount of O^{18} in freshwater systems varies by geographic location; as water masses move further from the coast, more of the heavier isotopes are precipitated out preferentially leaving behind higher concentrations of the lighter isotope O^{16} (Kendall and Coplen 2001). Because $\delta^{18}O$ values can vary in freshwater systems it is important to understand and anticipate how the system of interest is affected, and to know the contributing sources that affect $\delta^{18}O$ of the water. In the Grand Canyon, the $\delta^{18}O$ of the Colorado River is relatively consistent because of regularly-timed hypolimnial releases for hydropower production at Glen Canyon Dam. While the Grand Canyon has numerous tributaries that vary in baseline values of $\delta^{18}O$, these contribute relatively small amounts to the overall water budget and thus are unlikely to affect average values of $\delta^{18}O$ ratios of mainstem Colorado River water within the Grand Canyon.

Specific research objectives:

- Establish a method of obtaining $\delta^{18}O$ values from individual larval catostomid otoliths.
- Use $\delta^{18}O$ values to determine the average water temperatures experienced by *C. latipinnis* collected within the Grand Canyon in 2015 and use multiple linear regression analysis to assess the role of contributing environmental and temporal variables.
- Compare experienced temperatures with mean environment temperatures in the river and determine and use multiple linear regression analysis to assess the role of contributing environmental and temporal variables.
- Analyze the effect that average temperature experienced has on fish growth rates during early life stages, as measured by otolith microstructure analysis of individual larvae.

Methods

Field collection

A fine mesh seine (1m x 1m x 0.8mm) was used to collect age-0 catostomids in zero to low velocity habitats from the Colorado River within the lower Grand Canyon. Collection was conducted from river kilometer (RK) 289 to 449 during survey trips that were conducted to monitor Razorback Sucker spawning and recruitment in the Grand Canyon (Kegerries et al. 2016) (Figure 1). These trips occurred once per month (March – September) for the purpose of monitoring spawning and recruitment of Razorback Sucker in the Grand Canyon. Sampling for this project occurred from March to July, the period when wild catostomids are spawning and larvae were present. During each trip, larval fishes were collected from two to three low-velocity habitats approximately every 20 RK, equaling a maximum of 8 samples collected over 160 RK. Sampling sites were selected based on longitudinal position and the amount of low velocity habitat, which is considered high quality habitat for larvae and can be efficiently sampled. All age-0 fish collected were preserved in a 95% solution of ethanol and placed into a Whirl-Pak® with a field tag containing an alpha-numeric code (field number) and habitat code. Preservation of all fishes was necessitated because the small size of larval fishes precludes field identification. To ensure useful otoliths, it was necessary to preserve fishes in 95% EtOH as this solution maintains the crystalline structure of otoliths. Formalin, a common preservative, is acidic and erodes the calcium carbonate matrix of otoliths and was not used. Each sampling locality represents one collection. At each sampling location at least one digital photograph of the sampled habitat was taken and water temperature, salinity, conductivity, pH, and secchi disk measurements were

recorded.

Water samples for oxygen isotope analysis were collected during each sampling trip; however, water samples from March and April 2015 were compromised. Samples were collected mid channel at or near the RK where fishes were sampled. All water samples were collected with a large syringe and filtered with a sterile 0.45µm Whatman™ glass micro filter. Water samples were stored in a labeled acid washed plastic screw top vial or Whirl-Pak® and maintained in a cool and dark location. Water samples were transported to the University of New Mexico and analyzed at the Center for Stables Isotopes. Additional $\delta^{18}O$, collected from Colorado River tributaries between 2009 and 2012 were obtained with permission from Nearshore Ecology Final Report (Pine, W.E, unpublished data).

Temperature and discharge (*Q)* data were obtained from United States Geological Survey (USGS) stream gages (09380000 - Colorado River at Lee's Ferry, AZ, 09402300 - Little Colorado River above the mouth near Desert View, AZ, 09402500 - Colorado River near Grand Canyon, AZ , 09403000 - Bright Angel Creek near Grand Canyon, AZ, 09404115 - Havasu Creek above the mouth near Supai, AZ , 09404120 - Colorado River above National Canyon near Supai AZ, 09404220 – Colorado River above Spencer Canyon at river mile 246). Daily mean temperature was calculated for both water temperature and discharge data.

Laboratory – collections

At MSB, each sample was cleaned to remove debris and sediment, placed into labeled glass museum quality jars filled with fresh 95% EtOH. All specimens were identified to species by trained experts under polarized light and stereoscope

magnification with the use of larval fish guides and interactive computer based keys (Snyder 1981; Snyder and Muth 2004).

Once specimens were identified and separated by ontogenetic stage, larvae were randomly selected from collections of *C. latipinnis* that had large numbers of individuals from each monthly sampling (March – July). Because obtaining oxygen isotope ratios from larval otoliths is both novel and destructive, preference was given to the mesolarval ontogenetic stage because it was the most abundant in the samples. Using polarized light and a stereoscope with low-power magnification (10-40X) all six otoliths were dissected from selected larvae. Larval forceps were used for stabilization of the specimen and a 0.3 mm stainless steel BioQuip™ insect pin and vise were used for removal and transfer of otoliths. The left sagitta and lapillus otoliths were mounted on a labeled glass microscope slide microscope slide with Crystalbond™, and covered with a coverslip for use in age determination. Remaining sagitta and lapillus otoliths were placed into the individual sample holder holes of an aluminum ablation sample holder. Asteriscii, if present, were omitted from isotope and aging analysis because they form post hatch and are often comprised of polymorphic crystalline structures (Tomas and Geffen 2003; Hoff et al. 2016).

Otolith aging

Otoliths were aged under high magnification (500-1000X) by at least two readers. Each reader determined age by counting the number of visible daily annuli. This step was repeated by the other reader, and scores compared. If consensus was met (+/- 1 annulus) the age in days was recorded. If the independent ages were not within one annulus (1 day) the procedure was repeated by both readers. If ages were still not within one day, no

age was recorded and that sample was removed from analysis.

Growth rate calculation

Daily growth rates for individual larvae were calculated using Equation 1; the numerator is standard length at hatch is subtracted from the standard length at capture, the denominator is daily annuli revealed by age determination. Length at hatch for *C. latipinnis* is reported in a range from 10 to 11 mm standard length (Snyder and Muth, 2004); for this study 10.5 mm SL was used.

Equation 1

$$Growth\ rate = \frac{Length\ at\ capture\ (SL) - Length\ at\ hatch\ (SL)}{days\ old\ post\ hatch\ (annuli)}$$

Isotope analysis

Oxygen isotope ratio of otoliths were obtained by laser based gas chromatography isotope ratio monitoring mass spectrometry (GC-IRMMS) which allows for in-situ rapid analysis of small samples and spatial resolutions that would otherwise be infeasible using traditional extraction methods (Cerling and Sharp 1996; Sharp and Cerling 1996). For isotopic ($\delta^{18}O$) analysis, a "sample" consisted of either a sagitta or lapillus otolith and sometimes both from a single individual placed into the well of a custom sample holder. The sample holder is machined from aluminum barstock with final dimensions 12.8 mm diameter by 6.4 mm height. A total of 20 sample wells were CNC machined into one face of the sample holder with a total five rows each containing four sample wells arranged in a star pattern. Each well is approximately 1.0 mm deep by minimum diameter of 0.4 mm and maximum diameter of 0.7mm. The countersunk wells were designed to allow for line of sight imaging and target acquisition while simultaneously preventing the otoliths from

ejecting from the sample holder before being completely combusted by the laser. During sample analysis, the sample holder, containing a maximum of 20 samples, was placed into a sample chamber along with a small slab of Solnhofen limestone. Solnhofen limestone was used as an in-situ standard (Brand et al. 2014).

The sample chamber was designed at UNM's CSI to allow for analysis of small samples by minimizing the volume within the combustion chamber to approximately 6.6 ml, which was further reduced by the addition of the sample holder and limestone slab to approximately 1.8 ml. Simply, the chamber is a machined stainless steel cylinder with a solid bottom, two gas ports (entrance and exit) on either lateral side, and a screw top lid with a 30 mm diameter zinc selenide viewing window (Sharp et al. 2000) .

Laser

The sample chamber was placed below a Photon Machines Inc. FUSIONS 10.6 CO_2 laser, equipped with motorized motion control in all planes (X, Y, Z), minimum spot size of ~125 μm flat beam technology, and high resolution live view optics with motorized magnification and a 5.4 mm minimum field of view. Sample analysis was conducted at 100% zoom magnification, power setting of 25%, pulse width of 1 second, and 0.8 mm spot size. The 0.8 mm was sufficient to cover the entire sample and in nearly every example the entire sample was completely combusted. The same laser settings were used to analyze both samples and the Solnhofen limestone standard.

Sample produced CO_2 analysis

During sample acquisition a continuous flow of helium (He) gas was maintained through the sample chamber. Samples were analyzed heating samples with the laser,

which decarbonates the otolith material and produces CO_2. Laser produced CO_2 was carried by high flow rates (<350 ml/min) of He away from the sample chamber through stainless steel capillary tubing. Downstream of the sample chamber gasses passed through a Swagelok™ high purity inline particle filter and Nafion® water trap before entering a liquid nitrogen cryotrap. Sample CO_2 was frozen in the cryotrap for a total of two minutes, allowing the entirety of CO_2 to collect. After releasing the cryotrap, the CO_2 gas was carried to the 6-way valve, which in the inject position directed the flow to the gas chromatograph (GC) column. After the GC column the sample passed through another water trap before moving to the open split valve and was then injected into the mass spectrometer. All gas handling during this analysis was operated through a Thermo Fisher Scientific™ Gas Bench II. Sample acquisition and analysis methods were modeled from (Sharp et al. 2000) (Figure 2).

Three different Thermo Fisher Scientific Delta isotope ratio mass spectrometers Delta V, Delta V Plus, and 253 Plus were used in this study. Of these mass spectrometers, only the Delta V was unfavorable to use because of its lower range of sensitivity. Because the Delta V Plus and 253 Plus were capable of measuring sample peaks above 10 sVs these were most often used. Sample peaks below 3 sVs produced untrustworthy results and were exclude from further analysis. During analysis, two reference gas injections (CO_2) were placed at initiation of each sample acquisition and before and after each sample peak. Reference peaks were used to monitor for drift and a time-calculated correction was applied. Sample peaks were assigned an isotope ratio based on the second reference gas injection, unless manual correction was deemed necessary and then the nearest reference peak was used. Solnhofen standard (-4.1 ‰ $\delta^{18}O$) was analyzed in-situ using the same

methods for otolith analysis during each sample acquisition and resultant values were used to correct the sample values within each sample acquisition. Standards were generally measured at the beginning and end of each sample acquisition.

To correct for any difference in $\delta^{18}O$ fractionation caused by the laser between material types (aragonitic otoliths and calcitic limestone), I analyzed Solnhofen limestone and a lapillus otolith from a hatchery stock sub-adult *Xyrauchen texanus* by laser based GC-IRMMS and conventional acid digestion methods. The otolith was from a sub-adult (136 cm total length) reared at the US Fish and Wildlife Service's Southwest Native Aquatic Resources and Recovery Center in Dexter, New Mexico and was procured from American Southwest Ichthyological Researchers LLC (Barkalow et al. 2015). The resultant $\delta^{18}O$ values produced by acid digestion were used as the "true" values for each of the substrates (Solnhofen -4.1 ‰ $\delta^{18}O$, otolith -4.9 ‰). Comparing the laser obtained $\delta^{18}O$ values (Solnhofen -7.5 ‰ $\delta^{18}O$, otolith -7.4 ‰) to the conventionally obtained $\delta^{18}O$ produced the offset for the laser obtained samples. The offset for calcitic limestone was -3.4‰ while the offset for aragonitic otolith material was -2.5‰. The difference between offsets (-0.9 ‰) was used as a material fractionation correction factor and applied to all samples (otoliths).

Sample classification

Samples were classified into three categories representing natal habitat types, mainstem, travertine tributaries, and other tributaries. The mainstem of the Colorado River $\delta^{18}O_{SMOW}$ averages -14.6 ‰ and is temporally and spatially stable except during episodic monsoon floods (L.J. Crossey, University of New Mexico, personal communication). Tributaries, important spawning habitats for many Colorado River

native fishes (Weiss et al. 1998; Gorman and Stone 1999), have similarly stable $\delta^{18}O_{SMOW}$ but are isotopically distinct from the mainstem. Oxygen isotope signatures define two general tributary types available to suckers in the Grand Canyon. Travertine spring systems like the Little Colorado River (LCR) and Havasu Creek have the lowest $\delta^{18}O$ (mean = -11.5 ‰, SD = 0.40). Other tributaries (Bright Angel Creek, Shinamu Creek, and Tapeats Creek) have an intermediate $\delta^{18}O$ value (mean = -13.5 ‰, SD = 0.20). To calculate a relative temperature experienced from the $\delta^{18}O$ of a larva's otoliths, an environmental water $\delta^{18}O$ value must be used; larvae were assigned environmental water by a classification scheme similar to a quadratic discriminant analysis (QDA). The water $\delta^{18}O$ used to determine a larval fish's experienced temperature is assigned based on the Mahalanobis generalized squared distance ($D_j^2(X)$, or *M*-distance of a larva's assigned temperature (X_{FISH}) to mean temperature present in the environment water (j) during the time post hatch for each individual independently, i.e., using the three possible water $\delta^{18}O$ (mainstem, travertine, tributary) there are three possible temperatures that could be calculated from the $\delta^{18}O$ of a larva's otoliths(s), therefore the *M*-distance model was used to determine which of the experienced water temperatures was more likely given the temperatures present in those systems during a larva's development post hatch. This model was chosen because it allows for analysis of data with unequal variances (S_j) and prior probability ($PRIOR_j$). Prior probabilities for all classification runs were set equal to one. *M*-distance was calculated using Equation 2.

Equation 2

$$D_j^2(X) = \left(X_{FISH} - \bar{X}_j\right)' S_j^{-1} \left(X_{FISH} - \bar{X}_j\right) - 2\,log\left(PRIOR_j\right) + \log\left|S_j\right|$$

Where: $D_j^2 =$ Mahalanobis generalized squared distance.

$X_{FISH} =$ A larva's experienced temperature in *j* environment water.

$\bar{X}_j =$ The mean water temperature of *j* environment during lifetime of *FISH*.

$S_j^{-1} =$ The standard deviation of water temperature in *j* environment water .

