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THE INFLUENCE OF TEMPERATURE ON THE SEX DIFFERENTIATION PROCESS IN PELED *COREGONUS PELED* (GMEL.)

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ABSTRACT. The objective of this study was to examine the influence of water temperature (10, 17 and 21°C) on the sex differentiation process in peled. A high temperature, even a sub-lethal one, was not observed to have an influence on the sex ratio in this species; from day 81 after hatching until the end of the experiment the sex ratio in all three experimental groups was 1:1. It was noted, however, that thermal conditions determine the rate of sex differentiation and, in particular, of cytological differentiation. The first female-line cells, oogonia, were observed on day 102 after hatching in the peled kept in water at a temperature of 10°C. Cytological differentiation was observed in the fish that were reared in higher temperatures as soon as day 81 after hatching. The first symptoms of anatomical differentiation were noted at this time in fish from all experimental groups. Two types of gonads were observed in histological cross-sections; one had a thicker anterior part with the generative and somatic parts located separately (ovaries), and the other had small, spindle-like gonads (future testes). No cytological differentiation of the male sex cells was observed during the experiment.

Key words: PELED, (*COREGONUS PELED*), SEX DIFFERENTIATION, ENVIRONMENTAL CONDITIONS, TEMPERATURE

INTRODUCTION

The sex of the majority of the gonochoristic fish seems to be genetically programmed at the moment of fertilization, as it is in the higher vertebrates. In the case of the teleosts, there is no universal model of genetic sex determination (GSD), because at least nine types of chromosomal sex determination systems are known (Tave 1989). Morphologically differentiated sex chromosomes were located in only 30 out of about 1,000 cytogenetically analyzed species. It is worth emphasizing that either males (XX, XY) or females (ZZ, ZW) can be the heterogamous sex; the former being the case with some salmonids (Thorgaard 1983) and the latter with *Tilapia aurea* Steind. (Tave 1989). On the other hand, however, either male or female platyfish *Xiphophorus maculatus* Gűnth. can be the heterogamous sex, a fact that undermines the view that only genes placed on the sex chromosomes are responsible for genetic sex determination (Kallman 1984). A few theories of typical models of genetic sex determination were proposed on the basis of such observations, namely systems based on more than two

chromosomes, on sex determining polygeny and on the cooperation of autosomal genes (Reinboth 1983).

The influence of environmental factors on both sex determination and the process of sex differentiation was confirmed in fish. A lack of sufficient information concerning the mechanisms of these two processes poses additional problems in discerning between them, particularly when the environmental factor functions as a sex determinant (ESD) (Strüssman and Patiño 1999). The most important biotic and abiotic factors are food (Persov 1972), salinity (Roblin and Bruslé 1983), pH (Romer and Beisenherz 1996) and temperature (Conover and Kynard 1981, Baroiller et al. 1996). Initially, the term temperature-dependent sex determination (TDS) was applied to the phenomena as it occurs in reptiles and amphibians (Bull 1980). These processes were widely described for crocodiles, turtles and a few species of frog (Pieau 1996). Later this terminology was also applied to describe the phenomena which occurs in the process of sex determination in fish, although the mechanism itself seems to be different in the two cases (Strüssman and Patiño 1999).

The sexual thermolability of the teleosts has always concerned juvenile stages in which gonadal differentiation has yet to occur (Strüssman et al. 1996, Patiño et al. 1996). Studies on this subject were concerned with species characterized by undifferentiated gonochorism, i.e. those in which ovaries and testes develop from the germ of an ovary-like gonad (Davies and Takashima 1980). In *Oncorhynchus nerka* Walb. (indirect sex differentiation) the application of a high, sub-lethal temperature influenced the domination of the female (Craig et al. 1996). Sex differentiation in peled *Coregonus peled* (Gmel.) is called differentiated gonochorism. This is based on transforming an undifferentiated gonad into a female or a male gonad (Statova and Tomnatik 1970). The difference in sex formation and the fact that, to date, no sex chromosomes have been identified in the peled karyotype (2n = 76 chromosomes) (Jankun et al. 2001) shaped the objectives of this study.

The experiment presented in this paper was designed to analyze the influence of the water environment temperature on sex differentiation and the sex ratio in peled.

