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RESEARCH ARTICLE

Egg incubation and post-embryonic development in the red swamp crayfish *Procambarus clarkii* from the River Nile, Egypt

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Abstract

Under laboratory conditions, the incubated egg of ovigerous females, *P. clarkii* were observed during early autumn. Examined incubated eggs have black colors, characterize by spherical shape, averaged 2.143± 0.135mm in diameter before hatching, and have incubation periods varied from 11 to 19 days. After hatching the embryo is completely free, and undergoes several successive closely spaced molts. Stage 1 (Post-embryonic stage I) was characterized by globe-like carapace with uncomplete formation of appendages. The cephalothorax has black, dorsal hump containing the remaining vitellus. Eyes are sessile and rounded. Abdomen is arrow-like, lacking uropods, with naked telson without setae. At 2nd stage, it becomes elongated, with semicircular carapace, has dark pigment pedunculated eyes, covered entirely with red dots, with setose telson. At 3rd stage, it shows beginning of chromatophore pigmentation (with red dots on the whole organism), cephalothorax has almost reached it final anatomy. Stage 4 was characterized by first appearance of alimentary canal accompanied with pigmentation increases over the entire body, with relatively large-well defined telson and uropods. During stage 5th, the fully development eyes or mature eyes were formed; faint greenish colour on whole the body and red dots especially on abdomen were observed, showing independent locomotion far from mother´s abdomen. On the other hand, stages 6-9 have approximately same characters of stage 5, but with relatively developed growth. Stage 10showed the incidence or onset of external sexual dimorphism, characterized by first appearance for males modified pleopods(1st and 2ndpleopodes), and female 4 similar biramus pleopods on abdominal segments.

INTRODUCTION

Crayfishes are a group of crustaceans that live in different environments in the world, both in lotic systems and lentic, in addition to caverns, which makes them cosmopolitan organisms with a wide range of tolerance to environmental conditions (Hunner, 1994). Over 600 crayfish species are known to exist in the world, with at least 100 species in Australia and about 300 in the Americas (Holdich, 1993), mostly (85%) in North and Central America (Rojas,1998).

The red swamp crayfish, *Procambarus clarkii* (Girard, 1852), is an autochthonous speciesin the Northeast of Mexico and South Central USA (Hobbs *et al.*, 1989). It introduced into other countries and became worldwide and dominant freshwater organisms inalmost all areas it occupies (Henttonen and Huner, 1999). In Egypt,

Procambarus clarkii had been introduced during last decades of the 20th century. The only available explanation is that the initial access and colonization of *P. clarkii* started at commercial aquaculture farm in Giza (Manial- Sheiha), in the early 1980's, when the first immigrants of this species were introduced from USA. This project was shortly terminated due to administrative failure (Fishar, 2006).

However members of genus *Procambarus* inhabit temporary water bodies during dry season they can be seen in small holes in the soil, similar to the anteaters, which conduct to tunnels and chambers with sufficientmoisture for survive (Lopez, 2008). The crayfish has been adapted in various ways, according to environmental conditions that occur in the placesthey want to colonize. The first adaptation is their ability to spend a lot of time, even months, faced with the lack of water and breathe atmospheric oxygen (Huner, 1995). In some caverns environment it has been recorded that this organism exhibits a diminution in its effective breathing rate as a response to the decrease in the concentration of oxygen, and under supply of food (Mejia, 2010).

Crayfish like other decapods crustaceans, have some biological characteristics that make it potentially important species for aquaculture, among which: adaptation to conditions of captivity and handling; accept artificial feeds of different origins (shrimp, fishaquatic plants, vegetables) and can even be fed diets with vegetable protein (75%); have a relatively short life cycle (two years or less); breeds in captivity at early age (about four months), with first spawning had high survival rates (> 75%), with reproduction all year; and some females had more than one spawning per year(Hernández-Vergara and Pérez-Rostro, 2012).

The physiological characteristics of crayfishes allow them to adapt to extreme climatic variations, diversifying their potential habitats, ensuring reproduction and contributing to progeny survival under adverse conditions. This occurs under natural or artificial conditions, making them promising organisms for use in aquaculture systems (Gutiérrez- Yurrita, 1994; Rodriguez-Almaraz & Mendoza-Alfaro, 1999).

