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Relationships between Serum Levels of Gonadotropin, Oestradiol-17 β , and Vitellogenin in the Control of Ovarian Development in the Rainbow Trout

II. The Effects of Alterations in Environmental Photoperiod

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The present study investigates the effects of seasonally changing photoperiod regimes on the levels of serum gonadotropin, oestradiol-17 β , and vitellogenin (as indicated by phosphoprotein phosphorus and calcium measurements) on the time of spawning of female rainbow trout (*Salmo gairdneri*). This included a control regime and also seasonal cycles in which the increasing and decreasing components of the photoperiod were maintained but compressed into 6- and 9-month periods. Spawning in the control fish occurred in mid-January at the same time as fish in outside ponds under ambient conditions, whereas under the 6- and 9-month cycles it was advanced by approximately 12 and 6 weeks, respectively. Under both the control and experimental regimes, the primary change was an increase in serum levels of gonadotropin during the early stages of ovarian development, after which levels were reduced. This was followed by increased oestradiol-17 β levels which became particularly pronounced during the period of exogenous vitellogenesis. These changes in oestradiol-17 β appeared to stimulate vitellogenin production and release by the liver as evidenced by increases in serum calcium and phosphoprotein phosphorus levels. Subsequently, vitellogenin levels remained high until after spawning was complete. During the later stages of ovarian development oestradiol levels fell, thus signalling, probably by a negative feedback mechanism, a further and larger increase in gonadotropin levels presumably required for the control of final oocyte maturation and ovulation. This sequence of endocrine changes was similar under the shortened photoperiod cycles, although modifications were apparent in both their timing and duration, resulting in earlier spawning times.

The processes of oögonial multiplication, oögenesis, vitellogenesis, oöcyte maturation, ovulation, and oviposition constitute the seasonal reproductive cycle in salmonid fish and each requires precise coordination in order to produce a fertilizable oöcyte at a time of the year most favourable to its survival. It is now clear that a variety of geophysical parameters such as photoperiod, temperature, rainfall, and salinity as well as biological factors such as nutritional status play important roles in the timing of reproduction in teleost fish (see reviews by de Vlaming, 1972; Donaldson, 1975; Peter and Hontela, 1978). For salmonids in particular, photoperiod appears to be the major

controlling factor (Allison, 1951; Hazard and Eddy, 1951; Nomura, 1962; Henderson, 1963; Carlson and Hale, 1973; Kunesh *et al.*, 1974; McQuarrie *et al.*, 1978; Whitehead *et al.*, 1978a; Peter and Crim, 1979).

In all species of fish so far investigated, the initiation and subsequent modulation of the course of reproduction by environmental cues are achieved as a result of neuroendocrine changes involving the hypothalamus and the hormones of the pituitary gland and the gonads. Thus, changes in circulating levels of gonadotropin and gonadal steroids in response to altered environmental conditions have been reported

for both male and female rainbow trout (Breton and Billard, 1977; Billard *et al.*, 1978; Whitehead *et al.*, 1978a,b), carp (Billard *et al.*, 1978) and the goldfish (Gillet *et al.*, 1977, 1978; Hontela and Peter, 1978). Few of these studies, however, have investigated the effects of environmental changes on the interrelationships of the various endocrine mechanisms which are responsible for the control of all stages of the reproductive cycle. In view of this, the present study aims to investigate the relationship of serial changes in gonadotropin and oestradiol-17 β and phosphoprotein phosphorus and total calcium (as measures of vitellogenin) in female rainbow trout exposed to alterations in their normal seasonal light cycle.

MATERIALS AND METHODS

Three groups of previously immature female rainbow trout, initially weighing approx 1.3 kg, of the same Danish strain and stock and with a natural spawning time of January to February were each maintained in lightproof 800-liter aquaria as previously described (Whitehead *et al.*, 1978a,b). The water temperature was constant at 9° with dissolved oxygen of 100% saturation in the effluent and a pH of 6.6. The fish were fed with B.P. Nutrition Mainstream diet at a level of 0.5% body weight/day.

The first group of control fish was exposed to a normal 12-month seasonal light cycle with the daily light period increasing from April to the longest day on July 4, and thereafter decreasing to the shortest day on December 20 (Fig. 1). The other two groups received similar seasonally increasing and decreasing light cycles, but these were compressed into 9 and 6 months by accelerating the rates of increase and decrease of day length each week such that the longest and shortest days were reached on June 4 and October 20, respectively, for the 9-month regime, and on May 4 and August 8 for the 6-month regime (Fig. 1).

