

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

Efecto de la temperatura y de la concentración de alimento en la relación entre el crecimiento somático y la actividad de la enzima aminoacil ARNt sintetasa en nauplios de *Paracartia grani*



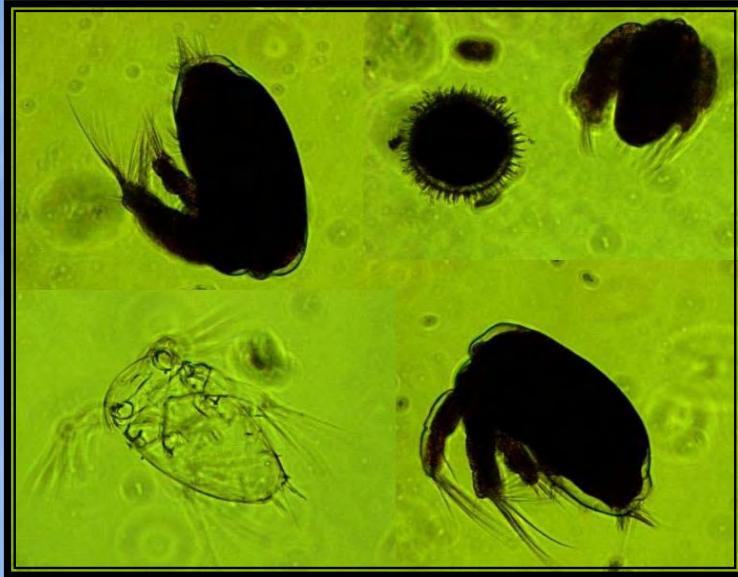
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# *Paracartia grani* (Sars, G.O. 1904)

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Typical of coastal area

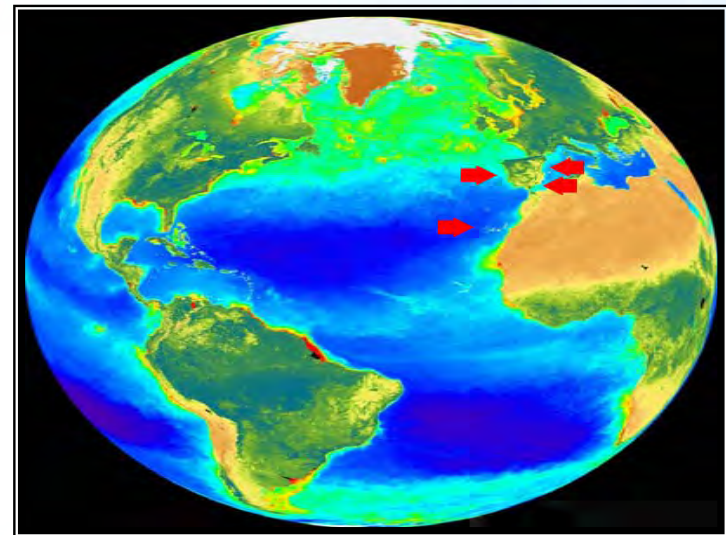
Conditioned:

Physical factors (temperature)

Biological factors (food)

*Acartiidae* are common in coastal and estuarine habitats worldwide.

An important component in the diet of a many of plankton species.



# OBJECTIVE

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The effect of temperature and food quantity on the growth of *Paracartia grani* (Sars 1904).

The relationship between direct growth rates and specific AARS<sub>situ</sub> activities to validate the AARS method as growth index for this species under different temperatures (12-28°C) and food quantity (0-880 µg C·L<sup>-1</sup>).



# METHODOLOGY



ICM  
Institute of  
Marine  
Sciences

*Paracartia grani*

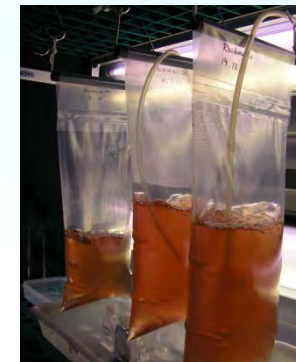


12h day: 12h night  
photoperiod

*Oxyrrhis marina*



*Rhodomonas baltica*

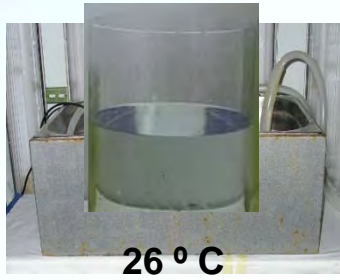
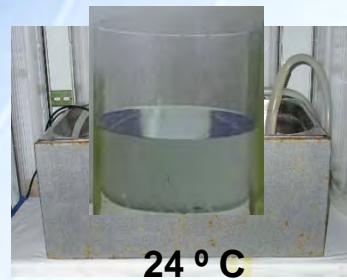
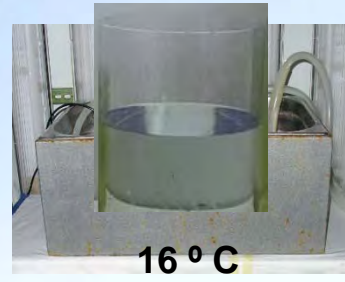


f/2 medium



24 hours → Eggs were collected and preserved in the fridge (4°C)

## Experiments at different temperatures



Eggs addition:  
(22.000-64.548)



16 hours

*Oxyrrhis marina*

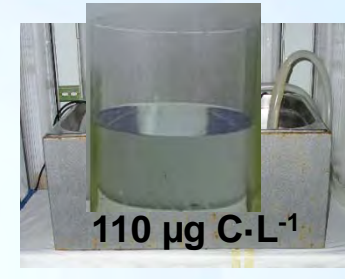
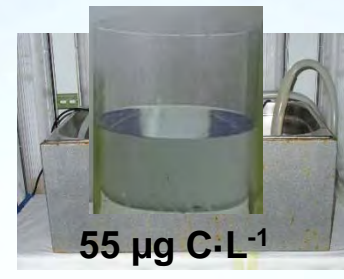
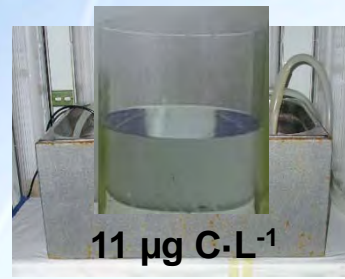
220-286  $\mu\text{gC}\cdot\text{L}^{-1}$

