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The environmental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin

Niall Bromage *, Mark Porter, Clive Randall

Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK

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1. Introduction

Arguably the most striking characteristic of fish is their seasonality, with the timing of developmental and maturational events dominated by and, in parallel, synchronised with seasonal changes in climate, daylength and food supplies. This coordination and the associated internal processes of control ensure that young fish are produced when environmental conditions are most suitable for their survival. Thus, the first-feeding fry of mid and higher latitude species are produced when temperatures and daylengths are increasing in the spring, whereas the corresponding stages of tropical and sub-tropical fish appear to be timed to the increased productivity which follows seasonal rainfall or movements in oceanic currents. Since gonadal development and subsequent gamete and embryo formation take a long time to complete, it is clear that gonadal recrudescence must have been initiated many months earlier to ensure that the production of first-feeding fry is coincident with seasonal improvements in climate and nutrient availability. Fish, along with most other seasonally breeding animals, rely on cues from the external environment to achieve synchronisation of maturational events with changing season. These cues are often referred to as “proximate” to distinguish them from the “ultimate” factors or environmental conditions, which are those under which ideally the young of

* Corresponding author.
E-mail address: nrb1@stir.ac.uk (N. Bromage).

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seasonally-breeding animals are produced and which give them the best chances of survival.

Although a number of environmental factors have been implicated as possible proximate cues including photoperiod, temperature, rainfall, food supplies and pheromones, it is the seasonally-changing pattern of daylength which is probably responsible for the cueing and timing of reproduction in the majority of fish. Certainly, it is the principal determinant of maturation in the salmonids, bass, the breams, mullet, flatfish, the sciaenids and serriolids, which collectively comprise the major intensively farmed species. Even the maturation and spawning of many sub-tropical and tropical species and also cyprinids, which existing literature would have us believe are cued by temperature or rainfall, almost certainly rely on photoperiod as a measure of daily and possibly calendar time.

Given the widespread importance of daylength, in this review, we will concentrate on the effects of various natural and artificial photoperiod regimes on reproduction. However, we will also consider the effects of some of the other environmental factors, as it is likely that as our knowledge of control measures in the wild progresses, we will find that maturation is influenced by a multiplicity of factors, some acting in concert with daylength as proximate cues of developmental events and others possibly only exerting permissive influences.

Also reviewed will be the neuroendocrine mechanisms by which the environment influences reproduction, in particular those relating to the pineal gland and melatonin, and how these processes might be used to measure and predict daily and seasonal time. We know that reproduction is profoundly affected by environmental change and that this is achieved by corresponding alterations in the activity of the gonadotropin releasing hormone (GnRH)–gonadotropic hormone (GtH)–gonadal axis. However, at present the linkage between the environment on the one hand and the controlling neuroendocrine cascade on the other remains unclear. There is considerable evidence for the involvement of the pineal hormone melatonin in these processes, but its role remains to be clarified. Data will be presented on how diel melatonin secretion is affected by photoperiod, temperature and other factors, how seasonal modifications of the melatonin profile affect reproduction and how these processes might interact with endogenous circadian and circannual rhythms. Also reviewed will be recent reports of the presence of melatonin binding and receptor activity in the brain and their co-localisation with the range of neurones implicated in the control of GnRH–GtH function. This will also consider the possible relationships of the various neuroendocrine and endogenous rhythmic processes to the array of clock and other genes which are thought to be involved in the measurement and co-ordination of daily and possibly calendar time.

Lastly, and arguably most importantly, we will review the uses of environmental and in particular photoperiod manipulation in finfish culture. Two types of application are particularly important here and both rely on the precision of the response of fish to environmental change and a clear understanding of the proximate and other factors that are involved. Firstly, hatcheries can use this knowledge to manipulate maturation and spawning time to produce all-year-round supplies of eggs and fry. Secondly, grow-out farms can construct programmes of environmental control, which prevent or delay maturation until after fish harvest, thus avoiding the deterioration in flesh quality, which

often accompanies maturation. Only by using this two pronged approach can grow-out farms produce the year-round continuity of supply of product of consistent size and quality, which is demanded by the retail markets.

2. Seasonally-changing light regimes

One of the first unequivocal demonstrations of the influence of daylength on the timing of reproduction in fish was the work of Hoover (1937) and Hoover and Hubbard (1937) on the brook trout (*Salvelinus fontinalis*); these authors showed that fish exposed to seasonal light cycles compressed into periods of time shorter than 1 year spawned 3–4 months earlier than control fish under ambient conditions. Further reports of the effects of advanced seasonal photoperiods on spawning time of brook trout were also made by Hazard and Eddy (1951), Corson (1955) and Carlson and Hale (1973). Modified seasonal photoperiods have also been shown to alter the timing of spawning in a wide range of other species including the rainbow trout, *Oncorhynchus mykiss* (Whitehead et al., 1978, 1983; Bromage et al., 1982a, 1993a,b; Bromage, 1995; Bromage and Duston, 1986; Bon et al., 1997, 1999); Atlantic salmon, *Salmo salar* (Taranger, 1993; Magwood et al., unpublished); Pacific salmon *Oncorhynchus* spp. (MacQuarrie et al., 1978, 1979; Beacham and Murray, 1993); the brown trout, *S. trutta* (Bromage et al., 1990); the European sea bass, *Dicentrarchus labrax*; gilthead bream, *Sparus aurata*; sole, *Solea solea*; turbot *Scophthalmus maximus* (Girin and Devauchelle, 1978; Imsland et al., 1997); the barbel, *Barbus barbus* (Poncin, 1989; Poncin, 1991); the summer flounder, *Paralichthys dentatus* (Watanabe et al., 1998); the sunshine bass, *Morone* sp. (Tate and Helfrick, in press); the striped trumpeter, *Latris lineata* (Morehead et al., 2000); and the Atlantic halibut, *Hippoglossus hippoglossus* (Smith et al., 1991; Björnsson et al., 1998). Spawning in rainbow trout, Pacific salmon and Altantic halibut has also been shown to be delayed if the seasonal photoperiod is extended into periods of time which are longer than one year (MacQuarrie et al., 1978, 1979; Bromage and Duston, 1986; Bromage et al., 1993a,b; Taranger, 1993; Björnsson et al., 1998).

