Behavioral and biochemical effects of hydrostatic pressure changes on *Acartia tonsa* (*Dana*, 1848) (Copepoda: Calanoida): A methodological approach

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Introduction

- Hydrostatic Pressure is a key variable influencing vital rates determining physiological limits on marine zooplankton
- Adaptations of Marine Zooplankton (i.e. vertical migration, food patches, niches)
- Buoyancy Control
- Technological Advances: Concept of relative density changes between fluid and device with depth

Potential Applications of Pressure Labs

- Effect of lipid compression on buoyancy control in copepods
- Effect of internal wave dynamics on copepod resting eggs in sediment
- Effect on bloom dynamics of diatoms (i.e. sinking and transfer rates)
- Effect on the degradation process as organic matter moves through the water-column to the deep-sea
- Effect on the physiology of vertically migrating species (e.g. medusae, *C. finmarchicus*)

Aim

- This study aims to test a new type of shallow-water pressure lab through a pilot study on the behavior and biochemistry of *Acartia tonsa* under varying physical conditions.

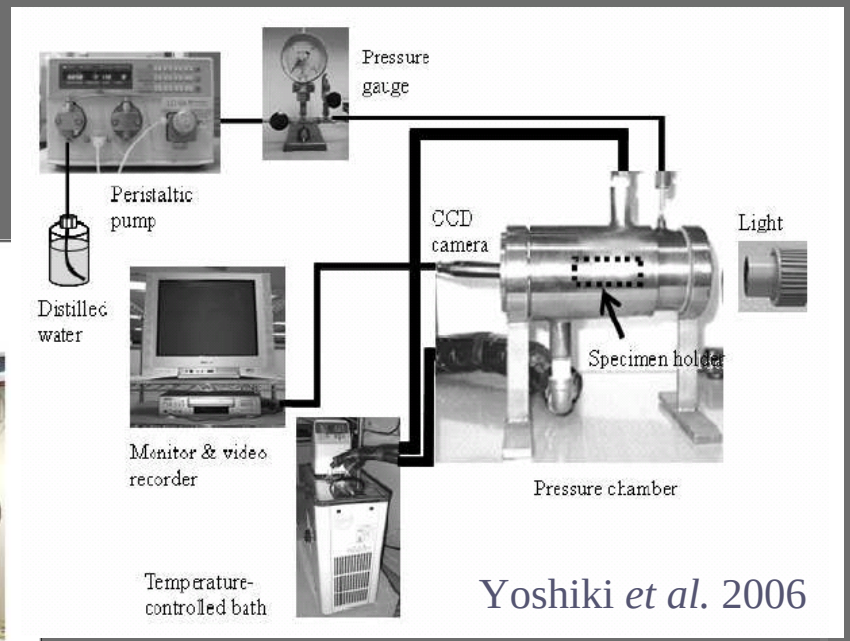
- These responses may be important influences on their ability to find and remain in a food patch at various depths.

Objective

- Expose *Acartia tonsa* to 4 different pressures in a step-wise fashion to simulate changes in depth.
- Questions addressed in this study:
  1. ***Do changes in pressure stimulate a behavioral response by Acartia tonsa?***
  2. ***Do changes in pressure elicit a biochemical response by Acartia tonsa over short temporal scales?***

Pressure Lab examples

Most previous pressure labs constructed of stainless steel



Yoshiki *et al.* 2006

TUHH
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High pressure technology at the Department of Ocean Engineering I of the Technical University Hamburg Harburg

All equipment is designed for the pressure range up to 550 bar

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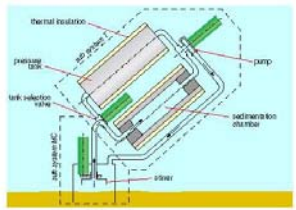
Pressure Laboratory DLI
40 l

Mimmit, electric motor for operation under pressure

Pressure Laboratory DLII
100 l

The slurper collects fluidic samples under pressure



Why use a shallow-water pressure lab?

