Photoperiod-induced phase-shifts of the endogenous clock controlling reproduction in the rainbow trout: a circannual phase–response curve

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Different groups of winter-spawning female rainbow trout that had been maintained under seasonally changing daylength and temperature were exposed to 2 months of continuous light at different times of the year. The same photoperiod produced advances in the time of spawning of up to 232 days and delays of up to 80 days, depending upon the timing of exposure in relation to the phase of the reproductive cycle. The proportion of fish spawning in each group varied from 18% to 100%, again dependent on the timing of exposure to continuous light. The photoperiod-induced changes in spawning time can be interpreted as phase-dependent phase-shifts of an endogenous circannual clock controlling maturation. It is proposed that long days, occurring earlier or later than they would under a natural photoperiod, were perceived as indications that the clock was running slow or fast, thus initiating corrective forward adjustments (advance phase-shifts) or backward adjustments (delay phase-shifts), respectively. Collectively, these responses can be described in the form of a circannual phase–response curve.

Introduction

In common with the vast majority of organisms inhabiting temperate and polar latitudes, reproduction in the rainbow trout (*Oncorhynchus mykiss*) is an annual event, with spawning confined to a brief (typically 6–8 week) period each year. This seasonality ensures that fry emerge at a time when the local environmental factors that dictate their survival (ultimate factors, for example temperature and availability of food) are at their most favourable. Breeding cannot be initiated instantly when ultimate factors become optimal since gonadal development may take a year or more to complete (Sumpter *et al.*, 1984; Bromage and Cumaratunga, 1988). The initiation and modulation of reproductive development is therefore timed with reference to earlier predictive environmental cues (proximate factors). In fish, both photoperiod and temperature have been identified as important proximate cues, but in salmonids, the primary environmental influence on reproductive timing is the seasonally changing daylength (Bromage and Cumaratunga, 1988; Bromage *et al.*, 1993).

It has long been assumed that annual rhythms in reproduction and other seasonal events are directly driven by seasonal changes in proximate factors, particularly daylength. This concept has been challenged in recent years and there is now a growing body of evidence that, in many long-lived species, seasonally changing daylength does not actively drive or induce reproductive development, but rather it acts as a zeitgeber to entrain an endogenous circannual rhythm of

reproductive function (Gwinner, 1986). To date the most compelling evidence for the existence of circannual rhythms in salmonids has been the demonstration of self-sustaining rhythms of gonadal maturation and spawning in female rainbow trout maintained under constant environmental conditions (Duston and Bromage, 1986, 1991). However, several additional criteria need to be satisfied to establish that an annual cycle is an endogenous circannual rhythm; the most important of these criteria is that the rhythm should possess a differential sensitivity to the phase-shifting effects of the zeitgeber according to the phase of the rhythm perturbed, that is, a phase–response curve should be available (Saunders, 1977).

There have been few reports of phase-dependent phase-shifts of circannual rhythms in response to a zeitgeber stimulus, and circannual phase–response curves have not been described for any organism. In female rainbow trout constant long (for example, 18 h light:6 h dark) or short (for example, 6 h light:18 h dark) daylengths can advance or delay maturation depending upon the timing of exposure in relation to the phase of the reproductive cycle, and the photoperiodic history of the fish (Duston and Bromage, 1987, 1988; Bromage *et al.*, 1993). The direction of change of daylength is the most important determinant of these effects which can be interpreted as corrective phase advances or phase delays of the circannual clock (Duston and Bromage, 1988; Randall *et al.*, 1991). A number of studies (reviewed in Bromage *et al.*, 1993) have shown that the reproductive response of rainbow trout to long-term exposure to continuous light is similar to the response to constant long photoperiods, suggesting that the fish interpret continuous light as long daylength. The ability to use periods of continuous light to manipulate spawning time could provide fish farmers with an extremely simple method

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for spreading the production of eggs. In this paper, the effects of exposure to 2 months of continuous light on the timing of maturation of female rainbow trout is reported and it is proposed that these effects can be interpreted as phase-dependent phase-shifts of an endogenous circannual clock controlling reproduction, which can be described in the form of a circannual phase-response curve.

