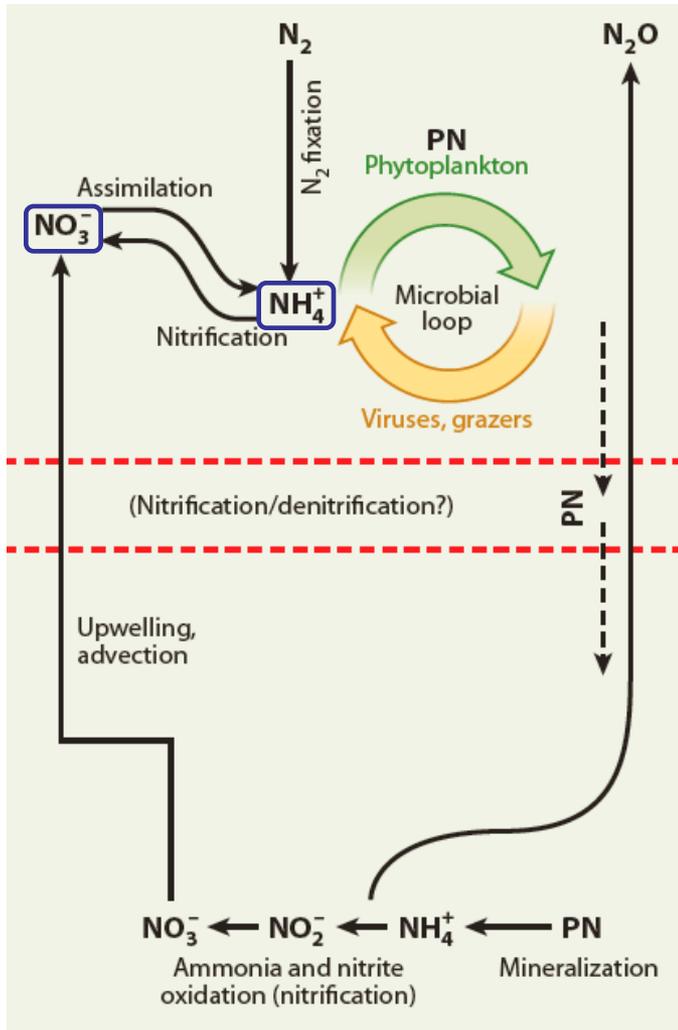


Is the $GDH/R_{NH_4^+}$ ratio in the mesozooplankton constant through different oceanic systems?

Fernández-Urruzola I., Packard T. and Gómez M.

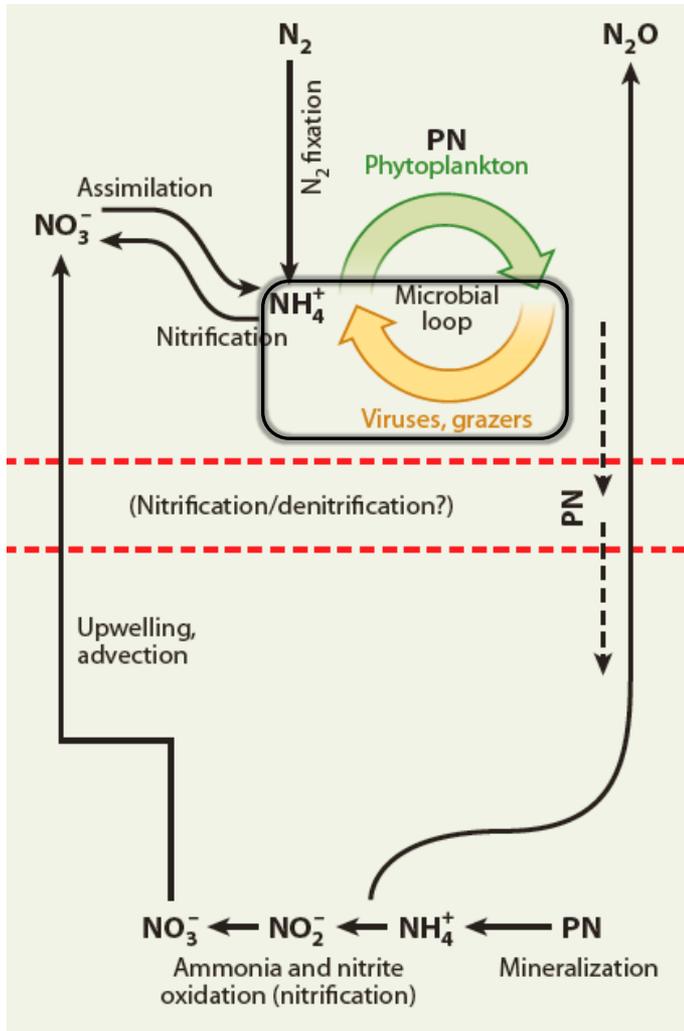
Institute of Oceanography and Global Change, Biological Oceanography Group, University of Las Palmas de Gran Canaria, Canary Islands, Spain. E-mail address: ifernandez@becarios.ulpgc.es



