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January 10, 2006

Re: Comment on the EIR for the LRDP, UC Santa Cruz Campus,
To Whom It May Concern:

This comment concerns the recent discovery of a new paedomorphic form of the California Giant salamander, *Dicamptodon ensatus*, that has been found in the Empire Cave. The assertions made in the Section 4.4 bioresources of the Draft EIR for the LRDP indicating that they are "rare" and it is "speculative to consider them genetically distinct" are completely erroneous given the wealth of data afforded by the parallel systems around the world on cave salamanders.

The assertions can be refuted specifically with reference to sections of my draft report, submitted to California Department of Fish and Game, along with my re-application for my permit to study these forms. A copy of this report has also been forward to the US Fish and Wildlife Office overseeing the Santa Cruz County area.

It is extremely likely that this is the cave salamanders of UC Santa Cruz cave system are a new form, genetically distinct from surface populations. It seems premature to make formulations for an LRDP that does not take into account this extremely high probability. We currently have studies underway to measure the levels of Mercury and other heavy metals in the stream larvae found on the UC Santa Cruz Campus (collaboration with Herpetology Class, UCSC and Michael Bank, Harvard University).

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A copy of my curriculum vitae is on file at the Ecology and Evolutionary Biology office, University of California, Santa Cruz.

Respectfully,
Barry Sinervo

Professor of Ecology and Evolutionary Biology

Synopsis of Sinervo's report, submitted to California Department of Fish & Game (1/11/2006) and to the USFWS (1/11/2006):

Introduction

The California Giant salamander, *Dicamptodon ensatus*, has a complex salamander life cycle throughout most of its range in California, including an aquatic larval stage lasting months after which the salmander metamorphoses into a terrestrial adult. Occasional reports of paedomorphic forms of *Dicamptodon*, which never metamorphose and breed as aquatic adults, are found in the literature (Nussbaum et al. 1983, Stebbins 1985), but these are rare. Paedomorphic salamanders are defined to be an adult that breeds with the aquatic morphology (Sinervo & Svensson 2003), usually at the same size as the normally terrestrial form, but often at a much earlier age at maturity. We have recently discovered a location in which the paedomorphic form is the most common morphotype. Empire Cave is a large cavern located in Cave Gulch (also known as Wilder Stream) on the UC Santa Cruz campus. During the winter months the cave floods periodically. After the water subsides, several pools are accessible from the bottom of the cavern. In these pools paedomorphic salamanders were recovered each year of sampling.

We tested whether the form is locally common in the cave by rearing a sample of larvae from the adjacent stream pools (larvae of the year, < 10 cm in length) up to metamorphosis or a size that is the cutoff for establishing a paedomorphic form (168 mm) (Nussbaum et al. 1983, Chaney 2004). We found a highly significant difference in the rate of paedomorphosis in the cave form relative to the rate of paedomorphosis in the adjacent stream. These results suggest that the local population inhabiting the caverns underneath the UC Santa Cruz campus represent a new population of paedomorphic salamander. If one extrapolates from the area available for sampling (Empire Cave) relative to the entire Karstic area of Marble that would provide underground galleries, it is clear that this paedomorphic population could be quite large.

Methods

We used published accounts and direct observations by UC researchers of salamanders recovered from Empire cave to determine the frequency of paedomorphic forms in Empire Cave.

We sampled the pacific giant salamander larvae from a pool immediately above the point at which Wilder Stream disappears underground into the Empire Cave system of galleries. We reared 20 larvae up to metamorphic size (15 Celsius, environmental chamber). Fresh water was collected from the pools of the Wilder Stream each week and all water was changed once per week. Larvae were fed dried tubifex worms. All larvae collected from the stream pool outside of the cave metamorphosed at a size ≤168 mm.

Results

Paedomorphic salamanders are a unique developmental form of a normally fully metamorphising amphibian species. The paedomorphic form breeds at an adult size, but retains its aquatic larval form. The Sinervo Lab has been sampling the cave population of *D. ensatus*, since the winter of 2001 (sampling in 2002, 2004, & 2006). In conjunction with Herpetology class, We surveyed major rain events during the winter months (on foot and with snorkeling geat), which allows one to sample a population of salamanders that enter the Empire Cave through small fissures from a cave complex of unknown size. Four paedomorphs from the Cave have been collected. A paedomorphic form is defined to be a larva of sufficient size to be

considered adult size (>168 mm snout-vent length, Nussbaum et al. 1983).

Based on the sampling we have conducted, if the cave system under UC Santa Cruz is extensive, the number of breeding paedomorphic salamanders could be considerable. The relatively small number of salamanders that have been recovered is because our ability to sample other areas of the entire cave complex is greatly restricted to a few fissures of Empire Cave. All other caves that can be accessed are above the water table. We can effectively only sample one small territory of a salamander in the area of the Empire Cave. Nevertheless, every year there is reliably one animal in this territory (the Empire Cave). The interpretation of a large population of paedomorphic *Dicamptodon* cave salamanders critically depends on the amount of permanent water in the cave complex during the summer months. Early well drilling around the UC Santa Cruz campus, suggests that this cave complex might indeed be quite extensive and there is even high-volume subterranean water flow during the summer months based on dye injection studies at sites upstream from drilling sites (early surveys of UC Santa Cruz).

