EFFECTS OF EGG BATH AND DAILY REMOVAL OF DEAD EGGS ON HATCHING SUCCESS AND PRODUCTION OF STAGE 2 JUVENILES DURING ARTIFICIAL INCUBATION IN NOBLE CRAYFISH (*ASTACUS ASTACUS* L.)

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Reçu le 23 juin 2005 *Received June 23, 2005*
Accepté le 12 décembre 2005 *Accepted December 12, 2005*

ABSTRACT

The effects of egg bath (iodine-detergent preparation) and daily removal of dead eggs on hatching success and production of juveniles in stage 2 were investigated during a short (sixteen days) artificial incubation (***AI***) of noble crayfish eggs.

At the beginning of ***AI***, eggs were in phase XII (pulsating heart appearance) and were incubated in 18 polyethylene 1liter jars (100 eggs/jar, egg density 4.5 eggs.cm^{-2}). Six different treatments were tested during ***AI***:

– **C**: control group without removal of dead eggs and egg bath;

– **R**: daily removal of dead eggs without egg bath;

– **R-LB**: daily removal of dead eggs, low frequency of egg bath (once every five days);

– **R-FB**: daily removal of dead eggs, frequent egg bath (once every three days);

– **LB**: without removal of dead eggs, with low frequency of egg bath;

– **FB**: without removal of dead eggs, with frequent egg bath.

Egg bath was performed by iodine-detergent preparation Jodisol (dose 2 ml.l^{-1} and exposition time 2 minutes).

Results showed a better hatching rate after removing dead eggs rather than using the egg bath. Three treatments (R; R-LB; R-FB) showed significantly better survival rates in stage 1 (86.3 ± 5.4%) and 2 (84.2 ± 5.4%) than control (74.3 ± 0.9% and 73.3 ± 0.5%, respectively). Two treatments (LB, FB) showed no statistically different survival rate in stage 1 and 2 (82.5 ± 5.5 and 80.7 ± 5.3%, respectively) than the other treatments.

After ***AI***, juveniles from all treatments were reared under controlled conditions. At the end of rearing period, juveniles reached a survival rate of 64.1 ± 0.5% with mean body length of 22.0 ± 1.6 mm and mean body weight of 266.0 ± 50.9 mg. Negative effects of ***AI*** on growth and survival of juveniles were not evidenced.

Key-words: *Astacus astacus*, artificial incubation, jar, juvenile production.

Article available at http://www.kmae-journal.org or http://dx.doi.org/10.1051/kmae:2006021

EFFICACITÉ DU BAIN DES ŒUFS ET DE L´ENLÈVEMENT JOURNALIER DES ŒUFS MORTS SUR L´ÉCLOSION ET LA PRODUCTION DES JUVÉNILES AU STADE 2 PENDANT L´INCUBATION ARTIFICIELLE DE L´ÉCREVISSE À PATTES ROUGES (*ASTACUS ASTACUS* L.)

RÉSUMÉ

L´efficacité du bain des œufs dans une solution d´iode – Jodisol, solution à 2 ml.l-^{1} – et de l´enlèvement journalier des œufs morts sur l´éclosion et la production des juvéniles au stade 2 a été observée au cours de la période courte (16 jours) de l´incubation artificielle (***IA***) d´ œufs de l´écrevisse à pattes rouges.

Au début de ***IA*** des œufs étaient à la phase XII (apparition de pulsations du cœur) et leur incubation s´est déroulée dans 18 flacons polyéthyléniques d´un litre (100 œufs/flacon, densité de 4,5 œufs.cm^{-2}). Six traitements différents ont été examinés pendant ***IA*** :

C : groupe témoin; pas d´enlèvement des œufs morts ; pas de bain des œufs ;

R : enlèvement journalier des œufs morts ; pas de bain des œufs ;

R-LB : enlèvement journalier des œufs morts ; fréquence de bain des œufs : une fois tous les cinq jours ;

R-FB : enlèvement journalier des œufs morts ; fréquence de bain des œufs : une fois tous les trois jours ;

LB : pas d´enlèvement des œufs morts, fréquence de bain des œufs diminuée ;

FB : pas d´enlèvement des œufs morts, fréquence de bain des œufs augmentée.

Le bain des œufs a été réalisé avec la solution d´iode – Jodisol (une dose est 2 ml.l-^{1}, une durée de bain est 2 minutes).