(~2 nauplii  $\text{mL}^{-1}$ )

**METHODOLOGY**

## *Experiments under different food concentrations*

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(~2 nauplii mL<sup>-1</sup>)

**METHODOLOGY**

# Nauplii growth experiments



Multisizer Coulter Counter



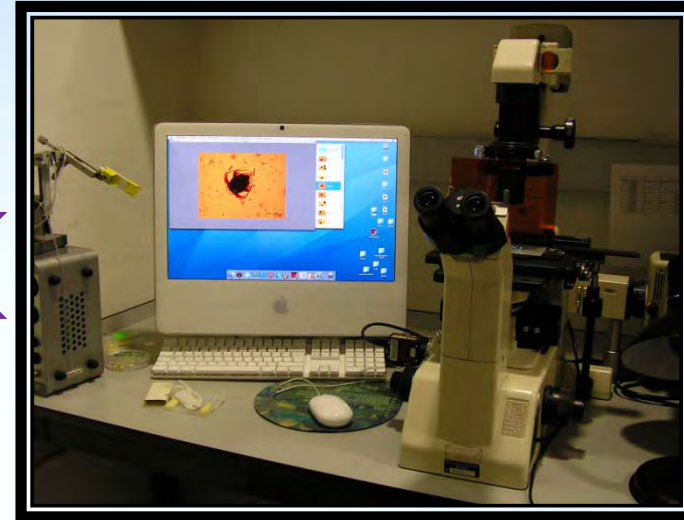
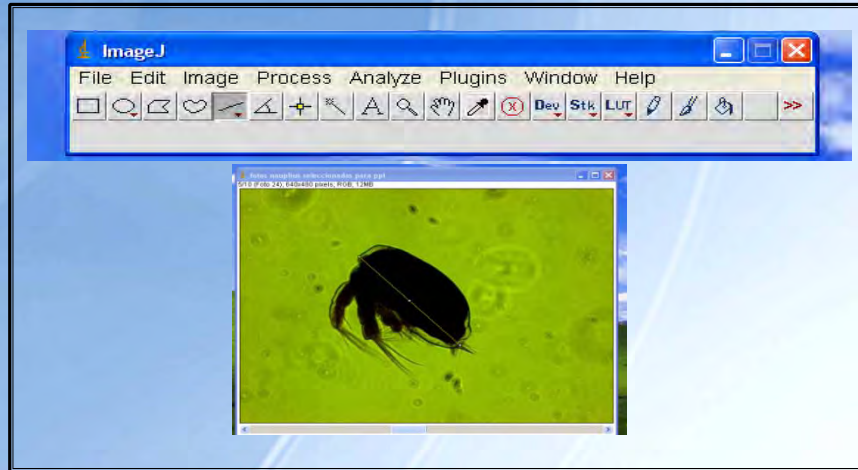
100 ml nauplii culture

Fixed with Lugol's acid (4%)

Measured of length and abundance

**METHODOLOGY**

# Length measurement and weight-specific growth calculations



**Dry weight (dw) / length**  
Durbin and Durbin (1978)  
*A. clausi*

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

W = dry weight ( $\mu\text{g}$ )  
L = prosome length (mm)



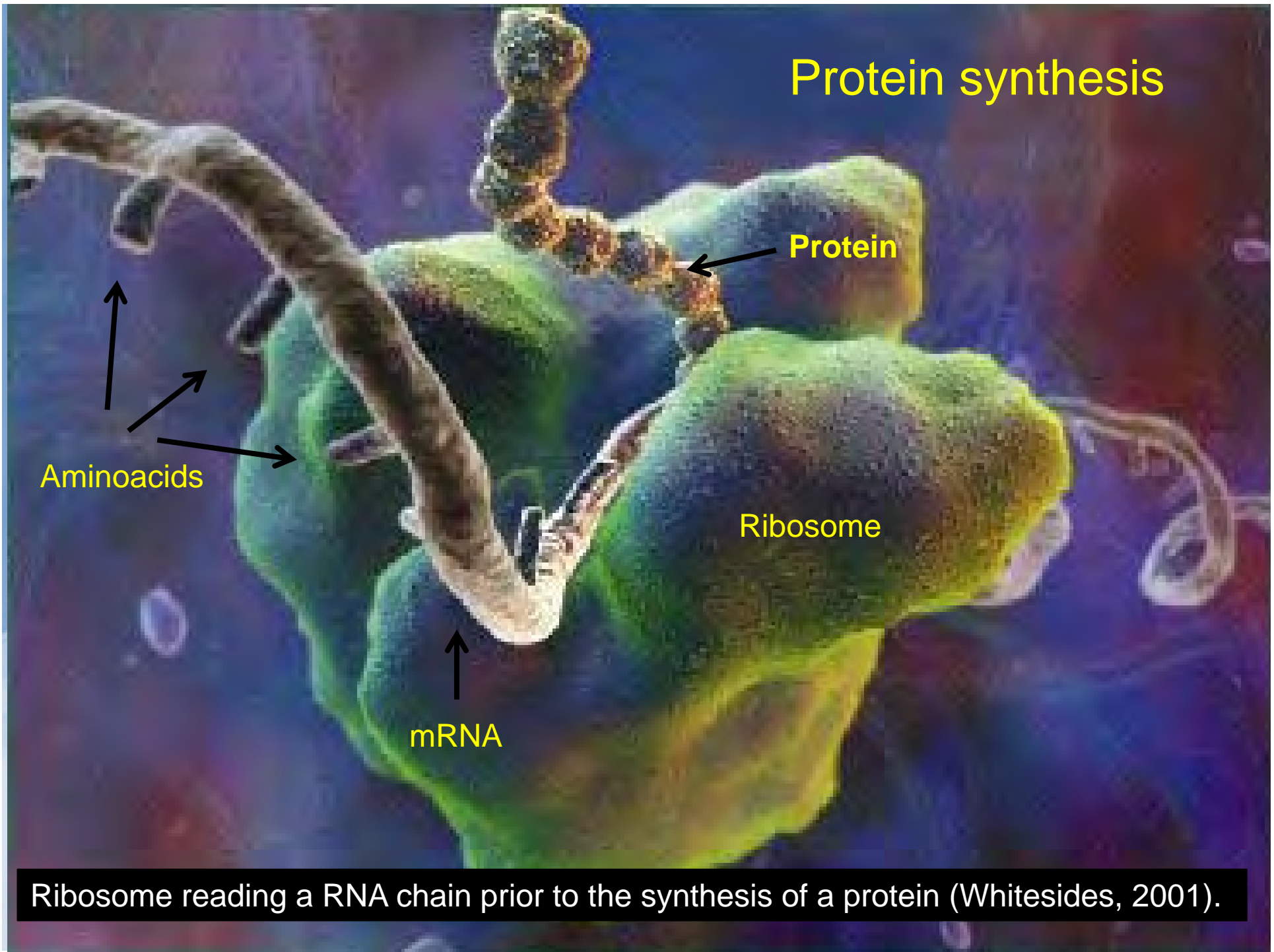
Converted in carbon (C) assuming a carbon/dry weight ratio of 0.40 (Postel et al. 2000).

