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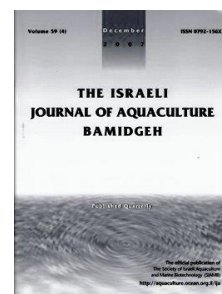
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Characterization of the Embryonic and Larval Development of Wild Pike-Barb (*Luciobarbus esocinus*) Biological Parameters, and the Possibility of Breeding

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Key words: *Luciobarbus esocinus*, pike-barb, artificial reproduction, embryonic and larval development, Euphrates River

Abstract

This study was conducted to obtain preliminary data on the possibility of breeding this species through artificial insemination and to determine the incubation period at different temperatures (°C) of hatchery water [20 (A1), 22 (A2), 25 (A3) and 26 (A4); (±1)] and to determine the morphological and larval growth parameters of *L. esocinus*. The blastoderm formed an embryonic shield 43 h (A1 and A2) and 36 h (A3 and A4) after insemination. The eggs hatched after 113 h (A1 and A2) and 61.5 h (A3 and A4) after insemination, and new larvae were observed. Exposing the newly fertilized eggs to various temperatures during the incubation period resulted in significantly longer duration of embryo formation, egg hatching and yolk sac consumption (h) at 20°C and 22°C than at 25°C and 26°C ($P < 0.05$). No significant differences were observed between eggs exposed to 20°C and 22°C and those exposed to 25°C and 26°C ($P > 0.05$). However, significant differences in day-degree of hatching were observed among all the groups ($P < 0.05$).

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Introduction

In aquaculture, the adaptation of new species is based on understanding the reproduction cycle, including larval stages (Kamler, 2005).

Spawning is controlled by endocrine mechanisms that are influenced by external conditions, such as seasonal changes in photoperiod and temperature. Some fish can spawn over a long period of time, e.g. several months, whereas others spawn over an interval of only a few weeks. Ecological features of spawning sites such as temperature, water flow, oxygen concentration, suitable substrate, and protective cover are extremely important for egg survival and development (Bagenal and Braum, 1978, Kamler, 2002). Several terminologies have been developed to describe the early life history stages of fishes, and these differ according to the developmental events that are considered fundamental. The basic stages consist of the egg or embryonic stages, from fertilization to hatching, the larval stage from hatching to the juvenile stage and attainment of complete fin-ray counts and the juvenile or post-larval stage (Demirsoy, 1993; Schreck and Moyle, 1990).

Luciobarbus esocinus, (pike barb) belongs to the Cyprinidae family and populate the Tigris and Euphrates river systems. This species can grow to an average weight of 60 kg and reach lengths ranging from 100–150 cm. It is of significant commercial value because its flesh is tasty. *L. esocinus* is a benthopelagic species that lives in large rivers and dams. However, details of its environmental requirements are unknown. The maximum recorded length and weight of *L. esocinus* are 230 cm and 140 kg respectively (Stone, 2007). This fish was formerly known as *Barbus esocinus* and was renamed *L. esocinus* in 2007 (Fricke et al. 2007).

The aim of the present study was to obtain preliminary data on the possibility of artificially breeding pike-barb as well as evaluating the incubation period of fertilized eggs, optimal water temperatures, and the larval period and growth of artificially spawned *L. esocinus*.

Materials and Methods

Study area and experimental layout. In this study, *L. esocinus* inhabiting the Karasu River were investigated. The Karasu River is a part of the upper Euphrates Basin located in the Kemaliye District of Erzincan Province, Turkey. For the incubation of fertilized eggs, we applied a hatchery mechanism adapted to a temperature-controlled aquarium. Water and air circulation were provided using a motor pump (Figure 1). Incubators were divided into 4 per aquarium with different temperatures (°C) [20 (A1), 22 (A2), 25 (A3) and 26 (A4); (± 1)]. In all the experiments, the water quality parameters were analyzed with a spectrophotometer, NOVA 60. Temperature, pH and dissolved oxygen were measured daily using a Hana HI model digital oxygen and pH meter.

