



## PECULIARITIES OF EGG STRUCTURE AND EMBRYONIC DEVELOPMENT OF GARFISH (*BELONE BELONE* (L.))

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**Abstract:** Peculiarities in egg morphometry and in the course of embryogenesis of the Baltic garfish (*Belone belone* (L.)) were studied. The eggs, measuring  $3.27 \pm 0.15$  mm in diameter and  $18.34 \pm 2.6$  mm<sup>3</sup> are equipped in viscous filamentous processes. The eggs lack the so-called structural lipids in the form of lipid vesicles, for which reason the embryonic disc is never located in the upper part of the egg and moves sideways during embryonic development. The perivitelline space, small (about 5% of the egg's volume) at first (after activation), expands gradually as the embryo grows to occupy up to 45% of the egg's volume when the embryo is fully formed. The embryonic development takes 340 D° (at 16°C). The Baltic garfish embryonic mortality is high and exceeds 90%. The larvae carry a residual yolk sac and are fully prepared to live independently in water and to feed efficiently. The structural and embryological peculiarities observed in the garfish development evidence an exceptional plasticity and adaptive potential of the species with respect to changing environmental conditions, which enables it to expand its natural range.

Key words: *Belone belone* (L.), egg structure, embryogenesis, embryonic locomotion

### Introduction

The garfish appears off the southern Baltic coast in spring and summer where, owing to a specific hydrological regime and shoreline physiography, it finds suitable conditions for spawning. Demel (1937) was the first to describe the species in the Polish waters. He followed the garfish migration route and observed the fish to head to spawn close to the shore off Reda where it lays eggs in shallow heated water of 5–6‰ salinity. Due to the particular shape of the garfish beak-like jaws, Demel jokingly called the species a “sea stork”.

The garfish inhabits brackish and marine waters of the North-East Atlantic from off Norway to Great Britain. It grows to 1 m in length and to more than 1 kg in weight. It reaches sexual maturity when 30–50 cm long, depending on the location.

The absolute fecundity varies greatly between individuals, from one to more than thirty thousand (Nikolskij 1954; Kompowski 1965; Rosenthal and Fonds 1973; Telofel and Militz 1996). The fecundity increases with fish age. Depending on the area, spawning begins in May–June to proceed occasionally until September, at 15–20°C. There are several batches (most probably 3, as evidenced by oocytes of different size found in different parts of the ovary; Nikolskij 1954). The eggs are laid on submerged vegetation, most frequently on shallows (Gašowska 1962), although plants growing at the depth of 12–18 m were observed to support garfish eggs as well (Berg 1949).

The large, greenish eggs measuring from 2.6 (Berg 1949) to 3.5 mm (Gašowska 1962) in diameter are covered

by 60–80 filamentous processes 8–18 mm long and 0.013 mm in diameter, spaced 0.6 mm apart. The processes facilitate attachment to plants and floating objects, while the greenish pigmentation makes the eggs rather difficult to spot.

Following 4–6 (most often 5) weeks of development, the eggs hatch to produce well-developed, large (9–13 mm long) larvae, strongly pigmented and coloured yellow-green (Berg 1949, Nikolskij 1954). Both larval jaws are short on hatching; subsequently, the lower jaw begins to elongate fast, while the upper jaw elongates much later, most probably as an adaptation to a change in feeding mode (Severcov 1939).

Fonds et al. (1974) studied effects of temperature (9–24°C) and salinity (10–45‰) on the embryonic development, larval growth, and vertebra count of the Wadden Sea garfish; they found 31–34‰ and 17.5–19.5°C to be optimal for the embryonic development of the species.

Westernhagen (1974) incubated the Baltic Sea garfish at very wide ranges of temperature (9–21°C) and salinity (5.6–36.2‰). He observed 9°C to be the lowest temperature at which the eggs could develop (but failed to hatch). The eggs did hatch following incubation at 12°C. Low salinity resulted in a high embryonic mortality, caused, i.a., by the fact that the egg membranes were prone to perforation. The high salinity tested (36.2‰) was not advantageous for the developing embryos, either, and the ensuing salinity reached 99%. Embryos incubated at lower salinities (5.6 and 10‰) were less motile than those incubated at higher salinities. According to Westernhagen (1974), 15–18°C and 15–33‰ are the optimal incubation conditions under which incubation takes 22 days, i.e., 396D°.