3. Constant photoperiod regimes

Although modifications in the rate of change of the annual seasonal light cycle induce profound alterations in the timing of maturation and spawning, it is evident that the increasing and decreasing components of the seasonally-changing daylength can be replaced by substituting daylengths or a combination of daylengths of constant lengths (Bromage et al., 1984, 1992, 1993a,b; Arnold, 1988; McCarty et al., 1990; Thomas et al., 1995; Carrillo et al., 1989, 1991, 1993; Taranger et al., 1991, 1998). The requirement for one or more different daylengths within an artificial photoperiod regime appears to vary with both the overall time it takes for the fish to undergo gonadal recrudescence, mature and spawn and also the time in the year in which it spawns. Thus, fish like the stickleback (*Gasterosteus maculeatus*) and many of the smaller cyprinids, which have short maturation cycles and spawn in the spring or early summer respond

positively to a single constant daylength of LD 16:8 (Baggerman, 1980; Borg et al., 1986). By contrast flatfish which also spawn in early summer but have longer cycles of maturation undergo maturation and spawn in response to short followed by long daylengths (Bye, 1984). Most salmonids spawn in Autumn or Winter with gonadal recrudescence occurring up to a year earlier, initially under a short and increasing daylength and then under a decreasing one. This is why initially long and then short constant daylengths are commonly used to programme reproductive development in this group of fish. Thus, rainbow trout, exposed to LD 18:6 from January to May followed by a sudden reduction to LD 6:18, spawn 3–4 months earlier than controls (Whitehead and Bromage, 1980; Bromage et al., 1984). Delaying the timing of the sudden increase to long days until February in this species results in a less pronounced advancement of spawning indicating that the timing of the increase is a significant determinant of the timing of maturation (Bromage et al., 1984; Duston and Bromage, 1987, 1988). The subsequent decrease to short days and its timing is also important, for trout which remain on long days throughout the year, although spawning earlier than controls, are less advanced than those which receive the "long-to-short" switch (Duston and Bromage, 1988).

Advancements of spawning following exposure to constant long and then short days have also been reported for the masu salmon (*O. masou*: Takashima and Yamada, 1984), the Atlantic salmon (Taranger et al., 1991, 1998, 1999a,b; Duncan et al., 1999), the brook trout (Henderson, 1963), the dace (*Leuciscus leuciscus*: Brook and Bromage, 1988), the brown trout (Bromage et al., 1990) and the European sea bass (Carrillo et al., 1989, 1991, 1993). Such regimes are also effective in advancing puberty (Bromage, 1987; Whitehead et al., 1983). Furthermore, as little as 1 month of long days during the early part of the cycle is all that is necessary to advance the timing of spawning in trout, masu salmon and sea bass (Bromage et al., 1984; Takashima and Yamada, 1984; Carrillo et al., 1989, 1991, 1993).

Constant daylengths have also been shown to delay spawning. Thus, constant short days either throughout the year or during the first 7 or 8 months of the year delay spawning as does exposure of fish to periods of constant long days after the summer solstice (Allison, 1951; Henderson 1963; Eriksson and Lundqvist, 1980; Whitehead and Bromage, 1980; Bromage et al., 1982b, 1990, 1993a,b; Gillet et al., 1998; Taranger et al., 1998; Kissil et al., 200).

4. Direction of change of daylength and photoperiodic history

During the earlier phases of work on photoperiodism in fish in the 1970s and 1980s, it was considered that daylength or the length of the light period was of critical importance in fish, as in some other photoperiodic organisms, and once a threshold daylength in the ambient seasonal light cycle was reached, then maturation would be induced. Certainly, in many higher vertebrates photoperiod serves to drive maturation with gonadal recrudescence initiated a short time after exposure to a specific critical daylength. However, in a number of the fish in which the effects of modified seasonal light cycles have been studied, spawning time appears not to bear a direct or constant

relationship to the prevailing daylength (Bromage and Duston, 1986; Bromage et al., 1993a,b). In these examples, spawning occurs at a different phase of the light cycle (i.e. a different daylength) depending on the degree of compression or extension of the seasonal light cycle to which the fish were exposed. Under compressed cycles, spawning is delayed with regards to the daylength (length of the daily light period) at which spawning occurs in control fish under ambient conditions, whereas it is advanced, i.e. occurs earlier, under extended cycles. Examples of such phase differences are to be found in the studies of Girin and Devauchelle (1978) on turbot, sea bass and gilthead bream, Macquarrie et al. (1978, 1979) on two Pacific salmon species, Bromage and Duston (1986) and Bromage et al. (1993a,b) on rainbow trout, Taranger (1993) on Atlantic salmon and Smith et al. (1991) and Björnsson et al. (1998) on Atlantic halibut. There are also variations in the timing of spawning of trout exposed to long and then short photoperiods at different times of the year with spawning time again not related in a consistent way with the onset of either the long or short daylengths (Bromage et al., 1992). It is of relevance here to mention that fish also continue to spawn under a range of year-round constant daylengths from LD 6:18 to LD 22:4 as well as continuous light (LL) and continuous dark (DD) (Bromage and Duston, 1986 and unpublished data; Bromage et al., 1993a,b). It would thus appear that the response of fish to light is not dictated solely by their exposure to a particular critical daylength.