No sufficient detailed studies on the life cycle of *Procambarus clarkii* in the Egyptian waters. Therefore, this study aims to through light on hatching, incubation period and post-embryonic development for this species under the prevailing environmental conditions in River Nile and its triturates in Egypt.

Materials and Methods

This study comprised follow up incubated eggs carried with ovigerous females from spawning (ovulation), incubation and first hatching in 13October 2013 to juvenile stages at 13 May 2014 under laboratory conditions in the Faculty of Science Al-Azhar University. Specimens were collected alive from River Nile tributaries at Al-Kanater Al- Khairiya, Qalyoubia Governorate and kept in water tanks containing fresh water (50 L), aerated with air pumps at room ambient temperature (21- 23ºC) and pH (7.3- 7.5). All animals fed on frozen fish meat. Water tanks changed at the end of each week, with aerated water under the same conditions.

All criteria used for stage determination were described based on external morphological changes observed in hatchlings (postembryonic stages) of *P. clarkii* after egg hatching according to Holdich and Lowery (1988).

Measurements of total length, standard length and egg diameter were determined by micrometric eye piece fixed in binuclear stereo microscope (Novel NTB-2B).

- Specific growth rate (SGR) in total length(mm) was calculated from the following equation:

$$SGR = 100 * \frac{Ln\ Lt - LnLi}{duratin}$$

Where Lt: final length mm, Li: initial length mm, and duration by days.

- Relative growth rate(increment rate) was calculated also from the following equation:

$$RGR = 100 * \frac{length\ increments}{intial\ length}$$

Results

A- Incubated eggs:

Incubated eggs are spherical in shape, varied from 1.93 to 2.31mm, and averaged 2.143± 0.135mm in diameter. All newly and incubated eggs have black color before hatching, transferred into opaque after hatching (Plate, I). After hatching, the hatcheries remain attached to female's pleopodes approximately one month in most cases (29-30 days).

The present results showed that, the incubation period for berried eggs varied from 11 to 23 days, and averaged 18 day.

It is worth to mention that, one of the ovigerous females ovulated on 11/11/ 2013 died in 17/11/2013. Five pleopodes carrying incubated egg (112) were cut out from the dead mother, and then put in aerated water tanks (Plate, I). The separated eggs were hatched after seven days from separation (14 from ovulation), of them 32 hatcheries (28.5 %) were recorded attached those pleopodes. The remaining un hatched eggs are spoiled. These ratios were less than recorded in other normal females.

B- Hatching:

Hatching takes place firstly by the appearance of fissure in the surface of the chorion, barely visible externally by naked eye. This fissure extends and covers the folds of the eyes and maxillae, followed by total rupture of the chorion and releasing out periopods before the total body (Plate, II). After hatching, embryos were released from incubated eggs.

C- Post embryonic stages (hatchlings):

The results of observed hatchlings (402 specimens) of the present study showed that, 13 post-embryonic stages were distinctly characterized morphologically and have remarkable colour changes, associated with regular increases in the total lengths at each stage as following:

- **Stage 1:**

This stage sometimes calls after "post embryonic stage 1" and takes one day or less. It is characterized by globe-like carapace with incomplete formation of appendages. The cephalothorax has black dorsal hump containing yolk remaining vitellus. The rest of body is translucent, with rounded sessile eyes. Abdomen has arrow-like form, with naked telson setae without uropods. Total length varied from 1.45 to 4 mm (2.32±1.46). All hatchlings remain attached to mother´s abdomen during stage (Plate, II).

- **Stage 2:**

This stage takes only one day. The body is elongated, with semicircular carapace, and has few yolk granules. Eyes are pedunculated with dark pigment. Red dots nearly cover the entire body. Telson's margins become setose. Total length varied from 2.6 to 4.1mm (3.48±0.78). Hatchlings remain attached to brood chamber (Plate, II).

- **Stage 3:**

This stage takes 5 days, and was characterized by the appearance of pigmentation (red dots on the whole body). Cephalothorax has almost reached to its final anatomy. Telson and uropods appear being separated with bristles at terminal ends (Plate, II). Total length ranged between 5.7 and 7.8 mm (6.5±1.14). Hatchlings still attached to the mother's abdomen.

