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The Pathologies of the Microstructure in Generative Organs of the Females of European Whitefish *Coregonus lavaretus* in Lake Imandra

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Abstract—The effects of different technogenic pollutants on the reproductive system of whitefish in Lake Imandra (Kola Peninsula) were studied from 1996 to 1999. The development and functioning of generative tissues in females of European whitefish *Coregonus lavaretus* were studied in impact zones and in the areas not subjected to high technogenic loads. The pathologies of histological structure were diagnosed in fish gonads. It was determined that gonad anomalies were found significantly rarer than pathologies in fish gills, kidneys, and the liver. The development of sexual cells in most females inhabiting the contaminated areas occurred without obvious tissue deviance.

INTRODUCTION

Intense transformation of natural ecosystems occurs as a result of commercial activity. The rate of succession processes significantly increases, and living organisms are forced to adapt in order to survive in the conditions formed. Water ecosystems are the collectors of all types of pollutants, and hydrobionts are relatively responsive to such changes. A wide diversity of pollutants input to water bodies, together with general toxic effects on living organisms, affect the processes of gametogenesis, particularly in fish. This causes disturbances in reproduction and the appearance of nonviable progeny, decreases reproductive potential of individuals, and causes injury to the fish resources of Russia (Luk'yanenko, 1990; Pavlov *et al.*, 1994; etc.). Conservation of species and of their capability for reproduction in the conditions of an intense anthropogenic load on natural ecosystems is presently one of the most important problems in biology.

Numerous works suggest the appearance of different anomalies in the development and functioning of reproductive systems of fish under the effects of different toxicants (Koshelev, 1978, 1980; Akimova and Ruban, 1996; Shatunovskii *et al.*, 1996; Moiseeva *et al.*, 1997; Chebotareva *et al.*, 1997; Akimova *et al.*, 2000). It is important not only to record the pathologies, but to reveal, if possible, the mechanisms of disturbances and fish adaptation to varying environmental conditions.

Lake Imandra, situated in the central part of the Kola Peninsula, is subject to a long anthropogenic impact from metallurgic and mining works. Intense development of these plants, their relatively small territory, imperfect technologies for emission control, and effluent treatments result in large amounts of pollutants coming into water bodies and negatively affecting their state. A series of works was devoted to the problem of the effects of pollutants on water quality and hydrobionts of water bodies in the northern part of the Kola Peninsula (Reshetnikov, 1968, 1980; Reshetnikov *et al.*, 1971; Belyaeva, 1976; Vladimirskaya, 1956; Moiseenko and Yakovlev, 1990; Moiseenko, 1997; Lukin, 1995). In these works, the primary emphasis was placed on the changes in the structural-functional arrangement of the fish part of the community.

The present study was aimed at the investigation of the state of gonads in females of whitefish *Coregonus lavaretus* in Lake Imandra, living under conditions of chronic pollution, and at revealing pathologies of the microstructure of the reproductive system.

MATERIAL AND METHODS

This work was based on the materials collected from 1996 to 1999. The whitefish *C. lavaretus* was the object of study. Fish were captured with a set of fixed gill nets from nylon monofilament of standard length 25 m and height 1.5 m and cell mesh 10, 12.5, 16, 22, 25, 30, 35, 38, and 45 mm. This provided capturing of fish 5 cm and more in length.

Size and weight characters, sex and stage of gonad maturity, fatness (in points), stomach fullness, and age were determined in fish. Functional state of generative organs was evaluated on the basis of pathologic-morphological analysis, including pathoanatomical and histological study. Gonads with different anomalies and with apparent signs of disturbance were taken for histological analysis. Gonads were fixed in Bouin's fluid and embedded into paraffin. The sections were stained with

Heidenhain's iron hematoxylin (Roskin and Levinson, 1957). Gonads from 109 females were analyzed. Special attention was focused on the study of the reproductive system in fish inhabiting impact zones in Lake Imandra: the region affected by waste waters of a copper-nickel plant (contamination with heavy metals, among which Zn, Cu, and Ni are of high priority); apatite-nepheline works (high concentrations in waste waters of highly-dispersed suspended matter, mineral salts, and flotation agents); and warmed waters of Kola NPP (part of the lake is used as a cooling water body).

