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Thermal and Developmental Ecology of Pupfish, *Cyprinodon*

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THERMAL AND DEVELOPMENTAL ECOLOGY OF PUPFISH, *CYPRINODON*

By

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Bachelor of Science - Biological Sciences
Arizona State University
2011

A thesis submitted in partial fulfillment
of the requirements for the

Master of Science – Biological Sciences

School of Life Sciences
College of Sciences
The Graduate College

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Abstract

Pupfish from the genus *Cyprinodon* are among the most endangered groups of fishes on the planet, with nearly 40% of species being threatened with extinction. These pupfishes are often assumed to be the most temperature tolerant of all fish, coping with temperatures as low as 0 °C and as high as 44 °C. However, conflicting data exist and certain measures of thermal tolerance are not markedly higher in pupfish compared to common game fish. Pupfish egg production and growth has been known to be hampered at temperatures well below what they apparently experience in nature. I addressed why eggs fail to hatch at high temperatures using reciprocal transplant experiments and measurements of oxygen consumption over the course of development. Parental thermal history appears to influence successful hatching more than egg incubation temperature. Eggs which are reared at the ecologically relevant temperature of 33 °C exhibit altered and unusual oxygen consumption patterns compared to eggs incubated at a lower temperature. I employed a capture-mark-recapture study and field experiments in a dynamic and hypervariable creek environment (Salt Creek, CA) to determine whether individual pupfish which experience temperatures as high as 40 °C survive long-term. Pupfish in Salt Creek appear to colonize warm environments during the spring when conditions can be favorable for growth and reproduction; however, these fish risk death by desiccation and heat exposure if they remain in warm environments during the summer months.

Acknowledgements

I would like to thank my graduate committee Frank van Breukelen, Stanley Hillyard, Jenifer Utz, Jef Jaeger, and Daniel Thompson. Their guidance and mentorship was influential and important throughout this research. I would also like to thank lab members including Joseph Hunt, Chalisa Pansin, Maja Aleksic and Lori McFadden. Each of them dedicated many hours to this work. The guidance and collaboration of the fish biologists at Death Valley National Park was also irreplaceable. I owe a special thanks to Jeff Goldstein, Ambre Chaudoin, and Kevin Wilson. Most importantly, I would like to thank my wife, Jessica Phelps, who demonstrated patience, empathy, and understanding during the completion of this work. I would also like to acknowledge the dozens of undergraduate researchers who assisted with the care of these fish in addition to those already listed above.

Dedication

This thesis is dedicated to these incredible fish, the Devils Hole Pupfish; *Cyprinodon diabolis*, the Ash Meadows Pupfish, *Cyprinodon nevadensis mionectes*, and the Salt Creek Pupfish, *Cyprinodon salinus*. I would also like to dedicate this work to those who came before me who dedicated themselves to the preservation of these unique fish and the Desert Southwest I love; those names include James Deacon, Stanley Hillyard, Harry Truman (who first preserved Devils Hole as a National Monument in 1952), and Dianne Feinstein (who introduced legislation and then worked tirelessly to make Death Valley a National Park). It is my hope that countless future generations will be able to marvel at these incredible fish, and that this thesis work will help ensure their continued persistence in the most extreme aquatic habitats on earth.

Table of Contents

Abstract	iii
Acknowledgements	iv
Dedication	v
Table of Contents	vi
List of Tables	vii
List of Figures	viii
Chapter 1: Introduction	1
Chapter 2: Influence of parent thermal history and egg incubation temperature on survival and oxygen consumption of pupfish eggs.....	6
Chapter 3: Pupfish growth, reproduction, and survival in a hypervariable environment	29
Chapter 4: Conclusions and future directions.....	65
Bibliography	67
Curriculum Vitae	75

List of Tables

Table 1: Influence of parent acclimation temperature and egg incubation temperature on hatch success percentage	17
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Table 2: Results from one way ANOVA with Fisher's LSD post-hoc, comparison of equivalent developmental stages at 28 °C and 33 °C	20
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List of Figures

Figure 1: Experimental design for egg survival experiments.....	13
Figure 2: Schematic of method used for egg oxygen consumption experiments	14
Figure 3: Description and photos of the four developmental stages used for categorizing embryos prior to oxygen consumption experiments	14
Figure 4: Incubation temperature and time to hatch	17
Figure 5: Incubation temperature and length at hatch.....	18
Figure 6: Oxygen consumption across development for pupfish eggs incubated at two ecologically relevant temperatures.....	19
Figure 7: Oxygen consumption versus oxygen partial pressure for representative individuals.	21
Figure 8: Method used for determining apparent critical partial pressure of oxygen.....	22
Figure 9: Oxygen consumption pattern for a representative individual.....	23
Figure 10: Example of pupfish marking system.....	32
Figure 11: Photographs of the ephemeral and headwaters habitats of Salt Creek, CA	33
Figure 12: Spatial overview of Salt Creek, CA with sampling locations and habitats	34
Figure 13: Enclosure placed in Salt Creek for breeding and heat exposure experiments.....	36
Figure 14: Temperature profiles of headwaters and ephemeral habitats of Salt Creek, CA.....	39
Figure 15: Growth rate of recaptured pupfish in Salt Creek, CA	40
Figure 16: Daily egg production per female for breeding enclosures.....	41
Figure 17: Sex ratios of detected for moving and stationary fish.....	42
Figure 18: Total length of male and female moving and stationary fish	43
Figure 19: Detected movement distance versus total length	44
Figure 20: Detected individual and mean movements detected in Salt Creek, CA.....	45

Figure 21: Mean number of fish observed in ephemeral habitats in automated photographs each month in Salt Creek, CA	46
Figure 22: Mean number of fish observed versus temperature in automated photographs	47
Figure 23: Detected stationary and moving fish for February.....	50
Figure 24: Detected stationary and moving fish for March	51
Figure 25: Detected stationary and moving fish for April	52
Figure 26: Detected stationary and moving fish for May	53
Figure 27: Detected stationary and moving fish for June	54
Figure 28: Detected stationary and moving fish for July	54
Figure 29: Detected stationary and moving fish for August	55
Figure 30: Detected stationary and moving fish for September	56
Figure 31: Survival during and following heat exposure.....	57
Figure 32: Photograph of fish kill observed in intermediate section of Salt Creek.....	58

Chapter 1: Introduction

Pupfish from the genus *Cyprinodon* are found in some of the most extreme temperature regimes fish are known to inhabit. *Cyprinodon julimes*, native to a modified spring system outside of Julimes, Mexico apparently experiences temperatures as high as 44 °C and no lower than 38 °C in nature (Carson et al., 2014). While the habitat of the Salt Creek Pupfish, *Cyprinodon salinus*, in the Salt Creek watershed of Death Valley National Park, CA can range from as low as 0 °C to above 40 °C depending on temporal and spatial factors (Brown & Feldmeth, 1971). The main pool of Devils Hole, home of the critically endangered Devils Hole Pupfish, *Cyprinodon diabolis*, rarely falls below 33 °C and portions of the water system can see temperatures as high as 38 °C during summer (Hausner, Wilson, Gaines, Suárez, & Tyler, 2013; Riggs & Deacon, 2002).

Surprisingly, despite their presence in many thermal spring systems, one measure of pupfish upper thermal limit does not appear to be much higher than those of familiar sport fish (Brown & Feldmeth, 1971; Currie, Bennett, & Beitinger, 1998). This despite the often touted assumption that pupfish are well adapted to hot temperatures (Brown & Feldmeth, 1971; C.H. Martin, Crawford, Turner, & Simons, 2016). The primary measurement of upper thermal limits measured in pupfish has been done by gradually warming the temperature (usually 0.2-1.0 °C per minute) until the fish die (e.g Brown & Feldmeth, 1971). This measure of upper thermal limit is referred to as the critical thermal maximum (CTM) and reflects the acute limits for survival during heat exposure. Depending on the species, time of year, and acclimation temperature the CTM of *Cyprinodon* pupfishes has been measured to range between 39-43 °C (Brown & Feldmeth, 1971). Though it should be noted that there has been no test of upper thermal limits for *Cyprinodon julimes*, which apparently does not have access to temperatures below 38 °C in

nature (Carson et al., 2014). Channel catfish and largemouth bass, though native to more temperate water systems, have been recorded as having CTMs as high as 42.1 °C and 41.8 °C, respectively, which is within the range of thermal maxima measured for pupfish (Brown & Feldmeth, 1971; Currie et al., 1998). Laboratory measurements of upper thermal limits show pupfish are not markedly more tolerant of high temperatures than familiar sportfish; yet adult pupfish are often observed in nature at temperatures well above 40 °C with no observed ill-effects. Importantly, it is not known whether the pupfish which were observed at temperatures > 40 °C survive. Some scientists still assume that pupfish are remarkably heat tolerant compared to other fish (C. H. Martin et al., 2016). However, chronic heat exposure affects organisms differently than an acute heat exposure (e.g. CTM). For instance, despite having CTM's equivalent to those of pupfish, channel catfish often die when exposed to temperatures above 32 °C for more than 24 hours (Currie et al., 1998). This measure of thermal maximum when fish are exposed to high temperatures for long periods (>24 hours) until death is often referred to as Incipient Upper Thermal Limit (IUTL). The effects of prolonged heat exposure have never been tested in pupfish.

While adult pupfish can apparently survive at least short periods above 40 °C, measured thermal tolerance of developing pupfish eggs is markedly lower. Successful reproduction and recruitment in the group is nearly non-existent above 32 °C (Deacon, Taylor, & Pedretti, 1995; Gerking & Lee, 1983; Minckley & Deacon, 1973). Growth is also hampered when pupfish are reared at temperatures above 32 °C (Gerking & Lee, 1983). The low reproduction and growth rate at these temperatures raises the question of whether pupfish are well adapted to the high temperatures that typify many of their habitats in nature. Despite years of research in the group, questions linger regarding their upper thermal tolerances for prolonged exposure. It is unclear

why pupfish eggs fail to develop at temperatures well below the CTM of adult fish and whether there are fitness consequences for adult pupfish exposed to prolonged high temperatures in nature. Over 40% of species in this group are threatened with extinction, so better elucidation of the thermal limits of these fish is warranted to inform effective conservation efforts (International Union on the Conservation of Nature, IUCN.org).

While numerous past studies have noted poor hatch success in *Cyprinodon* fishes above 32 °C, few if any have addressed underlying causes of hatch failure at high temperature. Past studies have reared adults and incubated eggs at the same temperature (Deacon et al., 1995; Gerking & Lee, 1983). Importantly, failure during oogenesis or spermatogenesis might be an underlying cause of low hatch success above 32 °C so separating parent thermal history from egg incubation temperature will prove informative. Alternatively, per the oxygen and capacity limitation of thermal tolerance hypothesis, increased metabolic requirements during development at high temperatures may make it difficult for the eggs to obtain enough oxygen from their localized environment to sustain growth (Pörtner, 2001). Examination of the oxygen requirements of eggs during development at high temperatures would lend insight into whether oxygen limitations play a role in reduced hatch success at temperatures above 32 °C.

Anecdotal observations have played a large role in the assumption that *Cyprinodon* fishes are remarkably heat tolerant. Unfortunately, it is not clear if prolonged or acute exposure to temperatures near CTM of pupfish (>35 °C) has any fitness consequences due to the methodologies used in past experiments. Measurement of CTM has limited ecological relevancy. Pupfish are likely exposed to temperatures above 35 °C for several hours per day, and this exposure may occur over the course of weeks. Possibly, such heat exposure might have medium or long term deleterious impacts on pupfish. CTM measurements, while useful, represent a

measure of upper thermal limit following a very short term exposure (Currie et al., 1998; Lutterschmidt & Hutchison, 1997). Advancements in marking methods now enable scientists to mark and recapture individual pupfish to test whether the pupfish which are observed in areas which exceed 35 °C remain in effective populations. It is possible that pupfish habitats which experience such temperature extremes could constitute sink environments e.g. pupfish observed at such high temperatures might not survive long-term (Amarasekare, 2004; Levin, 1974).

The purpose of my studies was to bridge the gaps in existing knowledge regarding the upper thermal limits of *Cyprinodon* fish. I utilized captive populations of *Cyprinodon diabolis* (made available due to concerns about possible hybridization) and its close sister species *Cyprinodon nevadensis mionectes* to conduct laboratory experiments on developing eggs. Rather than incubating eggs solely at the same temperature as parent fish were reared, I separated the two and quantified the effects of both on survival. I also documented the effects of incubation temperature on time to hatch and size at hatch. Finally, I used a novel method to measure oxygen consumption in individual pupfish eggs over the course of development at two ecologically relevant rearing temperatures. This method represents an improvement over past methods to assay oxygen consumption in fish eggs and gives us unique insight into the energetics of development at high temperatures in an endangered fish.

I employed a capture-mark-recapture study on *Cyprinodon salinus* in the hypervariable environment of Salt Creek in Death Valley National Park, CA to understand whether fish marked in the warmest portions of the creek remain in the effective population. In this creek system pupfish are exposed to wildly different thermal regimes depending on their spatial location within the stream. Some fish that colonize the lower, ephemeral portions of the creek could routinely experience temperatures above 35-40 °C for days or weeks. Conversely, fish which

remain in the headwaters of Salt Creek are unlikely to experience temperatures above 30 °C (Figure 16, unpublished National Park Service Data). I conducted this study using a marking system that allowed monitoring of individual pupfish, rather than batch marks, as has been used in the past (e.g. Dzul, Quist, Dinsmore, Gaines, & Bower, 2013). I augmented the mark-recapture monitoring with field experiments to understand whether egg production was significantly lower in the warmest parts of the creek and to examine the medium-term impacts of an ecologically relevant heat exposure on *C. salinus*. Monitoring longer term survival following exposure to fluctuating high temperatures present in native habitats could provide ecological insights that measuring CTM or IUTL might not.

Chapter 2: Influence of parent thermal history and egg incubation temperature on survival and oxygen consumption of pupfish eggs

This chapter is a slightly modified version of a previously published manuscript entitled Oxygen Consumption is Limited at An Ecologically Relevant Rearing Temperature in Pupfish Eggs (Jones et al., 2016).

In this chapter I use the plural nominative, “we” to identify myself and coauthors when writing in the first person, reflecting the collaborative nature of the work and as the chapter is presented in the literature.

Introduction:

Climate change has the potential to not only dramatically shift species distributions but also to threaten endemic species which cannot modify their range or easily colonize new environments (Hausner et al., 2014). The pupfish from the genus *Cyprinodon* are one such group, with many species restricted to small water systems or features (Hillyard, 2011; Hillyard, Podrabsky, & van Breukelen, 2015). Ash Meadows National Wildlife Refuge contains ~30 warm springs and seeps within a 162 km² area of the Mojave Desert. These wetlands provide critical habitat to a number of endemic and closely related pupfish species (Echelle & Dowling, 1992; Williams & Sada, 1985). The springs and seeps generally range from 21 °C to 35 °C (Hillyard, 2011). These springs are very diverse in size and flow. The result being that some waters may be well buffered against changes in temperature whereas other waters get very cold during winter when air temperatures are below 0 °C and very warm in summer when air

temperatures can exceed 45 °C. Many of these springs are inhabited by the Ash Meadows pupfish (*Cyprinodon nevadensis mionectes*). Another species found here is the critically endangered Devils Hole pupfish, *Cyprinodon diabolis*, which is restricted only to Devils Hole (Wales, 1930; Hausner et al., 2014). Devils Hole is a 3.5 x 22 m crack into the underlying aquifer within the Ash Meadows complex of springs in a detached portion of Death Valley National Park. While the main pool of Devils Hole is quite deep, in excess of 130 meters, most reproductive activity is limited to a shallow shelf which has a water depth of less than 1 m (Riggs & Deacon, 2002). The water in Devils Hole is characterized by features that might be considered inhospitable to most fish including high temperatures (main pool is 33.2 - 33.9 °C year round), low oxygen availability (~ 2 mg/l most of the year), and low primary productivity (Hausner et al., 2013; Karam, Parker, & Lyons, 2012; Wilson & Blinn, 2007). While the main pool in Devils Hole is remarkably stable thermally, the shallow shelf may experience extremes in temperature. For instance, typical daily minimum temperatures of 26 to 29 °C have been reported from areas of the shallow shelf during winter (Riggs & Deacon, 2002). Extreme low tides resulted in an excursion to a low temperature of 12.8 °C from December 15-17 2001 (Riggs & Deacon, 2002). Conversely, temperatures as high as 38 °C, but more commonly 34-36 °C, have been reported on the shallow shelf during the summer months (Hillyard, 2011; Riggs & Deacon, 2002).

Following resolution of a legal battle over water rights that imperiled the species, the estimated population size of *C. diabolis* increased to a level of 200-500 adult fish (Andersen and Deacon, 2001; Riggs and Deacon, 2002). However, a gradual decline, beginning in 1996, brought the population to a low point of only 35 adult individuals in 2013 (Hausner et al., 2013).

Although, the most recent census (April, 2016) found modest recovery with over 100 individuals counted, the species continues to be in a precarious situation.

Low recruitment of juveniles to the adult population could be a major factor in the recent population decline of *Cyprinodon diabolis* (Hausner et al., 2013). Measured during acute exposure to increasing temperature, adult pupfish have a critical thermal maximum of ~ 40 °C (Brown & Feldmeth, 1971). However, studies suggest a more narrow thermal tolerance of developing eggs and very low egg viability at temperatures typical of Devils Hole (Deacon et al., 1995; Gerking & Lee, 1983; Shrode & Gerking, 1977). It is unclear if the low hatch success rate at 32-33 °C is primarily due to parental effects or whether developing embryos simply die when exposed to these temperatures. While successful recruitment is thought to take place mainly in the spring, some breeding takes place year-round in Devils Hole and in other Ash Meadows springs. Depending on the time of year and spatial location in Devils Hole or other habitats, pupfish eggs may experience highly variable temperatures (Riggs & Deacon, 2002; Shrode & Gerking, 1977). Unlike adult fish, developing eggs are unable to choose between different thermal environments until they hatch. No data are available for how conditions typical of Devils Hole (low oxygen tensions and high temperatures) might affect the oxygen requirements of pupfish eggs.

The availability of a captive refuge population of fish *derived* from *C. diabolis* and a population of its closest relative, *C. nevadensis mionectes*, allowed us to examine how temperature affected oxygen consumption, hatching success, and development of eggs that were reared at environmentally-relevant temperatures. We use these data to address how a changing environment due to global climate change may affect the conservation of these endangered fishes.

