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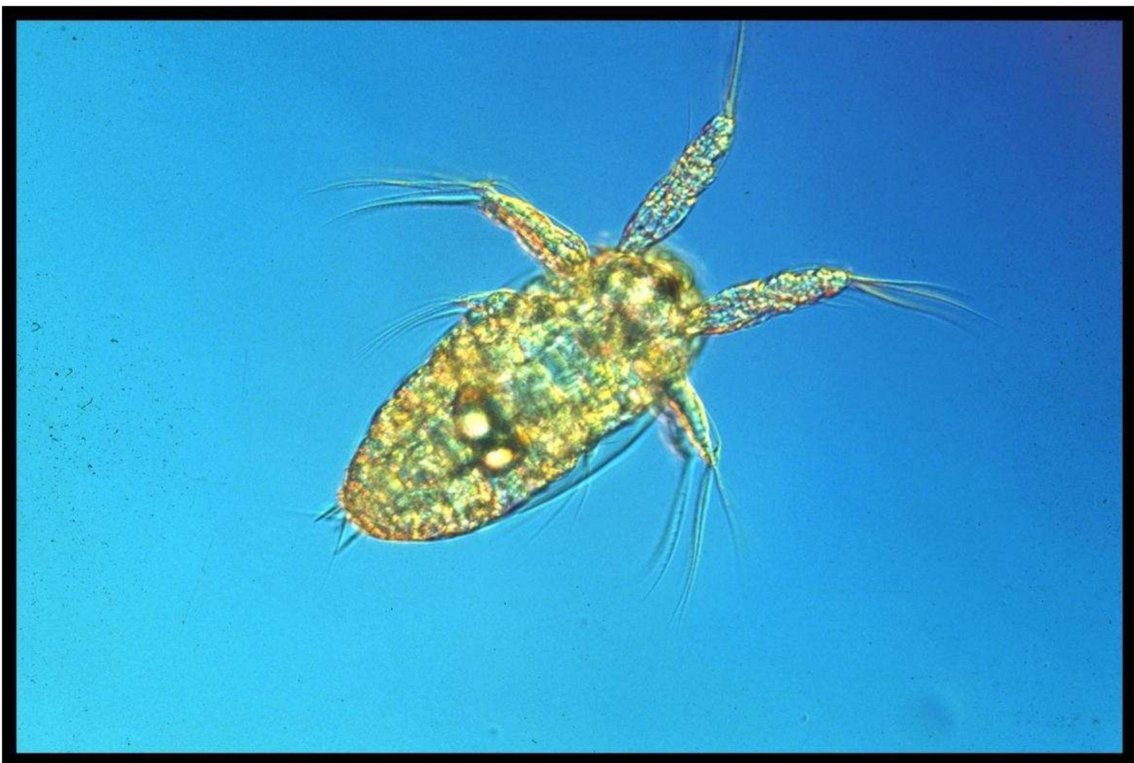
OCEANOGRAFÍA BIOLÓGICA

EFFECT OF TEMPERATURE ON *PARACARTIA GRANI* NAUPLII GROWTH RATES ESTIMATED THROUGH DIRECT AND INDIRECT METHODS



Memoria de Investigación presentada por la Lcda. Dña. Inmaculada Herrera Rivero

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Effect of temperature on *Paracartia grani* nauplii growth rates estimated through direct and indirect methods

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Abstract

Activity of the enzymes aminoacyl-tRNA synthetases (AARS) and weight-specific growth rates of naupliar stages of the planktonic marine copepod *Paracartia grani* were measured at different temperatures (12 to 28 ºC) in the laboratory, in order to assess the effect of temperature on both somatic growth and protein synthesis rates. A secondary aim consisted on the validation of the AARS method (Yebra and Hernández-León, 2004) as index of *in situ* growth rate for the naupliar stages. The nauplii were reared feeding on saturating concentrations of either the autotrophic algae *Rhodomonas baltica* or the heterotrophic dinoflagellate *Oxyrrhis marina*. Measurements of length growth rate and enzymatic activity were compared at the different temperatures. Temperature significantly affected somatic growth and AARS activities (r^2=0.95-0.99, $p<0.001$). Specific AARS activity showed a highly significant correlation (r^2=0.96, $p<0.001$) with the direct measurement of growth rates in the laboratory.

Key words: AARS, growth, nauplii, *Paracartia grani*, temperature

1. Introduction

Temperature can modify the life-history traits of copepods through its influence on growth and development rates (Huntley and Lopez 1992; Campbell et al. 2001; Hirst and Kiørboe 2002). Studies of copepods early developmental stages refer in general to the control exerted by predation (Landry 1978), food concentration (Paffenhöfer 1970; Klein Breteler and Gonzalez 1982; Berggreen et al. 1988; Tsuda 1994) or the effects of fluctuations in food availability (Calbet et al. 1997). There are several laboratory studies that analyze the effect of temperature on copepods growth rates (Durbin and Durbin, 1978; Kimmerer and Mckinnon 1987; Berggreen et al. 1988; Escribano and McLaren 1992; Huntley and Lopez 1992). They show that temperature affects growth, development time and final weight acquired in each copepodite stage. However, the effects of temperature on growth rates for early developmental stages of copepods (i.e. nauplii) are less well known (nauplii of *Acartia tonsa*: Berggreen et al. 1988; Leandro 2006; nauplii of *Acartia clausi*: Durbin and Durbin 1978; Klein Breteler 1994; nauplii of *Acartia grani*: Calbet et al. 1997).).

