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Chronology of embryonic development in Baltic herring *Clupea harengus membras*

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Abstract. Thirty stages were distinguished in the development of the Baltic herring embryo during the period from insemination to hatching. The age of embryos at these stages was expressed in relative time units (τ_0) and the timing of herring embryo development was compared with data for other teleosts. The chronology of Baltic herring's embryonic development was rather similar to that of salmonid fishes and differed from that of cyprinid fishes.

Key words: *Clupea harengus membras*, development chronology, temperature, heterochrony.

INTRODUCTION

Chronology and heterochronies of early development may have ecological and evolutionary importance (Emelianov, 1968; Balon, 1981, 1983; Paine & Balon, 1984; Fuiman, 1985). Correct comparisons of developmental chronology in poikilotherms, including fish, cannot be made on the basis of data expressed in astronomic time units (hours, minutes, etc.), or in "degree-hours" or "degree-days", as the species-specific development rate also depends on environmental factors (primarily on temperature), and this dependence is not linear (Dettlaff et al., 1987; Saat & Veersalu, 1996a).

The duration of one mitotic cycle during the period of synchronous cleavage divisions (τ_0) has been proved to be a proper unit to compare the duration of developmental processes in different species and at different temperatures (Dettlaff & Dettlaff, 1961; Dettlaff et al., 1987). The dependence of τ_0 on temperature has been revealed for more than 10 teleost species, including Baltic

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herring *Clupea harengus membras* L. (Saat & Veersalu, 1996a). The timing (in numbers of τ_0) of stages of normal development has been revealed for several species, including some freshwater and anadromous teleosts: rainbow trout *Oncorhynchus mykiss* (Walb.) (Ignatieva, 1991), Atlantic salmon *Salmo salar* (L.) (Gorodilov, 1983, 1990), carp *Cyprinus carpio* L. (T. Saat et al., unpublished), *Carassius gibelio* (Bloch) (Savolainen, 1985), and mud loach *Misgurnus fossilis* (L.) (Kostomarova, 1991). No such data are available for marine fish.

Kryzhanovskij (1956), Rannak (1961), and Ojaveer (1981, 1988) described the morphology of the embryonic development of some clupeid species, including Baltic herring. Ojaveer (1981) investigated the age of embryos of Baltic herring (expressed in degree-hours) at some stages and at three temperatures.

The aim of the present investigation was to compose a table of normal development for Baltic herring, reveal the timing of developmental stages (in numbers of τ_0), and compare the temporal pattern of development in Baltic herring with that of representatives of Salmoniformes and Cypriniformes.

MATERIAL AND METHODS

Gametes of 12 females and 12 males (range of total length (TL) 15.2–19.7 cm) of spring spawning Baltic herring (12 crosses) were used in experiments carried out in the spawning period (April–June). Fish were obtained from commercial trap net catches from Pärnu Bay (Estonia). Eggs and sperm were stripped from live "running" specimens and fertilized in water from the bay (salinity 4 ppt) at 10 °C. The water was changed every 2 days. Eggs attached to pieces of the fishing net or to the bottom of Petri dishes were incubated in 10–15 litre tanks (500–700 embryos per tank) with continuous aeration at 4 ppt salinity. The water temperature was 10.0 °C, which lies within the range of optimal temperatures for early development of this species (Saat & Veersalu, 1996a). The temperature was measured every 2–4 h. During the development of some batches of eggs some short-term fluctuations of temperature (between 9.5 and 12.3 °C) occurred. The age of embryos was expressed as multiples of τ_0 (τ_n/τ_0; τ_n, time of appearance of a particular character in minutes from insemination). The values of τ_0 at different temperatures (t) between 1 and 13 °C for Baltic herring embryos can be calculated as $\log \tau_0 = 2.4349 - 0.0684t$ (Saat & Veersalu, 1996a). At 10.0 °C, τ_0 equals 56.3 min. Approximately 30 embryos were sampled in every 0.25–2 τ_0 (more frequently until gastrulation) and investigated under a stereomicroscope. Stages of development were chosen according to those in tables of normal development of other teleost fish species (Gorodilov, 1983; Ignatieva, 1991; Kostomarova, 1991). Both living and preserved (in 4% formalin) embryos were examined and measured (TL in 0.1 mm) using a dissecting stereomicroscope; drawings were made using drawing apparatus. E. Ojaveer prepared most of the drawings. The relative linear rate of epiboly (rate of moving cell blast boarder over yolk towards the vegetal pole of the egg) was measured according to Ignatieva (1979).

