



FINAL REPORT

Devils Hole Pupfish Panel Review - March 14-15, 2007 Furnace Creek Inn, Death Valley National Park

Administrative Report

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**U.S. Department of the Interior
U.S. Geological Survey**

FINAL REPORT

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Objective and Process

Director Jon Jarvis of the Pacific West Region of the National Park Service asked the chair to address long-term monitoring of the Devils Hole pupfish population by assembling a panel of experts to provide recommendations on actions to be taken. The Director's request was prompted by what has been perceived as a somewhat precipitous decline in recent years of the Devils Hole pupfish population and concern about the impact and effect of human disturbance on the remaining fish in the population. The chair was instructed to assume that the pupfish population would not become extinct.

The chair assembled a panel of experts from a variety of disciplines to evaluate long-term monitoring of the pupfish population. The panel included:

Dr. Kumud Acharya - Desert Research Institute, Las Vegas
Mr. Mark Buktenica - Crater Lake National Park
Dr. Marlis Douglas - Colorado State University
Dr. David Hankin - Humboldt State University
Mr. Robert Hoffman - Forest and Rangeland Ecosystem Science
Center, U.S. Geological Survey
Dr. Stanford Loeb – University of Kansas
Dr. Douglas Markle – Oregon State University
Dr. David Prudic – Nevada Water Science Center, U.S. Geological Survey
Dr. Craig Stockwell - North Dakota State University
Dr. Andrea Woodward - Forest and Rangeland Ecosystem Science
Center, U.S. Geological Survey

Chair: Dr. Gary Larson - Forest and Rangeland Ecosystem Science Center,
U.S. Geological Survey (Emeritus)

Prior to the review, Death Valley National Park provided the panel with an extensive amount of literature about Devils Hole and the pupfish population. The review was convened on March 14 and a series of presentations were made to the panel to provide additional information about various aspects of the fish population and habitat. The presentations included a general review of the habitat and environmental setting (David Ek), hydrology (Jerry Fisk), a historical review of ecological studies (James Deacon), a review of current ecological studies (Michael Parker), reviews of the refugia and the genetic status of the fish population (Peter Dratch), a conceptual model of Devils Hole (Krystyna Stave), long-term monitoring (Dean Blinn), and population monitoring (Zane Marshall).

The panel met from 8:00 to 9:30 AM on March 15 to discuss what was learned about Devils Hole and the pupfish population. National Park Service staff and representatives from the Fish and Wildlife Service and State of Nevada joined the panel at 10:00 AM. To meet the request of NPS for expert recommendation, each panelist provided the audience with a preliminary summary of findings. The panel members sent the chair their individual recommendations and thoughts and these are attached as Appendix A.

Summary of Panel Findings

Monitoring in the Short Term

Dr. Dean Blinn presented a long-term monitoring program for Devils Hole. The plan contains three alternatives that vary in complexity and annual cost. Although the panel made several recommendations for long-term monitoring that included many elements of Dr. Blinn's plan, the panel unanimously recommended that a very limited monitoring effort be considered at this time owing to the extremely low abundance of the Devils Hole pupfish. In brief, the population should be disturbed as little as possible. The current supplemental feeding project was endorsed by most members of the panel because there was evidence that the productivity of Devils Hole has diminished. The basic monitoring project should include the measurement of on-site weather variables, water level and depth, water quality, bi-annual fish population estimates (maximum and minimum) and size classes of the fish, and limited sampling of the "shelf" for relative abundances of periphyton and prey taxa. Further, frequent high quality photographs of the shelf from

fixed points should be taken to document changes in the amount of allochthonous input and the size and color of algal mats on the shelf. Some panel members suggested using a web-cam system to better understand the frequency and intensity of disturbances to the system. With the exception of the water level recorder, on-going monitoring should avoid placing permanent equipment in Devils Hole. Sampling equipment should not be stored in the drainage area of Devils Hole. The panel also expressed concern about public access to Devils Hole. One panel member recommended moving the existing fence back by at least 25 m.

Some details and thoughts –

A weather station should be located near Devils Hole. The station should not be influenced by the fence surrounding Devils Hole. Weather data should include precipitation, air temperature, wind speed and direction, relative humidity, barometric pressure, UV, and net radiation.

Water quality data should include near-surface temperature, pH alkalinity, conductivity, dissolved oxygen, dissolved organic carbon, turbidity, nitrogen, and phosphorus. Seasonal samples should be taken from the inner, middle, and outer portions of the shelf and to a depth of at least 10 meters in the “Hole”. Concentrations of dissolved oxygen (DO) in the algal mats on the shelf should be measured (using a DO probe) during day and night when larval abundances are estimated. These data should be collected even if larval counts are temporarily suspended (see below).

Seasonal periphyton and benthic macro-invertebrate samples should be collected with minimal impacts on the shelf. The focus would be to document the dominant species and relative abundances of these taxa from the inner, middle, and outer portions of the shelf.

The panel greatly appreciated the dedication of those involved in the research and monitoring of Devils Hole. The panel had some concerns, however. For example, there appeared to be lack of a coherent understanding about the life-history of the Devils Hole pupfish (esp., reproduction; larval life-history requirements, survival, and recruitment; juvenile and adult survival; adult longevity; etc.), and fish population data did not include fish size. Additionally, because the impacts of spring sampling are unknown, a conservative approach would be to limit spring sampling activities. Perhaps

only the fall survey should be conducted until the population increases to average historical numbers, indicating a population in recovery. The panel also expressed concern about the use of chlorine solutions to sterilize dive suits owing to the potential of introducing toxic compounds into the ecosystem. This issue could perhaps best be dealt with by providing two complete sets of diving gear dedicated for use only in Devils Hole.

The panel was led to believe that a great deal of information about Devils Hole has been stored in file cabinets and not available to park management and researchers. These data need to be archived in a database that is part of an accessible data management system. The panel also expressed concern that major conclusions from the extensive research and monitoring efforts have not been published in peer-reviewed scientific journals. The panel recommended utilization of experts in various fields to summarize the most important findings and crucial facts for each subject. Furthermore, the panel recommended that the park:

1. Hire a fisheries biologist with biometric training to assemble these data and prepare, with appropriate co-authors, papers for peer-review and publication; and
2. Ask the authors to publish their research in peer-reviewed journals.

Publication in peer-reviewed journals to the extent possible would be important to NPS because gray literature would be removed from management decisions, policies, and actions.

The panel believed that all monitoring activities should be carefully documented (as one member put it, “to the smallest detail”). One panel member prepared a table showing activities and knowledge. This type of effort is essential for a long-term program to have a firm basis from which to understand what is known. Other panel members added to this notion, including documenting and classifying the questions being asked about Devils Hole and the pupfish population so as to avoid asking the same questions repeatedly without attempting to answer them and to avoid reinventing wheels.

Dr. Krystyna Stave presented a conceptual model of the Devils Hole ecosystem to the panel. Although complex, the model can be an excellent starting point for identifying and evaluating relationships of the major

components of the Devils Hole ecosystem. Several panel members were in favor of further development of the conceptual model (or other models).

Overall, panel members were somewhat skeptical of the conclusions drawn from the genetic evaluation of Devils Hole pupfish in refugia and suggest that alternative hypotheses should be considered to explain the existing data. One problem is that genetic markers used to evaluate hybridization are not diagnostic and thus do not allow the determination of introgression with certainty. The park would benefit by extending the genetic study using mitochondrial DNA sequence variation and microsatellite DNA loci. This would allow comparisons of the genetic compositions of the refugia populations to published data sets and to evaluate if detected patterns indeed represent introgression.

If any of the fish at the aquarium are “pure” Devils Hole fish, these should be isolated from the other fishes. If these fish, and those at Point-of-Rocks (POR), are reintroduced into Devils Hole, they should be checked for viral infections, bacterial pathogens, and parasites. Nonetheless, the panel was concerned about the reintroduction of Devils Hole pupfish from the refugia because these fish could have changed relative to body shape and behavior, e.g. aggressiveness. These fish could be competitively superior to those in Devils Hole and dominate progeny production. If such reintroductions occur, females would be preferred because they are less aggressive than males.

The history of all fish removed from Devils Hole should be documented. All Devils Hole and refugia mortalities should be preserved for genetic analyses. Removal of any additional fish from Devils Hole should be avoided in the short-term.

Little is known about the circulation of the water in Devils Hole. Given the apparent changes in water quality, an investigation of the circulation pattern(s) in Devils Hole is recommended. Furthermore, efforts should be made to compile and maintain records of ground-water withdrawals in the region that might impact Devils Hole and the surrounding systems, e.g., POR.

Standardization of temporal estimates of fish abundances and lengths was recommended by the panel. Sampling periods should be based on the life-history characteristics of the species.

Substrate composition of the shelf should be qualitatively assessed seasonally and after disturbance events. Removal of substrate from the shelf was not recommended by the panel, i.e., leave the removal of substrate to natural processes.

Short-term Monitoring of Point of Rocks Refugium

The panel expressed concerns about the pupfish population at POR as it did for the population in Devils Hole. The proposed short-term monitoring program at Devils Hole was recommended for the refugium.

Long-term Monitoring of Devils Hole

The panel expressed the need for expanded monitoring of Devils Hole once the pupfish population abundance returns to “normal”. Monitoring of the fish population should be designed around the life history of the species. Most panel members agreed that Dr. Blinn’s “Plan A” was appropriate, but this topic needs to be revisited by a panel of experts before implementation of the plan. Monitoring of the fish population should include egg and larval production, growth, age-specific mortality, longevity, deformities, and behavior and morphology.

The panel provided a wealth of information and research questions. This report is a summary intended to highlight the salient points for NPS management relative to long-term monitoring, but does not substitute for the detailed comments of panel members reported in Appendix A.

APPENDIX A

1. DR. KUMUD ACHARYA

Devil's Hole Pupfish Long Term Monitoring Panel Meeting 13-15 March, Death Valley, CA

Opinions by,
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Background

The Devils Hole Pupfish is an important legacy of the Southwest because it is unique to its location. The pupfish have provided clues to the development of the planet over the several million years. The Devils Hole pupfish has been an important part of the study into evolutionary biology and has provided valuable insight regarding the pathways followed by early ancestors over millions of years. The fish has ancestral relatives from the Middle East and Africa (Deacon 2006; Deacon & Baugh 1985; Mickley & Deacon 1975). Therefore saving this remarkable creature have huge implications. However, over the past 10 years, the pupfish population has fallen precipitously. The problem was exacerbated a couple of years ago when a set of fish traps was accidentally deployed killing a large portion of the population. The population estimate stands at <40 individuals (April 2006, NPS, Death Valley, CA) along with several dozen more at artificial 'refugium' at Hoover Dam. Biologists, aqua-culturists and scientists from various federal (NPS, FWS), county (Nye Co.) and academic institutions are in a race against time to identify the cause of this rapid decline.

The decline of the pupfish and correlations with food availability has often been debated. Limited life history information on the pupfish suggests that the growth and population is closely related to the availability of food. The small limestone shelf on which the fish spawn also provides a feeding ground for the fish. The shelf also provides a majority of the algae and much of the diatoms on which the fish feed. Earlier studies of the food habits of the Devils Hole pupfish determined that diatoms were the most important food in fall and summer. Also included among the pupfish food is amphipod *Hyaella*, an unidentified ostracod, and protozoans. A beetle, *Stenelmis*, a tubellarian, *Dugesia*, and a hydrobiid snail, *Tryonia* are much less commonly used as food.

Existing Data

Data on water quality, the food web, and other parameters related to fish life histories at Devils Hole over the years have been sporadic and uncoordinated. An analysis of the scant data available from 1984-2005 (NPS, Death Valley, CA) indicates that the pH in upper shelf area often fluctuates (unusual for a carbonate-based aquifer), average turbidity has been decreasing (perhaps due to reduced primary productivity) and nutrient concentrations (mainly nitrogen and phosphorus) are being depleted. For an extreme ecosystem like Devils Hole, already on the knife's edge, all or any one of these factors could affect the pupfish directly or indirectly via other organisms in the food web (invertebrates or algae). For example, it is thought that the ostracod, a zooplankton species which used to be common on the shelf and provided a food base for the pupfish when there was no primary productivity in the winter, are no longer found (NPS biologists, pers. comm.). A portion of the sparsely available Devils Hole limnological data is shown in Figure 1 below.

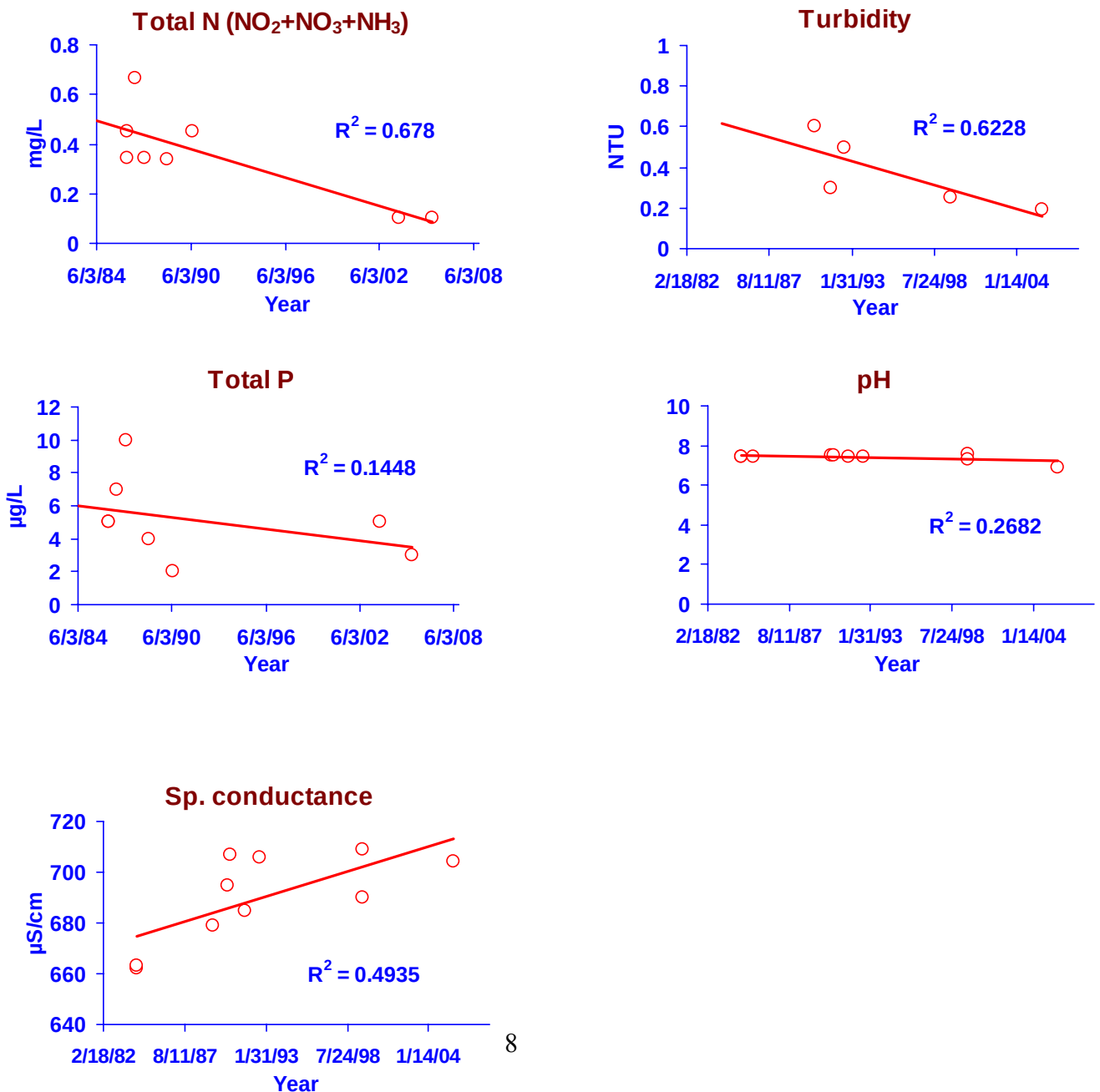


Figure 1: Preliminary data analysis of total N, total P, turbidity, pH and sp conductivity of Devil's Hole from 1980-2005. Data source USGS (courtesy of NPS, Death Valley, CA).

