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Fish biology

SEX DIFFERENTIATION IN WHITEFISH (*COREGONUS LAVARETUS* L.)

ZRÓŻNICOWANIE PŁCI U SIEI (*COREGONUS LAVARETUS* L.)

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Studies were carried out on gonad differentiation in whitefish (*Coregonus lavaretus* L.) reared in aquaria in conditions resembling the natural ones and fed ad libitum with alive zooplankton.

INTRODUCTION

Takashima et al (1980) (Takashima F., R. Patino, M. Nakamura, 1980); stated that sex differentiation in gonochoristic fishes is similar to sex differentiation in reptiles. Two phases are distinguished in course of sexual development: determination, when sex chromosomes of the joining gametes play the main role, and gonad differentiation, when steroid hormones are secreted which regulate the development to form either males or females. Persov (1972, 1975) distinguished the following stages of phenotipic sex determination in salmonids and sturgeons: 1) pregonial stage, in which the first sex cells are formed, migrate, and create gonad nuclei, 2) gonad formation, 3) sex differentiation-anatomic and cytological. The latter stage constitutes the subject of detail studies of biochemical, cytogenetic, endocrynologic and morphollgic character.

D'Ancona (1956), Yamammoto (1969), Persov (1975), and Hamaguchi (982) distinguished two types of sex differentiation. The first, i. e. direct sex differentiation, consists of an appearence of female or male sex cells in early,

undifferentiated gonads. The second is an indirect differentiation, in which female differentiation is initially observed in all specimens, while the secondary testis differentiation takes place only afterwards (Bruslé 1982).

Sex differentiation is observed in various stages of the ontogenesis, depending on the fish species. In some fishes it occurs already during embryonal life or immediately after hatching. In other, it is observed during larval stage (*Poecilla* – Takahashi 1974, *Oryzias* – Stach 1974) or later (*Tilapia* – Nakamura and Takahashi 1973, *Carassius* – Takahashi and Takano 1971). Sex differentiation in carp takes place in the first months of life (Natali and Natali 1947, Davies and Takashima 1980), and in lamprey and eel even later (Kuhlman 1975). Usually, sex differentiation is stepwise, with anatomic gonad differentition preceeding the cytological one. In some fish however, a reverse process is observed.

A number of observation on possible anatomic differentiation of fish gonads has been described. Persov (1975) observed differences in blood vessel system female and male gonads. In carp (Natali and Natali 1947) and tench (Długosz et al. 1983) gonad shape differs in females and males. Anatomic male differentiation may be suggested by intensive development of connective tissue in the gonad (Satoh 1974, Harrington 1975, Nakamura and Takahashi 1973, Takashima and Patino 1980, Shelton 1982) as well as by more intensive development of blood vessels and of lymphatic elements (Takahashi and Takano 1972). Robertson (1953) and Ashby (1957) stated that intensive development of the anterior gonad part may suggest female differentiation. Robertson (1953), Ashby (1957) and Lebrun (1977) noted tahat female gonads were more voluminous due to intensive karyokinetic divisions.

It is known that the environmental factores are highly significant for gonad morphogenesis. The most important are: temperature (Lebrun 1977), salinity (Roblin and Bruslé 1983), food (Persov 1972), density (Harrington 1975).

Lebrun (1977) stated that each temperature increase resulted in earlier sex differentiation. Kuhlman (1975) noted that optimal temperature for ovary development in eel amounted to 26°C, while testis differentiation required higher temperatures (D'Ancona 1957).

Cases of juvenile intersexuality in rainbow trout were connected with low temperatures, whereas male development with hogh temperatures (Ashby 1964). On the other hand, Padoa (1939) observed intersexuality in trout reared in higher temperatures. Intersexuality was also observed in whitefish. Długosz et al. (1987) noted this phenomenon in whitefish at constant temperature of 16°C and fed with dry feeds. Hence, knowledge of the time and course of gonad differentiation in fish, as also of the environmental conditions affecting this process, may be important for fishery management. Proper fish feeding, selection of temperature, or treatment with seroid hormones can cause sex inversion. Obviously, conscious control of the gonadogenesis cannot be

performed without extensive knowledge of this process in nature. Hence, the objective of this work was to follow up gonad differentiation in the whitefish reared in aquaria, in conditions similar to the natural ones.