In general, the direct method (Heinle 1969) is used to assess growth rates of copepods and early stages of this genus (nauplii and copepodites of *Acartia tonsa*: Berggreen et al. 1988; Leandro 2006; nauplii and copepodites of *Acartia clausi*: Durbin and Durbin 1978; Klein Breteler 1994; nauplii of *Acartia grani*: Calbet et al. 1997). Also, indirect methods have been used to estimate growth of calanoids on the basis of RNA content (*A. grani*: Saiz et al. 1998; *A. bifilosa*: Holmborn et al. 2008) and enzyme activities related to synthesis processes (aspartate transcarbamylase, ATC, Bergeron and Buestel 1979; Alayse-Danet 1980; Bergeron 1982; aminoacyl-tRNA synthetases, AARS, Yebra et al. 2005;

2006). In this work we used direct (body length) and indirect (AARS activity) approaches to determine the growth rate of nauplii of the calanoid copepod *Paracartia (Acartia) grani*. Recently, the AARS method has been successfully applied to assess growth rates 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). Thus, AARS activity seems to be a good candidate to be used as an index of *in situ* growth for *Paracartia grani*.

The species chosen, *Paracartia grani* (Sars G.O. 1904), is a copepod typical of coastal, semi-confined ecosystems, conditioned by a high degree of instability in physical (temperature) and biological (food) conditions (Calbet et al. 1996). *P. grani* has been recorded in different areas of the Atlantic Ocean, such as Canary Islands (Corral 1970; Vives 1982), Portuguese coast (Vilela 1972) as well as in the Western Mediterranean Sea (Guerrero et al. 1998, Saiz et al. 1998). This species (*Acartiidae* family) is so important because is considered to be one of the most abundant inside the oceanic systems; they are an important component in the diet of a great quantity of plankton species.

The present work was carried out on *Paracartia grani* nauplii with the aim of studying: 1) the effect of temperature on the nauplii growth rates and 2) the relationship between direct growth rates and specific $AARS_{situ}$ activities to validate the AARS method as growth index for this species under different temperatures (12-28ºC).

2. Methods

2.1 Parental cultures.

Paracartia grani Sars G.O. 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 20l transparent plastic tanks and 2l pyrex bottles respectively, at 20ºC with a 12h day: 12h night photoperiod. *P. grani* and *O. marina* were fed *Rhodomonas baltica* (Cryptophyceae, ESD = 6.6 µm), grown at 20ºC on f/2 medium (Guillard 1975). Every 24h, the eggs of *P. grani* were collected and preserved in the fridge (4ºC) until utilized for experiments (between 2-30 days).

2.2 Nauplii growth experiments.

We put seven water baths to acclimate to different temperatures (see Table 1). In each of them we introduced a plastic container with 10 litres of filtered seawater. Once the water reached the desired temperature we added the previously preserved eggs (22,000-64,548) to each experimental container. We allowed 16 hours for the eggs to hatch. Each group of nauplii was grown under food saturating conditions (Klein Breteler et al. 1994; Calbet et al. 1997; Almeda unpubl. data). The nauplii reared at 26 ºC were fed with *Rhodomonas baltica* (79,800 cels·ml^{-1}, 3,780 µgC·l^{-1} estimated from Calbet et al. 1997). The rest of experiments 12, 16, 19.8, 23.7, 24 and 28 ºC were fed with *Oxyrrhis marina* (1,000-1,300 cels·ml^{-1}, 220-286 µgC·l^{-1} estimated from Klein Breteler et al. 1994). Food concentration was measured daily with a Multisizer Coulter

Counter. Every 12-24h (depending on the experimental temperature) we took an aliquot of 100 ml from the nauplii culture and fixed it with lugol's acid (4%) for abundance and individual length measurement. Also, we took three replicates of approx. 1,000 individuals and froze them immediately in liquid nitrogen (-196 ºC) for biochemical assays. Sampling continued for 4-6 days, until the nauplii reached the stage VI (NVI).

2.3 Length measurement and weight-specific growth calculations.

Organism's were photographed with an Image Analysis System (dissecting microscope with camera). Prosome length (µm) was measured from pictures with Image software (Image/J). Individual biomass of *Paracartia grani* nauplii was estimated from length-dry weight (dw) equations given by Durbin and Durbin (1978) for *Acartia clausi*:

$$W = 19.04\ L^{2.849},\ r = 0.99$$

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

Weight in µg dry weight (dw) was converted in carbon (C) assuming a carbon/dry weight ratio of 0.40 (Postel et al. 2000).

