# Thermoregulation in Montane and Coastal Species Of Native Hawaiian Damselflies

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**Abstract**

Thermoregulation in native Hawaiian damselfly genus *Megalagrion* can be used to determine slight changes in temperature in regards to global warming. Hawaiian damselflies are an indicator species of habitat health and degradation. Through field and lab experiments the males and females of the *Megalagrion calliphya* and *Megalagrion xanthomelas* species were found to have different thermoregulatory processes due to their different colorations and place in which they inhabit. The temperature differences exhibited by different sexes and between two female color morphs show that there is a significant difference, and may be the cause of their different behavior choices.

**Introduction**

The endemic Hawaiian damselfly genus *Megalagrion*, order Odonata, family Coenagrionidae inhabits the most isolated archipelago in the world and is a spectacular group that is valuable for biological research. *Megalagri*on damselflies have radiated from a single ancestor into 23 species occupying a wide range of aquatic habitats (Polhemus 1997). Hawaiian damselflies have a diverse range of coloration from red, black, orange, yellow, blue, and green (Polhemus & Asquith 1996). In some species the males are different colors or shades than the females, which makes observation very interesting (Polhemus & Asquith 1996). They have undergone a spectacular adaptive radiation into many different habitats including streams, waterfalls, rainforest, reservoirs, cattle ponds and coastal anchialine ponds (Polhemus & Asquith 1996). Distribution and breeding habitats vary from species to species; some are restricted to high elevations, some to lower coastal elevations, and some even to the different islands (Polhemus & Asquith 1996).

Damselflies are a very important species within an ecosystem because they are both terrestrial and aquatic in different stages of their life; thus, they can be indicators of health in aquatic ecosystems (Polhemus 1997). A healthy aquatic ecosystem consists of native plant and animal species, with no pollution and other habitat degradation that threatens the existence of the damselflies (DiSalvo 2003). Additionally, Hawaiian damselflies are a sensitive and useful source to measure global change in hydrologic systems in Hawai'i because they display strong ecological interactions with other major components of communities along elevational gradients (USGS BRD).

A candidate endangered species, *Megalagrion xanthomelas* are often associated with anchialine ponds. Anchialine ponds are unique habitats because they have no coastal connection to the sea, but still demonstrate tidal fluctuations that influence salinity gradients due to a subsurface connection (Chai 1989). Due to many anthropogenic influences changing the anchialine pool ecosystems as well as the introduction of non-native predators such as mosquito fish and guppies, many rare anchialine pool species such as crustaceans and invertebrates are threatened and of concern.

In previous population density experiments conducted along the Hilo coast in the summer of 2004, Lori Tango found that there were more male *M. xanthomelas* perched in the sun while the female congregated in the shade. From these observations, she speculated that the two sexes have different thermoregulatory processes. Thermoregulation is the maintenance of temperature by active behavioral or physiological responses of an organism in its natural environment independent of the environmental temperature (May 1979). The ability of an organism to thermoregulate depends on the ability of the organism to control the amount of heat stored in the body. Light preferences and thermoregulatory processes may affect the color of males and female *Megalagrion*, and these patterns may also change with elevation. It was hypothesized that there would be a difference in thermoregulation rates between male and female *M. calliphya* and *M. xanthomelas*. It is also

hypothesized that the green female *M. calliphya* color morph will have a higher body temperature than the red female *M. calliphya* color morph.
Using both field and lab experiments the specific thermoregulation ability of both males and females in two different damselfly species, *M. xanthomelas* (coastal species) and *M. calliphya* (montane species) were examined. The majority of the testings were with *M. calliphya*, because they are the most commonly found species on Hawai'i Island and not a candidate endangered species like *M. xanthomelas*. This experiment helped to explain habitat choice, behavior, and evolution of color differences between male and females.

**Materials & Methods**

*Field Collection:*

Four sites on Hawai'i Island were selected to collect *Megalagrion* damselflies. *M. calliphya* was collected at eleven artificial pools, set up by Idelle Cooper in 2002 outside of the research station at Hawai'i Volcanoes National Park (HAVO) at about 3,500 feet and at a bog habitat above Kulani Prison at about 6,000 feet. Before and after collection, air temperature, cloud cover, precipitation & wind were quantifiably measured. The Beaufort Rain & Wind scales were used to measure the precipitation and wind. The damselflies were collected using fine meshed nets and transported into plastic containers with a few blades of grass after capture. Upon capture, color, sex and behavior were noted. *M. xanthomelas* was collected at anchialine pools at Ninole, Ka'u and at Kaloko-Honokohau Historical National Park (KAHO) in Kona. The same materials and methods for collecting *M. calliphya* were also used to collect *M. xanthomelas*.

*Lab Test Trials & Observations:*

After collecting male & female *M. calliphya*, they were transported back into the field lab in plastic containers or collecting nets to conduct the thermoregulation experiments. Before making the thermo-models, they were each weighed by placing a piece of paper over the live damselfly on a Denver Instrument Company A-200DS scale to measure their mass before exposing them to different light intensities to find out if desiccation from light exposure would have a great affect on their mass.

Depending on the damselflies caught, a pair of damselflies were selected to test. The testing is done on a green female & red female, a green female & red male or a red female & red male. After selecting a pair, the "Intermatic DT17C" timer was programmed to turn on and off the "plant grow" lamp for an hour, five minutes after starting the experiment. The "HOBO U12 J, K, S, T" thermocouple data logger was then programmed to a delayed start to give enough time to set up the rest of the experiment. The first part of the thermo-models using the "Onset 6' Beaded Type J" thermocouple was constructed by attaching it to the "HOBO" thermocouple data logger. One of the damselflies from the selected pair was chosen; then a tiny incision on its right side was made allowing to easily insert the tip of the thermocouple into the damselfly. These steps were repeated for the other damselfly. To complete the set of thermo-models, the two damselfly thermo-models along with one without a damselfly attached to measure the ambient air temperature was needed.

