Ann. Zool. Fennici 31:319–327
Helsinki 7 October 1994

ISSN 0003-455X

Testosterone and 17β-estradiol plasma fluctuations throughout spawning period in male and female rainbow trout, *Oncorhynchus mykiss* (Walbaum), kept under several photoperiod regimes

M. Pavlidis*, D. Dimitriou & A. Dessypris

Pavlidis, M., Dimitriou, D. & Dessypris, A., Division of Biochemistry, Cell and Molecular Biology and Genetics, Department of Biology, Athens University, Panepistimiopolis, Athens 15701, Greece. — * Present address: Institute of Marine Biology of Crete, P.O. Box 2214, Iraklion 71003, Crete, Greece*

Received 2 October 1993, Accepted 28 January 1994

Plasma levels of testosterone (T) and 17β-estradiol (E2) were measured in rainbow trout during the course of their first and second spawning period. Trout were exposed to normal photoperiod (Group C) and two static, artificial light regimes (Group A, 20L:4D from January to 15 May, then 4L:20D; Group B, 4L:20D from March to August, then 20L:4D). Identical patterns of E2 fluctuations were observed in all groups; E2 levels rose from 54 days prior to spawning and kept rising to reach a peak at 18 days prior to spawning, then decreased steadily reaching basal levels at ovulation. T levels rose from 54 days prior to spawning and peaked 6–8 days prior to spawning and then declined sharply before ovulation, reaching basal levels 6–12 days post spawning. T in male trout peaked 12–0 days prior to milt production. No correlation was found between the steroids levels and percentage of ovulated and spermiated fish. No difference was observed in plasma E2 levels carrying ripe or overripe eggs. T and E2 fluctuations were also observed at the population level (both mature and non-mature fish). These findings support the use of plasma T and E2 levels as an ancilliary predictor index/tool for the initiation of the spawning period and for the determination of the timing for human intervention (stripping).

1. Introduction

The annual reproductive cycle of the female rainbow trout can be divided into four physiological stages: previtellogenesis, endogenous vitellogenesis, exogenous vitellogenesis, and ovulation — spawning (Bohemen et al. 1981). Low levels of plasma sex steroids appear during previtellogenesis, 17β-estradiol (E2) starts rising during endogenous vitellogenesis, reaching high levels (as well as estrone and testosterone) during exogenous vitellogenesis, and a feedback in-

hibition mechanism between sex steroids and gonadotropin exist during oocyte maturation and ovulation (Scott & Sumpter 1983, Elliot et al. 1984, Fostier et al. 1983). The reproductive cycle of the male can be divided into three periods; quiscence, spermatogenesis and spermiation. During the last stages of spermatogenesis and the onset of spermiation high levels of T and 11-oxo-T have been reported (Scott et al. 1980, Fostier et al. 1982, Schulz 1984, Lou et al. 1986).

The ovary of Salmonids, lacks a hollow inner space and the ovulated eggs lie freely in the abdominal cavity. In natural environment, the eggs are held there and in response to the appropriate stimuli they are naturally spawned and fertilized. In captivity, the eggs very often undergo a gradual deterioration known as overripening (Craik & Harvey 1984a). Thus, the time of stripping of the eggs in relation to the date of ovulation seems to be significant factor of determining egg quality (Craik & Harvey 1984b). Furthermore, it appears to be related to the percentage of eggs which survive to hatching (Sakai et al. 1975).

Few studies on short term changes in plasma sex steroids associated with spawning (especially post-spawning) — and spermiation have been reported. (Fostier et al. 1978, Scott et al. 1983, de Mones et al. 1989). In males, at the onset of spermiation there is a decline in plasma androgen levels (Scott & Baynes 1982, Baynes & Scott 1985, Liley et al. 1986, Scott & Sumpter 1989).

In the present study plasma T and E2 in male and female rainbow trout taken at regular short intervals prior, during and post spawning were measured. The aim was to determine the precise timing of hormonal changes associated with ovulation and spermiation and to investigate the possible use of the hormonal levels as an ancilliary index/tool for the timing of stripping. The use of normal and static light photoperiod regimes for two successive years was hoped to add some insight in the complex relation of photoperiod — hormonal changes — spawning.