RESULTS AND DISCUSSION

The anomalies of the macrostructure of the gills, liver, and kidney in fish were found more often in the areas of Lake Imandra subjected to the most intense pollution than anomalies in generative organs (Akimova *et al.*, 2000; Sharova and Lukin, 2000). For example, the number of fish with liver pathologies varied in 1996 to 1998 from 3.6 to 38% depending on the studied region, and averaged 21.5%. The frequency of whitefish with kidney anomalies averaged 28.4% (1.3–65.6%) for the Lake. Destructive changes in gills, which were found in 30% of studied fish, were diagnosed as a rule after the appearance of initial changes in kidneys. Disturbances connected to changes in sexual glands were found to be significantly rare.

The number of whitefish with anomalies of generative organs varied from 2.6 to 14.9% depending on the region, and averaged 6.6% for the lake over the studied period. Anomalies were observed mostly in generative organs of females. Literature data suggest that male gonads, on one hand, are relatively resistant to the effects of external factors (Turdakov, 1972), but on the other hand, the organism of males more rapidly responds to the environmental changes in pollution conditions. Already, Nikolsky (1974) noted that varying environmental conditions caused the appearance of a large number of females. A similar phenomenon was observed in European cisco *C. albula* of Lake Imandra in the period of maximum anthropogenic load on the water body and in characters of the genus *Salvelinus* of Lake Saraslakki in the impact zone of Pechenganikel' Works (Lukin and Kashulin, 1991). Consequently, the resistance of males and females is different. Because of this, most attention was given in the process of study to the state of the reproductive system in females.

As a rule, gonads of whitefish females at maturity stages I and II are pale rose. The color becomes yellow-orange by stage III, and its intensity increases, as oocytes mature, at the expense of a greater amount of carotinoids possessing antioxidant capability, which is very necessary for preserving the quality of trophic material in ovicells (Fig. 1a). Gonads develop uniformly. The study of the microstructure of ovaries, together with normally developed oocytes, revealed destructive changes of sexual and somatic cells in females.

Resorption of previtellogenous oocytes. Females having ovaries with single resorbing oocytes of the period of cytoplasmic growth were found in all regions of Lake Imandra. Whitefishes with such a pathology dominated in the region affected by waste waters of copper-nickel works. Here, large zones of destruction were observed in three of eighteen studied individuals. These zones included about 40% of previtellogenous oocytes.

The importance of the period of previtellogenous growth of oocytes is mainly in the development of strong synthetic apparatus. It was considered in the past that in individuals not at sexual maturity, degeneration of sexual cells did not occur under the conditions of changing environmental quality, and only the duration of the period of cytoplasmic growth changed (Popova, 1977; Koshelev, 1978). Only single cases of resorption of previtellogenous oocytes were reported in the literature as a response to a certain type of water body pollution, particularly to intoxication with pesticides (Popova, 1978). It was shown in experiments (Lesniak and Ruby, 1982) that sublethal doses of hydrocyanic acid caused atresia in sexual cells at all stages of development, including previtellogenous oocytes. However, recent publications give strong evidence that total degeneration of oocytes of the period of cytoplasmic growth is not a rare phenomenon and is found relatively often among the gonad anomalies. Resorption of previtellogenous oocytes was observed in sturgeons in the Siberian rivers under the effect of effluent warm waters of power plants (Akimova and Ruban, 1992; Akimova *et al.*, 1995) and in the Sea of Azov under an increased level of technogenic pollution of the natural water body (Moiseeva *et al.*, 1997). Such a phenomenon was observed in whitefish of water bodies of Noril'sk-Pyasina system (Chebotareva *et al.*, 1997), and in whitefish of the Pasvik lake-river system (northwest part of Kola Peninsula) as a result of a chronic toxic effect of heavy metals on the fish organism (Akimova *et al.*, 2000). It must be emphasized that study of long-term dynamics of this process revealed a clear tendency for an increase in the frequency of this pathology.

Resorption of vitellogenous oocytes. Resorbing oocytes of vacuolization stage were found in gonads at maturity stages III and IV of most females. The number of degrading vitellogenous oocytes was small as a rule. However, oocytes of the elder generation that entered the period of trophoplasmatic growth were subjected to local resorption in 4 of 20 individuals inhabiting the region affected by waste water of *apatite-nepheline works*. This process covered about 50% of all vitellogenous oocytes in studied fish, and this could cause the absence of sexual cells at the final stage of development if the process of degeneration would progress. The state of such gonads probably attests to the impossibility of spawning of these individuals in the current year.

