Is the GDH/$R_{NH_4^+}$ ratio in the mesozooplankton constant through different oceanic systems?

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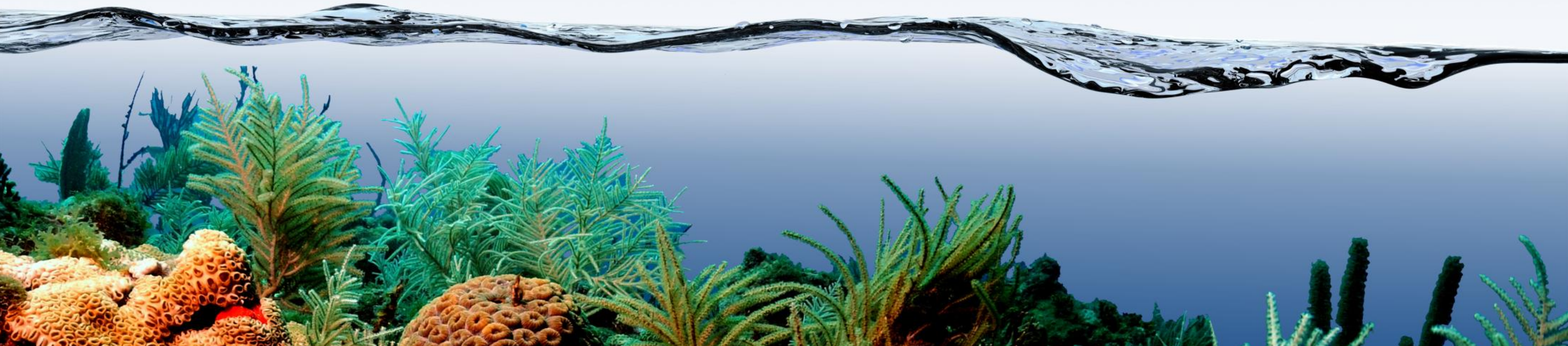
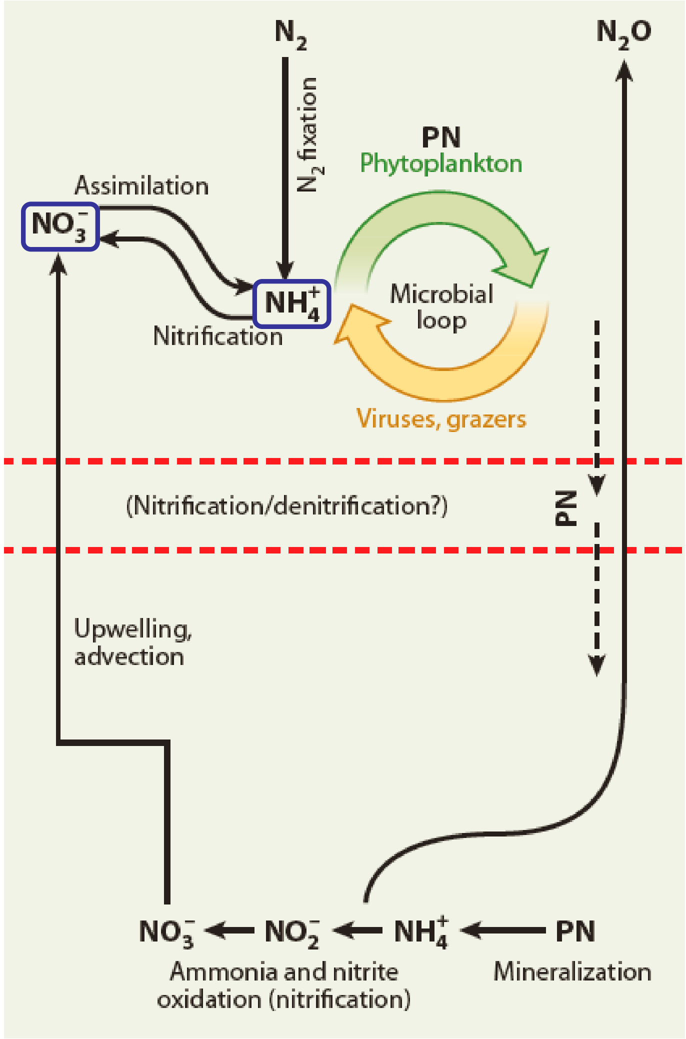


INTRODUCTION

Classical tenets of Nitrogen Cycle



Zehr and Kudela (2011)

(I) Nitrogen availability is **frequently limited** in ocean ecosystems.

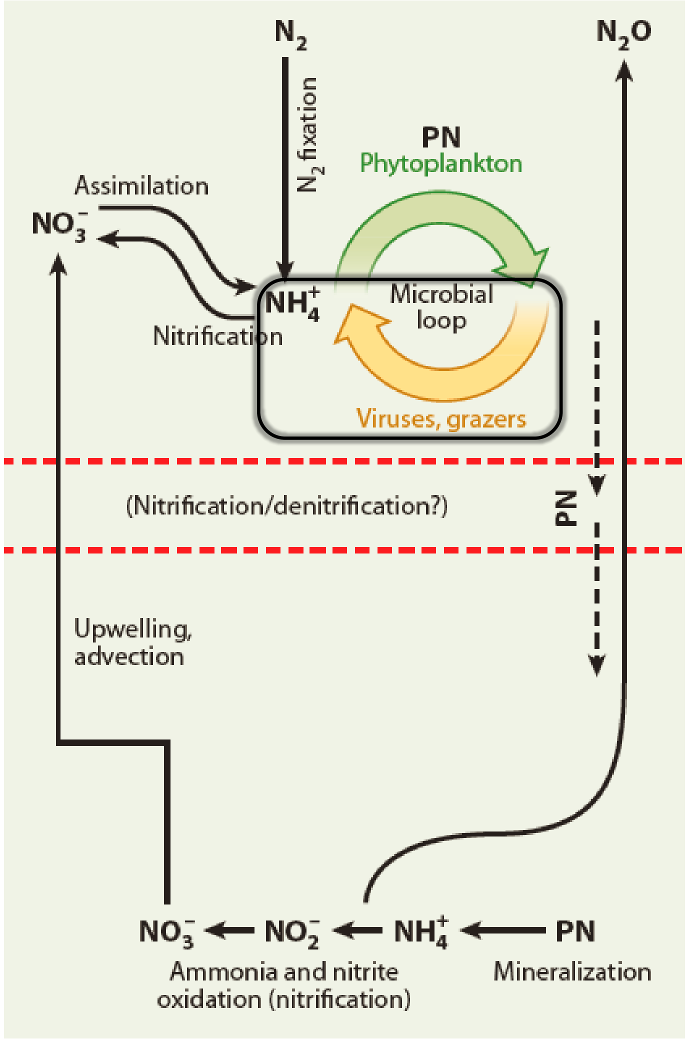
(II) Phytoplankton can use **inorganic** fixed-nitrogen compounds (NH_4^+, NO_3^-, NO_2^-), as well as some **organic** compounds (urea, free amino acids).