- **Stage 4:**

This stage takes 7 days. It is characterized by the first appearance of alimentary canal, with elongated antennae and movable mouthparts indicating starting of feeding. Pigmentation increases over the entire body. Telson and uropods are larger-well, defined (Plate, III). Total length ranged between 7.8 and 9.75mm (8.76±1.07). Hatchlings remain attached to the mother's abdomen.

- **Stage 5:**

This stage takes 7 days. The hatchlings of this stage are characterized by fully development eyes (stalked eyes). Body has faint greenish colour with red dots especially on abdomen (Plate, III). Total length ranged from 9.1 to 10.87 mm (10.28±0.49). Most of hatchlings move independently around brood chamber of their mother.

- **Stages 6 -9 :**

These stages have similar morphological characters, with complete post embryonic development, varied in color from faint greenish to pale red with red dots over the whole body (Plate, III). It has varied from11.2 to15.32mm in total length. The averages duration period were 14, 10, 11, 12 days for these four stages, respectively.

- **Stage 10 :**

This stage is characterized by first appearance of sexual dimorphism. Males have 1st and 2ndpleopodes, which modified to gonopodes, but females have similar five biramus pleopods on abdominal segments 1-5. In this stage the total length averaged (18.02±0.64) and ranged between 17.6 to 18.75mm. It takes 14 days.

- **Stage 11 :**

It takes same characters of stage 10, with total length ranged between 19.7-21.5 averaged 20.4±0.79mm. The duration of this stage extends to take 15 days.

- **Stages 12& 13:**

The hatchlings of these stages are very similar in their characters to the previous stage 11, with total length ranged between 21-27.5mm. From the previous results it was evident that this species has 10 post-embryonic stages to reach their sexual dimorphism.

Increment rate and specific growth rate:

The present results exhibited that, early post-embryonic stage showed high increment percentage which reached 114.86 % for 2nd stage in total length but decreased gradually in the following stages reached 3.04 in stage 12 with correlation coefficient R=-0.75439 (Table, 1, and Figure, 1). Survival rate for hatchlings of *P. clarkii* was sharply decreased in early stages till stage 5, but decreases slightly in the late stages (Figure, 2).

The maximum percentage for specific growth rate was 33.22, 31.09% recorded during the 2nd and 3rd stages of post-embryonic developmental stages, respectively. It decreases to minimum percentage (0.09 %) in stage 12 (Table, 1). Therefore, this species may be taking very fast descending pattern in SGR.

Table (1): Mortality percentages and increment rate of *Procambarus clarkii* hatchlings.

Stages	Survival rate %	Duration (Days)	Average length (mm)		Increment (%)		SGR % in standard length
			Total length	Standard length	Total length	Standard length	
1	------	less one day	2.32	------	-------	------	--------
2	-----	one day	3.48	2.63	50.00	------	-------
3	-----	5	6.50	5.47	86.78	107.98	31.80
4	90.7	7	8.76	7.75	34.77	41.68	3.03
5	77.4	7	10.28	9.11	17.35	17.55	1.00
6	56.0	14	11.20	10.13	8.95	11.19	0.66
7	43.8	10	12.80	11.18	14.29	10.37	0.31
8	39.0	12	14.63	12.50	14.30	11.81	0.48
9	33.2	11	15.32	12.80	4.72	2.4	0.09
10	15.7	14	18.02	14.98	17.62	17.03	0.62
11	9.4	15	20.38	17.00	13.10	13.48	0.39
12	7.9	28	21.00	17.90	3.04	5.29	0.15
13	3.2	-	25.33	21.70	20.62	21.23	0.30

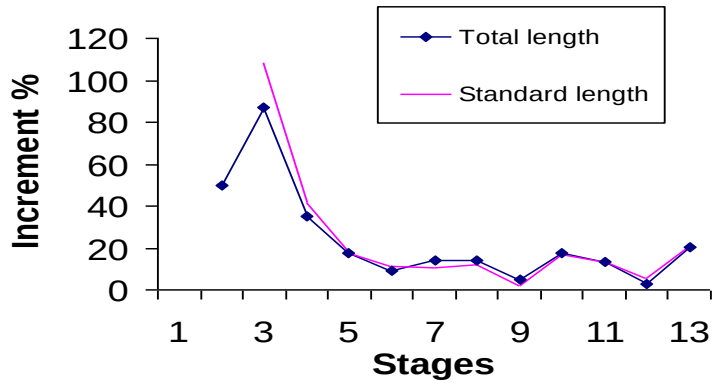


Figure (1): Increment rate of total length for hatchlings of *Procambarusclarkii*. (R=-0.75439)

