The effect of temperature on development and behavior of relict leopard frog tadpoles (Rana onca)

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THE EFFECT OF TEMPERATURE ON DEVELOPMENT AND BEHAVIOR OF RELICT LEOPARD FROG TADPOLES (*RANA ONCA*)

by

Jeffrey Abraham Goldstein

Bachelors of Science
University of Nevada, Las Vegas
2000

A thesis in partial fulfillment of the requirement for the

Master of Science Degree in Biological Sciences
School of Life Sciences
College of Sciences

Graduate College
University of Nevada, Las Vegas
Summer 2007
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JEFFREY ABRAHAM GOLDSTEIN

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THE EFFECT OF TEMPERATURE ON DEVELOPMENT AND BEHAVIOR OF RElict

LEOPARD FROG TADPOLES (RANA ONCA)

is approved in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE DEGREE IN BIOLOGICAL SCIENCES

Examination Committee Chair

Dean of the Graduate College

Examination Committee Member

Graduate College Faculty Representative
ABSTRACT

THE EFFECT OF TEMPERATURE ON DEVELOPMENT AND BEHAVIOR OF RELICT LEOPARD FROG TADPOLES (RANA ONCA)

by

Jeffrey Abraham Goldstein

Dr Stanley Hillyard, Examination Committee Chair
Professor of Biomedical Science
University of Nevada, Las Vegas

Relict Leopard frogs (Rana onca) are found in thermal springs with water temperatures in excess of 30°C at the source but it's unknown whether tadpoles require reintroduction sites with specific thermal conditions for development. Tadpole survivorship at 20, 25 and 30°C was 84, 93.8, and 66%, respectively, while none survived at 35°C. Time to metamorphosis was significantly shorter for 25°C acclimation group (62±8 days) followed by the 30°C (106±51 days) and 20°C (260±47 days) acclimation groups. Development was arrested in the 15°C acclimated group and survivorship declined to 63.8% after 191 days. However, 80% of the surviving larvae completed metamorphosis after temperature was increased to 25°C. When placed in a thermal gradient, larvae selected temperatures closest to their acclimation temperature. Maximum burst speed was greatest at 25°C for all but not the 30°C acclimation groups. Thus,
reintroduction sites require water temperatures between $20^\circ$ and $30^\circ$C during most of the year.
# TABLE OF CONTENTS

ABSTRACT................................................................................................................. iii  
LIST OF FIGURES.................................................................................................... vii  
ACKNOWLEDGMENTS ........................................................................................... viii  
CHAPTER 1  BACKGROUND INTRODUCTION ......................................................... 1  
CHAPTER 2  RANA ONCA INTRODUCTION ............................................................ 8  
  Range of Thermal Tolerance ............................................................................... 8  
  Thermal Effects on Metabolism ........................................................................... 9  
  Thermal Regulation ............................................................................................. 11  
  Predator Avoidance ............................................................................................ 12  
  Experimental Constraints .................................................................................... 13  
CHAPTER 3  METHODS AND MATERIALS ............................................................ 14  
  2005 Experiments ............................................................................................... 14  
    Collection and Maintenance ......................................................................... 14  
    Development Analysis ................................................................................... 16  
    Metabolic Rate ............................................................................................... 17  
    Thermal Preference ....................................................................................... 20  
    Predator Avoidance ....................................................................................... 24  
    Analysis .......................................................................................................... 25  
  2004 Experiments ............................................................................................... 25  
    Collection and Maintenance ......................................................................... 25  
    Development Analysis .................................................................................. 26  
    Metabolic Rate ............................................................................................... 26  
    Analysis .......................................................................................................... 26  
CHAPTER 4  RESULTS ........................................................................................... 27  
  2005 Experiments ............................................................................................... 27  
    Thermal Effects on Development .................................................................... 27  
    Metabolic Rate ............................................................................................... 30  
    Thermal Preference ....................................................................................... 30  
    Predator Avoidance ....................................................................................... 34  
  2004 Experiments ............................................................................................... 37  
    Thermal Effects on Development .................................................................... 37  
    Metabolic Rate ............................................................................................... 40  
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
CHAPTER 5  DISCUSSION................................................................. 42
CHAPTER 6  CONCLUSIONS ............................................................ 48
REFERENCES .................................................................................. 50
APPENDIX  TABLES ........................................................................ 54
VITA ................................................................................................. 56
LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1</td>
<td>Map of present <em>Rana onca</em> populations with historic range</td>
<td>2</td>
</tr>
<tr>
<td>Figure 2</td>
<td>Schematic of metabolic chamber</td>
<td>18</td>
</tr>
<tr>
<td>Figure 3</td>
<td>Example of oxygen consumption trial</td>
<td>19</td>
</tr>
<tr>
<td>Figure 4</td>
<td>Raw data from thermal preference trial showing edge effect</td>
<td>21</td>
</tr>
<tr>
<td>Figure 5</td>
<td>Effects of thermal acclimation on 2005 tadpole development</td>
<td>29</td>
</tr>
<tr>
<td>Figure 6</td>
<td>Effect of thermal acclimation on 2005 metabolic rate</td>
<td>31</td>
</tr>
<tr>
<td>Figure 7</td>
<td>Effect of thermal acclimation on 2005 thermal preference</td>
<td>32</td>
</tr>
<tr>
<td>Figure 8</td>
<td>Effect of thermal acclimation on 2005 thermal preference</td>
<td>33</td>
</tr>
<tr>
<td>Figure 9</td>
<td>Effect of thermal acclimation on 2005 maximum burst speed</td>
<td>36</td>
</tr>
<tr>
<td>Figure 10</td>
<td>Effect of thermal acclimation on 2004 tadpole development</td>
<td>39</td>
</tr>
<tr>
<td>Figure 11</td>
<td>Effect of thermal acclimation on 2004 metabolic rate</td>
<td>41</td>
</tr>
</tbody>
</table>

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ACKNOWLEDGMENTS

I would like to thank my entire advisory committee and all of the people who guided and assisted me through my graduate program. First and for most, I would like to thank my mentor, Dr. Stanley D. Hillyard, for his endless guidance, encouragement, and patience through the completion of this project. I am honored to have had the opportunity to work with and learn from Stan who is a very passionate educator and researcher.

I would like to thank Dr. Karen Hoff who provided the opportunity for me to further my education. I am ever indebt for her support and assistance with my experimental setup that lead to the completion of this project. Karen’s vast expertise with amphibian larvae and statistical knowledge was invaluable to my project. I would like to also thank the students in the Hillyard lab for their companionship and willingness to assist me in the lab throughout my research project. Especially, Keon Pierre for his assistance in the field when we were looking for potential reintroduction sites in 100 plus degree weather and Rachelle Mulford for her assistance with the tadpoles in the laboratory.

I would like to thank my committee member, Dr. Carl Reiber, for his guidance, and assistance with my project. I am also grateful for the space he graciously provided me with during my time at UNLV. I would like to also thank the Reiber lab, especially Jennifer Head and Teresa Mika, for their assistance and feedback.
with the presentation of my thesis defense along with their companionship within the lab.

I am grateful to my funding agency, Clark County Multiple Species Habitat Conservation Plan (MSHCP), for supporting this project.

