The relationship between ammonia excretion and GDH activity in marine zooplankton

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Abstract. The relationship between the ammonia excretion rate and glutamate dehydrogenase (GDH) activity was studied in marine zooplankton over the course of a bloom and in different size classes (100–200, 200–500 and 500–1000 μm). A weak correlation between GDH activity and ammonia excretion rate was observed when all data were pooled. Better relationships between the parameters were obtained by taking into account the substrate being metabolized, as deduced from the experimentally determined O/NH₄ ratio. There was also a positive correlation between the GDH/NH₄ and O/NH₄ ratios, suggesting that the former ratio was lower when the metabolic substrate being metabolized contained a high level of nitrogen. High GDH/NH₄ and high standard deviations were found when the in situ temperatures were low, while the ratio and standard deviations decreased at higher temperatures. Temperature probably had an indirect effect as a consequence of a better availability of nitrogen in the food when mixing took place in the water column. Differences in the GDH/ammonia ratio were also observed for different size fractions, largely because small animals had higher ammonia excretion rates. The composition of the metabolic substrate affected the observed GDH/NH₄ ratios because it led to variations in cellular glutamate concentrations, while enzyme activities were measured at maximal rates (Vₘₐₓ). It is suggested that this methodological limitation is probably the most important factor in determining the relationship between enzymatic activities and metabolic rates.

Introduction

Nitrogen has been recognized as a principal limiting nutrient for primary production in the ocean. Organic matter is converted into inorganic forms of nitrogen by animals and this is usable by primary producers, giving rise to the so-called regenerated production (Dugdale and Goering, 1967). Since the early study of Ketchum (1962), numerous studies have indicated that zooplankton excretion is an important source of nitrogen for phytoplankton. Eppley and Peterson (1979) reported that up to 95% of phytoplankton requirements are released by excretion by heterotrophs (bacteria, protists and metazooplankton) in the euphotic zone. Knowledge of zooplankton excretion is also of importance in order to assess their metabolic nitrogen budget.

The rate of nitrogen remineralization (ammonium release) has normally been obtained by the balance (bottle incubation) method in which the concentrations of compounds released by animals left in a closed vessel for a certain period of time are measured. Bidigare and King (1981) introduced an enzymatic assay method in which ammonium excretion rates could be estimated from glutamate dehydrogenase (GDH) activity. This enzyme has been found in relatively high levels in planktonic crustaceans, suggesting it to be the main step controlling ammonia release in these animals (Bidigare et al., 1982; King, 1984; Park et al., 1986; King et al., 1987). This technique reduces the problems associated with the incubation of zooplankton in a controlled environment and facilitates the acquisition of large data sets covering wide oceanographic areas. In addition, it enables the characterization of vertical profiles of ammonia excretion rates, so that
coupling relationships between zooplankton ammonia excretion and phyto-
plankton production can be assessed.

The GDH/ammonia ratio varied over a relatively narrow range (16.8–23.4) in
the studies of several authors (Bidigare and King, 1981; Bidigare et al., 1982; Park,
1986a; Park et al., 1986; King et al., 1987). The variability observed in other com-
parisons between enzymatic activities and physiological processes [e.g. the elec-
tron transport system (ETS)] was not commonly observed in early studies on
GDH, although there are relatively few reports in the literature. Moreover, one
can presumably find higher variability in GDH/ammonia ratios by increasing the
number of estimates in different trophic situations. In fact, Park (1986b) observed
that the GDH/ammonia ratio could be much more variable if it is measured in
copepods exposed to extreme situations of saturating feeding or starvation. In this
study, it changed from 18.2 to 103.5 after 4–5 days of starvation.