By removing the filamentous processes and thus improving somewhat the oxygen conditions, Fonds et al. (1974) considerably (by almost one-fourth) reduced the time needed for incubation. Rosenthal and Fonds (1973) followed embryonic and early larval development at

22°C. They counted heart contractions, the heart appearing as early as on day 3; the heartbeat rate was 36–30 per minute. The rate increased from one day to the next to reach the level of 160 contractions per minute just prior to hatching (day 11 of development). The embryo performed its first movements fairly late, at mid-point of the development, when the pigment appeared in the eyes. On day 7, the pectoral fins were formed, their movements ensuring transport of the perivitelline fluid, whereby the embryonic respiration was improved. The authors quoted noticed opercular movements, very rarely observed in embryogenesis of other fish species. In the opinion of Rosenthal and Fonds (1973), the movements are indicative of a very early development and functioning of gills.

As early as a few hours after hatching, the larvae were swimming, actively feeding, and aggregated in the central part of the aquarium with tails directed toward the centre of the aggregation; the position was termed as a “flight intention” by Rosenthal and Fonds (1973)

## Materials and methods

The study was carried out in June 2003 at a field laboratory in Jastarnia (Hel Peninsula).

Observations were made on eggs, collected from garfish (*Belone belone* (L.)) spawners caught in the Gulf of Gdańsk. Following dry fertilisation, the eggs were incubated at  $16 \pm 1^\circ\text{C}$  at salinities of 6, 12, and 34‰.

The garfish embryonic development was recorded with the aid of an equipment used earlier at the Department of Fish Anatomy and Embryology. The equipment consisted of a Nikon microscope (2× objective), a digital camera, a monitor, a VCR, and a computer (Winnicki and Korzelecka 1997).

Images of eggs and newly hatched larvae recorded were subsequently analysed and measured using

the Multiscan V. 13.01 image analysis software.

Two diameters of each eggs and an egg cell it contained were measured on reception mound formation. The two measurements were subsequently averaged, the average being used to calculate egg volume ( $V=4/3\times\pi r^3$ ), surface ( $S=4\times\pi r^2$ ), and the surface to volume ratio (S/V) of eggs and egg cells.

Immediately after hatching, the total length and width (h) of the larva as well as the yolk sac length (l). The yolk sac volume was calculated using the ellipsoid volume formula ( $V=\pi\times l\times h^2$ ).

**Embryonic movements:** all forms of locomotor activity in the garfish eggs at each stage of embryonic development were recorded and analysed. The heartbeat rate as well as the rate of somatic, fin, and opercular movements were expressed as the number of movements per minute.

The data were subjected to statistical processing using the Statistica 5.1 Pl. and Exel 97 software.

## Results

The perivitelline space formation took about 2 hours. At the time, the perivitelline space was occupying about 5% (from 3 to 10%) of the entire egg volume.

The eggs, measuring  $3.27 \pm 0.15$  in diameter, coloured green, are equipped with viscous processes enabling attachment to submerged vegetation. Individual eggs differ considerably: diameters of the smallest and the largest eggs were 2.95 and 3.70 mm (Figure 1), respectively, the respective volumes amounting to 13.43 and 26.51 mm<sup>3</sup>; thus the large egg may be even twice as big as the small ones (Figure 2, Table 1).

Egg cells, spherical in size, differ greatly in size, as evidenced by the range of their diameters (2.95–3.51 mm) and volumes (13.4–22,6 mm<sup>3</sup>). Thus the

volume of the large eggs may be almost twice as high as that of the small eggs.

Garfish eggs lack structural lipids in the form of lipid droplets; consequently, the embryonic disc that appears later moves sideways as much as the size of the perivitelline slit permit.

Accumulation, on the animal pole, of the material to form the embryonic disc from (reception mound) took 3.5 hours (from activation).

The course of embryogenesis (from activation to hatching) and the major structural changes taking place then are characterised in Table 2, listing the chronology of individual changes, and shown in photographs (figures 3–5).

The major findings resulting from the observations are as follows:

- Plasticity, making it possible for the eggs to adapt to varying salinity (from 6 to 34‰).
- The lack of pronounced differences in the rate of embryonic development at various salinities (except the extreme salinity values).
- A clearly reduced egg resistance to mechanic pressure in the low salinity water.
- Pigmentation: prior to the initial somatic movements, the emerging embryo showed the presence of green, followed by black pigmentation (Figure 4a,b,c).
- A considerable amount of storage material (yolk) enables the embryos to prolong their stay within the egg, whereby the hatching is delayed, but the larvae are fully capable to live independently and move in the water.
- Significant details of egg development include the lack of any clear-cut symptoms of water absorption immediately following egg lying; the animal (upper) pole of the egg features a small perivitelline slit that is gradually enlarged.

