Assessing Hypoxia Tolerance in the *Mytilus trossulus*

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Multidisciplinary-Undergraduate Research Conference 2008
UBC, Vancouver

Who are the Mytilus trossulus?

- The M. trossulus are common marine mussels
- Found along rocky shorelines of North America in the intertidal zone and span from high tidal zone to lowest tidal point.
- Colonize in clumps on rocks
- Are a foundation species - a dominant species in a habitat that are abundant and have major influence on the ecosystem
- Are also Indicator species – as they represent the overall health of an ecosystem and are influenced by environmental conditions: disease outbreak, pollution, species competition or climate change.

Who are the Mytilus trossulus

Phylum Mollusca

Class Bivalvia

Subclass Pteriomorphia

Order Mytiloida

Family Mytilidae

Genus Mytilus

Species trossulus

Mussels: An ideal model to study oxygen metabolism

- Reside in intertidal zone – subjected daily to extreme fluctuations in oxygen levels

- Sessile organisms - easier to minimize stress on animals resulting from captivity and testing procedures. No movement = no interference with equipment

- Mussels can live for extremely long periods in the presence of anoxia (no oxygen).

  - 35 days at 10°C !!!!!

Mussels: Overcoming adversity

- Mussels are well adapted to the problems face in low and high tides:
  - Filter feeding organisms that use delicate gills to trap nutrients including oxygen
  - In the submerged state, oxygen must be dissolved in water in order to be taken up by gills
  - Low tide mussels will clam up and employ strategies to survive hypoxia (low oxygen levels)

Overcoming Challenges in Oxygen Fluctuations

- Low tide poses special problems for organisms.
- Gill respiring animals have difficulty extracting oxygen from air
- For those lucky enough to have water, tidal pools are warmer
  - oxygen saturation in water is much lower, carbon dioxide dissolves readily into the pool and oxygen escapes it.
  - co-inhabitants compete for available oxygen

How do Mussel go without? How do they survive the dry spell?

With less oxygen, there comes a point where the cost of respiration exceeds the benefit of extracting oxygen.

The turning point is called the Pcrit – the critical oxygen pressure

The Pcrit represents the point where enviromental oxygen is too low for an organism to maintain current oxygen metabolism

When faced with oxygen shortage, an organism has a choice:

- To obtain energy from other sources
- To depress metabolism
- To do both of the above

Investigating Hypoxia Tolerance

- The purpose of this experiment is to further knowledge about the mechanisms mussels use to survive hypoxic conditions

NORMOXIA= normal oxygen levels

HYPOXIA = lower than normal oxygen levels

ANOXIA = no oxygen present

Methods

- 35 mussels were harvested in October 15th, 2007 to assess the Pcrit from mid-tidal zones at Locarno Beach
- 40 were collected Feb 1st, 2008 for the second phase of the experiment
- Mussel identified based on previous molecular genetic study of p53 gene (Muttray and Cox, 2005)

Methods continued

- Mussels were transported back to the Richards Lab at UBC:
  - Barnacles removed and shells cleaned from debris
  - Placed in a large aquarium and allowed to acclimate for a month at 12°C and 30ppt salinity.
  - 15 mussels from the first batch used to test setup and 16 were used to assess the Pcrit.