This contention is further supported by a series of experiments in trout in which the effects on spawning of different lengths of long and short days have been investigated (Randall et al., 1987, 1991a; Davies et al., 1991; Randall and Bromage, 1998). These show that any daylength can be considered long or short providing that the previous photoperiod to which the fish have been exposed is shorter or longer respectively. Randall et al. (1991a), Davies and Bromage (unpublished) and Randall and Bromage (1998) showed that daylengths as "long" as LD 13.5:10.5 or even LD 18:6 can be considered as 'short' if fish have previously been exposed to longer daylengths, i.e. LD 18:6 or LD 22:2, respectively. Even daylengths of LD 6:18 or LD 8.5:15.5 which are generally considered to be short can produce advancements of spawning if followed by exposure to even shorter daylengths of LD 2:22 or LD 1.5:22.5, respectively (Randall et al., 1987, 1991a; Davies et al., 1991; Davies and Bromage, unpublished; Randall and Bromage, 1998). Clearly, photoperiodic history and the direction of change of photoperiod is of far greater importance in the timing of maturation and spawning in fish than any concept of a rigid critical daylength. These data also indicate that the changing photoperiod is in some way modifying other internal processes (see Section 5).

The clear dependence of spawning time on photoperiodic history has lead inevitably to complications in the interpretation of results of experiments where the timing of introduction of fish into a specific experimental photoperiod may have been carried out at different times of the year from quite different ambient daylengths. Under such conditions, the response achieved will be dependent on the photoperiod from which the animals had been transferred at the beginning of the experiment. The range of responses which can be achieved is well illustrated in the work of Randall and Bromage (1998) and Randall et al. (1998, 1999) who transferred fish from ambient daylength to continuous light, LD 18:6 or LD 6:18 at regular intervals throughout the year. Spawning occurred over a number of months in all three experimental groups of fish.

5. Endogenous biological clocks or rhythms

From the foregoing discussion of the relative importance of photoperiodic history and critical daylength in the timing of fish reproduction, it is clear that the response to seasonal changes in daylength, exhibited by many fish, cannot be accommodated within classical concepts of photoperiodism as exemplified by many birds and mammals (Follett, 1984). A particular difficulty is the ability of fish to continue to spawn at approximately annual intervals under constant conditions of light, temperature, food, etc. (i.e. in the absence of any obvious changes in environmental cue). This has lead to suggestions that endogenous timing mechanisms may be in operation (Whitehead et al., 1978; Sundararaj et al., 1982; Bromage and Duston, 1986) and, because they relate to annual rhythms, they have been described as circannual (see Gwinner, 1986).

Full acceptance of a rhythm as circannual depends on a number of criteria being met: firstly, the rhythm must be represented over more than one and preferably several cycles; secondly, under constant conditions it should "free-run" with a periodicity which approximates to, but is significantly different from a year; thirdly, it should be entrainable by environmental factors or cues; fourthly, the rhythm should not be unduly influenced by variations in temperature; and lastly, the rhythm should exhibit a differential response to photoperiod; this is dependent on the phase of the rhythm which is exposed to the photoperiod cue, i.e. it should display a phase response curve. Many of the earlier studies on fish do not satisfy all of these criteria, generally because they had only considered data from a single reproductive cycle or that all conditions had not been maintained at constant levels throughout the experiments (see Bromage and Duston, 1986; Duston and Bromage, 1986, 1987, 1988, 1991 for further discussion). Amongst the earlier studies, only Sundararaj et al. (1982) working with the Indian catfish (*Heteropneustes fossilis*) provided good evidence of a self-sustaining circannual rhythm, although such a rhythm was also proposed for the stickleback (Baggerman, 1980).

Studies in this laboratory with trout have followed spawnings of fish maintained under constant condition for up to 5 years. Trout maintained under constant LD 6:18 exhibited circannual rhythms of gonadal maturation and spawning (Duston and Bromage, 1986, 1991), whose periodicity varied between fish, and in successive cycles in the same fish, ranging in length from 11–15 months. Under LD 18:6 or LL, the periodicity of the rhythm was much shorter (Bromage et al., 1984; Duston and Bromage, 1986). Similar free-running circannual periodicities in spawning have also been recorded for barbel (*B. barbus*) maintained under constant LD 8:16 (Poncin, 1991) and sea bass under constant LD 15:9 and LD 9:15 (Carrillo et al., 1993; Prat et al., 1999). Recently, further support that the apparent circannual rhythms of reproduction in fish were truly endogenous was provided by Randall et al. (1999); these authors described seasonal phase response curves in the spawning time of rainbow trout following exposure of groups of fish to long or short daylengths at a series of approximately monthly time points throughout the year (Fig. 1). Although a phase response curve was first suggested by Duston and Bromage (1991), their data was drawn from several experimental populations whereas those of Randall et al. (1999) comprised a single experiment.

The entrainment behaviour of these circannual cycles of reproduction also show considerable similarities to those of circadian rhythms. Thus, zeitgebers or cues designed

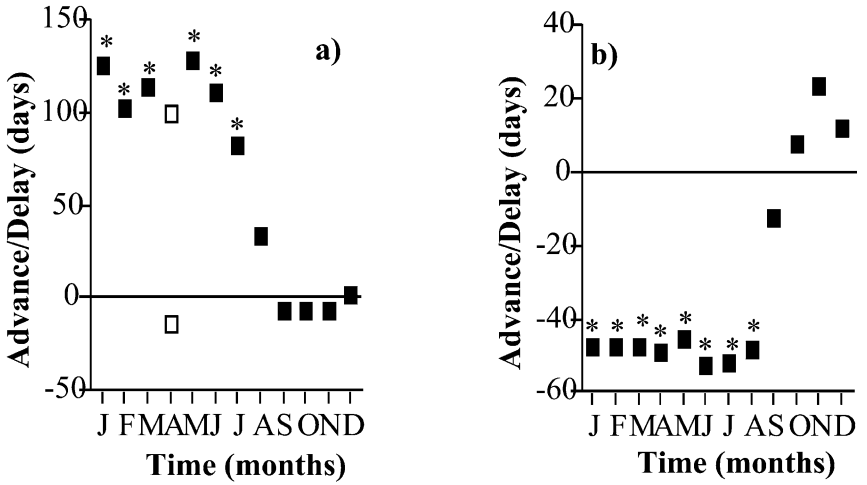


Fig. 1. Phase response curves illustrating the advance (+ive) or delay (−ive) in mean spawning time (first reproductive cycle) following transfer to either constant short (LD 6:18) (a) or long (LD 18:6), (b) days at monthly intervals. The rhythm split into two components/groups of fish during the first reproductive cycle of fish transferred to short days in April (open squares). * Significantly different from controls (horizontal line) at $p < 0.05$. Control spawning time February–April (from Randall et al., 1999).