$PRIOR_j =$ The prior probability of belonging to *j* environment water.

For clarity, Equation 3 was used to construct a posterior probability $Pr(j \mid X)$ of belonging to one of (*k*) groups and, while the results of these are equivalent to *M*-distance results, probability assignments from 0 to 1 are more easily interpreted. All classification was done in R-studio (Fan and J.Hyndman 2009).

Equation 3

$$Pr(j \mid X) = \frac{\exp\{-0.5\mathrm{D}_j^2(X)\}}{\sum_k \exp\{-0.5\mathrm{D}_k^2(X)\}}$$

All larvae with a probability greater than 0.60 of belonging to (*j*) environment water were assigned into one of the three sources (mainstem, travertine, or tributary). All larvae that had less than 0.60 probability of belonging to (*j*) environment water were classified as "unknown" and removed from further analysis.

Experienced temperature calculation

The temperature dependent equation developed by Grossman and Ku (1986, Equation 4) was used to calculate temperature experienced from results of the GC-IRMMS obtained $\delta^{18}O$ of a larval fish's otoliths. The assigned water $\delta^{18}O_{water\ (PDB)}$ was

subtracted from GC-IRMMS obtained $\delta^{18}O_{fish}$ of an otolith and the resultant value was multiplied by 4.38 and then subtracted from 20.6, resulting in a temperature in degrees Celsius.

Equation 4

$$T(°C) = 20.6\text{-}4.38(\delta^{\mathbf{18}}O_{fish} - \delta^{\mathbf{18}}O_{water(PDB)})$$

There are many different variations of the temperature dependent fractionation equation that have been derived for different types of biogenic calcium carbonate and species specific equations, however there are none currently developed for any catostomid. The equation by Grossman and Ku is widely cited as reliable for determination of temperature from otoliths of marine and freshwater fishes (Patterson et al. 1993; Guiguer et al. 2003; Høie et al. 2004).

To compare carbonate $\delta^{18}O$ values which are reported in the Pee Dee Belemnite (PDB) scale to water $\delta^{18}O$ which is reported in Standard Mean Ocean Water (SMOW) water $\delta^{18}O$ were rescaled to PDB by the relationship in Equation 5 (Friedman and O'Neil 1977).

Equation 5

$$\delta 18O_{(PDB)} = 0.99978 * (\delta 18O_{(SMOW)}) - 0.22$$

Additionally, relative difference in temperature experienced from the mean water temperature of the assigned environment ($Temp_{(diff)}$) water was computed by subtracting the mean environment water temperature from temperature experienced as determined by isotope analysis. All mean environment water temperatures were calculated independently for the time interval that each larva was present as indicated by the dates between the hatch date and date of capture.

Data analysis and visualization

Data analysis was conducted in program R accessed through R-studio version 1.0.136 (Fan and Hyndman 2009). All data visualization was done using the ggplot2 package (Wickham 2009). Prior to analysis, model assumptions were checked graphically and/or formally when appropriate. A quantile-quantile (Q-Q) plot was used to assess whether input data fit a normal distribution. If the data appeared to deviate from the normal distribution both a Shapiro-Wilks test and an Anderson Darling test were performed (Shapiro and Wilk 1965; Stephens 1979). Homogeneity of variance (HOV) plots, based on the Brown-Forsyth test, were used to assess the assumption of equal variance among groups (Heiberger 2017). Additionally, I performed both the Barlett and Fligner-Killen tests to formally assess the homogeneity of variance assumption (Conover et al. 1981). All mean water temperature and mainstem discharge data were independently calculated for the time interval that each larva was present as indicated by the dates between the hatch date and date of capture.

The difference in temperatures that larvae experienced among capture months (March – July) was assessed with a one-way analysis of variance ANOVA. A post-hoc Tukey's honest significant difference (HSD) was used to compare means between months. Additionally, a one-way ANOVA was performed to assess the difference between the temperatures experienced by larvae assigned to different environment waters (mainstem, travertine, or tributary). A post-hoc Tukey's HSD was used to compare means between groups. Further investigation into variables responsible for determining the temperature experienced by larvae was done with a multiple linear regression model. The explanatory variables chosen for the full model were river kilometer of capture,

mean mainstem water temperature, mean travertine tributary water temperatures (Havasu Creek, Little Colorado River), mean temperature tributary water temperature (Bright Angel Creek), month of capture, hatch date, annuli, mean mainstem discharge between hatch and capture dates, and mean daily fluctuation in mainstem discharge (*Q*) between hatch and capture date as determined by subtracting the daily minimum *Q* from the daily maximum *Q*. Backward stepwise model selection was performed using Akaike Information Criterion (AIC) scores. After low scoring variables were removed from the model the remaining explanatory variables were included in the final model.

The difference in $Temp_{(diff)}$ among month of capture (March – July) was assessed with a one-way ANOVA. A post-hoc Tukey's HSD was used to compare mean $Temp_{(diff)}$ between months. To assess differences in $Temp_{(diff)}$ between assigned and environmental water (mainstem, travertine, or tributary) a one-way ANOVA was used. A post-hoc Tukey's HSD was used to compare the mean $Temp_{(diff)}$ between groups. Further investigation into explanatory variables responsible for the $Temp_{(diff)}$ was modeled with multiple linear regression. The explanatory variables included in the full model were river kilometer of capture, mean mainstem water temperature, mean travertine tributary water temperatures (Havasu Creek, Little Colorado River), mean tributary water temperature (Bright Angel Creek), month of capture, hatch date, annuli, mean mainstem discharge, and mean daily fluctuation in mainstem discharge. Backward stepwise model selection was performed using (AIC) scores. After low scoring variables were removed from the model, the remaining explanatory variables were included in the final model.

To assess the difference in growth rates of larvae among capture months (March – July) was assessed with a one-way ANOVA. A post-hoc Tukey's HSD was used to

compare mean growth rate between months. The relationship between growth rate and experienced temperature and $Temp_{(diff)}$ were modeled with ordinary linear regression. Further investigation into other explanatory variables responsible for the variance observed between growth rate and temperature was modeled with a multiple linear regression. The explanatory variables chosen for the full model were experienced temperature, $Temp_{(diff)}$, river kilometer of capture, mean mainstem water temperature, mean travertine tributary water temperatures (Havasu Creek, Little Colorado River), mean temperature tributary water temperature (Bright Angel Creek), month of capture, hatch date, mean mainstem discharge, and mean daily fluctuation in mainstem discharge. Backward stepwise model selection was performed using (AIC) scores. After low scoring variables were removed from the model the remaining explanatory variables were included in the final model.

Results

Water Oxygen Isotopes

Fourteen mainstem Colorado River water samples were analyzed for $\delta^{18}O$ from 2015 (Table 1). Mainstem $\delta^{18}O_{SMOW}$ mean was -14.6 ‰ ± 0.31. The $\delta^{18}O$ data obtained from the Nearshore Ecology Project included years 2009, 2010, and 2012. Data were available in 2009 from May, July, August, September, and October. In both 2010 and 2012, data were only available from the month of September. A total of 13 travertine (Little Colorado River and Havasu Creek) $\delta^{18}O$ samples (mean, -11.5 ‰, SD, 0.4); (Table 2) and 18 tributary (Bright Angel Creek, Shinamu Creek, and Tapeats Creek) (mean = -13.5 ‰, SD = 0.2) (Table 3) samples were available. Because the three environment waters are relatively stable and distinct from each other (ANOVA: F_2, $P<0.05$) $\delta^{18}O$ of these

systems is sufficiently stable to allow for oxygen isotope thermometry within and between systems.

Water temperature and discharge

From the period in which larval catostomids were present, as indicated by the earliest hatch date (February 28th, 2015) to the last collection date (July 9th, 2015), daily mean water temperatures in the mainstem Colorado River ranged from 8.0–19.7°C, (mean, 12.5°C). In all months (February–July), mainstem water temperatures increased temporally and longitudinally (colder upstream and warmer downstream). Mean daily temperatures in Bright Angel Creek (tributary) ranged from 6.7–29.2°C, (mean = 15.9°C). Mean daily temperatures in travertine (Little Colorado River and Havasu Creek combined) ranged from 8.4–27.7°C (mean = 19.2°C). Travertine mean daily water temperatures were, on average, warmer than both the tributary and mainstem temperatures in every month. Tributary temperatures were more similar to mainstem temperatures for months February–May and became more similar to travertine temperatures during June and July (Figure 3).

Discharge of the three riverine systems is markedly different from each other; mainstem discharge varies by seasonal power demand and Glen Canyon dam operations while tributary and travertine systems vary mostly due to seasonal precipitation patterns (Figure 4). Mainstem Colorado River mean daily discharge ranged from 200.1–580.7 m^3/s, (mean = 346.0 m^3/s). Mean daily fluctuations in discharge due to peak power demands (i.e., hydropeaking) ranged from 102.5–158.7 m^3/s (mean = 124.7 m^3/s). Mean daily discharge in Bright Angel Creek (tributary) ranged from 0.2–5.8 m^3/s, (mean = 0.5 m^3/s). Mean daily discharge in travertine (Little Colorado River and Havasu Creek

combined) ranged from 1.8–61.7 m^3/s (mean = 5.2 m^3/s).

Age and development

Nearly all (95%) of the total selected larvae that were analyzed isotopically and aged (n = 88) were ontogenetically staged as mesolarvae; the remaining fish (5%; n = 5) were protolarvae. Mesolarvae were further subdivided into sequential sub-stages of development (flexion and postflexion). Within mesolarvae just under half (45%; n = 37) were identified as flexion and the remaining 46 were the latter developmental sub-stage, postflexion mesolarvae. Larvae ranged in age 17–57 days post-hatch. Mean age was 32 days post-hatch (SD = 11). Both sub-stages of mesolarvae were present April–July; there were no postflexion mesolarvae analyzed from March since only protolarvae were collected in March. Protolarvae (n = 5) ranged from 12.6–14.0 mm SL, (mean = 13.4 mm SL) and ages ranged from 17–20 days post-hatch (mean = 19 days). Flexion mesolarvae ranged from 12.6–15.6 mm SL, (mean = 14.4 mm SL) and ages ranged from 17–44 days post-hatch (mean = 26 days). Postflexion mesolarvae ranged from 13.9–18 mm SL, (mean =16.2 mm SL) ranged from 22–57 days post-hatch (mean = 37 days; Table 4)

Hatch dates ranged from February 28, 2015 to July 09, 2015. The distribution of hatch dates followed a bi-modal pattern, where the first and largest peak in hatching occurred around March 10 followed by a lull around April 7 and a second although lesser peak in hatching around April 29 (Figure 5).

Larval growth rates ranged from 0.07–0.28 mm/day, (mean = 0.16 mm/day, SD = 0.04). Larvae collected from March–May had the highest growth rates while larvae collected in June and July had the lowest growth rates (Figure 6). Growth rates did not significantly differ between developmental stages (Kruskal-Wallis ANOVA: $X^2 = 1.2$, df = 2, $P = 0.5$). Growth rates were negatively correlated with hatch date (Pearson's Correlation Coefficient: R = 0.41, $P < 0.05$).

Isotope analysis

A total of 200 *C. latipinnis* were selected for isotopic analysis. Unfortunately, a subset of these samples (n = 112) were deemed insufficient for analysis. Samples were excluded for two reasons: insufficient intensity peaks (too low < 3 sVs or too high > 10 sVs) or, less often, severe drift (indicated by deviation in values obtained for reference gas injections from known values). The remaining samples (n = 88) ranged in $\delta^{18}O_{PDB}$ from -8.8 to -14.8‰ (mean = -12.8 ‰, SD = 1.2).

Sample classification

Oxygen isotope ratios obtained from otoliths were used to calculate a range of possible temperatures experienced using the three mean different environment waters (mainstem, travertine, and tributary). These results were used to classify each larva into of the three potential environment water categories. Of the total larvae, slightly more than three quarters of them (76%; n = 67) were classified into one of the three environment waters and the remainder (n = 21) were unclassified. Slightly more than half (52%; n = 35) of the larvae were classified as mainstem. Travertine was next most commonly assigned water (n = 20) while the remainder (n = 12) were classified as tributary. Prior to

setting the assignment cutoff (60%) the removed individuals were classified as either mainstem (n = 11) or tributary (n = 10). Majority of individuals (78%, n = 52) that were classified had greater than or equal to 90% probability of belonging to their assigned group (Table 5).

Temperature experienced

Once larvae were classified, relative temperature experienced was calculated using the assigned water $\delta^{18}O_{PDB.}$ Relative temperatures experienced by larvae ranged from 8–25°C (mean = 16°C, SD = 4.5). Initial tests of significance revealed that there is a significant difference between the temperatures experienced by fish from different environment water (ANOVA: $F_{2,64} = 29.8$, $P < 0.05$) and by month (ANOVA: $F_{4,64} = 26.8$, $P < 0.05$). Further post-hoc analysis revealed that each environment water group is significantly different from each other (Tukey's HSD: $P < 0.05$); on average, fish assigned to the mainstem experienced the lowest temperatures while fish assigned to the tributaries experienced the warmest temperatures (Figure 7). Temporal variation in temperature experienced tracked increasing monthly water temperatures; fish captured in March experienced colder temperatures than all other months (Tukey's HSD: $P < 0.05$). After March the temperature experienced by larvae increased from April to July, however, not all subsequent months were significantly different from each other (Tukey's HSD: $P > 0.05$; Figure 8).

The results of the multiple linear regression models showed that the temperature experienced by larvae included all of the explanatory variables (n = 9) and accounted for nearly two-thirds (MLR: $R^2_{(adjusted)} = 0.65$) of variation observed in temperature experienced by larvae; however many of the variables (n = 8) included in the full model

were not indicated as significant ($P < 0.05$;Table 6). Month of capture was the only explanatory variable indicated as significant. Following stepwise AIC model selection the explanatory power of model improved slightly (MLR: $R^2_{(adjusted)} = 0.67$) and all remaining variables (n = 4; month of capture, hatch date, annuli, and river kilometer) were indicated as significant ($P < 0.05$;Table 6; Figure 9)

$Temp_{(diff)}$

The $Temp_{(diff)}$ experienced by larvae ranged from -8–8°C (mean = 1.2°C, SD =3.1). Initial tests of significance indicated a $Temp_{(diff)}$ between environment water groups (ANOVA: $F_{2, 64} = 17.0$, $P < 0.05$) and a slight difference by month of capture (ANOVA: $F_{4,64} = 3.1$, $P = 0.01$). Further post-hoc analysis revealed that $Temp_{(diff)}$ for travertine fish is different from both mainstem and tributary fish (Tukey's HSD: $P < 0.05$); there was no significant difference of $Temp_{(diff)}$ between mainstem and tributary (Tukey's HSD: P =0.18; Figure 10). There were no clear patterns of $Temp_{(diff)}$ varying by month of capture; June was significantly different from March and May (Tukey's HSD: $P < 0.05$) and all other comparisons were not significant ($P > 0.05$; Figure 11).

