

Proposal of a practical diet for juvenile astacid crayfish studies from the onset of exogenous feeding under controlled conditions

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ABSTRACT

Key-words:
astacid,
juvenile,
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Considering the knowledge on freshwater crayfish feeding requirements and other cultured crustaceans, a practical extruded diet was formulated with the aim to be used for juvenile astacid studies from the onset of exogenous feeding. According to a bifactorial design, the practical diet was compared with the diet which has allowed repeated good survival and growth results (control: feedstuff for trout combined with *Artemia* cysts) in groups and individually isolated crayfish in an 80-day experiment. Diets tested did not affect final survival rates and growth neither in grouped nor in isolated crayfish, being the highest survival rate (average: 93%) and growth values (average: 13.2 mm carapace length: *CL*, 578.1 mg weight: *W*, 3.52%.d⁻¹ specific growth rate: *SGR*, 1896.6% weight gain: *WG*) reached with the practical diet. Survival rates did not show significant differences neither among crayfish maintained in groups (86% for the practical diet and 81.7% for the control diet) nor among isolated crayfish (100% survival). Isolated crayfish had significantly higher growth (14.3 mm *CL*, 760 mg *W*) than the grouped crayfish (11.5 mm *CL*, 354 mg *W*). Results show the feasibility of the proposed practical diet as basis for further studies on astacid crayfish.

RÉSUMÉ

Proposition d'une nourriture pratique pour les études de juvéniles d'écrevisses dès la prise de nourriture exogène sous conditions contrôlées

Mots-clés :
astacidé,
juvénile,
alimentation,
nourriture
pratique

Étant donné les connaissances sur les besoins alimentaires des écrevisses d'eau douce et d'autres crustacés élevés, une nourriture extrudée pratique a été formulée dans le but d'être utilisée pour les études sur les juvéniles d'astacidés dès la prise de nourriture exogène. Selon un dispositif bifactoriel, la nourriture a été comparée à une alimentation qui a permis de bonnes survies et croissance (contrôle : nourriture pour truite avec des cystes d'*Artemia*) dans des expérimentations de 80 jours sur individus isolés ou en groupe. Les alimentations testées n'affectent pas les taux de survie finaux et la croissance que ce soit sur des écrevisses en groupe ou isolées, les taux de survie (moyenne : 93 %) et valeurs de croissance (moyenne : 13,2 mm de longueur de carapace : *CL*; 578,1 mg en poids : *W*; 3,52 %·jour⁻¹, croissance spécifique : *SGR*; 1896,6 %, en gain de poids : *WG*) sont les meilleurs avec l'alimentation pratique. Les taux de survie ne présentent pas de

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différences significatives que les écrevisses soient en groupes (86 % avec la nourriture pratique et 81,7 % avec le témoin) ou isolées (100 % de survie). Les écrevisses isolées ont une croissance significativement meilleure (14,3 mm *CL*, 760 mg *W*) que celles en groupe (11,5 mm *CL*, 354 mg *W*). Ces résultats montrent la faisabilité de cette nourriture pour des études ultérieures sur les écrevisses.

INTRODUCTION

One the main constraints to interpret and compare research results of different crustacean species is the lack of standardization in experimental methodology (D'Abramo and Castell, 1997). The recommendations proposed by New (1976) highlights the development of a standard reference to facilitate the comparison of data not only in nutrition research, but also in studies on crustacean culture technology such as general physiology, behaviour, disease and so on.

A standard reference diet for crustaceans (Castell, 1989), formulated for lobster (*Homarus* spp.), was evaluated in a wide range of crustaceans including some species of astacid crayfish (Celada *et al.*, 1989; Ackefors *et al.*, 1992). In addition to the poor results obtained on freshwater crayfish, the limited supply and high price of this diet discouraged its further use on crayfish research.