Les résultats démontrent que l´enlèvement des œufs morts est plus important pour le succès de l´éclosion que leur bain. Troix expériences (R ; R-LB ; R-FB) pendant lesquelles l´enlèvement des œufs morts a été réalisé chaque jour (pas de bain des œufs) montrent que la survie des œufs, des juvéniles au stade 1 (86,3 ± 5,4 %) et au stade 2 (84,2 ± 5,4 %) est plus grande en comparant avec le groupe témoin (74,3 ± 0,9 % au stade 1 et 73,3 ± 0,5 % au stade 2). Deux expériences (– LB, – FB) pendant lesquelles des œufs ont été baignés et l´enlèvement des œufs morts n´a pas été réalisé ne montrent pas de grande différence statistique de survie des juvéniles au stade 1 et au stade 2 (82,5 ± 5,5 et 80,7 ± 5,3 %) en comparaison avec les autres expériences.

Après l´incubation artificielle, les juvéniles de toutes les expériences ont été élevés en conditions artificielles. A la fin de la période d´élevage, les juvéniles ont gagné une longueur de 22,0 ± 1,6 mm et un poids de 266,0 ± 50,9 mg. Un taux de survie a été 64,1 ± 0,5 %. Aucun effet négatif de l´incubation artificielle n´a été observé.

Mots-clés : *Astacus astacus*, écrevisse à pattes rouges, incubation artificielle, flacon, production de juvéniles.

INTRODUCTION

Astacid crayfish have long embryonic development (6-9 months) in natural conditions (REYNOLDS *et al.*, 1992). During long embryonic development, hatching success has not been ensured (CARRAL *et al*., 2003). Frequent post-spawning egg losses have been mainly caused by aggressive interactions among animals and poor egg attachment (CELADA *et al*., 1988; TAUGBØL and SKURDAL, 1990a, b; POLICAR *et al.*, 2004). High

mortality of eggs was observed by CARRAL *et al.* (1988, 1992, 2004), MATTHEWS and REYNOLDS (1995), PÉREZ *et al*. (1998ab, 1999) and CELADA *et al.* (2004) during the last stages of development.

Many authors described and used ***AI*** of crayfish eggs under controlled conditions (MASON, 1977b; CUKERZIS *et al*., 1978; RHODES, 1981; CUKERZIS, 1988, 1989; CARRAL *et al*., 1992, 2004; MATTHEWS and REYNOLDS, 1995; JÄRVENPÄÄ and ILMARINEN, 1995; PÉREZ *et al*., 1998ab, 1999, 2003; CELADA *et al.,* 2000, 2001, 2004) in order to improve survival rate.

Various facilities were used to carry out ***AI***: Zuger jar (STREMPEL, 1973; KÖKSAL, 1988 and CUKERZIS, 1988); vertical incubator designed for salmonid eggs (MASON, 1977b); apparatuses based on a moving tray (JÄRVENPÄÄ and ILMARINEN, 1995); special flow incubators for crayfish in a semi-recirculation water system (CARRAL *et al*., 1988, 1992) and in a flow-through system (RHODES, 1981; MATTHEWS and REYNOLDS, 1995; CARRAL *et al*., 2004; CELADA *et al*., 1988, 2004; PÉREZ *et al*. 1998ab, 1999).

Two ways of ***AI*** are described, the so-called short and long artificial incubations. First method of ***AI*** covers only a short period (several days) of the final embryo development and before eggs remain attached to the maternal pleopods for several months (CUKERZIS *et al.*, 1988). Second type of ***AI*** takes several months and eggs are removed at earlier embryonic phases (CARRAL *et al.*, 1992, 2004; PÉREZ *et al.*, 1998ab, 1999, 2003; CELADA *et al.,* 2000, 2001, 2004). Short ***AI*** is less demanding for energy, time and space compared to long ***AI*** but incubated eggs are more dependent on maternal care and outdoor conditions, where hatching success is unwarranted for long time (CUKERZIS, 1988).

Generally*,* ***AI*** has several advantages such as a reduced dependence on females and minimizes maternal egg brooding problems such as egg losses (PÉREZ *et al.*, 1999). ***AI*** techniques provide control on ambient conditions such as water quality, elimination of predators and the reduction in transmission of pathogens from broodstock to offspring. During ***AI***, stage-2 juvenile production is obtained under controlled conditions making their subsequent collection easier (CARRAL *et al.*, 2003). Incorporation of artificial breeding techniques into crayfish farms could reduce food, energy and space expenses (CARRAL *et al.*, 1992; GONZÁLEZ *et al.*, 1993), since eggs can be incubated in high densities (JÄRVENPÄÄ and ILMARINEN, 1995).