Results of multiple linear regression modeling $Temp_{(diff)}$ that included all explanatory variables (n = 9) explained about one-fifth (MLR: $R^2_{(adjusted)} = 0.20$) of the variation observed in $Temp_{(diff)}$ experienced by larvae and all of the variables indicated no significant influence ($P > 0.05$). Following stepwise AIC model selection the explanatory power of the model improved (MLR: $R^2_{(adjusted)} = 0.26$) and all remaining variables (n=4; annuli, tributary mean temperature, mainstem mean *Q*, and mainstem mean temperature) were indicated as significant ($P < 0.05$; Table 7; Figure 12).

Growth rate analysis

The results of the multiple linear regression modeling larval Growth rates including all explanatory variables (n = 10) explained more than one-third (MLR: $R^2_{(adjusted)} = 0.41$) of the variation observed in larval growth rates, however, only two of the variables were significant ($P < 0.05$). Following stepwise AIC model selection the explanatory power of the model only slightly increased (MLR: $R^2_{(adjusted)} = 0.44$), however, all of the variables remaining (n= 4; hatch date, mainstem mean temperature, travertine mean temperature, and mean mainstem daily *Q* fluctuation) were significant ($P < 0.05$; Table 8; Figure 13).

Discussion

Glen Canyon dam has severely altered Colorado River water temperatures since its construction. This is because hypolimnial releases dampen seasonal variability and decrease the mean annual temperature (Stevens et al. 1997) relative to conditions before the dam was closed in 1963. From Glen Canyon dam the Colorado River spans nearly 450 RK and traverses the Grand Canyon before entering Lake Mead. Larval catostomids,

which are prone to passive downstream transport by water currents (Kennedy and Vinyard 1997; Robinson et al. 1998; Hedrick et al. 2009), are subject to water temperatures that are sub-optimal for growth and development (Clarkson and Childs 2000; Bestgen 2008). However, there are areas within the mainstem Colorado River, primarily in the lower 100 RK, where water temperatures are warmer and potentially sufficient for growth and development. Additionally there is potential for larval fish to experience warmer than average temperatures in tributaries and shoreline-associated, microhabitats (Robinson and Childs 2001; Childs et al. 2011; Ross et al. 2013). Isotopic analysis of larval *C. latipinnis* otoliths allowed for the reconstruction of an average temperature experienced by an individual larva from the time it hatched to the time it was collected. Temperatures experienced by larvae were within the range of environment water temperatures present in the tributaries and mainstem river and closely mirrored temporal variation displayed in water temperatures. While it is difficult to assess the accuracy of isotopically derived temperatures for wild-caught larvae, these results suggest that both the methods of obtaining $\delta^{18}O$ from a larval otolith and the subsequent determination of temperature experienced are likely valid.

Because there are isotopically ($\delta^{18}O$) distinct aquatic systems within the Grand Canyon it was important to be able to classify fish into environment water categories based on their oxygen isotope signatures. Determination of temperature experienced would otherwise likely lead to grossly underestimated water temperatures experienced for some fish (e.g., a larva collected in May that was classified as travertine had an experienced temperature of 15.4°C vs. 2.8°C had it been assumed to belong to mainstem). This classification scheme is by no means analogous to other studies

(Limburg et al. 2013) that have used stable isotopes and elemental ratios for determination of natal origin and or spawning location of juvenile and adult fish; rather this is an analysis of where a larva likely spent the majority of its life post-hatch. The majority of classified larvae were assigned with a very high probability of belonging to their assigned group. Approximately 85% of fish assigned to mainstem and travertine groups were assigned with greater than 90% probability. Tributary fish on average had the lowest probability assignment values (66%), likely due to the tributaries intermediate $\delta^{18}O$ value of -13.5 ‰ making it difficult to distinguish between true tributary fish and fish that have intermediate values as a result of emigrating from a travertine tributary into the mainstem. It is important to note that all fish were collected within the mainstem Colorado River, yet nearly 50% of the larvae were classified with a greater than 60% probability of having spent the majority of life post hatch in a tributary to mainstem. A drawback of this classification scheme is that it cannot discriminate between fish that spent a significant portion of their life in an isotopically distinct ($\delta^{18}O$) aquatic system before emigrating to another; which is a likely scenario in general because tributaries to Colorado River within the Grand Canyon are considered important for spawning and maintenance of native fishes (Weiss et al. 1998; Robinson et al. 1998) but especially for fish (n = 21) that were not classified. However, this confusion could potentially be addressed in future research by sampling larval fishes within and upstream of tributaries to greatly increase the known provenance and of larvae and the decrease range of potential water temperatures experienced by larvae.

The temperatures that larvae experience are greatly influenced by seasonal variability in temperature indicated by the month of capture and hatch date being the

most influential variables in the temperature experienced model (Table 7). These results are likely influenced by the fact that most larvae collected in later months were classified as the two warmest environments (tributary and travertine) and the smaller sample sizes available from months June and July (Figure 8). Additional variation of temperature experienced is explained by the age of a larva; older larvae experienced warmer temperatures. Possible explanations for influence of age over the temperature experienced can likely be broken into two categories, biotic and abiotic. Possible arguments for an abiotic explanation would be that as the fish increased in age, the water temperature similarly increased and by virtue of being present longer may have had a greater chance of being transported into warmer water. A biotic explanation may be that older larvae likely had increased swimming abilities (Ward et al. 2002; Korman et al. 2004) which allowed them to preferentially access warmer water temperatures especially in low velocity thermally graded habitats (e.g. backwaters, embayments, etc.). This hypothesis is supported by age being the most the most influential variable in the $Temp_{(diff)}$ model. Older larvae were more likely to have experienced warmer temperatures and, interestingly, mainstem and tributary fish were more likely to have experienced warmer than mean temperatures of their prospective environments while the majority of travertine fish experienced near or below average water temperatures (Figure 12). This result is a likely consequence of both abiotic and biotic influences. One possible biotic explanation for the lower $Temp_{(diff)}$ values for travertine fish may be that water temperatures in the travertine systems are more similar to optimal temperatures and thus fish do not preferentially access warmer microhabitats. A more simple explanation however, may be that temperatures within habitats used by larvae within the travertine

systems are more similar to the mean temperature.

Growth rates of larvae examined in this study were negatively correlated with temperature experienced, month of capture, and mean environment water temperature (Figure 13). This result was initially perplexing, primarily because it is contradictory to both basic ectothermic metabolic theory and empirical studies where growth rates increase with an increase in temperature (Pepin 1991; Charnov and Gillooly 2004; Bestgen 2008). Growth rate of wild caught larval fish is determined by subtracting the size at hatch from length at capture and dividing that by the number of days post hatch. Two of these variables (size at capture, and age) were obtained directly by measurement within this study. Size at hatch however, could not be measured directly in the field, and thus a well accepted mean size at hatch (11. 5 mm) was used (Snyder and Muth 2004). While it is expected that individuals likely deviate from the mean hatch size, only a systematic deviation would produce an inverse relationship between growth rate and temperature. Size at hatch for other fishes has been shown to increase at lower temperature (Ware 1975). Female fish that experience cooler temperatures may produce larger ova, which in turn produce larger larvae (Ware 1975; Pepin 1991). Additionally, egg incubation duration increases with cooler water temperatures (Pepin 1991; Gillooly et al. 2002) which may lead to larger larvae (Ware 1975). There is no way of empirically testing whether fish collected in this study hatched at larger sizes due to colder water temperatures, thus skewing the growth rate data, but this could explain the anomalous results. Further support is given by the results of the growth rate model which showed that hatch date was the variable having the greatest influence on growth rates; on average larvae with the earliest hatch dates had the highest growth rates.

Determining the relationship between water temperatures growth rates of larval catostomids was the main impetus for this project, however, given my results it is unlikely that any comparison between growth rates and other variables is valid. Understanding the relationship between temperature experienced by larvae and how that may influence their survival is often accomplished through laboratory experiments where carefully controlled conditions permit clear results. While fundamentally important, laboratory experiments fail to accurately represent the abiotic and biotic conditions present in the wild populations of interest and often these conditions have overwhelming influence over growth and survival. While some results were unexpected, the results presented here are an excellent example of why analysis of wild caught fish is important. Hopefully these results can provide both a novel method for analyzing the temperatures experienced by individual larvae and better insight into the thermal conditions that larvae experience within the Grand Canyon.

Given these findings, further investigation into the role of temperature and how that effects size at hatch of larval catostomids is warranted. Researchers often estimate impacts of altered ecosystems to wild populations of fishes and estimating altered temperature regimes on growth rate is profoundly important for early life stages. While the effect of variable hatch size on estimation of growth rates is minimized for later stages of development (juvenile, adult) estimation of larval growth rate is extremely important because maintenance of a population is dependent on their survival.

The novel approaches of this study provide greater insight into the variability of temperatures experienced by individual larval *C. latipinnis* throughout their development, and identified some of the abiotic and biotic variables that have significant influence over

growth and survival. This study used larvae collected throughout the lower 160 RK of the mainstem Colorado River where each individual collected has an unknown provenance and has potentially experienced a wide range of temperatures and habitats. Expansion of the sampling area and analysis of larvae caught further upstream in areas without travertine and regular tributaries would potentially constrain many of the complications experienced and provide for further validation of these techniques.

Literature cited

Albrecht, B. a., P. B. Holden, R. B. Kegerries, and M. E. Golden. 2010. Razorback sucker recruitment in Lake Mead, Nevada–Arizona, why here? Lake and Reservoir Management 26(4):336–344.

Barkalow, S. L. C., S. P. Platania, J. L. Kennedy, and M. A. Brandenburg. 2015. Natal Origin of San Juan River Razorback Suckers Collected in 2014. Final Report.

Bestgen, K. R. 2008. Effects of Water Temperature on Growth of Razorback Sucker Larvae. Western North American Naturalist 68(1):15–20.

Bestgen, K. R., G. B. Haines, and A. A. Hill. 2011. Synthesis of flood plain wetland information: Timing of razorback sucker reproduction in the Green River, Utah, related to stream flow, water temperature and flood plain wetland availability. Final Report. Pages 1-185

Bezzerides, N., and K. Bestgen. 2002. Status review of Roundtail chub (Gila robusta), Flannelmouth sucker (Catostomus latipinnis), and Bluehead sucker (Catostomus discobolus) in the Colorado River Basin. Colorado State University Larval Fish Laboratory 118(September):1–139.

Bozek, M. A., L. J. Paulson, and G. R. Wilde. 1990. Effects of ambient Lake Mohave temperatures on development, oxygen consumption, and hatching success of the razorback sucker. Environmental Biology of Fishes 27(4):255–263.

Brand, W. A., T. B. Coplen, J. Vogl, M. Rosner, and T. Prohaska. 2014. Assessment of international reference materials for isotope-ratio analysis (IUPAC Technical Report) 1. Pure and Applied Chemistry 86(3):425–467.

Bunch, A. J., R. J. Osterhoudt, M. C. Anderson, and W. T. Stewart. 2012. Colorado River Fish Monitoring in Grand Canyon , Arizona — 2012 Annual Report.

Campana, S. E. 1999. Chemistry and composition of fish otoliths: Pathways, mechanisms and applications. Marine Ecology Progress Series 188:263–297.

Campana, S. E. 2005. Otolith science entering the 21st century. Marine and Freshwater Research 56(5):485–495.

Carman, S. M. 2007. Bluehead Sucker Catostomus discobolus and Flannelmouth Sucker Catostomus latipinnis Conservation Strategy. Final Report.

Carter, J. G., R. A. Valdez, R. J. Ryel, and V. A. Lamarra. 1985. Fisheries Habitat Dynamics in the Upper Colorado River. Journal of Freshwater Ecology 3(2):249–264.

Cerling, T. E., and Z. D. Sharp. 1996. Stable carbon and oxygen isotope analysis of fossil tooth enamel using laser ablation. Palaeogeography, Palaeoclimatology, Palaeoecology 126(1–2):173–186.

Charnov, E. L., and J. F. Gillooly. 2004. Size and temperature in the evolution of fish life histories. Integrative and comparative biology 44(6):494–497.

Childs, M. R., R. W. Clarkson, and A. T. Robinson. 2011. Resource Use by Larval and Early Juvenile Native Fishes in the Little Colorado River , Grand Canyon , Arizona Resource Use by Larval and Early Juvenile Native Fishes in the. Transactions of the American Fisheries Society 127(September):620–629.

Clarkson, R. W., and M. R. Childs. 2000. Temperature Effects of Hypolimnial-Release

Dams on Early Life Stages of Colorado River Basin Big-River Fishes. Copeia 2000(2):402–412.

Conover, W. J., M. E. Johnson, and M. M. Johnson. 1981. A comparative study of tests for homogeneity of variances, with applications to the outer continental shelf bidding data. Technometrics 23(4):351–361.

Cross, W. F., C. V. Baxter, E. J. Rosi-Marshall, R. O. Hall, T. A. Kennedy, K. C. Donner, H. A. W. Kelly, S. E. Z. Seegert, K. E. Behn, and M. D. Yard. 2013. Food-web dynamics in a large river discontinuum. Ecological Monographs 83(3):311–337.

Degens, E., W. Deuser, and R. Haedrich. 1969. Molecular structure and composition of fish otoliths. Marine Biology 2(2):105–113.

Devereux, I. 1967. Temperature measurements from oxygen isotope ratios of fish otoliths. Science (New York, N.Y.) 155(770):1684–1685.

Dowling, T. E., W. L. Minckley, and P. C. Marsh. 1996. Mitochondrial DNA diversity within and among populations of razorback sucker (Xyrauchen texanus) as determined by restriction endonuclease analysis. Copeia (3):542–550.

Dudley, R. K., and S. P. Platania. 2007. Flow regulation and fragmentation imperil pelagic-spawning riverine fishes. Ecological Applications 17(7):2074–2086.

