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Photoperiodic history determines the reproductive response of rainbow trout to changes in daylength

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Abstract The mechanisms underlying the photoperiodic entrainment of the endogenous circannual rhythm of maturation in the rainbow trout were investigated by subjecting December-spawning fish to abrupt changes in daylength which varied in their timing or magnitude. These protocols advanced spawning by up to 4 months. Maturation occurred in sequence in fish maintained on 18L:6D from January and February, and in fish exposed to 18L:6D from December, January and February, followed by 6L:18D in May, indicating that the abrupt increases in daylength were effective entraining cues. 'Long' photoperiods of between 12 and 22 h applied in January, followed by shorter photoperiods of between 3.5 and 13.5 h from May, were equally effective in advancing maturation. Maturation was also advanced, though to a lesser extent, in fish maintained on photoperiods of 8.5 or 10 h from January, followed by a photoperiod of 1.5 h from May. In contrast, maturation was delayed in fish maintained under a constant 8.5-h photoperiod from January, and these fish also exhibited a desynchronization of spawning times characteristic of endogenous circannual rhythms in free-run. Collectively, these results indicate that photoperiodic history determines the reproductive response of rainbow trout to changes in daylength.

Key words Photoperiod · History · Reproduction · Circannual · Trout

Abbreviations *L* light · *D* dark

Introduction

The rainbow trout, *Oncorhynchus mykiss*, is a seasonally breeding teleost, which spawns for a brief (typically 6- to 8-week) period each year. In common with many other organisms inhabiting temperate and polar latitudes, the primary environmental influence on reproductive timing is the seasonally changing daylength (Bromage and Cumaranatunga 1988; Bromage et al. 1993).

On the basis of experiments demonstrating a stimulatory effect of either short or long days on gonadal maturation the rainbow trout has been variously classified as a 'short-day' animal (Breton and Billard 1977; Billard and Breton 1978; Billard et al. 1978; Peter 1981; Follett 1982) and a 'long-day' animal (Bromage et al. 1982; Elliott et al. 1984; Scott et al. 1984), terms which imply the existence of a 'critical daylength' above or below which reproductive development is initiated. However, there is now convincing evidence that, rather than actively driving or inducing reproductive development in this species, photoperiod acts as a zeitgeber to entrain an endogenous circannual rhythm of maturation (Duston and Bromage 1986, 1991; Randall et al. 1998); under constant conditions these rhythms 'free-run', exhibiting their innate circannual periodicity.

Photoperiod regimes comprising alternate periods of constant 'long' (usually 16L:8D or 18L:6D) and constant 'short' (usually 6L:18D or 8L:16D) days have provided a useful tool for investigation of the mechanisms underlying the photoperiodic entrainment of circannual rhythms. Duston and Bromage (1987, 1988) reported that exposure to a constant 'long' photoperiod (18L:6D) early in the reproductive cycle, followed 1.5–4.5 months later by an abrupt reduction to a shorter photoperiod, advanced spawning by up to 3–4 months. These studies emphasized the ability of abrupt reductions in daylength to act as entraining cues for the advancement in spawning time; the earlier the reduction in photoperiod occurred relative to the phase of the circannual cycle the greater was the advance in the timing of maturation (Duston and Bromage 1988).

In such studies it is apparent that the initial abrupt increase in daylength would also have served as an

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entraining cue, as evidenced by the 1- to 2-month advance in spawning time observed in fish subjected to a constant 18L:6D photoperiod from early in their reproductive cycle until spawning (Bromage et al. 1982, 1984; Duston and Bromage 1987, 1988). However, the effect of varying the timing of an increase in daylength on the entrainment of the circannual 'clock' controlling reproduction has not been investigated.

There is also evidence that the reproductive response of rainbow trout to a particular daylength may depend on its photoperiodic history. Thus, it has been suggested that a daylength of 10L:14D may be interpreted as a 'long' day when preceded by 6L:18D (Randall et al. 1987). Similarly, a daylength of 14L:10D appears to be interpreted as a 'short' day when preceded by 18L:6D (Duston and Bromage 1987). It has therefore been proposed that direction of change of daylength may be more important than absolute daylength for determining the timing of maturation and spawning in rainbow trout (Duston and Bromage 1987; Randall et al. 1987).

In order to clarify which features of the photoperiodic signal are utilized to entrain the endogenous circannual clock which ultimately determines the timing of reproduction in rainbow trout the present study investigated the effects of varying the timing of an increase in photoperiod, and varying the magnitude of an increase in photoperiod (i.e. varying the length of the 'long' day), on the timing of maturation and spawning in the female rainbow trout.

Materials and methods

Animals and husbandry

Rainbow trout from an established domesticated stock with a natural spawning period of November–December were used in all experiments. At the start of each experiment fish were transferred from ambient daylength (latitude 52°30N) to one of six circular 1200-l-capacity glass-fibre tanks enclosed in a wooden framework and lightproofed with industrial-grade black polythene sheeting. The fish were assigned to the tanks in a random manner. The tanks were supplied with constant temperature (7.5–8 °C) spring water and tungsten filament light bulbs which provided a light intensity of 25–30 lx at the water surface. Daylength was controlled by 24-h electronic time switches (Smith's Industries, London, UK). The tanks were covered with fine mesh netting to prevent fish escaping.

Individual fish were identified by plastic numbered tags (Charles Neal, East Finchley, London, UK) attached through the muscle at the base of the dorsal fin. When fish belonging to different year classes were maintained in the same tank different coloured tags were used to provide rapid identification of the age of a fish. Additionally, a Panjet (F.H. Wright, Dental MFG Company, West Dundee, UK) was use to dye mark (alcian blue, 1% w/v in water: Sigma Chemical Company, Poole, Dorset, UK) fish on their ventral surface to facilitate identification of year class in the event of a tag loss.