Blood samples were withdrawn under ethyl-4-aminobenzoate (1:20,000) anaesthesia from the Cuvierian vein of all control and experimental fish at the start of the experiment and each month thereafter. The blood samples were always taken between 1100 and 1500 hr and the serum obtained was stored at -20° until assay of gonadotropin using a homologous trout radioimmunoassay (Breton and Billard, 1977), oestradiol-17 β by radioimmunoassay (Whitehead *et al.*, 1978a), and total calcium and phosphoprotein phosphorus as indices of vitellogenin using methods

already described (Whitehead *et al.*, 1978a). Fish were also examined each month for signs of sexual maturation. For the purpose of this study spawning was said to have occurred when ripe eggs were expelled after gentle hand pressure on the abdomen (i.e., stripping of the fish).

Differences between means were tested statistically by either a Student's *t* test or an *F* test if the variances were dissimilar.

RESULTS

In the control fish spawning occurred in mid-January at exactly the same time as similar fish maintained outside in open ponds under naturally fluctuating environmental conditions. In contrast, spawning under the 9- and 6-month accelerated regimes was advanced by 6 and 12 weeks, respectively. Similar fecundities, egg sizes, fertilization rates, hatchabilities, and fry survival were recorded under each of the three photoperiod regimes.

As maturation proceeded gross changes were seen in the serum levels of gonadotropin, oestradiol-17 β , phosphoprotein phosphorus, and total calcium, although the timing of these changes was modified by the shortened light regimes when compared with the fish under the control photoperiod. All fish, irrespective of treatment, had similar levels of gonadotropin (9.0 ± 1.6 ng/ml), oestradiol-17 β (130 ± 15 pg/ml), phosphoprotein phosphorus (25 ± 7 μ g/ml), and total calcium (12 ± 2 mg%) in April at the start of the experiments. It might be mentioned, however, that the gonadotropin levels appeared to be falling from higher levels before sampling began in April; the variances of the mean values at this time are also suggestive of a mixed and changing population.

Under all regimes the initial endocrine change was an increase in serum gonadotropin (Fig. 2). This increase began at an earlier time and reached higher values in the fish under the shortened regimes, although in both control and experimental groups the peak values occurred in July after which they all fell to their lowest

FIG. 1. Photoperiod regimes under investigation: —, 12-Month cycle; --, 9-month cycle; ---, 6-month cycle.

FIG. 2. The sequential changes in levels of serum gonadotropin in female rainbow trout under each of the photoperiod regimes under investigation. —, 12-Month photoperiod cycle; ---, 9-month photoperiod cycle; ···, 6-month photoperiod cycle. Vertical bars represent $\pm$ SE. ●, Spawning under 12-month cycle; ○, spawning under 9-month cycle; △, spawning under 6-month cycle.

levels in October. In all cases the levels in July were significantly different from those in October ($P < 0.01$), where $n = 7, 5$, and 4 in the control, 9- and 6-month cycles, respectively.

The oestradiol-17 β levels were first seen to change in May and June in the shortened and control regimes, respectively, although a much more pronounced increase then occurred in July to August under all three photoperiods (Fig. 3). All showed their highest values in October although these were much reduced in size and duration under the two shortened light cycles, where levels of oestradiol fell toward basal values some 2–3 months before the controls. In all three cases the oestradiol levels in October were significantly different from those observed in April, (12 and 9 month, $P < 0.001$; 6 month, $P < 0.01$). Under the control 12-month cycle there was a significant negative correlation between serum gonadotropin and oestradiol during the months of July to February ($P < 0.001$, $r = -0.3569$, $n = 103$). Levels of these two hormones were also negatively correlated in fish under the 9-month cycle between July and January ($P < 0.05$, $r = -0.3617$, $n = 29$) and in fish under the 6-month cycle between July and December ($P < 0.001$, $r = -0.7497$, $n = 22$). No correlation was found between the gonadotropin and oestradiol levels of any of the groups during the earlier stages of development (April–July) although both hormones showed parallel increases during this period.

