

# **Presence and preferred thermal range of Chytrid fungus pathogens in UCSC's Forest Ecology Research Plot (FERP)**

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## **Abstract**

Many scientists believe we are in Earth's sixth mass extinction event (Berger et al. 1998; Ceballos et al. 2015; Lips et al. 2006; Wake D.B. & Vance 2008). Global climate change causes deviations in ecosystem characteristics such as temperature, and precipitation (Karl & Trenberth 2003). Different environments experience alterations in their natural cycles increasing susceptibility to insect or pathogen outbreaks (Dale et al. 2001). *Batrachochytrium dendrobatidis*, a fungal pathogen commonly known as Bd, is responsible for massive decline of amphibian populations around the globe (Pounds et al. 2006). *Batrachochytrium salamandrivorans* (Bsal), is a species of chytrid that is specific to salamanders and newts. Amphibians are bioindicators of the health of an ecosystem; tracking population status is vital to their communities. North America has the highest salamander biodiversity in the world- making Santa Cruz an acceptable case study site. This investigation sought to answer the following questions: Is there a presence of Bd and Bsal on UCSC's Forest Ecology Research Plot? What temperature ranges are associated with the presence of Bd and Bsal in this area? We sampled UCSC's Field Ecology Research Plot (FERP) for both Bd and Bsal using coverboard methodology. Bd and Bsal tested negative for all subjects: California slender salamander (*Batrachoseps* spp.), Yellow-eyed ensatina (*Ensatina eschscholtzii xanthoptica*), and the Rough-skinned newt (*Taricha granulosa*). No Bd or Bsal was detected on sampled species. Thermal analysis of salamander locations, under coverboards and parallel "natural cover", the likelihood of salamander presence increases with higher mean temperatures. Conversely, cover items with higher average daily maximum temperatures were less likely to have salamanders present.

## **Introduction**

Many scientists believe we are in Earth's sixth mass extinction event (Berger et al. 1998; Ceballos et al. 2015; Lips et al. 2006; Wake D.B. & Vance 2008). Human activity is the main force behind this global occurrence. The adverse contribution to global climate change causes deviations in ecosystem characteristics such as temperature, and precipitation. (Karl & Trenberth 2003). Different environments experience alterations in their natural cycles from climate change that directly and indirectly impact the survival and spread of herbivores and pathogens (Dale et al. 2001). For example, herbivorous insect populations in northern regions are directly affected by an increase in the length of the optimal temperature windows (Bale et al. 2002). Predators and competitors that regulate the abundance of pathogens and pests are indirectly impacted by change in climate. Forests have the highest average annual damage per hectare in the United States, as a result of insect and disease outbreak (Dale et al. 2001). This kind of pathogen-driven species loss was examined in the cloud forest of Costa Rica. Two species of toads endemic to Monteverde- the Golden Toad (*Incilius periglenes*) and Harlequin Toad (*Atelopus varius*)- went extinct. The cloud forest experienced a dry spell due to an increase in climate fluctuations. There

decline was linked to the outbreak of *Batrachochytrium dendrobatidis*, a fungal pathogen commonly known as Bd. This same fungus is responsible for massive decline of amphibian populations around the globe (Pounds et al. 2006).

Bd is a fungal pathogen known for its lethal effects in amphibians. Chytridiomycosis, the disease induced by the fungal pathogen, is evident from thickening in amphibian skin, hindered osmotic regulation, and a drop in electrolyte blood cell levels. (Whittaker & Vredenburg 2011). Many species of frogs are affected by the disease's virulence, even with low levels of initial infection (Skerratt et al. 2007). However, Chytridiomycosis does not always lead to mortality. For example, some boreal toad populations in the Rocky Mountains (*Anaxyrus [Bufo] boreas*) coexist with Bd showing an annual population decline of 5-7% rather than a dramatic depopulation (Pilliod et al. 2010). The Pacific chorus frog (*Pseudacris regilla*), located in the western Sierra Nevada mountains, was identified as a reservoir species for Bd in a study by Reeder et al. 2012. *P. regilla* could carry massive loads of Bd that were lethal to sympatric species. Salamanders such as *Ambystoma tigrinum*, Tiger Salamanders, were able to coexist and even clear Bd. (Davidson et al. 2003).