MATERIALS AND METHODS

The experiment lasted for 166 days. The materials consisted of about 1000 whitefish larvae, reared in three aquaria (50 l each). The aquaria were supplied with filtered lake water. Water temperature increased gradually from 7°C in February to 19.5°C in June 1986 (Fig. 1).

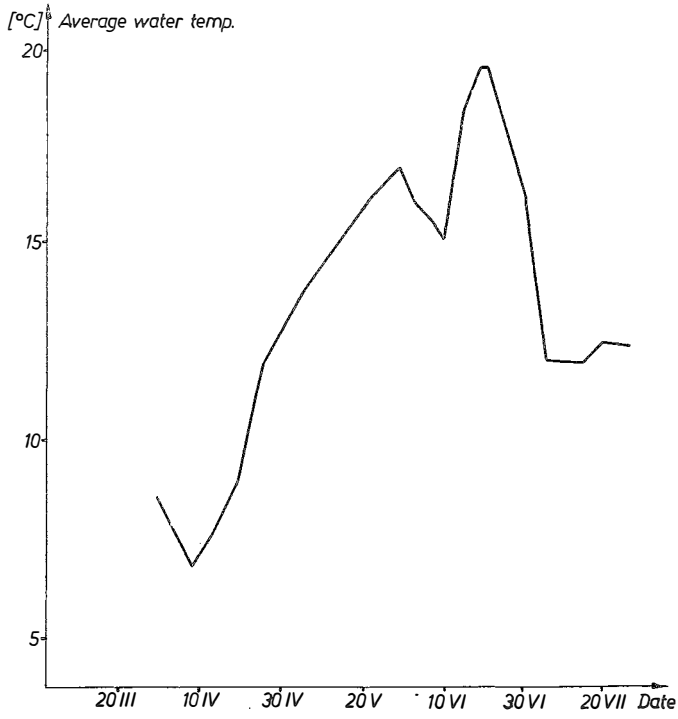

Fig. 1. Twenty four hours temperature average course of water during the whitefish breeding

Fish were fed with live zooplankton ad libitum. Feeding commenced on 13 February (three days after hatching).

Histological observations of whitefish gonads were carried out since 40 till 166 days after hatching. Totally, 87 fishes were studied. They were preserved in Bovin's liquid. Preserved fishes were transferred to alcohol of various

concentration, and then immersed in paraffin. Series of histological scraps, 6 - 10 μm thick, were stained with Meyer's hematoxylin with eosine. Ranges of fish lenght (L_t) and weight (W) in particular days of the experiment are given in Table 1.

Table 1

Description of the materials on every day of the experiment, with attention given to sample size (N), total fish length (l_t) and weigth (W)

Date	Weeks of life	Average water	Sample size (N)	Range of fish lenght (l_t) cm	Range of body weigth (W) mg
25.III - 31.III	7	8.6	3	1.9 - 2.3	40 - 76
1.IV - 7.IV	8	6.7	8	1.9 - 2.4	42 - 136
8.IV - 14.IV	9	7.7	4	2.3 - 2.6	97 - 133
15.IV - 21.IV	10	9.2	4	2.5 - 3.4	129 - 228
22.IV - 28.IV	11	12.0	4	2.8 - 3.4	167 - 314
29.IV - 5.V	12	13.2	8	2.3 - 4.2	84 - 550
6.V - 12.V	13	14.4	4	2.9 - 3.5	260 - 500
13.V - 19.V	14	15.6	8	2.9 - 4.1	260 - 460
20.V - 26.V	15	16.4	8	3.8 - 4.5	400 - 900
27.V - 2.VI	16	16.5	6	4.2 - 6.0	780 - 2000
3.VI - 9.VI	17	15.4	5	4.1 - 5.7	800 - 1500
10.VI - 16.VI	18	18.4	2	5.1 - 5.8	1000 - 1700
17.VI - 23.VI	19	19.5	10	4.6 - 6.0	800 - 1800
24.VI - 30.VI	20	17.1	4	5.2 - 6.3	1150 - 2500
1.VII - 7.VII	21	12.0	7	6.3 - 6.6	2500 - 3000
8.VII - 14.VII	22	12.0	7	5.3 - 6.7	1800 - 3200
15.VII - 21.VII	23	12.5	7	5.2 - 6.9	1800 - 3400
22.VII - 28.VII	24	12.5	7	4.7 - 7.0	1200 - 3600

RESULTS

From the 40th till the 166th day, after hatching the fish length (l_t) ranged from 1.5 to 7.0 cm, weight (W) from 15 to 3600 mg (Tab. 1).