Materials and Methods

Animals and husbandry

A series of five experiments was conducted over 4.5 years to assess the ability of 2 months of continuous light to modify the timing of maturation in female rainbow trout. All fish came from an established domesticated stock with a natural spawning period under ambient daylength (latitude 51°10'N) of November–January. Fish were maintained indoors in fibreglass D-ended tanks (length 3.1 m, width 1.65 m, water depth 0.7 m) with a centrally located partition. When necessary, the tanks were subdivided into eight sections with netted screens to allow different experimental groups to be segregated. Spring water was supplied at a rate of approximately 90 l min⁻¹ and water circulation and oxygen concentrations were increased by a large submersible air pump. Water temperature varied seasonally, consistent with external variations in environmental temperature. Over the 4.5 years, minimum and maximum temperatures recorded at sampling times were 6°C and 18°C, respectively. Natural light entered the building via plastic corrugated roofing and provided variable light intensity according to the external environmental conditions and time of day. Although the natural changes in light intensity were clearly attenuated by the roofing, on all occasions the changes in light intensity within the building accurately reflected the daily and seasonal changes occurring externally. Light intensities measured at the water surface of tanks maintained under ambient photoperiod (at about midday) generally ranged from about 200 to 600 lux, although values up to 800 lux were recorded on bright sunny days, and as low as 6 lux on one extremely overcast winter day. Continuous (24 h) illumination was supplied by cool white fluorescent tubes suspended 1 m above the tanks, providing a light intensity of approximately 1000 lux over the majority of the water surface. Covers made from fine mesh netting prevented fish from escaping.

The fish were fed daily with the recommended ration and pellet size of a commercial trout diet (Trouw Aquaculture, Northwich, Cheshire). A solution of malachite green (2 p.p.m., zinc free; Sigma Chemical Company Ltd, Poole, Dorset) was used occasionally to prevent the spread of fungal infections. All fish were starved for 24–48 h before any manipulative procedure.

Experimental design

At the start of Expt 1–4, up to 250 two-year-old virgin female rainbow trout, which were approaching their first natural spawning, were selected at random from the main broodstock. As the fish approached maturity they were examined every 2 weeks (more frequent examination is considered

too stressful for the fish) and the spawning times of individual fish recorded. In Expt 1, in which the treatments were due to begin before the first natural spawning time of the stock, the fish were randomly assigned to experimental groups. In Expts 2–4, in which the treatments began after the first natural spawning, approximately equal numbers of fish from each of the natural spawning dates were assigned to each of the experimental groups. In Expt 5, approximately 300 fish were randomly selected from the main broodstock in the summer after their first spawning (earlier segregation and examination of these fish were not possible for logistical reasons) and randomly assigned to each of the experimental groups. Groups of fish were always assigned to tank sections in a random manner.

In each experiment, groups of fish were exposed to 2 months of continuous light by transferring them from a tank maintained under ambient daylength to a tank maintained under continuous light, at the times shown in Table 1. At all other times the fish were maintained under ambient photoperiod. The numbers of fish transferred to continuous light at each time point were approximately 22, 26, 224, 200 and 100 for Expts 1–5, respectively. (Expts 3–5 were designed to provide commercial quantities of out-of-season eggs.) After the initial spawning, fish were examined at intervals of approximately 1 month until they again started to exhibit signs of maturity, and then every 2 weeks as they approached their second spawning. Mature females were recognized by distension and softening of the abdomen and the extrusion of the urogenital papilla which became swollen and reddish in colour. The point at which eggs had been ovulated into the abdominal cavity and could be expressed from the papilla by exerting gentle downward pressure on the abdomen was defined as the time of spawning for each individual. Mean spawning times were calculated using the day number on which each fish spawned (1 Jan = day 1). The mean natural spawning time was calculated by combining data from the first natural spawning in Expts 1–4 with that from other broodstock on the farm maintained under identical conditions over the same period. Expts 1, 2, 3, 4 and 5 were terminated on 8 Oct, 2 Jan, 16 Oct, 26 Apr and 25 Mar, respectively, at which times inspection of the fish indicated that no further fish would mature in advance of the forthcoming natural spawning times. All fish that had spawned up to, and including, 8 Oct in Expt 1, 10 Oct in Expt 2, and 16 Oct in Expt 3 were deemed to have matured in advance of the natural spawning season (Nov–Jan) to allow comparison between experiments.

