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Effect of temperature and food concentration on the relationship between growth and AARS activity in *Paracartia grani* nauplii

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ABSTRACT

The *in situ* activity of the enzymes aminoacyl-tRNA synthetases (AARS) and the growth rates of naupliar stages of the planktonic marine copepod *Paracartia grani* were measured in the laboratory under different temperature and food concentrations. We assessed the effect of these parameters on growth and protein synthesis rates of *P. grani* nauplii. Growth and protein synthesis rates of *P. grani* nauplii depended on temperature and food concentration. AARS activity is valid as an index of somatic growth for *P. grani* nauplii when growth is not limited by food availability. However, the relationship between protein-specific AARS activity and nauplii growth varied according to food availability levels. The degradation of proteins during starvation and/or the ß-oxidation of fatty acids affected the relationship between specific AARS activity and growth rates. The results presented here add to previous studies showing that the AARS activity is a useful tool for estimating somatic growth of this and other key copepod species. Nevertheless, further research is required to elucidate the validity of AARS activity as a universal proxy for growth.

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1. Introduction

The assessment of zooplankton production is a milestone in oceanography. Zooplankton is the main link between the primary producers and fisheries, and they are also important in the flux of energy and matter in the ocean. From this point of view, it is of interest to know which changes may produce temperature and food concentration variations on the growth rates of early developmental stages of key zooplankton species.

There are an increasing number of approaches to estimate growth rates in planktonic organisms such as copepods (Runge and Roff, 2000). Traditionally, the direct method (Heinle, 1966), based on length or weight increases, and the egg production rate method (EPR, Marshall and Orr, 1955) have been applied to assess copepod growth rates. EPR is currently the most used method to estimate copepod growth (Hirst et al., 2003) and it is rather sensitive to changes in environmental variables (Saiz et al., 1997). However, the assumption that EPR is comparable to the growth rates of the juveniles is often invalid (Hirst and Bunker, 2003). Also, adult females may lose or gain weight while producing eggs, and as such, EPR may not accurately represent growth of the female (Hirst and McKinnon, 2001). In addition, EPR measurements are labor consuming and involve a risk of introducing artifacts due to the handling of the animals (Jones, 1980).

In recent years, the use of biochemical methods as indices of growth in copepods has increased. These methods allow the assessment of zooplankton production on field collected organisms with less laboratory manipulation and are mainly based on either biomass ratios (e. g. RNA/DNA, Dagg and Littlepage, 1972; RNA/protein, Gorokhova, 2003; Saiz et al., 1998; Wagner et al., 2001) or the activity of enzymes involved in the process of growth (e.g. nucleoside diphosphate kinase NDPK, Berges et al., 1990; aspartate transcarbamylase ATC, Bergeron and Buestel, 1979; Biegala and Bergeron, 1998; chitobiase, Oosterhuis et al., 2000; Sastri and Roff, 2000). An enzymatic method, recently developed as index of copepod somatic growth, is based on the activity of the aminoacyl-tRNA synthetases (AARS, Yebra and Hernández-León, 2004). These enzymes catalyze the first step of the protein synthesis and their activity is significantly related to somatic growth in freshwater and marine crustaceans (*Daphnia magna*, Yebra and Hernández-León, 2004; *Calanus helgolandicus*, Yebra et al., 2005; *Calanus finmarchicus*, Yebra et al., 2006; *Euphausia superba*, Guerra, 2006).

In order to assess the effect of temperature and food concentration on their somatic growth and protein synthesis rates (AARS activity), as well as the relationship between both variables, we studied *Paracartia grani* nauplii. Copepods of the family *Acartiidae* are common in coastal and estuarine habitats worldwide (see Rosamma and Rao, 1985). They are mainly adapted to the high food concentrations normally found in estuaries and upwelled waters (Paffenhöfer and Stearns, 1988). As they are the principal link in the marine food web in some areas, there are many studies on growth of the genus *Acartia* (e.g. Berggreen et al., 1988; Bersano, 2000; Durbin and

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Durbin, 1978; Gorokhova, 2003; Klein Breteler and Gonzalez, 1982; Landry, 1978; Leandro et al., 2006; Saiz et al., 1998). However, their nauplii growth rates have rarely been described (Berggreen et al., 1988; Calbet and Alcaraz, 1997; Durbin and Durbin, 1978; Leandro and Tiselius, 2006).