Egg mortalities were found during the whole ***AI*** (CELADA *et al.*, 2001) but the majority of eggs were dead between the eyed stage and juvenile stage 2 (CARRAL *et al.*, 2004). Most of the egg mortalities coincide with terminal stages of embryogenesis (increased metabolic activities and the extraordinary physiological effort involved in hatching) and the raise of temperature (PÉREZ *et al.*, 1998ab). Organic contamination of water and spread of fungus caused losses of eggs during ***AI*** (MASON, 1977b; RHODES, 1981; CARRAL *et al.*, 2004). As prevention of fungal diseases, CARRAL *et al.* (2004) recommend to maintain good water quality and removal of dead eggs. MASON (1977b) and RHODES (1981) described successful use of malachite green, which is not licensed today, to decrease egg mortalities during ***AI***. CELADA *et al*. (2004) tested different doses and treatment frequencies of formaldehyde, hydrogen peroxide, sodium chloride and malachite green on eggs of signal crayfish (*Pacifastacus leniusculus* D.) during ***AI***.

KOUŘIL *et al*. (1998), HAMÁČKOVÁ and KOUŘIL (1997) recommend to successfully use the iodine-detergent preparation (Jodisol) instead of illegal malachite green during artificial incubation of different species of fish eggs.

The goal of our study was to establish the effect of egg bath (iodine-detergent preparation, Jodisol) and daily removal of dead eggs on hatching success and production of stage 2 juveniles using ***AI*** of eggs in the noble crayfish.

MATERIAL AND METHODS

On June 3rd 2004, 25 ovigerous females of crayfish *Astacus astacus* (mean total body length: 91.6 ± 9.8 mm, mean body weight: 23.9 ± 8.7 g and mean pleopodal fecundity: 87 ± 39.9 eggs) were caught by baited sticks in the Světlohorská reservoir. Environmental conditions of the Světlohorská reservoir were described in detail by POLICAR and KOZÁK (2005). All caught females were transported in two polystyrene boxes (30 dm^3 capacity, 10 dm^2 of bottom surface, containing 25 mm layer of wet grass). All transported females were placed in one fibreglass trough with 1.5 m^2 area and 25 plastic pipes as shelters. The eggs were removed and pooled from all females on June 4th. Females were stocked back into the Světlohorská reservoir after egg removal.

After removal, 1,800 eggs were stocked into eighteen jars. The embryonic development of removed eggs was assessed according to Celada *et al.* (1991). Eggs were detached in phase XII (pulsating heart appearance). Eggs were incubated in 1liter polyethylene jars (flow 1 $l.min^{-1}$) (Figure 1). Jars were parts of a recirculation water system containing storage and filtration tank, pump, stand of jars made of 18 one liter jars and water distribution system (Figure 2). Each jar had 22.0 cm^2 of bottom surface. Eggs were incubated in density 100 $eggs.jar^{-1}$ = 4.5 $eggs.cm^{-2}$. Dissolved oxygen and temperature were measured daily: temperature was 18.8 ± 0.75°C and dissolved oxygen was 8.5 ± 0.5 $mg.l^{-1}$. The parameters of water quality (pH, NH_3, NO_2^- and NO_3^-) were checked weekly on the 1st, 8th and 15th day of ***AI***. During incubation, the parameters of water quality were: pH = 7.6 ± 0.2, content of ammonium = 0.06 ± 0.002 $mg.l^{-1}$, nitrite = 0.0017 ± 0.000 2 $mg.l^{-1}$, nitrate = 0.2 ± 0.02 $mg.l^{-1}$.

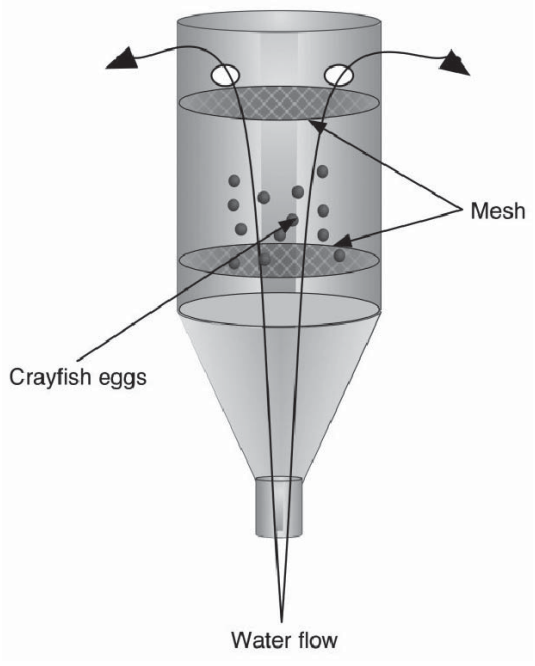

Figure 1
Detail of one liter jar.