2. Materials and methods

Rainbow trout (320 fish 15 month old, sex ratio 1:1), were reared together in outdoor raceways ($12 \times 1 \times 1.2$ m) at the National Hatchery of Louros (Epirus, Northwest Greece). The raceway water was supplied from an adjacent pondspring, assuring constant daily and minimal yearly fluctuations (11–14°C) and pH 7.4–7.8. The experimental stock was produced in September 87 from first spawning breeders (previously exposed to a 9-month seasonally compressed light cycle) and kept under natural photoperiod for 15 months (from the fry stage up to the start of the experiment). In January 1989 fish were divided into three groups (140 each) according to the photoperiod protocol used; Group A: 20L-4D from January to May 15, 4L-20D from May 16 to September, Group B: 4L-20D from March to August, 20L-4D from September to November and Group C: normal photoperiod (control).

Fish were fed twice a day by hand, with commercial pellets (Veronesi, Industria Mangini, Italy), at daily rate 3–4% of body weight. Broodstock was fed 1–2% of body weight, once a day; Fish were not fed on the day of sampling. Three series of experiments were peformed.

Series I (1989)

Ten fish were collected from each group at the end of each month from January to September 1989 between 9:30 and 13:00. Body weight, length, GSI was recorded and plasma was collected. The weight and colour of gonads together with the monthly levels of plasma steroids provided an index of the fish maturity stage and of the approximate timing of spawning period. Fish of Groups A, B were tagged and sampling was performed under light anaesthesia (MS 222) every 4th day for a period starting 8 days prior to the initiation of spawning period up to the end (Group A) or 25 days after the end (Group B) of the spawning period.

Series II (1990)

After the completion of the 1st spawning period fish of Groups B, C remained in the raceways under constant 20L:4D – 4L:20D and natural photocycle respectively. Six fish were collected from each group every two months from January to August 1990 between 9:30 and 13:00. The same criteria as above provided an index of the

fish maturity stage and of the approximate timing of spawning period. Fish were tagged and sampling was performed under light anaesthesia (MS 222) every 6th day from the initiation of spawning period up to 24 (Group B) or 36 days after the end (Group C) of the spawning period.

Series III (1991)

84 rainbow trout (11 month old, sex ratio 1:1) were transferred into outdoor raceways and reared under natural photoperiod and temperature. Ten fish were collected at the end of each month from January to September 1991 between 9:30 and 13:00. The same criteria as above provided an index of the fish maturity stage and of the appoximate timing of spawning period. A month prior to the expected initiation of spawning period, 12 fish were bled at the caudal vessels every 15 days in order to clarify if T, E2 levels may be used as an index for the timing of human intervention (stripping).

Spawning (Day 0) was assesed as the time when eggs flowed (freely) from the genital pore of the female trout, when gentle pressure was applied to the abdomen. Spermiation was defined as the period when sperm could be expressed manually from fish. The spawning period was defined as the time when ripe eggs could be stripped and its duration by examination of the population at weekly intervals. At the the end of spawning period no more mature females were present.

In all experiments an heparinized syringe was used to collect blood from the caudal vessels, which was centrifuged at 1600 g and then stored at –70°C until analyzed. Testosterone was analyzed by "Direct RIA" kit (Orion Diagnostica, Finland). The sensitivity was 0.1ng/ml. Cross reactivity was <0.05% and 1.8% for E2 and 11 -oxo -T respectively. Standards and some samples were determined in duplicate and if high values were found (approx. 20% of the samples) they were diluted (1:2–1:10) wih saline. Inter-and intra- assay precision (*CV*) was 8% and 6% respectively. 17β-estradiol was analyzed by Spectria coated tubes kit (ORION Diagnostica, Finland). The sensitivity was approximatelly 0.01 ng/ml. Cross reactivity was < 1.16%, 0.45% and 0.001% for estrone, estriol and T respectively. All samples were diluted 1:10 with "zero" standard. Intra and inter- assay precision (*CV*) was 5%–7% respectively. The Student *t*-test was used for statistical analysis.

3. Results

Results are presented as: a) Population steroid fluctuations, including values from all individual fish in each group (mature and non-mature) and b) individual steroid fluctuations centered around the time of spawning and spermiation (only ovulated and spermiated fish were included).

Spawning period: Timing-duration of spawning and percentages of stripped fish are shown in Table 1. Significantly higher numbers of ovulated females ($P < 0.025$) was recorded in Group A in comparison with Groups B, C (Series I) Group A fish attained spawning period 2 months prior to that of control. In Group B the spawning period was delayed one or 4 weeks (Series I and II respectively) compared to control (Group C). Higher mortality was observed in Groups A, C (20%, 21% respectively) compared to Group B (Series I, 9%, Series II, 8%).