Bd susceptibility varies among salamander species. *Batrachochytrium salamandrivorans* (Bsal), is a species of chytrid that causes Chytridiomycosis in salamanders and newts. Bsal has caused mass die-offs of the European Fire Salamander, *Salamandra salamandra*, in the Netherlands (Yap & Koo 2015). Bsal has only been documented in Asia, the Netherlands, Belgium, and Germany, although fear of intercontinental transmission has highlighted the dangers of the exotic pet trade (Yap & Koo 2015). Local transmission of Bsal, like Bd, is by release of aquatic fungi spores. Martel et al. in 2014 pioneered testing of different salamander species susceptibility, and found Bsal to be lethal to 12 of 24 tested salamanders. Few studies have been used to further research of Bsal.

It is important to continue to understand the spread of both Bd and Bsal in all organismal communities. Amphibians are bioindicators of the health of an ecosystem. Their survival depends on what their bodies exchange with their environment. North America has the highest salamander diversity. This biodiversity is threatened by the presence of major ports for the international amphibian trade (Yap & Koo 2015). The United States has 5 major ports for the salamander trade. California alone hosts 2 out of 5 (Yap & Koo 2015). To build the knowledge of Bd and Bsal distribution, I have conducted a case study in Santa Cruz, California. Data collection took place on the 16ha mapped and gridded Field Ecology Research Plot (FERP), located on UCSC's Natural Campus Reserve (Gilbert et al. 2010). In 2014 Bd was detected on amphibians sampled near UCSC's North Remote parking lot and within the campus's Upper Quarry (Barry Sinervo, unpublished data 2014). Both locations are within a 2 mile radius of UCSC's Natural Campus Reserve. On the FERP grid, 116 plywood coverboards were established in 2014-2015 (Horvath & Stevenson 2015). The herpetofauna of this area are surveyed biweekly during the rainy season (approx. Dec-May 2015 – 2017), though sampling duration has varied from year to year.

The study site coverboards are an established coversite for many herpetofauna in the area. The infrastructure and easy access to student volunteers has made this area a prime research location. The species of amphibians found on the FERP are the following: *Aneides flavipunctatus*, *Aneides lugubris*, *Batrachoseps spp.*, *Dicamptodon ensatus*, *Ensatina eschscholtzii xanthoptica*, *Taricha granulosa*, *Taricha torosa*, *Bufo boreas*, *Pseudacris sierra*, *Rana draytonii*, and *Rana boylei* (Horvath & Stevenson 2015). Due to the species' observed abundance, my study involved the California slender salamander (*Batrachoseps spp.*), Yellow-eyed ensatina (*Ensatina eschscholtzii xanthoptica*), and the Rough-skinned newt (*Taricha granulosa*). *Taricha granulosa* has a lethal reaction to Bsal (Martel et al. 2014). Little is known about the carrier capability of *Ensatina sp.*, and other salamanders present in the area. *Batrachoseps attenuatus* is speculated to be a carrier for Bd fungi outside of lab settings (Whittaker & Vredenburg 2011). This study will answer the following questions: Is there a presence of Bd and Bsal on UCSC's Forest Ecology Research Plot? What temperature ranges are associated with the presence of Bd and Bsal in this area? Chytrid fungus in culture can grow in temperatures from 4- 25 degrees Celsius, but has higher virulence and pathogenicity between 12- 23 degrees Celsius. (Whittaker & Vredenburg 2011). I hypothesis that if Bd and Bsal are present on the FERP, they will be found on amphibians that were present under both natural cover and coverboards in the fungi optimal temperature ranges.

## Methods

### *Field collection*

We searched for salamanders under 116 coverboards on the FERP as well as 116 paired "natural cover" counterparts. "Natural cover" was defined as log, up to 10m away from a coverboard, 3 cm to 50cm tall, and between 3 cm and 50 cm wide. We compared cover boards to natural cover to measure possible differences between artificial and naturally-occurring amphibian habitat (Fig. 1).

