Is the GDH/R_{NH4^+} ratio in the mesozooplankton constant through different oceanic systems?

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

(II) Phytoplankton can use **inorganic** fixed-nitrogen compounds \( \text{NH}_4^+, \text{NO}_3^-, \text{NO}_2^- \), 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) \( \text{NH}_4^+ \) is an intermediate product in decomposition of organic matter, which constitutes the most reduced form of N.

(II) \( \text{NH}_4^+ \) sustains a global average of 80 \% of the authtroph's requirements (Harrison, 1992), with the mesozooplankton responsible for 12 – 23 \% (Hernández-León, 2008).
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)?**

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 \(\text{NH}_4^+\) excretion.
(II) **Good correlation** with $\text{NH}_4^+$ excretion in several marine zooplankters.

**INTRODUCTION**

**Biochemistry of $\text{NH}_4^+$ in marine zooplankton**

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**Table I.** Correlation coefficients between GDH and $R_{\text{NH}_4^+}$ calculated in different works.

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

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

*Park et al. (1986)*
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).

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

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 II. Mean values of $\text{R}_{\text{NH}_4^+}$ and GDH activity of the zooplankton sampled during the different cruises.

<table>
<thead>
<tr>
<th>CRUISE</th>
<th>T (°C)</th>
<th>$\text{NH}_4^+$ Excretion ($\mu\text{mol} \text{NH}_4^+ \cdot \text{h}^{-1} \cdot \text{mg protein}$)</th>
<th>GDH Activity ($\mu\text{mol} \text{NH}_4^+ \cdot \text{h}^{-1} \cdot \text{mg protein}$)</th>
<th>GDH/$\text{R}_{\text{NH}_4^+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Camvalax I (n = 15)</td>
<td>20</td>
<td>0.46 ± 0.21 $\approx 0.48$</td>
<td>2.13 ± 1.07 $\approx 2.21$</td>
<td>5.43 ± 3.70</td>
</tr>
<tr>
<td>Malaspina LEGS 3 - 4 (n = 29)</td>
<td>19</td>
<td>0.30 ± 0.19 $\approx 0.33$</td>
<td>1.90 ± 0.92 $\approx 2.09$</td>
<td>9.57 ± 9.42</td>
</tr>
<tr>
<td>Malaspina LEG 7 (n = 61)</td>
<td>21</td>
<td>0.39 ± 0.32</td>
<td>2.39 ± 0.94</td>
<td>13.26 ± 15.67</td>
</tr>
<tr>
<td>Succession (n =15)</td>
<td>11</td>
<td>0.23 ± 0.20 $\approx 0.42$</td>
<td>1.93 ± 1.33 $\approx 3.52$</td>
<td>22.10 ± 18.59</td>
</tr>
</tbody>
</table>

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

Table IV. GDH/$\text{R}_{\text{NH}_4^+}$ Values from different works.

<table>
<thead>
<tr>
<th>Sample</th>
<th>GDH/$\text{R}_{\text{NH}_4^+}$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. lingvura (n = 16)</td>
<td>10.41 ± 4.84</td>
<td>Fernández-Urruzola <em>et al.</em> 2011</td>
</tr>
<tr>
<td>Mixed zooplankton</td>
<td>12.57 ± 11.11</td>
<td>Hernández-León and Torres, 1997</td>
</tr>
<tr>
<td>Mixed zooplankton (n=8)</td>
<td>23.4 ± 4.0</td>
<td>King <em>et al.</em>, 1997</td>
</tr>
<tr>
<td>Mixed zooplankton (n=10)</td>
<td>18.8 ± 6.72</td>
<td>Park <em>et al.</em>, 1987</td>
</tr>
<tr>
<td>Mixed zooplankton (n=5)</td>
<td>18.7 ± 4.3</td>
<td>Bidigare and King, 1982</td>
</tr>
<tr>
<td>C. finmarchicus (n=10)</td>
<td>16.8 ± 2.6</td>
<td>Bidigare and King, 1981</td>
</tr>
<tr>
<td>Mixed zooplankton (n=5)</td>
<td>18.7 ± 8.5</td>
<td>Bidigare, 1981</td>
</tr>
</tbody>
</table>

GDH/$\text{R}_{\text{NH}_4^+}$ 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

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

(I) Enzyme activities greater than physiological 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.
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Values in agreement with other works.