A black piece of construction paper was placed under the lamp to resemble the black lava rock substrate damselflies would normally perch on. The set of thermo-models were placed onto the black construction paper orientating the damselflies so they face the same direction, and are at the same height of 10cm above the substrate. The final step to set up the experiment was to place a wind block around the lamp and set of thermo-models using cardboard box with peep-holes so observations can be made while the experiment is being conducted. All of these steps to set up the experiment are done before the thermocouple loggers start logging (20min. is a sufficient amount of time).

After exposing the thermo-models for an hour to the different light intensities, the thermocouple data loggers were connected to a computer and data was imported into the "HOBOware" program. The "HOBOware" program provides a graph of the logged data from the whole experiment. To get a graph of specific times and intervals, the data was exported into "EXCEL," made into spreadsheets and graphs to be analyzed later.

After data was imputed into the computer, damselflies were removed from the thermocouples to be measured again on the scale. If both damselflies were alive after the experiment, they would be reused again to run another experiment. If they were dead, they were scanned using a "HP Scan Jet 5300C" scanner. The pair along with a ruler placed upside down was scanned into the "HP Precision Scanning" program. The damselflies were placed as straight as possible and labeled. The "dpi setting" was set at 1200, and it was scanned as a "color photograph." After scanning the image of the damselflies and part of the ruler, it was cropped, and saved as a "jpeg" file.

*Field Test Trials & Observations:*

After collecting males and females, they were set-up in the same way as in the lab trials, except they were turned up so the left side of their thorax would face the sun. The trial was only conducted for 10 minutes instead of the full hour. After the trials the data was also imported into the computer to make graphs and they were also scanned. The field comparisons were able to validate differences we saw in the lab between sexes and species.

*Statistical Analysis:*

Field and lab data was analyzed first with "Wilcoxon" tests in "SYSTAT 11.0" to see how many times one had a higher temperature than the other. Those numbers were then analyzed using "Z-test" in "SYSTAT 11.0" to determine "z" & "p" values and if there was a difference in thermoregulation rates between sexes and species.

**Results**

Through the data and graphs from the lab trials for *M. calliphya*, it was found that the ambient air temperature was always lower than the damselfly body temperature. *M. xanthomelas* was never tested in the lab, but for the outdoor sun trials the ambient air temperature was always lower than the damselfly body temperature for both *M. xanthomelas* and *M. calliphya*. In outdoor shade trials the ambient air temperature was higher than the damselfly body temperature for *M. xanthomelas*. The body temperatures for *M. calliphya* had increased slowly over time with each trial conducted in the lab. Experiments comparing a live damselfly with a dead damselfly had shown that the live damselflies had a higher body temperature than the dead damselflies for three replicates of female *M. calliphya*. When comparing green *M. calliphya* females to red *M. calliphya* females the green *M. calliphya* female morphs had a higher body temperature 8 out of the 10 lab trials with a "z" value of 2.878 and a "p" value of 0.004. The green *M. calliphya* females had a higher body temperature than the red *M. calliphya* females 2 out of the 3 outdoor trials with a "z" value of 0.832 and a "p" value of 0.405.

When comparing green *M. calliphya* females with red *M. calliphya* males, the green *M. calliphya* females had a higher body temperature than the red *M. calliphya* males in 9 out of the 11 lab trials with a "z" value of 3.235 and a "p" value of 0.001. In the outdoor trials, the green *M. calliphya* females had a higher body temperature than the red *M. calliphya* males 3 out of 3 trials with a "z" value of 3.848 and a "p" value of 0.000.

When comparing the red *M. calliphya* females with red *M. calliphya* males, the females had a higher body temperature than the red *M. calliphya* males 4 out of 5 of the lab trials with a "z" value of 2.035 and a "p" value of 0.042. In the outdoor trials comparing the red *M. calliphya* females with the red *M. calliphya* males, the red *M. calliphya* females had a higher body temperature 4 out of 6 times with a "z" value of 1.177 and a "p" value of 0.239.

The results from outdoor shade trials conducted on *M. xanthomelas* showed that the red *M. xanthomelas* male had a similar body temperature to the tan *M. xanthomelas* female. In the outdoor trials, the red *M. xanthomelas* male had a higher body temperature than the tan *M. xanthomelas* female.

**Discussion**

When conducting experiments in the lab, the data need to be analyzed very carefully. It had to be taken into consideration the possibility of complications the results may encounter when using artificial habitats to run the trials. The "plant-grow" lamp was used to replicate the sun, but the light was dispersed unevenly and the placement of the damselflies had a tremendous effect on individual body temperatures. The lamp also heated up very quickly, so desiccation of the damselflies under the lamp may have skewed the data slightly.

Although only a few outdoor trials were conducted, we were able to obtain more conclusive results than the lab trials. The first 15 minutes of the lab trials seem to correlate with the trials conducted outdoors, so when analyzing the data and graphs, only the first 15 minutes of the lab trials were used.

The green *M. calliphya* female morphs had a higher body temperature than the red *M. calliphya* females, which may be one reason the green female morphs are rarely found at higher elevations due to their coloration absorbing more heat at sites with higher maximum solar radiation. Results of temperature experiments on *M. calliphya* were similar to those for *M. xanthomelas*, which may be due to the similar colorations of both females and males. With the similar results of *M. calliphya* and *M. xanthomelas* we were able to conclude that may have been the reason the male *M. xanthomelas* were in the sun while the female *M. xanthomelas* were in the shade.

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