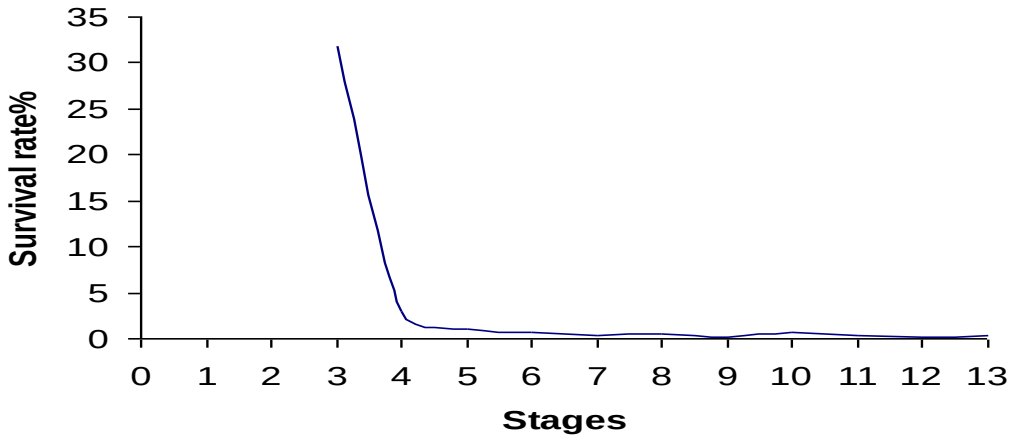


Figure (2): Survival rate percentages of post embryonic stages for fresh water crayfish *Procambarus clarkii*.

