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REGULARITIES AND ANOMALIES IN THE STRUCTURE OF GONADS IN COREGONID FISHES*

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Key words: coregonid fishes, ovaries, testes, anomalies in the structure of gonads.

Abstract

The anatomical structure of gonads in coregonids is characteristic for teleost fishes with follicular, synchronous group tape-like ovaries and acinose type, lobulated testes. In some alpine lakes in Switzerland and in water bodies in northern Poland, certain disorders and changes in the macroscopic structure of whitefish *Coregonus* sp. gonads have been observed in the pre-spawning period. In males, the most frequently observed alteration was the division of testes into a larger cranial lobe and a smaller caudal one connected by the spermatic duct, although this has been described as a normality for the species. However, some evident anomalies in the morphological structure of ovaries and testes have been noticed, such as adherence or adhesion of lobes to the peritoneal wall and lateral muscles, asymmetry in the size of lobes, their atrophy or deep narrowing of both gonads. There have also been noted cases of hermaphroditism among both fish fry and adult coregonids. The reasons why such anomalies appear remain unclear, although it has been documented that the formation and deformities of fish gonads can be strongly affected by environmental factors, chemical substances which produce crypto-endocrine effect, pathogens and parasites. The aforementioned changes may have also been an effect of interspecies hybridization, both natural and artificial, or a result of irresponsible actions undertaken by person involve in fishery practice.

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PRAWIDŁOWOŚCI I ANOMALIE W STRUKTURZE GONAD RYB SIEJOWATYCH

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Słowa kluczowe: ryby siejowate, jajniki, jądra, anomalia struktury gonad.

Abstrakt

Ryby siejowate cechują się posiadaniem charakterystycznych dla ryb kostnoszkieletowych anatomicznych planów budowy gonad, tj. pęcherzykowych jajników grupowo synchronicznych typu taśmowatego oraz acinozowych jąder typu zrazikowego. W kilku alpejskich jeziorach Szwajcarii oraz zbiornikach północnej Polski zanotowano zaburzenia i zmiany makroskopowej struktury gonad siei *Coregonus* sp. w okresie przedtarłowym. U samców najczęściej obserwowano separację jąder na większy płat kranialny i mniejszy kaudalny połączone nasieniowodem, co jednak uznano za prawidłowość dla tego gatunku. Obserwowano ponadto wyraźne anomalie struktury morfologicznej jajników i jąder, tj.: przyleganie bądź zrośnięcie płatów ze ścianą otrzewnej i mięśniami bocznymi, asymetrię wielkości płatów, ich atrofię czy wreszcie głębokie przewężenia obu gonad. Notowano również przypadki obecności osobników hermafrodytycznych wśród narybku, jak i u dorosłych siejowatych. Przyczyny tego typu nieprawidłowości pozostają niejasne, aczkolwiek udokumentowano, że na kształtowanie i deformacje gonad u ryb mogą istotnie wpływać czynniki środowiskowe, substancje chemiczne o działaniu kryptoendokrynowym, patogeny i pasożyty. Opisywane zmiany mogły być też wynikiem hybrydyzacji międzygatunkowej, tak naturalnej, jak i sztucznej będącej efektem nieodpowiedzialnych działań człowieka w warunkach hodowlanych.

The macro- and microscopic structure of gonads in teleost fishes

Gonads of teleost fishes, like those of all other vertebrates, appears as paired organs situated in the dorsal part of the body cavity near the mesentery. Although they always develop as paired organs, some adult individuals of certain species have single gonads (JOBLING 1995). Outermost, gonads are enveloped in a thin layer of fibrous connective tissue. In a transverse cross-section, they are typically oval or triangular structures. The left and the right gonad are usually clearly separated. However, sometimes they can be partly (e.g. in perch *Perca fluviatilis* L.) or completely (e.g. in guppy *Peocilia reticulata* Peters) joined (fusion) (BILLARD 1986). In some species, such as smelt *Osmerus eperlanus* (L.), testes and ovaries can be arranged in the body cavity asymmetrically, with distinct disproportion in the size of both parts of the gonads. Consequently, the lengths of oviducts or spermatic ducts running from either of the two gonads are likewise different (KOWALSKI et al. 2006). In sexually mature salmonid or coregonid fishes, oviducts undergo involution and during ovulation mature oocytes fall into the body cavity, from which they are released straight into water through a short, funnel-like duct and the genital aperture located just posterior to the anus (TAKASHIMA and HIBIYA 1995).

