Embryonic and Postembryonic Development of Freshwater Crayfish (*Astacus leptodactylus* Eschscholtz, 1823)

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Abstract

There is little knowledge about the egg development of freshwater crayfish (*Astacus leptodactylus*). Especially, some aspects of the postembryonic development of *A. leptodactylus* have been described in previous studies. However, all embryonic development of *A. leptodactylus* species, which has a long incubation period (5-6 month) has not been described. In this study, embryonic and post embryonic development of *A. leptodactylus* species was studied by a stereomicroscope under laboratory conditions which have similar temperature conditions with ones in their natural habitat in between 9^{o}C -15^{o}C. The eggs of this species hatched in 1608 degrees x days under these conditions. After 7-8 days of hatching, juvenile stage I reached to juvenile stage II with changing first moult. After 9-10 days of first moult, juvenile stage II reached to juvenile stage III with changing second moult.

Key words: Embryo, development, *Astacus leptodactylus*, Astacidae, crustacea

Tatlısu kereviti (*Astacus leptodactylus* Eschscholtz, 1823)' un Embriyonik ve Postembriyonik Gelişimi

Özet

Tatlısu kerevitlerinden *Astacus leptodactylus*'un yumurta gelişimi hakkında çok az bilgi vardır. Özellikle *A.leptodactylus*'un post embryonik gelişimin bazı yapıları tanımlanmıştır. Fakat diğer türlerden farklı olarak uzun bir inkübasyon süresine (5-6 ay) sahip olan *A. leptodactylus*' un embryonik gelişimleri tanımlanmamıştır. Bu çalışmada *A. leptodactylus*' un yumurta gelişimi, yumurtlamadan III. dönem larva aşamasına kadar, doğal ortamlarındaki sıcaklık şartlarına benzer olarak oluşturulan laboratuar şartları altında 9^{o}C ile 15^{o}C' ler arasında fotoğraflandırılarak çalışılmıştır. *A. leptodactylus*' un yumurtaları belirtilen şartlar altında 1608 gün/derece de açılmıştır. Yumurta açılımından 7-8 gün sonra, I. dönem larvalar ilk kabuklarını değiştirerek II. döneme ulaşmışlardır. İlk kabuk değişiminden 9-10 gün sonra II. dönem larvalar ikinci kabuklarını değiştirerek III. döneme ulaşmışlardır.

Anahtar kelimeler: Embriyo, gelişim, *Astacus leptodactylus*, Astacidae, Kabuklular

INTRODUCTION

The crayfish species in the world are threatened with population decline or extinction (Taylor 2002). Non-indigenous species in Europe now outnumber indigenous crayfish species 2:1, and Non-indigenous crayfish may dominate completely in the next few decades. A decline in the stocks of indigenous crayfish species has been recorded in many countries in the face of increasing populations of non-indigenous. In addition, indigenous crayfish are not only under the threat of non-indigenous crayfish but also habitat loss, deteriorating water quality, overfishing, climate change and crayfish plague (Holdich, 2009).

Freshwater crayfish *A. leptodactylus*, indigenous crayfish species of Europe have been harvested commercially in Turkey since the 1960s (Rahe and Soylu 1989). In 1985 and 1986, crayfish catch numbers collapsed catastrophically due to crayfish plague (Furst and Soderhall 1987; Baran and Soylu 1989; Timur 1990). Almost all populations are affected by this disease either disappeared completely or were dramatically reduced (Svoboda et al., 2012).

Decreasing stocks of indigenous crayfish should be increased in their natural and artificial areas, so that juvenile the culture of *A. leptodactylus* may be made improvement to the natural stocks. The success of the culture will be increased with the knowledge of embryonic and postembryonic development of species. Koksal (1984) studied embryonic and embryonic a post stage of *A. leptodactylus*. In the present study was presented whole embryonic and embryonic post stages of *A. leptodactylus.*

MATERIALS AND METHODS

The females of *A. leptodactylus* carrying sperm tube (20 individuals) were transferred to the laboratory from their natural habitat Egirdir Lake (Isparta, Turkey) in February 2011. The females were placed in each aquarium (70L) as two individuals. Water temperature of experiment was kept similar to their natural habitat (9-15 ^{o}C). The dissolved oxygen rate always ranged from 8 to 10 mg/l^{-1}. Animals were exposed to a 12: 12 h light/dark cycle. PVC tubes (15 cm diameter, 15 cm long) were used as a shelter in aquaria. The females were fed with shrimp feed. The live eggs were taken from the pleon of the mother with tweezers and put into petri dishes filled with tap water for observation in microscope every day. The photographs of eggs and larvae photographed with a stereomicroscope (Nikon SMZ-U DIA STAND). The embryonic phases were determined according to Celada et al. (1987; 1991). The post embryonic phases were determined according to Holdich (1992).