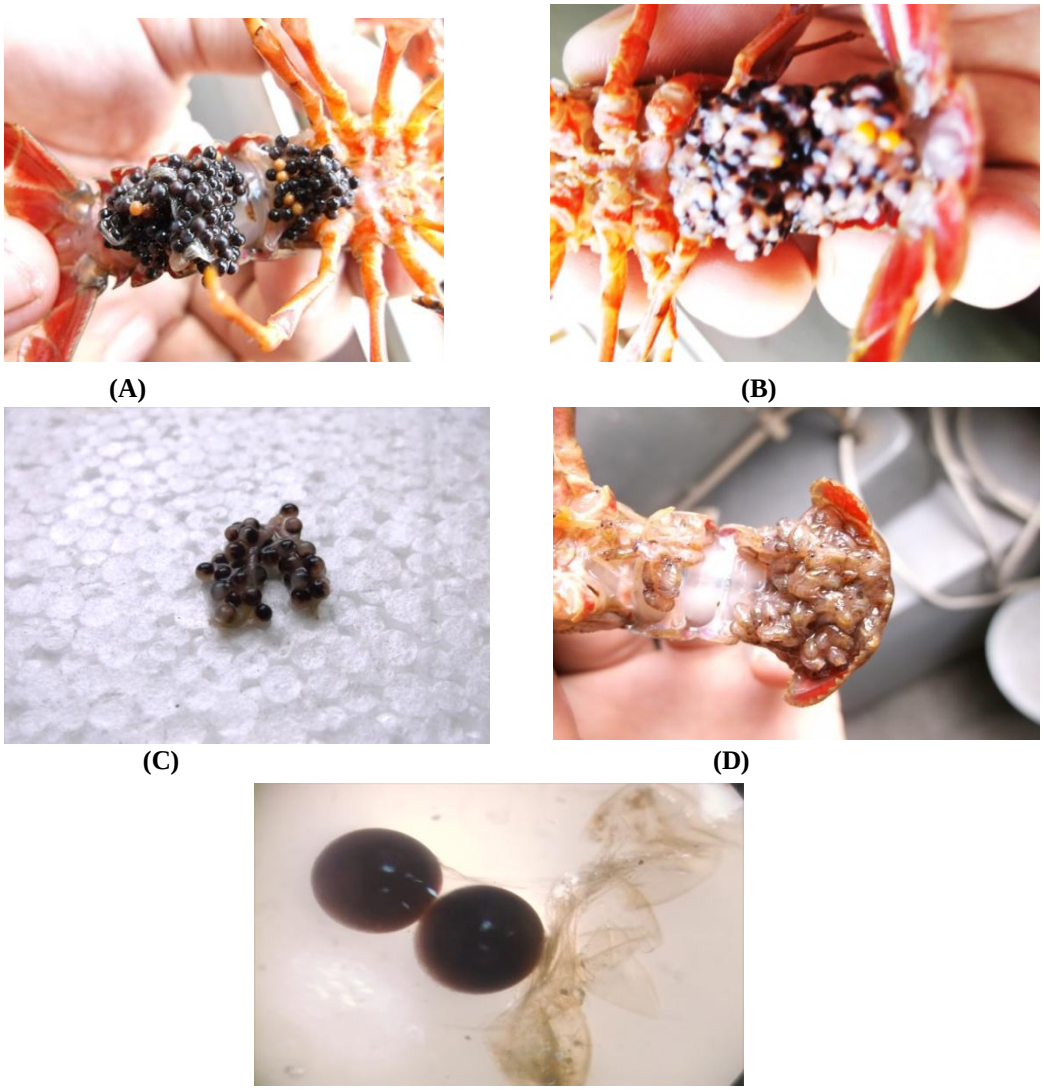
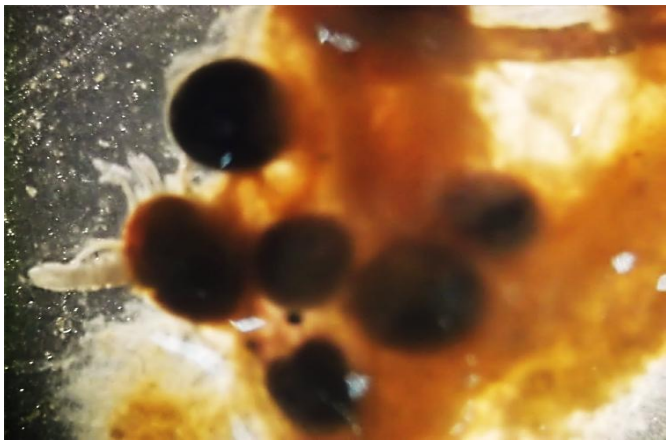
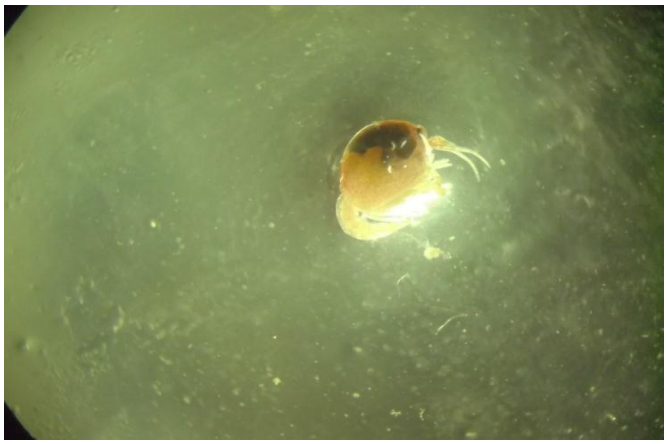


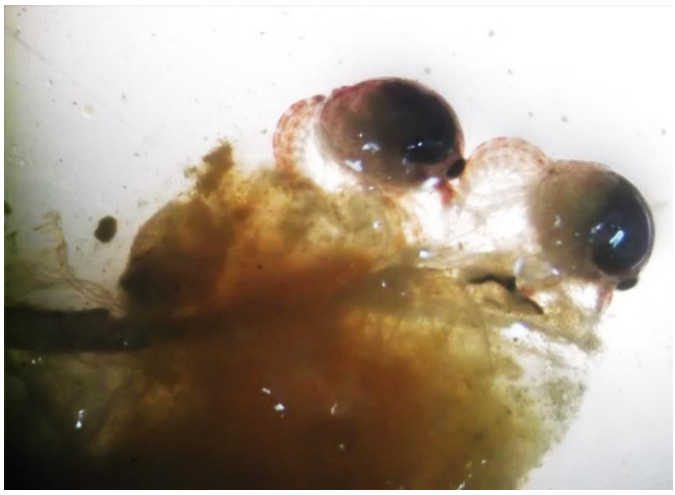
Plate I: Show: A- Incubated eggs of *Procambarus clarkii*, B- Incubated hatcheries, C- Isolated pleopodes carrying hatcheries, D- Maternal attachment of hatchlings, E- Isolated eggs just before hatching



