Use of three forms of decapsulated *Artemia* cysts as food for juvenile noble crayfish (*Astacus astacus*)

A. Kouba, J. Hamáčková, M. Buřič, T. Policar, P. Kozák

South Bohemia Research Center of Aquaculture and Biodiversity of Hydrocenoses, Faculty of Fisheries and Protection of Waters, University of South Bohemia in České Budějovice, Vodňany, Czech Republic

ABSTRACT: Summerling (six-month old) noble crayfish fed two forms of freshly decapsulated (FD) *Artemia* cysts more than tripled their weight during a 75-day experiment under controlled conditions. Final survival rates were at the level of 90%. Feeding commercially available industrially decapsulated cysts resulted in both poor growth and reduced survival (22.2%). Therefore FD *Artemia* cysts may be an effective diet for crayfish culture, but dehydrated industrially decapsulated (DID) cysts should be used with caution to avoid products of low quality. That should be considered especially in slow feeders such as crayfish because of leaching of nutrients from DID cysts. Costs of the processing of freshly decapsulated cysts are discussed.

Keywords: astacid crayfish; brine shrimp; decapsulation; live food

Various methods of using *Artemia* as feed are employed in both fish (Sorgeloos et al., 2001; Celada et al., 2007) and crustacean (Sorgeloos et al., 1998; Naegel and Rodríguez-Astudillo, 2004) culture. Moreover, decapsulation of *Artemia* cysts provides numerous benefits, including disinfection, improved hatchability and easy storage, and is less expensive and labour intensive than regular hatching (Bruggeman et al., 1980; Van Stappen, 1996). Even low-quality cysts can be used as a food source (Ribeiro and Jones, 1998), and decapsulated cysts preserve a higher energy content than freshly hatched nauplii (Vanhaecke et al., 1983).

Studies on African catfish and, to a lesser extent, on Asian catfish (Pector et al., 1994; Bardócz et al., 1999; García-Ortega et al., 2000; Hung et al., 2002), cyprinids (Vanhaecke et al., 1990; Harzevili et al., 2003; Kaiser et al., 2003) and some ornamental fish (Lim et al., 2002, 2003) have shown decapsulated *Artemia* cysts to be a good alternative to nauplii. Decapsulated cysts have also been used for the rearing of marine shrimp of the genera *Penaeus* (Kuban et al., 1983; Stael et al., 1995; Ribeiro and Jones, 1998) and *Metapenaeus* (Royan, 1980), freshwater prawns (Bruggeman et al., 1980), crabs (Davis et al., 2005) and crayfish (González R. et al., 2009a).

Overall, results of studies comparing feeding decapsulated cysts with feeding nauplii have been inconsistent. The variability may result from differences in feeding strategies of the cultured species (planktonic vs. benthic) as well as in the quality of decapsulated cysts. This quality may decline because of differences in the technology of harvesting or processing (Vanhaecke et al., 1990). There are many commercially decapsulated cyst feeds available and their quality and price vary widely.

We evaluated the survival and growth of noble crayfish (*Astacus astacus*), a threatened and vulnerable species with important socio-cultural, economic, and educational impacts (Souty-Grosset et

Supported by the Ministry of Education, Youth, and Sports of the Czech Republic (Project No. CZ.1.05/2.1.00/01.0024), by the Ministry of Agriculture of the Czech Republic (Project No. QH71305), and by the Grant Agency of the University of South Bohemia (Project No. 047/2010/Z).

al., 2006), fed two forms of freshly decapsulated (FD), and freeze-dried industrially decapsulated (DID) cysts.

