Methods

Nineteen male *Anolis carolinensis* lizards were obtained from Carolina Biological Supply and underwent identical treatment in the following manner.

**Maintenance**

The lizards were housed in individual plastic terraria numbered 1-19. The terraria were arranged in rows of 5 and 4, with each cage separated by a small potted plant. Line of sight was possible for lizards adjacent to one another. Rows were separated by cardboard. The lizards were placed on a diurnal cycle of 12 hours of light: 12 hours of darkness. Each cage contained an electrically heated rock, and was exposed to heat lamps 24 hours a day. Feeding took place every three days. Each lizard was given at least two live crickets at feeding. The cages were misted every day to provide drinking water and moisture.

**Endurance**

Endurance, time to exhaustion while running at a low consistent speed, was measured using a treadmill. The treadmill was set to approximately 0.5 km/hour. Testing occurred on two afternoons, with each lizard running both days. The longest endurance and fastest sprint speed of each lizard was used in analysis.

**Competitions**

In order to test for dominance, lizards were placed in paired competitions. The pairings were based on the mass of lizards so the effects of body size on dominance would be lessened, with the two largest lizards competing and so forth down to the two smallest lizards. The two lizards for each competition were placed in a novel arena. The first 20 minutes of each competition was monitored with a video camera. Pairings were maintained for approximately two weeks to allow further observations of dominance behavior and success in feeding competitions.

Dominance was ultimately determined as a combination of factors. The video of each fight, feeding competitions, and experimenter consensus were factored into the decision. In certain situations, the video did not display a clear winner, but during feedings, one of the lizards ate more of the crickets, or on multiple days following the initiation of the competition one lizard would appear to be more dominant. The appearance of dominance included dewlap displays, push-ups, and maintenance of position atop the heat rock.

Analysis would follow comparing endurance of a lizard
METHODS

We chose approximately thirty mussels of similar size for our trials. The mussels were purchased from a local seafood store, transferred to the lab, and held in a recirculating seawater aquarium at approximately 17 °C for up to 24 hours prior to use. We had one group of control mussels and three groups treated with varying ethanol concentrations. For the control group, seven mussels were selected and the gills exposed by inserting a knife and cutting the posterior and anterior adductor muscles, which made the valves fall away from each other. Then excess body mass was cut away and the gill was rinsed in seawater. Only one gill per animal was used in order to get the maximum number of different samples. No gill had been exposed for more than five minutes prior to testing.

For each mussel, 100μl of saline was applied to the gill. A one-centimeter length of clear ruler was placed directly on the gill and carmine particles were dropped on the gill forty-five seconds application of either saline or the appropriate ethanol solution. The number of seconds a carmine particle took to travel 3mm or more was recorded and normalized to μm/sec. A dissecting microscope was needed to observe carmine particle movement. The procedure was repeated for all mussels in the control group. We exposed mussel gills to three different concentrations of ethanol (Sigma Chemical) diluted in 3% NaCl; 1% (eight mussels tested), 0.5% (seven mussels tested), and 0.25% (eight mussels tested). We followed the same procedure for the non-control groups, applying ethanol to the gill instead of saline. All data was imported into a graphing program and a dose-response curve was created showing the mean and standard deviation for all groups.

How were differences determined?
MATERIALS AND METHODS

Subjects Use

(add Latin 1st name)

The blue mussels were purchased at a local seafood store where they were kept in a lobster tank until being transferred to a lab at Bates College. In the lab, the mussels were stored in a recirculating seawater aquarium for up to 24 hours prior to testing at approximately 17° C. The mussels were treated with caffeine that came from Sigma Chemical.

Experimental Design

It was originally predicted that caffeine would increase the CTR of blue mussels and that, as the concentration of caffeine increased, the carmine would move more quickly across the mussel gill. (The only variable in the experiment was the concentration of caffeine applied to the mussel gill. The control (n=7) was a 3.33% NaCl solution that resembled normal seawater salinity.