At the beginning of each experiment the fish were weighed and thereafter fed daily with the recommended ration and pellet size of a commercially available trout diet (Trouw Aquaculture, Northwich, Cheshire, UK). All fish were starved for 24–48 h prior to any manipulative procedure.

Photoperiod treatments

Three experiments were conducted: the first (Experiment 1) to investigate the effects of varying the timing of an increase in photoperiod, the second and third (Experiments 2a and 2b) to investigate the effects of varying the magnitude of an increase in photoperiod. The experiments consisted of a number of groups subjected to a variety of different photoperiods. Each group comprised approximately 25 individuals: in Experiment 2a these were all 2-year-old (2+) virgin fish at the start of the experiment, whereas each group in Experiments 1 and 2b consisted of approximately ten 2-year-old virgin fish, and fifteen 3-year-olds (3+), the latter having spawned naturally for the first time just prior to the start of the experiments. In Experiment 1 the effective n was reduced across all groups in April following a temporary interruption to the water supply which resulted in the death of 27 individuals.

Experiment 1

The effects of varying the timing of an increase to a constant long photoperiod were assessed by transferring fish from ambient daylength to the following photoperiod regimes (Fig. 1):

Group A – 18L:6D from December 23 until May 15 followed by 6L:18D until spawning.
Group B – 18L:6D from January 19 until May 15 followed by 6L:18D until spawning.
Group C – 18L:6D from January 19 until spawning.
Group D – 18L:6D from February 19 until May 15 followed by 6L:18D until spawning.
Group E – 18L:6D from February 19 until spawning.

Experiment 2a

The effects of varying the magnitude of an increase in photoperiod were assessed by transferring fish on January 19 from ambient daylength (8.5L:15.5D) to the following photoperiod regimes (Figs. 3, 4):

Group A – 22L:2D until May 6 followed by 13.5L:10.5D until spawning.
Group B – 20L:4D until May 6 followed by 11.5L:12.5D until spawning.
Group C – 18L:6D until May 6 followed by 9.5L:14.5D until spawning.
Group D – 16L:8D until May 6 followed by 7.5L:16.5D until spawning.
Group E – 14L:10D until May 6 followed by 5.5L:18.5D until spawning.
Group F – 8.5L:15.5D until spawning.

Experiment 2b

The effects of varying the magnitude of an increase in photoperiod were further assessed by transferring fish on January 17 from ambient daylength (8.5L:15.5D) to the following photoperiod regimes (Fig. 6):

Group A – 22L:2D until May 9 followed by 13.5L:10.5D until spawning.
Group B – 18L:6D until May 9 followed by 9.5L:14.5D until spawning.
Group C – 14L:10D until May 9 followed by 5.5L:18.5D until spawning.
Group D – 12L:12D until May 9 followed by 3.5L:20.5D until spawning.
Group E – 10L:14D until May 9 followed by 1.5L:22.5D until spawning.
Group F – 8.5L:15.5D until May 9 followed by 1.5L:22.5D until spawning.

The 8.5-h reduction in daylength in May utilized in Experiments 2a and 2b was selected because it approximates to the dif-

ference in daylength between the summer and winter solstices at the latitude at which the experiments were conducted.

Assessment of maturation and spawning

The 100-million-fold increase in oocyte volume which occurs in trout prior to ovulation is primarily due to the sequestration of yolk in the form of vitellogenin, a high-molecular-weight glycolipophosphoprotein which is synthesised by the liver (induced by oestradiol-17β) and released into the blood (reviewed by Tyler and Sumpter 1996). Vitellogenin levels peak at ovulation, at which time it is the major blood protein (Sumpter 1984). Calcium is an integral component of the vitellogenin complex and, consequently, serum calcium levels provide a useful and accurate index of vitellogenin levels in female rainbow trout (Elliott et al. 1984). The rate of maturation of each group was therefore assessed by measuring total serum calcium levels at approximately monthly intervals. Blood samples were withdrawn from the Cuverian sinus of anaesthetized fish (0.05% v/v 2-phenoxyethanol; Sigma) into 5-ml serum monovettes (Sarstedt, Leicester, UK); where groups consisted of two year classes, blood samples were either obtained from both 2+ and 3+ fish (Experiment 1) or were taken exclusively from 3+ fish (Experiment 2b). Following centrifugation, the resulting serum was stored at −20 °C until analysis for calcium. Serum calcium was determined fluorometrically using a Corning calcium analyser (model 940; Corning Scientific Instruments, Medfield, Mass., USA). Aliquots of pooled serum with a calcium content of approximately 30 mg 100 ml^{-1} were used for quality control; the inter- and intra-assay coefficients of variation were 4.87 and 2.80%, respectively.

Anaesthetized fish were examined at approximately monthly intervals outside the expected spawning period and at 2-week intervals as the fish approached maturity. The time of spawning for an individual fish was defined as the date on which eggs could be manually expressed from the genital papilla by exerting gentle downward pressure on the abdomen. The mean natural spawning time for each experiment was estimated from the spawning times of commercial numbers of the same stock maintained under natural daylight in adjacent ponds.

Statistical analysis

Homogeneity of variances was tested using the *F*-test (Fowler and Cohen 1987). Comparisons between spawning profiles for which the assumption of homogeneity was satisfied were performed by one-way analysis of variance (ANOVA) followed by a parametric multiple-comparisons *t*-test using the residual mean square from the ANOVA to provide a pooled estimate of the variance (Snedecor and Cochran 1980). Outliers were detected using Dixon's test (Sokal and Rohlf 1981). Calcium data, and spawning data for which assumptions of homogeneity were not satisfied, were analysed by the Kruskal-Wallis test followed by Dunn's multiple comparisons procedure (Zar 1984). The influence of age (2+ or 3+) on the spawning time of fish was analysed by two-way ANOVA. Differences in the proportion of fish attaining maturation were analysed by comparison of 95 and 99% confidence limits (Fowler and Cohen 1987).