Salamanders were measured, weighed, and photographed biweekly from DATE to DATE (*sensu* Horvath & Stevenson 2015). Skin swabs were taken from salamanders encountered during April and May 2016. A polyurethane foam dry swab (VWR International company) was used to stroke each salamander 30 times; ten times dorsally and ventrally, and five times on each laterum, spanning the majority of individual's body length (Sette et al. 2015). Gloves were changed between handling every specimen. We swabbed 34 specimens, 13 found under natural cover and 21 found under coverboards.

Swabs were kept in 5ml sterile tubes and stored at 4°C until DNA extraction and qPCR testing was performed. Samples were sent to Vance Vredenburg's lab at San Francisco State University to be tested for the presence of chytridiomycosis using qPCR following Boyle et al. (2004) and Hyatt et al. (2007). The volume of PrepMan Ultra extraction mixture was doubled to account for swabs larger than the standard M113.

In order to compare microhabitat between cover types in the field, 33 iButton programmable thermometers were placed under coverboards and natural cover items randomly

chosen from four categories: coverboards with salamander presence, coverboards with no salamander presence, natural cover with salamander presence, and natural cover with no salamander presence. Coverboard categories were based on biweekly surveys of salamander presence during the rainy season (approx. Dec-May 2015 – 2017) after coverboard establishment. Natural cover categories were based on the presence of salamanders during the swabbing period April-May 2016. The iButtons recorded temperatures for 30 days total (5/21 - 6/4 and 6/28 - 7/12). After the first deployment, only 29 iButtons were recovered due to animal interference. After the second deployment, only 22 iButtons were recovered.