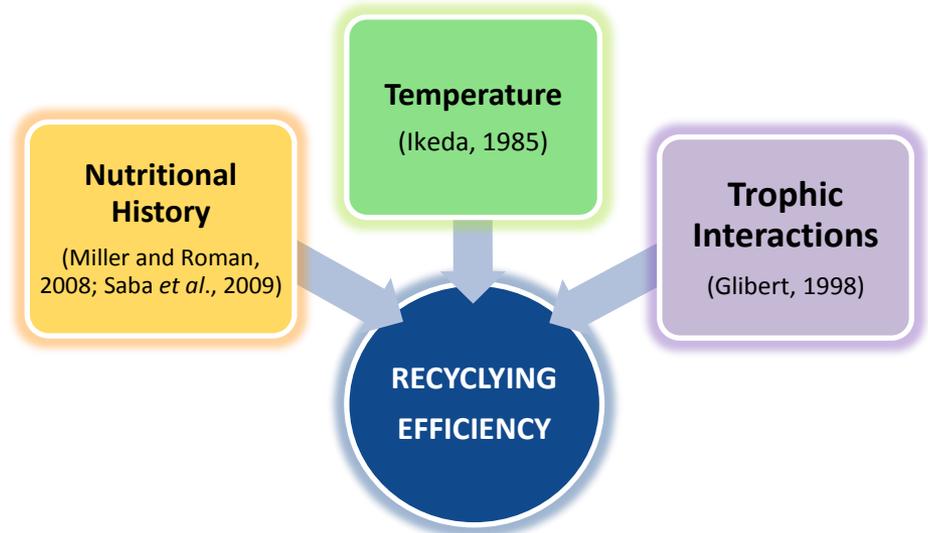


- (I) Nitrogen availability is **frequently limited** in ocean ecosystems.
- (II) Phytoplankton can use **inorganic** fixed-nitrogen compounds (NH_4^+ , NO_3^- , NO_2^-), as well as some **organic** compounds (urea, free amino acids).
- (III) Other pathways of N cycling (Denitrification, Anammox and 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).



- (I) NH_4^+ is an intermediate 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 autotroph's requirements (Harrison, 1992), with the mesozooplankton responsible for 12 – 23 % (Hernández-León, 2008).





WATER BOTTLE-INCUBATIONS



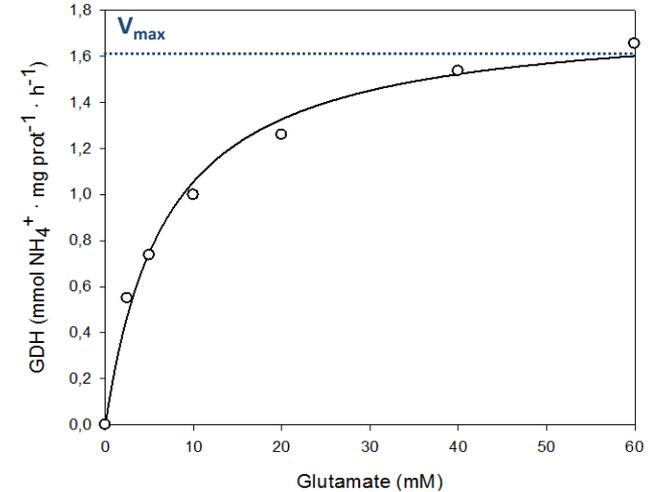
Direct measurement.

Low data acquisition rate.



Complicated by organism manipulation,
overcrowding and starvation.

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



ENZYMATIC ASSAYS (GDH)

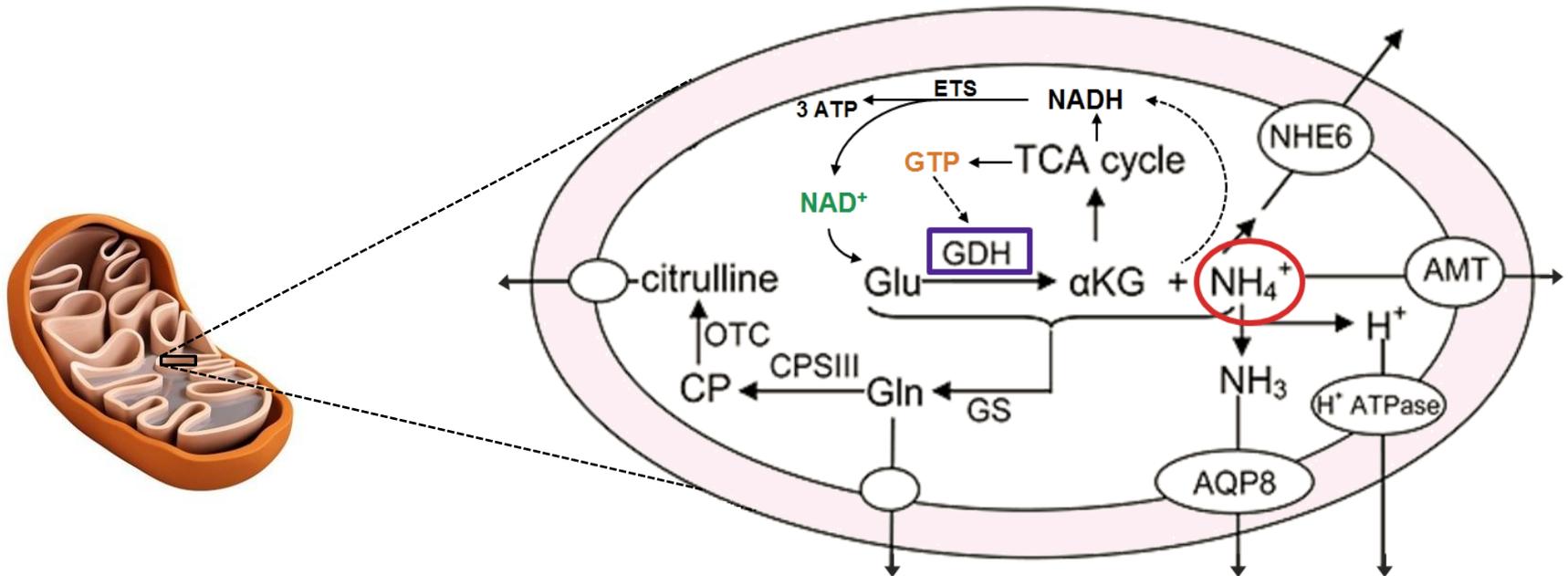
High data acquisition rate.