The resorption of sexual cells in fish can be a physiologically normal process if it is observed in spawned

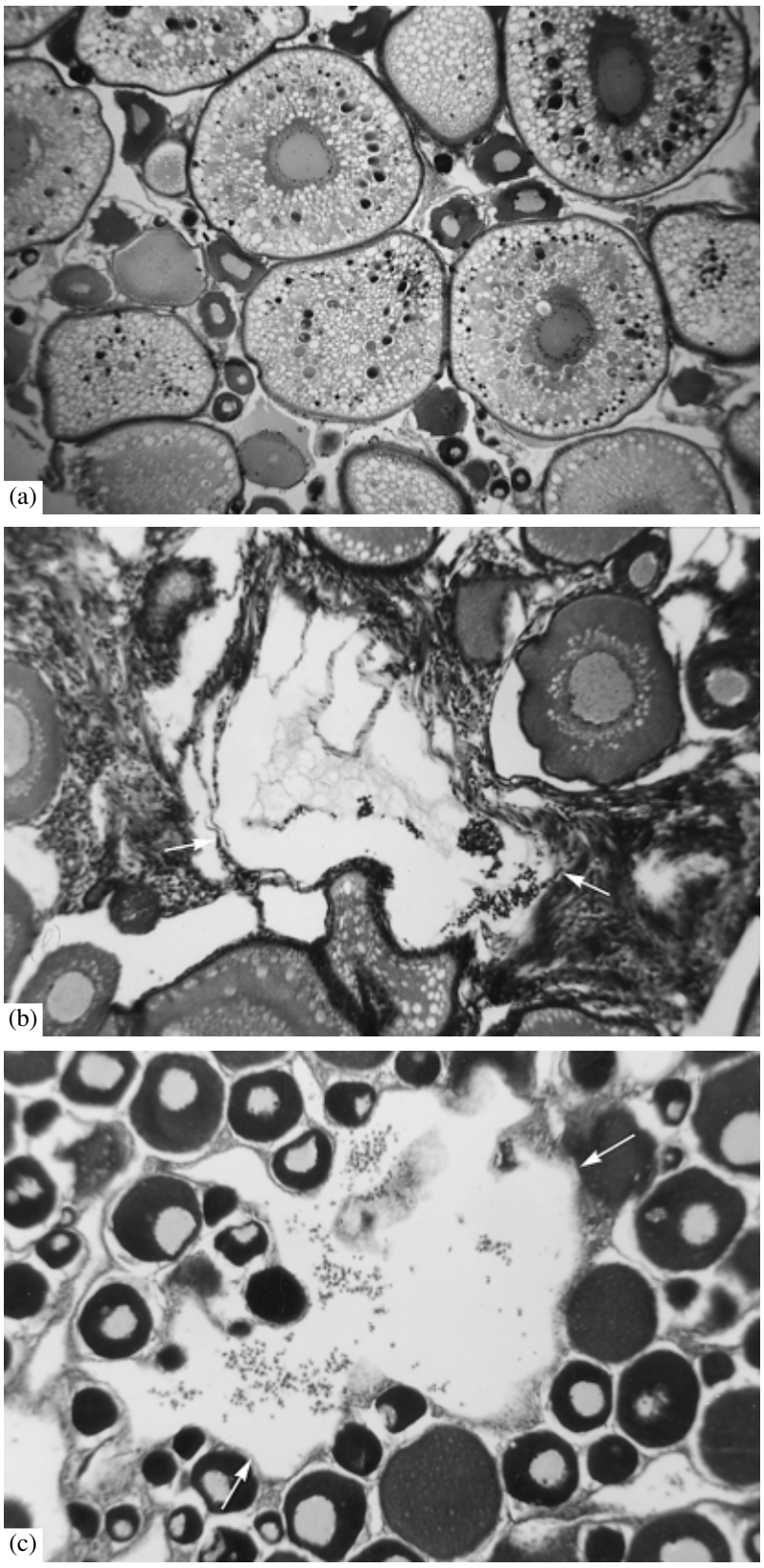

Fig. 1. Microstructure of gonads in whitefish females from polluted regions of Lake Imandra: (a) normal development of oocytes of the vitellogenesis period, magnification ×150; (b) lipoid degeneration (marked by arrows), magnification ×600; and (c) neoplasms (marked by arrows), magnification ×600.

