

## EFFECTS OF RATION SIZE AND TEMPERATURE ON MOULT INCREMENT AND METABOLIC PARAMETERS OF JUVENILE NOBLE CRAYFISH, *ASTACUS ASTACUS*

B. RENAI (1), F. GHERARDI (1) AND E. D'AGARO (2)

(1) Dipartimento di Biologia Animale e Genetica "Leo Pardi", University of Florence, Via Romana 17, 50125 Florence, Italy. (E-mail correspondence: [barbara.renai@unifi.it](mailto:barbara.renai@unifi.it); E-Mail: [gherardi@DBAG.UNIFI.IT](mailto:gherardi@DBAG.UNIFI.IT))

(2) Dipartimento di Scienze Animali, University of Udine, Via S. Mauro 2, 33010 Pagnacco (UD), Italy (E-Mail: [dagaro@dspa.uniud.it](mailto:dagaro@dspa.uniud.it))

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### ABSTRACT

A laboratory experiment was carried out to test the combined effects of ration size (1 vs 3% body weight (b.w.)) and temperature ( $15 \pm 2$  vs  $22 \pm 2$  °C) on moult increment and metabolic parameters of 80 juvenile noble crayfish (*Astacus astacus*). The maximum daily consumption (Cmax) and respiration rate (R) were used to calculate the growth scope (i.e. the difference between maximum daily energy consumption and energy costs at a given temperature). The conversion of R into a food-equivalent unit allowed the comparison with Cmax. Results showed that crayfish obtained the maximum moult increment when fed 3% b.w while temperature seemed to play a less relevant role on growth rate per moult, affecting only the moulting frequency. Crayfish *A. astacus* fed *ad libitum* showed a relative insensitivity to the metabolic parameters (oxygen uptake, R and Cmax) within the analysed range of temperatures, possibly as a reflection of this species distribution across a broad variety of habitats with different thermal regimes. In the present study, *A. astacus* displayed characteristics proper of a K-selected species, as slow to moderate growth.

**Key-words:** *Astacus astacus*, moult increment, temperature, ration size, maximum daily consumption, oxygen uptake, respiration rate.

### EFFETS DE LA RATION ALIMENTAIRE ET DE LA TEMPÉRATURE SUR L'AUGMENTATION DE LA MUE ET SUR LES PARAMÈTRES MÉTABOLIQUES DE LA JEUNE ÉCREVISSE NOBLE, *ASTACUS ASTACUS*.

### RÉSUMÉ

Une expérience de laboratoire a été conduite pour tester les effets combinés de la ration alimentaire (1 vs 3% du poids du corps (b.w.)) et de la température ( $15 \pm 2$  vs  $22 \pm 2$  °C), sur l'augmentation de la mue et des paramètres métaboliques de 80 juvéniles d'écrevisse noble (*Astacus astacus*). La consommation maximum journalière (Cmax) et le taux de respiration (R) ont été utilisés pour calculer un paramètre qualifiant la croissance (la différence entre la consommation maximum journalière d'énergie et les coûts énergétiques à une température donnée). La conversion de R en unités d'alimentation équivalent a permis la confrontation avec Cmax. Les résultats ont montré que les écrevisses avaient

l'augmentation maximum de mue quand leur ration alimentaire correspondait à 3% de leur poids corporel, tandis que la température semblait jouer un rôle moins élevé sur le taux de croissance de la mue, influençant seulement la fréquence des mues. Les écrevisses alimentées *ad libitum* ont montré une relative insensibilité aux paramètres métaboliques (demande d'oxygène, R et Cmax) dans l'intervalle de température analysée, sans doute en relation avec la distribution de cette espèce, dans une grande variété d'habitats avec différents régimes thermiques. Dans cet étude, *A. astacus* a montré des caractéristiques typiques d'une espèce K-sélectionnée, avec une croissance lente à modérée.

**Mots-clés:** *Astacus astacus*, augmentation de la mue, température, quantité de la ration, consommation maximum journalière, demande d'oxygène, taux de respiration.

## INTRODUCTION

Crayfish are a prominent component of many aquatic ecosystems. Despite of their importance as key species, knowledge of ecological requirements for most crayfish species is fragmentary or lacking (TAYLOR *et al.*, 1996), and only few bioenergetic studies have included crayfish (MOMOT, 1995). Together with fecundity (COREY, 1987; MUCK, 1995), growth rate is obviously one of the principal factors in population dynamics: large size is a prerequisite for crayfish to acquire and to maintain a shelter (RABENI, 1985) and to avoid predation. In particular, a better knowledge of growth rate at diverse food availabilities (ABRAHAMSSON, 1971) and at different temperatures seems essential for the conservation of indigenous crayfish species facing threats from both introduced species (PAGLIANTI *et al.*, 2004) and altered thermal habitats (WHITLEDGE and RABENI, 2003).