A wide variety of natural foods (mainly fresh or frozen animals and vegetables) and feedstuffs formulated for other aquatic species either alone or supplemented with natural foods (see review by González R. *et al.*, 2009a) have been tested to improve survival and growth rates of astacid juveniles from the onset of exogenous feeding. However, acceptable results have been only obtained by supplying live feed such as *Daphnia* or *Artemia* nauplii (Sáez-Royuela *et al.*, 2007; González À. *et al.*, 2008, 2011; González R. *et al.*, 2009a). Recently, González R. *et al.* (2009b) proved that decapsulated *Artemia* cysts can be better supplement than live nauplii, since cysts improve the performance of juvenile crayfish, allowing a reduction of labour and costs. These improvements on feeding schedules during the first months of independent life open the possibility of approaching a new step, the formulation of a specific practical diet for astacid crayfish.

MATERIAL AND METHODS

> CRAYFISH, FACILITIES AND EXPERIMENTAL PROCEDURE

An 80-day experiment was carried out in indoor facilities starting with stage 2 juvenile *Pacifastacus leniusculus* Dana (mean: 5.64 ± 0.05 mm carapace length and 28.94 ± 0.7 mg weight) hatched in the laboratory. Juvenile crayfish were maintained in groups at a density of 100·m⁻² and, in order to set adequate experimental conditions, isolation of crayfish was also considered to avoid possible influence of intraspecific behavioural interactions such as cannibalism. Following a bifactorial design, two diets were tested on groups (three tanks for each treatment, *N* = 25 per tank) and individually isolated animals (five replicates for each treatment). Crayfish in groups were held in opaque rectangular fibreglass tanks (0.25·m⁻²) at a density of 100·m⁻², whereas isolated animals were maintained in smaller tanks (0.125·m⁻²). In both cases, PVC pipes (4 cm long × 20 mm diameter) were used as shelter. Aerated artesian well water was supplied in open system, and each tank had its own water inlet and outlet. Quality parameters of the incoming water were pH 7.8, hardness 18 °f (French grades, calcium 72 mg·L⁻¹), total dissolved solids 205.2 mg·L⁻¹ and total suspended solids 13.9 mg·L⁻¹. Throughout the trial, oxygen content was measured in the tanks, ranging from

5.9 mg·L⁻¹ to 7.9 mg·L⁻¹ (average values around 7 mg·L⁻¹). Ammonia and nitrite were measured from water samples taken inside the tanks (values were always ammonia < 0.02 mg·L⁻¹ and nitrite < 0.05 mg·L⁻¹). Water temperature was maintained at 22 ± 1 °C. Photoperiod was natural (ca. 14 h light and 10 h dark). Tanks were cleaned twice a week.

> DIETS AND FEEDING

Based on the available knowledge on freshwater crayfish feeding and on recent advances, ingredients for a practical diet were chosen. Vitamins levels were included following the recommendations made by Conklin (1997) for different crustacean species and Akiyama *et al.* (1992) for shrimps. In the case of minerals, the information provided by Davis and Arnold (2000) was applied. The efficacy of the practical diet from the onset of exogenous feeding was compared with the diet which has allowed good results in previous studies: a feedstuff for trout supplemented with *Artemia* cysts (González R. *et al.*, 2009b, 2011). Crayfish were manually fed to excess (ca. 2.5% body weight per day) once a day.

In crayfish held both in groups and individually isolated, two diets were tested:

- Control: A dry diet for salmonids (T-NUTRA-0, Skretting, Trouw España SA. Cojobar, Burgos, Spain, composition data provided by the manufacturer: crude protein 54%, crude lipid 18%, crude cellulose 0.08%, ashes 12%, total phosphorus 1.8%, Vitamine A 10 000 UI·kg⁻¹, D₃ 1500 UI·kg⁻¹, E 150 mg·kg⁻¹, gross energy 19.3 kJ·kg⁻¹, pellet diameter 0.9–1.5 mm) supplemented with decapsulated *Artemia* cysts (cysts of INVE Aquaculture Nutrition, High HUFA 430 µm, Hoogveld 91, Dendermonde, Belgium). Cysts were decapsulated according to the method described by Van Stappen (1996). The initial amount of *Artemia* supplement was 500 cysts per crayfish per day (later increasing by 15% considering biomass values calculated every 20 days). Supplement assessment was performed from the number of cysts·g⁻¹ provided by the manufacturer.
- Practical diet: Ingredients were mixed and extruded using the stand-alone extruder Brabender E19/25 D (Diusburg, Germany) at a temperature range between 75 °C and 90 °C and then dried during 24 h at 30 °C. Later, pellets (1 mm diameter) received a coating of cod liver oil. Pellet size was adjusted to 2 mm. Diet composition is summarized in Table I.