As the timing of development varies markedly even within batches, the stages in the table of normal development are given for the most advanced embryos (cf. Dettlaff & Vassetzky, 1991).

RESULTS

We distinguished 30 stages of normal development of the spring spawning Baltic herring embryo. A brief description of each stage as well as the ages of embryos are presented in Table 1. Most stages are illustrated in Fig. 1.

Table 1. Stages of Baltic herring development ($\log \tau_0 = 2.4349 - 0.0684t$ at $t = 1–13$ °C; Saat & Veersalu, 1996a)

Stage	Age τ_n/τ_0 (mean ±SD, $n = 12$)	Description
0	0	Ovulated egg at the moment of insemination. Egg diameter 0.8–1.09 mm
0+	0.6 ± 0.5	Egg activation, narrow perivitelline space
1–	2 ± 0.5	Activated egg. Egg diameter (incl. well developed perivitelline space) 1.1–1.4 mm. Bipolar differentiation, formation of the germinal disc
1	3 ± 0.5	2 blastomeres
2	4 ± 0.5	4 blastomeres
3	5 ± 0.5	8 blastomeres
4	6 ± 0.5	16 blastomeres
5	7 ± 0.5	32 blastomeres. Cleavage furrow of the fifth cleavage division lies horizontally, blastomeres arranged in two layers
6	8 ± 0.5	64 blastomeres arranged in two or three layers
7	9 ± 0.5	128 blastomeres
8	12 ± 1	Morula
9	14 ± 1	Early blastula. Blastodisc edge is irregular
9+	20 ± 1	Late (high) blastula
10	23 ± 2	Beginning of epiboly. Edge of blastoderm is sharp and clear
11	26 ± 2	Blastoderm covers 1/3 of the egg surface, its rim starts to thicken
12	29 ± 3	Blastoderm covers 1/2 of the egg surface
13	41 ± 2	Blastoderm covers 4/5 of the egg surface; embryonic shield and axis formed
14	50 ± 2	Blastoderm covers yolk entirely
15	53 ± 5	Beginning of mesoderm segmentation, formation of the first pair of somites
16	57 ± 5	4 pairs of somites, formation of optic vesicles in advanced embryos
16+	60 ± 5	6 pairs of somites, formation of optic vesicles in late embryos. Kuppfer's vesicle emerges

Table 1. *Continued*

Stage	Age τ_n/τ_0 (mean ±SD, n = 12)	Description
17	65 ± 5	10 pairs of somites
18	77 ± 4	18 pairs of somites. Olfactory vesicles in advanced embryos
19	80 ± 3	20 pairs of somites. Olfactory vesicles in most embryos, auditory vesicles in advanced embryos. The size of Kuppfer's vesicle is maximal
20	84 ± 3	24 pairs of somites. Auditory vesicles in most embryos, cavity in olfactory vesicles and eye lens in advanced embryos
21	88 ± 3	26 pairs of somites. Most embryos have cavities in olfactory vesicles and eye lens. Trunk-tail mound has raised up
22	94 ± 3	30 pairs of somites. Trunk-tail growth has begun, formation of encephalomeres. Kuppfer's vesicle disappears
23	98 ± 3	33 pairs of somites. Formation of heart and otoliths
24	103 ± 3	36 pairs of somites. Irregular heart beats. Beginning of trunk-tail mesoderm segmentation. Inner cells can be seen in notochord
25	108 ± 4	40 pairs of somites. Regular heart beats
26	116 ± 4	45 pairs of somites. Formation of anus
27	133 ± 6	Pigment in eye periphery of most embryos. Buds of pectoral fins. 57 pairs (incl. 13 postanal) of somites. Mean TL of embryos is 3.5 mm. Embryos may hatch occasionally
27+	138 ± 6	Segmentation is complete. TL of embryos 4.0–5.0 mm. Pigment in eye periphery also in late embryos
28	185 ± 10	Yellow pigment and guanine in eyes. The mouth-opening emerges; beginning of hatching. Mean TL of embryos/prelarvae is 5.5 mm
29	202 ± 13	Pigment in intestine; head is detached from yolk. Mass hatching in our experiments. Mean TL of prelarvae at hatching is 6.0 mm
30	216 ± 13	Pectoral fins assume oblique position. Ear capsules enlarge, semicircular canals and endolymphatic duct form. Hatching continues. Mean TL of prelarvae is 6.3 mm