(III) Other pathways of N cycling (Denitrification, Anammox and DNRA) can occur in OMZ and sediments.

(Dugdale and Goering, 1967; Zehr and Ward, 2002; Brandes *et al*., 2007; Francis *et al*., 2007; Yool *et al*., 2007).

INTRODUCTION

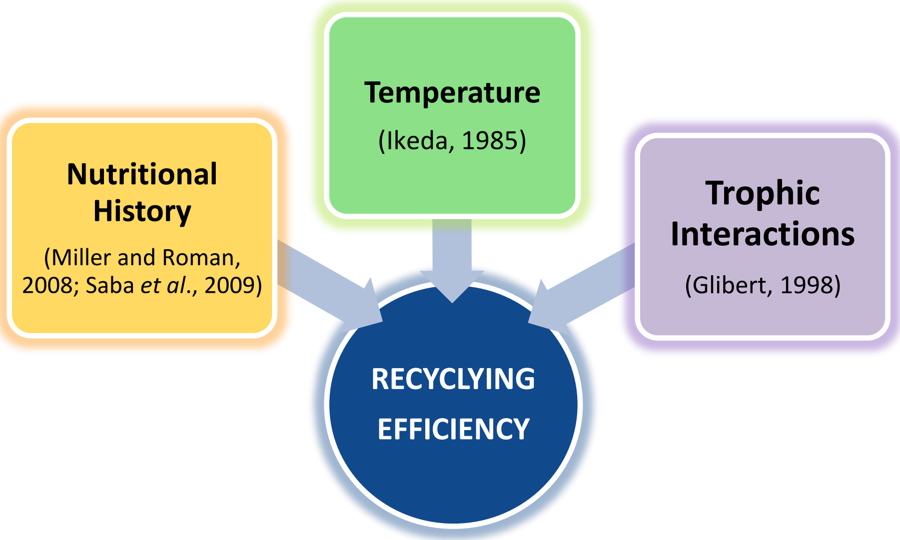
Importance of NH_4^+ in marine systems



Zehr and Kudela (2011)

(I) NH_4^+ is an intermeditate product in decomposition of organic matter, which constitutes **the most reduced form of N**.

(II) NH_4^+ sustains a **global average of 80 %** of the authotroph's requeriments (Harrison, 1992), with the mesozooplankton responsible for 12 – 23 % (Hernández-León, 2008).



Nutritional History

(Miller and Roman, 2008; Saba *et al.*, 2009)

Temperature

(Ikeda, 1985)

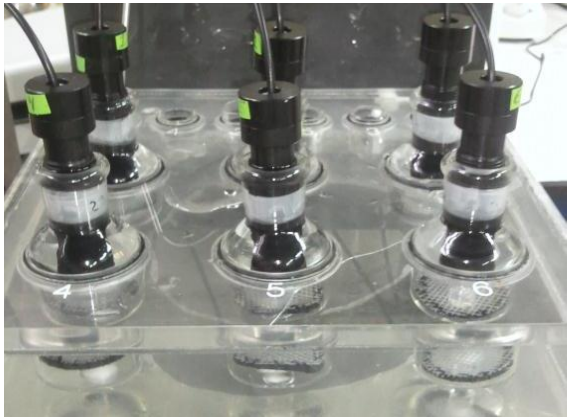
Trophic Interactions

(Glibert, 1998)

RECYCLYING EFFICIENCY

INTRODUCTION

How to determine NH_4^+ excretion in zooplankton?



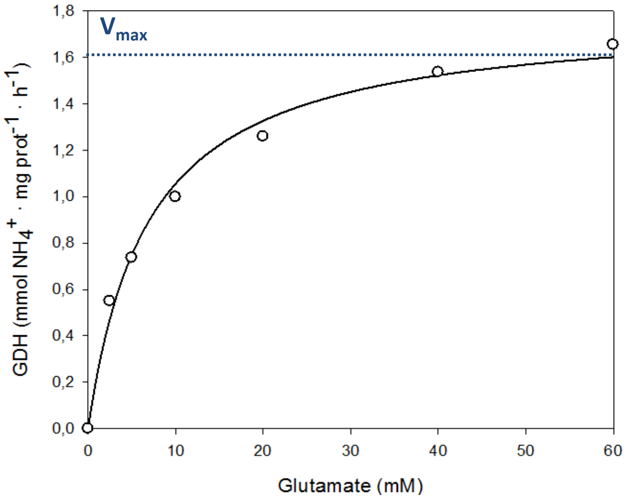
WATER BOTTLE–INCUBATIONS

✓ Direct measurement.

✗ Low data acquisition rate.

Complicated by organism manipulation, overcrowding and starvation.

(Mulling *et al*., 1975; Ikeda and Skjoldal, 1980; Bidigare, 1983)



ENZYMATIC ASSAYS (GDH)