Statistical analyses

Homogeneity of sample variances was tested using the *F*-test. When the assumption of homogeneity was satisfied, mean spawning times within an experiment were compared 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). When variances were found to be significantly heterogeneous ($P < 0.05$), comparisons were performed using the Kruskal–Wallis test followed by Dunn's multiple range procedure (Zar, 1984). The proportions

Table 1. The effects of exposure to 2 months of continuous light on spawning time in female rainbow trout and the percentage that matured

Experimental group	Timing of exposure to continuous light	Spawning period (mean date: number of days spawning advanced or delayed)*	Percentage maturing**
1/A	Sept 15–Nov 17	Apr 23–May 11 (May 2/ + 232) ^a	18 (2/11) ^a
1/B	Oct 15–Dec 16	Apr 23–May 22 (May 9/ + 225) ^a	21 (3/14) ^a
1/C	Nov 17–Jan 21	June 2–Aug 10 (June 25/ + 179) ^b	33 (5/15) ^a
1/D	Dec 16–Feb 17	June 17–Aug 10 (July 7/ + 166) ^b	85 (11/13) ^b
1/E	Jan 21–Mar 25	July 16–Sept 21 (Aug 4/ + 138) ^c	100 (18/18) ^b
1/F	Feb 17–Apr 23	Aug 24–Oct 8 (Sept 8/ + 103) ^d	25 (4/16) ^a
2/A	Jan 15–Mar 16	July 4–Aug 29 (July 23/ + 150) ^e	100 (23/23)
2/B	Feb 15–Apr 15	July 18–Oct 10 (Sept 1/ + 110) ^f	76 (16/21)
3	Jan 16–Mar 15	July 3–Oct 16 (July 17/ + 156)	96 (133/139)
4	July 21–Sept 22	Jan 23–Apr 26 (Feb 25/ – 67)	73 (125/171)
5/A	June 19–Aug 23	Dec 21–Jan 21 (Dec 28/ – 7) ^g	97 (105/108) [†]
5/B	July 19–Sept 20	Dec 21–Jan 21 (Jan 6/ – 17) ^h	98 (93/95) [†]
5/C	Aug 23–Oct 25	Mar 6–Mar 25 (Mar 10/ – 80) ⁱ	96 (110/114)

*Mean spawning dates were calculated using the day number on which each fish spawned (January 1 = day 1); advances/delays were calculated relative to the mean natural spawning time over the experimental period (Dec 20).

**Calculated from the number of fish that spawned 'out-of-season' relative to the number of fish surviving at the termination of each experiment (shown in parentheses); [†]denotes fish that spawned close to the natural spawning period.

Values with different superscripts within columns are significantly different ($P < 0.05$) within an experiment (a–d: Expt 1; e, f: Expt 2; g–i: Expt 5).

of fish spawning in each group were compared by the calculation of 95% confidence limits (Fowler and Cohen, 1987); if there was no overlap between the confidence limits of two proportions, they were considered to be significantly different. As the experiments were conducted in different years, statistical comparisons were performed only between groups from within the same experiment.