Results

Varying the timing of an increase to a constant long photoperiod (Experiment 1)

Spawning

The spawning times of the individual fish in each group are illustrated in Fig. 1. Groups in which the reduction in photoperiod in May was preceded by an increase to a 'long' daylength on December 23 (group A), January 19 (group B) or February 19 (group D), commenced spawning on August 26, August 26 and September 23, respectively; 91, 95 and 67% of the fish attained maturity over periods of 6, 8 and 6 weeks.

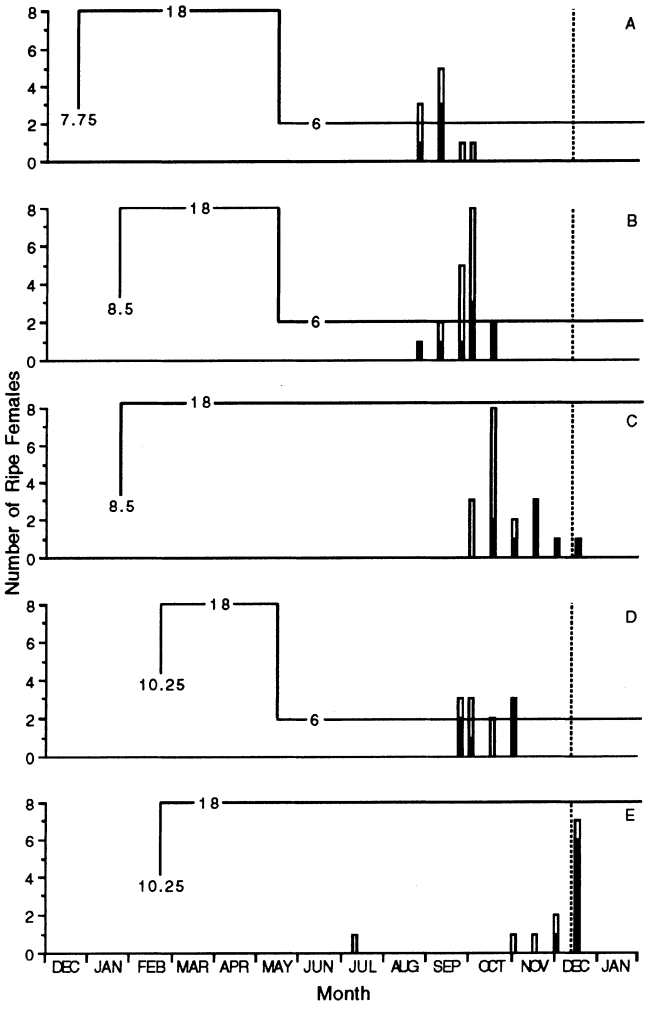

Fig. 1A–E The effects of five photoperiod regimes (Experiment 1) on the timing of maturation in female rainbow trout. The stacked histograms represent the number of ripe females on each sampling date; fish spawning for the first time are shown in *black*, those undergoing their second reproductive cycle in *white*. In December the histograms for group E represent an estimate of the spawning time. *Line graphs* indicate the photoperiod regime (hours of light/day) applied to each group. *Dashed vertical lines* indicate the mean spawning time of fish maintained under ambient daylength

Groups which received an increase in daylength on January 19 (group C) and February 19 (group E), but not a subsequent reduction, commenced spawning on October 7 and November 5, respectively, approximately 6 weeks later than their counterparts in groups B and D. In group C, 86% of the fish spawned over a period of 10 weeks. Unfortunately, an interruption to the water supply resulted in the death of all the fish in group E on December 2–3. Examination of mortalities on December 4 revealed that two had ovulated within the previous 12–13 days. The

remaining nine fish were assessed for maturity by visual inspection, measurement of oocyte diameter and ovary weight and calculation of the Gonadosomatic Index (GSI), which expresses gonad weight as a percentage of body weight {GSI = [gonad weight (kg)/body weight (kg)] × 100}. From these assessments, seven of the fish appeared close to ovulation (GSI = 6.3–14.7 and would probably have matured by the next or subsequent sampling dates (2–4 weeks). Two of the fish were assessed as immature (GSI = 0.25 and 0.57) and unlikely to spawn that year. On the basis of these estimates, which have been incorporated in Fig. 1, 85% of the fish in group E would have spawned over a period of 6–8 weeks.

The differences in the mean spawning times between groups were all significant at the $P \leq 0.001$ level with the exception of groups A versus B ($P \leq 0.01$), and groups B versus D and C versus D ($P \leq 0.05$), though it should be noted that the single fish which spawned in early July in group E was designated as an 'outlier' ($P \leq 0.01$) and hence was excluded from the statistical analysis to maintain homogeneity of variances. Although there was a tendency for 2+ rainbow trout to spawn later than 3+ fish, the effect of age on spawning time was not statistically significant ($P \geq 0.05$). There were no significant differences between the proportions of fish attaining maturity either between groups or between year classes within groups.