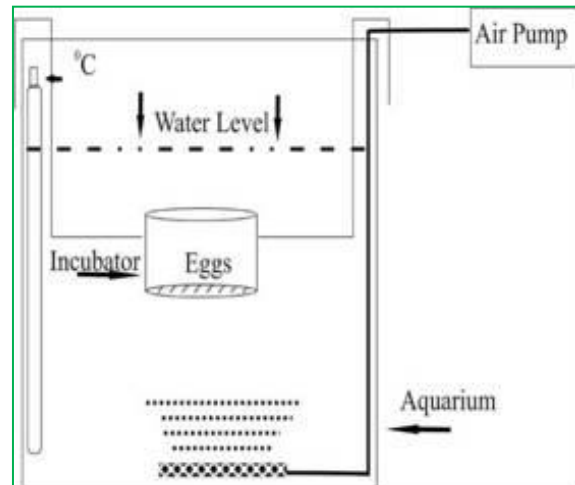


Fig. 1. Experimental scheme for determining the optimal incubation period for the fertilised eggs.

Wild brood stock handling and artificial insemination. Mature fish samples were caught from the Karasu River using gill nets (90 x 90 mm, 110 x 110 mm). Mature eggs and sperm were obtained from naturally spawning domesticated brood stock of *L. esocinus* at a temperature of 18°C and a natural photoperiod (12.59 h of daylight, 11.41 h of darkness) on 9 April, 2010. April and May are the normal spawning period for wild

pike-barb. The temperature, dissolved oxygen level, and pH of the water was 18°C, 7.5 mg/L and 8.2, respectively, at the time of capture of the broodstock.

Average weight of wild male broodstock (n=5) was 17.40 ±5.8 kg, and average total length was 75.30 ±15.07 cm in, whereas the average weight of the females (n=6) was 22.35 ±8.9 kg and 87.17 ±10.5 cm average total length. The fish (3 males + 2 females) were stripped in a domestic environment during the natural spawning period on 9 April, 2010. Spawning was achieved using the dry fertilization method. Eggs from each female were stripped into a dry dish, and sperm was added. A disinfected feather was used to gently mix the eggs and sperm. Water was added, and the mixture was stirred again. Next, an activating solution of 4 g of NaCl and 3 g of urea per liter of water was added to the mixture. Continuous stirring for 1.5 h in the same solution prevented adhesion (Al Hazzaa and Hussein, 2003). Fertilized and hardened eggs were carefully transported immediately to the hatchery aquaria at the Department of Aquaculture facility (within approximately 45 min). The reproduction of *L. esocinus* was conducted at the Department of Aquaculture, Kemaliye Hacı Ali Akın Vocational High School at Erzincan University, Turkey.

Egg size and larval growth. Egg diameter (mm) was measured under a dissecting microscope equipped with a micrometer eyepiece Leica EZ4 model. After stripping, 50 egg samples were used per observation. Upon hatching, 15 larvae were randomly sampled from each batch of eggs, and the total length (TL) was measured to the nearest millimeter. Length growth was expressed using the von Bertalanffy equation (Beverton and Holt, 1957):

$$L_t = L_\infty \times (1 - e^{-K \times (t - t_0)})$$

Where L_∞ is the asymptotic total length, L_t is the total length at age t , K is the growth curvature parameter and t_0 is the computed theoretical age at the time of hatching. Growth parameters were estimated according to the non-linear method using a spreadsheet in MS Office Excel 2007. Allometric growth was calculated as a power function of L_T using non-transformed data: $y = a L_T^b$, where y is the measured character, a is the intercept, b is the growth coefficient and L_T is the total length (Fuiman, 1983).

