Analysis of Methods to Measure Heart rate in *Mytilus edulis*

Kristin Pinho★ and Linda Auker, Ph.D.
Department of Biology, Misericordia University, Dallas, PA 18612

Introduction:

- The Atlantic blue mussel, common mussel, is a marine bivalve mollusk and is part of the family Mytilidae
- Wide distributional range due wide fluctuations in desiccation, salinity, temperature and oxygen tension
- Occupy the intertidal zone along the North American Atlantic coasts (Galaktionov *et al.*, 2015)
- For protection or food, mussels move by releasing byssal threads and use its foot to migrate
- Mussels feed using a filter-feeding mechanism
- Detritus and plankton are filtered from the water by cilia located inside the mussel
- Creates a current pulling in water and plankton (Koehler *et al.*, 2008)
- "Super-filters" that help conquer water pollution by taking in microplastics, pesticides and other water pollutants
- As water is filtered through gills, mussels store all the substances that pass through
- Used as bio-indicators of the health of the oceans, lakes and rivers
- Studies are conducted in various locations to monitor water quality
- After completion, researchers dissect mussels to determine chemical substances present in tissues (Kazour *et al.*, 2020)
- Studies are conducted to observe affects water pollution has on organisms that inhabit these locations
- Research will add to the existing knowledge on the affects water pollution from run-off has on the mussels, while observing the most efficient use of materials to collect data (Kazour *et al.*, 2020).
- Investigating the most effective approach for methods will be critical in providing a better foundation for future research.
- **For this experiment, there will be a significant difference in the heart rate of blue mussels when their environment is treated with glyphosate.**

Our objectives for this research project was:

1. **To examine the effects glyphosate has on mussel heart rate**
2. **To determine which methods and what materials were most effective when conducting research and obtaining results**

Materials & Methods

Tanks

- Three tanks were set up with different concentrations of Roundup weed killer
- 15 mussels placed in the control, 100 µg/L and 1000 µg/L tanks
- Figure 3 shows the three treatment tanks
- Salinity of the water was maintained at 30 psu
- Tanks were labeled in order to differentiate each treatment
- A drop of phytoplankton was administered into each tank on alternating days as a source of food
- Mussels were given one hour to acclimate to new environment
- 12 hours after the introduction of glyphosate, the HR were observed.

Concentration

- For the 100 µg/L and 1000 µg/L tanks, 2.5 L of saltwater (created using Instant Ocean) was mixed with 0.5 L of the specific concentrations
- The control tank was filled with 3.0 L of saltwater.

Paraffin Wax

- Paraffin wax was melted using a hotplate
- The hot wax was placed in a bowl and the mussel was secured to the bowl by the paraffin wax (Fig. 1)

Hot Glue Gun

- Holds the mussel to the bowl O-rings during monitoring
- Used to hold the HR sensor to the mussel
- Three 1.5 cm rings were stacked and glued using the glue gun (Fig. 4)
- Location that provides the clearest HR was located (Fig. 5)

PicoScope

- Baseline HR were obtained prior to the introduction of the treatments (Fig. 6)
- PicoScope 6 software used to observe the HR
- Ran in one second intervals Software detects HR by using the PicoScope2405 oscilloscope
- Graphically displays the electronic signals and changes over time
- Oscilloscope is connected to laptop and to AMP 03 Heartbeat monitor with an attached sensor that is placed onto the mussel (Fig. 2)
- Noninvasive by using infrared sensors to detect HR without additional stress
- 15 mussels from each tank were removed for one-minute HR monitoring

Figure 3: Mussels in glyphosate treatment tanks (control, 100 µg/L, 1000 µg/L)

Figure 4: O-rings hot glued to heartrate location on posterior side of mussel

Figure 5: Heart rate sensor connected to the mussel and secured by O-rings

Figure 1: Paraffin wax setup

Figure 2: PicoScope2405 oscilloscope connected to heartbeat monitor

Results:

Heart Rate

- Average beats per minute for mussels is 84 BPM
- Undetected for 100 µg/L concentration and 1000 µg/L concentration
- Very few peaks were observed
- Little activity in comparison to the baseline heartrates of the mussels (Fig. 7 and Fig. 8)
- Lack of peaks was a result of misplacement of the O-rings and the obstruction of glue