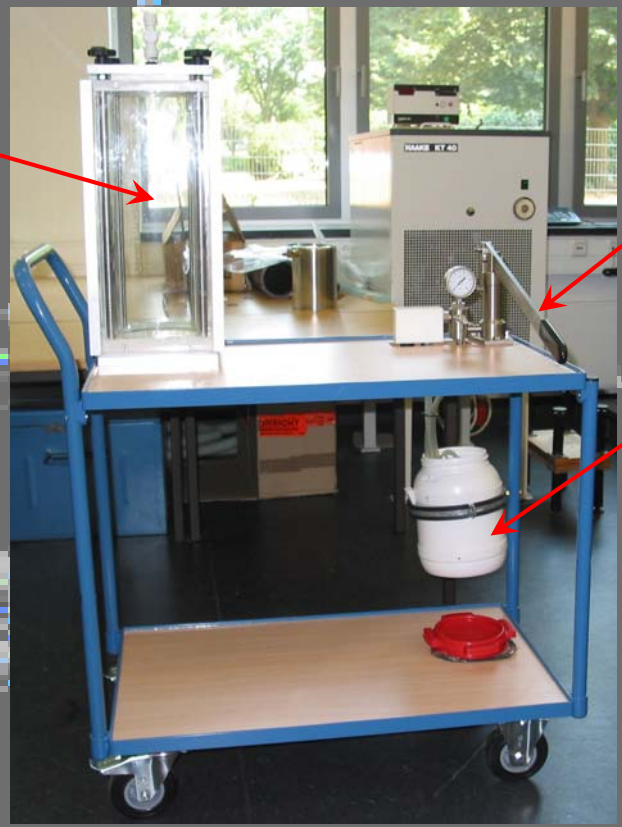
- The shallow-water lab is part of a relatively new series of pressure labs from extreme to simple or from deep to shallow
- Useful tool to simulate the ocean mixed layer
- Helps us to better understand what happens to those carried to depth
  - Survival?
  - Indices to indicate how animals succeed given change
    - i.e. Altering protein concentration, which may affect growth rates and reproductive potential.

Shallow-water Pressure Lab

Sample Chamber

Hand Pump

Reservoir



Material: Glass

Thickness: 1 cm

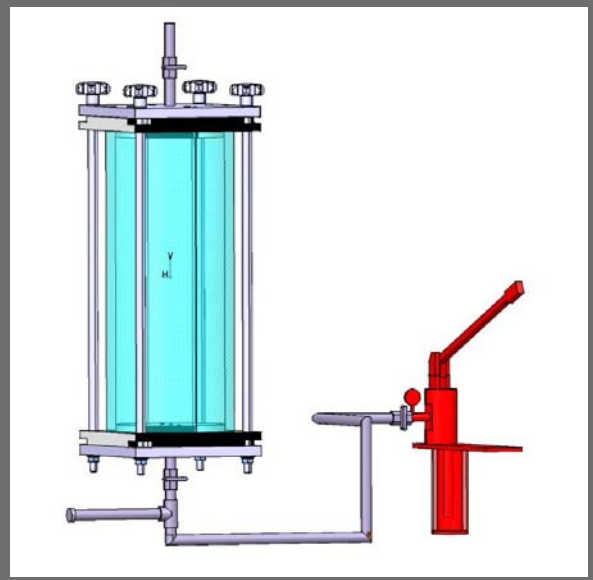
Max. Pressure:

10 Bar ~ 9.87 atm

Internal Volume: 9.4 L

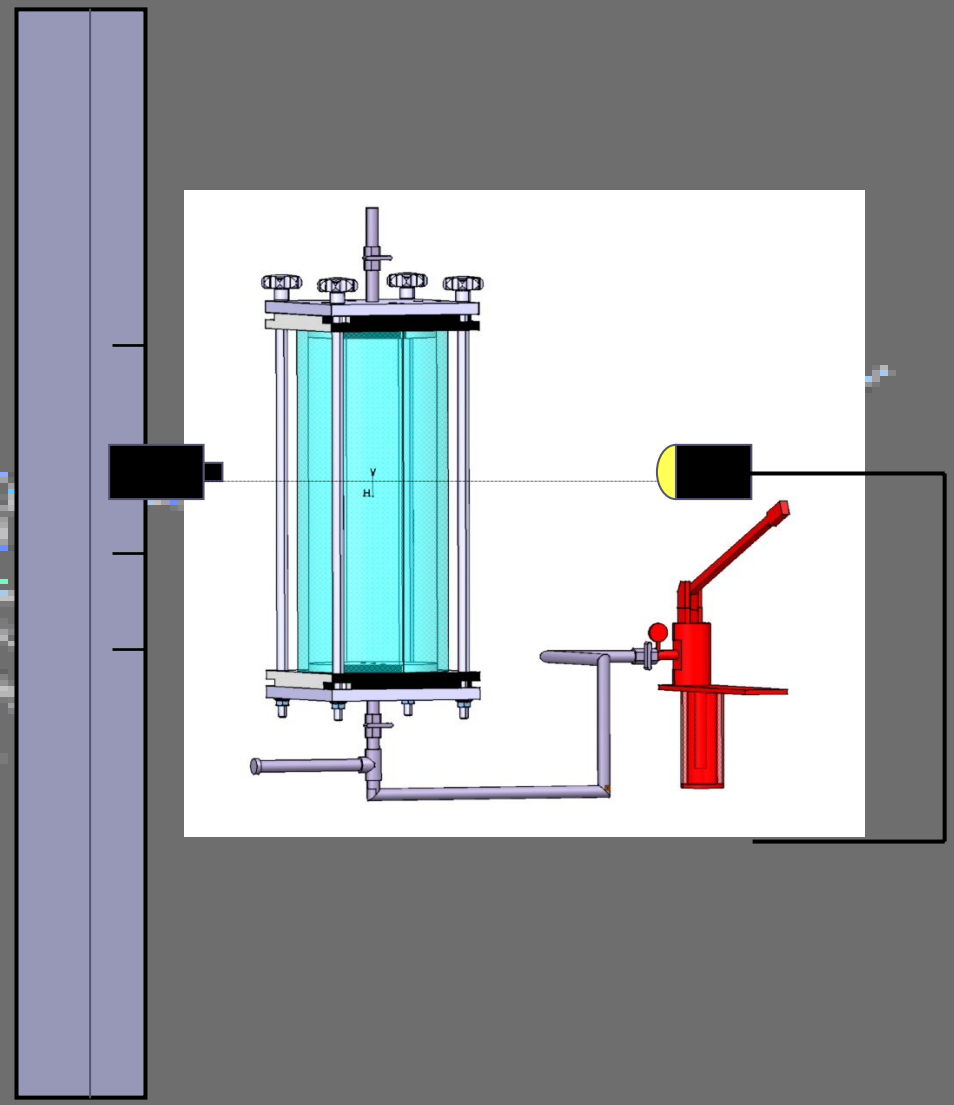
Height: 45 cm

Inner Diameter: 16 cm

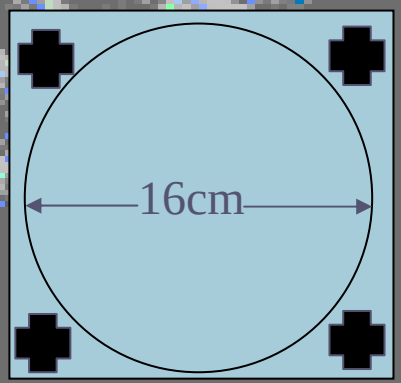


Experimental Setup

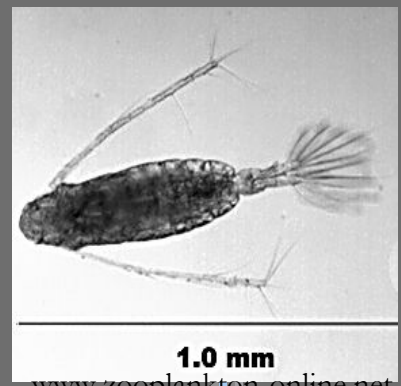
Side profile of camera, motion rail profiler, light source, and shallow-water pressure lab.



Top View



Experimental Design



- Target species: *Acartia tonsa*
- Pressure Steps: 1, 3, 6, 10 Bar
- Pilot Experiment: 22°C and 35 PSU
- Feeding History: Continuously Fed
- Biochemical Measurements_In triplicate:
  Carbohydrates & Protein
- Behavioral Measurements:
  Vertical Distribution &
  Passive Sinking (>10 data points)

Experimental Methods

- *A. tonsa* fed *Rhodomonas baltica*
- Approximately 200 CV copepodites l^{-1}
- Acclimation: 24 hours
- 12:12 Light/Dark Cycle