Methodology

Fish husbandry

Cyprinodon nevadensis mionectes and fish derived from a refuge population of *Cyprinodon diabolis* were maintained at the University of Nevada, Las Vegas beginning in 2010. Importantly, *C. n. mionectes* is likely more closely related to *C. diabolis* than it is to other *C. nevadensis* subspecies (Echelle & Dowling, 1992). Refuge fish were made available due to concerns about a potential hybridization event with *C. n. mionectes* (A. P. Martin, 2005; A. P. Martin, Echelle, Zegers, Baker, & Keeler-Foster, 2012). Briefly, the Point of Rocks Spring refugium (water temperature ~ 28 °C) was established using *C. diabolis* from another refugium in 1991 with additional pupfish added from Devils Hole (Karam et al., 2012). The population in the concrete pool was never very large, e.g., 77 pupfish in 2000. In 2005, 23/110 pupfish were found to possess a pelvic fin, a feature that *C. diabolis* typically lacks. However, pelvic fin development in pupfish is plastic and temperature sensitive (Lema & Nevitt, 2006). Other sources refer to these fish as hybrids between *Cyprinodon diabolis* and *Cyprinodon nevadensis mionectes* (Feuerbacher, Mapula, & Bonar, 2015). We refer to them here as “Refuge fish” since data supporting or refuting a hybridization event are non-conclusive and such debate is outside of the scope of this paper. Populations of fish from each species were maintained at ecologically relevant temperatures of 28 °C or 33 °C in fully aerated conditions. We chose 28 °C and 33 °C because the original refuge temperature at Point of Rocks Spring was 28 °C and Devils Hole is 33.2 to 33.9 °C in the main pool. Similar water temperatures are available in many of the other Ash Meadows springs (Brown & Feldmeth, 1971; Karam et al., 2012). Fish were fed Rio Grande Silvery Minnow Fish Food daily. Fish were provided an amount of food that they could

consume within 10 minutes. Temperature of the aquaria (40 liters) was checked daily. Partial or full water changes were done as required using dechlorinated water, though care was taken to avoid over-sanitization as from our experience microbiological communities present in the aquaria are vital for health of the fish. Average daily populations of approximately 200 fish per species were maintained. Aquaria typically contained ~20 adult pupfish for breeding purposes. Fish had been maintained and bred in our facility since 2010. As indicated later, breeding at 33 °C is very difficult and our limited success means that most fish were from 28 °C acclimated parents.

Egg collection, husbandry, and survivorship

Adult pupfish were acclimated to 28 °C or 33 °C for at least 3 weeks prior to any egg collection. Small squares (approximately 100 cm²) of a brown multicolor carpet were introduced into the aquaria. Pupfish were allowed to breed and carpets were removed several hours after introduction. The use of multicolor carpets appeared to reduce oophagy by the adults. We manually picked through the carpets to collect eggs. Carpets were then dried before reintroduction into the tanks to prevent uncollected eggs from developing.

Collected eggs were divided into treatment groups to discourage collection of data from a single clutch. Eggs were then incubated at either 28° or 33 °C (Figure 1. Incubation typically consisted of individual eggs in 6 well-plates in temperature-controlled incubators (e.g. for hatch success) or incubation in groups of up to 20 eggs in mesh-bottomed cylinders (e.g. for rearing of eggs for use in oxygen consumption experiments). All eggs were incubated in fully aerated conditions. Eggs were monitored daily for development and hatching. Upon hatch, the larvae were measured for total length (snout to tail tip) and head width.

Extremely low availability of viable eggs from 33 °C acclimated parents required that all subsequent described experiments used only eggs from 28 °C acclimated parents. These eggs were incubated at either 28 °C or 33 °C beginning on the first day of development.

Measurement of oxygen consumption

Prior to each experiment, eggs were rinsed in a 0.01% bleach solution to minimize bacterial activity and then categorized into one of four developmental stages based on observed morphology (Figure 3). Stage one eggs were comprised of early embryos from fertilization to gastrulation. Stage two eggs had a neural tube but lacked eyes and a beating heart. Stage three eggs had a beating heart and were in the pharyngula stage. In this stage, segmentation and blood circulation are present. Stage four eggs were primarily differentiated based on the presence of a well-developed and colored iris as the egg neared hatch and a well-developed heart. In general, our staging system corresponded to the following hours of described zebrafish development, (Stage 1 = 0 – 5.25 h; Stage 2 = 5.25 - 24 h; Stage 3 = 24 - 28 h; and Stage 4 = 48 - 72 h of zebrafish development; Kimmel, Ballard, Kimmel, Ullmann, & Schilling, 1995). Our observations suggest pupfish progress more slowly through the earlier developmental stages than do zebrafish.

We developed a system that allowed for measurement of oxygen consumption in a single egg (Figure 2). A single egg was placed into a depression slide with a Redeye oxygen sensor patch (a ruthenium complex that when excited exhibits a fluorescence decay proportional to oxygen availability, Ocean Optics, Dunedin, FL). A glass microscope slide and vacuum grease were used to seal the chamber. The metabolic chamber was placed onto a stationary Eppendorf Thermomixer R (Hamburg, Germany) with plate style attachment set to either 28 or 33 °C for

precise temperature control. A temperature probe was affixed to the plate style attachment and was kept in contact with the side of the metabolic chamber for precise temperature readings. The Thermomixer R is a Peltier-device based instrument and maintains very precise temperature control. The metabolic chamber had a total volume of 238 μ l. Prior to each experiment, the NeoFox system (Ocean Optics, Dunedin, FL) was calibrated using aerated water and water treated with sodium sulfite (depletes oxygen). Preliminary experiments using N₂-bubbled water demonstrated stable partial pressures of oxygen (PO₂) values over time, indicating that the system was closed and no air leaks were present.

Oxygen consumption was measured from the decrease in PO₂ and corrected for oxygen solubility at the experimental temperature and for the volume of the chamber with the egg. Oxygen consumption rates were calculated from the initial linear portion of the decline in PO₂ and consisted of the first hours' worth of data (when PO₂ values were high e.g. > 100 torr).

Critical PO₂ is defined as the partial pressure of oxygen where oxygen availability begins to limit oxygen consumption. We determined the relationship between oxygen consumption and PO₂ by determining oxygen consumption for 10 min periods and comparing that with the PO₂ at the start of that time period. As evidenced later, some pupfish eggs oxyconform. Therefore, there is no critical PO₂, *per se*. For the sake of comparison, we calculated an apparent critical PO₂ by fitting linear regressions to data points for the initial decline and the subsequent leveling off of oxygen consumption. Apparent critical PO₂ was calculated as the intercept between these two lines.

Statistical analyses were done using Fisher's LSD One-Way ANOVA comparisons for oxygen consumption, hatch size, and time to hatch experiments and chi-squared contingency tables for egg survival. Significance was determined at $p < 0.05$. Unequal sample sizes exist due

to the variable reproductive nature of the fish and due to cases in which an egg died while in the chamber (e.g. no heart beat following experiment or failed to develop further). Those data were discarded since it was impossible to tell exactly when the egg died while it was in the chamber. We used one way ANOVAs with Fisher's LSD post hoc tests to determine statistical differences. Unequal sample sizes have a limited effect on these ANOVAs. Nevertheless, we confirmed the inferences by using Wilcoxon Mann Whitney Tests when comparison sample sizes were unequal.

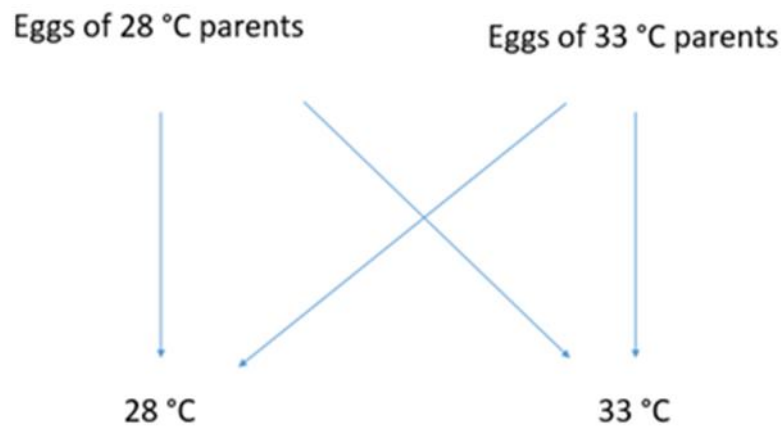


Figure 1: Experimental design for egg survival experiments

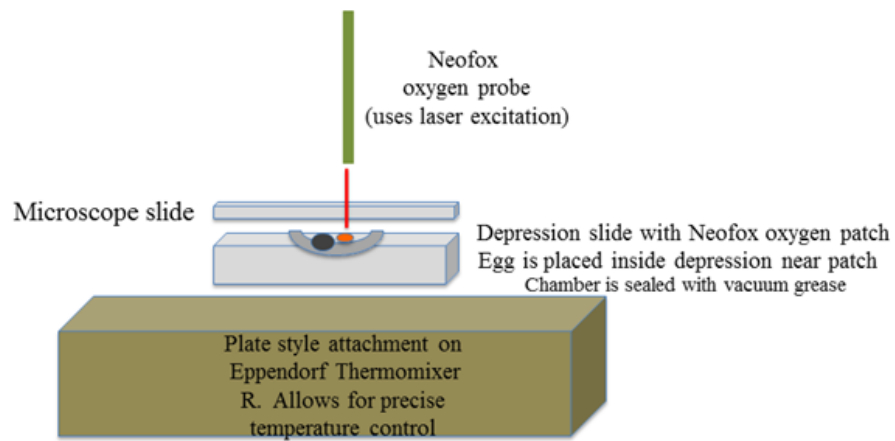


Figure 2: Schematic of method used for egg oxygen consumption experiments

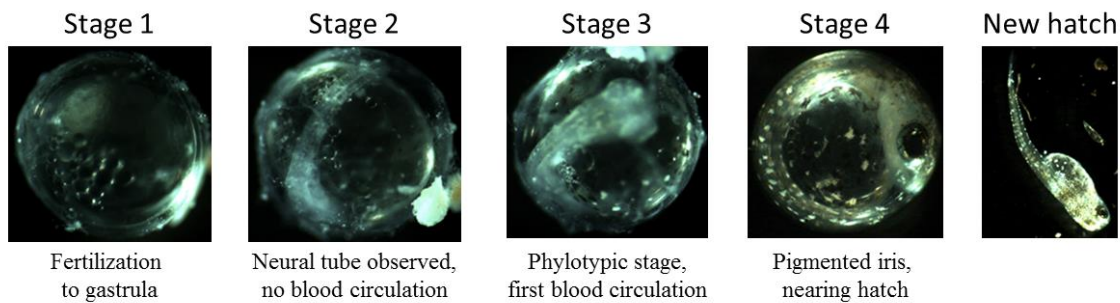


Figure 3: Description and photos of the four developmental stages used for categorizing embryos prior to oxygen consumption experiments

Results:

In both species, hatch success was greatest in eggs that were laid and reared at 28 °C (> 80% hatch; Table 1). Eggs laid by pupfish that had been maintained at 28 °C had reduced hatching success if the eggs were reared at 33 °C (e.g. 62.8 and 36.7% for Refuge and *C. n. mionectes*, respectively). While this level of success may be considered moderate, the hatching success from eggs laid by pupfish acclimated to 33 °C was much lower. Only one egg hatched

from over 100 Refuge eggs collected from 33 °C acclimated parents; Table 1). Egg hatch success was higher in *C. n. mionectes*; eggs laid at 33 °C hatched 24.6% and 17.6% of the time when incubated at 28 °C and 33 °C, respectively (Table 1).

Eggs hatched ~1 d more quickly when incubated at 33 °C compared to 28 °C (Figure 4). Despite decreased time to hatch at the higher temperature, larval size at hatch was the same regardless of incubation temperature or time to hatch. Larvae averaged ~4 mm at hatch (Figures 4 and 5). We did not observe any marked differences in the width of the larvae (head width was ~0.5 mm in all larvae).

Predictable declines in PO₂ were observed for single eggs in our oxygen consumption experiments (Figure 9). There were a few instances during the oxygen consumption experiments in which a stage 4 egg hatched during the experiment (Figure 9). In many cases, calculating precise oxygen consumption values for the newly hatched embryo was complicated by the larvae swimming throughout the chamber and often occluding the laser (Figure 9). In every instance upon hatch, oxygen consumption rates increased dramatically ($265 \pm 111\%$, n=4).

As might be predicted with a developing embryo and the resulting increased metabolically active tissue, oxygen consumption in 28 °C incubated eggs increased from stage one (early development) to stage four (near hatch) in both species (Figure 6a, c). Interestingly, oxygen consumption plateaued by stage 2 in both species when eggs were incubated at 33 °C. There is no blood circulation in these stage two eggs (Figure 6a, b). In Refuge eggs, this unexpected pattern is more extreme with oxygen consumption at stage two being 29.7% higher than at stage four (Figure 6d). At stage four, Refuge eggs incubated at 33 °C consumed 39.9% less oxygen than those exposed to 28 °C (Figure 6c, d, Table 2). Temperature coefficients reflect the temperature dependence on metabolic activities e.g. as temperature increases an activity is

expected to increase. In Refuge fish, eggs hatched at 4.8 d when reared at 33 °C and 5.8 d when reared at 28 °C even though the larvae were of similar size. The calculated Q_{10} temperature coefficient is thus 1.46 (Physiologyweb.com). Stage 4 oxygen consumption is 320.36 ± 27.41 nl $O_2 \cdot h^{-1}$ in Refuge eggs maintained at 28 °C. One would then expect, based on a temperature coefficient of 1.46, stage 4 eggs maintained at 33 °C to consume 387.5 nl $O_2 \cdot h^{-1}$. However, these eggs consumed 192.56 ± 14.60 nl $O_2 \cdot h^{-1}$.

We determined the relationship between oxygen consumption and PO_2 (Figure 7). We found significant variability in the relationships. At 33 °C, we found that oxygen consumption waxed and waned in stages 3 and 4. Some of this variation may be due to the relatively low oxygen consumption rates of eggs assayed at 33 °C (e.g. compare axes in Figure 7). However, this low rate of oxygen consumption would also be consistent with anaerobic metabolism. Some eggs oxyconform (e.g. Fig. 7C). As such, we are not comfortable with calculation of an absolute critical PO_2 . However, we are aware that oxygen consumption is less at lower tensions of oxygen. We determined *apparent* critical PO_2 values to allow for comparison with environmental oxygen availability (Figure 8). Apparent critical PO_2 values for eggs averaged ~120 torr. We were unable to calculate apparent critical PO_2 values for many eggs early in development due to low oxygen consumption rates.

		Refuge		<i>C. n. mionectes</i>	
		Parent acclimation temperature (°C)		Parent acclimation temperature (°C)	
		28	33	28	33
Egg incubation temperature (°C)	28	85.9% n = 78	0% n = 75	83.7% n = 86	24.6% n = 61
	33	62.8% n = 78	1.7% n = 57	36.7% n = 98	17.6% n = 51

Table 1: Egg hatching success for Refuge and *C. n. mionectes* at 28 °C or 33 °C from parents acclimated to those temperatures. Significance determined using a chi-squared contingency table at $P < 0.05$. Eggs hatch significantly less often when incubated at 33 °C when compared to those incubated at 28 °C. Eggs hatch significantly less often when parents are acclimated at 33 °C compared to eggs whose parents were acclimated to 28 °C. Due to low availability of eggs from fish reared at 33 °C, all subsequent experiments use eggs obtained from parents acclimated to 28 °C for at least three weeks.

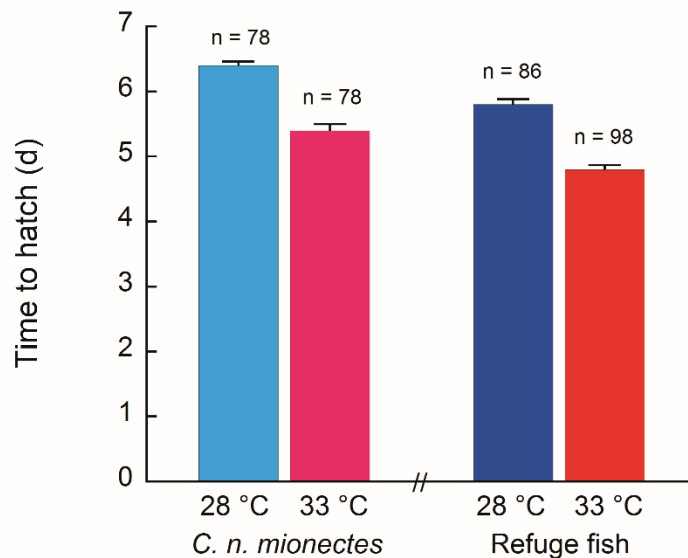


Figure 4: Post-fertilization time to hatch for eggs incubated at 28 °C and 33 °C. Eggs hatch on average one day earlier when incubated at 33 °C, $P < 0.05$ (Fisher's LSD). Error bars represent \pm standard error of the mean (SEM).

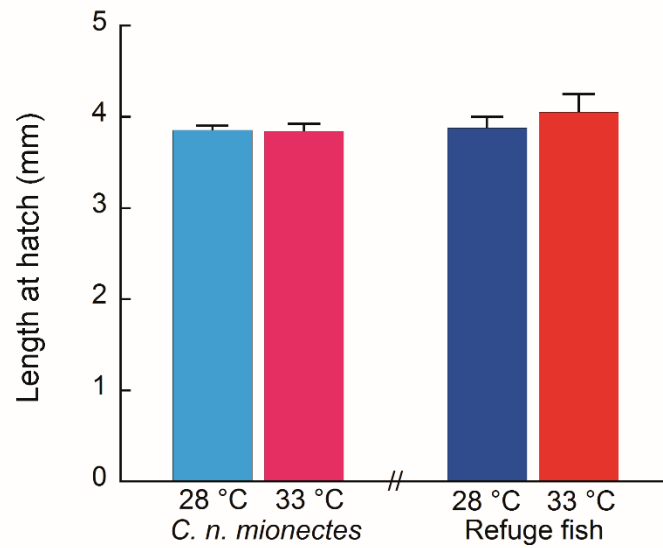


Figure 5: Larval length at hatch of eggs incubated at either 28 °C or 33 °C. No difference in hatch length due to incubation temperature. $P > 0.05$ (Fisher's LSD). $n = 13-23$. Error bars represent \pm SEM.