RESULTS

Embryonic and postembryonic development stages of *Astacus leptodactylus*, incubation period and incubation temperatures were given in Table 1 and Table 2.

Table 1. Embryonic development period of *A. leptodactylus* and incubation temperatures

	Incubation temperatures (^{o}C)	**Incubation period degrees x days (D^{o})**	**Incubation period (days)**
Embryonic development stage			
I	9	-	-
II	9	117	13
III-IV	9	270	30
V-VI	9	405	45
VII	11	600	60
VIII	11	870	87
IX	11	960	96
X	13	1078	98
XI	13	1111	101
XII	15	1476	123
XIII	15	1536	128
XIV	15	1608	134
Mean temperature	12		

Table 2. Post-embryonic development period of *A. leptodactylus* and incubation temperatures

	Incubation temperatures (^{o}C)	**Incubation period degrees x days (D^{o})**	**Incubation period (days)**
Post embryonic development			
Juvenile stage I	15	1608	134
Juvenile stage II	15	1713-1728	141-142
Juvenile stage III	15	1743-1758	150-151
Mean temperature	15		

Embryonic development

Phase I*:** New spawn egg. Cleavage process is not clear in the outer surface of egg. This phase was showed in Figure 1.1. ***Phase II*:** The eggs reached this phase at 117 degrees x days**.** The appearance of cleavage cells on the surface of egg are clear in this phase. This period has been photographed in Figure 1.2. ***Phase III*:** Blastosphere formation have been seen. In the present study, this period has been reached 270 degrees x days. (Figure 1.3).Phase IV*:** 270 degrees x days. The embryo with entodermal disc, mesoderm formative zone, cephalic lobes and thorocic abdominal plates. This period could not be photographed. ***Phase V*:** The eggs reached this phase at 405 degrees x days. Embryo with semicircular gastral furrow have been seen in this phase. (Figure1.4). ***Phase VI*:** 405 degrees x days. Embryo with circular gastral furrow. Photograph was not taken in this phase. ***Phase VII*:** In this study, the eggs reached this phase at 600 degrees x days. **a.** Embryo with existing blastopore was determined in Figure 1.5a. **b.** Blastopore (bl) in closing process was seen in Figure 1.5b. ***Phase VIII*:** The eggs reached this phase at 870 degrees x days. Embryo with mandibular anlangen (md) was shown in Figure 2.6 a. ***Phase IX*:** This phase was observed at 960 degrees x days. Embryo with naupliar appendages. at1, at2 and md is characterised in phase IX. (Figure 2.6 b). ***Phase X*:** 1078 degrees x days. Embryo with the anlagen of masticatory appendages (mx1, mx2, mxp1). The maxilliped 2 and maxilliped 3 are under the abdomen. The antennae (at2) have forked (Figure 1.7). ***Phase XI*:** In the present study, this period has been reached at 1111 degrees x days. Embryo with anlagen of pereiopods. The phase was shown in Figure 1.8. ***Phase XII*:** This period has been reached at 1476 degrees x days. Embryo with pulsating heart was observed in this phase. (Figure 1.9). ***Phase XIII*:** This period has been reached at 1536 degrees x days. Embryo with eye pigment was seen in this phase (Figure 2.10). ***Phase XIV*:** This period has been reached at 1608 degrees x days. Embryo with strongly developed posterior hepatopancreas lobes was shown in Figure 2.11. Shortly before hatching (Figure 2.12).