This variability in the relationship between enzymatic activities and physio-
logical rates might be due to factors which are inherent in the measurement of
enzymatic activities. Firstly, different species may characteristically have different
relationships between enzyme activity and physiological rate. This has not been
considered widely in previous research, but has been pointed out by Berges and
Harrison (1995) who observed that the half-saturation constants ($K_{m}$) of nitrate
reductase in marine phytoplankton varied between species. Park et al. (1986),
however, found that the apparent $K_{m}$ of GDH varied in relation to feeding con-
ditions in macrozooplankton. They explained this variability in terms of the regu-
latory role exerted by ADP. Increases in ADP levels can increase the $K_{m}$ as well
as the $V_{max}$. Secondly, size and biomass of the organisms might influence the
relationship, as stated by Bämstedt (1979) and Berges et al. (1993). In fact, some
of the strong relationships reported in the literature comparing enzymes and
physiological rates are flawed because of the influence of biomass. For example,
Park (1986a) and Park et al. (1986) expressed their results comparing GDH
activity and ammonia excretion on a volume basis, highly related to biomass (the
more biomass in a unit volume, the more excretion and the higher the enzyme
level). Thirdly, enzymes are measured in optimized in vitro assays at substrate sat-
uration and, therefore, at $V_{max}$. By contrast, the enzyme in vivo might not be oper-
ating at $V_{max}$ (e.g. limiting substrate levels). This is a limitation which is not fully
considered in the comparison between enzymes and physiological rates, although
it was noted by Park et al. (1986) working with GDH activity.

In order to validate the use of enzymatic activities as tools in biological
oceanography, it is important to know how measurements of enzyme activity at
$V_{max}$ relate to physiological processes. In order to evaluate the results of the rates
of ammonia release through the measurement of GDH activity, and to contribute
to the knowledge of the use of enzymes as predictors of physiological rates, we
conducted a series of incubations measuring both parameters. The purpose of the
study was to examine the factors affecting the relationship between GDH and
ammonia excretion in different trophic conditions, such as those produced during
the development of a late winter bloom in subtropical waters. A previous study
comparing respiration and ETS activity (Hernández-León and Gómez, 1996)
showed that food availability affected the respiration/ETS ratio. They suggested
that the variability in the relationship between enzymes and metabolic rates might be masked by differences in substrate saturation in nature and in vitro. This study reports results on GDH/ammonia ratios in relation to the amount of nitrogen being metabolized, the size of animals and the environmental temperature. The ratio oxygen consumed/ammonia excreted has been suggested as an index of nitrogen used for metabolism [see Omori and Ikeda (1984) for a review]. This ratio gives information about the relative composition of the substrates being consumed and has, therefore, been used as an indicator of the dietary composition and of the substrate used in vivo by the organisms.

**Method**

Mesozooplankton from the waters around Gran Canaria Island (Canary Islands) were collected by means of subsurface hauls using a WP-2 net (UNESCO, 1968). Experimental animals were obtained primarily during the winter and spring months (October 1988–June 1989) at a coastal station, east of Gran Canaria, in order to obtain data from the short annual enrichment of surface waters, the so-called late winter bloom. Temperature, chlorophyll a and in situ primary production (14C method) were measured at the same location. During this experiment, we also measured respiration in order to access values of the oxygen/ammonia ratio, which is an index of the relative amounts of nitrogen being metabolized by animals. A second set of data was obtained during two cruises in June 1990 and March 1991 (EMIAC 9006 and 9103) in the eddy field leeward of Gran Canaria Island (Aristegui et al., 1994). We used these experiments to determine the possible influence of the size of organisms on the GDH/ammonia ratio by using a WP-2 net (100 µm mesh size) and separating the 100–200, 200–500 and 500–1000 µm size fractions of zooplankton samples.

Experimental organisms were maintained in filtered seawater (Whatman GF/C) for a period of no longer than 3 h prior to incubation. Undamaged individuals were separated, but no species selection criteria were adopted except the elimination of the obviously carnivorous zooplankton. During the late winter bloom experiment, zooplankton used for incubations were mainly composed of copepods of the 200–500 µm size fraction. The organisms were siphoned into 1 l bell jars filled with filtered seawater. For each series, four bottles with animals together with three bottles containing filtered seawater as controls were incubated in the dark and in a water bath, near the in situ temperature (± 0.5°C). Because we were interested in measuring the oxygen consumed/ammonia excreted (O/N) ratio, we performed long incubations (18–24 h) in order to obtain accurate signals of oxygen depletion in the bottles. The water from the incubation bottles was siphoned for immediate ammonia determination, following the phenol hypochlorite method proposed by Parsons et al. (1984). Dissolved oxygen concentrations were measured according to the Winkler method described in Parsons et al. (1984) using a manual multipipette for titration. During the cruises, it was possible to use the method described by Bryan et al. (1976) using a photometric end-point detector and a digital microburette capable of dispensing 0.1 µl of sodium thiosulphate. The animals remaining in the bottles were collected on a
100 μm mesh and then immediately frozen in liquid nitrogen for the determination of enzyme activity and biomass.

