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PRECOCIOUS MALES OF CULTURED ATLANTIC SALMON, *SALMO SALAR* L. IN THE SECOND SPAWNING SEASON

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Background. Early maturation of salmon males (*Salmo salar* L.) affects the reduction of fish physical condition and culture results. On the other hand, precocious male can fertilize mature eggs. The aim of this study was to estimate the magnitude of precocious maturation and to evaluate semen characteristics in a group of cultured 1-year-old salmon.
Material and methods. A total of 145 salmon males belonging to a group of low growth rate specimens that had not smoltified during the first spawning season were sampled from the "Aquamar" Fish Farm (Miastko, Poland). The study was based on light microscopy examination of histological sections and a standard procedure of milt quality evaluation. The gonadal development stage was determined with Billard and Escaffre's 9-grade scale modified by Dziewulska.
Results. The mean fork length of males was 10.45 cm. Three groups of males were distinguished: non-maturing (stage I); beginning spermatogenesis (inactive substage II); and precocious (stages VI to IX plus maturing males classified as undergoing "attempted spermatogenesis"). The groups contained 72.4, 4.8, and 22.8% of the males examined, respectively. The gonadosomatic index recorded in the three respective groups ranged from 0.010 to 0.164 (mean 0.040); 0.050–0.155 (0.089); and 0.058–6.219 (1.358). The gonadosomatic index is not an accurate indicator of gonadal activity. The precocious males semen contained from 6.1 to 23.0 million spermatozoa per mm^3 (13.41 million on the average). Spermatozoa performing progressive movements constituted 80–90%.
Conclusion. Among non-maturing males and males beginning spermatogenesis, precocious individuals were detected, the latter produced semen of good quality.

Key words: fish, *Salmo salar*; maturity stage; precocious; gonadosomatic index; milt

INTRODUCTION

The salmonid life history is highly flexible, males of numerous species showing variable life cycles and reproductive strategies. Most salmonids are anadromous, i.e. they descend to the sea before maturation and home to rivers, as large adults, to

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spawn. Although some males can mature early, they do not migrate and, as small precocious individuals, compete for females, thus employing a "sneaking" reproductive tactic. This alternative life history trait is an adaptation to variable environmental conditions and maximises reproductive success. The precocious maturity is observed in various populations at different age and is regarded as depending on growth rate and fat reserves (Lejzerovič 1973, Saunders et al. 1982, Dalley et al. 1983, Baglinière and Maisse 1985, Dellefors and Faremo 1988, Rowe and Thorpe 1990, Skilbrei 1990, Rowe et al. 1991, Greenstreet et al. 1992, Clarke and Blackburn 1994, Adams and Huntingford 1997, Utrilla and Lobòn-Cerviá 1999). Occasionally, precocious males constitute as much as 80–100% of a population (Baglinière and Maisse 1985, Utrilla and Lobòn-Cerviá 1999). Whenever such a high proportion of precocious males is the case, particularly in cultures, it produces a considerable problem because early maturation decreases fish condition and increases mortality (Lejzerovič 1973, Mitans 1973, Lee and Power 1976, Dalley et al. 1983, Myers 1984). For this reason, the rate of early maturation and factors effecting it are studied.

The aim of this study was to estimate the magnitude of precocious maturation and to evaluate semen characteristics in a group of cultured salmon.

MATERIALS AND METHODS

This study involved a total of 145 male Atlantic salmon, S*almo salar* L. aged 1+, reared in the "Aquamar" Fish Farm (Miastko, Poland). The eggs were obtained from spawners from a fresh-water hatchery. Artificial spawning was carried out between 10 October and 24 November 1999. The eggs were incubated at the Stara Brda, in ambient water at 0.1–5°C. The eggs were eyed in early February; the larvae hatched in the second half of April. The fry began feeding in early May at Osowo where the fish were kept, at a constant temperature of about 8°C, during the first year of their life. Subsequently, the fish were transferred to Świerzenko and placed in ambient water temperature ranging from 0.1°C in winter to 15°C in summer. Those fish that smoltified in spring were released into rivers, the rest remaining at captivity. In autumn, the fish likely to reach smolt size the following spring were selected. The remaining fish, less likely to smoltify, were sampled in October (9 males), November (56), and December (80). A total of 145 specimens were collected for analyses.