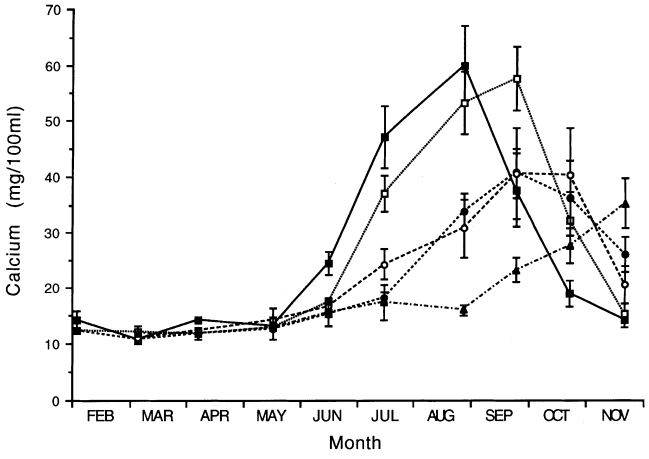

Fig. 2 The effects of five photoperiod regimes (Experiment 1) on the timing of changes in total serum calcium levels (mean ± SEM) during maturation in female rainbow trout; data combined from 2- and 3-year-old fish (——■—— group A, 18L:6D-Dec/6L:18D-May; ·····□····· group B, 18L:6D-Jan/6L:18D-May; ---●--- group C, 18L:6D-Jan; ---○--- group D, 18L:6D-Feb/6L:18D-May; ·-·-·-·- ▲ ·-·-·-·- group E, 18L:6D-Feb)

Total serum calcium

All groups exhibited significant changes ($P \leq 0.001$) in total serum calcium levels during the reproductive cycle. The timing of these changes was similar in 2+ and 3+ fish, and there was no consistent relationship between age and the amplitude of the calcium profiles. However, there were considerable differences in the timing of these changes (Fig. 2), consistent with the differences in spawning time between groups. Thus, calcium levels in group A began to increase between May and June, rose steeply between June and July, and peaked at about 60 mg 100 ml^{-1} in August; at the latter three time points levels were significantly higher ($P \leq 0.05$) in group A than in groups C, D and E.

Much shallower increases in serum calcium occurred in groups B–E between May and June, at which time levels were significantly higher ($P \leq 0.05$) in group B than in group E. In group B levels rose steeply through July and August and peaked at approximately 58 mg 100 ml^{-1} in September; these levels were significantly elevated ($P \leq 0.05$) compared to group C in July, group D in August, and group E at all three time points.

Serum calcium profiles were similar in groups C and D, exhibiting a more gradual increase than those observed in groups A and B, and attaining lower peaks of about 41 mg 100 ml^{-1} by September; calcium levels in groups C and D remained close to peak values in October, in contrast to the marked decline in levels observed in groups A and B by this time. Calcium levels in group E remained slightly higher than basal values from June to August, after which they gradually increased, reaching approximately 35 mg 100 ml^{-1} by the last sampling point in November; at this time levels in group E were significantly elevated ($P \leq 0.05$) compared to those in groups A and B, which were close to basal.

Varying the magnitude of an increase in photoperiod-I (Experiment 2a)

Spawning

The spawning times of the individual fish in groups A–E are illustrated in Fig. 3 and those of group F in Fig. 4a. When the photoperiod was reduced by 8.5 h on May 6 from 22L:2D (group A), 20L:4D (group B), 18L:6D (group C), 16L:8D (group D) or 14L:10D (group E) spawning commenced on August 17, August 31, August 17, August 17 and August 31, respectively, approximately 3 months in advance of the natural spawning period. The proportion of fish attaining maturity in groups A–E, respectively was 92, 96, 100, 96 and 82% over periods of 8, 8, 10, 10 and 8 weeks.

Group F (short days only) commenced spawning on December 20, approximately 1 month after natural spawning began (Fig. 4a). Spawning was desynchronized in group F relative to groups A–E, with 100% of the fish attaining maturity over a 20-week period.

There were no significant differences between the mean spawning times of groups A–E or between the proportions of fish attaining maturity in each group. Group F spawned significantly later than groups A–E ($P \leq 0.05$) and the variance of the spawning profile of group F was significantly greater ($P \leq 0.001$) than those of groups A–E.

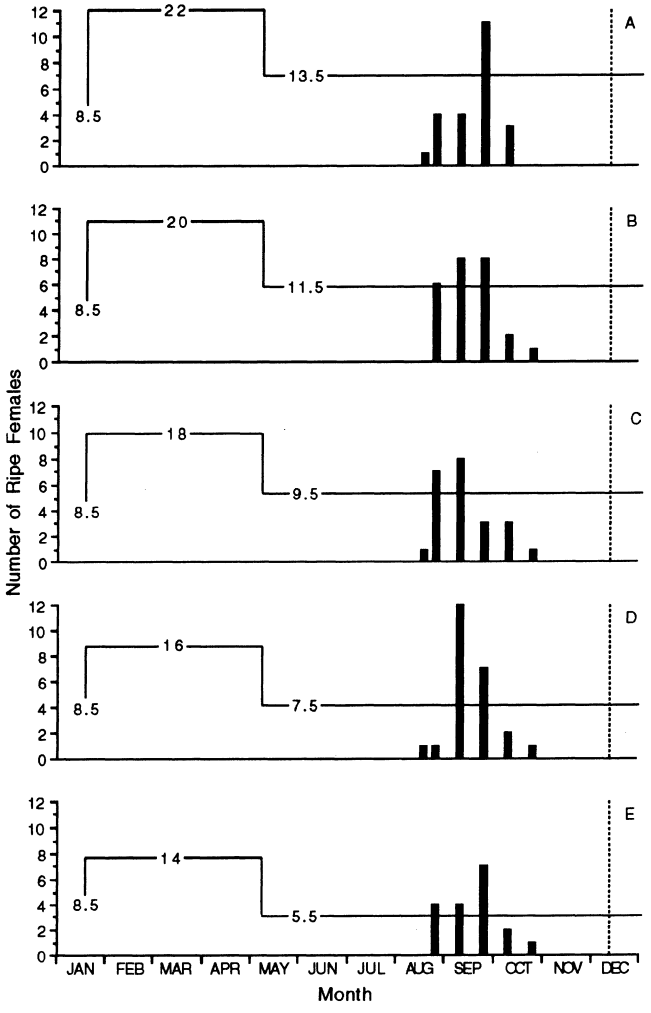

Fig. 3A–E The effects of five photoperiod regimes (groups A–E, Experiment 2a) on the timing of maturation in female rainbow trout. The histograms represent the number of ripe females on each sampling date. *Line graphs* indicate the photoperiod regime (hours of light/day) applied to each group. *Dashed vertical lines* indicate the mean spawning time of fish maintained under ambient daylength