MATERIAL AND METHODS

Peled eyed eggs were obtained from the Finnish Game and Fisheries Institute, Taivalkoshi Game and Fisheries Research Station in Finland. Once they had been transported to Olsztyn, the eggs were incubated in Weiss jars (at a water temperature

of 4°C). After hatching the larvae were reared in aquaria with a volume of 20 liters, a water flow of 1.5 l min^{-1} and a temperature of 10°C. The fish were fed *ad libitum* with *Artemia* sp. larvae and commercial artificial feed. The experiment began on day 39 after hatching and continued for two months. The fish were randomly divided into three experimental groups - group I was reared at a water temperature of 10°C, group II at 17°C and group III at 21°C. The fish were acclimatized to the higher temperatures for a period of several hours.

Samples for histological studies were taken four times following hatching on days 39 (12 May 1999 - 42 specimens), 60 (2 June 1999) and 81 (23 June 1999 – 14-18 specimens on each day) and 102 (14 July 1999 - 30 specimens from each temperature variant). After the fish had been sacrificed, they were measured (*longitudo totalis*) to the nearest ± 0.5 mm and weighed to the nearest ± 0.5 mg. The samples were preserved in Bouin's solution, rinsed with a 70% ethyl alcohol solution, dehydrated with the application of gradually increasing concentrations of ethyl alcohol solutions, kept in xylene and submerged in paraffin blocks. Histological sections were obtained by cutting the blocks with a Leica rotational microtome RM 2155 into 5 µm thick sections. The sections were then stained using HE (haematoxylin-eosin) (Zawistowski 1986) and analyzed under a light microscope. Growth data were statistically analyzed using one factor ANOVA correlation analysis.

RESULTS AND DISCUSSION

The average weight of the fish in the sample taken on day 39 after hatching was 27.5 mg and the average total length was 18.1 mm (Table 1). Undifferentiated gonads were observed in all the analyzed specimens (Photo 1). Apart from small somatic cells, the gonads contained primordial germ cells (15% of the sample specimens), primordial germ cells and gonocytes (55%) or only gonocytes (30%). Primordial germ cells (PGCs) were located in the middle of the gonad and were characterized by a large, distinctly visible dark nucleus which was surrounded by a narrow strip of clear cytoplasm. The gonocytes were considerably smaller than the PGCs, and the line between the nucleus and the cytoplasm was less distinct.

On day 60 after hatching (2 June 1999), the average weight of the fish from group I was 119.5 mg and the average total length was 25.5 mm (Table 1). The gonads of all the specimens were characterized by a greater volume than previously, although no significant differences in their anatomy were noted. The gonads of about 79% of the

TABLE 1

Fish body weight, length and sex ratio troughout the experiment.
Values with the same letter index in rows do not significantly differ (P > 0.05)

	Experimental groups (temperature of water)					
	10°C		17°C		21°C	
	12 May 1999 – 39th day after hatching					
Body weight (mg)			27.8 ± 10.8			
Total body length (mm)			18.1 ± 1.89			
	02 June 1999 – 60th day after hatching					
Body weight (mg)	119.3 ± 26.28[a]		153.4 ± 59.15[b]		147.4 ± 57.83[b]	
Total body length (mm)	25.5 ± 2.50[a]		29.1 ± 3.57[a]		28.2 ± 3.38[a]	
	23 June 1999 – 81st day after hatching					
Body weight (mg)	246.8 ± 98.67[a]		600.0 ± 184.76[b]		514.0 ± 220.34[b]	
Total body length (mm)	34.7 ± 3.86[a]		45.3 ± 4.46[b]		42.3 ± 8.26[b]	
Sex	♀	♂	♀	♂	♀	♂
Sex ratio	50	50	50	50	50	50
	14 July 1999 – 102nd day after hatching					
Body weight (mg)	807.0 ± 296.06[a]		1857.3 ± 684.07[b]		2532.0 ± 894.19[b]	
Total body length (mm)	49.5 ± 6.43[a]		62.7 ± 8.68[b]		65.0 ± 9.01[b]	
Sex	♀	♂	♀	♂	♀	♂
Sex ratio	50	50	50	50	50	50