Females

Population fluctuations: Changes in plasma concentrations of T, E2 at population level are

Table 1. Timing, duration of spawning period and percentage of stripped rainbow trout kept under normal and artificial photoperiod regimes. Spawning period (ASP), alteration in the initiation of spawning period, compared to control (Group C); MF, percentage of ovulated females; SM percentage of spermiated males. * = Not recorded.

Series	Group	Timing	Duration (days)	ASP (days)	MF (%)	SM (%)
I	A	14.09–26.10	32	–60	76	91
1989	B	20.11–30.11	10	+7	46	93
	C	13.11–18.12	25	–	50	*
II	B	19.10–28.11	40	+25	75	61
1990	C	24.09–19.11	56	–	85	75
III						
1991		17.12–3.02	48	–	32	*

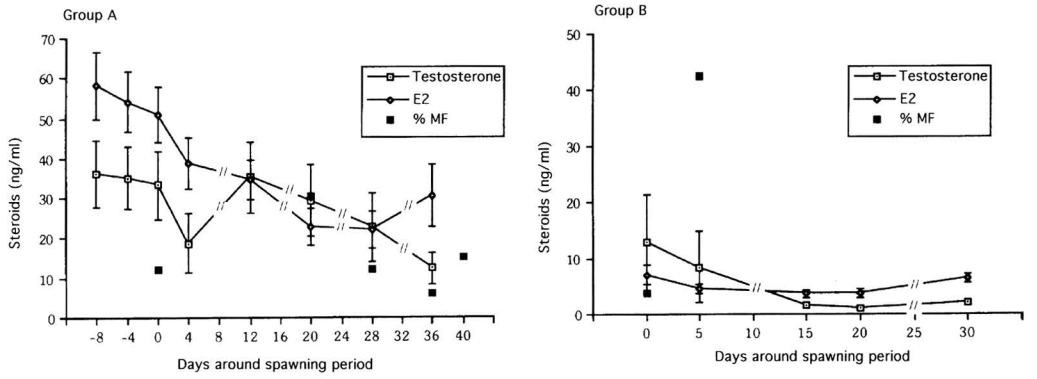

Fig. 1. Changes in plasma testosterone and estradiol (E2) levels ($\bar{x} \pm SE$) (n = 10–33) throughout the 1st spawning period in a population of female rainbow trout (Series I) kept under artificial photoperiod regimes. 0 – initiation of spawning period; % MF – percentage of ovulated females. Symbol II means that no sampling was performed or data are not available.

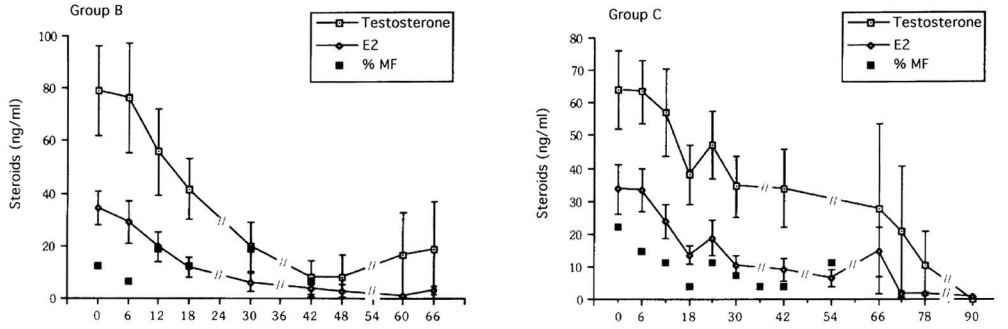

Fig. 2. Changes in plasma testosterone and estradiol (E2) levels ($\bar{x} \pm SE$) (n = 5–28) throughout the 2nd spawning period in a population of female rainbow trout (Series II). 0 – initiation of spawning period; % MF – percentage of ovulated females. Symbol II means that no sampling was performed or data are not available.

illustrated in Figs. 1, 2. In all series of experiments similar patterns of E2 fluctuation were observed; E2 levels were decreasing steadily 8 days prior to the initiation of spawning period, reaching basal levels 10 days prior to the end of spawning period. The peak of E2 was statistically significant higher than the level observed in day +12 (Fig. 1, Group A and Fig 2, Group B) + 15 (Fig. 1, Group B) or + 18 (Fig. 2) after the initiation of spawning period. T levels declined at the onset of the spawning period, reaching basal levels shortly before or after the end of the spawning period. The decline of T levels was more gradual in Group B (Series I and II) and similar in all groups 12 days after the start of spawning period. Statistically significant higher values of T ($P < 0.001$), were observed in Series II groups compared with Series I.