Edmonds, J. S., N. Caputi, and M. Morita. 1991. Stock Discrimination By Trace-Element Analysis of Otoliths of Orange Roughy (Hoplostethus-Atlanticus), a Deep-Water Marine Teleost. Australian Journal of Marine and Freshwater Research 42(4):383–389.

Elsdon, T., B. Wells, S. Campana, B. Gillanders, C. Jones, K. Limburg, D. Secor, S. Thorrold, and B. Walther. 2008. Otolith Chemistry To Describe Movements And Life-History Parameters Of Fishes (1):297–330.

Epstein, S., R. Buchsbaum, H. A. Lowenstam, and H. C. Urey. 1953. Revised Carbonate-Water Isotopic Temperature Scale. Geological Society of America Bulletin 64(11):1315.

Fan, S., and R. J.Hyndman. 2009. R Development Core Team, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing.

Friedman, I., and J. R. O'Neil. 1977. Data of Geochemistry. Geological Survey Professional Paper 440–KK(Sixth Edition):1–117.

Fry, B. 2006. Stable Isotope Ecology. Page Libro. Springer Science & Business Media.

Gillooly, J. F., E. L. Charnov, G. B. West, V. M. Savage, and J. H. Brown. 2002. Effects of size and temperature on developmental time. Oikos:70–73.

Gorman, O. T., and D. M. Stone. 1999. Ecology of spawning humpback chub, Gila cypha, in the Little Colorado River near Grand Canyon, Arizona. Environmental Biology of Fishes 55(1):115–133.

Govoni, J. J., G. W. Boehlert, and Y. Watanabe. 1986. The physiology of digestion in fish larvae. Environmental Biology of Fishes 16(1–3):59–77.

Grossman, E. L., and T.-L. Ku. 1986. Oxygen and carbon isotope fractionation in biogenic aragonite: Temperature effects. Chemical Geology: Isotope Geoscience section 59:59–74.

Guiguer, K., R. Drimmie, and M. Power. 2003. Validating methods for measuring δ18O and δ13C in otoliths from freshwater fish. Rapid Communications in Mass Spectrometry 17(5):463–471.

Hedrick, T. N., K. R. Bestgen, and K. D. Christopherson. 2009. Entrainment of semi-buoyant beads and razorback sucker, Xyrauchen texanus, larvae into flood plain wetlands of the middle Green River, Utah. Utah Division of Wildlife Resources.

Heiberger, R. M. 2017. HH: Statistical Analysis and Data Display: Heiberger and Holland.

Hoff, G. R., D. J. Logan, D. F. Markle, G. R. Hoff, D. J. Logan, D. F. M. Notes, G. R. Hoff, D. J. Logan, and D. F. Markle. 2016. Transactions of the American Fisheries Society Notes : Otolith Morphology and Increment Validation in Young Lost River and Shortnose Suckers. Transactions of the American Fisheries Society 8487(June):37–41.

Høie, H., E. Otterlei, and A. Folkvord. 2004. Temperature-dependent fractionation of stable oxygen isotopes in otoliths of juvenile cod (Gadus morhua L.). ICES Journal of Marine Science 61(2):243–251.

Holden, P. B., and C. B. Stalnaker. 1975. Distribution and abundance of mainstream fishes of the middle and upper Colorado River basins, 1967-1973. Transactions of the American Fisheries Society 104(2):217–231.

Houde, E. D. 1989. Comparative growth, mortality, and energetics of marine fish larvae: temperature and implied latitudinal effects. Fishery Bulletin 87(3):471–495.

Kegerries, R., B. Albrecht, E. I. Gilbert, W. H. Brandenburg, A. L. Barkalow, H. Mohn, R. Rogers, M. McKinstry, B. Healy, J. Stolberg, E. Omana Smith, and M. Edwards. 2016. Razorback Sucker Xyrauchen texanus research and monitoring in the Colorado River inflow area of Lake Mead and the lower Grand Canyon, Arizona and Nevada. Final report prepared by BIO-WEST, Inc., for the U.S. Bureau of Reclamation, Upper Colorado Region, S.

Kendall, C., and T. B. Coplen. 2001. Distribution of oxygen-18 and deuterium in river waters across the United States. Hydrological Processes 15(7):1363–1393.

Kennedy, T. A., J. D. Muehlbauer, C. B. Yackulic, D. A. Lytle, S. W. Miller, K. L. Dibble, E. W. Kortenhoeven, A. N. Metcalfe, and C. V. Baxter. 2016. Flow management for hydropower extirpates aquatic insects, undermining river food webs. BioScience 66(7):561–575.

Kennedy, T. B., and G. L. Vinyard. 1997. Drift ecology of western catostomid larvae with emphasis on Warner suckers, Catostomus warnerensis (Teleostei). Environmental Biology of Fishes 49(2):187–195.

Kitagawa, T., T. Ishimura, R. Uozato, K. Shirai, Y. Amano, A. Shinoda, T. Otake, U. Tsunogai, and S. Kimura. 2013. Otolith ??18O of Pacific bluefin tuna Thunnus orientalis as an indicator of ambient water temperature. Marine Ecology Progress Series 481(Anon 2010):199–209.

Korman, J., S. M. Wiele, and M. Torizzo. 2004. Modelling effects of discharge on habitat quality and dispersal of juvenile humpback chub (Gila cypha) in the Colorado River,

Grand Canyon. River Research and Applications 20(4):379–400.

Ligon, F. K., W. E. Dietrich, and W. J. Trush. 1995. Downstream Ecological Effects of Dams: A geomorphic perspective. BioScience 45(3):183–192.

Limburg, K. E., T. A. Hayden, W. E. Pine, M. D. Yard, R. Kozdon, and J. W. Valley. 2013. Of travertine and time: Otolith chemistry and microstructure detect provenance and demography of endangered humpback chub in Grand Canyon, USA. PLoS ONE 8(12):1–18.

Marsh, P. C. 1985. Effect of incubation temperature on survival of embryos of native Colorado River fishes. Southwestern Naturalist 30(1):129–140.

Marsh, P., and W. Minckley. 1989. Observations on recruitment and ecology of razorback sucker: lower Colorado River, Arizona-California-Nevada. The Great Basin Naturalist:71–78.

Matta, M. E., I. J. Orland, T. Ushikubo, T. E. Helser, B. A. Black, and J. W. Valley. 2013. Otolith oxygen isotopes measured by high-precision secondary ion mass spectrometry reflect life history of a yellowfin sole (Limanda aspera). Rapid Communications in Mass Spectrometry 27(6):691–699.

McCormick, J. 1977. White sucker (Catostomus commersoni) embryo development, and early growth and survival at different temperatures. Journal of the Fisheries Board of Canada 34(7):1019–1025.

Miller, R. R. 1961. Man and the Changing Fish Fauna of the American Southwest. Papers of the Michigan Academy of Science. Arts, and Letters 46:365–404.

Minckley, W. L. 1991. Native fishes of the Grand Canyon region: An Obituary? Proceedings of a Symposium, May 24-25, Santa Fe, New Mexico:124–177.

Modde, T., R. T. Muth, and G. B. Haines. 2001. Floodplain wetland suitability, access, and potential use by juvenile razorback suckers in the middle Green River, Utah. Transactions of the American Fisheries Society 130(6):1095–1105.

O'Connor, M. I., J. F. Bruno, S. D. Gaines, B. S. Halpern, S. E. Lester, B. P. Kinlan, and J. M. Weiss. 2007. Temperature control of larval dispersal and the implications for marine ecology, evolution, and conservation. Proceedings of the National Academy of Sciences of the United States of America 104(4):1266–71.

Panella, G. 1971. Fish otoliths: daily growth layers and periodical patterns. Science (New York, N.Y.) 173(2):1124–1127.

Papoulias, D., and W. L. Minckley. 1990. Food limited survival of larval razorback sucker, Xyrauchen texanus, in the laboratory. Environmental Biology of Fishes 29(1):73–78.

Patterson, W. P., G. R. Smith, and K. C. Lohmann. 1993. Continental paleothermometry and seasonality using the isotopic composition of aragonitic otoliths of freshwater fishes. Climate Change in Continental Isotopic Records. American Geophysical Union. Pages 191–202

Pepin, P. 1991. Effect of temperature and size on development, mortality and survival rates of the pelagic early life history stages of marine fish. Canadian Journal of Fisheries and Aquatic Sciences 48(Beyer):503–518.

Robinson, A. T., and M. R. Childs. 2001. Juvenile growth of native fishes in the Little Colorado River and in a thermally modified portion of the Colorado River. North American Journal of Fisheries Management 21:1(March 2015):135–146.

Robinson, a T., R. W. Clarkson, and R. E. Forrest. 1998. Dispersal of larval fishes in a regulated river tributary. Transactions of the American Fisheries Society 127(5):772–786.

Ross, Robert, P., and S. Vernieu, William. 2013. Nearshore Temperature Findings for the Colorado River in Grand Canyon , Arizona — Possible Implications for Native Fish. United States Geological Survey (USGS).

Sabo, J. L., K. Bestgen, W. Graf, T. Sinha, and E. E. Wohl. 2012. Dams in the Cadillac Desert: Downstream effects in a geomorphic context. Annals of the New York Academy of Sciences 1249(1):227–246.

Shapiro, S. S., and M. B. Wilk. 1965. An Analysis of Variance Test for Normality (Complete Samples). Biometrika 52(3/4):591.

Sharp, Z. D., V. Atudorei, and H. Furrer. 2000. The effect of Diagensis on oxygen isotope ratios of biogenic phosphates. American Journal of Science 3000:222–237.

Sharp, Z. D., and T. E. Cerling. 1996. A laser GC-IRMS technique for in situ stable isotope analyses of carbonates and phosphates. Geochimica et Cosmochimica Acta 60(15):2909–2916.

Snyder, D. E. 1981. Contributions to a guide to the cypriniform fish larvae of the Upper Colorado River system in Colorado. US Bureau of Land Management, Biological Sciences Series 3, Denver CO.

Snyder, D. E., and R. T. Muth. 2004. Catostomid fish larvae and early juveniles of the upper Colorado River Basin - Morphological descriptions, comparisons, and computer-interactive key. Colorado Division of Wildlife Technical Publication 42:110.

Stephens, M. A. 1979. Tests of fit for the logistic distribution based on the empirical distribution function. Biometrika 66(3):591–595.

Stevens, L. E., J. P. Shannon, and D. W. Blinn. 1997. Colorado River benthic ecology in Grand Canyon , Arizona, USA: dam, tributary and geomorphological influences. Regulated Rivers Research and Management 13:129–149.

Tomas, J., and A. J. Geffen. 2003. Morphometry and composition of aragonite and vaterite otoliths of deformed laboratory reared juvenile herring from two populations. Journal of Fish Biology 63(6):1383–1401.

USFWS. 2002. Razorback sucker (Xyrauchen texanus) Recovery Goals: amendment and supplement to the Razorback Sucker Recovery Plan. U.S. Fish and Wildlife Service, Mountain-Prairie Region (6), Denver, Colorado.

Ward, D. L., O. Eugene Maughan, S. a. Bonar, and W. J. Matter. 2002. Effects of Temperature, Fish Length, and Exercise on Swimming Performance of Age-0 Flannelmouth Sucker. Transactions of the American Fisheries Society 131(Minckley 1991):492–497.

Ware, D. M. 1975. Relation Between Egg Size, Growth, and Natural Mortaility of Larval FIsh. Journal of the Fisheries Research Board of Canada 32(12):2503–2512.

Webb, R. H., D. L. Wegner, E. D. Andrews, R. A. Valdez, and D. T. Patten. 1999. Downstream Effects of Glen Canyon Dam on the Colorado River in Grand Canyon: A Review. The Controlled Flood in Grand Canyon:1–21.

Weiss, S. J., E. O. Otis, and O. E. Maughan. 1998. Spawning ecology of flannelmouth sucker, Catostomus lattipinnis (Catostomidae), in two small tributaries of the lower Colorado river. Environmental Biology of Fishes 52(4):419–433.

Wickham, H. 2009. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.

Yarnell, S. M., G. E. Petts, J. C. Schmidt, A. A. Whipple, E. E. Beller, C. N. Dahm, P. Goodwin, and J. H. Viers. 2015. Functional Flows in Modified Riverscapes: Hydrographs, Habitats and Opportunities. BioScience 65(10):963–972.

Zeigler, J. M., and G. W. Whitledge. 2011. Otolith trace element and stable isotopic compositions differentiate fishes from the Middle Mississippi River, its tributaries, and floodplain lakes. Hydrobiologia 661(1):289–302.

Table 1. Oxygen isotope analysis results for mainstem Colorado River water samples collected and analyzed in 2015 organized by month and river kilometer.

Year	Month	River Kilometer	$\delta^{18}O$ ‰
2015	May	309.8	-14.8
2015	May	385.6	-14.8
2015	May	391.1	-14.9
2015	June	309.8	-15.0
2015	June	358.6	-15.0
2015	June	391.1	-14.8
2015	June	449.2	-14.5
2015	July	296.6	-13.9
2015	July	313.2	-14.4
2015	July	384.0	-14.4
2015	July	391.1	-14.4
2015	July	432.9	-14.3
2015	July	426.5	-14.6

Table 2. Oxygen isotope analysis results from travertine (Little Colorado River and Havasu Creek) stream water samples organized by year, month, site, and river kilometer. All data that appears below was collected and analyzed by the Nearshore Ecology Group and used with permission.

Year	Month	Site	River Kilometer	$\delta^{18}O‰$
2009	May	Little Colorado River	99.5	-11.6
2009	July	Little Colorado River	99.5	-11.2
2009	July	Havasu Creek	253.0	-12.0
2009	August	Little Colorado River	99.5	-11.6
2009	August	Havasu Creek	253.0	-11.9
2009	September	Little Colorado River	99.5	-11.1
2009	September	Havasu Creek	253.0	-12.1
2009	October	Little Colorado River	99.5	-11.8
2009	October	Havasu Creek	253.0	-12.2
2010	September	Little Colorado River	99.5	-9.7
2010	September	Havasu Creek	253.0	-11.7
2012	September	Little Colorado River	99.5	-11.0
2012	September	Havasu Creek	253.0	-11.8

Table 3. Oxygen isotope analysis results from tributary (Bright Angel Creek, Shinumo Creek, and Tapeats Creek) water samples organized by year, month, site, and river kilometer. All data that appears below was collected and analyzed by the Nearshore Ecology Group and used with permission.