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) ?

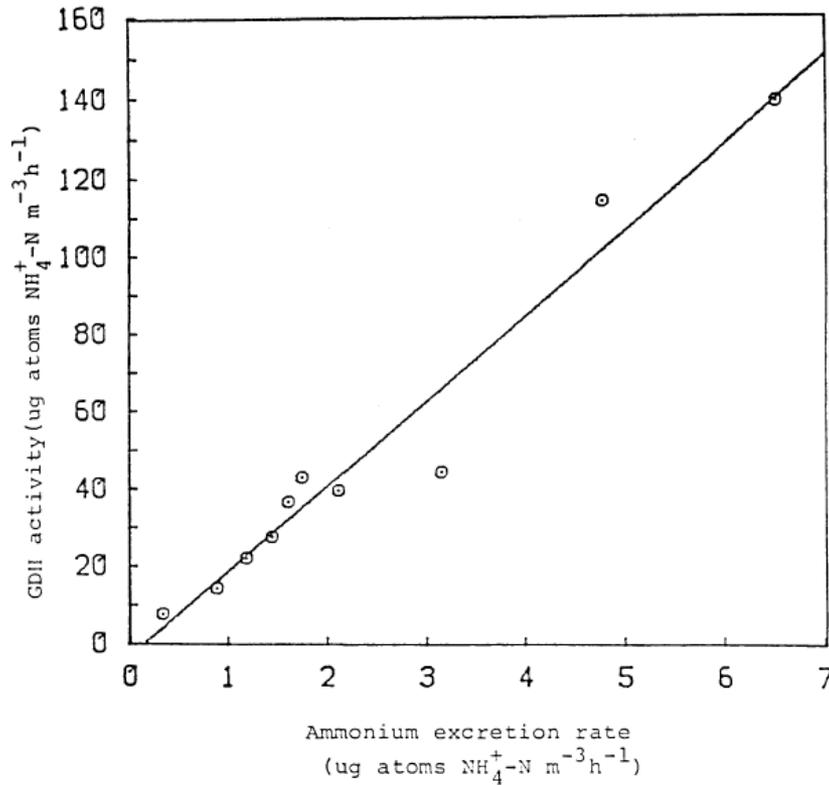


Modified from Yuen and Chiew (2010)

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

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

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



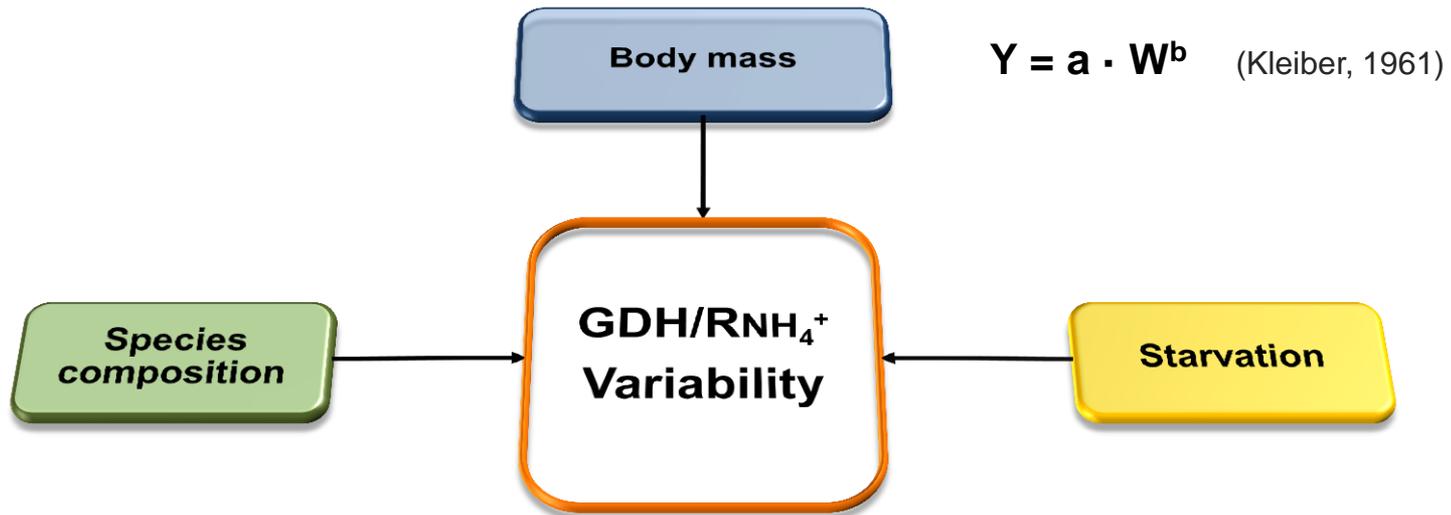
Park *et al.* (1986)

Table I. Correlation coefficients between GDH and RNH_4^+ calculated in different works.