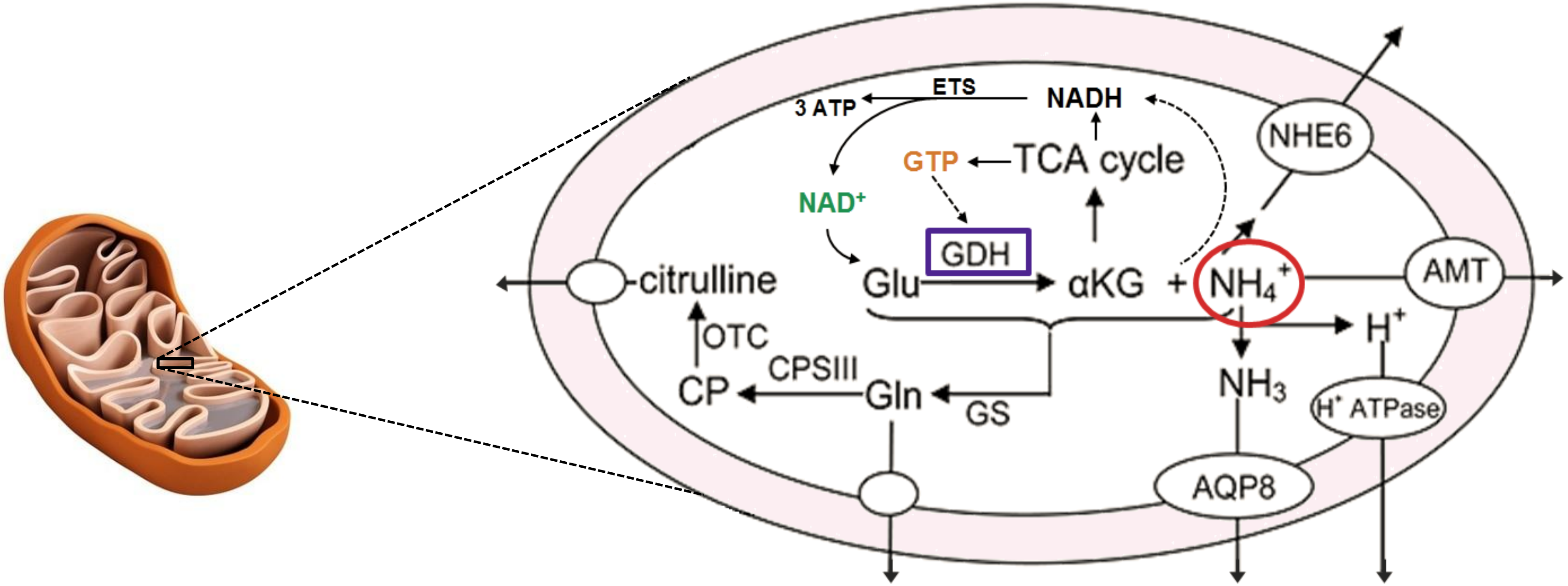
High data acquisition rate.

Measurement of potential NH_4^+ excretion.

Variability in the GDH/RNH_4^+ ratio.

(Bidigare and King, 1981)

Why Glutamate dehydrogenase (GDH, EC 1.4.1.3) ?



Modified from Yuen and Chiew (2010**)**

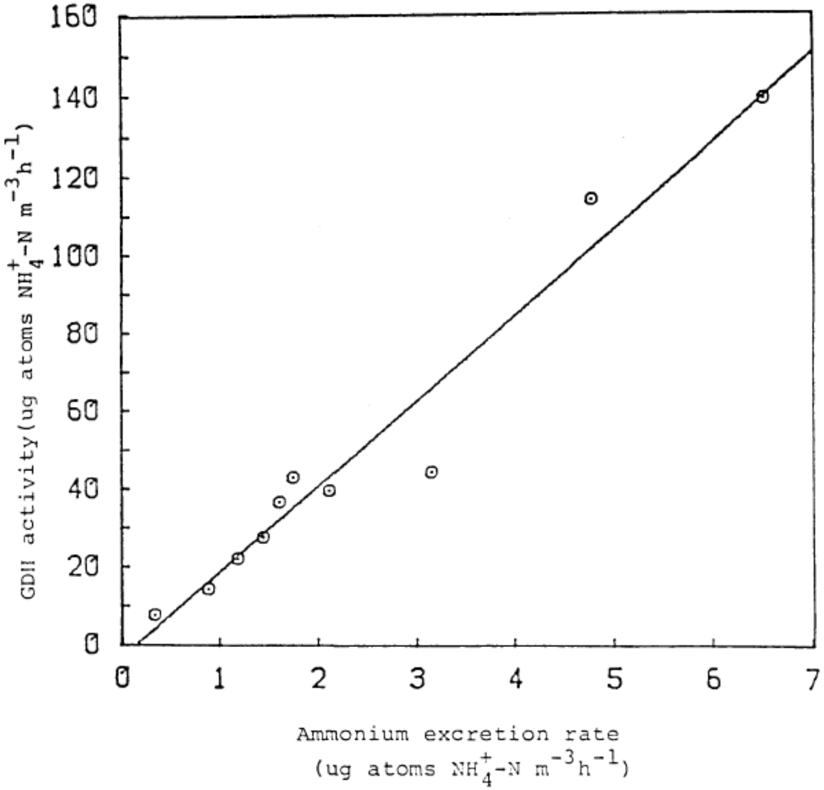
(I) GDH is found in **high levels** in planktonic crustaceans (Regnault, 1987)**.**

⇨ Its role in amino acids catabolism agues for its control over a great proportion of NH_4^+ excretion.

INTRODUCTION

Biochemistry of NH_4^+ in marine zooplankton

(II) Good correlation with NH_4^+ excretion in several marine zooplankters.



Park *et al.* (1986)

Table I. Correlation coefficients between GDH and $R_{NH_4^+}$ calculated in different works.

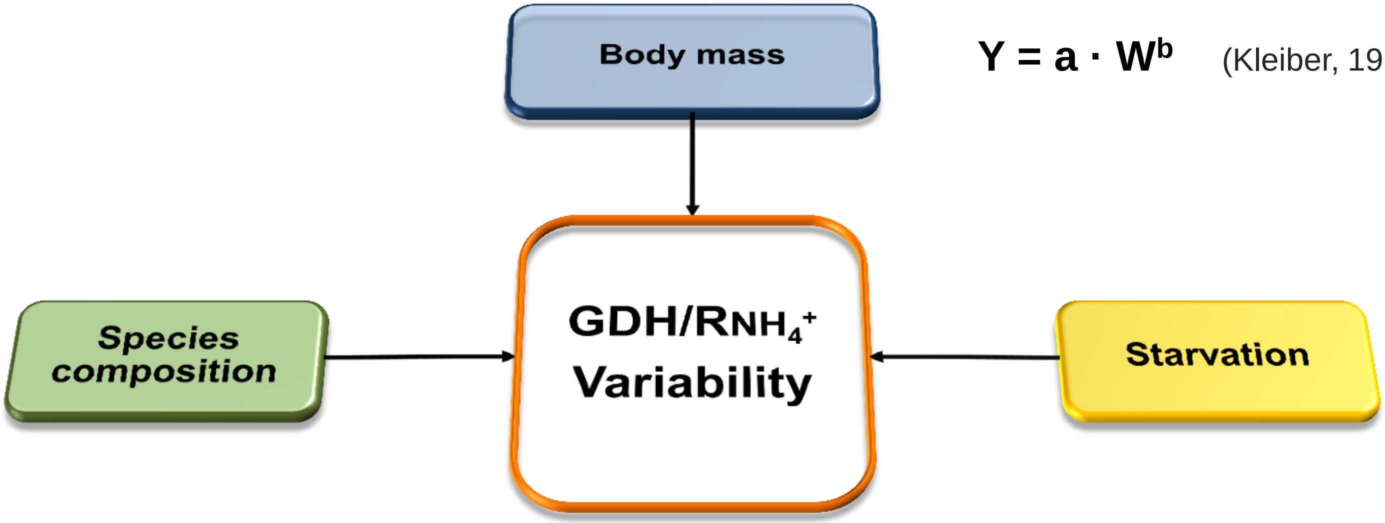
SAMPLE	r^2	REFERENCE
P. flexuosus (n=7)	**0.92**	Bidigare and King (1981)
Macrozooplankton (n=10)	**0.98**	Park *et al.* (1986)
L. lingvura (n=41)	**0.87**	Fernández-Urruzola *et al.* (2011)

But… Is that relationship constant in all the situations?