Based on an analysis of the data available from 1980-2005 (NPS, Death Valley, CA), personal communications with park managers, and scientists with prior experience at Devils Hole, the following conclusions seem plausible:

- Fish diets include zooplankton, benthic micro-invertebrates, periphyton, algae (mostly diatom) depending on time of year. A common zooplankton in the past (ostracod), is now not seen on the spawning shelf.
- The Hole is generally light limited year round. Winter months are severely light limited (no primary productivity) which coincides with the minimum annual average in fish population (food reliance on zooplankton).
- Average annual turbidity *seems to be* decreasing.
- Nitrogen concentration *may be* reducing. Earlier data indicate that the concentrations were above 0.3 mg/L but the recent numbers are less than 0.15 mg/L.
- *Perhaps* average primary productivity is decreasing. The Hole *appears to be* shifting to ultra-oligotrophy. Also, due to a change in nutrient levels, community composition of the algae *might* also be shifting. However, it is unclear whether primary productivity is causing the change in nutrient concentrations or vice versa.
- The upper layer of the Hole may be turning acidic which is unusual for a carbonate based aquifer. The impact of low pH may not be significant if the turnover rate of water is good. However, there is no data to verify water circulation patterns and rates.
- Specific conductivity *appears to be* increasing.
- Life history characteristics of DH pupfish are not well understood.
- Genetic information of the DH pupfish is limited.

From the above, it is safe to say that the food web of the Devils Hole ecosystem appears to be changing. The data abundantly indicate that the quality and availability of the food for the pupfish may have been reduced. Therefore the decline of the pupfish population appears to be related to the food limited condition.

However, the overall lack of information concerning the structure and function of the lower trophic levels in the Devils Hole hydrologic system represents a potential management issue and also a nightmare for understanding fundamental science for pupfish population decline. The lower trophic levels in the system are largely unaddressed at this point; predictions of how these habitats will respond to anthropogenic or natural stresses are therefore difficult based on existing data. The case can be made here that understanding the capacity of primary and secondary producers to respond to environmental change is important for understanding the overall food web of the Devils Hole.

Recommendation

Based on numerous talks presented at the long term monitoring panel meeting, published literature and a preliminary data analysis (above), I conclude that the understanding of basic limnology including water quality, habitat characteristics and the food web of the Devils Hole is critical before any restoration measures can be undertaken. In addition to the food web and limnology of the hole, it is also important to understand the life history characteristics of the pupfish and their genetic information. However, due to a limited number of pure-breed Devils Hole pupfish remaining, life history and genetic studies should be conducted using abundantly available hybrid species from refugium elsewhere. Following are the list of recommended limnology, physical habitat and food web studies at Devils Hole in the near future that I see are critical:

Water quality and physical habitat

1. Substrate composition: Monitor substrate composition on the spawning shelf biannually (winter and summer) and following a major storm and/or earthquake etc.
2. Water Level: Monitor water depth on the spawning shelf of Devils Hole continuously.
3. Water Temperature: Monitor water temperature throughout the spawning shelf. Existing monitoring of temperature should be continued but should be expanded beyond the shelf area and into 1, 5, 10 and 25 meters below in the cavern where the pupfish are likely to hang out, bi-monthly or monthly.
4. Solar Energy: Monitor light energy on the entire water surface throughout the year continuously.
5. Physico-chemical Measurements: Monitor dissolved oxygen, % oxygen saturation, water temperature, pH, and specific conductance over the spawning shelf and at several locations across the southern portion of the shelf. Install at least three YSI or hydrolabs (one each at the upper and lower shelves and third at the much deeper pool area (approx. 10 meters) for continuous monitoring.
6. Measure reduced sulfur (H_2S) within the sediment-water interface. Continuous monitoring for at least one 48-h period bimonthly is recommended. In addition, monthly measurement of H_2S is recommended at 3-4 locations on the shelf.
7. Total Nitrogen and total Phosphorus: Measure concentrations of total nitrogen and total phosphorus in the water column over the spawning shelf monthly.
8. Total Organic Carbon: Measure concentrations of total organic carbon in the water column over the spawning shelf bimonthly.

9. Sediment nutrient analysis: Measure C, N and P of the sediments on the shelf biannually.
10. Continuous Recording Weather Station and Earthquakes: Continuously measure air temperature, rainfall, wind speed, and wind direction at Devils Hole. Also, document seismic, climatic, and human disturbances. It is recommended that a weather station be installed there permanently.
11. Measure all the trace and heavy metals on the shelf area biannually.
12. Measure water circulation and mixing pattern of DH water using an ADCP (Acoustic Doppler Current Profilers).

Biology

1. *Algae*

Primary productivity: Measure primary productivity for at least two 24 hour periods during the longest day of the year (likely high primary productivity time seasonally). Also, monthly monitoring of primary productivity is suggested.

Filamentous algae: Monitor the mass and composition of benthic algae on the spawning shelf. Also, rank substrate embeddedness on the spawning shelf. This should be done monthly from April to September and twice in winter (December and February).

Phytoplankton and diatoms: Monitor the mass and composition of suspended algae and diatoms (including the calcite-attached ones) on the spawning shelf. This should be done monthly from April to September and twice in winter (December and February).

2. Allochthonous Material: Monitor the allochthonous carbon input into the Devils Hole ecosystem bimonthly and after measurable rainfall events.
3. *Dugesia dorotocephala* (flatworm): Monitor the density and distribution of *D. dorotocephala* on the spawning shelf seasonally (February, April, July, and October).
4. Benthic Invertebrates: Monitor invertebrate densities on the spawning shelf seasonally (February, April, July, and October).
5. Microbiological Analyses: Measure total coliform and fecal coliform in the water column over the spawning shelf biannually (June/July and December/January).

References:

- Deacon, J.E. (2006). The world and Devils Hole. Las Vegas Sun, Review Journal, article by Rake, L.
- Deacon, J.E., and T.M. Baugh. 1983. Annual report on Devils Hole: 1983. Report to the National Park Service, Death Valley National Monument. 18 pp.
- Minckley, C.O., and J.E. Deacon. 1975. Foods of the Devils Hole pupfish, *Cyprinodon diabolis* (Cyprinodontidae). Southwestern Naturalist 20(1):105-111.

2. MR. MARK BUKTENICA

April 5, 2007

Gary Larson, Chair
Devils Hole Long-Term Monitoring Plan Review

Dear Gary:

A lot of good work has been done toward understanding and managing Devils Hole and its resident pupfish. I appreciated the efforts made by cooperating managers and scientists to summarize and present this information prior to and during our meeting at Death Valley National Park, March, 2007. With all that is known about the Devils Hole system, there remain many unanswered questions. A long-term monitoring program can help answer some of these questions and guide management and research. An approach that you, I, and others found useful at Crater Lake was to develop a conceptual model (box, line, and arrow cartoon) of ecosystem components and processes depicting how we believed the system worked. We then developed hypotheses relative to the lake, and management concerns, to help guide what components and processes to monitor.

A conceptual model of the Devils Hole ecosystem could be further developed. Hypotheses to help guide monitoring might include: Human activity (e.g. water withdrawal or climate change) have impacted Devils Hole and its resident pupfish; A change in natural disturbance (floods, earthquakes) has impacted pupfish habitat and/or survival; A genetic bottleneck has led to reduced fitness, reproductive success, and survival; Pupfish are declining in abundance because the system has experienced a change in productivity.

The draft monitoring plan developed by Dean Blinn and others would go a long way toward addressing these hypotheses. I would recommend Level One sampling with some additions as described below.

- Describe spatial and temporal variability in chemical, physical, and biological parameters. The existing data indicate a great deal of spatial and temporal variability horizontally. This variability should be characterized horizontally (as it has for several parameters) and vertically when appropriate. Although scientists in the program believe there is little variation in chemistry and physical parameters with depth, it is important to verify this. Understanding that it is important not to “over task” SCUBA divers with too many jobs per dive - could the safety diver for the pupfish counts carry a hydrolab and collect a couple of water samples without compromising safety or the counts? Chemistry, temperature, chlorophyll, and light transmission or turbidity would be interesting to include in vertical sampling. Consider adding alkalinity (regularly) and ions (annually) to chemistry sampling. If resources are available the characteristics in the Blinn plan that are sampled biannually should be sampled more regularly (monthly or seasonally). Once

horizontal, vertical, and temporal variability are better understood, sampling stations and frequency can be reduced in number to index sites.

- Monitor flood and earthquake-generated waves. Natural disturbance has been part of the long-term history of the shelf and may be important to maintaining substrate conditions and processes that also support pupfish. I recommend water level sensors at the center and ends of the pool to evaluate disturbance events. Video of these events would be extremely valuable. A video camera could be triggered by a rapid change in pool height. The same triggering device could initiate a cell phone or radio alarm to Park or Refuge Headquarters to initiate post event sampling or mitigation.
- Transmit important data parameters (minimally climate and water level) to Park or Refuge Headquarters by radio or cell phone for rapid information dissemination and to alert managers and scientists to disturbance events and initiate post-event sampling or mitigation.
- Develop a data management system early in the program to include historic data, validate and archive continuing data collection, and disseminate information among cooperators.
- Additions to substrate monitoring. Monitor bed elevation of the shelf (in addition to water surface elevation). In addition to annual sampling, monitor substrate composition and bed elevation after disturbance events.
- Monitor water column and benthic primary production or chlorophyll. Note the hydrolab and chlorophyll sensor that has been used over the shelf is measuring water column chlorophyll not benthic chlorophyll.
- Monitor genetic structure of the pupfish in Devils Hole and refuges.
- Fish counts were presented as annual max and min counts. Recommend standardizing by season e.g. fall (September, October, and November) to reduce variability.
- Research life-history of pupfish as per comments by Markle and Hankin, and incorporate into monitoring program if appropriate.
- Suggested additions to climate station: solar radiation, UV, relative humidity, and barometric pressure.
- Monitor for radio nucleotides at an appropriate time interval. (At some point they will show up).
- Other management considerations:

- > Modify the existing gate and fence, and minimize structures in the “hole” and allow for unrestricted water and substrate movement during disturbance events.
- > Initiate reduced but parallel monitoring program for refuges and mimic Devils Hole conditions to the extent possible. Transmit data and alarm conditions for temperature and flow via radio or cell phone to Park or Refuge Headquarters.

It has been a pleasure working with this group and I commend all of the hard work that has gone into understanding and managing Devils Hole and toward the persistence of this unique pupfish. May the latter endeavor be fruitful! I hope some of the above comments are useful. Feel free to contact me in the future for further input or clarification.

Sincerely,

/s/ Mark Buktenica
Aquatic Ecologist
Crater Lake National Park

3. DR. MARLIS DOUGLAS

Thoughts on a Monitoring Protocol for Devils Hole Pupfish (*Cyprinodon diabolis*) **With Emphasis on Genetic Management of the Species**

Submitted by

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16-April-2007

I. INTRODUCTION

Decline of Devils Hole Pupfish (*Cyprinodon diabolis*) population over the past five years to unprecedented lows in 2006 gave rise to serious concerns about the long-term persistence of the species in its native habitat. Concurrent with this downward trend was the discovery that refuge populations in Point-of-Rocks and “other” refugia might be introgressed by Ash Meadows Pupfish, *Cyprinodon nevadensis*, a species that occurs in natural geographic proximity to Devil’s Hole (Martin 2005, unpubl.). A genetically compromised refugium population of the wild Devils Hole Pupfish is deemed less suitable for supplementation or reestablishment purposes.

A panel of scientists was informed about various aspects of Devil’s Hole environment and the biology of the pupfish during a two-day workshop in Death Valley National Park (14-15 March 2007). Based on available information (i.e., presentations, unpublished reports and peer-reviewed publications), the panel was asked to derive recommendations for a long-term monitoring program for the Devils Hole Pupfish.

My recommendations were derived from available information (as above) which I utilized to evaluate alternative hypotheses regarding life history and evolution of Devils Hole Pupfish. I formulated these hypotheses based on my understanding of the aquatic ecosystems in the arid southwest, and the unique ichthyofauna associated with these habitats. My perspectives are derived from 10+ years of research on desert fishes and are augmented by my training as an ichthyologist, conservation geneticist and evolutionary biologist.

First, I list my recommendations for the monitoring of Devils Hole Pupfish and briefly described specific issues I consider relevant to the long-term survival and ultimate recovery of the species. These are based on anecdotal observations about the ecology of the species. Selected topics, pertaining to conservation genetics of Devils Hole Pupfish, are then addressed in more detail. I review pertinent published information and offer

alternative hypotheses that accommodate these data and which are also supported by previous findings.

II. GENERAL RECOMMENDATIONS FOR MONITORING DEVILS HOLE PUPFISH

1. Focus and Emphasis

- (1) Main focus should be on the biology of Devils Hole Pupfish. This should entail recording and tracking of basic life history data (e.g., population census, age structure) so as to generate a baseline against which potential (suspected) changes can be contrasted. Experts on pupfish biology should be consulted to identify pertinent biological parameters that should be recorded. Methods may need to be developed for monitoring of biological parameters that are unique to the specific circumstances of Devil's Hole.
- (2) Efforts should be made to also monitor other biological components of Devil's Hole, such as algal and invertebrate communities. Some suggestions provided below.
- (3) Less emphasis should be placed on monitoring physico-chemical parameters. Those that are monitored should be important to the biology of the Devils Hole Pupfish. A rationale for de-emphasizing physico-chemical factors is provided below.