Finally, I would like to thank my parents, Jay and Esther Goldstein, for all of their unconditional love, support and encouragement during my graduate studies. I would like to thank my sisters, Nicole Goldstein and Siovhan Goldstein, for their assistance with my studies and their willingness to listen to my multiple talks. I am truly blessed to have such a great family.
CHAPTER 1

BACKGROUND

Once there was an abundance of unique species of amphibians found throughout the deserts of North America, but recently there has been an increase in population extinctions which are not clearly understood. These extinctions may be due to environmental changes, human manipulations of their habitat such as diverting water for irrigation (Bradford, 2002) or the introduction of non-native species (Hayes and Jennings, 1986). For a variety of ranid frogs, it is believed that non-native fish may prey on native species eggs and larvae or out-compete the native species for nutrient resources (Adams, 1999). Some combination of these factors is believed to be responsible for the decline in numbers and local extinctions of the Relict Leopard frog (*Rana onca*).

*R. onca* is part of the *Rana pipiens* (Leopard frog) complex which is found from the northern half of Central America to Canada in North America (Stebbins and Cohan, 1995). Cope (1875) was the first to describe an adult *R. onca* female observed within a spring draining into the Virgin River in Washington County, Utah. At the time, *R. onca* were commonly found in relatively high numbers around thermally influenced springs along the Virgin River from Hurricane, Utah to the convergence with the Colorado River into Black Cannon (Jaeger et al., 2001). *Rana onca* were also found along the Muddy River.
Figure 1: Map showing the location of *R. onca* after rediscovering the population at Corral Spring. Populations at Corral and Reber Springs have since been lost leaving only 5 native populations (Blue Point, Rogers, Boy Scout, Salt Cedar, and Bighorn Sheep Spring). Dark line outlines *R. onca*'s known historic range where reintroduction will take place. Map obtained from Relict Leopard Frog Conservation Team 2005.
drainage in Nevada (Jaeger et al., 2001). In 1935, Hoover Dam started to
impound water from the Colorado River and by 1938 Lake Mead extended 110
miles upstream, submerging a number of the thermal springs along the Virgin
and Colorado River. The formation of Lake Mead also produced a large barrier
isolating the populations along the Virgin River from populations within the Black
Canyon portion of the Colorado River. R. onca was last observed in their natural
habitat in 1950 and was believed to have gone extinct sometime after that point
(Jennings, 1988). The species was officially listed as extinct in 1982; however in
1991 a population was rediscovered at Corral Spring, Nevada followed thereafter
by 6 other locations (Figure 1; Jaeger et al., 2001). It was first believed that the
newly discovered populations found in the region were a disjunct population of
lowlands leopard frog, Rana yavapaiensis, (Jaeger et al., 2001). R. yavapaiensis
is found within perennial streams and ponds throughout southern and
northwestern Arizona, portions of south eastern California and southwestern New
Mexico (Platz and Frost, 1984). However, Jaeger et al. (2001) determined that
the frogs found along the Virgin River and within Black Canyon were indeed R.
onca by comparing their mitochondrial DNA and morphological features with a
variety of southwestern ranid species including R. yavapaiensis.

With the loss of two populations (Corral Spring, Nevada in 1995, and Reber
Spring, Arizona in 2001) and the reduction in population size and usable habitat
at the other sites (Bradford et al., 2004; Jaeger et al., 2001), R. onca is once
again a threatened species that has been petitioned for endangered status under
the Federal Endangered Species Act of 1973. Currently, there are only five
known native sites, 2 located near the Overton arm of Lake Mead (Blue Point and Rogers Spring) and 3 located within Black Canyon just south of Hoover Dam (Boy Scout, Salt Cedar, and Bighorn Sheep Spring; Jaeger et al., 2001). Their population size is estimated at 1100 adult frogs at all 5 native sites combined, with the majority of the frogs are found at one site (Bighorn Sheep Spring, Black Canyon, Nevada) placing them at a severe risk of stochastic extinction (Relict Leopard Frog Conservation Team, 2005).

Two major reasons for their decline are a loss of usable habitat with the encroachment of dense vegetation and the introduction of non-native species such as fish, crustaceans, and turtles (Adams, 1999; Bradford et al., 2004; Courtenay and Deacon, 1983; Jennings, 1988; Jennings and Hayes, 1994). Adult frogs prefer areas with light vegetation near the shore line of the spring for hunting prey and evading predators (Harris, 2006). In 1991, the Lake Mead National Recreation Area (LMNRA) removed wild burros from the Overton Arm to prevent the trampling and subsequent erosion of the spring drainage to assist in the preservation of the springs (person communication Ross Haley, NPS). Since 1991, the springs have been overrun by native vegetation making it difficult for *R. onca* to evade predation and search for food (Harris, 2006). Discarded exotic fish such as mollies, guppies, and cichlids have been released in the springs on the Overton arm of Lake Mead, Nevada and have devastated the frog population at these sites (Courtenay and Deacon, 1983; Jennings and Hayes, 1994). The fish will initially feed on *R. onca* eggs and hatchlings, and once the tadpoles become too large for the fish to consume, they will then compete with the
tadpoles for food. The combination of introduced species and the decrease in usable habitat space is decreasing the likelihood of survival at all life stages. (Adams, 1999; Harris, 2006)

Currently, all *R. onca* sites are associated with perennial, thermally influenced springs with water temperatures at the spring’s sources in excess of 30°C (Bradford et al., 2004). In general, as the spring water moves further down the drainage, water temperature begins to decrease until it approximates ambient temperature which varies seasonally from 15°C during the winter months to as high as 30°C in the summer as well as a function of daily fluctuations in temperature, humidity, and precipitation. Water temperatures along the spring drainage vary with changes in solar radiation by variations in vegetative cover, water depth, and flow rates. This sets up a range of thermal environments where *R. onca* can select from; however, the tadpoles must endure the higher thermal conditions when development lasts into the summer months. Currently, it is unknown whether the five remaining frog populations are restricted to a specific temperature range (thermal obligates) during tadpole development or weather they are able to adapt to the cooler conditions found at majority of potential reintroduction sites within their known range.

For *R. onca* to persist and increase in population size, the frogs must be able to successfully produce offspring that reach reproductive age. Adults have been observed calling, a series of clucks or chuckles, and mating takes place in late January through April and once again in November (Bradford et al., 2005). *Rana onca* produce zygotes through external fertilization of eggs laid in a globular
mass bound to a vegetative stems located within shallow slow moving pools with water temperatures around 16°C (Bradford et al., 2005; NPS unpublished data). Eggs, in the wild, hatch after approximately 7 days, and the hatchlings lay on the bottom of the pool until they are capable of swimming to a preferred microhabitat (Relict Leopard Frog Conservation Team, 2005). After metamorphosing, juvenile frogs will consume a large quantity of food to increase their rate of growth until reaching reproductive maturity, which takes approximately one year (Bradford et al., 2005).

It is believed that thermal tolerance range for anuran larvae gets narrower and warmer as the larvae approaches metamorphosis (Dupré and Petranka, 1985). Larval thermal tolerance ranges are also generally narrower than the ranges for anuran eggs from fertilization to hatching (Moore, 1939; 1949; Zweifel, 1968). Moore (1939; 1949) and Zweifel (1968) studied the effects of temperature on successful hatching of eggs of a number of Ranid species ranging from southern Canada to northern Mexico. Eggs collected from a species northern range were capable of tolerating cooler temperatures then their southern counterparts; however, the southern species were capable of tolerating warmer temperatures then their northern counterparts. However the ranges for normal hatching of eggs of the *R. pipiens* complex spanned between 18 to 27°C independent of their origin. For example, Zweifel (1968) found that eggs of the Chiricahua leopard frog (*Rana chiricahuensis*) from southern Arizona normally developed within a range from 12 to 31.5°C which is a thermal span of 19.5°C. While few experiments have been done on their larvae, *R. onca* would fall under
the "warm" class where development occurs quickest at warmer temperatures and they are capable of withstanding warmer temperatures than their northern relatives (Ruibal, 1962). Thus, minimal thermal conditions must be met for egg and larval development to proceed toward metamorphosis and eventually into adult frogs. The present study was initiated to determine the thermal sensitivity of *R. onca* during larval development and its effects on survival in specific thermal ranges representing their historic habitat range. This study also investigated the influence of thermal conditions during development on the ability of larvae to avoid predation and/or influence their thermal preference.
INTRODUCTION

Range of Thermal Tolerance

One strategy for the conservation of *R. onca* is to increase the number of populations within the frog's historical range. However, for re-introduction to succeed, reproduction is essential. Species in the *R. pipiens* complex have adapted to a variety of different thermal habitats (Hillis, 1988). *R. onca* must withstand a variety of thermal conditions throughout its habitat; however, temperatures during the summer months seldom drop below 25°C except during heavy rains (Western Regional Climate Center, 1971-2000). Tadpoles of *R. onca* must endure these higher thermal conditions during development, but it is unknown whether they are able to develop normally under cooler conditions at potential re-introduction sites.

