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ORIGINAL ARTICLE

Embryonic and larval development of garpike from the Adriatic Sea

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Abstract

Although information about embryonic and larval development of garpike, *Belone belone* (Linnaeus, 1761), is present in the published literature, the bulk of research concerns garpike from the northeastern Atlantic Ocean and the Baltic Sea. The present work describes the embryonic and larval development of garpike, *Belone belone*, from the Adriatic Sea, and methods used for incubation of fertilized eggs in aquarium conditions. Because garpike is, as suggested by some authors, divided into subspecies, we conclude that some differences in embryonic development could also be expected. In the present study, eggs were fertilized using the dry fertilization method and were incubated in a tank equipped with aeration and constant sea water flow. Salinity and content of dissolved oxygen were constant, and the temperature varied between 19.4 and 22.3°C. Eggs were spherical, measuring 3071.9 ± 75.73 μm in diameter. Yolk sacs were homogeneous and did not contain oil globules. The first larvae hatched 329 h and 47 min after fertilization. Absorption of the yolk sac occurred 17 h – 48 h after hatching and the total length of newly hatched larvae was 9.78 mm. The peculiarities observed in the embryonic and early larval development are evidence of an exceptional plasticity and adaptive potential, which could be considered as helpful features in extending the natural range of occurrence of this species.

Key words: Adriatic Sea, *Belone belone*, embryonic development, larval development.

INTRODUCTION

The garpike, *Belone belone* (L. 1761), is distributed in the eastern Atlantic Ocean and the Mediterranean Sea. It is a pelagic, marine and brackish water fish that lives close to the surface where it feeds mostly on small fish (Jardas 1996.).

It has a very elongated and laterally flattened body. Garpikes are oviparous gonochorists and have large eggs (3.0–3.5 mm) with long tendrils for attaching onto objects in the water (Breder & Rose 1966). In the Adriatic Sea it spawns in coastal waters from October to May (Jardas 1996). Garpikes can be caught during the whole year but are mostly caught in January and February. The garpike does not have large economic value in Croatian fisheries. The Croatian garpike catch in 1994 was 88 t, whereas in 2003 it was only 3 t (Jardas 1996.).

Information on early development stages of garpike from the Baltic Sea can be found in Rosenthal and Fonds (1973), Fonds *et al*. (1974), Westernhagen (1974) and Korzelecka-Orkisz *et al*. (2005). Such data are lacking for the garpike from the Mediterranean area, including the Adriatic Sea.

According to Collette and Parin (1986), three subspecies of *B. belone* can be distinguished, each with different areas of distribution. A nominal subspecies, *B. belone belone* (L. 1761), is distributed in the northeastern Atlantic from France northwards, *B. belone euxini*

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Günther, 1866 in the Black Sea and the Sea of Azov, and *B. belone gracilis* Lowe, 1839 in the southern northeast Atlantic and the Mediterranean, including the Adriatic Sea. These subspecies are distinguishable by meristic characteristics, such as vertebrae counts and fin ray counts in the dorsal fin.

The aim of the present work is to describe embryonic and early larval development of *B. belone* from the Adriatic Sea, to provide descriptions of the early life history stages of this species and to point out peculiarities in development that could be evidence of an exceptional adaptive potential of this species. Furthermore, differences in embryonic characteristics between those garpike observed in the present study, and those provided in previous studies for Baltic waters could provide further evidence of the separation of the subspecies, as proposed by some authors.

MATERIALS AND METHODS

Broodstock individuals were caught in Karinski ždril in the Karin Sea (middle Adriatic) on 6 June 2005 using a shore trawl net. Of 22 surviving specimens, five were chosen for broodstock: two males (65.2 cm total length TL, 60.8 cm TL) and three females (49.4 cm TL, 46.6 cm TL, 40.8 mm TL).

Embryonic development was observed under a binocular microscope. The diameters of the eggs were measured. Embryogenesis was examined at different time intervals.

Eggs were artificially fertilized with sperm using dry fertilization. A random sample of 48 eggs was separated for observation 7 h and 17 min after fertilization. Egg and yolk diameter were measured using an ocular micrometer and a binocular microscope. All eggs that were chosen for observation were ‘shaved’ using tweezers and a hypodermic needle, because eggs are equipped with sticky fila-