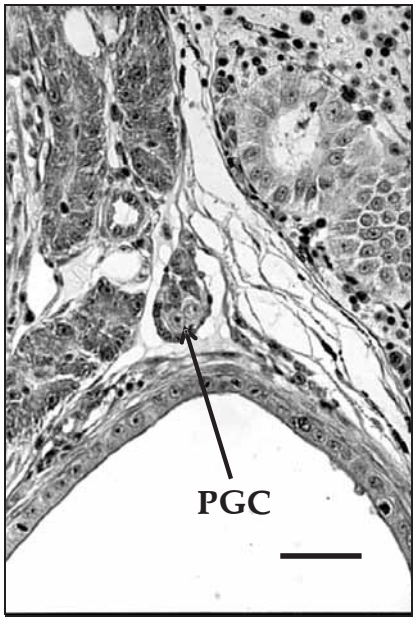

Photo 1. Undifferentiated peled gonad on day 39 after hatching. PGC – Primordial Germ Cells. Bar = 25μm.

specimens contained only gonocytes, in 14% primordial germ cells were also present in addition to gonocytes and 7% had only primordial germ cells. Although peled from groups II and III were, on average, 3 mm longer and weighed about 30 mg more than the fish reared at 10°C (Table 1), their gonads were similar and contained the same categories of germ cells.

Some changes in the gonad shape and structure were observed in the sample taken on day 81 after hatching (23 June 1999). One half of the peled from each experimental group had gonads with a thicker anterior part with the generative and somatic parts located separately, while the other half had small spindle-like gonads and their germ cells were dispersed

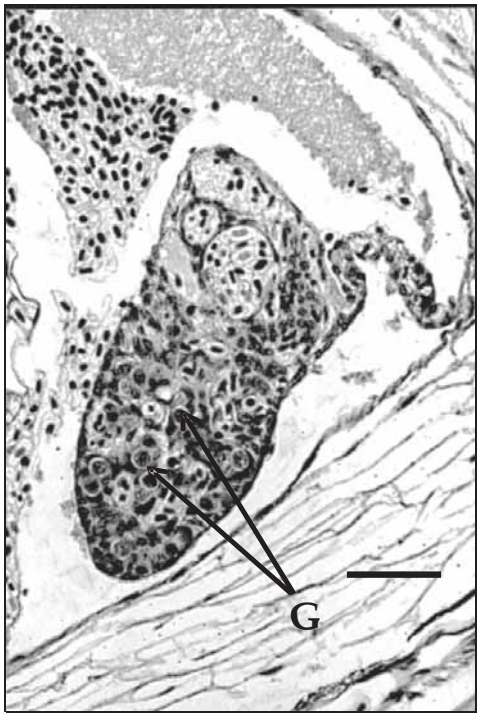

Photo 2. Female gonad of peled reared at 10°C on day 81 after hatching. G - Gonocytes. Bar = 25μm.

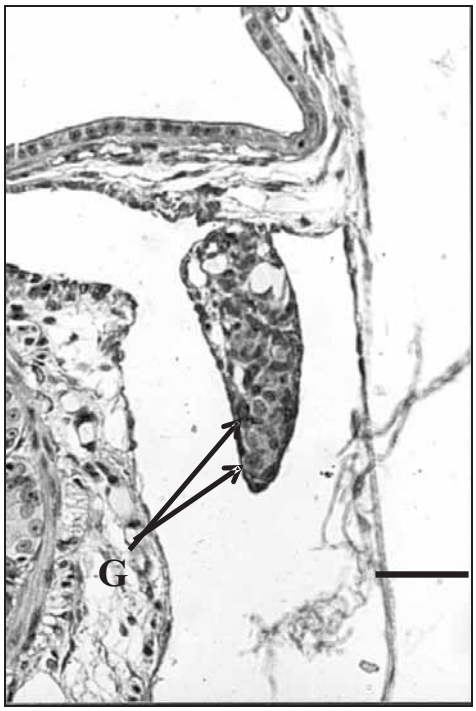

Photo 3. Male gonad of peled reared at 10°C on day 81 after hatching. G - Gonocytes. Bar 1 = 25μm.

throughout the volume. No germ cell differentiation was observed in the future ovaries (Photo 2) or testes (Photo 3) of the fish reared at 10°C; they all contained only gonocytes. This category of cells was also observed in male gonads of fish from group II and III. The first stages of cytological differentiation were observed in females kept at higher temperatures; the ovaries of about 30% of the peled contained oogonia and individual oocytes in the prophase of the first meiotic division (Photo 4).