MATERIAL AND METHODS

Summerlings (six-month old noble crayfish) were held for 45 days at water temperatures of 9–12°C to synchronize moulting. One hundred and thirty-five specimens (mean weight 313.3 ± 36.2 mg; range 250–380 mg) were used for a 75-day experiment under controlled conditions. The experiment was divided into three periods which took 25 days. Crayfish were reared in 9 glass aquaria (14.3 l, bottom area 0.075 m^2 at a density of 200 crayfish per m^2) fed by water from a recirculating system comprising a reservoir with bio-filter, pump, piping, and the aquaria. The flow rate was 0.5 l/min. The volume of the recirculating system was 850 l, and water lost due to semi-weekly cleaning and evaporation was replaced by tap water. Two bricks (28.5 ×13.5 × 6.5 cm), each with 39 cross holes (26 and 13 holes with a profile of 1 × 3 cm and 1 × 1 cm, respectively), were placed in each aquarium to provide shelters for the crayfish. The light/dark regime was 12 h:12 h.

Water temperature was maintained by three 300 W aquarium heaters placed in the reservoir and monitored hourly by automatic temperature loggers. Dissolved oxygen level was measured twice a day, and pH was measured daily. During the experiment, water temperature, oxygen content and pH were 20.0 ± 0.9°C, > 7.5 mg/l, and 7.7 ± 0.2, respectively. Other water quality parameters, checked bi-weekly, were: NO_3-N, 4.37 ± 1.38 mg/l; NO_2-N, 0.01 ± 0.01 mg/l; and NH_4-N, 0.10 ± 0.07 mg/l.

Crayfish were fed three forms of decapsulated *Artemia* cysts. Processing of freshly decapsulated cysts (Ocean Nutrition™ Brine shrimp eggs, Salt Lake City, USA) was done according to methods described by Van Stappen (1996) (FD-Van Stappen) and Adámková (1999) (FD-Adámková). In both cases, all recommended parameters were applied. FD cysts were prepared every three days and refrigerated. The last treatment was fed freeze-dried industrially decapsulated cysts produced in China and purchased from EasyFish (Rohatec, Czech Republic). All treatments were tested in triplicate. Crayfish were fed once a day in excess, so that some cysts remained uneaten. Moulting was determined daily by the visible presence of a shed exoskeleton or part thereof on the bottom.

Every 25 days, surviving crayfish were counted and individually weighed with an electronic balance to the nearest 0.1 mg after removal of excess water with absorbent tissue. Survival rates were calculated as percent survival from the initial sample and arc-sine transformed prior to statistical analysis using Statistica software 8.0 for Windows (StatSoft, Prague, Czech Republic). Results were subjected to analysis of variance after assessing for normality and homoscedasticity by Kolmogorov-Smirnov and Levene's tests, respectively. Tukey's multiple comparison was used as a *post hoc* test. For all statistical tests, P values ≤ 0.05 were considered significant. All data are presented as the mean ± SD.

RESULTS AND DISCUSSION

At least around a half of crayfish fed FD cysts moulted during the last five days of the first period (8.0 ± 0.0 and 7.3 ± 1.5 visible exoskeletons in crayfish fed FD-Van Stappen and FD-Adámková cysts, respectively). The next periods of moulting were noted in the second half of the following, and during the last two thirds of the third period in FD-Van Stappen group (8.3 ± 2.5 and 10.0 ± 2.6 exoskeletons, respectively). Crayfish moulted identically in the FD-Adámková group (8.0 ± 2.6 and 10.3 ± 1.5 exoskeletons in the second and third period, respectively). A similar pattern was also observed in crayfish fed DID cysts (6.0 ± 3.0 exoskeletons) between the 21st and 24th day; nevertheless, moulting was infrequent in the second and third period (1.3 ± 0.6 and 0.7 ± 0.6 exoskeletons, respectively). Crayfish fed FD cysts more than tripled in weight over the 75 days. Crayfish reared on DID cysts increased in weight during the first 25 days but their values were significantly lower. Thereafter growth was very poor in this group. Growth during the first 25-day period could be partly attributed to the increased water temperature relative to the acclimation period. This is in agreement with previous studies which pointed out an importance of water temperature for growth in crayfish (Reynolds, 2002; Kozák et al., 2009). Final survival rates of crayfish fed FD were at the level of 90% (Table 1) but they were much lower in crayfish fed DID cysts (22.2%). These losses occurred especially in the third period. Cannibalisms were occasionally observed on some dead crayfish in crayfish reared on DID cysts.