Regarding the histological structure of fish gonads, they are composed of two types of cells, i.e. germ cells from which mature gametes (eggs or spermatozoa) are formed, and somatic cells, which support, nourish and regulate the activity and development of germ cells. Apart from cells, gonads also contain acellular components, such as basement membranes (NAGAHAMA 1983). Ovaries of teleost fishes, in contrast to other vertebrates, are predominantly follicular structures, which contain a distinct cavity (ovocell), into which oocytes are released during ovulation. Ovarian lamellae containing developing oocytes typically stretch alongside this ovarian cavity (HOAR 1969). Functionally, the basic component of an ovary are ovarian follicles, whose arrangement is similar in all teleost fishes. Centrally located oocytes are enveloped in a cytoplasmatic membrane and yolk layer, known also as *zona radiata* or the chorion. This layer is surrounded by a follicular theca, innermost composed of one layer of granular cells and outermost – of a layer of secretion cells producing steroids, which are accompanied by fibroblasts, collagen fibers and blood vessels (TAKASHIMA and HIBIYA 1995).

In teleost fish males, two types of microscopic structure of testes are distinguished: tubular and lobular (BILLARD 1986). In tubular testes, characteristic for such fish as percids, canaliculi (tubes) create a regular systems of structures, in which spermatozoa are formed in the peripheral part of a testis. During the final stage of spermatogenesis, they are gradually directed to the central part of a gonad (GRIER et al. 1980). In turn, lobular testes, characteristic for cyprinids, salmonids and coregonids, are composed of numerous and irregular in shape ampules (lobules), separated by a thin layer of connective tissue (Figure 1 a, c). In this case, spermatozoa are formed in ampules and during spawning they move to a large canal in the central part of a testis. Histological sections of testicular ampules during the pre-spawning period in European whitefish (Figure 1b) and vendace (Figure 1d) reveal a high degree of similarity. The central part of the structure of gonad lobules is full-filled with mature spermatozoa, while their walls are rather thin at that time, with numerous and distinctly marked Sertoli cells. The primary function of Sertoli cells is to control the development and physiological functions of germ cells. Sertoli cells are a source of hormonal substances secreted to the lumen of seminal ampules. They also act as phagocytes consuming apoptotic or damaged spermatids occurring during spermatogenesis (SCHULZ et al. 2010).

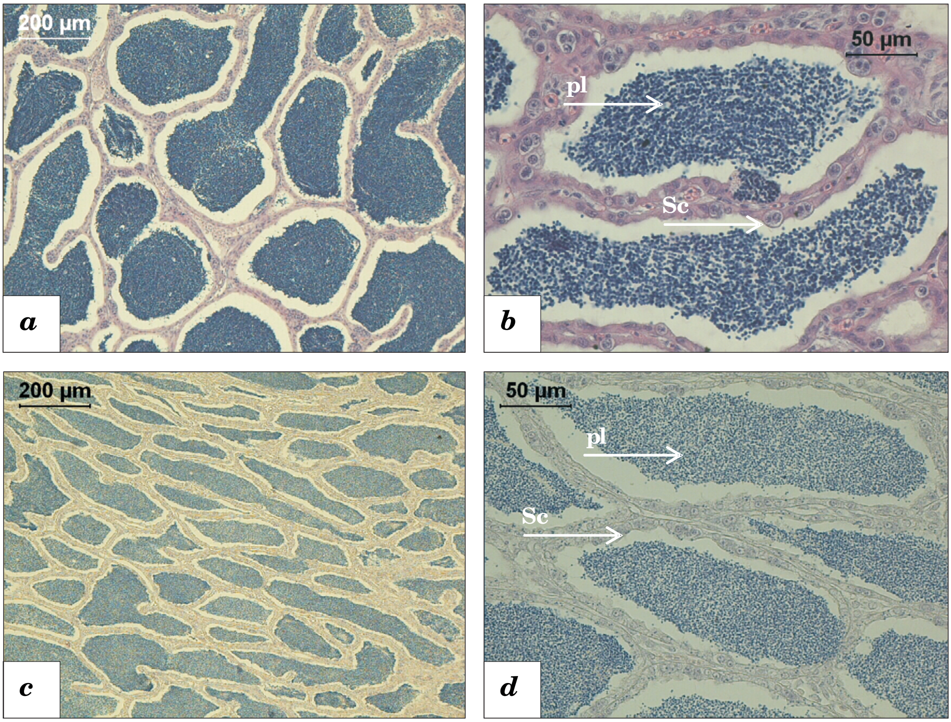

Fig. 1. Histological cross-section of testis: *a, b* – whitefish; *c, d* – vendace. Descriptions: pl – spermatozoa, Sc – Sertoli cells.

