EFFECTS OF DEAD EGG REMOVAL FREQUENCY ON STAGE 2 JUVENILE PRODUCTION IN ARTIFICIAL INCUBATION OF *AUSTROPOTAMOBIUS PALLIPES* LEREBOULLET

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ABSTRACT

In artificial incubation (AI) of astacid species, which have a long embryonic development, eggs have to remain during long periods without maternal care and it is recommended to remove the dead ones. With the aim to test the effect on final stage 2 juvenile production of different removal frequencies, four treatments were tested: removal every 4 days, every 7 days, every 10 days and no removal. Using an egg density of 2.2 eggs/cm^2, stage 2 juvenile production rate was significantly better (70.3%) with removal every four days than with less frequent removals. However there were not detected statistical differences between the control (no egg removal) and the other treatments. The relatively low incubation density (2.2 eggs/cm^2) could partially explain the general good results achieved, even when dead eggs were not removed.

Key-words: White-clawed crayfish, artificial incubation, juvenile production.

EFFETS DE LA FRÉQUENCE D'ENLÈVEMENT DES ŒUFS MORTS SUR LA PRODUCTION DE JUVÉNILES STADE 2 DANS L'INCUBATION ARTIFICIELLE D'*AUSTROPOTAMOBIUS PALLIPES* LEREBOULLET

RÉSUMÉ

Lors de l'incubation artificielle (IA) d'espèces d'astacides, qui ont un développement embryonnaire long, les œufs doivent rester longtemps sans soins maternels et il est recommandé d'enlever les morts. Dans le but de tester l'effet de différentes fréquences d'enlèvement des œufs morts sur la production finale de juvénile stade 2, quatre traitements ont été testés : enlèvement tous les 4 jours, tous les 7 jours, tous les 10 jours et sans enlèvement. Avec une densité d'œufs de 2,2 œufs/cm^2 les taux de juvéniles stade 2 atteints lorsque l'enlèvement a été effectué tous les quatre jours ont été considérablement meilleurs (70,3 %) que ceux obtenus avec enlèvements moins fréquents. Cependant, il n'y a pas de différence statistique entre les résultats des incubations sans enlèvement des œufs morts, et les autres. La densité d'incubation relativement basse (2,2 œufs/cm^2) pourrait expliquer partiellement les bons résultats atteints, même lorsque les œufs morts n'ont pas étés enlevés.

Mots-clés : Écrevisse à pattes blanches, incubation artificielle, production de juvéniles.

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INTRODUCTION

Astacid crayfish culture has acquired an extraordinary interest in most European countries owing to their extraordinary gastronomic quality and high demand and also to the environmental situation derived from the severe decline of European crayfish stocks. Undoubtedly, the development and improvement of breeding techniques must play an important role in conservation plans for threatened species in order to provide juveniles for restocking actions, but also for grow-out purposes.

Under maternal incubation, aggressive interactions among breeders, handling, poor egg attachment and female diseases led to egg detachment and mortality (CELADA *et al*., 1988, 2001a; WOODLOCK and REYNOLDS, 1988; CARRAL *et al*., 1994). Also death of females results in a total loss of pleopodal eggs. Considering the relative low egg production, between 20 and 250 eggs per female (REYNOLDS *et al*., 1992) and a long incubation period of astacid species (6-9 months in wild habitats), any possibility to reduce the risk of egg losses deserves special interest. In this sense, artificial incubation (AI) allows for an independence of females and eggs losses derived from absence of maternal care can be minimized. Other advantages of this technique, such as availability of juveniles for collection, staggered production of different batches and avoided transmission of pathogens from broodstock to offspring, are described by CARRAL *et al*. (2003).

Our research team has developed AI techniques and proved its feasibility in the white-clawed crayfish (PÉREZ *et al*., 1998a, 1998b, 1999). Depending on stripping time and temperature of incubation, eggs remain in artificial devices for long periods of time, up to 106 days (PÉREZ *et al*. 1998a). Thus, periodic removal of dead eggs and stage 1 juveniles is usually carried out trying to avoid that fungal growth over them could have negative effects on healthy eggs surrounding, and to maintain water quality. However, this delicate operation requires a lot of labour and the viable eggs could be damaged, giving rise to new losses.

Trying to reduce an excessive handling of the eggs and thus the labour, in the present trial were tested four removal frequencies of dead eggs and stage 1 juveniles in order to know their effects on final juvenile production.