Temperature quotient ( $Q_{10}$ )

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

$R_1$  and  $R_2$  = Growth rates  
 $T_1$  and  $T_2$  = Temperatures



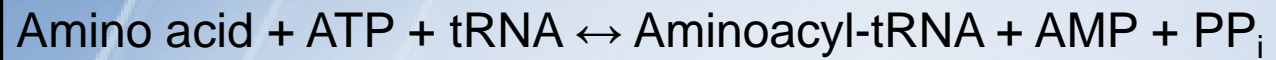


Ribosome reading a RNA chain prior to the synthesis of a protein (Whitesides, 2001).

# Aminoacyl-tRNA synthetases (AARS)

## Biochemical assays

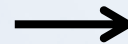
Aminoacyl-tRNA synthetases



Reagent Sigma-P7275

2 NADH

2 NAD<sup>+</sup>



Decrease in  
absorbance at  
340 nm

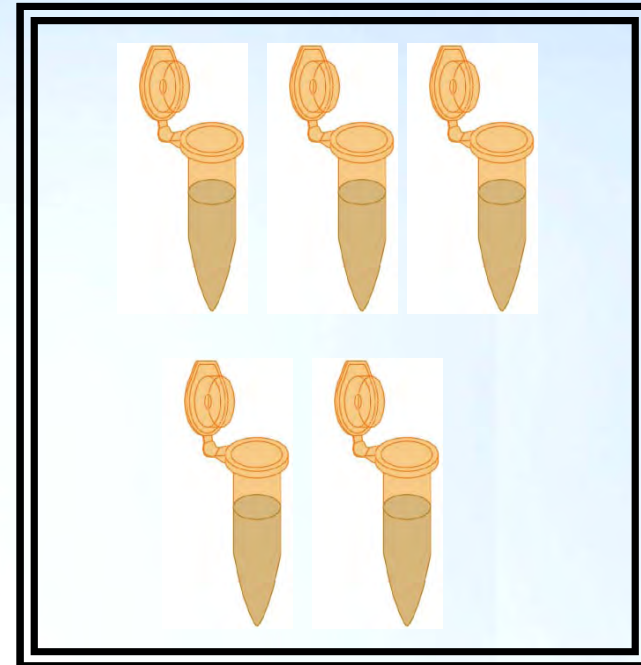
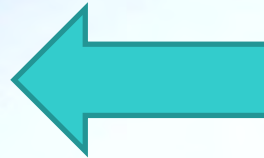
Chang et al., 1984 modified by Yebra & Hernández-León 2004

# Aminoacyl-tRNA synthetases (AARS)

## Biochemical assays



5000 rpm , 10 min, 0 °C

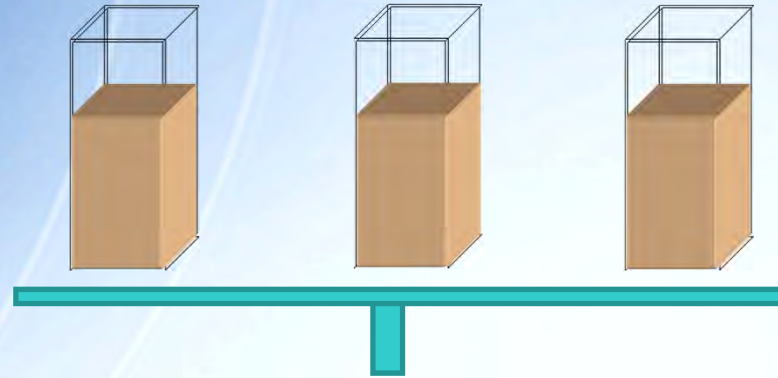


Homogenized



Tris-HCl buffer (20 mM, pH 7.8)

## Biochemical assays

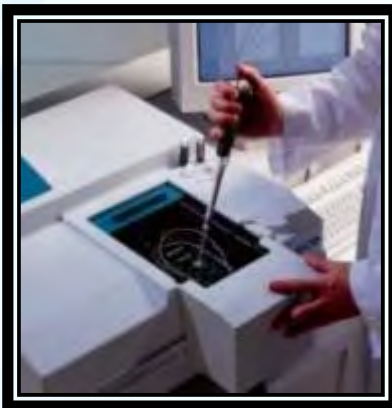


250  $\mu$ l supernatant



200  $\mu$ l Pyrophosphate (PPi reagent)

300  $\mu$ l MilliQ water



340 nm UV  
10 min  
25 °C

## Biochemical assays

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

(Yebra et al. 2004)

$\text{V}_{\text{rm}}$  = Volume of the reaction mixture in ml

6.22 = Millimolar absorptivity of NADH at 340 nm

2 = Number of moles of  $\beta$ -NADH oxidized per mole of PPI consumed

**Temperature correction**

$$\text{AARS}_{\text{situ}} = \text{AARS}_{\text{incubation}} \cdot \exp[E_a \cdot (T_{\text{incubation}} - T_{\text{situ}})^{-1} \cdot R^{-1}]$$

