Preliminary result of respiratory kinetics in zooplankton samples: MALASPINA 2010

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INTRODUCTION

The respiratory oxygen consumption (**R**) is a physiological process that occurs wherever the oxygen and the organic matter are present. It is catalyzed by the enzymatic activity of the respiratory electron transfer system (**ETS**). This activity, in turn, is controlled by the availability of substrates, i.e., nicotinamide adenine dinucleotide (**NADH**) and nicotinamide adenine dinucleotide phosphate (**NADPH**). The **Enzyme Kinetic Model (EKM)** argues that, according to the theory of Michaelis- Menten, the maximum activity of these enzymes and the availability of their substrates control the *in vivo* activity of the ETS enzymes at constant temperatures (Packard and Gomez, 2008). Up to now, no study has measured the NADH and NADPH intracellular levels and applied them to the EKM in the zooplankton. In the present work, we have measured the intracellular level of substrates and calculated the kinetics constants.

Field samples were collected during three legs of the Malaspina 2010 oceanographic cruise (Fig 1). The physiological measurement of respiration were accomplished on board in well fed and in 24h starved organisms. The enzyme activities and kinetics and intracellular substrates levels were determined back at laboratory.

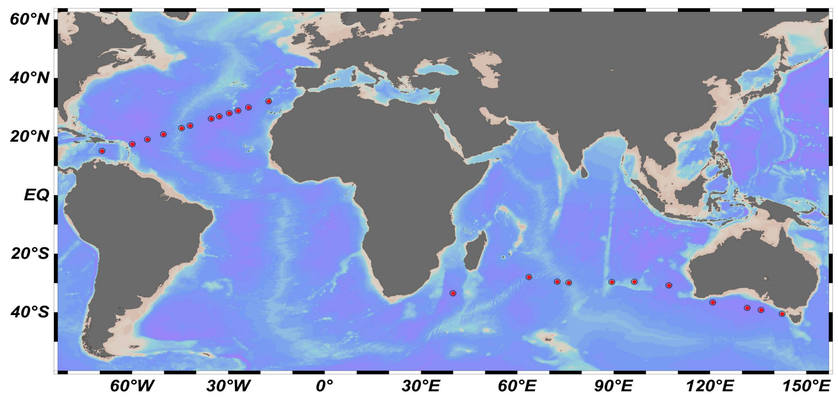

Fig 1. Cruise track of the R/V Hesperides during the LEG 3, 4 and 7.

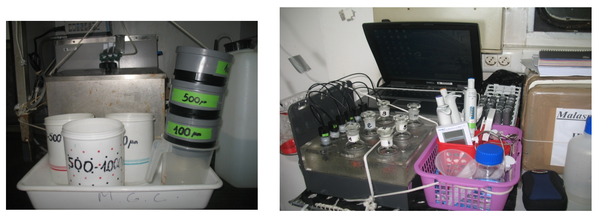

Fig 2. Size fractionation and on board incubation system.

Fig 3. 100 µm mesh size WP-2 net used for sampling.

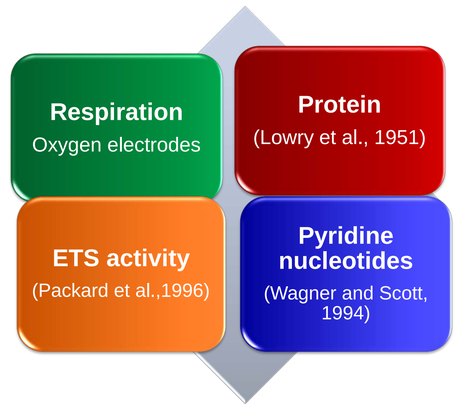

Fig 4. Analytical methods

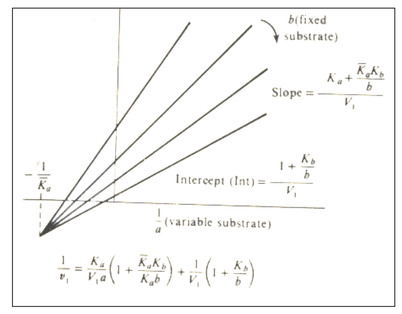

Fig 5. Lineweaver-Burk reciprocal plot for calculating the kinetic parameters in bisubstrate reactions.