Field monitoring performed by the Lake Mead National Recreation Area (LMNRA) found tadpoles in areas with temperatures around 25°C (unpublished data). I hypothesized that temperatures at or above 25°C would allow for the shortest time to metamorphosis. Rapid development to metamorphosis can increase survivorship by reducing the chances of confronting aquatic predators as tadpoles. Insufficient production of heat shock or metabolic protein can
influence survival, so I hypothesized that tadpoles reared at or above $25^\circ$C would have the highest survival. Work done on other frog species has shown larval mass to be inversely related to temperature (Álvarez and Nicieza, 2002; Smith-Gill and Berven, 1979), so I hypothesized that mass at metamorphosis would decrease as acclimation temperature increases.

**Thermal Effects on Metabolism**

Because reproduction occurs in late winter when the ambient temperatures are still cool but development occurs under warming temperatures (Bradford et al., 2005), the effects of temperature on tadpole development may be the best indicator of suitability of potential reintroduction sites. Amphibian body temperature varies with surrounding environmental thermal conditions (poikilothermic) and as the temperature changes so does their metabolism and behavioral activity. Amphibians are capable of regulating their body temperature by selecting from a variety of microhabitats with different thermal conditions. In general, for the normal development of tadpoles into adults, the surrounding water temperature must be within a particular thermal range, which is currently unknown for *R. onca*. When temperatures fall below their preferred thermal range, their rate of development and metabolism will decrease. If temperature decreases further, their development may become arrested. However, if the temperatures rise above this range, tadpoles can experience respiratory distress which can lead to a dramatic decrease in survivorship (Ultsch et al., 1999).
As with all aquatic poikilotherms, as temperatures increase there is generally an increase in tissue metabolism and tadpole activity; however, as temperature increases there is a decrease in oxygen solubility in water so that at a given partial pressure of oxygen \((P_o_2)\), less oxygen will be available. Below a certain \(P_o_2\) \((P_{o_2}_{crit})\), tadpoles are unable compensate for reduced \(P_o_2\) or reduced oxygen availability were the tadpoles become starved for oxygen. Above \(P_{o_2}_{crit}\), tadpoles can utilize multiple methods to compensate for increased oxygen demand and decreased oxygen availability at warmer temperatures. For instance, tadpoles can increase ventilation rate to increase the amount of water flowing across the gills, increase blood flow through gill capillaries, and/or rise to the waters surface to breathe atmospheric oxygen, a process called bobbing (Wassersug and Seibert, 1975; West and Burggren, 1982). In all instances, warmer temperatures may increase metabolic energy requirements with more of the energy diverted towards maintaining adequate oxygen levels at the expense of development.

To evaluate the effects of thermal acclimation on tadpole metabolic rate, we chose routine metabolic rate (RMR) as opposed to standard metabolic rate (SMR). RMR provides a measure of the oxygen requirement for maintenance metabolism and spontaneous activity with short term measurements as opposed to SMR that measures maintenance metabolism with a minimum activity. SMR is determined in an open system which takes in completely oxygenated water from a large reservoir and passes it through a flow-through chamber housing the tadpole. Measurement of SMR requires a longer acclimation to a metabolic chamber than measurement of RMR to minimize spontaneous activity and
diurnal variation in tissue metabolism (Gordon et al., 1982). Because of equipment limitations, RMR was measured in a closed system and the depletion of oxygen was recorded before reaching the $P_{O_2}^{crit}$ (Noland and Ultsch, 1981).

Tadpoles acclimated to colder temperatures may up-regulate their metabolic rate to promote development while producing enough energy to swim, avoid predation, and digest food. This can be done by increasing the quantity or type of metabolic proteins to compensate for the decrease in protein activity due to low temperatures, or by aggregating together to increase surface area which can enhance the absorption of radiant heat, if available (Brattstrom, 1962). Similarly, tadpoles acclimated to warmer temperatures may down-regulate the synthesis of metabolic proteins while increasing their thermal tolerance (Brown, 1969; van der Have, 2002). For this reason, I hypothesized that the cooler acclimated tadpoles will be less proficient at metabolically compensating to warmer waters than tadpoles acclimated to warm waters.

Thermal Regulation

Unlike eggs, tadpoles are capable of selecting a variety of microhabitats to regulate their body temperature to increase their developmental rate to metamorphosis. Changes in solar radiation from, full sun exposure to complete shading, with differences in vegetative densities and variations in water depth can provide a wide range of temperatures for thermal regulation (Ultsch et al., 1999). To further increase body temperature above surrounding water temperatures, tadpoles can obtain a greater surface area by huddling together to
increase the intensity of solar radiation absorbed from the sun (Brattstrom, 1962). The behaviors linked to thermal preference, may be influenced by temperature acclimation, or due to differences in neural temperature sensors for the different acclimation groups (Hoff et al., 1999; Spray, 1986). I hypothesized that tadpoles will select for temperatures that are nearest to their developmental temperature.

**Predator Avoidance**

Tadpoles must avoid predation from a variety of aquatic predators such as non-native fish and dragonfly nymphs along with birds and mammals (Courtenay and Deacon, 1983; Jennings and Hayes, 1994). There are multiple methods utilized by tadpoles to avoid predation by these different predators. Tadpoles evolved elaborate integument patterns to blend into their surroundings and will hide under vegetative debris, large rocks and/or crevices (Hoff et al., 1999). However, if the tadpole is detected by a predator, it must be able to evade predation. Tadpoles are not known for endurance type swimming to outrun a predator; rather, they utilize short bursts of high speed swimming to move out of the predator's path (Chovanec, 1992). Because of the differences in activity at the different temperatures, I hypothesized that tadpoles, regardless of their rearing temperature, when placed in cooler waters will have a slower maximum burst speed than when placed in warmer waters. I also hypothesized the maximum burst speed of warmer-acclimated tadpoles will be quicker than the cooler acclimated tadpoles.
Experimental Constraints

There were a few constraints due to the threatened status of *R. onca*. Experiments were limited to only two replications and the initiation of experiments was dependent on the arrival of tadpoles from the LMNRA. Due to a delay in funding, the first set of experiments (2004) began with tadpoles further along in development and acclimated to 21°C for 48 days before their arrival. On the other hand, the second set (2005) began with tadpoles earlier in development with only ten days of 21°C aquatic conditions. The 2005 experiments cover a greater scope of development and are presented first. For both groups there was a limited amount of time to perform all of the experiments due to the rapid development of some of the acclimation groups.
CHAPTER 3

MATERIAL AND METHODS

2005 Experiments

Collection and Maintenance

The National Park Service, NPS, collected multiple *R. onca* egg clutches (permit # S24407) from Bighorn Sheep Spring, Nevada (Figure 1) on January 21, 2005 by gently separating each egg clutch in half with a plastic spoon. The clutch half that was still attached to the vegetation was left behind while the free half was gently placed in a plastic container completely filled with spring water from that site to prevent agitation damage. The water temperature at the time of collection was 16.5°C. The eggs where housed at 21°C in 120-gallon aquaria at the Lake Mead National Recreation area facility in Boulder City, Nevada where they hatched on January 25, 2005.