After they had been sacrificed, the fish were fixed in Bouin's solution. The fish sampled in December were stripped for milt by pressing the abdomen before fixation; the genital vent was dried to prevent contamination of the semen with water; care was taken to avoid contamination with urine and faeces. In the 8 milt-running males, the proportion of motile spermatozoa was determined, in triplicate, during examination of the sperm under a polarised light microscope. Spermatozoa motility was observed in the activating solution (119 mM $NaHCO_3$ and culture water). The spermatozoa concentration was assessed in the Bürker chamber. Subsequently, the fish were measured (fork length) and weighed, following which the gonads were removed.

The Fulton condition factor (CF = whole fish weight [g] x fish length^{-3}[cm] x 100%) and gonadosomatic index (GSI = gonad weight [g] x of whole fish weight $^{-1}$ [g] x 100%) were calculated. The fish were divided into length classes (6.0–6.9; 7.0–7.9; 8.0–8.9 cm, etc). The gonads intended for histological examination were selected based on their gonadosomatic index. Microscopic examinations involved the fish of the lowest GSI in each length classes and of GSI higher than 0.050. A total of 65 gonads were analysed. Histological slides were made using the standard paraffin technique. The 3–6 µm sections were cut from the mid-part of the gonad, stained with Heidenhain haematoxylin, and examined under a Zeiss Jenaval light microscope. The stage of gonadal development was determined with Billard and Escaffre's (1975) 9-stage scale modified by Dziewulska (2002). When spermatogenesis in the gonads had already been finished, the type of maturation was determined with additional criteria. The evaluation was supported by the appearance of lobules, i.e. by the presence of residual spermatozoa or peeled off cells in the lobule lumen.

Significance of differences in body lengths of male maturity groups was tested with t-test. All analyses were performed at significance levels of 0.05 using the STATISTICA software.

RESULTS

The mean fork length (±SD), weight, and Fulton condition coefficient of the fish studied were 10.45 cm (±1.22), 13.85 g (±4.73), and 1.17 (±0.10), respectively. The length was found to range from 6.8 to 13.4 cm, the weight and condition coefficient ranges being 3.7–28.2 g and 0.88–1.46, respectively. The gonad weight ranged from 1.50 to 926.30 mg (mean 45.40 ± 122.72 mg), while the gonadosomatic index varied between 0.010–6.219 (mean 0.367 ± 0.971). Values of the parameters in individual length classes are shown in Table 1.

Table 1

Major characteristics of males studied by length class (±SD)

Length class [cm]	No. of fish	Fish fork length [cm]	Fish weight [g]	Fulton condition coefficient	Gonad weight [mg]	Gonadosomatic index
6.0–6.9	1	6.8	3.7	1.18	6.1	0.164
7.0–7.9	1	7.5	7.6	1.33	2.5	0.045
8.0–8.9	16	8.51 ± 0.28	7.10 ± 0.99	1.15 ± 0.10	60.12 ± 150.46	0.636 ± 1.609
9.0–9.9	30	9.50 ± 0.26	9.79 ± 0.99	1.14 ± 0.08	68.69 ± 110.98	0.701 ± 1.154
10.0–10.9	40	10.45 ± 0.30	13.75 ± 1.46	1.21 ± 0.10	40.75 ± 93.07	0.290 ± 07.17
11.0–11.9	40	11.32 ± 0.28	16.80 ± 1.82	1.16 ± 0.10	44.72 ± 168.05	0.238 ± 0.896
12.0–12.9	15	12.26 ± 0.28	21.03 ± 2.76	1.14 ± 0.10	8.29 ± 2.82	0.040 ± 0.013
13.0–13.9	2	13.25 ± 0.21	26.85 ± 1.91	1.16 ± 0.14	6.85 ± 1.91	0.026 ± 0.009
Total	145	10.45 ± 1.22	13.85 ± 4.73	1.17 ± 0.10	45.40 ± 122.72	0.367 ± 0.971

The males differed in the degree of the advancement of their testes development. Based on that, the fish were divided into three groups: non-maturing (stage I);

beginning spermatogenesis (inactive substage II); and maturing (stages VI to IX and mature with "attempted spermatogenesis").