(A)



(B)



(C)

(D)

(E)

(F)

Plate II: Shows: Post-embryonic development of *Procambarus clarkii*, A-Hatched eggs, B- Free first instar, C &D- Second stage, E- Stage 3, F- Ecdysis, and old cuticle of successive molting.

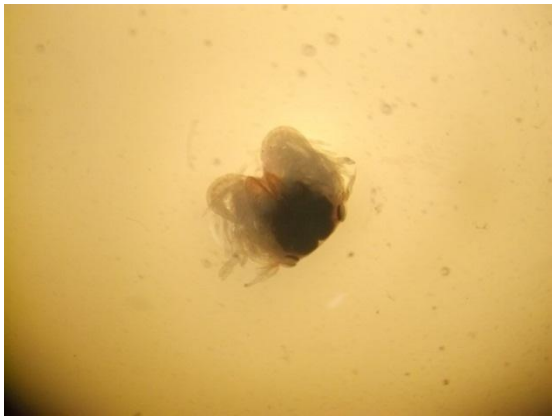
(A)

(B)

(C)

(D)

(E)



(F)

Plate III: Shows A and B: Graduation in colour change in hatchlings(stage 4,5), C- Arrow donates to apolysis in hatchlings like adults, D: - Stages 6-9 and F: hatching deformation.

Discussion

Crayfishes like other decapods crustaceans, have some biological characteristics that make them potentially important species for aquaculture, among which include: adaptation to conditions of captivity and handling; accept artificial feeds of different origins (shrimp, fish aquatic plants, vegetables) and have a relatively short life cycle takes two years or less(Huner and Lindqvist, 1995; Ibrahim and Khali, 2009). Early studies indicated that these organisms can breed in captivity at early age (about four months), and first spawning had high survival rates (> 75%), with

reproduction all year, and some females had more than one spawning per year (Hernandez-Vergara and Perez-Rostro, 2012).

The results reported in the present study clearly show the period from egg-laying till hatching varied from 11 to 23 days, and averaged 18 days during October (21-23 ºC) which agreement well with Gherardi and Barbaresi (2000) and Hernández-Vergara and Pérez-Rostro (2012) who reported that incubation and maternal attachment in crayfish within three weeks.

The development of the hatchlings of *P.clarkii* was carried out through 13 successive stages. After hatching, metamorphosis takes place, and undergoes several molts, varied from one or less day at 1st, 2nd stages to 5 days at 3rd stage. After that, they molted periodically fast enough to reach immature appearance at stage 10 after 61 days (7 days for 4th and 5th stages, 14days for 6th and 7th then 12, 11 days for 8th and 9th stages). These results agree to some extent with those mentioned by Gherardi and Barbaresi (2000) who stated that young molted periodically every 10 days. On contrast, Huner and Barr (1991) reported that this species molts and reaches its immature appearance after two to three weeks. The occurrence of hatchlings attached to their mother during early stages (1-5) may be attributed to protection between mother's pleopods. However, they leave their mothers, feed voraciously at the following stages, and reach sexual dimorphism at stage 10 after six weeks from hatching.

The results of the present study indicated that this species has 9 post-embryonic stages and reached sexual characteristic at stage 10. It increases in size from initial egg diameter varied from 1.93 to 2.31mm, and averaged 2.143± 0.135mm to juveniles with size up to 1.8 cm. These results are very close to that reported by Montemayor *et al.* (2010) for *Procambarus regiomontanus*, which has eight embryonic stages and eight post-embryonic stages, with an average juvenile size of 2 cm. But on contrast to that mentioned by Garcya-Guerrero *et al.* (2003) on *Cherax quadricarinatus* and *C. destructor* which have two post-embryonic stages and a juvenile stage only.

The present results indicated that, *P. clarkii* has the longest post-embryonic stages within its genus. This may be due to lower embryonic development periods than a cold water species mentioned by Holdich and Lowery (1988).

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