2. Time Interval for Monitoring

Any short or long-term monitoring must be designed with the lifespan of Devils Hole Pupfish in mind. One advantage of working with a short-lived species is that population trends occur quickly and over a short time span, as opposed to endangered species such as Humpback Chub, *Gila cypha*, with 40+-year life spans (see Douglas and Marsh, 1996). While a compressed life history interval for the Devils Hole Pupfish can be advantageous for monitoring, it also complicates protection and recovery of the species, in that very limited time is available to identify appropriate management actions, particularly in the presence of dire circumstances, such as the current one.

3. Short Term Monitoring (weeks to months):

Goal: To maintain alive the remaining individual pupfish in Devil's Hole.

Below is a list of potential limiting factors (based on information provided during the workshop), as well as mechanisms on how these might affect pupfish, and what (if any) management actions should be considered.

Possible Limiting Factor	Mechanism	Management Action
Trophic limitations	Mortality through starvation; insufficient energy for successful reproduction	Supplemental feeding (already implemented)
Global warming, temperature increase	Temperature limitations exceeded for metabolic and physiological processes	No apparent solution for pupfish in wild habitat
<u>Intrinsic</u> factors altering physico-chemical environment	Manifold effects on metabolism and physiology	No apparent solution for pupfish in wild habitat
<u>Extrinsic</u> factors altering physico-chemical environment	Manifold effects on metabolism and physiology	Identify cause, find remedies to mitigate effects

Potential Monitoring Activities:

Devi's Hole should be inspected frequently, preferably daily but at least weekly, so as to visually evaluate the environment and its resident pupfish population, with an eye towards obvious changes. Standardized digital photographs of the shelf environment could serve as an archive against which presumed changes can be judged. The same approach (i.e., frequent visitation with digital archiving) should be applied to refugial habitats and populations. Visual inspection and digital documentation are inexpensive, non-invasive methods to ascertain overall condition of Devil's Hole and associated refugia, and to detect within a reasonable time-frame any potentially catastrophic changes.

4. Mid- to Long Term Monitoring (months to years):

Goal: To stabilize and increase the pupfish population in Devil's Hole.

Possible Limiting Factor	Mechanism	Management Action
Adult survival	Mortality; failure to reproduce	Increase understanding of pupfish biology, habitat requirements, life history
Individual reproductive success	Insufficient resources for reproduction (trohic levels, spawning habitat, potential mates)	
Successful recruitment	Low survival of larvae or juveniles	

Potential Monitoring Activities:

Census and length-frequency estimates provide qualitative (rather than quantitative) data to infer adult survival, ongoing reproduction and recruitment of juveniles. Given the short live-cycle of the pupfish, census/ length-frequency estimates should be performed bi-monthly. The length-frequency data record age-structure and potential shifts in mortality

rates of specific life history stages. Experimental trials for propagation of Devils Hole Pupfish should be intensified, with initial tests first using surrogate species.

III. SPECIFIC RECOMMENDATIONS FOR DEVILS HOLE PUPFISH MONITORING

1. Increase Understanding of Devils Hole Pupfish Biology

(A) Peer-reviewed and unpublished literature on Devils Hole Pupfish and Devil's Hole

As pointed out during the work shop, much of the research conducted either on Devils Hole Pupfish or its habitat has not been published in the peer-reviewed literature.

- (1) Compile a comprehensive list of all available literature on Devils Hole Pupfish and its habitat.
- (2) If necessary, subject unpublished reports to peer-review so as to ascertain validity of reported information.
- (3) Combine available literature in a digital database that can be accessed by researchers.
- (4) Utilize experts in various fields to summarize the most important findings and crucial facts for each subject.
- (5) Identify information needs based on summarized information. Again, main focus should be increasing the biological knowledge-base of Devils Hole Pupfish, and secondarily on a better understanding of the physico-chemical environment of Devil's Hole.

(B) Surrogate Species

Information on the biology of **closely related species** of pupfish should also be gleaned.

- (1) Ask experts to compile available information on the biology of closely related species of pupfish. Focus primarily on reproductive/ trophic ecology, early life history, and population structure, demography, and dynamics.
- (2) If certain traits are shared amongst all pupfish considered, it might be prudent to assume that Devils Hole Pupfish would similarly share that particular trait.
- (3) If considerable variation occurs among different species for a given trait, then each variant should be considered as a potential template for Devils Hole Pupfish. This would be potentially invalid if specific conditions make it highly unlikely that a particular variant would apply at Devil's Hole.

- (4) Generate a matrix from these data that reflects potential attributes and traits that might describe the biology of Devils Hole Pupfish. Qualify this information by clearly identifying level of uncertainty about each trait.
- (5) Use this matrix to visualize a hypothetical ecology of Devils Hole Pupfish, listing alternatives where appropriate.
- (6) Use matrix to identify information needs and to initiate studies that fill voids in the matrix and/ or examine specific aspects of pupfish biology.

I advise against the derivation of (or heavy reliance upon) a conceptual model. While modeling tools can be effective in certain situations, they are often less accurate at predicting the manner in which an organism responds to environmental change, particularly when solid information on the basic life history traits of the species are lacking. The latter are the data needed for the model, and if indeed they are lacking (or incomplete and in need of estimation), then predictions from the model will be deficient.

(C) Environmental Parameters

Physical and chemical parameters of Devils Hole are easier to measure than are biological attributes of the resident pupfish. However, I doubt that more detailed physico-chemical measurements will elucidate why Devils Hole Pupfish have declined so drastically and how the population can be stabilized and increased in the immediate future. The salient problem (again) is that we know very little about the biology of Devils Hole Pupfish, and given this, it will be difficult to relate a change in physical or chemical parameters to an appropriate biological response. At best, one could guess. However, this is unsatisfactory and would not provide answers that could be translated into direct management actions. Simply put, correlation does not represent causation.

(D) Reproduction, Artificial Propagation and Experimental Trials

Currently, there are insufficient numbers of Devils Hole Pupfish to permit removal of individuals for experimental studies. Similarly, it would be prudent to evaluate if indeed removal for propagation and/ or establishment of new refugial populations is advisable. One concern is that a critical minimum number of individuals must be maintained in the population to sustain and encourage natural reproduction. Low-fecundity in Devils Hole Pupfish females, and potential low sperm viability of males would hamper reproductive success in both natural and artificial habitats. Reproductive success also varies among individuals or mating pairs, and because of this, only a subset of adults may in fact contribute offspring to the next generation.

To alleviate the low genetic diversity in Devils Hole Pupfish, some might consider spawning minimally-related individuals (i.e., those with lowest genetic similarities). However, this would prevent mate-choice, a pupfish behavior that has been shaped over evolutionary time to increase individual fitness. This aspect would be crucial to the long-term persistence of a population. Also, paired matings are impractical for Devils Hole Pupfish because individual marks cannot be applied, thus making it difficult to distinguish individual fish that could be associated with a particular genotype.

A hatchery-based breeding scheme for Devils Hole Pupfish is not recommended. The main focus should instead be to increase the population size of Devils Hole Pupfish (both in wild and refugial populations). Once large and stable populations are secured, a population genetic approach could then be employed to assess genetic diversity and relatedness. Here, the difficulty is that genotyping an insufficient number of individuals per population, particularly with highly variable markers such as microsatellites, will lead to serious stochastic errors. This is discussed below.

Another concern is that Devils Hole Pupfish seem unable to adequately survive their removal and transport to refugia, which in turn limits the ability to initiate new refugial populations.

(E) Trophic Ecology

Pupfish in general and Devils Hole Pupfish specifically are adapted for existence in very marginal habitats. Food availability in these systems is generally very limited, and over evolutionary time it could have led to local extinctions. A slight alteration in food supply/availability may be sufficient to push a population to the brink of extinction (or beyond). Anecdotal information hints at potential changes in trophic conditions of Devil's Hole (e.g., Owl digestive pellets and feces; allochthonous deposition into Devil's Hole curtailed by fence).

Assuming food supply is a limiting factor at Devils Hole; supplemental feeding is a good short-term management action (and indeed has already been implemented).

(F) Refugia Populations

An important question is whether refugia populations indeed represent genetically admixed populations or if observed patterns match equally well with an alternative hypothesis. Those traits that promote concerns about genetic introgression in refugial populations include (a) spontaneous growth of pelvic fins, (b) increase in aggressive behaviors, and (c) shift in allele frequencies at two highly variable microsatellite loci.

To determine if refugial populations have been introgressed by alien genes, it is important to ascertain those attributes that distinguish Devils Hole Pupfish from other pupfish species, particularly *C. nevadensis*. Genetic interrelationships among Death Valley Pupfishes have been assessed by various researchers using different approaches (see summary below). However, phylogenetic relationships are difficult to infer, due in large part to the relative recent evolutionary histories of the different forms.

IV. CONSERVATION GENETICS OF DEVILS HOLE PUPFISH

1. Genetic Diversity and Conservation

An underlying premise of conservation genetics is that elevated genetic variability will enhance the probability of a population to survive in an unpredictable environment.

This is because it supplies a population with the evolutionary potential to adapt to altered conditions (Vignieri et al., 2006). The take-home message is that the evolutionary potential of populations must be protected to preserve biodiversity.

Since biotic diversity is the product of "descent with modification," the question of "what to conserve" is often reduced to taxonomy and phylogenetics. A known taxonomy should be a prerequisite for conservation plans (Avice and Hamrick, 1996). In situations where such data were lacking, conservation efforts done with best intentions often bring more harm than good (see Avice, 1992; Moritz, 1994a). Hence, to manage biodiversity, groups with independent evolutionary histories must first be identified (Mayden and Wood 1995). These have been variously categorized as "species" (Cracraft, 1983; Frost and Hillis, 1990), "subspecies" (Avice and Ball, 1990; O'Brien and Mayr, 1991), or "evolutionary significant units" (Ryder, 1986; Dizon et al., 1992). In addition, genetically divergent populations are being increasingly recognized as appropriate for conservation, regardless of their taxonomic status (Moritz, 1994a). This approach allows diversity to be conserved before it is formally recognized hierarchically and often before a species goes extinct.

Traditionally, taxonomy was based only on phenotypic traits, and only recently have molecular methods become applicable to decipher evolutionary relationships or distinguish genetically distinct populations. It is here that conservation genetics (*sensu* Vrijenhoek et al., 1986) has a role in identifying cryptic variation towards which management action can be directed (as per Avice, 1989; Quattro et al., 1996).

2. Microsatellite DNA Loci as Molecular Markers

(A) Characteristics of Microsatellite (msat) DNA

Microsatellite loci offer several advantages over other types of molecular markers, in that the former are abundant and highly variable (Ashley & Dow 1994, Burke 1994). Loci are characterized by tandem repeats of short, specific motifs of one to six nucleotides, and are recognized as among the fastest evolving of DNA sequences (Weber and Wong 1993). They have been successfully used to investigate microgeographic divergence of fishes (Douglas *et al.*, 1999b), and were able to detect high levels of genetic differentiation when mtDNA and allozymes failed to do so (Brunner *et al.*, 1998). In addition, msat loci have become a useful tool for management of fish stocks (Bentzen *et al.*, 1996; Ruzzante *et al.*, 1997; Douglas *et al.*, 1999b).

A disadvantage to msats (Burke, 1994) is that loci have to be developed for a particular target species. This is often a time-consuming and expensive proposition. A second disadvantage is the limited and controversial application they offer for phylogenetic interpretations (Feldman *et al.*, 1997; Goodman, 1997).

(B) Msats and Sample Size

Because msat loci have rapid mutation rates and often yield large number of alleles, they are well suited for projects that examine genetic or demographic population structure. However, a large number of alleles also necessitate large sample sizes, so as to determine an accurate reflection of genotypic frequencies. Sample sizes between 30 and 50 individuals are considered necessary for precise estimation of genetic distances and this value also depends on numbers of loci, numbers of alleles, and the range in allele size (Ruzzante, 1998; Estoup *et al.*, 2000). While resampling procedures often allow larger sample sizes to be simulated from the data, they do not overcome those potential biases induced by small sample sizes. This relates to the comments (above) regarding msats and introgression within refugia populations. For example, Martin and Wilcox (2004) found limited evidence of recent bottlenecks in Ash Meadows Pupfish based upon microsatellite data, a fact they attributed to the low number of loci analyzed.

(C) Msats and Detection of Introgression

Microsatellite loci also have limited use for detecting introgressive hybridization because they are rarely diagnostic for a specific species. This is due to the very nature of microsatellite DNA that allows this marker to be highly suitable for population-level analysis, namely a high mutation rate that leads to a high number of alleles within any given population. But, microsatellite loci most often consist of multiple alleles that differ in frequency. The size-ranges of these alleles often overlap as a result and shared alleles are both common and non-diagnostic.

(D) Specifics about *Cyprinodon* msats

Several of the results reported by Martin and Wilcox (2004) are noteworthy with regard to characteristics of their microsatellite loci. For one, patterns of diversity among the six microsatellite loci in *C. diabolis* were unusual. This species was fixed (or almost so) for a single allele at each of four loci. However, the remaining two (i.e., D1 and D15) revealed considerable diversity. Three hypotheses were offered as equally valid explanations for this situation: (1) locus-specific differences in mutation rate, (2) locus-specific differences in mutational variance, and (3) locus-specific effective population sizes.

Hypothesis 1 (differences in mutation rate) could indicate that D1 and D16 are in areas of the genome subjected to diversifying selection (as opposed to purifying selection in most parts of the genome). In other words, mutations that generate new alleles are “encouraged” in these regions. This phenomenon is known from MHC loci where higher allele diversity is beneficial for immune responses against quickly evolving pathogens.

However, if indeed these loci are in fast evolving areas of the genome, then the appearance of novel alleles would not be surprising. These could have mutated towards the shorter allele size ranges considered “characteristic” for *C. nevadensis*. Selection

against excessive numbers of repeats (i.e., allele length) would force mutations towards lower numbers of repeats.

3. Genetic Interrelationships of Death Valley Pupfishes

(A) Vicariance versus Local Adaptation

Is genetic divergence among pupfish populations in Death Valley the result of natural selection (i.e., adaptation to local conditions) or does it instead reflect isolation and lack of gene flow (i.e., vicariance, population bottlenecks and founder events).

Genetic evaluations of Death Valley pupfishes based on proteins or mtDNA did not reveal substantial genetic divergence between *C. nevadensis* and *C. diabolis* (Turner, 1974; Echelle and Dowling, 1992; Duvernell and Turner, 1998). Not surprisingly, recent studies employing fast-evolving microsatellite DNA loci did report genetic divergence among Death Valley Pupfish (Martin and Wilcox, 2004). Moreover, Martin and Wilcox (2004) found population divergence to correspond with elevational but not geographic distances. They attributed this pattern to longer isolation of high elevation habitats, reasoning that these would be the first areas to become separated when the water table dropped due to a drying climate. This hypothesis would suggest that vicariance, rather than local adaptation, drives population divergence of pupfishes in Death Valley.