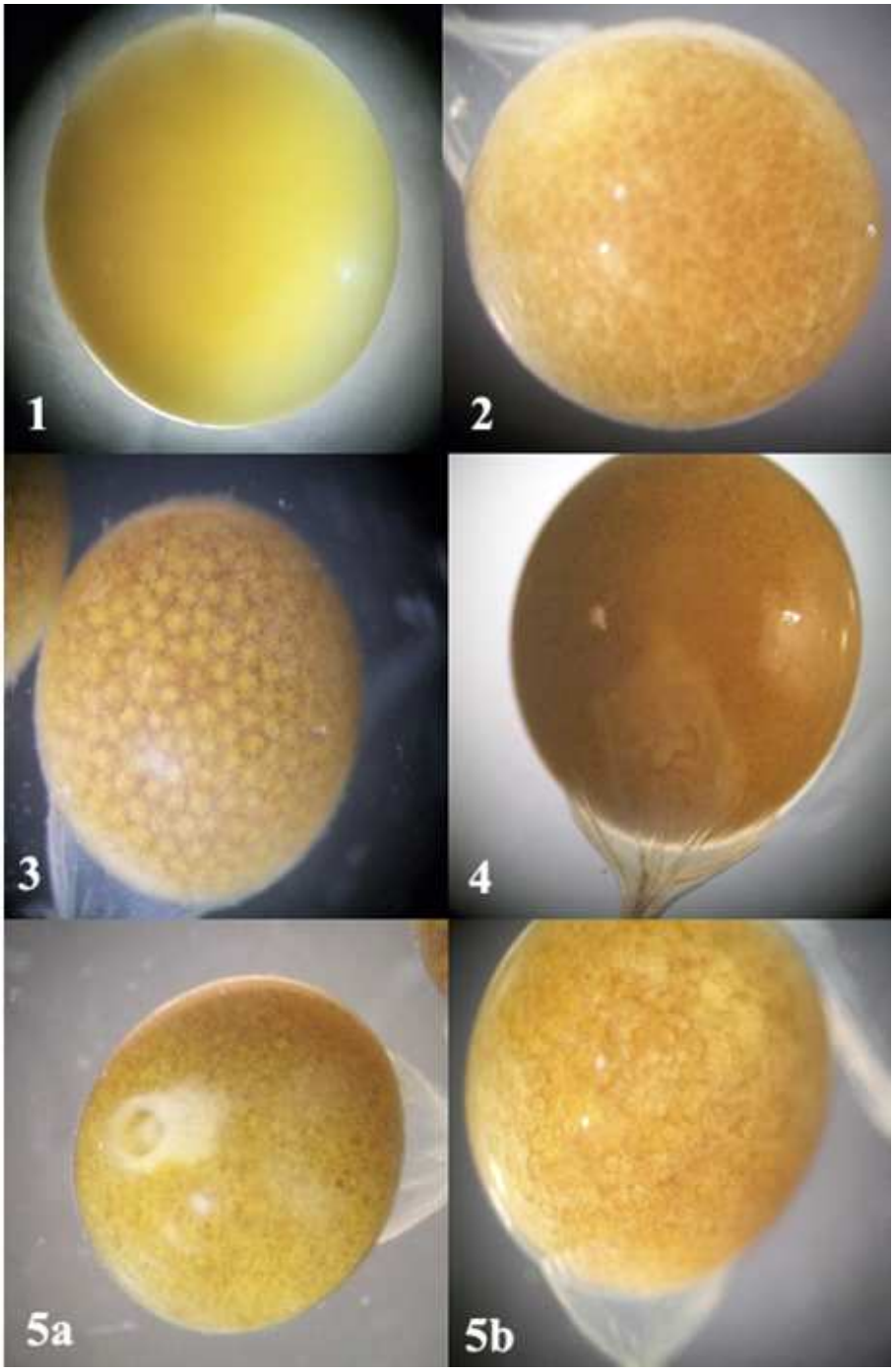

Figure 1. The embryonic development of *Astacus leptodactylus. Phase I*: New spawn egg. (Figure 1.1), *Phase II*: The appearance of cleavage cells on the surface are clear. (Figure 1.2), *Phase III*: Blastosphere (Figure 1.3), *Phase V*: Embryo with semicircular gastral furrow. (Figure 1.4), *Phase VII*:. Embryo with existing blastopore (Figure 1.5a); Blastopore (bl) in closing process (Figure 1.5b).

