
Small-scale variability of zooplankton pyruvate kinase activity in the Gironde Estuary plume (Atlantic French Coast): A case study under unusually low freshwater discharge

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Abstract:

Pyruvate kinase (PK) activity measurements are used to assess the role of carbohydrates in global feeding of mesozooplankton communities inhabiting an estuary plume. As a consequence of a remarkably low freshwater discharge rate, the sea surface layers of the area under estuarine influence showed a very moderate salinity fall and a nearly total depletion in nitrates, whereas higher levels of these nutrients were found in deeper, more saline, layers. Small-scale PK activity variations in mesozooplankton appear to be closely correlated to nitrate integration values within the water column. The results were analysed in comparison with literature reports. The study produced a coherent overall interpretation, which strongly supports the reliability of this new biochemical tool in detecting assimilation of trace carbohydrates in the diet of mesozooplankton.

Keywords: inorganic nutrients; chlorophyll; phytoplankton; zooplankton; carbohydrates; pyruvate kinase; Bay of Biscay; Gironde Estuary

1. Introduction

The assessment of food particle transfer to the zooplankton component has long been a major concern of biological oceanographers (Banse, 2002). Also, the classical food chain concept, based on phytoplankton growing on inorganic nutrients and consumed by mesozooplankton, the bulk of which is made up of copepods, has dominated studies on life in pelagic systems. Consequently, the issue of the significant role of bacteria (Azam *et al.*, 1983) and the microbial loop, the increasing number of elements confirming the importance of small-sized organisms (Morales *et al.*, 1991) and the diversity of auto- and heterotrophic pico-, nano- and microplankton (Jochem, 2003) has led to a progressive abandonment of the classical concepts and, instead, a consideration of the trophic interrelationships within pelagic systems as 'food webs'. There is, however, no conceptual conflict between both systems, rather they should be visualized as a continuum (Legendre and Rassoulzadegan, 1995).

Among the multiple processes at work within pelagic systems, the most fundamental is the formation, from inorganic nutrients, of organic matter, as the basic production of living organisms, i.e. photosynthesis in phytoplanktonic cells. Photosynthesis creates the basic product of one of the most important classes of organic molecules, carbohydrates. In view of food web complexity, it has recently been suggested (Bergeron and Herbland, 2001) to assess the role of carbohydrates in global feeding of mesozooplankton communities at a cellular level, by analysing the unique pathway, common to all organisms, of the targeted metabolic step implicated in the relevant process. In the present study, the relevant process is digestion and assimilation of autotrophic phytoplankton cells, and the targeted metabolic step is the final step of carbohydrate catabolism (glycolysis), catalysed by the enzyme, pyruvate kinase (PK).

PK is the terminal enzyme and a key regulator of glycolysis. It is inhibited by ATP and by acetyl-CoA, so it is not active during fat and protein metabolism. In an earlier field trial of this new biochemical tool, Bergeron and Herbland (2001) showed that PK specific activity variations in zooplankton were, to various degrees, linked to both inorganic nutrients and phytoplankton biomass, expressed through chlorophyll *a* (Chl *a*) concentrations. The presented results offer new insights into PK activity variability at a small scale and, moreover, within the special context of a low nutrient-enrichment rate.

2. Material and methods

Like previous results presented in 2001 by Bergeron and Herbland (1994, in the Bay of Biscay, NE Atlantic, with special attention to the Gironde Estuary plume), the present data were obtained during a fisheries oceanography cruise aboard the French R.V. *Thalassa*, mainly devoted to the study of European anchovy (*Engraulis encrasicolus* L.) abundance, spatial distribution and ecology (PEGASE 97, from 6 May to 3 June 1997). Less than 2 days (24–26 May) were allocated to a more extensive assessment of a special study area within the plume of the extensive Gironde Estuary, an important breeding ground for anchovy in this region. A sampling grid of 15 stations, spaced 3 n. m. (5556 m) from each other (GIR area: Fig. 1), was designed to assess the small-scale ecological features of the plume. For comparison, a second similarly constructed sampling grid of 15 stations, where anchovy is usually also found breeding, was located beyond the slope of the continental shelf, i.e. in typically oceanic waters, called by fishermen "Fer à Cheval" area (FAC: cf. insert in Fig.1). At each station:

- temperature and salinity profiles were recorded with a CTD (SeaBird SBE 19);
- water samples were collected with five Niskin bottles at standard depths (0, 10, 20, 30 and 40 m) in GIR, six bottles in FAC (0, 10, 20, 30, 50, and 100m) to estimate inorganic nutrients (nitrate and silicate), according to the methods of Strickland and Parsons (1972) and Chl *a* concentrations (Yentsch and Menzel, 1963);
- mesozooplankton samples were collected by a vertical tow of a WP2 net (200 μm mesh size) from the bottom to the surface (or 200 m depth to the surface in FAC).