Caffeine was tested at concentrations of $10^{-2}$ (n=5), $10^{-3}$ (n=7), $10^{-4}$ (n=7), and $10^{-5}$ M (n=6).

CTR was measured as a carmine solution was applied to the mussel’s gill. This speed was measured in mm/sec and then normalized to um/sec.

Protocol for Collecting Data

The caffeine was diluted in a 3.3% saline solution into concentrations of $10^{-2}$ M, $10^{-3}$ M, $10^{-4}$ M, and $10^{-5}$ M. With a dissecting knife, a mussel was opened and half of its shell was discarded. The remaining gill was treated with 20 µl of saline solution or caffeine, and after 30 seconds the excess solution was dumped off. A small section of a ruler with mm markings was set on the gill next to the area that was treated. With the mussel being held flat, 2 µl of carmine solution was deposited at the same location as the caffeine solution. Using a dissection microscope, a particle of carmine that was moving across the gill was picked and the time that it took for it to move three millimeters across the gill was measured.

Data Analysis

The ciliary transport rate was converted to µm/sec. The mean and standard deviations for each group were calculated and compared. Means were considered significantly different if, when graphed, the negative standard deviation of one was higher than the positive standard deviation for another.
RESULTS

The concentrations of d-tubocurarine ranging from $4.0 \times 10^{-8}$M to $4.0 \times 10^{-2}$M produced visible effects on the force of contraction of the gastrocnemius muscle in frog 1 and 2 (Figure 1). Five minutes after the injection of $4.0 \times 10^{-8}$M d-tubocurarine, the force of contraction was 13% and 3% less than the control for frogs 1 and 2, respectively. As the dosage increased the force of contraction continued to decrease (37% and 9% at $4.0 \times 10^{-6}$M; 93% and 14% at $4.0 \times 10^{-4}$M in frogs 1 and 2, respectively, and 66% at $4.0 \times 10^{-2}$M in frog 2). In frog 1, the $4.0 \times 10^{-2}$M solution was not tested as a 93% decrease suggested near paralysis of the muscle. These data indicate that increasing concentrations of d-tubocurarine did have an effect on the force of contraction produced in the gastrocnemius muscle.

![Graph showing force of contraction vs. concentration](image)

Figure 1. The concentrations of d-tubocurarine ranging from $4.0 \times 10^{-8}$M to $4.0 \times 10^{-2}$M produced visible effects on the force of contraction of the gastrocnemius muscle in frog 1 and frog 2. A control was measured without the addition of d-tubocurarine. The force of contraction was measured using the computerized oscilloscope. As dosage increased the force of contraction continued to decrease. Increasing concentrations of d-tubocurarine decrease and eventually inhibit muscle contraction.

Discussion

- Proper way to refer
- Comparing magnitudes - great!
- Noting a trend - great!
- Conclusion - move to
- Discussion

Move lower and box in entire Figure.
RESULTS

The mussels exposed to $10^{-5}$, $10^{-3}$, and $10^{-4}$ M caffeine had mean value transport rates and standard deviations that were similar to that of the control (Fig. 1). $10^{-5}$ M (n=6): 184.88 μm/sec, SD=52.79 μm/sec; $10^{-4}$ M (n=7): 196.43 μm/sec, SD=56.59 μm/sec; $10^{-3}$ M (n=7): 155.80 μm/sec, SD=96.73 μm/sec; control (n=7): 190.48 μm/sec, SD=46.46 μm/sec. Mussels treated with $10^{-2}$ M caffeine had a very low mean CTR and very high standard deviation (n=5; 46.15 μm/sec, SD=103.19 μm/sec), and it was the only group with a significant change in CTR compared to the control.

Figure 1: Mean ciliary transport rate (± SD) of carmine particles over the gill of intact blue mussels (*Mytilus edulis*) after the application of caffeine (for $10^{-4}$, $10^{-3}$, and control n=7; $10^{-2}$ n=5; $10^{-5}$ n=6).