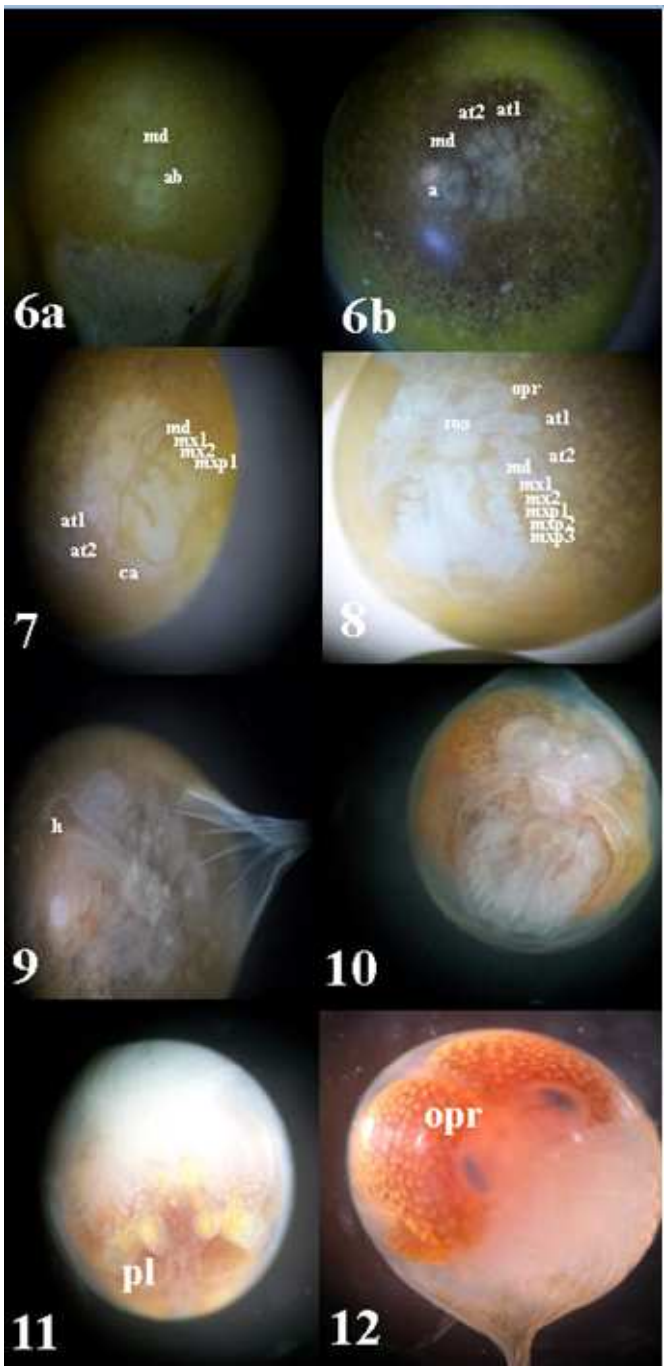

Figure 2. The embryonic development of *Astacus leptodactylus. Phase VIII*: Embryo with mandibular anlangen (md) (Figure 2.6 a), *Phase IX*: Embryo with naupliar appendages (Figure 2.6 b), *Phase X*: Embryo with the anlagen of masticatory (Figure 2.7), *Phase XI*: Embryo with anlagen of pereiopods (Figure 2.8), *Phase XII*: Embryo with pulsating heart (Figure 2.9), *Phase XIII*: Embryo with eye pigment (Figure 2.10), *Phase XIV*: Embryo with strongly developed posterior hepatopancreas lobes (Figure 2.11), Shortly before hatching (Figure 2.12).

Post-embryonic development

***Juvenile Stage I*:** In this study, Stage I juveniles hatched from egg (1608 degrees x days). Stage I juveniles have sessile eyes, the carapace is globose, 4 pleopods present on each somite, the rostrum is small and curved ventrally. The telson is large and roundish. There is a small notch in the centre of the posterior telson margin. The large chelipeds are equipped with strongly recurved hooks on the propodus and dactylus. (Figure 3.1a). The juvenile crayfish is connected to the mother via the telson thread. One end of the telson thread is attached to the posterior hooked spines of the telson of Stage I juveniles. The other end is connected to the egg shell which is attached to the maternal pleopods (Figure 3.1b). No feeding activity. The juveniles changed its first moult after 7-8 days (Figure 3.2a), and they reached to stage II. (Figure 3.2b).