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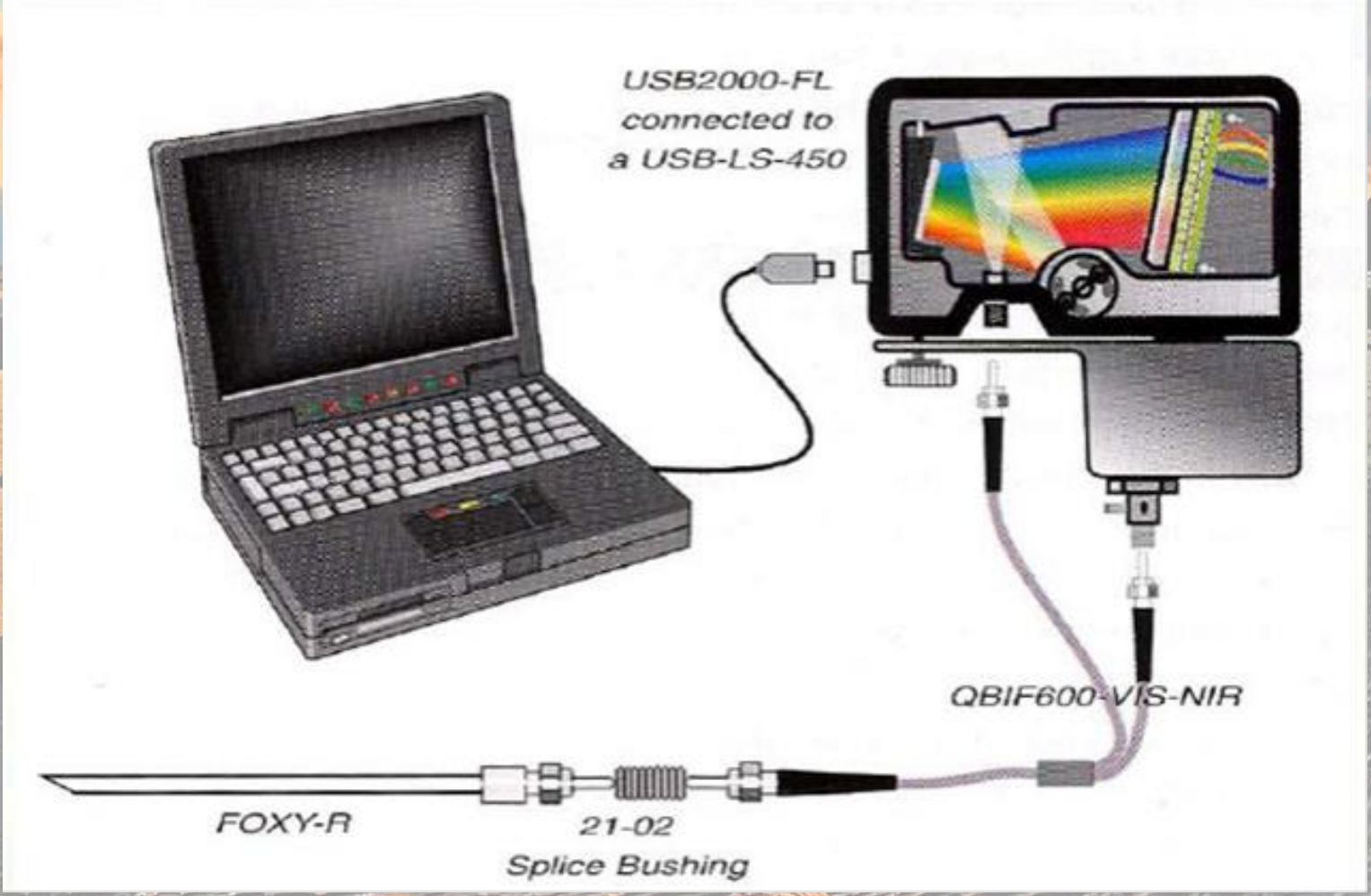
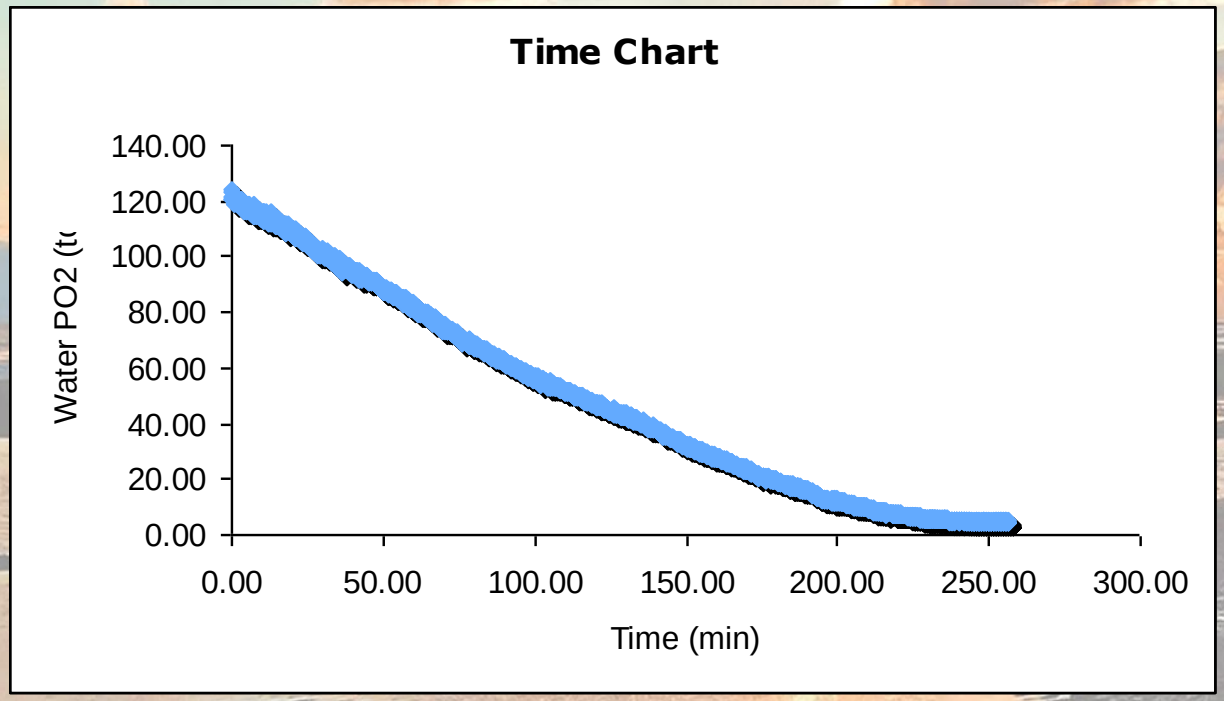
Measuring Oxygen Consumption