to advance or delay the rhythm characteristically produce less advance or delay than might be expected (see Section 4; Bromage and Duston, 1986; Bromage et al., 1993a; Smith et al., 1991; Björnsson et al., 1998). Such differences in phase are typical of true endogenous rhythms.

If one accepts that endogenous mechanisms underlie the timing of reproduction in fish, then it is easier to understand how the altered seasonal and constant photoperiod regimes exert their effects, why photoperiodic history and the direction of change of daylength are important and why the same photoperiod can induce different effects if administered at different times of the year. Under natural conditions, the circannual rhythm or clock would be fully in phase with the ambient light cycle as a result of ongoing re-entrainment by the seasonally-changing daylength. Long days earlier in the year, particularly if followed by short days, would be expected to lead to corrective phase advances of the rhythm because it will have been perceived as running behind real time. Conversely, short days early in the year or long days after the Summer Solstice would both be perceived as the clock being ahead of real time and would be followed by phase delays in the rhythm; in a similar fashion compressed and extended seasonal light cycles would lead respectively to phase advances and delays of the endogenous rhythm.

6. Photoperiod–temperature interactions

There is considerable evidence to suggest that the maturation of many carps and other cyprinids, catfish and other tropical and sub-tropical species is affected by temperature (de Vlamming, 1972; Sundararaj and Vasal, 1976; Billard, 1981–1982; Davies et al.,

1986; Horvath, 1986; Munro, 1990). For other cyprinids and also *Fundulus heteroclitus* both high temperatures and long daylengths appear important (de Vlamming, 1972; Billard, 1981–1982; Taylor, 1986; Munro, 1990). However, there is continuing controversy as to whether the temperature effects are direct ones and hence could be considered 'permissive' or whether temperature is acting as a proximate cue in its own right. A major problem with much of these data is the failure by many of the investigators to experimentally differentiate photoperiodic from temperature effects.

In a similar way, much of the work with salmonids has been conducted in borehole or spring waters which have constant water temperatures (8–11°C), again raising the question of the relative importance of temperature and photoperiod in the timing of reproduction. Davies and Bromage (1991) confirmed, at least as far as the rainbow trout is concerned, that photoperiod is the principal determinant of the timing of maturation, with water temperature providing only a moderate influence. These authors showed that long days followed by short days produced similar 3–4 month advancements of spawning in fish maintained in river water, which had seasonally varying temperatures (2–21°C), to those maintained in constant 9°C borehole water; there was, however, a modifying influence of temperature with the fish maintained in river water, irrespective of whether they were photoperiodically advanced or controls under ambient daylength, spawning 3–4 weeks after the corresponding groups held under constant temperatures. Whether this modest delay was due to a supplementary cueing by temperature is not known. However, it is more likely that the effects of temperature were achieved by direct effects on the ovary, as similar delays in spawning have been reported for trout held at low temperatures (Morrison and Smith, 1986; Bromage and Cumaranatunga, 1988; Nakari et al., 1988).

There are also other complications with temperature relating to its effect on the final maturation of the oocytes, ovulation and oviposition. Davies and Bromage (unpublished) found in trout, maintained in river water and photoperiodically induced to spawn in mid-summer during periods of very high ambient temperatures, that the eggs undergoing final maturation in the ovary appeared to overripen whilst still within the gland and before ovulation had occurred, resulting in no eggs being released.

A more detailed study of the effects of higher temperatures on maturation and spawning in the rainbow trout was carried out by Pankhurst et al. (1996). These authors held groups of broodstock trout at temperatures ranging from 9°C to 21°C for the 3 months before the predicted spawning times. Egg production was normal up to 15°C but significantly reduced above this temperature. Furthermore, no eggs survived from broodstock maintained above 15°C.

High temperatures have also been reported to inhibit ovulation and reduce fertility in sturgeon (*Acipenser transmontanus*) broodstock maintained at constant 15°C or 18°C (Webb-Brewer et al., 1999). Temperatures of 12°C or 16°C delayed maturation in Arctic charr (*Salvelinus alpinus*: Jobling et al., 1995) and those above 6°C reduced egg production and egg and larval survivals in Atlantic halibut (Brown et al., 1995). All these effects appear to be direct ones on gonadal development. Temperatures above 14°C also appear to delay ovulation in Atlantic salmon (Taranger and Hansen, 1993; Duncan et al., 1999; Taranger et al., 1999b). Taranger et al. (1999b) showed this to be clearly due to temperature by reducing the temperature of the water supply to the