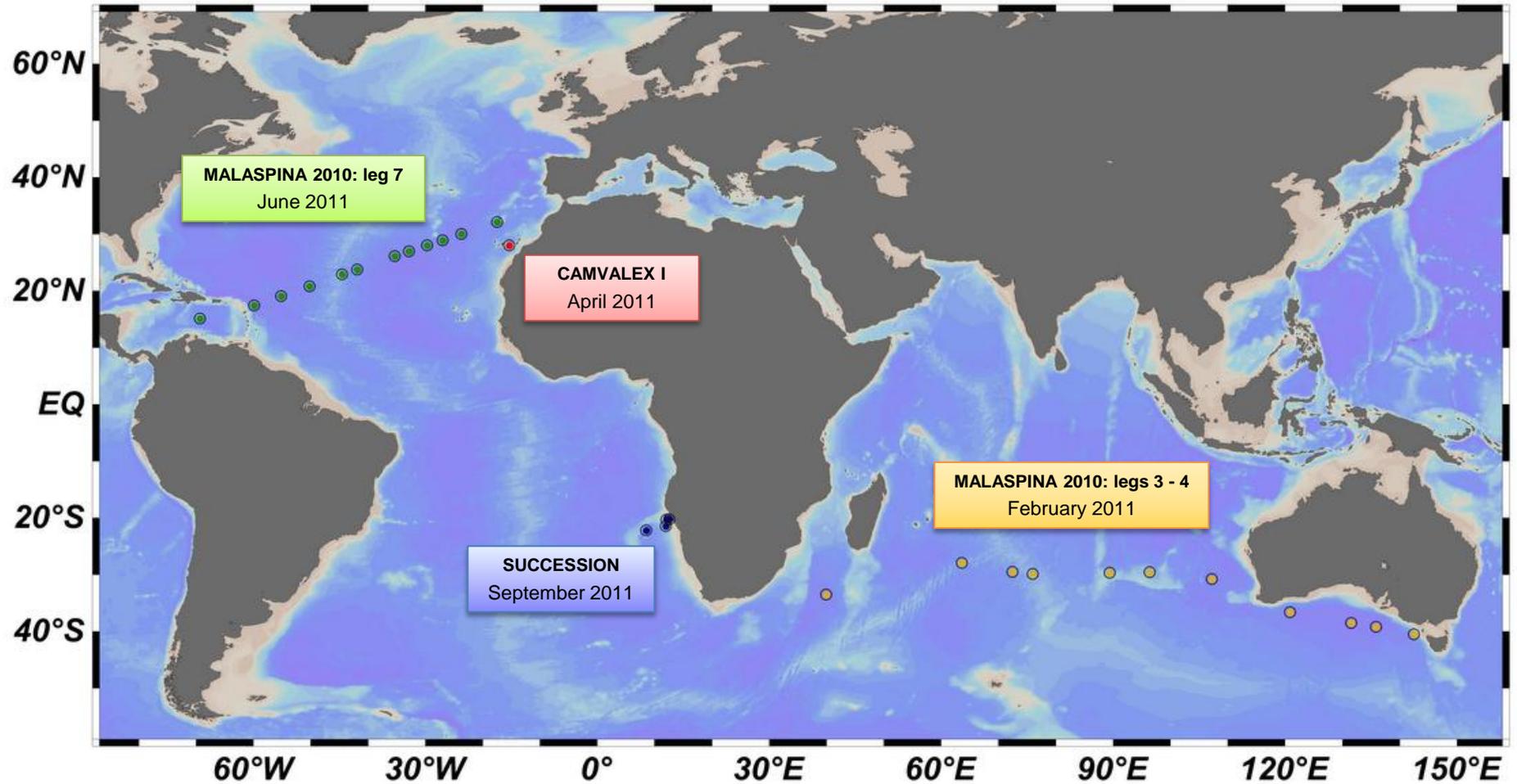
SAMPLE	r^2	REFERENCE
<i>P. flexuosus</i> (n=7)	0.92	Bidigare and King (1981)
Macrozooplankton (n=10)	0.98	Park <i>et al.</i> (1986)
<i>L. lingvura</i> (n=41)	0.87	Fernández-Urruzola <i>et al.</i> (2011)

But... Is that relationship constant in all the situations?

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



- 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).



SIZE FRACTIONATION



> 1000 μm

500 – 1000 μm

100 – 500 μm

PHYSIOLOGICAL ANALYSES ON BOARD

(Holmes *et al.*, 1999)

Storage in criovials at -80 °C

GDH Activity

(Bidigare and King, 1981)

Intracellular
Substrates

(Glutamate and NAD⁺)

Protein
mass

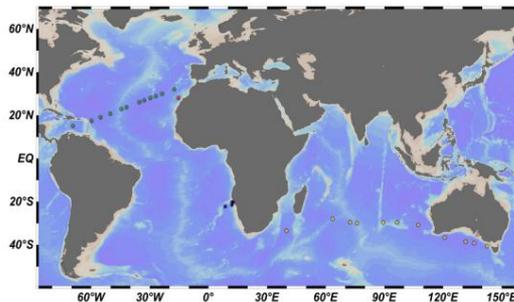
(Lowry, 1951)

Laboratory work at institute

Data analysis



150 m sampling



RESULTS AND DISCUSSION

Global averages from the different cruises

Table III. Mean values of RNH₄⁺ and GDH activity of the 0 plankton sizes of zooplankton from different cruises.