Results

Spawning

Although some groups received continuous light just before their initial (winter) spawning in Expt 1, there were no significant differences ($P > 0.05$) between the mean spawning times of any of the groups at this stage (in Expts 2–5, treatments began after the first spawning). The timing of the second spawning period for each experimental group was either advanced or delayed compared with the mean natural spawning time (Table 1). The direction and magnitude of the alterations in spawning time were dependent on the timing of the continuous light period relative to the phase of the reproductive cycle (Table 1).

In Expt 1, the differences in the timing of the second spawning period between groups were statistically significant ($P < 0.05$) except for groups A versus B and C versus D (Table 1). The mean spawning times of the two groups in Expt 2 were also significantly different ($P < 0.05$), as were those of the three groups in Expt 5 ($P < 0.05$).

There were marked differences in the proportion of fish responding with a change in spawning time, again according to the phase of the reproductive cycle at which the continuous

light treatment was applied (Table 1). In Expt 1, the proportion of fish attaining early maturity was significantly lower ($P < 0.05$) in groups A, B, C and F than in groups D and E. Other differences were not significant.

The majority of individuals in each group became reproductively mature for the second time within about 8 weeks, consistent with the natural spawning period of 6–8 weeks for fish maintained under ambient conditions. In contrast, fish exposed to continuous light from February–April in Expt 2, and July–September in Expt 4, spawned over 12 weeks. This desynchronization was most marked in Expt 2, in which small numbers of fish spawned at each time point between 18 July and 10 October; of the five remaining fish, four spawned between 7 Nov and 5 Dec, close to the beginning of the natural spawning period.

Phase-response curve

A phase-response curve has been constructed, describing the changing magnitude of the phase-shifts (advance or delay in the timing of maturation) that occurred in response to 2 months of continuous light applied at different phases of the circannual cycle (Fig. 1). Each phase-shift is plotted against the mid-point of the continuous light period to which it relates. The advance or delay (in mean spawning time) was calculated relative to the mean natural spawning time (20 December) over the experimental period.

Discussion

An important property of endogenous clocks, and a prerequisite for entrainment, is that they possess a differential

Fig. 1. Phase-response curve illustrating phase-shifts (advance or delay in mean spawning time) of an endogenous circannual rhythm caused by exposing female rainbow trout to 2 months of continuous light at different times of the year in Expt 1 (■: exposure in Sept–Apr, before and after the first natural spawning), Expt 2 (□: exposure in Jan–Apr after the first natural spawning), Expt 3 (●: exposure in Jan–Mar after the first natural spawning), Expt 4 (○: exposure in July–Sept after the first natural spawning) and Expt 5 (▲: exposure in Jun–Oct after the first natural spawning). WS: winter solstice, SS: summer solstice.

sensitivity to the phase-shifting effects of the zeitgeber (Aschoff, 1965; Gwinner, 1973, 1986; Saunders, 1977; Brady, 1979; Daan, 1982). A particular time cue therefore causes phase-shifts of different magnitude and sign depending on the phase at which the endogenous rhythm is perturbed. In the present work, rainbow trout exposed to 2 months of continuous light exhibited advances or delays in spawning time depending upon the timing of exposure relative to the phase of the reproductive cycle. Periods of continuous light, applied in advance of the significant increase in daylength that occurs under the natural seasonal cycle, advanced maturation, whereas periods of continuous light applied after the natural occurrence of long days delayed maturation. These modifications in spawning time can be interpreted as the overt expression of corrective phase advances or phase delays of an endogenous circannual clock controlling maturation; these occur when the clock is perceived to be running 'slow' or 'fast' with respect to the perceived time of year indicated by the change to a new daylength (continuous light). By plotting these phase-shifts against the time when the periods of continuous light were applied, a circannual phase-response curve has been obtained that describes the phase-shifting effects of 2 months of continuous light applied at different phases of the circannual cycle. The apparent availability of a circannual phase-response to 2 months of continuous light provides strong additional evidence to support the hypothesis that reproduction in rainbow trout is ultimately controlled by an endogenous circannual clock that, under natural conditions, is entrained by the seasonal changes in daylength.

