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Early gametogenesis of the Volkhov whitefish, *Coregonus lavaretus baeri*

Vera A. Bogdanova

State Research Institute on Lake and River Fisheries, Fishery Laboratory, emb. Makarova 26, Saint-Petersburg, Russia

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The first detailed examination of the gonadal development of Volkhov whitefish juveniles was performed. Morphology and sequence of germ cells and gonad development are presented. Thirty days after hatching in the gonad anlagen, primordial germ cells began to divide. Sex differentiation occurred 82 days after hatching. Gonia in ovaries proliferated rapidly, some of them entered meiotic prophase developing into oocytes. In two weeks, the developing oocytes had reached the 'early perinucleolus stage' and began the cytoplasmatical growth. At the same time, in ovaries of some females, cysts with male germ cells appeared. Ten days later, all whitefish females had hermaphroditic features. In male gonads few germ cells were dispersed singly amongst stromal tissue. The reason for the mass hermaphroditism among Volkhov whitefish females may lie in the environmental circumstances such as high temperature during the study period, or the specificity of the early gametogenesis in this whitefish species.

Introduction

The Volkhov whitefish (*Coregonus lavaretus baeri*), also known as "sigolov", earlier held the first place in fishery of Lake Ladoga. It mainly inhabits the southern part of this lake. The main natural spawning areas of that whitefish were situated in Lake Iljmenj and the Msta river. A spawning migration of the whitefish has gone up the Volkhov river. This way became blocked up after the construction of the Volkhov dam. Recently the reproduction of the Volkhov whitefish is mainly carried out by the Volkhov hatchery, but its abundance is strongly reduced. In spite of the fact that the Volkhov whitefish is a rare, disappearing species and is included in the Russian Red Book, some characters of its biology and, in particular, gametogenesis, are poorly investigated. There are fragmentary data on the gonads in adult fish only (Anpilova & Chernikova 1969). Research of other whitefish has demonstrated different specific characters at different stages of early gametogenesis and sex differentiation in different species (Statova & Thomnatic 1970, Selukov 1985, Zacharova 1997, Bogdanova 2002). The detailed research of these processes is important for the knowledge about the specificity of reproductive function of the Volkhov whitefish, which, in turn, is necessary for organisation of monitoring of reproduction in aquaculture, and also at elaboration of management methods of their reproductive function, e.g., hormonal sex reversion (Donaldson & Hunter 1982, Foyle 1993). The aim of this study

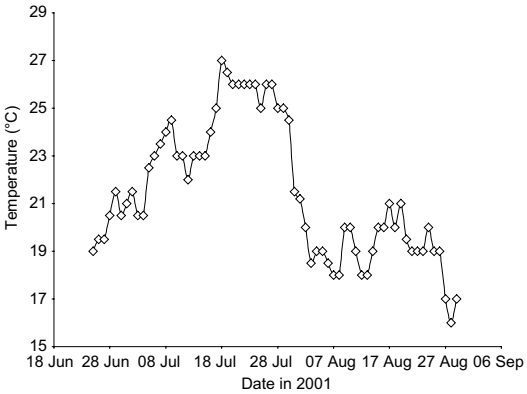

Fig. 1. The water temperature in tanks during the Volkhov whitefish rearing in 2001.

was a detailed histological examination of the gonadal development in the Volkhov whitefish in age from 30 to 128 days including the indifferent stage, sex differentiation and development after sex differentiation in female and male.

Material and methods

The material for the studies of early gonado- and gametogenesis of the Volkhov whitefish was collected in the experimental hatcheries of the Leningrad region. In 2001, the material collection began on 11 July when the juveniles (aged 82 days) were transported from the Volkhov hatchery at the base "Priladogskye". The experimental rearing temperature was registered (Fig. 1). In 2002, the material for the whitefish gonadal development study was collected in the base Motornoye starting on 16 May (aged 30 days). The juveniles were reared in basins and fed with artificial feed. For the histological analysis of gonads the Bouin's fixative was used. Larvae were totally fixed and measurements of such material were performed. Juveniles were measured immediately after catching and gonads were fixed. Histological analysis was performed using standard methods. Histological cross-sections (7–8 μm) were stained with haematoxylin. Developmental stages of larvae were identified according to Evropeitzeva's classification (1949). Estimation of the gonad condition was performed from the upper part of the cranial part of both lobes. The larvae specimens were serially sectioned. In total, 65 specimens were histologically studied (Table 1).