In this work, we focused on the effect of temperature and food quantity on the growth and AARS activity of *P. grani* (Sars, 1904) nauplii. In order to assert the use of this enzyme as a proxy for growth rates in the ocean, a parallel response of its activity and rates should be expected.

2. Material and methods

2.1. Parental cultures

P. grani Sars, 1904 (Copepoda: Calanoida) and *Oxyrrhis marina* (heterotrophic dinoflagellate, equivalent spherical diameter, ESD = 16.9 μm) were obtained from continuous cultures maintained at the Institute of Marine Sciences (ICM, Barcelona, Spain). They were kept in 20 L transparent plastic tanks and 2 L pyrex bottles respectively, at 20 °C with a 12:12 h photoperiod. *P. grani* and *O. marina* were fed with *Rhodomonas baltica* (Cryptophyceae, ESD = 8 μm), grown at 20 °C on f/2 medium (Guillard, 1975). Every 24 h, the eggs of *P. grani* were collected and refrigerated (4 °C) until used for experiments (between 2 and 30 days).

2.2. Experiments at different temperatures

Organisms were acclimated at different temperatures using six water baths (Table 1). In each of them we introduced a plastic container with 10 L of filtered sea water. Once the water reached the desired temperature we added the previously refrigerated eggs to each container and allowed 16 h for them to hatch. Each group of nauplii (~2 nauplii mL^{-1}) was grown under food saturating conditions (Calbet and Alcaraz, 1997). The nauplii were fed with *O. marina* (1000–1300 cells·mL^{-1}; 220–286 μg C·L^{-1}, assuming 215.8 pg C·$cells^{-1}$ from Klein Breteler and Schogt, 1994). Food concentration was measured daily with a Multisizer Coulter Counter. Every 12–24 h (depending on the experimental temperature) we took an aliquot of 100 mL from the nauplii culture and fixed it with Lugol's acid solution for abundance and individuals' length measurement. Three replicates of approx. 1000 individuals were sampled daily and frozen immediately in liquid nitrogen (−196 °C) for AARS activity assays. Sampling continued for 4–6 days until the nauplii reached the stage VI (NVI).

2.3. Experiments under different food concentrations

Nauplii of *P. grani* were acclimated at 20 °C in seven water baths. Eggs were allowed to hatch during 24 h, and a similar amount of nauplii (~2 nauplii mL^{-1}) were incubated in 10 L plastic containers. Each group of nauplii was grown under different concentrations of *O. marina* (Table 2). Food concentration was measured daily with a Multisizer Coulter Counter. Every 24 h we took three aliquots of 50 mL from the nauplii culture and fixed them with Lugol's acid solution for abundance and individuals' length measurement. Three replicates of approximately 1000 individuals were sampled and frozen immediately in liquid nitrogen (−196 °C) to assess AARS activity. Sampling continued for 5–8 days, until the nauplii reached stage NVI.

2.4. Growth calculations

Organisms fixed in Lugol's were photographed using a camera connected to a dissecting microscope at 40× magnification. Prosome length (μm) was measured from pictures with Image/J software. Individual biomass of *P. grani* nauplii was estimated from the length-dry weight (dw) equation given by Durbin and Durbin (1978) for *Acartia clausi*:

$$W = 19.04 \cdot L^{2.849}, \ r^2 = 0.98$$

where W is body weight in μg dw and L is prosome length in mm.

Dry weight (dw) was converted to carbon (C) assuming a carbon/dry weight ratio of 0.40 (Postel et al., 2000). Weight-specific growth rates (G, d^{-1}) were calculated as the slope of ln(weight) increases over time.