The relationship between water temperature and growth rate was previously analysed in some Australian crayfish (MILLS, 1989; MORRISSY, 1990; SMALLRIDGE and GEDDES, 1991; VILLAREAL, 1991; ROUSE and KARTAMULIA, 1992; KING, 1994; AUSTIN, 1995; JONES and ROMAIRE, 1995) and North American species as: *Orconectes rusticus* (cf. MUNDAHL and BENTON, 1990), *O. virilis* (cf. MOMOT, 1984; WETZEL and BROWN, 1993), *O. immunis* (cf. WETZEL and BROWN, 1993) and *Pacifastacus leniusculus* (WESTMAN, 1973). However, only few studies (VERHOEF *et al.*, 1998; PAGLIANTI and GHERARDI, 2004) considered the effects of temperature on the separate components of growth. The life cycle of crayfish progresses in fact through a series of moults interspersed by intermoult during which the animals increase in size. Growth is therefore a function of both length increment per moult and the number of moults completed per unit time. Consequently, in addition to measuring changes in wet weight and cephalothorax length over time (GEDDES *et al.*, 1988; ACKEFORS *et al.*, 1995; JONES *et al.*, 1996), growth rate should be analysed in terms of the frequency of moults and the increase in size after a given moult (moult increment) (HARTNOLL, 1982).

The maximum daily consumption and the respiration rate are measured to obtain the growth scope. This represents the difference between maximum daily energy consumption and energy costs at a given temperature, being the amount of absorbed energy potentially available for growth at that temperature (WARREN and DAVIS, 1967; JOBLING, 1997; ZWEIFEL *et al.*, 1999). A high growth scope, as the growth rate, would represent an important advantage among crayfish being size positively related to fecundity (COREY, 1987; MUCK, 1995), shelter acquisition (RABENI, 1985) and predation, because fishes often prey selectively on smaller crayfish (PROBST *et al.*, 1984; GARVEY *et al.*, 1994). Maximum daily consumption and respiration rate are two components of the Wisconsin fish bioenergetics models (HEWETT and JOHNSON, 1992), which identify the relationship between growth scope of a species and temperature. The Wisconsin fish bioenergetics models have been already used with invertebrates, e.g. mysids (RUDSTAM, 1989) and crayfish (WHITLEDGE and RABENI, 2003).

The work aimed at testing the combined effects of feeding rate and temperature on growth rate and metabolic parameters of *Astacus astacus* juveniles cultured in a battery system with recirculating water. The results of this study can be used to develop growth models for the juveniles of this species in order to summarise overall growth performance and to optimise culture conditions. Furthermore, the identification of the nutritional and environmental conditions, that maximise energy utilisation by crayfish (VILLAREAL, 1991), will benefit production in commercial ponds. In fact the noble crayfish (*Astacus astacus*) is the only indigenous species cultured to a large extent in several European countries and the juveniles are extensively cultured for restocking purposes (HOLDICH, 1993). *Astacus astacus* has been exploited for centuries due to its world-wide recognition as being a culinary delicacy (SPITZY, 1973; BRINCK, 1975; HOLDICH and LOWERY, 1988; WESTMAN *et al.*, 1990). This species naturally occurs in at least 20 European countries ranging from Italy and Greece in the south to the Scandinavian peninsula and Finland in the north, and from France in the west to Russia in the east.

## MATERIAL AND METHODS

### Moult increment and growth regression model

*Astacus astacus* juveniles (0+) were obtained from the First Bavarian Crayfish Hatchery at Augsburg (Germany) and were grown from May to July 2002 under laboratory conditions at a natural L/D photoperiod. Eighty crayfish (initial mean weight:  $0.42 \pm 0.02$  g and mean cephalothorax length:  $12.78 \pm 0.14$  mm) were randomly allotted into 4 rectangular plexiglas aquaria (40x40x20 cm), each containing 25 litres of dechlorinated tap water. Water-recirculation was maintained by a pump at  $60 \text{ l}\cdot\text{min}^{-1}$  provided with a filter allowing catabolite filtering and water oxygenation. Submersed heaters (25W), controlled by a thermostat or coolers (Teco RA 200), were used to maintain water temperature at the constant ( $\pm 1$  °C) desired value. Crayfish (20 juveniles per aquarium) were reared for 70 days at two different temperatures ( $15 \pm 2$  and  $22 \pm 2$  °C) and fed either 1 or 3% of the total body weight (b.w.) with a formulated feed. Proximate analysis was performed following the methods of AOAC (1990) and gross energy by an adiabatic calorimeter. The experimental diet was made of 40.95% DM of crude protein, 7.42% DM of lipid and 1.24% DM of crude fibre.

Following the experimental protocol of PAGLIANTI *et al.* (2004), juveniles were held individually in glass canning jars of  $8 \text{ cm}^2$  in order to prevent cannibalism and fights and to provide a given amount of food to each crayfish. Twenty jars were submerged in a horizontal position in adjacent four aquaria and were closed with a net (mesh size: 2 mm) to allow water circulation. This system let us to follow the individual growth of each specimen under the same rearing conditions (i.e. food quantity, temperature, and water quality). Animals were acclimated to feeding regimes and temperatures for one week prior to testing.

Twice a week the water in each jar and in each aquarium was totally siphoned out and replaced, taking care to remove any uneaten food, shed exoskeleton, and dead animals, and the prescribed quantity of nutrients was weighed and put in every jar. Once a week, crayfish were carefully blotted with paper towel to remove excess moisture and then weighed (to the nearest 0.001 g) and measured for their cephalothorax length (Cl) (accurate to 0.1 mm). Moulting was determined by the presence of shed exoskeletons or part thereof within the culture jars.