Results

The gonad development of the Volkhov whitefish in the postembryonic period

The main morphogenetic processes in 30-day-old Volkhov whitefish (III larval development stage) relate to the secondary symmetric caudal fin formation: upwards curvative of the urostyle and total fin ray formation in all fins (Fig. 2). At the beginning of gonadogenesis the reproductive system was characterized by the gonad anlagen and division of the primordial germ cells (PGC). The gonad formation begins from the enlarging of the coelom epithelium and mesenchyme cells, as a result of which the genital folds form along the dorsal coelom wall. At the cross-sections of larvae, the gonad anlage, primodial germ cells and germ cells of first orders in the mitotic divisions, known as gonia, were observed (Fig. 3). PGC were identified by large size and sharp borders of nucleus and cell. The mean diameter of PGC in the Volkhov whitefish larvae was 12.7 ± 0.4 μm

Table. 1. The mean total length and body mass ± SE of the studied Volkhov whitefish at different ages.

Age (days)	Date	*n*	Total length (cm)	Body mass (g)
30	16 May 2002	5	0.17 ± 0.003	0.023
40	26 May 2002	4	0.21 ± 0.01	0.035
60	16 June 2002	3	0.28 ± 0.02	0.12
82	20 April 2001	8	4.5 ± 0.26	1.2 ± 0.12
90	21 July 2001	10	5.4 ± 0.17	0.9 ± 0.01
100	31 July 2001	10	6.1 ± 0.31	1.7 ± 0.25
120	18 August 2001	15	9.2 ± 0.24	6.3 ± 0.55
130	28 August 2001	10	10.0 ± 0.12	7.6 ± 0.44

Fig. 2. A Volkhov whitefish larva of the III developmental stage.

and that of nucleus 8.5 ± 0.2 μm. The cells had characteristically lightly stained cytoplasm and 1–2 nucleoli were present (Fig. 3). PGC began to occur at the level of 9–11 myomeres. Sizes of gonia were 9.8 ± 0.2 μm with a nucleus diameter of 7.3 ± 0.2 μm. In the nucleus there were from one to six or even more nucleoli.

Ten days later (age 40 days), the Volkhov whitefish larvae reached a standard length (SL) of 21–22 mm and were determined at IV prejuvenile developmental stage. In the gonad formation the processes of the stroma development (Fig. 4) and gonia proliferation continued.

At the age of 60 days, changes in the exterior appearance of specimens (SL = 25–30 mm) were caused by their passing to the juvenile developmental stage. The juveniles had definitive features formed, i.e., the beginning of scale formation was noted. In that age the processes of the stroma development and increase in number of gonia continued. PGC occurred rarely. The average number of the germ cells on cross section was 3–6.

