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The ovarian development and spawning time of *Salmo gairdneri* R. reared in advanced and delayed annual photoperiod cycles at naturally fluctuating water temperature in Finland

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The development of rainbow trout ovaries, and changes in plasma vitellogenin and Ca^{2+} concentration was investigated for two annual photoperiod cycles, one advanced and another delayed for 1.5 months. The natural yearly water temperature fluctuated between 22° and 0.2°C.

Ovarian development, the increase in plasma vitellogenin and total calcium concentration was significantly faster in the advanced than in the delayed and control group fish. The fish in the advanced photoperiod cycle matured earlier than normally, but they laid their eggs first during the normal spawning time (in May), when the water temperature had increased to over 4°C. At the end of the experiment the oocytes of the delayed photoperiod group fish were about 30% smaller than those of the advanced group fish. Besides, ovarian development in these fish was abnormal to some extent, part of the oocytes being necrotic.

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1. Introduction

Photoperiod is considered to be the most effective environmental factor in controlling the reproductive cycle of salmonid fish (Alison 1951, Henderson 1963, Carlson & Hale 1973, Whitehead et al. 1978b, 1980 and McQuarrie et al. 1978, 1979). There is also some information available on the effects of water temperature on the gonadal growth (Titarev 1975, Morrison & Smith 1986).

The annual changes in salmonid fish gonads during the reproductive cycle have been reasonably well described (Hurley & Fisher 1966, van den Hurk & Peute 1979, Bry 1981 and Schulz 1984), but there are only a few morphometrical descriptions of the effects of different annual photoperiod cycles on the ovarian development of rainbow trout (Nakari et al. 1987). Most of the studies on the effects of altered photoperiod cycles have been carried out at a constant or reasonably high water temperature, using an autumn spawning stock. The environmental conditions are quite different from those here in Finland, in the nothernmost parts of the rainbow trout's range.

In Finland the breeding results of farmed spring spawning rainbow trout have recently been poor,with <50% of the eggs hatching. Here the yearly lake water temperatures fluctuate between 22° and 0.2°C, and in winter the lakes are covered with ice; fish are farmed in extremely cold water, and in almost total darkness (Virtanen & Soivio 1984). The gonadal maturation of spring spawning fish occurs during the cold winter time. By advancing the photoperiod cycle we endeavoured to advance gonadal development, as carried out successfully by many workers in the past (Billard & Breton 1977, Whitehead et al. 1978b, McQuarrie et al. 1978, 1979, Boulier & Billard 1984, Scott et al. 1984, Duston & Bromage 1986), thereby bringing the ovaries to a more advanced stage during the autumn, before the annual decrease in the water temperature. The effects of the delayed photoperiod cycle at these natural water temperature conditions were also tested.

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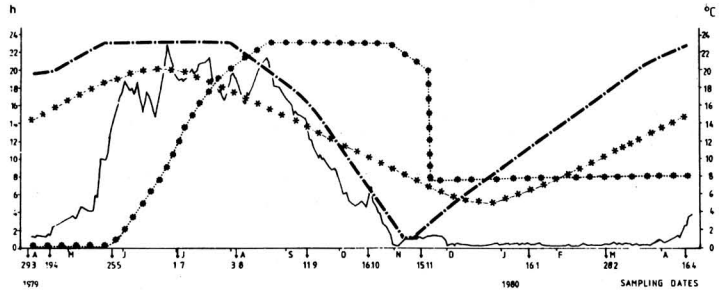

Fig. 1. The annual photoperiod cycles for advanced (—·—·—) and delayed (• • • • • •) experimental fish together with the normal cycle in central Finland (• • • • • •), and experimental water temperature (————). Sampling dates are marked by arrows.

The effects of the different photoperiod cycles on the spawning time was clarified using gonadal weight, plasma vitellogenin and total calcium concentration, as well as morphometrical measurements of ovaries, as parameters. The gonadal weight is at its maximum just before or during spawning (McQuarrier et al. 1978, 1979, Whitehead et al. 1978a, b, 1980, van Bohemen & Lambert 1981, De Vlaming et al. 1984). The same holds true for the plasma vitellogenin and total calcium concentration of normal females (Woodhead 1968, Whitehead et al. 1978a, b, 1980, van Bohemen & Lambert 1981), because one atom of calcium is associated with every protein phosphate group in the vitellogenin complex, the precursor of the yolk proteins of oocytes. Thus, as maturation proceeds gross changes occur in the plasma vitellogenin and total calcium levels (Wallace 1970).