(B) MtDNA Analyses

An analysis of mitochondrial genome restriction sites failed to resolve phylogenetic relationships among *C. diabolis* and *C. nevadensis* subspecies (Echelle and Dowling, 1992). MtDNAs from different subspecies were no more divergent than were individuals from the same locality (0 to 2 site differences). The only exception was *C. n. mionectes*, which exhibited three to five site differences when compared to other members of the species. All estimates of intraspecific sequence divergence were less than 0.65%. Interspecific levels of sequence divergence were much greater than intraspecific values, which is to be expected. However, this fact did not hold true for *C. diabolis* versus the various subspecies of *C. nevadensis*, where 0.32-0.49% sequence divergence was found.

Examining phylogenetic relationships, Echelle and Dowling (1992) found that taxa from Ash Meadows-Death Valley formed a cluster of highly similar forms in which *C. salinius* was the most divergent (1.2-1.3% sequence divergence). However, *C. diabolis* clustered among the various subspecies of *C. nevadensis*. In the maximum parsimony analysis, *C. diabolis* formed an unresolved trichotomy with *C. nevadensis* subspecies, whereas in the strict consensus tree the mtDNAs of *C. diabolis* clustered within a clade composed of the various subspecies of *C. nevadensis* (Echelle and Dowling, 1992).

Duvernell and Turner (1998) examined mtDNA sequence variation at the ND2 gene and the control region among pupfish populations in the Ash Meadows area. Interestingly, subspecies of *C. nevadensis* were differentiated from each other as much as they were from *C. diabolis*. Each of several populations was fixed for a single haplotype,

including *C. diabolis* and *C. n. pectoralis* from Indian Springs and School Springs. *Cyprinodon n. mionectes* showed two unique haplotypes each for Point of Rocks and Big Springs populations. Albeit Duvernell and Turner (1998) did not state percent sequence divergence among specific haplotypes, overall pair-wise divergences ranged from 1-9 mutational steps, corresponding to 0.3%-2.5% sequence divergence. As depicted in dendrograms, *C. diabolis* and *C. nevadensis* differed by one or two base substitutions.

Furthermore, the phylogenetic analysis by Duvernell and Turner (1998) clustered *C. diabolis* within the *C. nevadensis* clade, rendering the latter paraphyletic. Based on Duvernell and Turner's (1998) analysis, *C. diabolis* and *C. nevadensis* are not reciprocally monophyletic, which precludes their designation as separate ESUs (sensu Moritz, 1994).

These analyses suggest that certain taxa in the Ash-Meadows-Death Valley area are of very recent evolutionary origin, in agreement with Miller's (1981) suggestion that *C. diabolis* is only 10,000-20,000 years old (or even younger). This stands in contrast with the high morphological divergence found between *C. diabolis* and other pupfishes. *Cyprinodon diabolis* was considered one of the most distinctive of all pupfishes, with small body size, lack of pelvic fins, reduced sexual dimorphism, and lack of male territorial behavior considered as characteristic traits (Miller, 1948; Liu, 1969; Soltz and Naiman, 1978).

(C) Microsatellite DNA Analyses

Martin and Wilcox (2004) examined variation across six microsatellite loci among nine *Cyprinodon* populations from Ash Meadows (NV) area. Pairwise *F_{st}* (and *R_{st}*) values among the nine populations did not consistently reflect higher genetic similarity among populations assigned to the same subspecies (Martin and Wilcox, 2004). For example, the population from Marsh (recognized as *C. m. pectoralis*) when compared with one of the four *C. n. mionectes* populations showed lower pairwise *F_{st}* values than it did when compared to other *C. m. pectoralis* populations. This underscores that *F_{st}* and *R_{st}* values have limited use in determining taxonomic distinctiveness.

Martin and Wilcox (2004) found population divergence to correspond with elevational but not geographic distance. They attributed this pattern to longer isolation of high elevation habitats, reasoning that these would be the areas first to become separated as the water table dropped as the climate become drier (i.e., vicariance driving population divergence). Genetic distance was negatively correlated with linear geographic distance which seems to contradict an isolation-by-distance model. However, the four mid- and one high-elevation habitat (the latter being Devil's Hole) are all in close geographic proximity, whereas the low elevation habitats are scattered. Interestingly, two distinct subspecies of *C. nevadensis* are identified, one (*C. n. mionectes*) being associated with the low elevation springs, whereas the other (*C. n. pectoralis*) is recognized in the mid-elevation springs. Distribution of *C. n. mionectes* effectively straddles the range of *C. n. pectoralis*.

4. Refugia Populations

(A) Differentiation between Refugia and Wild Population

Wilcox and Martin (2006:319) conclude that “The observed differences for behavioral and morphological characters may signal the effects of genetic drift and selection acting on heritable variation OR may reflect phenotypic plasticity, or both.” They then ask whether this reflects divergent evolution or ecophenotypic variation (2006:319). They even consider the possibility that the Devils Hole population and the two refugia populations (i.e., School Springs and Point of Rock) could be incipient species, in that they are “reproductively isolated and inhabit distinct environments with unique selection regimes”. In my opinion, this is too extreme of a viewpoint. If one followed this logic, most populations in captivity or under artificial propagation would be considered as “incipient species.”

(B) Invasion of Refugia by *C. nevadensis*

How could *C. nevadensis* have invaded the Points of Rocks refuge in the first place? Several hypotheses can be invoked, including “small fish navigating the outflow and passing through the gravel filter into the pool” (Martin, 2005), or transfer of *C. nevadensis* individuals from Ash Meadows to Points of Rocks refuge, either by accident or intentionally (Martin, 2005). These all seem rather *ad hoc*.

(C) Genetic Patterns and Interpretation

Some alleles found in microsatellite loci D16 and D1 from the 2005 Point of Rocks sample were not detected in the 1998 sample. Does the presence of these alleles indeed suggest hybridization (i.e., are the alleles diagnostic for *C. nevadensis*?).

For locus D16, the answer is ‘no.’ Allele 400 (the alleged diagnostic allele for *C. nevadensis* at this locus) was also detected in *C. diabolis* in the 1998 analysis (Martin and Wilcox, 2004). Thus, it is shared between two species and thus cannot be considered diagnostic for either.

The situation is a little more complicated for locus D1, simply because of its much larger allele size range and greater number of alleles, often at low frequencies. Three of the four “*C. nevadensis*” alleles were not detected in the 1998 sample of *C. diabolis*. However, allele 278 was at a high frequency in the 1998 sample. One of the novel alleles of the 1998 sample (i.e., allele 282) differs by one repeat motif (= 4 base pairs) from allele 278, and by three (= 12 base pairs) from the next shortest (allele 294).

In the 1998 sample, there are four locations where neighboring alleles differ by two motifs (= 8 base pairs). However, there are gaps between adjacent alleles in the 2005 sample of two, three or eight motifs (8-28 base pairs). The mutations in locus D1 do not appear stepwise (i.e., 4 base pairs on a regular basis). Instead, any number of motifs may seemingly be added or deleted during DNA replication. Thus, an alternative hypothesis to

explain the “jumps” in allele size ranges from the 1998 vs. 2005 Point of Rocks samples is that locus D1 does not mutate in a step-wise fashion.

5. Phenotypic Plasticity and Local Adaptation

(A) Ecophenotypes or Ecotypes

Phenotypically distinct forms in different environments can either represent ecophenotypes or ecotypes (defined in Douglas et al., 1999a). Ecophenotypes are generally considered different forms of a single species that emerge in response to a particular habitat, and thus represents phenotypic plasticity. In contrast, ecotypes represent genetically distinct populations that have diverged from other populations due to selective pressures of the environment. They thus represent evolutionarily independent lineages that are adapted to local conditions.

In commercially important salmonids, distinct forms were often considered ecophenotypes (Douglas and Brunner, 2002), whereas similar variants in non-game fishes (including pupfish) have been described as distinct taxa and granted specific or sub-specific status based on morphological characteristics. Interestingly, Wales (1930) considered the possibility that *C. diabolis* could actually represent an ecophenotype of *C. nevadensis*, rather than a distinct taxon.

(B) Translocations and Local Adaptation versus Phenotypic Plasticity

Populations of imperiled species translocated into new habitats or artificial refugia often exhibit changes in morphology and behavior. Underlying mechanisms for these phenotypic shifts are not well understood. Novel selective pressures induced by environmental differences between natural and artificial habitats are sometimes invoked to explain the alterations. Selection would alter genotype frequencies in the population and may favor phenotypes that are poorly suited to survive in the wild. For conservation efforts, where the ultimate goal is to reestablish the species in its natural environment, such evolutionary changes could be detrimental to recovery. However, these observed shifts in traits could also simply be due to phenotypic plasticity within the species, and thus would not reflect underlying genetic changes.

(C) Phenotypic Plasticity in *Cyprinodon*

Rapid shifts in phenotypic characteristics have been observed in Devils Hole pupfish, with refugial populations developing characteristics generally recognized in adult specimens of closely related pupfish. Compared to other pupfishes, Devils Hole Pupfish exhibit a paedomorphic phenotype characterized by small body size, proportionally large head and eyes, absence of pelvic fins and vertical stripes in mature males (Wales 1930, Miller 1948). These are juvenile traits not observed in sexually mature individuals of other pupfish species.

Martin and Wilcox (2006) emphasize that to better understand mechanisms underlying these shifts, experiments would be needed to examine explicitly the relative importance of genes versus plasticity. In other words, a ‘common garden’ experiment was suggested, where Devils Hole Pupfish would be exposed to conditions prevalent in various *C. nevadensis* habitats, and *C. nevadensis* to conditions characteristic for Devil’s Hole. The latter experiment is exactly what Lema and Nevitt (2006) performed (see below). Furthermore, the refugial populations do approximate a common garden experiment where Devils Hole Pupfish are raised under habitat conditions (i.e., more food, lower temperatures) prevalent for *C. nevadensis*.

(D) Experiments by Lema and Nevitt

Lema and Nevitt (2006) examined how pupfish morphology responds developmentally to variation in environmental conditions. They employed a surrogate species, the Amargosa River Pupfish (*C. nevadensis amargosae*) and tested if phenotypic traits characteristic to Devils Hole Pupfish could be experimentally induced by regulating food rations. In laboratory trials, they limited food availability to slow the growth rate in Amargosa River Pupfish so as to approximate that observed in Devils Hole Pupfish. Amargosa River Pupfish on a restricted diet did indeed develop the paedomorphic phenotypes characteristic for Devils Hole Pupfish. In a second set of trials, Lema and Nevitt (2006) showed that depression of thyroid hormone levels can induce similar phenotypic shifts. An unintentional outcome of this study was that slight temperature differences among replicates produced significant morphological variation in developing pupfish.

With these experiments Lema and Nevitt (2006) provided a mechanistic model that described the manner in which the environmental parameters that distinguish Devil’s Hole (i.e., low productivity and elevated temperatures that reduce thyroid hormone production during early development) induce the paedomorphic phenotype characteristic for Devils Hole Pupfish.

These studies strongly support the hypothesis that phenotypic differences among wild and refugia Devils Hole Pupfish are due to phenotypic plasticity, and not to natural selection.

6. Artificial Propagation and Genetic Management

(A) Supplementation

The ability of supplementation to mitigate declines or extirpation of natural populations is itself controversial (Schramm and Piper, 1995, and references therein). Supplementation as a management tool cannot be a viable alternative to the difficult task of solving ecosystem disturbances (Bowles, 1995). Efforts to improve natural habitats should instead be foremost. In addition, recovery efforts are ill-fated if genetic diversity is not sustained. Supplementation has a great potential to inflict adverse genetic or ecological effects on natural populations (Busack and Currens, 1995; Leary et al., 1995;

Waples and Do, 1994). Further, each aquatic system and each component population is unique, thus requiring tailored experiments and operating practices to evaluate both. Accordingly, expectations of supplementation range from a panacea for solving population declines to a curse that will accelerate declines and seal the fate of imperiled populations (Bowles, 1995).

(B) Genetic Management of Pupfish

Wilcox and Martin (2006) stressed that populations under artificial propagation should consist of enormous numbers of individuals so as to minimize loss of genetic diversity through drift. Founder effects can be limited by (a) establishing a refugium population with sufficiently large numbers of individuals, and (b) regularly transferring individuals from the wild population to the refugium, particularly during early stages of population establishment (Wilcox and Martin, 2006).

I strongly support these recommendations, particularly the argument that large populations are necessary to sustain genetic diversity within the population.

(C) Suitability of Refugia Pupfish for Supplementation

Lema and Nevitt (2006) pointed out that fish raised in refugia might express higher metabolic demands (food and oxygen consumption rates) and thus would not be suitable for release into the energetically challenging environment of Devil's Hole. They suggested instead that refugial rearing conditions should mimic the Devils Hole environment.

However, it appears that Devil's Hole represents an environment that pushes pupfish to their physiological limits (i.e., minimal food, temperatures almost too elevated for successful reproduction), and these conditions will severely hamper successful propagation of Devils Hole Pupfish. Since environmental conditions modulate developmental parameters, one option would be to supplement the Devils Hole population with early life history stages of refugial fish. This could be tested as a viable option by raising a subset of larvae under limiting conditions (of course, sufficient numbers of refugial individuals must first be produced).

I do not think that mimicking environmental conditions of Devils Hole (i.e., low productivity, high temperatures) will successfully increase artificial propagation of Devils Hole Pupfish because these are the very factors that apparently limit survival and reproductive success in Devil's Hole. **Thus I disagree with the suggestion that Devils Hole Pupfish be raised under restricted conditions.**

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4. DR. DAVID HANKIN

Thoughts on Pupfish Workshop: Dave Hankin, 20 March 2007

With the abundance of Devils Hole pupfish down to something under 40 individuals, it is impossible for me to imagine that long-term monitoring will play any critical role in the possible recovery of the species. If, however, good fortune or blind luck causes the pupfish population to rebound (e.g., if current supplemental feeding substantially improves conditions for survival), then long-term monitoring might prove to be a valuable tool for assessing the causes for fluctuations in abundance of DH pupfish. Almost all of the discussions of long-term monitoring were devoted to tracking changes in the physical/chemical environment faced by the pupfish and/or to the biological environment (temporal and interannual fluctuations in abundance of prey items and in energy production or inputs). Others on the workshop panel are far more qualified than I to judge the merits of various monitoring protocols, so I instead comment below on those areas of importance where I feel I may have some contribution of my own to make.

Priority I – Immediately explore the possibility, even if remote, that Point of Rocks fish are NOT Hybrids.

At the August 2006 meeting, after (1) reading Andy Martin's report on the apparent hybridization between DH and Ash Meadows pupfish that apparently took place at Point of Rocks Refuge, after (2) talking with Andy Martin about this topic, after (3) seeing vigorous presumptive hybrids being reared at Shark Reef Aquarium and at Willow Beach Fish Hatchery, and after (4) talking with the Shark Reef manager, staff at Willow Beach, and Arcadia, I was firmly convinced that the individuals currently reared at Shark Reef and Willow Bank are indeed hybrids. At the March 2007 meeting, however, after I showed Andy Martin's report to Doug Markle, both Doug and Marlis expressed skepticism that there was, in fact, compelling evidence of hybridization.