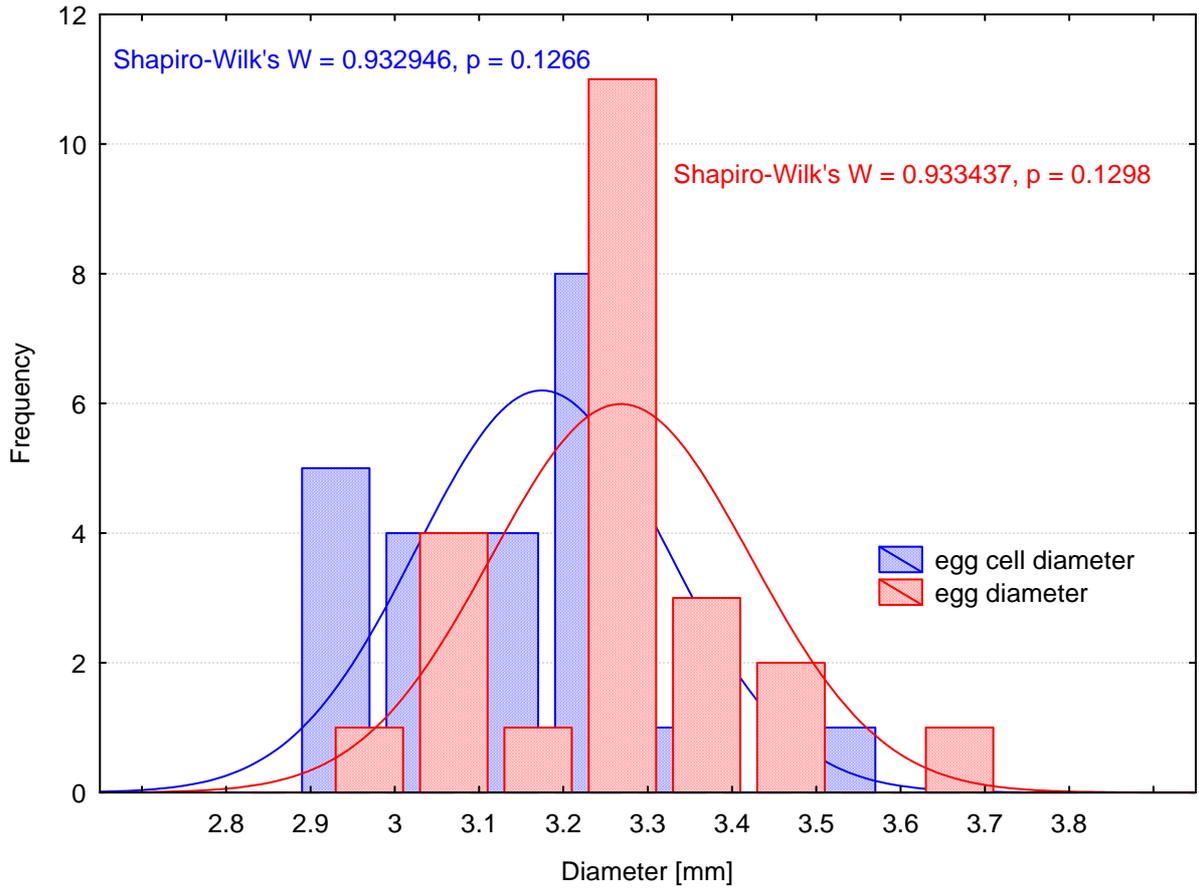


Figure 1. Distribution of garfish (*Belone belone* (L.)) egg cell and egg diameter

