Batrachochytrium dendrobatidis Sampling of *Emys marmorata* in a Sierran Pond

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Introduction

As global temperatures increase, the severity and frequency of emerging infectious diseases in wildlife may also increase (Pounds et al. 2007). One such emerging infectious disease, Chytridiomycosis, caused by the fungus *Batrachochytrium dendrobatidis*, has been correlated with the decline and extinction of many amphibian species worldwide (Lips et al. 2006). *B. dendrobatidis* has now been reported from 13 amphibian families including frogs, toads, and salamanders, in the wild and in captivity (Daszak et al. 2003). *B. dendrobatidis* can be detected in water samples (Kirshtein et al. 2007), and is significantly more likely to occur in frogs living in permanent water bodies and streams than in ephemeral or terrestrial habitats (Kriger and Hero 2007). Berger et al. (2004) reports that temperature is directly related to the onset of chytridiomycosis, with a higher mortality rate occurring at 17° C and 23° C than at 27° C.

Longcore et al. (1999) reports that chytrid can be cultured in the laboratory in tryptone agar without keratin or keratin derivatives, and that chytrid is harbored in the ventral keratinized skin of adult amphibians. The thickness of the keratinized skin on amphibians, often referred to as alpha-keratin, a soft form of keratin, varies per species.

In addition, chytrid fungus may be harbored in a reservoir host such as in the African clawed frog (*Xenopus laevis*) and American bullfrog (*Rana catesbeiana*) (Daszak et al. 2003). Although relatively benign to the host, the pathogen may occur in high prevalence in the host and is usually enzootic (present at a reasonably constant level but not leading to the demise of the population) (McCallum 2005).

Although *B. dendrobatidis* has not yet been detected in non-amphibian hosts, it is possible that other poikilotherm animals with keratinized surfaces could harbor infections and play a role in the persistence and spread of disease (Berger et al. 1999). For example, turtles have alpha-keratin on the interstices of their epidermis (Alexander 1970, Alibardi 2003), which we postulated could possibly support chytrid fungus. This investigation was conducted to assess whether there is potential for the western pond turtle (*Emys marmorata*), a co-inhabitant with many amphibian species in ponds and streams, to harbor the chytrid fungus in alpha keratin. If this is so, could it also be possible for turtles infected with *B. dendrobatidis*, to serve as a reservoir host for the fungus? In our study we sampled 23 individuals for the presence of the chytrid fungus from an ephemeral pond known to support *B. dendrobatidis*.

Methods

The study site is located in the Plumas National Forest in Butte County, California. The average annual maximum temperature reported for the area, averaged over 52 years, was 24° Celsius (75.2° Fahrenheit), while the average minimum temperature is 9° C (48.8° F) (Western Regional Climate Center - wrcc@dri.edu).

Turtle Population Survey: Visual surveys of *E. marmorata* were conducted sporadically between 2005 and 2006, with timed focused surveys conducted between April-August 2007. Visual surveys were conducted with binoculars during the daytime between the hours of 11:00 am to 4:00 pm, when the highest number of individuals can be observed. Surveys were also conducted during summer nights, when turtle eye-shine illuminated by lights is quite apparent.

Surveys were conducted using 8 x 42 roof-prism binoculars from a bank elevated 3 feet above the waters' edge. A visual reference starting point for the survey was established and the edge of the water/emergent vegetation was used a perimeter reference line. Each survey consisted of visual scanning transects; first the perimeter was scanned, then the interior of the water body. The water body was small enough that the bankside vegetation could provide a reasonable visual reference to prevent double-counting individuals. After each survey was conducted the number of turtles observed was recorded. A total of 11 surveys were conducted in 2007. Each survey consisted of at least three visual scans, each separated by 5 to 10 minutes. The direction of visual scanning transects was reversed for each survey. We averaged the total of all turtles recorded during each survey by dividing by the number of scans.

Chytrid Sampling: On July 16, 2007, Trish and Greg Tatarian, Amy Fesnock, Cindy Roberts and Maria Cisneros conducted the first phase of a mark recapture survey of *E. marmorata*. Between 2:30 pm and 6:00 pm, turtles were captured by hand, primarily using a dipnet, then placed at the edge of the pond in 10-gallon holding containers partially filled with pond water. Turtles were then removed individually and swabbed for chytrid fungus, using the methods described by Livo (2004). Fresh nitrile gloves were used when handling each individual to avoid cross-contamination. Swabs of the skin were taken of each individual on the ventral portions of the limbs and abdomen and under the marginal scutes. Individuals were then measured, sexed, aged, and weighed according to Holland (1994). Individuals were marked by notching the marginal scutes, as described by Reese (1996) and adopted from Holland 1994. We placed individuals according to their size (i.e., small, medium, medium-large and large) into other 10-gallon containers, each with no more than 6 turtles per container and held them until capturing was complete.

