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The fate of the yolk syncytial layer during postembryonic development of *Stenodus leucichthys nelma*

Ekaterina A. Kondakova[1,*] & Vera A. Bogdanova[2]

[1)] *Saint Petersburg State University, Universitetskaya Emb. 7/9, RU-199034 Saint Petersburg, Russia; and Saint Petersburg Branch of VNIRO (GosNIORKH, named after L.S. Berg), Makarova Emb. 26, RU-199053 Saint Petersburg, Russia (*correspondig author's e-mails: 23eak@mail.ru, katekondakova1989@gmail.com)*

[2)] *Saint Petersburg Branch of VNIRO (GosNIORKH, named after L.S. Berg), Makarova Emb. 26, RU-199053 Saint Petersburg, Russia*

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The yolk syncytial layer (YSL) is a multifunctional transient structure of teleost embryos and larvae. The YSL performs nutritional, morphogenetic and immune functions. However, the data on the YSL structure in different teleost species is fragmented. We aimed to characterize YSL of the postembryonic inconnu (*Stenodus leucichthys nelma*) along with the depletion of maternal reserves by analyzing serial histological sections. At hatching, the yolk complex was located anterior to the liver and contained a large oil globule (OG) in the front region, 1–2 additional OG, and the yolk itself. The YSL region around the OG was striated, the region surrounding the yolk was regionalized in an apico-basal direction. The yolk syncytial nuclei (YSN) were large and complex-shaped. The yolk was exhausted by approximately the 17th day post hatching (dph). One–two large OG remained in the yolk complex until the programmed death of the YSL at 31 dph. The organization of the larval yolk complex of the inconnu was typical for Coregonidae. The abundance of comet-shaped YSN was its peculiarity.

Introduction

The inconnu (*Stenodus leucichthys nelma*) is a new and interesting species for cold-water aquaculture. Inconnu is a semianadromous species, but also residential forms are found in some waterbodies. It inhabits the rivers flowing to the Arctic Ocean (Chereshnev *et al*. 2002).

The ontogeny of the digestive system and yolk sac of this species should be studied because it would allow improving rearing conditions. This data will also contribute to fundamental knowledge of teleost ontogenetic diversity.

The YSL (periblast) of bony fish is a transient multifunctional structure vital for embryonic and postembryonic development. The YSL is an active component of yolk complex. The latter is a constituent of the yolk sac formed by ectodermal and mesodermal derivatives. Importantly, YSL and many other "extraembryonic" structures are defined by multiple nuclei of variable sizes, shapes and ploidy levels. The YSL forms during the blastula stage (Chu *et al*. 2012, Takesono *et al*. 2012), while further playing an active role in epiboly (reviewed by Lepage & Bruce 2010, Bruce 2016, Eckerle *et al*. 2018, Bruce &

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Heisenberg 2020). It has various morphogenetic, immune and nutritional functions; for instance, it contributes to yolk metabolism and performs transport of ions from yolk to developing definitive structures (Fu *et al*. 2019, Fu *et al*. 2019, Carvalho & Heisenberg 2010, Kondakova *et al*. 2019).

The literature data as well as our earlier results define the common principle of the teleost YSL organization. However, there are some species-specific structural and functional features determined by the anatomy of embryos and post-embryos, organization of yolk mass and probably some other factors (Kunz 2004, Jaroszewska & Dabrowski 2011, Kondakova *et al*. 2017, Kondakova *et al*. 2019).

Unlike other Coregonidae, the inconnu is a piscivore, which is one of the factors that determine specific early developmental, structural and functional features of its digestive system. To breed it successfully in artificial conditions, these features should be taken into account.

The objective of our study was to describe postembryonic development of an inconnu YSL from hatching to the fully active feeding. To this end, we used serial paraffin sections in parasagittal, frontal and transverse planes to determine species-specific features of the inconnu's YSL. In this paper, we also expand on the earlier data on yolk utilization and YSL structure during the development of the inconnu (Bogdanova 1977, Kondakova *et al*. 2017).

