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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.

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.

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)

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):

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

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 2
Paracartia grani nauplii somatic growth (d^{-1}), protein synthesis rates [$spAARS_s$ ($nmPPi \cdot mg\ prot^{-1} \cdot h^{-1}$) and individual $AARS_s$ ($nmPPi \cdot ind^{-1} \cdot h^{-1}$)] at different food concentrations ($\mu g\ C \cdot L^{-1}$). n is the number of either individuals sized or samples analyzed.

Food concentration ($\mu g\ C \cdot L^{-1}$)	Somatic growth (d^{-1}) (r^2, n)	$spAARS_s \pm SE$ (n) ($nmPPi \cdot mg\ prot^{-1} \cdot h^{-1}$)	individual $AARS_s \pm SE$ (n) ($nmPPi \cdot ind^{-1} \cdot h^{-1}$)
0	−0.01 (0.604, 454)	59.60 ± 5.18 (12)	0.003 ± 0.000 (12)
11	0.03 (0.677, 678)	59.02 ± 12.24 (10)	0.004 ± 0.001 (10)
55	0.34 (0.945, 740)	46.51 ± 4.43 (12)	0.004 ± 0.001 (12)
110	0.49 (0.935, 459)	46.03 ± 6.84 (12)	0.005 ± 0.001 (12)
220	0.64 (0.989, 299)	34.80 ± 11.03 (11)	0.006 ± 0.001 (11)
440	0.68 (0.991, 482)	32.24 ± 3.12 (9)	0.004 ± 0.001 (9)
880	0.61 (0.945, 465)	34.29 ± 4.60 (10)	0.006 ± 0.001 (10)

Temperature (°C) had a significant positive effect on growth rates (G, d^{-1}) of nauplii (Fig. 2A):

$$G = -0.188 + 0.038 \cdot T,\ r^2 = 0.984,\ p<0.001 \quad (1)$$

Also $spAARS_s$ ($nmPPi \cdot mg\ prot^{-1} \cdot h^{-1}$) and individual $AARS_s$ ($nmPPi \cdot ind^{-1} \cdot h^{-1}$) were significantly affected by temperature (Fig. 2B, C):

$$spAARS_s = -42.51 + 5.44 \cdot T,\ r^2 = 0.942,\ p<0.001 \quad (2)$$

$$AARS_s \cdot ind^{-1} = -0.010 + 0.001 \cdot T,\ r^2 = 0.852,\ p<0.001 \quad (3)$$

The Q_{10} value obtained within the 16 and 26 °C range was the same for growth rate and specific $AARS_s$ (2.1) and was 2.4 for individual $AARS_s$.

3.2. Effect of food concentration on nauplii rates

Weight-specific growth rates (slope of regression lines in Fig. 3) varied from −0.01 to 0.68 d^{-1} with increasing food concentrations (Fig. 4A) and the average $spAARS_s$ ranged between 32.24 and 59.60 $nmPPi \cdot mg\ prot^{-1} \cdot h^{-1}$ (Table 2). The average $AARS_s$ per individual increased from 0.003 to 0.006 $nmPPi \cdot ind^{-1} \cdot h^{-1}$ (Table 2) and presented high variability within experiments. The assumption

