JUVENILE PRODUCTION OF THE FRESHWATER PRAWN *CRYPHIOPS*
CAEMENTARIUS (DECAPODA: PALAEMONIDAE) UNDER
LABORATORY CONDITIONS IN COQUIMBO, CHILE

PRODUCCION DE JUVENILES EN CONDICIONES DE LABORATORIO DEL
CAMARON DE RIO CRYPHIOPS CAEMENTARIUS (DECAPODA:
ALAEMONIDAE) EN COQUIMBO, CHILE

Jaime Meruane^{1*}, Miguel Rivera, Cristina Morales, César Galleguillos
& Hidetsuyo Hosokawa²

¹Departamento de Acuicultura, Facultad de Ciencias del Mar, Universidad Católica del Norte,
Casilla 117, Coquimbo-Chile.

²Laboratory of Fish Nutrition, Faculty of Agriculture, Kochi University, Monobe, Nankoku 783-8502, Japan,

*Corresponding author. Email: jmeruane@ucn.cl

ABSTRACT

Two culture experiments from 1995 until 1997 were carried out with larvae obtained from gravid females of *Cryphiops caementarius* (Molina 1782), to define a methodology in order to produce juvenile using controlled conditions of water temperature, salinity and feeding. Time in culture of larval development and the survival rate were determined. Two distinct types of culture systems were studied. Both systems incorporated a standard alimentation using nauplius of *Artemia* sp. plus microalgae; however the second system included, in addition, a supplement of fish and chicken eggs. The decrease in survival rate in both cultures systems was related to morphological changes in each stage of development. Juveniles were obtained after 98 days of culture with the first culture system, whereas in the second culture system, the time to reach the first juvenile stage was 62 days. In addition, an increase in the values for metamorphosis synchronism induced a good amount of metamorphosed animals (juveniles) during the time involved in this study.

KEYWORDS: Chile, freshwater prawn, larvae, culture technology, metamorphosis, juvenile.

RESUMEN

Con larvas obtenidas de hembras grávidas de *Cryphiops caementarius* (Molina 1782), se realizó dos experiencias de cultivo entre 1995 y 1997. Estas experiencias fueron diseñadas con el propósito de definir una metodología que permitiera producir juveniles de la especie, utilizando condiciones controladas de temperatura, salinidad y alimentación. Durante este estudio se determinó el desarrollo larval, su duración y la tasa de supervivencia. La disminución en la tasa de supervivencia en ambos cultivos fue coincidente con los cambios morfológicos en cada estado de desarrollo. Durante el primer cultivo, el estado de primer juvenil fue alcanzado a los 98 días. Durante la segunda experiencia, el tiempo empleado en la obtención del primer estado de juvenil fue de 62 días. Durante ésta, se utilizó un suplemento alimenticio consistente en pescado y huevos de gallina. Otro de los resultados importantes logrados fue el aumento en los valores de sincronía en la metamorfosis, lo cual permitió obtener una importante cantidad de animales metamorfoseados (juveniles) durante el tiempo involucrado en el estudio.

PALABRAS CLAVES: Chile, camarón de río, larvas, tecnología de cultivo, metamorfosis, juveniles.

INTRODUCTION

Cryphiops caementarius (Molina 1782) (Decapoda: Palaemonidae), is a freshwater prawn distributed from the South of Perú (10°S) to Northern central Chile (30°S) (Bahamonde & Vila 1971, Castro 1966, Elias 1960, Viacava *et al.* 1978). Ripe and gravid females move downstream seeking estuarine conditions to spawn. After hatching larvae stay in the estuarine zone and/or the sea, returning to the river as juveniles (Norambuena 1977, Rivera & Meruane 1987). This conduct has been reported for several species of the genus *Macrobrachium* with prolonged developmental pattern (New & Singholka 1984, Jaliha *et al.* 1993, New 1995, 2005). In Chile, the commercial production of freshwater prawn is not very developed, yet. The culture companies get the juveniles directly from rivers, instead of producing them in a hatchery. In order to incorporate this activity as an industry, it is necessary to count with abundant and constant juvenile production. It is necessary ensure culture success in spite of the availability of "seeds" from nature.