First, the presence of pelvic fins, used by Andy's student as presumptive evidence of hybridization, is not a definitive indicator of hybridization because, as noted at the March workshop, the character is plastic and apparently is more likely to be expressed under favorable rather than stressed conditions. I have no firsthand knowledge of this, but had assumed (incorrectly, I guess) that the character was fixed (absent) in DH pupfish whereas it was generally present in Ash Meadows pupfish and in the presumptive hybrids. Also, it took me quite a while to even "find" the pelvic fins on the presumptive hybrids at Willow Beach. They are laughably small fins, probably of little functional use in any event.

Second, the microsatellite allele frequencies at the two variable loci examined by Andy Martin exhibit some very strange patterns. Although collected data are certainly consistent with an assertion that the allele frequencies of the individuals present in Point of Refuge were statistically different from those of pure DH pupfish, the analysis of collected data was preliminary and a colleague (phylogeneticist) at my own institution tells me that the Rst measure is not universally accepted, and it is clear to me that

substantial differences between DH and Point of Refuge might develop within a relatively few generations just due to (a) founder effects (a very small population originally used to start the refuge population), (b) differences in selection regimes between the two environments, and (c) further divergence that might be caused by differential reproductive success of individuals present in the refuge.

Thus, there is a possibility, perhaps not entirely remote, that the presumptive hybrids now present at Willow Beach and Shark Reef are not hybrids but are instead pure DH pupfish. This possibility needs to be followed up immediately. I recommend that Eric Anderson, NMFS SWFSC in Santa Cruz (eric.anderson@noaa.gov) be contacted ASAP and asked to provide his initial impression of Andy Martin's earlier report. Eric is a young genius or near genius with special expertise in hybridization and its diagnosis. At the same time, Andy Martin should be consulted to see if he has ever carried through with any more sophisticated analyses of collected data. Again, my colleague had a few suggestions, but Andy is probably well aware of the kinds of things that might be done.

If the presumptive hybrids are, in fact, just "exceptionally vigorous" pure DH pupfish, then they may be at least as valuable as the fish remaining in the Hole. Until this is settled, they should be treated as if they are pure DH pupfish and attempts should be made to increase their total population size far beyond the current numbers and to do so as rapidly as possible. If they prove to be hybrids in fact, which is what I personally suspect given their greatly superior performance in a lab setting and given their much greater aggressiveness, then they should be used immediately to create second generation backcrosses with DH pupfish, to begin a process of potential reintroduction of, say, 15/16 DH pupfish back into the Hole.

Priority II – Mine existing count data and explore new methods for generating index values for assessment of trends in abundance

No future long-term monitoring effort will generate substantial insight into those environmental factors that lead to poor (or good) pupfish reproduction, larval survival, recruitment, or juvenile or adult survival, unless the basic life history of the DH pupfish is described in a more analytical manner that submits to modeling and statistical analysis. I guess that I must be dense, because after having sat through two DH pupfish workshops, I remain very confused regarding the annual cycle of reproduction, larval life history, growth and maturation. I believe that several key steps could be taken to improve this situation, perhaps quite rapidly:

1. Compile ALL of the existing length frequency data into data files that could be shared and analyzed by others, and develop a brief report consisting of length frequency histograms for individual years, presented vertically, with the same x axis limits. That is, suppose that length frequencies are available for March, June, October and December in a particular year. On one graph, place the histogram of March on the top of the page, June immediately below, October below that, and December at the bottom, with the same range of length covered on both axes. (I

suppose this would actually just be three or four “categories” of size, but I guess that’s all we have.)

2. Explore more defensible methods for expressing the fish counts made in the Hole, especially those made using scuba. The methods vaguely described for working up the scuba dive count data collected by two divers did not make good sense to me. Without any investment of time to think this through, I think that the best count for assessment of trends would be to take the average total count by each diver and to add to this the “surface” count. Revised data could then be plotted to see if they showed that same apparent pattern of decline beginning circa 1995. Personally, I did not find that date to be well-defined and my hunch would be that a different manipulation of the count data would generate different conclusions regarding when the most recent long-term decline began.
3. Summarize whatever data are available concerning “larval” counts, including a summary of protocols used for these counts.
4. Consider supporting a good biometrician/fishery scientist to make some sense out of the information collected in items 1-3.

Priority III – Identification of “Human Factors” possibly linked with decline, assuming that it began circa 1995.

I was alarmed by the scuba talk in which it was stated that all dive gear was chlorinated prior to use in the Hole. Although that would certainly be a good way to prevent introduction of exotic pests, I’d like to know how the gear is rinsed before divers enter the Hole. Introduction of chlorinated gear can’t possibly be a good idea.

I’m sure there are many other similar issues that are worthy of scrutiny.

5. MR. ROBERT HOFFMAN

COMMENTS AND RECOMMENDATIONS

I appreciate the opportunity to participate in the Devils Holes Monitoring Workshop and Program Review, March 13–15, 2007. The focal species (Devils Hole Pupfish, *Cyprinodon diabolis*) of this review is at a critical stage in its survival in Devils Hole. I commend the National Park Service and staff at Death Valley National Park for their concern for and response to the present situation at Devils Hole.

The Devils Hole Pupfish was first described as a species in 1930 by JH Wales. The water quality and biotic community of Devils Hole and the ecology of the Devils Hole Pupfish have been studied on an on-going basis since the species was listed as endangered in 1967. However, these research and monitoring activities have been somewhat temporally disjunct and of varying intensity. The workshop and review presentations of past investigations were thorough and detailed although it became apparent to me that much of the information has been summarized primarily relative to time and sampling dates (which I think contributes to the apparent fragmentation of results) and has not been systematically synthesized. The following items summarize my primary concerns and recommendations.

1. In the short-term, the park and NPS should focus on recovery of the Devils Hole Pupfish population. All unnecessary activities in Devils Hole should be discontinued until population numbers increase sufficiently to indicate population recovery. In this context, census counts should be continued especially in spring and summer. The park and NPS might also consider ecosystem enhancements that promote and support successful Devils Hole Pupfish reproduction and recruitment (such as installation of artificial lights to increase productivity; installation of a relatively shallow artificial shelf to provide a secondary site for reproduction and rearing; continued feeding of the present pupfish population).
2. All available data from previous Devils Hole investigations need to be gathered and entered into a single primary Devils Hole Research and Monitoring Database. Once this database is completed, the data should be re-analyzed to provide a synthesis of long term trends in the physical, chemical, and biological characteristics of Devils Hole, and for the identification of potential correlative relationships between ecosystem parameters. Metadata should be completed once the primary database has been constructed.
3. All questions concerning the status and trends of the water quality and biotic community of Devils Hole, and factors that potentially contribute to the continued persistence and health of the Devils Hole Pupfish should be archived into a second database. This database will serve to better organize ongoing concerns, research, and investigations concerning Devils Hole and the Devils Hole Pupfish; and will act as an active checklist of questions that have or have not been explored or resolved. Such a database can help to end the circular and somewhat chaotic nature of repeatedly asking the same questions without documenting their resolution or lack of resolution.

4. A better understanding of the food and rearing requirements of larval Devils Hole Pupfish is needed. This is an extremely important link in the connection between Devils Hole Pupfish survival, life history requirements, and ecosystem dynamics, especially productivity. I encourage park resource managers to focus on this aspect of the Devils Hole Pupfish life stage after the population is stabilized and has moved into recovery. Perhaps in the interim what is known about the early life history of other pupfish species could help with generally conceptualizing the early life history of Devils Hole Pupfish.
5. Once the Devils Hole Pupfish population is stabilized and showing signs of recovery, a long term monitoring plan should be initiated. A plan that was presented by Dean Blinn at the workshop appears to contain all of the necessary elements for successfully monitoring the conditions in Devils Hole. These elements include:
 - A. Weather (air temperature, precipitation, barometric pressure, wind speed and direction);
 - B. Shelf substrate composition;
 - C. Water level and temperature;
 - D. Solar energy input;
 - E. Physicochemical parameters (Dissolved Oxygen, percent saturation, pH, H₂S, specific conductance)
 - F. Nitrogen and phosphorus;
 - G. Biotic parameters (algae, allochthonous carbon input, microbial community, benthic macroinvertebrates, larval and adult pupfish densities).
6. I recommend that the Devils Hole biotic community (especially algae and benthic macroinvertebrates invertebrates) be sampled per the methods described in Blinn, D.W., K.P. Wilson, and D.B. Herbst, 2000, Devils Hole energetics/community relationships: Death Valley National Park, California, Annual Report 6 December 2000, Project No. 7530, 37 pages.
7. Park resource managers should continue to develop a detailed conceptual model for the Devils Hole ecosystem, perhaps using the model developed by Yuri Graves and Krystyna Stave (Department of Environmental Studies, University of Nevada Las Vegas).
8. Apparent disagreement concerning the genetic status of pure Devils Hole Pupfish and so-called hybrids at various refuge sites needs to be resolved. See the comments of other panel members concerning this issue.
9. Finally, I recommend that all activities to be considered as part of any long term monitoring or short term research plan be evaluated for their potential physical impacts on the Devils Hole ecosystem, especially the Devils Hole Pupfish population in general and larvae specifically.

6. DR. STANFORD LOEB

March 21, 2007

Dr. Gary Larson, Organizer
Devil's Hole Monitoring Workshop
USGS Forest and Rangeland Ecosystem Science Center
3200 SW Jefferson Way
Corvallis, Oregon 97331

RE: Thoughts about long-term monitoring at Devil's Hole.

Dear Gary,

The following is a summary of my thoughts concerning the long-term monitoring of the Devils Hole environment/ecosystem. The situation at the Devil's Hole is primarily focused on the unique species of pup fish (*Cyprinodon diabolis*) that is found only at this location. Decisions about what actions need to be taken to insure the survival of this unique species form the justification for the long-term monitoring plan.

There are six areas of interest associated with the long-term monitoring of Devil's Hole and the survival of the pup fish (*Cyprinodon diabolis*). These include the following: (1) the access area for the public; (2) the amount and type of equipment that should be within/near the aquatic system; (3) the important parameters to measure continuously to assist in understanding the population dynamics of the pup fish; (4) a full review, collection and synthesis of information collected to date associated with the Devil's Hole system; (5) improved life history of the Devil's Hole pup fish; and (6) further genetic analyses of *Cyprinodon diabolis* and other related pup fish in the region.

1. Public Access to Devils Hole

As part of the National Park System, Devil's Hole should be accessible to the public to some extent. However, the need to protect the aquatic system and the pup fish that resides within it is paramount. I strongly believe that the fence be moved further away from the aquatic system at least another 25 meters. Should the decision be made to include a video webcam monitoring device at the site to assist in monitoring the system, a link could be established outside the fence for the public to view (a still camera webcam could also be used like the one used for Old Faithful in Yellowstone National Park). Currently, the close proximity of the fence to the aquatic system poses a potential vulnerability to the pup fish should someone throw something into the water or drop material on the ground that might be washed into the aquatic system.

2. Equipment in the Near Vicinity of Devils Hole

There has been in the past a great deal of equipment within and around the aquatic system of Devils Hole. Optimally, the less equipment within the water and in the near

vicinity of the water would be recommended. The one piece of equipment that might be necessary to maintain within the aquatic system would be the water level measuring device (already in place). While there are techniques other than a stilling well to measure the water level, the continuity in the data collection by having one methodology may supersede alternative methods. This continuity in methodology may also have important legal aspects associated with it; however, I am not qualified to evaluate that aspect.

3. Parameters to be Measured as Part of a Long-Term Monitoring Plan

The specific parameters that I would recommend to be collected as part of a long-term monitoring program include physical (e.g., climate and water level in Devil's Hole), chemical (e.g., wet and dry atmospheric deposition and water within Devil's Hole), and biological (e.g., pup fish population and periphyton community composition) elements. Many of these elements were identified by Dean Blinn in his presentation at the meeting. In this summary of my thoughts, I will emphasize those elements that I believe are critical to the underlying purpose of a long-term monitoring plan for the Devil's Hole ecosystem. I feel these important parameters would include the following:

1. The population size of the Devils Hole pup fish measured at least twice a year to estimate the maximum and minimum population numbers.
2. Continuous measurement of the water level of the aquatic system.
3. The composition of the periphyton community at different times of year.
4. A webcam system to better understand the frequency and intensity of disturbances to the ecosystem (e.g., flooding and subsequent sediment input).

4. Collation of All Information Collected to Date Concerning Devil's Hole

All past studies, published and unpublished reports, maintenance activities logs, dive records and changes in dive protocols (with dates of any changes) and all other information pertaining to Devil's Hole and the general region need to be assembled and continuously updated. There is a tremendous amount of information that needs to be collected and, once collected, reviewed and synthesized.

For example, during the meeting, Zane Marshall presented a review of the SCUBA work used to determine the population size of the Devils Hole pup fish. During that presentation, he mentioned that size frequency data was collected during these efforts. Unfortunately, no one seems to have reported or analyzed these data which would be very valuable to help understand the life history of these fish.

A second example illustrates how having all the data collected into one "library" is essential. During the meeting both James Deacon and Michael Parker presented information and graphs concerning how the population size of the Devil's Hole pup fish has varied over time. In Deacon's presentation, his graph showed the maximum and minimum population sizes for the fish population from 1972 through 2002 (Figure 1).

Annual max/min Population Size 1972-2002

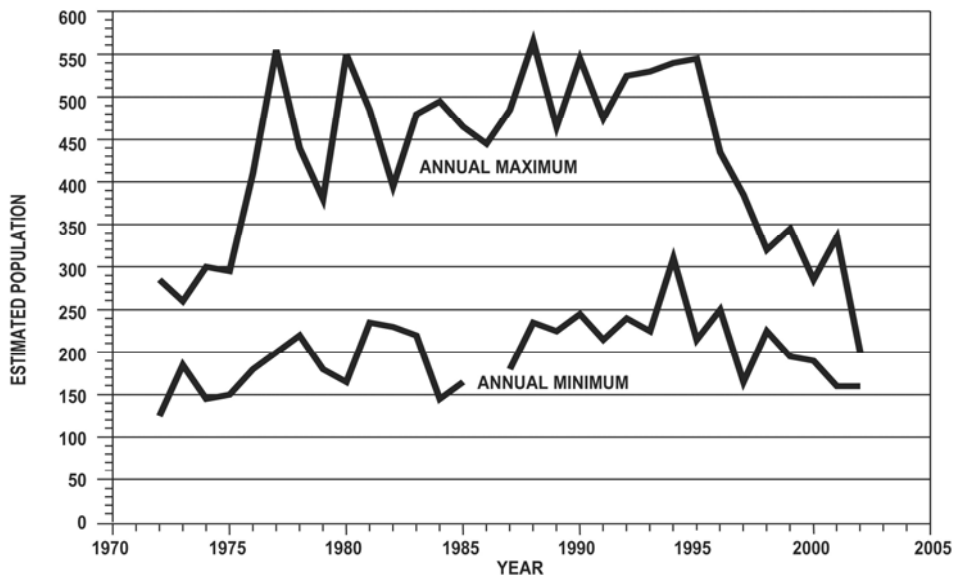


Figure 1. Annual population size (maximum and minimum) of the Devil's Hole pup fish (1972-2002) (From Deacon 2007 presentation).