The phosphoprotein phosphorus and calcium levels exhibited parallel changes within each of the three light regimes and thus may be considered together (Figs. 4 and 5). Under each regime there was a significant positive correlation between serum levels of these two parameters throughout the whole cycle (12 month, $P < 0.001$, $r = 0.8411$, $n = 75$; 9 month, $P < 0.001$, $r = 0.9511$, $n = 52$; 6 month, $P < 0.001$, $r = 0.9641$, $n = 43$). The control and experimental fish had similar values until July

after which levels were increased earlier and attained higher levels in the shortened light cycles; maximum levels were reached in November, December, and January under the 6- and 9-month shortened and control cycles, respectively. At these times, the levels were significantly different from their respective values in April in each case ($P < 0.001$).

Between July and October, serum levels of oestradiol and phosphoprotein phosphorus/total calcium were positively correlated (12 month, $P < 0.001$, $r = 0.6448$, $n = 26$; 9 month, $P < 0.001$, $r = 0.7469$, $n = 20$; 6 month, $P < 0.01$, $r = 0.6297$, $n = 14$). No such correlation was observed either earlier (April–July) or later (November–February) in each cycle.

DISCUSSION

The present study demonstrates that the advancement of spawning in the female rainbow trout, achieved by condensing the natural yearly light cycle into either 6 or 9 months, occurs as a result of a modification in the timing, but not the overall sequence of changes in gonadotropin, oestradiol, and vitellogenin which is observed during the normal reproductive cycle.

Under all regimes the primary change was an increase in serum levels of gonadotropin during the earlier stages of ovarian development. Similar increases have also been demonstrated in the brown trout by Billard *et al.* (1978). This early increase in gonadotropin would appear to be responsible for the stimulation in oestradiol-17 β levels which occurred in June to July under all the light cycles. A similar induction of oestradiol has been reported after gonadotropin administration in the rainbow trout (Billard *et al.*, 1978). As the experiment was not begun until April it is not possible to comment on the gonadotropin levels before this date. The falling values in April, however, and the relatively high variances of the mean together with the unpublished observations of Breton that marked but tran-

FIG. 3. The sequential changes in levels of serum oestradiol-17 β in female rainbow trout under each of the photoperiod regimes under investigation. —, 12-Month photoperiod cycle; --, 9-month photoperiod cycle; ---, 6-month photoperiod cycle. Vertical bars represent $\pm$ SE. ●, Spawning under 12-month cycle; ○, spawning under 9-month cycle; ◐, spawning under 6-month cycle.

FIG. 4. The sequential changes in levels of serum phosphoprotein phosphorus in female rainbow trout under each of the photoperiod regimes under investigation. —, 12-month photoperiod cycle; ---, 9-month photoperiod cycle; ···, 6-month photoperiod cycle. Vertical bars represent $\pm$ SE. $\bullet$, Spawning under 12-month cycle; $\circ$, spawning under 9-month cycle; $\odot$, spawning under 6-month cycle.

FIG. 5. The sequential changes in levels of total serum calcium in female rainbow trout under each of the photoperiod regimes under investigation. —, 12-Month photoperiod cycle; —, 9-month photoperiod cycle; ---, 6-month photoperiod cycle. Vertical bars represent $\pm$ SE. $\uparrow$, Spawning under 12-month cycle; $\circ$, spawning under 9-month cycle; $\bullet$, spawning under 6-month cycle.

sient fluctuations in levels occur in March suggest that gonadotropin may be exerting a significant influence at this early stage of the reproductive cycle.

Although there was little difference in the timing of the early increase in gonadotropin under the three cycles, much higher levels were produced under the 6-month regime than the other cycles presumably in response to the increased amount of light to which the fish had been exposed. A relationship between gonadotropin levels and the amount of light has been reported by O'Connor (1972), although questions have been raised about the sensitivity of this assay. In birds, pituitary gonadotropin content decreases under long days (20L:4D) but not under short days (6L:18D), long days thus stimulating gonadotropin release (Follett *et al.*, 1967).

In view of the higher gonadotropin levels it is possibly surprising that the serum oestradiol values attained in the fish under the 6-month regime were lower than either of the other two cycles. It has recently been shown, however, that shortened photoperiods earlier in the year serve to diminish the absolute level of oestradiol-17 β attained and curtail the period of its increase (Whitehead and Bromage, 1980). Under the 9-month regime again the maximum level of oestradiol achieved and the duration of this response were reduced when compared to control values. Under all three regimes the timing of the onset of increases in gonadotropin and oestradiol was only moderately changed, possibly as a result of the similar light cycle to which all the fish had been exposed before the beginning of the experiment in April. Recently we (Whitehead and Bromage, unpublished data) have shown that the ovarian development may be influenced in part at least by the photoperiod during the previous reproductive cycle.