Most importantly, the cave population of *Dicamptodon ensatus* appears to be fixed on the paedomorphic adult stage (4 of 4 larvae from the cave paedomorphic). In contrast, of 20 larvae sampled from the adjacent stream that were reared beyond the critical size of 168 mm, only 1 remained paedomorphic but eventually transformed at a size of 168 mm size, the cutoff for paedomorphosis. All of the remaining 19 transformed into terrestrial forms before the size of 168 mm was reached (average size was 138 mm, range 115-168). The sample of stream salamander larvae was obtained 100 m upstream of the Empire Cave Drainage, in the Cave Gulch Stream. Thus, the available evidence suggests that paedomorphic *Dicamptodon* salamanders from Empire Cave are much more common in the cave than the adjoining stream (Chi-square = 19.04, 1 d.f.. $P < 0.001$). Moreover, the statistical analysis supports that it is significantly more paedomorphic than adjacent populations.

The morphology of the salamander is quite unique. All cave salamanders recovered are patternless and grey in coloration, compared to the highly patterned terrestrial form that is very dark in coloration. One cave salamander became darker in the laboratory, after being reared on tubifex worms, suggesting that the grey color is from feeding on food without pigment (e.g., the pale cave isopods and amphipods described below).

Other Paedomorphic records for Santa Cruz County. A few other paedomorphic salamanders have been recovered from Santa Cruz County (on record at the Museum of Vertebrate Zoology, UC Berkeley), however, all of these have been collected in streams that are also downstream of known Marble deposits (Chaney 2004). We also surveyed the salamanders of Wilder Creek, in pools below the cave boil of Empire Cave. We found a few individuals with the light grey color, consistent with the hypothesis that breeding adults in the Empire Cave release these forms into the cave stream course, and some of these larvae are pushed out of the cave during winter storms. Thus, our observations on the light colored morphs of larvae could readily explain the occurrence of paedomorphosis outside of the cave in downstream pools (e.g, the aforementioned museum collections.).

Observations on feeding ecology. The cave salamander feeds on cave isopods and amphipods located in the pools. The number and density of isopods and amphipods are more than enough to sustain growth to metamorphic size and adult reproduction. Population dynamic studies of amphipods in other ecosystems suggest that growth is very rapid at 12 degrees C (Sinervo & Doyle 1990), the temperature of the cave. The amphipods and isopods feed on detritus washed in from sinkholes, which are abundant across the UC Santa Cruz campus. The amphipods and isopods are often found in gour pools, which are conspicuous limestone pools of permanent standing water, that provide a refuge from predation. The furthest accessible portions of Empire

Cave have a prominent series of cave gours with amphipods in abundance. The pools were salamanders have been observed are have hundreds of each species.

Observations on unique behaviors of the paedomorphic salamanders. These remote pools where amphipods might find refuge might provide a realiable food source during the summer months of low water flow. Given the demonstrable ability of paedomorphic cave salamanders (from Empire Cave) to climb and move on dry substrates (Sinervo personal observations), it is likely that after a salamander has eaten all of the locally available isopods and amphipods in its summer pool, it could move on to the next pool upstream or downstream by short trips out of the water. Thus, the salamanders can probably move and even feed during the summer months. During the flooding in the winter a salamander could move across the entire cave system, given the low flow rates through the cave and high water levels. Thus, the cave system under UC Santa Cruz campus is very extensive and sufficient to support a large breeding population of several hundred to a few thousand salamanders, given the number of sinkholes, underground stream beds and across stream fissures indicated by the sinkhole patterns in the published campus geological reports and campus maps of the north-south fracture system (www.es.ucsc.edu/~es10/fieldtripUCSC/cave.html).

Discussion

Based on other paedomorphic salamanders, the available evidence supports the hypothesis that the Empire Cave *Dicamptodon* are a genetically distinct population segment, given that the proportion of paedomorphic salamanders is fixed at 1.0 in the Empire Cave. The only other *Dicamptodon* in species in the genus that is completely fixed on the paedomorphic form is *Dicamptodon* copei of the Olympic peninsula, Washington State. Furthermore, the caves are old (Tinsley 1985), and two endemic species of aquatic invertebrates, a cave amphipod (*Stygobromus mackenziei*) and an as yet undescribed isopod (*Calasellus n. sp.*), are both endemic to the cave system (CDFG 2001) (Ubick 2001). Thus, it is reasonable to anticipate that other organisms, like a derived aquatic salamander, will be adapted to the Santa Cruz Karst Ecosystem.

Potential threats. Paedomorphic salamanders are common in karst formations around the world. Essentially every one of these species is endangered or threatened, and some have gone extinct. Most notably the Olm, *Proteus anguinus*, of Slovenia has been wiped out from up to 90% of the historic caves because of hydrological contamination from pollutants of human origin. A population of *P. anguinus* has been transplanted to the Pyrenees of France to ensure its long-term survival. I will be studying this translocatd form, in May 2006. I will obtain additional data on the ecology of cave paedomorphic salamanders during this visit (a collaboration with Dr. Jean Clobert, Centre Nationale pour les Reserches Scientifique).