The most striking aspect of the phase-response curve is the transformation from an 80 day delay phase-shift, caused by exposure to continuous light from August to October, to a 232 day advance phase-shift, caused by exposure to continuous light from September to November. It should be noted, however, that the September to November exposure did not affect the timing of the subsequent spawning (approximately 2 months later), rather it advanced spawning in two of the fish in the following year. Presumably, the circannual clock had become entrained by the naturally decreasing daylength at this stage and hence the perception of the fish of a long photoperiod gradually switched from that indicating that their circannual clock was running fast (corrected by a delay phase-shift) to that indicating that their clock was running slow (corrected by an advance phase-shift). Abrupt changes from phase delays to phase advances are characteristic of circadian phase-response curves (Saunders, 1977).

In contrast to phase-response curves for circadian systems, which are usually constructed by the application of light pulses to a free-running rhythm under constant conditions, and followed for a number of cycles, the circannual phase-response curve in the current work describes the effects of periods of continuous light applied within a seasonally changing photoperiod and temperature regimen over a single reproductive cycle. Consequently, all the fish in each group were synchronized to the ambient light cycle before exposure to continuous light, rather than free-running with an endogenous periodicity (and hence, becoming gradually desynchronized with local time). To our knowledge, there has been only one attempt to derive a circannual phase-response curve from the results of experiments on animals maintained under constant conditions. Duston and Bromage (1988) combined their results with those of Bromage *et al.* (1984) to describe the effects (advance or delay in the timing of maturation) of exposing female rainbow trout to varying periods of constant long (18 h light:6 h dark) days, reduced abruptly to a constant short (6 h light:18 h dark) photoperiod at different times of the year. The two studies used different strains of rainbow trout maintained under different conditions, and with different natural spawning times, but, as they were both conducted under constant temperature and photoperiod, the circannual clocks of the fish in each group would have been free-running when exposed to the reduction in daylength. It is of interest that the resultant phase-response curve, although with fewer points, was broadly similar in appearance to that described in the present study (that is, reductions in daylength in advance or behind those of the natural light cycle advanced or delayed maturation, respectively). However, as Aschoff (1965) points out, such qualitative similarities between different response curves are to be expected.

Experiments in which rainbow trout were exposed to long, followed by short, daylengths in advance of these daylengths occurring naturally suggest that the advance in spawning time observed results from two separate phase advances of the circannual rhythm caused by the increase from ambient daylength to a long day (perceived as premature arrival of long days) and the subsequent decrease in daylength to a short day (perceived as premature arrival of short days) (Duston and Bromage, 1988; Randall *et al.*, 1991). Thus, the direction of change of daylength is the feature of the photoperiodic signal

responsible for the entrainment of the circannual rhythm that controls reproduction; absolute daylength and the magnitude of change of daylength are of comparatively little importance (Randall *et al.*, 1991; Bromage *et al.*, 1993). Furthermore, when rainbow trout are maintained under constant daylength for long periods, the spawning times of individuals become desynchronized, characteristic of individual rhythms free-running with various periodicities (Bromage *et al.*, 1984; Duston and Bromage 1986, 1987, 1988; Randall *et al.*, 1991). Similarly, continuous light applied throughout the reproductive cycle results in a desynchronization of individual spawning times, again characteristic of individual circannual rhythms free-running under constant conditions (Bourlier and Billard, 1984a,b; Bromage *et al.*, 1984; Duston and Bromage, 1986). In contrast, in the present work, 2 months of continuous light usually resulted in a synchronized pattern of spawning, indicating that the increase from ambient photoperiod to continuous light and the decrease from continuous light to ambient daylength 2 months later also acted as separate entraining cues. It is, therefore, proposed that each phase-shift obtained from the results of the present study represents the sum of the response to two entraining cues.