INTRODUCTION

Variability in the GDH to NH_4^+ excretion ratio

Some factors could affect the relationship between GDH activity and NH_4^+ excretion!!



Body mass

$Y = a \cdot W^b$ (Kleiber, 1961)

Species composition

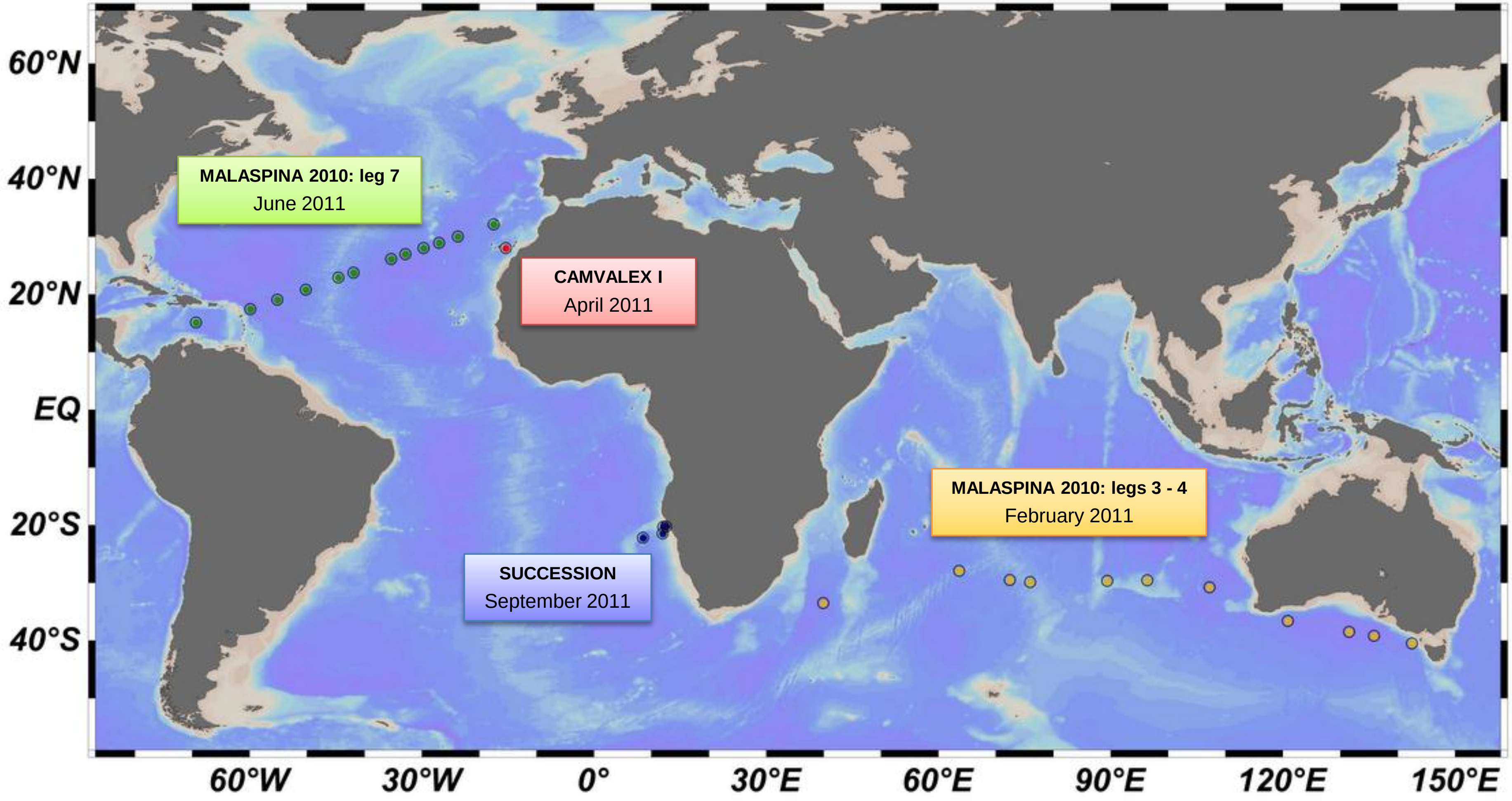
GDH/RNH_4^+ Variability

Starvation

- Physiology and biochemistry should share **scaling exponent** (Berges et al., 1993).
- Changes in **nutritional state** lead to increase the variability (Park, 1986; Hernández-León and Torres, 1997; Fernández-Urruzola *et al*., 2011).

MATERIAL AND METHODS

Location and Sampling



MALASPINA 2010: leg 7
June 2011

CAMVALEX I
April 2011

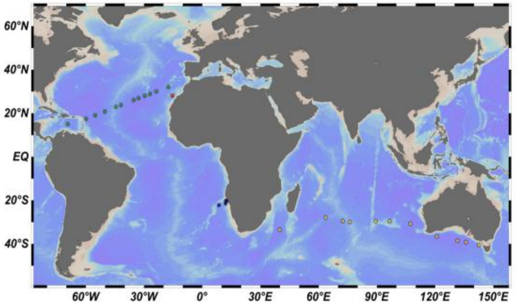
MALASPINA 2010: legs 3 - 4
February 2011

SUCCESSION
September 2011

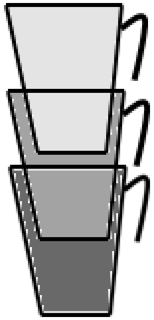
MATERIAL AND METHODS

Experimental design

150 m sampling



SIZE FRACTIONATION



> 1000 µm

500 – 1000 µm

100 – 500 µm

PHYSIOLOGYCAL ANALYSES ON BOARD

(Holmes *et al.*, 1999)

Storage in criovials at -80 ºC

GDH Activity

(Bidigare and King, 1981)

Intracelullar Substrates

(Glutamate and NAD$^+$)

Protein mass

(Lowry, 1951)

Laboratory work at institute

Data analysis

RESULTS AND DISCUSSION

Global averages from the different cruises

Table III. Mean values of $R_{NH_4^+}$ and GDH activity for the zooplankton sampled during the different cruises.