> DATA COLLECTION AND ANALYSIS

Every twenty days, crayfish were counted and survival rates calculated. After removing excess water with tissue paper, all survivors were weighed and measured individually. Subsequently, animals were gently returned to their respective tanks. Carapace length (CL) was measured with a digimatic calliper (to the nearest 0.01 mm) and wet individual weight (*W*) was determined by means of a precision balance (to the nearest 0.1 mg). Specific growth rate (SGR) and weight gain (WG) were expressed as $(\ln W_t - \ln W_i) \times 100/T$ and $(W_t - W_i)100/W_i$, respectively, where *W_t* is the mean final weight (mg), *W_i* is the mean initial weight, and *T* is the period (days). At the end of the experiment, survival percentages were calculated and all surviving crayfish were weighed and measured individually.

Results were examined by analysis of variance (ANOVA) using the computer program SPSS 16 (SPSS Inc. Chicago, Ill., USA). Duncan test was applied to compare means at the *P* < 0.05 level of significance. Percentages were arcsine-transformed prior to statistical analysis.

RESULTS AND DISCUSSION

Final survival and growth values (80 days) are presented in Table II. Diets tested did not affect final survival rates and growth values neither in grouped animals nor in individually isolated

Table I

Composition of the practical extruded diet.

Tableau I

Composition de l'aliment extrudé.

Ingredients (%)	
Fish meal ¹	61.48
Corn meal ²	13.0
Cod-liver oil ³	3
Soy lecithin ⁴	0.1
Cholesterol ⁵	0.5
Ascorbyl monophosphate ⁶	0.04
Choline chloride ⁶	0.5
Dicalcium phosphate ⁶	1.0
Dried decapsulated <i>Artemia</i> cysts ⁷	15.0
Carboxymethylcellulose ⁸	3.0
Astaxanthin ⁹	0.1
Vitamin premix ¹⁰	0.28
Mineral premix ¹¹	2.0
* Proximate composition (%)	
Moisture	8.3
Crude protein	50.22
Lipids	12.14
Carbohydrates	12.33
Ashes	13.23
Fiber	3.78
Gross energy (kJ·g ⁻¹)	19.33

* It was calculated from the known composition of each ingredient

¹ BIOMAR Iberia / PROAQUA Nutrición, Dueñas (Palencia), Spain.

² ADPAN, Siero-Asturias, Spain.

³ ACOFARMA, Terrassa (Barcelona), Spain.

⁴ BIOVER NV/SA Brujas, Belgium.

⁵ Sigma-Aldrich Chemie GMBH, Steinheim, Germany.

⁶ NUTRAL SA, Madrid, Spain.

⁷ *Artemia* cysts INVE Aquaculture Nutrition, High HUFA 430 µm, Dendermonde, Belgium.

⁸ HELM IBERICA SA, Madrid, Spain.

⁹ BIOMAR Iberia / PROAQUA Nutrición, Dueñas (Palencia), Spain.

¹⁰ (mg·100 g⁻¹ premix): Thiamine 2142.9; Riboflavin 1892.9; Niacin 7142.9 ; Pyridoxine 1785.7; Pantothenic acid 3785.7; Biotin 35.7; Folic acid 571.4; Cyanocobalamin 7.1; Myoinositol 14285.7; Retinol 53.7; α -tocopherol 2382.1; Cholecalciferol 392.86; Naphthoquinone 312.43; Ethoxyquin 3571.43.

¹¹ (mg·100 g⁻¹ premix): CoCl₂ 4; CuSO₄·5H₂O 250; FeSO₄ 4000; MgSO₄·7H₂O 28398; MnSO₄·H₂O 650; KI 67; Na₂SeO₃ 10; ZnSO₄·7H₂O 13193.

animals. Figures 1 and 2 show the carapace length and weight, respectively, throughout the 80 days when crayfish were fed the control diet or the practical diet. In the different checks, no differences in carapace length and weight were found between the tested diets in both isolated and grouped crayfish. Regardless of whether the animals were kept isolated or in groups, no differences were detected between the practical diet (average: 93.0% survival, 13.19 mm CL, 578.12 mg W, 3.52%·d⁻¹ SGR and 1896.6% WG) and the control diet (average: 90.8% survival, 12.72 mm CL, 536 mg W, 3.44%·d⁻¹ SGR and 1748.1% WG).

