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Additional supply of decapsulated *Artemia* cysts for various periods in intensive rearing of juvenile crayfish (*Pacifastacus leniusculus*, Astacidae)

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ABSTRACT

Key-words: *astacid crayfish, juvenile, feeding, stocking density*

The additional supply of live feeds such as *Artemia* remains indispensable in juvenile astacid rearing from the start of independent life. Considering that cost and work could be reduced by restricting this supply, a 100-day experiment was carried out with stage 2 *Pacifastacus leniusculus* to evaluate the effects of different administration periods of decapsulted *Artemia* cysts as supplement to a dry diet at two stocking densities. Using a bifactorial design, six treatments differing in the time at which the cysts were withdrawn (after 20, 30 or 50 days of experiment) and the stocking density (100 or 200 crayfish·m^{-2}) were conducted. Survival rates did not show significant differences among groups, with final figures averaging 81%. Crayfish receiving cysts up to day 50 showed faster growth (around 13.8 mm carapace length and 610 mg weight at the end of the experiment) than the rest, significant differences being recorded from day 60 of experiment onwards. The 20-day and 30-day supplies resulted in similar growth values. There were no significant differences in survival or growth between 100 and 200 crayfish·m^{-2}. This study shows that decapsulated *Artemia* cysts can be withdrawn at day 20 (allowing to feed a dry diet as the sole food thereafter) and that faster growth can be obtained if cysts are supplied up to day 50. Furthermore, a stocking density of 200 crayfish·m^{-2} can be advised under the mentioned feeding conditions.

RÉSUMÉ

Alimentation complémentaire avec des cystes décortiqués d'*Artemia* pendant différentes périodes de l'élevage intensif de juvéniles d'écrevisses (*Pacifastacus leniusculus*, Astacidae)

Mots-clés : *écrevisse, juvénile, alimentation, densité d'élevage*

L'alimentation complémentaire avec de la nourriture vivante comme *Artemia* reste indispensable dans l'élevage de jeunes écrevisses dès le début de la vie libre. Considérant que le coût et le travail pourraient être réduits par une réduction de cette alimentation, une expérience de 100 jours a été menée au stade 2 de *Pacifastacus leniusculus* pour évaluer les effets de différentes périodes d'alimentation avec des cystes décortiqués d'*Artemia* à deux densités d'élevage. Selon un dispositif bifactoriel, six traitements différents dans le temps de retrait des cystes (après 20, 30, 50 jours d'expérimentation) et de densité d'élevage (100 ou 200 écrevisses·m^{-2}) ont été suivis. Les taux de survie ne présentent pas de différence significative entre groupes, avec des taux finaux moyens de 81 %.

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Les écrevisses recevant des cystes jusqu'à 50 jours présentent une croissance plus rapide (autour de 13,8 mm de longueur de carapace et 610 mg de poids à la fin de l'expérience) que les autres, ces différences étant significatives à partir de 60 jours. Le nourrissage de 20 et 30 jours conduit à des croissances similaires. Il n'y a pas de différence significative de survie et de croissance entre les deux densités d'élevage (100 ou 200 écrevisses·m^{-2}). Cette étude montre que les cystes décortiqués d'*Artemia* peuvent être supprimés après 20 jours (permettant l'emploi d'une nourriture sèche seulement par la suite) et qu'une croissance plus rapide peut être obtenue si les cystes sont délivrés jusqu'à 50 jours. De plus, une densité d'élevage de 200 écrevisses·m^{-2} peut être recommandée dans ces conditions de nourriture.

INTRODUCTION

One of the main constraints to intensifying astacid culture has been the unpredictable and usually poor survival and growth rates of juveniles during the first months of independent life. As feeding is a decisive factor in this critical period, a wide variety of foods has been tested in experiments carried out under controlled conditions (see review by González *et al.*, 2008). Among them, several trials have shown that the supply of dry diets as the sole food from the onset of exogenous feeding yields poor survival or growth rates (*e.g.* Ackefors *et al.*, 1989; Taugbøl and Skurdal, 1992; Celada *et al.*, 1993; Ulikowski *et al.*, 2006; Sáez-Royuela *et al.*, 2007). Recently, acceptable survival and growth rates were recorded by means of the additional supply of live *Artemia* nauplii to a commercial dry diet for salmonids (Sáez-Royuela *et al.*, 2007; González *et al.*, 2008), thus allowing for the first time reliable possibilities of intensification. Later, González *et al.* (2009) advised the use of decapsulated *Artemia* cysts instead of nauplii, as they can support a faster crayfish growth and their production offers important advantages.