The temperature quotient (Q_{10}) of growth rates and AARS activities was calculated as: $Q_{10} = (M1/M2)^{10(T2-T1)}$, where M2 and M1 are the rates of the studied processes at temperatures T2 and T1 (°C), respectively. In order to use a 10 °C range, we calculated the Q_{10} between 16 and 26 °C.

2.5. AARS activity assay

Frozen samples were homogenized in Tris–HCl buffer (20 mM, pH 7.8) and centrifuged (10 min, 0 °C). AARS activity was assayed following the method of Yebra and Hernández-León (2004), slightly modified as follows: 250 μL of each sample supernatant was added to a mixture containing 200 μL of pyrophosphate (PPi) reagent (P-7275, from Sigma) and 300 μL of Milli-Q water at room temperature. The absorbance of the reaction mixture was monitored at 340 nm for 10 min at 25 °C. The aminoacylation of the tRNA releases PPi, which produces an oxidation of NADH. This is registered as a decrease in absorbance (dA). The NADH oxidation rate (dA·min^{-1}) was converted to PPi release rate (AARS activity, nmPPi·mL^{-1}·min^{-1}) using the equation (1) in Yebra and Hernández-León (2004):

$$n\text{mol PPi} \cdot h^{-1} \cdot \text{sample mL}^{-1} = \left(\text{dA} \cdot \min^{-1} \cdot 10^3 \cdot 60\right) \cdot (V_{rm} \cdot 6.22 \cdot 2)^{-1}$$

where V_{rm} is the volume of the reaction mixture in mL, 6.22 is the millimolar absorptivity of NADH at 340 nm and 2 is the number of moles of β-NADH oxidized per mole of PPi consumed.

AARS activity was corrected for the in situ temperature of each experiment by applying an activation energy of 8.57 kcal·mol^{-1} (Yebra et al., 2005) to the Arrhenius equation in order to obtain the *in situ* activity ($AARS_s$).

Protein content of the samples was measured following the Lowry et al. (1951) method adapted for micro-assay by Rutter (1967), using Bovin Serum Albumin as standard (A-4503, from Sigma).

3. Results

3.1. Effect of temperature on nauplii rates

Weight-specific growth rates (slope of each regression line in Fig. 1), varied from 0.28 to 0.85 d^{-1} between 12 and 28 °C (Table 1). The protein-specific $AARS_s$ ($spAARS_s$, Table 1) ranged from 24.35 to 106.44 nmPPi·mg $prot^{-1}$·h^{-1} and the individual $AARS_s$ increased from 0.003 to 0.019 nmPPi·ind^{-1}·h^{-1}.

Table 1
Paracartia grani nauplii somatic growth (d^{-1}) and protein synthesis rates [$spAARS_s$ (nmPPi·mg $prot^{-1}$·h^{-1}) and individual $AARS_s$ (nmPPi·ind^{-1}·h^{-1})] at different temperatures (°C). n is the number of either individuals sized or samples analyzed.

T (°C)	Somatic growth (d^{-1}) (r^2, n)	$spAARS_s$ ± SE (n) (nmPPi·mg $prot^{-1}$·h^{-1})	individual $AARS_s$ ± SE (n) (nmPPi·ind^{-1}·h^{-1})
12	0.28 (0.990, 382)	24.35 ± 0.69 (9)	0.003 ± 0.000 (9)
16	0.41 (0.987, 480)	49.13 ± 7.04 (9)	0.006 ± 0.001 (9)
19.8	0.54 (0.987, 188)	50.26 ± 1.81 (8)	0.007 ± 0.002 (8)
24	0.70 (0.970, 299)	96.79 ± 5.08 (6)	0.019 ± 0.007 (6)
26	0.85 (0.973, 430)	102.23 ± 19.72 (6)	0.016 ± 0.005 (6)
28	0.85 (0.962, 597)	106.44 ± 13.32 (9)	0.017 ± 0.007 (9)

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