Individual fluctuations: T, E2 fluctuations in female fish, centered around the time of ovulation are illustated in Fig. 3. Models of E2 changes were indentical in all Groups. E2 levels increased 54 days prior to ovulation, peaked 18 days ($P < 0.005$) before ovulation and then decreased steadily, reaching basal levels at ovulation, where they remained low for further 48 days. Similar T changes were observed in all Groups. T levels increased from 54 days up to 6–8 days prior to

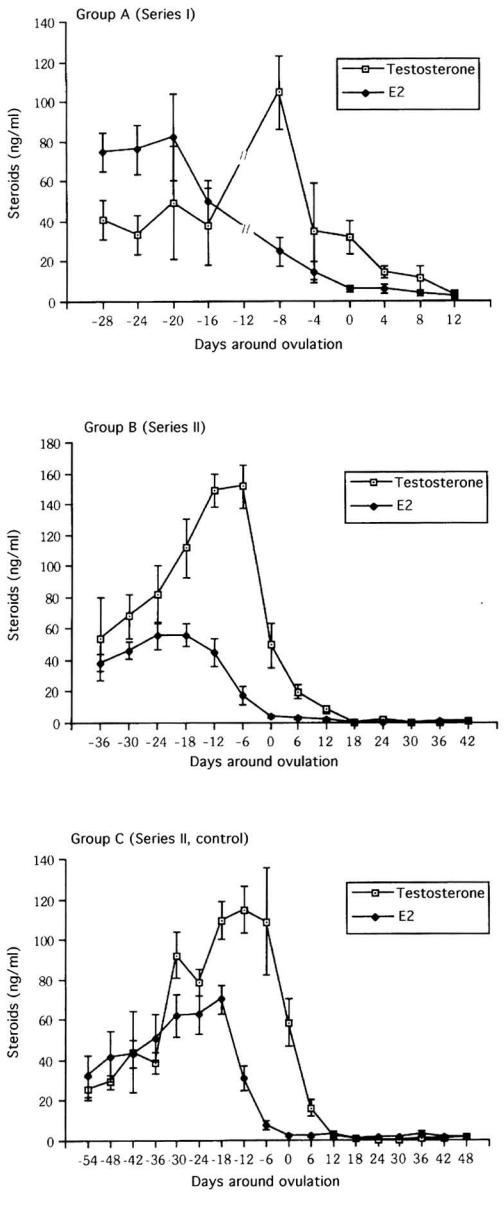

Fig. 3. Changes in plasma testosterone and estradiol (E2) levels ($\bar{x} \pm SE$) (n = 4–17)in individual females centered around the time of ovulation. 0 – ovulation. Symbol II means that no sampling was performed or data are not available.

ovulation (peak value), ($P < 0.005$) decreased sharply before ovulation, reaching basal levels 6–12 days after ovulation up to 48 days post ovulation.

Males

T plasma changes at the population level are illustrated in Figs. 4, 5. T showed a small decline from 6 days prior to the onset of spermiation up to 8 days post-spermiation, and then remained steadily low (3.4–21.2 ng/ml). T plasma changes in male fish, centered arround the time of first sperm appearance, are illustrated in Fig. 6. In Groups A, ($P < 0.025$) and C (NSD) T peaked at day 0 (first appearance of milt by gentle pressure on the abdomen) and then declined steadily. However, in Group B (Series II) T peaked 12 days ($P < 0.01$) prior to milt production. No correlation was found between T levels and percentage of spermiated males.

Series III

T, E2 levels were low 5–3 months prior to the start of spawning period reached a peak 67–47 days prior to the start of spawning period and declined thereafter (Fig. 7). These results are in accordance to those of series I, II. The high value of T (20.6±9.2 ng/ml) observed 65 days after the start of spawning period were due to the presence of 3 tardive mature fish.

4. Discussion

Few studies have been conducted on the hormonal changes accompaning the last stages of rainbow trout reproductive cycle. Fostier et al. (1978) reported the highest levels of E2 few weeks before spawning and a drop to basal levels prior to ovulation. Over the course of first ovulation period in 14 female rainbow trout T levels peaked 8 days prior to ovulation (276 ng/ml) (Scott et. al. 1983), decreased markedly before ovulation, more steadily thereafter, reaching basal levels 28 days post ovulation. Similarly E2 fell to 15–17 ng/ml 12 days prior to ovulation to basal levels (2–3 ng/ml), at 4 days prior to ovulation, remaining low up to 32 days post ovulation. Mones et al. (1989) studied estrogen production 5–50 days post ovulation and found low but detectable levels of E2 and a peak (5.5 ng/ml) within the first month post ovulation. Our results are in agreement with the above mentioned although there