(Yebra et al. 2005)

$$E_a = 8.57 \text{ kcal} \cdot \text{mol}^{-1}$$

**AARS<sub>in situ</sub> activity**

# Biochemical assays

Protein content



Lowry et al. (1951) → Rutter (1967)



5000 rpm ,10 min, 0 °C

200 µl supernatant



1000 µl Rutter

→ Sol A: Na<sub>2</sub>CO<sub>3</sub> 0.90 M  
NaOH 0.45 N  
Tartaro Na-K 2%  
→ Sol B: CuSO<sub>4</sub> · 5H<sub>2</sub>O

After 10 min



100 µl FOLIN → 1:1 Folin : Water

After 40 min



750 nm Spectrophotometer

Bovin Serum Albumin (BSA) → protein standard  
5 and 500 (µgproteins · mL<sup>-1</sup>)

# RESULTS & DISCUSSION

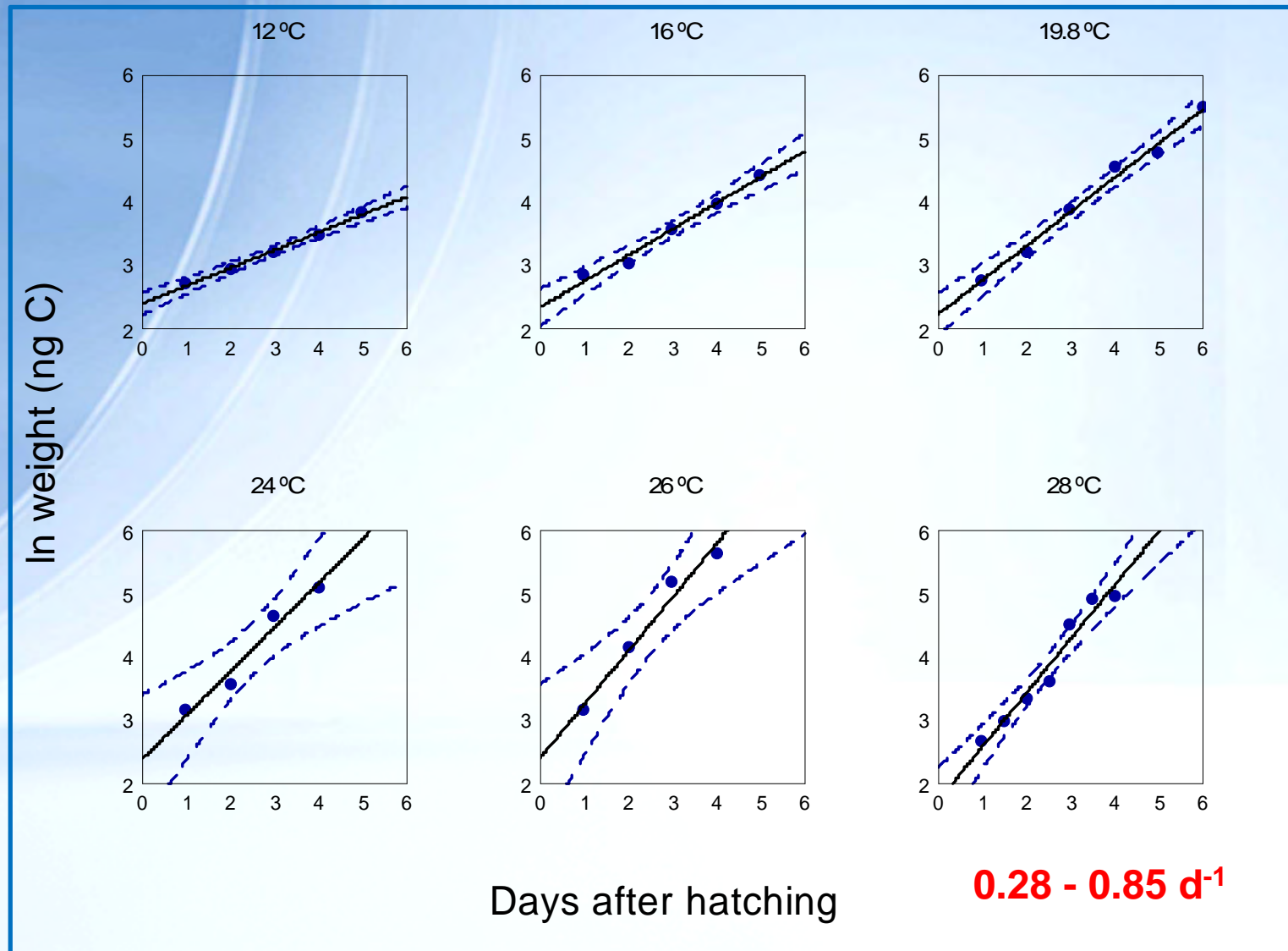


Fig 1. *Paracartia grani* nauplii. Carbon content (ng C) increases at different temperatures.