CRUISE	T (º C)	NH_4^+ Excretion (µmol $NH_4^+ \cdot h^{-1} \cdot$ mg protein)		GDH Activity (µmol $NH_4^+ \cdot h^{-1} \cdot$ mg protein)		GDH/$R_{NH_4^+}$
Camvalex I (n = 15)	20	0.46 ± 0.21	**≈ 0.48**	2.13 ± 1.07	**≈ 2.21**	5.43 ± 3.70
Malaspina LEGS 3 - 4 (n = 29)	19	0.30 ± 0.19	**≈ 0.33**	1.90 ± 0.92	**≈ 2.09**	9.57 ± 9.42
Malaspina LEG 7 (n = 61)	21	0.39 ± 0.32		2.39 + 0.94		13.26 ± 15.67
Succession (n =15)	11	0.23 ± 0.20	**≈ 0.42**	1.93 ± 1.33	**≈ 3.52**	22.10 ± 18.59

⇨ **Temperature** influences on both metabolism and biochemistry: *correction via* ***Q_{10} factor*** *needed !!*

Table IV. GDH/$R_{NH_4^+}$ Values from different works.

Sample	**GDH/$R_{NH_4^+}$**	**Reference**
L. lingvura (n = 16)	**10.41 ± 4.84**	Fernández-Urruzola *et al*., 2011
Mixed zooplankton	**12.57 ± 11.11**	Hernández-León and Torres, 1997
Mixed zooplankton (n=8)	**23.4 ± 4.0**	King *et al*., 1997
Mixed zooplankton (n=10)	**18.8 ± 6.72**	Park *et al*., 1987
Mixed zooplankton (n=5)	**18.7 ± 4.3**	Bidigare and King, 1982
C. finmarchicus (n=10)	**16.8 ± 2.6**	Bidigare and King, 1981
Mixed zooplankton (n=5)	**18.7 ± 8.5**	Bidigare, 1981

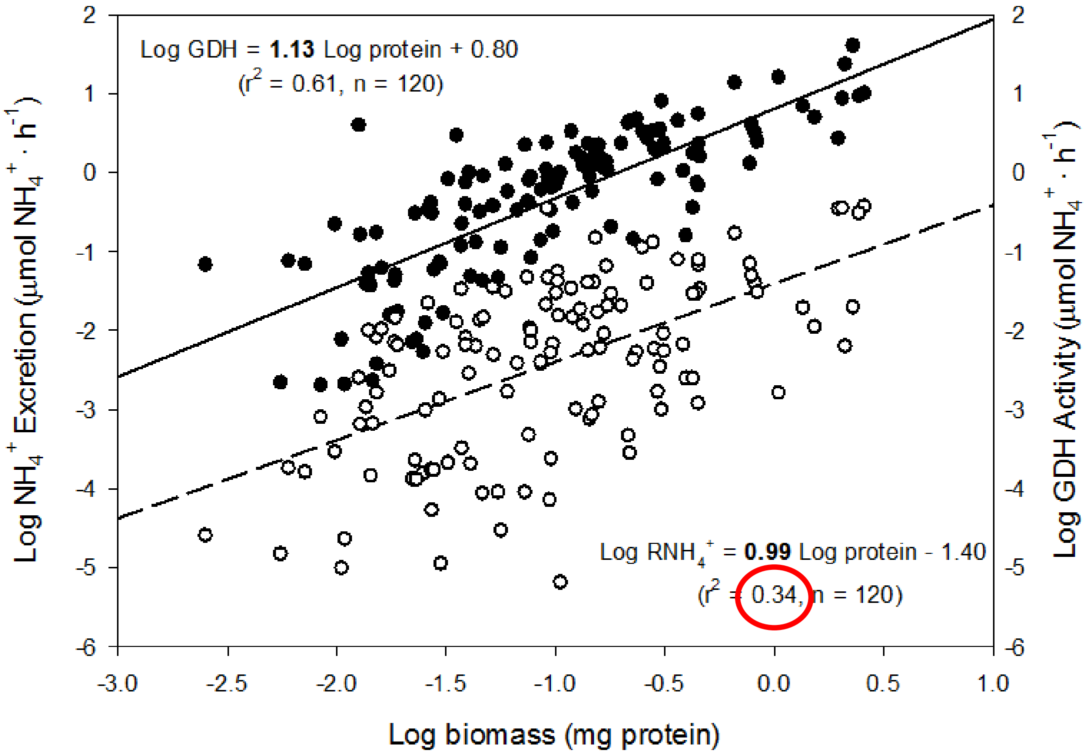
GDH/$R_{NH_4^+}$ values one order of magnitude higher when completely starved.

Park (1986), Fernández-Urruzola *et al*. (2011)

Do these metabolic rates really follow the Kleiber's Law?

RESULTS AND DISCUSSION

Effect of biomass on GDH/RNH$_4^+$ ratio



(I) Enzyme activities **greater** than physiologycal rates.

Potential measurement!

(II) NH$_4^+$ excretion data **more scattered**.

Methodological artifacts?

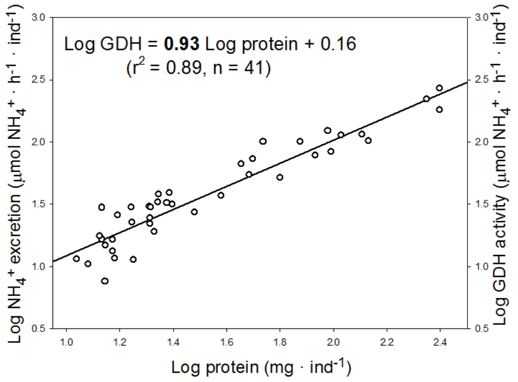
(III) **Isometric relationship** with biomass of both scaling exponents. ($b = 1$, $p < 0.05$)

Values in agreement with other works.

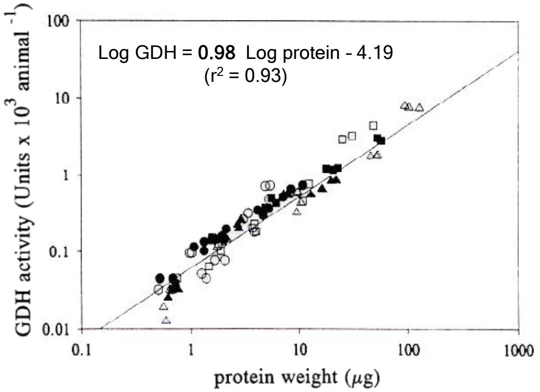
The effect of biomass acts in a similar way in both enzimatic and physiolocal rates. Thus, it is not seem to be the main factor on the GDH/RNH$_4^+$ ratio variability.