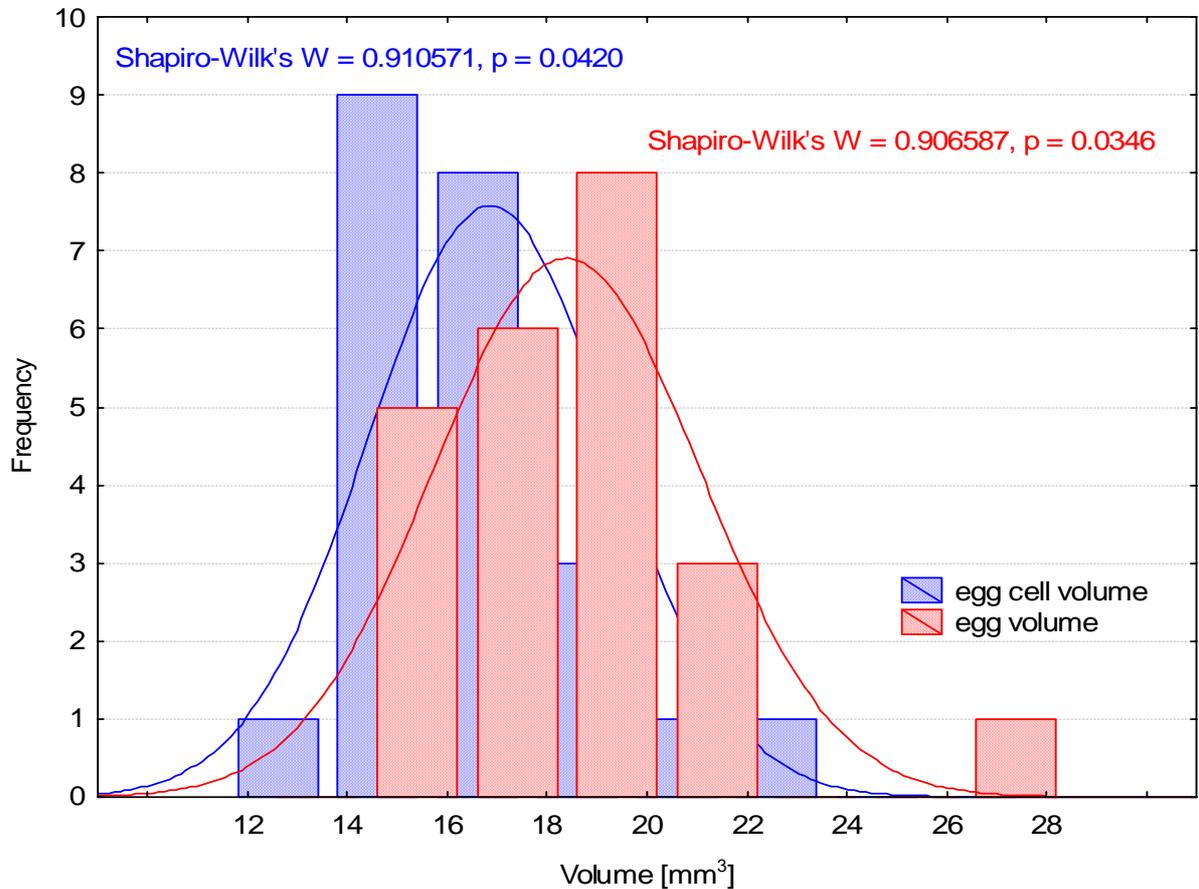


Figure 2. Distribution of garfish (*Belone belone* (L.))egg cell and egg volume

Table 1. Dimensions of garfish (*Belone belone*) eggs and egg cells ( $\bar{x} \pm SD$ )

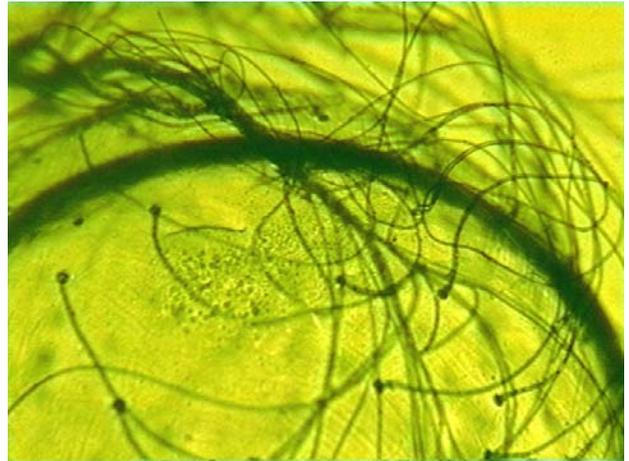
Number of eggs [n]	Diameter [mm]		Volume, V [mm <sup>3</sup> ]		Surface, S [mm <sup>2</sup> ]		S/V ratio	
	Egg	Egg cell	Egg	Egg cell	Egg	Egg cell	Egg	Egg cell
23	3.27 ±0.15	3.17 ±0.15	18.34 ±2.66	16.81 ±2.42	33.85 ±3.60	31.67 ±2.99	1.84 ±0.13	1.88 ±0.63

Table 2. The history of embryogenesis in garfish (degree-days, D°)