Throughout vitellogenesis and final oocyte maturation under all three photoperiod regimes there was a significant negative correlation between gonadotropin and oes-

tradiol levels, confirming negative feedback of oestradiol on gonadotropin during this stage of oocyte development, irrespective of photoperiod treatment. The increases in oestradiol under all three regimes were followed by a stimulation of vitellogenin levels in the blood as evidenced by parallel increases in both serum calcium and phosphoprotein phosphorus. The induction of vitellogenin synthesis by the liver as a result of oestrogen treatment has been reported for several species of fish (Bailey, 1957; Plack *et al.*, 1971; Emmerson and Peterson, 1976; Terkatin-Shimony and Yaron, 1978; Elliot *et al.*, 1979). The present work both supports the correlation previously observed between calcium and phosphoprotein phosphorus (Elliot *et al.*, 1979) and confirms the 1:1 ratio of these parameters in the vitellogenin moiety.

The increased oestradiol and consequent vitellogenin levels indicate the onset of an active exogenous vitellogenesis in the oocytes in the ovary. Broadly, the timing of these changes is in accord with the timing of vitellogenesis suggested by other workers (Peute *et al.*, 1978; Lambert *et al.*, 1978; Crim and Idler, 1978) and with histological demonstration of Stage 6 oocytes (van den Hurk and Peute, 1980) in the ovaries of parallel groups of trout with similar spawning times. Precise correlation with this data is difficult, as it is likely that the onset and duration of vitellogenesis will vary according to the strain of trout studied. The highly significant correlation between oestradiol and calcium and phosphoprotein phosphorus levels from July to October was not seen later in the cycle suggesting that once vitellogenin synthesis has been initiated by oestradiol it continues despite the return of oestradiol to basal values. Similar findings have been made by Elliot *et al.* (1979) after treatment of fish with exogenous oestradiol-17 β .

Serum levels of calcium and phosphoprotein demonstrate the first major differences in the timing of the onset of increases under the different photoperiod regimes.

Thus, levels were raised in July to August under the 6- and 9-month regimes, but not until August–September in the control fish under the 12-month cycle. Furthermore, the maximum levels attained were higher and were attained at a faster rate under the shortened regimes. Whether this is due to an increased synthesis of vitellogenin under the shortened regimes, a temporary reduction in the ability of the ovarian follicles to sequester the egg protein, or a combination of both is not known.

As levels of oestradiol increased and vitellogenesis progressed under all three regimes, gonadotropin levels gradually returned to low values. Similar results have been observed in the brown trout by Billard *et al.* (1978). Under both the shortened cycles this decrease in gonadotropin levels as oestradiol increases occurred earlier than under the control cycle. As oestradiol levels fall during the latter stages of vitellogenesis, a surge in gonadotropin levels was observed probably related to the final stages of oocyte maturation and ovulation in each case. Under the 6-month cycle the mean oestradiol levels were apparently still falling at spawning although the monthly sampling procedure inevitably includes a mixed population of preovulatory and ovulated fish. Observation of individual fish values confirms the coincidence of high gonadotropin and low oestradiol levels at spawning as indicated by other studies (Fostier *et al.*, 1978; Whitehead *et al.*, 1978a,b; Crim and Idler, 1978). The continued increase of gonadotropin values even after ovulation and spawning in salmonid fish has also been reported by Crim *et al.* (1975) and Jalabert and Breton (1980), and may be due to the low levels of oestrogens present at this time.

Although present under all photoperiod cycles the second and much higher increase in levels of serum gonadotropin occurred earlier under the shortened regimes than under the control cycle, thus indicating that this change is important in the control of the later stages of maturation and the timing of final spawning.

Thus, we see a similar relationship in the profiles of gonadotropin, oestradiol-17 β , and vitellogenin under the experimental and control light cycles although there are marked alterations in the timing and extent of these changes which are clearly related to the overall control of reproductive development and spawning. The accelerations in development provoked by shortened light cycles raises important questions concerning the photoperiodic mechanisms involved in the recognition of environmental change and their subsequent interpretation by the neuroendocrine system. Preliminary data suggest that it is the amount of light received per day and its relationship to photosensitive phases in the reproductive rhythms of fish, rather than its rate of change, which is of importance in the overall control of ovarian development. These aspects are currently under investigation.

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