RESULTS AND DISCUSSION

Effect of biomass on GDH/RNH_4^+ ratio



Fernández-Urruzola *et al.* (2011)



Berges *et al.* (1993)

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Potential measurement!

(II) NH_4^+ excretion data **more scattered**.

Methodological artifacts?

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Values in agreement with other works.

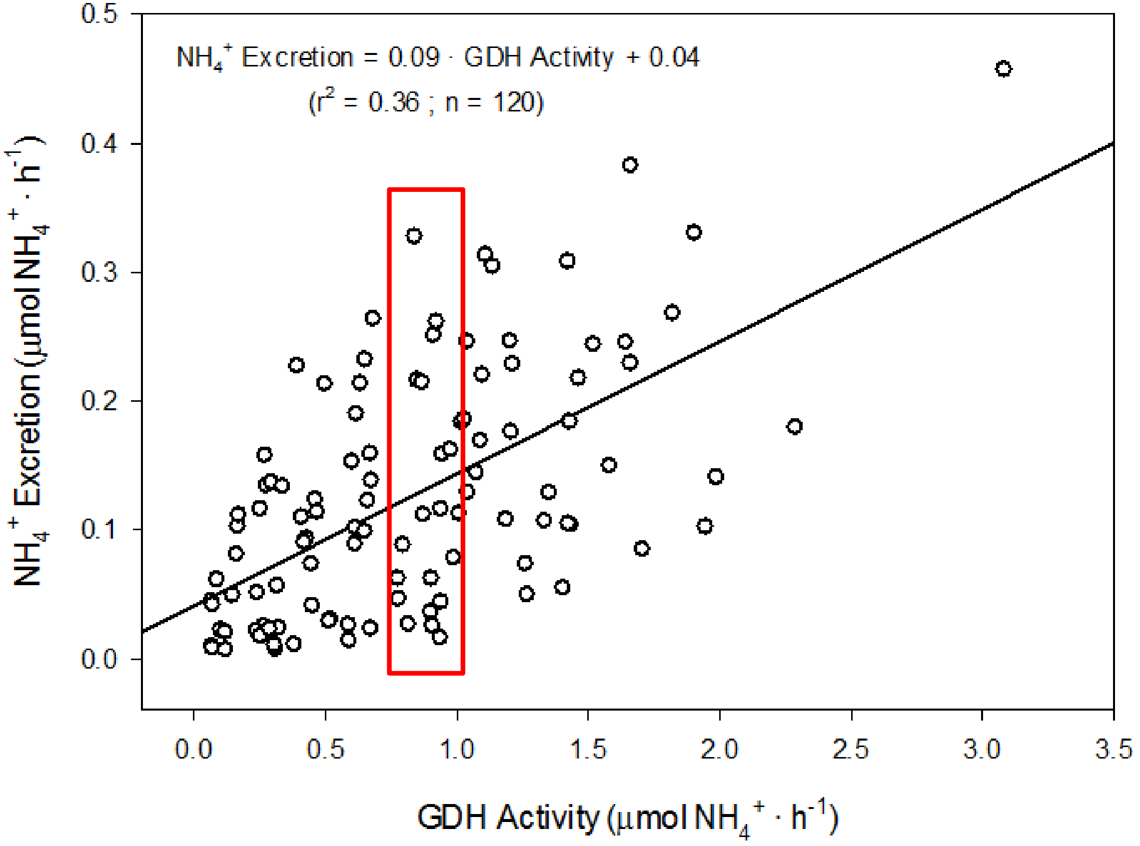
Table V. Scaling exponents for NH_4+ excretion rates summarized from the literature.

Organisms	Mass variable	NH_4^+ Scaling exponent	Reference
Ocean zooplankton	Dry weight	**1.00**	Blazka *et al*., 1982
Ocean zooplankton	Dry weight	**1.00**	Vidal and Whitledge, 1982
Crangon crangon	Dry weight	**0.79**	Regnault and Batrel, 1987
Ocean zooplankton	Carbon	**0.84**	Ikeda 1988
Ocean zooplankton	Nitrogen	**0.65 - 0.80**	Ikeda and Skjoldal, 1989
Mixed zooplankton	Carbon	**0.92**	Schmeider, 1990

The effect of biomass acts in a similar way in both enzimatic and physiolocal rates. Thus, it is not seem to be the main factor on the GDH/RNH_4^+ ratio variability.

RESULTS AND DISCUSSION

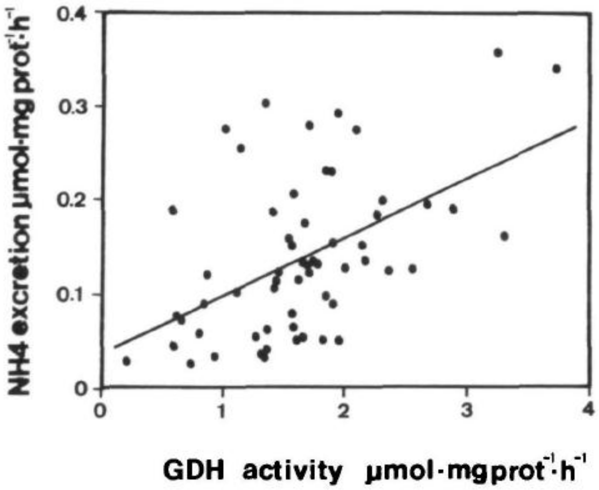
GDH activity to NH_4^+ excretion relationship



The correlation between both variables is far from the stronger values found in culture conditions. Nevertheless, the slope is still in the same range (0.02 – 0.1).

Field data from waters around the Canary Islands showed a similar pattern.