Due to the scarcity of information on the nutritional requirements of juvenile astacids in their first stages, available dry diets are ineffective as the sole food. Thus, the additional supply of live feeds such as *Artemia*, which would constitute the source of unknown factors (Sáez-Royuela *et al.*, 2007), still remains indispensable under controlled conditions. At this point, it should be considered that both cost and work could be reduced by means of restricting the supply periods of *Artemia* cysts. Thus, the determination of crayfish optimum age for the withdrawal of cysts would be a key to the further development of astacid culture. The aim of this study was to evaluate the effects of different periods of *Artemia* cysts supply on survival and growth of juvenile crayfish at two stocking densities.

MATERIALS AND METHODS

> CRAYFISH, FACILITIES AND EXPERIMENTAL PROCEDURE

A 100-day experiment was carried out under controlled conditions with stage 2 juvenile *Pacifastacus leniusculus* (Dana, 1852). A total of 342 crayfish (5.16 ± 0.04 mm carapace length and 28.8 ± 0.4 mg weight, $n = 75$) were placed in 18 fiberglass tanks (0.125 m^2, 20 L water). According to a bifactorial design, six treatments differing in the period in which the cysts were withdrawn (after 20, 30 and 50 days of experiment) and the stocking density (100 and 200 crayfish·m^{-2}) were conducted. Jointed sections of PVC pipe (4 cm long × 20 mm diameter) were provided as shelter (4 groups of 4 sections for 100 crayfish·m^{-2} and 7 groups for 200 crayfish·m^{-2}).

Aerated artesian well water was supplied in a flow-through system, and each tank had its own water inlet (flow rate: 250 mL·min^{-1}) and outlet, provided with a 200 μm mesh filter outlet. The parameters of incoming water quality were measured weekly, averaging: pH 7.9, hardness 5.2 °dH (calcium 32.3 mg·L^{-1}), total dissolved solids 110 mg·L^{-1} and total suspended solids 46.4 mg·L^{-1}. Throughout the trial, dissolved oxygen content in the tanks was measured every second day with a Sension 6 Dissolved Oxygen Meter of Hatch Company, and values were around 7 mg·L^{-1} (range 6.2–8.3). Ammonia and nitrites were measured once a week from water samples taken inside the tanks with a Pocket-Photometer Lasa Aqua; values were always ammonia $<$ 0.02 mg·L^{-1} and nitrites $<$ 0.05 mg·L^{-1}. Water temperature was registered daily using maximum–minimum thermometers and maintained at 22 ± 1 °C. Photoperiod was natural (corresponding to February–May). Tanks, filters and shelters were cleaned twice a week.

> FEEDING AND SUPPLEMENTATION PERIODS

Crayfish were manually fed once daily in excess (*ca*. 3% body weight) a dry diet for salmonids (T-Nutra 0, Skretting, Trouw España S. A. 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%, Vitamin A 10 000 UI·kg^{-1}, D_3 1500 UI·kg^{-1}, E 150 mg·kg^{-1}, pellet diameter 0.9–1.5 mm). In addition, decapsulated *Artemia* cysts were supplied for different periods from the start of the trial (cysts of INVE Aquaculture Nutrition, High HUFA 430 μm, Hoogveld 91, B-9200 Dendermonde, Belgium). Cysts were decapsulated following the method described by Van Stappen (1996). According to González *et al.* (2009), initial supply was performed at 500 cysts per crayfish per day at the beginning of the trial, being increased later by 15% every 20 days. Fresh stocks of cysts were prepared every three days and stored at 4 °C, and supplement assessment was performed from the number of cysts·g^{-1} provided by the manufacturer. A gradual reduction of cysts inputs was carried out during the 20 days prior to each withdrawal: the amount was halved for 10 days and halved again for the next 10 days. Animals did not receive any live feed from withdrawal onwards.

> DATA COLLECTION AND ANALYSIS

Every 20 days survivors were counted and survival rates calculated. A sample of juveniles from each replicate was also collected for recording carapace length (*CL*) and weight (*W*) individually. The sample size varied according to stocking density, *i.e.* 7 and 13 juveniles (100 and 200 crayfish·m^{-2}, respectively). Total crayfish weight in each tank was also recorded and biomass (B; expressed as g of crayfish·m^{-2}) was calculated. Number of juveniles lacking one or both chelipeds was registered. At the end of the experiment (100 days), all surviving crayfish were counted, measured and weighed. Carapace length (*CL*) was measured with digimatic calliper (to the nearest 0.01 mm). After removing excess water with tissue paper, individual wet weight (*W*) was determined using a precision balance (to the nearest 0.001 g). Specific growth rate (*SGR*) and weight gain (*WG*) were expressed as $(\ln W_t - \ln W_i) \times 100/T$ and $(W_t - W_i) \times 100/W_i$ respectively, where W_t is the final weight (mg), W_i is the initial weight, and T is the duration of the trial (days).