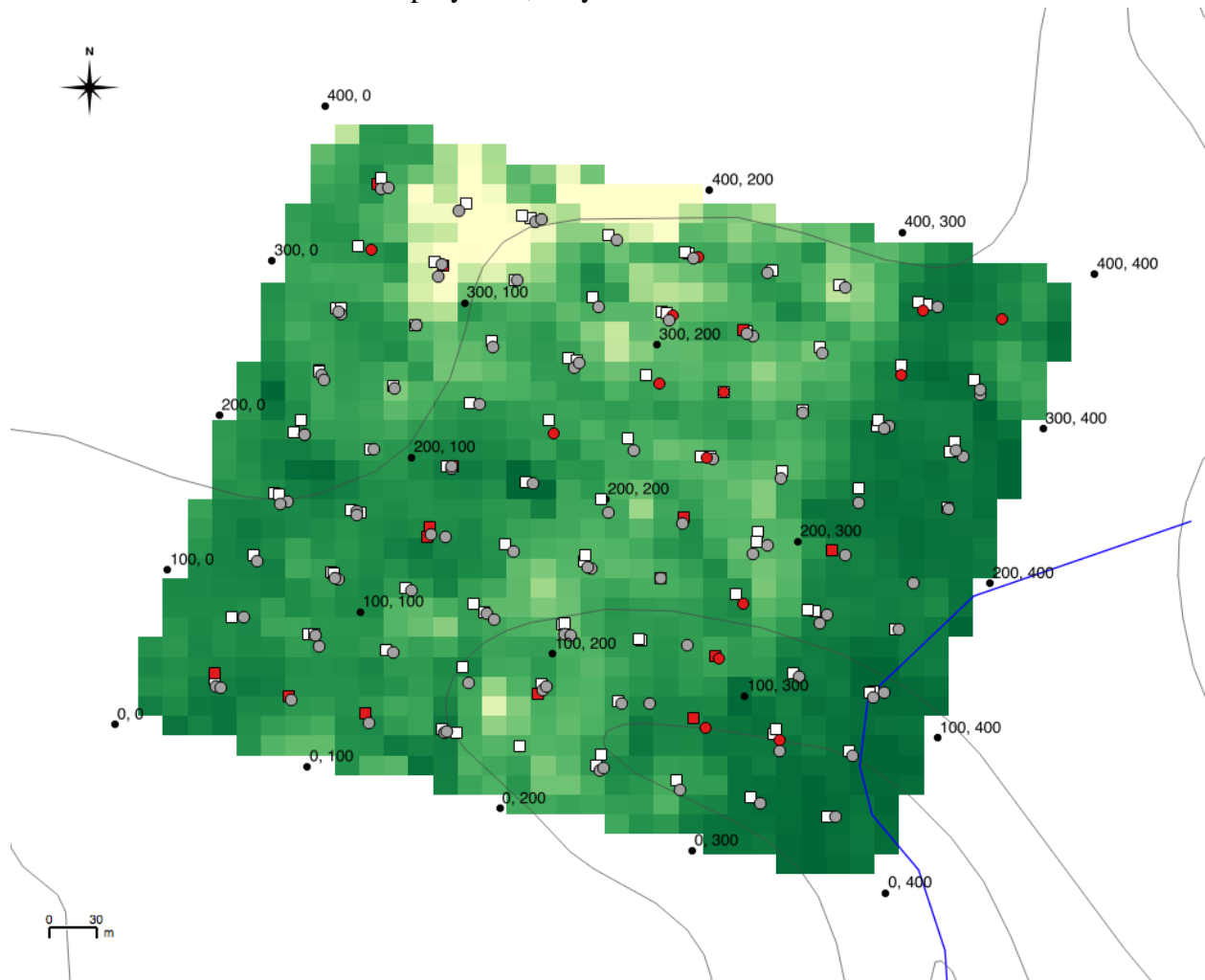


Figure 1. Map of the FERP. Percent canopy openness is shown in greenscale (denser canopy is green, more open canopy is yellow). Coverboards are indicated by squares, while natural cover is shown with circles. Cover objects where iButtons were deployed are indicated in red.

### *Climate and Terrain Data*

A canopy coverage analysis was conducted on UCSC’s FERP. Photographs of the canopy were taken at 20 meters distances from each other in a grid pattern throughout the FERP September 2014 to June 2015 using a high resolution digital camera and a 180° fisheye lens.

The computer program ImageJ was used to analyze light entering through the canopy by calculating the amount of tree foliage in each photo (Austenfeld & Beyschlag 2012). Photos were set to black and white in order to be properly analyzed (Greg Gilbert unpublished data). Quantum GIS was used to generate maps of the UCSC FERP (<https://ferp.ucsc.edu/research/data/>), including canopy coverage, and cover objects .

### *Statistical Analysis*

All statistical analysis was performed using R statistical software (2017). We compared iButton temperature data across cover type and salamander presence category using ANOVA.

We performed a generalized linear model (GLM) to assess whether temperature predicts salamander presence across cover types. We only included the cover objects which were the site of iButton thermometer deployment. For all models, the response variable was salamander presence or absence under each cover object throughout the salamander surveys performed November 2015 - May 2016. We performed a GLM with a binomial distribution. We included cover type (coverboard vs. natural cover) as a fixed effect in all the models, as the thermal properties seemed to vary between coverboards and natural cover. We compared models using average daily temperature, average minimum daily temperature, average maximum daily, and the square of each of those variables as model factors (Table 1). Average daily temperature, average minimum daily temperature, and average maximum daily temperature were collinear (Pearson test: mean and min temp: 0.75, mean and max temp: 0.47, and min and max temp: 0.51). Wald's test was used to compare AIC's to select the best model.

	<b>Response variable</b>	<b>Model factors</b>	<b>Fixed effect</b>	<b>AIC</b>
Model 6	Salamanders (0/1)	(Avg. max daily temp) <sup>2</sup>	Cover type	127.96
Model 5	Salamanders (0/1)	Avg. max daily temp	Cover type	126.55
Model 4	Salamanders (0/1)	(Avg. mean daily temp) <sup>2</sup>	Cover type	144.87
Model 3	Salamanders (0/1)	Avg. mean daily temp	Cover type	144.18
Model 2	Salamanders (0/1)	(Avg. min daily temp) <sup>2</sup>	Cover type	155.72
Model 1	Salamanders (0/1)	Avg. min daily temp	Cover type	155.72
Model 0	Salamanders (0/1)		Cover type	153.72

Table 1. Model factors included in the GLMM models we compared.