Two hundred and thirty eight tadpoles from this group were obtained from the NPS on February 3, 2005 with a developmental stage (Gosner, 1960) of 25 and a mass between 25-80 mg (37 ± 20 mg). The Gosner staging system (1960) was produced by looking at specific visual changes during development from fertilization to the completion of metamorphosis based on *Bufo valliceps* raised at 25°C. There are four main phases of development within the staging system: embryo, hatchling, tadpole, and metamorph. The tadpole stage of development
occurs between stage 25 and 41 with metamorphosis starting at stage 42. Metamorphosing frogs were placed in a shallow-water holding tank until the NPS retrieved the frogs for reintroduction.

The tadpoles were randomly divided into five groups, ranging from 46 to 50 individuals, each placed in a 30-gallon (113.6 L) aquarium (74 cm x 30 cm x 46 cm) filled with Las Vegas tap water at 22°C. Water temperature was increased or decreased by 1°C per day until acclimation temperatures of 15, 20, 25, 30, and 35°C were obtained. *Rana onca* tadpole care and experimentation procedures were approved and regulated by the University of Nevada, Las Vegas Animal Use and Care Committee under protocol number R701-0204-187.

Prior to receiving the tadpoles, the aquaria were filled with Las Vegas tap water treated with Stress Coat® (Aquarium Pharmaceuticals, Inc., Chalfont, PA, USA) per product recommendation to neutralize chlorine and allowed to equilibrate overnight to room temperature (22°C). Cycle Biological Aquarium Supplement (Rolf C. Hagen Corp., Mansfield, MA, USA) was added per product recommendation to maintain low nitrogenous waste values. Each aquarium contained gravel covering approximately 1/3 of aquarium bottom with an under gravel biological sponge filter and synthetic vegetation. Water was oxygenated with a tetratec AP200 air pump (Tetra/Second Nature, Blacksburg, VA, USA) that filtered atmospheric air through an air stone, and water quality was maintained with a Tetra Whisper 20 power filter (Tetra/Second Nature, Blacksburg, VA, USA) minus the activated charcoal. Thermal conditions were maintained with either a ViaAqua (Commodity Axis Inc, Camarillo, CA, USA) titanium tube electric
aquarium heater (VA200T) or an AquaChill Industries LTD (Vernon, BC, Canada) cooling unit (model # AE3414YA). To prevent metamorphosing larvae from drowning, a plastic shelf was placed just below water level.

Tadpoles were fed frozen spinach *ad libitum* to avoid the effects of starvation (Merilä et al. 2004) and hardboiled egg albumen was added as a source of protein twice a week and removed after 2 days. Aquaria were cleaned twice a week with a gravity tube until a quarter of the water was removed and replaced with freshly conditioned water. The sides of the aquaria were cleaned with an aquarium scrub pad monthly. Tadpoles of the same thermal acclimation group were reared together in the same aquarium, without dividers, to permit social interactions to simulate a natural situation. The number of tadpoles per tank was limited to 50 individuals to insure restricted space (John and Fusaro, 1981) or overcrowding (Browne et al., 2003; Martinez et al., 1996) did not effect developmental rate and mass.

**Development Analysis**

Developmental stage and mass were recorded 2 to 3 times per week until the initiation of metamorphosis (stage 42). The Gosner staging system (1960) was produced by looking at specific visual changes during development from fertilization to the completion of metamorphosis based on *Bufo valliceps* raised at 25°C. There are four main phases of development (embryo, hatchling, tadpole, and metamorph). The tadpole stage of development begins at stage 25 with the internalization of the gills extends to stage 41 where the front limbs emerge.
Metamorphosis begin at stage 42 and extend to stage 46 which the complete reabsorption of the tail. Staging occurred by placing tadpoles individually on a moistened Kimberly-Clark lab tissue to view under a stereoscope. Snout vent and total body length were measured to the nearest millimeter with a translucent plastic ruler. The tadpole was then placed in a tared 100ml beaker filled with approximately 20ml of aquarium water and weighed to the nearest milligram on a Mettler Toledo (Columbus, OH, USA) PM 400 electronic scale.

Metabolic rate

Routine metabolism was measured at 15, 25, and 35°C for the 15, and 25°C acclimation groups. Temperature was regulated with a thermal regulator mounted to an insulated chamber that housed the apparatus which consisted of a Gilson (Middletown, WI, USA) Minipuls 2 peristaltic pump, tadpole chamber and oxygen sensor (Figure 2). Tadpoles were placed in a 130 or 500ml chamber depending on tadpole size to permit free unrestricted movement. Attached to opposite sides of the lid were an outflow and an inflow tube which permitted the peristaltic pump to mix the chamber contents as it recirculated the water. An Instech Laboratories, Inc. (Plymouth Meeting, PA, USA) polarographic oxygen electrode was spliced about 4cm from the start of the outflow line. The partial pressure of oxygen was recorded with Sable Systems Inc. (Las Vegas, NV, USA) Redox 4 oxygen analyzer and recorded on a computer with Sable Systems Inc. (Las Vegas, NV, USA) DATACAN software.
Figure 2: Schematic of metabolic chamber. A tadpole is placed in the tadpole chamber which is completely filled with oxygenated water that is circulated with a peristaltic pump. Spliced into the outflow line of the tadpole chamber is an oxygen (O$_2$) sensor measuring the partial pressure of oxygen (PO$_2$) recorded on a computer (Figure 3). The entire setup is placed in an insulated chamber with a thermal regulator to maintain the experimental temperature.
Figure 3: Change in partial pressure of oxygen (PO2) with time as a mechanism to measure metabolism in an individual tadpole acclimated to 25°C within a 25°C chamber ran sequentially. Readings were in the order of Line A, B, then C. Line A has a slope of -1.085 mmHg/min with an $R^2 = 0.99$, line B has a slope of -1.182 mmHg/min with an $R^2 = 0.99$ and line C has a slope of -0.756 mmHg/min with an $R^2 = 0.96$. 

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Each trial consisted of three 20 minute readings, and the reading with the lowest slope ($P_{O_2}$ vs time) was used to minimize the activity component of metabolism (line C in Figure 3). Noland and Ultsch (1981) looked at $P_{O_2}$crit in *Rana pipiens* (Northern Leopard Frog) and found that $P_{O_2}$crit for tadpoles between stages 25 and 39 and between 22 and 32°C was below 50 mmHg. So we arbitrarily chose 100 mmHg as the minimum oxygen tension permitted at the start of the third reading. The rate of oxygen consumption ($\dot{V}O_2$) was calculated from the slope for the depletion of oxygen, measured as the decline in $P_{O_2}$, tadpole mass, chamber volume, and oxygen solubility using equation 1. $\dot{V}O_2$ was corrected to standard temperature and pressure (STP).

$$\frac{\text{solubility (ml O}_2/\text{ml H}_2\text{O)} * \text{chamber volume (ml H}_2\text{O)} **}{\text{mass (g)} * 160 \text{mmHg} ***} \times \text{slope (mmHg/hr)} = \dot{V}O_2 (ml/g*hr)$$

Equation 1: Formula used to calculate standardized routine metabolism for volume of oxygen consumed (* from reference appendix table 1, ** 130 or 500ml, *** corrected to STP).