Table 1. Survival and growth values (mean ± SD) for noble crayfish (*Astacus astacus*) fed freshly decapsulated cysts prepared according to Van Stappen (1996) (FD-Van Stappen), Adámková (1999) (FD-Adámková), and dried industrially decapsulated (DID) *Artemia* cysts for 75 days

Parameter	Diet	Days			
		0	25	50	75
Body weight (mg)	FD-Van Stappen	314.1 ± 0.6[a]	442.0 ± 16.2[a]	700.1 ± 24.6[a]	1023.5 ± 64.5[a]
	FD-Adámková	312.7 ± 3.3[a]	447.6 ± 6.6[a]	706.8 ± 20.2[a]	1050.6 ± 37.2[a]
	DID	313.0 ± 1.7[a]	367.9 ± 17.3[b]	382.4 ± 20.1[b]	385.5 ± 46.0[b]
Survival (%)	FD-Van Stappen	100.0 ± 0.0[a]	93.3 ± 6.7[a]	91.1 ± 10.2[a]	88.9 ± 10.2[a]
	FD-Adámková	100.0 ± 0.0[a]	95.6 ± 3.8[a]	93.3 ± 6.7[a]	93.3 ± 6.7[a]
	DID	100.0 ± 0.0[a]	91.1 ± 10.2[a]	75.6 ± 7.7[a]	22.2 ± 3.8[b]

Values with differing letters within each parameter in columns were significantly different ($P \leq 0.05$)

Freshly decapsulated *Artemia* cysts can be effective as food for juvenile crayfish, but it is limited by the cost. However, supplementing *Artemia* products to dry diets can improve survival and growth in crayfish, as has been found for *Artemia* nauplii (González et al., 2008, 2009b). Moreover, according to González et al. (2009a), using freshly decapsulated cysts as a supplement, rather than *Artemia* nauplii, supported significantly higher growth of *Pacifastacus leniusculus* compared to nauplii.

Decapsulated *Artemia* cysts are a suitable food for fish larvae when dried at 35–40°C (Vanhaecke et al., 1990; Pector et al., 1994; Lim et al., 2002; Harzevili et al., 2003). However, protein solubility and enzyme activity decreased in cysts treated at 96°C, and it negatively influenced fish growth (García-Ortega et al., 2000). Processing at higher temperatures also resulted in increased leaching of nutrients (Ribeiro and Jones, 1998), which should be taken into account for slow feeders such as crayfish. In these respects, the quality of many commercially available products is uncertain.

Furthermore, costs of the preparation of FD cysts are mainly represented by the consumption of bleach liquid (solution of sodium hypochlorite). Methodology recommended for Czech culturists was written by Adámková (1999). This method is based on using a commonly known bleach liquid Savo Original (Bochemie, Bohumín, Czech Republic) which is very well obtainable in the market. However, decapsulating one kilogram of *Artemia* cysts is very expensive (1100–1400 CZK/kg) in this case. On the other hand, costs of decapsulation performed by Van Stappen (1996) using Savo Original and commercially available solution of sodium hypochlorite are several times lower (250–350 and 80–120 CZK/kg, respectively).

To sum up, freshly decapsulated *Artemia* cysts can be used effectively as an exclusive diet in juvenile crayfish or as a supplement to a dry diet. Costs of the procedure of decapsulation should be considered carefully. Usually, low priced, industrially dried decapsulated cysts may also be used, either exclusively or as supplemental feeds, but they should be used with caution, monitoring animal growth and survival to avoid products of low quality.

Acknowledgements

We deeply appreciate the assistance of the Lucidus Consultancy for English correction of this manuscript.