# RESULTS & DISCUSSION

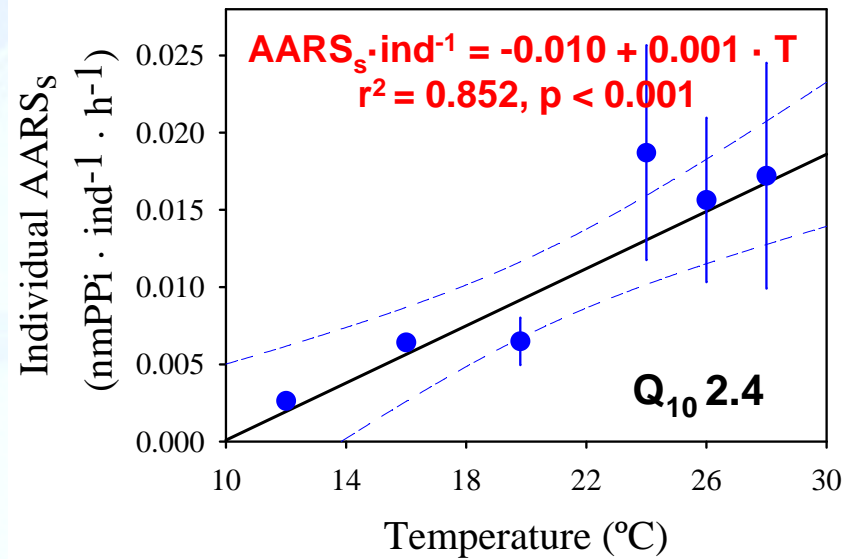
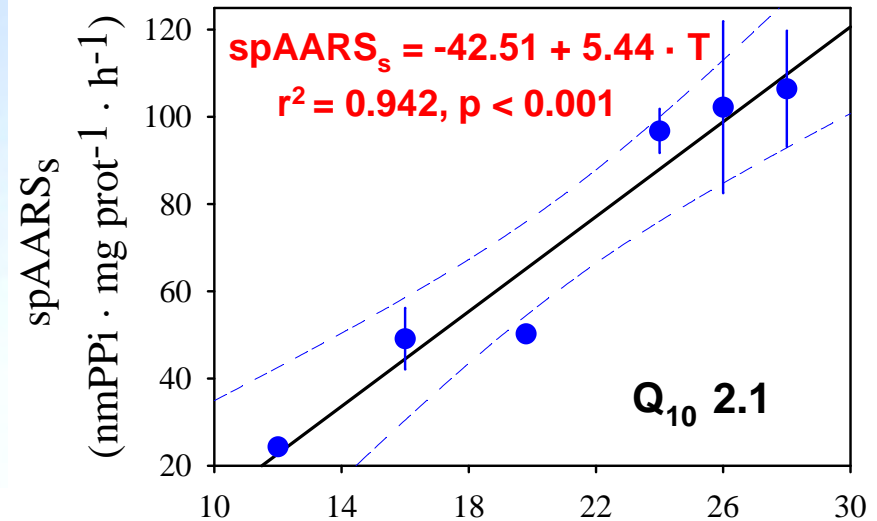
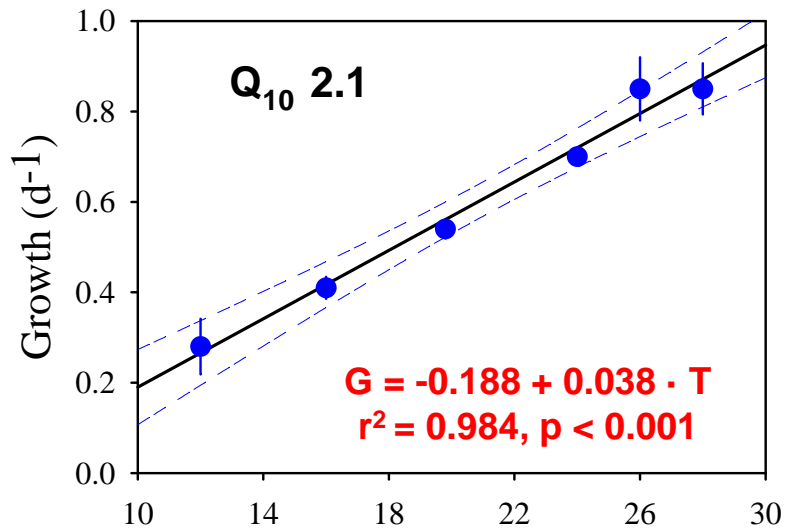
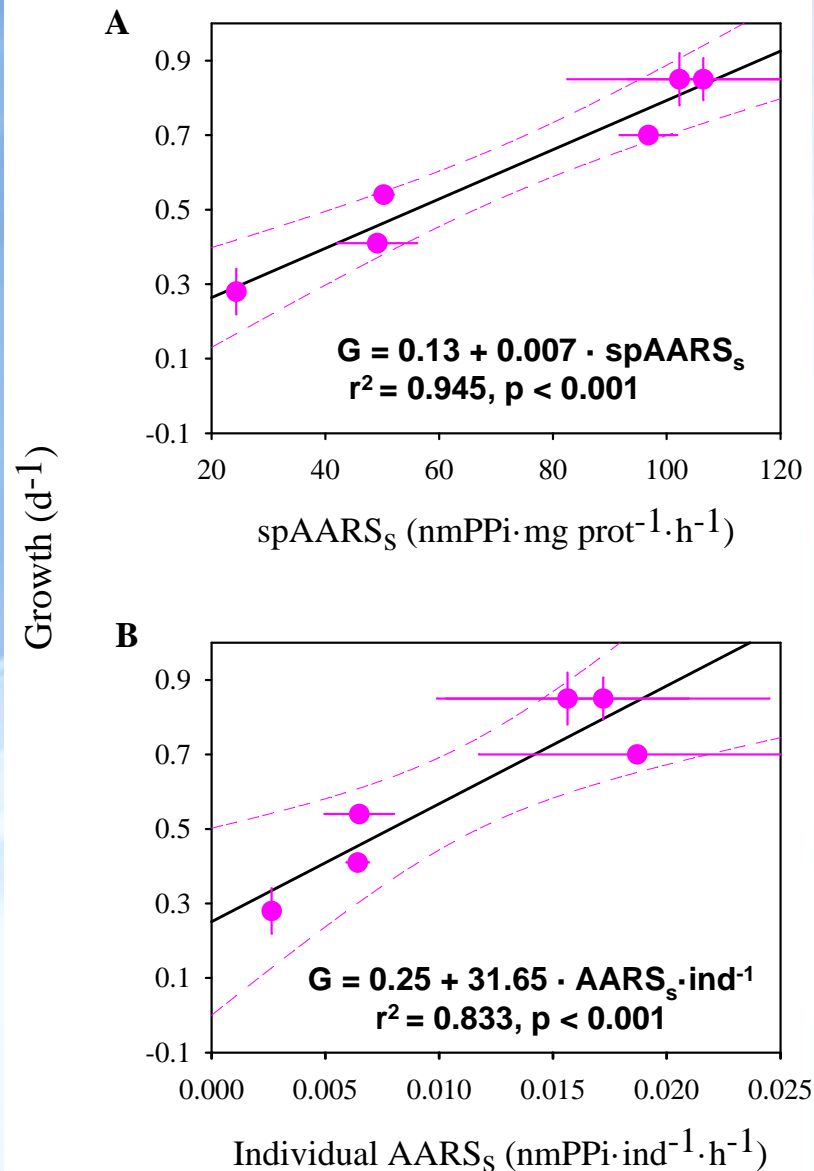


Fig 2. *Paracartia grani* nauplii. Effect of temperature on A) growth (d<sup>-1</sup>), B) spAARS<sub>s</sub> (nmPPi · mg prot<sup>-1</sup> · h<sup>-1</sup>), C) individual AARS<sub>s</sub> (nmPPi · ind<sup>-1</sup> · h<sup>-1</sup>).