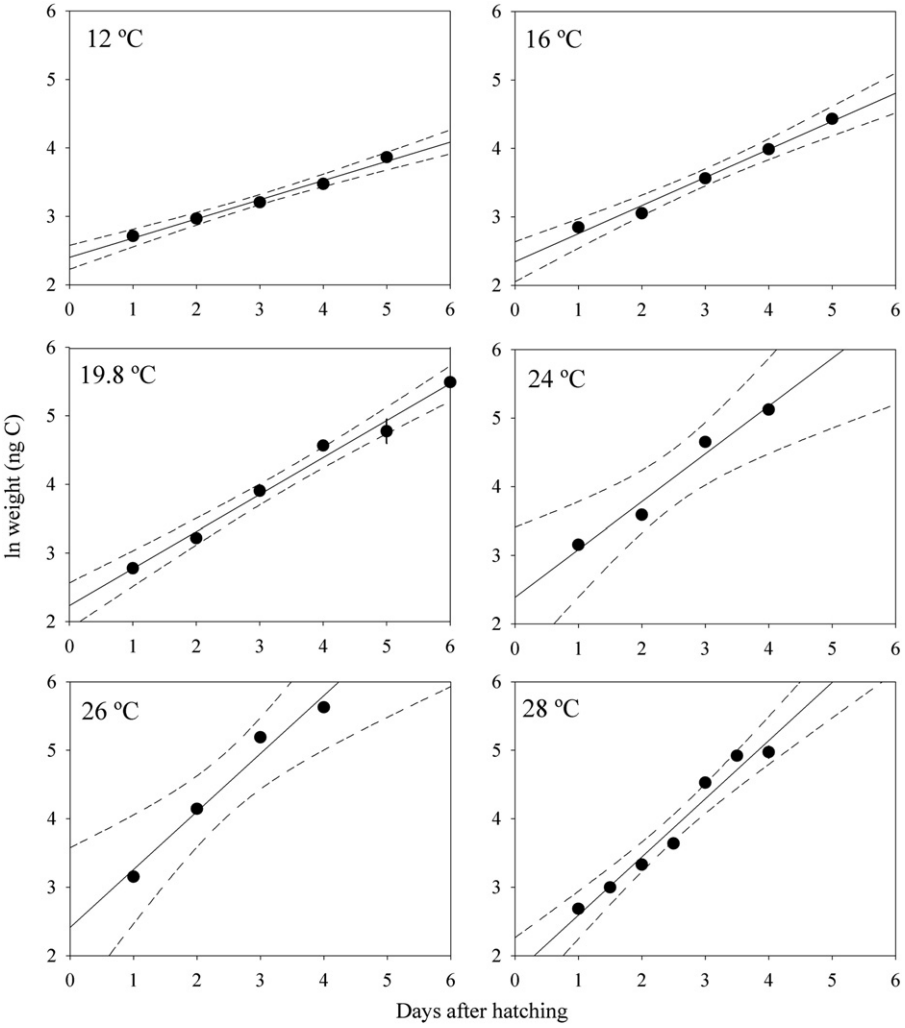

Fig. 1. *Paracartia grani* nauplii. Carbon content (ng C) increases at different temperatures.

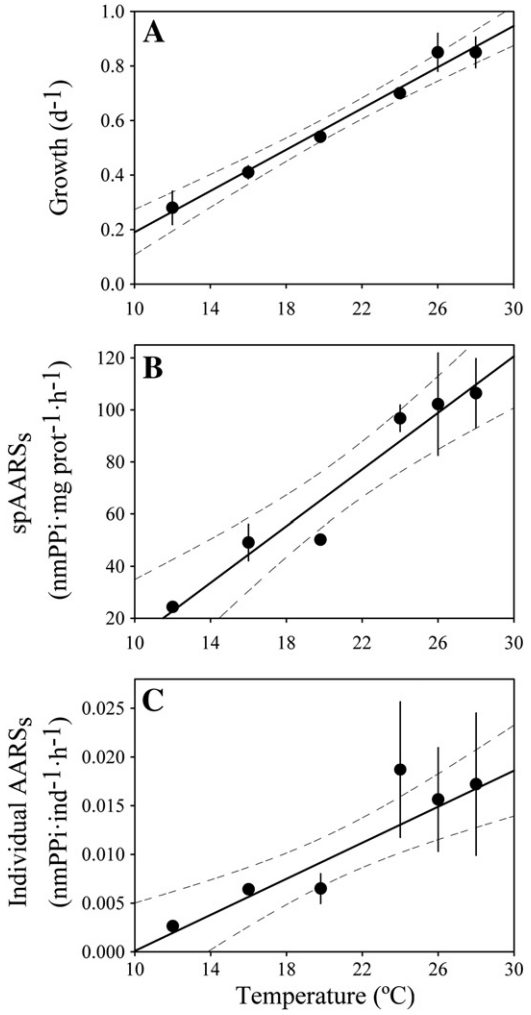

Fig. 2. *Paracartia grani* nauplii. Effect of temperature on A) growth (d^{-1}), B) $spAARS_s$ ($nmPPi \cdot mg\ prot^{-1} \cdot h^{-1}$), C) individual $AARS_s$ ($nmPPi \cdot ind^{-1} \cdot h^{-1}$).

that the aliquots frozen for biochemical assays contained a fixed amount of 1000 nauplii was not always correct, as observed on the protein content of the sample replicates (data not shown). This was mostly noted in the growth experiment conducted at a food concentration of 440 $\mu g\ C \cdot L^{-1}$, which was excluded from fit calculations in Fig. 4C.