**Thermal Preference**

For the thermal preference experiments, a thermally regulated racetrack system (Sable System Inc., Las Vegas, NV, USA), was used. The racetrack (Figure 4A) has a 2cm thick aluminum floor (66 cm long x 6 cm wide), which served as a thermal conductor to establish and maintain a linear gradient, and Plexiglas sides (6 cm tall). The aluminum floor was connected at each end of the
Figure 4: A) An image of the thermal racetrack B) A graph of a single thermal preference trial with the presence of edge effects. Each point represents the amount of time spent at that particular location in the racetrack. The set temperature range for the racetrack is 20 to 30°C with the tadpole spending a large portion of time at around 26°C (arrowhead).
The racetrack was filled approximately 2 cm deep with water obtained from the aquarium associated with the tadpole being tested. Initially, four thermocouples located 1 and 20 cm from either end were used, but the combination of the shallow water and the thick aluminum base maintained a linear thermal gradient so effectively that three thermocouples were sufficient for verifying the gradient. The temperature of each location selected by the tadpole was verified with a thermometer after each experiment.

The location of the tadpole was recorded with the use of 64 LED sensors located 1 cm apart and 1 cm above the bottom of the racetrack. When the LED beam was disrupted, the location would be transmitted to a Linear Activity Detector, LAD, (Sable System Inc., Las Vegas, NV, USA) set to the LadScan setting which integrated the signal and sent the data to a computer equipped with the LAD scan utility software. To prevent interference from external light sources and to reduce stress levels, a cover was placed above the racetrack during experimental runs.

Thermal selection was evaluated with two thermal regimes, 10 to 20°C and 20 to 30°C, with a subset of 10 individuals from each acclimation group.
Individuals were observed only once for each trial within each temperature range. During a trial, one tadpole was gently placed in the center of the chamber (15°C and 25°C respectively for the two gradients) and allowed to freely move around for fifteen minutes (900 s). This was found to be a sufficient amount of time for tadpoles to select a preferred temperature range. The location of each tadpole within the experimental temperature ranges was determined by dividing the chamber into three sections, the middle third covered the mean temperature and the upper and lower thirds the warmer or cooler ends. For the 10 to 20°C gradient the lower third represented temperatures below 13.3°C and the upper third temperatures above 16.6°C. For the 20 to 30°C gradient the lower third represented temperatures below 23.3°C and the upper third temperatures above 26.6°C. Output from the LAD was binned into three groups equaling 22 cm in length for analysis and location was classified as to whether the tadpole remained in the center or selected the warmer or cooler end of the chamber. For example, the trial depicted in Figure 4B shows the location of each bin within the 20 to 30°C gradient along with the temperature range found with each bin. It can be seen that the individual remained in the center third of the gradient. During normal activity, tadpoles periodically swam to either end of the chamber producing an edge effect shown in Figure 4B. The edge effect seen in Figure 4B had no affect on the overall results for thermal preference, so no adjustments to the data were warranted.

Stage and mass were recorded after each experiment. To prevent any effects of the brief exposure to the variations in thermal conditions, tadpoles were
returned to their tanks for a minimum allotment of 48 hours between different thermal gradient trials.

**Predator Avoidance**

Tadpole selection and setup was similar to the thermal selection experiment except there was a uniform temperature of 15, 20, 25, 30, or 35°C. The water was agitated prior to each experiment to ensure the water temperature was constant across the entire track. Maximum burst speed was used to measure predator avoidance because the quicker the tadpoles are able to move out of the path of an advancing predator the greater their chances are of surviving a predator's advances. Maximum burst speed was obtained by applying an electrical stimulation of 80 V for 12 ms with metallic electrodes connected to a Grass Technologies (West Warwick, RI, USA) SD9 stimulator. The electrodes were placed at one end of the racetrack and the tadpole was maneuvered so its tail was between the electrodes while facing the center of the track. Once the tadpoles appeared calm (usually less than 10 s) the Grass stimulator was activated to elicit a burst speed response. Tadpole location was recorded by Microsoft (Redmond, Wa, USA) HyperTerminal software every 100 ms with the use of the LAD set to the racetrack mode. Maximum burst speed was determined by looking at the longest distance traveled in any 500 ms period. A subset of 10 individuals from each acclimation group was run to evaluate maximum burst speed for each of the above experimental temperatures, and each individual was used only once for each trial at each experimental
temperature. No adverse effects on tadpole development were observed with this procedure.

Analysis

Statistical analysis used Statview 4.5 (SAS Institute Inc., Cary, NC, USA). All experimental data were analyzed with an ANOVA factorial design with Bonferroni/Dunn for post hoc t-test unless noted. The significance level for Post hoc comparisons was $\alpha = 0.05$.

2004 Experiments

Collection and Maintenance

Tadpoles were collected from the same site as the 2005 acclimation group described above. The tadpoles were reared at the Lake Mead National Recreation Area Facility in Boulder City, Nevada in 50-gallon aquaria at 21°C from egg masses collected in early March. The eggs hatched on March 16, 2004 and were fed spinach twice a week and egg albumin once a week during that time. Tadpoles were received in our laboratory on May 3, 2004 (48 days post-hatch) at which time they ranged from stage 25 to 35 with masses ranging from 298 mg to 1.03g. Tadpoles were placed in two thirty-gallon aquaria at room temperature (22°C) and allowed one day to acclimate to their new surroundings. The tadpoles were randomly divided into five equally sized groups and the temperatures were then adjusted to the selected acclimation temperatures of 15, 20, 25, 30, or 35°C. Adjustment of acclimation conditions, aquarium setup and
animal care were all performed in the method as described in the 2005 experiments section of this chapter.

Development Analysis

Once the aquaria reached their acclimation temperature, each acclimation group was monitored on a daily basis. Developmental staging and mass were monitored in the same manner as described in the 2005 experiments section of this chapter.

Metabolic Rate

Oxygen consumption was used to estimate routine metabolism at 15, 20, and 30°C for the 15, 20, and 25°C acclimation groups. Metabolic setup and data collection were obtained by the same methods described in the 2005 experiments section of this chapter.

Analysis

Analysis of the development and metabolic rate data were performed in the same manner as stated in the 2005 experiments section of this chapter.
CHAPTER 4

RESULTS

2005 Experiments

Thermal Effects on Development

The numerical data and statistical comparison for survivorship, time to
metamorphosis and mass at metamorphosis are presented in Appendix, Table 2.
Out of the five acclimation groups, only the 20, 25, and 30°C acclimation groups
proceeded successfully to metamorphosis without further manipulating thermal
conditions. The 25°C acclimation group had the highest survivorship and the
shortest time to metamorphosis (Figure 5). Survivorship for the 20°C acclimation
group was not significantly different from the 25°C group; however the 20°C
group took over four times longer to reach metamorphosis. For the 30°C
acclimation group, tadpole survivorship was significantly lower and time to
metamorphosis was significantly longer when compared to the 25°C group. The
average mass of the tadpoles at metamorphosis for the 20 and 30°C acclimation
groups were not significantly different from the 25°C acclimation group; however,
they were significantly different from each other.

On post-hatch day 191, the 15°C acclimation group had an average increase
of only 2 stages, with a mass of 580 ± 160 mg and frequency of fatalities was
rising. Due to this lack of development, we decided to treat them as if they were
experiencing an over-wintering event. By utilizing the average regional daily temperatures from the Western Regional Climate Center, we determined that temperatures increase in the general area by a rate of 1°C every five days. Temperatures were increased at this rate until reaching 25°C since the 25°C acclimation group had the highest survivorship and developmental rate. It took a total of 50 days to reach 25°C with 80% of the remaining tadpoles (n = 30) surviving during the thermal increase. After the 15°C acclimation group reached 25°C, 96% of the remaining tadpoles (n = 24) survived to metamorphosis in 41 ± 12 days at a mass of 4.83 ± 1.08 g. Of interest, this time course was not significantly different from the 25°C acclimation group.