Year	Month	Site	River Kilometer	$\delta^{18}O‰$
2009	July	Bright Angel	142.1	-13.4
2009	July	Shinumo Creek	175.7	-13.4
2009	July	Tapeats Creek	216.1	-13.7
2009	August	Bright Angel Creek	142.1	-13.6
2009	August	Shinumo Creek	175.7	-13.4
2009	August	Tapeats Creek	216.1	-13.6
2009	September	Bright Angel Creek	142.1	-13.8
2009	September	Shinumo Creek	175.7	-13.6
2009	September	Tapeats Creek	216.1	-13.8
2009	October	Bright Angel Creek	142.1	-13.8
2009	October	Shinumo Creek	175.7	-13.5
2009	October	Tapeats Creek	216.1	-14.0
2010	September	Bright Angel Creek	142.1	-13.2
2010	September	Shinumo Creek	175.7	-13.0
2010	September	Tapeats Creek	216.1	-13.5
2012	September	Bright Angel Creek	142.1	-13.4
2012	September	Shinumo Creek	175.7	-13.1
2012	September	Tapeats Creek	216.1	-13.6

Table 4. Larval *C. latipinnis* collected from Colorado River within Grand Canyon in 2015 organized by collection month, river kilometer, development stage, standard length, annuli, and hatch date.

Month	River K	Development	Length (SL)	Annuli	Hatch Date
March	314.0	Protolarvae	13.8	20	3-Mar
March	314.0	Protolarvae	13.6	20	3-Mar
March	369.7	Protolarvae	12.6	17	8-Mar
March	391.1	Protolarvae	14	18	8-Mar
March	434.5	Protolarvae	13	19	9-Mar
March	314.0	Flexion Mesolarvae	14.3	25	26-Feb
March	314.0	Flexion Mesolarvae	15.4	20	3-Mar
March	369.7	Flexion Mesolarvae	15.1	28	25-Feb
March	369.7	Flexion Mesolarvae	14.2	21	4-Mar
March	391.1	Flexion Mesolarvae	14.4	23	3-Mar
March	391.1	Flexion Mesolarvae	14.1	18	8-Mar
March	391.1	Flexion Mesolarvae	15.4	24	2-Mar
March	391.1	Flexion Mesolarvae	15	19	7-Mar
March	391.1	Flexion Mesolarvae	15.6	22	4-Mar
March	391.1	Flexion Mesolarvae	15.4	24	2-Mar
March	391.1	Flexion Mesolarvae	14.2	17	9-Mar
March	391.1	Flexion Mesolarvae	14.4	17	9-Mar
March	391.1	Flexion Mesolarvae	15.1	19	7-Mar
March	391.1	Flexion Mesolarvae	15.2	17	9-Mar
March	391.1	Flexion Mesolarvae	14.6	24	2-Mar
April	385.4	Flexion Mesolarvae	14.6	27	24-Mar
April	385.4	Flexion Mesolarvae	14.7	26	25-Mar
April	385.4	Flexion Mesolarvae	14.6	32	19-Mar
April	385.4	Flexion Mesolarvae	14.8	26	25-Mar
April	385.4	Flexion Mesolarvae	13.6	30	21-Mar

Month	River K	Development	Length (SL)	Annuli	Hatch Date
April	385.4	Flexion Mesolarvae	14.8	29	22-Mar
April	391.1	Flexion Mesolarvae	15.2	44	8-Mar
April	391.1	Flexion Mesolarvae	15.4	42	10-Mar
May	385.4	Flexion Mesolarvae	14.4	24	22-Apr
May	385.4	Flexion Mesolarvae	13.8	20	26-Apr
May	385.4	Flexion Mesolarvae	14.4	21	25-Apr
May	385.4	Flexion Mesolarvae	14.2	20	26-Apr
May	385.4	Flexion Mesolarvae	15.3	22	24-Apr
May	385.4	Flexion Mesolarvae	13.9	19	27-Apr
June	449.0	Flexion Mesolarvae	14	34	12-May
June	449.0	Flexion Mesolarvae	14.6	30	16-May
June	449.0	Flexion Mesolarvae	14.1	35	11-May
June	449.0	Flexion Mesolarvae	13.3	36	3-Jun
July	296.1	Flexion Mesolarvae	13.9	39	31-May
July	296.1	Flexion Mesolarvae	13.7	28	11-Jun
July	296.1	Flexion Mesolarvae	13.9	24	15-Jun
July	296.1	Flexion Mesolarvae	13.3	34	5-Jun
April	385.4	Post-flexion Mesolarvae	17.2	45	6-Mar
April	385.4	Post-flexion Mesolarvae	16.3	31	20-Mar
April	385.4	Post-flexion Mesolarvae	16.5	33	18-Mar
April	385.4	Post-flexion Mesolarvae	17.4	43	8-Mar
April	385.4	Post-flexion Mesolarvae	15.3	31	20-Mar
April	385.4	Post-flexion Mesolarvae	16	36	15-Mar
April	385.4	Post-flexion Mesolarvae	16.2	36	15-Mar
April	385.4	Post-flexion Mesolarvae	16.6	41	10-Mar
April	385.4	Post-flexion Mesolarvae	17.6	34	17-Mar
April	296.1	Post-flexion Mesolarvae	16.1	36	16-Mar

Month	River K	Development	Length (SL)	Annuli	Hatch Date
April	391.1	Post-flexion Mesolarvae	17.3	40	12-Mar
April	391.1	Post-flexion Mesolarvae	17.4	39	13-Mar
April	391.1	Post-flexion Mesolarvae	16.9	36	16-Mar
May	309.8	Post-flexion Mesolarvae	16	35	10-Apr
May	385.4	Post-flexion Mesolarvae	16.3	26	20-Apr
May	385.4	Post-flexion Mesolarvae	15.7	22	24-Apr
May	385.4	Post-flexion Mesolarvae	15.8	24	22-Apr
May	385.4	Post-flexion Mesolarvae	15	26	20-Apr
May	385.4	Post-flexion Mesolarvae	15.3	33	13-Apr
June	391.1	Post-flexion Mesolarvae	14.6	33	12-May
June	391.1	Post-flexion Mesolarvae	16.5	33	12-May
June	391.1	Post-flexion Mesolarvae	15.7	37	8-May
June	391.1	Post-flexion Mesolarvae	13.9	35	10-May
June	391.1	Post-flexion Mesolarvae	16	37	8-May
June	391.1	Post-flexion Mesolarvae	16.7	36	9-May
June	391.1	Post-flexion Mesolarvae	16.6	46	29-Apr
June	391.1	Post-flexion Mesolarvae	15.7	57	18-Apr
June	449.0	Post-flexion Mesolarvae	15	52	24-Apr
June	449.0	Post-flexion Mesolarvae	16.2	44	2-May
June	449.0	Post-flexion Mesolarvae	16.2	31	15-May
June	449.0	Post-flexion Mesolarvae	14.2	30	16-May
June	449.0	Post-flexion Mesolarvae	14.6	44	2-May
June	358.6	Post-flexion Mesolarvae	17.2	36	8-May
June	358.6	Post-flexion Mesolarvae	17.8	51	23-Apr
June	358.6	Post-flexion Mesolarvae	15.5	42	2-May
June	358.6	Post-flexion Mesolarvae	16.5	41	3-May
June	358.6	Post-flexion Mesolarvae	15.8	40	4-May
June	358.6	Post-flexion Mesolarvae	16.6	45	29-Apr

Month	River K	Development	Length (SL)	Annuli	Hatch Date
June	358.6	Post-flexion Mesolarvae	17	56	18-Apr
July	296.1	Post-flexion Mesolarvae	17.5	39	31-May
July	296.1	Post-flexion Mesolarvae	16.4	41	29-May
July	296.1	Post-flexion Mesolarvae	16.44	35	4-Jun
July	296.1	Post-flexion Mesolarvae	16.6	47	23-May
July	296.1	Post-flexion Mesolarvae	18	37	2-Jun
July	296.1	Post-flexion Mesolarvae	14.7	40	30-May
July	296.1	Post-flexion Mesolarvae	14.85	31	8-Jun

Table 5. Classification results (modified quadratic discriminate analysis) of *C. latipinnis* larvae. The classification scheme used the $\delta^{18}O‰$ from each fish to determine water temperatures experienced and compared that to environment temperatures present. Fishes that had a greater than 60% probability of belonging to mainstem, travertine, or tributary were assigned to that group. All fishes with less than 60% probability were assigned unknown.

Classification	**$\delta^{18}O‰_{(otolith)}$**	**$Pr_{(mainstem)}$ %**	**$Pr_{(travertine)}$ %**	**$Pr_{(tributary)}$ %**
mainstem	-12.9	99	0	1
travertine	-10.6	0	100	0
travertine	-11.1	0	100	0
mainstem	-12.8	98	0	2
unknown	-12.3	50	0	50
mainstem	-13.6	100	0	0
mainstem	-12.4	68	0	32
unknown	-12.3	51	0	49
mainstem	-12.4	63	0	37
mainstem	-13.1	100	0	0
mainstem	-13.8	100	0	0
travertine	-11.3	0	97	3
travertine	-10.8	0	100	0
mainstem	-13.7	100	0	0
travertine	-10.7	0	100	0
mainstem	-14.2	100	0	0
mainstem	-13.5	100	0	0
mainstem	-13.8	100	0	0

Classification	$\delta^{18}O‰$	$Pr_{(mainstem)\ \%}$	$Pr_{(travertine)\ \%}$	$Pr_{(tributary)\ \%}$
mainstem	-14.3	100	0	0
mainstem	-14.1	100	0	0
mainstem	-11.2	100	0	0
mainstem	-10.6	100	0	0
mainstem	-10.9	100	0	0
mainstem	-10	100	0	0
mainstem	-11.5	100	0	0
mainstem	-10.8	100	0	0
mainstem	-11.1	100	0	0
mainstem	-11.8	100	0	0
mainstem	-14.3	100	0	0
mainstem	-13	100	0	0
mainstem	-11.7	100	0	0
mainstem	-13.7	100	0	0
mainstem	-12.9	100	0	0
mainstem	-12.8	100	0	0
tributary	-12.4	36	0	64
mainstem	-13.8	100	0	0
mainstem	-13	100	0	0
mainstem	-13.2	100	0	0
mainstem	-13.4	100	0	0

Classification	$\delta^{18}O‰$	$Pr_{(mainstem)\ \%}$	$Pr_{(travertine)\ \%}$	$Pr_{(tributary)\ \%}$
mainstem	-14.8	100	0	0
mainstem	-14	100	0	0
mainstem	-13.5	100	0	0
mainstem	-13.5	100	0	0
mainstem	-14.2	100	0	0
mainstem	-14.1	100	0	0
mainstem	-14.1	100	0	0
mainstem	-13.3	98	0	2
travertine	-13.6	0	100	0
mainstem	-13.7	99	0	1
mainstem	-13.9	100	0	0
mainstem	-14.3	100	0	0
mainstem	-14.3	100	0	0
travertine	-13.3	0	100	0
travertine	-13.5	0	100	0
travertine	-13	0	100	0
travertine	-14.4	0	100	0
travertine	-13.3	0	100	0
travertine	-12.6	0	100	0
travertine	-11.5	0	100	0
travertine	-14.4	2	86	12

Classification	$\delta^{18}O‰$	$Pr_{(mainstem)\ \%}$	$Pr_{(travertine)\ \%}$	$Pr_{(tributary)\ \%}$
mainstem	-14.3	85	0	15
mainstem	-14.3	100	0	0
travertine	-14.6	1	84	15
unknown	-13.5	51	0	49
unknown	-12.2	50	0	50
unknown	-12.4	49	0	51
unknown	-13	44	5	52
unknown	-11.9	56	0	44
unknown	-8.8	51	0	49
unknown	-12.2	59	0	41
unknown	-12.1	44	0	56
unknown	-12	42	0	58
tributary	-12.8	32	0	68
unknown	-12.7	45	0	55
unknown	-13.3	49	0	51
unknown	-13.2	41	0	59
unknown	-13	59	0	41
mainstem	-12.5	72	0	28
unknown	-12.9	49	0	51
unknown	-12.6	46	0	54
tributary	-13	39	0	61

Classification	**$\delta^{18}O$‰**	**$Pr_{(mainstem)}$ %**	**$Pr_{(travertine)}$ %**	**$Pr_{(tributary)}$ %**
tributary	-12.8	30	0	70
unknown	-12.5	56	0	44
unknown	-12.4	46	0	54
travertine	-12.8	0	100	0

Table 6. Multiple linear regression analysis results for both initial (full) model and final (reduced) temperature experienced model. Multiple linear regression was used to evaluate the explanatory variables significantly affecting temperature experienced (response variable) by *C. latipinnis* larvae. Backward stepwise AIC model selection was performed to produce the final (reduced) model.

	Reduced Model – AIC stepwise				**Full Model – all variables**			
Variable	*Estimate*	*Std. error*	*t-value*	*Pr (>| t |)*	*Estimate*	*Std. error*	*t-value*	*Pr (>| t |)*
(Intercept)	5.86 e^{05}	2.08 e^{05}	2.812	0.006	7.842 e^{05}	3.952 e^{05}	1.984	0.052
River kilometer	0.0290	0.0138	2.103	0.039	0.0188	0.0255	0.738	0.464
Month	10.4200	2.8230	3.691	0.000	9.9380	3.9630	2.508	0.015
Hatch date	-0.2908	0.1034	-2.812	0.006	-0.3892	0.1962	-1.984	0.052
Annuli	-0.2415	0.1090	-2.216	0.030	-0.3022	0.1676	-1.803	0.077
Mainstem mean temp		-	-	-	-3.9790	12.7200	-0.313	0.756
Tributary mean temp	-	-	-	-	2.1860	3.2390	0.675	0.503
Travertine mean temp	-	-	-	-	0.4250	5.3150	0.08	0.937
Mainstem mean *Q*	-	-	-	-	-0.0958	0.1313	-0.73	0.469
Mainstem mean daily *Q* flux	-	-	-	-	0.2018	0.3317	0.608	0.545
Adjusted R^2				**0.67**				**0.65**
ANOVA	DF	*F-value*	***P-value***		DF	*F-value*	***P-value***	
	(4,62)	35.5	$2.09e^{-15}$		(9,57)	15.02	$3.62e^{-12}$	

Table 7. Multiple linear regression analysis results for both initial (full) model and final (reduced) $Temp_{(diff)}$ model. Multiple linear regression was used to evaluate the explanatory variables significantly affecting temperature difference between experienced and environment temperatures (response variable) by *C. latipinnis* larvae. Backward stepwise AIC model selection was performed to produce the final (reduced) model.