We obtained the temperature coefficient (Q_{10}) of weight-specific growth by means of the following equation:

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\left(\frac{10}{T_2 - T_1}\right)}$$

Were R1 and R2 are the growth rates at temperatures T1 and T2.

Table 1. Different characteristics of each experiment. Somatic growth rates (d^{-1}) correspond to the slope of weight increases shown in Figure 2.

T (ºC)	**Food type**	**Food concentration ($\mu gC \cdot l^{-1}$)**	**Prosome length (µm) range**	**Prosome length (µm) Mean ± SD**	**Somatic growth (d^{-1}) (R^2, n)**	**spAARSsitu ± SD (n) (nmPPi·mg protein^{-1}·h^{-1})**
12	*Oxyrrhis marina*	220-286	102.96 - 242.84	145.07 ± 28.63	0.28 (0.99, 382)	24.35 ± 2.97 (9)
16	*Oxyrrhis marina*	220-286	105.59 - 246.05	161.69 ± 35.95	0.41 (0.99, 480)	49.13 ± 13.42 (9)
19.8	*Oxyrrhis marina*	220-286	107.47 - 302.52	168.12 ± 47.54	0.54 (0.99, 188)	52.66 ± 8.40 (15)
23.7	*Oxyrrhis marina*	220-286	102.09 - 397.73	179.78 ± 58.81	0.68 (0.98, 219)	101.01 ± 2.91 (9)
24	*Oxyrrhis marina*	220-286	105.20 - 304.49	192.42 ± 61.77	0.70 (0.97, 299)	96.79 ± 15.09 (6)
26.6	*Rhodomonas baltica*	3,780	98.36 - 357.53	178.63 ± 60.13	0.80 (0.97, 297)	122.91 ± 33.86 (9)
28	*Oxyrrhis marina*	220-286	107.46 - 347.04	179.22 ± 55.91	0.85 (0.96, 597)	131.45 ± 17.00 (9)

2.4. Biochemical assays.

In the laboratory frozen samples were homogenized in Tris-HCl buffer (20 mM, pH 7.8) and centrifuged (10 min, 0ºC). AARS activity was assayed following the method of Yebra and Hernández-León (2004), slightly modified as follow: 250 µl of the samples supernatant was added to a mixture containing 200 µl of pyrophosphate (PPi) reagent (Sigma, P-7275) and 300 µl of Milli-Q water. The absorbance of the reaction mixture was monitored at 340 nm for 10 min at 25 ºC. 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 ($dA \cdot min^{-1}$) was converted to PPi release rate (AARS activity, $nmPPi \cdot ml^{-1} \cdot min^{-1}$) with equation (1) in Yebra and Hernández-León (2004):

$$\text{nmol PPi} \cdot h^{-1} \cdot \text{sample ml}^{-1} = (dA \cdot min^{-1} \cdot 10^{3} \cdot 60) \cdot (V_{rm} \cdot 6.22 \cdot 2)^{-1}$$

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.

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

Protein content of the same samples was assessed following the Lowry et al. (1951) method adapted for micro-assay by Rutter (1967). Using Bovin Serum Albumin (BSA) as the protein standard.

3. Results

The mean prosome length increases were linear over time ($p<0.05$, Fig.1). The prosome length obtained in the laboratory ranged between 98.36 µm (NI) and 397.73 µm (NVI). In spite of having used a different food type for the experiment at 26ºC, it did not show differences in the pattern of growth with regard to the other experiments. We estimated that temperature had a significant positive effect on body length increases of nauplii ($r^2=0.99$, $p<0.05$).