Pyridine Nucleotides

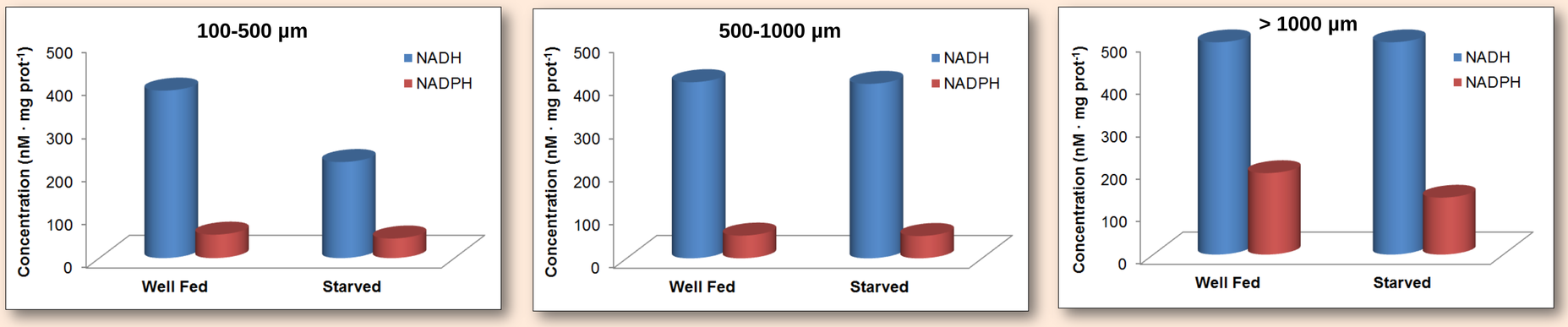

Fig 6. Protein specific NADH and NADPH levels in well-fed and starved zooplankton. The specific concentration of both NADH and NADPH were not significantly different in well-fed zooplankton (p>0.001) of the three size fractions. Levels of NADH were only significantly different between well-fed and starved conditions in 100-500 µm size fraction.

ENZYME KINETIC MODEL

$$R_o = \frac{Vmax \cdot [S1 \cdot S2]}{K1 \cdot Kia + K2 \cdot [S1] + K1 \cdot [S2] + [S1 \cdot S2]}$$

Vmax: Maximum velocity of the reaction
S1, S2: Substrate concentration of NADH and NADPH
K1, K2: Michaelis constant of NADH and NADPH
Kia: Apparent dissociation constant

Table II. Kinetic constants of NADH and NADPH in two size fractions of zooplankton, There are no significative differences between the equilibrium constant (K_m) and the apparent dissociation constant (K_{ia}) for NADH and NADPH when well fed and starved organisms are compared. The maximum velocity (Vmax) of the reaction decreases in starved zooplankton. Data are given as mean ± SD. Numbers in parentheses represent the number of data used to calculate the average values.

Size	K_m (µM) for: NADH	K_m (µM) for: NADPH	K_{ia} (µM)	Vmax (µmol $O_2 \cdot h^{-1} \cdot$ mg $prot^{-1}$)
Well Fed				
100- 500 µm	242.73 ± 49.99 (5)	30.17 ± 4.84 (5)	404.18 ± 205.27 (3)	4.73 ± 1.47 (5)
500- 1000 µm	211.78 ± 60.54 (4)	21.94 ± 6.08 (3)	589.63 ± 92.38 (3)	3.57 ± 1.10 (4)
Starved				
100- 500 µm	273.00 ± 230.43 (2)	24.94 ± 29.88 (2)	427.96 ± 54.79 (2)	3.78 ± 1.98 (3)
500- 1000 µm	209.02 (1)	43.66 (1)	476.78 (1)	2.37 (1)

Table I. Protein specific rates of respiratory oxygen consumption (R) and potential respiration (Φ) both in well-fed and in starved zooplankton. Data are given as means ± SD.

Size	n	R (µmol O_2/ h · mg prot)	Φ (µmol O_2/ h · mg prot)	R / Φ
Well Fed				
100- 500 µm	38	2.584 ± 2.426	2.190 ± 1.317	0.888 ± 0.621
500- 1000 µm	29	2.303 ± 1.637	2.494 ± 1.743	0.923 ± 0.939
>1000 µm	15	1.351 ± 1.077	1.239 ± 0.542	1.09 ± 1.987
Starved				
100- 500 µm	9	3.053 ± 2.173	3.323 ± 2.021	0.919 ± 1.075
500- 1000 µm	6	1.452 ± 1.189	2.073 ± 0.759	0.700 ± 1.566
>1000 µm	---	---	---	---

CONCLUSSIONS

I. NADH levels remained nearly constant from well-fed to starvation conditions in 500-1000 µm and >1000 µm size fractions. However, they decreased in 100-500 µm size fraction. Highest size classes might have a larger reserve and 24 h of starvation might not challenge them.

II. Specific respiratory oxygen consumption decreases with size fraction, both in well-fed and starvation conditions.

III. There are no significant differences in the kinetics constants between well-fed and starved organisms.

B/O Hespérides

References

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