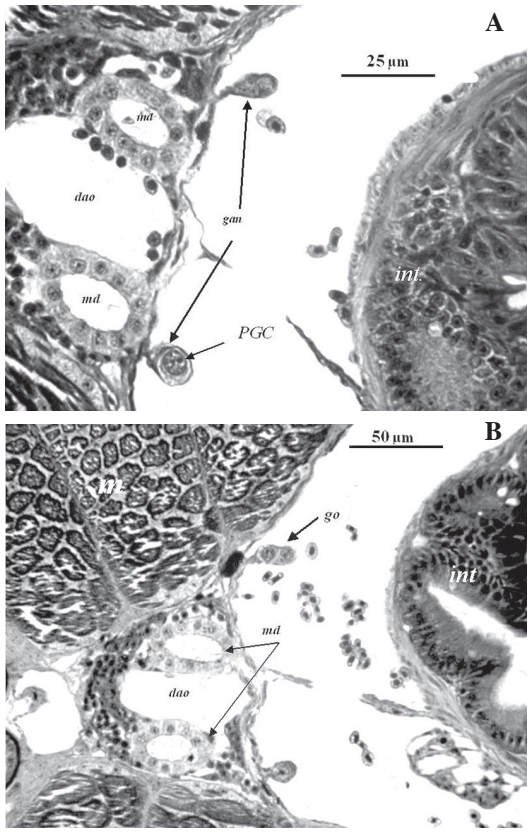

Fig. 3. Cross-section of a 30-day-old larva. — **A**: The gonad anlagen (gan) consist of a ridge of peritoneum and contain primordial germ cells (PGC). — **B**: Gonia (go) in the gonad anlage. md = mesonephric duct, dao = dorsal aorta, int = intenstine.

The gonad development of the Volkhov whitefish juveniles

In some fish of 82 days of age (with body mass more than 1.5 g) the sex differentiation towards female began. In the dorsolateral part of the future female gonads the number of germ cells was increasing. Some gonia passed to the early meiotic prophase (Fig. 5). The females' gonads often developed asymmetrically, the left lobe being mostly more advanced. In other fishes, the gonad condition was considered as indifferent.

At the age of 90 days, in developing females the part of gonia in gonads was in the premeiotic transformation condition. In some fishes that process was just beginning, in others it was more advanced. Occasionally, the metaphase and anaphase stages of mitotic divisions could be seen.

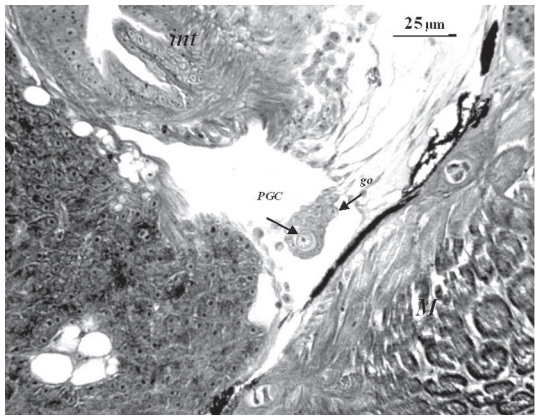

Fig. 4. Cross-section of a 40-day-old larva. Among somatic cells of gonad there are primordial germ cells (PGC) and gonia (go). m = lateral body muscles, int = intenstine.

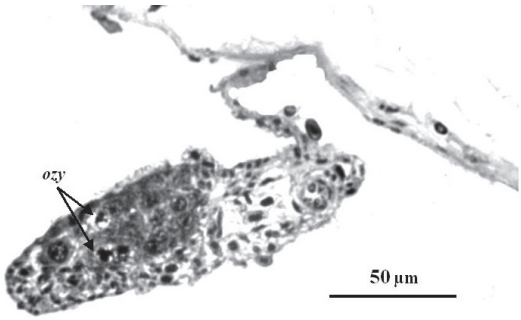

Fig. 5. Cross-section of a newly differentiated ovary at 82 days. In the dorso-lateral part of ovary the germ cells concentrate, some of which are in zygotene stage (ozy).

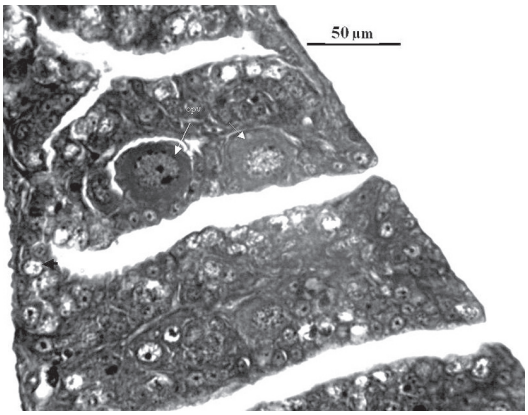

Fig. 6. Ovary of a 100-day-old whitefish. Most germ cells have entered meiotic prophase to become previtellogenous oocytes (opv). The ovary has the forming lamellae.