CRUISE	T (° C)	NH ₄ ⁺ Excretion (μmol NH ₄ ⁺ · h ⁻¹ · mg protein)	GDH Activity (μmol NH ₄ ⁺ · h ⁻¹ · mg protein)	GDH/RNH ₄ ⁺
Camvalex I (n = 15)	20	0.46 ± 0.21	2.13 ± 1.07	5.43 ± 3.70
Malaspina LEGS 3 - 4 (n = 29)	19	0.30 ± 0.19	1.90 ± 0.92	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	1.93 ± 1.33	22.10 ± 18.59

⇒ **Temperature** influences on both metabolism and biochemistry: *correction via Q₁₀ factor needed !!*

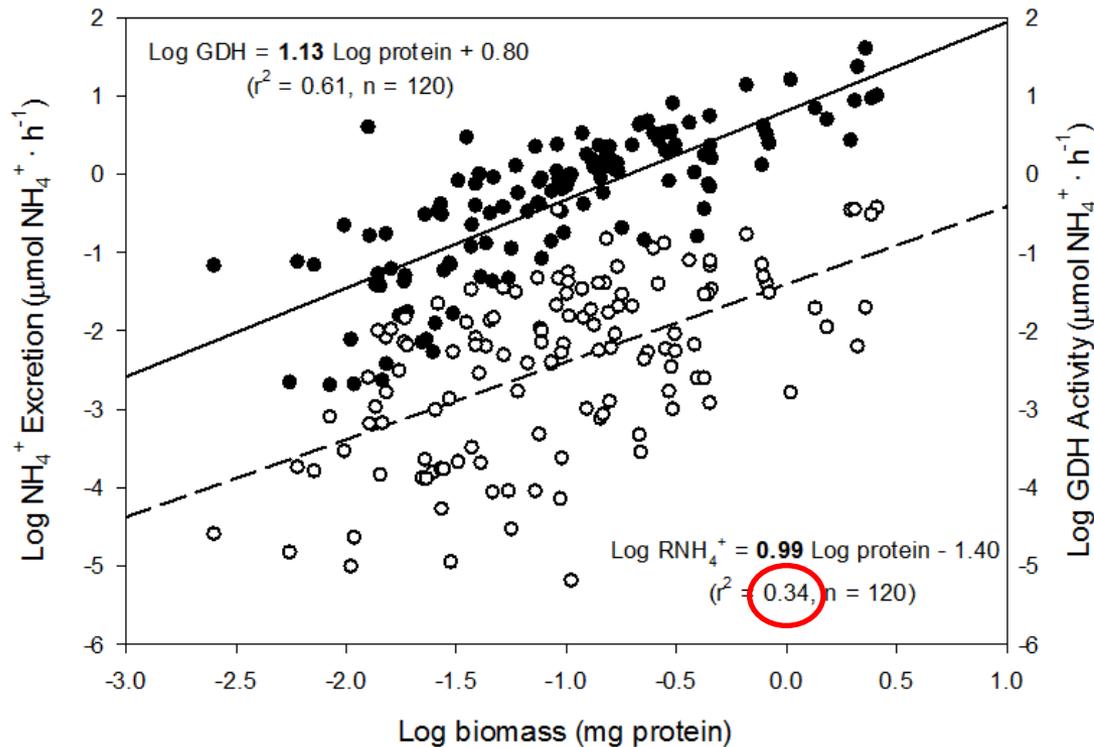
Table IV. GDH/RNH₄⁺ Values from different works.

Sample	GDH/RNH ₄ ⁺	Reference
L. lingvura (n = 16)	10.41 ± 4.84	Fernández-Urruzola <i>et al.</i> , 2011
Mixed zooplankton	12.57 ± 11.11	Hernández-León and Torres, 1997
Mixed zooplankton (n=8)	23.4 ± 4.0	King <i>et al.</i> , 1997
Mixed zooplankton (n=10)	18.8 ± 6.72	Park <i>et al.</i> , 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/RNH₄⁺ 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?



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

Potential measurement!

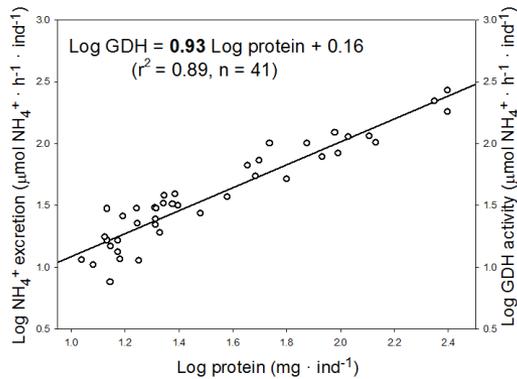
(II) NH₄⁺ excretion data **more scattered**.

Methodological artifacts?

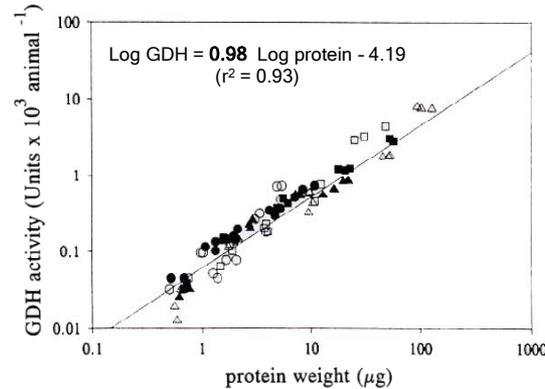
(III) **Isometric relationship** with biomass of both scaling exponents. ($b = 1$, $p < 0.05$)

Values in agreement with other works.

The effect of biomass acts in a similar way in both enzymatic and physiological rates. Thus, it is not seem to be the main factor on the GDH/RNH₄⁺ ratio variability.



Fernández-Urruzola *et al.* (2011)



Berges *et al.* (1993)

Table V. Scaling exponents for NH₄⁺ excretion rates summarized from the literature.

Organisms	Mass variable	NH ₄ ⁺ Scaling exponent	Reference
Ocean zooplankton	Dry weight	1.00	Blazka <i>et al.</i> , 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	Schneider, 1990

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

Potential measurement!