The Barton Springs Salamander of Austin Texas faces similar difficulties of surface water contamination and is considered threatened. I had opportunity to conduct a site visit of this salamander in 1994. All of the known surface habitats of the Barton Springs salamander are found within Zilker Park. On April 30, 1997 the U.S. Fish and Wildlife Service (USFWS) added the Barton Springs salamander (*Eurycea sosorum*) to the list of endangered and threatened wildlife which receive federal protection under the Endangered Species Act. The available habitat of the Barton Springs Salamander is smaller in size to the potential subsurface cave complex under UC Santa Cruz that would serve as habitat for paedomorphic *Dicamptodon*. Observations on the Barton Springs Salamander are also illuminating about the likelihood of observing adults. In many years only 1-4 adults are observed. The remainder of the population

resides in deep fissures and is likewise inaccessible as is the case with paedomorphic Empire Cave *D. ensatus* salamanders.

Ecology, life history, and density. It is important to realize that all troglodític vertebrates (fish and salamanders) are found at low density, given the low input of resources into a cave ecosystem, and the generally low growth rate of their food supply in the cave, and the low temperature regimes experienced by cave organisms on an annual basis. In the case of the Empire Cave the food for the paedomorphic *Dicamptodon* is largely a cavernicolous isopod species and an amphipod species (Ubick 2001). In the case of vertebrates like blind cavefish, a given cave form may live to be in excess of 60-80 years. This life history greatly buffers recruitment dynamics over long time scales and allows for such forms to exist at low population densities. Based on growth rate studies at 3 different temperatures, we estimate that the largest adult recovered from the Cave is in excess of 20 years old (Sinervo et al. in prep.). These long-lived adults can breed over decades and recruit progeny over many, many years. However, it makes them particularly vulnerable to extinction. All aquatic amphibians are extremely sensitive to a wide range of chemicals, which are routinely used in urban settings such as that found on the UC Santa Cruz campus.

Data on the actual density of aquatic cave vertebrates is in fact difficult to obtain (given most of these ecosystems are very very remote and virtually inaccessible). However, during my tenure at Indiana University, I taught an evolutionary biology course at Indiana University and we made yearly field trips to visit the blind cave fish populations adjacent to the Indiana University campus, Bloomington, IN. Each year, we surveyed a 1.5 km section of blind cave fish habitat along the drainage of Blue Springs Cave, the longest subterranean navigable waterway in North America. We recorded the numbers of adult cavefish from a boat during "blue water" conditions when the visibility is superb. This population blind cavefish is at extremely low density (< 1 fish/ 25-50 m), despite their small size (8-10 cm). The paedomorphic salamanders of Empire cave are considerably larger (>168 mm) and nearly an order of magnitude more massive. Thus, the density of adult paedomorphic *Dicamptodon* salamanders are expected to be > 50 m. The habitat in Empire Cave that is inundated by winter rains is 50 m long, and there is one adult salamander in the cave on a yearly basis. This cave system extends up to areas under Science Hill, UC Santa Cruz, and down into Wilder Ranch. The other tributaries draining the karst formation are equally extensive. Thus, the number of salamanders could number in the hundreds of adults, given published accounts of the fracturing of the Karst features and the extensive jointing system in the block of Marble underlaying the UC Santa Cruz Campus: (www.es.ucsc.edu/~es10/fieldtripUCSC/images/maps/fracture.jpg.

During the summer months a small puddle the size of 20-40 litres in volume would be sufficient to sustain an adult. Thus, the adults do not need large bodies of water during the summer, only large numbers of water pools, which are very common in the cave complex under UC Santa Cruz, given the abundance of recorded sinkholes.

Summary Opinion. The Empire Cave populations of *D. ensatus* may be a new species, given that it is fixed for paedomorphosis and the form is common in the area of the karst topography that we can sample (Sinervo et al. in prep.). Is is also clear that this population reflects a distinct population segment of *D. ensatus.* The karst topography as a whole (Marble formation under University of California, Santa Cruz) is extensive enough in area that it might support a very large population (several hundred to thousands based on the known fracturing pattern, sinkholes, and likely presence of many underground galleries that are confluent with one another). Genetic analyses comparing the cave salamanders and the surface salamanders are critically needed to determine if the cave salamander is a new species. However, the available ecological and comparative data already supports this hypothesis.

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Response to Comment Letter I-71

Response to Comment I-71-1. The presence of only the neotenic form of D. *ensatus* does not confirm that the population is genetically distinct, nor does it preclude the species from breeding with non-neotenic D. *ensatus* in the vicinity of Empire Cave. As discussed on page 4.4-28 of the Draft EIR, neotony is known in all populations of *Dicamptodon* (Nussbaum et al. 1983; Good 1989). Genetic analysis has confirmed that both paedomorphic and adult forms occur within the same species (Daugherty et al. 1983; Good 1989; Steele et al. 2005). Thus, without genetic evidence or breeding experiments outside of Empire Cave, it is impossible to state with certainty that speciation has occurred and it is likely that this is not a distinct species.