Material and methods

In 2017 and 2018, we obtained the eggs of the inconnu from the fish farm located on Lake Sukhodolskoye in the Leningrad region. We fertilized the mix of eggs stripped from several females with milt from 7–10 males per one female. In 2017, fertilization took place on 3 November at 4.8 °C and in 2018 on 30 October at 7.4 °C. The eggs were incubated in the Weiss apparatus for 178–180 days, and the larvae were kept in tanks with the natural temperature regime. The water temperature during the main incubation period was 0.2 °C.

In 2018, the larvae hatched on 5 May at 5.9 °C. At hatching (dph = 0), we sampled and fixed the newly hatched yolk-sac larvae (n = 3). In 2019, hatching occurred on 29 April at 6.8 °C. We sampled 2, 3, 3, 2, 3, 2, 3 and 3 larvae, 6, 10, 13, 17, 22, 25, 28 and 31 days after hatching, respectively. The total number of the larvae was 24. Larvae with any developmental abnormalities were excluded.

At the fish farm, we fixed the larvae in Bouin's solution. In the laboratory, we washed the larvae and stored in 70% ethanol, dehydrated and embedded into the Paraplast (Leica) according to the standard procedure (Merkulov 1961). We made parasagittal and frontal 6-µm thick sections with Leica SM 2010R sliding microtome (Leica Microsystems, Germany). We stained sections with Carazzi's hematoxylin and eosin (Biovitrum). We also used the slides from our previous study in 2015–2016 (Kondakova *et al*. 2017).

Results

In the inconnu larva, the yolk complex is anterior to the liver (Figs. 1 and 2A), and its shape changes during the postembryonic development. At hatching it was pear-shaped (Fig. 1A and 2A), by day 6 after hatching its anterior region became almost rectangular (Fig. 1B). As the yolk mass disappeared, the posterior region of the YSL was located above the liver (Fig. 1C). The analysis of the samples obtained at short time intervals showed that changes in the YSL organization were slow and smooth.

By the time of hatching, the oil globules (OG) coalesced in the anterior region of the yolk complex. One or a couple of additional OG could still be present within the solid eosinophilic yolk mass. The YSL cytoplasm around the OG was striated (Fig. 2B). The YSL partially separated the OG from the yolk (Fig. 2A). The YSL region surrounding the yolk in apico-basal direction had 2 distinguishable zones: the basal zone was heterogeneous and filled with the yolk inclusions, and the apical zone was homogenous. The larvae were provided food on the day of hatching. During the mixed feeding period, the YSL thickened, the amount of yolk inclusions in the basal YSL region increased, and the polarization became more prominent (Figs. 1 and