Growth rates (d^{-1}) relative to food concentrations (C, $\mu g\ C \cdot L^{-1}$) followed a saturation curve (Ivlev's equation, 1955) expressed by the function:

$$G = 0.65 \cdot \left(1 - e^{(-0.013 \cdot C)}\right),\ r^2 = 0.986,\ p<0.001 \quad (4)$$

where 0.65 is the maximum growth rate (d^{-1}) and 0.013 is a constant that indicates the rate at which growth approaches the maximum rate. Naupliar growth became saturated at a food concentration level of 220 $\mu g\ C \cdot L^{-1}$ (Fig. 4A).

Individual $AARS_s$ ($nmPPi \cdot ind^{-1} \cdot h^{-1}$) activities also increased with increasing food concentration (Fig. 4C), following a logarithmic model:

$$AARS_s \cdot ind^{-1} = 0.0032 + 0.0004 \ln(C),\ r^2 = 0.907,\ p<0.05 \quad (5)$$

In contrast, specific $AARS_s$ ($nmPPi \cdot mg\ prot^{-1} \cdot h^{-1}$) exhibited three different values in relation to food concentration (Fig. 4B). Specific AARS showed maximum values (59.31 ± 0.29) from 0 to 11 $\mu g\ C \cdot L^{-1}$, while between 55 and 110 $\mu g\ C \cdot L^{-1}$ the average $spAARS_s$ was 46.27 ± 0.24. Above 220 $\mu g\ C \cdot L^{-1}$ $spAARS_s$ remained low and rather constant (33.78 ± 0.78).

3.3. Relationship between nauplii growth and protein synthesis rates

Positive significant relationships were found between growth rates (d^{-1}) and both $spAARS_s$ ($nmPPi \cdot mg\ prot^{-1} \cdot h^{-1}$) and individual $AARS_s$ ($nmPPi \cdot ind^{-1} \cdot h^{-1}$) activities under food saturating conditions within the 12–28 °C range (Fig. 5):

$$G = 0.13 + 0.007 \cdot spAARS_s,\ r^2 = 0.945,\ p<0.001 \quad (6)$$

$$G = 0.25 + 31.65 \cdot AARS_s \cdot ind^{-1},\ r^2 = 0.833,\ p<0.001 \quad (7)$$

A positive relationship between growth rates (d^{-1}) and individual $AARS_s$ ($nmPPi \cdot ind^{-1} \cdot h^{-1}$) was also found within the 0–-4000 $cells \cdot mL^{-1}$ (0–880 $\mu g\ C \cdot L^{-1}$) food concentration range (Fig. 6B):

$$G = -0.59 + 204.46 \cdot AARS_s \cdot ind^{-1},\ r^2 = 0.951,\ p<0.001 \quad (8)$$

However, the relationship between growth rates (d^{-1}) and $spAARS_s$ activities ($nmPPi \cdot mg\ prot^{-1} \cdot h^{-1}$) was negative (Fig. 6A):

$$G = 1.49 - 0.025 \cdot spAARS_s,\ r^2 = 0.958,\ p<0.0001 \quad (9)$$

Specific $AARS_s$ activities ($nmPPi \cdot mg\ prot^{-1} \cdot h^{-1}$) also showed a negative relationship with nauplii individual biomass ($\mu g\ protein-s \cdot ind^{-1}$), presenting higher $spAARS_s$ activities and lower individual biomass in starved organisms, and lower enzyme activities and higher protein content in the nauplii growing at food saturating levels (Fig. 7A):

$$spAARS_s = 64.3 - 165.0 \cdot \text{individual biomass},\ r^2 = 0.490,\ p<0.0001 \quad (10)$$

The relationship between daily growth rates and $spAARS_s$ in relation to the levels of food availability showed three different relationships (Fig. 7B):