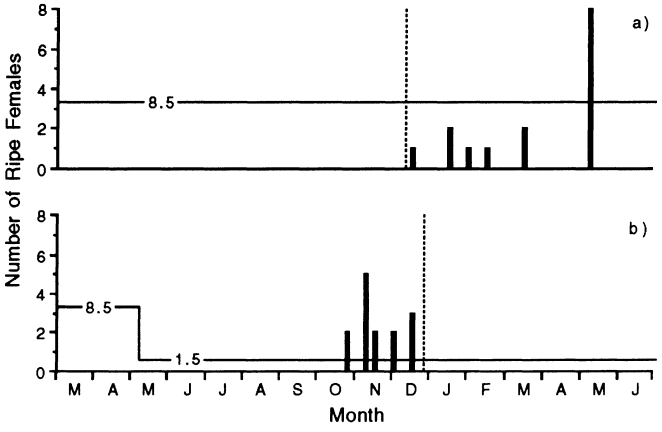

Fig. 4 Comparison of the effects of exposure to **a** constant 8.5L:15.5D (ambient daylength at the start of the experiment in January) or **b** 8.5L:15.5D from mid-January to early May, followed by 1.5L:22.5D until spawning, on the spawning time of female rainbow trout. Data in the upper figure (**a**) relate to group F from Experiment 2a and the lower figure (**b**) to group F from Experiment 2b. The *dotted lines* indicate the mean natural spawning time in each experiment

Total serum calcium

All groups exhibited significant changes ($P \leq 0.001$) in total serum calcium levels during the reproductive cycle. The timing of the changes in calcium levels was virtually identical in groups A–E (Fig. 5), consistent with the similarity in spawning times in these groups. Calcium levels in groups A–E began to rise between May and June, were significantly higher ($P \leq 0.05$) than basal values by July, and continued to rise steeply until peaking at approximately 70 mg 100 ml^{-1} in August (group C) or 45 mg 100 ml^{-1} (group A), 61 mg 100 ml^{-1} (group B), 54 mg 100 ml^{-1} (group D) and 57 mg 100 ml^{-1} (group E) in September. A rapid decline in serum calcium occurred in groups A–E between September and October, and levels were approaching basal by November. Significant differences in calcium levels between groups were only detected during the early part of the annual cycle; levels in group B in April, and group C in both April and June, were significantly elevated ($P \leq 0.05$) compared with those in group E.

Group F exhibited a much shallower rise in serum calcium than groups A–E (Fig. 5), consistent with the later spawning time of this group and the desynchronization between individuals. Calcium levels first increased significantly ($P \leq 0.05$) above basal values between September and October, and had reached 27 mg 100 ml^{-1} by the last sampling point in January.

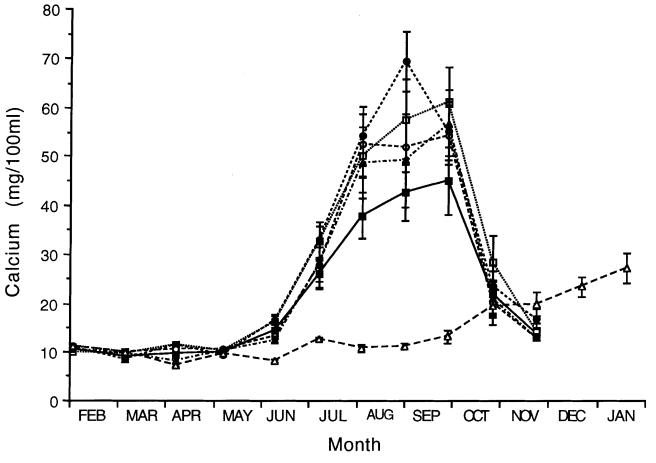

Fig. 5 The effects of six photoperiod regimes (Experiment 2a) on the timing of changes in total serum calcium levels (mean ± SEM) during maturation in female rainbow trout (—■— group A, 22L:2D/13.5L: × 10.5D; .....□..... group B, 20L:4D/11.5L:12.5D; - - -●- - - group C, 18L:6D/9.5L:14.5D; - - -○- - - group D, 16L:8D/7.5L:16.5D; ..... ▲ ..... group E, 14L:10D/5.5L:18.5D; - - -△- - - group F, 8.5L:15.5D)

Varying the magnitude of an increase in photoperiod-II (Experiment 2b)

Spawning

The spawning times of the individual fish in each group are illustrated in Fig. 6. When the photoperiod was reduced by 8.5 h on May 9 from 22L:2D (group A), 18L:6D (group B), 14L:10D (group C) and 12L:12D (group D) spawning commenced in all groups on August 18, approximately 3 months in advance of the natural spawning period. Unfortunately, an interruption to the water supply on June 26 caused the death of all but two of the fish in group B which subsequently spawned on August 18 and September 14. No examination of mortalities was possible on this occasion.