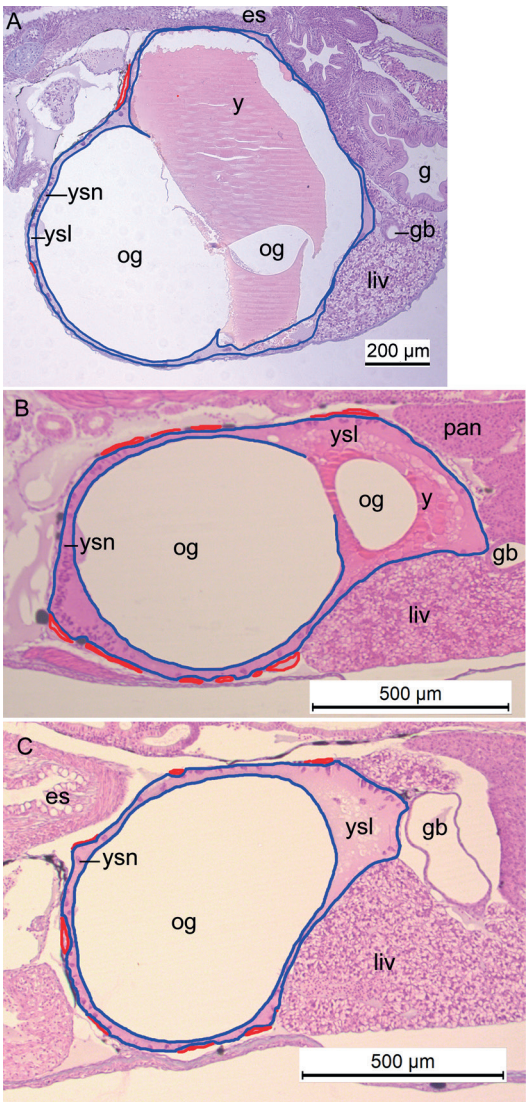

Fig. 1. Sequential developmental stages of the yolk complex (parasagittal sections); the contours of the yolk syncytial layer (YSL) and blood vessels are shown with blue and red lines, respectively. — **A**: hatching (dph = 0). — **B**: day 13 after hatching. — **C**: day 17 after hatching. Abbreviations: es = esophagus, g = gut, gb = gall bladder, liv = liver, og = oil globule, pan = pancreas, y = yolk, ysl = yolk syncytial layer, ysn = yolk syncytial nucleus.

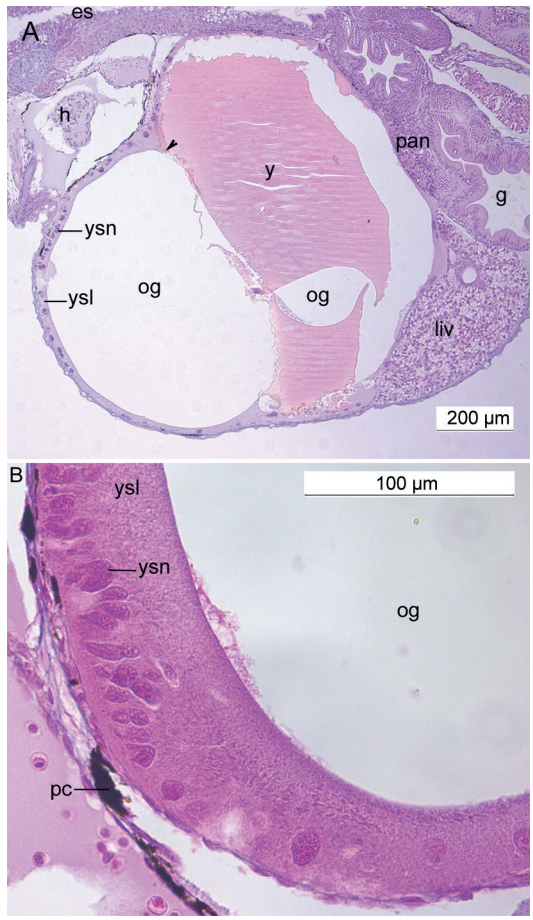

Fig. 2. The yolk complex at hatching (dph = 0) and at dph = 6; parasagittal sections. — **A**: at hatching: large oil globule occupies the anterior region of the yolk complex, additional OG are present within yolk mass, the YSL partially separates the OG from the yolk (arrowhead), the YSL region surrounding yolk in apico-basal direction. — **B**: day 6 after hatching: striated YSL cytoplasm around the OG and large YSN of diverse sizes and shapes as compared with that of blood cells. Abbreviations: es = esophagus, h = heart, g = gut, liv = liver, og = oil globule, pan = pancreas, y = yolk, ysl = yolk syncytial layer, ysn = yolk syncytial nucleus, pc = pigment cell.

3A). The posterior YSL region adhered to the pancreas, liver, and gall bladder. The YSL penetrated between the adjacent structures (Fig. 3B). Thin layer of the connective tissue separated the yolk complex from definitive digestive organs. Numerous blood vessels with a well-visible endothelium and pigment cells contacted the YSL (Figs. 1, 2A, 3B and 4).