## Results

FERP surveys from 3/15/2015 to 1/20/2017 found 66 *B. attenuatus*, 0 *D. ensatus*, 244 *E. eschscholtzii*, 6 *T. granulosa*, and 0 *T. torosa* for a total of 317 amphibians. Surveys of natural cover performed from 4/9 to 5/7/2016 found 7 *B. attenuatus*, 2 *D. ensatus*, 4 *E. eschscholtzii*, 0 *T. granulosa*, and 0 *T. torosa* for a total of 14 amphibians. Quantitative PCR testing showed that all of the 34 salamander swabbed on 4/9/2016, and 4/24/2016 were negative for both Bd and Bsal.

Figure 2 shows the average daily minimum, mean, and maximum temperatures collected during the two rounds of iButton deployment for cover board and natural cover items both with and without salamanders. Locations with salamander presence ( $\geq 1$ ) and salamander absence (0) for both displayed a trend of salamander absence. cover board and natural cover, were within the same temperature range- except for the average max temperatures. Average max temperatures generally displayed a trend of salamander absence.

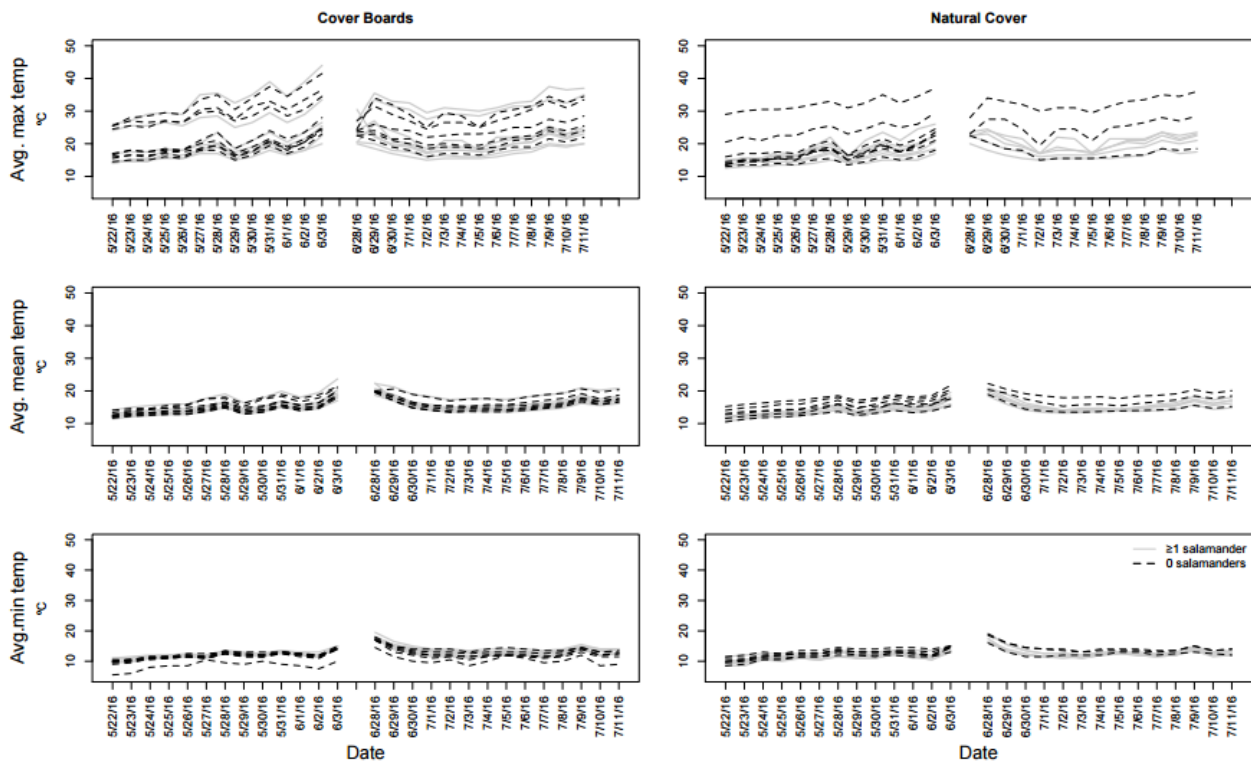


Figure 2. Daily maximum temperatures, daily mean temperatures, and daily minimum temperatures recorded by 33 iButton programmable thermometers from May-June 2016. The left column of figures show temperatures recorded under cover boards. The right column of figures show temperatures recorded under natural cover items.