Moult increment (Mi), in terms of wet weight and Cl, was defined as:

$$(1) \text{ Mi}_w (\%) = N_m^{-1} \cdot 100 \sum_{i=1}^{N_m} (W_t - W_0)_i \cdot (W_t^{-1})_i$$

where  $W_t$  and  $W_0$  were respectively the final and initial wet weights (mg) for each moult and  $N_m$  was the number of moults for each juvenile; and

$$(2) Mi_w (\%) = N_m^{-1} \cdot 100 \sum_{i=1}^{N_m} (Cl_t - Cl_0)_i \cdot (Cl_t^{-1})_i$$

where  $Cl_t$  and  $Cl_0$  were respectively the final and initial Cl (mm) for each moult and  $N_m$  was the number of moults for each juvenile.

Besides, we represented the  $\text{Log}_{10}$  of weight against the time as recommended by HARTNOLL (1982) and HOPKINS (1992).

### Standard aerobic metabolism

At the end of the growth trial, ten juveniles were randomly selected from the group of animals fed 3% b.w. This feeding rate can be considered as the *ad libitum* level, since part of the ration remained always uneaten into the experimental jars.

Oxygen uptake rates were determined at the two temperatures ( $15 \pm 2$  vs  $22 \pm 2$  °C using 5 crayfish per treatment), under a natural L/D photoperiod, using the respirometer method with a dual-chamber open system (LAMPERT, 1986). The instrument was composed of a respirometric chamber with one animal and a chamber without crayfish used as a control. The scheme of flow-through respirometer used in this trial was the same described in PAGLIANTI *et al.* (2004). Every 15 minutes, the water coming from the chambers can be directed to two alternate electrodes (LAMPERT, 1986).

Before the experiment started, each crayfish was starved for one day (CECH, 1990) and was housed in a cylinder of net that was inserted into the respirometer chamber to avoid any movement. This method allowed us to obtain the rate of oxygen uptake and therefore provided an estimation of energy expenditure for maintenance standard metabolism, i.e. the metabolism at minimum activity level (FRY, 1957). Juveniles were acclimatised to the experimental condition for 45 minutes. Each experimental session lasted 90 minutes.

Oxygen uptake (Ou) (LAMPERT, 1986) and Respiration rate (R) at each temperature were calculated as follows:

$$(1) Ou (\text{mg O}_2 \cdot \text{h}^{-1}) = (D \cdot S \cdot F)$$

where D was the dissolved oxygen uptake estimated as the difference between the average value measured in the control chamber, in each 15 minutes interval, and the average value revealed, by the same probe, in the experimental chamber during previous and successive intervals; S was the solubility of oxygen in water in equilibrium with air at 760 mm Hg pressure and 100% relative humidity ( $10.07 \text{ mg O}_2 \cdot \text{l}^{-1}$  at  $15$  °C and  $8.74 \text{ mg O}_2 \cdot \text{l}^{-1}$  at  $22$  °C) and F was the water flow ( $810 \text{ ml} \cdot \text{h}^{-1}$ ) in both experimental and control chambers. Oxygen uptake rate was calculated comparing measurements obtained with the same electrode in different times (with or without juveniles), rather than with different probes at the same time, to avoid the inclusion in the analysis of differences simply due to the probes (PAGLIANTI *et al.*, 2004);

$$(2) R (\text{g O}_2 \cdot \text{g}^{-1} \text{ d}^{-1}) = (Ou \cdot W_a^{-1}) d$$

where Ou was the Oxygen uptake rate,  $W_a$  was the *A. astacus* weight and d indicated 24 hours.

Respiration rate was converted to Joule-equivalent unit using a multiplier of  $13.724 \text{ J} \cdot \text{g O}_2^{-1}$  (ADAMS and BRECK, 1990) and then into feed-equivalent units ( $\text{g feed} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$ ) by dividing by the energy density of nutrients ( $19181.58 \text{ J} \cdot \text{g}^{-1}$ ). This conversion into feed-equivalent units ( $\text{g feed} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$ ) allowed us to compare directly between R rate data and maximum daily consumption (Cmax) data.

### Maximum daily consumption

At the end of the growth experiment, we measured the  $C_{max}$  rates of 10 juveniles fed 3% b.w. and reared at  $15 \pm 2$  and  $22 \pm 2$  °C (five per treatment). The weight of feed consumed was estimated in terms of dry weight at 60 °C (24 hours) as:

$$C_{max} (g \cdot g^{-1} \cdot d^{-1}) = (W_s - W_r) \cdot W_a^{-1}$$

where  $W_s$ ,  $W_r$  and  $W_a$  were respectively the dry weights of supplied (24 h earlier) and recovered feed and body weight of crayfish.

Weight of supplied feed ( $W_s$ ) value was calculated considering feed losses (for leaching and disintegration) due to the flowing water into the jars. In order to determine the quantity of nutrient loss, 20 jars filled only with a fixed quantity of feed, ranging from 0.009 g to 0.036 g, were inserted into the aquaria (10 per aquarium) at the same time.

Weight of supplied feed was computed from the linear equation  $y = ax + c$  representing the nutrient dissipation into the water, where  $x$  was the dry weight of supplied feed 24 h earlier and  $y$  was  $W_s$  considering feed losses.

Nitrite, nitrate, ammonium, and phosphate concentrations were measured using Aquamerck® (Darmstadt, Germany) reagent kits.