On board, the collected macrozooplankton were separated by sieving through a 5-mm mesh. The samples were homogenised in iced distilled water with a Polytron[®] grinder. Then, 2.5-ml aliquots were immediately frozen in liquid nitrogen and preserved in this medium until the end of the cruise. Thereafter, they were stored at $-80\text{ }^{\circ}\text{C}$ until analysis in the laboratory. After thawing, the crude extract was homogenised again with a Potter–Elvehjem and centrifuged (10 min at 4000 rev min^{-1} , $3\text{ }^{\circ}\text{C}$). A 200- μl aliquot of the supernatant fluid was used for enzyme assay or protein determination.

PK activity was estimated according to the widely used method of Bücher and Pfeleiderer (1955), under conditions previously defined (Bergeron and Herbland, 2001). Briefly, the product of the enzyme reaction (pyruvate) serves as a substrate for a second enzyme, lactatedehydrogenase (LDH), added in excess to the mixture. The functioning of LDH requires the presence of the reduced form of nicotinamide adenine dinucleotide (NADH) as co-factor, which is oxidized; its progressive disappearance can be followed by monitoring the decrease in its specific absorbance at 340 nm. Thus, PK activity assay is performed using a recording spectrophotometer, with a water-jacketed cuvette-holder for temperature control (25°C). Enzyme activity, proportional to the rate of change in absorbance, was expressed as specific activity ($\mu\text{mol NADH oxidized min}^{-1}\text{ mg}^{-1}\text{ protein}$). Protein was estimated by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

3. Results

The aim of the study was an attempt at evaluating the small-scale variability of mesozooplankton PK specific activity facing environmental conditions, therefore the first step must be a comparison of variability levels observed in both GIR and FAC study areas. This comparison shows a highly significant difference (Student *t*-test: $t = 4.708$, $p < 0.001$) in PK specific activity means (Table 1), another noteworthy point is the difference in standard deviations showing a greater homogeneity in the FAC area and permitting to note that there is no remarkable small-scale variability of PK specific activity in this area of the open sea. In contrast, the PK specific activity appears to be fairly variable in the GIR area. Therefore the following part of the "Results" section will be exclusively devoted to the GIR area, i.e. a marine area under influence of the freshwater discharge from the large Gironde estuary (Fig. 1).

In a water column 25–45 m deep, the vertical hydrological structure showed a thermo-halocline of around 15–25 m in depth. Temperature variations were rather intense, ranging from ~12.5 °C at depth to 16 °C and up to 17 °C at the surface. In contrast, differences in salinity from bottom to surface were weak, ranging between 35.4–35.5 and 34.95–35.20. The relatively high salinity values measured in the surface layers may be related to especially low freshwater discharge rates from the Gironde—around 300 m³ s⁻¹ (daily flow rates data were provided by the 'Port Autonome de Bordeaux')—rates of two-fold or greater are generally observed at that time of the year (Bergeron, 2004). Obviously, the consequences are a depletion of inorganic nutrient supplies to the neritic region located off the estuary mouth. In both distinct water masses above and below the pycnocline, inorganic nutrient concentrations presented different patterns. In surface layers, nitrate levels were either totally depleted or very low (0.1 μM maximum), whereas values were higher (between 2 and 4 μM) in bottom water. For silicate, such a robust pattern did not appear: values <1 μM were rare in the upper layer and ranged from 2 to 3 μM in the deep layer. Chl *a* concentrations were generally < 1 μg.l⁻¹ at 0, 10 and 30 m depths, but higher peak values, ranging from 1 up to 3 (more at two stations with a maximum value 3.3 μg.l⁻¹), were clearly located in the thermocline and nutricline (Plounevez and Champalbert, 1999) at the 20 m depth layer, presumably where nitrate is mostly being utilized.