Swab samples were analyzed by Pisces Laboratory (Boulder, Colorado which assayed the sampled DNA for the presence of *B. dendrobatidis* ribosomal RNA Intervening Transcribed sequence (ITS) region. Each polymerase chain reaction (PCR) run included two laboratory culture controls, positive *B. dendrobatidis* DNA, and negative non-batracho chytrid fungus DNA (both supplied by Joyce Longcore to Pisces Laboratory). A control with water was also run.

Environmental temperature sampling: Two Hobo H8 temperature data loggers (Onset Computer Corp., Pocasset, MA) in watertight containers were deployed at the site to measure temperatures in degrees Celsius. One aquatic temperature logger was placed at a depth of 2 feet in water on the shore of the pond, attached to a line. The air temperature logger was deployed one foot from the ground on the southwest side of the pond in the shade to record ambient air temperature.

Results

Visual Counts: Between 3 and 95 *Emys marmorata* were observed at the pond between 2005 and 2007. Turtles were observed in the pond every month. Turtles were not detected in the pond only when ice covered it. The greatest numbers observed were typically in the months of May through August.

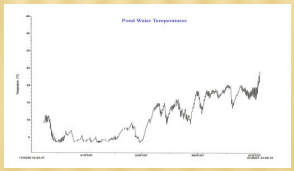
A total of 25 turtles were captured; 24 were marked and 1 was not marked due to the small size (34 mm plastron). Pictures of all individuals were taken of the dorsal and ventral shell. Of the 25 captured, 17 were females, 6 were males and 2 were unknown. We captured age classes between one year and approximately 12 years, with carapace lengths ranging between 34 mm and 122 mm (Table 1).

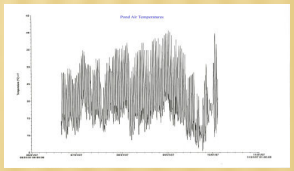
Table 1: Age, Carapace length and Plastron length of Population Sample

	Average	Range
Years	5.8 years	1 – 12 years
carapace	84.6 mm	34 -122 mm
plastron	76.36 mm	21 – 106 mm

***Chytrid Sampling*: None of the swab samples taken from *E. marmorata* returned a positive PCR signal for *B. dendrobatidis* ribosomal RNA (Pisces Molecular 2007).**

Environmental Temperatures: Aquatic temperatures varied between 3.5° C and 20.1° C. Terrestrial temperatures varied between -4.72° C and 34.85° C before July 2007. Between June 1st and July 17th temperatures greater than 28° C occurred between 2 and 9 hours per day on 26 separate days. Temperatures greater than 30° C occurred between 2 and 7 hours per day on 14 separate days (Figures 1 and 2). By September, the pond was dry.





Discussion

This study was conducted to determine whether *Emys marmorata* could either be infected with *B. dendrobatidis*, or could serve as an asymptomatic host reservoir for the chytrid fungus. Although alpha-keratin occurs on the skin of *E. marmorata*, it did not harbor the fungus in the 23 individuals sampled at this site in 2007. This negative finding for chytrid on *E. marmorata*, at a pond in which *B. dendrobatidis* has been detected within a portion of the resident frog population, does not support the notion that *E. marmorata* either contracts or serves as a host reservoir for this emerging infectious disease. However, there is some evidence that frogs may shed the fungus at certain times of the year (P. Johnson, pers. comm.), and our samples were collected in a single day. Additionally, Pisces Laboratory recommends a minimum of 30 samples per site in order to have a 95% confidence interval of finding a 5% infection rate. While our data certainly suggests that turtles do not harbor chytrid, we believe further testing – more individuals and different times of year – may be warranted.

Air and water temperatures at the study site were suitable to sustain *B. dendrobatidis*, with water temperatures below 20°C. Although air temperatures on occasion reached lethal conditions for the fungus (greater than 27°C (Berger, et al. 2003)), they did not match the duration shown in laboratory settings to be lethal to the fungus (Berger, et al. 2003).

A more complete knowledge of the hosts of *B. dendrobatidis* is one essential element in the understanding of the spread and, hopefully, management of the disease. Although other aquatic species such as turtles co-occur with amphibians in suitable habitat, the most obvious potential reservoir species for *B. dendrobatidis* appear to be other anuran and amphibian species (McCallum 2005). The fungus affects a wide variety of frog species, however population declines and species extinctions have occurred in only a few. To determine if other species are infected and potentially acting as reservoirs, we will sample *Pseudacris regilla* at the same site in the future.

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Acknowledgements

The authors wish express our appreciation to Cindy Roberts, US Forest Service, for her support to this project and her commitment as a wildlife biologist, and Maria Cisneros for her helpful assistance with survey work.