The GDH assay was run as described by Bidigare and King (1981) and Bidigare et al. (1982), with certain modifications: 1 ml of the crude homogenate was diluted in Tris buffer (pH 8.8) and centrifuged (0–4°C) for 10 min at 4000 r.p.m. An aliquot (0.5 ml) of the supernatant was mixed with NAD and ADP solutions in a Perkin-Elmer 551-S UV/VIS spectrophotometer equipped with a thermostated 1 cm water-jacketed cuvette. When the absorbance reading at 340 nm was stable, the assay was initiated by adding glutamate and recording changes in absorbance for 2 min. The total volume in the cuvette was 3.0 ml and the initial NAD, ADP and glutamate concentrations were 1.2, 2.0 and 40 mM, respectively (Bidigare et al., 1982). Soluble protein was used as the index of biomass and was measured by the folin-ciocalteu method (Lowry et al., 1951) using bovine serum albumin (BSA) as the standard.

In order to compare the ratio between GDH activity and ammonia excretion in relation to temperature for the different cruises, we normalized it by taking 0% as the coldest temperature and 100% as the warmest. For example, during the late winter bloom experiments, temperature varied between 18 and 22.4°C. A temperature of 18°C was taken as 0% and 22.4°C as 100%.

Results

Metabolized nitrogen

Ammonia excretion rates, GDH activities and GDH/ammonia ratios obtained during the course of the late winter bloom are shown in Figure 1. Mixing in the water column took place from the end of December to mid-April. Temperature fell from 20.55°C in December to 19.02°C in January, while it rose from 18.1°C in mid-April to 19.5°C at the beginning of May. Average ammonia excretion rates were more variable than average GDH activities. The latter were consistent during the development of the bloom. As a consequence of this, GDH/ammonia ratios were highly variable, ranging between 1.2 and 42.5. The regression between both measurements showed quite a low correlation coefficient (Figure 2, Table I) if we compare this with the results reported in the literature [r = 0.92, n = 7 in Bidigare and King (1981); r = 0.94, n = 5 in Park (1986a); r = 0.98, n = 10 in Park et al. (1986)]. This difference might have been due to differences in methods. While their incubations were quite short (2–4 h), ours were longer (see Method). In addition, we had a larger number of estimates (n = 59), which covered different trophic situations during the course of the late winter bloom. The ammonia excretion rates obtained were, however, in agreement with values reported by other authors (Table II).

No relationship was found between chlorophyll or primary production and the GDH/ammonia excretion ratio. In order to study the influence of nitrogen being metabolized by organisms, we calculated the O/N ratios using the respiration rates obtained in the same experiments (Figure 3). O/N variability was high (6.2–73.5) indicating a succession of shifts between protein and lipid or carbohydrate substrates being metabolized (Conover and Corner, 1968; Nival et al., 1974; Ikeda,
Relationship between ammonia excretion and GDH activity

At the end of the late winter bloom, when stratification was established, the O/N ratio remained stable and low. The similarity between the values of the GDH/ammonia ratio (Figure 1c) and the O/N ratio (Figure 3) was reflected in a significant correlation between both parameters (Figure 4, Table I). The variability

Fig. 1. Time course of (a) ammonia excretion, (b) glutamate dehydrogenase (GDH) activity and (c) the GDH/ammonia ratio during the development of the late winter bloom. Mixing in the water column took place from the end of December to mid-April. Vertical bars are standard deviations.
of the GDH/ammonia excretion ratio appeared to be related to the nitrogen being metabolized by the organisms and driven by a higher ammonia excretion at lower values of the O/N ratio. In fact, when the metabolic substrates of animals were low in nitrogen content (high O/N ratio), the ammonia excretion rate was low (Figure 5). In contrast, as the nitrogen being metabolized increased, it was also reflected in a higher rate of ammonia release. However, the relationship between the GDH/ammonia ratio and the nitrogen metabolized (O/NH₄) by the organisms could be (although not necessarily) flawed because this relationship is driven by a common mass denominator. Therefore, we represented the relationship of Figure 2 taking into account the O/N ratio for each experiment (Figure 6). Quasi-parallel regressions were obtained in relation to the amount of nitrogen being metabolized. Higher O/N ratios showed lower ammonia excretion rates than the lower O/N ratios (protein-based metabolism) for a given enzymatic activity.