- Mussels were placed in respirometer and allowed a 24h recovery to de-stress prior to collecting data
- Respirometer was connected to a Foxy Probe – transmits blue LED light at ~470nm and quenching of signal is read by a spectrometer, USB2000-FL Fluorescence Spectrometer,
- Using OOISensors Software oxygen consumption was assessed using the Stern-Volmer equation.
- Mussels were allowed a 24h recovery to de-stress prior to collecting data

Apparatus Setup

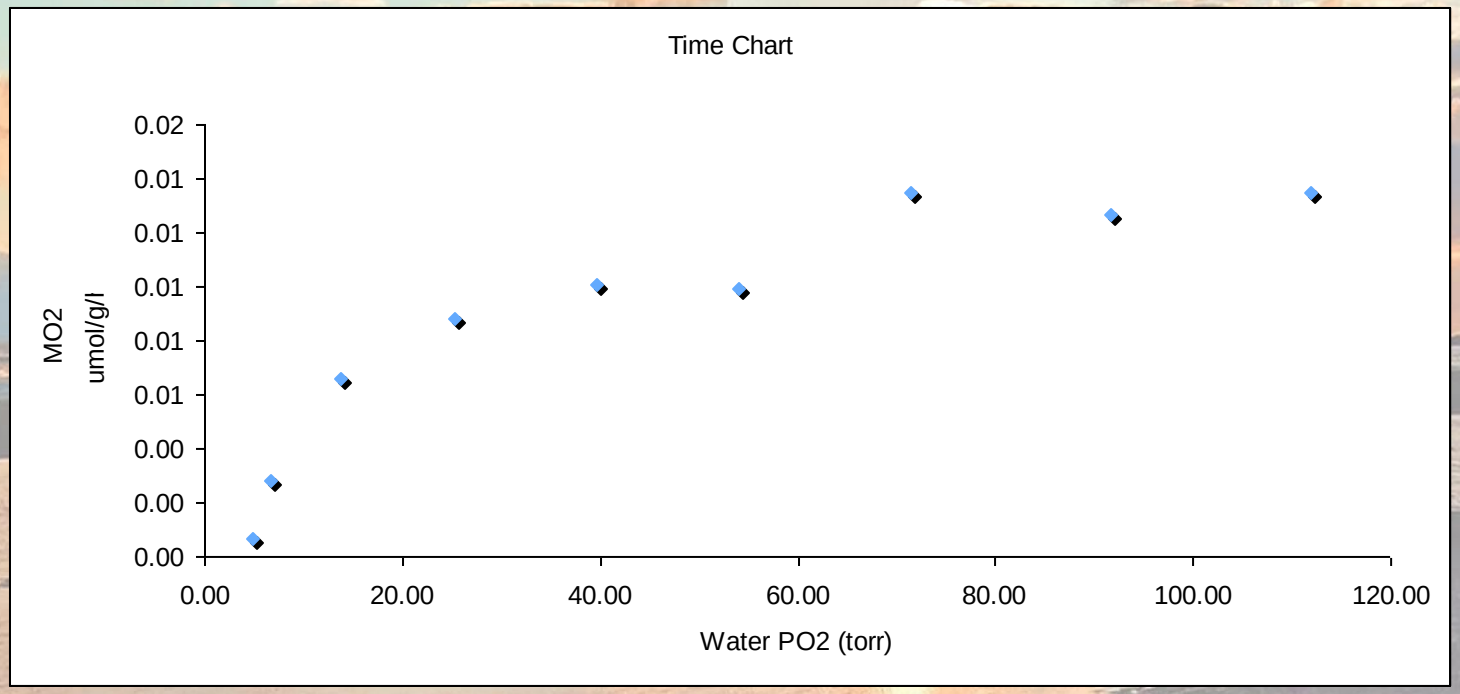


Results on Oxygen Consumption



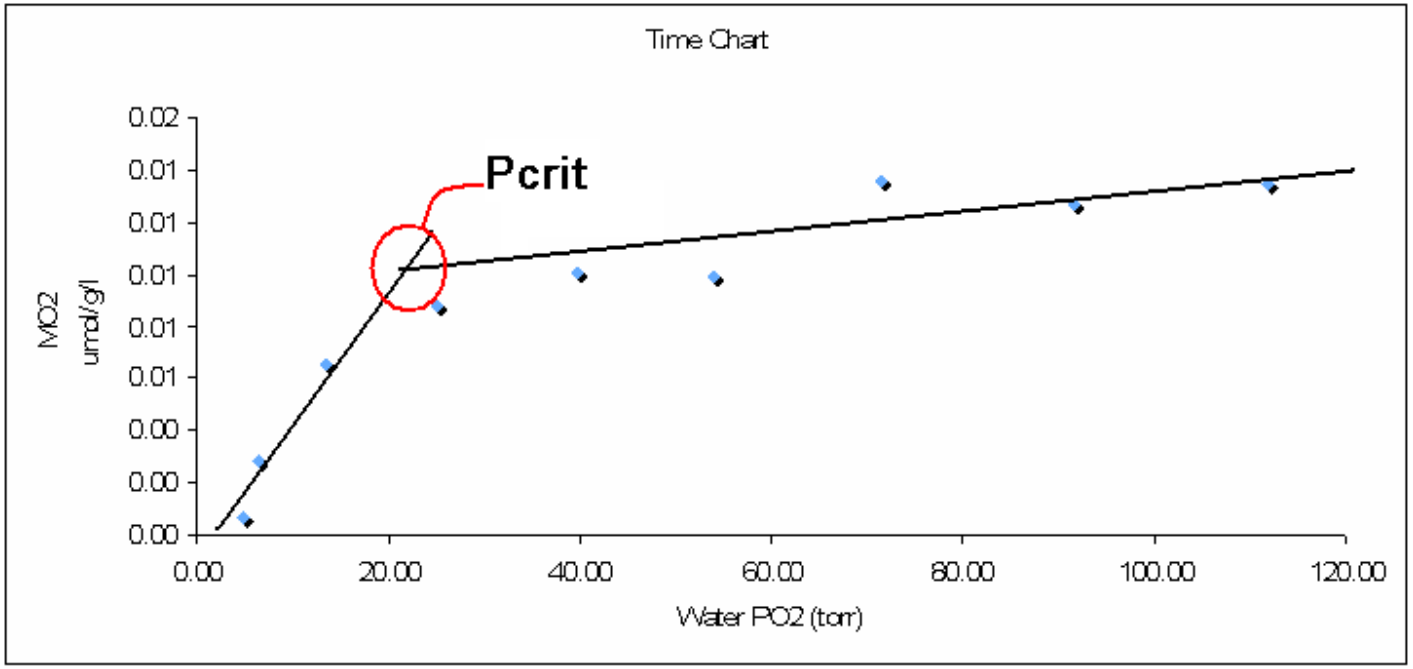
- 16 mussels were tested in a closed respirometer and allowed to consume the limited available oxygen until they ceased to respire
- The above graph shows the consumption of oxygen when a mussel is placed in a sealed respirometer.
- For reference, 125 torr ~ 16% O_2 , which is the amount of oxygen in air.

Converting Oxygen Consumption Rate to calculate Pcrit



- The above graph shows the molecular oxygen consumption rate calculated from the previous slide .
- Consumption of Molecular Oxygen Consumption Rate relative to Oxygen Pressure yields the Pcrit using regression lines.