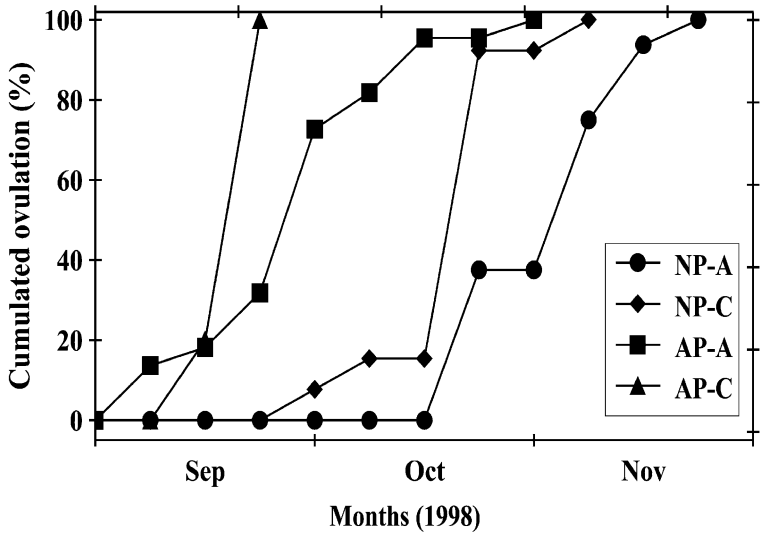


Fig. 2. Effects of accelerated (AP:LL from February–May and then LD 8:16) and ambient (NP) photoperiod on cumulative spawning of previously immature Atlantic salmon maintained under ambient (A) or cooled (C) water supplies (from Taranger et al., 1999b).

broodstock from 14°C to 8°C; only the broodstock at the lower temperature ovulated (Fig. 2). This effect occurred in both photoperiodically advanced and ambient light maintained broodstock.

In a similar way, Carrillo et al. (1991, 1993) reported that sea bass, which had been programmed to spawn in September using a modified photoperiod, failed to spawn naturally unless the water temperatures were reduced below 16°C or the fish received LHRHa injections. By contrast, fish in which final maturation was adjusted so that it occurred a little later in the Autumn or Winter, when ambient water temperatures had cooled, were able to spawn naturally without temperature or hormonal manipulation. Clearly, in all these species of fish the high temperatures were in some way interfering with the normal processes of final maturation, ovulation and/or oviposition.

7. Effects of salinity

There is little published data on the effects of changed salinity on the timing of reproductive development. This is surprising given the migrations between fresh and sea water and sea and fresh water that many fish undergo. Zanuy and Carrillo (1984) and Haddy and Pankhurst (2000) reported no effects of a range of salinities on reproductive development, respectively, in European seabass and black bream (*Acanthopagrus butcheri*). By contrast reduced salinity slowed ovarian development in the striped mullet (*Mugil cephalus*) (Tamaru et al., 1994). Recently, Magwood et al. (1999) showed that ovulation in Atlantic salmon was advanced in fish transferred to fresh water up to 3–4 months before the time of expected ovulation compared to broodstock maintained in sea

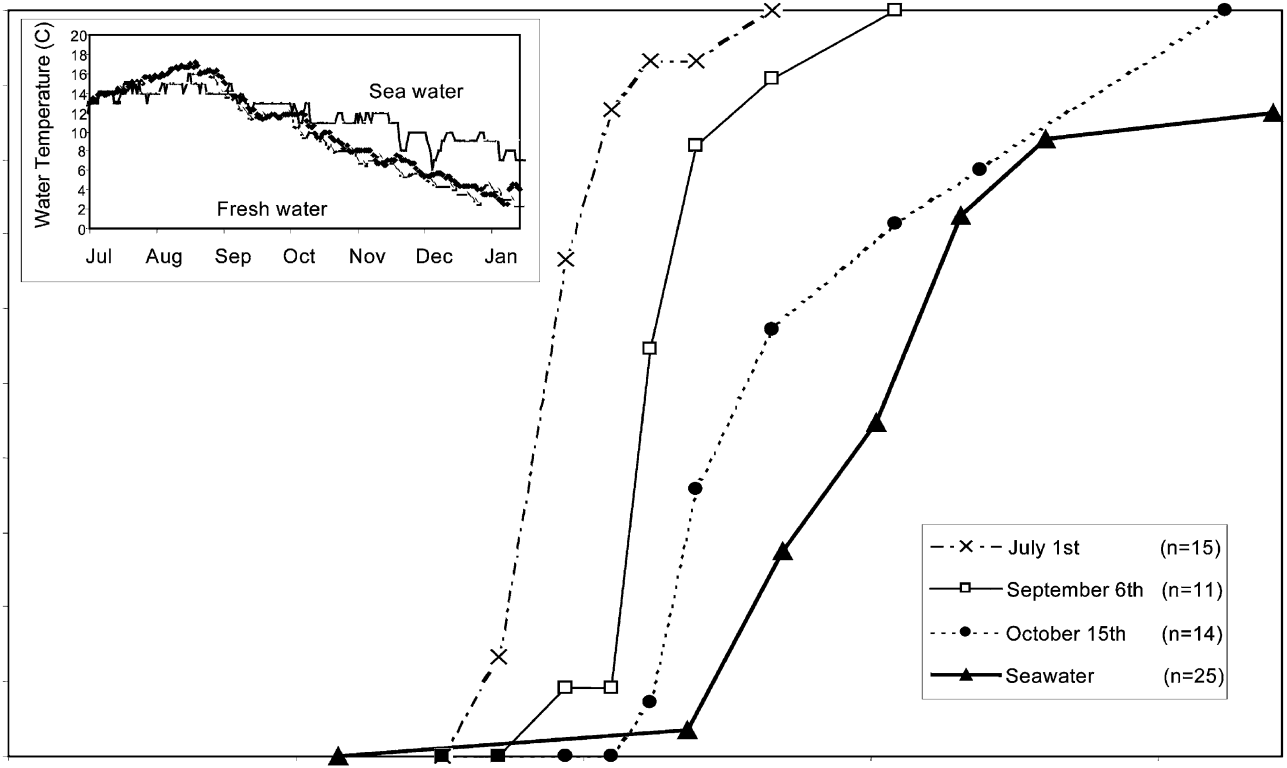


Fig. 3. Effects of transfer from sea water to fresh water in July 1st, September 6th or October 15th on cumulative spawning times of maturing Atlantic salmon grilse. A further group was maintained in sea water (from Magwood et al., 1999).

water (Fig. 3). Furthermore, the spawning period of the fish maintained in sea water was also less synchronised than those transferred to fresh water. Thus, it appears that salinity may act as a subsidiary or permissive cue to photoperiod in some species.