Starvation level (0–11 $\mu g\ C \cdot L^{-1}$):

$$G = -0.141 + 0.0024 \cdot spAARS_s,\ r^2 = 0.458,\ p = 0.095 \quad (11)$$

Intermediate level (55–110 $\mu g\ C \cdot L^{-1}$):

$$G = -0.027 + 0.008 \cdot spAARS_s,\ r^2 = 0.146,\ p = 0.351 \quad (12)$$

Saturation level (>220 $\mu g\ C \cdot L^{-1}$):

$$G = -0.57 + 0.038 \cdot spAARS_s,\ r^2 = 0.799,\ p = 0.003 \quad (13)$$

4. Discussion

We assessed the effect of temperature and food concentration on somatic growth (calculated from length measurements) and protein synthesis rates (AARS activity) of *P. grani* nauplii. A strong relationship was observed between growth rate and specific AARS activity at saturating food concentration and at a wide range of temperatures. However, this strong relationship was not observed at different food levels, except for those incubated at saturation. High specific AARS activities were found at low growth rates under limiting food concentration and low individual biomass. Early nauplii stages do not feed but develop consuming lipid reserves. Whether this feature

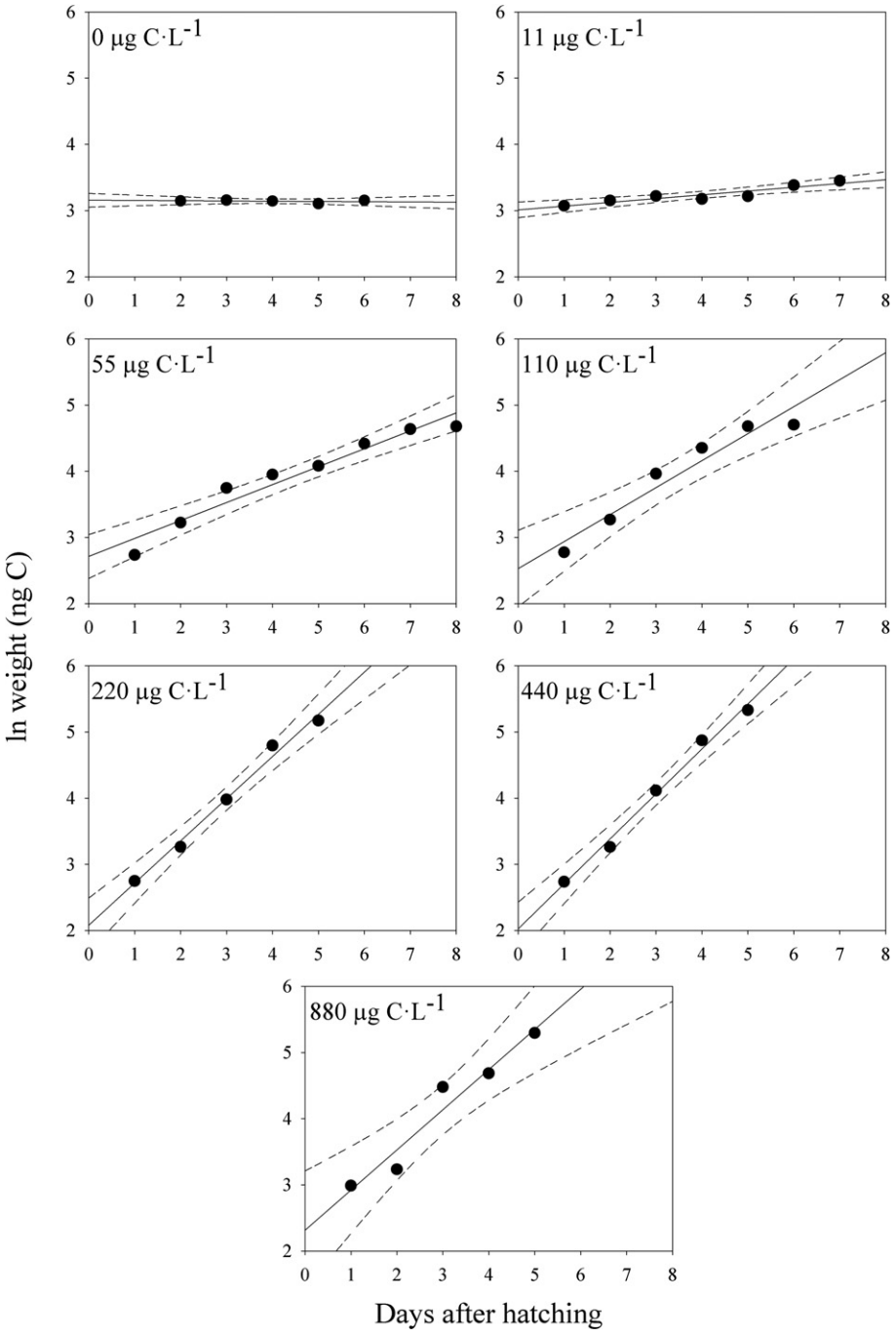

Fig. 3. *Paracartia grani* nauplii. Carbon content (ng C) increases under different food concentrations.

influences the relationship between growth rates and specific AARS activity seems the key to understanding the high activity observed in the present and other works (e.g., Holmborn et al., 2009) at low levels of food and growth.