Hernández-León and Torres (1997)

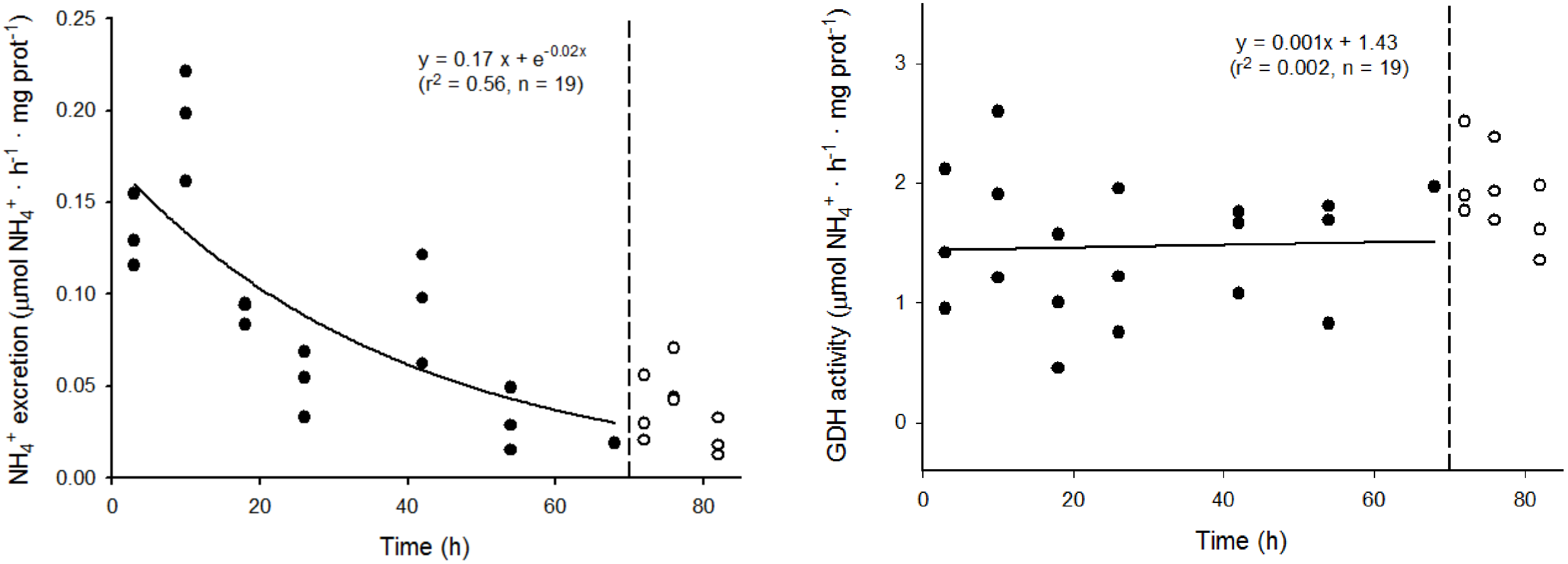


The correlation should increase if we select points with the same nutritional state.

RESULTS AND DISCUSSION

GDH activity to NH_4^+ excretion relationship

- Rapid fall of nitrogen release after depletion of the food source (Mayzaud, 1976; Ikeda and Skjodal, 1980).
- GDH does not vary significantly with environmental changes, proving its constitutive nature.



Fernández-Urruzola (2011)
Herrera *et al.* (2011)

RESULTS AND DISCUSSION

GDH activity to NH_4^+ excretion relationship

- Rapid fall of nitrogen release after depletion of the food source (Mayzaud, 1976; Ikeda and Skjodal, 1980).
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Table VI. Effect of starvation on the Michaelis constant for glutamate, and on the GDH/$R_{NH_4^+}$ and R_{O_2}/$R_{NH_4^+}$ ratios.

Starvation (h)	Apparent Km (mM)	GDH/$R_{NH_4^+}$	O_2 consumption (μmol $O_2 \cdot h^{-1} \cdot$ mg protein^{-1})	R_{O_2}/$R_{NH_4^+}$
4	4.69 ± 0.69	11.23 ± 4.46	0.61 ± 0.09	4.57 ± 1.90
10	12.1 ± 5.9	9.74 ± 2.98	1.29 ± 0.32	6.66 ± 1.65
18	8.55 ± 4.04	11.14 ± 5.93	0.5 ± 0.15	5.51 ± 2.47
26	19.44 ± 10.8	30.87 ± 25.28	0.6 ± 0.11	11.51 ± 1.73
42	4.67 ± 0.79	16.28 ± 1.55	0.2 ± 0.18	2.13 ± 0.29
54	5.04 ± 0.27	64.72 ± 51.3	0.22 ± 0.22	7.07 ± 3.25
68	2.84	102.41	0.24	12.9
72	4.89 ± 3.45	67.89 ± 29.5	0.55 ± 0.05	15.49 ± 8.6
76	5.95 ± 2.48	39.1 ± 5.09	0.74 ± 0.43	14.12 ± 2.96
82	6.25 ± 2.51	89.1 ± 34.58	0.59 ± 0.36	47.2 ± 15.5

Fernández-Urruzola *et al.* (2011)