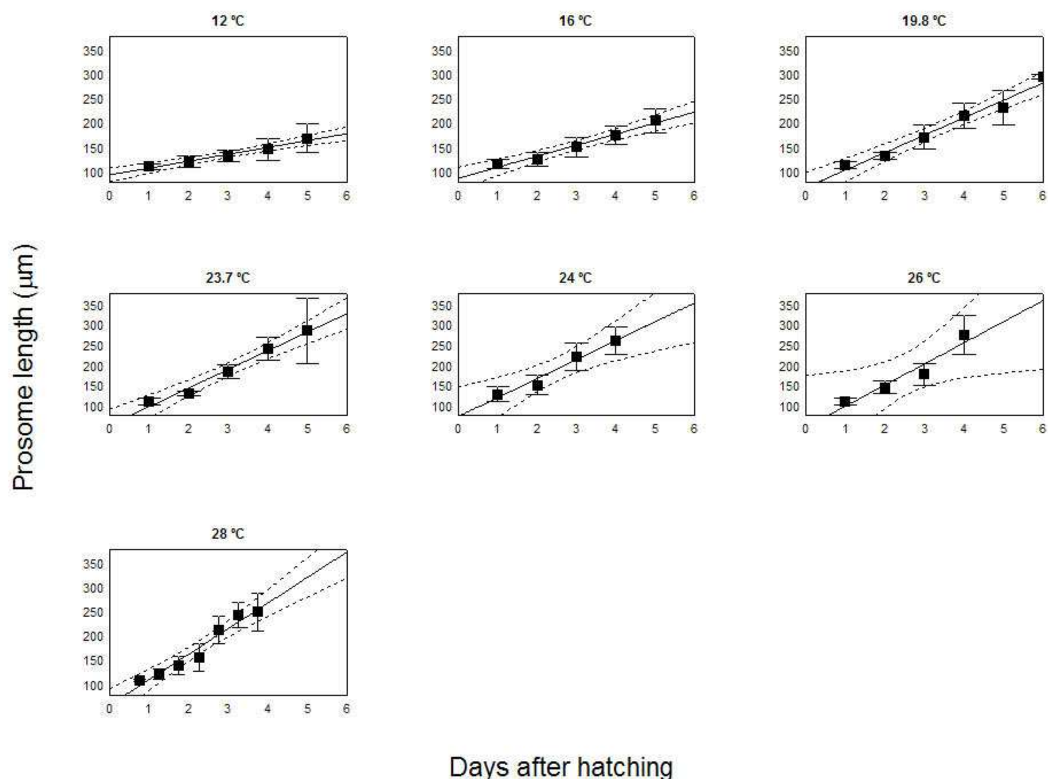

Fig.1. *Paracartia grani* nauplii prosome length (µm) increases during each growth experiment.

In order to determine the weight-specific growth rate of *P. grani*, the prosome length was converted to dry weight. The weight-specific growth rates (slope of

each graph in Fig. 2), varied from 0.28 to 0.85 d^{-1} (Table 1), which corresponds to a Q_{10} value to 2.0. The average $spAARS_{situ}$ (Table 1) ranged from 24.35 to 131.45 $nmPPi \cdot mgprotein^{-1} \cdot h^{-1}$, which corresponds to a Q_{10} value to 2.9.

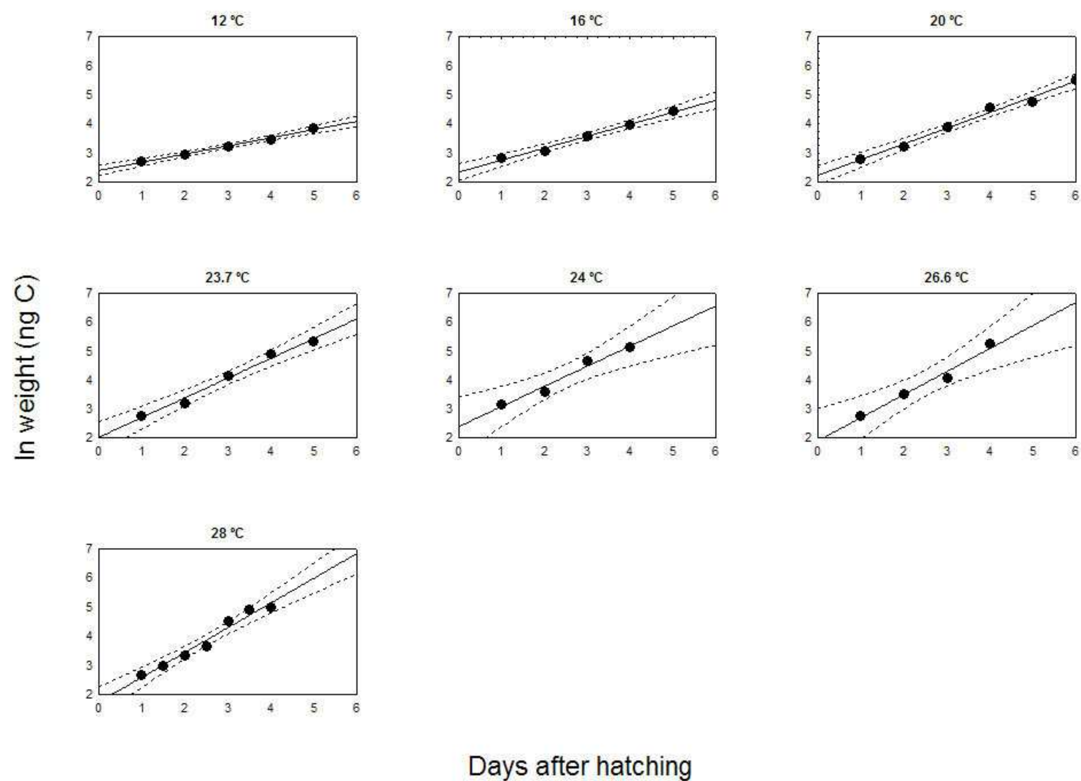

Fig.2. *Paracartia grani* nauplii carbon weight increases (calculated from Fig.1) during each growth experiment.