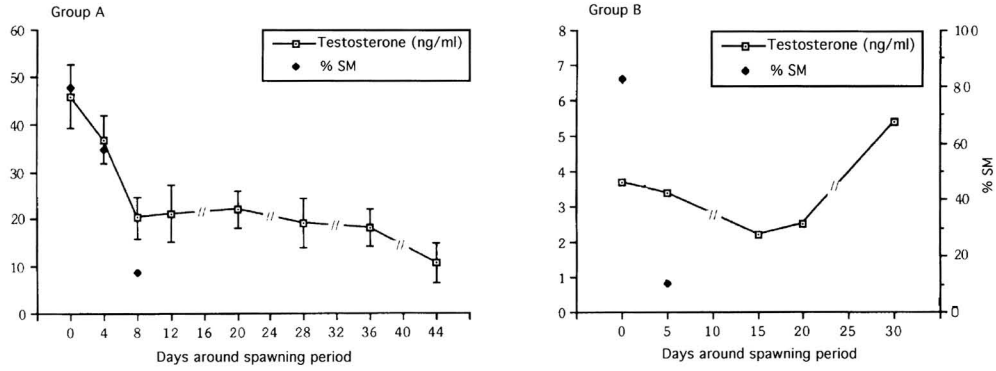

Fig. 4. Changes in plasma testosterone levels ($\bar{x} \pm SE$)(n = 7–28) throughout the 1st spawning period in a population of male rainbow trout (Series I) kept under artificial photoperiod regimes. 0 – initiation of spawning period; % SM – percentage of spermiated males. Symbol II means that no sampling was performed or data are not available.

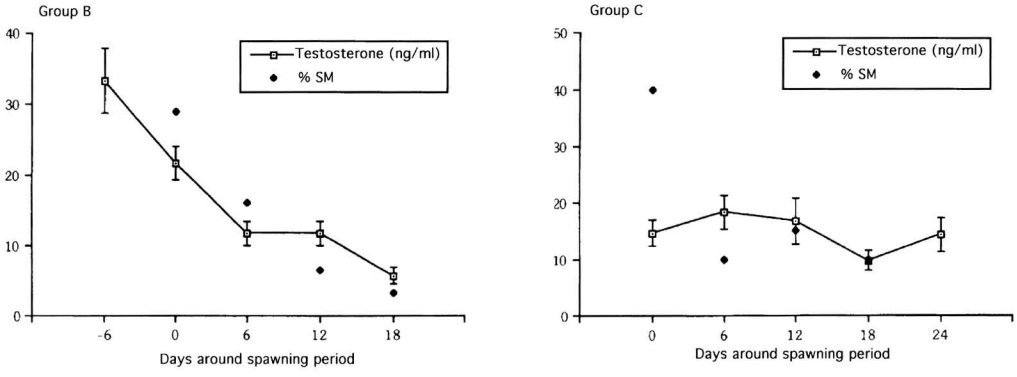

Fig. 5. Changes in plasma testosterone levels ($\bar{x} \pm SE$) (n = 11–32) throughout the 2nd spawning period in a population of male rainbow trout (Series II). 0 – initiation of spawning period; % SM – percentage of spermiated males.

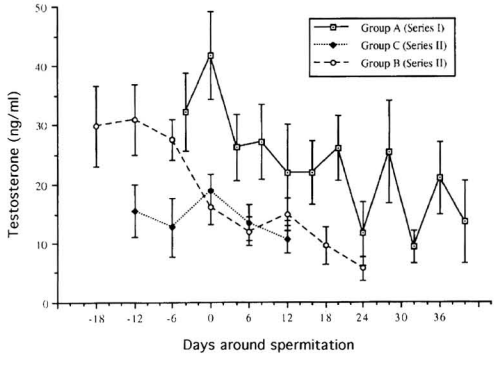

Fig. 6. Changes in plasma testosterone levels ($\bar{x} \pm SE$) (n = 6–21) in individual males centered around the time of spermiation (first appearance of sperm). 0 – spermiation.