8. Effects of fish size / nutritional status

There is considerable circumstantial evidence that the relative availability of food significantly affects the ability of animals to mature. Fish in oligotrophic waters are said not to spawn as frequently as their relatives in richer environments. Experimentally, maturity levels can also be reduced by feeding lower ration levels (Jones and Bromage, 1987; Thorpe et al., 1990; Burton, 1991). Overall these data suggest that fish must be of a threshold or critical size or stage of development or have certain levels of energy supply or storage depots for maturation to occur. These hypotheses beg the question as to how fish measure their size or well-being and when do they measure it in order to make the decision to mature. It would not appear to be their size at spawning time because in annually spawning fish, invariably spawning and non-spawning groups include a full range of fish sizes. There are also experimental data to suggest that the assessments of size or energetic or nutritional status occurs at an early stage of maturational development (Duston and Saunders, 1992). Thus, Bromage and Jones

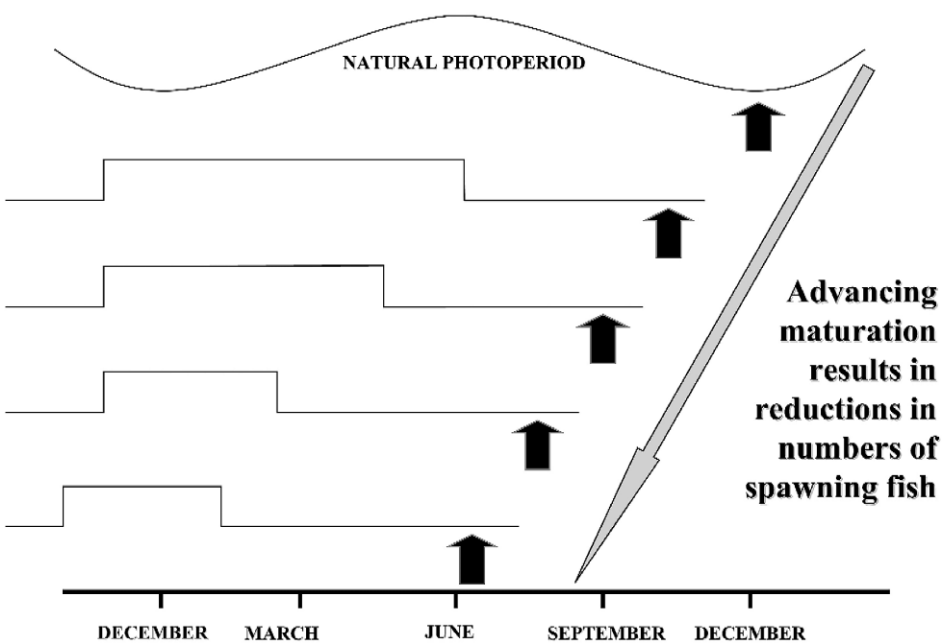


Fig. 4. Effects of long-short daylengths on the timing of spawning and percentage of trout undergoing maturation in that reproductive cycle. The greater the degree of advancement of spawning, the fewer fish mature.

(1991) and Bromage et al. (1992) showed that experimental reductions in ration during the first 3–4 months after the first spawning of rainbow trout were more effective in reducing the percentages of maturing fish at the second spawning than the same restriction given later in the annual cycle. Similar results have also been reported for salmon (Rowe and Thorpe, 1990; Thorpe et al., 1990; Duston and Saunders, 1999).

There is also evidence from studies of photoperiod advancements of spawning that increasing numbers of fish fail to spawn, the earlier the manipulation is made during the reproductive cycle (Duston and Bromage, 1987, 1988; Taranger et al., 1991, 1998, 1999a,b; Randall and Bromage, 1998; Fig. 4). Thus, it would appear that fish have to be at a minimum or critical size or energetic or nutritional status at a specific stage of maturational development in order for them to be able to respond to photoperiodic or other proximate cues. From this, one might conclude that size or energy status has a permissive role in reproductive development. This concept has also given rise to ideas of 'windows of opportunity' or 'gating of rhythms' (McCormick and Naiman, 1984; Thorpe, 1986, 1989; Duston and Bromage, 1988; Duston and Saunders, 1999) at which time a decision to mature is made on the basis of some measure of nutritional or energetic sufficiency.

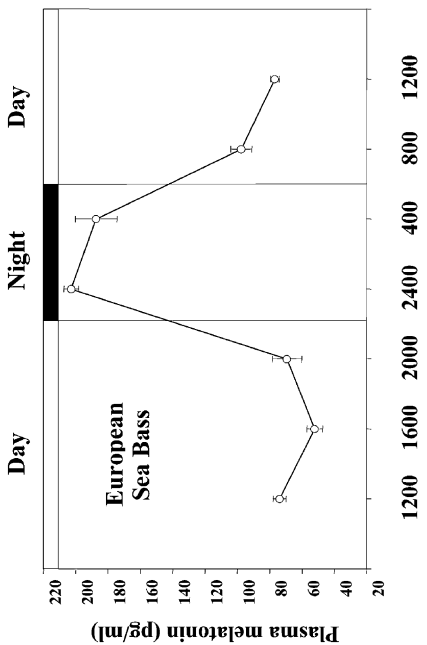
How measurements of size or energetic status are achieved is not known. However, the frequently suggested involvement of lipid stores (Rowe et al., 1991), which are thought to be a particularly good indicator of nutritional status in fish, has implicated leptin, growth factors, cortisol and growth hormone amongst others as possible metabolic indicators of growth or energetic status. It is thought that one or more of these factors may in some way interact with the hypothalamic–pituitary mechanisms, which ultimately control puberty and subsequent spawnings.