Formation of the anatomical and histological structure of gonads in coregonid fishes

According to TAKASHIMA et al. (1980), sex differentiation in gonochoristic fish follows a similar course as in reptiles. The two phases are distinguishable during the development of the reproduction system: determination, when the main role is played by sex chromosomes, and differentiation of gonads, when steroid hormones are released to regulate the proper development of male or female gonads. Under laboratory conditions, an analysis of the formation of anatomical structure of gonads and their cytological differentiation in European whitefish has been described by DLUGOSZ and DEMSKA-ZAKES (1992). The experiment realized based on material obtained during artificial reproduction of spawners captured in Isąg Lake (the Mazurian Lake District).

Sex differentiation in European whitefish, similarly to other Salmoniformes (HURK VAN DEN and SLOF 1981, SACOBIE and TILLMANN 2005) ongoing through the two stages, and the anatomical differentiation of gonads was preceded by cytological differentiation. At the same time, female gonads were always larger than male ones and were characterized by much higher intensity of karyokinetic divisions of their cells. The primordial germ cells (PGC) in gonadal anlagae of European whitefish larvae were observed from 47th day of post-embryonic development. In turn, ovaries with distinct protoplasmatic oocytes were visible in histological sections from 108th day after hatching, when the fish reached the total length from 4.2 to 4.8 cm and weighed between 780 and 1100 mg (Figure 2a). Sex differentiation towards becoming male fish was much slower as no typical testes with spermatogonial cells were observed until the termination of the experimental rearing, i.e. until 166th day after hatching (Figure 2b) (DLUGOSZ and DEMSKA-ZAKES 1992).

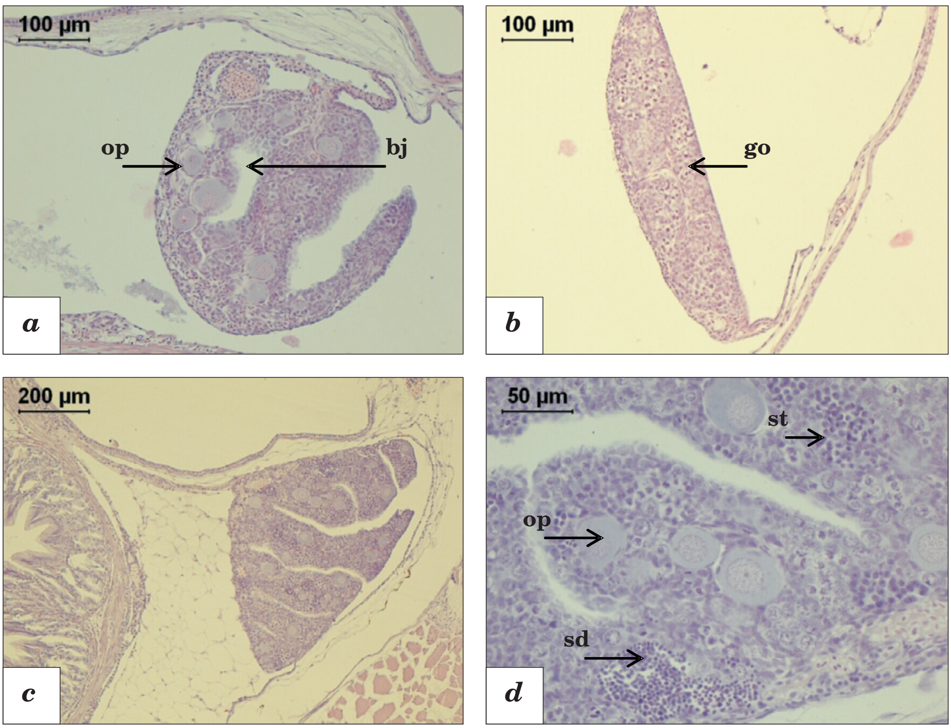

Fig. 2. Histological cross-section of coregonids gonads: *a* – an ovary whitefish on 133th day of post-embryonic development; *b* – a testis of whitefish on 166th day after hatching; *c, d* - a gonad of a hermaphroditic individual (a hybrid of whitefish and peled), containing female and male germ cells. Descriptions: bj – ovarian lamellae, go – gonocytes, op – previtellogenic oocytes, sd – spermatids, st – spermatocytes

A very similar course of sex differentiation has been observed in peled *Coregonus peled* Gmel., in which gonadal anlagae transform directly into male or female gonads, which means that they are differentiated gonochoristic species. In an experiment concerning analysis of potential influence of varied rearing temperature (10, 17 and 21°C) on formation of gonads in this species, first symptoms of anatomical differentiation of gonads were noticed on 81st day of post-embryonic development in all experimental groups. At the same time, presence of first cells of the female sex line, i.e. oogonia, was confirmed in fish reared in water of the temperature close to optimal and sublethal. It was also found out that a rise in the temperature, by making the growth rate of fish more dynamic, affected directly the time and rate of sex differentiation of peled (KRÓL et al. 2003).