Growth was evaluated on the basis of length during the larval period. The absolute growth length (AGL), relative growth length (RGL), specific growth rate of length (SGRL), instantaneous growth length (IGL), characteristic growth length (CGL) and development rate (V) with the biological process rate (Q_{10}) were computed using the following formulae (Bertalanffy, 1938; Chugunova, 1963; Pawlak and Hanumara, 1981; Atay, 1989; Kamler, 2002; Çetinkaya et al. 2005):

$$AGL = L_2 - L_1, \quad RGL = \left[\frac{(L_2 - L_1)}{L_1} \right] \times 100, \quad SGRL = \left[\frac{\ln\left(\frac{L_2}{L_1}\right)}{t(day)} \right] \times 100$$

$$, \quad CGL = \left[\frac{\ln(L_2) - \ln(L_1)}{0.4343} \right] \times t(day) \times L_1 \quad IGL = b \times [\ln(L_2) - \ln(L_1)]$$

Volume (mm^3) of the ellipsoidal larval yolk sac at hatching was calculated with the following formula (Heming & Buddington, 1988):

$V = 0.1667 \times \pi \times L \times H^2$, where H is the height and L is the length of the yolk sac mass.

$V = t^{-1}$, where t is the time (days) from fertilization to the mass occurrence of a given developmental stage. In addition,

$$Q_{10dev} = \left(\frac{V_2}{V_1} \right)^{\frac{10}{(t_2-t_1)}}, \text{ where } V_1 \text{ is the developmental rate at a lower temperature (} t_1 \text{)}$$

and V_2 is developmental rate at a higher temperature (t_2) (Belehradek, 1930).

Statistical analysis. The effects of different temperatures on the duration of embryo formation, egg hatching, day-degree and yolk sac consumption were analyzed by one way ANOVA using the SPSS Statistical Software System, Version 16.0 (SPSS, Chicago, IL, USA). When the effects were significant, Duncan's Multiple Range test was conducted for multiple comparisons of means. Differences with $P < 0.05$ were considered statistically significant. For descriptive statistical analysis, we used the mean and standard deviation.

Results

Water quality parameters in incubators. No significant differences in the different physico-chemical parameters of overlying water were observed among various treatments. The temperatures of incubation aquariums were held at 20°C, 22°C, 25°C and 26°C for A1, A2, A3 and A4, respectively. The physical and chemical parameters of the hatchery water at different temperatures, dissolved oxygen, pH, alkalinity, hardness and ammonia, nitrite and nitrate levels are shown in Table 1.

Table 1. Physico-chemical parameters of hatchery water at different temperatures.

Temperature	Dissolved Oxygen, Mg/ml	pH	Alkalinity (as CaCO ₃) Mg/ml	Hardness (as CaCO ₃) mg/ml	NH ₄ , mg/ml	NO ₂ , mg/ml	NO ₃ , mg/ml
20	7.73-8.25	7.65-8.05					
22	7.12-8.18	7.44-7.85					
25	6.25-7.17	7.07-7.12	100-125	115-120	0.02-0.2	0.01-0.03	0.08-0.10
26	6.15-7.07	6.95-7.02					

Incubation period. In this study, the diameter of the fertilized water-hardened eggs of *L. esocinus* was 3 ± 0.05 mm ($n=10$). The chorionic membrane was adhesive, spherical, transparent, and straw-colored. The yolk was a paler yellow color, without oil globules (Figure 2). The eggs were demersal. Perivitelline space measured approximately 0.4 mm. During the hatchery period, the blastoderm formed an embryonic shield 43 h (A1 and A2) and 36 h (A3 and A4) after insemination. The blastoderm covered almost half of the egg 67 h (A1 and A2) and 43 h (A3 and A4) after insemination, and the embryo was formed (Figure 3). Some myomeres could be recognized at 89 h (A3 and A4) and 50.5 h (A3 and A4) after insemination. The eggs hatched at 113 h (A3 and A4) and 61.5 h (A3 and A4) after insemination, and new larvae were observed at 5.14–5.63 degree days (A1 and A2) and 2.46–2.36 degree days (A3 and A4) (Table 2). Incubating the newly fertilized eggs at various temperatures resulted in significantly longer duration of embryo formation, egg hatching, and yolk sac consumption (h) at 20°C and 22°C than at 25°C and 26°C ($P < 0.05$). However, no significant differences were observed between 20°C and 22°C as well as between 25°C and 26°C ($P > 0.05$). In contrast, for day-degree of hatching, significant differences were observed among all the groups ($P < 0.05$) (Table 2).