For the 35°C acclimation group, symptoms of malnourishment and weakness started to show nine days after reaching 35°C with a drop in mass and activity, even with continuous food availability. To minimize fatalities, temperature was reduced in 2°C increments every 15 days until reaching 30°C as was done with the previous year's experiments (subheading 2004 Data). The first decrease in temperature occurred 10 days after the acclimation temperature reached 35°C. Despite the decrease in temperature, all of the tadpoles briefly exposed to 35°C did not reach metamorphosis, with the last tadpole surviving until 143 days post hatch at stage 27 with a mass of 271 mg.
Figure 5: Effect of thermal acclimation on tadpole development in 2005: A) survival to metamorphosis, B) time to metamorphosis, and C) mass at metamorphosis. Asterisks (*) = Because of the lack of development at 15°C (solid bars), rearing temperature was increased to 25°C (cross-hatch bars) to simulate an overwintering event. The open area between the bars represents changes occurring as temperature was raised. Number of individuals per aquarium is shown on top axis. Statistical comparisons are described in the text. Bars containing the same letter are not significantly different while those with a different letter are significant (p < 0.05). Each bar shows mean ± standard error.
**Metabolic Rate**

Metabolic rates for the 15 and 25°C acclimation groups are shown in Figure 6. Between chamber temperature of 15 and 25°C, both acclimation groups had a significant increase in VO₂ with Q₁₀ values of 2.34 and 1.63, respectively. When the chamber temperature was further increased to 35°C, the VO₂ of 15°C acclimation group significantly increased with a Q₁₀ of 2.76; however, there was not a significant increase in the 25°C acclimation group. Thus, the 15°C acclimation group was significantly more sensitive to the warmer temperatures than the 25°C acclimation group.

**Thermal Preference**

The thermal preference data for the 15, 20, 25 and 30°C acclimation groups are shown in Figures 7 and 8. When the thermal gradient was between 10 and 20°C, tadpoles from all thermal acclimation groups preferred temperatures greater than 16.6°C (Figure 7). Locomotor activity of the 30°C acclimation group was greatly reduced in the 10 to 20°C gradient, so larvae that moved to the cooler end of the racetrack (<13.3°C) tended to stay there. Nonetheless, a significantly greater amount of time was spent above 16.6°C. In contrast, with a thermal gradient between 20 to 30°C, the 15 and 20°C acclimation groups preferred temperatures less than 23.3°C, while the 25 and 30°C acclimation groups still preferred the warmer end of the race track which was greater than 26.6°C (Figure 8). In comparison to the other acclimation groups, the 30°C acclimation group spent significantly less time at temperatures below 23.3°C.
Figure 6: Oxygen consumption rate showing the changes in metabolic rate between the 15 and 25°C acclimation group. Thermal acclimation has a significant effect on oxygen consumption between 15 and 35°C. The 15°C acclimation group is significantly more sensitive to 35°C than the 25°C acclimation group. There is also a significant increase in oxygen consumption between 15 and 25°C chamber conditions for both acclimation groups but only the 15°C acclimation group is significantly different between 25 and 35°C. The asterisk (*) shows points that are significantly different from the 25°C chamber temperature for each acclimation group (p ≤ 0.05). Each point represents a mean ± standard error. (n=10)
Figure 7: The effect of thermal acclimation on thermal preference for the 15, 20, 25, and 35°C acclimation groups when placed in a 10 to 20°C gradient. Within this temperature range, each acclimation group significantly preferred temperatures greater than 16.6°C. Bins where tadpoles spent a significantly greater amount of time are marked with an asterisk (*) ($P < 0.05$). Each bar represents a mean ± standard error. (n=10)
Figure 8: The effect of thermal acclimation on thermal preference for the 15, 20, 25, and 35°C acclimation groups when placed in a 20 to 30°C gradient. Within this temperature range, the 15 and 20°C acclimation group significantly preferred temperatures less than 23.3°C while the 25 and 30°C acclimation group preferred temperatures greater than 26.6°C. Bins within an acclimation group with a significantly greater time are marked with an asterisk (*). The bin representing the coolest end of the racetrack with the significantly shortest time is marked with a double asterisk (**) (P ≤ 0.05). Each bar represents a mean ± standard error. (n=10)
Predator Avoidance

Table 1 shows the mean stage and mass of the tadpoles at the time of the experiments. Because of the variations in development rate at the different acclimation temperatures, some acclimation groups had larger standard deviation for stage and mass. For example, the 25°C acclimation group had a larger standard deviation because they were further in development when measured at 15°C (stage of 34 ± 1.1 and at a mass of 1.72 ± 0.45 g) than at the other experimental temperatures, which were performed at an earlier stage (29.1 ± 1) and lower mass (541 ± 237 mg). Thermal acclimation had an effect on maximum burst speed across a range of thermal conditions shown in Figure 8. Regardless of their acclimation regime, tadpole maximum burst speed was significantly slower when placed in 15°C thermal conditions than the 20, 25, and 30°C thermal conditions. However, the 30°C acclimation group was significantly slower at the 20°C thermal conditions. The slowest group at 15°C was the 30°C acclimation group which moved a short distance at a slow rate after stimulation possibly caused by thermal cold shock. The 15, 20 and 25°C acclimation groups followed a similar trajectory as temperature was increased with a peak speed for all three groups at the 25°C experimental temperature. The 30°C acclimation group had a trajectory shifted to the right of the other three groups with a maximum burst speed peaking at 30°C. When the thermal conditions are combined for each acclimation group, the 25°C acclimation group was significantly faster than any of the other acclimation groups.
Table 1: Average stage and mass (± standard deviation) of tadpoles during maximum burst speed experiments. Tadpoles throughout the experiment averaged a stage of 27.9 ± 2.1 with a mass of 460 ± 420 mg. The large standard deviation for the 25°C acclimation group was due to their rapid development to metamorphosis.

<table>
<thead>
<tr>
<th>Acclimation Group</th>
<th>Stage</th>
<th>Mass (mg)</th>
<th>Range (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15°C</td>
<td>27.5 ± 0.6</td>
<td>511 ± 132</td>
<td>285 – 755</td>
</tr>
<tr>
<td>20°C</td>
<td>26.9 ± 0.8</td>
<td>261 ± 69</td>
<td>148 – 435</td>
</tr>
<tr>
<td>25°C</td>
<td>30.4 ± 2.4</td>
<td>835 ± 595</td>
<td>209 – 2.585</td>
</tr>
<tr>
<td>30°C</td>
<td>26.3 ± 0.8</td>
<td>129 ± 37</td>
<td>72 – 197</td>
</tr>
</tbody>
</table>
Figure 9: This figure shows the effect of thermal acclimation on maximum burst speed. The asterisk (*) shows the points that are significantly slower than the 25°C experimental temperature within each acclimation group (p < 0.05). The double asterisk (**) shows the acclimation group that is significantly faster than the other acclimation group (p < 0.05). Each point represents a mean ± standard error. (n = 10, except † = 4)
2004 Results

Thermal Effects on Development

Figure 10 shows the effects of thermal acclimation on survivorship, time to metamorphosis and mass at metamorphosis for the 2004 acclimation groups. As with the 2005 group, only the 20, 25, and 30°C acclimation groups proceeded successfully to metamorphosis without further manipulating thermal conditions. Survivorship for all of the groups was high although the 25°C acclimation group was significantly lower than the other groups with only 78% of the tadpoles surviving to metamorphosis. For the 20 – 30°C groups, the time to metamorphosis was not significantly different between the 25 and 30°C acclimation groups; however they were both significantly shorter than the 20°C acclimation group. Mass was inversely proportional to temperature; the 20°C acclimation group had the greatest mass followed by the 25, than 30°C acclimation group, respectively.