We found a temperature dependence of growth rates for nauplii of *P.* grani (Fig. 3). The growth rates (d^{-1}) varied significantly according to temperature (ºC):

$$\text{weight-specific growth} = -0.1582 + 0.0358 \cdot T$$

$$(R^2 = 0.99, p<0.001)$$

Specific $AARS_{situ}$ activities ($nmPPi \cdot mgprotein^{-1} \cdot h^{-1}$) were also highly correlated to temperature (ºC):

$$spAARS_{situ} = -64.15 + 6.84 \cdot T$$

$$(R^2 = 0.95, p<0.001)$$

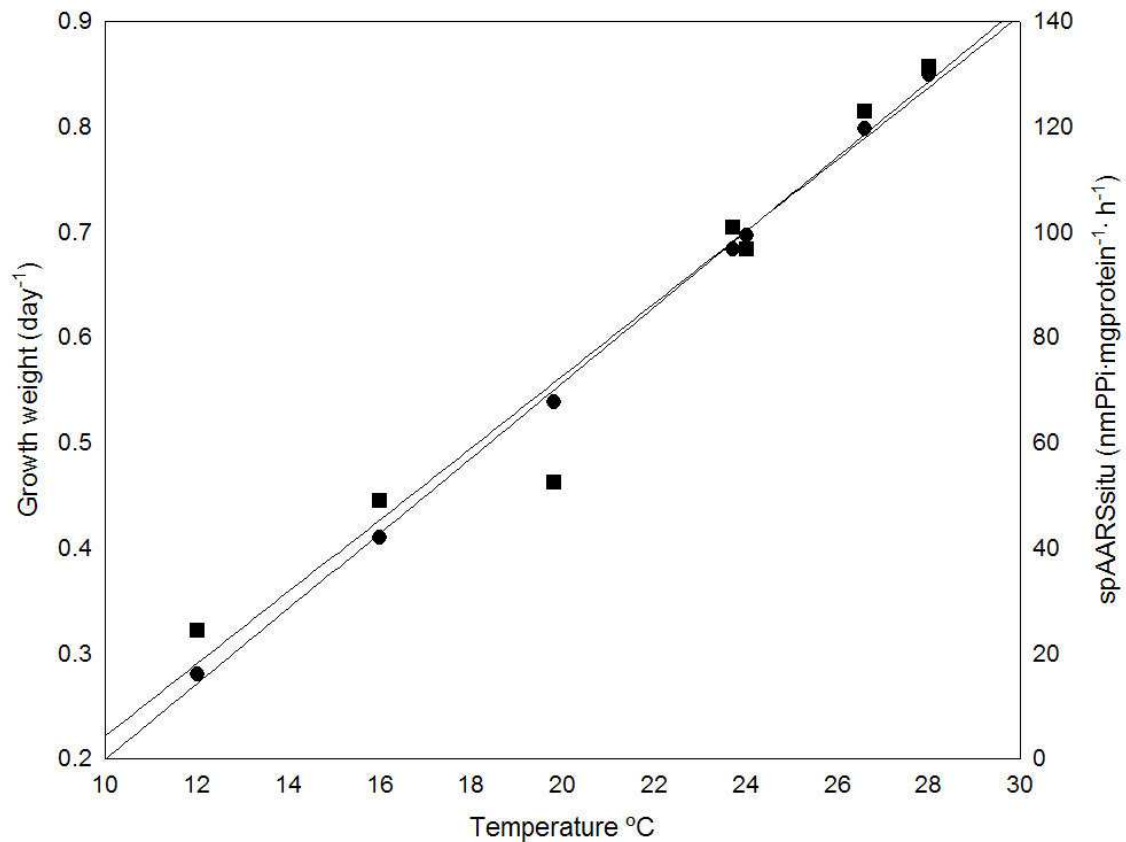

Fig.3. *Paracartia grani* nauplii growth variation with temperature, measured as a) weight-specific growth rates (day^{-1}), circles, left axis; and b) $spAARS_{situ}$ ($nmPPi \cdot mgprotein^{-1} \cdot h^{-1}$), squares, right axis.