On 47th day of life the first, primordial sex cells were observed in the gonad nuclei. The fishes were then 1.9 - 2.3 cm long and weighed 40 - 76 mg (Fig. 2). Primary sex cells were concentrated mostly in the middle, posterior-wise part of the gonad. On 103rd day, intensive karyokinetic divisions were noted in a specimen 4.0 cm long, weighing 600 mg. Two gonad parts were easily distinguishable; a somatic one, located at the side of body cavity, and a generative one, observed at the opposite side (Fig. 3).

On 108th day after hatching, oocytes in the diplont stage of the meiotic prophase, and single follicular cells were noted in a whitefish 4.8 cm long, weighing 1100 mg (Fig. 4). At the same time the primary groove appeared, which later on results in the ovary lobes (Fig. 5). Since 136th day, oocytes of

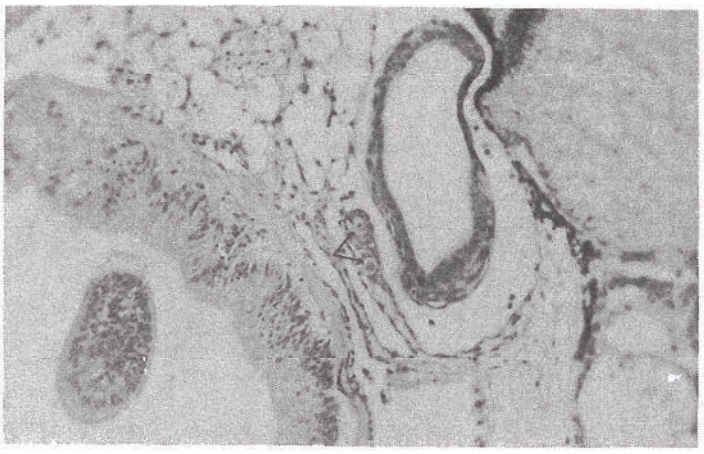

Fig. 2. The 47th day after hatching (1986.03.28). Visible gonad blastema with two primary sexual cells; l_t – 2.3 cm, W – 76 mg (enl. 200x).

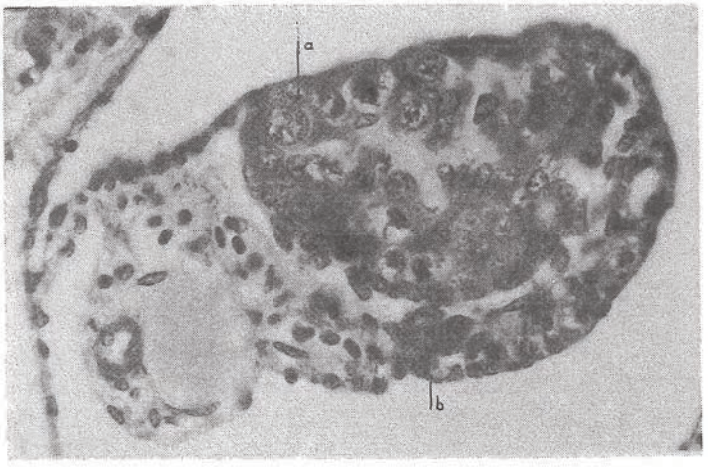

Fig. 3. The 103rd day of experiment (1986.05.23) l_t – 4.0 cm, W – 600 mg. Visible two parts of gonad (generative –a and somatic one –b) and numerous meiotic divisions (enl. 800x).