Table 2. Incubation period of *L. esocinus* at different temperatures.

Incubation periods	Temperature Sets	Mean±SD	95% Conf. Interval for Mean	
			Lower Bound	Upper Bound
Embryo formation time (Hour)	A ₁	66.50±0.88 ^b	65.41	67.59
	A ₂	66.53±0.83 ^b	65.50	67.56
	A ₃	43.04±0.31 ^a	42.66	43.42
	A ₄	42.88±0.28 ^a	42.54	43.22
Egg hatching time (Hour)	A ₁	112.48±0.53 ^b	111.83	113.13
	A ₂	112.94±0.19 ^b	112.70	113.18
	A ₃	61.53±0.38 ^a	61.06	62.00
	A ₄	61.48±0.19 ^a	61.24	61.72
Day/Degree of hatching rate	A ₁	5.63±0.03 ^d	5.59	5.66
	A ₂	5.14±0.01 ^c	5.12	5.15
	A ₃	2.46±0.02 ^b	2.44	2.48
	A ₄	2.36±0.01 ^a	2.35	2.38
Consumption of yolk sac time (Hour)	A ₁	280.00±1.58 ^b	278.04	281.96
	A ₂	279.50±1.12 ^b	278.11	280.89
	A ₃	210.50±0.71 ^a	209.62	211.38
	A ₄	210.50±0.57 ^a	209.80	211.20

Differences among average values are indicated by different alphabets (a–d) in the same line (ANOVA and Duncan's test, $P < 0.05$); A₁ = 20°C, A₂ = 22°C, A₃ = 25°C and A₄ = 26°C.

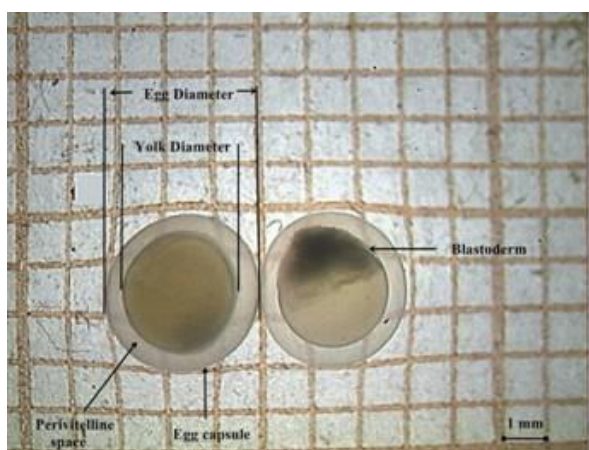


Fig. 2. Blastula development and an egg of *L. esocinus*.

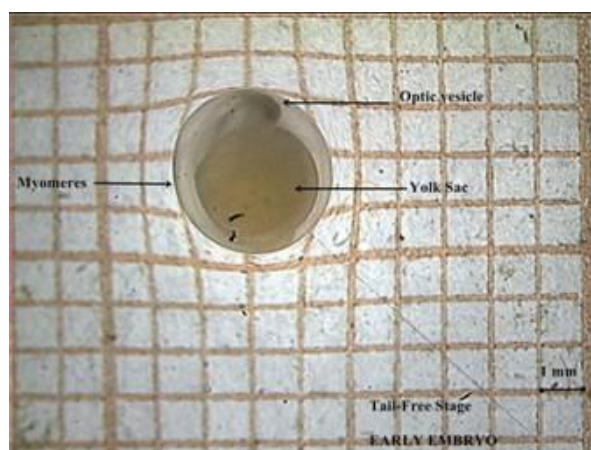


Fig. 3. Early embryo of *L. esocinus*.

