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Effects of Different Water Temperatures on the Hatching Time and Survival Rates of the Freshwater Crayfish *Astacus leptodactylus* (Esch., 1823) Eggs

Hamdi Aydın[1,*], M. Kamil Dilek[1]
[1] Kocaeli University, Gazenfer Bilge College, Department of Fisheries, 41670 Karamürsel, İzmit, Turkey

* Corresponding Author: Tel.: +90. 262 454 34 51; Fax: +90. 262 454 10 78;
E-mail: aydin@kou.edu.tr

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Abstract

In this study, freshwater crayfish (*Astacus leptodactylus,* Esch, 1823) eggs were incubated starting seven weeks after spawning, at three different temperatures (11.8 ºC, 16±1ºC and 20±1ºC) and the results were compared with respect to hatching time and survival rate. The eggs hatched between days 116 and 125, 88 and 97, and 69 and 74, at 11.8 ºC, 16 ±1 ºC, and 20 ±1 ºC, respectively. The survival rates at these temperatures were 22.4%, 46.9% and 32.5%. In conclusion, it was found that *A. leptodactylus* eggs could hatch 1.5 months earlier than under natural conditions by increasing water temperature, starting in March, up to 20 ºC, but the optimum temperature for maximum hatching rate was found to be around 16ºC.

Key Words: Freshwater crayfish, *Astacus leptodactylus*, hatching time, survival rate, water temperature.

Introduction

Most of Turkey's freshwater reservoirs are inhabited by A*stacus leptodactylus,* Esch. 1823. It is known as the Turkish, Galicia, swamp, pond or long-clawed crayfish. Until 1984, freshwater crayfish had an important role as a high-quality live export product, but after 1985 production declined dramatically in most lakes and dams as a result of the crayfish plague (*Aphanomyces astaci*) (Harlıoğlu, 2004). Under natural conditions *A. leptodactylus* reaches first sexual maturity at age of three years. Mating occurs during October and November when the water temperature is 7–12 ºC. Incubation of the eggs takes five to six months in warm climates, and six to eight months in cold climates. Low temperature prolongs the incubation period and delays hatching, which takes place between late May and end of June. After hatching, the young remain with their mother for about 20–25 days. During this period, the juveniles molt once and then leave their mothers to begin to live independently in shallow waters (Köksal, 1988).

As with other species, the egg incubation and larval feeding periods are the most critical parts of freshwater crayfish culture. Many researches have studied artificial incubation of freshwater crayfish eggs (Mason, 1977; Rhodes, 1981; Carral *et al.*, 1988; Matthews and Reynolds, 1995; Perez *et al.*, 1998 and 1999; Henryon and Purvis, 2000 and 2003). Successful egg incubation affects production directly and it is well known that water temperature has a great deal of control over breeding, egg development and molting in crayfish (Westin and Gydemo, 1986; Köksal 1988; Dube and Portelance 1992; Harlıoğlu 1999; Türkgölü 2000). A long incubation period (from 7 to 9 months) in *Astacidae* species, may result in high mortality rates of up 70% (e.g., Rhodes 1981; Perez *et al*., 1998, 1999; Henryon and Purvis, 2000). This is presumably because the eggs of the species have a long development period, including a period of winter diapause. This has the potential to increase the risk of infection and mechanical damage during artificial incubation, while development during the period of winter diapause requires a specific thermal regime (Henryon and Purvis, 2000).

Many studies have been carried out on artificial incubation of crayfish eggs and to obtain early-hatched larvae by increasing water temperature. It has been reported that different methods can be used for crayfish egg incubation, involving the use of zug-jars, meshed materials, tanks and maternal brooding (Ackefors, 1989; Leonard *et al.*, 2001).

In some European countries, both native and newly-introduced species have been cultured to support natural stocks of crayfish (Vorburger and Ribi, 1999; Savolainen *et al*., 2003), whereas in Turkey, studies on crayfish culture have been very limited. Ackefors (1989) reports that by gradually increasing the water temperature to 18–20 ºC starting in March, it becomes possible to hatch eggs of *A. leptodactylus* two months earlier than those held at natural conditions. Similar results are recorded by Matthews and Reynolds (1995) for *Austropotamobius pallipes* eggs. The main aim of this study was to investigate effect of increasing water temperature on hatching time and survival rates of *Astacus leptodactylus* (Esch 1823) eggs.