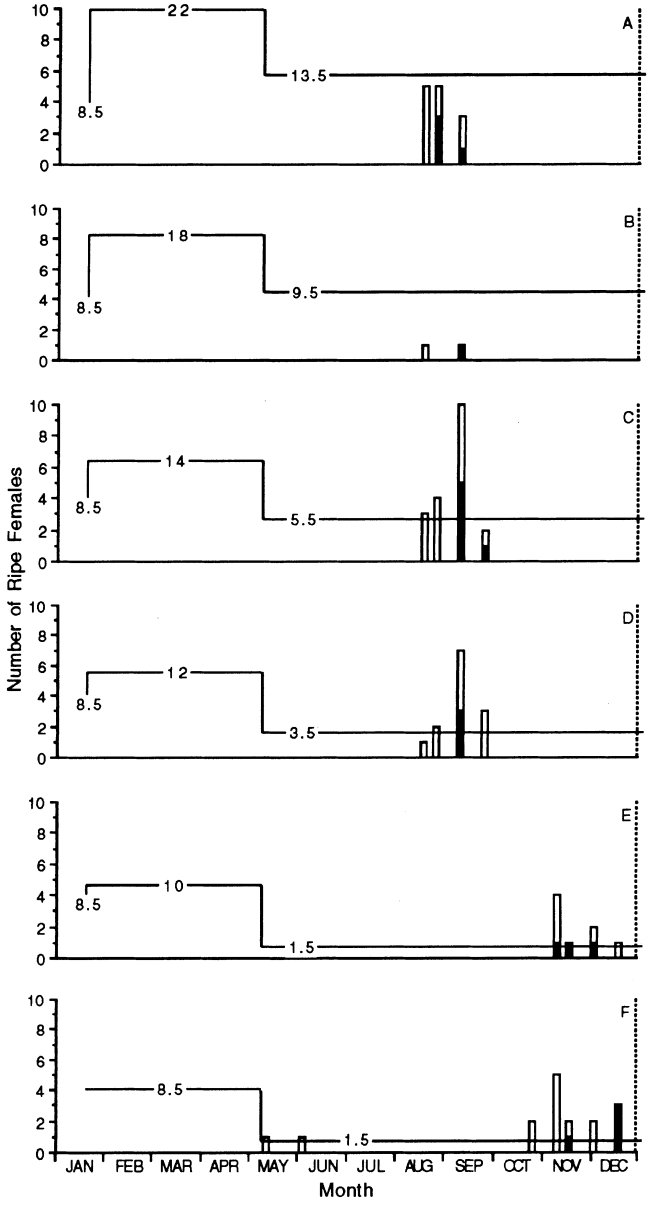

Fig. 6A–F The effects of six photoperiod regimes (Experiment 2b) on the timing of maturation in female rainbow trout. The stacked histograms represent the number of ripe females on each sampling date; fish spawning for the first time are shown in *black*, those undergoing their second reproductive cycle in *white*. *Line graphs* indicate the photoperiod regime (hours of light/day) applied to each group. *Dashed vertical lines* indicate the mean spawning time of fish maintained under ambient daylength

When the photoperiod was reduced to 1.5L:22.5D in early May from 10L:14D (group E) or 8.5L:15.5D (group F) spawning commenced on November 9 and October 24, respectively, in both cases in advance of the natural spawning period. Notably, group F also commenced spawning almost 2 months in advance of fish which had been maintained under a constant short photoperiod in Experiment 2a, and exhibited a synchronized spawning profile relative to fish maintained under constant short days (Fig. 4). The proportion of fish attaining maturity in groups A, C, D, E and F was estimated as 87, 79, 65, 62 and 71% over periods of 4, 6, 6, 6 and 8 weeks, respectively.

There was only one statistically significant difference (groups A versus D; $P \leq 0.05$) between the mean spawning times of groups A–D and no significant difference between those of groups E and F. There were, however, highly significant differences ($P \leq 0.001$) between the mean spawning times of both groups E and F when compared with groups A–D, though it should be noted that the two fish which spawned in early May and early June in group F were designated as 'outliers' ($P \leq 0.01$) and hence were excluded from the statistical analysis to maintain homogeneity of variances. There was a significant effect of age on spawning time ($P \leq 0.01$), the mean spawning time of 3+ fish occurring approximately 2 weeks earlier than that of 2+ fish. Between-group differences in the proportion of fish attaining maturity were not significant but there were significant differences between year classes within groups: the proportion of 3+ fish maturing was significantly greater than that of 2+ fish in groups C ($P \leq 0.01$), D ($P \leq 0.05$) and E ($P \leq 0.01$).

Total serum calcium

All groups exhibited significant changes ($P \leq 0.001$) in total serum calcium levels during the reproductive cycle. However, there were considerable differences in the timing of these changes (Fig. 7), consistent with the differences in spawning time between groups. The timing of the changes in calcium levels was virtually identical in groups A and C (Fig. 7a), consistent with the similarity in spawning times. Calcium levels in these groups increased between March and May, were significantly higher ($P \leq 0.05$) than basal values by June, and continued to rise at a moderate rate until they peaked at approximately 33 mg 100 ml^{-1} (group A) and 36 mg 100 ml^{-1} (group C) in early August, prior to a decline towards basal values in September. Compared to group