# RESULTS & DISCUSSION



Yebra et al., 2005

*Calanus helgolandicus*  
 $R^2=0.55$

Yebra et al., 2006

*Calanus finmarchicus*  
 $R^2 = 0.55$

Fig 5. *Paracartia grani* nauplii. Relationship between growth rates ( $d^{-1}$ ) and A) specific AARS<sub>s</sub> activities ( $nmPPi \cdot mg \text{ prot}^{-1} \cdot h^{-1}$ ), B) individual AARS<sub>s</sub> ( $nmPPi \cdot ind^{-1} \cdot h^{-1}$ ) at different temperatures ( $^{\circ}C$ ).

# RESULTS & DISCUSSION

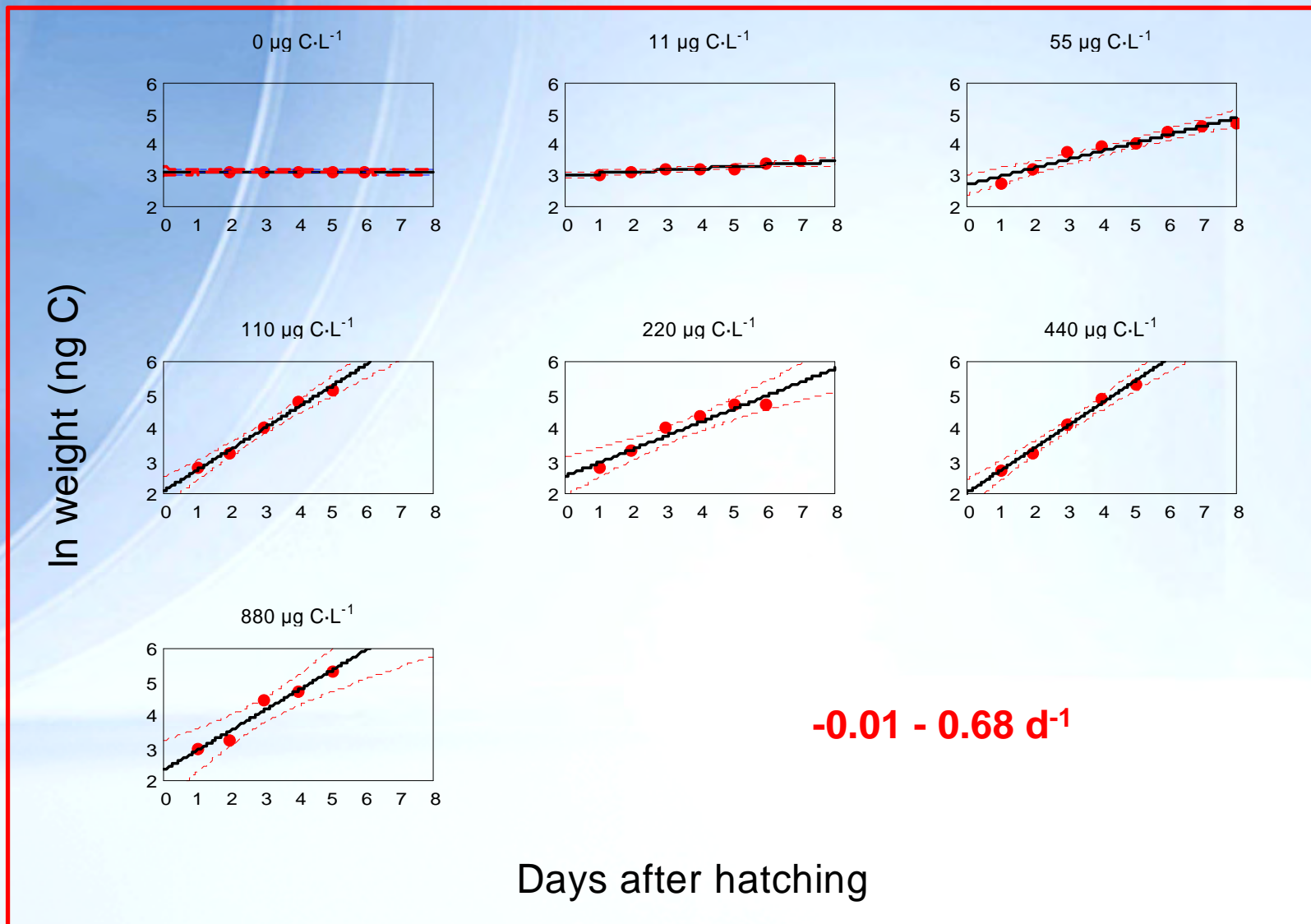


Fig 3. *Paracartia grani* nauplii. Carbon content (ng C) increases under different food concentrations.

