Respiration and ammonium excretion metabolism in oceanic mesozooplankton:

The relationship between physiology, enzyme activities and intracelular substrates.

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Introduction

Both respiration (R) and ammonium excretion ($R_{NH_4^+}$) are critical processes in marine ecology, serving to counter balcance productivity and synthesis. Furthermore, this balance controls the efficiency of the net production. Enzymatic assays are important tools for monitoring the zooplankton metabolism. True, their requirement substrate saturation yields potentials rather than the actual rates. Nevertheless, measuring substrates and employing enzymatic intracellular substrate control leads to actual rates. Here with mesozooplankton we find the substrate concentrations (pyridine nucleotides and glutamate) are directly proportional to the nutritional state. We describe the spatial variability of the biomass-specific R and $R_{NH_4^+}$ in mesozooplankton collected during the Malaspina 2010 cruise, and we compare these values with the enzymatic activities associated to each process (i.e. ETS and GDH activities, respectively). Finally, we study the effect of short-term starvation (24 hours) on the mesozooplankton community.

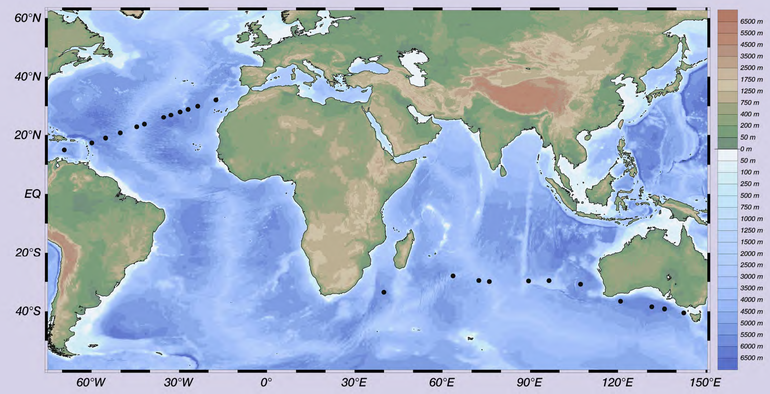

Figure 1. Locations where the mesozooplankton was sampled for physiological studies during the Malaspina-2010 cruise. A total of 23 stations were considered along the North Atlantic (Leg 7) and Indian Oceans (Legs 3-4).