Parker's presentation also presented a summary of the population size between 1994 and 2006 (Figure 2). What is difficult to understand is that the correlation analysis done by Parker indicates a strong correlation between time (year) and declining population size.

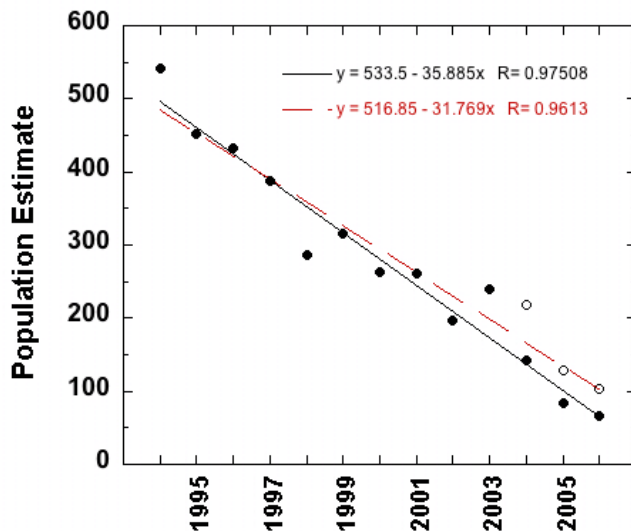


Figure 2. Population estimate for the Devil's Hole pup fish (1994-2006) (From Parker 2007 presentation).

These two graphs do not use the same values for (1) fish population size (estimates) or (2) time. Parker's population numbers appear to be average annual values while Deacon presents both annual maximum and minimum population numbers. Furthermore, Parker uses a shorter time series (13 years) compared to Deacon (31 years). Parker's analysis appears to lead to the conclusion that since 1994 to 2006, the pup fish population has been steadily declining. However, when I analyzed the minimum population size of the pup fish over 31 years (1972-2002), the minimum number of pup fish is very weakly correlated with time ($R = 0.298$) and the slope of the line is positive (+1.39). This analysis would indicate that the minimum population size has been fairly constant (mean = 198.6) for the past 31 years and slightly increasing.

What is important is that it is difficult to determine when the fish population appeared to begin to drop dramatically. At first glance, it would appear that the population began to drop dramatically in 1995. However, that may not be the case. It may be that the perturbations to the Devils Hole ecosystem that resulted from the heavy precipitation event in 2004 had a serious impact on the minimum population size of the pup fish. Although Deacon's data on minimum population size between 1994 and 2002 demonstrate a decreasing size (slope = -14.75), his 31 year record appears to demonstrate the minimum population size has undergone significant increases and decreases but has not significantly changed during that period. Also, the correlation between the minimum population size and time using Deacon's 1994-2002 data is weaker than that found by Parker ($R = -0.763$ compared to $R = 0.975$).

Not having all the data to conduct a thorough analysis of the population size over time is a clear demonstration of the critical need to have a repository for all relevant information concerning this ecosystem. It is impossible to understand what is going on with the fish population and therefore impossible to make an informed decision as to how to assist in this fish's survival.

5. Better Understanding of the Devils Hole Pup Fish Life History

In order to provide conditions or maintain conditions that are favorable to the continued survival of the Devils Hole pup fish, a much better understanding of its life history is required. At the present time, it will be difficult to do any manipulations with the existing population due to its unusual small size. In lieu of using the Devil's Hole species, other populations of pup fish in the region (e.g., King's Pool) should be examined in regards to their life history characteristics. While the life history of these other species of pup fish may not be identical to that of the Devils Hole species, there should be sufficient similarities to assist in better understanding many aspects of the Devil's Hole life history (e.g., spawning time, spawning habitat preferences, life span and shifts in dietary preferences during their life span).

Current efforts to propagate the Devils Hole pup fish outside their resident aquatic system have not been successful. In order to possibly achieve that ability, a clear understanding of the fish's life history is essential.

6. Further Genetic Analyses of *Cyprinodon diabolis* and Other Related Pup Fish

I am not a geneticist. This point does not preclude me from realizing the critical need to have a much better understanding of the genetics of this endangered species, *Cypinodon diabolis*. Earlier attempts to develop satellite populations of the Devils Hole pup fish have encountered problems believed to be associated with hybridization. While hybridization may have occurred, there is some speculation as to the plasticity of this species' physical morphology and behavioral traits. A clear understanding of the genetic identity and the diversity this species naturally has at various genetic loci is essential to identifying the occurrence of hybridization.

As part of the effort to better understand the genetics of the Devils Hole pup fish, additional genetic analyses should be conducted on the other pup fish in this region. The relationship between these fish in this region would assist in determining many interesting aspects about these populations. These aspects include the timing of when these populations arrived at their different habitats, the genetic diversity among these populations and the relatedness of the different populations.

I am happy to have been able to participate in this workshop. If you have any questions concerning the comments I have made above, I would be happy to discuss them further with you.

Respectfully,

Stanford L. Loeb

7. DR. DOUGLAS MARKLE

Devils Hole Monitoring Workshop, 13-15 March 2007; Summary remarks – Douglas F. Markle, 25 June 2007

The management need for monitoring a population is to understand the factors driving changes in population numbers. The classic ecological factors are weather or climate, food, predators and disease. These factors interact with the population's demographic structure to influence development, growth, reproduction, and death. The actual monitoring strategies and protocols depend on prior knowledge of the species and system, costs and logistics. Prior to, and during, the Monitoring Workshop, the Panel was given information about the species and system. The following remarks summarize my concerns and my views on approaches to structure monitoring around key life history processes.

The species

Differences in average mitochondrial DNA between Ash meadows pupfish and Devils Hole pupfish (DHP) suggest isolation up to 0.5 million year ago, but this is probably an overestimate since there appear to be shared alleles from the common ancestor (Echelle et al 2005 Copeia 2005(2):320-339). These authors suggest that previous estimates of 10,000 – 20,000 years are not unreasonable and that the best estimate is “anytime from the last few thousand years to about 500,000 years”.

The DHP is a food-limited dwarf that survives at or near physiological limits. Skeletal and otolith deformities have been noted. When removed from the system, DHP, if they survive, typically grows larger, may grow pelvic fins or buds, and becomes more aggressive. This apparent plasticity is not unusual. Populations of Amargosa pupfish differ in aggressiveness with those reared under a fluctuating temperature regime showing higher aggression than fish reared under a stable temperature regime. Temperature, salinity and genetics also appear interact to affect aggressive behavior and neural development (Lema 2006. *Hormones and Behavior* 50:183-193).

We were presented with an analysis that implied hybridization in the Point of Rocks refuge population. The refuge population was sampled in 1998 and 2005 and both DHP and Ash Meadows pupfish (both considered nominally “pure”) were also sampled. The refuge population could be expected to lose alleles either because of sampling artifact (an allele present in both years was sampled in 1998 but missed in 2005) or selection and drift (an allele present in 1998 was lost from the refuge population because the bearer(s) of the allele left no progeny with the allele). The refuge population could also be expected to gain alleles either because of sampling artifact (an allele present in both years was missed in 1998 but sampled in 2005) or hybridization (new alleles are added from the new parent species). Sorting out these possibilities requires at least two loci and we were shown data for two microsatellites loci.

One microsatellite locus (D16) had 6 alleles in 1998 and 5 in 2005 – all alleles were either DHP-only alleles or shared alleles (this is slide 19 in the Dratch Powerpoint presentation and is mislabeled “D1”). The 2005 sample had 2 missing alleles and one new DHP allele, so all of the changes were due to sampling artifact, selection or drift. The other locus (D1) was more diverse, had 12 alleles in 1998 and 9 in 2005 with 7 lost and 4 new “Ash Meadows” alleles found in 2005. Additions could be hybridization or sampling artifact (if the 4 “Ash Meadows” alleles were really shared alleles and not unique Ash Meadows alleles) and losses due to sampling artifact, selection or drift. The hybridization hypothesis was reinforced with the story that, on an unspecified date, a technician saw a pupfish of unknown sex and size outside the Point of Rocks refuge and, thinking it had escaped, returned it to the refuge. Such a fish could, at most, contain 2 of the 4 needed “Ash Meadows” alleles. Since it is highly unlikely that one locus could show hybridization and another not, and since we were not presented information on genetic linkages or covariance with morphology, the simplest explanation for these two loci is that the changes showed sampling artifact, selection or drift. Hybridization is certainly still a possibility but these data require some rather complex assumptions to reach that conclusion. Under the circumstances, less equivocal evidence should be required before accepting that conclusion.

Fish from the Point of Rocks refuge were removed from the drainage basin and transplanted to Willow Beach National Fish Hatchery and Shark Reef Aquarium at Mandalay Bay Casino.

Refuge recommendations.

If a refuge protocol and strategy exists, it needs to acknowledge that all refuge fish are likely to change quickly. Even if genetically identical to DHP, a fish one generation removed from Devils Hole could be larger and more aggressive if it had more food or different environmental conditions. Even if reintroduced to DH after one generation, such a fish could be competitively superior and dominate progeny production, although its progeny would likely revert to the typical DHP. Reintroduction strategies, other than an extirpation reintroduction, need to be articulated based on refuge location (in or out of basin), generations removed from DH, and sex (females may be preferable to males).

All refuge populations should be considered to represent Devils Hole pupfish, monitored (see below), and ranked for the purpose of reintroduction. Ranking criteria should minimally consider generations removed from DH and basin location.

Removal of fish from the drainage basin should be considered an extreme measure requiring extreme quarantine measures, including water treatment sufficient to prevent inoculation by pathogens.

Every living fish, including known or suspected hybrids, with an ancestry to DH, and that has been removed from DH, should be trackable, its security known and demonstrable, and its potential exposure to viral and bacterial pathogens and parasites documented.

All mortalities should be saved for monitoring analyses.

Morphological, behavioral and genetic changes in refuge fish should be better understood. There should be well-thought out contingencies made prior to management decisions.

Demographic monitoring recommendations.

All DHP populations need to be monitored for egg and larval production, growth, age-specific mortality, longevity, deformities, behavior and morphology. As demonstrated to the Panel, some of these metrics will be difficult to obtain in some locations, notably Devils Hole. Prior to setting up any demographic monitoring protocol, a thorough synthesis of all available data is needed. Such a synthesis should include information from other Death Valley pupfishes, especially Ash Meadows, as well as all of the published and unpublished data on DHP. This synthesis must be sensitive to the needs of an endangered species monitoring program and to the dynamics of fish life histories. Life history monitoring must include the interplay of age and growth on interpretation of “fish counts”. For example, any count of a life history stage must include the age or size range being counted and any known or suspected bias in those counts due to availability or “catchability” of the method. For example, if a larval count effectively samples fish from birth to age 4 weeks, frequent (< 4 week intervals) counts will double-count some fish. The monitoring must also account for behavioral differences in availability to the counting method. For example, the maximum population estimate (PopmaxD1 and PomaxD2) from the “master data base for Devil's Hole pupfish population counts”, shows the first count exceeds the second count by an average of 110 fish over the period of record from April 1972 to September 2006. Further this difference has changed over time. When the population was low in the 1970's and recently, there is

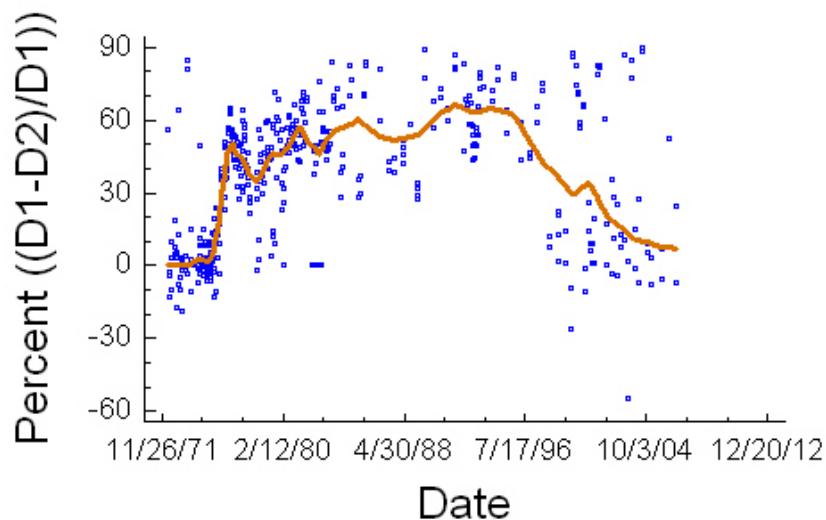


Figure 1. Percent difference between first (PopmaxD1) and second (popmaxD2) surface plus dive count estimates. Red line is Lowess regression with a 10% spread (N=440).

little difference in average counts (Fig. 1), though there is greater variance in the percent difference. This suggests density-dependent behavior, such that individuals are less available to the method on the second dive at high densities. They are presumably hiding. Why don't fish hide when densities are low and, if they hide, why do they hide on some surveys but not others (the greater variance at low densities)? Changes in availability to the method, such as hiding, could also be seasonal and should be better understood, and at least modeled with error estimates. The absolute differences show similar trends. The initial rise in both metrics occurred in 1974-75 and the recent decline began in 1995-1996.

It is curious to note that the North Pacific Ocean shifted from a cool to a warm regime in 1976 and from a warm to a cool regime in 1998 (Fig. 2). The approximate coincidence of changes in a major weather forcing function and DHP has to be tempered with known habitat changes such as the cessation of groundwater pumping in 1976.

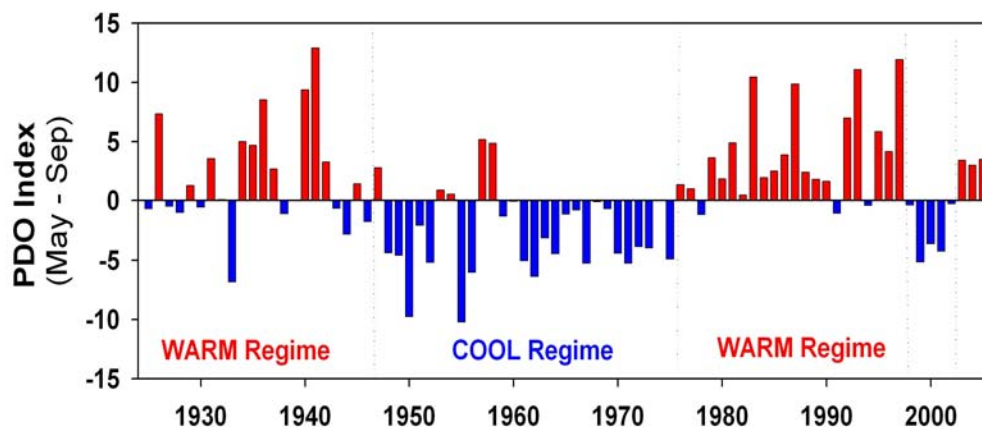


Figure 2. Long term trends in the Pacific Decadal Oscillation (<http://www.nwfsc.noaa.gov/research/divisions/fed/oeip/ca-pdo.cfm>).