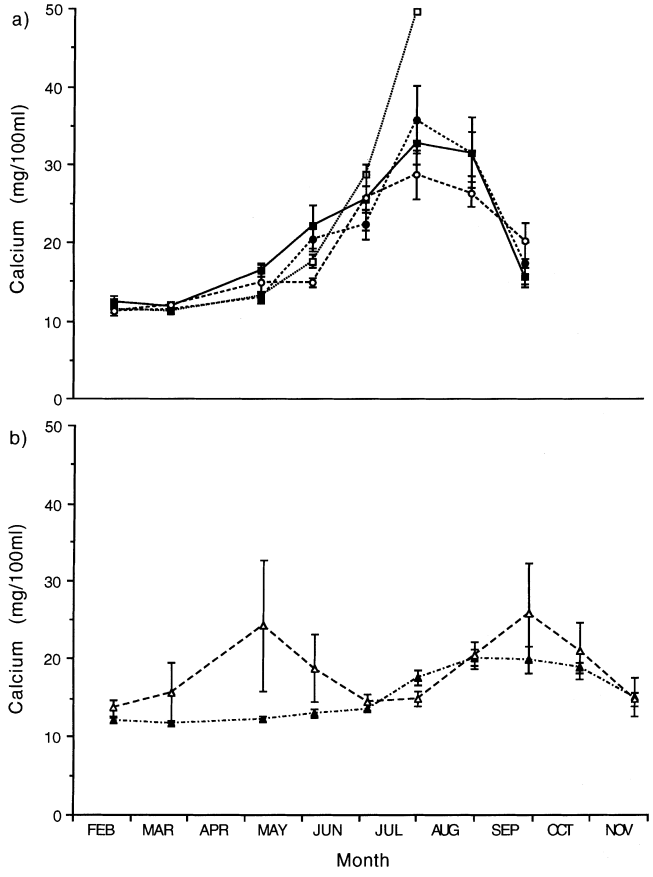

Fig. 7a, b The effects of six photoperiod regimes (Experiment 2b) on the timing of changes in total serum calcium levels (mean ± SEM) during maturation in female rainbow trout. **a** —■— group A, 22L:2D/13.5L:10.5D; .....□..... group B, 18L:6D/9.5L:14.5D; - - -●- - - group C, 14L:10D/5.5L:18.5D; - - -○- - - group D, 12L:12D/3.5L:20.5D. **b** ..-..-..▲..-..-.. group E, 10L:14D/1.5L:22.5D; - - -△- - - group F, 8.5L:15.5D/1.5L:22.5D). For clarity, error bars are omitted from the last two samples of group B (when $n = 2$ due to mortalities)

E, levels were significantly elevated ($P \leq 0.05$) in group A in May, and in groups A and C in June, July, early August and late August. Calcium levels in groups A and C were also significantly higher ($P \leq 0.05$) than those in group F in July and early August and levels in group A were significantly elevated ($P \leq 0.05$) compared to group F in late August. The timing of the changes in calcium levels in group B was comparable to that in groups A and C (Fig. 7a), consistent with the similarity in spawning times between the two surviving fish in group B and those in groups A and C. Peaking at about 29 mg 100 ml^{-1} in early August, group D exhibited a similar serum calcium profile to groups A and C (Fig. 7a), except that the first significant increase ($P \leq 0.05$) above basal values occurred approximately 1 month later, between June and July; calcium levels in group D were significantly higher ($P \leq 0.05$) than those in group E in July and group F in early August.

Compared with groups A–D, significant ($P \leq 0.05$) changes in serum calcium occurred 1–3 months later in groups E and F (Fig. 7b), consistent with the later spawning times in the latter two groups. The first significant rise ($P \leq 0.05$) in calcium levels in group E occurred between July and early August and extended into a broad peak lasting until late October and attaining a maximum of only 20 mg 100 ml^{-1} prior to a decrease in levels between October and November. Two early spawning fish in group F caused a transient increase in mean serum calcium levels in May, but these two fish were not representative of the sample population and hence the difference between group F and groups A–E was not statistically significant at this time. The first significant increase ($P \leq 0.05$) in calcium levels in group F occurred in August with levels gradually increasing to peak at about 26 mg 100 ml^{-1} in late September, before commencing a symmetrical decline in October and November.

Discussion

The results demonstrate that exposure to a constant 'long' photoperiod early in the reproductive cycle, followed by an abrupt reduction to a shorter daylength after 3–4 months, can advance the time of spawning in the rainbow trout by up to 4 months. This is in general agreement with previous studies on the rainbow trout (e.g. Duston and Bromage 1987, 1988), and the masu salmon (Takashima and Yamada 1984). The timings of the seasonal changes in serum calcium (measured as an index of vitellogenin) were consistent with the differences in the timing of maturation between groups, indicating that the modifications in spawning time were mediated by changes in the timing of the endocrine events controlling reproduction. Similar variations in calcium profiles, consistent with differences in spawning times, have been reported in previous studies (e.g. Duston and Bromage 1987, 1988).

If one accepts that maturation in the rainbow trout is ultimately under endogenous control (Duston and Bromage 1986, 1991; Randall et al. 1998), it follows that any modification in spawning time can be interpreted as the overt expression of either a phase advance or a phase delay of the endogenous circannual clock (Duston and Bromage 1987, 1988; Randall et al. 1988). In the present study, the changes in spawning time reflected either a single phase advance, elicited by a single change in photoperiod (e.g. Experiment 1, group C), or two separate phase advances, elicited by two changes in photoperiod (e.g. Experiment 1, group B). The fish interpreted each change in photoperiod (premature arrival of long or short daylengths) as an indication that their clock was running 'slow' and compensated with a corrective forward adjustment.

The action of abrupt reductions in photoperiod as entraining agents for the endogenous circannual rhythm of reproduction in rainbow trout has been reported previously (Duston and Bromage 1988). Experiment 1 confirmed that abrupt increases in daylength also act as entraining cues. Spawning occurred in sequence in fish

exposed to an increase in photoperiod in December, January and February, followed in each case by a decrease in daylength in May. Similarly, spawning occurred earlier in fish exposed to a constant long photoperiod (no reduction in May) from January than in those subjected to the same photoperiod from February. The timing of the increase to a long photoperiod is therefore an important determinant of spawning time. Variations in the timing of artificial changes in daylength also influence the timing of smoltification (saltwater adaptation) in Atlantic salmon, *Salmo salar* (Duston and Saunders 1990; Thrush et al. 1994) and reproduction in some higher vertebrates (e.g. sheep: Sweeney et al. 1997a).