Artificial productions of postlarvae or juvenile in hatcheries have been attempted by several authors using different techniques. However, the main problems found were the variability in the number of larval stages, length of developmental period, food, environmental requirements and morphological changes of larvae during their life cycle. Sanzana (1976) described the first three stages of development. Munaylla (1977) obtained *C. caementarius* postlarvae with 17 zoeal stages. Viacava *et al.* (1978) studied the larval survival, with salinity variation and with and without feeding, only during the first and second stages of development. Hernández (1981) reached the 4° stage of mysis but without a description of larval stages. Schmiede *et al.* (1982) carried out larval development at experimental conditions during 130 days and with 16 moult cycles. Rivera *et al.* (1983) maintained larvae during 153 day reaching the 10° zoeal stage. Rivera & Meruane (1987) studied the effects of salinity and food on larval survival and metamorphosis of *C. caementarius* and found that the first juvenile stage was reached with a reduction of 10‰ at 80 days of culture.

The purpose of this study was to develop the technology culture for larvae of *Cryphiops*

caementarius under controlled conditions through adjusting and modifying existing techniques to obtain the complete cycle until the first juvenile stage.

MATERIALS AND METHODS

An experimental hatchery for the production of juveniles of *Cryphiops caementarius* was set up at the facilities of the Universidad Católica del Norte, Coquimbo (29° 58'S; 71° 21'W), Chile. It included one water treatment room, which had a series of cartridge filters (25, 10, 5 and 1 µm), a water heater exchanger and UV equipment (QR-80) for sterilization. During ontogeny, *Cryphiops caementarius* needs different salinity concentrations. Seawater was obtained from La Herradura Bay (29° 58'S; 71° 21'W) using of two lift pumps (ESPA Flipper 1,5" Model 75), while freshwater was obtained from the domestic supply. These water sources were used to prepare water at desired salinities (Table I). The air was carried from the blower room and added permanently to the tanks through a 3/4" (1,9 cm) PVC hose. Another room was available to prepare the feed for larvae (microalgae and *Artemia* nauplii). The larviculture room had 2 tanks of 1000 L of capacity, operating alternatively so that one remained as a reservoir tank to be used the next day. Water and feeding conditions during the larval culture are shown in Table I.

Females with fertilized eggs were obtained from a prawn farm and also from the natural environment, and transported to the experimental laboratory. Here they were placed individually in 100 L tanks with freshwater and continuous aeration. The spawning occurred during the first night, and early in the morning larvae were removed, examined and transported to the larviculture room.

Two cultures at different times were developed. The first started with a density of 23 larvae L⁻¹, and added feed was *Artemia* at 1 nauplii mL⁻¹, microalgae at 50,000-150,000 cel mL⁻¹, to 25°C water temperature, salinity at 20‰ and 100‰ daily of water exchange. The second culture started at the same salinity, temperature and food conditions, but at a density of 40 larvae L⁻¹. In addition, this second culture received triturated fresh fish flesh and boiled chicken eggs as nutritional supplements. In both cultures, the salinity values were decreased gradually in relation to the developmental stage of larvae with the purpose to get juveniles (Table I).

TABLE I. Culture conditions for production of *Cryphiops caementarius* juvenile.TABLA I. Condiciones de cultivo para la producción de juveniles de *Cryphiops caementarius*.

Stages	Water conditions			Feeding conditions		
	Daily exchange (%)	Temperature (°C)	Salinity (‰)	Microalgae (cell m ⁻¹)	Artemia (nauplii mL ⁻¹)	Artificial food (g L ⁻¹)
Zoea 1	100	25	10	80,000	1,5	-,-
Zoea 2	100	25	15	80,000	1,5	0,01-0,02
Zoea 3	100	25	20	80,000	1,5	0,01-0,02
Zoea 4	100	25	25	100,000	1,5	0,03-0,05
Zoea 5	100	25	25	150,000	2,0	0,03-0,05
Zoea 6	100	25	30	150,000	2,0	0,03-0,05
Zoea 7	100	25	30	150,000	2,0	0,05-0,07
Zoea 8	100	25	30	150,000	2,0	0,05-0,07
Zoea 9	100	25	30	150,000	2,0	0,05-0,07
Zoea 10	100	25	30	150,000	2,0	0,05-0,07
Zoea 11	100	25	30	100,000	3,0	0,09-0,12
Zoea 12	100	25	30	100,000	3,0	0,09-0,12
Zoea 13	100	25	30	100,000	3,0	0,09-0,12
Zoea 14	100	25	30	100,000	3,0	0,09-0,12
Zoea 15	100	25	30	100,000	3,0	0,09-0,12
Zoea 16	100	25	25	80,000	3,0	0,14-0,16
Zoea 17	100	25	20	80,000	3,0	0,14-0,16
Zoea 18	100	25	15	50,000	3,0	0,14-0,16
Juvenile	100	25	5	-,-	-,-	0,20-0,22