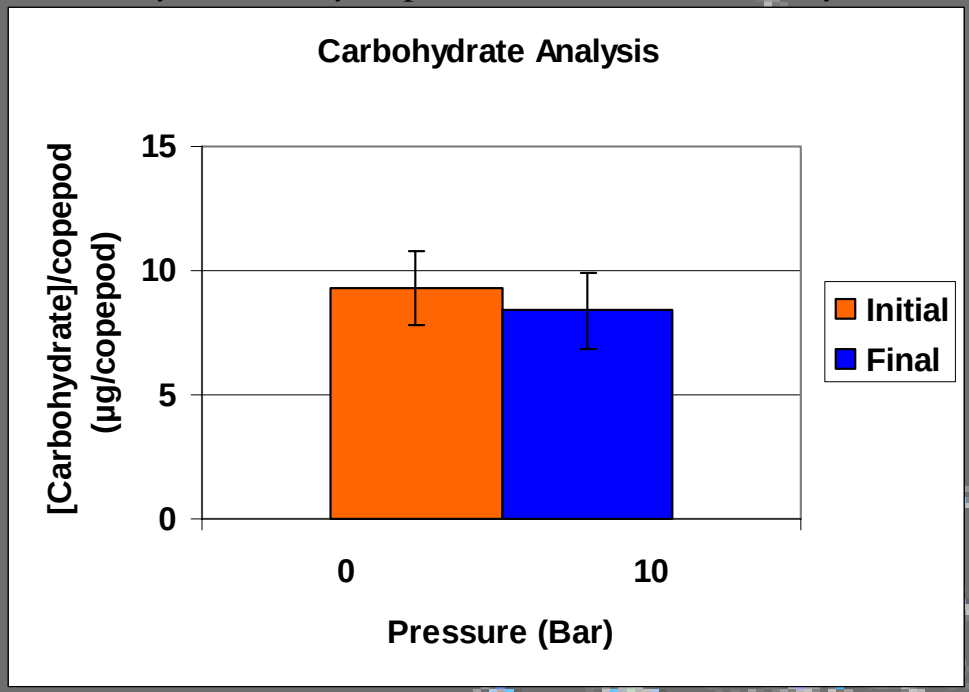
  Light intensity: 5 µEin m^{-2} s^{-1}

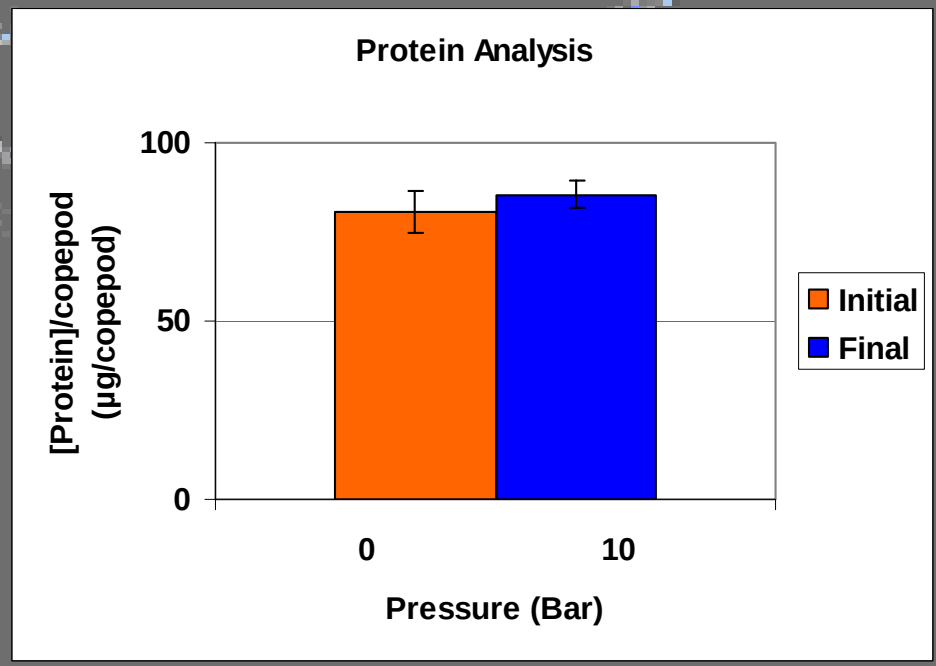
http://shigen.lab.nig.ac.jp/alga

- SONY Handycam ® (DCR-PC6E) panned every 10 cm in a vertical direction using LINOS® X-95 motion rail profiler
  - 25 fps
- Average time to apply pressure: 4.5 mins
- Total recording time for each pressure step: 60 mins
- Total recording time for experiment: 3.5 hrs

Biochemical Results Carbohydrate & Protein

Carbohydrate analysis performed as described by Dubois *et al.* (1956) and Herbert *et al.* (1971).