For both diets tested, individually isolated crayfish had final higher survival rate and growth values than the grouped crayfish (Table II). Figures 3 and 4 show the changes in carapace length and weight, respectively, throughout the 80 days when crayfish were maintained in groups or isolated. Since the first check (20 days), isolated crayfish grew faster than the grouped crayfish. At the end of the experiment (80 days) and regardless of the diet supplied, isolated crayfish had significantly higher growth (average: 14.3 mm CL, 760 mg W, 3.94%·d⁻¹

Table II

Final survival and growth values (80 days) of juvenile crayfish held in groups or individually isolated, fed the control diet or the proposed practical diet.

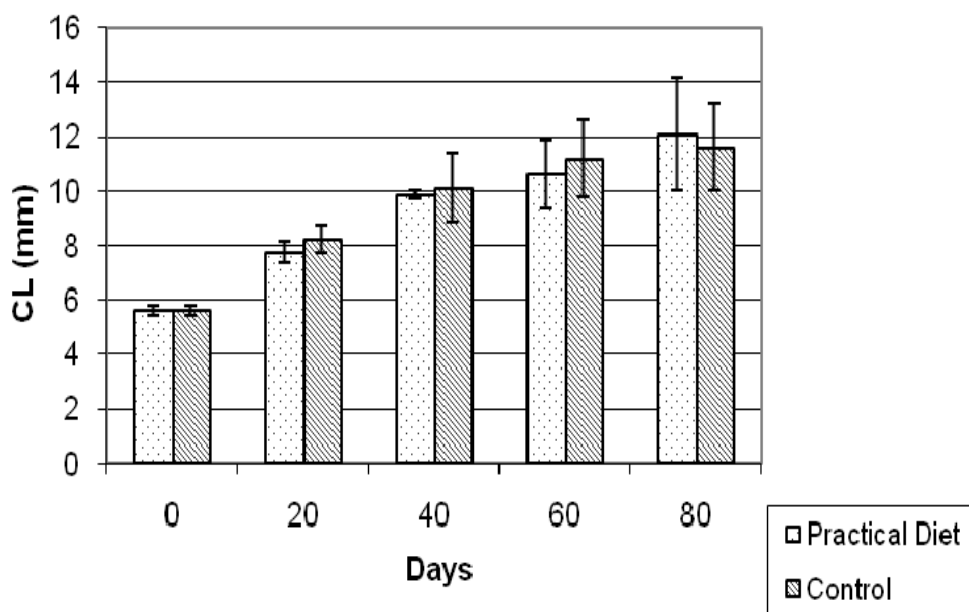
Tableau II

Survie finale et croissance (à 80 jours) des juvéniles d'écrevisses tenues en groupe ou isolées nourries avec la nourriture témoin ou proposée.

	Control diet		Practical diet	
	Groups	Isolated	Groups	Isolated
Survival (%)	81.7 ± 5.8 ^a	100 ^b	86.0 ± 3.40 ^a	100 ^b
CL (mm)	11.31 ± 0.21 ^a	14.14 ± 0.26 ^b	11.82 ± 0.28 ^a	14.57 ± 0.98 ^b
W (mg)	330.65 ± 20.75 ^a	741.40 ± 123.32 ^b	377.65 ± 27.38 ^a	778.60 ± 171.09 ^b
SGR (%)	2.97 ± 0.07 ^a	3.91 ± 0.32 ^b	3.07 ± 0.10 ^a	3.99 ± 0.66 ^b
WG (%)	1033.8 ± 61.45 ^a	2462.5 ± 610.3 ^b	1204.9 ± 94.6 ^a	2590.4 ± 591.8 ^b

Values are mean ± mean standard error.

Values in the same row having different superscript are significantly different ($P < 0.05$).

**Figure 1**

Mean carapace length CL (\pm SD) of juvenile crayfish fed the control diet or the practical diet in the checks throughout the experimental period.