Yolk-sac larval period. The length of the first-day larva was 8.5 mm. No eye pigmentation and active swimming were observed. Eye pigmentation was established after the second day, and its color crystallized on the third day (Figure 4). Body pigmentation of larva occurred after the 8th day. In addition, the larva could swim semi-actively, and the fins were visible to naked eye (Figure 5). The gas bladder could be observed after the 11th day along with highly active swimming (Figure 6). After the 15th day, the larva had consumed three quarters of its yolk sac, and the pectoral, ventral and dorsal fins could be observed (Figure 7). The morphological parts of *L. esocinus* are shown in Figure 8. The survival rate of larva was high.

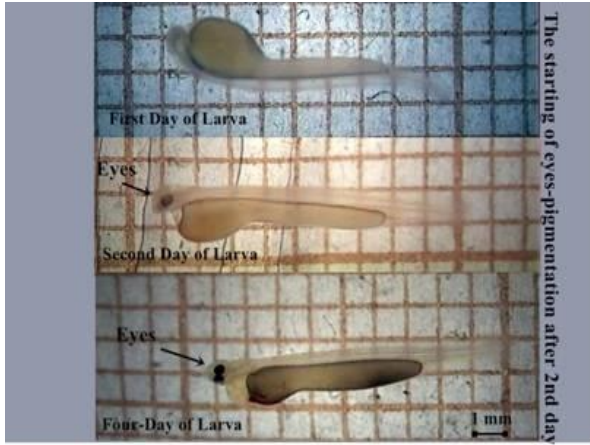


Fig. 4. First, second, and fourth day larvae of *L. esocinus*.

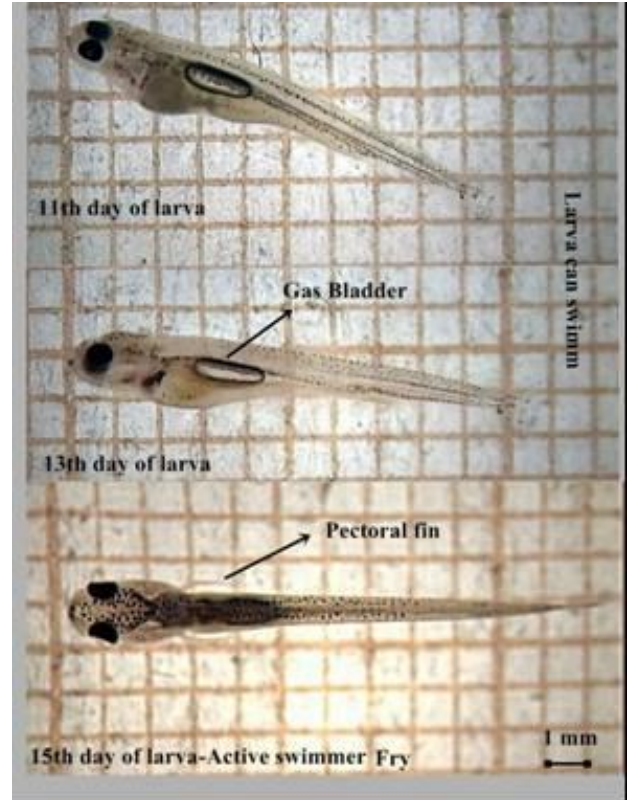


Fig. 6. Establishment of the gas bladder and 11th and 15th day larva of *L. esocinus*.