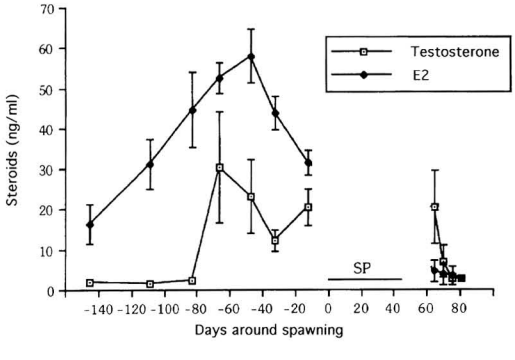

Fig. 7. Changes in plasma testosterone and estradiol (E2) levels ($\bar{x} \pm SE$) (n = 10–12) in Series III female rainbow trout exposed to normal photoperiod.

were differences: a) in environmental conditions (water temperature – quality, latidute), b) management (type of tank, rearing of male-female fish together etc.), c) strain and number of examined fish. We studied two succesive reproductive cycles (1st, 2nd) and we used various photoperiodic regimes, a possible advantage. Thus, we can speculate that a general pattern on T, E2 fluctuations exists throughout ovulation: T is increasing from 54 days up to 8–6 days (peak value) prior to ovulation, followed by an abrupt decrease and remainig low up to 48 days post-ovulation. High E2 levels are observed 20–18 days prior to ovulation, decreasing steadily thereafter, reaching basal levels 4 days prior to ovulation.

T and E2 plasma fluctuations were also observed at the population level. E2 fluctuation pattern was very similar in all groups; E2 levels started decreasing steadily 8–5 days prior to the initiation of the spawning period, reaching basal levels at the end of the spawning period. Slight differences occurred in T profil of the various groups. Higher T levels were observed throughout spawing, of the second time spawners, when compared with the first time spawners.

In adult trout, the interstitial and theca cells exhibit a maximal Hydroxy Steroid Dehydrogenase activity at the time of meiotic maturation and ovulation (Van Den Hurk & Peute 1979). Aromatase activity also declines during the same period (Bohemen & Lambert 1981). The progressive switching off of enzymes at the time preceeding ovulation could explain the observed drop in E2 (Scott et al. 1983). It is also possible the falling levels of E2 20–18 days prior to ovulation, contribute to the removal of a negative feedback mechanism of E2 on gonadotropin (GtH) secretion, allowing an initial rise of GtH. A direct negative effect on ovarian sensitivity to maturational GtH can not be excluded (Fostier et al. 1983). Scott et al. (1983) suggested that the rising levels of GtH may suppress the C21–C19 desmolase and/or induce the 20β-hydroxy-steroid oxidoreductase activity in the steroid secreting cells, stimulating the rapid increase of progestagens with a concominant fall in androgen levels. In vivo and in vitro studies confirmed this suggestion and lead to the assumption that increasing GtH levels act on follicles ready to undergo maturation by stimulating the production of androgens whose aromatization to E2 is depressed and by modulating the enzymes implicated in the synthesis of the maturation inducing steroid 17α, 20β-0H-Progesterone (Zohar et al. 1986). This may expain the dramatic decrease in T plasma levels observed 12–6 days prior to ovulation.

Photoperiod altered the timing of spawning. The effects of photoperiod on spawning time are generally associated with changes in the levels and timing of peak values of plasma E2, T (Duston & Bromage 1987). However, the E2 pattern of fluctuations in the last stages of the reproductive cycle (during spawning period) is not influenced by the artificial photoperiod used. E2 fluctuations observed in all groups were almost indentical in spite of the shift in spawning time, indicating that a) light maintains its effect on spawning period at the early and middle stages of the reproduction cycle and b) the endocrinological events associated with the last stages, essential for the success of the maturation course, are not affected by photoperiod.

In male rainbow trout spermiation, defined as the release of motile sperm from the genital pore by hand-stripping, and milt production seems to be under hormonal control (Billard et al. 1982, Baynes & Scott 1985, Nagahama 1987). T fluctuations were observed during the spermiation period in all groups, with differences in the range of fluctuations, in absolute values and in the timing of peak T value.

Scott et al. (1980) and Scott & Baynes (1982) reported maximum T levels at the onset of spermiation, although Whitehead et al. (1979) reported a constant increase from 4 ng/ml (April) to 20 ng/ml (January) — spermiation occurred in December. In our study T ranged 20.8–42.2 ng/ml, from 4 days prior to milt production up to 8 days after [Group A] whereas in Group B (Series II) it ranged 24–36.6 ng/ml, from 18 days prior up to 8 days prior to milt production. The above concentrations are similar to those observed at the final stages of spermatogenesis. In contrast, lower concentrations of T values were observed in groups B (Series I) and C (controls). It is not clear if these differences can be attributed to the photoperiod protocol used. It seems that high T levels occur shortly before or at the onset of

spermiation. 11-KT peaks just before noticable amounts of sperm are produced, but thereafter both T and 11-KT levels decline while sperm production is still rising (Baynes & Scott 1985). It is generally assumed (Scott & Baynes 1982, Nagahama 1987) that the fall in T levels observed during spermiation may be explained by the combined action of gonadotropin and testicular somatic elements to stimulate the production of steroidal mediator (s).