### STATISTICAL ANALYSES

Moult increment were analysed using two-way ANOVA, while one-way ANOVA was used to test the significance of differences for each treatment. We analysed frequency data by G-test.  $\log_{10}$  transformed weight and CI data were combined to produce a CI-weight, a weight- $C_{max}$  and a weight-R relationship and a growth model of weight vs time and CI vs time for each temperature and nutrient ration, using regression analysis (Pearson correlation coefficient). Regression coefficients (slopes) were compared using analysis of covariance (ANCOVA) with pair wise comparisons of slopes after Bonferroni corrections to reduce Type I errors (ZAR, 1990). P values of less than 0.05 were considered statistically significant.

### RESULTS

#### Survival and growth regression model

Juvenile survival, ranging from 65 to 95%, was not affected by the ration size (G-test=1.856, df=1,  $P > 0.1$ ) and by the temperature (G=0.015, df=1,  $P > 0.1$ ).

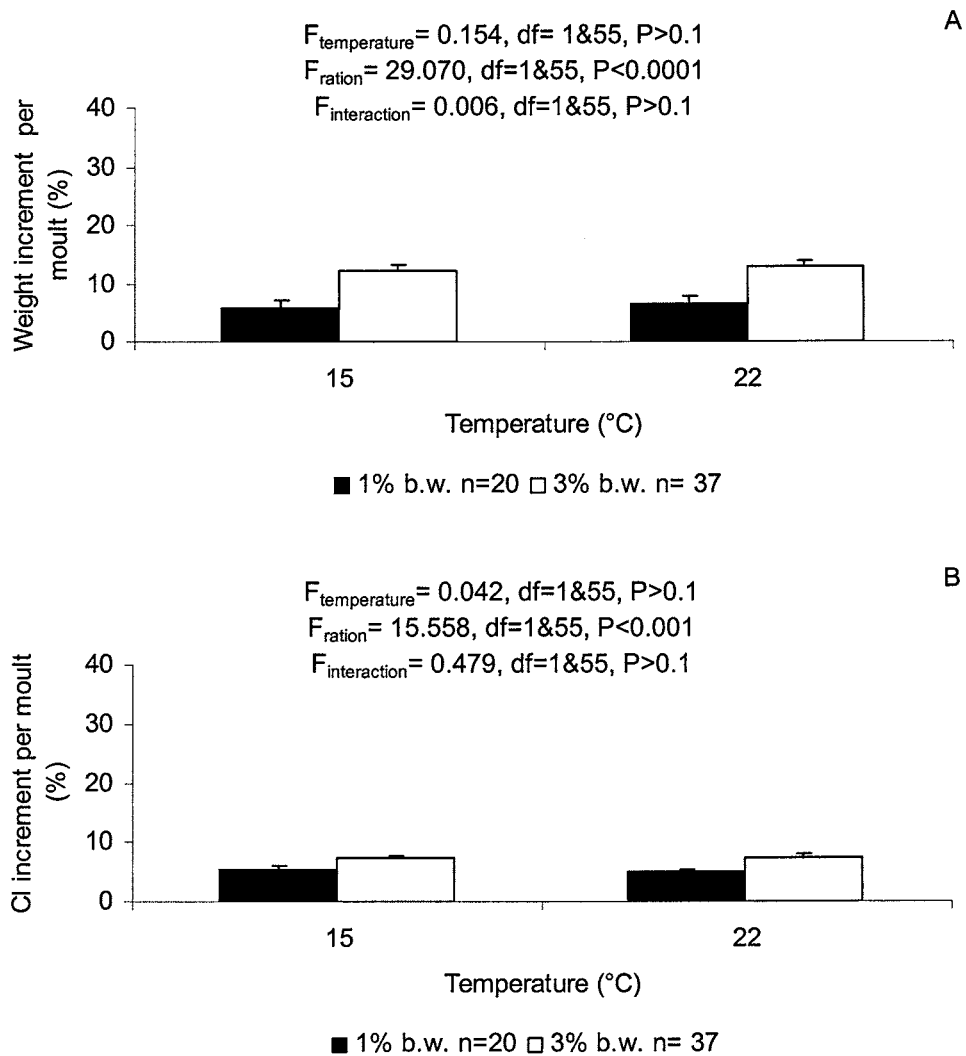
Individuals fed 3% b.w. at 22° C showed a significantly higher increase of body weight ( $F=56.472$ ,  $df=1&63$ ,  $P < 0.001$ ) (final weights: 1% b.w. & 15°C,  $0.588 \pm 0.02$  g; 3% b.w. & 15°C,  $0.756 \pm 0.03$  g; 1% b.w. & 22°C,  $0.630 \pm 0.02$  g; 3% b.w. & 22°C,  $0.840 \pm 0.05$  g).

The linear regression model of  $\log_{10}$  weight (mg) and  $\log_{10}$  CI (mm) vs time showed a significant correlation only in the juveniles fed 3% b.w. ( $\log_{10}$  weight at 15 °C,  $r=0.208$ ,  $df=208$ ,  $P < 0.01$ ;  $\log_{10}$  weight at 22 °C:  $r=0.317$ ,  $df=208$ ,  $P < 0.001$ ;  $\log_{10}$  CI at 15 °C:  $r=0.235$ ,  $df=208$ ,  $P < 0.001$ ;  $\log_{10}$  CI at 22 °C:  $r=0.356$ ,  $df=208$ ,  $P < 0.001$ ).