### Table I. Relationships between ammonia excretion and glutamate dehydrogenase (GDH) activity for all the experiments performed in the present work. The different relationships observed in Figure 6 at the different O/N ratios, as well as the regression between the GDH/ammonia excretion and the O/N ratio of Figure 4, are also given. In order to calculate the regression equations, data points inside the dashed lines in Figure 6 were excluded

<table>
<thead>
<tr>
<th>O/N</th>
<th>Regression</th>
<th>r</th>
<th>P</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>5–10</td>
<td>NH₄ = 0.071 + 0.078 · GDH</td>
<td>0.812</td>
<td>0.003</td>
<td>10</td>
</tr>
<tr>
<td>10–15</td>
<td>NH₄ = 0.053 + 0.043 · GDH</td>
<td>0.803</td>
<td>0.001</td>
<td>18</td>
</tr>
<tr>
<td>15–25</td>
<td>NH₄ = 0.040 + 0.038 · GDH</td>
<td>0.474</td>
<td>0.25</td>
<td>8</td>
</tr>
<tr>
<td>&gt;25</td>
<td>NH₄ = 0.019 + 0.022 · GDH</td>
<td>0.335</td>
<td>0.4</td>
<td>9</td>
</tr>
<tr>
<td>All data</td>
<td>NH₄ = 0.036 + 0.062 · GDH</td>
<td>0.509</td>
<td>0.001</td>
<td>59</td>
</tr>
<tr>
<td>All data</td>
<td>GDH/NH₄ = 3.918 + 0.666 O/NH₄</td>
<td>0.834</td>
<td>0.001</td>
<td>52</td>
</tr>
</tbody>
</table>
Relationship between ammonia excretion and GDH activity

Table II. Values of ammonia excretion rates (μmol NH₄⁺ mg⁻¹ dry weight day⁻¹) obtained by different authors. We calculated dry weight (DW) from protein (PROT) biomass using the linear regression DW = 1.445 + 4.283 × PROT (r = 0.900; n = 306; P < 0.001)

<table>
<thead>
<tr>
<th>Area</th>
<th>Temperature (°C)</th>
<th>Excretion rate</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central Pacific</td>
<td>20</td>
<td>0.19-1.29</td>
<td>Eppley et al. (1973)</td>
</tr>
<tr>
<td>Northwest African upwelling</td>
<td>14-15</td>
<td>0.83-3.44</td>
<td>Herbland et al. (1973)</td>
</tr>
<tr>
<td>Georges Lake</td>
<td>27</td>
<td>3.02-3.24</td>
<td>Ganf and Blazka (1974)</td>
</tr>
<tr>
<td>Gulf of Guinea</td>
<td>16-28</td>
<td>0.64-1.80</td>
<td>Le Borgne (1977)</td>
</tr>
<tr>
<td>Northwest African upwelling</td>
<td>14-16</td>
<td>0.39-0.54</td>
<td>Le Borgne (1979)</td>
</tr>
<tr>
<td>Mid. Atlantic Bight</td>
<td>–</td>
<td>0.23-0.79</td>
<td>Harrison et al. (1983)</td>
</tr>
<tr>
<td>Canary Islands</td>
<td>20</td>
<td>0.28-1.04</td>
<td>Hernández-León (1986)</td>
</tr>
<tr>
<td>Canary Islands</td>
<td>18-22</td>
<td>0.11-3.27</td>
<td>Present work</td>
</tr>
</tbody>
</table>

The influence of zooplankton size

Table III shows the GDH/ammonia ratios for the different size classes. The lowest ratios were found in the smallest size fraction, while the two larger size classes displayed higher and comparable ratios, although with high standard deviations. The average values of the O/N ratios were not significantly different for the smallest size fractions. However, the relationship between this ratio and the ammonia excretion rates was similar to the ones observed during the late winter bloom with higher ammonia excretion coinciding with low O/N ratios (Figure 5). This fact again indicates higher ammonia release when the metabolic substrate is nitrogen based. The different GDH/ammonia ratio in the smallest size fraction seems to be the result of increased ammonia excretion by small animals compared to the larger size fractions. The higher specific metabolic rates of small zooplanktonic forms is quite a common result in the literature (Le Borgne, 1986).

