Recent Advances on Freshwater Crayfish Nutrition: Proposal of a Practical Diet for Juvenile Astacid Crayfish Studies from the Onset of Exogenous Feeding under Controlled Conditions

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Abstract

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 either in grouped or 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 either among crayfish maintained in groups (86 % for the practical diet and 81.7% for the control diet) or 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.

Keywords: *Astacid, juvenile, feeding, practical diet*

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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, Carral, Gaudioso, Temiño & Fernández 1989; Ackefors, Castell, Boston, Räty & Svensson 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., Celada, González Á., García, Carral & Sáez-Royuela 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, Carral, Celada, Pérez & González 2007; González A, Celada, González R., García, Carral & Sáez-Royuela 2008; González R *et al.*, 2009a). Recently, González R., Celada, Carral, González Á., Sáez-Royuela & García (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/ 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 x 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 ºf (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 ºC. Photoperiod was natural (ca. 14 hours light and 10 hours 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, Dominy & Lawrence (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; González R., Celada, García, Carral, González Á. & Sáez Royuela 2010). 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, E-09620, 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 10000 UI kg^{-1}, D_3 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, B-9200 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 estimated 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 ºC and 90 ºC and then dried during 24 hours at 30 ºC. 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 1.

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).

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Table 1. Composition of the practical extruded diet

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 *Artemia* 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 gr^{-1}	19.33

* It was calculated from the known composition of each ingredient

Specific Growth Rate (SGR) and Weight Gain (WG) were expressed as (ln W_t-ln W_i) x

[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, Riedstr.2, D-89555 Steinheim, Germany
[6] NUTRAL S.A, Madrid, Spain.
[7] *Artemia* cysts INVE Aquaculture Nutrition, High HUFA 430 µm, Hoogveld 91, B-9200 Dendermonde, Belgium.
[8] HELM IBERICA SA., Madrid, Spain.
[9] BIOMAR Iberia / PROAQUA Nutrición, Dueñas (Palencia), Spain.
[10] (mg/100 g premix): Thiamine 2142.9; Riboflavin 1892.9; Niacin 7142.9 ; Pyridoxine 1785.7; Pantothenic acid (B5) 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 premix): $CoCl_2$ 4; $CuSO_4$-$5H_2O$ 250; $FeSO_4$ 4000; $MgSO_4$-$7H_2O$ 28398 mg; $MnSO_4$-H_2O 650; KI 67; Na_2SeO_3 10; $ZnSO_4$-$7H_2O$ 13193.

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 2. Diets tested did not affect final survival rates and growth values either in grouped animals or in individually isolated 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 2). 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} 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).

Table 2. 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.

	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 ($P < 0.05$)

In previous studies (Celada *et al*., 1989; Celada, Carral, Gaudioso, González, Lopez-Baissón & Fernandez 1993; Sáez-Royuela, Carral, Celada & Muñoz 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.

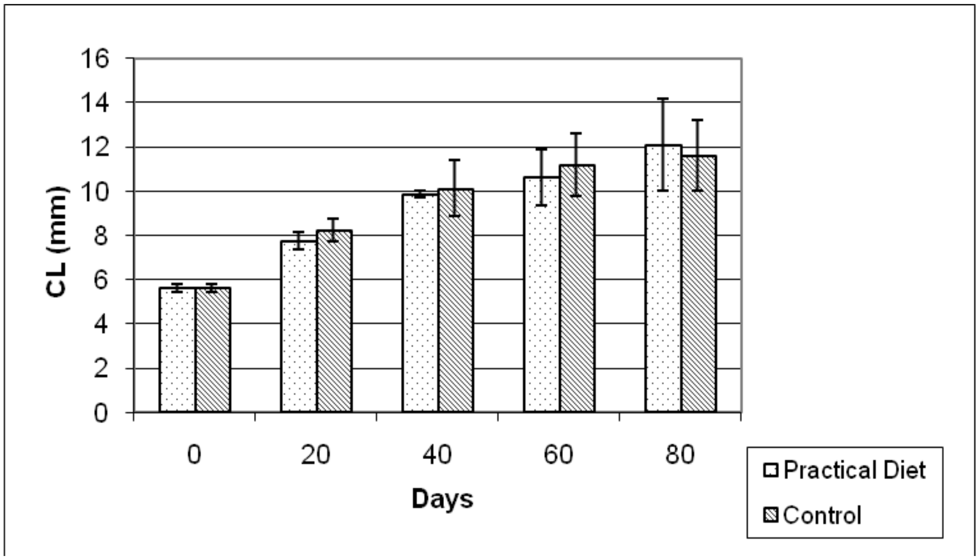

Figure 1. Mean carapace length (± SD) of juvenile crayfish fed the control diet or the practical diet in the checks throughout the experimental period.