Tanks were controlled every day and accumulated survival was registered during all culture cycle. The larval stages were determined and quantified

according to previous descriptions made by Rivera *et al.* 1987. Larval stage endpoints were when 50% of larvae developed into the next stage (Table II).

TABLE II. Duration of each larval stage.

TABLA II. Duración de cada estado de desarrollo larval.

Stages	Duration in days	
	First culture	Second culture
Zoea 1	3	3
Zoea 2	13	2
Zoea 3	6	3
Zoea 4	6	4
Zoea 5	5	3
Zoea 6	4	4
Zoea 7	6	4
Zoea 8	6	6
Zoea 9	4	4
Zoea 10	5	2
Zoea 11	6	3
Zoea 12	5	5
Zoea 13	5	4
Zoea 14	5	3
Zoea 15	6	3
Zoea 16	5	3
Zoea 17	5	3
Zoea 18	3	3
First juvenile	Day 98	Day 62

RESULTS

FIRST CULTURE

The daily survival rate of *Cryphiops caementarius* larvae showed that the main decrease was coincident with each of the changes in the developmental stages. A high mortality was observed between zoea 2 to zoea 5, probably due to changes made in the feeding system. At this point the quality, quantity and the frequency of food supply should be adjusted to avoid a lengthening of zoea 2 stage. The prolongation of this stage demonstrated an increase in mortality during 10 to 20 days of culture. This is associated with the natural mortality due to developmental changes of these individuals. The average moulting time for zoea 2 is 4 to 5 days. In the culture process mortality diminishes once each of the factors involved in the development and growth of larvae are regulated; such as quality and quantity of food,

culture water quality and stable water temperature. As seen in Fig. 1 the daily larval mortality during 100 days of culture displayed an accumulated value close to 50%, and the first postlarvae or juvenile was obtained at 98 days. The highest mortalities were observed during 10 to 30 days of culture. At this time larvae were very sensitive to handling (water replacement) and to the adjustment of external factors as food, temperature and salinity. This indicated that the mortality increases with lengthening of time in zoea 2 stage is observed. As the larvae continued its development and growth, the culture conditions became less significant for larval survival. However, an increase in nutritional requirements and consequently an increment in metabolic waste products during the progression of larval development necessitate that the control of water quality be the first priority. The increase in mortality during days 45 and 50 could be results from the metamorphosis induction through changes in culture water salinity (Fig. 1).

FIGURE 1. Daily mortality of *C. caementarius* larvae during both cultures.

FIGURA 1. Mortalidad diaria de larvas de *Cryphiops caementarius* en ambos cultivos.

FIGURE 2. Total production of *C. caementarius* juvenile during the second culture.

FIGURA 2. Producción total de juveniles de *Cryphiops caementarius* durante el segundo cultivo.

The duration of the zoea 2 stage was 13 days (Table II), situation that increased the mortality. The next stages of development were inside of the normal ranges of time, according to previous investigations carried out by Rivera *et al.* (1987).

All these information indicated that the most critical moments of the larval development of these prawns are directly related to morphological changes associated to a new developmental stage.

Adjustment of the quantities and frequencies of feeding, the standardization of the values of water temperature, and the maintenance of culture water conditions are the factors that need to be evaluated during the second larval mass culture in order to establish if is possible a synchronism in larval stage changes, which apparently would lead to an increment of the survival rate and probably a decrease of the larval cycle time.