The ANCOVA analysis did not show differences between regression coefficients (slopes) calculated on juveniles fed 3% b.w. (weight:  $t=0.360$ ,  $df=414$ ,  $P > 0.05$ ; CI:  $t=1.740$ ,  $df=414$ ,  $P > 0.05$ ), suggesting that the temperature does not influence the overall growth rate.

### Moult increment and length-weight relationship

A raise in the temperature did not exert any influence on the growth rate per moult (weight:  $F=0.154$ ,  $df=1\&55$ ,  $P>0.1$ ; CI:  $F=0.042$ ,  $df=1\&55$ ,  $P>0.1$ ). The maximum moult increment of both body weight (%) and CI (%) was obtained when juveniles were fed 3% b.w. (weight:  $F=29.070$ ,  $df=1\&55$ ,  $P<0.0001$ ; CI:  $F=15.558$ ,  $df=1\&55$ ,  $P<0.001$ ). No significant interaction was found between temperature and ration size (weight:  $F=0.006$ ,  $df=1\&55$ ,  $P>0.1$ ; CI:  $F=0.479$ ,  $df=1\&55$ ,  $P>0.1$ ) (Figure 1A,B).



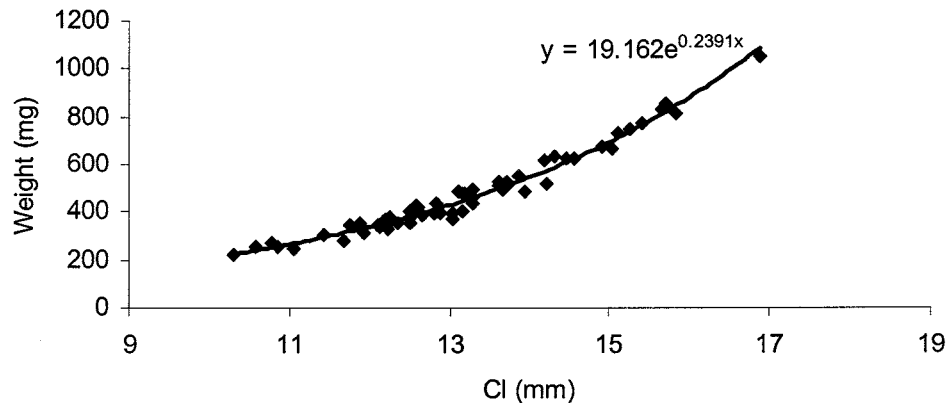
**Figure 1**

Weight (A) and cephalothorax length (CI) (B) increment (in%) per moult (mean and SE) of *A. astacus* juveniles.

**Figure 1**

Augmentation du poids (A) et de la longueur du cephalothorax (CI) (B) (en%) par mue (valeur moyenne et SE) des *A. Astacus* juvéniles.

$\text{Log}_{10}$  mean weight (mg) and  $\text{Log}_{10}$  mean CI (mm) were used to calculate a CI-weight relationship for each treatment (1%: at 15 °C,  $r=0.972$ ,  $df=12$ ,  $P<0.01$ ; at 22 °C,  $r=0.993$ ,  $df=11$ ,  $P<0.01$ ; 3%: at 15 °C,  $r=0.983$ ,  $df=17$ ,  $P<0.01$ ; at 22 °C,  $r=0.972$ ,  $df=17$ ,  $P<0.01$ ). The CI-weight relationship fitted exponential growth function (Figure 2). ANCOVA did not show significant differences between regression coefficients calculated for each treatment (i.e. slopes) (1%:  $t=0.150$ ,  $df=23$ ,  $P>0.05$ ; 3%:  $t=0.499$ ,  $df=34$ ,  $P>0.05$ ; 15 °C:  $t=0.514$ ,  $df=29$ ,  $P>0.05$ ; 22 °C:  $t=1.274$ ,  $df=28$ ,  $P>0.05$ ).



**Figure 2**

Relationship between cephalothorax length (CI) and weight of *A. astacus* juveniles surviving until the end of the growth experiment. Data of all rearing conditions were pooled because the relationship did not vary among temperature-ration size combinations.

**Figure 2**

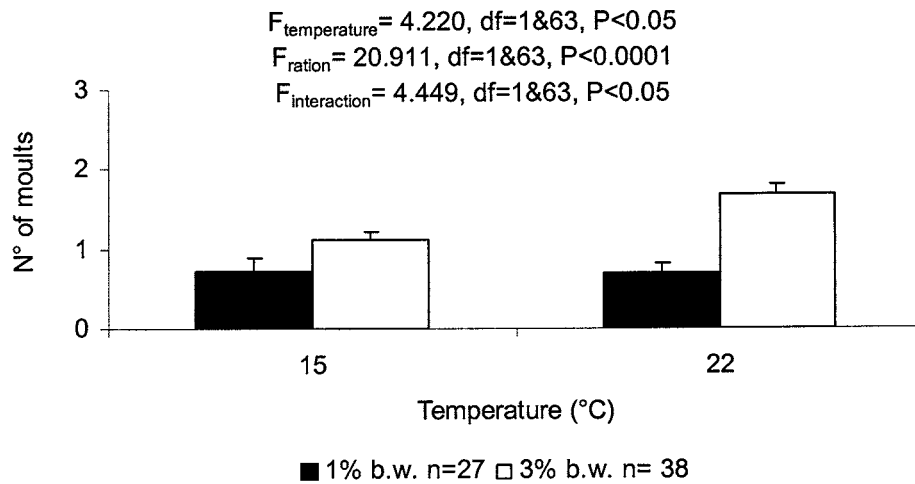
Relation entre la longueur du cephalothorax (CI) et le poids des *A. Astacus* juvéniles survivant jusqu'à la fin des expérimentations de croissance. Les données relatives aux conditions d'élevage sont agglomérées parce que la relation ne variait pas en fonction des combinaisons température-taille de ration.