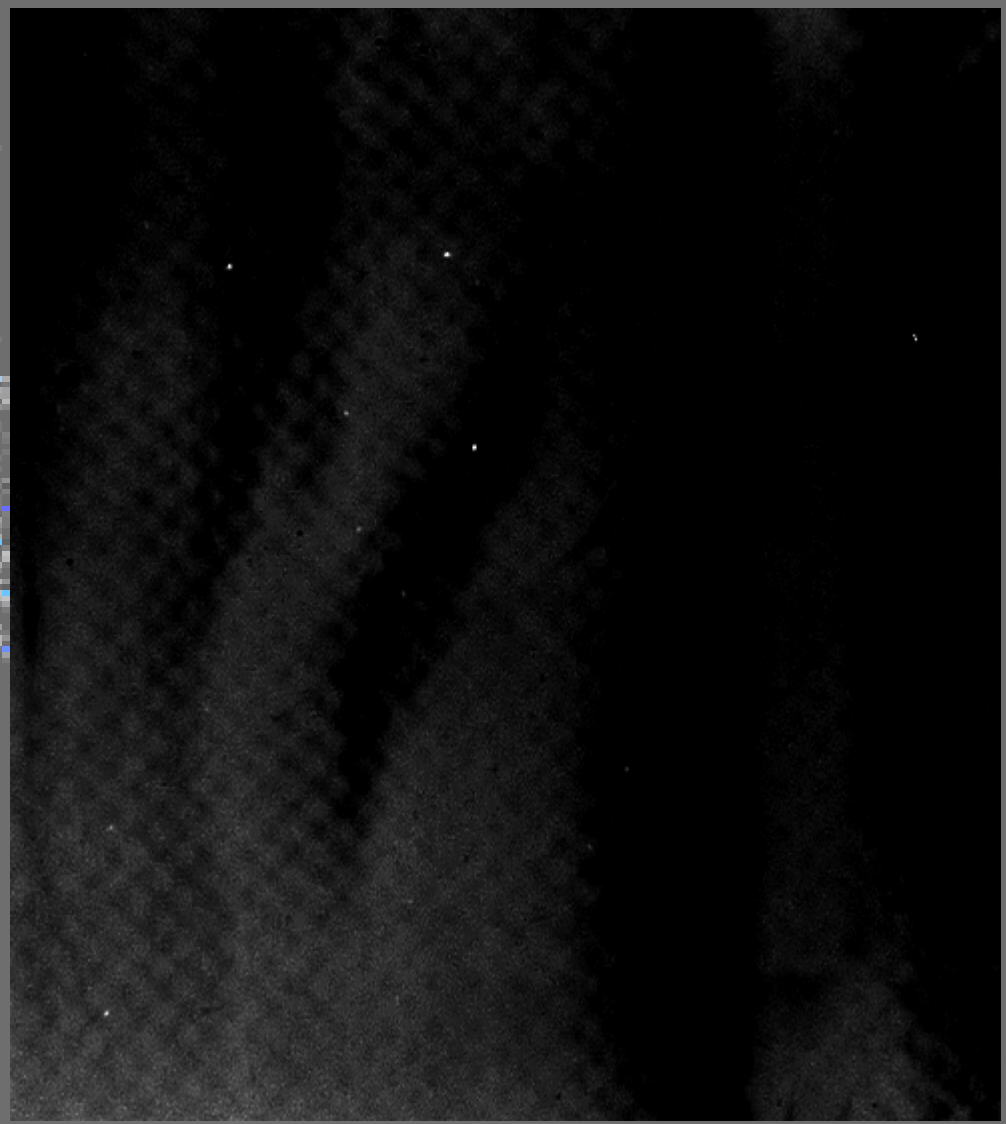


Protein analysis performed using Sigma-Aldrich bicinchoninic acid protein assay kit (product code: BCA-1 & B 9643).