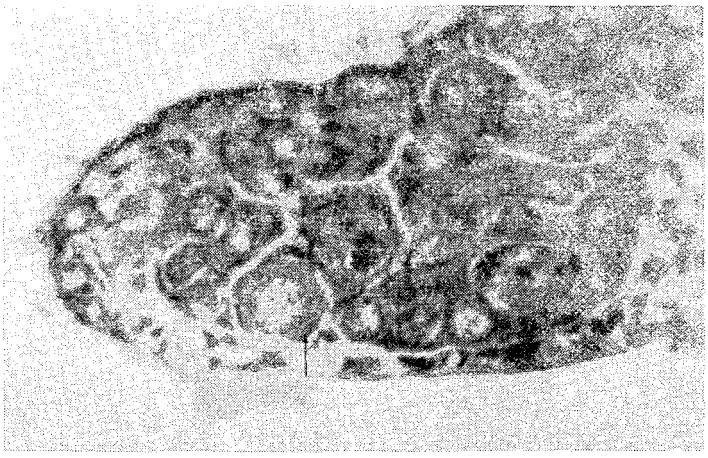

Fig. 4. The 108th day after hatching (186.05.28). Visible oocyte at meiotic prophase of diplont stage and singular follicular cells surrounding an oocyte (enl. 800x)

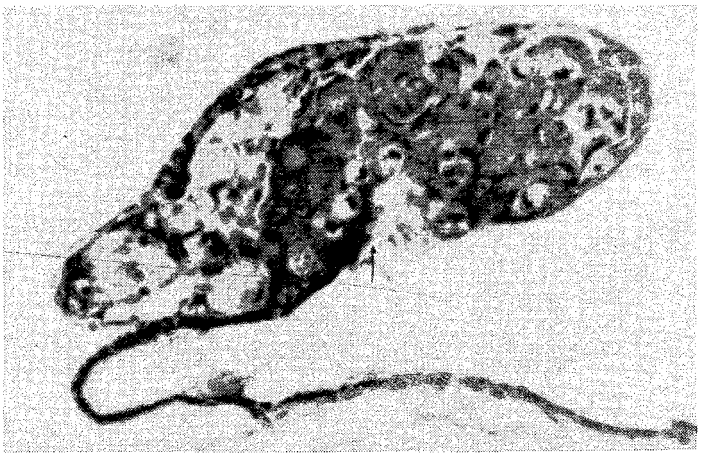

Fig. 5. The whitefish gonad age 108 days (l_t 4.8 cm, W — 1100 mg). Visible meiotic divisions and the primary groove profile (enl. 500x).

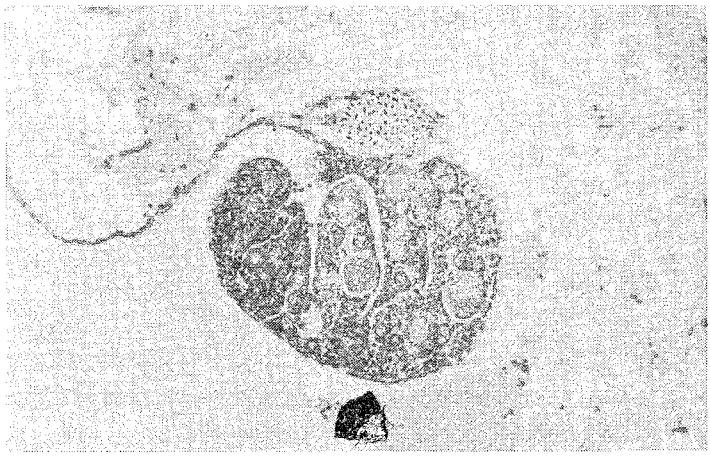

Fig. 6. A picture of 113 days old whitefish gonad (l_t 5.2 cm, W – 1150 mg). ovary lobes are being formed (enl. 200x)