The coincidence of these two events underscores both a monitoring and analytical challenge at Devils Hole, namely that the sample size for long term analyses has N=1. We can not perform critical experiments. For example, we do not know if the population would have recovered in 1976 with a lowered water level, and whether it would have recovered more quickly or more slowly. There are biological reasons to presume that they would not have recovered, but the common garden experiment requires a replicate which does not exist.

Other important demographic metrics like mortality can be grossly estimated from size frequency data using catch curves, but more refined estimates could only come from refuge populations.

All natural mortalities that can be collected from Devils Hole or refuges should be saved. If possible, these individuals should be monitored for pathogens, parasites, skeletal and otolith deformities, fluctuating asymmetry (left-right differences in morphology that may indicate developmental problems), genetics, size, sex, fecundity and age.

Further work on ageing should be conducted using refuge populations and employing both otoliths and size frequency data. Otoliths should be polished and increment verification attempted.

All carcasses, DNA, otoliths, and microscopic slide preparations should be archived for at least 20 years and the data link to each maintained.

Remote monitoring of Devils Hole DHP should be explored. The feasibility of solar powered underwater camera(s) should be explored.

Environmental monitoring recommendations

Among the classic forcers of population change are weather and food. Environmental monitoring should have, as its focus, those variables most likely to influence fish growth and mortality. As with demographic monitoring, the identification of environmental monitoring variables should follow a synthesis of available information to identify potential key factors. For example, among the myriad of weather and climatic factors that could be important, previous work has shown seasonal input of potentially important allochthonous materials to Devils Hole. This input may be wind-driven and provides a reason to better understand wind speed and directions.

Remote monitoring of Devils Hole environment through daily still photographs of the spawning shelf should be explored.

The current crisis situation

Because there is currently no coherent long term monitoring, it is going to be difficult to identify factors that might be responsible for initiating the 1995-96 decline. Potential factors might come from the proposed synthesis of existing data and identifying those factors is important. However, the current situation requires extreme measures in my opinion and is the highest priority.

Feeding fish in Devils Hole is a reasonable response since it is possible that the long-term cycles of population numbers are food related. In the early 1970's artificial feeding was accomplished with a lighted artificial shelf while this year it involves aquarium flake food. We can expect that this action could change growth rates, fecundity, aggressiveness and morphology, but it should do it in a relatively uniform manner for all current residents of Devils Hole. Introduction of refuge fish, especially males, should be discouraged at the moment because their aggressiveness could provide a distinct

reproductive advantage over current residents and could dominate production of all progeny.

If there is evidence of recruitment failure of the 2007 year class, preparations for introducing refuge populations into Devils Hole need to be made immediately and should be in place by September 2007 at the latest. Introductions should be prioritized and the plan should be ready to introduce fish as early as late winter 2008.

8. DR. DAVID PRUDIC

United States Department of the Interior

U. S. GEOLOGICAL SURVEY
NEVADA WATER SCIENCE CENTER
2730 N. Deer Run Road
Carson City, Nevada 89701
March 29, 2007

Gary Larson
Research Ecologist
USGS Forest and Rangeland Ecosystem Science Center
3200 SW Jefferson Way
Corvallis, Oregon 97331

Dear Gary:

Thank you for inviting me to be part of the Devils Hole Pupfish workshop that was held March 13 and 14, 2007. I am not sure that I can contribute much to the long-term monitoring needs at Devils Hole in relation to the life cycle and health of the Pupfish that reside within the hole. I have given some thought to monitoring environmental measures of climate and ground water.

I took the liberty of graphing a time series of the number of Pupfish in Devils Hole by month and year. I found that the number of Pupfish is less in March and April following the winter months and between 1972 and 2001 generally ranged from 120 to 250 fish. I also found that the number of fish increased markedly during the summer months with maximum populations from July through October that exceeded 400 fish from 1976-96. Starting in 1997, there is a marked decline in the summer population, whereas the winter population remained within the historical range until after 2004 such that the summer populations are now much closer in number to the winter populations. Both declined markedly after 2004.

The Pupfish population in 1972-75 was low but not as low as the current population. The low population in 1972-75 has been correlated to nearby ground-water pumping that caused the water level in Devils Hole to decline exposing the shelf that the fish use to feed and breed. The water level in the hole rose following a court order to stop nearby ground-water pumping and the Pupfish population increased with the rise in water level, particularly the summer population.

We also know that the current population decline is not caused by a similar decline in water level observed in 1972 as the water level is sufficiently high to cover the shelf at all times (except following the 2004 flash flood that deposited detritus on the shelf). The water level has been continuously recorded in Devils Hole since at least 1972. Such monitoring of the water level must be continued indefinitely into the future.

I am not sure of the meaning but I find it interesting that the Pupfish population during the summer declined prior to a decline in population during the winter. Presently, the cause of decline in summer population is a mystery but it could be the result of a loss of primary food supply, fewer eggs and/or larvae that reached maturity, disease in the Pupfish or in the critters it eats, or some combination of all.

Knowing the food supply that the fish need to survive seems critical and perhaps more importantly, if there has been a change in the summer food supply. Additionally, knowing the conditions that promote egg laying, fertilization, and larvae development also seems important. Much of what needs to be monitored at Devils Hole as far as food supply, egg laying, fertilization, and larvae development is beyond my knowledge so I will limit myself to relatively simple environmental measures related to changes in water within and the climate of Devils Hole.

My suggestions for long-term monitoring are as follows:

1. Continue recording the water level in Devils Hole. Newer technology using duplicate pressure transducers could be used to replace the old stilling well and float in Devils Hole if the structure for the stilling well and maintenance of it is harmful to the fish. The advantage of pressure transducers are that they do not require a large stilling well, can be put in a place that would result in minimal disturbance to the shelf, and the data are compatible with modern computers. The disadvantages of them are that they record at a fixed interval—usually minutes or hours and generally have greater error in the measure of head (error depends on sensitivity of pressure transducer). Water levels in the hole are sensitive to earth quakes and the magnitude and oscillations of water levels caused by them would be less precise using a pressure transducer than a pen trace on paper even if the transducer is capable changing its sampling frequency. If transducers are used to replace the stilling well and float, I recommend that the pressure transducers be attached to a fixed structure (like a bracket attached to the wall) as opposed to it being allowed to dangle from a wire. Any lateral movement of the pressure transducer and the shrink and stretch of the wire may affect the measurement of head.

2. Monitor temperature, dissolved oxygen, dissolved organic carbon, and the concentration of dissolved salts (specific conductance is often used but you may wish to identify individual compounds) in water on the shelf and at a location deeper in Devils Hole. These measures seem prudent because they are relatively cheap and temperature in particular is easy to measure. Air temperature in southern Nevada has been increasing since the early 1970's and this trend is likely to continue into the foreseeable future. The increasing air temperature may have an effect on the types of organisms that live on the shelf of Devils Hole because air temperature likely affects the temperature of shallow water over the shelf. Dissolved oxygen is important because the fish cannot survive without it and we should know its trend.

3. Monitor weather data at the surface of Devils Hole. Weather data may be necessary in understanding how changes in our climate may be affecting the food supply and the

environment of the shallow water on the shelf. The weather station should include typical climate measures—precipitation, temperature, and wind speed and direction. I also recommend that the station include barometric pressure (already being measured for the pressure transducers and is for analysis of water-level trends), net radiation and if ideally, other measures needed to calculate evapotranspiration. The station does not need to be placed in the hole rather; I recommend that it be placed on the surface away from the hole and in an area that would not be affected by the fence itself. I suggest that “dust” samples also be collected with time and analyzed for organic content to determine if there is a change in the quantity and type of debris that is blown into Devils Hole seasonally and with time. I am not certain how frequently the samples should be analyzed.

4. Compile and maintain records on ground-water withdrawals in the region that is thought to contribute discharge to nearby Ash Meadows. These records are being kept by the State of Nevada but every effort should be made to continue analysis of trends in water levels at Devils Hole. Such an analysis is important because water-level declines at Devils Hole caused by distant pumping (unlike the nearby pumping that created the decline in the late 1960’s and early 1970’s) will be slow and prolonged and even if the pumping stops after the water level has declined below the court mandated level, declines from distant wells will continue for some time because of the delayed effect caused by storage in the aquifers.

5. Identify the area that contributes runoff, debris, and surface food supply that may contribute to Devils Hole and determine if it changes. Also, monitor the frequency of runoff and debris into the hole. The contributing area for runoff and debris could be determined using ground-based Lidar. The Lidar survey need not be done but every 5 years and would be sufficient to determine if the contributing area changes and if the land surface including Devils Hole is rising or falling because of tectonic activity. I suggest an annual vegetation survey in March or April over an area greater than the contributing area. This survey would identify the abundance and type of vegetation that may be a source of food to the Pupfish or the other critters. Finally, some means is needed to monitor the frequency of runoff and volume of debris that enters the hole from storms similar to August 2004. Such a process likely occurred in the past but the volume entering Devils Hole could be increasing with time as erosion on the surface may result in an expanding area that contributes runoff and debris.

6. I am unsure of its importance but mining in Nevada has increased Mercury concentrations in the atmosphere that is then deposited across the landscape. I am not certain if the U.S. EPA sampling stations in Beatty, Amargosa Valley, and at US Ecology analyze for Mercury but this may be something to consider. Have tissue samples from the Pupfish been analyzed for Mercury?

Sincerely,

David E. Prudic
Hydrologist

9. DR. CRAIG STOCKWELL

Devils Hole Thoughts.

During the panel there was considerable discussion regarding the genetic data. The issue of particular interest is whether and to what extent the POR population had been “compromised” due to hybridization. This is important because if some of the POR fish are actually pure *C. diabolis*, then the prospects for population recovery are much improved.

One problem for the group was that the genetic data presented were not particularly compelling in regard to hybridization. As I compared the presentation to the original report, it became apparent that some of the figures were mislabeled. For instance, the first figure showed allele frequencies of locus D-1 for *C. diabolis* and *C. nevadensis*. This graph shows that alleles appear to fall out by size with relatively little overlap. Thus, this would seem to be a reasonable marker for detecting hybridization. However, the next figure is portrayed as from D-1 as well to compare allele frequencies from 1998 to 2005. This figure does not match up and is actually presenting data for another locus that is less diagnostic.

After another more thorough look at the data presented by Martin (2005), I am convinced that hybridization has occurred at POR. However, I still am not completely convinced that the hybridization has been complete. Martin (2005) presents data that reports for 35 inspected fish, none were pure *C. diabolis*. If true, this would indicate that the entire population is probably compromised. I think however given the stakes, that additional markers should be considered. There are some patterns that not consistent. For instance, one *C. diabolis* allele (D16 -400) actually increases in frequency at POR from 1998 to 2005.

Thus, I would suggest that additional markers should be applied and/or developed. Markers available:

Duvernell and Turner (1998) surveyed mt-DNA haplotype diversity and found considerable variation among the Ash Meadows pupfish. These markers could be used to further evaluate the hybrids. Because mt-DNA is maternally inherited, these data will not be sufficient. For instance, hybrids with *C. nevadensis* mothers would be detected, but hybrids where the mother was *C. diabolis* would go undetected. Nevertheless, such data combined with the other markers may allow a better examination of the hybridization issue now and in the future.

Likewise, markers developed by Duvernell and Turner (1999) could provide additional information. This work would also not be definitive because the markers are not fixed between POR and *C. diabolis*. Nevertheless, POR has considerably higher genetic variation compared to *C. diabolis*. Thus, these markers could likewise be used.

Further, our lab has developed a battery of markers for the White Sands pupfish which may or may not have been evaluated for the Ash Meadows fishes. Thus, these could be potentially applied. The biggest problem for any new genetic work will be to have access to DNA from *C. diabolis* and the other Ash Meadows fishes.

Future Genetic Surveys:

Given the recent event of hybridization, regular genetic surveys should be conducted in the future to detect any similar events more rapidly. The surveys in 1998 and 2005 reveal a rapid case of hybridization and apparent selection against *C. diabolis*. Thus, surveys over a 5 to 7 year period are not sufficiently regular. I would suggest genetic surveys be conducted at the refuge sites every two years. If possible a small number of pure *C. diabolis* should be regularly surveyed. Again, development of additional markers should be pursued to make such diagnoses a little more definitive. Further, if evidence of hybridization is detected the entire population should be subsequently surveyed to rapidly identify any remaining pure *C. diabolis*.

Documentation:

What has happened over the last 30 years in terms of research, maintenance and management? Karam (a student with Dr. Michael Parker) reconstructed the introduction history for refuge populations using notes stored in Dr. Jim Deacon's office. Perhaps a similar and equally thorough set of data collection can be applied to all other aspects of Devils Hole pupfish biology. For instance, the decline appears to be relatively linear since about 1995. Has there been any particular activity that changed around this time period? Such questions did not seem to have any satisfactory answers. It could however be possible with some research to fill some of the knowledge gaps. This being said, it may be useful for all activities regarding *C. diabolis* to be registered in a central data bank. This would include all research, management and maintenance.

Building up the current population:

Ultimately, the short-term goal should be to take appropriate actions to increase the probability of population recovery. There was much learned during the last major decline in the 1970's that could be used to "grow more fish". In my view this is the most critical issue. It could be that the best action is "no action", but such a decision could also lead to the ultimate loss of this species. Thus, I would suggest a more aggressive analysis of the potential management actions that could take place. In my view, it seems that attempting to breed these fish in captivity has had very limited success. Further, by removing fish from such a small population, a demographic threshold could be reached leading to extinction. Thus, I would strongly recommend against removing any of the remaining adults unless there were strong evidence of a rapid die-off occurring.

Further, I would consider activities that could be done to enhance larval survival and increase reproductive activities. 1) Establishment of a caged nursery area could be pursued where larvae could be shielded from the risks of cannibalism. 2) Consider providing additional breeding habitat by suspending an artificial shelf in the deep pool and providing artificial light so that algae will grow. Something akin to this

approach was apparently successfully used in the 1970's to help recover the population (Dr. Jim Deacon, personal communication).

Future abiotic and biotic surveys:

I think the outline provided by Dr. Blinn provides an excellent outline for a monitoring protocol. I think a similar approach should be taken not only for Devils Hole but all of the refuge sites as well.