Anomalies in the morphological structure of gonads

Irregularities in the development of the reproduction system may occur as early as the stage of gonad formation. Anomalies during the anatomical sex differentiation have been found, for example, in hybrids of European whitefish and peled reared in cage culture (DEMSKA-ZAKES and MAMCARZ 1996). These alterations most often included occurrence of both male and female gonads, i.e. testes and ovaries, in the same individual. There were also cases when male germ cells were found next to previtellogenic oocytes in typical ovaries (Figure 2 c, d).

Our analysis of the quality of semen and the work undertaken towards improvement of its cryopreservation based on anadromous whitefish spawners from Łebsko Lake (CIERESZKO et al. 2008) demonstrated the presence of two separate, morphologically clearly distinct testicular lobes in males of this species. These consisted of a larger (cranial) and smaller (caudal) part, the latter located close to the anus (Figure 3a). Additionally, through the application of computer-assisted sperm analysis (CASA), we compared parameters of semen obtained from two fragments of testes in fish captured in two water bodies, located far from each other, that is from Łebsko Lake on the Baltic Sea coast and Gaładuś Lake, lying near the Polish--Lithuanian border. The histological sections of both fragments of testes was likewise examined. No significant differences in the histological structure or quality of semen sampled from the cranial and caudal parts of testes were found (HLIWA et al. 2008, 2010).

Similar results obtained from macroscopic observations of gonads and anomalies in their morphological structure in three ecotypes of *Coregonus* sp. during the pre-spawning period were reported by BERNET et al. (2004). In oligotrophic alpine Lake Thun (Switzerland), characteristic "separation of lobes in gonads", repeating in many examined individuals, was observed and regarded as a normal event in whitefish. The macroscopic and microscopic analysis of gonads demonstrated that 35%, i.e. 281 out of 808 examined fish,

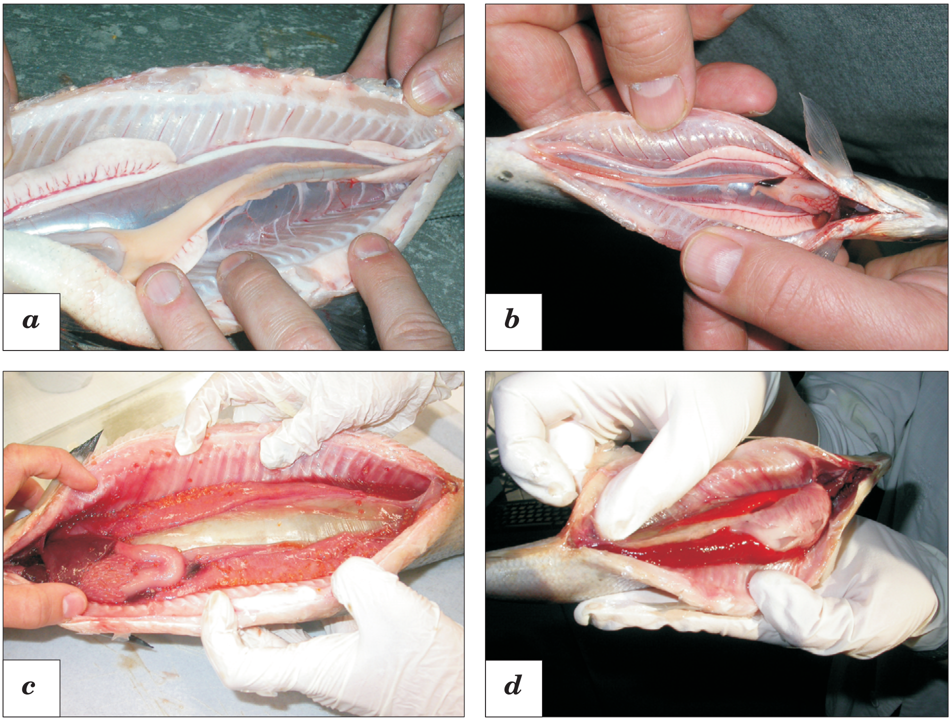

Fig. 3. The gonads of coregonids: *a* – a macroscopic image of whitefish testes during the pre-spawning period; visible is the division of gonads into the cranial (larger) and caudal (smaller) lobe; *b* – a macroscopic image of vendace testes; *c* – a macroscopic image of whitefish ovaries during the post-spawning period, visible are single oocytes remaining in the body cavity of a female; *d* – a macroscopic image of vendace ovaries during the pre-spawning period