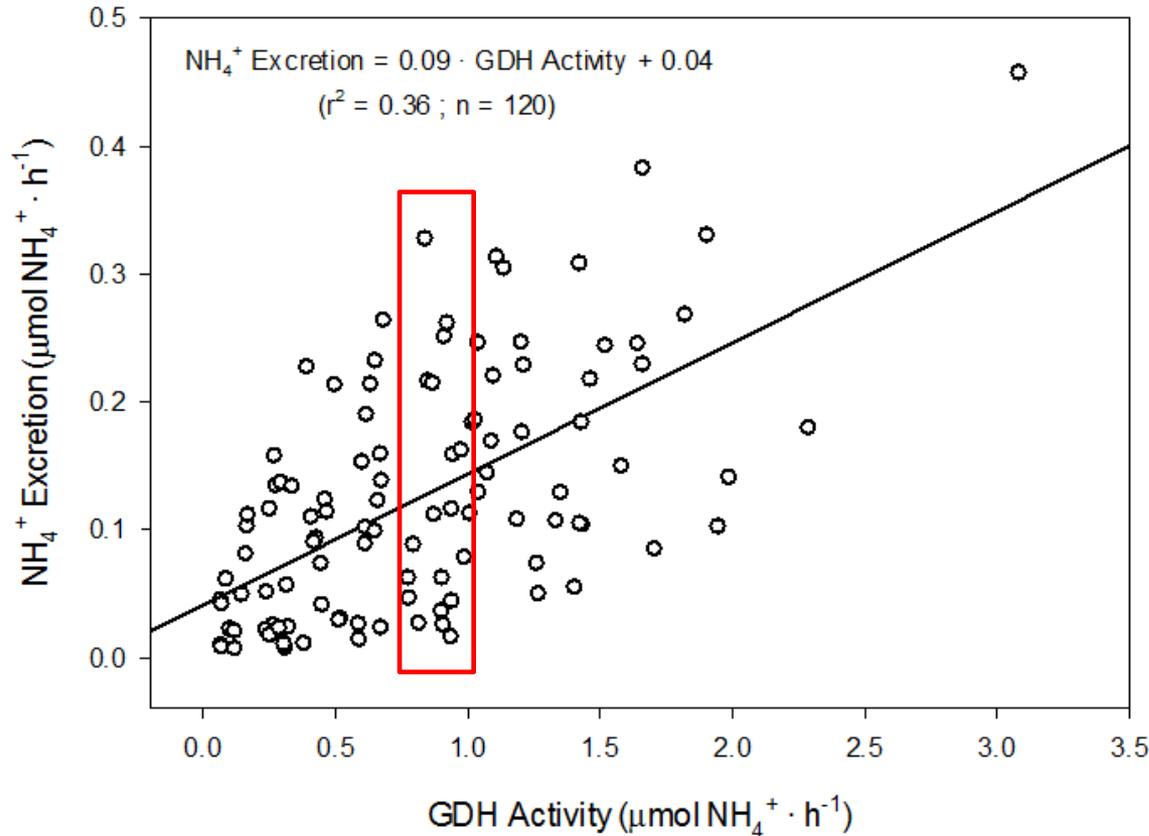
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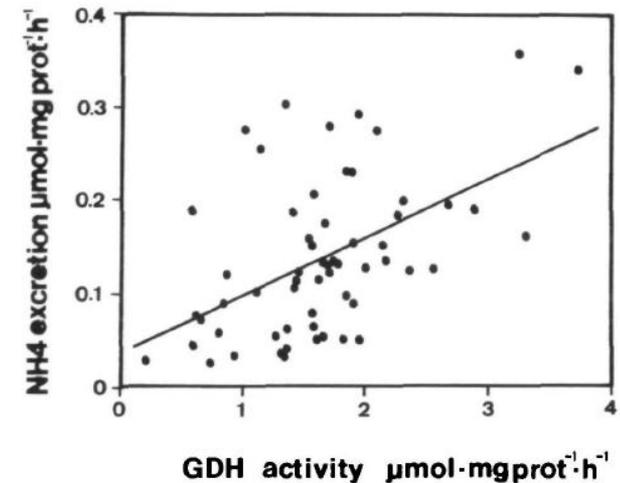


The correlation should increase if we select points with the same nutritional state.