# RESULTS & DISCUSSION

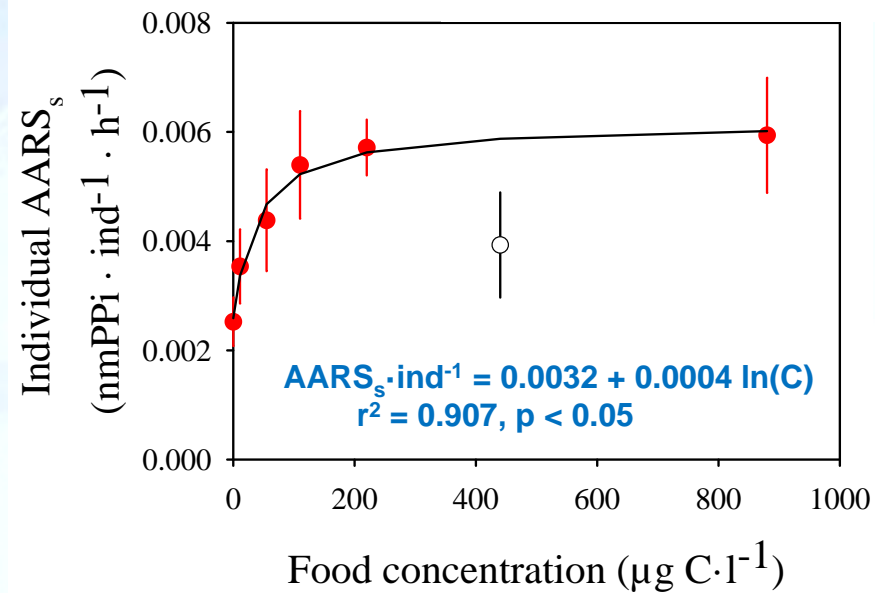
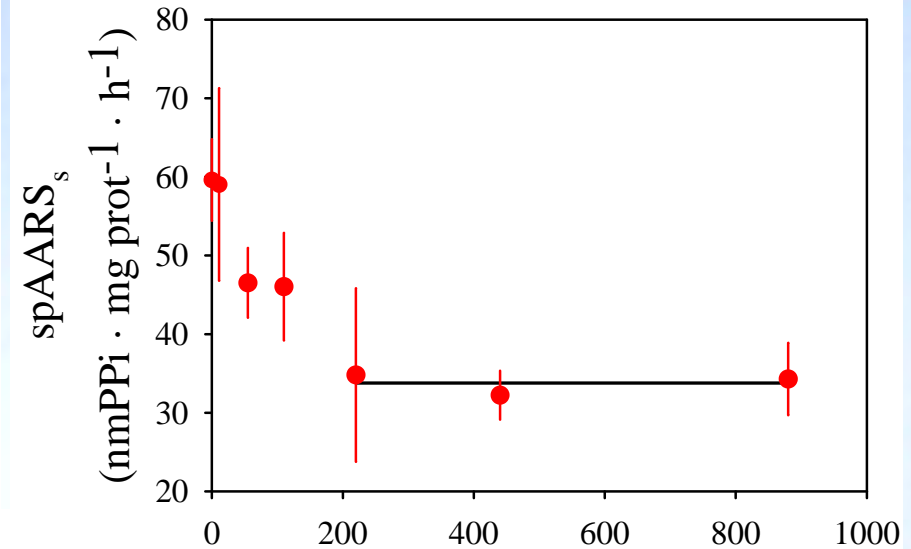
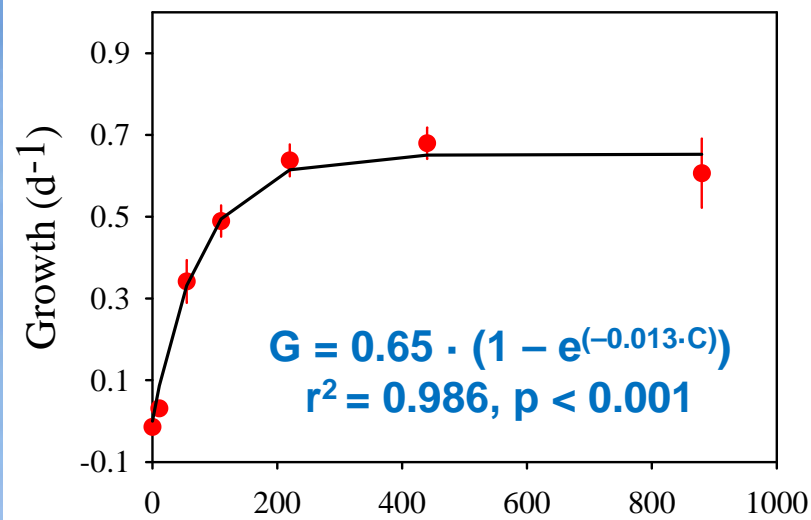


Fig 4. *Paracartia grani* nauplii. Effect of food concentration on A) growth ( $d^{-1}$ ), B) spAARS<sub>s</sub> (nmPPI · mg prot<sup>-1</sup> · h<sup>-1</sup>), C) individual AARS<sub>s</sub> (nmPPI · ind<sup>-1</sup> · h<sup>-1</sup>); open circle: value not included in fit (see text).