The mean body length of male maturity groups is given in Table 2. The difference in body length between males in I and II maturity stage was not significant ($P > 0.05$), while males of two mentioned groups were longer than maturing one, differences were significant ($P < 0.05$).

Table 2

Major characteristics of males studied by gonadal maturity stage (±SD and range)

Stage of maturation	No. of fish	Fish fork length [cm]	Fish weight [g]	Fulton condition coefficient	Gonad weight [mg]	Gonadosomatic index
I	105	10.69 ± 1.22 6.8–13.4	14.81 ± 4.67 3.70–28.20	1.17 ± 0.10 0.88–1.46	6.09 ± 2.72 1.50–13.80	0.040 ± 0.029 0.010–0.164
II	7	10.73 ± 1.25 8.60–12.20	15.24 ± 5.26 7.50–22.80	1.19 ± 0.06 1.11–17.30	12.16 ± 3.68 7.20–17.30	0.089 ± 0.043 0.050–0.155
VI	1	8.50	8.80	1.43	547.30	6.219
VII	2	10.25 ± 1.34 9.30–11.20	12.60 ± 3.68 10.00–15.20	1.16 ± 0.11 1.08–1.24	407.35 ± 102.88 334.60–480.10	3.252 ± 0.133 3.159–3.346
VIII	14	9.83 ± 0.76 8.80–11.90	11.04 ± 3.03 7.30–19.10	1.14 ± 0.10 0.98–1.41	250.78 ± 215.56 34.40–926.30	2.024 ± 1.314 0.310–4.850
IX	12	9.38 ± 1.04 8.10–12.40	9.51 ± 3.87 5.50–21.40	1.11 ± 0.07 0.99–1.24	13.99 ± 6.05 7.20–28.70	0.164 ± 0.99 0.058–0.448
AS	4	10.55 ± 0.59 9.70–11.00	13.30 ± 3.06 9.70–16.40	1.12 ± 0.11 1.00–1.23	10.48 ± 3.76 8.30–16.10	0.079 ± 0.017 0.058–0.098
VI–IX and AS	33	9.73 ± 0.95 8.10–12.40	10.75 ± 3.47 5.50–21.10	1.14 ± 0.10 0.98–1.43	152.79 ± 204.53 7.20–926.30	1.358 ± 1.602 0.058–6.219
Total	145	10.45 ± 1.22 6.80–13.40	13.85 ± 4.73 3.70–28.20	1.17 ± 0.10 0.88–1.46	45.40 ± 122.72 1.50–926.30	0.367 ± 0.971 0.010–6.219

AS, attempted spermatogenesis

Lobules of the first group, made up by 72.4% of the males, contained only type A spermatogonia and Sertoli cells. The gonads weighed 1.50–13.80 mg (mean 6.09 ± 2.72 mg). The gonadosomatic index in that group ranged from 0.010 to 0.164 (mean 0.040 ± 0.029) (Table 2). Those males did not mature in the second reproductive season. In the second group males (in inactive substage II), the presence of type A spermatogonia and some cysts of type B spermatogonia were observed. The cells were seldom undergoing divisions. The males costituted 4.8% of fish in this group. The mean gonad weight and mean GSI were 12.16 mg (range 7.20–17.30 mg) and 0.089 (range 0.050–0.155), respectively. The remaining males (22.8%) matured precociously in the second spawning season. Gonads of some of them contained

certain, low numbers of maturing cell ("attempted spermatogenesis"). The mean gonad weight and mean GSI were 10.48 mg (range 8.30–16.30 mg) and 0.079 (range 0.058–0.098), respectively. Gonads of 20.1% of males matured in the typical manner. The gonad weight and GSI reached 926.30 mg and 6.219, respectively. The gonadosomatic index was not an accurate indicator of gonadal activity because the GSI's of various stages were found to overlap.