Synchronous cleavage divisions (stages 2–7) are followed through blastulation and epiboly. The relative linear rate of epiboly (Ignatieva, 1979) was 0.03–0.04 mm τ_0^{-1}. The segmentation of trunk mesoderm was, as in other fishes, synchronous, with 1.48 ± 0.03 τ_0 between the formation of successive pairs of somites. Somitogenesis was complete (59–60 pairs of somites) at stages 27–27+.

There was a high variation in the chronology of the development of embryos of the same progeny, as well as between progenies, especially at later stages. Hatching typically started at stage 28 (185 τ_0 from fertilization), and its timing was, as in other fishes, very variable. Hatching of externally normal (but at various stages of development) embryos was observed between 133 and 230 τ_0 (125 and 216 hours, respectively; stages 27–30 of development). Yolk was exhausted by 313–338 τ_0 (294–317 h) after fertilization.

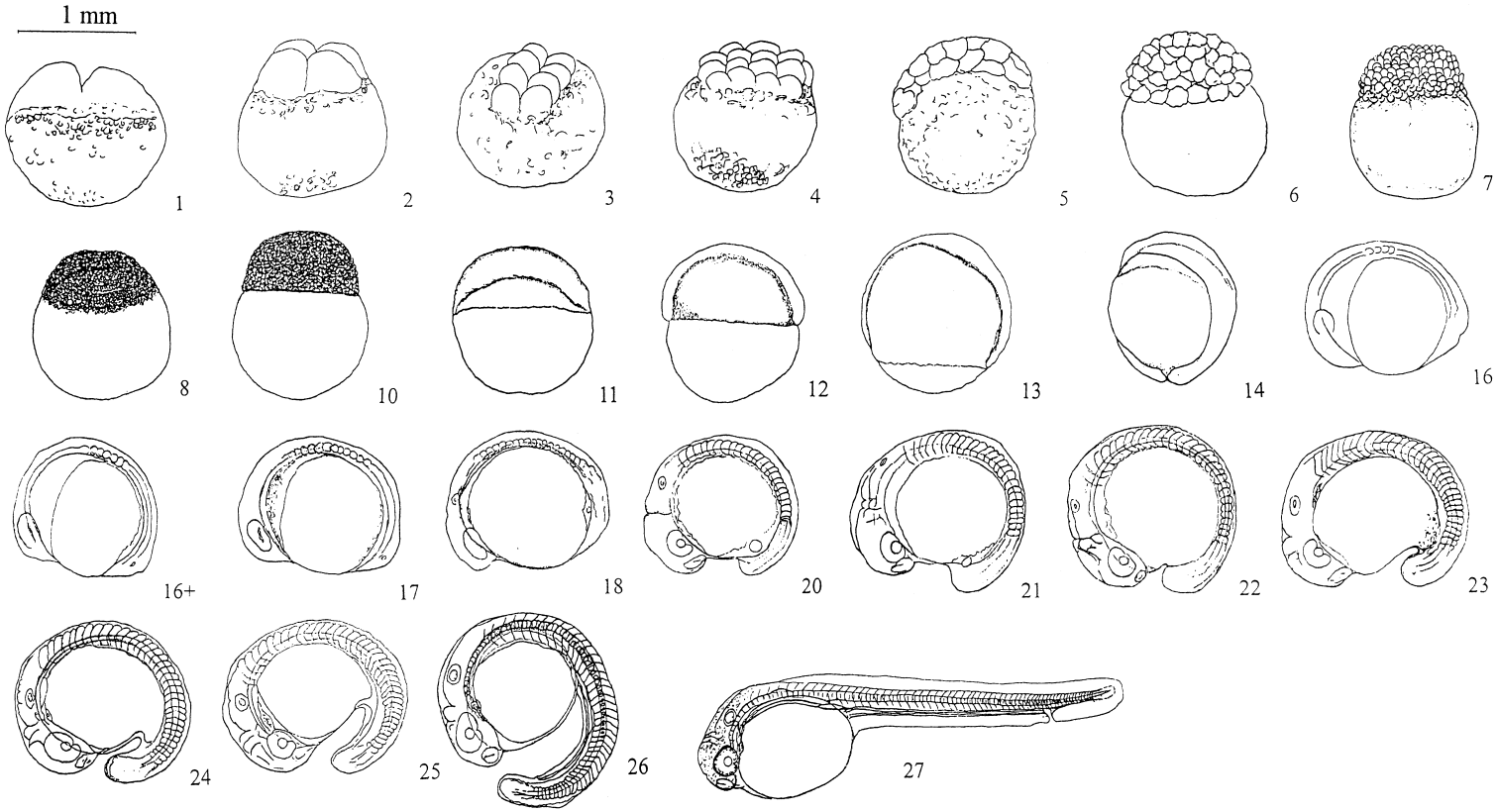

Fig. 1. Stages of the development of Baltic herring's embryo. Drawing numbers (from 1 to 27) correspond to stage numbers (see description in Table 1). Drawings 7–27 made by E. Ojaveer.

DISCUSSION

Timing of Baltic herring development

The morphological pattern of the development of Baltic herring has been investigated by Kryzhanovskij (1956), Rannak (1961), and Ojaveer (1988). Our results are in good accordance with these earlier studies.

The timing of development in Table 1 can be recalculated into astronomic time units (hours, minutes) at any temperature between approximately 5 and 12 °C, as the ratios of τ_n/τ_0 remain constant within the range of optimal temperatures for early development (Dettlaff & Dettlaff, 1961; Dettlaff et al., 1987). The upper optimal temperature for Baltic herring embryos is 12–13 °C (Saat & Veersalu, 1996a), and severe abnormalities of embryos have been detected at 17 °C (Ojaveer, 1981). The lower optimal temperature for Baltic herring embryos is approximately 5 °C. For example, stage 24 (heartbeats) could be detected at the age of 103 τ_0 at 10 °C (this study) and 103 τ_0 at 7 °C (calculated from Ojaveer, 1981). The corresponding values for 3 and 17 °C were 126 and 70 τ_0, respectively (calculated from data in Ojaveer, 1981, 1988).