Fig. 3. Variability of the O/N ratio during the development of the late winter bloom. Observe the parallelism with the time course of the GDH/ammonia ratio in Figure 1.
The influence of temperature

The relationship between the GDH/ammonia ratio and normalized temperature (see Method) during the late winter bloom in the Canary Islands showed highest average values (and highest standard deviations) at the lowest temperatures (Figure 7a). The high standard deviations are the consequence of the continuous shift in the amount of metabolized nitrogen (Figure 3) during the development of the late winter bloom. During the post-bloom period, when stratification took place in the water column and temperatures were highest, the GDH/ammonia ratio was low (and had low standard deviations). Pooled data of different size fractions (Figure 7b) showed similar results, with low average values and standard deviations at higher temperatures. Thus, the influence of temperature on the variability of the GDH/ammonia ratio seems to be an indirect effect. The trophic environment of organisms at which they were acclimatized was the determining factor in the variation of the ratio, as was stated above in the study of the O/N ratio.

Discussion

Comparisons between GDH and ammonia excretion observed in the literature show strong correlations and the similarity in values of GDH/ammonia excretion ratios reported by different authors have suggested an important applicability of this enzyme in field studies in oceanography. However, our more detailed study, involving measurements in different trophic situations, has shown that this ratio is...
not constant. This apparent discrepancy is primarily a function of how the different authors have presented their results. Bidigare and King (1981), Park (1986a) and Park et al. (1986) expressed their results on a volume basis (per m³), so that the biomass per unit volume dominated the value. Thus, the greater the range of

Fig. 5. Ammonia excretion rates versus the ratio O/N (a) during the late winter bloom and (b) for the different size fractions studied.
Fig. 6. Relationship between ammonia excretion and GDH activity of Figure 2 in relation to the oxygen consumed/ammonia excreted (O/N) ratio. Observe the parallel regressions found with increasing y-intercepts as the O/N ratio decreases. The six data points inside the dashed lines were excluded from regressions in Table I.

Biomass, the better will be the relationship between GDH activity and ammonium excretion. Park (1986b), however, found large differences in the GDH/ammonia excretion ratio when animals were starved or exposed to high levels of food. Berges et al. (1993) were also critical and suggested that GDH activity should be a poor predictor of metabolic activity, although in their studies no attempt was made to examine the relationships. The argument of non-coincidence between the so-called scaling exponent (the slope of the regression between biomass and enzyme activity or ammonia excretion) of their enzyme determinations and the classical values of the scaling exponent in the literature should not be used to derive such conclusions. Rather, better calibrations between the measurements are needed, avoiding the influence of biomass in the enzyme–excretion relationship.

Table III. Average values of the GDH/ammonia and oxygen/ammonia ratios for the different size fractions on incubations carried out during June 1990 (9006) and March 1991 (9103) cruises

<table>
<thead>
<tr>
<th>Size fraction (μm)</th>
<th>GDH/NH₄</th>
<th>O/NH₄</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9006</td>
<td>9103</td>
</tr>
<tr>
<td>100-200</td>
<td>2.93 ± 1.47</td>
<td>8.93 ± 3.53</td>
</tr>
<tr>
<td>200-500</td>
<td>12.57 ± 11.11</td>
<td>21.97 ± 10.56</td>
</tr>
<tr>
<td>500-1000</td>
<td>20.73 ± 16.17</td>
<td>16.19 ± 2.03</td>
</tr>
</tbody>
</table>
GDH/ammonia excretion ratios were elevated as a consequence of an increased amount of metabolized nitrogen. Low O/N ratios are expected to be found immediately after feeding on a diel scale, shifting the metabolism to a routine or active value (sensu Brett, 1964). Organisms tend to maximize the
ingestion of nitrogen (Cowles et al., 1988) and it has been recognized that they aggregate in the water column, at or near the depth of the assimilation number maximum, where cells are actively growing (Roman et al., 1986). It is also known that these organisms have diel feeding rhythms, as recently observed using the gut fluorescence method (Simard et al., 1985; Durbin et al., 1990; Atkinson et al., 1992; Dam and Peterson, 1993). Increased feeding activity must produce a short-term increase in the metabolic level of zooplankton (e.g. specific dynamic action), and an increase in oxygen consumption and ammonia excretion, although probably with some degree of uncoupling (Checkley et al., 1992). The rate of ammonia release probably increases with the diel increases in feeding activity and follows the changes in the O/N ratio after feeding. Our results show an influence of the metabolized nitrogen by organisms in the GDH/ammonia excretion ratio with higher values coinciding with the late winter mixing period (January–March). This is a similar result to the finding of Hernández-León and Gómez (1996) who observed a positive relationship between the R/ETS ratio and primary production. In both cases, the availability of food seemed to influence the metabolic rate/enzyme activity ratio. Moreover, the relationship between GDH activity and the ammonia excretion rate showed a degree of scatter which could be partially explained by differences in O/N ratios (Figure 6). However, no relationship was found with other indices of available food such as chlorophyll and primary production. This fact might be due to the inadequacy of such indices as proxies of nitrogen available for organisms. Unfortunately, we did not measure any parameter related to the nitrogen available in the water column.