### Number of moults

The number of moults per individual, ranging from 1 to 3, was greater in juveniles reared at 22 °C than at 15 °C ( $F=4.220$ ,  $df=1\&63$ ,  $P<0.05$ ) and fed 3% b.w. than 1% b.w. ( $F=20.911$ ,  $df=1\&63$ ,  $P<0.0001$ ). Moreover, we recorded a significant interaction between temperature and feeding rate ( $F=4.449$ ,  $df=1\&63$ ,  $P<0.05$ ) (Figure 3). A higher number of moults was found at 22 °C and 3% b.w. ( $t=3.404$ ,  $df=36$ ,  $0.001<P<0.01$ ). No difference was found in crayfish fed 1% b.w. reared at different temperatures ( $t=0.092$ ,  $df=25$ ,  $P>0.1$ ). The duration of the intermoult period was not analysed due to the small number of moults (see Figure 3).

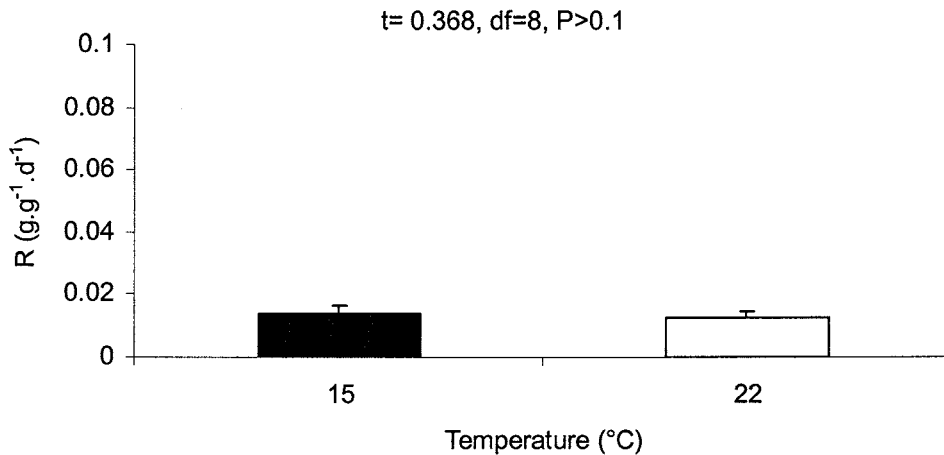
### Respiration and maximum daily consumption

Aerobic metabolism of juveniles fed 3% b.w. was not influenced by different temperatures ( $t=0.368$ ,  $df=8$ ,  $P>0.1$ ) (Figure 4). R ranged from  $0.006 \text{ g O}_2\text{-g}^{-1}\text{-d}^{-1}$  to  $0.023 \text{ g O}_2\text{-g}^{-1}\text{-d}^{-1}$  with a mean value of  $0.013 \text{ g O}_2\text{-g}^{-1}\text{-d}^{-1}$ . Body weights were negatively correlated to respiration rates in juveniles reared at 22 °C ( $\text{Log}_{10}$  weight vs  $\text{Log}_{10}$  R:  $r=-0.978$ ,  $df=8$ ,  $P<0.01$ ).



**Figure 3**  
 Number of moults (mean and SE) during the growth experiment for *A. astacus* juveniles.

**Figure 3**  
 Nombre de mues (valeur moyenne et SE) durant l'expérimentation de croissance pour les *A. astacus* juvéniles.