Figure 1
Détail d'un flacon d´un litre.

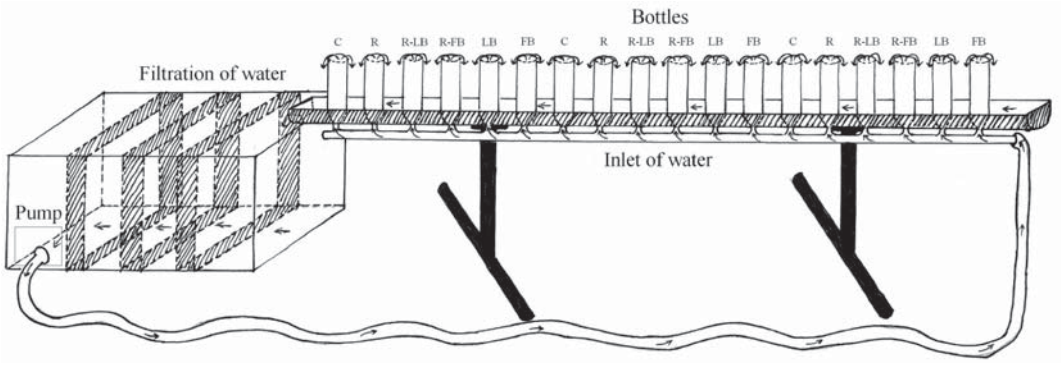

Figure 2
Description of the recirculation system.

Figure 2
Système de recyclage utilisé pour les expériences.

Six different treatments with three replicates were tested:

C: control group without removal of dead eggs and egg bath;

R: daily removal of dead eggs without egg bath;

R-LB: daily removal of dead eggs, low frequency of egg bath (once every five days);

R-FB: daily removal of dead eggs, frequent egg bath (once every three days);

LB: without removal of dead eggs, with low frequency of egg bath;

FB: without removal of dead eggs, with frequent egg bath.

Egg bath was performed by iodine-detergent preparation, Jodisol, dose 2 $ml.l^{-1}$ and with the exposition time of 2 minutes according to KOUŘIL *et al*. (1998). During removal of dead eggs, all eggs were taken from the jar to a flat saucer. Eggs were checked, dead eggs were removed and live eggs were put back into the jar. Daily egg mortality was observed in all treatments. At the end of ***AI***, productions of stage 1 and 2 juveniles were determined in separate jars for each treatment, respectively. Productions of stage 1 and 2 juveniles among each treatment were compared using ANOVA (Statgraphic, Tukey test, $P < 0.05$).

After the production of stage 2 juveniles, animals from all treatments were stocked into two rearing troughs, 1.5 m^2 in each area, under controlled laboratory conditions. At the beginning of rearing, 600 stage 2 juveniles (initial density of 400 individuals in the stage 2 per 1 m^2 of rearing area) were stocked in each trough. Juveniles were reared under the same conditions described by POLICAR and KOZÁK (2004) till the end of the 1st growing season (September 29th). At the end of the rearing period, mean body length, weight and survival rate were measured as described by POLICAR and KOZÁK (2004). Growth and survival rate of juveniles from ***AI*** were compared to the normal hatched juvenile rearing by POLICAR and KOZÁK (2004).

RESULTS

First hatching was observed on June 20th (***AI*** 17th day) and stage 2 on June 27th. Thus, hatching was observed after 16 days with ***AI*** and first moult (production of stage 2) of juveniles occurred after 23 days.

Table I

Mean survival rates (% ± SEM) of *Astacus astacus* eggs to hatching and to stage 2 juveniles for each treatment.

Tableau I

Taux de survie des œufs d´*Astacus astacus* à l´éclosion et au stade 2 juvéniles pour toutes les expériences.

Treatment	Hatching (%)	Success rate to stage 2 (%)
Control	74.3 ± 0.9 a	73.3 ± 0.5 a
LB	82.3 ± 4.0 ab	80.3 ± 4.0 ab
FB	82.6 ± 6.0 ab	81.0 ± 5.7 ab
R-LB	86.0 ± 5.3 b	83.3 ± 4.9 b
R	86.3 ± 6.0 b	84.3 ± 5.7 b
R-FB	86.6 ± 5.9 b	85.0 ± 5.9 b

Different superscript shows significant differences of hatching rate and success rate to stage 2 between treatments (ANOVA; Tuckey test; P< 0.05).