Figure 1

Longueur moyenne de la carapace CL (avec déviation standard) des juvéniles d'écrevisses nourris avec la nourriture témoin ou proposée pendant la période de l'expérimentation.

SGR and 2526.4% WG) than the grouped crayfish (average: 11.5 mm CL, 354 mg W, 3.02%·d⁻¹ SGR and 1119.3% WG). In previous studies (Celada *et al.*, 1989, 1993; Sáez-Royuela *et al.*, 1995), crayfish in groups had higher growth than those isolated. This fact was explained by the poor quality of the diets provided, which was the only food available for isolated animals, whereas the maintained in groups had a food supplement provided by cannibalism. In the present experiment, the use of an improved diet allowed that isolated crayfish, free from the stress generated by foraging competition and aggressive behavior with mates, could better express their growth potential.

The proposed practical diet enabled similar performance after 80 days in terms of survival and growth than the control diet previously used by González R. *et al.* (2009b, 2011). Considering that the *Artemia* cysts availability guarantee the success of astacid crayfish breeding from

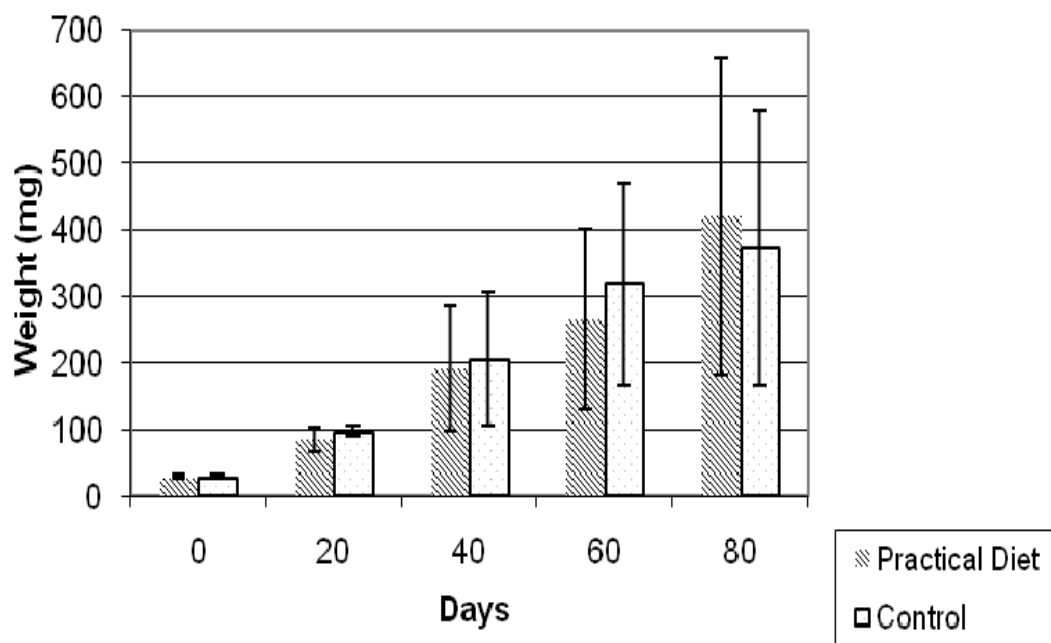


Figure 2
 Mean weight (\pm SD) of juvenile crayfish fed the control diet or the practical diet in the checks throughout the experimental period.

Figure 2
 Poids moyen (avec déviation standard) des juvéniles d'écrevisses nourries avec la nourriture témoin ou proposée pendant la période de l'expérimentation.