experienced a variety of pathological changes. In general, anomalies appeared more frequently in males (40% of captured individuals) than in females (26%), and predominantly in the age groups 3+ to 5+. The morphological changes in gonads included adherence or adhesion of lobes to the peritoneal wall and lateral muscles, distinct asymmetry in size of both gonads, atrophy, segregation of lobes into subunits or their deep narrowing. Moreover, the histological analysis revealed a small percentage (1.1%) of hermaphroditic individuals, in which testicular and ovarian tissues were present, lying sequentially next to one another in gonads, or such individuals, in which one strip consisting of ovarian tissue and another one being testicular tissue were found, or finally a mosaic type of gonads, in which oocytes were set in gonads which morphologically resembled a typical testis or else spermatozoa were present in ovaries.

The morphological asymmetry of female gonads during the pre-spawning period was observed by HEESE (1990) in whitefish *Coregonus lavaretus*

(L. 1758) from the Gulf of Pomerania and the Szczecin Lagoon. In this case, the left lobe of a gonad in females was typically 15-20% shorter than the right one. However, in females of whitefish captured in the coastal area of the Łeba River or in females of the vendace *Coregonus albula* (L.), related to whitefish, and caught in Gaładuś and Hańcza Lakes in the Suwałki Lake District, no such anatomical changes in ovaries and testes were found (Figure 3 b, c, d) (HLIWA et al., unpublished).

Causes of macro- and microscopic changes in the structure of coregonids gonads

Until now, changes in the morphological structure of gonads have frequently been reported for salmonid fishes (FITZSIMONS and CAIRNS 2000, KINNISON et al. 2000, DZIEWULSKA and DOMAGAŁA 2009) and for coregonids, phylogenetically related to the former family (URBACH et al. 2008, BITTNER et al., 2009, HLIWA et al. 2010). The causes of this type of observed anomalies remain unclear, although it has been documented that the formation and deformities of fish gonads as well as the sex structure of fish natural populations can be affected by a variety of environmental factors, mainly the temperature (TESSEMA et al., 2006, BAROILLER et al. 2009), chemical substances which produce crypto-endocrine or toxic effects (JOBLING et al. 1998, NOAKSSON et al. 2001) or even parasites (WICKLUND and BYLUND 1994).

One of the most frequent genetically based cause, which may considerably determine the development and the final macroscopic structure as well as functions performed by fish gonads is interspecies hybridization (CHEVASSUS 1983). Similar dates and places of spawning as well as the spawning behavior itself favour natural and uncontrollable hybridization of coregonid fishes. Such hybridization, however, is frequently a result of thoughtless and irresponsible actions of people in aquaculture. Back in the 1980s, in eutrophic Legińskie Lake, 15.2% of hybrids of indigenous whitefish *Coregonus lavaretus* L. and introduced peled *Coregonus peled* Gmel. revealed aplasia or atrophy of one of the gonads (DEMSKA-ZAKES and MAMCARZ 1996). Moreover, hermaphroditic specimens possessing one female and one male gonad were found, and the histological assays confirmed that in gonads of fish aged 3+ there were both previtellogenic and vitellogenic (during the vacuolization stage) oocytes as well as male spermatogonial cells. In turn, BOGDANOVA (2002) described such changes in *C. peled* × *C. nasus* hybrids as degeneration of maturing oocytes or/and disrupted differentiation of ovaries.

All things considered, it cannot be excluded that a specific division into two asymmetric lobes, observed in whitefish specimens from Polish (HLIWA et al.

2010) or Swiss lakes (BERNET et al. 2004, URBACH et al. 2008) is a side-effect of long-lasting hatchery practice, including such techniques as using a limited number of spawners for artificial reproduction or selecting spawners according to phenotypes, thus diminishing genetic diversity of breeding populations (WEDEKIND 2002). Negative effects can also appear due to maintaining spawners in water recirculatory systems or using inadequate artificial feeds, which leads to certain 'domestication' of this fish species. The latter hypothesis is noteworthy because vendace living in the wild, which is not subjected to breeding practice, has not been observed to undergo segregation of testes or other atypical changes in the macroscopic image of gonads.

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