Variation in the rate of development among embryos of the same progeny is a rather common phenomenon in fish development, and the timing of stages in the tables of normal development is therefore given for the most advanced embryos (Dettlaff & Vassetzky, 1991). Asynchrony in embryo development may result from both genetic and environmental factors. In the case of Pacific herring *Clupea pallasi* it has been shown that in unfavourable oxygen (Dushkina, 1988) and salinity conditions (Griffin et al., 1998) hatching is significantly delayed. In Baltic herring, the highest variation appeared in the hatching time. A wide variation in the hatching time (stage) of Baltic herring embryos was also observed by Kryzhanovskij (1956) and Ojaveer (1981).

Hatching in Baltic herring seems to be shifted to earlier stages as compared with another subspecies, Atlantic herring (*Clupea harengus harengus* L.). According to Johnston (1993), hatching of Atlantic herring embryos occurs 384 h after fertilization at 8 °C. As the τ_0 dependence on temperature has been proved to be species specific (see Saat & Veersalu, 1996b for references), we used the τ_0 values for Baltic herring (Saat & Veersalu, 1996a) for recalculations, and got 354 τ_0, or over 100 τ_0 more than in Baltic herring. At the same time, the rate of somitogenesis in the two subspecies of *C. harengus* is rather similar, with mean intervals between the successive pairs of somites 1.48 ± 0.03 τ_0 in Baltic herring, and 1.52 (1.43–1.58) τ_0 in Atlantic herring (5–12 °C; recalculated from data in Johnston et al., 1995). Differences in the hatching time (stage) may be caused, at least in part, by adaptations to different water salinity (see Vetemaa & Saat, 1996 and Griffin et al., 1998 for references).