Most of the males studied were immature and remained at stage I. In late October, one male only was precocious, at a stage corresponding to maturation stage VI. In early November, most dwarf males completed spermatogenesis or were close to completing it (stages VII, VIII or "attempted spermatogenesis"), some of them being spent (stage IX) or just beginning the process (inactive substage II). In mid-December, the advancement of gonadal development was similar to that observed in November.

The volume of milt stripped from the fish (at stage VIII) ranged from 0.01 to 0.37 ml (mean 0.21 ± 0.14 ml). The milt contained from 6.1 to 23.0 million spermatozoa per mm^3 (mean 13.41 ± 5.09 million). The spermatozoa performing progressive movements made up 80–90%.

DISCUSSION

In selected group of fish studied, in addition to males with gonads at resting- and precocious state, there was a small percentage of males (4.8%) undergoing spermatogenesis (i.e. in inactive substage II of maturation). Those males were not expected to complete spermatogenesis that year. During the reproductive season, in sea trout growing in natural streams only one out of 33 in their second year of life, represented inactive substage II (Dziewulska 2001). In younger (7-month-old) sea trout at the beginning of spawning season 5.8% males represented inactive substage II. Moreover, the same number of males had their gonads at active substage II (Dziewulska and Domagała 2003). Actually, because of the lack of comparable samples from natural environment, it is difficult to evaluate if the presence of males in inactive substage II during second spawning season in the studied group is the result of the culture condition or natural state of gonadal development of some males.

The maturation of a small number of germ cells in gonads of salmonid fishes, called "attempted spermatogenesis" was also recorded by other authors. According to Murza and Hristoforov (1983, 1984) such type of maturation is typical but not obligatory stage of reproductive system development and is characteristic for young fish, maturing for the first time, what often takes place in the first year of life (Murza 1985, Dziewulska 2001, Dziewulska and Domagała 2003). In north-west Poland males of the sea trout showing signs of "attempted spermatogenesis" constituted 3.5% of the fish sampled (Dziewulska and Domagała 2003). With age the number of males maturing that manner decreases (Murza and Hristoforov 1983, 1984). The relative number of sea trout males "attempting spermatogenesis" in their second year of life (3%) was similar (Dziewulska 2001) as the respective number of such salmon males in the present study.

Among fish studied, 22.8% of males matured precociously in the second spawning season. This percentage is not low because the males were selected from a low growth rate group. In other areas, the percentage of precocious males varied considerably between different salmonid populations and years of study (Nævdal 1983, Dellafors and Faremo 1988). The fish growing in cold climate reach the maturation at parr stage not earlier than in the second year of their life (Dalley et al. 1983, Myers 1984, Murza and Hristoforov 1984, Dellafors and Faremo 1988, Skilbrei 1990). Norwegian salmon populations showed 0–43.0% of males being precociously mature in the second reproductive cycle (Skilbrei 1990). In warmer climate, the process was observed as early as in the first year of the fish life. In the second year of life, it affected a high proportion of the fish, even in wild populations. In northern Spain, such fish accounted for 86.0–95.0% of the population (Utrilla and Lobòn-Cerviá 1999), as many as 100% being recorded in France (Baglinière and Maisse 1985).

The basic parameters of precocious males' milt were good. The number of spermatozoa per mm^3 was high, similarly to values recorded by other authors in such individuals (Kazakov 1979a, b). The precocious males' semen, compared to that of adults, revealed the precocious males to contain higher concentrations of spermatozoa (Daye and Glebe 1984, Vladić and Järvi 2001, Vladić et al. 2002). Some studies showed the semen to contain a higher proportion of motile spermatozoa, a higher sperm energy load as expressed by the ATP content (Vladić and Järvi 2001), and longer duration of spermatozoa motility (Kazakov 1979b, Daye and Glebe 1984, Vladić 2000).

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