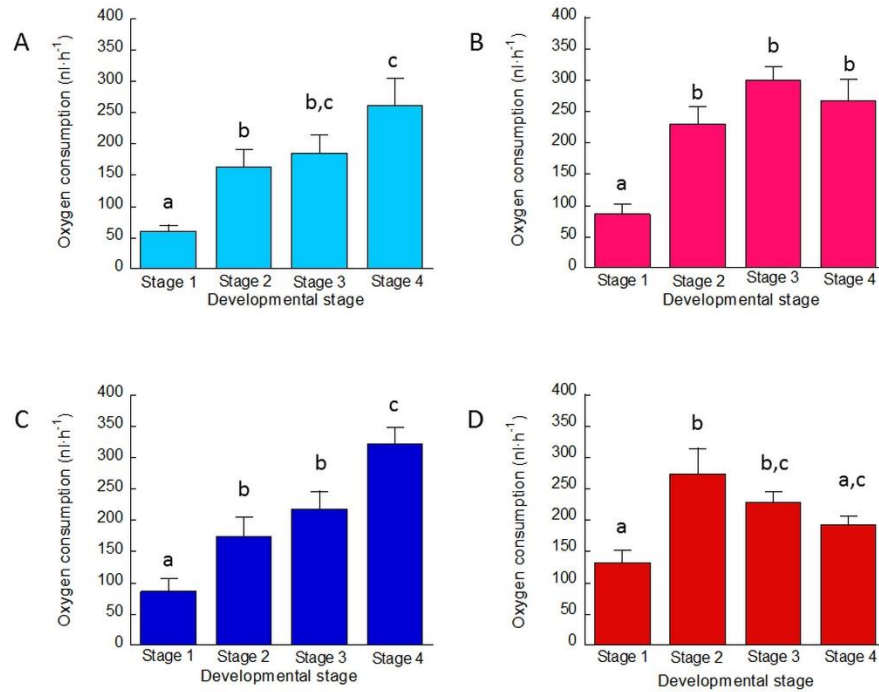


Figure 6. Oxygen consumption across the developmental stages for A) *C. n. mionectes* eggs incubated at 28 °C, B) *C. n. mionectes* eggs incubated at 33 °C, C) Refuge eggs incubated at 28 °C, D) Refuge eggs incubated at 33 °C. Eggs incubated at 33 °C in most cases did not consume significantly more oxygen than those incubated at 28 °C at equivalent developmental stages, while spending less time in the egg. Different superscripts within panels denotes significance at $P < 0.05$ (Fisher's LSD). $n = 5-12$. Error bars represent \pm SEM.

Comparison	Mean difference (33 °C minus 28 °C) nl·h ⁻¹	P-value
28° <i>C. n. mionectes</i> Stage 1(n=6) vs. 33° <i>C. n. mionectes</i> (n=6) Stage 1	25.4	0.5918
28° <i>C. n. mionectes</i> Stage 2(n=8) vs. 33° <i>C. n. mionectes</i> (n=6) Stage 2	66.1	0.1411
28° <i>C. n. mionectes</i> Stage 3(n=5) vs. 33° <i>C. n. mionectes</i> stage 3(n=9)	114.1	0.0154
28° <i>C. n. mionectes</i> Stage 4(n=9) vs. 33° <i>C. n. mionectes</i> Stage 4(n=7)	7.2	0.8619
28° Refuge Stage 1(n=6) vs. 33° Refuge Stage 1(n=6)	46.4	0.3381
28° Refuge Stage 2(n=9) vs. 33° Refuge Stage 2(n=11)	108.4	0.0051
28° Refuge Stage 3(n=12) vs. 33° Refuge Stage 3(n=12)	10.7	0.5369
28° Refuge Stage 4(n=11) vs. 33° Refuge Stage 4(n=11)	-127.8	0.0006

Table 2: Results from one way ANOVA with Fisher's LSD post-hoc, comparison of equivalent developmental stages at 28 °C and 33 °C.

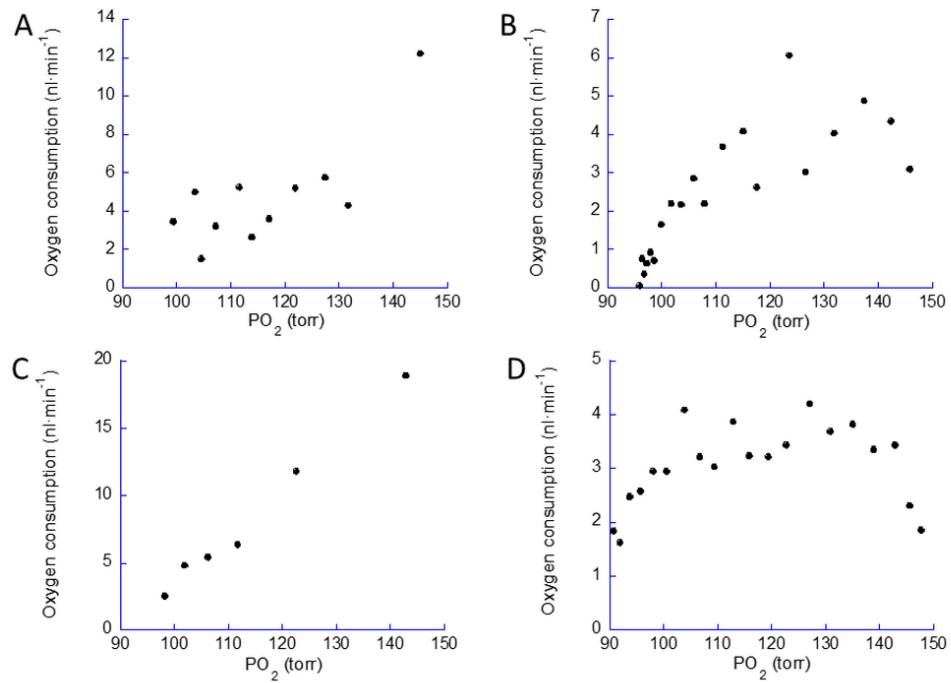


Figure 7: PO₂ (partial pressure of oxygen) vs. oxygen consumption (nl/min) for Refuge eggs laid by 28 °C acclimated parents. A and B) Stage three eggs incubated at A) 28 °C and B) 33 °C. C and D) Stage four eggs incubated at C) 28 °C and D) 33 °C. Initial oxygen consumption is higher at 28 °C and some eggs (e.g. A and C) appear to oxy-conform.

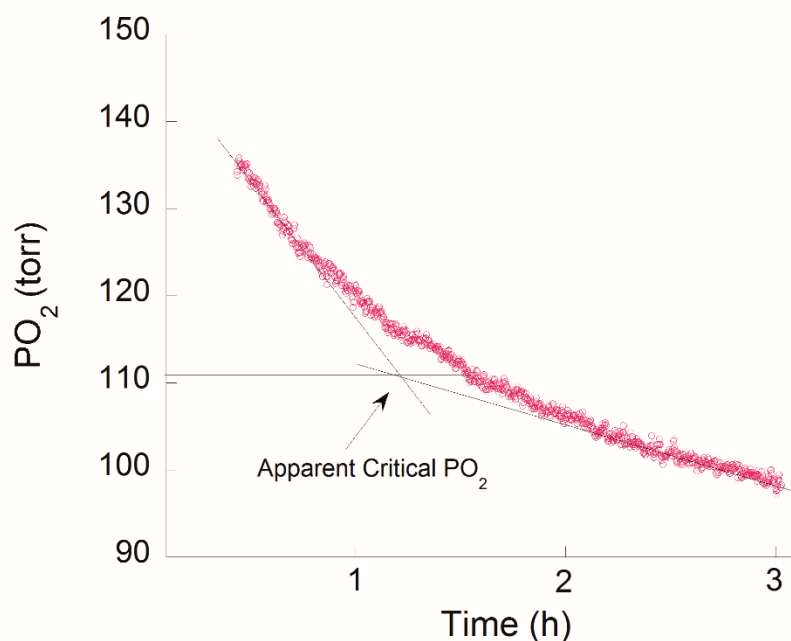


Figure 8: Typical apparent critical PO₂ determination post-experiment. The apparent critical PO₂ for each experiment was calculated by determining the intersect of two linear portions of the PO₂ vs. time curve (initial decrease and subsequent leveling off) graphed using least sum of squares regression. Apparent critical PO₂ values were ~120 torr.

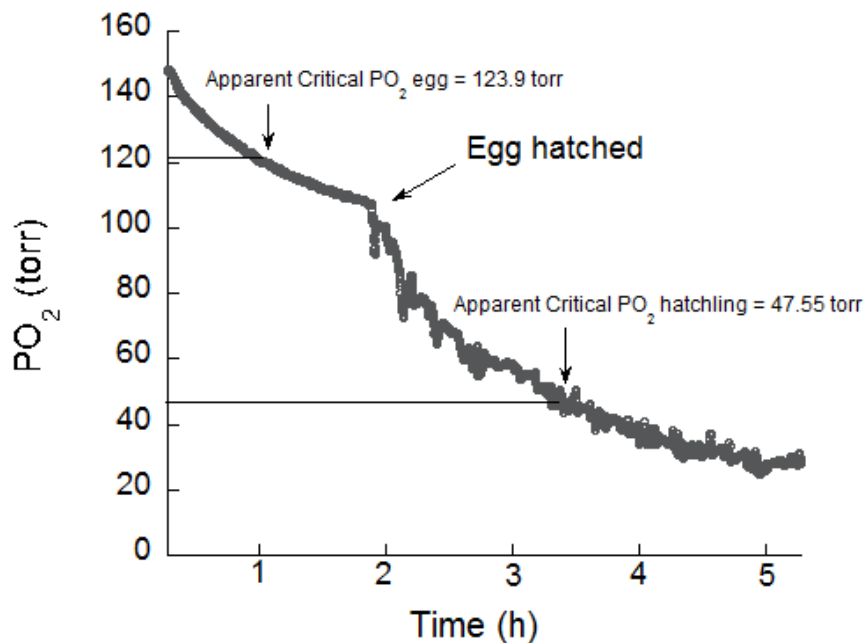


Figure 9: Partial pressure of oxygen over time for a Stage 4 Refuge egg nearing hatch and post hatch. Results show a typical experimental run. In cases when the egg hatched during the experiment, oxygen consumption increased $265 \pm 111\%$, $n=4$.

Discussion:

While others studies have noted that pupfish egg viability is hampered at temperatures typical for Devils Hole and other warm springs (Deacon et al., 1995; Shrode & Gerking, 1977), this study separated the effects of incubation temperature from parent acclimation temperature in *C. n. mionectes* and Refuge fish derived from *C. diabolis*. Parent acclimation temperature played an important role in determining hatch success for pupfish eggs. Parents acclimated to 28 °C laid eggs that had modest to good hatch success regardless of incubation temperature. However, very few eggs were laid by parents maintained at 33 °C and the survival of these eggs was markedly decreased at both incubation temperatures. We found these eggs generally failed to develop past stage one. These data indicate that low recruitment at temperatures typical of many of the warm

springs in Ash Meadows may be the result of failure during oogenesis or spermatogenesis. We speculate that even small thermal heterogeneities and microclimates present in an environment like Devils Hole could have profound implications for successful breeding of pupfish.

Importantly, the relative lack of success of eggs from higher temperatures may explain the previously observed seasonal recruitment to the adult population in Devils Hole; recruitment appears highest in cooler parts of the year despite at least some continuous spawning throughout the year (Riggs & Deacon, 2002).

One could question if transgenerational thermal histories played a role in our results. In a study on a related species, parent thermal history influenced temperature dependent growth rate in larval *C. variegatus* (Salinas & Munch, 2012). *C. variegatus* are routinely found in waters in southern Texas that may be 40 °C or higher (Bennett & Beitinger, 1997). One might expect these fishes to reproduce well at higher temperatures. Unfortunately, pupfish from the Death Valley complex of pupfishes may be less temperature tolerant. When these adult pupfish are maintained at temperatures above 32 °C, successful breeding is extremely limited (Feuerbacher et al., 2015; Minckley & Deacon, 1973; Riggs & Deacon, 2002; Salinas & Munch, 2012; Shrode & Gerking, 1977). Our similar poor breeding success from 33 °C acclimated pupfish required that we use eggs from only 28 °C acclimated parents for all but the initial hatch success experiments. Thus, we are unable to adequately address if transgenerational thermal histories are important here.

Cannibalization of larval pupfish by adults has been observed (Deacon et al., 1995). We tested the possibility that incubation temperature might alter length at hatch, which could be an important factor in controlling predation of larvae. A previous study on *Cyprinodon macularius* reported that incubation temperature can alter length at hatch in pupfish (Kinne & Kinne, 1962).

We found no evidence that incubation temperature is affecting length at hatch in our larvae, at least at these two temperatures which are relevant for springs in the Ash Meadows complex (Hillyard, 2011; Riggs & Deacon, 2002). We also observed no difference in head width. Pupfish eggs incubated at 33 °C hatched one day earlier than those incubated at 28 °C (Figure 4). Interestingly, in other species there may be a tradeoff between time of development and body size at hatch (Blueweiss et al., 1978; Mueller et al., 2015; Mueller, Joss, & Seymour, 2011). In our experiment, we found no such relationship.

The method we developed to measure oxygen consumption of individual eggs allowed continuous measurement over a several hour period. Previous efforts at measuring oxygen consumption in eggs typically involved numerous eggs being measured at the same time (e.g. Barrionuevo and Burggren, 1999). Advantages to measuring oxygen consumption in single eggs are that individual variation may be assessed and there is no need to try to synchronize development amongst all of the eggs. Eggs were typically on or within a millimeter of the NeoFox patch. An advantage of close proximity of the egg to the Neofox patch may be in reducing boundary layer effects. Here, limited diffusion may occur around an object and these boundary layer effects result in a transient underestimation of oxygen consumption (Bang, Grønkjær, & Malte, 2004). Boundary layer effects appear to be resolved after ~15 min e.g. our several hour experimental period would not be unduly influenced by boundary layer effects. We note that boundary layer effects are oftentimes exacerbated and could limit oxygen delivery when multiple eggs are placed in the same area.

Increased temperature often results in increased oxygen consumption rate and a decrease in larval size at hatch in fish (Mueller et al., 2015, 2011). However, our Refuge eggs exhibited depressed oxygen consumption at 33 °C even though they hatched more quickly and at the same

size when compared to eggs incubated at 28 °C (Fig. 6 C, D). Based on thermal coefficient considerations and the timing of hatch, we might predict oxygen consumption at 33 °C should have been more than twice what we measured in our Stage 4 Refuge eggs.

One possibility to explain how reduced oxygen consumption rates reconcile with a faster developmental trajectory and resulting in a similar sized larva is the use of anaerobic metabolism. We note that maximal oxygen consumption rate occurred by stage two in 33 °C reared eggs. This point precedes blood circulation. Recently, our group reported unexpected oxygen consumption patterns in adult pupfish acclimated to 33 °C (Heuton et al., 2015). Adult pupfish exhibit extended periods of little or no oxygen consumption that may be as long as 149 min continuous. This paradoxical anaerobism occurs despite the availability of sufficient ambient oxygen. These adult pupfish apparently produce ethanol as an alternative product of metabolism. Perhaps the unexpectedly reduced oxygen consumption in eggs incubated at 33 °C is the result of a similar process.

Another possible explanation for the unexpectedly low oxygen consumption rates may be limitations to gas exchange at the higher temperature. Indeed, such a model is supportable by Fig. 6B, where *C. n. mionectes* oxygen consumption plateaus by stage 2. However, we note that Refuge eggs consume significantly more oxygen at stage 2 compared to stage 4 (Fig. 6C-D). It is unlikely that changes to the chorion could occur over the course of development that would drastically alter O₂ diffusion into the egg as these same changes would likely make hatching much more difficult.

We determined an *apparent* critical PO₂ value of ~120 torr for our fish eggs (Fig. 8). Below this PO₂, one might expect marked depression of oxygen consumption. Depending on their spatial and temporal placement in water systems like Devils Hole, eggs could be exposed to

oxygen conditions ranging from chronic hypoxia to periods of hyperoxic or hypoxic conditions (Bernot & Wilson, 2012). Oxygen levels in the main pool of Devils Hole are generally low with values reported to be between 42 to 63 torr (Bernot & Wilson, 2012). However, microhabitats within the shallow shelf may offer more variable oxygen availabilities depending on algal communities and diel light fluctuations. Barrionuevo and Burrgren (1999) report similar critical PO_2 values for zebrafish embryos maintained at high temperatures. Diel-cycling hypoxia occurs in many aquatic habitats worldwide. If these high apparent critical PO_2 values are the norm for developing warm water fish embryos, there is the potential that many fish species experience oxygen limitations at some point during development in the egg. When an egg hatches, there is marked increase in oxygen consumption indicating that embryos are limited by oxygen availability while in the egg (Fig. 3).

Breeding in Devils Hole occurs almost exclusively on the shallow shelf. Seasonal differences in the availability of sunlight limits food production in winter and causes very high breeding ledge temperatures in the summer (Riggs & Deacon, 2002). The result is that pupfish reproduction is essentially restricted to when food is available and temperatures are moderate. We contend that warmer water temperatures and/or the accompanied reduction in oxygen solubility may negatively impact reproduction and successful egg development. Census data show the percentage of the population observed on the shelf ranged from over 60% during the winter to 40% during the summer between 1974-1981. However, between 1993-2003, the percentage on the shelf declined to about 30% during the winter and less than 10% during the summer (Riggs & Deacon, 2002). This change in localized distribution of the pupfish coincides with a trend towards warmer ambient temperatures over the past 30 years (www.climate.gov). The correlation between warm temperatures and access of fish to the shallow shelf would

indicate that high temperatures limit the reproductive success of these pupfish, which our findings here support. When combined with the poor success of eggs at higher temperatures, global climate change may be markedly deleterious to these pupfish. Climate models predict that the average air temperatures in the Southwest could rise by 3.9 – 6.7° C this century (Merideth, 2001). Presumably, this large temperature increase will result in significant increases in shelf water temperatures. If climate scientist predictions are correct, warmer winter temperatures and early summers will narrow the interval during the spring when temperatures, oxygen solubility, and food availability are favorable for reproduction and successful egg development. Global climate change may thus be a contributing factor to the decline in *C. diabolis* numbers. However, it is important to note that some authors suggest depletion of the underlying aquifer poses a more immediate and serious threat to the conservation of these fishes (Hausner et al., 2016).