Table 1 Embryonic development of the garpike

Time	Stage	Description	Temperature (°C)
00.00	Fertilzation		15.2
07.15	Morula	Clearly noticeable morula stage	22.5
21.30	Blastula	Noticable blastula stage and growth of perivitelline space	22.0
39.00	Neurula	End of neurula stage, formation of notochord	22.0
55.00	Embryo	Optic vesicles visible, beginning of heart cavity formation, somites clearly visible	21.5
60.00	Embryo	14 somites visible, appearance of Kupffer cells	21.0
72.00	Embryo	Heart begins contracting (27/min)	21.0
80.00	Embryo	Pigmentation, melanophores on head and eyes, heart 52/min	20.5
90.00	Embryo	Heart 85/min	20.5
100.00	Embryo	Formation of pectoral fins, heart 103/min	20.1
113.00	Embryo	Melanophores along the spine and scattered on tail tip, yolk sac circulatory system formed, heart 108/min	19.8
123.00	Embryo	Circulation of blood vessels visible, heart 100/min	19.3
135.00	Embryo	Clearly visible pectoral fins, heart 112/min	19.5
155.00	Embryo	Melanophores down full length of yolk sac	19.8
200.00	Embryo	Pectoral fins functional	20.8
230.00	Embryo	Tail tip touches the head, heart 130/min	21.00
250.00	Embryo	Jaw developed and mobile, heart 150/min	21.2
277.00	Embryo	Operculum functional, heart 154/min	21.8
330.00	Free larvae	Hatching	22.6

ments approximately 8–20 mm long. During further examination, notes were taken describing the condition of the embryo, and in later stages the frequency of heart and jaw (after the mouth opened) contractions. Standard morphometric measures were also taken.

Fertilization was achieved at a temperature of 15.2°C and salinity of 35 (transport conditions), and eggs were acclimated to the incubation temperature and salinity (22.1°C, 37.8, respectively). The temperature regime during the incubation varied in accordance with the environmental conditions because the tanks were not tempered. The incubation tanks were equipped with an aeration unit providing an air flow of 20 L/h.

RESULTS

Egg diameters were sorted into six (from 2950 to 3200 μm) and yolk into five length categories (from 2850 to 3050 μm). In a sample of 10 eggs, values of yolk diameters in proportion to egg diameters fluctuated between 93.4 and 98.3%. Garpike eggs obtained in this study were spherical and transparent, and the yolks were homogeneous, unsegmented and did not contain oil globules. The average diameter of newly spawned eggs was 3071.9 ± 75.73 μm, with a yolk diameter of 2921.3 ± 53.22 μm in a salinity of 35–37.8. The chorion of the eggs was quite thick, with numerous long tendrils.

The first appearance of the morula stage (Fig. 1) was observed approximately 7 h after fertilization (Table 1), while blastula was first observed after 21 h 30 m. At this time, an intensive growth of perivitelline space was observed. After 55 h, formation of optic vesicles and somites, and the beginning of formation of the heart cavity were observed. The heart started beating after approximately 72 h. At 113 h, the yolk–sac circulatory system was formed, and at 135 h, pectoral fins were clearly visible. At 230 h, the embryo occupied three-quarters of the yolk–sac circumference. Jaws were completely developed and mobile after 250 h (Table 1).

The first larvae hatched 329 h 47 min after fertilization. Newly hatched larvae had functional jaws, operculum and pectoral fins, and were capable of active feeding. Absorption of the yolk sac occurred 17 h – 48 h after hatching, and immediately thereafter the larvae started catching air bubble in order to form a swim bladder. The total length of newly hatched larvae was 9.78 mm.

This study was based mostly on embryonic development, as opposed to larval development. Therefore, larvae were not fed, which resulted in high incidence of death. Newly hatched larvae were exposed to stress because of the lack of oxygen.

Morphometric changes of garpike larvae during the first 72 h after hatching are presented in Table 2. Because of the high mortality of larvae and rapid cell lysis, only seven were measured correctly. The values shown in the table are mean values of each morphometric parameter taken from measured larvae.

Appearance of the blue–green pigmentation in larvae was consistent with the absorption of the yolk sac and the beginning of the extension of the lower jaw. The pigmentation first appears on the operculum and ventral side, then spreads to the head and finally to the whole body.

DISCUSSION

Research conditions in our experiment differed from those of other studies, such as von Westernhagen (1974), Rosenthal and Fonds (1973) and Korzelecka-Orkisz *et al*. (2005), and were ambiental rather then fully controlled. The variations in temperature might have proved to be an advantage because they provided temperature and salinity conditions close to those of the natural environment. A limitation of the present in comparison to other studies is the existence of only one series of incubated eggs, as opposed to two (Rosenthal and Fonds 1973), three (Korzelecka-Orkisz *et al*. 2005) and 36 (von Westernhagen 1974).

Our results show that 24 h old larvae had bigger yolk

Table 2 Morphometric changes of garpike larvae during the first 72 h after hatching (in mm)

Hours after hatching	Lt	Ls	Lc	O	La	a	b	Hmax	Hm
0 (3 larvae)	9.78	9.0	1.85	0.55	5.9	1.9	1.08	1.48	0.5
24 (2 larvae)	10.5	9.5	1.83	0.53	6.88	2.33	0.70	1.3	0.4
72 (2 larvae)	13.97	13.05	3.25	0.8	8.93	–	–	1.08	0.3

Lt, total length; Ls, standard length; Lc, head length; O, eye diameter; La, pre-anal length; a, yolk sac length; b, depth of yolk sac; Hmax, greatest body depth; Hmin, smallest body depth). Number of measured larvae for each time interval is shown in parentheses.