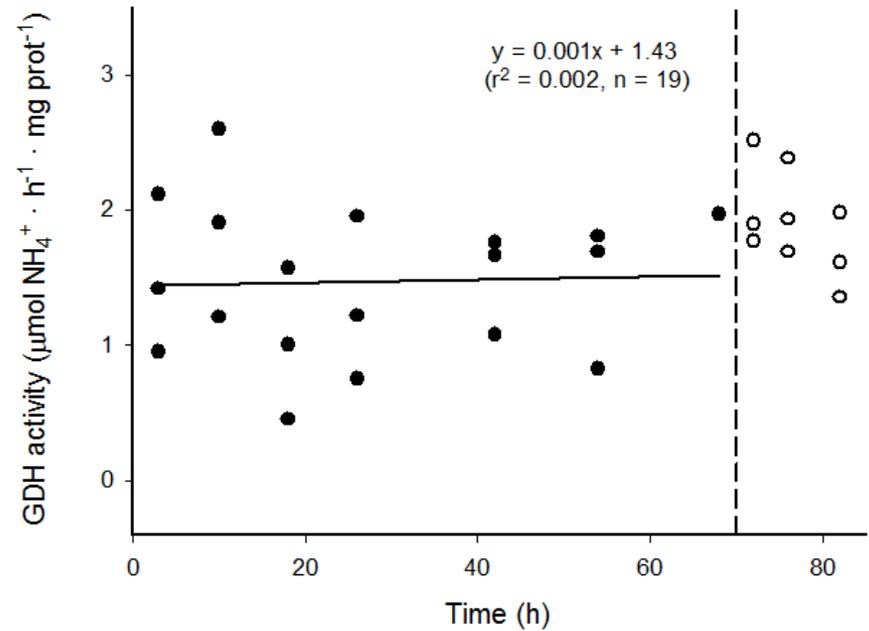
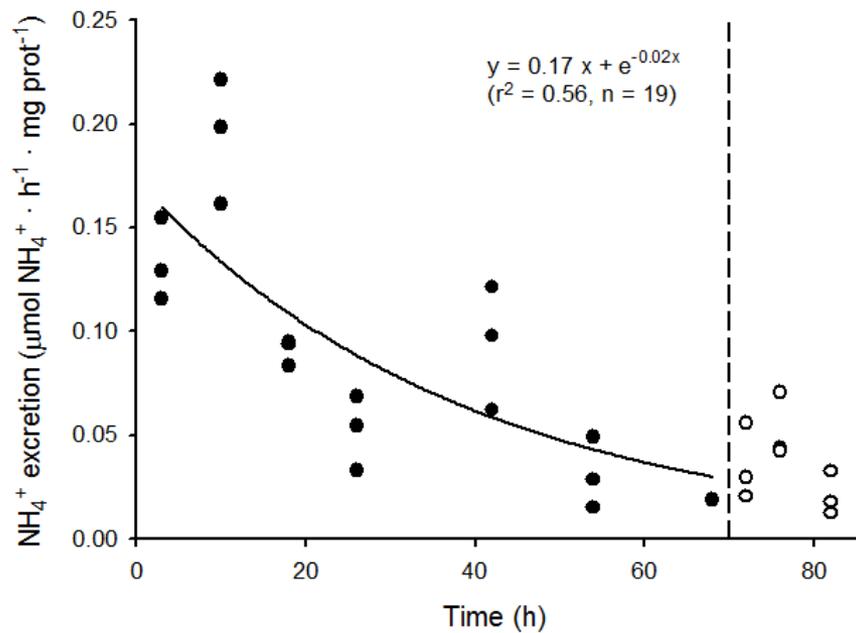
The correlation between both variables is far from the stronger values found in culture conditions. Nevertheless, the slope is still in the same range (0.02 – 0.1).

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

Hernández-León and Torres (1997)



- 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.



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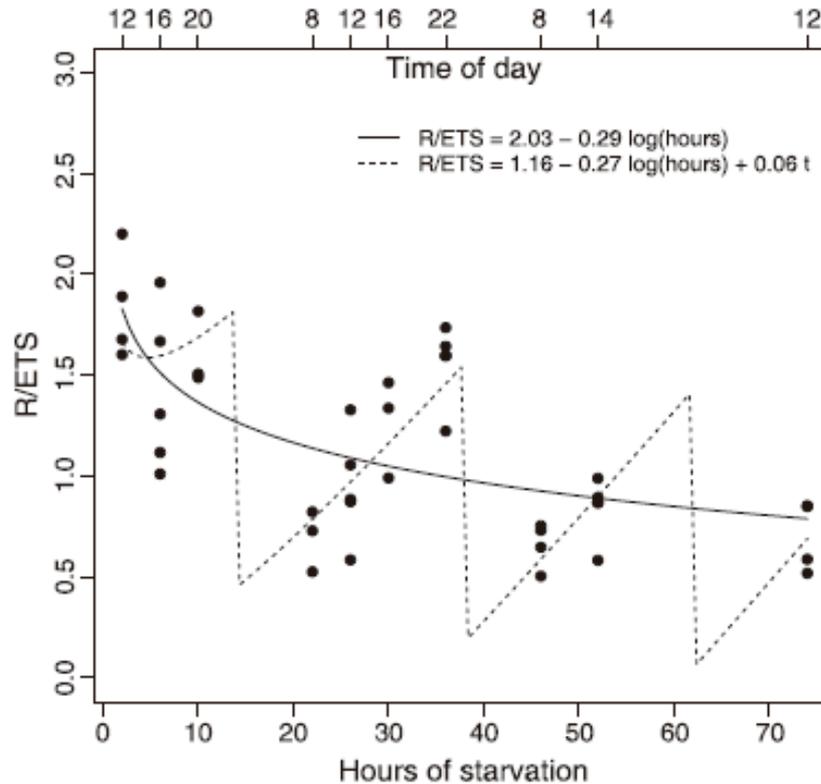
Table VI. Effect of starvation on the Michaelis constant for glutamate, and on the GDH/ RNH_4^+ and $\text{RO}_2/\text{RNH}_4^+$ ratios.

Starvation (h)	Apparent K_m (mM)	GDH/ RNH_4^+	O_2 consumption ($\mu\text{mol O}_2 \cdot \text{h}^{-1} \cdot \text{mg protein}^{-1}$)	$\text{RO}_2/\text{RNH}_4^+$
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