Taken together, our data suggest that egg viability may be limiting success in the warmer springs that characterize pupfish habitats. Parental thermal histories may be more important in dictating success than is egg incubation temperature. The data suggest oxygen consumption may not fully explain the energetic story of developing pupfish eggs, especially at high temperatures. A better understanding of a putative role of anaerobic metabolism in these eggs is warranted. Further work at better understanding microclimates of oxygen availability in water systems like Devils Hole may allow for better elucidation on what factors limit successful hatching of pupfish eggs.

Chapter 3: Pupfish growth, reproduction, and survival in a hypervariable environment

Despite widespread interest in the conservation of pupfish species, much of the current knowledge regarding pupfish thermal limits and habitat use in the wild is anecdotal in nature. Scientists would visit a pupfish habitat several times during a year, note whether fish were observed, and take temperature measurements (e.g. Sada & Deacon, 1995). Assumptions were made that fish that were observed in temperatures $>40^{\circ}\text{C}$ in nature were well adapted to cope with these thermal regimes (Brown & Feldmeth, 1971). However, whether these individuals which were exposed to temperatures $>40^{\circ}\text{C}$ survived long term was unknown. The small size of the fish precluded common methodologies for identifying and following individual fish inhabiting different thermal environments to quantify survival. Despite years of research on these fishes, their upper thermal limits remain somewhat unknown. Measures such as upper avoidance temperature, incipient upper thermal limit (IUTL), and long-term survival following ecologically relevant heat exposures have not been tested in most species. Better elucidation of pupfish response and survival in different thermal regimes is important to informing mitigation and conservation efforts as climate change impacts their already endangered habitats.

The unique stream environment of Salt Creek, home to *C. salinus* and located in Death Valley National Park, CA provides an opportunity to test assumptions regarding pupfish thermal limits and habitat use in the wild. Temperatures in Salt Creek vary widely depending on spatial location in the creek and season. In the headwaters, pools are deep and generally more thermally stable ranging from 6°C during the winter to no warmer than 30°C in the summer (unpublished National Park Service data, Figures 12 and 14). In contrast, in the lower portions of Salt Creek, temperatures can surpass 40°C as early as May, and temperatures of $35\text{--}40^{\circ}\text{C}$ are common at that time (Figure 14). In general, pupfish reproduction is very limited above 32°C (Deacon et al.,

1995; Gerking & Lee, 1983; Shrode & Gerking, 1977). However, pupfish that colonize the lower portions of the creek experience more favorable breeding and growth temperatures earlier in the season than pupfish which remain in the headwaters (Sada & Deacon, 1995; Figure 14). My study of the pupfish in this system provides an opportunity for understanding why these fish move into hot, ephemeral environments and whether those fish remain in the effective population as the creek quickly warms and dries during the early summer.

I utilized fluorescent Visual Implant Elastomers (VIE) and coding to mark and recapture individual pupfish in the hypervariable environment of Salt Creek in Death Valley National Park. This approach enabled me to quantify individual movement patterns and growth rates, and to quantify which individuals remain detectable in the population and which individuals did not. I conducted field experiments aimed at measuring important fitness correlates in headwaters and ephemeral portions of Salt Creek such as breeding and survival following a heat exposure. This study provides resource managers with a better knowledge of the unique habitat at Salt Creek and how fish use different parts of the creek. Ephemerality and variability are likely to increase in the Mojave Desert; knowledge provided by this study will be important for management decisions since ephemeral habitats are likely to increase at Salt Creek, while permanent habitats could decrease (Archer & Predick, 2008; Nickus et al., 2010). I hypothesized that ephemeral habitats at Salt Creek were low quality sink habitats while headwaters and intermediate sections comprise pupfish sources and are most favorable for pupfish reproduction, growth, and survival.

Methodology:

Capture-mark-recapture study

I used a computer-generated coding system that enabled me to give unique marks using VIE to up to 7,000 potential individuals (Northwest Marine Technology, Shaw Island, WA). Each pupfish individual was injected with up to three different colors in up to seven different body locations (Figure 10). Fish were anesthetized using a 0.033 ml/l clove oil solution, then sex and total length were determined. Individuals captured which were less than 25 mm were not marked due to small body size. I characterized the habitats at Salt creek as either headwaters (small area with many pupfish near NPS monitoring station and source of creek, depth greater than 50 cm), intermediate habitats (generally thermally stable and permanent, greater than 25 cm in depth), and ephemeral habitats (temporary pools that can fluctuate widely in temperature during a day or season, depth less than 25 cm) (Figures 11 and 12). Temperatures were recorded continuously in several sections of the ephemeral habitats at Salt Creek (Figure 12). Wilderness regulations precluded temperature recording in the headwaters or intermediate areas of Salt Creek, but the National Park Service provided me temperature data from past years, and a representative year is shown in Figure 12.

Over the course of the study 1,671 Salt Creek Pupfish were marked; 445 fish from the headwaters, 427 fish from intermediate habitats, and 799 fish from ephemeral habitats (Figure 11). The overall study period lasted from November of 2015 through September of 2016 and fish were marked from November of 2015 through April of 2016. If a recaptured fish was found during either a marking or a recapture session, its marks were noted and its position within the creek was recorded. Recaptured fish were measured for total length to determine growth since it was last captured. Movements of individuals and presence-absence in the population was noted

once I returned to the laboratory. There were some instances when a recaptured fish had lost one or two of its marks, making it unidentifiable. These fish were not included in the data analyses. Similarly, length recordings were subject to error and so when determining growth rate of pupfish, I did not include fish that were recaptured within one month of their initial capture. This ensured that the calculated growth rate was not being overly influenced by an observer's error when measuring a fish. Not including fish recaptured within one month of initial capture helped avoid erroneously large growth rates which could be due to an observer error of $\pm 1-2$ mm. For instance, if an observer measured a fish of the same size but the measurement was subject to 1 mm of error one week after initial capture, this fish would incorrectly be recorded as having a large growth rate of 1 mm per week. Only including fish that were captured more than one month after initial capture minimized erroneously large growth rates which were likely only due to observer error. It is likely that observer error was the same throughout the course of the study based on analysis of marked fish recorded lengths.

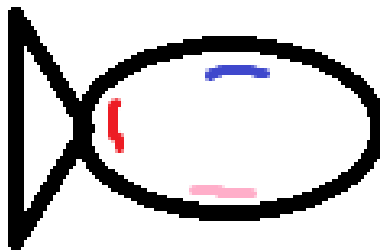


Figure 10: Example of pupfish marking system. Three marks were made per individual. Using six different colors and seven different body locations, several thousand unique individuals can be coded for using Visual Implant Elastomers.



Figure 11: A) Headwaters habitat of Salt Creek and B) Ephemeral habitat at Salt Creek. The ephemeral portion of the creek pictured in panel B had evaporated by June 9th, 2016.



Figure 12: Aerial overview of the Salt Creek system. Red shaded areas represent ephemeral habitats, green shaded areas represent intermediate habitats, and blue areas represent the headwaters of Salt Creek. Stars represent marking and sampling locations and are labeled with the number of individuals marked. Circles represent areas which were sampled while searching for marked individuals.

Breeding experiments

March is generally considered to be the start of breeding season for Salt Creek Pupfish (Dzul et al., 2013). Enclosures were constructed of PVC pipes/joints and mesh fabric. Enclosures measured 55 cm x 25 cm x 25 cm and were roughly the same size as ten gallon aquaria (Figure 13). The mesh was small enough to prevent fish from escaping, but allowed algae and invertebrates access e.g. food was available to fish while they were in the enclosures. One enclosure was placed in the intermediate section of Salt Creek, one enclosure was placed in the headwaters section of Salt Creek, and two enclosures were placed in the ephemeral sections of

Salt Creek during March of 2016. Enclosures which were placed in the headwaters or intermediate habitats were tied to a PVC pipe section anchored into the creek substrate and were made to float by tying pipe insulation to the top parts of the enclosure (Figure 11). In the ephemeral sections of creek, the enclosures were not completely covered by water and were simply placed on the substrate. Fish were placed into these enclosures in the same densities and sex ratios (20 fish if fully submerged, 12 fish if partially submerged, 1:3 M/F). Two equal sized breeding carpets were placed in all enclosures to allow for egg deposition. A synthetic plant was placed in each enclosure to provide cover for fish. Enclosures were left in the creek for five days and eggs were counted and removed from the breeding carpets each day. Mean egg production per female fish was calculated using these egg counts. Following a five-day period fish were released into the creek in the same area in which they were captured. Marked fish were not used for these breeding experiments. The breeding enclosures were also placed in the headwaters of Salt Creek in September since breeding was not detected in the headwaters enclosures in March.



Figure 13: Enclosure placed in the creek for use in breeding and heat shock experiments.

Survival following heat exposure experiment

Eighty fish were captured from the headwaters of Salt Creek in September 2016 and subjected to an ecologically relevant heat exposure of 35-40 °C for one, two, or four hours. Twenty control fish did not receive any heat shock and were immediately moved to an enclosure within the headwaters for monitoring. Fish were separated into four groups of twenty. Groups of twenty fish were placed in five gallon buckets initially held at the temperature of the headwaters section of creek (~25 °C) and hot water (heated using a portable camp stove) was added to each bucket to gradually raise the temperature of the water. Rate of warming was ~0.5 °C per minute. For measuring the duration of the heat shock, a stop watch was started for each bucket when the temperature reached 35 °C, but heating continued until each bucket was ~40 °C. This method of heat exposure roughly approximates the heating of the ephemeral sections of Salt Creek in May

and June during a warm day. Following heat exposure fish were cooled to creek temperature gradually by replacing water in the buckets with water from the creek. Fish were then moved to enclosures in the headwaters section of the creek to monitor for survival for seven days following this exposure. Enclosures were visited each day, expired fish were noted daily and removed from the enclosures.

Camera monitoring of ephemeral sections of the creek

Three cameras were placed on the boardwalk in the heavily visited, ephemeral area of Salt Creek and aimed at the creek to monitor fish numbers. Temperature loggers were also placed at each camera station in the creek following waterproofing. Pictures were taken of the creek and corresponding temperatures recorded every hour while there was water in this section of creek, from October 4, 2015 to June 9, 2016. For each picture at each camera station, the number of fish were counted, with care taken so that only fish were counted and not invertebrates or other objects.

Results:

Movement of pupfish at Salt Creek was not detected until February, which corresponds to longer days and warming ephemeral habitats (Figure 14). I recaptured 109 Salt Creek Pupfish which were positively identifiable to the individual e.g. fish retained all three marks. Recapture rate of pupfish initially marked in headwaters habitat was highest over time. The percentage of fish recaptured from the ephemeral habitats declined drastically as the summer progressed and the total area of the creek diminished. This is despite regular sampling throughout the entirety of the creek. Out of 109 individuals, 71 pupfish were captured in the same location in the creek where they were originally captured. Growth rate was significantly higher for fish recaptured in

the ephemeral habitats at Salt Creek compared to headwaters (Figure 15). Similarly, breeding as measured by eggs laid per female each day during March using mesh enclosures was significantly higher in the ephemeral habitats (Figure 16). Breeding was also assessed in the headwaters of Salt Creek in the fall when temperature profiles in the headwaters were near those measured downstream in March. No breeding was detected in the headwaters of Salt Creek in either March or September. No breeding behavior was observed in the headwaters throughout the course of this study during sampling trips.

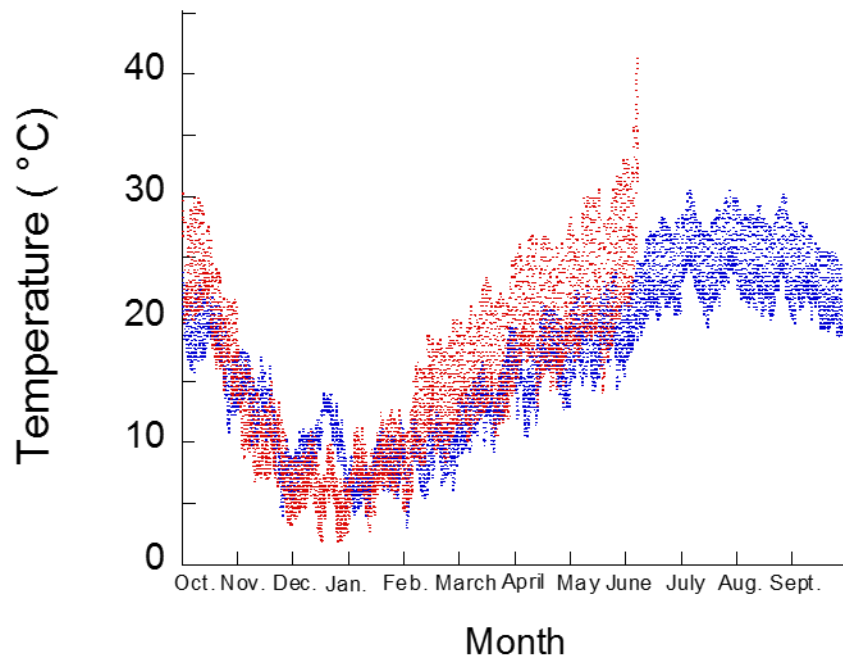


Figure 14: A comparison of temperatures recorded in the headwaters and ephemeral habitats of Salt Creek. Blue trace represents headwaters temperatures and red trace represents temperatures from ephemeral habitats. Data for ephemeral habitats was recorded by an ibutton temperature logger over the course of sample season until the water in the creek evaporated completely in June. Data for headwaters habitat are taken from National Park Service monitoring data in the headwaters for 2011. Wilderness regulations precluded monitoring temperature of headwaters during 2015-2016 sampling season but data from 2011 appears representative for the headwaters.

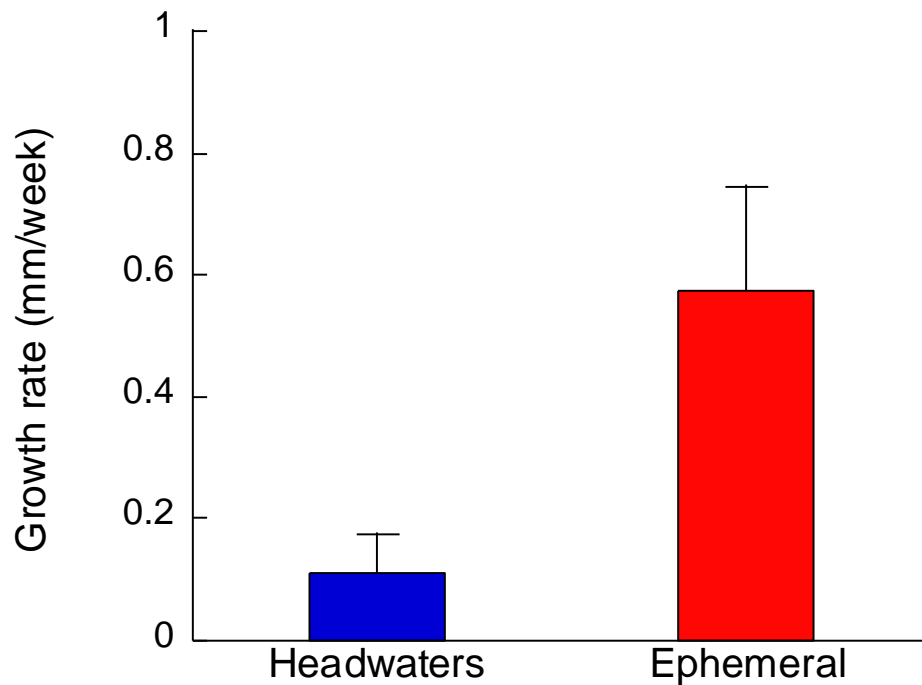


Figure 15: Growth rate of individual non-moving fish recaptured in headwaters and ephemeral habitats at Salt Creek from February to June. N=23 for headwaters, N=17 for ephemeral. To reduce the influence of human error during measurement on these data only fish that were recaptured 27 days or more following their initial capture were included in this analysis. Pupfish growth rate is higher in ephemeral habitats, $P < 0.05$ (Fisher's LSD). Error bars represent standard error of the mean.

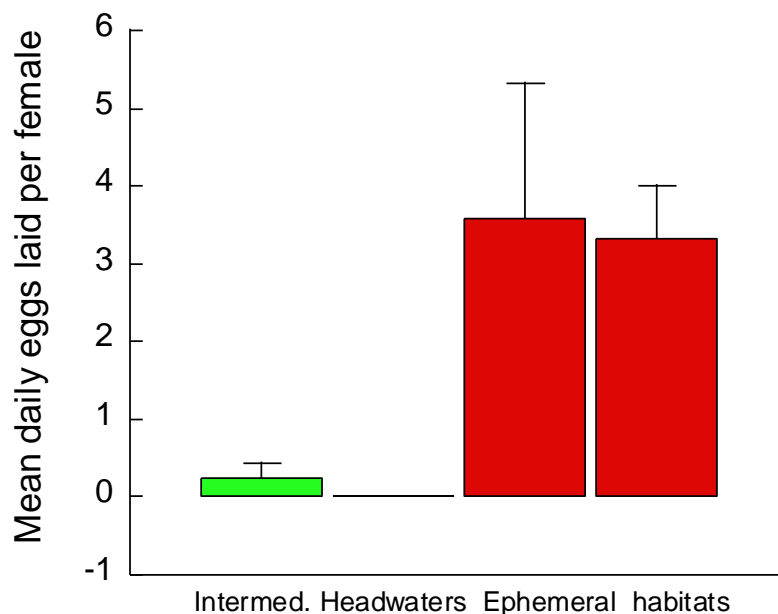


Figure 16: Egg production from breeding enclosure experiments during March, often considered the breeding season for *Cyprinodon salinus*. One enclosure was placed in the headwaters, one enclosure in the intermediate sections of creek (intermed.) and two were placed in ephemeral habitats. More eggs are laid in the ephemeral habitats during March, $P < 0.05$ (ANOVA Fisher's LSD Post hoc). Errors mars represent standard error of the mean. No eggs were laid in the headwaters enclosures during the experiments. Breeding was never observed in the headwaters of Salt Creek during sampling trips.