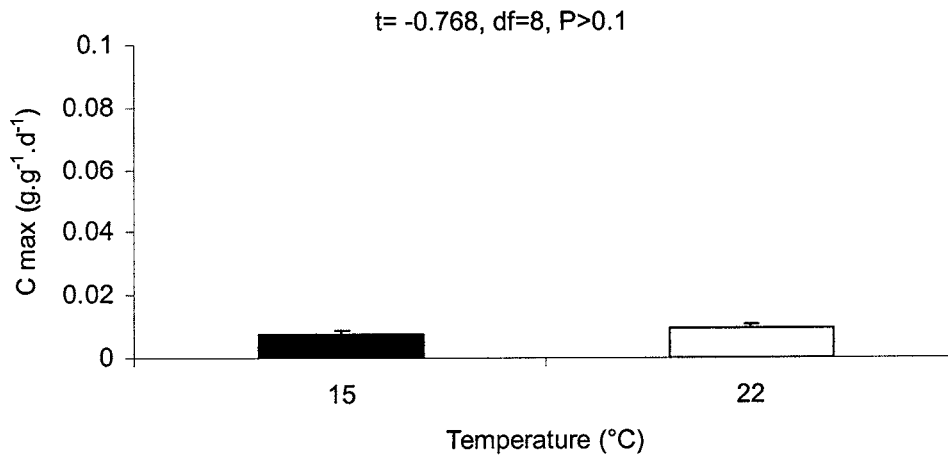


**Figure 4**  
 Respiration rates (mean and SE) of *A. astacus* juveniles fed 3% b.w..

**Figure 4**  
 Taux de respiration (moyenne et SE) des *A. Astacus* juvéniles alimentés avec 3% b.w..

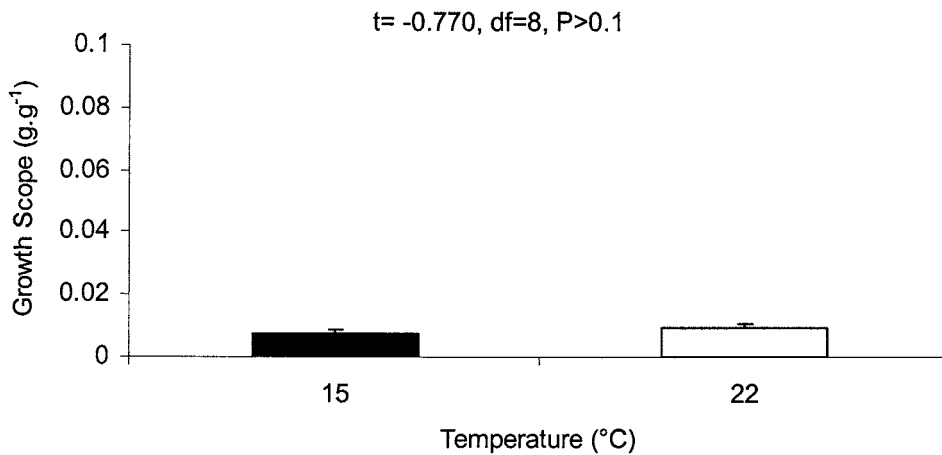
Maximum daily Consumption ( $C_{\text{max}}$ ) ranging from  $0.0024 \text{ g}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$  to  $0.0133 \text{ g}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$  with a mean value of  $0.0085 \text{ g}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ , did not change with temperature ( $t=-0.768, df=8, P>0.1$ ) (Figure 5). No correlation was found between weight and  $C_{\text{max}}$  at the two temperatures ( $\text{Log}_{10} \text{ weight vs } \text{Log}_{10} C_{\text{max}}$ , 15 °C:  $r=-0.588, df=8, P>0.1$ ; 22 °C:  $r=0.548, df=8, P>0.1$ ). Growth scope ( $C_{\text{max}} - R$ ) was not influenced by temperature ( $t=-0.770, df=8, P>0.1$ ) (Figure 6).





**Figure 5**  
Maximum daily consumption (mean and SE) of *A. astacus* juveniles fed 3% b.w..

**Figure 5**  
Consommation maximum journalière (moyenne et SE) des *A. Astacus* juvéniles alimentés avec 3% b.w..



**Figure 6**  
Growth scope (maximum daily consumption – respiration rate) (mean and SE) of *A. astacus* juveniles fed 3% b.w..

**Figure 6**  
Extension de la croissance (consommation maximum journalière – taux de respiration) (moyenne et SE) des *A. Astacus* juvéniles alimentés avec 3% b.w..

At the end of the experiment, nitrite, nitrate, ammonium, and phosphate were practically absent in the water, silicate concentration and pH ranged 0.3-0.7 mg.l<sup>-1</sup> and 7-8, respectively, without any difference among aquaria. Calcium concentration for every aquaria was: 1) 46, 2) 44-46, 3) 46-50 and 4) 50-54 mg.l<sup>-1</sup>.

## DISCUSSION

The life cycle of crayfish progresses through a series of moults interspersed by intermoult, during which the animals increase in size. Growth is therefore a function of both length increment per moult and the number of moults completed per unit time. Size increase during moult period results from many factors and, combined with changes in the frequency of moulting, makes the analysis of crayfish growth particularly complex.

Temperature, along with other abiotic factors (LOWERY, 1988), is known to have a large effect on the survival and growth of crayfish in natural environments. In *Astacus astacus* juveniles we found that temperature had an influence on moulting frequencies only, as already shown in *Austropotamobius pallipes* (LOWERY, 1988). According to NYSTRÖM (2002), the optimal thermal range for noble crayfish is relatively wide, from 16 to 24 °C that allows this species to adapt to a broad variety of habitats, from cold rivers to warmer lakes. This may explain the limited thermal dependence in the range 15 - 22 °C of juvenile *A. astacus* survival and growth, as revealed by our study.