4.1. Effect of temperature

Acartia species present isochronal development and exponential growth when reared at ad libitum food concentrations, and it has been shown that both growth and development rates depend on temperature (Klein Breteler and Schogt, 1994; Leandro and Tiselius, 2006; Leandro et al., 2006; Sekiguchi et al., 1980). Temperature had a positive effect on the naupliar growth and on the protein synthesis rates of these species when they were fed *ad libitum*. These naupliar growth rates were similar to those of other *Acartia* species nauplii (Table 3). The results obtained by Berggreen et al. (1988) and Leandro and Tiselius (2006) for *Acartia tonsa* agree with the rates of *P. grani* observed by Calbet and Alcaraz (1997) and those found in the present work within the 10–18 °C temperature range. However, at 22 °C *A. tonsa* grew faster (Leandro and Tiselius, 2006) than *P. grani*. This might be due to the different quality of the food supplied (Table 3), but also to the different responses that these species may have with increasing temperature. For example, *A. tonsa* is distributed worldwide (Kouwenberg, 2011), while *P. grani* is found in coastal NE Atlantic and Mediterranean Sea waters (Walter and Boxshall, 2011). Also, the temperature quotients (Q_{10}) observed for both growth and protein synthesis rates in *P. grani* (2.1 between 16 and 26 °C) were lower than the Q_{10} values reported by Leandro and Tiselius (2006)

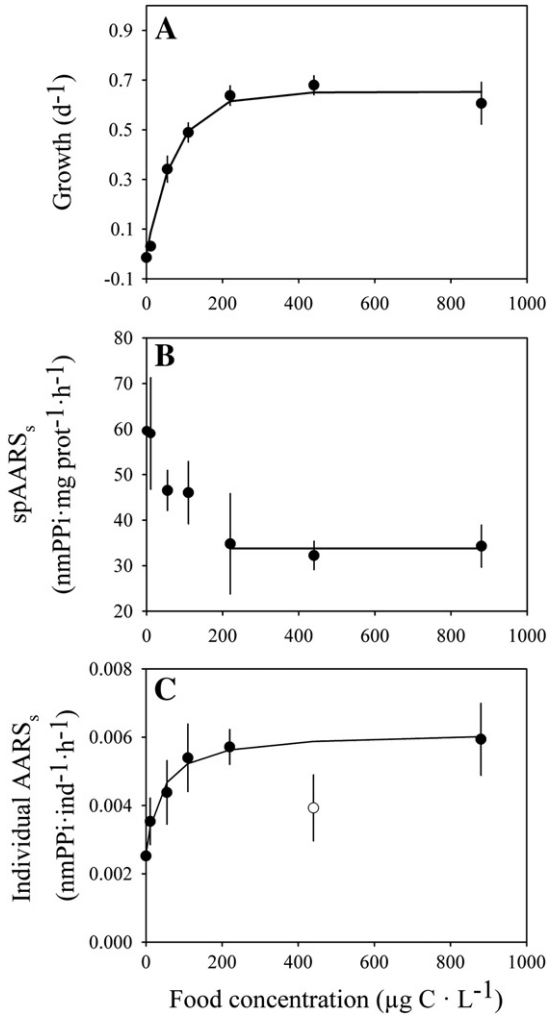

Fig. 4. *Paracartia grani* nauplii. Effect of food concentration on A) growth (d^{-1}), B) $spAARS_s$ ($nmPPi \cdot mg\ prot^{-1} \cdot h^{-1}$), C) individual $AARS_s$ ($nmPPi \cdot ind^{-1} \cdot h^{-1}$); open circle: value not included in fit (see text).