Phase-response curves for circannual rhythms cannot be expected to provide as accurate a picture of the underlying clock as those described for circadian systems because of the long time-scale involved. After a perturbation, circadian rhythms typically pass through a series of transients before a steady state phase-shift is attained (Saunders, 1977; Pittendrigh, 1981). Since circannual rhythms may also undergo transient cycles during re-entrainment (Gwinner, 1986), phase-response curves obtained during a single cycle, as in the present study, may not be fully representative of the phase-shifting effects of the zeitgeber stimulus on the clock. Clearly, the presence of transient cycles can be investigated only by maintaining fish under constant photoperiods for several consecutive cycles after exposure to the signal. However, the availability of single cycle response-curves for particular stocks of fish held under natural conditions could prove of considerable value to fish farmers wishing to plan their future production of out-of-season eggs in order to make optimum use of their facilities.

A number of previous studies have demonstrated the effectiveness of artificial photoperiod regimens for manipulating the timing of maturation of salmonid fish (for recent reviews, see Bromage and Cumaranatunga, 1988; Scott, 1990; Bromage *et al.*, 1993). In each case, the reported changes in spawning time can also be interpreted as the overt expression of phase-shifts of a circannual clock caused by photoperiod acting as a zeitgeber. Several of these studies used continuous light: advances in maturation have been reported in rainbow trout (Bromage *et al.*, 1984; Duston and Bromage, 1986) masu salmon, *Oncorhynchus masou* (Takashima and Yamada, 1984) and Atlantic salmon, *Salmo salar* (Taranger *et al.*, 1991) exposed to continuous light from early in the reproductive cycle, whereas delays in spawning time have been observed in rainbow trout (Shiraishi and Fukuda, 1966; Bourlier and Billard, 1984a,b), brook trout, *Salvelinus fontinalis* (Shiraishi and Fukuda, 1966) Atlantic salmon (Taranger *et al.*, 1991), sockeye salmon, *Oncorhynchus nerka* (Combs *et al.*, 1959; Shiraishi and Fukuda, 1966), and amago salmon, *Oncorhynchus rhodurus* (Shiraishi and Fukuda, 1966) exposed to continuous light

during the latter half of the reproductive cycle. Although most of these investigations used much longer periods of continuous light than in the present work, the changes in spawning time can also be interpreted as differential phase-shifts of a circannual clock, dependent on the timing of exposure to continuous light, suggesting that the underlying mechanisms controlling reproduction are similar in a variety of salmonid species.

There have been only a few reports of phase-dependent phase-shifts of circannual rhythms in response to a zeitgeber stimulus in other species. Gwinner (1971) observed that transferring willow warblers (*Phylloscopus trochilus*) from ambient daylength to 18 h light:6 h dark or 12 h light:12 h dark, either advanced or delayed moulting depending upon the time of year when the transfer occurred. Similarly, testicular development of starlings (*Sturnus vulgaris*) was either enhanced, inhibited or unaffected by exposure to 1 month of continuous light or constant darkness, the response again depending upon the time of year of the transfer from ambient daylength (Gwinner, 1973). Circannual rhythms in moulting, and in testicular size, have been demonstrated in willow warblers (Gwinner, 1971, 1973) and starlings (Schwab, 1971), respectively, and hence these results can be interpreted in terms of differential phase-shifts of a circannual clock (Gwinner, 1973, 1986).

