Final Project Report For Clark County Desert Conservation Program
Project #2003-NPS-230-P-2004-16, Temperature Acclimation/Oxygen Consumption in *Rana onca* larvae

PROJECT REVIEW:

What measurable goals did you set for this project and what indicators did you use to measure your performance? To what extent has your project achieved these goals and levels of performance?

This project combined a laboratory evaluation of the effects of temperature on growth and development of the aquatic larval phase (tadpoles) of the Relict Leopard Frog life cycle, with acute measures of acclimation to specific temperatures spanning the range of temperatures of current occupied as well as potential frog habitat. A field study to examine summer (lowest water) conditions for 21 springs and seeps on Gold Butte that might be considered as localities for new frog populations is included in the project also. The indicators used to measure performance were completion of the planned studies with sufficient sample size for statistical validity, as well as analysis of the results and publication in appropriate professional journals.
Most of the laboratory studies were completed, but not until the end of January 2006 due to delay of funding that caused a mismatch of funding availability with the natural breeding cycle of the frog. For scientific validity, studies done in 2004 had to be repeated in 2005 to make sure the temperature effects on very early stage tadpoles (missing from the 2004 studies) were included.
Growth and development studies were completed and data analyzed showing a clear peak for most rapid development and highest survivorship at 25 degrees Celsius. Oxygen consumption studies were also completed as were temperature preference studies. Preliminary analysis of both showed some acclimation to rearing temperature. These preliminary analyses were presented in earlier reports on this project and are summarized in the attached presentation. Preliminary evaluation of Gold Butte water sources was also completed and presented in earlier reports. The preliminary analyses do not include final data collection in late 2005 and early 2006. Sprint speed studies, to examine whether temperature might affect capacity for predator evasion, were completed but no analysis was done before the termination of funding.
No final analyses or publications have yet resulted from these studies.

Graphs, photographs and presentations of preliminary analyses are included in previous reports. Preliminary results and project status are summarized in the attached presentation.

Did the project encounter internal or external challenges? How were they addressed? Was there something Clark County could have done to assist you?

Challenges to completion of the project related only to the mismatch of funding availability to the natural life cycle of the frog. Additional funding and a time extension of several months would result in completed final analyses and manuscripts submitted for publication to appropriate professional journals.

What lessons did you learn from undertaking this project?

- To assure completion of research projects funding must start with predictability so that staff recruitment, arranging for appropriate facilities and other project logistics can be accomplished at the correct time and within budget. When the timing of funding availability can not be assured or weather or other forces beyond the control of either funding entity or project proponent intervene to the detriment of project completion, clear mechanisms for changes to scope of work or funding (including timelines and due dates) should be identified. In the identification of such mechanisms priority must be given to completion of projects so that conservation is advanced.
- With the stipulations above I would not hesitate to encourage others to find ways to work with resource management agencies and funding entities to pursue conservation research.

What impact do you think the project has had to date?

Preliminary results of the studies performed have already provided information to the National Park Service to assist in fine-tuning their *Rana onca* captive rearing program. Description of summer water availability has also assisted the Relict Leopard Frog Conservation Team in identification of sites that might be further explored as potential frog habitat.

There have been no final analyses or publications from this research.

Is there additional research or efforts that would complement or add to your project that could be conducted?

Final analyses of laboratory data and publication of results in appropriate professional journals would complete the project.

Additional investigation of field sites would be required to assure adequacy of habitat for new frog populations.

FORMAL REPORT:
Executive Summary:

Featured Project and Type: **Project #2003-NPS-230-P-2004-16, Temperature Acclimation/Oxygen Consumption in *Rana onca* larvae.** This is a research project on a Covered species.

Species Addressed: Rana onca (Relict Leopard Frog)

Summary Project Description: This project combined a laboratory evaluation of the effects of temperature on growth and development of the aquatic larval phase (tadpoles) of the Relict Leopard Frog life cycle, with acute measures of acclimation to specific temperatures spanning the range of temperatures of current occupied as well as potential frog habitat. A field study to examine summer (lowest water) conditions for 21 springs and seeps on Gold Butte that might be considered as localities for new frog populations is included in the project also.