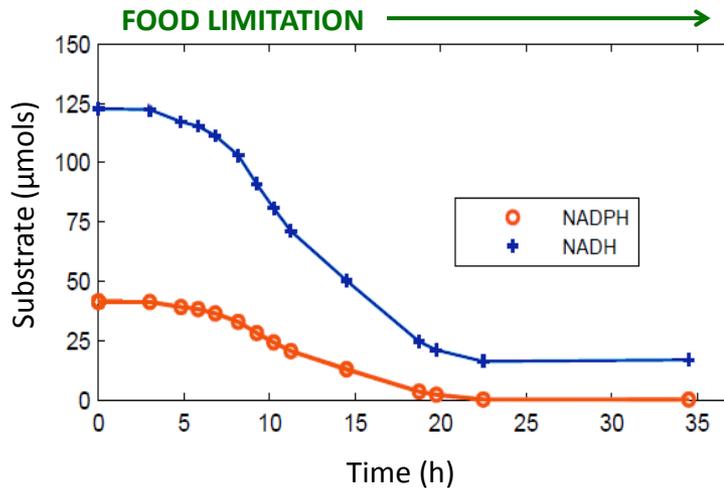
RESULTS AND DISCUSSION

GDH activity to NH_4^+ excretion relationship

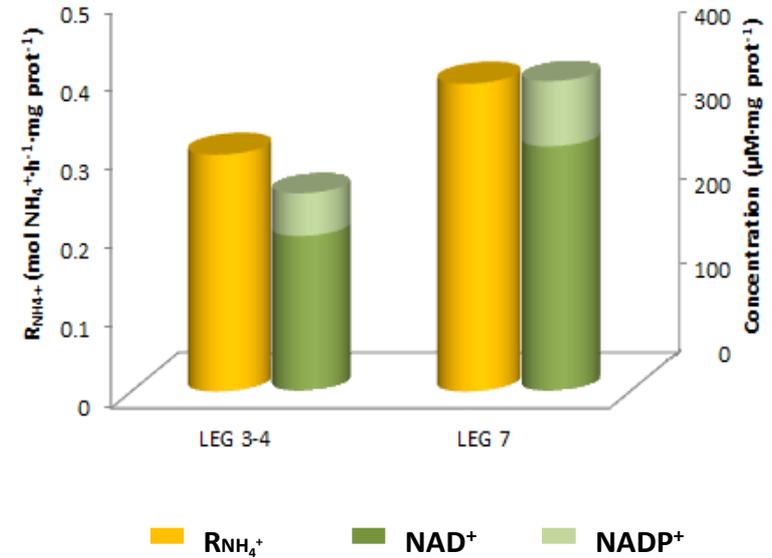
- Rapid fall of nitrogen release after depletion of the food source (Mayzaud, 1976; Ikeda and Skjodal, 1980).
- GDH does not vary significantly with environmental changes, proving its constitutive nature.



The intracellular substrates
might be the answer !!



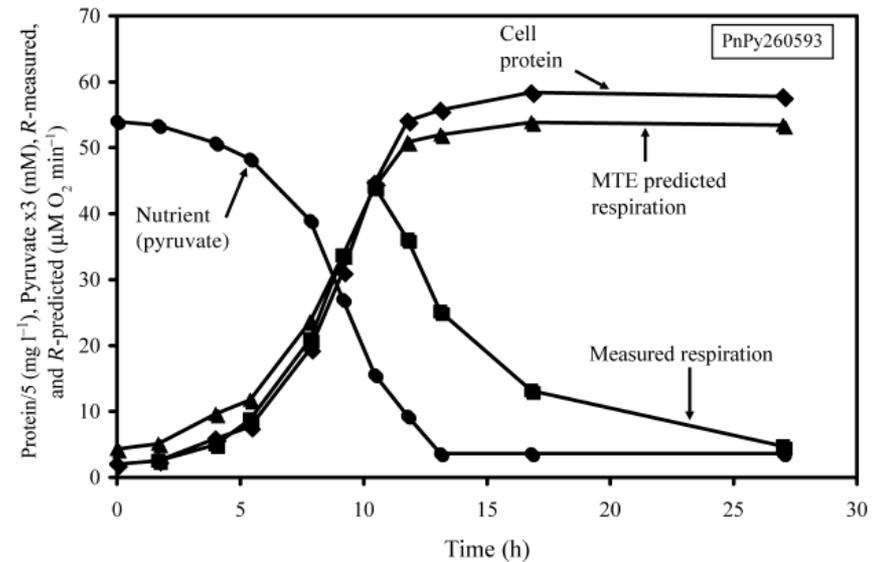
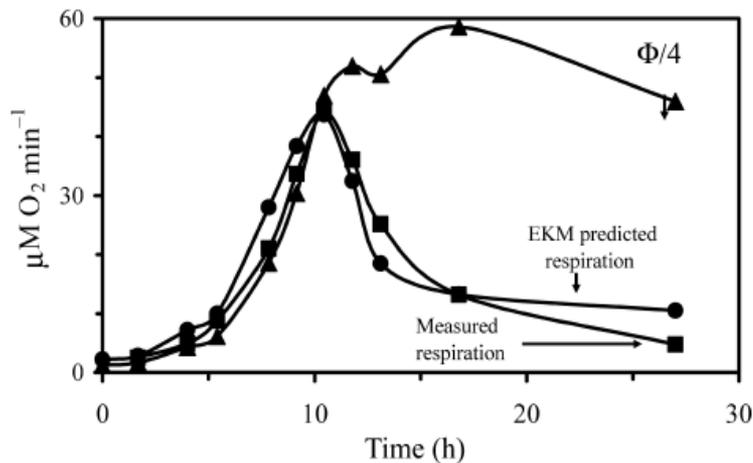
Aguiar-González *et al.* (2012)



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

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

$$\text{RNH}_4^+ = \frac{\text{GDH} \cdot ([S_1] \cdot [S_2])}{\text{KS}_1 \cdot K_{ia} + \text{KS}_2 \cdot [S_1] + \text{KS}_1 \cdot [S_2] + [S_1] \cdot [S_2]}$$



Packard and Gómez (2008)

- (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 enzymatic 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.

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I also thank to MALASPINA 2010 (CSD-20080077) and SUCCESSION projects for inviting me to participate in their cruises.



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