The mechanisms underlying the photoperiodic entrainment of the circannual clock controlling reproduction were further investigated in Experiments 2a and b by subjecting fish to 'long' photoperiods varying from 10 to 22 h; the differences in the magnitude of the May reduction in photoperiod inherent in the design of a preliminary experiment (Randall et al. 1987) were eliminated by decreasing the daylength by 8.5 h (approximating to the difference in daylength between the summer and winter solstices) in all groups. Spawning was advanced in all 'long' day groups compared to fish maintained under ambient conditions, with similar spawning times recorded for the three directly comparable groups in Experiments 2a and b. Similar advances in spawning time occurred in response to 'long' photoperiods of between 12 and 22 h, which represented increases from the ambient daylength in January of between 3.5 and 13.5 h. Thus, a 'long' photoperiod of 12 h was as effective as daylengths of up to 22 h for the advancement of spawning. Moreover, maturation was independent of the length of the 'short' photoperiod, which ranged from 3.5 to 13.5 h in groups exposed to 'long' photoperiods of between 12 and 22 h. Similarly, Takashima and Yamada (1984) reported virtually identical advances in the timing of maturation in masu salmon exposed to 6-, 8- or 12-h photoperiods after maintenance on LL from December to April (although a reduction to 18 h was less effective). These results support the proposition that it is the change in photoperiod (i.e. an increase or decrease) which is most important for the entrainment of the endogenous clock controlling reproduction in the female rainbow trout, rather than the absolute daylength, or the magnitude of the change in photoperiod.

Although advanced compared to fish maintained under ambient conditions, fish exposed to a 'long' photoperiod of only 10 h did not commence spawning until about 12 weeks after those subjected to daylengths of 12 h or more. Analogous results were obtained in a previous study (Randall et al. 1987), but there remained the possibility that the differences in spawning time were attributable to the differences in the magnitude of the changes in photoperiod. In Experiments 2a and b, however, the magnitude of the decrease in photoperiod was constant in fish subjected to 'long' daylengths of between 10 and 22 h, and there was no difference in the effects of increases in photoperiod varying between 3.5 and 13.5 h. In contrast, fish subjected to a 1.5-h increase in photoperiod in January spawned nearly 3 months later than those exposed to a 3.5-h increase. Although rainbow trout may be able to discriminate between daylengths of 10 and 12 h it is more likely that the fish were unable to detect a single abrupt increase in daylength of only 1.5 h and hence the advance in spawning time was due solely to the decrease in photoperiod in May. Certainly the 1- to 2-month advance achieved with a 'long' photoperiod of 10 h in both Experiment 2b and a previous study (Randall et al. 1987) equates with that attributed to a single change in photoperiod (an increase) in Experiment 1 and previous studies (e.g. Duston and Bromage 1987, 1988). Abrupt changes in photoperiod do not, of course, occur under natural conditions, in which the annual reproductive cycle is entrained by the seasonally changing daylength. However, the stability of the circannual clock in response to small but abrupt (rather than gradual) changes in photoperiod may protect it from the effects of 'noise' inherent in the environmental signal, such as coloured water during flooding or extensive cloud cover, which may otherwise be perceived as late dawn or early dusk and cause a phase-shift of the clock.

Clearly, to distinguish the effects of an increase in photoperiod to 10 h in January from that of a decrease in daylength in May it is necessary to repeat the experiment with a control group maintained on a constant 10L:14D photoperiod from January. However, further support for the conclusion that the advance in spawning time in groups exposed to 'long' days of 10 h was due solely to the subsequent reduction in daylength in May is provided by the important observation that maturation can be advanced even in fish which do not experience any increase in daylength in advance of the natural light cycle. Thus, spawning was advanced by about 2 months relative to the natural spawning period in fish maintained from January on a daylength of only 8.5 h (approximating to ambient daylength at the time) followed by a reduction to 1.5L:22.5D in May. This is similar to the advances obtained in fish exposed to 'long' days of 10 h, followed by 'short' days of 6L:18D (Randall et al. 1987) and 1.5L:22.5D (Experiment 1). Conversely, spawning was delayed and occurred over an extended period in fish maintained under a constant 8.5L:15.5D photoperiod from January (Experiment 2a). This suggests that the reduction in photoperiod in May provided a cue which both advanced and synchronized maturation. In contrast, fish maintained on constant daylength exhibited a desynchronization of spawning times, characteristic of free-running circannual rhythms. To our knowledge, the ability to perceive a photoperiod typical of mid-winter as a 'long' day (when followed by an even shorter photoperiod) has not been reported for any other organism.

The results of this study show clearly that the direction of change of daylength is responsible for the entrainment of the endogenous circannual clock which controls reproduction in the female rainbow trout; daylength per se (absolute daylength), and the magnitude of change in daylength, are of little importance in the entrainment process. This implies that the rainbow trout reads daylengths comparatively, with reference to the preceding photoperiod, rather than absolutely. The response of the fish to a particular daylength therefore depends on the previous photoperiod(s) experienced, that is, their photoperiodic history. In Experiment 2, for example, 14L:10D and 12L:12D were interpreted as 'long' days after an increase from 8.5L:15.5D in January, but similar photoperiods (13.5L:10.5D and 11.5L:12.5D) were perceived as 'short' days after a decrease from either 22L:2D or 20L:4D in May. Similarly, a reduction in daylength from 8.5L:15.5D to 1.5L:22.5D was interpreted as a decrease from a 'long' to a 'short' photoperiod. There have been no comparable studies in other species of fish, but the reproductive response to a particular daylength has also been shown to be dependent on recent photoperiodic history in a number of mammals (e.g. sheep: Sweeney et al. 1997b) and birds (e.g. Japanese quail, *Coturnix coturnix japonica*: Robinson and Follett 1982), and photoperiodic history may also be important in some plants (Breeman 1993). Thus, although the precise mechanisms underlying the response to photoperiod may vary between species, photoperiodic history appears to be important for seasonal reproduction in a variety of organisms.

In conclusion, any photoperiod may be perceived by rainbow trout as 'long' or 'short' providing it is longer or shorter than that to which they have been previously exposed. The traditional concept of a rigid 'critical' daylength for reproductive function is therefore untenable for this species. Utilizing direction of change of daylength is clearly consistent with the requirement for accurate synchronization of a circannual cycle in reproductive function since the same daylength occurs twice a year.

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