Project Status/Accomplishments: Growth and development studies were completed and data analyzed showing a clear peak for most rapid development and highest survivorship at 25 degrees Celsius. Oxygen consumption studies were also completed as were temperature preference studies. Preliminary analysis of both showed some acclimation to rearing temperature. These preliminary analyses were presented in earlier reports on this project and are summarized in the attached presentation. Preliminary evaluation of Gold Butte water sources was also completed and presented in earlier reports. The preliminary analyses do not include final data collection in late 2005 and early 2006. Sprint speed studies, to examine whether temperature might affect capacity for predator evasion, were completed but no analysis was done before the termination of funding.

Partners: National Park Service: Lake Mead National Recreation Area

Project Contact: Stanley Hillyard, University of Nevada School of Dental Medicine and UNLV Biology Department and Karin Hoff

Funding: $81,000

Completion Date or Status: Incomplete due to mismatch of timing of funding availability and natural life cycle of the frog.

Documents/Information Produced:
See attached summary presentation made by Jeffrey Goldstein (UNLV graduate student in Biology) for the Disappearing Amphibian Populations Task Force meeting in January 2006.

No publications have yet resulted from this project.

Two (2) Project Photos: Photographs below illustrate an egg mass with developing tadpoles (top) and an adult frog (bottom; courtesy of Ross Haley, National Park Service Lake Mead NRA).

Introduction:

Description of the Project: This project combined a laboratory evaluation of the effects of temperature on growth and development of the aquatic larval phase (tadpoles) of the Relict Leopard Frog life cycle, with acute measures of acclimation to specific temperatures spanning the range of temperatures of current occupied as well as potential frog habitat. A field study to examine summer (lowest water) conditions for 21 springs and seeps on Gold Butte that might be considered as localities for new frog populations is included in the project also.

Background and Need for the Project:

The relict leopard frog, *Rana onca* has been extirpated from most of its range, and some of the few remaining Rana onca populations have been extirpated within the last several years. It is likely that fewer than 1000 adult frogs remain. Rana onca is currently under petition for Federal listing under the ESA.

Water sources for many sites where frogs occurred most recently are geothermally influenced, with water temperatures between 16° and 55° C at the spring head.

The LMNRA/NPS has embarked on a program to increase the number of frogs and the number of populations, but, identification of suitable habitat is confounded by the varied thermal environments in which the frogs currently occur. It is not known whether frogs from very warm springs can be moved safely to springs with much cooler water, or whether frogs reared under the current captive rearing protocol can be introduced successfully to new habitats with very different water temperatures. It is not known either whether frogs from the different populations are sufficiently genetically different that their preferred thermal regimes will significantly compromise efforts to introduce RLFs to new or historical sites. The proposed study will measure oxygen consumption of *Rana onca* larvae in different thermal environments in order to make an acute determination of temperature tolerance using thermal acclimation and stress level.

Thermal Acclimation: In general, anurans are quite plastic in their response to temperature and will acclimate more quickly than, and to a wider range of temperature than many other taxa. However, temperature tolerance and temperature preference vary among species and within species among populations. In addition, measures of temperature tolerance and temperature preference are known to correlate with acclimation to specific temperatures (reviewed in Ultsch, et al., 1999).. In an extreme range of thermal conditions, it may be possible to introduce animals to inappropriate thermal environments or to move animals too quickly into conditions different from rearing temperature regimes.

Measures of thermal acclimation and thermal tolerance: Many traditional physiological studies of thermal tolerance have used measures that require considerable stress to the animals (critical thermal maximum and critical thermal minimum) and whose value in defining a useful thermal regime for animals in the wild is limited. Behavioral measures of temperature preference (preferred body temperature, PBT) are useful for defining the center of the tolerance range, but do not define the long-term consequences of particular thermal regimes; which may include dramatic effects on time to metamorphosis, size at metamorphosis, and survival through the larval period. Acute measure of metabolic activities, such as oxygen consumption, may be useful in defining stress level and acclimation, and when combined with behavioral measures such as ventilation rate (Stuenkel and Hillyard, 1981), have the added benefits of not requiring high stress levels and being sufficiently early indicators of stress that experiments can be terminated without harm to the animals.