Materials and Methods

The broodstocks of freshwater crayfish used in this study were obtained from İznik Lake, Bursa. Beginning in November 2002, 300 broods (200 female, 100 male) crayfish with a mean size of 114.4 ±10,5 mm total length (TL) were collected using fyke nets (pinters) and transported to the Sapanca Inland Aquaculture Research and Production Center of İstanbul University. Brood crayfish were stocked into two concrete ponds (200 x 100 x 60 cm) with 40 cm water depth for mating and spawning, then provided with plastic shelters. Spring water with constant temperature (11.8°C) was supplied to the two concrete ponds. The major water quality parameters are presented in Table 1.

Mating started on 22 November 2002 in these concrete ponds, and 94% of the crayfish deposited their eggs by 21 January 2003. After the males mated, they were removed and transported to other ponds. Gravid females were kept approximately 7 weeks in these ponds at 10±2°C water temperature. On 1 March 2003, gravid females were selected and stocked into ten egg incubator tanks of 230 x 50 x 60 cm in the hatchery. For each temperature group (11.8, 16±1 and 20±1°C) with two replications, 5 gravid crayfish were placed into each basket.

It is impossible to count crayfish eggs without removing them, since a large female can carry as many as 400 eggs in her abdomen (Köksal, 1988). Therefore in order to estimate average fecundity, eggs were stripped separately from the right-hand-side (RHSP) and left-hand-side (LHSP) pleopods of 20 buried females representing experimental animals and then counted. The counts from the two sides of each animal were compared using a paired t-test, and no significant differences were found. Eighty-four (84) of the broods were used in the trial at the three different temperatures. For each water temperature, a group of eggs was removed from the RHS pleopods only, counted, and then gravid females were placed in the incubation baskets. For each temperature group, along with two separate lines of 10 eggs, baskets were separated, and into Group I, 27 (2,813 eggs), Group II, 28 (2,816 eggs) and Group III, 29 (2,828 eggs) gravid crayfish were placed (Table 2).

Two pieces of plastic pipe with diameters of 70 mm and 150 mm were set up as hiding places for crayfish in each egg incubation basket. The baskets were covered with styrofoam plates to protect them from light. Gravid females were kept there for about a week to acclimate them to their new environment. Starting on 8 March 2003, the first groups of females were kept at 11.8 ºC. The water temperature was gradually increased (1 °C every two days) for the second and the third groups. Electric heaters (4.5 kW) were used to increase water temperature.

Crayfish were fed fresh trout meat twice a week and the uneaten food was cleaned up regularly. Water temperature was taken daily and dissolved oxygen content was measured twice a week, respectively. To compare the effect of water temperature on incubation period, ambient water temperature in İznik Lake was also measured at the same time. The development of crayfish eggs was observed and noted every week.

Results

Hatching began earliest in the third group, at 20±1ºC, between days 69 and 74 (16–21 May). In the second group, at 16±1ºC, hatching occured between days 88 and 97 (4–13 June), and in the first group, at

Table 1. Water quality analysis parameters of Sapanca Research Centre

Parameters		Parameters	
Total Hardness	120 mg/l	Aluminum	0.069 mg/l
Calcium (Ca^{2+})	46.89 mg/l	Carbon dioxide (CO_2)	5.3 mg/l
Magnesium (Mg^{2+})	7.29 mg/l	Dissolved oxygen (DO)	minimum 7.5 mg/l
pH	7.66-8.00	Water Temperature	11.8 °C

Table 2. Survival rate of crayfish eggs incubated in different water temperatures

Groups		Water Temp . (ºC)	Gravid Females (n)	Initial Number of eggs	Number of larvae at the end of study	Survival Rate (%)	Average Incubation Time (days)
Group-I	a	11.8	13	1404	304	21.6	
(11.8°C)	b	11.8	14	1409	329	23.3	120±4
Group-II	a	16 ±1	13	1406	681	48.4	
(16±1ºC)	b	16 ±1	15	1410	641	45.4	92±4
Group-III	a	20 ±1	14	1413	482	34.1	
(20±1ºC)	b	20 ±1	15	1415	438	30.9	71±2

11.8ºC, hatching took place between days 116 and 125 (2–11 July). The study continued until the juveniles left the mothers. The incubation period in İznik Lake continued for approximately 5 months at a monthly mean temperature of 7.7–22.8ºC (Figure 1), and the first larvae were observed on 14 June 2003.

At the end of the study, the highest survival rate was obtained with the second group (46.9%), i.e., at 16±1ºC, and the lowest survival rate was obtained with the first group (22.4%) or at 11.8ºC (Table 2).

During the study, 5 gravid females in the first group, 4 in the second and 7 in the third died due to crayfish plague. Lesions were observed on the ventral side of the cephalo-thoracic, abdominal, and anal areas, and on the joints of the legs.