REFERENCES

Adámková I. (1999): Procedure of decapsulation of dormant *Artemia* cysts and their use in aquaculture. Edice metodik VÚRH JU 58, 10. (in Czech)

Bardócz T., Kovács É., Radics F., Sándor Zs. (1999): Experiments for the improved use of decapsulated *Artemia* cysts in intensive culture of African catfish larvae. Journal of Fish Biology, 55, Suppl. A, 227–232.

Bruggeman E., Sorgeloos P., Vanhaecke P. (1980): Improvements in the decapsulation technique of *Artemia* cysts. In: Persoone G., Sorgeloos P., Roels O., Jaspers

E. (eds): The Brine Shrimp Artemia. Ecology, Culturing, Use in Aquaculture. Vol 3. Universa Press, Wetteren, Belgium, 261–269.

Celada J.D., Carral J.M., Rodríguez R., Sáez-Royuela M., Aguilera A., Melendre P., Martín J. (2007): Tench (*Tinca tinca* L.) larvae rearing under controlled conditions: density and basic supply of *Artemia* nauplii as the sole food. Aquaculture International, 15, 489–495.

Davis J.A., Wille M., Hecht T., Sorgeloos P. (2005): Optimal first feeding organism for South African mud crab *Scylla serrata* (ForskÍl) larvae. Aquaculture International, 13, 187–201.

García-Ortega A., Verreth J., Van Hoornyck A., Segner H. (2000): Heat treatment affects protein quality and protease activity in decapsulated cysts of *Artemia* when used as starter food for larvae of African catfish *Clarias gariepinus* (Burchell). Aquaculture Nutrition, 6, 25–31.

González Á., Celada J.D., González R., García V., Carral J.M., Sáez-Royuela M. (2008): *Artemia* nauplii and two commercial replacements as dietary supplement for juvenile signal crayfish, *Pacifastacus leniusculus* (Astacidae), from the onset of exogenous feeding under controlled conditions. Aquaculture, 281, 83–86.

González R., Celada J.D., Carral J.M., González Á., Sáez-Royuela M., García V. (2009a): Decapsulated *Artemia* cysts as dietary supplement for juvenile crayfish (*Paciastacus leniusculus*, Astacidae) at different food supply frequencies from the onset of exogenous feeding under controlled conditions. Aquaculture, 295, 200–204.

González R., Celada J.D., García V., González Á., Carral J.M., Sáez-Royuela M. (2009b): The artificial incubation of crayfish eggs: review and report from an experimental study concerning the effects of offspring (maternal or artificial incubation) on the survival and growth of juvenile signal crayfish (*Pacifastacus leniusculus*, Astacidae). Reviews in Fish Biology and Fisheries, 19, 167–176.

Harzevili A.S., De Charleroy D., Auwerx J., Vught I., Van Slycken J. (2003): Larval rearing of chub, *Leuciscus cephalus* (L.), using decapsulated *Artemia* as direct food. Journal of Applied Ichthyology, 19, 123–125.

Hung L.T., Tuan N.A., Cacot P., Lazard J. (2002): Larval rearing of the Asian catfish, *Pangasius bocourti* (Siluroidei, Pangasiidae): alternative feeds and weaning time. Aquaculture, 212, 115–127.

Kaiser H., Endemann F., Paulet T.G. (2003): A comparison of artificial and natural foods and their combinations in the rearing of goldfish, *Carassius auratus* (L.). Aquaculture Research, 34, 943–950.

Kozák P., Buřič M., Kanta J., Kouba A., Hamr, P., Policar T. (2009): The effect of water temperature on the number of moults and growth of juvenile signal crayfish *Pacifastacus leniusculus* Dana. Czech Journal of Animal Science, 54, 286–292.

Kuban F.D., Wilkenfeld J.S., Lawrence A.L. (1983): Survival and growth of *Penaeus setiferus* L. and *Penaeus aztecus* Ives larvae fed *Artemia* beginning at the protozoea-two substage versus the mysis-one substage. Journal of the World Mariculture Society, 14, 38–48.