A significant range of intraspecific variation in plasma T, E2 levels was observed between individual parent females prior and the onset of spawning and the extend of the variation was higher in T. The variation is unlikely to be attributed to differences in the photoperiod (it appeared in all groups) or to nutrition. It may reflect the normal biochemical counterpart of well documented variation in e.g. fecundity, egg size and biochemical composition of fertilized eggs, observed previously in rainbow trout and other salmonids (Craik & Harvey 1984a, b). The effects, if any, of such variation in egg quality, hatching success and larval survival remain to be investigated.

It is well known that the time of stripping in relation to the time of ovulation is an important factor in determining the hatching success of rainbow trout eggs. Therefore knowledge of the time of ovulation in each parent female is essential for the achievement of maximum egg viability (Craik & Harvey 198b). Sakai et al. (1975) found that the hatching percentage declined gradually from over 70% when eggs were stripped up to 10 days post ovulation to zero for more than 30 days after ovulation. Craik & Harvey (1984b) showed that hatching percentages of eggs from one fish felt from >90% to zero between 6 and 10 days after ovulation, without any appreciable changes in gross composition of the eggs. Furthermore, they reported differences in the biological composition between ripe and overripe eggs. In the present sudy no differences were observed in the plasma E2 levels between mature and overripe individual females.

In rainbow trout the beginning of oocyte maturation (when meiosis is resurned) to ovulation (when the oocytes are released into the body cavity) takes about 50 degree (°C) days (that is, at 10°C this would take 5 days) (Bry 1981). In our study T peaked 6–8 days prior to handstripping which corresponds to the critical period between ovulation and spawning Measurements at the population level (by examining 10–12 fish), at regular intervals one month prior to the expected day of the initiation of the spawning period may contribute to existing techiques.

Acknowledgments. Grants from the Ministries of Agriculture and Industry-Energy-Technology (General Secretariat of Research and Technology), are gratefully acknowledged as well as advice and support from cadres of both Ministries. We are indepted to Drs Theochari, A. Strantzali, K. Sepheriades for invaluable help and to Dr M. Typas for help in manuscript preparation and to to Mrs M. Dimitriadou for secretarial help.

References

Baynes, S. M. & Scott, A. P. 1985: Seasonal variation in parameters of milt production and in plasma concentrations of sex steroids of male rainbow trout (Salmo gairdneri). — General and Comparative Endocrinology 57: 150–160.

Billard, R., Fostier, A., Weil, C. & Breton, B. 1982: Endocrine control of spermatogenesis in teleost fish. — Canadian Journal of Fisheries Aquatic Sciences 39: 65–79.

Bohemen, Gh. G. van & Lambert, J. G. D. 1981: Estrogen synthesis in relation to estrone, estradiol and vitellogenin plasma levels during the reproductive cycle of the female rainbow trout, Salmo gairdneri. — General and Comparative Endocrinology 45: 105–114.

Bohemen, Gh. G. van & Lambert, J. G. D. & Peute, J. 1981: Annual changes in plasma and liver in relation to vitellogenesis in the female rainbow trout, Salmo gairdneri. — General and Comparative Endocrinology 44: 94–107.

Bry, C. 1981: Temporal aspects of macroscopic changes in rainbow trout (Salmo gairdneri) oocytes before ovulation, and of ova fertility during the postovulation period: effect of treatment with 17a -hydroxy- 20β - dihydroprogesterone. — Aquaculture 24: 153–160.

Craik, J. C. A. & Harvey, S. M. 1984a: Biochemical changes associated with overripening of the eggs of rainbow trout Salmo gairdneri Richardson. — Aquaculture 37: 347–357.

— 1984b: Egg quality in rainbow trout: the relation between egg viability, selected aspects of egg composition, and the time of stripping. — Aquaculture 40: 115–134.

Duston, J. & Bromage, N. 1987: Constant photoperiodic regimes and the entrainment of the annual cycle of reproduction in the female rainbow trout (Salmo

gairdneri). — General and Comparative Endocrinology 65: 373–384.