Oxygen uptake is strictly related to temperature. Losses due to metabolic functions are important components of the energy budget in all the organisms and are usually measured by oxygen consumption. PROSSER (1991) showed that both the activity and metabolism had a logarithmic increase with temperature, following the principle of Arrhenius. A similar trend was found by PAGLIANTI *et al.* (2004) in *A. italicus*: juveniles of this species had a higher oxygen uptake rates with the increased temperature (16 °C, 20 °C, and 24 °C). WHITLEDGE and RABENI (2003) observed a positive relationship between temperature (18 °C, 22 °C, 26 °C, and 30 °C) and respiration rate of five *Orconectes* species with a different distribution in Missouri, U.S.A. Our results showed no positive relationship between temperature and respiration rate in contrast with PAGLIANTI *et al.* (2004) and WHITLEDGE and RABENI (2003), but the occurrence of the noble crayfish in water bodies with different thermal regimes may explain this discrepancy. In fact, several invertebrates do not follow in their metabolism the Arrhenius' logarithmic trend, but their oxygen uptake is maintained at a relatively constant value within the environmental temperature range in which they usually live (NEWELL and NORTHCROFT, 1967; VERNBERG and VERNBERG, 1972; NEWELL, 1976; BÜCKLE *et al.*, 1994; GUTIÉRREZ-YURRITA, 2000). This was also observed by FRY (1957) in some species of freshwater fishes.

Studies carried out on fishes showed that maximum daily consumption is also influenced by temperature:  $C_{max}$  initially increases with temperature, peaking or reaching a plateau at some intermediate values ( $T_{max_C}$ ), and then declines rapidly as temperature approaches the upper thermal tolerance limit for the species (KITCHELL *et al.*, 1977; JOBLING, 1993; ELLIOT, 1994; HAYWARD and ARNOLD, 1996; ZWEIFEL *et al.*, 1999). Consequently, growth scope is maximised at temperature slightly lower than  $T_{max_C}$ , declining precipitously under  $T_{max_C}$ . A raise in the temperature did not exert any influence on the  $C_{max}$  and growth scope in *A. astacus* reared at 15 °C and 22 °C in disagreement with the results of WHITLEDGE and RABENI (2003) to *Orconectes* genus. As just seen for respiration rates, the wide optimal thermal range of the noble crayfish could be the probably explanation of these differences.

Food availability is another important factor that influences survival and growth in freshwater species. The present study showed a significant increase of juvenile *A. astacus* growth performance with a higher ration size in agreement with the results obtained by others authors (D'AGARO, 2004). CHAKRABORTY *et al.* (1995) observed that the pattern of energy allocation of the common carp, *Cyprinus carpio*, varied with ration size. Increasing the ration level up to an optimal level, a larger amount of ingested energy is available to the crayfish for growth. Several studies have clearly shown that the relationship between growth and ingestion is a non linear curve (BRETT and GROVES, 1979). It is also known from other research works carried out with lobster and crayfish species that the growth

and survival performances may be adversely affected if the containers are too small. In our experiment the stocking density was of 125 individuals.m<sup>-2</sup> with no adverse effects on growth and survival of juvenile crayfish. In agreement with these findings, GYDEMO and WESTIN (1989) showed that the density could be increased to about 100 individuals.m<sup>-2</sup> if shelters were in excess and D'ABRAMO *et al.* (1985) to 200 individuals.m<sup>-2</sup> if proper feed is given.

Values of increments per moults in *A. astacus* (range: 0.54-19.93%, mean: 10.37%), were similar to the values found in *A. italicus* by PAGLIANTI and GHERARDI (2004) that however contrast with other studies showing higher increments of crayfish species, such as *Cherax destructor* (VERHOEF *et al.*, 1998) reared in laboratory conditions. Moreover the number of moults was lower than expected, probably due to the physical confinement of crayfish in small compartments (GOYERT and AVAULT, 1978) that however had the advantage to prevent cannibalism and fights and to provide a given amount of food to each crayfish.

Discrepancies between different studies might be explained by the chemical characteristics of the used diet. In fact, a diet with a low level of proteins can reduce moult increments and extend the intermoult period in decapod crustaceans (HARTNOLL, 1982), as found in *C. destructor* (JONES *et al.*, 1996). Protein requirements of crayfish vary from 20 to 50% (GODDARD, 1988): *C. tenuimanus* growth is slow at protein levels below 20%, while at levels above 30% no significant increase in growth rate was achieved (TSVETNENKO *et al.*, 1995). An optimal diet protein level of 20% was also reported for *Procambarus clarkii* (HUNER and MEYERS, 1979). The diet used in our experiment had a rather high value of crude proteins (40.95% DM), even if a diet based on zooplankton may induce better growth than most pelleted diets (MITCHELL and COLLINS, 1989; JONES *et al.*, 1995; AUSTIN *et al.*, 1997; VERHOEF *et al.*, 1998). It is also evident that the availability of different feed sources is more important than the dietary protein level as reported for pond-reared *C. tenuimanus* (VILLARREAL, 1988). Previous study (D'AGARO *et al.*, 2004) suggested that *Elodea canadensis* could be used in the aquaculture practice as a cheap supplemental food in order to increase the growth and the survival in summerling noble crayfish.

Besides these factors, in agreement with VERHOEF *et al.* (1998), we hypothesise that an inherent diversity in the life-cycle strategy between species may be in large part responsible for differences in crayfish growth rate and in metabolic responses. While *r*-selected species, such as *C. destructor*, show rapid growth, early maturity, and a shorter life span (HONAN and MITCHELL, 1995), both *A. italicus* and *A. astacus* display characteristics proper of a *K*-selected species, as longevity, slow to moderate growth, low fecundity, and low juvenile survival rates (TAMKEVICIENE, 1988; HONAN and MITCHELL, 1995).

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