The best hatching rate (86.3 ± 5.4% average, with minimum 86.0 ± 5.3% and maximum 86.6 ± 5.9%) and production of stage 2 juveniles (84.2 ± 5,4% average, with minimum 83.3 ± 4.9% and maximum 85.0 ± 5.9%) were observed in treatments (R; R-FB and R-LB), where dead eggs were removed daily. On the contrary, the lowest hatching rate (74.3 ± 0.9%) and production of stage 2 juveniles (73.3 ± 0.5%) were observed in the control group without manipulation (no removal of dead eggs and no egg bath). Treatments LB and FB did not show a significant difference in the hatching rate (82.5 ± 5.5% average, with minimum 82.3 ± 4.0% and maximum 82.6 ± 6.0%) and production stage 2 juvenile (80.7 ± 5.3% average, with minimum 80.3 ± 4.0% and maximum 81.0 ± 5.7%) than others treatments (Table I). Daily cumulative survival rate of incubated eggs and juveniles in the different treatments are shown in Figure 3.

At the end of juvenile rearing, mean body length (22.3 ± 1.7 mm and 21.7 ± 1.4 mm, respectively), body weight (278.6 ± 55.9 mg and 254.0 ± 46.9 mg, respectively) and survival rate (64.1 ± 0.5%) were found in both rearing troughs. Similar growth and better survival of juveniles were observed using ***AI*** in comparison to the normal hatched juveniles (POLICAR and KOZÁK, 2004).

DISCUSSION

After ***AI***, a high hatching rate was observed in all treatments compared to the values attained by STREMPEL (1973) and CUKERZIS (1988) after similar long ***AI*** of *Astacus astacus*. On the contrary, MASON (1977b) achieved a better hatching rate (90-98%) and success rate to stage 2 juveniles (88%) in similar long ***AI*** of *Pacifastacus leniusculus*.

Good results in all treatments observed in the present experiment were probably due to the high quality of the water during ***AI***, which eminently influences survival of incubated eggs (MASON, 1977b; RHODES, 1981; CARRAL *et al.*, 2004; PÉREZ *et al.*, 1998b).

AI lasted a short period but according to CARRAL *et al.* (2004) and PÉREZ *et al.* (1998ab) included the big part of total egg losses. Most of the egg losses occurred in the control group, without any treatment. Egg bath by preparation Jodisol caused less egg losses than those which were noted in the control group, however without a significant difference. Three treatments, where dead eggs were removed daily (without reference to egg bath), showed significantly less egg losses than other treatments.