All experimental groups were tested in triplicate (1 tank per replicate). Data were examined by analysis of variance (one way ANOVA) for significant differences among treatments using the computer software SPSS 15.0 (SPSS Inc., Chicago, Ill., USA). Kolmogorov–Smirnov and Levene's tests were used to assess normality and homogeneity of variances of data, respectively. Duncan's test was used to compare differences. Level of significance was $P < 0.05$. Percentages were arcsine-transformed prior to statistical analysis.

RESULTS

Survival rates did not show significant differences among groups during the trial (Figure 1). As Table I shows, final values ranged from 76.9 to 86.7%.

Crayfish receiving cysts up to day 50 showed a faster growth than the rest (averaging 13.8 mm *CL*, 610 mg *W*, 3.05%·day^{-1} *SGR* and 2025% *WG* at the end of the experiment), being significant differences recorded from day 60 of experiment onwards (Figure 2). The 20-day and the 30-day supplies resulted in similar growth values among them (Table I).

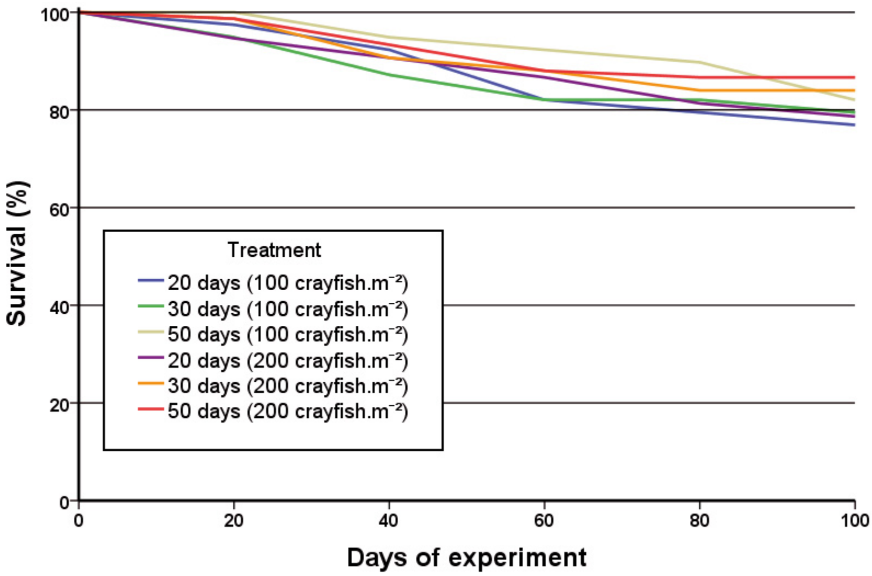

Figure 1
Survival rates of juvenile crayfish receiving an additional supply of decapsulated Artemia *cysts for different periods throughout 100 days from the onset of exogenous feeding.*

Figure 1
Taux de survie jusqu'à 100 jours après le début de la prise de nourriture exogène d'écrevisses juvéniles recevant une nourriture complémentaire de cystes décortiqués d'*Artemia* pendant différentes périodes.

Table I
Final survival and growth values (mean ± SEM) of juvenile crayfish receiving an additional supply of decapsulated Artemia *cysts for different periods throughout 100 days from the onset of exogenous feeding.*

Tableau I
Survie finale et valeurs de croissance (moyenne ± SEM) à 100 jours après le début de la prise de nourriture exogène de juvéniles d'écrevisses recevant une nourriture complémentaire de cystes décortiqués d'*Artemia* pendant différentes périodes.