At 100 days, in developing ovaries some oocytes had begun cytoplasmatical growth. A small part of females had well-developing ovarial lamellae at this age (Fig. 6). In other females ovaries were poorly developed anatomically, and in the generative part of one of the females, cysts with male germ cells were found.

At 120 days, among ten studied individuals there were three males. In five of seven fishes with gonads characterised as the ovarian type, spermatogenesis processes were noted. The disturbances were well expressed and appeared in both gonad lobes. The testicular tissue development was followed by anatomical changes of gonads; in particular, connective tissue parts uncharacteristic for ovaries appeared. In gonads of some fishes, intensive development of the testicular cells was detected, and female germ cells rarely occurred in histological sections (Fig. 7). In gonads of other individuals the cysts with the male germ cells were rare and in the generative tissue oogonia and oocytes at different stages of oogenesis prevailed. In two females, the cysts with the male germ cells were not found. One of them (body mass 3.1 g, length 7.2 cm) had small gonads in which the anatomical and cytological differentiations were beginning. In another female (body mass 7.6 g, length 8.7 cm), the disturbance of the anatomical ovary structure with the connective tissue parts present was noted. This can indirectly indicate possible presence of testicular elements in other gonad parts. On the basis of the analysis of the studied samples, it is possible that at a certain developmental level of gonads, females pass into the intersexual condition. In males, the gonad stroma formation took place, and few spermatogonia were present.

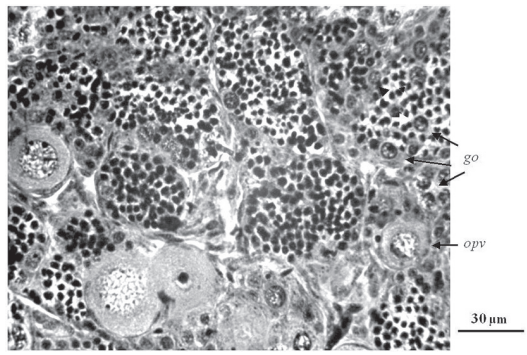

Fig. 7. Testis-ovary at 120 days. There are many cysts with male germ cells. The previtellogenous oocytes (opv) are few. (go = gonia).

Among the 130 days old juveniles there were 4 males and 6 females. All the females had hermaphroditic characters. Two groups of the hermaphroditic specimens were distinguished. In one group there were specimens with strong testis development; the formation of the cysts contained cells of different phases of spermatogenesis and different connective tissue expansions were observed. In those specimens the process of oogenesis was delayed, the number of the early oocytes was small or they were almost absent in some sections. In the other group oogenesis prevailed, recruitment and growth of the previtellogenous oocytes took place, while cysts with the testicular cells were few.

Preliminary investigation of whitefish at the age of 390 days demonstrated that active oogenesis began (Fig. 8), and in some ovaries resorption of testicular tissue was detected.

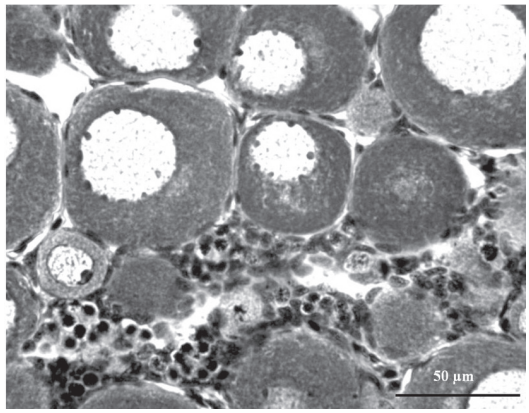

Fig. 8. Germinative part of an ovary of a 390-day-old Volkhov whitefish containing gonia, oocytes of early meiotic prophase and oocytes in a phase of cytoplasmatical growth.