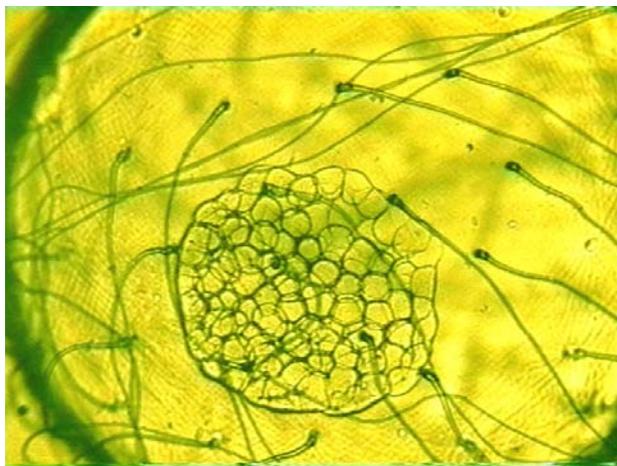
Time from fertilisation (degree-days, D°)	Developmental stage
2.3	Reception mound formation
3.3	2 blastomeres (Fig. 3b)
5.6	8 blastomeres
17	Morula (Fig. 3c,d)
34	¼ epiboly
49	¾ Epiboly
71	Blastopore closure; embryo with emerging cephalic part and distinct 4 myomeres in the posterior part
84	Appearance of egg balls; heartbeat begins
116	Eyes visible, blood vessel network on the yolk sac, initial movements of the embryo
212	Pectoral fin movements
324	Opercular movements
340	Hatching (Fig. 8)



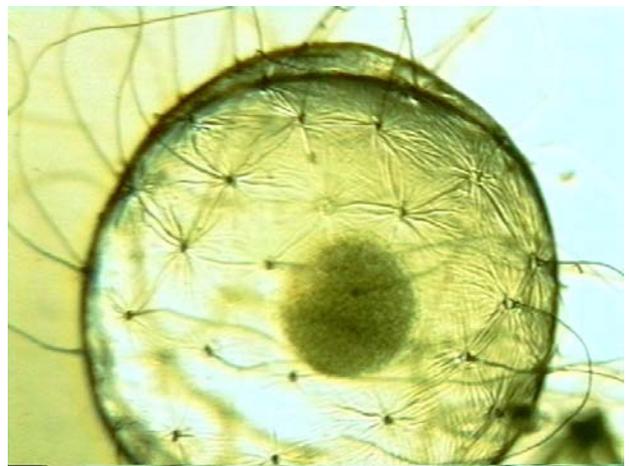
3a - Egg activation and reception mound formation



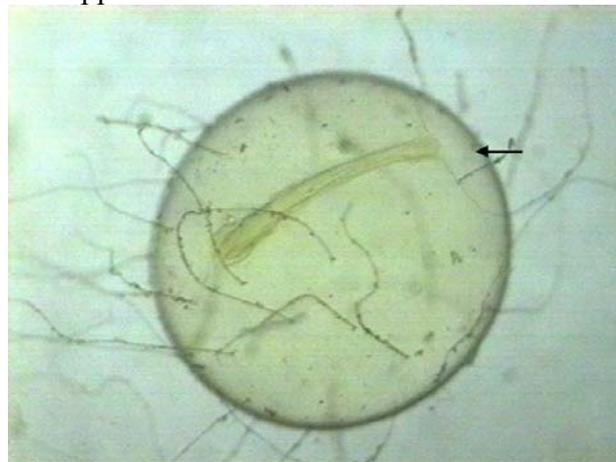
3b - Two-blastomere stage



3c - Coarse morula in upper view



3d - Fine morule in lateral view



3e - Blastopore closure (←, unenclosed yolk fragment)

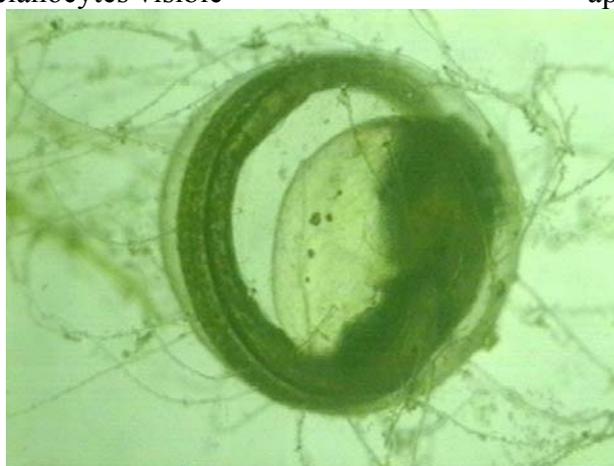
Figure 3. Stages of garfish embryonic development:



4a - Green coloration of embryo's body; aggregations of melanocytes visible



4b - Melanophores scattered; eye pigment appears



4c - Embryo coloured dark-brown

Figure 4. Changes in embryonic coloration



Figure 5. An embryo immediately before hatching

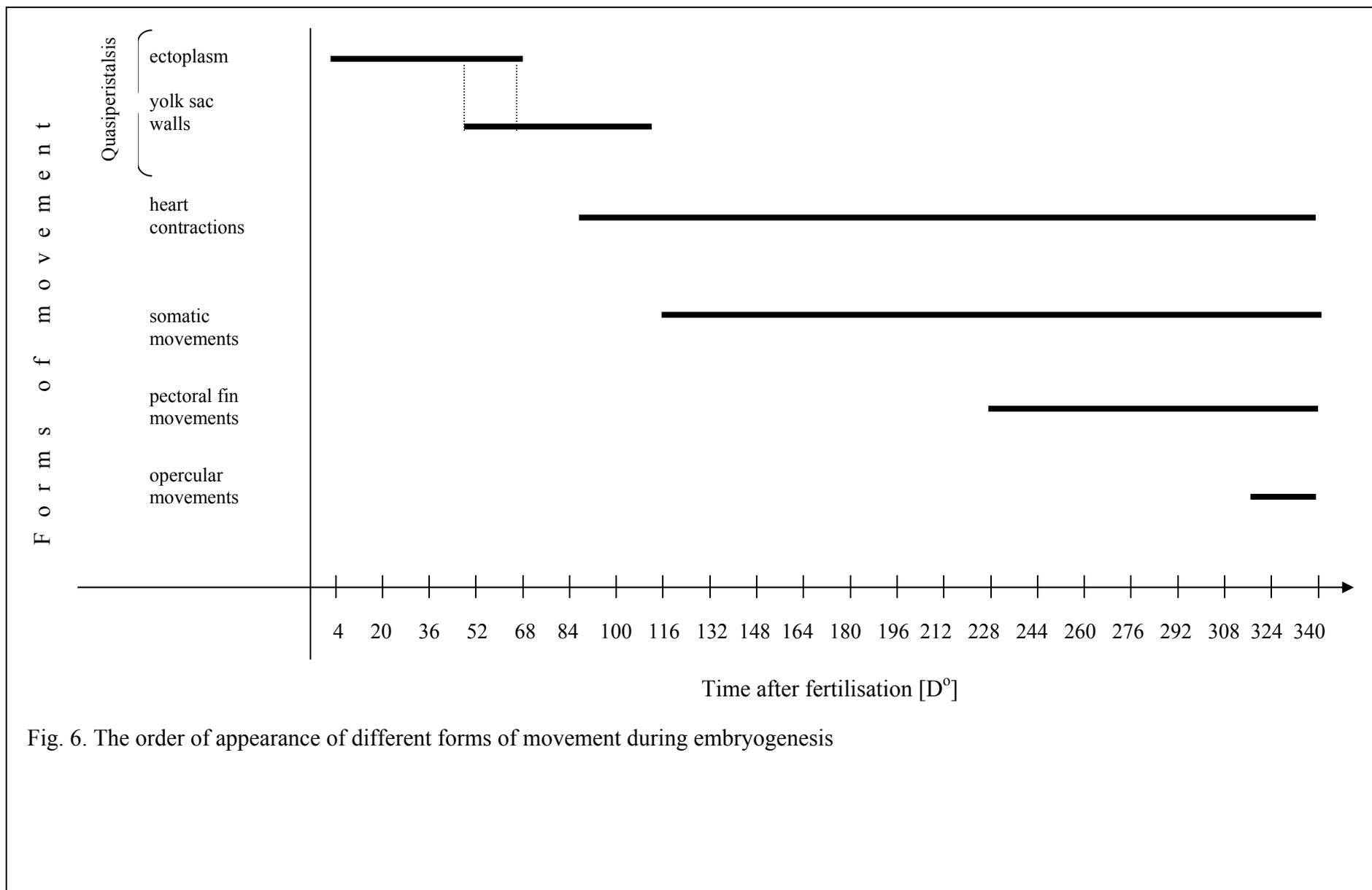


Fig. 6. The order of appearance of different forms of movement during embryogenesis

**E m b r y o n i c   m o v e m e n t s**  
(Figure 6)

- Quasi-peristalsis: intensive, as much as the perivitelline space dimensions permit; the ectoplasm contraction waves around the yolk sphere move the embryonic disc, and the embryo, within the perivitelline space.
- Contractions of the budding cardiac muscle appear on 84 D°; the contractions are slow at first (about 28 /minute). The frequency of contractions increases gradually: as of 142 D° until hatching the heart contracts at a rate of 100 /minute (Figure 7).
- Somatic contractions: the trunk muscle activity is low and becomes still lower when the pectoral fins begin moving.
- Opercular movements: initially irregular, stabilise their rhythm prior to hatching.