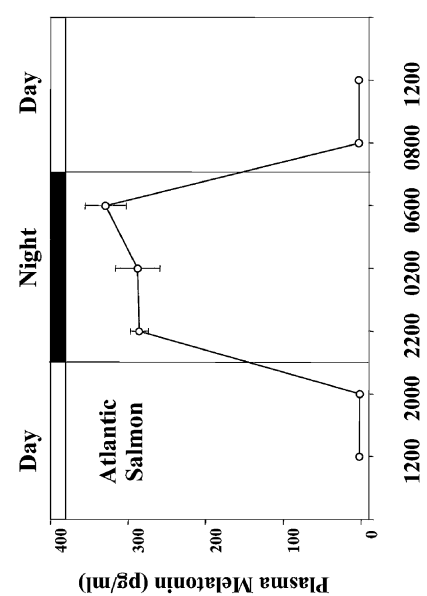
9. Melatonin secretion and the timing of reproduction

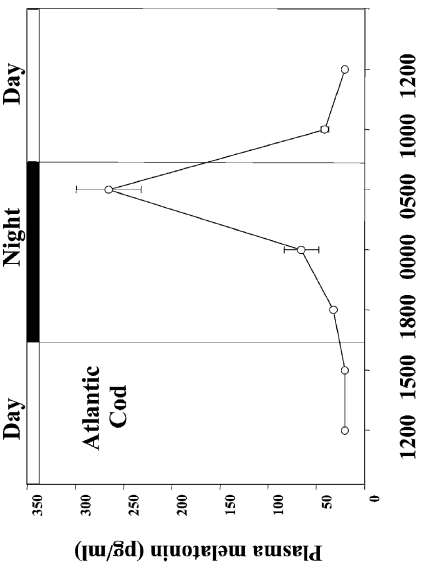
9.1. Diel and seasonal changes in melatonin

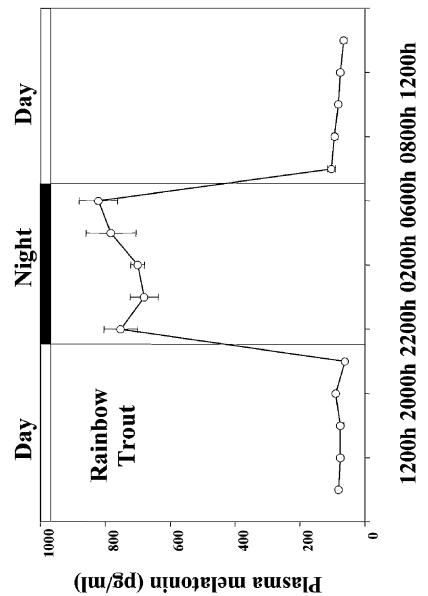
Despite widespread recognition of the importance of photoperiod in the timing of reproduction and extensive knowledge of the hypothalamic–pituitary mechanisms involved in its control, it is only recently that we have begun to understand how animals obtain information on the daily and seasonal changes in daylength. In mammals, it is now clear that information on daily and calendar time is provided by the changing patterns of secretion of the indoleamine hormone melatonin from the pineal gland (Bartness and Goldman, 1989; Bartness et al., 1993). In all vertebrates so far examined, including fish, levels of circulating melatonin are raised during the night and fall to basal during the day. Fig. 5 shows the diel profile of change of plasma melatonin in the

Fig. 5. Diel profiles of plasma melatonin levels (Mean ± SEM; pg/ml) in Atlantic salmon, rainbow trout, European sea bass and Atlantic cod. In some cases the error bars are too small to be depicted (from Porter et al., 1999a,b; Carrillo et al., unpublished; Dahle et al., unpublished; Porter et al., 2001b).


Day
Night
Day
European
Sea Bass
Plasma melatonin (pg/ml)
220
200
180
160
140
120
100
80
60
40
20
1200 1600 2000 2400 400 800 1200
Time (h)


Day
Night
Day
Atlantic
Salmon
Plasma Melatonin (pg/ml)
400
300
200
100
0
1200 2000 2200 0200 0600 0800 1200
Time (h)


Day
Night
Day
Atlantic
Cod
Plasma melatonin (pg/ml)
350
300
250
200
150
100
50
0
1200 1500 1800 0000 0500 1000 1200
Time (h)


Day
Night
Day
Rainbow
Trout
Plasma melatonin (pg/ml)
1000
800
600
400
200
0
1200h 2000h 2200h 0200h 0600h 0800h 1200h
Time (h)

rainbow trout, Atlantic salmon, European sea bass and Atlantic cod; Gern et al. (1978a) and Sánchez-Vásquez et al. (1997) have also reported such changes in rainbow trout and European sea bass, respectively. Although there are differences between these and also other species in the timing and amplitude of the changes in levels of melatonin in relation to nightfall (cf. the profiles of the salmonids and that of the cod: Fig. 5), in general, the duration of the nocturnal increase in plasma melatonin levels is directly proportional to the length of the night and hence provides a direct transduction of night length. Similar diel patterns of circulating melatonin are also seen in the common carp (*Cyprinus carpio*: Kezuka et al., 1988); goldfish (*Carassius auratus*: Kezuka et al., 1992; Iigo et al., 1994a), pike (*Esox lucius*: Fálcon et al., 1989), brook trout (Zachmann et al., 1992a) and three-spined stickleback (Mayer et al., 1997). Exposure of fish experimentally to either constant long or short days leads to a curtailment or extension of the duration of raised melatonin levels, respectively (Randall et al., 1991a,b).

Following exposure to light or return to darkness from light, plasma melatonin levels in trout return to values normally seen during the day or night, respectively within 30 min of treatment (Randall et al., 1991b; Alvarino et al., 1993a,b). Similar response times are also seen in other species indicating the ubiquity of plasma melatonin's transduction of altered daylength. In all circumstances, it would appear that the diel changes in melatonin secretion result from alterations in activity of pineal serotonin *N*-acetyltransferase (NAT), which is a key enzyme in the biosynthetic pathway for melatonin (Fálcon et al., 1987, 1992; see also the excellent review of Ekström and Meissl, 1997).