(3.66 between 10 and 22 °C). However, the use of Q_{10} values calculated across different temperature ranges could result in errors when comparing the temperature effects on physiological rates, as the Q_{10} has been shown to be temperature dependent, decreasing when temperature rises (Almeda et al., 2010).

4.2. Effect of food availability

As expected, growth rates of *P. grani* depended on the food availability. At low food concentration a low growth rate was observed in *P. grani* nauplii. This fact might be explained by a decreased efficiency of food capture at low food concentration, as observed for other congeneric species (Paffenhöfer and Stearns, 1988: *A. tonsa*), suggesting their adaptation to the high food environments in which they are mainly found (estuarine areas and coastal waters, Alcaraz, 1977; Villate, 1982). At high food concentrations growth rate became stable for *P. grani* nauplii, as was previously observed for this (Calbet and Alcaraz, 1997: *A. grani*) and other species (Berggreen et al., 1988: *A. tonsa*) at the same temperature 18 °C, but feeding on a different food type (*R. baltica*). There are several studies on the effect of the quality and food concentration on the egg-production rates, growth and development of the genus *Acartia* (Berggreen et al., 1988; Calliari and Tiselius, 2005; Hassett, 2004; Stottrup and Jensen, 1990). However, the effect of the food type on the growth of early stages of copepods is scarcely studied. The prey used in this study

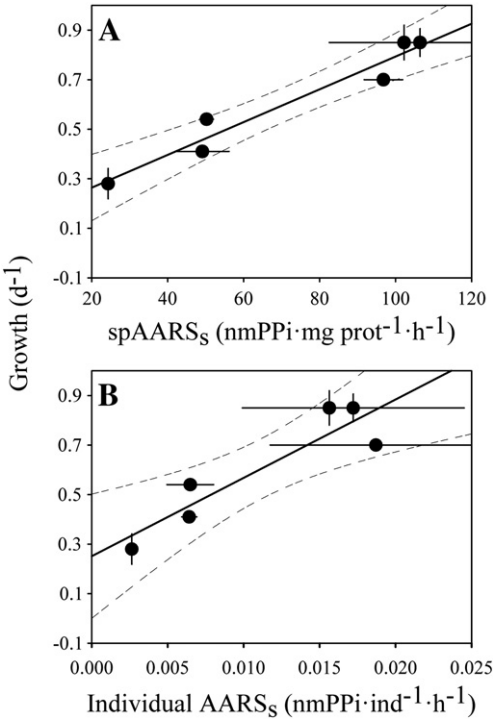

Fig. 5. *Paracartia grani* nauplii. Relationship between growth rates (d^{-1}) and A) specific $AARS_s$ activities ($nmPPi \cdot mg\ prot^{-1} \cdot h^{-1}$), B) individual $AARS_s$ ($nmPPi \cdot ind^{-1} \cdot h^{-1}$) at different temperatures (°C).

(*O. marina*) is considered a high-quality food for *Acartia* species (Klein Breteler and Schogt, 1994; Kleppel et al., 1998), although other studies used different prey (*R. baltica* or a mixture of the diatom *Thalassiosira weissflogii* and the cryptophyte *Rhodomonas* sp.). It has been shown that diet modification affects *Acartia* species growth,

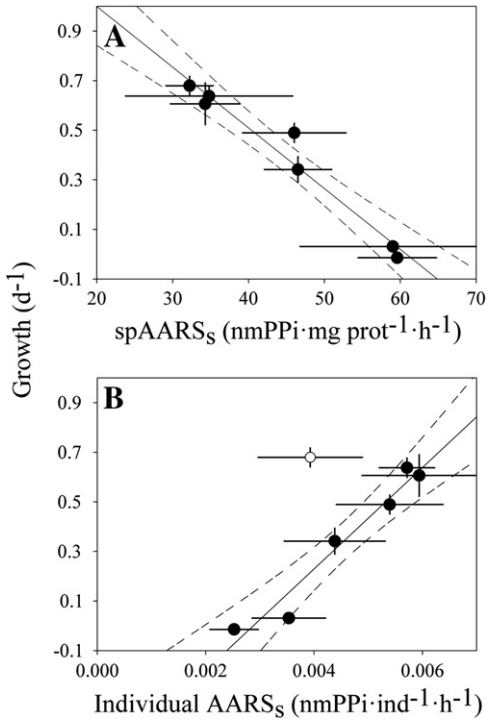

Fig. 6. *Paracartia grani* nauplii. Relationship between growth rates (d^{-1}) and A) specific $AARS_s$ activities ($nmPPi \cdot mg\ prot^{-1} \cdot h^{-1}$), B) individual $AARS_s$ ($nmPPi \cdot ind^{-1} \cdot h^{-1}$) under different food concentrations ($\mu g\ C \cdot L^{-1}$); open circle: value not included in fit (see text).