Results

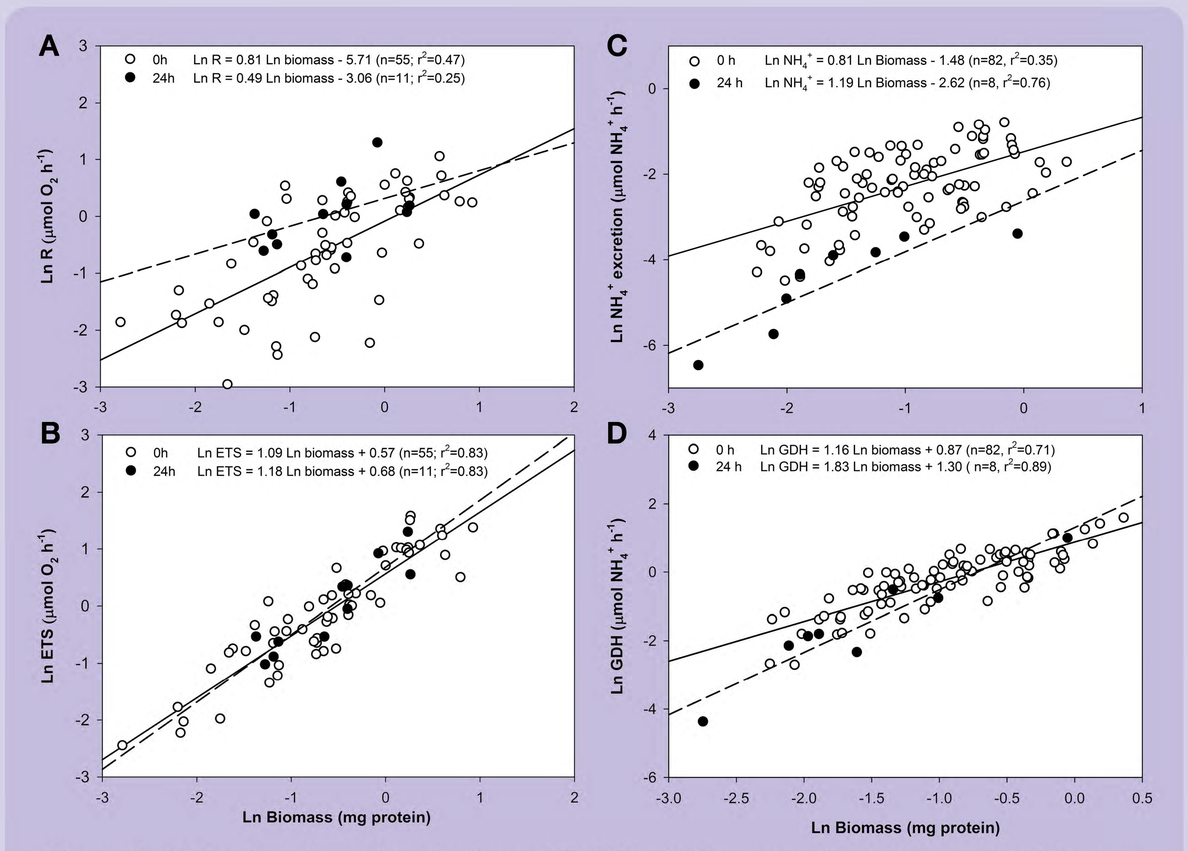

Figure 2. (A) Log-transformed respiration and (B) ETS activities *versus* log-transformed protein mass, just after the capture of organisms (open circles) and under starvation (filled circles). Panels C and D show the relationship between NH_4^+ excretion and GDH with biomass, respectively.

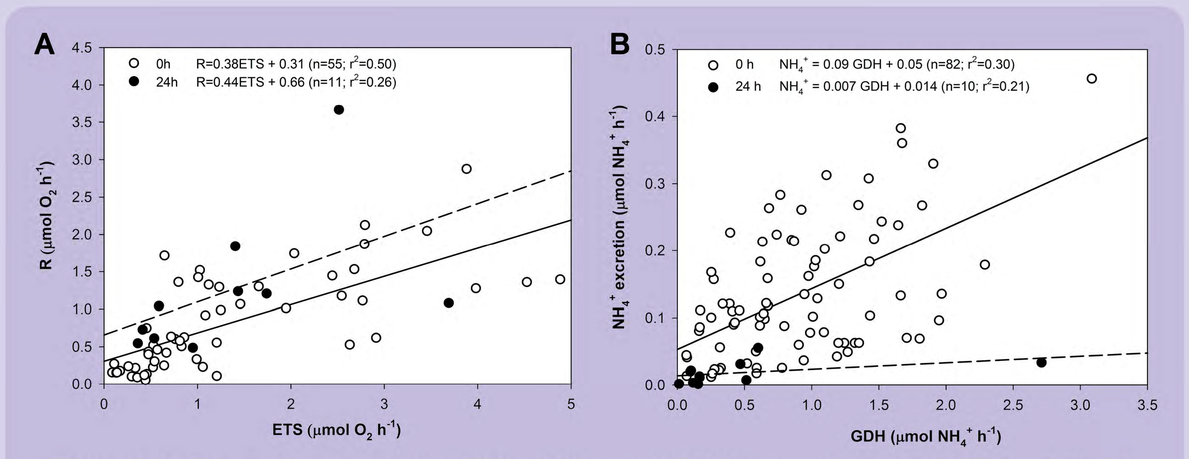

Figure 3. (A) Respiration rates against ETS activities, and (B) NH_4^+ excretion against GDH activities, in both natural conditions (open circles) and under starvation (filled circles).