The lack of development and the increase in observable malformations in the 15°C acclimation group caused us to become concerned about their health. So we decided to treat this group as if they were experiencing an over wintering event. The 15 degree acclimation group was divided into two groups on post hatch day 189 to demonstrate the effects of a short and long over-wintering event. The short over wintering group began their acclimation regime 191 days post hatch while the long over-wintering group began 226 days post hatch. The acclimation temperature of both groups was increased by 1°C every five days until reaching 25°C with 100% of the tadpoles surviving to metamorphosis. The
short over-wintering group reached metamorphosis before the long over-wintering group; however, the long over-wintering group reached metamorphosis in a shorter time after temperatures reached 25°C. The long over-wintering groups also had a mass statistically greater than the short over-wintering group.

The 35°C acclimation group was reduced to 30°C after 14 days because of the declining health of the tadpoles. After decreasing the temperature, the tadpoles began to increase in mass and activity with improved health allowing 94% of the tadpoles to survive to metamorphosis. They took a significantly longer time to reach metamorphosis with a significantly lower mass than the 30°C acclimation group.
Figure 10: Effect of thermal acclimation on 2004 tadpole development: A) survival to metamorphosis, B) time to metamorphosis, and C) mass at metamorphosis. Because of the lack of development at 15°C (solid bars), rearing temperature was increased to 25°C (cross-hatch bars) to simulate an over-wintering event. The open area between the bar and cross hatch represents the time it took for the temperature to rise. The 15°C acclimation group started to increase to 25°C one month prior to the increase of the 15°C acclimation group. Because of the decrease in health at 35°C the temperature was reduced to 30°C after 14 days. Number of individuals per aquarium is shown on top axis. The asterisk (*) in Figure A shows survival to metamorphosis is significantly different from the other acclimation groups (p < 0.05). In Figure B and C, bars containing the same letter are not significantly different while those with a different letter are significant. Each bar shows mean ± standard error.
Metabolic Rate

Metabolic rate for the different acclimation groups is shown in Figure 11. Oxygen consumption for all thermal groups was significantly lower at 15°C than the 20 and 30°C camber temperature, which was consistent with the lack of development in the 15°C acclimation group. The increase from 15 to 20°C produced a significant increase in oxygen consumption; however, there was no difference between the acclimation groups at either temperature. The $Q_{10}$ for $\dot{V}O_2$ of the 15, 20 and 25°C acclimation groups was 4.2, 2.8 and 3.4 respectively. Oxygen consumption rates did not significantly change when the chamber was further increased to 30°C.
Figure 11: Oxygen consumption ($\dot{V}O_2$) of tadpoles showing the changes for the 15, 20, and 25°C acclimation groups. Thermal acclimation does not have an effect on oxygen consumption between 15 and 30°C; however, there is a significant increase in oxygen consumption between 15 and 20°C chamber conditions. The asterisk (*) shows the points which are significantly different from the 20°C chamber temperature for each acclimation group ($p \leq 0.05$). Each point represents a mean ± standard error (n=10).
CHAPTER 5

DISCUSSION

Frogs within the *R. pipiens* complex are found within a wide range of thermal habitats where each species evolved adaptations to survive. Results of the present study indicates *R. onca* have adapted to the seasonal variations in temperature found along the Virgin River drainage and Colorado River within Black Canyon. Survivorship for *R. onca* tadpoles falls rapidly below 20°C and above 30°C, and within this 10°C range the time to metamorphosis is significantly shorter at 25°C.

The general thermal span for developing ranid eggs (Moore, 1939, 1949; Zweifel, 1968) is around twice that for developing *R. onca* tadpoles. This is understandable since tadpoles are capable of selecting for the most optimal conditions for development from a variety of different microhabitats containing a wide range of thermal conditions (Freidenburg and Skelly, 2004). Eggs on the other hand are immobile and must be able to tolerate a wider range of thermal conditions which is dependent on their parent’s choice of habitat. This places a strong selection for eggs with a tolerance for a wider range of thermal conditions. This corresponds nicely with adult *R. onca* egg placement in the wild and tadpole development. Eggs of *R. onca* are usually found in cooler areas of the spring with temperatures around 16°C, on the other hand tadpoles are usually found in
warmer waters around 20 to 27°C (personal communication, Cristina Velez, NPS).

At the cooler temperatures there is an abundance of oxygen; however, there is also a reduction in the rate of development, rate of metabolism, and activity. The reduction in development was most dramatic for the 15°C acclimation group with an increase of only 2 stages in 191 days, which is similar to other ranid species during an over wintering response (Collins and Lewis, 1979; Herreid II and Kinney, 1967). If *R. onca* was introduced into a cooler environment where water temperature never exceeds 15°C, the tadpoles would have most likely never reached metamorphosis. The tadpoles could die from old age, but most likely they would have been easy prey for aquatic predators due to the reduced burst speeds observed at the cooler temperatures.

The 15 and 20°C acclimation groups showed an increase frequency in malformations when compared to the 25 and 30°C acclimation groups. For instance, we noted in 2004 that apoptosis of the tissue between the digits of the hind limbs was not occurring while cartilage development and elongation of their phalanges continued for the tadpoles acclimated to 15°C. Also, scoliosis occurred at a higher frequency in the 15 and 20°C acclimation groups for both years when compared to the other acclimation groups. In either case these malformations in the 15 and 20°C acclimation groups are more likely caused by the effects of cooler temperatures on developmental processes than on the tadpoles’ genetic makeup. Many genetic processes occur during development in a well orchestrated series of events. The loss of coordinated biochemical events
caused by the sensitivity of transcription or function of a few proteins to colder temperatures can delay or prevent selective processes (Murata and Yamauchi, 2005). However, temperature independent processes, which are also uncoupled with the events of any temperature sensitive processes, can continue without temperature affecting development processes leading to a host of malformations.

Metabolic rates for poikilotherms are dependent on their thermal surroundings and as temperatures begin to increase so does their metabolic rate and food requirement. However, oxygen availability is inversely related to water temperature forcing the tadpoles to allocate more energy towards breathing instead of developing towards metamorphosis. Tadpoles are able to avoid hypoxia by actively searching for cooler microhabitats located in the shade or in shallow water which may be cooled through evaporative cooling, and where breathing in surface oxygen is energetically less costly (Feder and Moran, 1985). This was seen when the 35°C acclimated tadpoles spent a greater amount of time resting on a plastic shelf located near the water’s surface so the tadpoles can increase frequency of bobbing for air with minimal energy. However, the tadpoles had to travel to the bottom of the tank where the food was located.

After a few days at 35°C, a decrease in mass, abdominal girth, and activity was noted leading to the decrease in acclimation temperature to 30°C. Even with the reduction in thermal conditions, none of the tadpoles reached metamorphosis. A combination of decreased oxygen availability and the increase in metabolic demands from increased respiration and actively searching for cooler temperatures may have lead to respiratory stress and decreased
appetite (Ultsch et al., 1999). Heat shock protein transcription is activated to prevent protein degradation when thermal conditions become excessive for normal cellular processes (Hubbard and Sander, 1991). The inactivation or delayed activation of heat shock protein transcription can lead to an irreversible degradation and misfolding of proteins at the higher temperatures. Regardless of the causes leading to the fatalities observed for the 35°C acclimation group, the brief exposure to a 35°C environment is more demanding for tadpoles exposed earlier than later in development with majority of the 2004 tadpoles surviving to metamorphosis.