2. Material and methods

The experiment was carried out in a private fish farm belonging to the Savon Taimen Co., at Rautalampi (central Finland), in the years 1979–1980. Juvenile, spring spawning female *Salmo gairdneri* (R.) (at the beginning of the experiment 2-year-old) were reared in the farm's routine manner. The experimental fish were transferred in March 1979 from an outdoor earth pond into two concrete troughs (3.6×2.1 m, water depth 1 m, 200 fish per group) with an artificial lighting device (8×100 W bulbs) inside the lightproof lids. After the transfer water flow and fish density were kept equal to the control group, which was reared in an outdoor earth pond (100 m²). In the tested annual photoperiod cycles the maximal daylengths were either 1.5 months advanced or 1.5 months delayed compared to the natural one (control group). The daylengths in the advanced and delayed group were simulated in the covered troughs by means of electrical timers adjusted once per week (see Fig. 1). The differences in the shortening daylength are due to technical difficulties. The natural lighting conditions in the water, during the wintertime almost total darkness under the ice, and in the spring several thousand lux at the water level, were taken into account. Water temperatures fluctuated naturally (22°–0.2°). For practical reasons the photoperiod cycle of the delayed group was changed to 8L:16D in December 1979. Water temperatures, day lengths, and sampling dates are given in Fig. 1.

There were 11 sampling times altogether. The starting samples were taken on the 29 of March 1979, when the fish were brought inside into the test troughs. The last sampling was made on the 16 of April 1980. The mortality during the experiment was negligible. The test strippings were started at the beginning of April.

For sampling, the fish were immobilized by a blow to the head, measured and weighed. Blood samples were aspirated by heart puncture into heparinized syringes. Plasma was separated without delay by Beckman Microfuge B (3 minutes), frozen in liquid nitrogen and stored at –20° until analyzed. The ovaries were quickly dissected and weighed (always the middle part), and fixed by Smith's formol bichromate method (Pantin 1946), embedded in paraffin wax, and sectioned at 7 µ. The sections were stained with Masson-Gomori (Romeis 1932).

From each fish the diameter and zona radiata of 10 oocytes were measured with the help of an eyepiece micrometer under a low power light microscope.

Vitellogenin was assayed by measuring its phosphoprotein phosphorus content (Boehringer Corporation Test Handbook, 1970), with the following modifications; from 100 µl of plasma the protein precipitate was isolated and washed successively with organic solvent to remove lipid (Henry et al. 1974). The remaining protein pellet was assayed according to the Boehringer method. The phosphoprotein phosphorus levels thus obtained were converted to vitellogenin levels as descibed by Whitehead et al. (1978a, b).

The total calcium concentration of the plasma was determined photometrically with Waco reagent kits (Waco Pure Chemical Industries Ltd, Japan, Cod. No. 276-21809).

All values are expressed as mean ± *SE* of means measured for each sample, (*n*). A statistical comparison to the control was made by Student's *t*-test, which is acceptable for this kind of data.

3. Results

The ovarian weights are expressed as gonadosomatic indices (GSI = % of the total body weight) (Fig.