Enormous yolk syncytial nuclei (YSN) of very diverse shapes were characteristic of the inconnu YSL. In addition to rounded and elliptical, there were YSN of complex shapes, such as lobed and "comet-shaped", i.e. elongated with numerous thin projections on one side. The abundance of these YSN is a characteristic feature of

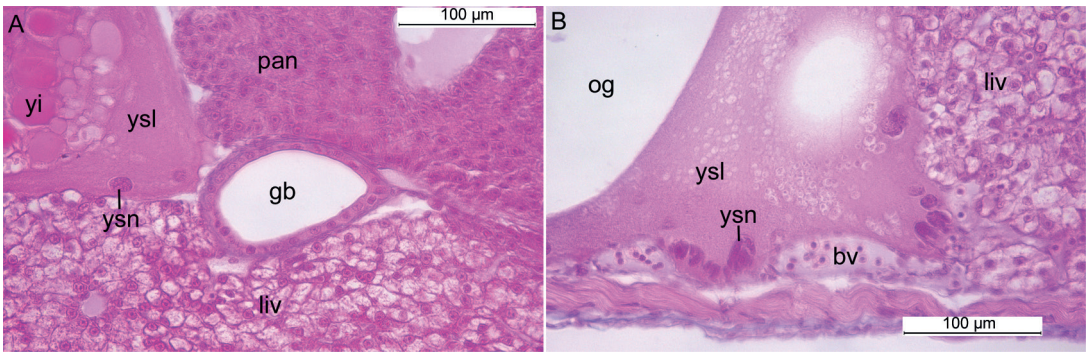

Fig. 3. The posterior yolk syncytial layer (YSL) region (dph = 13). — **A**: parasagittal section; apicobasal regionalization is evident. — **B**: frontal section. Abbreviations: bv = blood vessel, gb = gall bladder, liv = liver, pan = pancreas, og = oil globule, yi = yolk inclusion, ysl = yolk syncytial layer, ysn = yolk syncytial nucleus.

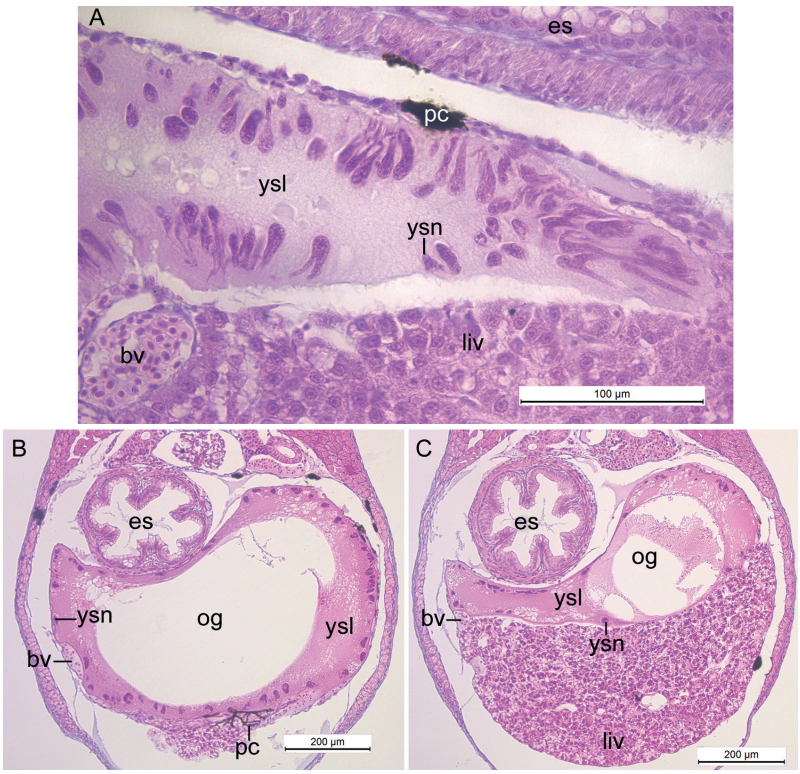

Fig. 4. The yolk syncytial layer (YSL) after yolk depletion. — **A**: day 28 after hatching (parasagittal section): yolk syncytial nuclei (YSN) of diverse sizes and shapes in the posterior tip of the YSL. — **B** and **C**: transverse sections of the same larva at different levels along the anteroposterior axis (day 17 after hatching). Abbreviations: bv = blood vessel, es = esophagus, k = kidney, liv = liver, m = muscles, ysl = yolk syncytial layer, ysn = yolk syncytial nucleus, pc = pigment cell, og = oil globule, ysl = yolk syncytial layer, ysn = yolk syncytial nucleus.