***Juvenile Stage II*:** Juvenile stage II have the following appearance. There is less yolk in the body, and the thoracic region is not as globose as in juvenile stage I. The eyes are equipped with a short stalk. rostrum emerging between eyes (3.2a, 2b, 2c). Many setae occur on the body surface. The telson, in particular, is equipped with long plumose setae. Feeding activity. Total length and weight of the juvenile stage II were determined as 9-11 mm and 30-60mg respectively. In the present study, juvenile stage II changed its second moults after 9-10 days, and they reach to stage III. (Figure 3.3a).

***Juvenile Stage III*:** After the second postembryonic moult, the crayfish of juvenile stage III look more or less like miniature versions of the adults (Figure 3.3a). The uropods have unfolded, and the tail fan is complete (Figure 3.3a). The plumose setae of the telson are somewhat shortened and the same type of plumose setae occurs on the margin of the uropods (Figure 3.3a). The gonopores become invisible under steromicroscope. Total length and weight of the juvenile stage III were determined as 12-14 mm and 60-90mg respectively.

Figure 3. Post-embryonic development of *Astacus leptodactylus.Juvenile Stage I*: (Figure 3.1a, 1b), *Juvenile Stage II* (Figure 3.2a, 2b, 2c), *Juvenile Stage III*: (Figure 3.3a)

DISCUSSION

Embryonic development

In the present study, water temperatures of experiment was attempted to keep equivalent those of their natural habitat. In this conditions, incubation period for *A. leptodactylus* was determined as 1608 degrees x days (D^o) at 9- 15 ^{0}C. Zehnder, (1934) determinated as 8 mounths at 6-12 ^{o}C embryogenesis of *Astacus astacus* which belongs to this genus. For same species, Palows et al. (2010) indicated that embryogenesis of *A.astacus* lasted 2100 D^o at 8 ± 2^{o}C. Celada et al., (1991) described as 82 days (1271 D^o) at 15.5 ^{o}C embryonic processes for *Austropotamobius pallipes*. Celada et al. (1987) determined as 70 days (1085 D^o) at 15.5 ^{o}C embryonic processes for *Pacifastacus leniusculus*. However, Mason (1978) reported 1387 D^o embryonic processes for this species. All of above species are coldwater species and these species spawn in autumn-winter and hatching takes place in spring-summer. Incubation processes for these species is long.

In previous studies in warm water species, incubation processes indicated as 40 days 760 D^o at 19 ^{o}C for *Cherax destructor* (Sandeman and Sandeman, 1991), 28-31 days (728-806 D^o) at 26.0 ^{o}C for *Cherax quadricarinatus* (Garcia Guerrero et al., 2003), 17 days (388 D^o) at 22.8 ^{o}C for *Procambarus clarkii* (Suko,1956). Differences between incubation periods of the same species might be sourced from applied temperature difference.

When it is looked at the embryonic characters; in the present study, embryo with semicircular gastral furrow was observed at 405 D^o at 9 ^{o}C for *A. leptodactylus*. Palows et al. (2010) observed first semicircular germs of the embryo at 592 D^o at 8 ±2 ^{o}C for *A. astacus*. In these two studies both, although almost the same temperature practice was applied until this stage, same incubation processes were not observed for that stage. The reason of this might be their being from different species. Embryo with semicircular gastral furrow was described as 434 D^o at 15.5 ^{o}C for *A. pallipes* (Celada et al., 1991), at 201.5 D^o at 15.5 ^{o}C for *P. leniusculus* (Celada et al., 1987).

In this study, while closed blastopore was observed about 600 D^o at 9-11 ^{o}C for *A.leptodactylus*, Palows et al. (2010) observed at 928 D^o at 8 ±2 ^{o}C for *A. astacus*. The increase of difference between incubation processes in these studies can be sourced from practicing ever-increasing temperature in the present study. Celada et al. (1987) indicated closed blastopore at 294.5 D^o at 15.5 ^{o}C for *P. leniusculus*. Celada et al. (1991) observed this stage at 480.5 D^o at 15.5 ^{o}C for *A. pallipes*. Garcia Guerrero et al. (2003) determined closed blastopore 104-156 D^o at 26 ^{o}C for *C. quadricarinatus*.