Discussion

Quite a few studies have been conducted on artificial incubation of freshwater crayfish, especially on summer-spawning species. Mason (1977), Carral *et al*. (1988 and 1992), Rhodes (1981) and Perez *et al*. (1998 and 1999) investigated separating the eggs from the females in the late phases of embryonic development during artificial incubation of *Pacifastacus leniusculus* and *Austropotamobilus pallipes*. They obtained better or similar success in comparison to maternal incubation. Leonard *et al.* (2001) report that because of their high fecundity and short incubation period, summer spawning crayfish species do not need artificial incubation. According to Carral *et al.* (1992) and Perez *et al.* (1999), the incubation period of winter spawning crayfish species takes more than 150 days. Köksal (1988) reports that incubation period of *A. leptodactylus* takes 5–6 months in temperate climates and 6–7 or even 8 months in cold climates. In İznik Lake, the incubation period begins in January and goes on until the first week of June. In the current study, the first crayfish egg was observed in the first week of January in the lake, and these eggs underwent incubation until the second week of June. Regarding the reproductive biology of Astacus species, Balık and Ustaoğlu (1983), Soylu (1984), Cukerzis (1988), Köksal (1988), Laurent (1988), Alderman and Wickins (1990), Köksal *et al*. (1992) and Ackefors (1989, 2000) report similar findings.

Researchers emphasize the importance of maintaining gravid females for approximately one month under natural conditions before stocking them at high temperatures (Mason, 1977; Huner and Lindqvist, 1985; Köksal, 1985; Westin and Gydemo, 1986; Celeda *et al*., 1988). Recently, Türkgülü (2000) reported that controlled incubation of *A. leptodactylus* gravid females at relatively high temperatures should commence approximately two months after spawning. In present study as well, incubation of gravid females of crayfish at different water temperatures began about 1.5 months after spawning.

Among the gravid females that were kept at three different water temperatures, the earliest hatching of eggs occurred in the group held at 20±1ºC, followed by those in the second (16±1ºC) and first (11.8ºC) groups. Köksal (1988) and Ackefors (1989) report that crayfish larvae can be produced by incubating *A. leptodactylus* eggs at gradually increasing water temperatures between 18–20ºC, about two months earlier than in natural waters. In the current study, hatching time changed with water temperature, and eggs that were kept at the highest temperature (20±1ºC) hatched 47–51 days earlier than eggs kept at the lowest temperature (11.8ºC).

Köksal (1988) and Ackefors (1989) claim that the optimum water temperature for incubation of *A. leptodactylus* eggs ranges between 16–18ºC. Türkgülü (2000) stocked gravid females of *A. leptodactylus* at 14, 16, 18, and 20ºC water temperatures, and obtained the highest hatching rate at 16ºC. The results of our study, in which the highest hatching or survival rate of 46.9% was obtained at 16±1ºC, are in agreement

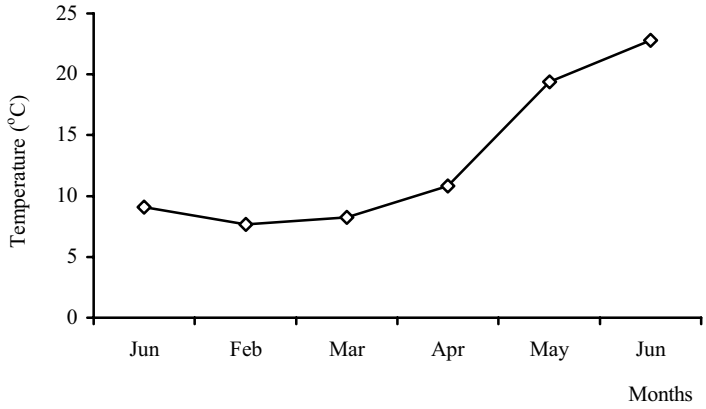

Figure 1. Monthly variations in mean water temperature in Lake İznik (2003).

with the Türkgülü's (2000) results. Differences between survival rates at different temperatures were significant ($P<0.05$).

Sixteen gravid females died of crayfish plague. Therefore, because still persists in Lake İznik crayfish from there should not be used for broodstock or stocking purposes for other lakes.

In conclusion, it was observed that during incubation of *A. leptodactylus* eggs, larvae could be obtained 1.5 months earlier than under natural conditions by increasing water temperature starting in March to 20ºC. However, due to the risks associated with increasing the water temperature earlier, we concluded that there would not be much benefit obtained through such a process. Because water temperature rises gradually under natural conditions, reaching 20–22ºC in the summer (Figure 1), the egg development rate increases accordingly. Moreover, keeping crayfish eggs at a constant water temperature of 11–12ºC during the incubation period not only prolonged the incubation period but also lessened the survival rate.

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