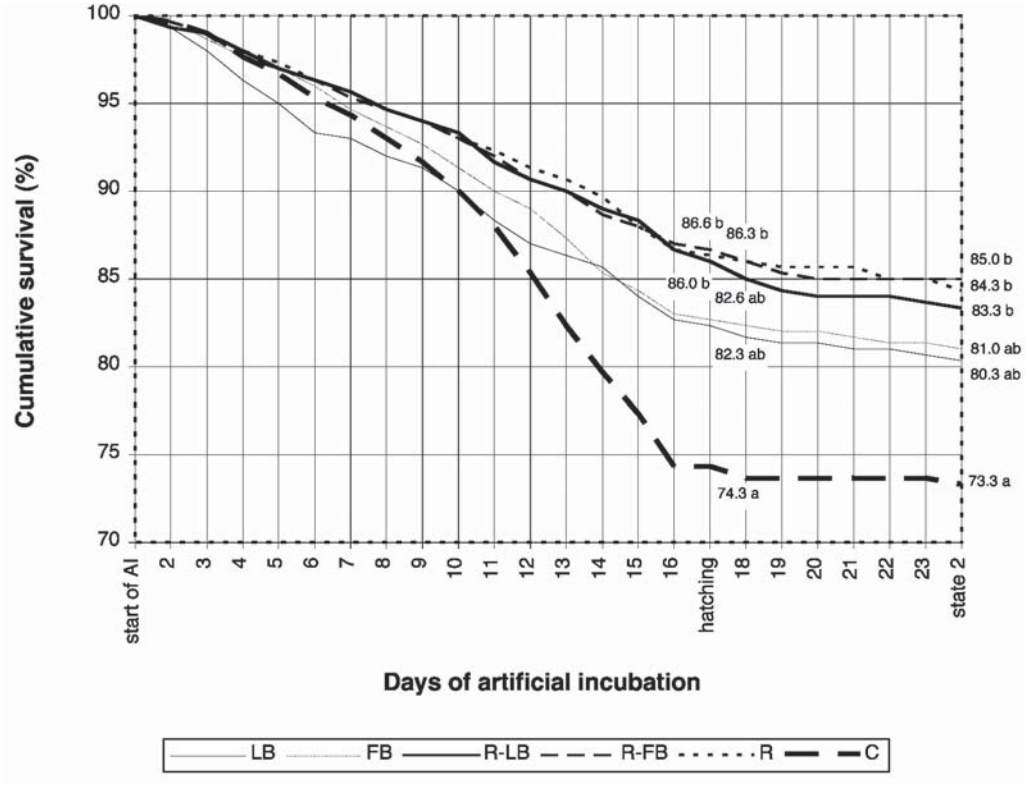

Figure 3
Daily cumulative survival rate of eggs and juveniles during artificial incubation.

Figure 3
Taux de survie cumulé journalier des œufs et des juvéniles pendant l´incubation artificielle.

According to our results we agree with MASON (1977b), RHODES (1981) and CELADA *et al*. (2004) on the possibility to successfully use antifungal preparations to achieve a higher hatching success. CELADA *et al.* (2004) successfully used antifungal preparations under lower egg density (2.2 eggs.cm^{-2}) and higher egg density (6.6 eggs.cm^{-2}). High survival rate was observed under low egg density (2.2 eggs.cm^{-2}) even without antifungal preparation, like in CARRAL *et al*. (1988, 1992), PÉREZ *et al*. (1998ab, 1999; 2003); CELADA *et al*. (2000; 2001; 2004). Under higher egg density (6.6 eggs.cm^{-2}), high efficiency was obtained with the administration of 4,500 ppm of formaldehyde or 15 ppm of malachite green. However, the use of malachite green on edible animal species has been banned in the European Union by means of the regulation 2377/90/EEC (CELADA *et al*., 2004).

On the other side, our results showed that removal of dead eggs had a higher effect to protect incubated eggs. This could be caused by the low concentration or short exposition time of the antifungal preparation Jodisol. A lower dose and a shorter exposition time of Jodisol than the values recommended by KOUŘIL *et al.* (1998), HAMÁČKOVÁ and KOUŘIL (1997) were used for incubation of fish eggs.

Periodical removal of dead eggs is effective in protecting egg incubation against fungi (CARRAL *et al.*, 2004). CARRAL *et al.* (2004) used a lower egg density (2.2 eggs.cm^{-2}) however recommended using a higher egg density for a more exclusive demonstration of

the effect of dead egg removal on the efficiency of egg incubation. The higher efficiency of the methods that use the removal of dead eggs compared to the egg bath by Jodisol could be due to the higher egg density in our study. Most likely the removal of dead eggs helped to decrease the fungal disease propagation. On the contrary, the selected dose and exposition time of Jodisol were insufficient to protect the incubated eggs against fungi diseases. In the future, it will be important to choose a higher dose or exposition time of Jodisol to ensure the vitality of incubated eggs.

Use of ***AI*** in crayfish reproduction had no negative effects on the achieved growth and survival of juvenile at the end of their rearing (SÁEZ-ROYUELA e*t al*., 1995). These authors found a lower survival rate of signal crayfish juveniles from artificial incubation during the first 80 days of their rearing with no significant differences in survival and growth rates at the end of their rearing compared to those from maternal incubation. Our performed ***AI*** had no negative effect on the growth and survival rate of juveniles at the end of their rearing, too. Prematurely separated stage 2 juveniles in signal crayfish influenced lower growth but no lower survival rate than normal juveniles (Mason, 1977a).

Removal of dead eggs was a more effective method for increasing the hatch rate than egg bath during the short ***AI***. It may be concluded according to our results that the use of antifungal preparations can protect incubation eggs against fungal diseases. Therefore we recommend the search for other applications of antifungal bathing to be used during ***AI*** of crayfish eggs.

ACKNOWLEDGEMENTS

This investigation was supported by the Ministry of Education of the Czech Republic, project no. MSM 6007665809 and by the Ministry for Agriculture of the Czech Republic, project no. NAZV QF 3028. Authors of this paper gratefully thank colleague Petra Vorlíčková for the translation of the French abstract.

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