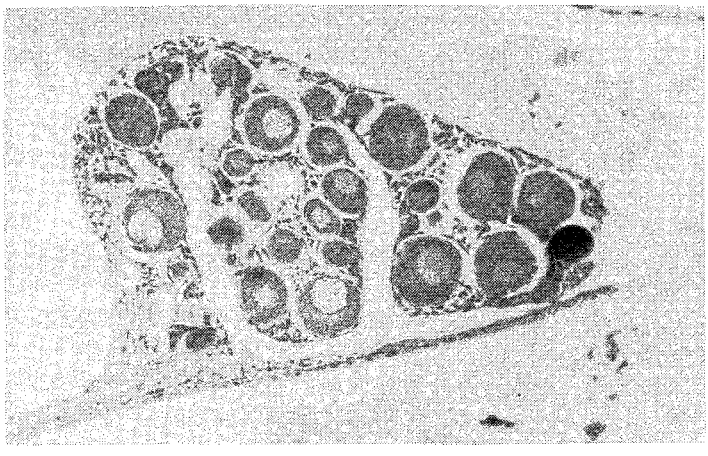

Fig. 7. A whitefish gonad at the 166th day after hatching (l_t 6.9 cm, W – 3200 mg (enl. 200x).

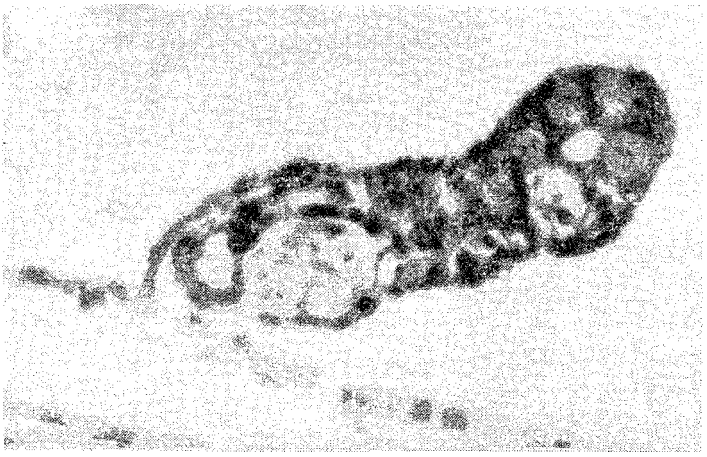

Fig. 8. The 113th day of experiment (l_t 4.9, W – 1600 mg). Visible gonad blastema with primary sexual cells (enl. 800x)

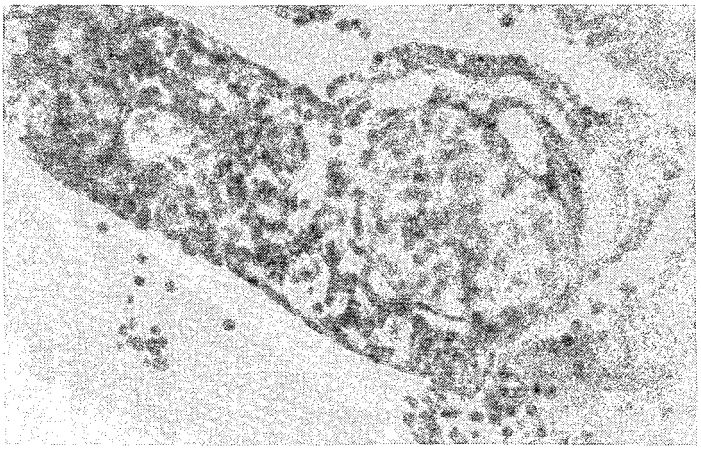

Fig. 9. Picture of the whitefish gonad at age 166 days (L_t 7.0 cm, W 3600 mg (enl. 500x).

protoplasmatic growth were observed (Fig. 6, 7). On the other hand, only gonad nulei with 1-2 primary sex cells were observed until 166th day in a number of specimens (Fig. 8, 9). No intensive mitotic divisions were observed in these gonads.

DISCUSSION

Appearance of primary sex cells and their migration to the forming gonads nuclei is observed in different periods of ontogenesis in particular fish species. Place of their concentration in a gonad also differs. In carp, primary cells were observed in head-wise part (Davies and Takashima 1980), in perch in a middle part (Zelenkov 1981), and in rainbow trout in an anterior and middle part (Takashima and Patino 1980). Primary sex cells in whitefish concentrated in a middle – posterior wise gonad nucles (Fig. 2). The same was observed in salmon (Persov 1975).