RESULTS AND DISCUSSION

GDH activity to NH_4^+ excretion relationship

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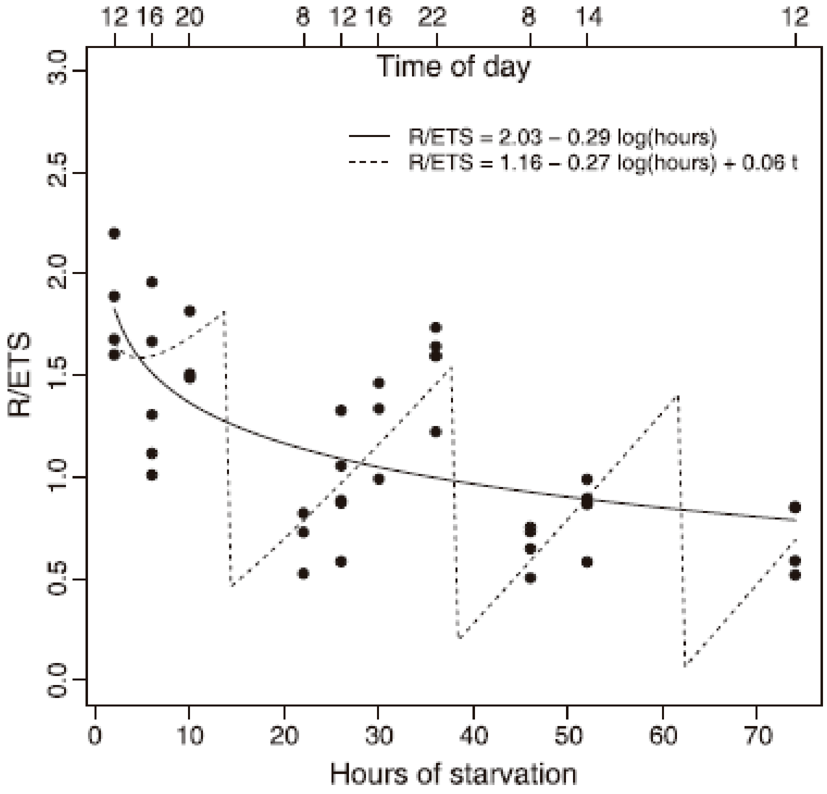
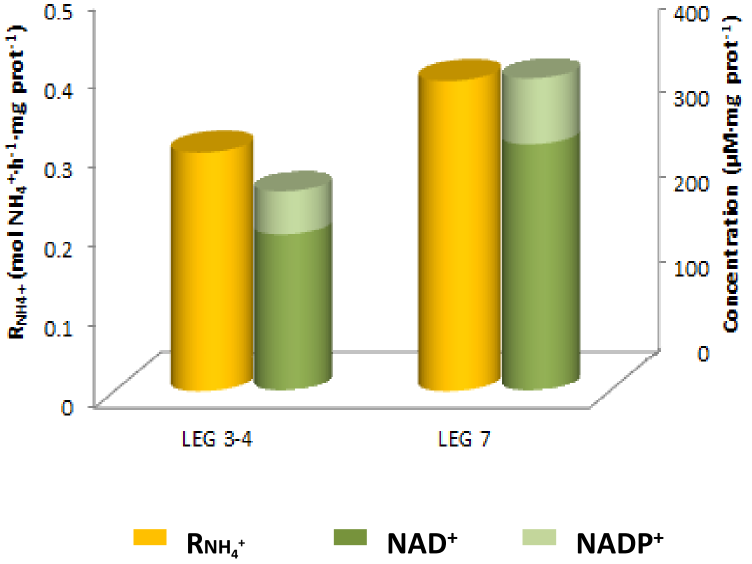
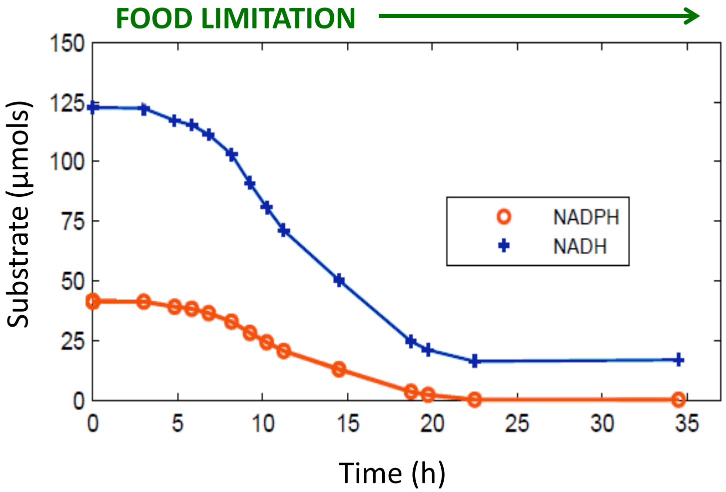


RESULTS AND DISCUSSION

Intracellular substrate levels as a key factor

The intracellular substrates might be the answer !!





Roy and Packard (1998) found a decrease in intracellular substrates concentrations with food source limitation. As a concequence, the actual enzimatic rates would also decrease.

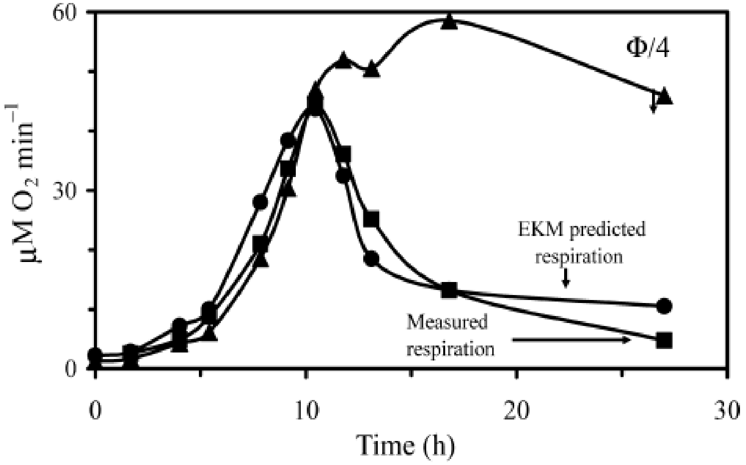
Aguiar-González *et al.* (2012)

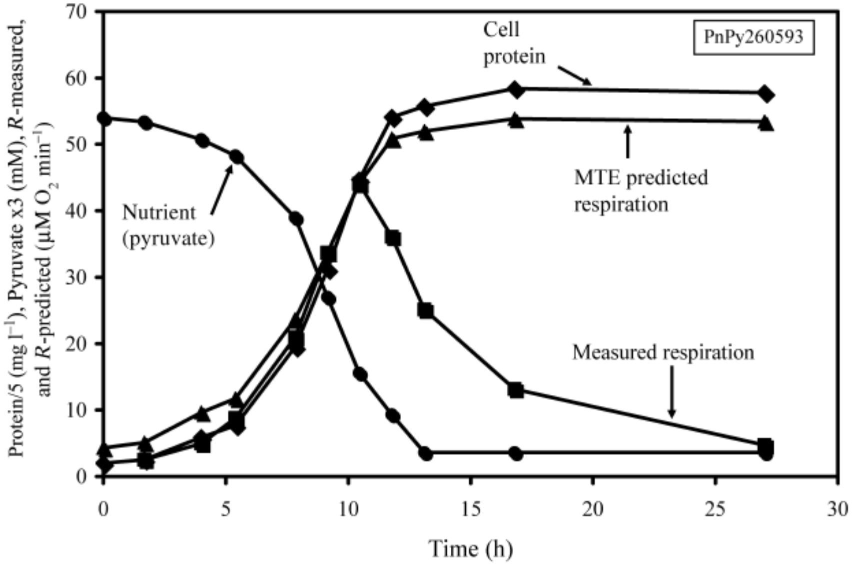
RESULTS AND DISCUSSION

Future work

An enzyme kinetic-based model should predict the *in vivo* RNH_4^+ on natural samples of zooplankton from different productivity areas.

$$\mathbf{RNH_4}^+ = \frac{\mathrm{GDH} \cdot ([S_1] \cdot [S_2])}{KS_1 \cdot K_{ia} + KS_2 \cdot [S_1] + KS_1 \cdot [S_2] + [S_1] \cdot [S_2]}$$





Packard and Gómez (2008)

SUMMARY

(I) GDH/RNH_4^+ is not constant in all the marine ecosystems sampled so far. However, the ratios argue that the zooplankton communities are in a healthy physiological state.

(II) Starvation causes NH_4^+ excretion and GDH activity to diverge more than does biomass.

(III) Intracellular substrate levels should explain the variability between the physiological and enzimatic rates. As a result, a kinetic-based model would predict *in vivo* NH_4^+ excretion rates better than other theories based in biomass, such as the MTE.

AWKNOLEDGEMENTS

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