Intent of proposed experiments: The studies focused on acute measures of metabolic rate. The results of these studies may be used to determine stress level and acclimation time for animals reared at one temperature and transferred to another. This will inform the development of management procedures for transfer of captively reared frogs. The growth rates at different rearing temperatures may also provide information to better define most efficacious rearing protocols and preferred temperature regimes. This will provide guidance for description, discovery and creation of habitat and restoration/change of existing habitat.

In addition to evaluating the ability of larvae of the Relict Leopard Frog to acclimate to different water temperatures, we will further the objective of discovering and creating new habitat by examining 21 spring sites for their suitability as relict frog habitat. All the springs are on public land and water rights are designated for wildlife use.

Elements of the MSHCP addressed: In collaboration with the National Park Service the following elements of the Clark County MSHCP were identified as addressed by this project:

Species covered

1. Relict leopard frog (*Rana onca)*

Habitat/Ecosystem(s) covered

1. Spring Ecosystem
2. Desert Riparian/Aquatic Ecosystem

Location of Project(s)

1. Springs occupied or previously occupied by *Rana onca*
2. Springs potentially suitable as habitat for *Rana onca*
3. Boulder City Wetlands Park & other potential man-made refugia
4. Park Service facility

Threats Addressed

1. Species threats

1 (13) Aquatic Resources

Threat 1301 - Lowland riparian habitat degradation and modification associated with channelization.

Threat 1302 - Changes in habitat quality due to changes in water flows resulting from groundwater pumping.

Threat 1303 – Decreased water availability to support riparian habitat.

Threat 1304 – Changes in water quality from grazing and agriculture.

(14) Springs

Threat 1401 – Habitat degradation resulting from spring diversion and modification.

Threat 1402 – Habitat degradation from spring outflow diversion.

Threat 1403 – Habitat degradation due to decreased spring flows resulting from groundwater pumping.

Threat 1404 – Changes in water quality resulting from grazing and agriculture.

Threat 1405 – Reduced flows resulting from overutilization by animals.

(15) Exotic, Subsidized and Parasitic Species

Threat 1501 – Habitat degradation and population decreases resulting from introductions, competition and encroachment of exotic species.

(17) Illegal or unauthorized activities

2. Ecosystem threats
   1 (13) Aquatic Resources

      Threat 1301 - Lowland riparian habitat degradation and modification associated with channelization.

      Threat 1302 - Changes in habitat quality due to changes in water flows resulting from groundwater pumping.

      Threat 1303 – Decreased water availability to support riparian habitat.

      Threat 1304 – Changes in water quality from grazing and agriculture.

   (14) Springs

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      Threat 1404 – Changes in water quality resulting from grazing and agriculture.

      Threat 1405 – Reduced flows resulting from overutilization by animals.

Methods and Materials:

Procedures for thermal acclimation experiments: The thermal environment of the currently occupied habitats was characterized. Rana onca tadpoles were maintained at temperatures spanning the thermal range of the species for their entire larval life. Tadpoles in the different treatments were selected at random from those available. Growth rate, time to metamorphosis and size at metamorphosis were recorded. Tadpoles were placed in plexiglass oxygen consumption chambers kept at several different temperatures. After stable rates of oxygen consumption have been attained, continuous recording were made for 30 minutes to quantify the metabolic rate using a Sable Systems Read-Ox 4 dissolved oxygen analyzer and Sable Systems software. Detailed procedures are documented in Hillyard (2002).

We used 5 temperature treatments and 20 tadpoles in each treatment. Temperatures were determined after evaluating temperature regime in occupied and potential habitat and consulting with the RLFCT. Tadpoles were randomly assigned to the treatment groups. Individuals were weighed and developmental stage determined once per week. Rearing will follow NPS protocols and food was not limiting.

Metabolic rate: At least 3 times during larval life, oxygen consumption was measured at each of the 5 rearing temperatures.