The two highly synchronous processes in fish embryo development, several first cleavage divisions (with an interval of 1 τ_0) and trunk mesoderm segmentation (Dettlaff et al., 1987), can be relatively easily followed in live embryos. The number of somite pairs served as one of the main morphological characters in the table of normal development of Baltic herring (Table 1), as well as in other fishes

(Dettlaff & Vassetzky, 1991). The time of the appearance of some other characters was quite variable and not fixed to a particular number of somites. For example, the formation of auditory and olfactory vesicles could be detected in embryos with a different number (18–25) of pairs of somites. At the same time, eye lenses and cavities in olfactory vesicles could usually be detected in embryos with 26 pairs of mesodermal somites. Kryzhanovskij (1956) found that these latter characters appeared simultaneously with the 24th pair of somites. However, his experiments were carried out at 14 °C, which is suboptimal for Baltic herring embryos (Saat & Veersalu, 1996a).

Comparison with other teleosts

The timing of cleavage (until blastulation) is rather similar in different teleost species (Table 2). The period from insemination to the first cleavage in Baltic herring is longer than in species reproducing in fresh water (Saat & Veersalu, 1996a). The timing of the further embryonic development of Baltic herring seems

Table 2. Timing of embryonic development in some teleost species (τ_n/τ_0)

Stage, period	*Clupea harengus membras*	*Oncorhynchus mykiss*	*Salmo salar*	*Carassius gibelio*	*Cyprinus carpio*	*Misgurnus fossilis*
2 blastomeres	3	2.5	2.15	2	2	2
16 blastomeres	6	5.5	5.15	5	5	5
Beginning of epiboly	23	29	34	22	22	20
Epiboly 50%	29	52	60	32	32	28
End of epiboly	50	72	72	40	42	38
1 pair of somites	53	48	57	46	46	42
Optic vesicles	57	56	66	50	50	46
10 pairs of somites	66	57	66	56	54	50
Auditory vesicle	76	72	78	62	51	52
Heart beats	103	96	99	84	80	80
Eye pigmentation	133	112	120	86	86	92
Body pigmentation	202	200	201	98	98	~150
Hatching	202	272	283	176	170	102
Duration of epiboly	27	43	38	18	20	18
Beginning of epiboly – 1 pair of somites	30	19	23	24	22	22
Relative linear rate of epiboly, mm/τ_0	0.035	0.14*	–	–	0.07*	0.07*
Mean diameter of unfertilized egg, mm	1.0	4.5	5.0	1.5	1.2	1.2

Sources: *O. mykiss*, Ignatieva, 1991; *S. salar*, Gorodilov, 1983, 1990; *M. fossilis*, Kostomarova, 1991; *C. gibelio*, Savolainen, 1985; *C. carpio*, Soa, 1987; T. Saat, unpublished.
* Ignatieva, 1979.

to be more similar to that of salmonids than cyprinids (Table 2). This reflects the phylogenetic closeness of clupeid and salmonid fishes. However, the timing of several stages of embryonic development in Baltic herring differs from that of salmonids. In some cases, these differences are probably due to the different size of eggs. In Atlantic salmon and rainbow trout the period of blastulation is longer, epiboly starts later and lasts longer than in species with smaller eggs; the mesoderm segmentation starts before half of the yolk is overgrown (Table 2). The relative linear rate of epiboly in freshwater fishes is correlated with the egg size (Table 2). However, the rate of epiboly in Baltic herring is very low as compared with freshwater species (Table 2).

Another process that proceeds slower in Baltic herring than in freshwater (or anadromous) species is mesoderm segmentation. Intervals between the appearance of successive pairs of somites in Baltic and Atlantic herring are approximately 1.5 τ_0, but in carp, loach, Atlantic salmon, and rainbow trout they almost equal 1.0 τ_0, and in chum salmon 1.1 τ_0 (Ignatieva, 1979; Gorodilov, 1990).

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Räime (*Clupea harengus membras*) lootelise arengu kronoloogia

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Pärnu lahe koelmutelt püütud räimede munarakud seemendati ja inkubeeriti püsival temperatuuril. Räime loote arengus viljastamisest kuni koorumiseni eristati 30 arengustaadiumit. Loodete vanus erinevatel arengustaadiumitel väljendati suhtelistes ajaühikutes (τ_0). Räime loodete arengu ajaline käik oli sarnane lõhilaste omale, ent erines karpkalaliste omast.