the inconnu YSL. Some YSN also had long thin constrictions or "bridges". As the yolk complex volume decreased during mixed feeding, the YSN concentrated at the periphery perpendicular to the apical surface (Figs. 2B and 4A).

The yolk was exhausted by approximately the 17th day after hatching. One or two large OG remained in the yolk complex (Figs. 1C and 4B–C).

On day 31 after hatching, we observed the pycnotic YSN and apoptotic bodies. The OG was still present in the YSL remnant.

From hatching up to day 22, all larvae had notably large unstained regions in hepatocytes that indicated lipid inclusions. From day 22 onwards, the amount of lipids in the liver decreased. Individual variations in the lipid content were observed as well.

Discussion

The position of the yolk complex (in relation to the liver) differs among teleost species. The yolk complex can be in direct contact with the liver or be separated from it (Kunz 2004). In the inconnu, as in other Coregonidae, the yolk complex is anterior to the liver. There is a layer of connective tissue between the YSL and liver, as in *Coregonus alpinus, Coregonus peled, Coregonus muksun* and *Coregonus nasus* (Kunz 1964, 2004, Kondakova *et al*. 2017).

The structural and functional regionalization is a characteristic feature of the teleost YSL. It is determined by the yolk organization, the presence of one or more OG and interaction with adjacent structures. In the postembryonic Coregonidae, this regionalization is evident. In the inconnu, the YSL cytoplasm had different characteristics in the regions around the OG, and a solid yolk mass. Unlike the yolk complex of some other teleost fishes, the coregonid YSL did not separate the OG from the yolk completely (Mani-Ponset *et al*. 1994, 1996, Poupard *et al*. 2000, Ostaszewska 2005, Kondakova *et al*. 2019).

The inconnu YSL interacts with numerous yolk sac vessels. In Coregonidae, the yolk circulation is formed by a subintestinal yolk vein and its capillaries (Bulanov 1979). Several studies showed the contact of YSL with pigment cells (Mani-Ponset *et al*. 1996, Poupard *et al*. 2000, Kondakova *et al*. 2016, 2017, 2019).

The giant size and an extremely complex shape of the YSN are characteristic of Coregonidae. In the inconnu, the YSN are elongated, rounded, elliptical, lobed or of diverse irregular shapes. The "comet-shaped" YSN are present in all studied coregonids, *Pterophyllum scalare* (Perciformes), and numerous in *Coregonus nasus* (Kunz 1964, 2004, Kondakova *et al*. 2017), but their abundance is a noticeable feature of the inconnu YSL.

Typically, the teleost larvae deplete the yolk first, and the OG remain for a longer period of time (Bogdanova 1977, Kunz 1964, 2004, Mani-Ponset *et al*. 1996, Poupard *et al*. 2000).

In this study, we show the pyknosis of the YSL remnant in Coregonidae for the first time. The data on the YSL programmed death is fragmented. The YSN of *Salmo trutta fario* undergo pyknosis (Walzer & Schönenberger 1979a, 1979b). In *Cyprinus carpio,* unlike in *Danio rerio*, the morphologically intact and pyknotic YSN are simultaneously present in the YSL remnant (Kondakova & Efremov 2014, Kondakova *et al*. 2016).

The yolk complex organization of the postembryonic inconnu is typical for Coregonidae. The prevalence of elongated comet-shaped YSN is its lineament (Kunz 1964, 2004, Kondakova *et al*. 2017).

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