The variation of the GDH activity to ammonium excretion ratio with the O/N ratio gives rise to the question of what we really measure when we assay the enzyme activities. From the work by King and Packard (1975) to the present, the study of the relationship between respiration and ETS activity has shown an average 3- to 4-fold variability in the R/ETS ratio, which is similar to the variability that we observed in the average GDH/ammonia ratio (Figure 1c). This fact has reduced the usefulness of such an enzyme assay in determining physiological rates in nature. Sources of errors to explain this common variability could be grouped into two methodological limitations related to the way we measure the metabolic rates and enzyme activities. The observed changes in the O/N ratio might indicate an important source of error in our experimental procedure which might partially explain the scatter in the relationships as well as in the ratios shown. When animals have been feeding in situ (low O/N ratios) and are allowed to starve in filtered seawater during the bottle incubation, relatively high average (or integrated) rates of ammonia release will be measured, whereas GDH activity in the animals at the end of incubation might be relatively low, because metabolic rates and enzyme activities vary in parallel as the animals begin to starve (Bämstedt, 1980; Ikeda and Skjoldal, 1980; Skjoldal et al., 1984; Hernández-León and Gómez, 1996). In contrast, if animals had been acclimated to a low-food environment, or they had empty guts because of intermittent feeding (high O/N ratios), then when they are transferred to filtered seawater, the initial ammonia excretion rates and GDH activities will be low, and the decreases between the start and the end of the experiment much less. In such a case, the GDH/ammonia ratio would tend to be
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higher in experiments with well-fed copepods. The observation that starvation leads to an increase in the O/N ratio during the course of an incubation (Skjoldal et al., 1984) supports our suggestion. Thus, we cannot recommend the bottle incubations if metabolic rates and enzyme activities are to be compared. Furthermore, the ratios themselves are of no use if the slope of the regression between both variables is not unity (see Hernández-León and Gómez, 1996).

An even more important factor in the relationship between enzymatic activities and physiological rates might be the way in which enzyme activities are measured. The activities are measured under substrate saturating conditions, therefore at the maximal rate ($V_{\text{max}}$). In nature, it is likely that $V_{\text{max}}$ is not often achieved so that the complexity in the relationship between both parameters is increased. In the present study, the O/N ratio has been used as an index of the metabolic substrate used by the organisms. An increased amount of nitrogen being metabolized might lead to an increase in the availability of the substrate (glutamate) used by the enzyme GDH. Zooplankton glutamate levels might actually reflect the entire nitrogenous nutrition of the organism (Park et al., 1986). Therefore, if the enzymatic activity is measured at $V_{\text{max}}$, and organisms have different levels of glutamate in their cells in nature or in the incubation bottle (as observed by the O/N ratios), then one might predict that there would be different relationships between GDH and ammonia excretion depending on the level of substrate. Figure 6 demonstrates something of this complex relationship. The different regressions obtained showing increased values of the y-intercept might correspond to increasing levels of substrate in the organisms. This type of observation may help us to interpret the differences in the values of the y-intercepts observed in published relationships between metabolic rates and enzyme activities in areas of different productivity (e.g. Aristegui and Montero, 1995).

Finally, despite the possible interspecific differences in enzyme activity, our results, together with those of Hernández-León and Gómez (1996), suggest that the methodological limitation imposed by the measurement of enzymes at substrate saturation is probably the most critical factor in order to obtain good relationships between enzymes and physiological rates. The influence of biomass (as suggested by Berges et al., 1993) may have only a minor role. One way to solve this general methodological limitation might be a proper calibration avoiding the influence of biomass and measuring the amount of substrate present in the organisms being sampled (e.g. glutamate for GDH activity). This procedure could open new insights into the use of enzymes in biological oceanography.

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Relationship between ammonia excretion and GDH activity


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