Twenty pupfish individuals were recaptured downstream of their original marking site and seventeen individuals were captured upstream of their marking location. Most detected movements by pupfish individuals were less than 0.5 km; however, three fish moved 2.4 km upstream from ephemeral habitat into the headwaters. No difference in movement patterns between male and female pupfish or different size classes was detected in this study (Figures 17 and 18). Similarly, there was no correlation between length of fish and distance moved in Salt Creek (Figure 19). No seasonal movement pattern was apparent for detected movers, but some large movements were detected from ephemeral habitats into intermediate or headwaters habitats

(Figure 20). Game cameras detected significantly more fish in the ephemeral habitats in May and June compared to all other months (Figure 21). These months were warmer on average and so more fish were detected by the cameras at temperatures above 30 °C (Figure 22).

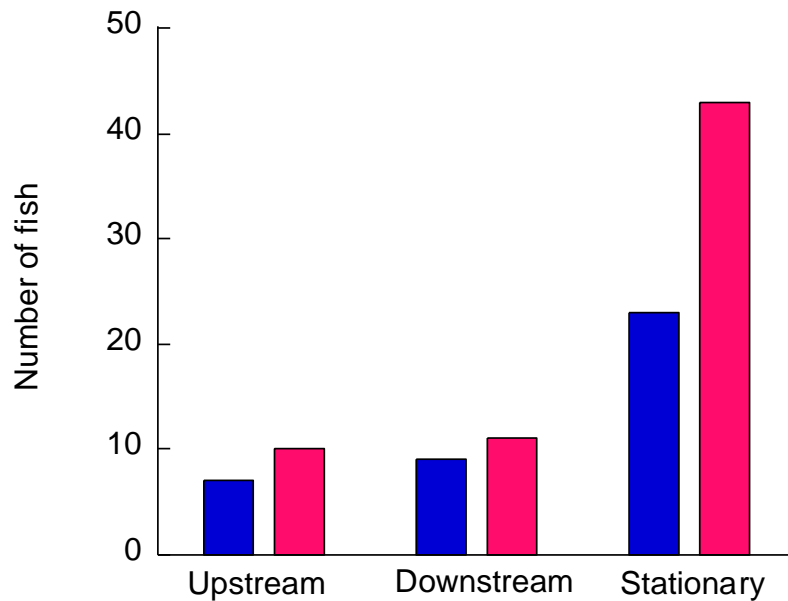


Figure 17: Numbers of males and females with detected movement history in Salt Creek. No significant difference in sex ratios between movers and stationary fish (Chi-squared, $P > 0.05$)

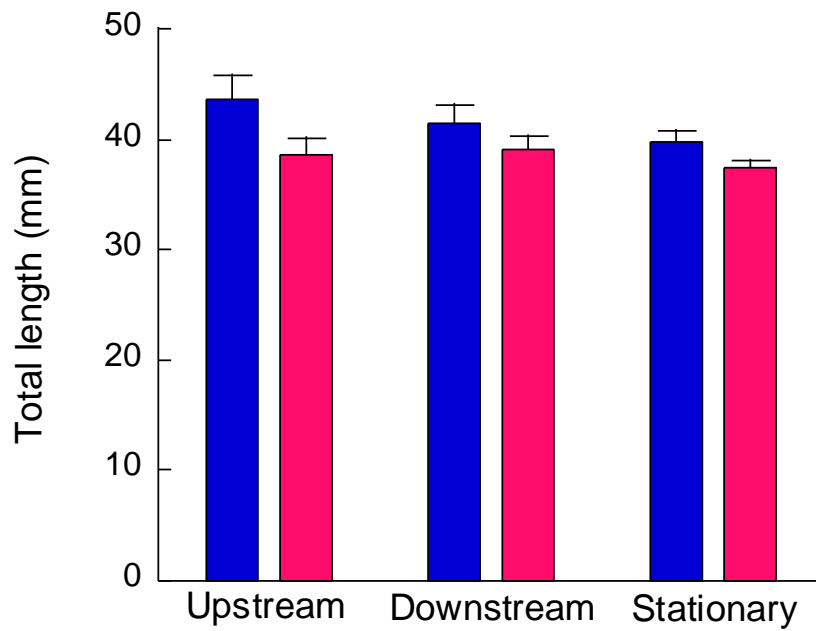


Figure 18: Total body length of movers and stationary fish separated based on sex. Pink bars represent female fish and blue bars represent male fish. No significant difference was found between movers and stationary fish of each sex. $P > 0.05$ (Fisher's LSD). Error bars represent standard error of the mean.

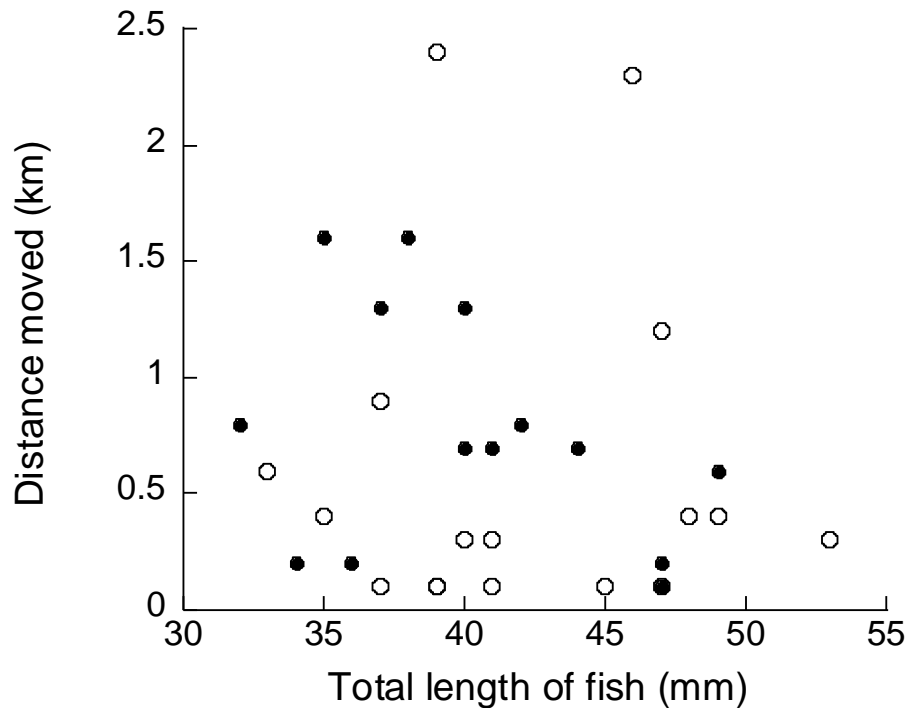


Figure 19: Detected distance moved in kilometers plotted against total length of fish in millimeters. Closed dots represent downstream movements and open dots represent upstream movements. There is no correlation or a very weak negative correlation between length of fish and distance moved downstream. There is no correlation between size of fish and distance moved upstream. $R = -0.276$ for downstream movements, $R = 0.020$ for upstream movements.

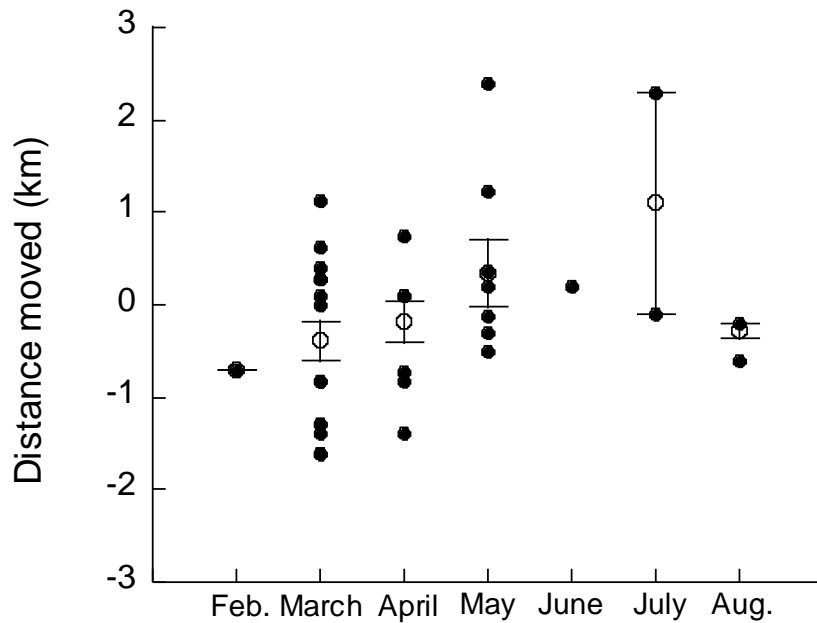


Figure 20: Detected individual and mean movements for recaptured Salt Creek Pupfish. Stationary individuals (~65% of recaptured fish) are not shown. Open circles represent mean movements \pm standard error of the mean. Closed circles represent individual movers. Positive values represent upstream movement and negative values represent downstream movements. In February, only two movers of the same distance were detected. In June, only one mover was detected. While some individuals did make large upstream moves as the creek dried and warmed, mean upstream detected movement did not increase significantly over the course of the summer.

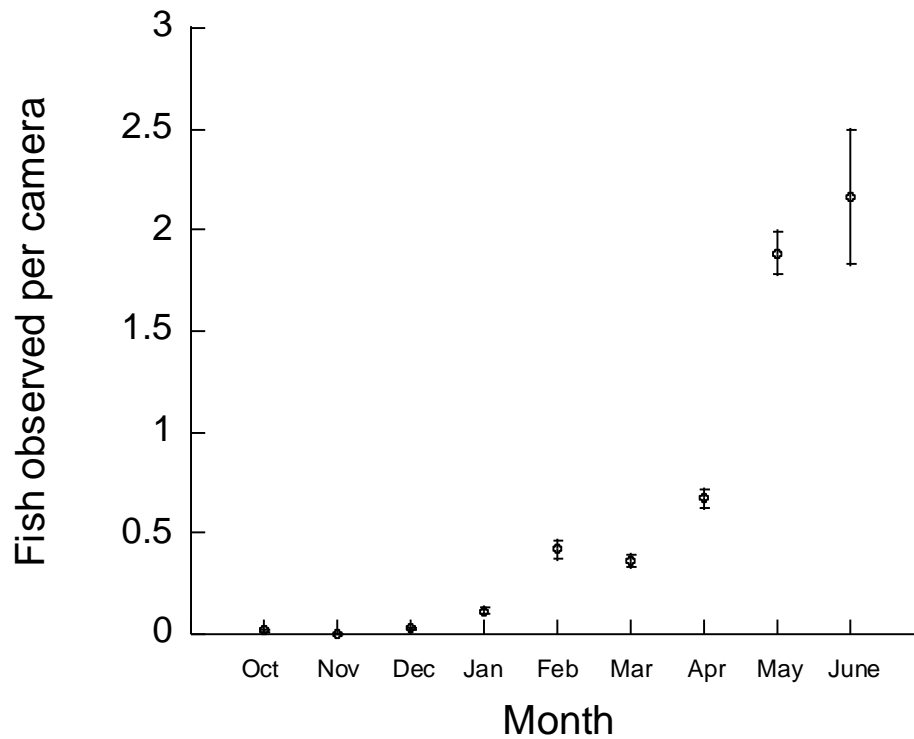


Figure 21: Mean number of fish observed each month by three automated game cameras placed in the ephemeral section of Salt Creek. Pictures were taken hourly during periods when ephemeral sections of the creek contained water. Fish initially appear to colonize these areas in February and the greatest number of fish were observed by cameras in May and June.

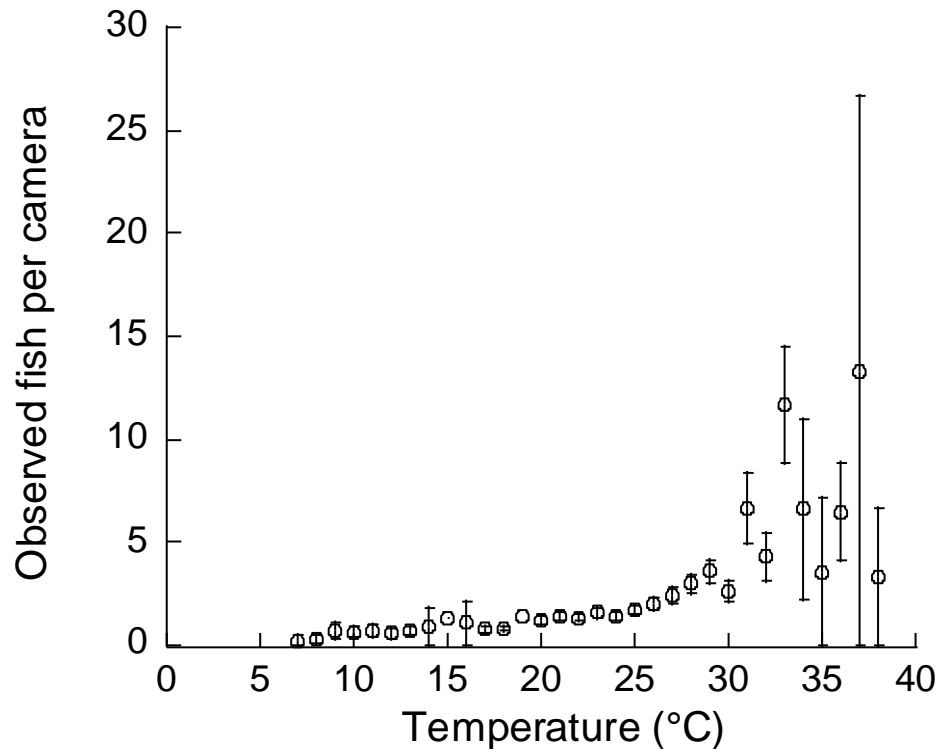


Figure 22: Observed fish per camera plotted against recorded temperature. This data was taken from February when fish first colonized ephemeral sections of creek until June when this portion of the creek dried. The highest number of fish were observed on the game cameras between 30-40 °C, which occurred mainly in May and June.

In February, two fishes from the headwaters had moved 0.73 km downstream into an ephemeral portion of the creek (Figure 23). In March, marking continued in the ephemeral sections of the creek and most recaptured fish were stationary in the headwaters and ephemeral sections of the creek (Figure 24). Movement of individuals was detected but no pattern was apparent. One fish moved upstream from the intermediate sections of the creek into the headwaters (Figure 24). One fish each from the headwaters and intermediate sections of the creek made large moves downstream into ephemeral habitats and there were several shorter

movements by individuals both downstream and upstream within the ephemeral sections of the creek (Figure 24). Fish were curiously absent, or present in low densities, for all sampling occasions between March and August in intermediate reaches of Salt Creek despite cooler temperatures being available in these reaches. In April, all detected movements were fish moving within the ephemeral stretches of the creek (Figure 25). In May, two large upstream movements were detected including one that was ~2.4 km from the furthest downstream marking location into the headwaters of the creek (Figure 26). Four fish remained stationary in the ephemeral sections of the creek while 3 fish moved further downstream into ephemeral sections (Figure 26). For June through August, safety restrictions based on high ambient temperatures prohibited extensive sampling, but an effort was made to sample throughout the creek during these months. In June, only one marked fish was detected which had made a small movement ~0.25 km upstream (Figure 27). It should be noted that this fish was found in a pool that had no visible surface connectivity to the rest of the creek and very likely perished as the creek continued to dry and warm. In July, three fish were recaptured. One stationary fish in the headwaters, one long distance mover from the ephemeral section into the headwaters, and one short downstream movement from the headwaters into the intermediate section of the creek (Figure 28). In August, only downstream movements from the headwaters into intermediate sections of creek were detected (Figure 29). In September, another large upstream movement was detected from the furthest downstream marking area into the headwaters (Figure 30). All other recaptured fish in September were stationary.

In the heat exposure experiments, no fish died during or following exposure in either the control treatment (no heat exposure), or in the 1 hour exposure to 35-40 °C. One fish out of nineteen died in the days following exposure to 35-40 °C for two hours. Eight out of twenty fish

exposed to 35-40 °C for four hours died during the exposure itself (Figure 31). Four additional fish died in the seven days following the four-hour exposure. Only 40% of fish exposed to 35-40 °C for 4 hours survived for seven days following exposure.

On one sampling trip in July, a large fish kill was observed in the intermediate area of Salt Creek (Figure 32). Dissolved oxygen levels were 0.85 mg/l and temperature was 26 °C when the kill was detected at 9:00 AM. Hypoxia could be an issue for fish which inhabit intermediate habitats during the summer as spring algal blooms decay.

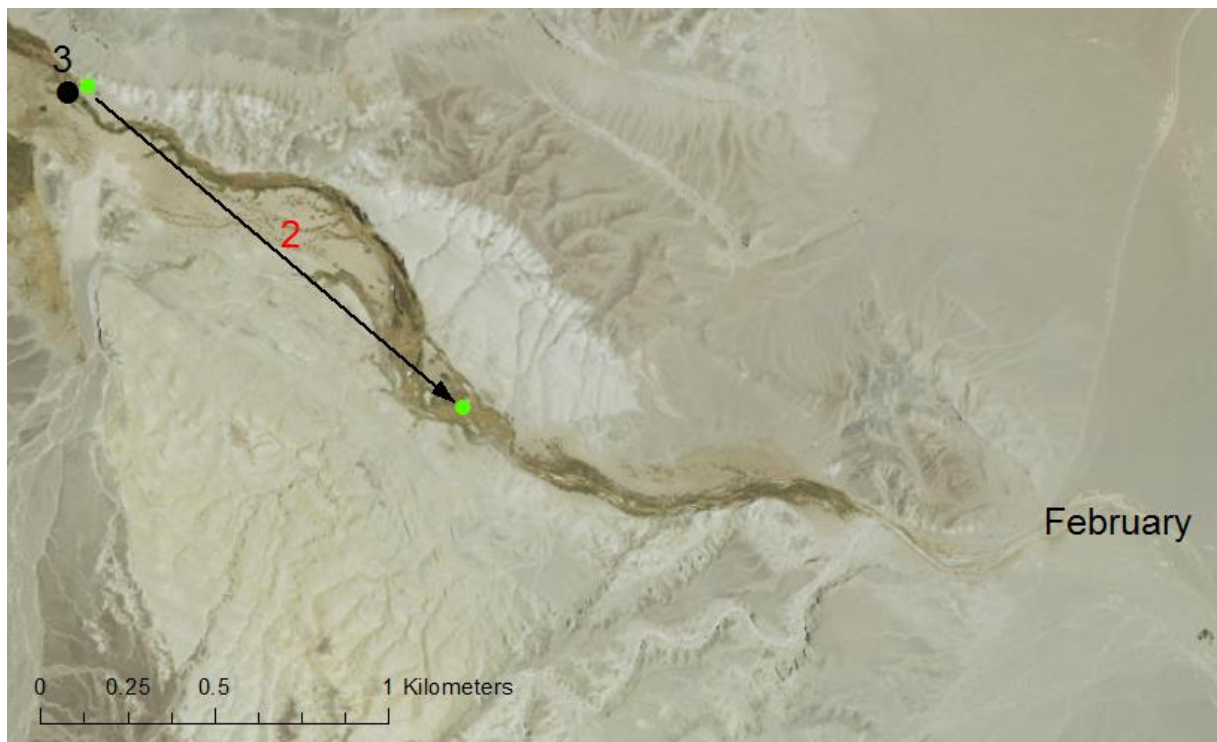


Figure 23: Documented recaptures and movements for the month of February. Black circles denote stationary individuals and green circles denote moving individuals. Number labels denote number of individual fish with that movement detected. Month label denotes furthest downstream extent of Salt Creek for that month.