# RESULTS & DISCUSSION

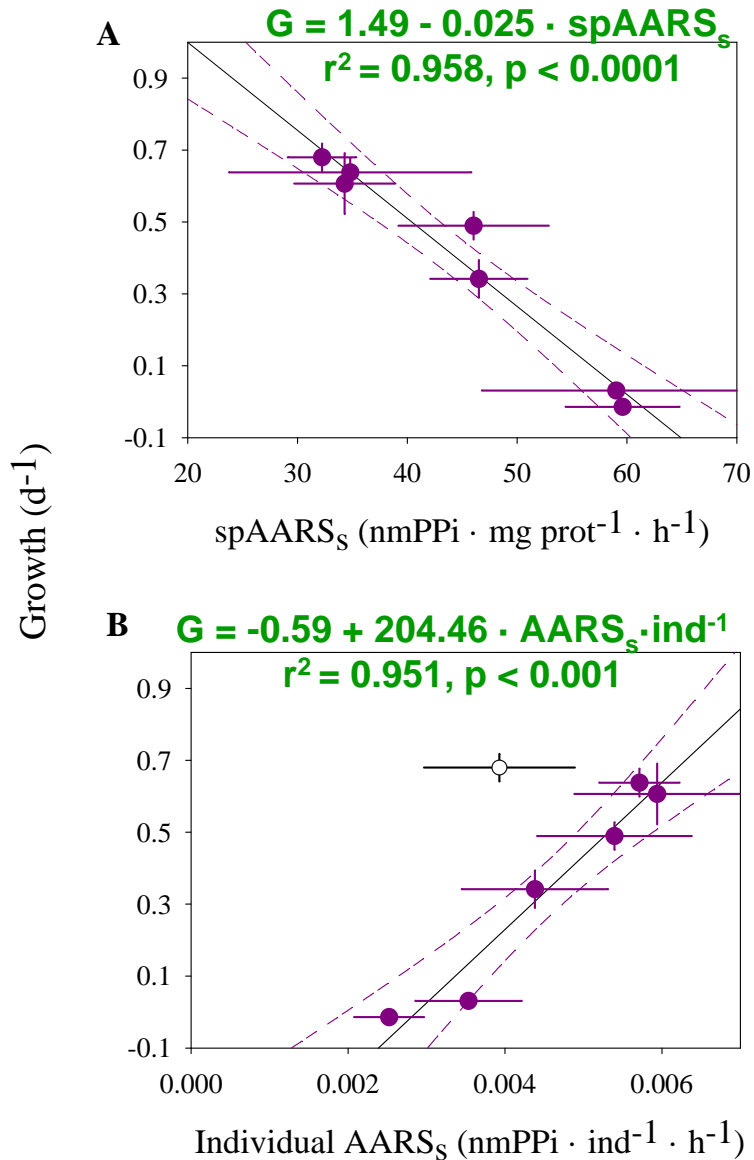


Fig 6. *Paracartia grani* nauplii. Relationship between growth rates ( $\text{d}^{-1}$ ) and A) specific  $\text{AARS}_s$  activities ( $\text{nmPPi} \cdot \text{mg prot}^{-1} \cdot \text{h}^{-1}$ ), B) individual  $\text{AARS}_s$  ( $\text{nmPPi} \cdot \text{ind}^{-1} \cdot \text{h}^{-1}$ ) under different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

## RESULTS & DISCUSSION

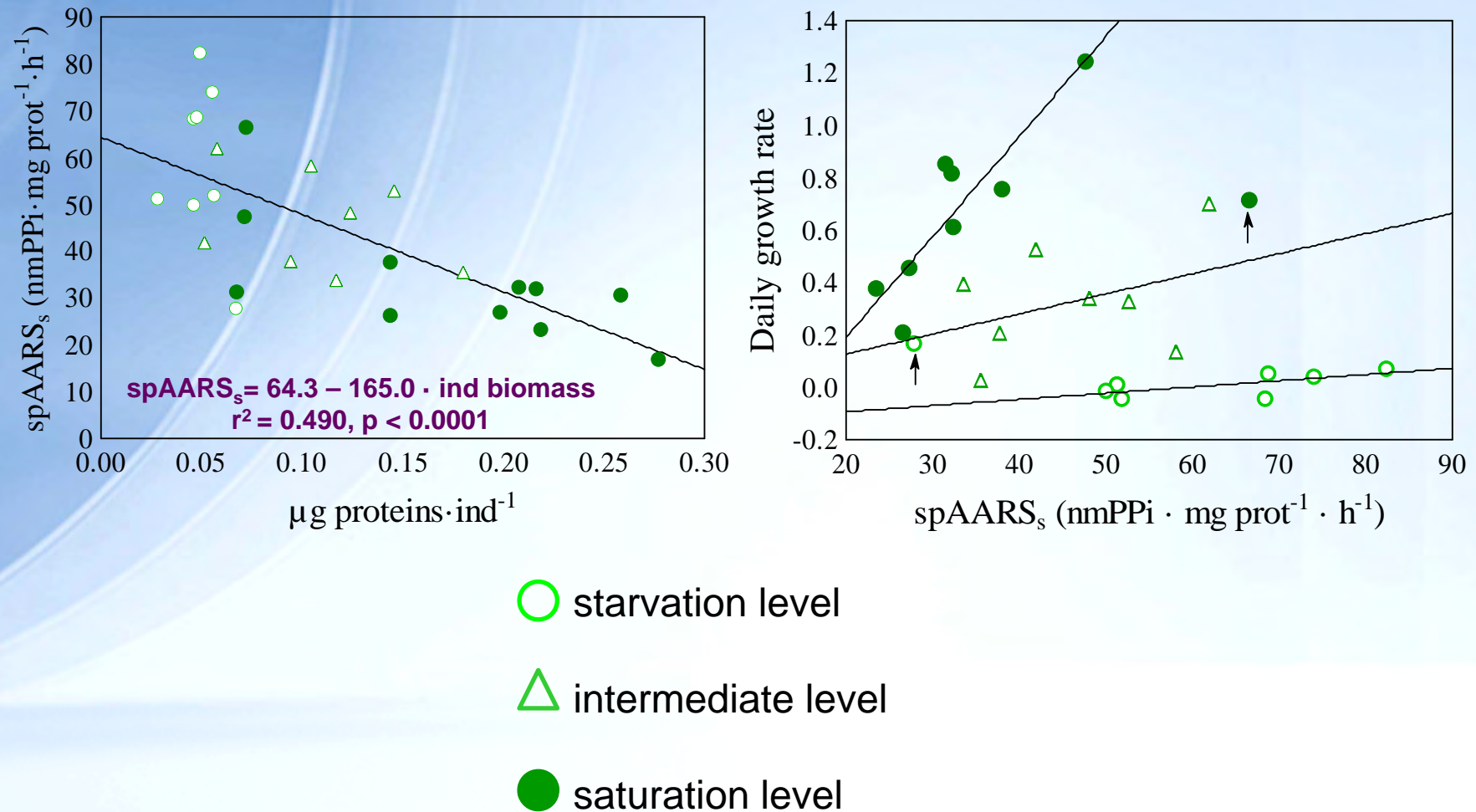


Fig 7. *Paracartia grani* nauplii. A) Relationship between specific AARS<sub>s</sub> activities (nmPPi · mg prot<sup>-1</sup> · h<sup>-1</sup>) and individual biomass (μg proteins · ind<sup>-1</sup>); B) Relationships between daily growth rates (d<sup>-1</sup>) and specific AARS<sub>s</sub> activities (nmPPi · mg prot<sup>-1</sup> · h<sup>-1</sup>) under different food concentrations.

## CONCLUSIONS

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Growth and protein synthesis rates of *Paracartia 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.

The results presented here add to previous studies showing that the AARS<sub>s</sub> activity is a useful tool for estimating somatic growth in copepods.

# ACKNOWLEDGMENTS

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**Thank you for your attention!**





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