Table 1. Mean values (± SD) of biomass-specific NH_4^+ excretion and respiration, as well as the enzymatic rates responsible for these processes (ETS and GDH, respectively) in size-fractionated zooplankton. The resultant R/ETS, GDH/NH_4^+ and O/N ratios are also shown. The incubations were perfomed just after the net hauls, and after 24 h under starvation conditions.

	Size	NH_4^+	GDH	GDH/NH_4^+	R	ETS	R/ETS	O/N
	µm	µmol NH_4^+ h^{-1} mg $prot^{-1}$	µmol NH_4^+ h^{-1} mg $prot^{-1}$		µmol O_2 h^{-1} mg $prot^{-1}$	µmol O_2 h^{-1} mg $prot^{-1}$		
0 h	*100 - 500*	0.36 ± 0.22	2.15 ± 0.91	9.14 ± 8.63	0.80 ± 0.51	1.98 ± 0.79	0.66 ± 0.49	0.29 ± 1.74
	500 - 1000	0.38 ± 0.29	2.54 ± 0.99	9.89 ± 8.18	1.26 ± 0.98	1.68 ± 0.61	0.79 ± 0.63	3.32 ± 2.48
	> 1000	0.27 ± 0.20	2.24 ± 1.30	12.15 ± 8.89	0.82 ± 0.53	1.39 ± 0.59	0.50 ± 0.40	3.04 ± 2.51
24 h	*100 - 500*	0.06 ± 0.04	0.80 ± 0.36	11.31 ± 3.43	2.02 ± 1.33	2.01 ± 0.67	1.08 ± 0.66	33.67 ± 22.50
	500 - 1000	0.09 ± 0.09	1.26 ± 1.05	14.98 ± 14.64	2.24 ± 1.09	1.74 ± 0.61	1.28 ± 0.43	24.89 ± 16.83
	> 1000	-	-	-	-	-	-	-

Table 2. Mean values (± SD) of biomass-specific metabolic rates and enzyme activities for each leg and size fraction. The resultant O/N ratios are also shown. No significant differences between legs were found on the $R_{NH_4^+}$ ($p > 0.05$), although respiration seemed to be larger on leg 4.

Size	LEG	NH_4^+	GDH	R	ETS	O/N
µm		µmol NH_4^+ h^{-1} mg $prot^{-1}$	µmol NH_4^+ h^{-1} mg $prot^{-1}$	µmol O_2 h^{-1} mg $prot^{-1}$	µmol O_2 h^{-1} mg $prot^{-1}$	
100 - 500	*Leg 3*	0.40 ± 0.18	1.88 ± 0.79	1.50 ± 0.83	1.70 ± 0.49	3.75 ± 2.67
	Leg 4	0.28 ± 0.14	1.84 ± 1.04	1.23 ± 0.49	2.15 ± 0.37	7.67 ± 2.81
	Leg 7	0.35 ± 0.24	2.30 ± 0.92	2.07 ± 0.97	1.51 ± 1.11	5.91 ± 4.90
500 - 1000	*Leg 3*	0.56 ± 0.50	2.89 ± 1.91	1.32 ± 0.84	1.98 ± 0.64	2.35 ± 2.58
	Leg 4	0.39 ± 0.36	2.19 ± 0.74	1.67 ± 1.48	2.27 ± 0.31	4.28 ± 5.47
	Leg 7	0.44 ± 0.38	2.56 ± 0.83	1.01 ± 0.87	1.15 ± 0.23	2.61 ± 2.80
> 1000	*Leg 3*	0.38 ± 0.24	2.29 ± 1.81	0.77 ± 0.50	1.42 ± 0.56	2.02 ± 1.84
	Leg 4	0.22 ± 0.12	1.27 ± 0.74	1.22 ± 0.58	1.56 ± 0.94	5.54 ± 4.01
	Leg 7	0.24 ± 0.20	2.61 ± 1.13	0.25	0.9	1.04