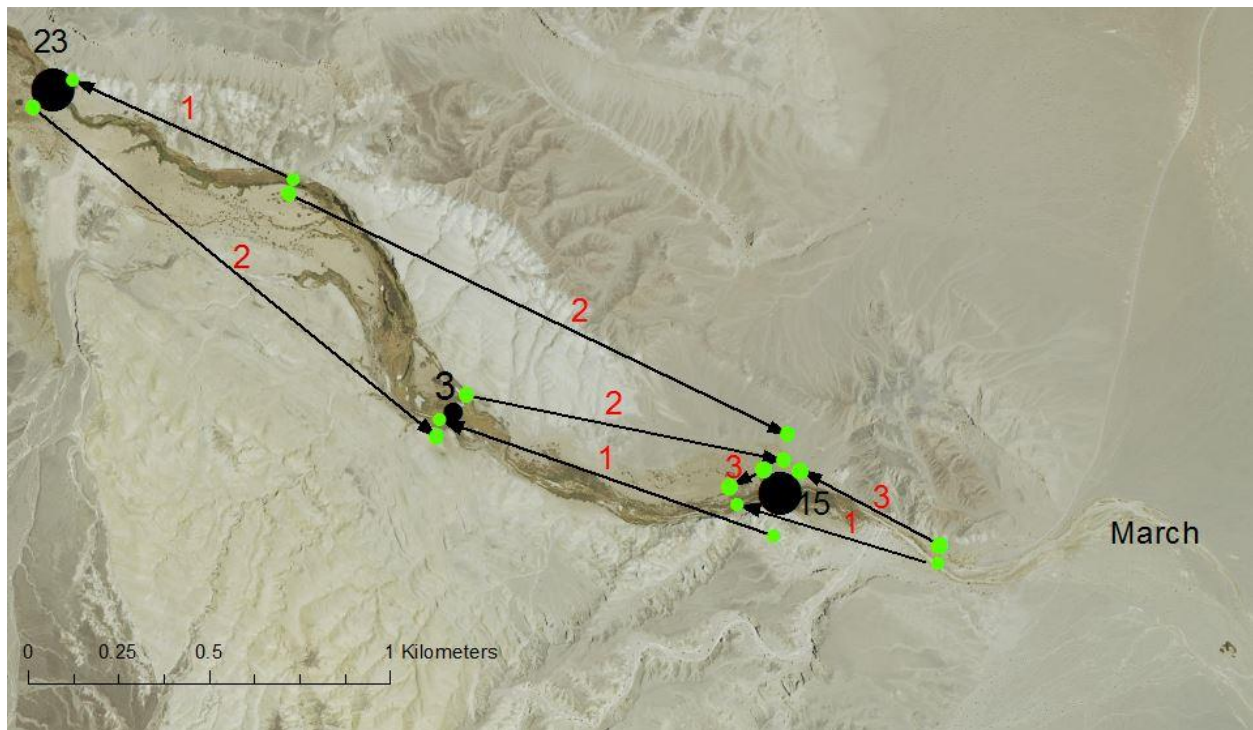


Figure 24: Documented recaptures and movements for the month of March. Black circles represent stationary recaptures and green circles denote moving individuals. Labels represent number of fish with that recapture history, red labels for moving fish and black labels for stationary fish. Month label represents lowest stretch of the creek.

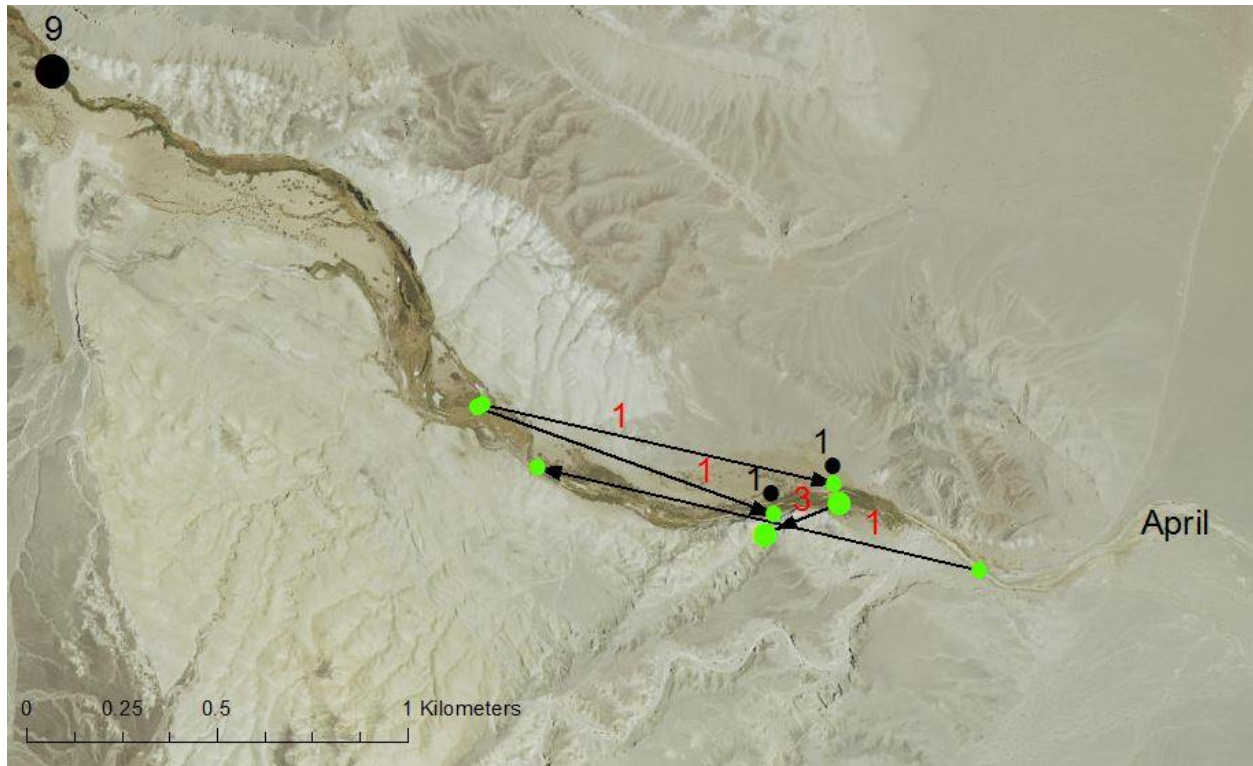


Figure 25: Documented recaptures and movements for the month of April. Black circles represent stationary recaptures and green circles denote moving individuals. Labels represent number of fish with that recapture history, red labels for moving fish and black labels for stationary fish. Month label represents lowest stretch of the creek.

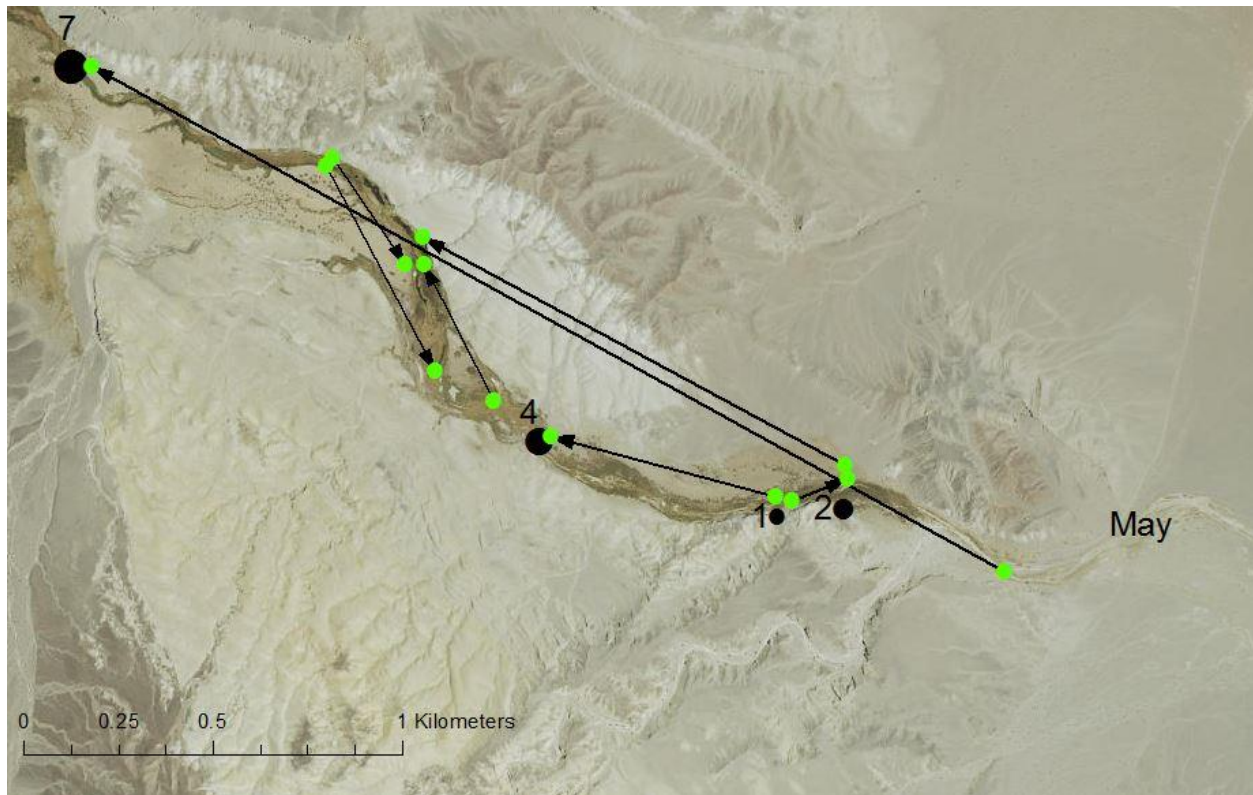


Figure 26: Documented recaptures and movements for the month of May. Black circles represent stationary recaptures and green circles denote moving individuals. Black labels represent number of stationary recaptures at each location. All fish movements for May were single fish only. Month label represents lowest stretch of the creek.

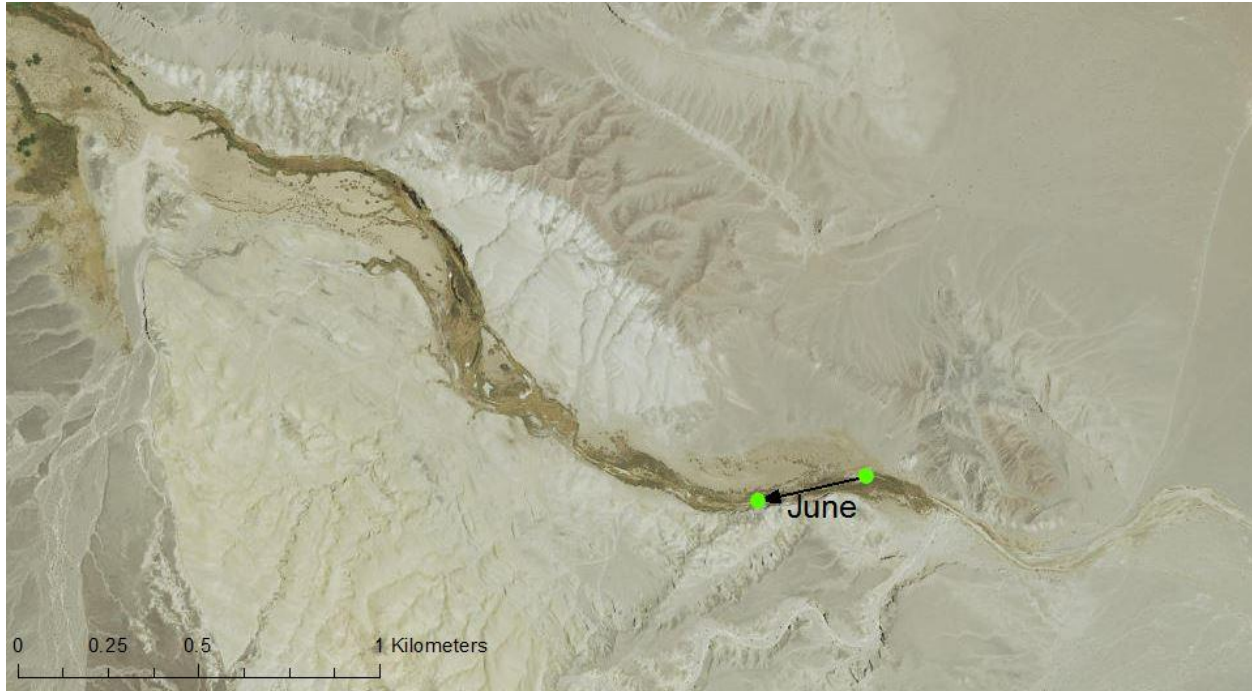


Figure 27: Documented fish movement for the month of June. Only a single fish was recaptured during this month. Month label represents lowest wetted stretch of Salt Creek.

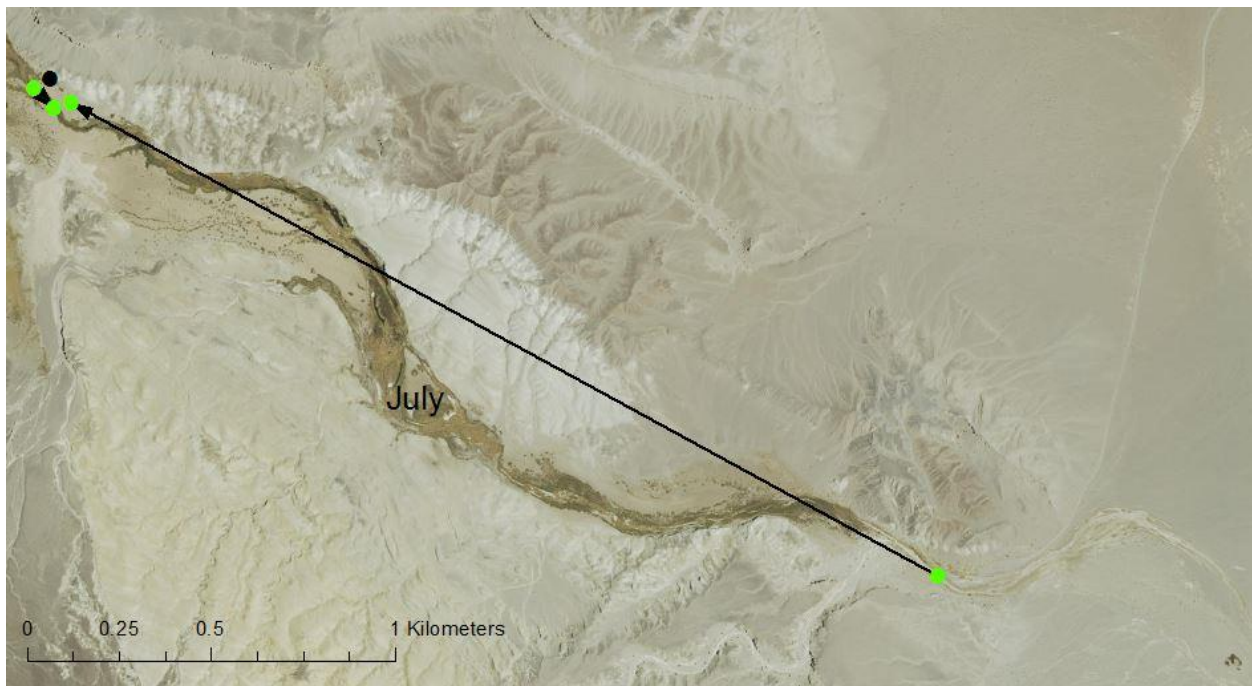


Figure 28: Documented fish movement and recaptures for the month of July. Black circle represents a stationary fish. Green circles and arrows represent moving fish. Only three fish were recaptured during the month. Month label represents lowest wetted stretch of Salt Creek.