Temperatures under cover objects where salamander presence was never recorded are shown with dashed black lines, and solid grey lines for cover objects where salamanders have been found.

Recorded maximum daily temperature, mean daily temperature, and minimum daily temperatures were all significantly different between coverboards and natural cover. Only maximum daily temperature and mean daily temperature were significantly different between the cover objects with salamanders present and those without salamanders present (Fig. 3). Results for the GLM support this. Both average mean daily temperature and mean daily temperature were significant predictors of salamander presence, however average mean daily temperature was a stronger predictor (Model 5 AIC: 126.55) than mean daily temperature (Model 3 AIC: 144.18).

The models predict that for both natural cover and cover boards, the likelihood of salamander presence increases with higher mean temperatures (Fig. 4). When temperatures were higher, coverboards were more likely to have salamanders under them than natural cover. Conversely, cover boards with higher average daily maximum temperatures were less likely to have salamanders present. Cover boards experienced higher daily maximum temperatures than natural cover items.

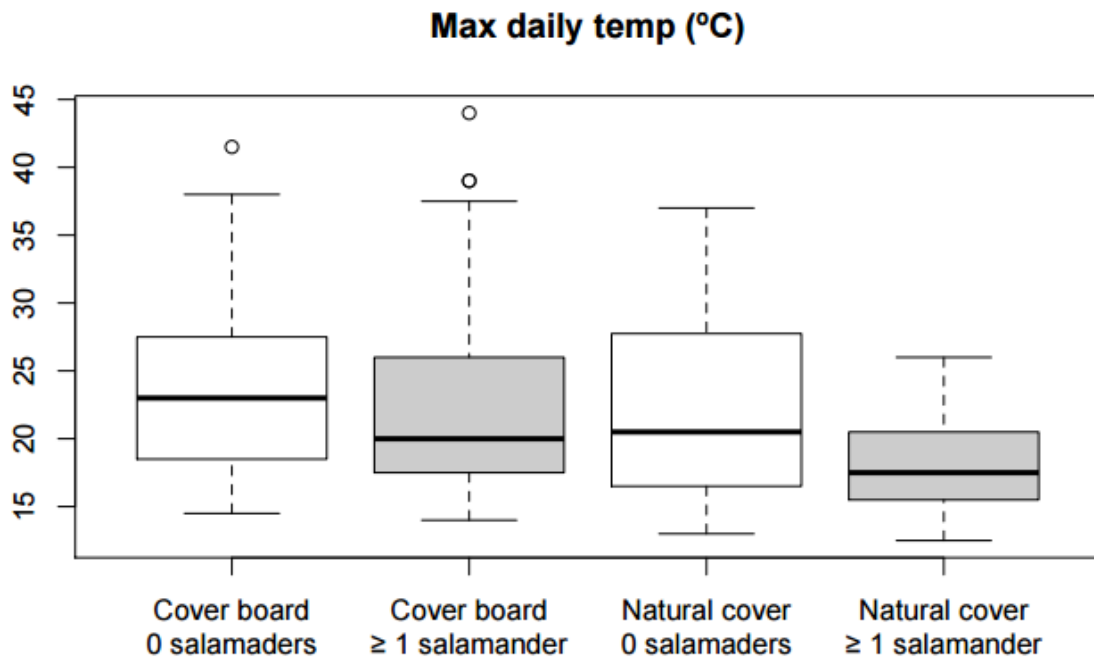


Figure 3. Boxplots of daily maximum temperatures, daily mean temperatures, and daily minimum temperatures by cover type and cover items with salamander presence and with no salamander presence throughout the FERP surveys.