**M a s s   h a t c h i n g** occurred after 340 D°.

The newly hatched, fully pigmented **l a r v a e** measured  $11.99 \pm 0.22$  mm and were equipped with a residual  $1.08 \text{ mm}^3$  yolk sac. The larvae were very active; their heartbeat rate amounted to about 140 contractions/minute. The larvae were basically ready to live freely and independently in the water column (Figure 8)

## Discussion

The details of morphology and function in the course of embryonic development of garfish spawning off the southern Baltic shores (Gulf of Gdańsk) fully corroborate observations and findings reported by many authors during the last few decades of the 20<sup>th</sup> century (Demel 1937; Demel 1948; Berg 1949; Nikolskij 1954; Kompowski 1965; Kompowski 1965b; Rosenthal and Fonds 1973; Fonds et al. 1974; Westerhagen, 1974; Westerhagen et al. 1975; Dorman 1989; Dorman 1991; Zaporowski 1996).

Despite the abundance of relevant information, however, it is difficult to find a more comprehensive theoretical discussion of a number of morpho-

functional peculiarities of the garfish embryonic development. There have been no attempt, either, to answer the question why those peculiarities are specific for the garfish and what is their biological importance.

It was those questions that prompted the present study, aimed at attempting – based on the relevant information contained in the literature – to find reasonably justified and in-depth answers to certain basic questions on cause-effect relationships governing the peculiarities encountered at the most important reproductive stage, i.e., the embryogenesis. It was also attempted to cast the peculiarities observed against a more general background of life strategy evolved by the garfish, a doubtless very interesting species.

The most important morpho-functional embryogenetic peculiarities of the garfish include the large egg dimensions, low fecundity, a specific structure of egg membranes, coloration, dimensions, and specificity of the perivitelline space, diversity of forms and physiological dynamics of embryonic locomotion, and the considerably extended embryonic development.

Following a fairly detailed analysis, it is easy to conclude that all those peculiarities are directly or indirectly related to salinity at which the eggs are to develop.

Thus, the garfish eggs, as opposed to eggs of the vast majority of marine fish, are large, which is obviously translated into the absolute fecundity, low in the species (Nikolskij 1954).

The large eggs, a treat for predators, need protection provided, on the one hand, by the membranes equipped with special attachment instruments in the form of filamentous processes easily adhering to submerged plants; another means of protection is saturation of the yolk with biliverdin (Caglar 1945), whereby the eggs is efficiently camouflaged.