MATERIAL AND METHODS

Adult *A. pallipes* freshwater crayfish caught at the end of September in different populations of the province of León (Spain) were used in this study. The animals were transported to indoor experimental facilities and placed in square fibreglass tanks (100 × 100 × 30 cm, 1 m^2 bottom surface) with 20 cm of water depth and provided with shelters where mating and spawning took place. Eggs from 78 females were removed from maternal pleopods and used for the experiment. Phases of embryonic development were determined in accordance with the criteria of CELADA *et al*. (1991). Eggs were detached in phase VIII (embryo with mandibular rudiments, 20th January, mean of 77 days after spawning and 582 degree days). Experimental artificial incubation devices described by CARRAL *et al*. (1992) provided with a 1 l/min flow-through were used. The parameters of water quality were: pH = 8.1; hardness = 5.2ºd (calcium: 32.3 mg/l); total dissolved solids content = 108.5 mg/l, and total suspended solids content < 0.5 mg/l. Samples of water were taken weekly to analyse the content of ammonium and nitrite. Dissolved oxygen was measured daily.

Four treatments were performed:

– Removal of eggs every 4, 7 and 10 days.

– No egg removal.

Temperature was 10 ± 1ºC until eggs reached the eye stage (phase XIII, 1,142 degree days), when it was raised to 15 ± 1ºC up to final stage 2 juvenile production. Egg density was 2.2 eggs/cm^2.

Trying to prevent fungus transmission, dead eggs and stage 1 juveniles were removed using blunt forceps previously disinfected. In order to obtain data on evolution of viability, eggs were counted inside the incubation devices without handling every twenty days until hatching started. Then final number of stage 1 and stage 2 juveniles was quantified. Final efficiency rates were measured as number of stage 2 juveniles obtained from initial number of eggs.

Each treatment was tested on three replicates of 100 eggs. Arc-sine transformation of results was made and then examined by analysis of variance (ANOVA) using the STATISTICA 4.0. computer program. Mean comparison was tested using the Newman-Keuls test. The significance level was $P < 0.05$.

RESULTS

Parameters of water quality did not show alterations throughout the experiment and in all cases nitrite and ammonium were under 0.015 mg/l and 0.02 mg/l respectively. Dissolved oxygen was between 7 and 9 mg/l.

First hatchings were observed on 2nd April (mean of 149 days after spawning, 1,382 degree days) and stage 2 juveniles from 11th April (mean of 158 days after spawning, 1,517 degree days). Thus, artificial incubation lasted 81 days (51.2% of total embryonic development).

Hatching and stage 2 juvenile rates in the different treatments are included in Table I. Best mean hatching (85.0%) and stage 2 juvenile production rates (70.3%) were obtained when removal of dead eggs was performed every 4 days, being significantly better than the rest of treatments tested. However, there were no observed statistical differences when eggs were not removed and when this practice was made every 7 or 10 days.

Table I

Mean survival rates (% ± SEM) of *A. pallipes* eggs to stage 1 (hatching) and to stage 2 juvenile in each treatment.

Tableau I

Taux moyens de survie (% ± ESM) des œufs d'*A. pallipes* au stade 1 (éclosion) et au stade 2 juvénile dans chaque traitement.

Removal frequency	Hatching (%)	Stage 2 (%)
Every 4 days	85.0 ± 0.0	70.3 ± 2.8^a
Every 7 days	74.3 ± 8.5	56.7 ± 4.5^b
Every 10 days	77.7 ± 4.1	56.7 ± 3.9^b
No removal	78.7 ± 3.9	61.3 ± 2.7^b

Most mortality was concentrated in the last stages of embryogenesis (Figure 1), mainly from phase XIII-XIV (embryo with strongly developed eye pigment - embryo with strongly developed hepatopancreatic lobes) and the stage 2 ranging between 29.7 and 43.3% (mean 38.7%). Between hatching and stage 2 juvenile the mean mortality was 17.7%.