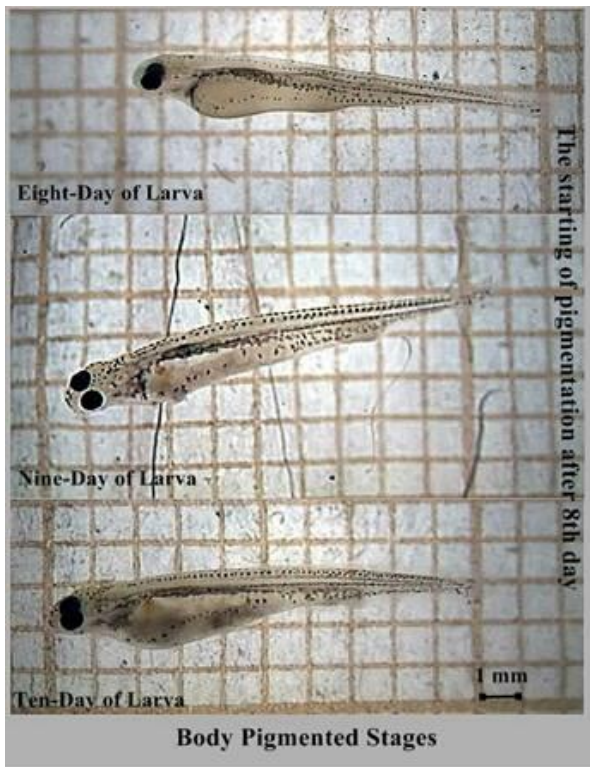


Fig. 5. 8th, 9th and 10th day larvae of *L. esocinus*, showing active body pigmentation.

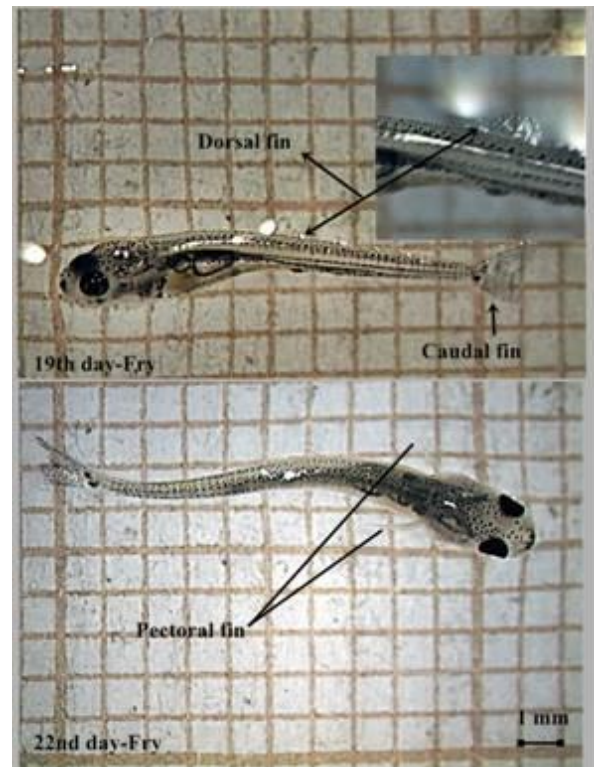


Fig. 7. Dorsal, caudal and pectoral fins of *L. esocinus*.

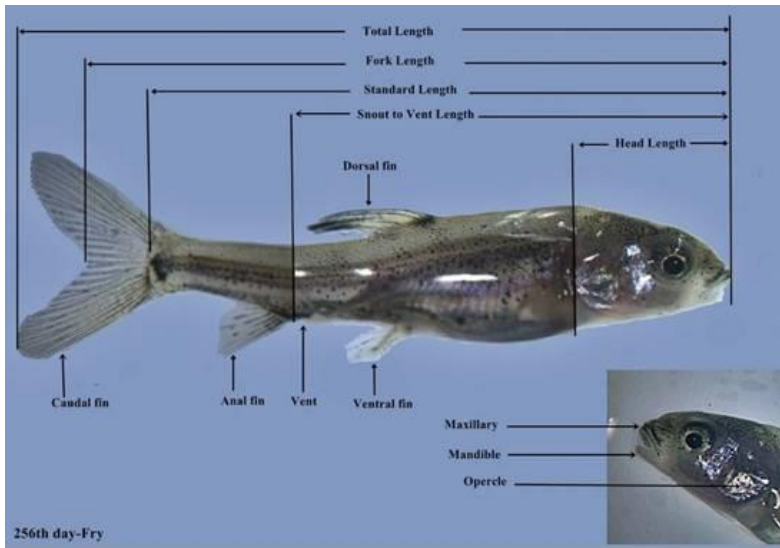


Fig. 8. Morphological parts of *L. esocinus* after 25th day.