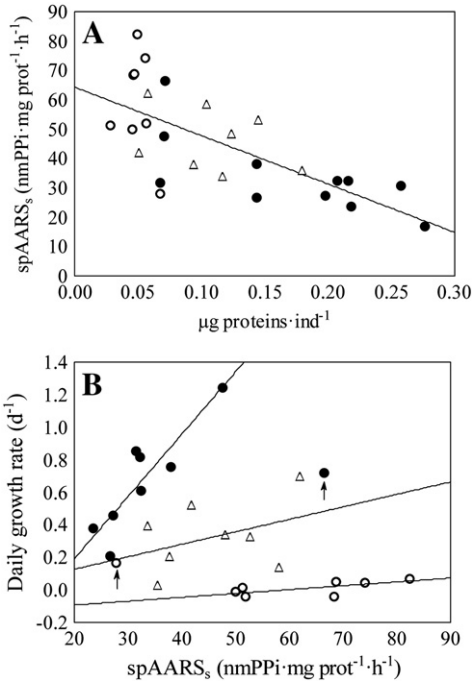

Fig. 7. *Paracartia grani* nauplii. A) Relationship between specific $AARS_s$ activities (nmPPi·mg prot^{-1}·h^{-1}) and individual biomass (µg proteins·ind^{-1}); B) Relationships between daily growth rates (d^{-1}) and specific $AARS_s$ activities (nmPPi·mg prot^{-1}·h^{-1}) under different food concentrations (open circles: starvation level, triangles: intermediate level, filled circles: saturation level); arrows: values not included in fit.

development, production, and nutritional composition (Ismar et al., 2008; Teixeira et al., 2010). Nevertheless, the growth rate of naupliar stages of *P. grani* at 18 °C (0.50 d^{-1}, calculated from Eq. (1)) was comparable to those observed on nauplii of this and other congeneric species (Table 3) despite the different prey supplied as food. This fact suggests that food quality might not strongly affect nauplii growth rates of these species under saturating food concentrations.

Table 3
Summary of published *Acartia spp.* nauplii growth rates under saturating food levels.

Temperature (°C)	Growth rate (d^{-1})	Species	Food type	Reference
10	0.19	*Acartia clausi*	*Rhodomonas* sp.	Leandro et al., 2006
10	0.19	*Acartia tonsa*	*Rhodomonas* sp.	Leandro and Tiselius, 2006
12	0.28	*Paracartia grani*	*Oxyrrhis marina*	This work
15	0.27	*A. clausi*	*Rhodomonas* sp.	Leandro et al., 2006
15	0.37	*A. tonsa*	*Rhodomonas* sp.	Leandro and Tiselius, 2006
16	0.41	*P. grani*	*O. marina*	This work
16	0.41	*A. tonsa*	*Rhodomonas baltica*	Berggreen et al., 1988
18	0.46	*P. grani*	*R. baltica*	Calbet and Alcaraz, 1997
18	0.45	*A. tonsa*	*R. baltica*	Berggreen et al., 1988
18	0.46	*A. clausi*	*Rhodomonas* sp.	Leandro et al., 2006
18	0.54	*A. tonsa*	*Rhodomonas* sp.	Leandro and Tiselius, 2006
19.8	0.53	*P. grani*	*O. marina*	This work
20	0.42	*A. clausi*	*Rhodomonas* sp.	Leandro et al., 2006
20	0.61–0.68	*P. grani*	*O. marina*	This work
22	0.42	*A. clausi*	*Rhodomonas* sp.	Leandro et al., 2006
22	0.88	*A. tonsa*	*Rhodomonas* sp.	Leandro and Tiselius, 2006
24	0.70	*P. grani*	*O. marina*	This work
26	0.85	*P. grani*	*O. marina*	This work
28	0.85	*P. grani*	*O. marina*	This work

4.3. Relationship between somatic growth and protein synthesis rates

Somatic growth and protein synthesis rates in *P. grani* nauplii were affected by temperature and food concentration. We found positive relationships between somatic growth and protein synthesis rates of nauplii feeding at ad libitum food concentrations. This is in agreement with the significant correlations previously observed between somatic growth rates and specific $AARS_s$ activities in copepodites and adults of other calanoid species (Yebra et al., 2005, 2006), both in laboratory and field experiments.