SECOND CULTURE

During this culture, an important mortality between the stages of zoea 2 and zoea 5 also occurred, although considerably minor than in the first culture conditions. This second culture was properly controlled incrementing the quantity of food (fish and egg) delivered *ad libitum*. This was carried out by a redistribution in the frequency of *Artemia* feeding (one in the morning, another in the afternoon and one during the night), and maintaining the microalgae concentration constant. New microalgae at densities of 50,000 cell/mL⁻¹ were added after each total replacement of water (Table I).

The feeding regime probably diminished the permanency of larvae in each stage, especially during the zoea 2, exhibited notably decrease in larvae mortalities (Fig. 1).

Around day 30 of culture a mass infection with filamentous bacteria from the natural source of seawater was detected and caused an abrupt increment in the mortality rate (Fig. 1) in all the culture tanks. Once the microorganisms were identified, an antibiogram was carried out and the bacteria were found to be sensitive to Tetracycline® at a concentration of 3 mg/L⁻¹. Larvae were incubated with Tetracycline® for one minute and immediately after the treatment, the larvae were seeded in previous disinfected tanks with microfiltered and sterilized water, producing a massive moulting of the animals during the day, which eliminated great

part to the bacteria. A preventive treatment with Tetracycline® was done every one month approximately, and until the larvae reached the stage of zoea 10. After that massive contamination with bacteria the system did not undergo any other alteration.

The first juvenile was obtained after 62 days of culture and 36 days earlier than in the first culture (Fig.1). The juvenile production increased progressively, and after 19 days they were between 200 and 300 new juveniles daily, and from the 22nd day after metamorphosis, over 4,000 individuals were obtained (Fig. 2).

DISCUSSION

The harvested larvae in both cultures showed a normal behavior with respect to similar experiments done by Rivera & Meruane (1987) and available information on cultivation of larvae of *M. rosenbergii* done in others countries, especially from Southeast Asia (New & Singholka 1984, New 1995; 2005).

C. caementarius and *M. rosenbergii* belong to the Family Palaemonidae. In general, they have similar requirement during their development and growth. However required temperature and salinity differ. *M. rosenbergii* completes larval development at temperatures between 27 to 30°C (New & Singholka 1984), while *C. caementarius* does so at 18 to 20°C (Bahamonde & Vila 1971, Báez *et al.* 1983, Elias 1960, Nava 1980, Rivera *et al.* 1983, Rivera & Meruane 1987). They also require different salinities (New & Singholka 1984, Rivera & Meruane 1987). These species have larval stages that naturally occur in estuaries or the sea, while for metamorphosis freshwater is required. If these conditions are not regulated, the larvae experiment frequent moults that do not always led to a change of developmental stage, but delaying the metamorphosis. Postponement of metamorphosis causes high growth variability, mortality and vulnerability to diseases.

The first culture allowed to develop a methodology to obtain a good quantity of larvae (> 50% of viability) coming from ovigerous females gathered at the natural environment or from the culture ponds. During this research it was also possible to obtain adequate larval survivals until the premetamorphic

stage (> 50%), even considering the delay of intermoult period due to an insufficient feeding of larvae, detection and treatment of potential illness, search of inducer that permits the metamorphosis and one correct management for adaptation of juveniles to mass culture tanks conditions. The first culture culminated with ≈ 400 juveniles produced after 98 days under laboratory conditions and valuable information to be applied during the second cultivation was gotten.

The improvement in key factors detected during the first culture and applied during the second experiment resulted in the attainment of juveniles 36 days before the first experiment. Interestingly the metamorphosis synchronism was incremented, permitting a sustained production of juveniles (4,120) during the time involved in this study. For this reason is necessary to improve even more the synchronism rate thereby achieving better percentages of metamorphosis in each one of the cultivations to be carried out. Other factors which need more investigation are the determination of the composition, ration and frequency of diets, the identification of new alternatives to the natural diets used until now, the water culture conditions (biological and chemical), as well as the identification of inducers and their effects on the metamorphosis and their synchronism.

Our results shows, the importance of factors such salinity, temperature and feeding conditions on larval production and subsequent juvenile yield. The achieved methodology assures the production of animals and could provide prime material (juvenile) produced at controlled conditions to farmers producing culture and to those that do so in the near future.

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