When comparing the results obtained applying direct and indirect methods (Table 1), we found a positive significant relationship between somatic weight-

specific growth rates (d^{-1}) and average $spAARS_{situ}$ activities (nmPPi·mg protein^{-1}·h^{-1}) within the 12-28ºC temperature range (Fig. 4):

$$\text{weight-specific growth} = 0.1947 + 0.005 \cdot spAARS_{situ}$$

$$(R^2 = 0.96, p < 0.001)$$

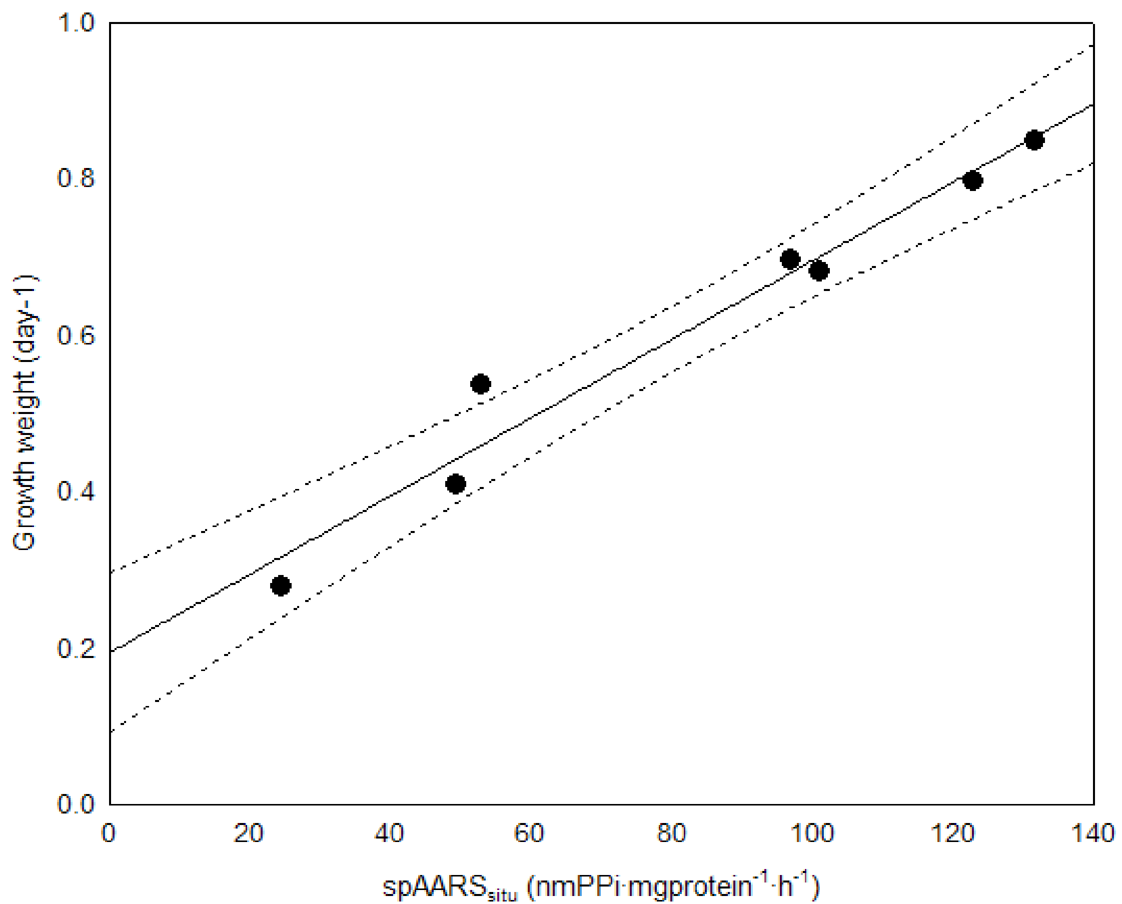

Fig.4. Correlation between weight-specific growth rates (day^{-1}) and specific $AARS_{situ}$ activities (nmPPi·mg protein^{-1}· h^{-1}) of *Paracartia grani* nauplii. Dashed lines: 95% confidence limit the regression.

4. Discussion

This work took place within the frame of the international program GLOBEC (Global Ocean Ecosystem Dynamics), which from 1995 has as fundamental aim to understand how climate change affects the abundance, diversity and productivity of the marine populations in order to assess the effects of global change on the fisheries of commercial interest. With this aim GLOBEC focuses on the study of the zooplankton. Why the zooplankton? Because these small animals adrift in the ocean are the main link between the primary producers and the fisheries. From this point of view, it is very important to know what changes may produce temperature variations in the growth rates of early developmental stages of key zooplankton species.

We focused on copepods of the family *Acartiidae* because they are common in coastal and estuarine habitats in all oceans of the world. They are thought to be mainly adapted to high food concentrations which are found in estuaries and upwelled waters. For example *Acartia tonsa* cannot obtain sufficient food for reproduction on the middle and outer shelf of Georgia (USA), where food concentrations are usually low, because their clearance rates decrease when food concentrations fall below a certain level (Paffenhöfer and Stearns 1988). Their wide distribution in space and time may owe a lot to the fact that a number of *Acartia* species are known (1) to produce resting eggs which lie dormant in the sediment and allow them to appear suddenly in the plankton when conditions are favorable (e.g. Uye 1983; Lindley 1990; Naess 1991; Belmonte 1992) and (2) to be transported in ships' ballast water to other parts of the world (Hirakawa 1988). The keeping of these copepods alive in the laboratory is