	Reduced Model – AIC stepwise				**Full Model – all variables**			
Variable	*Estimate*	*Std. error*	*t-value*	*Pr (>| t |)*	*Estimate*	*Std. error*	*t-value*	*Pr (>| t |)*
(Intercept)	124.08554	48.95071	2.535	0.013785	$4.67e^{04}$	$4.18e^{05}$	0.112	0.911
Annuli	0.1716	0.0422	4.064	0.0001	0.0156	0.0177	0.88	0.382
Mainstem mean temp	-14.8582	5.9934	-2.479	0.0159	-22.1700	13.4400	-1.649	0.105
Tributary mean temp	6.0568	2.3441	2.584	0.0121	6.3070	3.4240	1.842	0.071
Travertine mean temp	-	-	-	-	3.3580	5.6190	0.598	0.553
Mainstem mean *Q*	-0.1127	0.0445	-2.536	0.0138	-0.0853	0.1388	-0.614	0.542
Mainstem mean daily *Q* flux	-	-	-	-	-0.0397	0.3507	-0.113	0.910
River kilometer	-	-	-	-	-0.0064	0.0270	-0.236	0.814
Month	-	-	-	-	-0.2509	4.1900	-0.06	0.952
Hatch date	-	-	-	-	-0.0231	0.2074	-0.111	0.912
Adjusted R^2				**0.26**				**0.20**
ANOVA	DF	*F-value*	***P-value***		DF	*F-value*	***P-value***	
	(4,62)	6.814	0.001		(9,57)	2.866	0.007	

Table 8. Multiple linear regression analysis results for both initial (full) model and final (reduced) larval growth rate) model. Multiple linear regression was used to evaluate the explanatory variables significantly affecting growth rate (response variable) of *C. latipinnis* larvae. Backward stepwise AIC model selection was performed to produce the final (reduced) model.

	Reduced Model – AIC stepwise				**Full Model – all variables**			
Variable	*Estimate*	*Std. error*	*t-value*	*Pr (>| t |)*	*Estimate*	*Std. error*	*t-value*	*Pr (>| t |)*
(Intercept)	-6.31E+03	1.31E+03	-4.807	1.01E-05	$-6.54e^{3}$	$1.56\ e^{3}$	-4.193	$9.90\ e^{-5}$
Hatch date	0.0031	0.0007	4.316	0.0000	0.0032	0.0008	4.193	0.0001
Mainstem mean *Q*	-	-	-	-	-0.0013	0.0016	-0.821	0.4151
Mainstem mean daily *Q* flux	-0.0055	0.0166	-3.335	0.0014	-0.0041	0.0038	-1.068	0.2902
River kilometer	-	-	-	-	-0.0001	0.0003	-0.323	0.7476
Month	-	-	-	-	-0.0206	0.0274	-0.752	0.4554
Mainstem mean temp	0.213	0.0616	3.460	0.0010	0.2580	0.1559	1.655	0.1036
Tributary mean temp	-	-	-	-	0.0273	0.0401	0.682	0.4983
Travertine mean temp	-0.0106	-0.0200	-4.265	0.0001	-0.1474	0.0663	-2.222	0.0303
Experienced temp	-	-	-	-	0.0013	0.0020	0.661	0.5112
$Temp_{(diff)}$	-	-	-	-	0.0011	0.0019	0.557	0.5800
Adjusted R^2				**0.43**				**0.41**
ANOVA		DF	*F-value*	***P-value***		DF	*F-value*	***P-value***
		(4,62)	13.85	$3.96e^{-08}$		(10,56)	5.584	$9.99e^{-6}$

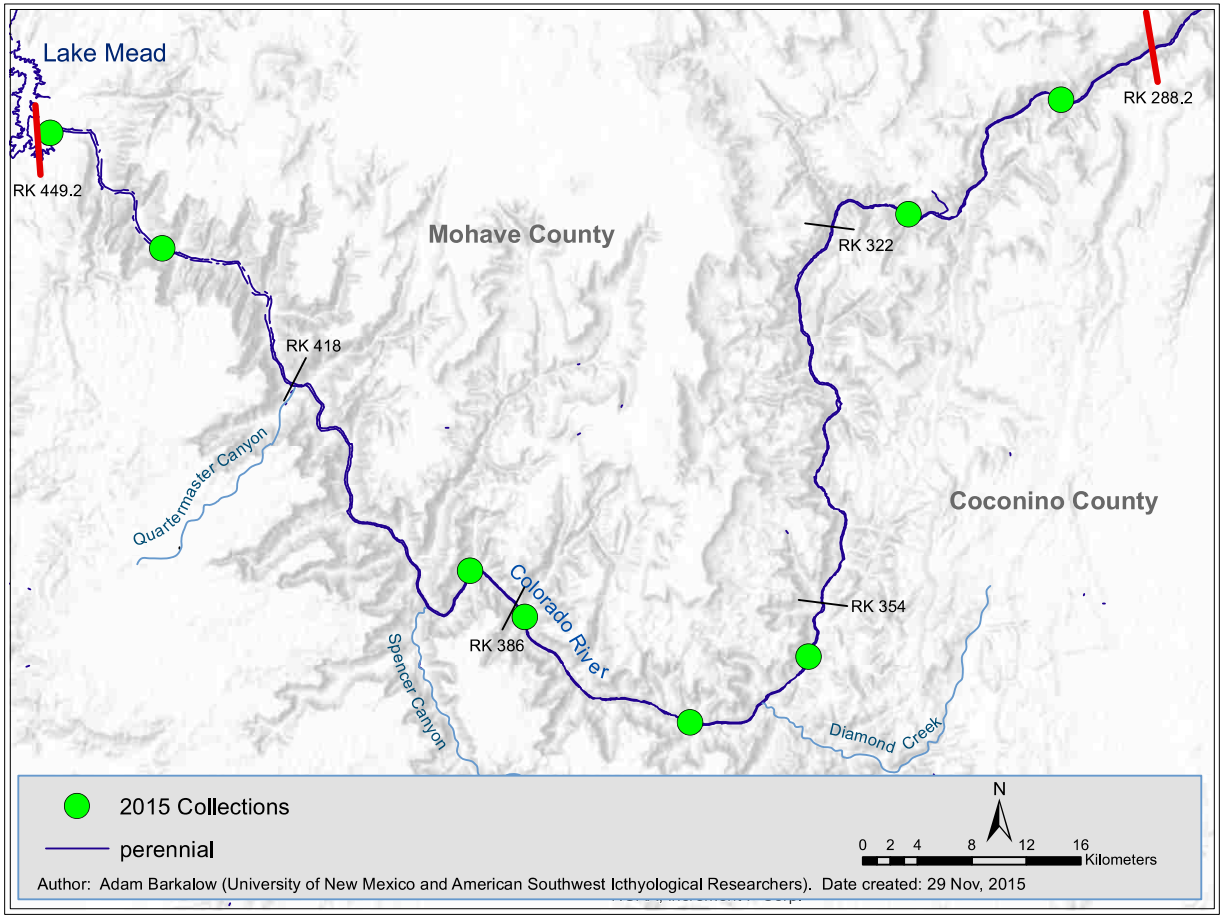

Figure 1. Study area encompassed 160 river kilometers (RK) of the Colorado River within Western Grand Canyon from RK 288.2 near Lava Falls rapid to RK 449.2 near Pearce Ferry. Larval fish collection sites are indicated by green circles and study area boundaries are indicated by red lines.

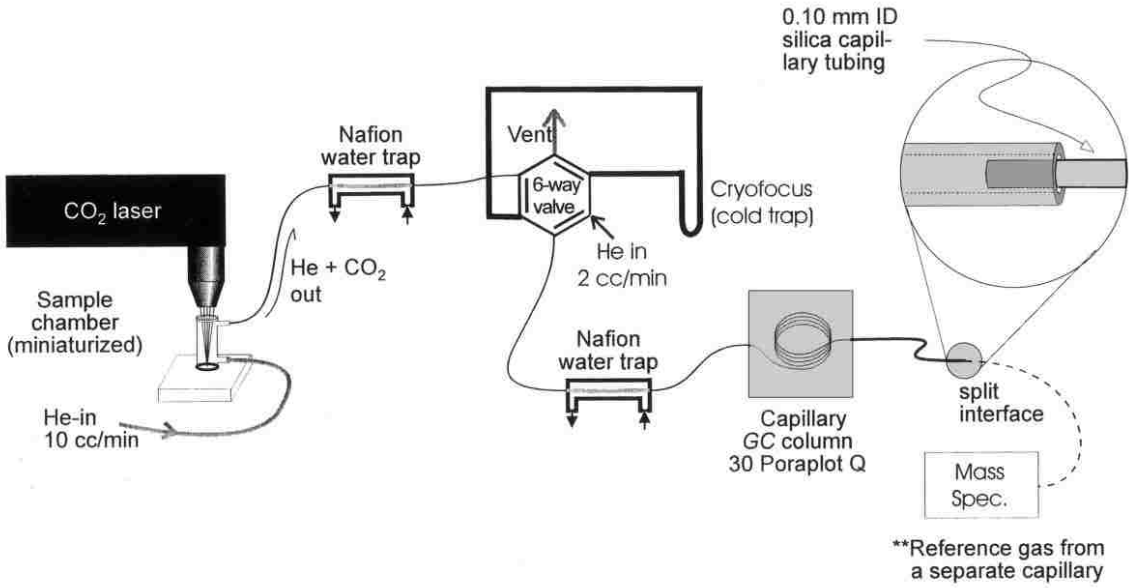

Figure 2. Diagram of GC-IRMS CO_2 acquisition for small carbonates samples from Sharp et al. 2000, used with permission.

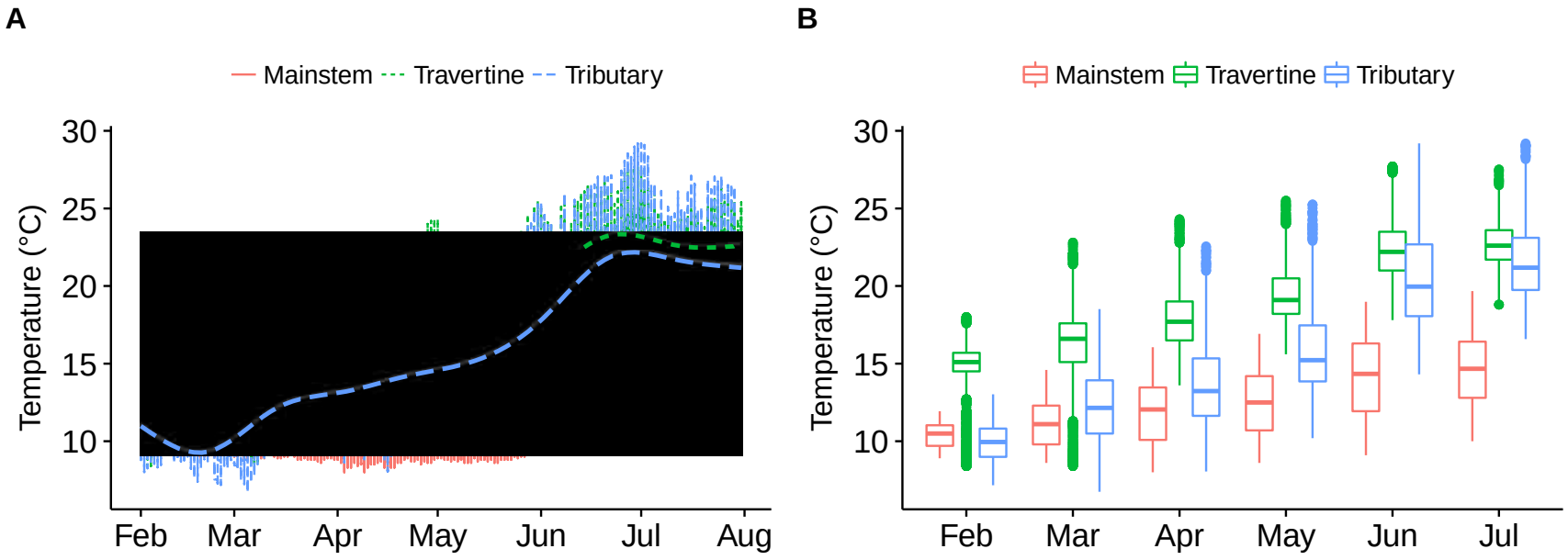

Figure 3. Water temperature data in degrees Celsius for the mainstem Colorado River, travertine tributaries, and tributary during the period when larval *C. latipinnis* were present. Data are presented in a continuous format in panel A and summarized in boxplots in panel B. Mainstem data are the combined mean daily temperatures recorded by USGS gages (09380000 - Colorado River at Lee's Ferry, AZ, 09402500 - Colorado River near Grand Canyon, AZ , , 09404120 - Colorado River above National Canyon near Supai AZ, 09404220 – Colorado River above Spencer Canyon at river mile 246. The travertine data is the combined mean daily temperatures recorded by USGS Gages(09402300 - Little Colorado River above the mouth near Desert View, AZ and 09404115 - Havasu Creek above the mouth near Supai, AZ). The tributary data is the mean daily temperature data recorded by USGS gage 09403000 - Bright Angel Creek near Grand Canyon, AZ.