Converting Oxygen Consumption Rate to calculate Pcrit



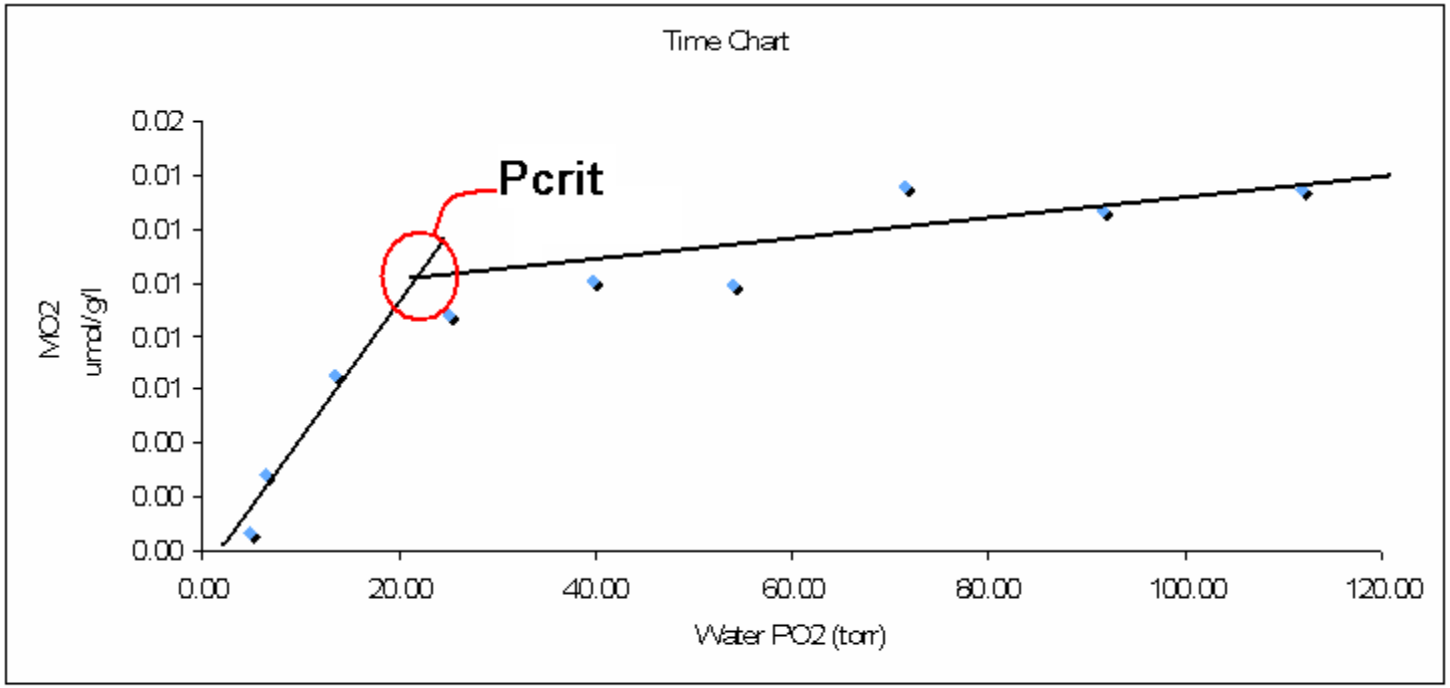
- Using regression lines to determine the consumption of Molecular Oxygen Consumption Rate relative to oxygen pressure.

Results of Pcrit Assessments

- Pcrit is the threshold pressure of oxygen in which the mussel cannot sustain usual metabolic consumption rate of oxygen

- From data results, it was assessed that the mussels had a mean critical oxygen tension of 20.94± 2.09 torr ~ 2.7% atmospheric oxygen

What does this mean? What happens after Pcrit



Phase II of Mussel Hypoxia Study

- Now that we have data on the transition point in the oxygen metabolism and we know what their threshold is.
- Very little is known about the emergency mechanisms that mussels employ when faced with an oxygen crises
- Studies have focused on long term effect which show that Mussels will reduce metabolic activity – requiring fewer resources and producing less toxic byproducts

The Emergency Response

- The second portion is to assess metabolic markers measure ATP, Phosphoarginine, Octopine (the invertebrate equivalent of lactate).
- This will show whether the mussel's primary survival mechanism is to immediately lower metabolism or switch to alternate pathways to get their energy
- For example, if ATP levels are the same before and after Pcrit, the muscle has not lowered metabolic activity.

The Emergency Response

- At this point 40 mussels have been acclimating:
  - 10 will be used at normoxia - control
  - 10 will be subjected at 20 torr
  - 10 will be subjected to 12 torr (2 standard deviations below mean)
  - 10 subjected to complete anoxia

- Study is ongoing to acquire data to determine ATP, Phosphoarginine, Octopine in Mussel Tissues
- Data will allow further or understanding into how the Mytilus trossulus's uses emergency metabolic coping mechanisms to hypoxia stresses

Acknowledgment

- I would like to thank the audience for their attention
- The Richards Labs for use of their facilities
- Dr. Jeff Richards PhD for his patience and guidance.

Images of mussels are altered pictures. Originals were obtained from the index found on http://www.wallawalla.edu/academics/departments/biology/rosario/inverts. D. Cowles (2002)

Image for OOSensor Foxy probe can be found at:
http://www.instrumentregister.com/2006/09/oximeter-blood-infrared.html . (2006)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Questions?