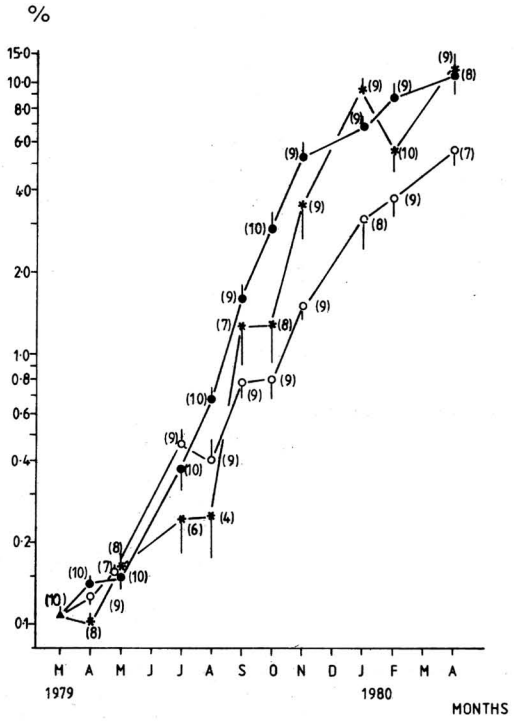

Fig. 2. The gonadosomatic indices of the rainbow trout females in the advanced ●, delayed o, and normal * annual photoperiod cycle, and of the starting samples ▲. In parenthesis the number of fish.

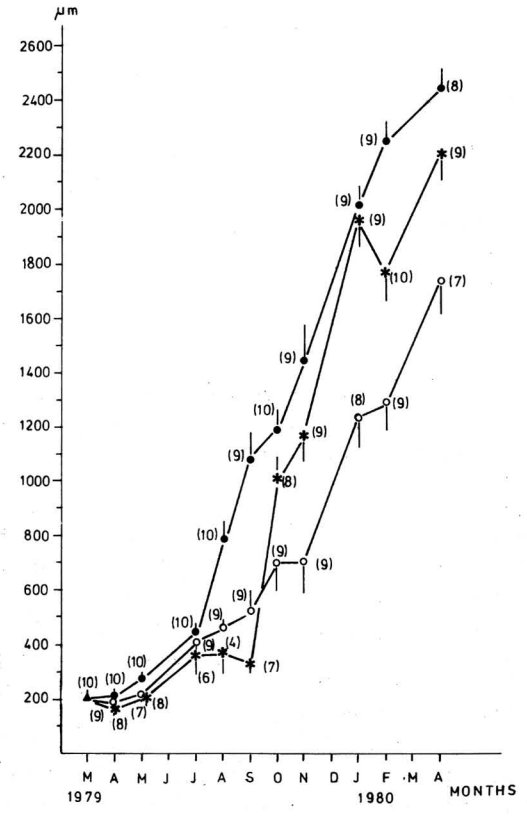

Fig. 3. The oocyte diameters of rainbow trout in the advanced ●, delayed o, and normal * annual photoperiod cycle, and of the starting samples ▲. In parenthesis the number of fish.

2). At the beginning of the experiment the ovaries were small and ribbonlike, and started to grow rapidly after the end of May. In the advanced group this was preceded by one month's (April) plateau, beginning from the light (long day) induction. Similar plateaus can be seen in other groups, after their light inductions (July). The shortening day lenght induces an identical plateau both in the delayed and in the normal group. In July the GSI values of the delayed group fish were significantly higher ($P<0.001$) than those of the other groups. The ovaries of the control group fish were the smallest ones, and their rapid increase did not start until after August. From August onwards the ovaries of the advanced group fish were significantly heavier than those of the delayed ones ($P<0.001$). The GSI value of the control group was between that of the advanced and delayed group. The oocytes grew quite similarly (Fig. 3), and the effect of the advanced photoperiod cycle was seen from August onwards, the oocytes of these fish being significantly ($P<0.001$) bigger than those of the delayed and control group ones. It should be noted that the ovarian development of the control group fish was not as steady as that of the other group fish. This is reflected in all the measured parameters. The reason for this is unknown.

The oocytes of the delayed group fish grew quite poorly up to November, when the photoperiod was changed to 8L/16D. After that they started to grow a little faster, but right up to the end of the experiment their development was significantly ($P<0.001$) poorer than that of the other fish groups.

Simultaneously with the oocyte growth the zona layer became thicker. This thickening was significantly ($P<0.001$) faster in the fish of the advanced group than in those of the others (Fig. 4). The zona radiata began to form in June, during the warm water period, when ovarian growth began to accelerate rapidly.