Swimming speed: Ten tadpoles from each rearing temperature were placed in a custom designed (Sable Systems International) race track and induced to swim in bursts using mild

electrical stimulation. Position/velocity were recorded with light sensors at 1 cm increments.
Temperature selection: At least once during larval life, tadpoles were placed in a custom designed (Sable Systems International) thermal gradient chamber with low/high temperatures spanning the 5 rearing temperatures in 10 degree increments. Position of tadpoles were recorded for 30 minutes to determine temperature selection.

The following hypotheses were tested.

Hypothesis 1: Rearing temperature will not affect Oxygen consumption or swimming speed at any temperature.

Hypothesis 2: Rearing temperature will not affect growth, development or survivorship.

Hypothesis 3: Rearing temperature will not affect temperature selection.

Procedures for examining suitability of springs as frog habitat:
Environmental parameters at the spring sites were measured following Bradford, et al. (2003). Twentyone springs were characterized during late spring and summer when water levels are likely to be lowest.
The twentyone springs are located in remote areas of Gold Butte. Travel time from Las Vegas is 6 hours or more with approximately half the distance over very rough roads. Four trips were required for a preliminary evaluation of the springs. The list of springs is shown in appendix I; the environmental metrics used for evaluation are shown in appendix II.

Results and Evidence of the Results: Please see attached presentation for summary of results to date. Preliminary results for all hypotheses except those relating to swimming speed are presented. No final analyses have been completed.

Evaluation/Discussion of Results: Final analysis must be completed before results can be discussed with confidence. The results and analyses must be published in appropriate professional journals. Preliminary results suggest that *Rana onca* have thermal preferences and some capacity to acclimate. These results could influence the NPS captive rearing program and the selection of sites for new frog populations.

Conclusion: These studies are substantially complete and have followed the intent of the original proposal closely, but final data analyses are not complete and no publications have yet resulted.

Recommendations:
Based on preliminary results, suggestions have been made to the RLFCT on fine-tuning of the NPS captive rearing program and considerations for frog larvae in evaluating potential sites for new frog populations. No formal recommendations can be made until data analyses are complete.

Literature Cited
No publications have resulted from this study to date.
Literature cited for the original proposal is still applicable.

1. Center for Biological Diversity and Southern Utah Wilderness Alliance. 2002. Petition to List the Relict Leopard Frog (Rana onca) as an endangered species under the Endangered Species Act.
2. Hoff, K.vS., A. R. Blaustein, R.W. McDiarmid and R. Altig. 1999. Behavior: Interactions and their consequences. In: Tadpoles, the biology of anuran larvae. R.W. McDiarmid and R. Altig, eds. Univ. of Chicago Press.
3. Ultsch, G, D.F. Bradford and J. Freda (1999) Physiology: Coping with the environment. In: Tadpoles, the biology of anuran larvae. R.W. McDiarmid and R. Altig, eds. Univ. of Chicago Press.
4. Van Maaren, C.C., J. Kita and H. V. Daniels (2002) Temperature tolerance and oxygen consumption rates for juvenile Southern Flounder Paralichthys lethostigma acclimated to five different temperatures. UJNR Technical Report No. 28.
5. Hillyard. S.D. (2002). Metabolic rate of Devil's Hole Pupfish. Research proposal to the NPS. Funded for 2002-2004. Not yet executed due to low fish numbers.
6. Steunkel , E.L. and S.D. Hillyard. (1981) The effects of temperature and salinity acclimation of metabolic rate and ormoregulation in the pupfish *Cyprinodon salinus*. Copeia 1981(2): 411-417.
7. Bradford, D. F. , A.C. Neale, M. S. Nash, D. W. Sada and J. R. Jaeger (2003) Habitat patch occupancy by toads (Bufo punctatus) in a naturally fragmented desert landscape. *Ecology,* 84(4), 2003, pp. 1012–1023

Appendix I. Springs to be characterized:

Gold Butte Springs	TNC/BLM owned water rights
Water/Well Name	Site/Location
Gold Butte Spring	Sec. 17, T19S-R70E
Horse Spring	Sec. 24, T18S-R70E
Cat Claw Spring	Sec. 29, T19S-R69E
Granite Spring	Sec. 17, T19S-R70E
Grapevine Spring	Sec. 34, T19S-R70E
Maynard Spring	Sec. 20, T19S-R69E
Red Rock Spring #2	Sec. 18, T17S-R70E
Mud Spring	Sec. 25, T17S-R70E
Summit Spring	Sec. 13, T19S- R70E
Connelly Spring	Sec. 31, T19S-R71E
Walker Spring	Sec. 20, T20S-R69E
Twin Springs	Sec. 19, T20S-R70E
Garden Spring	Sec. 11, T19S-R70E
Greasewood Reservoir	Sec. 33, T17S-R71E
Gann Spring	Sec. 15, T20S-R69E
Liston Spring	Sec. 18, T18S-R70E
Pierson Spring	Sec. 34, T19S-R70E
Perkins Spring	Sec. 11, T19S-R69E
Quail Spring	Sec. 22, T19S-R69E
Rattlesnake Spring	Sec. 13, T20S-R69E
Red Rock Spring #3	Sec. 18, T17S-R70E

Appendix II. Environmental Metrics for use in characterizing springs:

A. **PATCH SIZE METRICs**
   a. Surface water linear extent (%)
   b. Surface water area (log10 [m2 + 10])
   c. Emergent-type vegetation linear extent (%). Indicator taxa: *Typha*, *Eleocharis*, *Scirpus*, *Mimulus*, *Anemopsis*; *Juncus* & *Carex* if in stream channel.
   d. Native riparian trees linear extent (%). Indicator taxa: *Salix*, *Populus*, *Fraxinus*.
   e. Riparian shrubs/herbs linear extent (%). Indicator taxa: *Baccharis*, *Pluchea*, *Vitis*, *Allenrolfea*, *Equisetum*; *Juncus* or *Carex* if outside stream channel.
   f. Phreatophytes linear extent (%). Indicator taxa: *Prosopis*, *Chilopsis*.
   g. Riparian zone area (log10 [m2 + 10]). Zone boundaries delineated by indicator taxa.

B. **ISOLATION METRICS**
   a. Distance (Euclidian) to nearest patch (log10 of km)
   b. Distance (via drainage network) to nearest patch (log10 of km)
   c. Distance (Euclidian) to nearest occupied patch (log10 of km)
   d. Distance (via drainage network) to nearest occupied patch (log10 of km)

C. **ENVIRONMENTAL METRICS**
   a. Elevation (m)
   b. Linear extent of channel entirely overgrown (< 1 m height) with vegetation (%)
   c. *Tamarix* spp. (exotic plant) linear extent (%)
   d. Bedrock substrate cover (%)
   e. Predominate substrate grain size (median of 5 categories: <0.1, 0.1-0.5, 0.5-8, 8-30, >30 cm)
   f. Predator presence/absence (bullfrogs [*Rana catesbeiana*], crayfish, or fish) (0, 1)
   g. pH
   h. Electrical conductivity (log10 of µS/cm)
   i. Water depth, mean (cm)
   j. Wetted perimeter width, mean (m)
   k. Submerged or floating vegetation cover, including filamentous algae, mean (%)
   l. Emergent vegetation within 15 cm of point, mean (%)
   m. Vegetation cover over water, mean (%)
   n. Vegetation cover (<1 m high) over adjacent land, mean (%)
   o. Plot substrate size, for granular substrate, mean (log10 of cm)
   p. Bedrock substrate cover, mean (%)
   q. Water temperature, 0.5 m offshore, 2 cm deep (oC)

D. **PERSISTENCE OF WATER METRIC**
   a. Presence/absence of taxa indicative of persistent water (0, 1)

E. GEOGRAPHIC METRICS

a. X coordinate (km), centered on mean (UTM - 659); approximately corresponds to longitude.
b. Y coordinate (km), centered on mean (UTM - 3989); approximately corresponds to latitude.

F. TEMPORAL METRICS

a. Year of habitat sampling
b. Day of year of habitat sampling