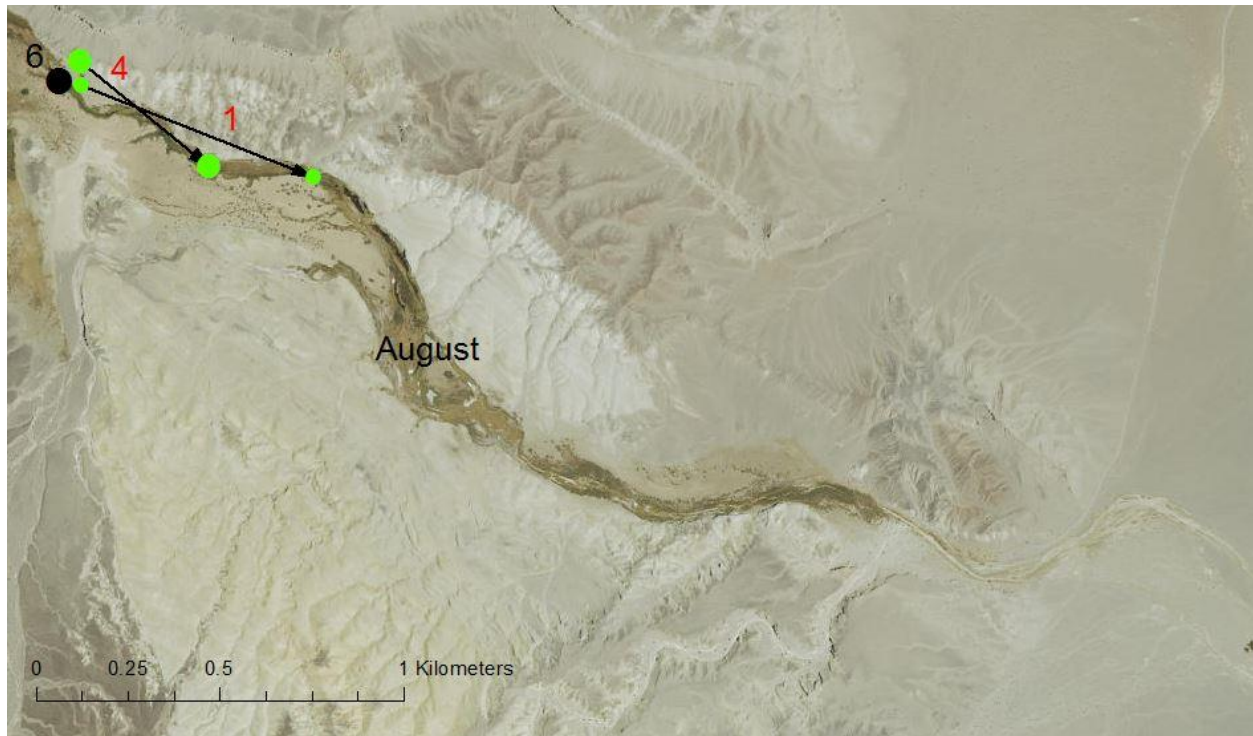


Figure 29: Documented fish movement and recaptures for the month of August. Black circles represent stationary fish and green circles and arrows represent fish movements. Labels represent number of fish with that recapture history. Month label represents lowest wetted stretch of Salt Creek.



Figure 30: Documented recaptures and fish movement for the month of September. Closed circle represents stationary fish, green circle and arrows represent moving fish. Black label represents the number of stationary fish recaptured at that location. Only a single moving fish was detected in September. Month label represents lowest wetted reach of Salt Creek.

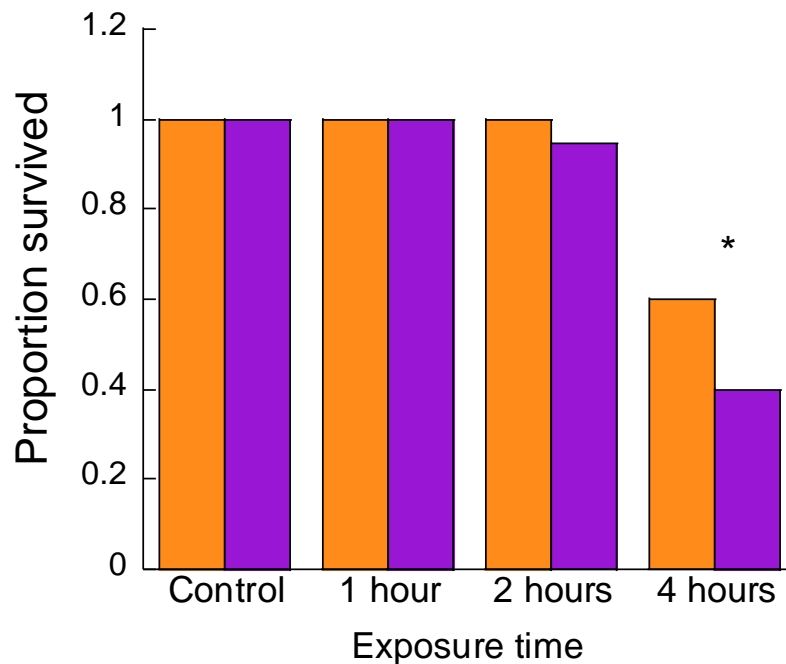


Figure 31: Proportion of *C. salinus* individuals that survive following heat exposure. Fish were exposed to 35- 40 °C from 0 to 4 hours and survival was monitored using enclosures placed in the creek for one week. Orange bars represent survival during initial exposure, while purple bars represent survival for one week following exposure. Test for statistical significance was performed using Chi squared contingency test. Individuals exposed to 35- 40 °C for 4 hours had a survival rate of less than 50% one week after exposure. Asterisk denotes statistical significance, $P < 0.05$.



Figure 32: Fish kill observed in the intermediate marking location at Salt Creek in July of 2016. Dissolved oxygen was 0.85 mg/l.

Discussion:

Pupfish from the genus *Cyprinodon* are often cited as having some of the widest thermal tolerances of any fish species. Sheepshead Minnow, *Cyprinodon variegatus*, and several species from the Death Valley species complex of pupfishes have been reported to be tolerant of temperatures above 40 °C on a short-term basis (Bennett & Beitinger, 1997; Brown & Feldmeth, 1971). Measured upper lethal thermal limits of the Salt Creek Pupfish are reported to be between 41 °C and 42 °C when temperature is raised at a gradual rate until fish die (Brown & Feldmeth, 1971). Importantly, despite being often touted as perhaps the most temperature tolerant fishes the recorded critical thermal maximum of *Cyprinodon* fishes is not markedly higher than common game fish which inhabit habitats which are generally far cooler than those inhabited by pupfishes (Brown & Feldmeth, 1971; Currie et al., 1998).

Several different approaches have been used to measure upper thermal limits in fish (Becker & Genoway, 1979; Currie et al., 1998; Fields Robert; Lowe, 1987). I contest, as numerous other authors have, that there are weaknesses associated with each of these methods (Becker & Genoway, 1979; Currie et al., 1998; Lutterschmidt & Hutchison, 1997). Incipient upper thermal limit (IUTL) measures a chronic effect but ignores the variability that is often present in aquatic habitats. Critical thermal maximum (CTM) measures the effects of a dynamic, albeit constantly warming temperature. Thus, upper thermal tolerance is difficult to measure in an ecologically relevant way. For instance, at my chosen study area, Salt Creek, fish in the ephemeral sections of the creek are exposed to high temperatures in excess of 40 °C for a several-hour period before temperatures drop at night (Figure 14). This work aimed to better understand the long-term effects of fish which are exposed to prolonged high temperatures just below their CTM through 1) assessing longer term survival following a heat exposure and 2) assessing whether fish that colonize ephemeral habitats return to permanent sections of the creek. This study is relevant not only to better understanding the biology of *Cyprinodon salinus*, but to also understanding fisheries in general since variability and temperatures of aquatic systems are expected to increase as a result of global climate change (Archer & Predick, 2008; Ficke, Myrick, & Hansen, 2007).

A previous study aimed to examine movement patterns of *C. salinus* during the spring breeding system (Dzul et al., 2013). This study detected substantial movement within the ephemeral reaches of Salt Creek and limited movement from the lower reaches of Salt Creek into permanent habitats. Likewise, colonization of ephemeral habitats from upstream areas was detected but rare (Dzul et al., 2013). This study utilized a batch mark system method e.g. fish were given marks that corresponded to habitats rather than individual marks. My work had a few

advantages, a longer sampling period allowed for analysis of movement patterns over a longer time scale including during and following the harsh summer months. Additionally, I utilized an individual marking system which enabled me to determine precise movements of each recaptured individual and to analyze growth patterns of individuals inhabiting different spatial patterns of Salt Creek (Figure 10).

While both downstream and upstream movement of individuals was detected throughout Salt Creek during the study period, 65% of fish remained stationary in Salt Creek after marking (Figures 20, 23-30). Fish appeared to colonize the ephemeral sections of Salt Creek in February and March but these colonizers appear to represent a low percentage of the overall population (Figure 23 and 24). Only six fish out of over 800 marked in headwaters and intermediate stretches of the creek were later detected in ephemeral habitats. Most of the fish which were recaptured from ephemeral habitats were also marked in those areas (Figures 24-26). Most recaptured fish were fish that were marked and remained stationary in the headwaters (Figures 23-30). This had been reported previously in Salt Creek Pupfish (Dzul et al., 2013). Fish densities increased in ephemeral areas from February through June despite no increase in detected movement into these areas (Figures 20-27). I speculate that many of the fish observed by our game cameras in May and June were fish that were spawned in these habitats. Only 3 fish out of 799 total marked in the furthest downstream marking area in March were detected later in permanent sections of Salt Creek. These fish were detected separately in the headwaters and intermediate sections in May, July, and September. These fish moved 2.3-2.4 km upstream from the lower reaches of Salt Creek into permanent sections of creek. This is the first time that an upstream movement so large was detected in Salt Creek Pupfish. I note that the three fish which returned to permanent sections of Salt Creek were detected in the ephemeral sections no later

than March, when temperatures in these sections did not exceed 30 °C (Figure 14). The possibility exists that these three fish never experienced the extreme high temperatures which become common in ephemeral sections of Salt Creek in May and June.

I initially hypothesized that Salt Creek functioned as a classical source-sink system for pupfish with ephemeral sections of Salt Creek functioning as sinks habitats and the headwaters functioning as a source habitat (Amarasekare, 2004; Levin, 1974). The temperature regimes present in ephemeral sections of Salt Creek in May and June are very near the CTM of *C. salinus* and outside the thermal limits for successful reproduction (Brown & Feldmeth, 1971; Gerking & Lee, 1983). However from mid-February through April the temperatures present in the ephemeral sections of the creek are very favorable for pupfish growth and reproduction (Shrode & Gerking, 1977; Figure 15). Individuals that initially colonize the downstream reaches of Salt Creek early in the year could also benefit from relatively low population densities according to fish count data obtained from game cameras placed in ephemeral portions of the creek (Figure 21). While Wilderness regulations precluded me from placing game cameras in the headwaters or intermediate sections of the creek for comparison, observations and previous reports indicate that population densities in Salt Creek are generally lower in ephemeral sections (Sada & Deacon, 1995). Recaptured pupfish in the ephemeral sections of Salt Creek grew faster than those recaptured in the headwaters (Figure 15). Additionally, breeding experiments conducted throughout Salt Creek in March showed that reproduction is likely higher in ephemeral sections of the creek, at least during spring (Figure 16). No eggs were collected from breeding enclosures placed in the headwaters of Salt Creek in either March or September of 2016, and no breeding was observed during sampling sessions in the headwaters. These data suggest that there are some fitness benefits for pupfish which colonize the ephemeral sections of Salt Creek in the spring.

There appears to be several risks for fish which inhabit ephemeral sections of Salt Creek, whether they are colonizers or fish which were spawned there. One obvious risk for pupfish which live here is desiccation. There were some large upstream movements detected by individuals which were initially marked in the ephemeral habitats of Salt Creek. However, most fish marked in these areas were not detected anywhere in the creek after March (Figures 24-30). While I did not recover any marked dead fish from these areas, pupfish mortality was observed throughout the ephemeral areas on most sampling sessions. Many of these marked fish may have perished as the creek evaporated and warmed. In our heat exposure experiments, fish exposed to 35-40 °C for four hours experienced greater than 50% mortality within one week of exposure (Figure 31). This temperature regime becomes common in the ephemeral sections of the creek in June (Figure 14). Early season ephemeral colonizers experience favorable conditions for growth and reproduction but risk desiccation and unfavorable temperature conditions if they remain in these areas into the summer months. While pupfish reproduction tends to be high in ephemeral habitats, it is unclear how many new recruits successfully escape desiccation and heat exposure in the summer months. Follow up studies may prove beneficial to test whether very young fish escape ephemeral habitats in higher percentages than do adult fish. Researchers have been successful in marking fish as small as 8 mm using visual implant elastomers, so juvenile pupfish can be marked successfully by an experienced researcher (Frederick, 1997).

Most recaptured pupfish in Salt Creek remained stationary in their original marking location (~65%). A small proportion of fish moved between habitats at Salt Creek (Figures 23-30). Instead of a classical source-sink system, the Salt Creek Pupfish seem to be a partially migratory species (e.g. Chapman, Brönmark, Nilsson, & Hansson, 2011). Many fish seem to live their entire lives stationary within the headwaters of Salt Creek, while a few fish make large

downstream and upstream movements. It is possible that lack of favorable breeding or foraging conditions within the headwaters mediate migration into ephemeral habitats for some individuals (Figures 15 and 16). Risks associated with migrating into a temporary habitat include desiccation and heat exposure. These risks could explain why many fish live their entire lives within the permanent headwaters of Salt Creek. Further investigation into the genetic makeup, nutritional status, and individual physiology of fish living in the ephemeral and permanent habitats of Salt Creek might provide insight into why some individuals move and others remain stationary.

Habitat suitability differs not only spatially across Salt Creek, but also temporally. The ephemeral sections of the creek appear to be the most favorable pupfish habitats during the spring, but the intermediate and headwaters sections of the creek constitute the only favorable habitat for pupfish during the hot summer months. I observed a large fish kill of several hundred individuals located in the intermediate sections of Salt Creek in July, likely due to nominally hypoxic conditions. Even certain permanent sections of the creek may not be suitable for pupfish survival year-round (Figure 32). Curiously, I was unable to detect any breeding in the headwaters of Salt Creek during this study and it is unclear whether any breeding takes place there. Breeding is readily observed throughout ephemeral sections of the creek but was not observed throughout the study period in the headwaters. It is possible that breeding in the headwaters takes place over the summer months when access to those habitats is difficult. However, I note that no eggs were collected from breeding enclosures placed in the headwaters during September when temperatures should have been suitable for reproduction and some proportion of females should have been carrying mature eggs (Figure 16; Lee, Heins, Echelle, & Echelle, 2014; Shrode & Gerking, 1977). Contrary to the original hypothesis, ephemeral habitats are favorable for certain aspects of pupfish life cycles such as growth and reproduction.

Salt Creek in Death Valley, CA represents a dynamic inland fisheries environment marked by tremendous diel, seasonal, and spatial variation (Dzul Maria C.; Gaines, 2012; Miller, 1943; Williams & Sada, 1985). Temporal influences control habitat quality throughout the creek with ephemeral habitats being favorable in the spring and headwaters habitats providing refuge for fish during the summer. Fish population dynamics throughout the creek differ between and within the year and it is likely that some factors controlling movement and fish distribution are overlooked (Sada & Deacon, 1995). Individual movements did not appear to be affected by fish size or sex (Figures 17, 18 and 20). All habitats at Salt Creek are potentially important to maintenance of the overall pupfish population. While no large-scale migrations from ephemeral habitats into permanent habitats were detected during this study, a few fish were able to escape the ephemeral stretches before the creek evaporated. Future efforts should focus on examining physical characteristics which could influence pupfish habitat choice such as oxygen and food availability. Long-term monitoring of intermediate stretches of the creek should identify which pools can act as summer refuges for pupfish. Marking and recapturing juvenile pupfish which were spawned in ephemeral stretches of Salt Creek will help quantify how many juveniles escape ephemeral habitats as the creek dries. Such work will be important for identifying how to best conserve and manage a unique creek system which will likely be altered by climate change substantially (Archer & Predick, 2008; Ficke et al., 2007; Nickus et al., 2010)

Chapter 4: Conclusions and future directions

In the second chapter, I focused on a methodological improvement for measuring oxygen consumption in developing fish eggs and separated the effects of parent thermal history from incubation temperature on developing eggs (Jones et al., 2016). Parental thermal history is important in determining overall hatch success of developing eggs. Eggs reared at the ecologically relevant temperature of 33 °C displayed altered patterns of oxygen consumption despite no evidence of altered developmental outcomes. Based on this work and previous work in the laboratory, it appears that anaerobic metabolism is especially important for pupfish which live at 33 °C, the most available temperature within the important Devils Hole system (Heuton et al., 2015; Jones et al., 2016). Further work should aim at a better understanding the overall role of anaerobic metabolism both in eggs and adult fish at ecologically relevant high temperatures. Utilization of the described method for measuring oxygen consumption in single eggs will enable other researchers to measure individual variations in aerobic cost of development. Pupfish remain an ideal organism for examining the effects of life at high temperatures and the role of anaerobic processes as a component of overall metabolism.

In the third chapter, I focused on understanding individual movement patterns and habitat utilization within a hypervariable creek system inhabited by the Salt Creek Pupfish, *Cyprinodon salinus*. I employed a marking method which for the first time allowed for identification of individual rather than batch movements of pupfish. I also used field experiments to test fish reproduction and survival in the creek. Unexpectedly, I found that there are potential fitness benefits for fish which colonize and live in ephemeral habitats at Salt Creek. Fish reproduction is highest in ephemeral areas during the spring breeding season, and fish grow faster in these habitats. However, fish in these ephemeral habitats risk death by desiccation and heat exposure

when they leave the permanent habitats at Salt Creek. Further work is needed to identify whether new recruits from ephemeral waters escape desiccation and heat exposure. Habitat modeling for Salt Creek would prove useful for understanding how climate change might alter population dynamics and habitats available to these endangered fish.

Bibliography

- Amarasekare, P. (2004). The role of density-dependent dispersal in source-sink dynamics. *Journal of Theoretical Biology*, 226(2), 159–168. <http://doi.org/10.1016/j.jtbi.2003.08.007>
- Andersen, M. E., & Deacon, J. E. (2001). Population size of Devils Hole pupfish (*Cyprinodon diabolis*) correlates with water level. *Copeia*, 2001(1), 224. [http://doi.org/10.1643/0045-8511\(2001\)001\[0224:psodhp\]2.0.co;2](http://doi.org/10.1643/0045-8511(2001)001[0224:psodhp]2.0.co;2)
- Archer, S., & Predick, K. (2008). Climate change and ecosystems of the southwestern United States. *Rangelands*, 30(3), 23–28. Retrieved from [http://www.srmjournals.org/doi/full/10.2111/1551-501X\(2008\)30%5B23:CCAEOT%5D2.0.CO%3B2](http://www.srmjournals.org/doi/full/10.2111/1551-501X(2008)30%5B23:CCAEOT%5D2.0.CO%3B2)
- Bang, A., Grønkjær, P., & Malte, H. (2004). Individual variation in the rate of oxygen consumption by zebrafish embryos. *Journal of Fish Biology*, 64(5), 1285–1296. <http://doi.org/10.1111/j.1095-8649.2004.00391.x>
- Barrionuevo, W. R., & Burggren, W. W. (1999). O₂ consumption and heart rate in developing zebrafish (*Danio rerio*): influence of temperature and ambient O₂. *American Journal of Physiology- Regulatory, Integrative, and Comparative Physiology*, (276), R505–R513.
- Becker, C. D., & Genoway, R. G. (1979). Evaluation of the critical thermal maximum for determining thermal tolerance of freshwater fish. *Env. Biol. Fish*, 4(3), 245–256. <http://doi.org/10.1007/BF00005481>
- Bennett, W. A., & Beitinger, T. L. (1997). Temperature Tolerance of the Sheepshead Minnow, *Cyprinodon variegatus*. *Copeia*, (1), 77–87.