Discussion

The 30-day-old Volkhov whitefish larvae were at the stage III of larval development. Their reproductive system, similarly to that of the other whitefishes at that age (Mostovskaya & Polyakova 1986, Selukov 1985, Zacharova 1997), was characterized by first PGC divisions, first gonia generation formation and beginning of gonadogenesis. At passing to the juvenile stage of development mainly gonia were in the developing gonads, PGC occurred only rarely. First features of the gonadal differentiation to female direction were found in fishes of 82 days at body mass of more than 1.5 g. In the salmonids the anatomical gonad differentiation precedes the cytological differentiation (Persov 1975, Murza & Christoforov 1991). In the Volkhov whitefish, in the dorso-lateral part of the gonad, the gonia concentration preceded the appearance of clear characteristics of sex differentiation to female. Differently to female whitefish, in future males of that age the somatic gonadal part, as a rule, was less developed, the germ cell number was smaller and they were dispersed across the whole volume of gonads. The asymmetrical gonad development was frequently detected in Volkhov whitefish, which possibly is a relatively typical phenomenon in the early ontogenesis of whitefish. The gonadal asymmetry in the early ontogenesis of some salmonid species has also been described (Persov 1975, Murza & Christoforov 1991).

The cytological differentiation of gonads began in whitefish at the age of 82 days, and after 20 days gonads of future males and females had clear anatomical and cytological differences. Thus, original sex differentiation in the Volkhov whitefish juveniles, as in most salmonids, goes directly into male or female. However, at further gonadal development in female, the disturbances indicating abnormality of sex differentiation were found. After 100 days, in ovaries of some females the cysts with the male germ cells were noted and after a further 10 days hermaphroditism in females occurred widely while in the gonad development of males no abnormalities were detected. The testis cell appearance in ovaries of the Volkhov whitefish juveniles indicates an ability of the gonia to conserve bisexual potention. Obviously, disturbance of the mechanism of sex differentiation in female Volkhov whitefish led to the stimulation of gonia development toward male direction and formation of testis parts in ovaries.

The coregonids are considered as gonochorists, i.e. species with a direct type of gonad differentiation. Hermaphroditism relatively seldom occurs in Siberian coregonids such as *Coregonus peled, C. muksun, C. nasus,* or *C. autumnalis*, the gametogenesis of which is well studied (Kuzmin 1975, Selukov 1989, Zacharova 1997). Reasons for hermaphroditism could be related to hybridisation or inbreeding (Bogdanova 1997), although in the present study, these reasons were hardly probable. In whitefish, *C. lavaretus sensu lato*, the hermaphroditism is more often detected. Cases of hermaphroditism were noted in *C. lavaretus baunti* (Muchomedjarov) under starvation (Anpilova 1965). Mass hermaphroditism was found by Konradt (1969) in the Chud whitefish, *Coregonus lavaretus maraenoides* (Poljakov 1874). The author related that disturbance to the deterioration of environmental circumstances and the high water temperature in Lake Chud. In Volkhov whitefish juveniles, the high tempera-

ture in the middle of summer in 2001 might be the reason to the hermaphroditism.

In considering the possible reasons for the observed massive disturbances in gonad differentiation in Volkhov whitefish the specificity of the early gametogenesis should not be excluded either. Principally different types of gonad differentiation may exist in genetically close species of the same genera. For example, there are species like *Oncorhynchus kisutch* (Walbaum) with the direct sex differentiation and *O. gorbuscha* (Walbaum) in which the juvenile hermaphroditic stage exists during the sex differentiation process (Persov 1975).

According to the preliminary data, in the beginning of the next season at the age of 390 days, in many Volkhov whitefish females the testicular cells were absent and processes possibly relating to the testicular tissue resorption were observed. However, in some females, many male germ cells remained among the ovarian cells. Further studies are needed to find out the reasons to the mass hermaphroditism among the Volkhov whitefish females, the details of the development of this phenomenon and its influence on oogenesis and fecundity.

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