In this study, the volume of the ellipsoidal larval yolk sac measured at hatching was 13.61 mm³. Oil globules were not observed in the yolk sac, which occupied three quarters of the abdomen of the larva. Length of the larva was 11.5 mm after consumption of yolk.

According to the von Bertalanffy equation, formula for length growth was determined using L_{∞} , a , b , K and t_0 :

$$L_t = 10.77 [1 - e^{-0.343 (t + 1.5002)}] \text{ (Table 3).}$$

Table 3. Growth parameters for the larva of *Luciobarbus esocinus*.

Parameters	Mean±SD.
Length of First Hatching Larva (mm)	8.5±0.22
Length of Post Larva (mm)	11.50±0.34
Volume of Larval Yolk sac (mm ³)	13.61±0.85
Absolute Growth of Length (AGL)	3
Relative Growth of Length (RGL)	0.35
Specific Growth Rate Length (SGRL)	2.02
instantaneous Growth of Length (IGL)	0.21
Characteristic Growth of Length (CGL)	38.54
a	3.13
b	0.71
K	0.343
L_{∞}	10.77
t_0	-1.5002
$Q_{10Dev} (A_1-A_2)$	2.87
$Q_{10Dev} (A_3-A_4)$	1
$Q_{10Dev} (A_1-A_4)$	2.76

In this study, using regression analysis a strong relationship was observed between temperatures and duration of embryo formation, hatching, and yolk sac consumption. (Table 4, Figure 9, 10).

Table 4. Regression analysis between temperatures and various incubation parameters.

Incubation parameters	a	b	R^2
Temperatures-Embryo Formation Times	-4.661	163.1	0.89; P<0.05
Temperatures-Hatching Times	-10.10	322.1	0.89; P<0.05
Temperatures-Yolksac Consumption Times	-13.72	564.1	0.90; P<0.05

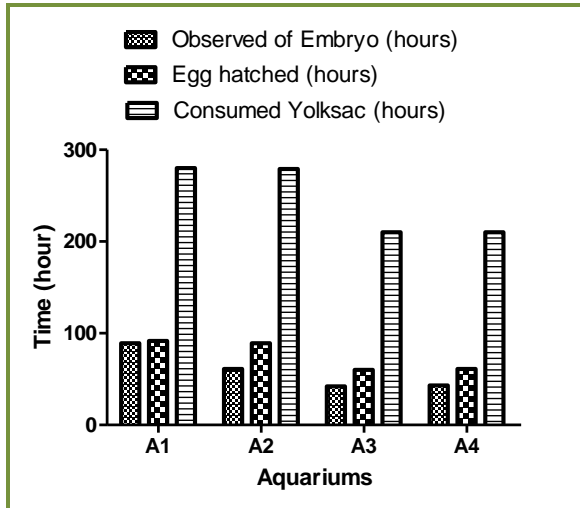


Fig. 9. The times of Embryo, hatching and consumed yolksac at different temperatures.

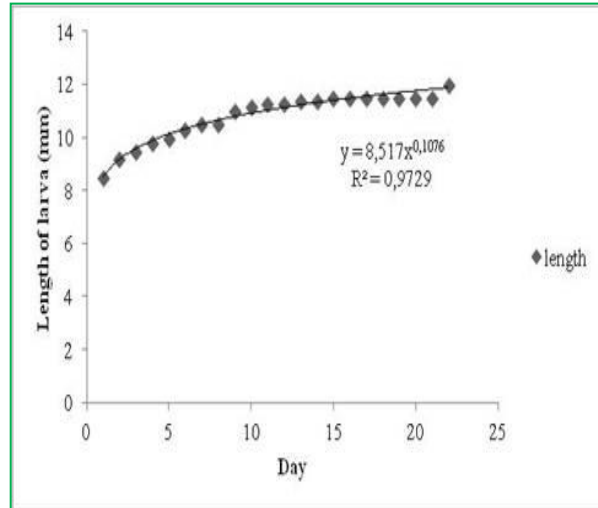


Fig. 10. The relationship between day and length of larva.