Regarding mesozooplankton, firstly, it should be noted that biomass values (expressed in terms of mg protein m⁻³) were predominantly homogenous and normal for such an area and at that stage of the seasonal cycle (mean = 6.4, S.D. = 0.7, $n = 9$), whereas six stations showed values ≥ 10 mg.m⁻³, and a single value reaching 16.7. It is noteworthy that the spatial distribution of biomass did not show any clear or coherent pattern. Despite the closeness of the 15 stations within the study area, mesozooplankton PK specific activities were somewhat variable (Fig. 1), ranging from 0.2 to 1.2 μmol min⁻¹ mg⁻¹ protein. In comparison with measurements in other situations, which demonstrated general variations within the range of 0–2 μmol min⁻¹ mg⁻¹ protein (Bergeron, 2004), exceptionally high levels up to 3 μmol min⁻¹ mg⁻¹ protein and slightly more in only one data set among seven (Bergeron *et al.*, unpublished data), a number of values were notably high. As the aim of measuring PK activities is the estimation of algae-produced carbohydrate assimilation rates, it seems consistent to search for a relationship between PK activities and Chl *a* concentrations (mean value integrated for the water column). In fact, there was no significant correlation (Table 2), but, in contrast, a very close correlation is found with nitrate concentrations. This correlation looks so strong that it must be searched out whether any source of bias does not introduce a spurious trend. First, as there is some variation in depth in the

sampled area and as the deeper layers are the nitrate-richest ones, the calculation was made by eliminating values measured at the deepest layer (40 m depth) which had not been systematically sampled because of impossibility in some stations: the correlation retains almost the same significance ($\text{NO}_3_{\text{up.lay.}}$ in Table 2). Second, it may be tested whether the highest value recorded at each station does not originate by itself the observed trend and it does prove to be not the case (NO_3_{max} in Table 2). Moreover, to eliminate the possible influence of zooplankton biomass variability which, given the trophic environment, may induce quantitative variations in food availability, total PK activity per unit volume of sea water (m^3) was calculated and correlated with nitrate concentration: the significance level was even higher (Table 2), this being partly due to a small, but non-significant ($r = 0.220$), positive correlative trend between zooplankton biomass and nitrate concentration. Therefore the correlation between nitrate and PK, at least in this area and under these environmental conditions, appears to be very strong. Finally, mesozooplankton PK activity was not correlated with silicate (Table 2).

4. Discussion

The pronounced difference in homogeneity between FAC and GIR study areas may most probably be ascribed to the radically different physical processes responsible of their nutrient enrichment. The FAC area is supplied thanks to deep waters upwelled by large-scale tidally-induced internal waves (Mazé, 1987; New and Pingree, 1990). This explains, for example, that nitrate concentrations in the nutricline vary within a very narrow range, from 5.5 to 6.2 μM in the 15 stations. It may by the way be noted that the variations observed in PK specific activity (Table 1) between both study areas are in accordance with overall differences in nitrate concentrations. In contrast, nutrients are brought to the GIR area by the potentially more short-term varying outflow from the Gironde estuary.

That mesozooplankton PK activity is not correlated with silicate in the GIR area is not surprising as Si is mainly used by diatoms; in the present study, the N:Si ratio is far below the threshold ($\sim 1:1$) permitting diatoms production (Martin-Jézéquel *et al.*, 2000; Roberts *et al.*, 2003). Previous studies carried out in the same area (Sautour *et al.*, 1996; Herbland *et al.*, 1998; Labry *et al.*, 2002) showed that diatoms are absent or negligible at this period of the annual cycle and that small-sized phytoplankton cells are dominant.