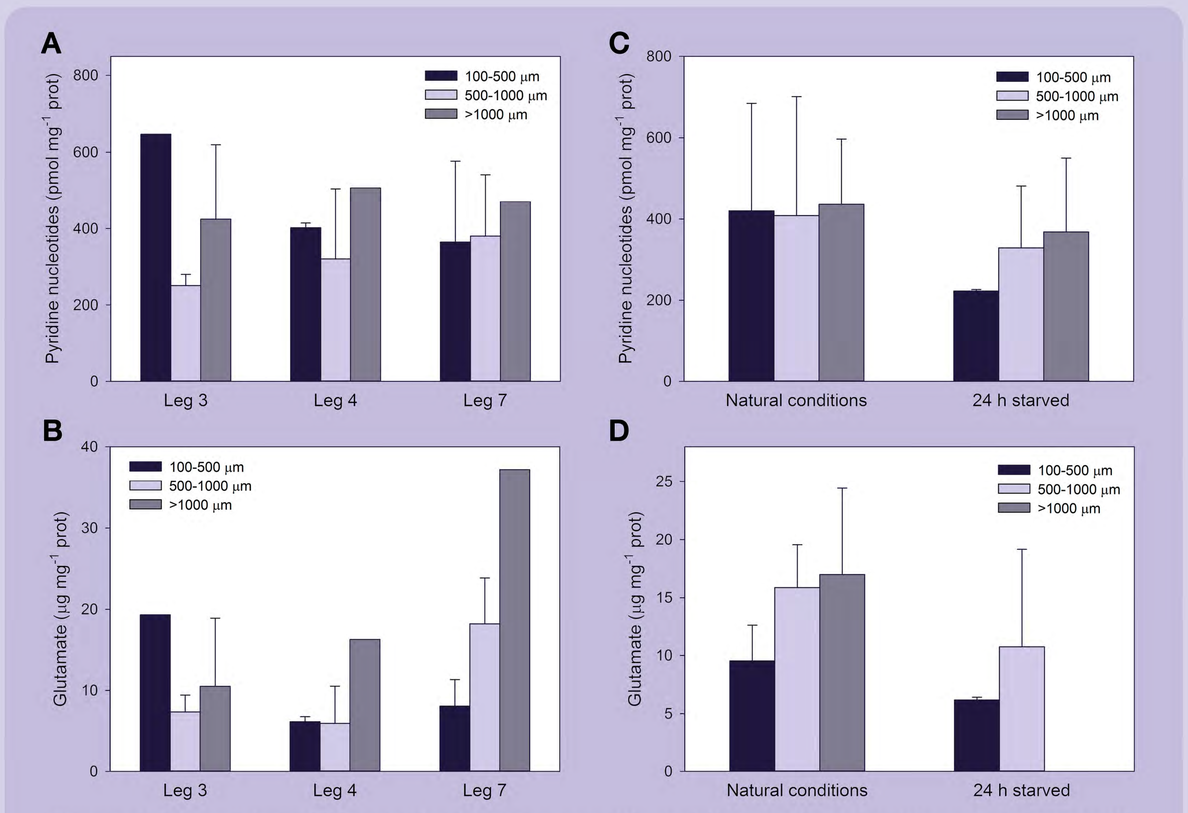

Figure 4. Comparison between the biomass-specific pyridine nucleotides (A) and glutamate (B) concentrations of the three legs sampled during the Malaspina cruise. Panels C and D show a decrease in the substate concentrations in starved organisms.

Summary

1. The relationship of biomass with enzymatic rates is more isometric than with physiological rates (Fig. 2). The latter showed more scatter as it is affected by complex biological control mechanisms (hormonal, behavioral, etc.) and methodological artifacts.

2. Starvation causes the ammonium excretion to diverge more from the potential rate than does respiration (Fig. 3). Furthermore, it involves a decrease in the intracellular concentration of pyridine nucleotides and glutamate of 27 and 40%, respectively (Fig. 4).

3. No clear pattern in mesozooplankton metabolism was found between regions. This is likely due to the heterogeneity in the productivity regimes recorded within each leg.

Acknowledgements

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