undoubtedly of great interest to obtain better information on their biology. Because they are the principal link in the marine food chain in some areas, there are many studies about growth in copepods of the genus *Acartia* (Paffenhöfer 1970; Durbin and Durbin 1978; Landry 1978; Klein Breteler and Gonzalez 1982; Kimmerer and Mckinnon 1987; Berggreen et al. 1988; Escribano and McLaren 1992; Huntley and Lopez 1992; Tsuda 1994; Saiz et al. 1998; Campbell et al. 2001; Hirst and Kiørboe 2002; Leandro et al. 2006). However, growth in the early developmental stages has rarely been described (Durbin and Durbin 1978; Berggreen et al. 1988; Calbet et al. 1997; Leandro et al. 2006). The most important factors controlling stage duration of copepods are temperature, food quantity and food quality (Cook et al. 2007). In this work we focused on temperature. *Paracartia grani* distribution range covers from 12 to 28 ºC, hence we studied the effect of temperature within this range on growth rates of this species nauplii assessed through direct and indirect methods under food-saturating conditions.

Traditionally, the direct method (Heinle 1966), based on length or weight increases measurement, has been applied to assess copepodites growth rates. Weight-specific growth rates of other species of the genus *Acartia* have been previously calculated with the direct method or the egg production method (Hirst et al. 2003, and references in Table 2). The direct weight-specific growth rates increased linearly with temperature over the range tested in the present study.

Table 2. Summary of weight-specific growth rates of *Acartia spp.*

Temperature ºC	**Growth rate day^{-1}**	**Species**	**Reference**
10	0.19	Acartia tonsa	Leandro et al. 2006 ♣
10	0.13	Acartia tonsa	Leandro et al. 2006 ♦
12	0.28	Acartia (P) grani	**This work** ♣
12	0.17	Acartia clausi	Huntley and Lopez 1992 ♦
12	0.13	Acartia tranteri	Kimmerer and Mckinnon 1987 ♦
14.6	0.00	Acartia (P) grani	Rodriguez et al. 1995 ♠
15	0.37	Acartia tonsa	Leandro et al. 2006 ♣
15	0.27	Acartia tonsa	Leandro et al. 2006 ♦
16	0.41	Acartia tonsa	Berggreen et al. 1988 ♥
16	0.14	Acartia (P) grani	Rodriguez et al. 1995 ♠
16	0.03	Acartia (P) grani	Rodriguez et al. 1995 ♠
16	0.41	Acartia (P) grani	**This work** ♣
16.9	0.20	Acartia (P) grani	Rodriguez et al. 1995 ♠
17.1	0.08	Acartia (P) grani	Rodriguez et al. 1995 ♠
17.7	0.35	Acartia (P) grani	Rodriguez et al. 1995 ♠
17.8	0.03	Acartia (P) grani	Rodriguez et al. 1995 ♠
18	0.46	Acartia (P) grani	Calbet et al. 1997 ♥
18	0.45	Acartia tonsa	Berggreen et al. 1988 ♥
18	0.54	Acartia tonsa	Leandro et al. 2006 ♣
18	0.36	Acartia tonsa	Leandro et al. 2006 ♦
18	0.59	Acartia (P) grani	Rodriguez et al. 1995 ♠
18	0.33	Acartia clausi	Huntley and Lopez 1992 ♦
18	0.25	Acartia tranteri	Kimmerer and Mckinnon 1987 ♠
19.8	0.54	Acartia (P) grani	**This work** ♣
20.3	0.29	Acartia (P) grani	Rodriguez et al. 1995 ♠
22	0.88	Acartia tonsa	Leandro et al. 2006 ♥
22	0.49	Acartia tonsa	Leandro et al. 2006 ♦
23.7	0.68	Acartia (P) grani	**This work** ♣
24	0.70	Acartia (P) grani	**This work** ♣
25	0.20	Acartia (P) grani	Rodriguez et al. 1995 ♠
26.6	0.80	Acartia (P) grani	**This work** ♣
28	0.85	Acartia (P) grani	**This work** ♣

♣Nauplii ♥Nauplii and Copepodites ♦Copepodites ♠Estimated from female egg production rate

The growth rates observed for *P. grani* are similar to those reported for *Acartia tonsa* at the same temperature (Table 2). On the other hand, probably due to their different habitats (Kimmerer and Mckinnon 1987) *Acartia tranteri* presents lower growth rates at similar temperatures. Differences between studies may also be due to the different feeding conditions. In this work we reared *P. grani* nauplii under food saturation with *Oxyrrhis marina* (except at 26 ^{0}C that was fed with *Rhodomonas baltica*), whilst Berggreen et al. (1988, nauplii and copepodites) and Calbet et al. (1997, nauplii) fed the cultures with different concentrations of *R. baltica.* Also*,* Leandro et al. (2006) fed the cultures of nauplii with *R. baltica* but the copepodites with *Rhodomonas sp. a*nd *Thalassiosira weissflogii* at saturating food concentrations; and Kimmerer and Mckinnon (1987) used a mixture of batch-cultured *Tetraselmis chui*, *Phaeodactylum tricornutum*, and *Isochrysis galbana*. Also, we can found differences with study done by Rodriguez et al. 1995, because they used adult cultures and they estimated the growth rate as egg production rate and the females production not always match growth of the earlier stages.