Discussion

The eggs and yolk sac of pike-barb, *L. esocinus*, were incubated in an experimental hatchery system at different temperatures. The embryonic development of eggs, hatchability, and growth of the yolk sac, were studied under controlled conditions. The results showed that the time from fertilization to hatching decreased in relation to increase in temperature. This observation is in agreement with results obtained for other fish species (Kucharczyk et al. 1997; Michal, 2008; Abd El-Hakim, 2009). However, temperature also influences embryonic development and affects survival and efficiency of yolk utilization (Fuiman, 2002; Kamler, 2002, 2008). As in many other cyprinids, larval development of *L. esocinus* was similar and early eye pigmentation formed in the larval stage rather than during the embryonic phase. Similar results were found in *B. xanthopterus*, *B. grypus* and *B. sharpeyi* (Pyka et al. 2001). While the first embryonic formation of the egg was observed after 67 h (A1 and A2) and 43 h (A3 and A4) of incubation in this study, it has also been observed in another study after 65 h of incubation (Pyka et al. 2001).

During isometric growth, $b = 1$, allometric growth is positive when $b > 1$ and negative when $b < 1$ (Gisbert, 1999). The growth of *L. esocinus* larva was negative with an allometric growth of $b = 0.71$ in this study.

The SGRL is defined as the percentage of daily growth of fish at a time. SGRL is determined using various parameters, such as physiology, morphology and biomass (James and Drenovsky, 2007); therefore, factors such as weight, length and height can be used to express specific growth rate. In this study, the mean standard length of fish from all groups was used to calculate specific growth rate. The SGRL was 2.02 after 15 days in the larval period. Absolute, relative and instantaneous growths are a simple measurement of the total change in size over a specific period of time. Absolute growth is slow when fish are small and increases as they grow larger (Brett, 1979; Jones, 2002). In this study, absolute, relative, and instantaneous growths in length were 3, 0.35 and 0.21, respectively, during the larval period of *L. esocinus* (Table 3).

Q_{10} is a thermodynamic expression for elucidating the effects of temperature and is commonly used to standardize a rate of change in response to temperature to a common index. It describes the response of biological processes to a 10°C change in temperature (Schmidt-Nielsen, 1997). Due to the complexity of cellular structure and metabolism, the acute thermodynamic response to temperature cannot be predicted from the first principle. However, this response is determined to a significant extent by the temperature sensitivity of weak bonds, and the typical Q_{10} values are 2–3 (Hochachka and Somero, 2002). In addition, a compilation of Q_{10} values within species to elucidate the acute effect of temperature on resting metabolic rate in teleost fish suggest a mean Q_{10} of 2.36 (Clarke and Johnston, 1999). $V = t^{-1}$, where t is the time (days) from

fertilization to the mass occurrence for a particular developmental stage. To confirm the number of times the biological process rate (in this case development) theoretically increases with temperature, 10°C increases in the temperature coefficient Q_{10} , had already been applied to V. In this investigation, the $Q_{10\text{dev}}$ of A_1 - A_2 and A_1 - A_4 was determined to be 2.87 and 2.76, respectively during the larval period of *L. esocinus* (Table 3). The values of Q_{10} in this study reflect results in common carp (1.7-4.5) and grass carp (1.5-3.7) at 20, 24, 28 and 32°C (Korwin-Kossakowski, 2008) and in common carp (2.36) (Kaushik et al., 1982).

The processes of artificial fertilization, embryonic development and growth of *L. esocinus* larvae were monitored in the present study. The rate of fertilization was low, whereas the rate of egg development and hatching as well as survival of larvae was high. Artificial insemination achieved with a sperm mixture. *L. esocinus* is endemic to the Euphrates basins, and it reproduces naturally. The short embryonic and larval periods of the pike-barb are considered an advantage for aquaculture of such warm-water fish. This investigation provides new information on the embryonic and larval development of fish in *Barbus* genus of the Cyprinidae family.

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