Reproductive activity in sheep also appears to be under endogenous circannual control (Ducker *et al.*, 1973; Howles *et al.*, 1982; Almeida and Lincoln, 1984; Karsch *et al.*, 1989), and there is a growing awareness that the effects of photoperiodic change on the timing of reproduction in this species can also be interpreted in terms of phase-shifts of an endogenous clock (Malpoux *et al.*, 1988, 1989; Jackson *et al.*, 1989; Malpoux and Karsch, 1990; Wayne *et al.*, 1990; Woodfill *et al.*, 1994). For example, Malpoux *et al.* (1989) reported that increases in daylength in advance or behind those of the natural light cycle, respectively, advanced or delayed the breeding season in sheep. One interpretation of these observations is that the sheep perceived the premature arrival of long days as an indication that their endogenous clock was running slow and, therefore, compensated with a corrective forward adjustment (phase advance), whereas the late appearance of long days was perceived as an indication that their clock was running fast, initiating a corrective backward adjustment (phase delay). The similarity of these phase-dependent phase-shifts in sheep to those described in the present work for rainbow trout suggests that similar mechanisms are involved in the timing of seasonal events in these species.

It is of interest that varying the position of the continuous light period in relation to the phase of the reproductive cycle not only affected the time of spawning, but also produced marked differences in the proportion of fish that spawned out of season. The reasons for this differential response to the same photoperiod are unclear, although similar variations in the percentage of fish spawning out of season associated with the timing of a change in photoperiod have been reported for rainbow trout (Duston and Bromage, 1988) and Atlantic salmon (Taranger *et al.*, 1991). Duston and Bromage (1988) proposed that such variations could be explained by a 'gating' model, whereby fish would mature in a particular year only if they had reached a certain threshold stage of reproductive

development or size while the circannual clock was at a specific (gate-open) phase of the circannual cycle; fish that did not attain this threshold had to wait for the gate to re-open the following year. Thorpe (1986) suggested an analogous model of developmental regulation in the Atlantic salmon, whereby the 'decision' to mature or smolt depends on a metabolic assessment taken at seasonally critical times. Clearly, such hypotheses can be stringently tested only by long-term studies under constant conditions.

Although we have presented here the most comprehensive circannual phase-response curve so far described for any organism, with the intention of stimulating the initiation of more stringent long-term studies under constant conditions, it is acknowledged that the data presented in this work do not provide unequivocal evidence of the availability of a circannual phase-response curve, because the studies were conducted over a single reproductive cycle under seasonally changing temperature and photoperiod. However, variations in temperature are known to produce only minor modifications in the spawning time of rainbow trout (Davies and Bromage, 1991). The predominance of photoperiod over temperature as a zeitgeber for the entrainment of reproduction was confirmed in the present study by the manipulation of spawning time, such that it occurred during the summer, at the peak of the seasonal temperature cycle, rather than in the winter, at the nadir of the temperature cycle. The seasonally changing photoperiod might have been expected to re-entrain the endogenous circannual rhythm after exposure to continuous light, thus adjusting spawning back to the natural time. This was clearly not the case for the many fish that exhibited considerable advances and delays in spawning time (although it was not possible to maintain fish that failed to attain early maturity in Expt 1 until the subsequent natural breeding season). This result implies that the entraining action of the gradual seasonal changes in photoperiod was overridden by that of the large abrupt changes in daylength which resulted from exposure to 2 months of continuous light. In this context, it is notable that in a parallel experiment, most fish (of the same stock and maintained under identical conditions) that failed to respond to 2 weeks or 1 month of continuous light with an advance in spawning time observed in other fish subjected to the same treatments, eventually spawned at the natural time (C. Randall, N. Bromage and J. Symes, unpublished). Whether these fish were merely insensitive to such short periods of continuous light, or were physiologically incompetent to mature when their clock was advanced by exposure to continuous light (that is, had not reached the threshold stage of development) and re-entrained to the natural photocycle when returned to ambient daylength, is not clear.

In conclusion, the work presented here demonstrates that exposing female rainbow trout to 2 months of continuous light can both advance and delay spawning time depending upon the time of exposure relative to the natural spawning time. These data are consistent with continuous light eliciting phase-dependent phase-shifts of an endogenous circannual clock, which can be represented in the form of a phase-response curve. The apparent availability of a circannual phase-response curve strongly supports the contention that reproduction in rainbow trout is not driven directly by daylength, but is ultimately controlled by an endogenous circannual clock.

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