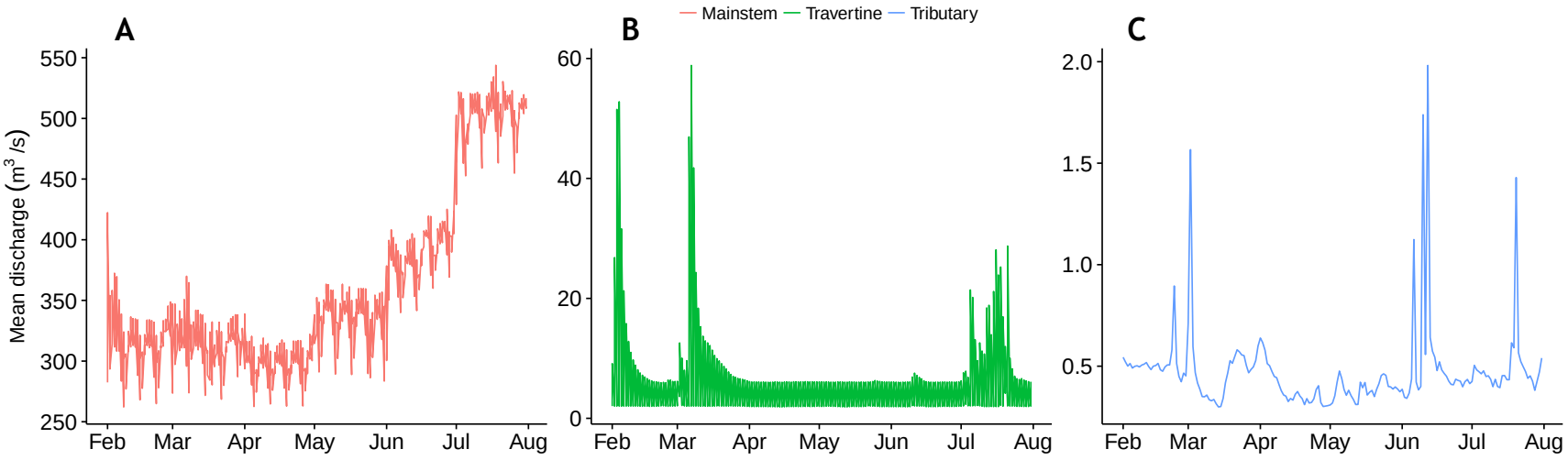

Figure 4 (A) Combined daily stream discharge recorded by USGS gages (09380000 - Colorado River at Lee's Ferry, AZ, , 09402500 - Colorado River near Grand Canyon, AZ 09404120 - Colorado River above National Canyon near Supai AZ, 09404220 – Colorado River above Spencer Canyon at river mile 246), (B). Combined daily stream discharge recorded by USGS gages 09402300- Little Colorado River above the mouth near Desert View, AZ and 09404115 - Havasu Creek above the mouth near Supai, AZ (C) Daily stream discharge recorded by 09403000 - Bright Angel Creek near Grand Canyon, AZ, All data presented in A, B, and C were recorded during the period of time when *C. latipinnis* larvae used in this study were present., The discharge data is presented in m^3/s and scaled independently for each system displayed (mainstem, travertine, and tributary).

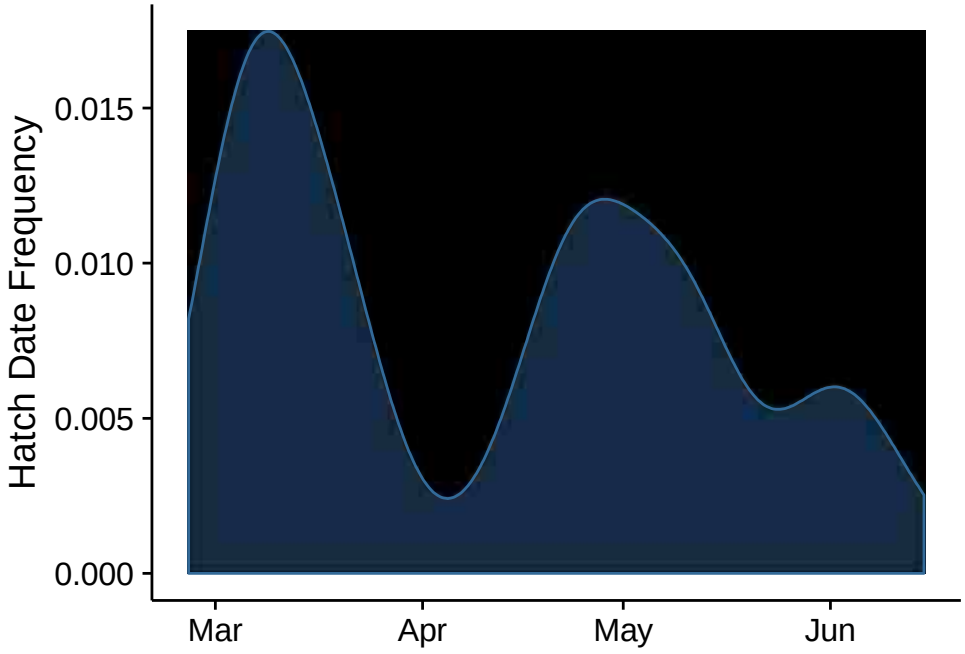

Figure 5. Frequency plot of the distribution hatch dates for *C. latipinnis* used in this study. Hatch dates were calculated by subtracting age in days from date of collection.

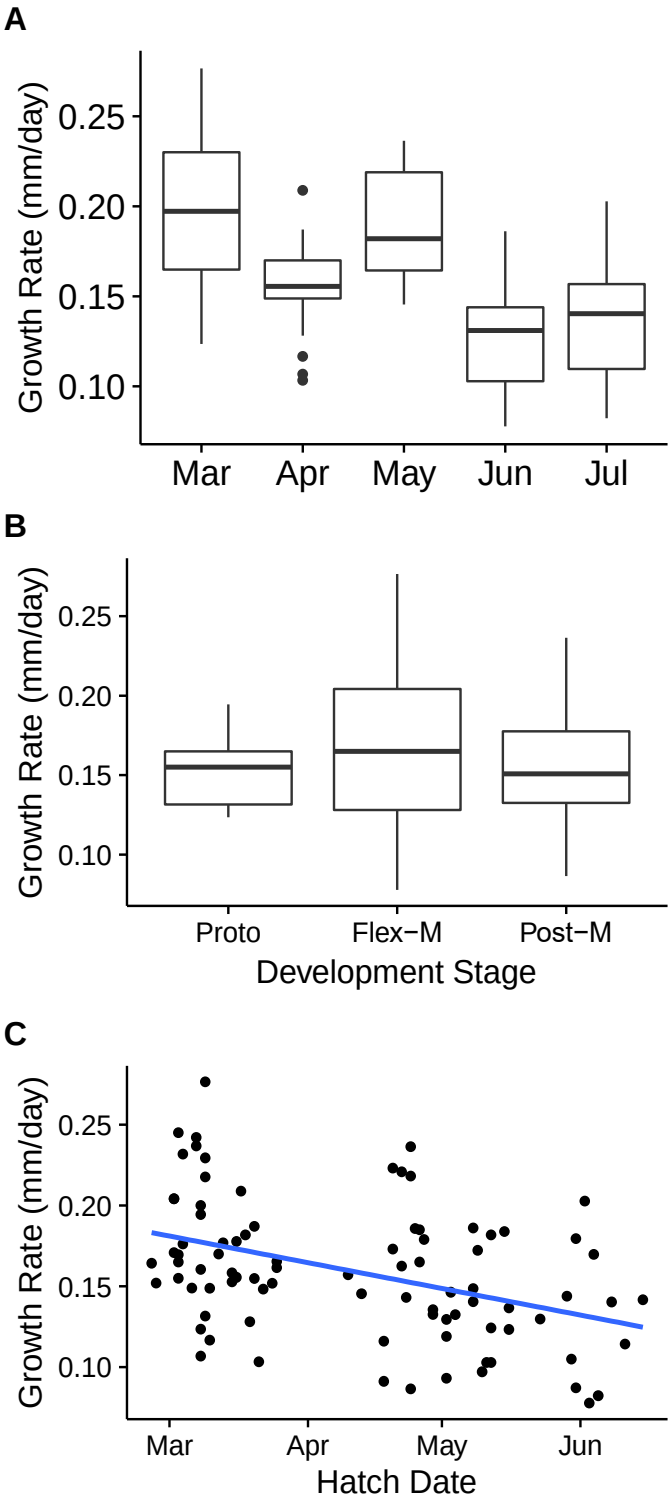

Figure 6. Comparison of growth rates (mm/day) of *C. latipinnis* displayed by month of capture (A), development stage (B), and hatch date (C).

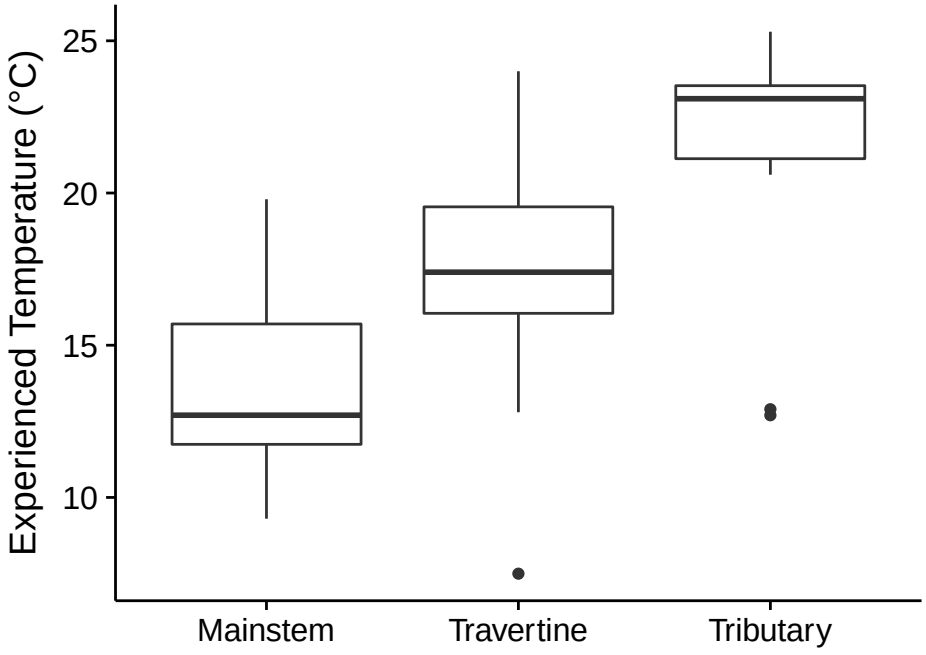

Figure 7. Comparison of temperature experienced estimated by analysis of oxygen isotope ratios of otoliths from *C. latipinnis* used in this study by the environment water (mainstem, travertine, tributary) into which they were classified. Black dots indicate outliers.

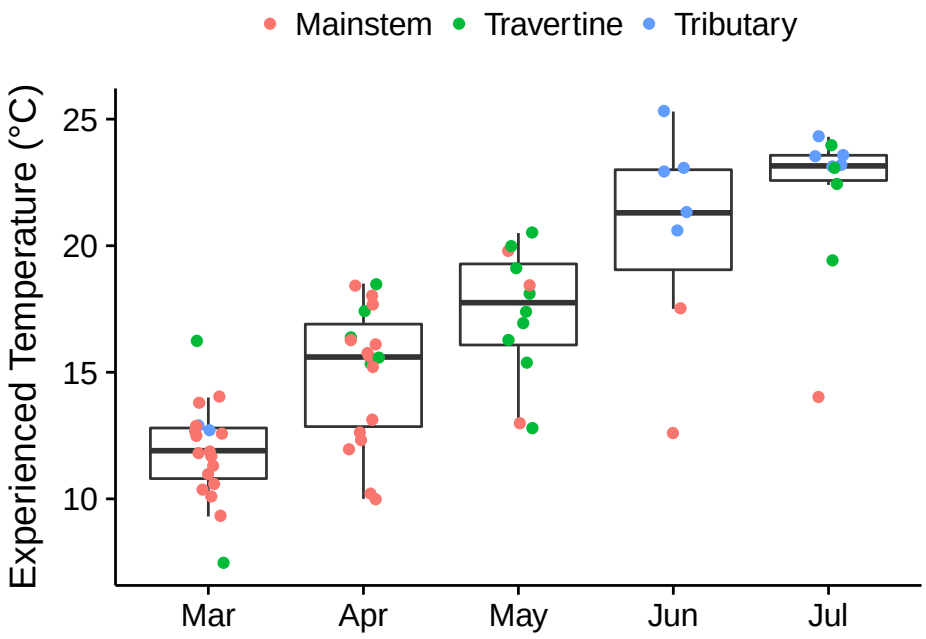

Figure 8. Comparison of temperature experienced (estimated by analysis of oxygen isotope ratios of otoliths from *C. latipinnis*) by month collected. Single points represent individual larvae that are classified to source water conditions (mainstem, travertine, tributary).

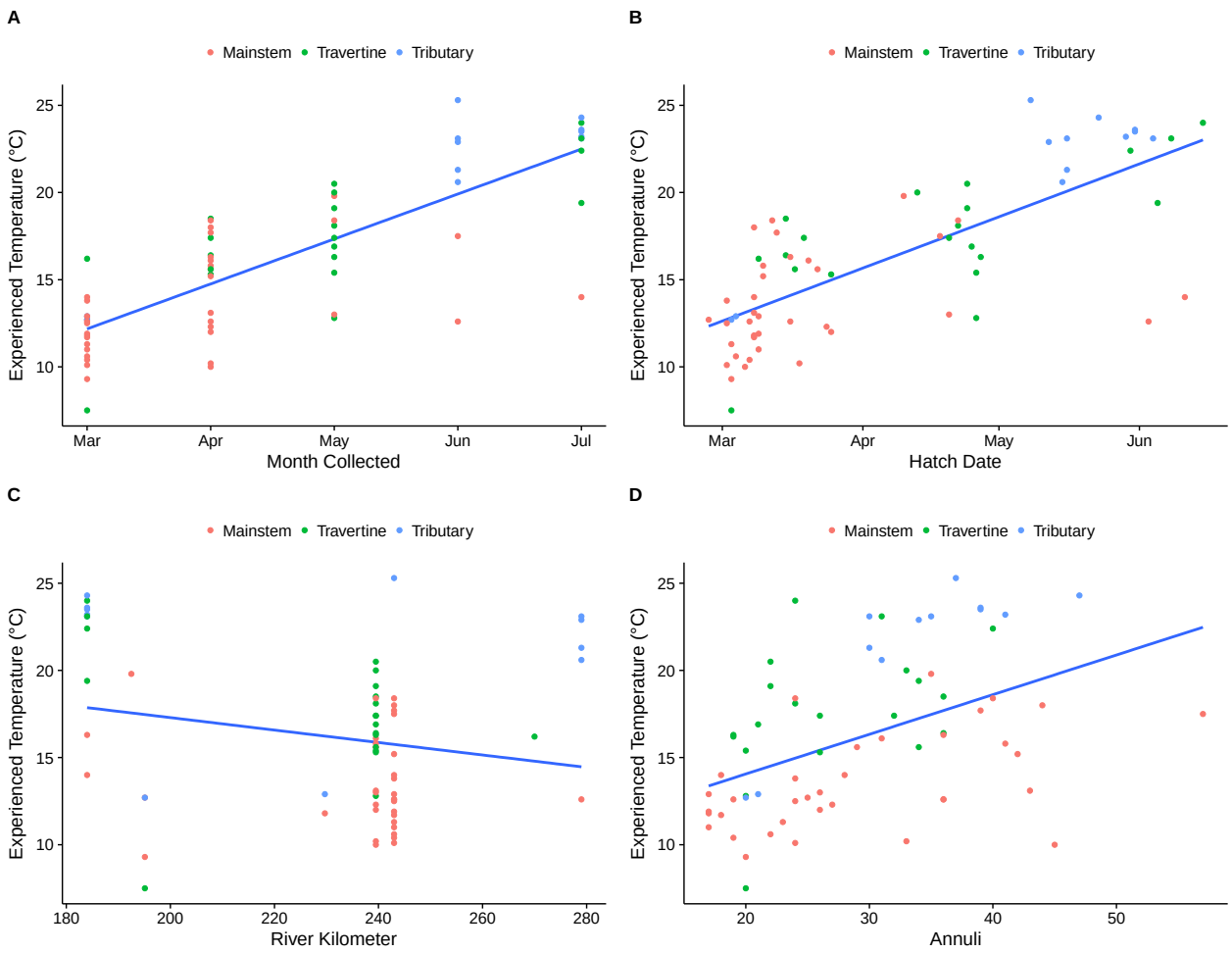

Figure 9. Relationship of temperature experienced (estimated by analysis of oxygen isotope ratios of otoliths from *C. latipinnis*) by variables remaining in the reduced (final) model, A) month collected, B) hatch date, C) river kilometer, and D) annuli. Single points represent individual larvae that are classified to source water conditions (mainstem, travertine, tributary)