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Table V. Scaling exponents for NH$_4^+$ excretion rates summarized from the literature.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Mass variable</th>
<th>NH$_4^+$ Scaling exponent</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ocean zooplankton</td>
<td>Dry weight</td>
<td>1.00</td>
<td>Blazka et al., 1982</td>
</tr>
<tr>
<td>Ocean zooplankton</td>
<td>Dry weight</td>
<td>1.00</td>
<td>Vidal and Whitledge, 1982</td>
</tr>
<tr>
<td>Crangon crangon</td>
<td>Dry weight</td>
<td>0.79</td>
<td>Regnault and Batrel, 1987</td>
</tr>
<tr>
<td>Ocean zooplankton</td>
<td>Carbon</td>
<td>0.84</td>
<td>Ikeda 1988</td>
</tr>
<tr>
<td>Ocean zooplankton</td>
<td>Nitrogen</td>
<td>0.65 - 0.80</td>
<td>Ikeda and Skjoldal, 1989</td>
</tr>
<tr>
<td>Mixed zooplankton</td>
<td>Carbon</td>
<td>0.92</td>
<td>Schmieder, 1990</td>
</tr>
</tbody>
</table>
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.

The correlation should increase if we select points with the same nutritional state.
- 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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- GDH does not vary significantly with environmental changes, proving its constitutive nature.

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

<table>
<thead>
<tr>
<th>Starvation (h)</th>
<th>Apparent Km (mM)</th>
<th>GDH/RNH$_4^+$</th>
<th>O$_2$ consumption (μmol O$_2$ · h$^{-1}$ · mg protein$^{-1}$)</th>
<th>RO$_2$/RNH$_4^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>4.69 ± 0.69</td>
<td>11.23 ± 4.46</td>
<td>0.61 ± 0.09</td>
<td>4.57 ± 1.90</td>
</tr>
<tr>
<td>10</td>
<td>12.1 ± 5.9</td>
<td>9.74 ± 2.98</td>
<td>1.29 ± 0.32</td>
<td>6.66 ± 1.65</td>
</tr>
<tr>
<td>18</td>
<td>8.55 ± 4.04</td>
<td>11.14 ± 5.93</td>
<td>0.5 ± 0.15</td>
<td>5.51 ± 2.47</td>
</tr>
<tr>
<td>26</td>
<td>19.44 ± 10.8</td>
<td>30.87 ± 25.28</td>
<td>0.6 ± 0.11</td>
<td>11.51 ± 1.73</td>
</tr>
<tr>
<td>42</td>
<td>4.67 ± 0.79</td>
<td>16.28 ± 1.55</td>
<td>0.2 ± 0.18</td>
<td>2.13 ± 0.29</td>
</tr>
<tr>
<td>54</td>
<td>5.04 ± 0.27</td>
<td>64.72 ± 51.3</td>
<td>0.22 ± 0.22</td>
<td>7.07 ± 3.25</td>
</tr>
<tr>
<td>68</td>
<td>2.84</td>
<td>102.41</td>
<td>0.24</td>
<td>12.9</td>
</tr>
<tr>
<td>72</td>
<td>4.89 ± 3.45</td>
<td>67.89 ± 29.5</td>
<td>0.55 ± 0.05</td>
<td>15.49 ± 8.6</td>
</tr>
<tr>
<td>76</td>
<td>5.95 ± 2.48</td>
<td>39.1 ± 5.09</td>
<td>0.74 ± 0.43</td>
<td>14.12 ± 2.96</td>
</tr>
<tr>
<td>82</td>
<td>6.25 ± 2.51</td>
<td>89.1 ± 34.58</td>
<td>0.59 ± 0.36</td>
<td>47.2 ± 15.5</td>
</tr>
</tbody>
</table>

Fernández-Urruzola et al. (2011)
- 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!!

Roy and Packard (1998) found a decrease in intracellular substrates concentrations with food source limitation. As a consequence, the actual enzymatic rates would also decrease.
An enzyme kinetic-based model should predict the \textit{in vivo} $\text{RNH}_4^+$ on natural samples of zooplankton from different productivity areas.

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