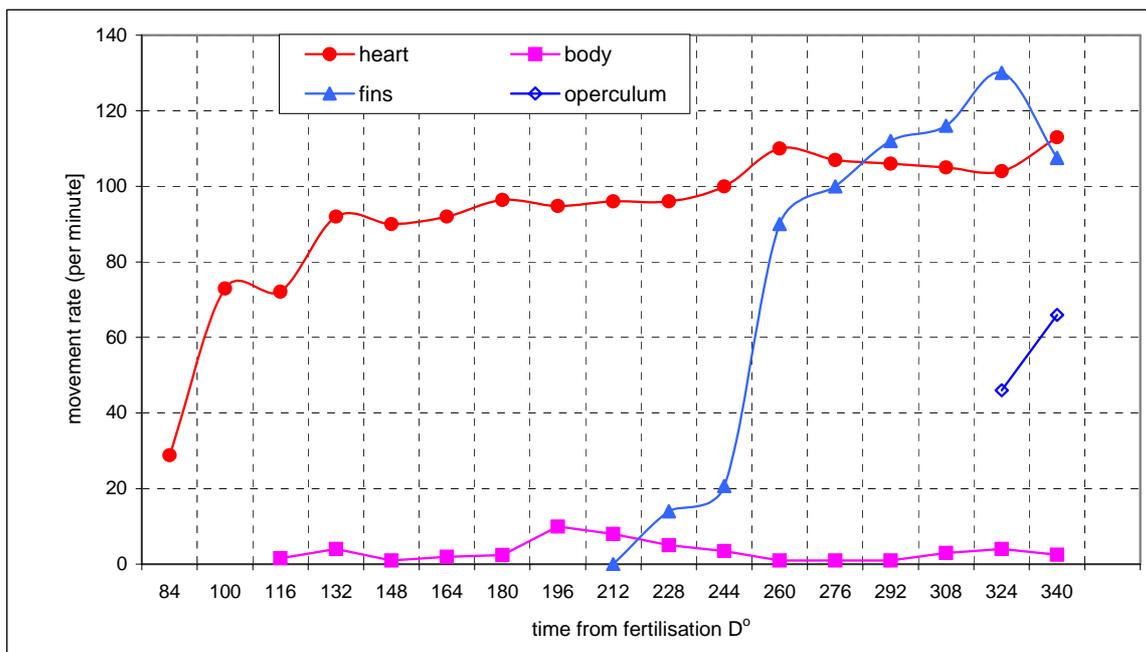


Figure 7. Duration of different forms of embryonic movements and rate of movements of various structures



Figure 8. A newly hatched larva with residual yolk sac

Suspending the eggs on submerged vegetation prevents their emergence to the surface in saline water and sinking to the bottom at low salinity, and density, of the water.

Important is also the fact that movements of the thalli that support the attached eggs produce water streams facilitating respiration of the developing embryos (later during morphogenesis).

In addition to their large size, the garfish eggs evacuating from the ovaries

are only slightly smaller than the activated eggs. That means that the eggs are only slightly (by an average of 3-4%) larger than their egg cells. Consequently, the perivitelline slit is very small during fertilisation, so – unlike in most other fish species – the eggs do not absorb much water initially after evacuation and thus are not hydrated (Bogucki 1930, Zotin 1961, Winnicki, Korzelecka 1997, Formicki et al. 2002).

Interestingly, however, although the egg does not increase in size with time after activation, the perivitelline space volume increases gradually throughout the embryogenesis, to reach about 45% of the entire egg volume immediately before hatching, i.e., after 320°D.

A question then arises where did the water come from, as it was not absorbed by the egg during spawning?

There seems to be only one answer: the water filling the perivitelline slit and the perivitelline space later on has been already stored in the egg cell (to be exact, in its integral component, the yolk).

The basic cause has to be sought in the fact that the garfish lay eggs in highly saline waters (up to 30‰ in the Atlantic); those eggs would be incapable of absorbing, during activation, enough water by means of only the suction pressure of hydrophilous colloids released from cortical alveoles to the perivitelline slit (Bogucki 1930); besides, the high salinity would be detrimental for the delicate embryonic structures rinsed by the saline perivitelline fluid.

Therefore, the garfish evolved a solution in the form of the future embryo and the new individual being supplied with enough “fresh” water originating from the water stored in the maternal organism and transferred to the yolk of the developing embryo during the final stage of oogenesis. That enabled the future embryonic structure to become independent of the salinity of the surrounding water and to ensure the building materials for the embryo.

The egg dimensions themselves greatly hamper the embryonic gas exchange, particularly at final stages of embryogenesis, as the surface to volume ratio (1.84 in the garfish) is not overly advantageous. The necessity to counteract those numerous shortcomings resulted in the development of numerous and various forms of active embryonic movements, manifested as a conspicuous quasi-peristalsis and, superimposed at the final stage, active movements of the large budding heart, accelerated heartbeat of the

actual heart, and the movements of the operculum emerged towards the end of embryogenesis, which marks the onset of the gill function.

Finally, a peculiar detail of the garfish embryogenesis is a certain extension of the period of embryonic development at the expense of the larval stage duration. The embryos leaving the egg membranes is a fully developed individual capable of independent life in the water column, almost devoid of the yolk sac that could hamper movement in the water column. Obviously, such larva is fully capable of independent exogenous efficient feeding. It is at that time (just prior to and during hatching) the embryo assumes intensive dark-brown coloration, adequate to its surroundings, and loses the green pigment that would make the body easy to spot against the grey backdrop of the illuminated water column.

Without any doubt, the peculiarities of the structure and the related physiological details seem to be associated with direct and indirect effects of elevated salinity and the species' attempts to overcome the contradictions, to find an appropriate location in the sea, and to adapt to the hydrological regime and sociobiological conditions prevailing in various ecological niches.

To sum up, the peculiarities observed in the garfish egg structure, dimensions of individual components, their formation and spatial arrangement, egg coloration and attachment structures as well as a significant temperature tolerance and the very high tolerance to salinity are evidence of an exceptional plasticity and adaptive potential of the species making it capable of extending its natural range of occurrence.

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