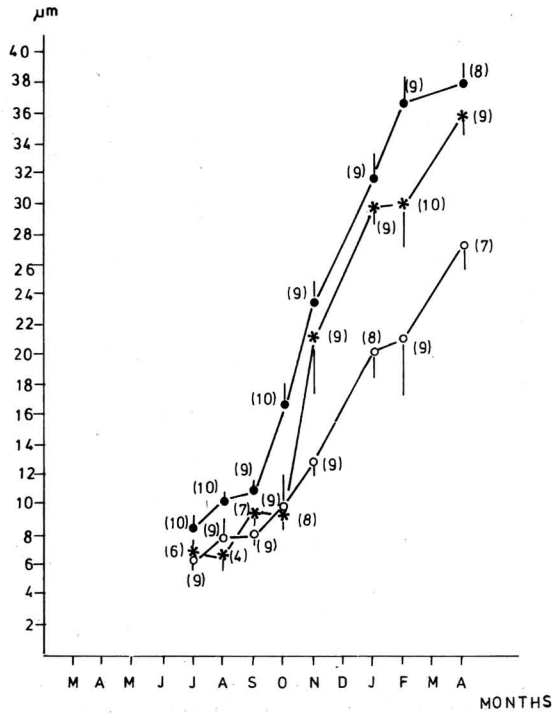

Fig. 4. The thickness of the zona layer of oocytes of the rainbow trout in the advanced ●, delayed o, and * normal annual photoperiod cycle. In parenthesis the number of oocytes measured.

The plasma vitellogenin and calcium concentration of every fish in all groups stayed at a rather steady low level until July–August (Fig. 5a & b), though they were higher in the advanced group fish. After August the concentrations started to increase in pace with oocyte size, significantly faster in the advanced, than in the other fish groups. The increase in vitellogenin concentration of the advanced group fish slowed down a little during the coldest winter period, but after February the concentration levels of both vitellogenin and calcium were doubled up to April 1980. The concentrations of delayed and control group fish also increased, but the values were significantly ($P<0.001$) lower than those of the advanced group ones.

Between January and February 1980 in the control group fish a decrease appeared to occur in almost all the measured parameters which was statistically not significant. The reason for this is not clear.

The test stripping of the fish was started at the beginning of April, but at that time only about 40% (20 fish) of the advanced group fish were running their eggs. At the beginning of May, 99% of this fish group were laying their eggs, but in the delayed group only one fish was doing so. After the beginning of May the control group fish also started to spawn. The roe was fertilized and incubated, and the hatching of the advanced group fish, which were spawning for the first time, was twice as good (losses only 22%) as those of the farm's normal brood fish (4–6-year-old) (losses 40%).

4. Discussion

During the reproductive cycle the oocyte development of rainbow trout can be divided into five stages (Lambert et al. 1978 and van den Hurk & Peute 1979): previtellogenic, vitellogenic, mature, post-ovulatory, and atresia. During the vitellogenic stage, yolk globules appear in the oocyte cytoplasm, anda zona radiata, situated between the follicle cells and oocyte, begins to form and increase in thickness quite rapidly (Plack & Fraser 1971 and Le Menn 1976). As maturity progresses, the oocytes become filled with yolk, and their diameters increase rapidly, this also causing a rapid increase in gonadal weight. In mature ovaries the oocytes are filled with yolk. Vitellogenesis ceases once the oocytes reach their full size.

In the advanced group fish, yolk started to accumulate in the oocytes very rapidly after July, when the water temperature had reached its summer level, and accumulation was completed after the coldest midwinter. During the midwinter the increase in vitellogenin concentration slowed down a little. This could not be seen either in the GSI values or in the oocyte sizes.

In fish, vitellogenesis ceases once the oocytes reach their full size. Such oocytes eventually undergo maturation and ovulation after an appropriate hormonal stimulus (Masui & Clarke 1979). Although the oocytes of the advanced group fish had reached, according to the morphometrical measurements, their full size earlier, they did not go further through the ovulatory and post-ovulatory phases until the natural spawning time. This was evidently due to the cold water temperature. The spring spawning stock we used for the experiment spawn at a water temperature of over 4°, and so indeed did the fish in the advanced photoperiod group. We have shown in our previous paper (Nakari et al. 1987) that in Finland the spawning of rainbow trout can be advanced by advancing the photoperiod cycle, if the fish are kept at a constant water temperature of 10°. Titarev (1975) has shown