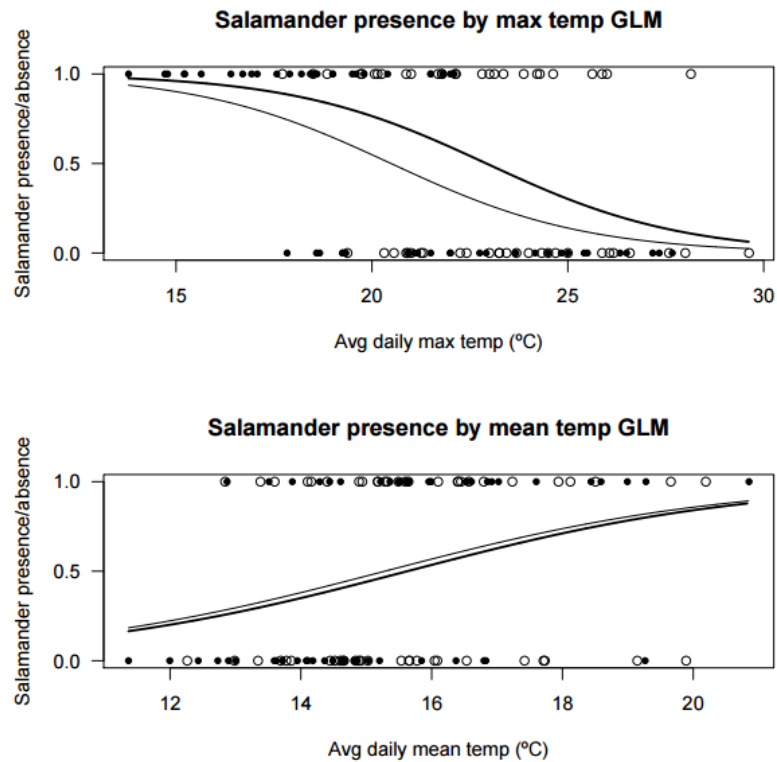


Figure 4. Model predictions for the two most significant generalized linear models (GLM). The model which includes daily average max temperature is above and the model which includes mean daily temperature is in the lower panel. The prediction for natural cover items is shown in the lighter line, and the prediction for cover boards is the thicker line.

## Discussion

### *Bd* & *Bsal*

We found no positive *Bd* or *Bsal* individuals on the FERP. This could be attributed to the small sampling time frame and size. *Bd* was located in historical samples of Santa Cruz county from the early 1970s (Sette et al. 2015; Padgett-Flohr & Hopkins 2009). The results do not necessarily represent all possibilities for the *Bd* pathogen- it may still be present in or around the sampled area. A disease in a susceptible population can be categorized in two ways- epizootic or enzootic. Epizootic disease pathogens are novel to an area and may cause extreme damage to populations once introduced because of native populations lacking proper defenses. Enzootic diseases decline in virulence over time and are absent or present in a population but no longer harmful. Chytridiomycosis is likely enzootic on the FERP to susceptible species, such as *Batrachoseps attenuatus*. Field *B. atrachoseps attenuatus* tested positive for *Bd*, but populations remained stable with seasonally variable infection rates (Cummer et al. 2005; Weinstein et al.



2009). *B. attenuatus* had 100% mortality in the lab when collected with Bd, but appear to be able to shed the pathogen in higher temperatures and drier conditions (Weinstein et al. 2009).

It is possible that *B. attenuatus* populations act as vectors of Bd transmission to more susceptible species on the FERP during colder seasons. A dead California Red- legged frog (*Rana draytonii*) tested positive for Bd and was located in UCSC's arboretum were a small population aggregate to breed (Carla Sette, per comms. 2017) . *R. draytonii* are considered vulnerable (IUCN 2008) and are especially difficult to find in Santa Cruz. It is crucial to continue to monitor the population abundance and health in order to preserve the Santa Cruz county population. Additional sampling of the FERP, the surrounding area, and sampling of *Rana draytonii*- is necessary for further investigation.

### *Temperature analysis*

Temperature analysis revealed that coverboards were more likely to have salamanders under them, when temperatures were higher than natural cover. This is likely because of the shorter sampling time frame for the natural cover. A higher sample size could provide further insight as to the thermal preferences of FERP species. Time during temperature measurements could also be lengthened to better understand the fluctuation of temperature fluctuations and salamander preference. A long-term monitoring program of FERP temperatures would further our understanding of species thermal preferences. Extreme temperatures exhibited by the coverboards also suggest that coverboard monitoring protocol may be better suited for reptiles rather than amphibians.

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