Protein-specific and individual $AARS_s$ activities of *P. grani* nauplii showed patterns similar to growth at different temperatures when feeding ad libitum. We also observed a similar pattern between growth rates and individual $AARS_s$ in relation to food availability. However, contrary to expectations, the relationship between specific $AARS_s$ activities and growth rates was negative (Fig. 6A). *P. grani* nauplii specific $AARS_s$ activities also showed a negative relationship with individual biomass (Fig. 7A), and presented three different relationships with growth, corresponding to organisms either starved, under food saturating conditions or growing at intermediate food levels (Fig. 7B). Under starvation (0–11 µg C·L^{-1}), *P. grani* nauplii metabolic activities were maintained at the expense of accumulated endogenous energy reserves, resulting in either negative or almost nil growth rates (−0.01–0.03 d^{-1}) and low individual $AARS_s$, but very high specific $AARS_s$ activities. On the contrary, nauplii feeding on saturating food concentrations showed a clear relationship between growth rates and specific $AARS_s$ activity. This observed variability could be due to one or, most probably, different factors combined, such as i) food limitation of growth and protein synthesis rates, ii) the relative importance of the protein turnover rates in relation to the 'de novo' protein synthesis rates of the nauplii, which would lead to anomalously high $spAARS_s$ values on organisms under starvation, as previously observed in *A. bifilosa* females by Holmborn et al. (2009), and *Oithona davisae* nauplii by Yebra et al. (2011); iii) the body size of the nauplii (as suggested by the relationship observed between $spAARS_s$ activity and individual biomass) and iv) the different developmental stages reached by the nauplii in each food treatment. In this sense, under low food conditions nauplii may not reach their first feeding stage, and the pre-feeding stages might have different protein metabolism and growth pathways that the feeding ones. Protein growth is defined as the change in mass of the protein pool and can be either positive or negative depending on the relative balance between protein synthesis and degradation (see Fraser and Rogers, 2007). Thus, a first case to explain the observed results could be related to the fact that in actively feeding organisms (above 55 µg C·L^{-1} in this study), rates of protein synthesis would be greater than protein degradation and hence somatic growth will occur. However, in starved organisms (0–11 µg C·L^{-1} in this study) proteins are degraded faster than they are synthesized (Hawkins, 1985). Previous studies on the effect of the frequency of feeding on both overall growth and protein metabolism in the European lobster showed elevated protein synthesis rates but also elevated degradation, resulting in high turnover and reduced growth (Mente et al., 2001). Then, the observed high protein-specific AARS values could be explained by the coupling of the nauplii protein mass decrease due to starvation (or insufficient food for growth) and their high turnover rates. A second case could be methodological as non-feeding organisms may use their lipid reserves to survive. The ß-oxidation of fatty acids could produce PPi during the assay which would interfere in our method because we measure AARS activity as PPi release rate. This precludes the application of this method on organisms living on the degradation of their own lipid reserves. These problems should be tested by measuring enzyme activity using radioactive substrates. In any case, this mismatch between

specific AARS activity and growth rates due to artificially induced low food concentrations would not likely occur in the field for this species, considering that typical particulate organic carbon values observed in their coastal habitats lay above those experimental food levels (Duforet-Gaurier et al., 2010; Gardner et al., 2006; Huntley and Boyd, 1984).

Summarizing, both growth and protein synthesis rates of *P. grani* nauplii depended on temperature and food concentration. AARS activity is valid as index of somatic growth for *P. grani* nauplii when growth is not limited by food availability. However the degradation of proteins during starvation and/or the use of lipids as fuel in prefeeding nauplii affected the relationship between specific AARS activity and growth rates. The results presented here add to previous studies showing that the $AARS_S$ activity is a useful tool for estimating somatic growth in copepods. Nevertheless, further investigations are required to elucidate the possible effects of size, lipids and protein metabolism in order to use AARS activity as a universal proxy for growth.

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