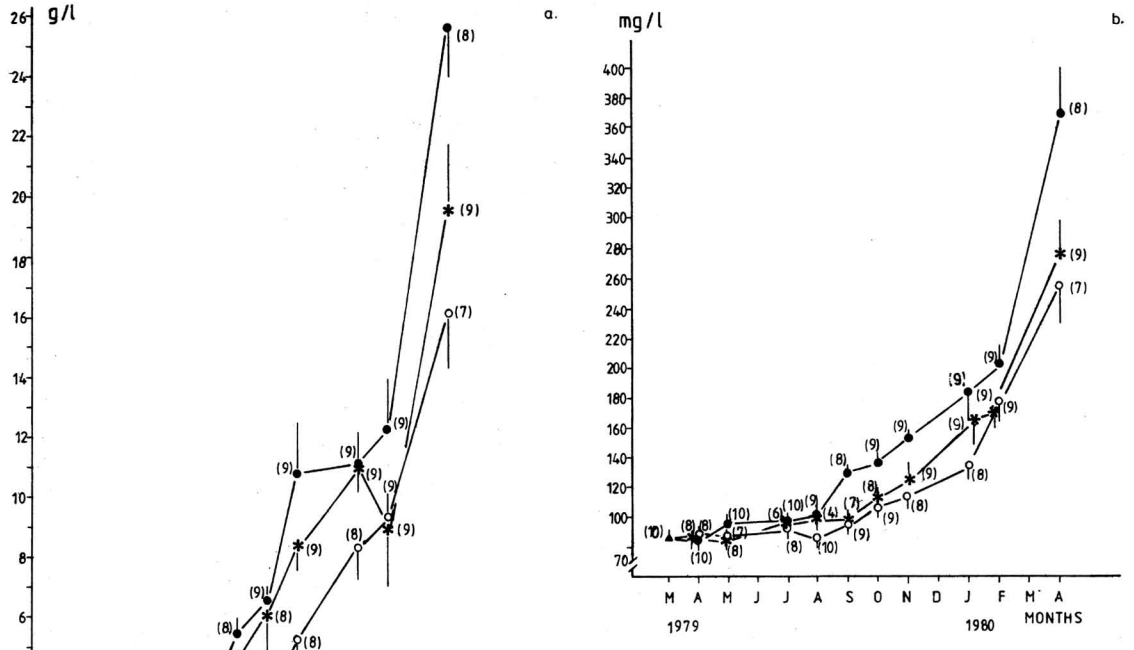

Fig. 5. The a) vitellogenin and b) total calcium concentration of rainbow trout plasma in the advanced ●, delayed ○, and normal ✻ annual photoperiod cycle, and of the starting samples ▲. In parenthesis the number of fish.

that warm industrial waste waters make it possible to reduce the time at which trout become mature. Morrison & Smith (1986) have shown that the spawning of rainbow trout can be delayed if the fish are kept in rather cold water. The effects of different photoperiod cycles are not discussed in these two papers.

The lake water started to warm up in April. The increases in the plasma vitellogenin and calcium concentrations between February–April can be explained by increased metabolic rate in the higher temperature. The sharp increase was seen in fish of every group, but most clearly in the advanced group fish. There were no great changes in GSI values or oocyte sizes during that time. The increase in water temperature allows the completion of the final vitellogenesis and the oocytes undergo the maturation and ovulation for which the increase in water temperature is the right stimulus.

We think that if the oocytes are developed to a certain phase before the annual cold period, they can continue their development. Those oocytes, however, which do not reach this phase begin to atrophy. This was the case with our delayed group fish, which had considerable amounts of necrotic oocytes in their ovaries after December.

In Finland the water temperature, fluctuating as it does, is a very important factor for the spawning of the fish. Though the roe of the advanced group fish according to the morphometrical measurements and GSI values seemed to be ready for spawning earlier than normally, the low water temperature (at that time 0.4°) appears to inhibit the completion of the final vitellogenesis, and thus the earlier spawning.

Although the advanced group fish did not spawn earlier than normal, the hatching results were twice as good and the quality of the roe was improved significantly. It should also be noted that these fish were spawning for the first time, when roe development under normal rearing conditions is still rather poor (farmer's statement).

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