- Bernot, M. J., & Wilson, K. P. (2012). Spatial and Temporal Variation of Dissolved Oxygen and Ecosystem Energetics in Devils Hole, Nevada. *Western North American Naturalist*, 72(3), 265–275. <http://doi.org/10.3398/064.072.0301>
- Blueweiss, A. L., Fox, H., Kudzma, V., Nakashima, D., Peters, R., & Sams, S. (1978). Relationships between Body Size and Some Life History Parameters Published by : Springer in cooperation with International Association for Ecology Stable URL : <http://www.jstor.org/stable/4215748>. *Oecologia*, 37(2), 257–272.
- Brown, J., & Feldmeth, C. (1971). Evolution in constant and fluctuating environments: thermal tolerances of desert pupfish (Cyprinodon). *Evolution*, 25(2), 390–398. Retrieved from <http://www.jstor.org/stable/2406931>
- Carson, E. W., de la Maza-Benignos, M., de Lourdes Lozano-Vilano, M., Vela-Valladares, L., Banda-Villanueva, I., & Turner, T. F. (2014). Conservation genetic assessment of the critically endangered Julimes pupfish, *Cyprinodon julimes*. *Conservation Genetics*, 15(2), 483–488. <http://doi.org/10.1007/s10592-013-0548-x>
- Chapman, B. B., Brönmark, C., Nilsson, J. Å., & Hansson, L. A. (2011). The ecology and evolution of partial migration. *Oikos*, 120(12), 1764–1775. <http://doi.org/10.1111/j.1600-0706.2011.20131.x>
- Currie, R. J., Bennett, W. A., & Beitinger, T. L. (1998). Critical thermal minima and maxima of three freshwater game-fish species acclimated to constant temperatures. *Environmental Biology of Fishes*, 51(2), 187–200. <http://doi.org/10.1023/A:1007447417546>
- Deacon, J. E., Taylor, F. R., & Pedretti, J. W. (1995). Egg viability and ecology of the Devils Hole Pupfish: Insights from captive propagation. *The Southwestern Naturalist*, 40(2), 216–

- Dzul, M. C., Quist, M. C., Dinsmore, S. J., Gaines, D. B., & Bower, M. R. (2013). Coarse-scale movement patterns of a small-bodied fish inhabiting a desert stream. *Journal of Freshwater Ecology*, 28(1), 27–38. <http://doi.org/10.1080/02705060.2012.718250>
- Dzul Maria C.; Gaines, D. B. F. J. R. . Q. M. C. . D. S. J. (2012). Evaluation of Otoliths of Salt Creek Pupfish (*Cyprinodon salinus*) for use in Analyses of Age and Growth. *The Southwestern Naturalist*, 57(4), 412–416. <http://doi.org/10.1894/0038-4909-57.4.412>
- Echelle, A. A., & Dowling, T. E. (1992). Mitochondrial-DNA Variation and Evolution of the Death-Valley Pupfishes (*Cyprinodon*, *Cyprinodontidae*). *Evolution*, 46(1), 193–206.
- Feuerbacher, O. G., Mapula, J. A., & Bonar, S. A. (2015). Propagation of Hybrid Devils Hole Pupfish × Ash Meadows Amargosa Pupfish. *North American Journal of Aquaculture*, 77(4), 513–523. <http://doi.org/10.1080/15222055.2015.1044057>
- Ficke, A. D., Myrick, C. A., & Hansen, L. J. (2007). *Potential impacts of global climate change on freshwater fisheries. Reviews in Fish Biology and Fisheries* (Vol. 17). <http://doi.org/10.1007/s11160-007-9059-5>
- Fields Robert; Lowe, S. S. . K. C. W. G. S. . P. D. P. (1987). Critical and Chronic Thermal Maxima of Northern and Florida Largemouth Bass and Their Reciprocal F 1 and F 2 Hybrids. *Transactions of the American Fisheries Society*, 116(6). [http://doi.org/10.1577/1548-8659\(1987\)116<856:CACTMO>2.0.CO;2](http://doi.org/10.1577/1548-8659(1987)116<856:CACTMO>2.0.CO;2)
- Frederick, J. L. (1997). Evolution of Fluorescent elastomer injection as a method for marking small fish. *Bulletin of Marine Science*, 61 (2)(2), 399–408.

- Gerking, S. D., & Lee, R. M. (1983). Thermal Limits for Growth and Reproduction in the Desert Pupfish *Cyprinodon n. nevadensis*. *Physiological Zoology*, 56(1), 1–9.
- Hausner, M. B., Wilson, K. P., Gaines, D. B., Suarez, F., Scoppettone, G. G., & Tyler, S. W. (2014). Life in a fishbowl: Prospects for the endangered Devils Hole pupfish (*Cyprinodon diabolis*) in a changing climate. *Water Resources Research*, 50, 7020–7034.
<http://doi.org/10.1002/2013WR014049>.Received
- Hausner, M. B., Wilson, K. P., Gaines, D. B., Suárez, F., Scoppettone, G. G., & Tyler, S. W. (2016). Projecting the effects of climate change and water management on Devils Hole pupfish (*Cyprinodon diabolis*) survival. *Ecohydrology*, 9(4), 560–573.
<http://doi.org/10.1002/eco.1656>
- Hausner, M. B., Wilson, K. P., Gaines, D. B., Suárez, F., & Tyler, S. W. (2013). The shallow thermal regime of Devils Hole, Death Valley National Park. *Limnology & Oceanography: Fluids & Environments*, 3, 119–138. <http://doi.org/10.1215/21573689-2372805>
- Heuton, M., Ayala, L., Burg, C., Dayton, K., McKenna, K., Morante, A., ... Breukelen, F. van. (2015). Paradoxical anaerobism in desert pupfish. *Journal of Experimental Biology*, 218, 3739–3745. <http://doi.org/10.1098/rsbl.2009.0501>
- Hillyard, S. D. (2011). Life in hot water: The desert pupfish. In A. P. Farrell & D. Stevens (Eds.), *Encyclopedia of Fish Physiology: From Genome to Environment* (pp. 1831–1842). London: Academic Press.
- Hillyard, S. D., Podrabsky, J. E., & Breukelen, F. van. (2015). Desert Environments. In R. Riesch, M. Tobler, & M. Plath (Eds.), *Extremophile Fishes: Ecology, Evolution, and Physiology of Teleosts in Extreme Environments* (pp. 59–83). <http://doi.org/10.1007/978-3->

- Jones, A. C., Lim, D., Wayne-Thompson, J. J., Urbina, N., Puentedura, G., Hillyard, S., & Breukelen, F. Van. (2016). Oxygen Consumption is Limited at an Ecologically Relevant Rearing Temperature in Pupfish Eggs. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, 325(8), 539–547. <http://doi.org/10.1002/jez.2048>
- Karam, A. P., Parker, M. S., & Lyons, L. T. (2012). Ecological Comparison between Three Artificial Refuges and the Natural Habitat for Devils Hole Pupfish. *North American Journal of Fisheries Management*, 32(2), 224–238. <http://doi.org/10.1080/02755947.2012.672870>
- Kimmel, C. B., Ballard, W. W., Kimmel, S. R., Ullmann, B., & Schilling, T. F. (1995). Stages of embryonic development of the zebrafish. *Developmental Dynamics*, 203(3), 253–310. <http://doi.org/10.1002/aja.1002030302>
- Kinne, O., & Kinne, E. . (1962). Rates of development in embryos of a cyprinodont fish exposed to different temperature-salinity-oxygen combinations. *Canadian Journal of Zoology*, 40(2), 231–253. <http://doi.org/10.1139/z62-025>
- Lee, J. S., Heins, D. C., Echelle, A. F., & Echelle, A. A. (2014). Annual ovarian cycle and other reproductive traits of female Red River Pupfish (*Cyprinodon rubrofluviatilis*) in the Red River drainage of Texas. *The Southwestern Naturalist*, 59(1), 9–14. <http://doi.org/10.1894/F02-RJE-37.1>
- Lema, S. C., & Nevitt, G. a. (2006). Testing an ecophysiological mechanism of morphological plasticity in pupfish and its relevance to conservation efforts for endangered Devils Hole pupfish. *The Journal of Experimental Biology*, 209(Pt 18), 3499–509. <http://doi.org/10.1242/jeb.02417>

- Levin, S. A. (1974). Dispersion and Population Interactions. *The American Naturalist*, 108(960), 207–228. <http://doi.org/10.1086/282900>
- Lutterschmidt, W. I., & Hutchison, V. H. (1997). The critical thermal maximum: history and critique. *Canadian Journal of Zoology*, 75(10), 1561–1574. <http://doi.org/10.1139/z97-783>
- Martin, A. P. (2005). *Genetic analysis of C. diabolis: hybridization with C. nevadensis in the Point of Rocks Refuge. Report to the Nevada Department of Wildlife.*
- Martin, A. P., Echelle, A. a., Zegers, G., Baker, S., & Keeler-Foster, C. L. (2012). Dramatic shifts in the gene pool of a managed population of an endangered species may be exacerbated by high genetic load. *Conservation Genetics*, 13(2), 349–358. <http://doi.org/10.1007/s10592-011-0289-7>
- Martin, C. H., Crawford, J. E., Turner, B. J., & Simons, L. H. (2016). Diabolical survival in Death Valley: recent pupfish colonization, gene flow and genetic assimilation in the smallest species range on earth. *Proceedings of the Royal Society B: Biological Sciences*, 283(1823), 20152334. <http://doi.org/10.1098/rspb.2015.2334>
- Merideth, R. (2001). *A Primer on Climate Change and Climatic Variability in the Southwest*. Tucson, AZ.
- Miller, R. (1943). *Cyprinodon salinus*, a new species of fish from Death Valley, California. *Copeia*, 1943(2), 69–78. Retrieved from <http://www.jstor.org/stable/1437768>
- Minckley, C. O., & Deacon, J. E. (1973). Observations on the reproductive cycle of *Cyprinodon diabolis*. *Copeia*, 1973(3), 610–613.
- Mueller, C. A., Eme, J., Manzoni, R. G., Somers, C. M., Boreham, D. R., & Wilson, J. Y. (2015).

- Embryonic critical windows: changes in incubation temperature alter survival, hatchling phenotype, and cost of development in lake whitefish (*Coregonus clupeaformis*). *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, 185(3), 315–331. <http://doi.org/10.1007/s00360-015-0886-8>
- Mueller, C. A., Joss, J. M. P., & Seymour, R. S. (2011). The energy cost of embryonic development in fishes and amphibians, with emphasis on new data from the Australian lungfish, *Neoceratodus forsteri*. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, 181(1), 43–52. <http://doi.org/10.1007/s00360-010-0501-y>
- Nickus, U., Bishop, K., Erlandsson, M., Evans, C. D., Forsius, M., Laudon, H., ... Thies, H. (2010). Direct Impacts of Climate Change on Freshwater Systems. In M. Kernan, R. W. Battarbee, & B. R. Moss (Eds.), *Climate Change Impacts on Freshwater Systems* (1st ed., pp. 38–64). Wiley-Blackwell.
- Pörtner, H. (2001). Climate change and temperature-dependent biogeography: Oxygen limitation of thermal tolerance in animals. *Naturwissenschaften*, 88(4), 137–146. <http://doi.org/10.1007/s001140100216>
- Riggs, A. C., & Deacon, J. E. (2002). Connectivity in desert aquatic ecosystems : The Devils Hole Story. In *Spring-fed Wetlands: Important Scientific and Cultural Resources of the Intermountain Region*. Retrieved from www.wetlands.dri.edu
- Sada, D. W., & Deacon, J. E. (1995). *Spatial and Temporal Variability of Pupfish (genus Cyprinodon) Habitat and Populations at Salt Creek and Cottonball Marsh, Death Valley National Park, California*.

- Salinas, S., & Munch, S. B. (2012). Thermal legacies: Transgenerational effects of temperature on growth in a vertebrate. *Ecology Letters*, 15(2), 159–163. <http://doi.org/10.1111/j.1461-0248.2011.01721.x>
- Shrode, J. B., & Gerking, S. D. (1977). Effects of Constant and Fluctuating Temperatures on Reproductive Performance of a Desert Pupfish, *Cyprinodon n. nevadensis*. *Physiological Zoology*, 50(1), 1–10.
- Wales, J. H. (1930). Biometrical Studies of Some Races of Cyprinodont Fishes, from the Death Valley Region, with Description of *Cyprinodon diabolis*, n. sp. *Copeia*, 1930(3), 61–70.
- Williams, J. E., & Sada, D. W. (1985). Status of Two Endangered Fish, *Cyprinodon nevadensis mionectes* and *Rhinichthys osculus nevadensis*, from Two Springs in Ash Meadows, Nevada. *The Southwestern Naturalist*, 30(4), 475–484.
- Wilson, K. P., & Blinn, D. W. (2007). Food Web Structure, Energetics, and Importance of Allochthonous Carbon in a Desert Cavernous Limnocrone: Devils Hole, Nevada. *Western North American Naturalist*, 67(2), 185–198. [http://doi.org/10.3398/1527-0904\(2007\)67\[185:FWSEAI\]2.0.CO;2](http://doi.org/10.3398/1527-0904(2007)67[185:FWSEAI]2.0.CO;2)

Curriculum Vitae

Alexander Charles Jones

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Education

University of Nevada Las Vegas, NV

-M.S. Biological Sciences

-GPA 3.93/4.0

-Research related to the ecology and physiology of desert pupfish, *Cyprinodon sp.*

-Thesis title: Thermal limits and developmental physiology of desert pupfish

Arizona State University Tempe, AZ

-B.S. Biological Sciences (Biological Conservation and Ecology).

-GPA 3.72/4.0

-Magna Cum Laude Graduation

Achievements

-Mentor and supervisor to over 18 undergraduate volunteer researchers and employees in the husbandry and research of endangered pupfish at University of Nevada, Las Vegas (UNLV)

-Successfully managed over 5 research projects aimed at understanding pupfish physiology and ecology

-Skilled at public outreach and engagement. Previous experience as outreach specialist for non-profits and the National Park Service. Reached over 10,000 individuals while enrolled at UNLV with research regarding desert pupfish.

-Nevada NASA Space Grant Consortium Higher Education Fellowship Recipient

-Desert Studies Center-Judith A. Presch Scholarship for Desert Research Recipient

-UNLV Graduate Student Association Research Sponsorship Recipient

-University of Nevada Las Vegas Graduate Recruitment Scholarship

Experience

University of Nevada, Las Vegas, Fish Biologist and Husbandry Specialist, August 2014-present

-Supervise undergraduate researchers in the husbandry and research of endangered pupfish

-Manage research projects in the field and laboratory

-Present research findings to colleagues at scientific meetings

-Prepare technical reports and scientific manuscripts for publication

-Apply for and obtain research permits from numerous federal and state agencies

University of Illinois, Ratsnake Biological Technician, April-August 2014

-Track Ratsnake movement and habitat use via radio telemetry

-Manage and enter data

-Care for captive Ratsnake population

-Follow security protocols on Dept. of Energy field site

-Provide injections and medical care to Ratsnakes following surgery

National Park Service, Great Basin National Park, Park Ranger, April-October 2013

- Presented and develop educational programs on a variety of park resources
- Assisted park visitors at visitor center and throughout park
- Assisted resource management division in Great Basin Rattlesnake telemetry project

Great Basin Institute, Education and Outreach Specialist, January 2012- March 2013

- Presented and developed interpretive and educational programs relating to natural and cultural resources of Lake Mead
- Helped to implement and develop programs aimed at getting high school and junior high kids to recreate responsibly on their public lands
- Managed public educational hiking program for Lake Mead National Recreation Area
- Roved Lake Mead NRA providing informal interpretation to visitors
- Assisted with resource management projects
- Acted as frontline representative of the National Park Service

Central Arizona College, Math and Science tutor, August 2009- January 2012

- Tutored students in Math courses ranging from basic arithmetic through Calculus
- Tutored students in Science courses ranging from GED science to advanced biology
- Planned and hosted study sessions for advanced science classes
- Provided customer service
- Tracked and entered learning center usage data

National Park Service, Great Basin National Park, Park Guide, May-August 2010

- Developed and presented educational programs related to natural and cultural resources of the Great Basin
- Assisted park visitors in visitor centers and throughout park
- Assisted in resource management projects
- Collected and account for fee monies

Scientific Publications and Presentations

Jones A, Lim D, Wayne-Thompson J, Urbina N, Hillyard S, van Breukelen F. Oxygen Consumption is limited at an ecologically relevant rearing temperature in pupfish eggs. Journal of Experimental Zoology Part A. 2016.

Jones A, Crowder C, Mosher K. Description of a new, previously unknown population of an endangered desert fish, *Poeciliopsis occidentalis occidentalis*. The Southwestern Naturalist. 2016.

Jones A, Hunt J, Pansin C, Aleksic M, Chamnong O, Hillyard S, van Breukelen F. A Pup's life: Pupfish growth, reproduction, and survival in a hypervariable environment. Desert Fishes Council 2016 Annual Meeting. Oral Presentation.

Jones A, Lim D, Hillyard S, van Breukelen F. The influence of temperature on reproductive success and oxidative metabolism in desert pupfish. James Deacon Special Symposium. Desert Fishes Council 2015 Annual Meeting. Oral Presentation.

Jones A, Lim D, Hillyard S, van Breukelen F. Oxygen consumption in single eggs of a desert pupfish. Experimental Biology 2016 Annual Meeting. Poster Presentation. Abstract available in the FASEB Journal. March 2016.

Professional Presentations

February 2013- Nevada Department of Wildlife Project Aquatic WILD Teacher Workshop:

“Chilling with the Chubs: Threats faced by native fishes in the Colorado River watershed”

September 2012-Great Basin Institute Parks Climate Challenge Teacher Workshop: “Climate Change at Lake Mead: What we know now and how deserts might be affected”

April 2011- Arthur Carhart National Wilderness Training Center: “Wilderness at Lake Mead: Management issues and opportunities for ‘urban’ Wilderness Areas in the Mojave Desert”