Thermal acclimation had an effect on tadpole behavior in regards to temperature preference and predator avoidance. Tadpoles of most species generally select for microhabitats with the warmest temperatures to shorten their developmental time to metamorphosis. *R. onca* tadpoles can choose from a variety of microhabitats within the spring’s runoff, allowing them to select for their preferred thermal condition. Depending on the thermal acclimation of the tadpole they preferred temperatures at around 20°C or above 26.6°C. The 15 and 20°C acclimation groups may have increased the quantity of metabolic proteins to allow enough energy production for development. This might explain the preference for temperatures around 20°C; even though it would take at least 9 months to reach metamorphosis. In contrast, the tadpoles acclimated to 25 and 30°C preferred temperatures above 26.6°C with metamorphosis occurring in less than a 2 month period for the 25°C acclimation group. The reduced time to
metamorphosis would reduce predation leading to an overall increase in survivorship.

In their native habitats, tadpoles must avoid a variety of native predators, such as dragonfly nymphs, a variety of birds, and ring-tailed cats, along with recent introductions of exotic fish, crayfish, and turtles. (Jennings, 1988; Jennings and Hayes, 1994) Tadpoles are not known for long distance endurance swimming; however, short bouts of energy can propel them away from a predator’s path (Chovanec, 1992). Thermal acclimation has an affect on maximum burst speed where cooler acclimated tadpoles will have a greater myofibrillar ATPase activity permitting them to move at a quicker speed than warmer acclimated tadpoles at cooler temperatures (Watkins, 2000). Also, regardless of tadpole acclimation, tadpole performance is best between a range of temperatures that are not much different from each other. In the current study, the 30°C acclimation group had the lowest speeds at 15°C where they moved a very short distance or not at all. This may be due to the decrease in myofibrillar ATPase activity and protein. Larvae of different amphibian species respond differently to a variety of thermal conditions were developmental and current temperatures have an effect on maximum burst speed (Watkins, 2000). *R. onca* is affected by changes in thermal conditions most notably when placed into 15°C waters where conditions are just too cold for tadpoles to effectively avoid predation due to the significant reduction in metabolic rate and locomotor function.
Within the 25 to 30°C range, tadpoles develop normally to metamorphosis with a rapid developmental rate. Tadpoles at 15 and 20°C had increases in visual malformations and decreased activity, while tadpoles at 35°C struggled to obtain enough oxygen from its surroundings for normal development. The age of the tadpole at the time of thermal acclimation also has a great effect on survival of tadpoles, with the older tadpoles being less sensitive to thermal changes then younger tadpoles. The older tadpoles may have been exposed to warmer temperatures before their acclimation regime began where they were capable of producing enough of the proper heat-shock proteins allowing the tadpoles acclimated to 35°C in 2004 to survive to metamorphosis. When temperatures are optimal, development properly proceeds to metamorphosis with relatively low occurrences of malformations and a decreased probability of predation.
CHAPTER 6

CONCLUSION

When looking for a reintroduction site within a known habitat range for any given ranid, one should look at the different stages within their life cycle. The most sensitive stage should be the determining factor for which habitats to use. The experiments described here show *R. onca* is definitely a thermal obligate where they are restricted to a specific range of temperature to develop metamorphosis regardless of developmental temperatures. *R. onca* tadpoles preferred temperatures between 20 and 30°C with an optimal thermal condition for development and predator avoidance at 25°C. Water temperatures which remained at 15°C for most of the year are not suitable habitat for *R. onca* tadpole development due to the decrease in survivorship, development rate, metabolic rate, and maximum burst speed along with an increase in malformations. This may be reason for the lack of reproductive success at the reintroduction sites with cold spring water sources. Water temperatures at the spring's source at or above 35°C are able to support *R. onca* reproduction because egg deposition and tadpole development can occur further down stream where water temperatures are cooler. To ensure the tadpoles are capable of metamorphosing into adults, *R. onca* reintroduction sites should be associated
with thermally influenced hot springs or water sources with thermal conditions at
or above 20°C for at least 9 months of the year.

Adults have a preference for laying their eggs in slow flowing pools with water
temperature around 16°C. Since the eggs are immobile, they must tolerate a
wider range of thermal conditions. This places a strong selection for eggs with a
tolerance for a wider range of thermal conditions. A few days after hatching they
are able to actively search for a preferred habitat to allow for a rapid development
to metamorphosis. *R. onca* tadpoles are usually found in a variety of habitats
with water temperatures ranging from 20 to 27°C (personal communication,
Cristina Velez, NPS). Looking at all of the experimental data, everything points
to an optimal acclimation at 25°C; however, temperatures between 20 and 30°C
are acceptable for development.

In the future, it would be interesting to determine the genes that are
temperature sensitive, and at what temperature do the different subtypes of heat-
shock protein start synthesizing. This will give a better understanding about how
protein regulation and how different environmental conditions can affect
developmental outcome. It would be interesting to determine if tadpoles prefer
different temperatures during different stages of development. If so, would this
allow differently staged tadpoles to select for different habitats to increase
development rates to prevent competition for resources and/or overcrowding?
Also, is this change in temperature selection due to changes in thermal sensitivity
of sensory nerves as development proceeds to metamorphosis?
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### Table 1: Shows the effect of temperature on oxygen solubility of water.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>$O_2$ ml/L$_{H_2O}$</th>
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Table 2: The effects of thermal acclimation on survival to metamorphosis, mass at metamorphosis, and time to metamorphosis on tadpole development in the 2005 experimental group. Because of the lack of development at 15°C (stage 27.6 @ 191 days), rearing temperature was increased to 25°C to simulate an over-wintering event. The breakdown of temperature increase is shown as a subset of the Total 15°C row. Because of the decrease in health at 35°C, the temperature was reduced to 30°C after 10 days; however, only 35% survived past 60 days post-hatch and the last tadpole survived to 143 days post-hatching without metamorphosing. Asterisks (*) shows significant difference from the 25°C acclimation group (p < 0.05). Mass and Days represents a mean ± standard error for each acclimation group.

<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th>Count</th>
<th>Survival</th>
<th>Mass (g)</th>
<th>Time (Days)</th>
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<tbody>
<tr>
<td>Total 15</td>
<td>47</td>
<td>48.9%*</td>
<td>4.83 ± 1.08</td>
<td>282 ± 12*</td>
</tr>
<tr>
<td>15</td>
<td>47</td>
<td>63.8%*</td>
<td>0.58 ± 0.16</td>
<td>191*</td>
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<tr>
<td>15 → 25</td>
<td>30</td>
<td>80.0%</td>
<td>1.10 ± 0.76</td>
<td>50</td>
</tr>
<tr>
<td>15 @ 25</td>
<td>24</td>
<td>95.8%</td>
<td>4.83 ± 1.08</td>
<td>41 ± 12</td>
</tr>
<tr>
<td>20</td>
<td>50</td>
<td>84.0%</td>
<td>4.92 ± 1.14</td>
<td>260 ± 47*</td>
</tr>
<tr>
<td>25</td>
<td>48</td>
<td>93.8%</td>
<td>4.40 ± 0.72</td>
<td>62 ± 8</td>
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<tr>
<td>30</td>
<td>47</td>
<td>66.0%*</td>
<td>3.90 ± 0.84</td>
<td>106 ± 51*</td>
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<tr>
<td>35</td>
<td>46</td>
<td>0.0%*</td>
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<tr>
<td>Total</td>
<td>238</td>
<td>58.8%</td>
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</tbody>
</table>
VITA

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