out individuals and at establishing of the final fecundity. It can also be a pathological process if mass resorption of sexual cells occurs in the period of gametogenesis (Faleeva, 1965; Persov, 1975; Koshelev, 1978; Makeeva, 1992; etc.). According to modern views, different types of gametogenesis and corresponding types of spawning exist in fish (Koshelev, 1984). Asynchronous development of oocytes in the period of vitellogenesis is typical of the whitefish genus in natural conditions, which is smoothed by the beginning of maturity stage IV (Dryagin, 1949; Lapitskii, 1949; Koshelev, 1984). Homogeneity of oocytes in the process of maturation is achieved at the expense of the resorption of sexual cells highly advanced in development. In relation to this, resorption of single oocytes is considered as a norm in fish gametogenesis (Kuz'min and Chuvatova, 1975). However, many researchers consider that the mass resorption of oocytes in the period of vitellogenesis is the response of the reproductive system in females to changes in the water environment quality and suggests the disturbances of the conditions for reproduction (Faleeva, 1965; Koshelev, 1984; Akimova and Ruban, 1992, 1996; Belova *et al.*, 1993; Moiseeva *et al.*, 1997; Chebotareva *et al.*, 1997; Akimova *et al.*, 2000).

What is the cause of mass resorption of oocytes of trophoplasmatic growth? The period of vitellogenesis is characterized by an accumulation of yolk, which has dual origin in some fish: endo- and exogenous. Yolk is used subsequently as trophic material in the process of embryogenesis (Aizenshtadt, 1984). The granules of endogenous yolk are synthesized within an oocyte, but the most part of yolk proteins has an extragonad origin. And, the main reserves of the ovicell are formed at the expense of synthetic activity in other organs. The molecules of vitellogenin, synthesized by liver cells and transported by the blood stream into a developing follicle, are the precursors of exogenous yolk (Aizenshtadt, 1984; Murza and Khristoforov, 1991). The intensity of vitellogenous growth of oocytes depends in part on vitellogenin concentration in the blood (Semenov, 1980; Khristoforov and Murza, 1998). Consequently, The disturbance of yolk deposition in developing sexual cells because of low concentrations of vitellogenin, due to by the decrease in functional activity of the liver (low synthesis of vitellogenin), can cause the resorption of oocytes of the period of trophoplasmatic growth. Dixon (1975) demonstrated in experiments with sublethal (0.05 mg/l) concentrations of cyanide that the degeneration of a great number of hepatocytes occurred in rainbow trout *Salmo gairdneri* under the effect of hydrocyanic acid, and this resulted in the deposition of yolk in oocytes. A similar regularity was observed in the fish studied in our work. A great number of resorbing oocytes of the period of trophoplasmatic growth was observed in the case of liver disease and the appearance in it of fat dystrophy and connective-tissue growths.

However, high levels of vitellogenin in the blood do not provide in themselves intensive including of this compound into ovicells (Khristoforov and Murza, 1998). Gonadotropic hormones are known to significantly decrease the concentration of vitellogenin in the blood of females and to stimulate its sorption by oocytes (Aizenshtadt, 1984; Khristoforov and Murza, 1998). However, it is supposed that thyroxin, triiodthyronin, insulin, and hormone of growth, increasing sensibility of ovaries to gonadotropins and controlling vitellogenesis, are also involved in the regulation of this process (Tyler, 1991; Kaufman, 1994). For example, injections of thyroxin were successfully used to prevent degeneration of sexual cells in sturgeon fishes (Smith, 1982). This is an example of the fact that the animal organism acts as a united whole in actually all manifestations of adaptive activity, including the realization of reproductive function. Hence, the complex of an organism's structures, normal functioning of which depends largely on the quality of the environment, is required for successful realization of different aspects of reproduction.

Fat dystrophy. The disturbances of gonad structure were observed in whitefish in addition to anomalies connected to the resorption of oocytes. Growth of fatty tissue was found in the place of degenerating generative tissue in one of the ovaries of a whitefish female in the impact zone of *apatite-nepheline works*, gonads of which were at maturity stage III (Fig. 1b). A large number of resorbing vitellogenous oocytes was found in this tissue.

Lipoid degeneration appears as a result of disturbances in lipid metabolism and is caused by an excessive accumulation of reserve lipids in a cell. The processes participating in the development of cell changes typical of dystrophy include decomposition: the destruction of cytoplasmic lipoproteins resulting in a disappearance of cell structures and cell destruction (Strukov and Serov, 1985). The damage caused by the toxin is one of the causes of degenerative obesity. Among these substances, compounds of arsenic and phosphorus are most toxic (Kokuricheva, 1976), and they are present in excess in the region of Lake Imandra subjected to the effect of effluents of apatite-nepheline works. Such an effect can be attributed to the capability of these compounds to change the intensity of oxidative processes in organisms affecting fat utilization. The replacement with fatty tissue leads to topical disproportion in surrounding cells, causing the squeezing of tissue and the progress of fat dystrophy in neighboring cells. The process of fatty tissue growth in the studied ovary was accompanied by a resorption of oocytes and could ultimately cause the physiological atrophy of an organ.