The last sample was taken on day 102 after hatching (14 July 1999). The average weight of the fish reared at the lowest temperature was 807 mg and the average total length was 49.5 mm. The fish from groups II and III grew faster (Table 1). For the first time differentiated germ cells, oogonia, were observed (5% of specimens) in the ovaries of the fish from group I. The decided majority of female gonads (95%) contained only gonocytes, as did the testes. No cytological changes were observed in the gonads of the fish kept at 17°C in comparison to the previous sample. Only gonocytes were observed in the testes of all the males and in 62% of the females reared at the highest water tem-

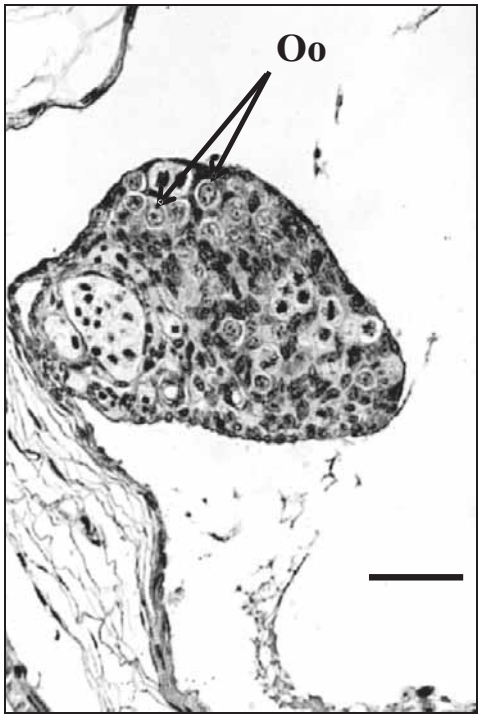

Photo 4. Female gonad of peled reared at 21°C on day 81 after hatching. Oo - Oogonia. Bar = 25μm.

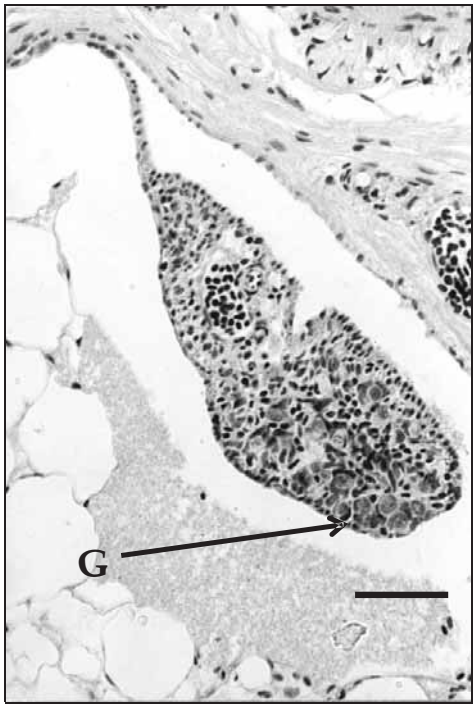

Photo 5. Male gonad of peled reared at 21°C on day 102 after hatching. G - Gonocytes. Bar = 25μm.

perature (Photo 5). The remaining females from this group exhibited either oocytes in the prophase of meiotic division (23%) or previtellogenic oocytes (15% of females) (Photo 6). The ovaries and testes of the fish in all experimental groups grew in volume.

Low or high sub-lethal temperatures influence the domination of either sex in many fish species, despite the existence of the GSD system. Studies conducted on Atlantic silverside *Menidia menidia* L. proved that a low sub-lethal temperature influences the process of sex differentiation towards the female sex (Conover and Fleisher 1986). Similar observations were made in *Patagonina hatcherii* Eigenm., pejerrey *Odontesthes bonariensis* Val., *Odontesthes argentinensis* Cuv. et Val. (Strüssmann et al. 1996, 1997). Conversely, an increase in the number of males was noted in *Oreochromis mossambicus* Peters (Strüssmann and Patiño 1995). Not only low sub-lethal but also high sub-lethal temperatures can modify the process of sex differentiation. Applying high sub-lethal temperatures in Nile tilapia *Oreochromis niloticus* L. (Baroiller et al. 1995) and