Some authors are of the opinion that concentration of primary sex cells in head or middle-posterior part of the gonad may reflect further sex determination. According to Lebrun (1977), gonocyte concentration in an anterior part in *Salmo gairdneri* usually develops into an ovary, whereas dispersion of the cells over the whole length of gonad nucleus results in male gonad.

No such phenomena were observed in our experiments, although anatomic differentiation always preceeded cytological one. More karyokinetic divisions and, thus, higher gonad volume were observed in future ovaries (Fig. 5, 8). The same was observed for other species by Robertson (1953), Ashby (1957) and Leburn (1977).

It should be underlined that in whitefish females cytologic differentiation took place just after anatomic, while oocyte growth and development occurred simultaneously with formation and growth of the ovary (Fig. 5, 6). No cytological sex differentiation was noted in whitefish males in course of the experiment. Thus, period between anatomic and cytological differentation was longer in whitefish males than in females.

Statova and Tomnatik (1970) stated that gonad formation in peled males may last until end of the first year of life. The same was observed by Persov (1962, 1965) in Salmon, and by Ashby (1957) and Dimćeva-Grozdanova(1968) in rainbow trout. In our experiment all whitefish females possessed ovaries with oocytes in the protoplasmatic growth since the 108th after hatching, although the fish length ranged from 4.2 to 4.8 cm, and weight from 780 to 1100 mg.

In view of this, it may be assumed that sex differentiation was determined by age as well as size. Data from the available literature suggest that the sexualization moment in particular fish species depends on a defined body size (Kuhlman 1975, Epler and Bieniarz 1981). However, many authors (Ashby 1957, Takahashi and Takano 1971, Davies and Takashima 1980) are of the opinion that this period depends on fish age. Our studies suggest that in case of whitefish, these contradicting opinions should be verified taking into account strict correlation between fish age and size (May 1967, McKay and Mann 1969, Abel 1973, Terlecki 1973).

Two gonad parts may be distinguished when the oocytes enter a final stage of the meiotic prophase: the generative and the somatic one (Fig. 3). Single follicular cells also appear at this time; these from a compact envelope along with oocyte growth (Fig. 4).

In some specimens only gonad nuclei with primary sex cells were observed until 166th day of the experiment (Fig. 8, 9). No intensive karyokinetic divisions, characteristic of female differentiation, were observed in the these specimence. Hence, it may be assumed that they will develop into males. In view of this, it can also be stated that female and male sex developed separately until 166th day of the experiment (Fig. 7, 9).

CONCLUSIONS

1) In whitefish, the sex cell concentrated mainly in middle-posterior wise part of the gonad.

2) Male and female sex developed separately. Female gonads were always bigger than male gonads, and were characterized by more intensive karyokinetic divisions.

3) Sex differentiation in whitefish, as in other salmonids, took place in two stages, anatomic differentation preeceding cytologic one.

4) Two parts were distinguishable in whitefish ovary: generative and somatic one.

5) Apperance of a primary groove in the gonad, which later on results in ovary lobe formation, was observed when the oocytes entered final stage of the meiotic prophase and when single follicular cells appeared around the oocyte.

6) All whitefish females possessed ovaries with oocytes in the phase of protoplasmatic growth since 108th day of the experiment. L_t range from 4.2 to 4.8 cm and weight from 780 to 1100 mg.

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ZRÓŻNICOWANIE PŁCI U SIEI (*COREGONUS LAVARETUS L.*)

STRESZCZENIE

Badano przebieg różnicowania się gonad siei (*Coregonus lavaretus L.*) hodowanej w warunkach akwaryjnych zbliżonych do naturalnych. Przy użyciu mikroskopu świetlnego prowadzono obserwacje histologiczne gonad larw i młodocianych osobników siei. Pierwsze pierwotne komórki płciowe („pkp") obserwowano w 47dniu po wylęgu, przy długości osobników 1,9-2,3 cm i masie 40-151 mg. Samice dostrzeżono w wieku 103 dni. Do 166 dnia prowadzenia eksperymentu, u części osobników widziano tylko zawiązki gonad z pojedynczymi „pkp". Mniejsza objętość tych gonad oraz brak intensywnych podziałów kariokinetycznych sugerować może o ich ukierunkowaniu męskim.

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