The phenomenon of lipoid degeneration was also observed in other organs, most often in the liver. Judging from all these facts, the appearance of fat dystrophy is the typical response of an organism to the effect of

toxic substances, be it heavy metal or organic pollutant. Available literature data report that a similar disease was recorded in the generative tissue of separate females of cisco *C. sardinella* and peled *C. peled* from water bodies of the Noril'sk-Pyasina system subjected to the effect of heavy metals (Chebotareva *et al.*, 1997) and in males of Russian sturgeon *Acipenser gueldenstaedti* from the Volga River contaminated with organochlorine compounds (Akimova and Ruban, 1996).

Neoplasms. The disturbance of sexual and somatic cells of the ovary was accompanied sometimes by the appearance of neoplasms, microscopically similar to cysts (Strukov and Serov, 1985). We found cysts in gonads of whitefish females captured in the region affected by waste water of *copper-nickel works.* The parenchyma of the neoplasm had the form of a cavity restricted by a capsule with a loose structure (Fig. 1c). This could be a sign of the malignant transformation of a cyst and could cause the growth of the latter into adjacent cells and subsequent destruction of the generative tissue.

Multiple cystic formations of local type were found in the other whitefish female, and this allowed us to classify this as ovarian polycystic disease. Cyst parenchyma consisted of empty cells filled with a reticular structure, between which groups of blood corpuscles were found. Normal development of oocytes of the period of cytoplasmic growth was observed in gonad parts, untouched by pathological changes. Such cysts are apparently formed in the process of the transformation of egg-bearing plates and are the result of connective-tissue growth in an ovary. The analysis of literature data suggests that cystic formations are a rare phenomenon, but an analogous an anomaly in the ovary structure was also recorded in other taxonomic groups of fish, particularly in females of sturgeons from the Sea of Azov (Moiseeva *et al.*, 1997) and sturgeon males from the Siberian rivers and the Volga River (Akimova *et al.*, 1995).

The aggregates of blood corpuscles participating in the process of resorption were found in parts of gonads with intense destruction of cells, accompanied by the resorption of previtellogenous and vitellogenous oocytes and the appearance of neoplasms. Similar phenomena were observed by other researchers also (Akimova *et al.*, 1995; Akimova and Ruban, 1996; Chebotareva *et al.*, 1997).

Change of time of sexual maturation. The response of whitefish to heat pollution in the region of warmed waters of Kola NPP was specific. The change in the character of sexual maturation of fish was observed in this zone, warmed throughout the year. As a rule, transition from gonad maturity stage II in individuals attaining maturity for the first time occurred in late June–July, and the coefficient of maturity in the norm did not exceed 1% in females. In females feeding in the region of warmed water, development of gonad reached stage III in May, and the coefficient of maturity comprised 3.5%. The complex of sexual cells was presented by oocytes of different phases of vitellogenesis. Accumulation of yolk occurred in oocytes of the elder generation, whereas oocytes in gonads of females from other regions were in the early phase of cytoplasm vacuolization.

The acceleration of vitellogenesis was apparently determined by a longer growing period and favorable conditions for feeding in this zone. A similar phenomenon was first recorded by T. I. Moiseenko (Kryuchkov *et al.*, 1985) and attested the existence of a certain range of ecological plasticity for the period of yolk accumulation.

Hence, the following gonad pathologies under the effect of different toxicants were found in whitefish females from Lake Imandra: the resorption of oocytes of the previtellogenesis period, resorption of oocytes of the period of trophoplasmic growth, lipoid degeneration, and cystic formations. Factors affecting the fish organism can have specific manifestations, diagnosed pathologies can be attributed to certain types of pollution, and the cause of their appearance can be revealed on this basis.

It should be noted in conclusion that determined pathologies of whitefish organs were not widely spread. The percentage of fish with gonad anomalies was not high in the conditions of chronic stress, and development of sexual cells in most females inhabiting polluted regions went without deviation from the norm. High values of individual absolute fecundity were observed in most individuals, which retained the ability for reproduction. This attests to their high reproductive capability. Taking into account the level of anthropogenic load on the water body, we could expect more serious and deep changes in the development of female gonads. This situation suggests a high degree of protection of generative organs in whitefish females from Lake Imandra, directed at the preservation and supporting the population abundance.

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