catfish *Ictalurus punctatus* Raf. (Patiño et al. 1996) skewed the sex differentiation towards the male. In *Oncorhynchus nerka* Walb. applying a high temperature during the period of sex differentiation influenced the dominance of the female (Craig et al. 1996). These studies were conducted on species characterized by undifferentiated gonochorism. In peled, however, the process of sex differentiation is direct, a fact which is confirmed by the present studies and also by the observations of Reshetnikov and Mukhachev (1989). Presumably, this was why water temperature, even a sub-lethal one (21°C), was not observed to have had an impact on the process of sex differentiation. Similarly, the sex ratio remained 1:1 from day 81 after hatching until the end of the experiment in all groups. Morphologic and structural differences in the gonad structure of peled were noted in the present study on day 81 after hatching in all experimental groups. At this moment, gonads with a thicker anterior part and generative and somatic parts located separately (ovaries) and small spindle-like gonads (testes) were observed. Cytological differentiation occurred at this time in the fish kept at higher water temperatures (17 and 21°C); the first female germ cells, or oogonia, appeared. This cell category was visible only on day 102 after hatching in females that were reared in water at a temperature of 10°C, which distinctly proves that environmental conditions, in this case water temperature, influences the rate of the sex differentiation process. Reshetnikov and Mukhachev (1989) are of the same opinion. This fact is also supported by studies of other fish species: common carp *Cyprinus carpio* L. (Pospisil and Smisek 1971); grass carp *Ctenopharyngodon idella* (Val.) (Jensen and Shelton 1983); pikeperch *Sander lucioperca* (L.) (Demska-Zakes and Zakes 1995).

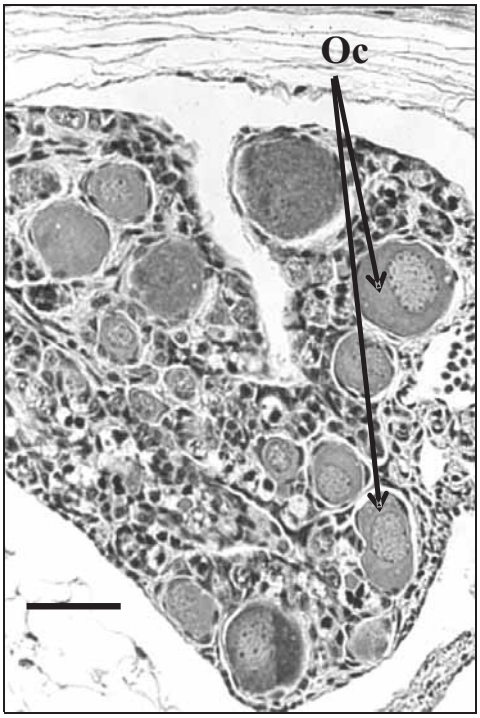

Photo 6. Female gonad of peled reared at 21°C on day 102 after hatching. Oc - Previtellogenic Oocytes. Bar = 25μm

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STRESZCZENIE

WPŁYW TEMPERATURY NA PROCES DYFERENCJACJI PŁCI U PELUGI *COREGONUS PELED* (GMEL.)

Badania nad zależną od temperatury determinacją płci prowadzono dotychczas na gatunkach ryb, u których występuje gonochoryzm niezróżnicowany, a dyferencjacja płci przebiega w sposób pośredni. U pelugi, gatunku będącego obiektem badań, dyferencjacja płci przebiega w sposób odmienny – niezróżnicowane zawiązki gonad przekształcają się bezpośrednio w gonady samicze lub samcze. W niniejszej pracy nie zaobserwowano wpływu subletalnie wysokiej temperatury wody na stosunek płci u pelugi (tab. 1). Potwierdzono natomiast, iż wzrost temperatury środowiska poprzez zwiększenie tempa wzrostu, wpływa pośrednio na tempo różnicowania się płci. Symptomy anatomicznej dyferencjacji gonad zauważono w 81 dniu po wykluciu u ryb ze wszystkich grup doświadczalnych (fot. 2, 3). W tym samym czasie nastąpiło także różnicowanie cytologiczne komórek płciowych pelug przetrzymywanych w wyższych temperaturach (17 i 21°C) (fot. 4). U ryb podchowywanych w najniższej temperaturze wody (10°C) pierwsze żeńskie komórki płciowe – oogonia obserwowano dopiero w 102 dniu po wykluciu. Do czasu zakończenia eksperymentu nie odnotowano różnicowania się męskich komórek płciowych – spermatogoniów.

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