We observed at 960 D^o at 9-11 ^{o}C embryo with nauplius shape for *A.leptodactylus*. Palows et al. (2010) was be able to this stage 1200 D^o at 8 ±2 ^{o}C for *A. astacus*. Celada et al. (1987) indicated to this stage at 403 D^o at 15.5 ^{o}C for *P. leniusculus*. Celada et al. (1991) observed at 589 D^o at 15.5 ^{o}C for *A. pallipes*. Garcia Guerrero et al. (2003) determined this stage 182- 234 D^o at 26 ^{o}C for *C. quadricarinatus*. It is observed that embryonic development processes of warm species are faster than cold species.

In the present study, active heart was seen at 1476 D^o 9-15 ^{o}C for *A.leptodactylus*. Active heart was observed about 1500 D^o at 8 ±2 ^{o}C for *A. astacus* (Palows et al., 2010),

682 D^o at 15.5 ^oC for *P.leniusculus* (Celada et al., 1987), 775 D^o at 15.5 ^oC for *A.pallipes* (Celada et al., 1991), 338-390 D^o at 26 ^oC for *C. quadricarinatus* (Garcia Guerrero et al., 2003).

We observed pigmented eyes 1536 D^o at 9-15 ^oC for *A.leptodactylus*. Pigmented eyes were observed 1800 D^o at 8 ±2 ^oC before short a time from hatching for *A. astacus* (Palows et al., 2010), 852.5 D^o at 15.5 ^oC for *P.leniusculus*.(Celada et al., 1987), 992 D^o at 15.5 ^oC for *A. pallipes* (Celada et al., 1991), 416-468 D^o at 26 ^oC for *C. quadricarinatus* (Garcia Guerrero et al., 2003).

In this study, embryo with strongly developed posterior hepatopancreas lobes was observed at 1608 D^o. This stage was determined at 945.5 D^o for *P.leniusculus* (Celada et al., 1987), at 1116 D^o for *A. pallipes* (Celada et al., 1991).

Postembryonic development

In the present study, juvenil stage I reached 1608 D^o at 15 ^oC. Garcia Guerrero et al. (2003) indicated that observed to post-embryo I (juvenil stage I) at 832-936 D^o 26 ^oC for *C. quadricarinatus*. In this study, The juvenile stage I changed its first moult after 7-8 days at 1713-1728 D^o and they reached juvenile stage II. Total length and weight of the juvenile stage II were determined as 9-11 mm and 30-60mg respectively. Garcia Guerrero et al. (2003) determined post-embryo II (juvenil stage II) at 962-1066 D^o 26 ^oC for *C. quadricarinatus*. Sandeman and Sandeman (1991) indicated that juvenile stage II was reached 7 to 8 days later the hatchling at 19 ^oC for *Cherax destructor*. In the present study, juvenile stage II changed its second moults after 9-10 days at 1743- 1758 D^o, and they reach to stage III. Total length and weight of the juvenile stage III were determined as 12-14 mm and 60-90mg. Garcia Guerrero et al. (2003) indicated that juvenile (juvenil stage III) at 1092 D^o 26 ^oC for *C. quadricarinatus*. Sandeman and Sandeman (1991) indicated that juvenile stage II was reached 14 days later juvenile stage II at 19 ^oC for *Cherax destructor*.

The incubation period of *Astacus leptodactylus* eggs was 4.5 month in the present study. Development structures of whole embryonic and post- embryonic for *Astacus leptodactylus* species were taken photograph and described as degrees x days and days at in this study.

Abbreviations

a, anus; ab, abdomen; at1, first antenna; at2**,** second antenna; bl, blastopore; md, mandible; mx 1, first maxilla; rnx 2, second maxilla; mxp 1, first maxilliped; mxp 2, second maxilliped; mxp 3, third maxilliped; opr, optic rudiment; pl, posterior hepatopancreas lobes; h, heart; ros, rostrum.

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