The temperature dependence of growth rates for nauplii of *P. grani* corresponded to Q_{10} values of 2.0 and 2.9. These values are lower than reported by Leandro et al. (2006) for *A. tonsa*. They found a Q10 of 3.66 for nauplii and 3.12 for copepodites within the same temperature range. The differences found between the two species could be due to the experimental conditions.

In recent years, the use of biochemical assays as indices of growth in copepods has increased. These methods allow for the assessment of potential secondary

production on field collected organisms with less laboratory manipulation. Examples of these indices are:

(1) The RNA:DNA ratio analysis. There is often a positive correlation between copepods RNA content and growth rate, but in several studies the relationship has been shown to lack sufficient specificity to reliably predict growth rates (e.g. Dagg and Littlepage 1972; Ota and Landry 1984). RNA content is predominantly a function of body size, as is growth rate in many taxa (Båmstedt and Skjoldal 1980; Banse and Mosher 1980). Also, DNA content per unit biomass generally decreases with increased body size (e.g. Sulkin et al. 1975), and with increased growth rate (as a function of diminished cell volume, e.g. Ota and Landry 1984; Bulow 1987), although relationships are generally weak. However, a positive correlation between the RNA/DNA ratio and somatic or reproductive growth rate has been observed (e.g. *Paracalanus* sp.: Nakata et al. 1994; *Sciaenops ocellatus*: Westerman and Holt 1994; *Acartia grani:* Saiz et al. 1998; *Calanus* finmarchicus: Wagner et al. 1998, 2001; Bersano 2000; *A.* bifilosa: Gorokhova 2003; *A tonsa*: Speekmann et al. 2007). Biomass ratios may be useful as species-specific and age or stage-specific condition factors. Nevertheless, while such ratios may reflect the past character of tissue accumulation, and may even show correlation with current or past growth rate, they are not measures of growth rate itself (Runge and Roff 2000).

(2) Enzyme activities related to synthesis processes like the aspartate transcarbamylase, (ATC), Bergeron 1983, 1986). A number of studies have established that in plant and animal tissues or in whole organisms the specific activity of ATC can be correlated with cellular division and somatic and germinal

growth rates (Kim and Cohen 1965; Stein and Cohen 1965; Brothers et al. 1978; Bergeron and Buestel 1979; Alayse-Danet 1980; Bergeron and Alayse-Danet 1981; Bergeron 1982; Mathieu et al. 1982; Chim 1983). However, ATC activity has not been shown to be correlated with somatic or female copepod productivity (Hernández-León et al. 1995).

Another method used as a potential index of somatic growth is based on the activity of the aminoacyl-tRNA synthetases activity (AARS, Yebra and Hernández-León 2004). These enzymes catalyse the first step of the protein synthesis and their activity presents a positive relationship with somatic growth in metazoan such as cephalopods (Houlihan et al. 1990.) and fishes (Sveier et al. 2000); as well as 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; *Paracartia grani,* this work).

In this work we showed that specific $AARS_{situ}$ activities ($nmPPi \cdot mgprotein^{-1} \cdot h^{-1}$) were affected by temperature ($Q_{10} = 2.9$). When we compared *P. grani* with other calanoid species grown at similar temperatures (Yebra et al. 2005) we found both higher specific $AARS_{situ}$ activities and growth rates (d^{-1}) than for *Calanus helgolandicus*. This could be explained by the fact that our experiments were performed with nauplii not with copepodites and adults as in Yebra et al. (2005). Early stages present faster growth rates than copepodites in order to minimize the risk of mortality by predation (Vilela 1972). In addition, *Calanus spp*. are temperate species which accumulate proteins and lipids on the later stages of their development in order to survive during the winter months.

Contrarily to that, *Acartia spp*. produce resting eggs as overwintering strategy (Uye 1985).

The AARS activity of nauplii (NI-NVI) incubated in the laboratory showed a highly significant correlation (r^2=0.96) with the direct measurement of weight-specific growth rates. In previous studies, the correlations between direct measurement of growth and AARS activities were also significant [*Daphnia magna* r^2=0.69 (Yebra and Hernández-León 2004), *Calanus helgolandicus* r^2=0.55 (Yebra et al. 2005), *Calanus finmachicus* r^2=0.49 (Yebra et al. 2006)], and included both laboratory and field experiments. For more than two decades, zooplankton ecologists have been looking for a valid index of growth in zooplankton. The results presented here add to previous studies which suggest that the AARS activity is a good index for estimating somatic growth of *Paracartia grani* and other key zooplankton species.

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