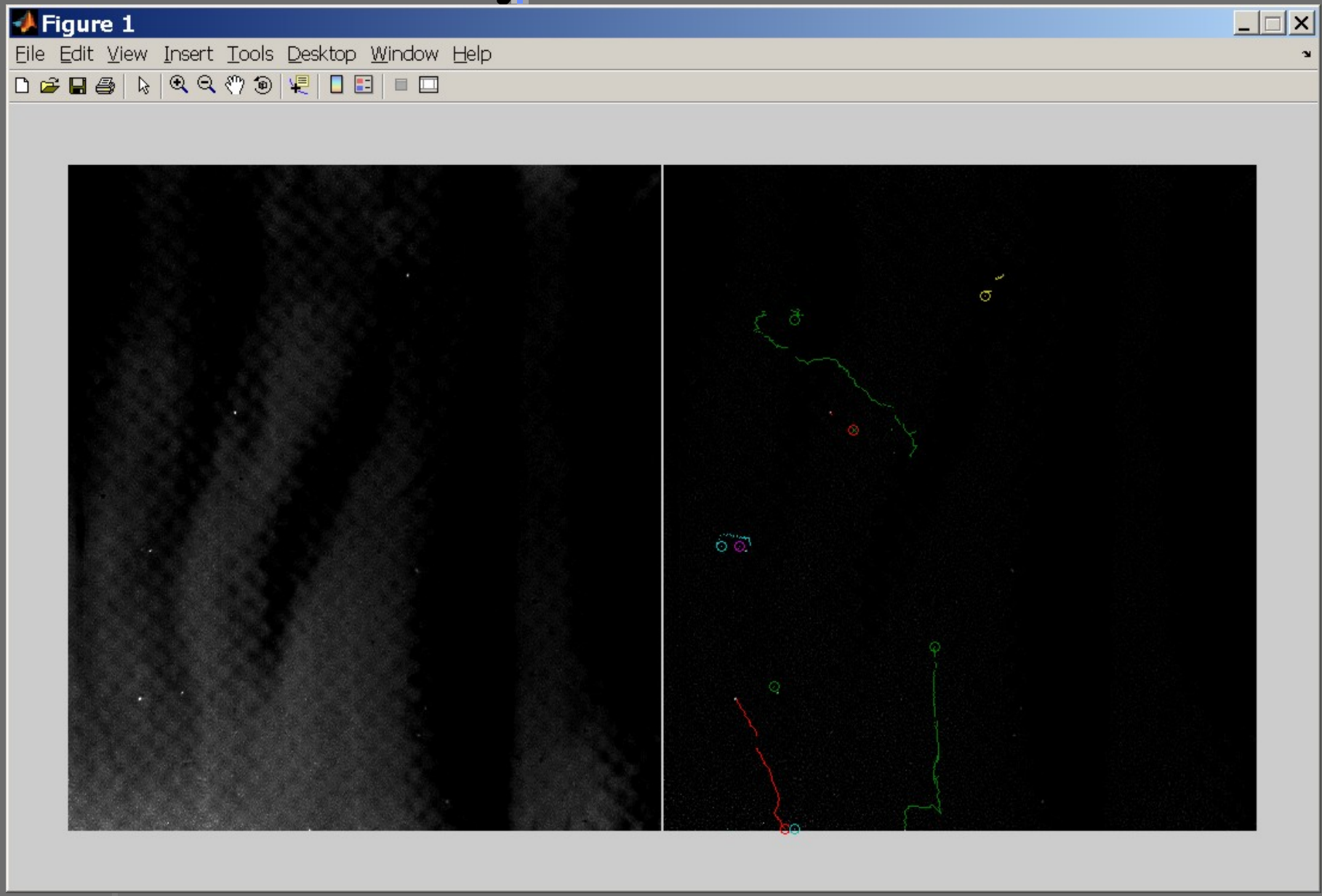
Video Analysis of Behavior

Using VirtualDub & MATLAB:

- 1. Levels
- 2. Grayscale
- 3. Invert
- 4. Locate particles in frame
- 5. Connect particles in neighboring frames using search radius & linear extrapolation of position
- 6. Calculate motion (distance/time)