	100 crayfish·m^{-2}			200 crayfish·m^{-2}		
	20 days	30 days	50 days	20 days	30 days	50 days
Survival (%)	76.9 ± 4.4^a	79.5 ± 6.8^a	82.1 ± 2.6^a	78.7 ± 4.8^a	84.0 ± 6.1^a	86.7 ± 5.8^a
***CL* (mm)**	12.9 ± 0.3^a	12.7 ± 0.4^a	14.2 ± 0.4^b	12.9 ± 0.3^a	12.7 ± 0.3^a	13.5 ± 0.3^b
***W* (mg)**	483 ± 19^a	445 ± 41^a	650 ± 61^b	484 ± 40^a	452 ± 21^a	571 ± 37^b
***SGR* (%·day^{-1})**	2.82 ± 0.04^a	2.73 ± 0.10^a	3.11 ± 0.10^b	2.82 ± 0.09^a	2.75 ± 0.05^a	2.99 ± 0.07^b
***WG* (%)**	1580 ± 67^a	1447 ± 143^a	2163 ± 212^b	1585 ± 140^a	1472 ± 74^a	1888 ± 130^b
Biomass (g·m^{-2})	38.6 ± 2.6^a	36.1 ± 4.0^a	55.7 ± 6.6^b	75.4 ± 2.3^c	75.4 ± 2.4^c	98.7 ± 7.5^d

* *CL* carapace length, *W* body weight, *SGR* specific growth rate, *WG* weight gain.
* Values followed by the same superscript are not significantly different ($P < 0.05$) from other in the same line.

Regarding biomass, values with 200 crayfish·m^{-2} were always significantly higher than those recorded with 100 crayfish·m^{-2} (Figure 3). Furthermore, final biomass figures with the 50 day-supply were significantly higher than those obtained with the 20-day or the 30-day supplies at the same stocking density.

Percentages of crayfish lacking one or both chelipeds did not show significant differences among groups throughout the trial. At day 100, values were from 5.3 to 7.2%, whereas in previous controls the range was 5.8–8.4%.

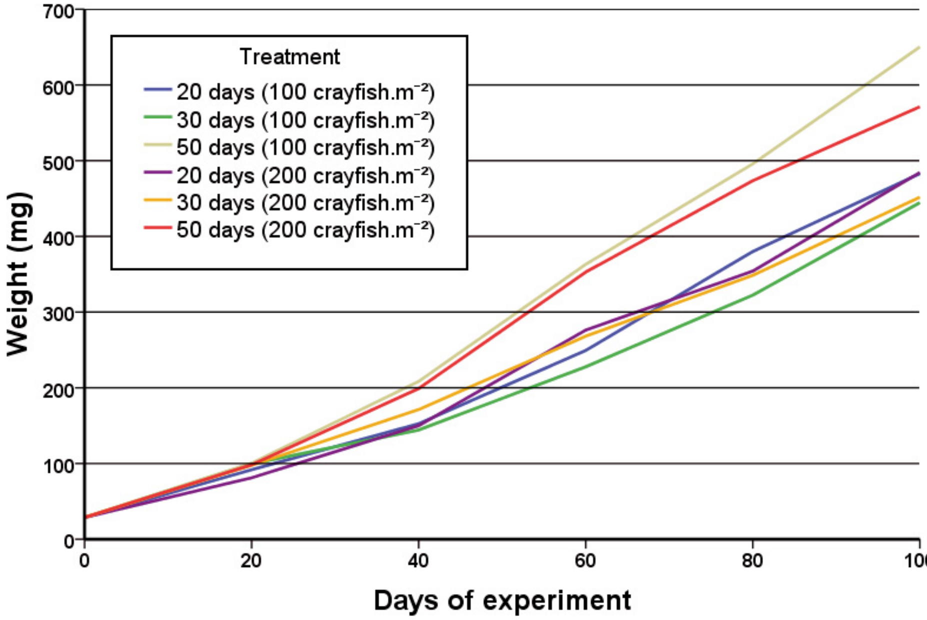

Figure 2
Weight of juvenile crayfish receiving an additional supply of decapsulated Artemia *cysts for different periods throughout 100 days from the onset of exogenous feeding.*

Figure 2
Poids jusqu'à 100 jours après le début de la prise de nourriture exogène d'écrevisses juvéniles recevant une nourriture complémentaire de cystes décortiqués d'*Artemia* pendant différentes périodes.