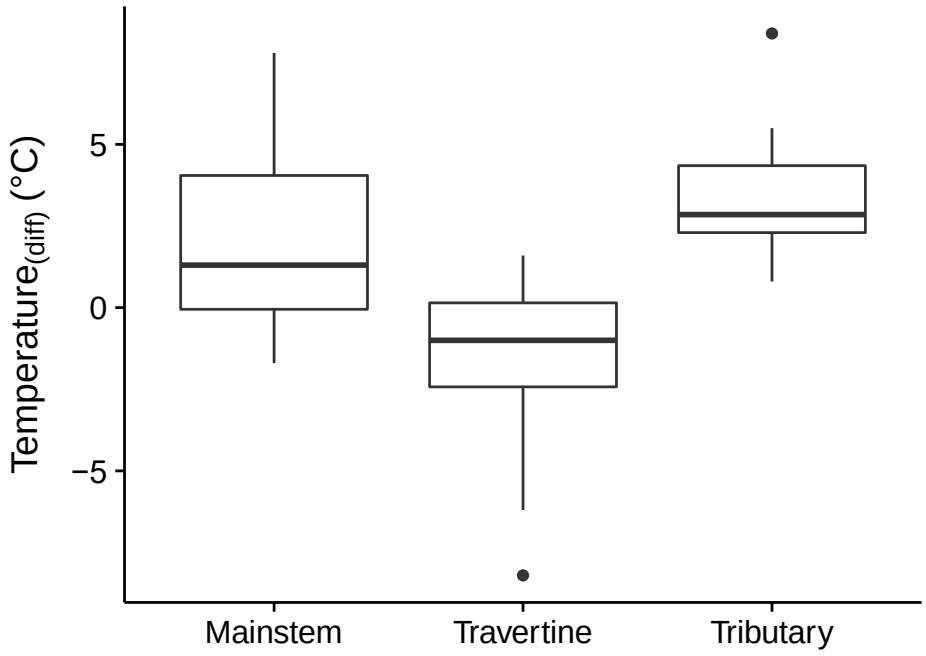

Figure 10. Comparison of $Temp_{(diff)}$ estimated by analysis of oxygen isotope ratios of otoliths from *C. latipinnis* classified by the source water (mainstem, travertine, tributary). Box plots are composed of data from each fish classified to individual environment waters. Black dots indicate outliers.

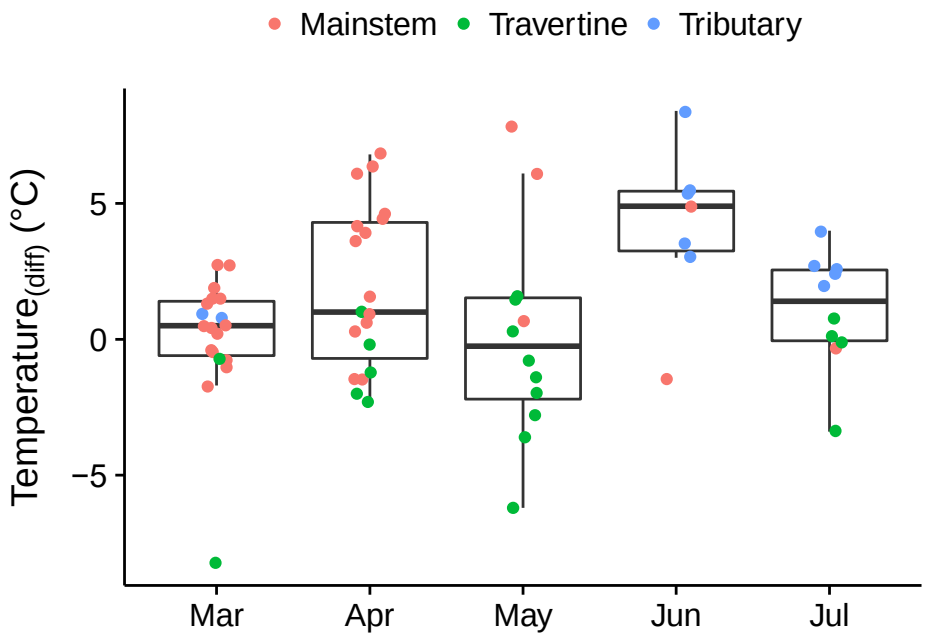

Figure 11. Comparison of $Temp_{(diff)}$ estimated by analysis of oxygen isotope ratios of otoliths from *C. latipinnis* by collection month. Points are color coded according to environment water and represent individual larvae classified to source water (mainstem, travertine, tributary).

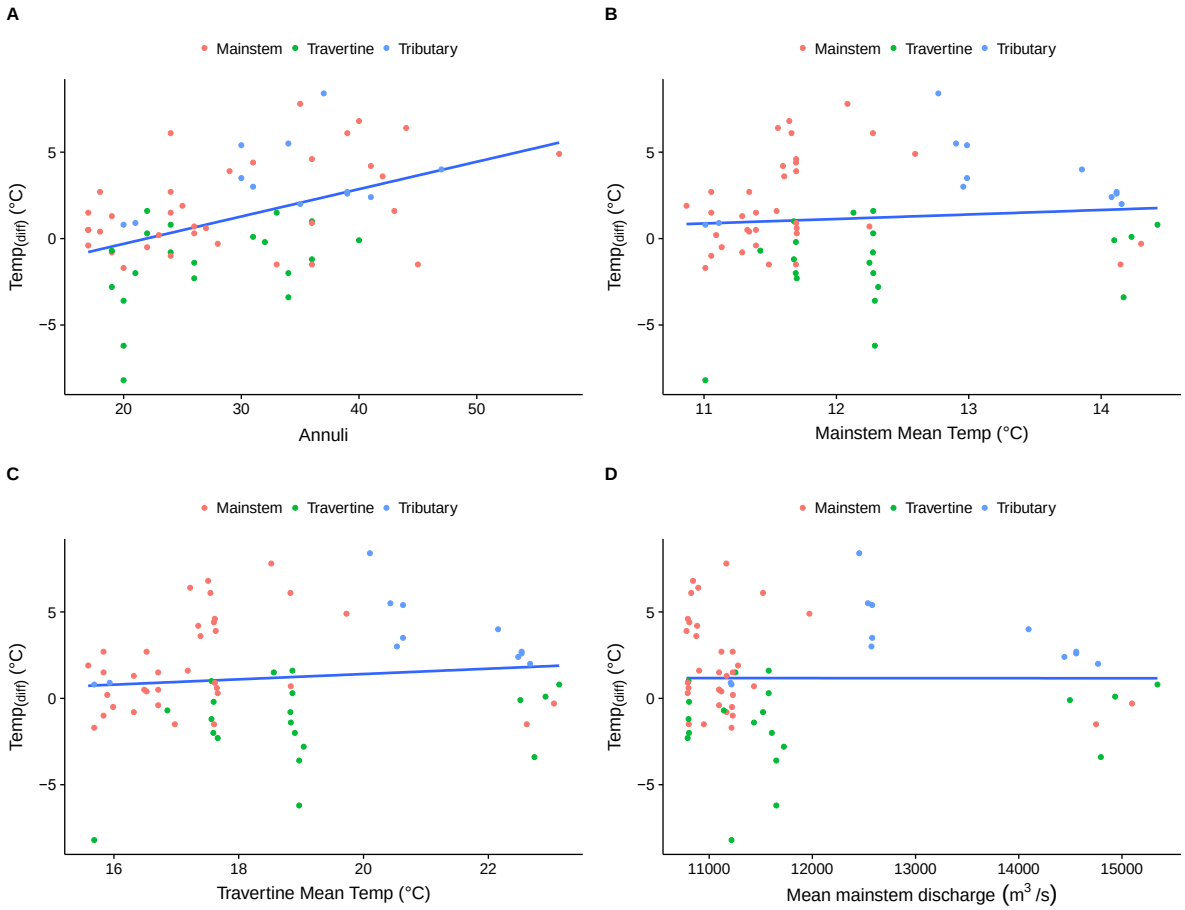

Figure 12. Relationship of Temp*(diff)* (estimated by analysis of oxygen isotope ratios of otoliths from *C. latipinnis* used in this study) by variables remaining in the reduced (final) model, A) annuli, B) mainstream mean temperature C) travertine mean temperature, and D) mean mainstem discharge. Points are color coded according to environment water and represent individual larvae belonging to the water (mainstem, travertine, tributary) in which they were classified.

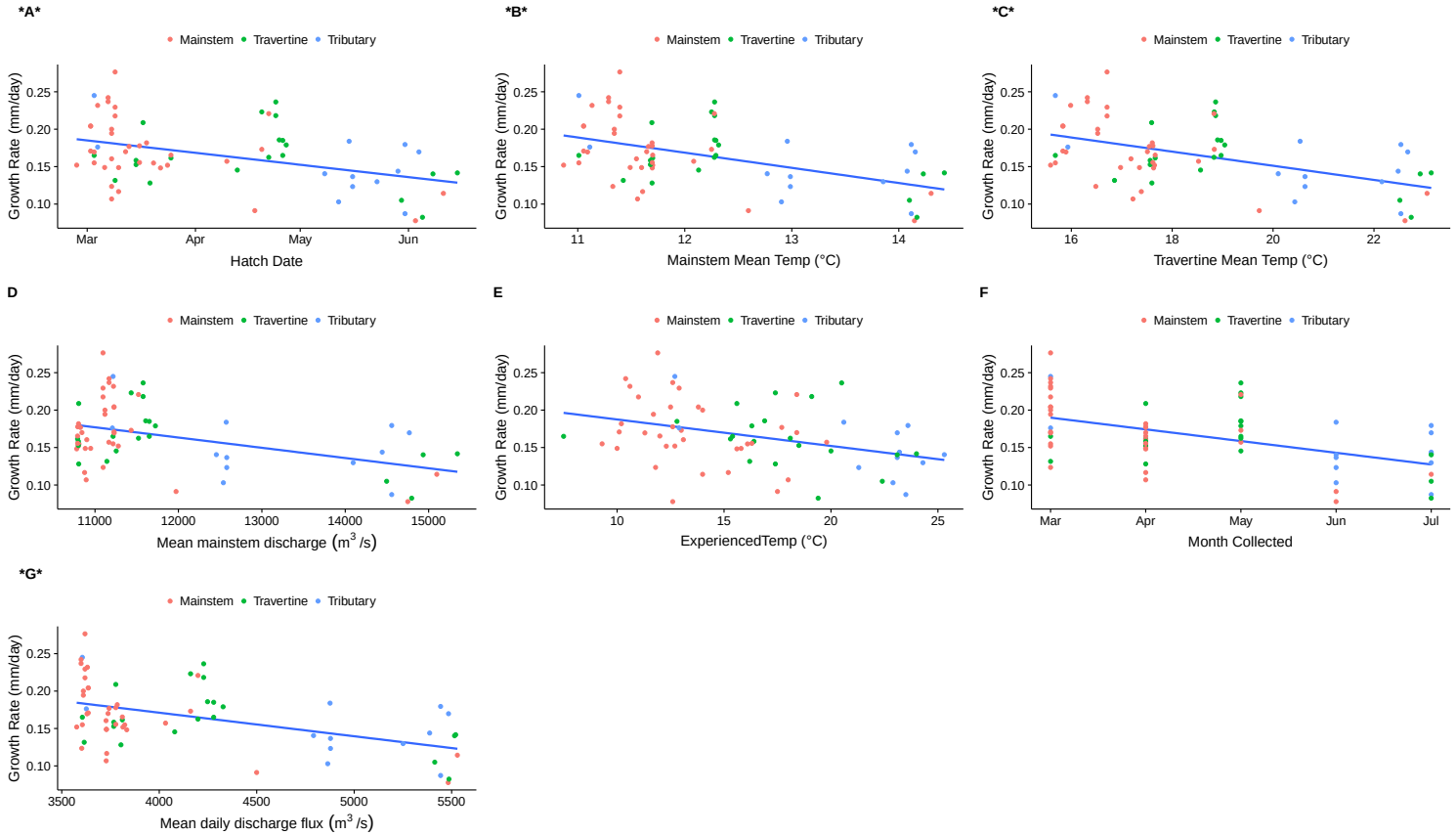

Figure 13. Relationship of growth rates from *C. latipinnis* used in this study by variables remaining in the reduced (final) model. A) hatch date, B) mainstem mean temperature, C) travertine mean temperature) mean mainstem discharge, E) experience temperature, F) month collected, and G) mean daily discharge. Points are color coded according to environment water and represent individual larvae belonging to the water (mainstem, travertine, tributary) in which they were classified.

This page was intentionally left blank.