Elliot, J. A. K., Bromage, N. R. & Springate, J. R. C. 1984: Changes in reproductive function of three strains of rainbow trout exposed to constant and seasonally-changing light cycles. — Aquaculture 43: 23–34.

Fostier, A., Weil, C., Terqui, M., Breton, B. & Jalabert, B. 1978: Plasma estradiol -17β and gonadotropin during ovulation in rainbow trout (Salmo gairdneri R). — Annales de biologie animale biochimie biophysique 18(4): 929–936.

Fostier, A., Billard, R., Breton, B., Legendre, M. & Mariot, S. 1982: Plasma 11-oxotestosterone and gonadotropin during the beggining of spermiation in rainbow trout (Salmo gairdneri R). — General and Comparative Endocrinology 46: 428–438.

Fostier, A., Jalabert, B., Billard, R., Breton, B. & Zohar, Y. 1983: The gonadal Steroids. — In: Hoar, W. S., Randall, D. J. & Donaldson, E. M. (eds.), Fish physiology, Vol. IX: 277–372. Academic Press, New York, London.

Liley, N. R., Breton, B., Fostier, A. & Tan, E. S. R. 1986: Endocrine changes associated with spawning behavior and social stimuli in a wild population of rainbow trout (Salmo gairdneri). I. Males. — General and Comparative Endocrinology 62: 157–167.

Lou, S. W., Aida, K., Hanyu, I., Sakai, K., Nomura, M., Tanaka, M. & Tazaki, S. 1986: Endocrine profiles in the males of a twice-annually spawning strain of rainbow trout, Salmo gainderi. — General and Comparative Endocrinology 64: 212–219.

de Mones, A., Fostier, A., Cauty, C. & Jalabert, B. 1989: Ovarian early postovulatory development and oestrogen production in rainbow trout (Salmo gairdneri R from a Spring-spawning Strain. — General and Comparative Endocrinology 74: 431–441.

Nagahama, Y. 1987: Gonadotropin action on gametogenesis and steroidogenesis in teleost gonads. — Zoological Sciences 4: 209–222.

Sakai, K., Nomura, M., Takashima, F. & Oto, M. 1975: The over-ripening phenomenon of rainbow trout – II. Changes in the percentage of eyed eggs hatching rate and incidence of abnormal alevins during the process of overripening. — Bulletin Jpn. Soc. Fish 41: 855–860.

Schulz, R. 1984: Serum levels of 11-oxotestosterone in male and 17β-estradiol in female rainbow trout (Salmo gairdneri) during the first reproductive cycle. — General and Comparative Endocrinology 56: 111–120.

Scott, A. P. & Baynes, S. M. 1982: Plasma levels of sex steroids in relation to ovulation and spermiation in rainbow trout (Salmo gairdneri). — In: Richter, C. J. J. & Goos, H. J. Th. (eds.), Reproductive physiology of fish: 103–106. PUDOC, Wageningen. The Netherlands.

Scott, A. P. & Sumpter, J. P. 1983: The control of trout reproduction: basic and applied research on hormones. — In: Rankin, J. C., Pitcher, T. J. & Duggan, R. T. (eds.), Control processes in fish physiology: 200–220. Croom Helm, London & Camberra.

— 1989: Seasonal variations in testicular germ stages and in plasma concentrations of sex steroids in male trout (Salmo gairdneri) maturing at 2 years old. — General and Comparative Endocrinology 73: 46–58.

Scott, A. P., Bye, V. J., Baynes, S. M. & Springate, J. R. C. 1980: Seasonal variations in plasma conentrations of 11-ketotesterone and testosterone in male rainbow trout (Salmo gairdneri R.). — Journal of Fish Biology 17: 495–505.

Scott, A. P., Sumpter, J. P. & Hardiman, P. A. 1983: Hormone changes during ovulation in the rainbow trout (Salmo gairdneri Richardson). — General and Comparative Endocrinology 49: 128–134.

Van Den Hurk, R. & Peute, J. 1979: Cycle changes in the ovary of the rainbow trout (Salmo gairdneri, with special reference to sites of steroidogenesis. — Cell Tissue Research 99: 289–306.

Whitehead, C., Bromage, N. R., Breton, B. & Matty, A. J. 1979: Effect of altered photoperiod on serum gonadotropin and testosterone levels in male rainbow trout. — Journal of Endocrinology 81: 139–140.

Zohar, Y., Breton, B. & Fostier, A. 1986: Short-term profiles of plasma 17α–hydroxy, 20β-dihydroprogesterone levels in the female rainbow trout at the periovulatory period. — General and Comparative Endrocrinology 64: 189–198.