However, all monitoring activities should be carefully documented down to the smallest detail. Also, sampling should be very carefully evaluated against the potential impacts. For instance, allochthonous materials appear to be important. Thus, any sampling to evaluate such inputs may in of itself deprive the system of needed nutrients. However, to limit such activities would not be prudent either. Thus, perhaps such activities could be mitigated by compensating for the "lost material" by adding in a similar amount of material.

Recovery and onward:

Assuming that this population recovers, I believe that some serious thought should be given to setting up a back-cross population. There is now some limited data to suggest that hybridization could be useful in the context of genetic restoration. It is less clear if the recent decline is directly due to genetic impacts. One hypothesis is that this species has accumulated a considerable genetic load (mutational meltdown), but it is not clear how such a meltdown would actually manifest in terms of the shape of a population decline. However, if one refuge population could be used say at POR to test this hypothesis, it may be worthwhile. Genetic restoration was recently used to manage the Florida panther and the same general situation may well exist for Devils Hole pupfish. At this point to try hybridization may be considered too risky, but once a few viable refuge populations are re-established, I think it would be good to pursue the genetic rescue approach with one of these populations.

10. DR. ANDREA WOODWARD

Devil's Hole Pupfish Recommendations

I thank the organizers of the Devil's Hole Pupfish Monitoring Review for inviting me to participate. The situation is fascinating and population recovery is a daunting challenge. Although I am not an aquatic biologist, I hope the following recommendations will be helpful.

1. Data Analysis

I understand that the meeting was meant to be focused on long-term monitoring, but unless the reasons for population decline are understood, there will be nothing to monitor for much longer. So I will start by recommending some data analysis.

People who work at Devil's Hole and are otherwise familiar with the situation likely know the long-term story as second nature. For those of us who were new to the area, the data presented during the meeting were scattered through time and were not put into a chronological context. It seems that most studies were conducted in the 1970s and then recently, coinciding with low population numbers, a difference of nearly 30 years. Influences on the productivity of the Devil's Hole ecosystem were presented (e.g., allochthonous input, solar radiation, substrate), but perhaps the nature of these relationships depends on the background weather, air pollution, etc. I made a quick effort to begin putting together contextual parameters with pupfish numbers to look for patterns (Table 1).

Pupfish numbers seem to be related to abundance of algae and algae seem to be related to dry summers (high insolation) during the early part of the record, with the exception of 1976 and 1977. However, large algal mats in these years could be related to the presence of owls. Wilson and Blinn (2001) found a strong correlation between total annual precipitation and maximum number of pupfish, which they attributed to inputs during rain events. However, both maximum and minimum pupfish numbers declined in 1979, 1982 and 1997, the only years with both a wet summer and winter. This very crude evaluation of the role of weather should be refined to look at local records and details of timing and magnitude of large events.

It also seems likely that flood and seismic events are extremely important for providing inputs, cleaning gravel from the shelf, scouring algae off of the shelf, and renewing spawning substrate. It would be interesting to look through historic records to identify when these happened. Are the two floods that occurred in 2004 typical? Did something similar happen in 1996 or 1997 when the maximum population began to decline? I expect that most people associated with Devil's Hole have a sense of the answer. Also, the frequency and size of events can also be looked at in terms of wet and dry phases of the Pacific Decadal Oscillation climate pattern. If we began a dry phase in 1998 or so, what are expectations for frequency of future flood events based on data from earlier dry phases?

Recent especially sharp declines coincide with removal of gravel from the shelf by people, exacerbated by the trap accident in 2004. Maybe gravel removal as it has been done is disruptive in the short term even if it is beneficial in the longer term. What has happened in the past when the shelf was inundated with gravel and no action was taken to remove it?

Finally, perhaps more can be done with the pupfish count data. For example, when did the number of pupfish on the shelf begin to decline? Does the timing coincide with population decline?

I recommend that something like this table be filled in with local data based on measurements and the recollection of people who visit the site frequently, including maintenance staff. Water chemistry and other data from the many publications about Devil's Hole should also be included. I found daily records of maximum and minimum temperature and precipitation with notes about significant events recorded at Amargosa Farms on the NOAA NCDC website (COOP station). Unfortunately the data have not been summarized beyond scanning in the raw data sheets. Nevertheless, the information is likely worth the effort required to summarize it unless there are data from a site closer to Devils Hole.

I also recommend that money be spent on contracting with someone to analyze, integrate and synthesize the data. It should not be left up to busy park staff.

Table 1. Beginnings of chronology

Year	Pup-min	Pup-max	Algae	Owls present	Summer ppt anomaly	Winter ppt anomaly	Floods	Seismic Events	No. fish on shelf	Visibility clear days	Visibility hazy days	Sulfates in ppt	Ammon/nitrate in ppt	Ozone	Human activities
1972	127	286	Hi		3	-60									Lights, platform present; gage moved
1973	184	253	Hi		-20	25									Lights removed
1974	143	304	Hi		-2	-10			Hi						
1975	148	294	Hi	X	-8	-5			Hi						
1976	180	410	Hi	X	62	-62			Hi						
1977	198	553	V.hi		25	130			Hi						
1978	219	441	Hi		-8	60			Hi						Water reaches court minimum
1979	179	378	Lo		20	80									
1980	165	548	hi		-12	30									
1981	237	484	Hi	X	0	-45									Platform removed
1982	229	396	Lo		20	110									
1983	218	481	Lo		75	-60									
1984	143	497	Lo?		45	-25									
1985	164	467			0	40									
1986		447			3	-25									
1987	180	484			-12	20									
1988	236	566			0	0									
1989	223	463			-5	-60									(Intense drought)
1990	244	544			5	-25					↓	↓	↓		

1991	215	474			0	25					↓	↓	↓		
1992	238	524			-10	145					↓	↓	↓		
1993	226	528			-22	-40			0		↓	↓	↓		
1994	309	541			-15	75			0		↓	↓	↓	0	
1995	217	543			-30	0			0		↓	↓	↓	0	
1996	252	433			-10	-65			0		↓	↓	↓	0	
1997	164	387			30	80		Lo	0		↓	↓	↓	0	
1998	226	320			10	-15		Lo	0		↓	↓	↓	0	
1999	197	344			10	-60		Lo	0		↓	↓	↓	0	
2000	190	286			-10	5		Lo	↑	0	↑	↑	↑	↑	
2001	159	282						Lo	↑	0	↑	↑	↑	↑	Gravel removed - April
2002	161	197	Lo					Lo	↑	0	↑	↑	↑	↑	
2003	153	303	Hi?					Lo	↑	0	↑	↑	↑	↑	
2004	123	142	Hi?				2	1	↑	0	↑	↑	↑	↑	Trap accident
2005	88	84	Lo												Gravel removed – February; metal frame altered
2006	38	85	Lo												

Notes:

- summer and winter precipitation are expressed as anomalies from average and were estimated from a very small graph presenting a summary for the entire Mojave;
- visibility, sulfate, N data are from Grand Canyon National Park;
- pupfish numbers may not have come from the final version of data.

2. Stochastic Model

Assuming that the data analyses produce hypotheses regarding why the pupfish are declining rather than a definitive answer, I recommend that the hypotheses be explored with a model. Tony Starfield has done some interesting work with objective-driven, simple models, which can be built, tested and revised fairly quickly. He has demonstrated that useful results can be obtained with fewer data than are intuitively necessary coupled with sensitivity analyses. I will attach an example related to another endangered population. The model that has already been created may be useful, but my impression from the presentation is that it *does* attempt to model the entire system in detail and thereby has become a complicated black box whose results may be hard to accept by people not involved in the details of its creation.

If the model has stochastic elements (e.g., frequency and timing of flood and seismic events) it can be run repeatedly to indicate the probability of the various possible outcomes. This is a method for addressing the problem that Devil's Hole is an $n=1$ situation. That is, the response of Devil's Hole is only one example of a response to a set of environmental variables and does not indicate what else is possible and with what probability.

Based on my experience during a recent training course, I highly recommend contracting with Tony Starfield to build one of his Rapid Assessment models for Devil's Hole. He would probably need to work on and off with park staff and other knowledgeable folks for a couple of weeks. The Chernoff (1985) model and the Stave model presented at the meeting contain values for needed parameters. The model could be made more sophisticated with time, but I guarantee that much will be learned from the rapid assessment approach.

3. Long-term Monitoring

In general, I think Blinn's list of high priority measurements makes sense (identified by him at the meeting):

- Substrate composition
- Water level (including depth above substrate)
- Water temperature
- Physicochemistry
- Weather station
- Allochthonous C
- Benthic macroinvertebrates
- Larval and adult pupfish

I might add nitrogen because N may be limiting for spirogyra on one hand, but is increasing in precipitation on the other.

A fruitful way to determine what to monitor is to consider which data you wish you had already been monitoring to explain the current population decline. My own short list would include:

- Weather
- Disturbance (human and natural)
- Air quality (but not necessarily at the site; its synoptic and data can be obtained from others)
- Algae
- Substrate on shelf
- Water depth over shelf
- Water temperature over shelf
- Pupfish numbers and size distribution

Given that resources for monitoring are always limiting, I would put more emphasis on habitat quality than inhabitants (other than pupfish).

It seems that the substrate and algae composition on the shelf are dynamic and would require frequent monitoring. However, high frequency of monitoring will increase the cost of monitoring and may increase disturbance of the site. This could be mitigated by using minimally disruptive methods such as ocular estimates and/or photos to describe algae and substrate.

I recommend that a “Site Visit Log” be instituted for anyone who visits the site for any reason. The data sheet would include at least:

- Name of observer, date, time of day
- Description of the algae (e.g., categories of cover maybe)
- Description of the substrate (e.g., cover of sand, gravel, cobble, etc.)
- Evidence of any type of disturbance
- Weather at the time of the visit
- Description of the activity undertaken by the observer (reason for visit)

I thank you again for including me in this review, and I wish you the best for solving this problem and applaud your dedication.

Andrea Woodward
USGS-Forest and Rangeland Ecosystem Science Center
206-526-6282 x 332

APPENDIX B

Reviewer No. ____EGS__

Review Completion Date: _6/7/07_____

**Forest and Rangeland Ecosystem Science Center
Peer-Review Form**

Title: Devils Hole Pupfish Panel Review - March 14-15, 2007, Furnace Creek Inn, Death Valley National Park

Author(s): Larson, GL; Acharya, K; Buktenica, M; Douglas, M; Hankin, D.; Hoffman, R; Loeb, S.; Markle, D; Prudic, D; Stockwell, C.; Woodward, A

Product #: 1847

Do you wish to remain anonymous? No:

General Review Comments: (Responses required for questions 1-3).

1. Does the overall rigor of the manuscript meet the standards of current scientific practice? Potential areas for comment include but are not limited to scientific merit, originality, presence/absence of critical references, and appropriateness of approach.

Yes

2. Does the manuscript effectively communicate the main points?

Yes

3. Requirements or suggestions for key improvements to the manuscript (Specifically identify improvements that you consider requirements in order to distinguish them from suggestions).

No requirements

4. Is the paper relevant to practitioners and scholars in the area of discipline? Should other work in this area be cited or discussed? (Response is optional)

Yes

Reviewer No. ____EGS_
Review Completion Date: _6/7/07_____

Publication Recommendation

x Acceptable ☐ Acceptable with revisions as noted ☐ Not acceptable*

*if checked, please state rationale (comments above can be referenced)

Additional Comments (optional):

Specific Comments: Please provide specific comments in the format provided below.

Page No.	Line No.	Comment

I find this paper to be appropriate for the intended audience. It provides a good summary of the panel results. The pupfish issue is a difficult one and was handled well by the panel members and chair.

The paper might benefit from a numbered or bulleted approach to making recommendations. For example, a list of suggested parameters to monitor would be easier to follow than in the current text form in one paragraph. Parameters to monitor:

- Dissolved Oxygen
- pH
- Conductivity
- etc.

Alternatively, a table might work well.

I cannot agree more with the panel recommendation that the pupfish work be subjected to peer-review and published. Several panel members provided preliminary analyses and suggestions using extant data. I believe a stronger case should be made for “get the data analyzed”. This is discussed briefly with the note that considerable data exist in files and that this should be cataloged and put into a database. I suggest the panel chair make a stronger case for the analysis – This is implied by the recommendation that a fish biologist with a strong biometric background be hired.

Reviewer No. ____EGS__

Review Completion Date: _6/7/07_____

I base my remark above on the intriguing connection Doug Markle made with the PDO – this is a repeated pattern for many organisms (and fire frequency) in western North America. Surely enough data exists to test this hypothesis, perhaps even using the Ocean temperature data directly from NOAA. In addition, there must be enough data to examine weather factors as related to the pupfish population – It probably does not matter what weather station is used as long as it is in a desert ecosystem. The panel members made a number of very fine suggestions for directions this data analysis might take.

Please return this form to:

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Reviewer B

Review Completion Date: 21 June 2007

**Forest and Rangeland Ecosystem Science Center
Peer-Review Form**

Title: Devils Hole Pupfish Panel Review - March 14-15, 2007, Furnace Creek Inn, Death Valley National Park

Author(s): Larson, GL; Acharya, K; Buktenica, M; Douglas, M; Hankin, D.; Hoffman, R; Loeb, S.; Markle, D; Prudic, D; Stockwell, C.; Woodward, A

Product #: 1847

Do you wish to remain anonymous? NO

General Review Comments: (Responses required for questions 1-3).

1. Does the overall rigor of the manuscript meet the standards of current scientific practice? Potential areas for comment include but are not limited to scientific merit, originality, presence/absence of critical references, and appropriateness of approach.

The manuscript presents the views and comments of well-qualified scientist researching a critical conservation problem. However, the format in which this material is presented does not qualify it for publication per se. For journal acceptance it would need to be revised significantly.

2. Does the manuscript effectively communicate the main points?

Yes, but again this needs to be read and revised by somewhat competent to do so.

3. Requirements or suggestions for key improvements to the manuscript (Specifically identify improvements that you consider requirements in order to distinguish them from suggestions).

Revise from its present form to include the normal divisions of a published paper: Abstract; Introduction; Presentation of data, including materials and methods; Discussion, with implications for future management; Summary and conclusions, Literature cited.

Reviewer B

Review Completion Date: 21 June 2007

4. Is the paper relevant to practitioners and scholars in the area of discipline? Should other work in this area be cited or discussed? (Response is optional)

This is a highly unique matter relating to the continued existence of a species of desert fish. The material presented represents the best current thinking of excellent practitioners and scientists in this field of conservation biology, and will be helpful to future practitioners as similar conservation matters occur. It should be made available to the scientific community.

Publication Recommendation

☐ Acceptable ☒ **Acceptable with revisions as noted** ☐ Not acceptable*

*if checked, please state rationale (comments above can be referenced)

Additional Comments (optional):

Specific Comments: Please provide specific comments in the format provided below.

Page No.	Line No.	Comment

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June 21, 2007