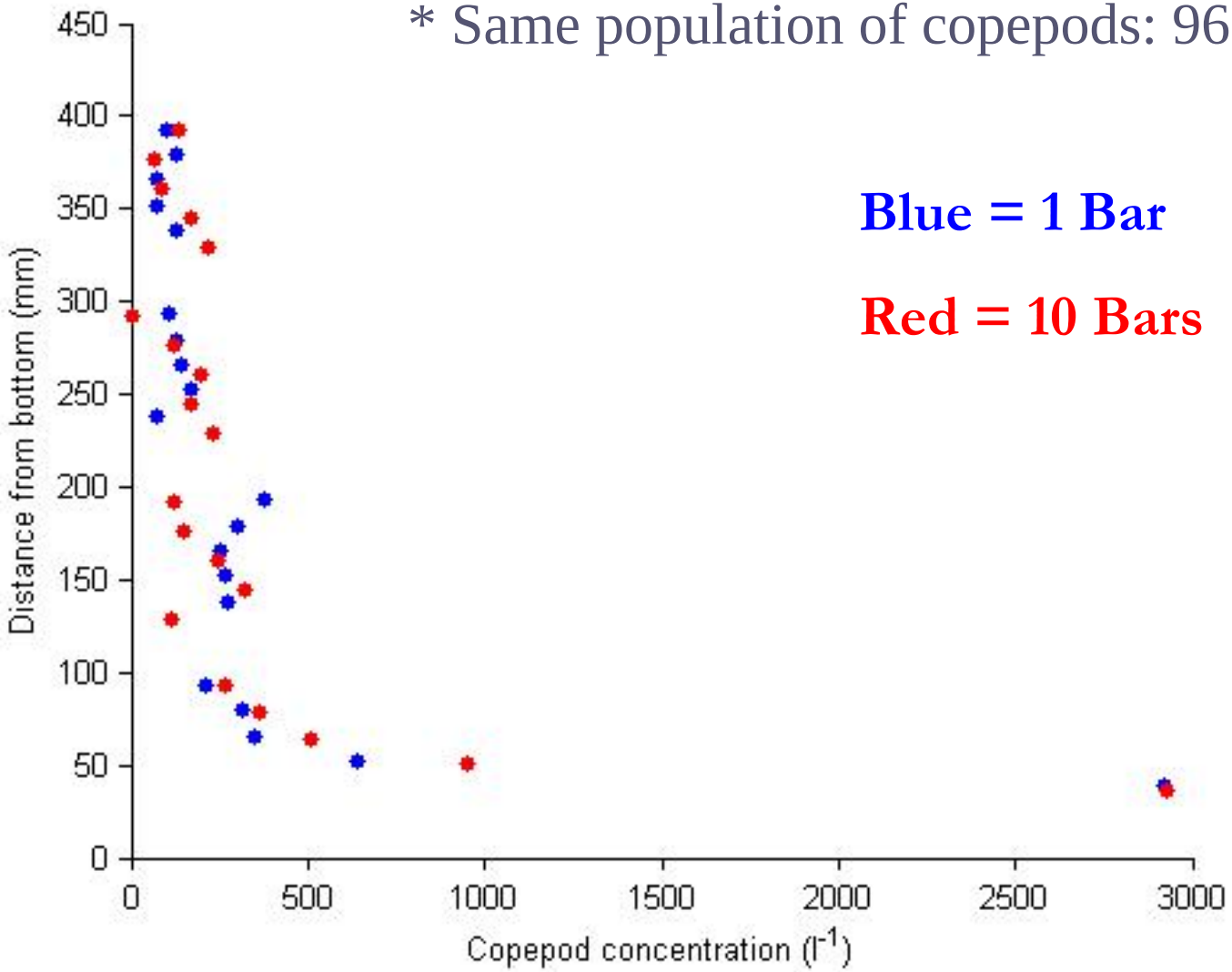
Video Analysis of Behavior Output



Video Analysis of Behavior

Distribution

* Same population of copepods: 9600

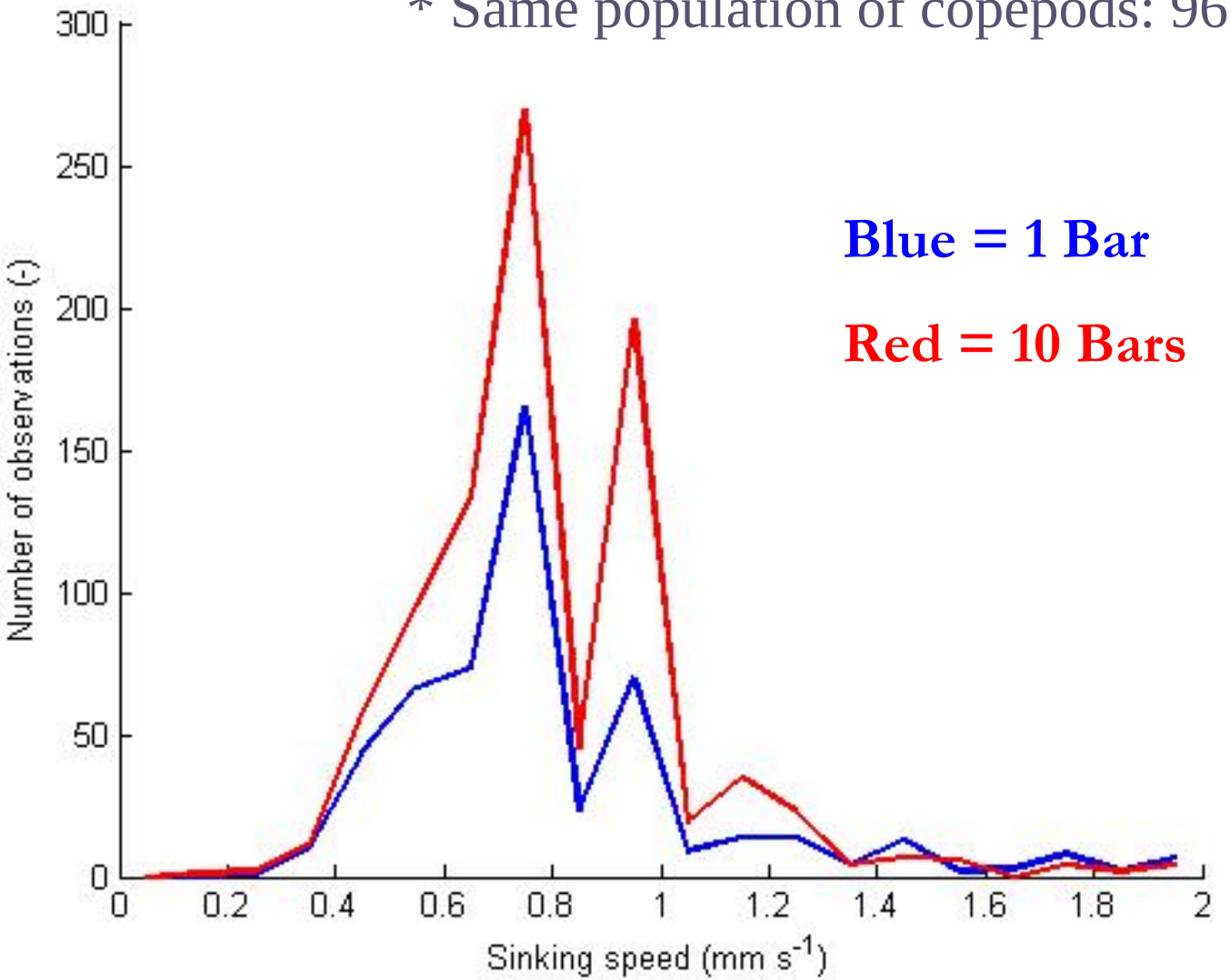


Blue = 1 Bar

Red = 10 Bars

Video Analysis of Behavior Sinking

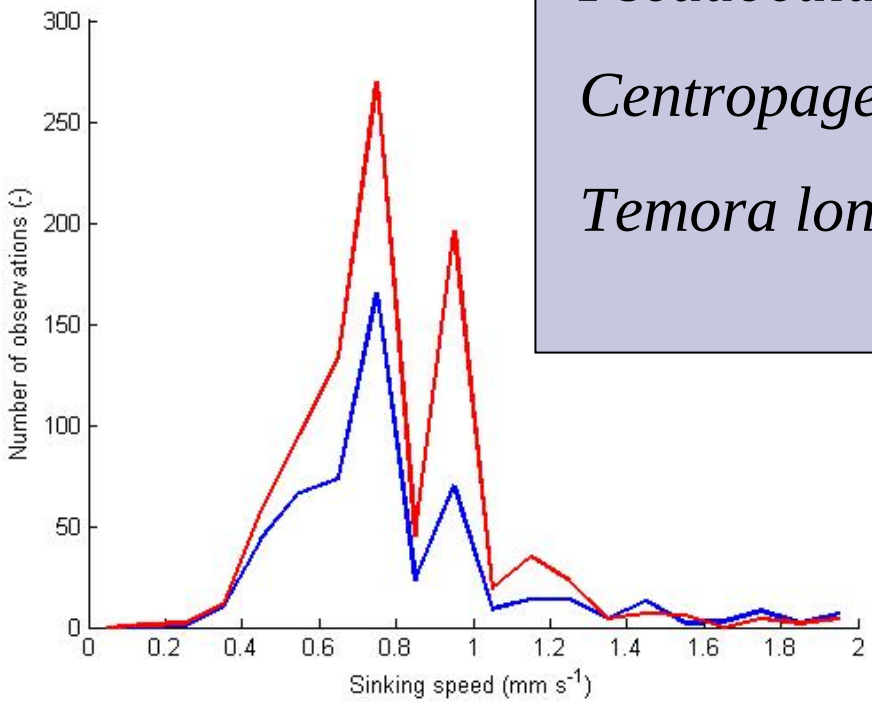
* Same population of copepods: 9600



Sinking Speed

Species	*Prosome length mm*	*mms^{-1}*
Acartia clausi	0.91	0.27
Paracalanus parvus	0.67	0.60
Centropages typicus	1.27	1.0
Pseudocalanus elongatus	0.92	1.1
Centropages hamatus	1.03	1.4
Temora longicornis	0.97	2.5

Tiselius & Jonsson MEPS 1990



***Acartia tonsa* prosome length range**

0.6-1.2 mm

Summary…

- Carbohydrate concentration decreased after pressure treatment.
- Protein concentration increased after pressure treatment.
- However, there were no significant differences among biochemical measurements at 0 and 10 Bars.
- No pressure effect demonstrated by the distribution or sinking video analyses.
- However, it seems that greater numbers of copepods were observed to passively sink at 10 bars as compared to 1 bar.

Take Home Message

The shallow-water pressure lab allows investigations into biological effects of pressure that still remain unresolved for marine zooplankton.

Future Activities:

- Larger species
- Better video quality
- Longer recording time

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