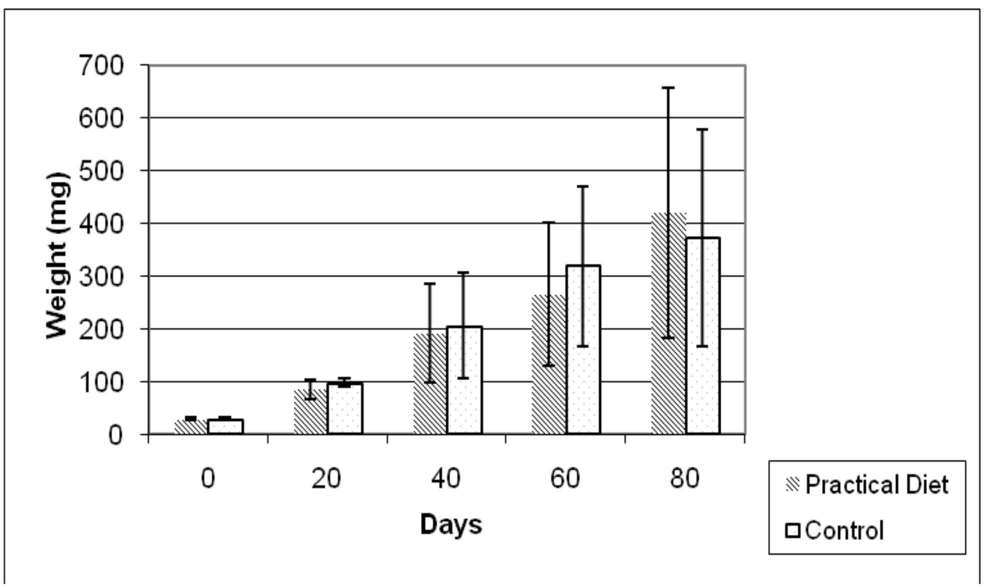

Figure 2. Mean weight (± SD) of juvenile crayfish fed the control diet or the practical diet in the checks throughout the experimental period.

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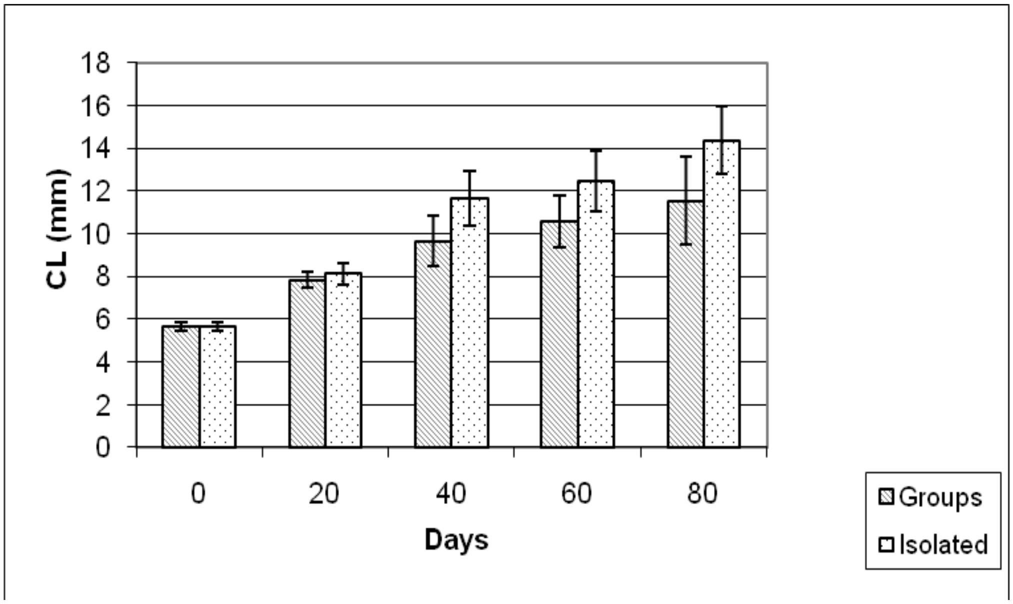

Figure 3. Mean carapace length (± SD) of juvenile crayfish held in groups or isolated in the checks throughout the experimental period.

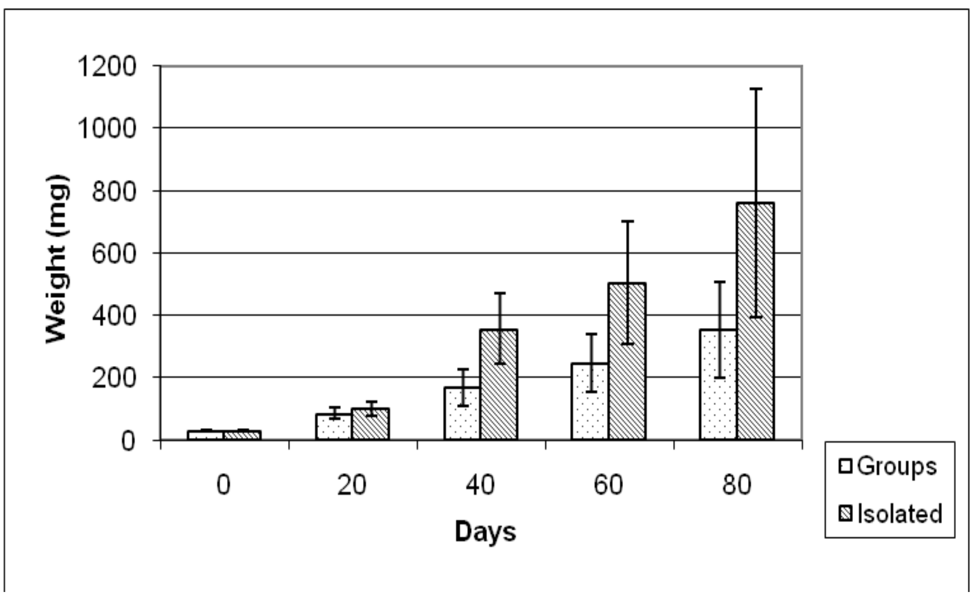

Figure 4. Mean weight (± SD) of juvenile crayfish held in groups or isolated in the checks throughout the experimental period.

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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, 2010). Considering that the *Artemia* cysts availability guarantee the success of astacid crayfish breeding from 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 performance of juvenile crayfish, allowing an important saving of 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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