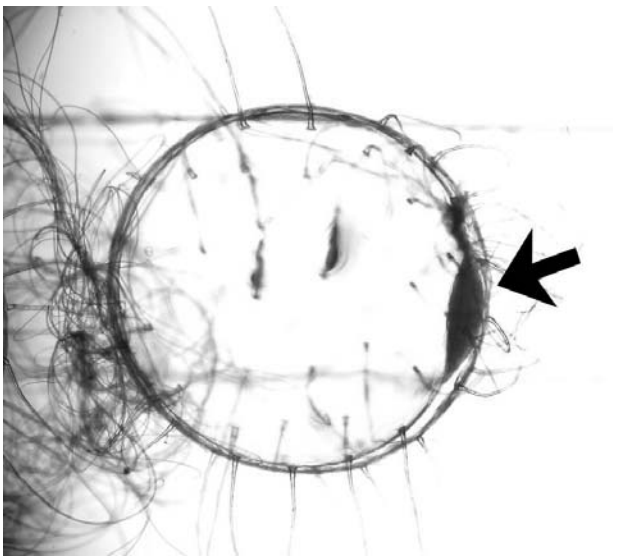

Figure 1 The first appearance of the morula stage (indicated by arrow) was observed approximately 7 h after fertilization.

sac volumes than newly hatched ones. These values would probably be in favor of newly hatched larvae if a larger sample were observed. In this case, faster consumption of the yolk sac could be explained by embryonic movement, insufficient circulation, stress and other biotic and abiotic factors.

After mass hatching of larvae, when there were more than 50 individuals in the tank, characteristic behavior was noticed: the larvae gathered in a small school in the middle of the tank with caudal parts turned toward the middle. This type of schooling behavior was also observed by Rosenthal and Fonds (1973). There is no primordial dorsal fin, but developing fin rays are already visible in the true dorsal and anal fins. The absence of a primordial dorsal fin could be explained by the advanced respiratory capabilities of larvae on hatching (Rosenthal & Fonds 1973). There is a very deep pre-anal primordial fin. The anus is situated approximately two-thirds of the body length from the snout, which is in agreement with observations by Rosenthal and Fonds (1973).

Embryo pigmentation described in this study differs from that presented by Rosenthal and Fonds (1973). In this study, pigmentation first occurred on the head and in the eyes (orange and brown pigment), whereas Rosenthal and Fonds (1973) note that pigmentation first occurred around the yolk sac circulatory system and the heart. In the present study, pigmentation occurred prior to the initial somatic movements (approximately 10 h previously), which is in agreement with the findings of Korzelecka-Orkisz *et al*. (2005). The emerging embryo in the study by Korzelecka-Orkisz *et al*. (2005) has green, followed by black pigmentation. This is not the case in the present study: we initially observed orange and brown pigment followed by black pigmentation. Ehrenbaum (1905) states that some days before hatching, green pigment spots appeared and that this moss-green pigment was arranged in transverse stripes. He also observed that some small pigment aggregations exist on the yolk. Korzelecka-Orkisz *et al*. (2005) observed that just prior to and during hatching the embryo exhibits intensive dark-brown coloration, and loses the green pigment. Furthermore, Rosenthal and Fonds (1973) note that larva exhibited distinctive pigmentation, and were almost completely covered by large numbers of yellow and black cromatophores.

In the present study, only one Kupffer cell appeared 60 h after fertilization, approximately at the 14 somite stage, while Rosenthal and Fonds (1973) describe the appearance of several Kupffer's vesicles at the 3–8 somite stage.

These differing characteristics of the embryonic development of the garpike from the Baltic Sea and of the garpike from the Adriatic Sea could also be a result of separation into subspecies. According to distribution maps of subspecies provided by Collette and Parin (1986), Baltic garpike is considered to be a nominal *B. belone belone* subspecies, while garpike from the Adriatic Sea represents *B. belone gracilis* subspecies.

The embryonic period of garpike is very long, but larvae hatch with fully functional pectoral fins and gills. The jaw is mobile three days before hatching. The yolk sac remains from 17 to 48 h after hatching, but larvae are capable of active feeding immediately upon hatching. These adaptations increase chances of survival for newly hatched larvae and compensate for the low fecundity of garpike. Larvae also hatch with different yolk sac volume to body length ratios.

The garpike eggs, as opposed to eggs of the vast majority of marine fish, are large, which is obviously translated into the low absolute fecundity (Nikolskij 1954). A peculiar detail of the garpike's embryogenesis is a certain extension of the period of embryonic development at the expense of the larval stage duration. The embryo leaving the egg membranes is a fully developed individual capable of independent life in the water column. It is also fully capable of independent, exogenous and efficient feeding.

The peculiarities observed in the garfish egg structure, the dimensions of individual components, their formation and spatial arrangement, the egg coloration and attachment structures, as well as the garpike's significant temperature tolerance are evidence of an exceptional plastic-

ity and adaptive potential, which could be considered as helpful features in extending the natural range of occurrence of this species.

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