Lim L.C., Cho Y.L., Dhert P., Wong C.C., Nelis H., Sorgeloos P. (2002): Use of decapsulated *Artemia* cysts in ornamental fish culture. Aquaculture Research, 33, 575–589.

Lim L.C., Dhert P., Sorgeloos P. (2003): Recent developments in the application of live feeds in the freshwater ornamental fish culture. Aquaculture, 227, 319–331.

Naegel L.C.A., Rodríguez-Astudillo S. (2004): Comparison of growth and survival of white shrimp postlarvae (*Litopenaeus vannamei*) fed dried *Artemia* biomass versus four commercial feeds and three crustacean meals. Aquaculture International, 12, 573–581.

Pector R., Tackaert W., Abelin P., Ollevier F., Sorgeloos P. (1994): A comparative study on the use of different preparations of decapsulated *Artemia* cysts as food for rearing African catfish (*Clarias gariepinus*) larvae. Journal of the World Aquaculture Society, 25, 366–370.

Reynolds J.D. (2002): Growth and reproduction. In: Holdich D.M. (ed.): Biology of Freshwater Crayfish. Blackwell Science, Oxford, England, 152–191.

Ribeiro F.A.L.T., Jones D.A. (1998): The potential of dried, low-hatch, decapsulated *Artemia* cysts for feeding prawn post-larvae. Aquaculture International, 6, 421–440.

Royan J.P. (1980): Decapsulated brine shrimp cysts – an ideal feed for shrimps in aquaculture. Indian Journal of Marine Sciences, 9, 125–126.

Sorgeloos P., Coutteau P., Dhert P., Merchie G., Lavens P. (1998): Use of brine shrimp, *Artemia* spp., in larval crustacean nutrition: a review. Reviews in Fisheries Science, 6, 55–68.

Sorgeloos P., Dhert P., Candreva P. (2001): Use of the brine shrimp, *Artemia* spp., in marine fish larviculture. Aquaculture, 200, 147–159.

Souty-Grosset C., Holdich D.M., Noël P.Y., Reynolds J. D., Haffner P. (eds) (2006): Atlas of Crayfish in Europe. Patrimoines Naturels. Vol. 64. Muséum National d'Histoire Naturelle, Paris.

Stael M., Sanggontanagit T., Van Ballaer E., Puwapanich N., Tunsutapanich A., Lavens P. (1995): Decapsulated cysts and Artemia flakes as alternative food sources for the culture of *Penaeus monodon* postlarvae. In:

Lavens P., Jaspers E., Roelants I. (eds): Larvi'95 – Fish and Shellfish Larviculture Symp., Gent, Belgium. European Aquaculture Society Special Publication 24, 342–345.

Vanhaecke P., Lavens P., Sorgeloos P. (1983): International study on *Artemia* XVII. Energy consumption in cysts and early larval stages of various geographical strains of *Artemia*. Annales Societé Royale Zoologie de Belgique, 113, 155–164.

Vanhaecke P., De Vrieze L., Tackaert W., Sorgeloos P. (1990): The use of decapsulated cysts of the brine shrimp *Artemia* as direct food for carp *Cyprinus carpio* L. larvae. Journal of the World Aquaculture Society, 21, 257–262.

Van Stappen G. (1996): Use of cysts. In: Lavens P., Sorgeloos P. (eds): Manual on the Production and Use of Live Food for Aquaculture. FAO Fisheries Technical Paper 361. FAO, Rome, 107–136.

Received: 2010–01–25
Accepted after corrections: 2010–09–02

Corresponding Author

Ing. Antonín Kouba, University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemia Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zátiší 728/II, 389 25 Vodňany, Czech Republic
Tel. +420 383 382 402, fax +420 383 382 396, e-mail: akouba@frov.jcu.cz