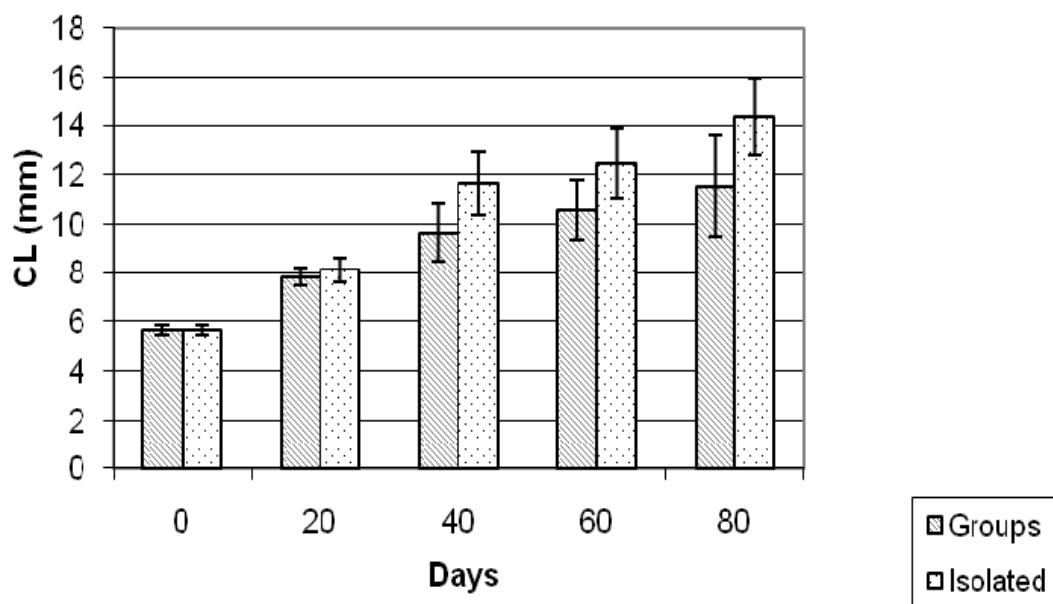


Figure 3
 Mean carapace length (\pm SD) of juvenile crayfish held in groups or isolated in the checks throughout the experimental period.

Figure 3
 Longueur moyenne de la carapace CL (avec déviation standard) des juvéniles d'écrevisses tenues en groupes ou isolées pendant la période de l'expérimentation.

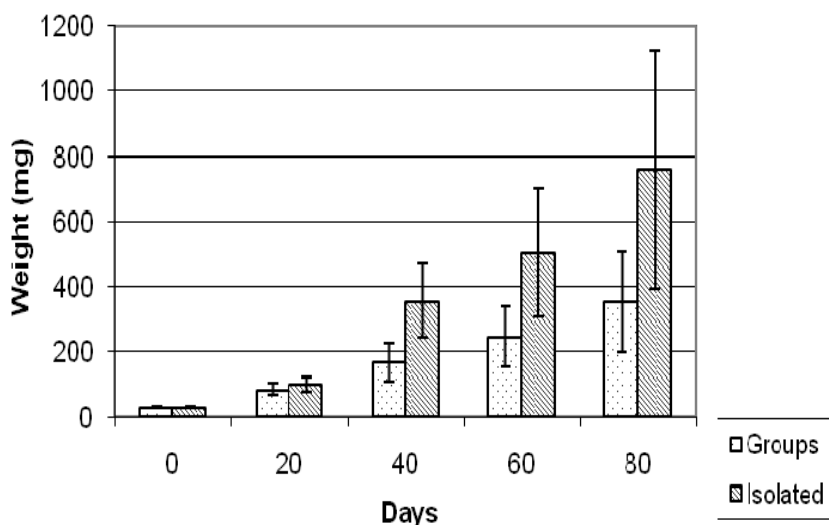


Figure 4

Mean weight (\pm SD) of juvenile crayfish held in groups or isolated in the checks throughout the experimental period.

Figure 4

Poids moyen (avec déviation standard) des juvéniles d'écrevisses tenues en groupes ou isolées pendant la période de l'expérimentation.

the onset of exogenous feeding, a small amount of cysts was included in the practical diet. Despite the significant reduction of cysts compared to those supplied in the control diet, both diets did not show differences in the performance of juvenile crayfish, allowing an important saving of brine shrimp cysts. In addition, this practical diet avoids the need of combining several foods and can be handled as a commercial dry diet. In order to make feasible its use and following the suggestions made by D'Abramo and Castell (1997), the practical diet was formulated with a small number of ingredients, all easily available, minimizing the eventual inability to get regularly a particular ingredient. From the good results obtained, the proposed practical diet show its feasibility to be used as basis for further studies on freshwater crayfish under controlled conditions, preventing distortion or masking of experimental results caused by a deficient feeding.

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