Discussion & Conclusion

Paraffin wax

- Drew up concerns regarding the affects it may have on the mussels
- Placing hot wax on the mussel just before collecting HR measurements may impact the HR
- Extreme heat can stress the mussels
- Process was very long as we needed to set up the hotplate, beaker and foil and wait for the wax to melt

Hot glue gun

- Technique was successful when securing the HR sensor to the mussel
- The heat may add stress to the mussels, thus causing an increase or decrease to the normal HR
- Mussels must lay out to dry before using the glue gun
- Water affected how successful the hot glue worked in securing the mussels to the plate and to the O-ring

O-rings

- Useful when holding the sensor to the mussel for HR detection
- Location on the mussel that provides the best HR detection must be found before gluing
- O-ring placement wasn't accurate
- Would often fall apart with force Curvature on the shell did not provide a steady surface for sensor to adequately read the HR
- There were very few HR peaks when observing the treatment tanks with the concentrated glyphosate (Fig. 7 and Fig. 8)
- May have resulted from insufficient methods that were utilized throughout this research
- Future research to be conducted to improve hot glue and O-ring's methods
- Methods provided results but had minor flaws
- Further research should consider the effects some methods may have on the mussels being studied.

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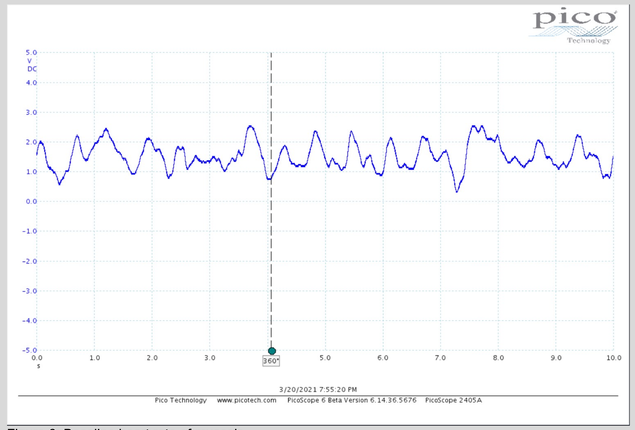

Figure 6: Baseline heart rate of mussels

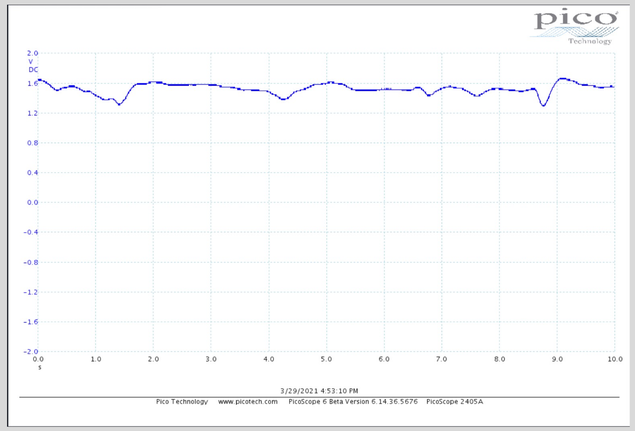

Figure 7: Heart rate of mussel in 100 µg/L concentration

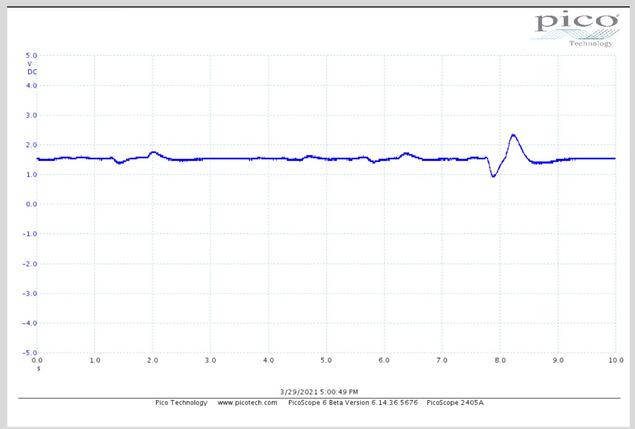

Figure 8: Heart rate of mussel in 1000 µg/L concentration

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