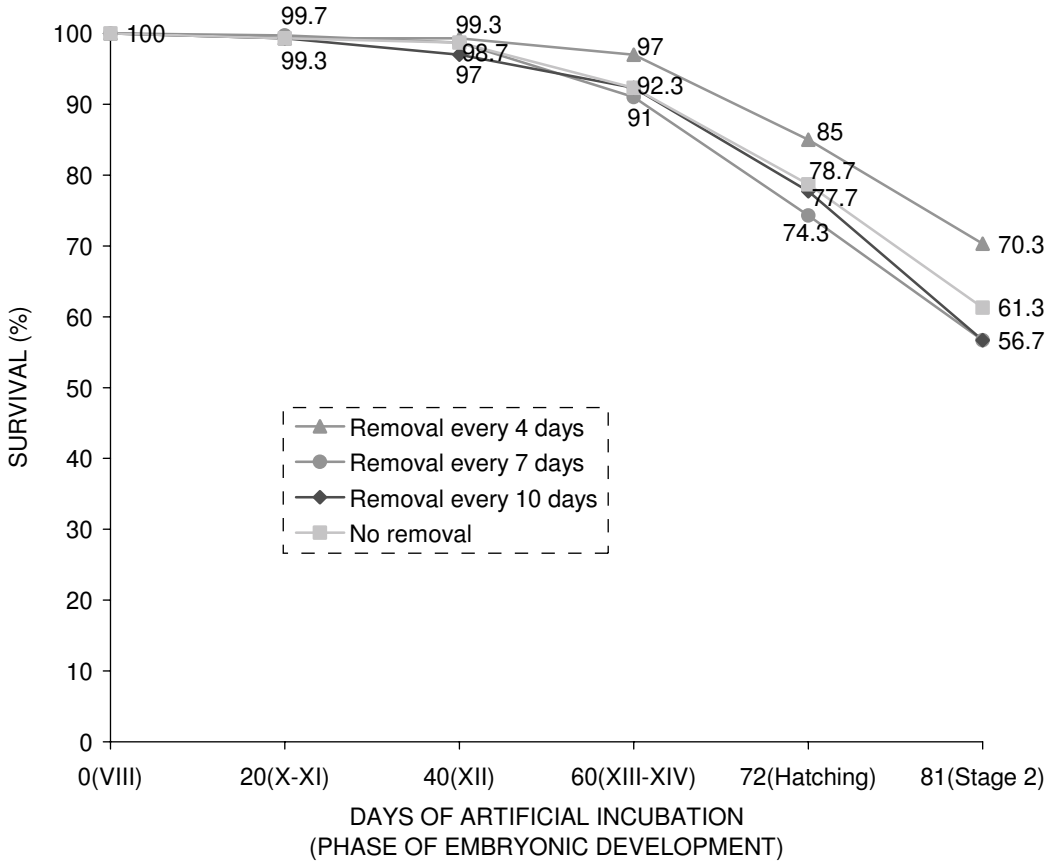

Figure 1
Development of the survival rates in each treatment.

Figure 1
Développement des taux de survie dans chaque traitement.

DISCUSSION

Most of artificial incubation attempts have had short duration, between 7 and 30 days, as eggs were removed from females at advanced phases of embryogenesis (STREMPEL, 1973; MASON, 1977; CUKERZIS, 1988; KÖKSAL, 1988). In these circumstances, egg removal could be unnecessary if water quality is maintained. However, EVANS *et al*. (1993) working with the parastacid *C. destructor*, reported important losses (68%) after 30 days of incubation even when eggs were daily removed, which were attributed to poor water quality. In contrast, water quality problems did not overcome in our case and removal of dead eggs and stage 1 juveniles every 4 days allows for the lower egg mortality (29.7%).

Egg mortalities ranging between 38.7% and 29.7% were concentrated in all treatments between the eyed stage and juvenile stage 2 in agreement with the results reported by MASON (1977), RHODES (1981), CARRAL *et al.* (1992), MATTHEWS and REYNOLDS (1995), PÉREZ *et al*. (1998a, 1998b) and CELADA *et al*. (2001b). Before eggs reached the eyed-stage 56 days of AI have elapsed. During this period statistical differences were not found between treatments. Main egg losses coincided not only with the embryonic stage but also with the raise of temperature. A strong increase in metabolic activity at terminal stages of embryogenesis has been hypothesized as one of the causes of reduced egg viability (PÉREZ *et al*. 1998a, 1998b). In addition, the last 25 days of incubation at 15ºC led to an increased fungal growth in all replications. Although no statistical differences were found, under these circumstances egg removal every 7 and 10 days resulted in lower stage 2 juvenile production than treatment without removal. This could suggest that handling when fungus development has spread could have negative effect on healthy eggs. In fact, most of dead eggs broke when removal was practiced making this work more difficult and releasing organic wastes to the water.

It should be emphasized that these results were obtained with relatively low incubation densities (2.2 eggs/cm^2) in which most of the eggs were not in contact with others. This could partially explain the general good results achieved, even when dead eggs were not removed. However, with higher occupation loads, the situation could be worse since fungal proliferation could be easy with eggs in close contact. In this case, the periodic removal of dead eggs could be combined or suppressed with the administration of antifungal substances. Therefore, it would be necessary to carry out studies guided to determine effective antifungal agents and doses.

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