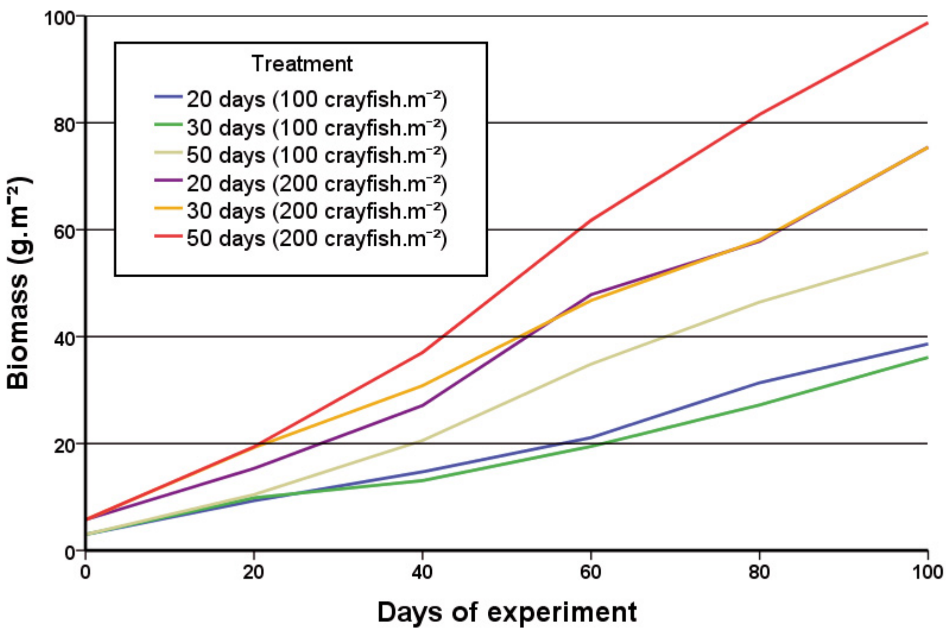

Figure 3
Biomass of juvenile crayfish receiving an additional supply of decapsulated Artemia *cysts for different periods throughout 100 days from the onset of exogenous feeding.*

Figure 3
Biomasse jusqu'à 100 jours après le début de la prise de nourriture exogène d'écrevisses juvéniles recevant une nourriture complémentaire de cystes décortiqués d'*Artemia* pendant différentes périodes.

DISCUSSION

Several studies have recorded high survival rates of juvenile astacids with no supply of live feeds, but crayfish used were older than in the present experiment. For instance, crayfish at the start of the trials were stage 5–6 (around 3 months) in Ackefors *et al.* (1992), 4 months in Westman (1973), 6 months in Fernández *et al.* (1983), 1 year in Emadi (1974) or 1–1,5 years in Wolf (2004). However, when the same feeding conditions have been tested in juveniles from the start of independent life (stage 2), survival and growth rates drastically decreased (see review by González *et al.*, 2008). This fact has been mainly attributed to a nutritional deficiency related to unknown essential factors that can be provided by live *Artemia* nauplii or *Daphnia* (Sáez-Royuela *et al.*, 2007) or decapsulated *Artemia* cysts (González *et al.*, 2009). The present results show that need for these factors could be satisfied once juveniles have received decapsulated *Artemia* cysts for an initial period of 20 days, since longer supplies did not improve the survival. Therefore, the subsequent juvenile rearing would be feasible even feeding only a dry diet, which offers several advantages such as less labour, reliable supply and easy storage. Moreover, if the supply of cysts is extended up to day 50 faster growth can be obtained as indicated by the present results, which are in the same range as previous studies in which live *Artemia* (Nyström, 1994; González *et al.*, 2008, 2011) or decapsulated *Artemia* cysts (González *et al.*, 2009) were supplied for longer.

Even though nutritional requirements of newly independent astacids are still unknown, the dietary lipid contents usually advised for other crustaceans (4–12%) are lower than that of the dry diet for salmonids used in this study (18%). With this in mind, future research on nutrition of juvenile astacids through specific practical diets should also include fat content adjustment.

Regarding the stocking density for the intensive rearing of juvenile astacids, the best results have been obtained with 100 crayfish·m^{-2} (Savolainen *et al.*, 2004; González *et al.,* 2010) or less (Ackefors *et al.*, 1989; Nyström, 1994), whereas higher densities resulted in higher biomass but reduced survival and growth rates. In the present study, the feeding conditions allowed an increase from 100 to 200 crayfish·m^{-2} with no reduction in survival or growth, biomass values being doubled. The low and similar percentages of juveniles lacking chelipeds (a density dependant factor related to mortality) suggest that no magnification of agonistic behaviour took place in the tanks with the higher density.

In summary, this study shows that decapsulated *Artemia* cysts can be withdrawn at day 20 and that a faster growth can be obtained if cysts are supplied up to day 50. Furthermore, a stocking density of 200 crayfish·m^{-2} can be advised under the mentioned feeding conditions.

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