The highly significant correlation, linking zooplankton PK activity to nitrate integrated values in the water column, is of major interest and warrants more consideration and discussion, especially with the lack of a relationship with Chl *a*. A previously collected data-set (Bergeron and Herbland, 2001), under differing surface salinity variations (30 to slightly >34), had already shown varying links of PK activity with either NO_3 or Chl *a*. These results were obtained from the same area and at the same season as those presented here, but on another year and it must be emphasised that the hydrobiological context was radically different. In the present study, as a consequence of the low freshwater outflow rate of the Gironde (see above), the lowest surface salinity value did not fall below 34.95; therefore, it is not surprising that the inorganic nutrient supply was so weak and that NO_3 was almost or totally depleted in the surface layer above the thermocline. Moreover, it is worth noting that deficiencies in nutrients (Moal *et al.*, 1978), notably nitrogen (Granéli *et al.*, 1999), have been shown to induce significant enrichment of phytoplankton cells in carbohydrates, with varying effects, besides, on metabolism, growth and reproduction of copepods (Van Nieuwerburgh *et al.*, 2004; Augustin and Boersma, 2006). In deeper layers, higher concentrations in high salinity waters were available to sustain photosynthetically working cells involved in basic carbohydrate production. According to previous studies in the Gironde Estuary plume (Artigas, 1998; Artigas *et al.*, 2000), the small-sized cells, mentioned above, are the basis of a complex food web, where the microbial loop is likely to play a crucial role. However, phytoplanktonic cells are by far the carbohydrate-richest components (Mayzaud and Martin, 1975; Moal *et al.*, 1978) available for primary grazers. The microzooplankton community efficiently preys on these components (Sautour *et al.*, 2000) and, for so small organisms, it may be reasonably assumed that growth and turnover rates are of the same order of magnitude (Sieburth *et al.*, 1978). For instance, growth rates of algae and ciliates (grazers of small phytoplanktonic cells) are comparable and much higher than those for copepods (Gismervik *et al.*, 1996; Hansen *et al.*, 1997), whereas the typical size of ciliates (10–40 μm) is within the size range of copepod preys (Hansen *et al.*, 1994; Gismervik *et al.*, 1996). Recent experimental works carried out in mesocosms are illustrative of such mechanisms (Stibor *et al.*, 2004a; Vadstein *et al.*, 2004). The omnivorous feeding behaviour of copepods is now widely

recognised (Kleppel, 1993). Whether mesozooplankton (i.e. 92–98% copepods, according to a study carried out on the same cruise by Plounevez and Champalbert (1999)) feed directly on phytoplankton or on protozoans and small metazoans, enriched in carbohydrates through their grazing activity, the apparent direct link between PK activity and nutrient availability implies a very close coupling between biologically produced carbohydrates at the primary levels and both ingestion and assimilation by copepods of the prey component, whatever its nature (Stibor *et al.*, 2004b). To sum up, it appears that some processes within the pelagic food web, such as consumption of smallest living particles stemming from primary productivity, may be so speedy that they escape most classical observation means and the use of enzyme activity could be able to evidence such processes.

Further results, obtained in different oceanographic contexts, are in course of study (Bergeron *et al.*, unpublished data, as mentioned above) and will show that, more often than not, PK activity and nutrient concentrations relationships are not so simple. Nevertheless, the present results support the basic concept (Bergeron and Herbland, 2001) of the potential usefulness of PK activity measurements in samples of mesozooplankton. The challenge is to define strict conditions for the implementation of an assessment tool for a process not readily, or occasionally not determined at all by other currently used methods. These methods may be excessively time-consuming, difficult to apply, because they require sorting and handling of very small and delicate organisms, or inadequately represent the whole process, if, for example, a method is only applicable to single or selected species. Scrutinizing enzyme activities in a mesozooplankton sample may be considered as searching for "common denominators, analogous to the venerable chlorophyll" (Banse, 1992), which is to reveal a metabolic step specifically and exclusively involved in the studied process. If pyruvate kinase activity measurements fulfil, at least partially, "the absence of routine measurement of rates of community ingestion" (Banse, 1992), a useful new tool is made available to biological oceanographers.

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	mean	s.d.	n
GIR	0.626	0.327	15
FAC	1.084	0.138	15

Tab. 1 Comparison of mean values and standard deviations of PK specific activity in mesozooplankton sampled in both study areas.

PK	variable	R	p =
spe. act.	Chl <i>a</i>	-0.304	0.271
spe. act.	Si(OH) ₄	0.0094	0.9736
spe. act.	NO ₃	0.7095	0.0037
spe. act.	NO ₃ <i>up.lay.</i>	0.6863	0.0055
spe. act.	NO ₃ <i>max</i>	0.6747	0.0066
act.m ⁻³	NO ₃	0.8511	0.00016

Tab. 2 Relationships between mesozooplankton PK activity (specific activity or total activity per m³) and chlorophyll *a* or nutrient concentrations: correlation coefficients (R) and significance levels (p).

Legend of the figure

Fig. 1 Location of sampling stations (3 n m spacing) of the GIR study area in the Bay of Biscay (in insert, where are also indicated the 200 m isobath and the location of the FAC study area with a small trapezium) and spatial pattern of mesozooplankton PK specific activity.

