Brandon Sanchez, MESc Student

Testing tail muscle performance of larval wood frogs (Rana sylvatica)

Research Highlights

- Climate change has the capability to change phenotypic change as a result of rapid development in ectotherms.
- This study is the first documented use of electromyography on wood frog larvae.
- Results suggests that neuromuscular recruitment of tail muscle fibers in larval wood frog tadpoles is positively associated with development rate.

Research Summary

In cases where an organism's phenotype determined by their genetic influences counters its environmental influences, a countergradient variation occurs. Countergradient variation also demonstrates diminishing phenotypic change along a gradient such as temperature. Decades ago, ecologists discovered that populations of both fish and amphibians displayed countergradient patterns in temperature specific development rates. Within some range of temperatures, all ectotherms develop faster when temperature increases. But it was also discovered that populations from colder natal environments (farther north in fish and higher in elevation in frogs) would develop faster than their counterparts when placed in a common garden experiment at a specific temperature.

On one level, this pattern of population divergence makes sense. Most animals are ectotherms and most undergo metamorphosis. In their embryonic and larval phases, there is a strong premium on getting to metamorphosis quickly so that they can undergo seasonal migrations, increase in body size prior to the onset of winter, avoid pond drying or other seasonal events, and reach adulthood to begin reproducing. The fact that individuals from colder environments have that challenge compounded seems clear. But these findings also show that some populations, e.g., those from warmer environments, do not develop as fast as they could. Why? Countergradient variation suggests a tradeoff, but despite many studies of this phenomenon it wasn't until recently that evidence emerged what that might be. Yale College graduate Kaija Gahm published a study which clearly shows that wood frog (*Rana sylvatica*) larvae from warmer populations have lower swimming burst speeds by 20%. These findings imply, but do not demonstrate, that muscle physiology may be compromised by rapid development. While some evidence that extremely rapid development in ectotherms can compromise tissue function exists, there is lack of studies demonstrating if the neuromuscular recruitment of muscle fibers is similarly compromised.

Research Summary cont.

The purpose of this study is to take this line of research to the next step and determine whether there is evidence of a trade-off between development rate and neuromuscular recruitment of muscle fibers that will drive countergradient variation in development rates.

Beginning February 2022, I collected wood frog embryos across seventeen ponds including nine ponds at Yale-Myers Forest and five ponds from Madison, Connecticut. Twelve embryos from all ponds were put into four clutches with three embryos in each. Each clutch corresponded to the egg mass they were collected from. Embryos were kept in a temperature-controlled animal room kept on a 12:12 hr light:dark cycle at 12°C. After hatching, tadpoles are reared individually in glass jars. Throughout their growth, embryos and tadpoles had their development rate recorded.

Once the tadpoles reached a suitable size, the neuromuscular recruitment of their tail muscle fibers was measured using electromyograms (EMGs). EMGs record the electrical activity of muscle tissue by inserting electrodes into a muscle which can be used to measure how well their tail muscles are working. EMGs were recorded using bipolar hook electrodes made from insulated alloy silver wires, formed into a hook, and threaded into 29 G hypodermic needles. The electrodes were implanted deep below the right hand lateral surface of the tail muscle on non-anesthetized tadpoles. Following the electrode implant, tadpoles were placed in a custom experimental arena followed by a gentle tap on their tail to simulate a predator attack. The resulting "burst" swimming allowed us to record the tadpoles neuromuscular activity in its tail.

The results from these experiments show that EMG procedures can be done on wood frog tadpoles, something that has yet to be accomplished, and that the neuromuscular recruitment of tail muscle fibers is positively correlated with the development rate of tadpoles. Thus, revealing that while a tadpole's burst speed depreciates the faster it develops, the tails neuromuscular functions do not. This difference could be due to the varying shapes of tails tadpoles have that may change how fast a tadpole swims but not its muscle function or a behavioral change when exposed to a variety of extreme temperatures during development. Further studies using EMG alongside tadpole tail morphometrics is needed to understand this line of questioning better. As species evolve in response to climate change, revealing the structure of thermal tradeoffs is fundamental to understanding the consequences and responses of species living in a rapidly changing world.