An important development in our understanding of the possible role of melatonin secretion in seasonal reproduction in fish was the work of Randall et al. (1995a,b) on the seasonal changes of diel plasma melatonin profiles in the Atlantic salmon. Measurements taken at the Winter and Summer Solstices, the Spring (Vernal) and Autumn (Fall) Equinoxes and at intermediate times (Randall et al., 1995; Randall et al., unpublished) showed that on all occasions throughout the year, the duration of raised melatonin values faithfully represented the length of the night and hence, the seasonally changing pattern of daylength. This change is diagrammatically represented in Fig. 6 (from Porter et al.,

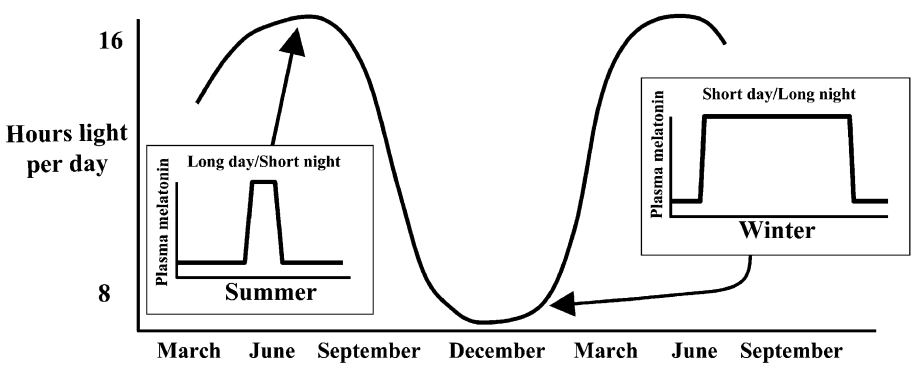


Fig. 6. Diagrammatic representation of the seasonal changes in diel profile of plasma melatonin. This provides information on daily and calendar time (from Porter et al., 1999c).

1999c). In this way, all organ systems including those involved with the control and timing of reproduction would be provided with information on daylength and its seasonal change and hence, calendar time.

9.2. Effects of light intensity on melatonin secretion

Following exposure of fish to light, levels of melatonin in both the plasma and pineal fall to basal values within 30–45 min (Randall et al., 1991b; Zachmann et al., 1992a; Alvarino et al., 1993a,b). Similar effects have also been reported with in vitro pineal preparations (Max and Menaker, 1992; Porter et al., unpublished). Both in vivo and in vitro, these reductions occur in a dose dependent manner. Zachmann et al. (1992a) working with brook trout showed that exposure at mid-dark to an illumination of 2 lux at the water's surface was sufficient to reduce plasma melatonin values to 50% of the night-time levels of controls; 20 lux lighting reduced levels fully to day-time values. Working with rainbow trout in vivo, Alvarino et al. (1993b) showed that 1 h light pulses of either 1 or 10 lux intensity reduced melatonin levels to 33% or 22% of the previous scotophase (night-time) values, respectively. Similar reductions in plasma melatonin values following progressive increases in illumination during the dark period are also seen in the Atlantic salmon (Fig. 7 from Porter et al., 2001a; see also Porter et al., 1999a,b) and rainbow trout (Futter et al., 1999).

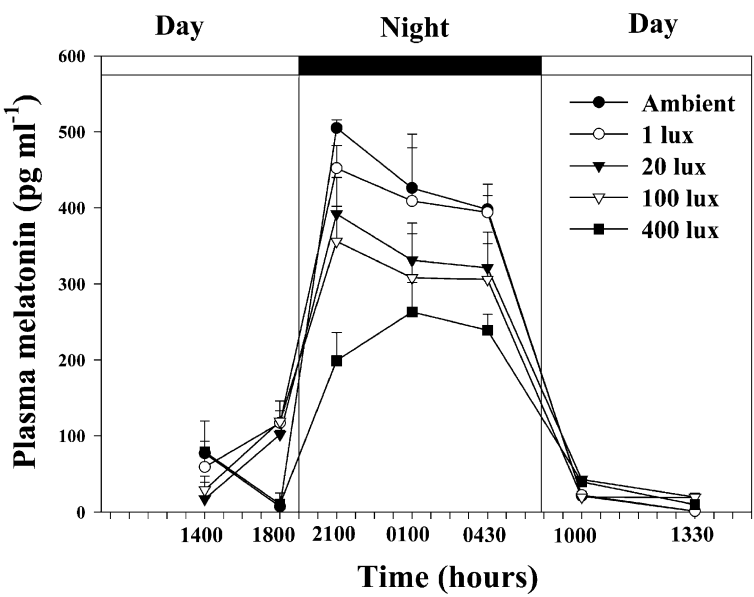


Fig. 7. Diel profiles of plasma melatonin levels (Mean ± SEM; pg/ml) under ambient daylength and under different light intensities (1, 20, 100 and 400 lux) of additional night-time illumination administered 1 h before dusk and until 1 h after dawn each day. In some cases, the error bars are too small to be depicted (from Porter et al., 1999a, 20001a).

A major question relating to light intensity is what levels of illumination are perceived as 'day' and what are seen as 'night'. Irrespective of whether the effects of daylength on reproduction are achieved by neural or hormonal pathways (see Section 9.5), there must be a threshold of illumination above or below which it is considered day or night, respectively. This question assumes a greater significance when lights are being used on commercial farms to artificially increase daylength. It is even more problematic when the lights are being used on cages where the artificial illumination has to be superimposed on the ambient solar daylight which is almost always far brighter than the brightest artificial lights (see Section 11 for further discussion of this subject).

9.3. Temperature effects on melatonin secretion

Aside from light and daylength, melatonin secretion is also influenced by temperature. In general, night-time melatonin levels are lower at reduced temperatures and increase at higher ones (Randall et al., 1995a,b). Fig. 8 shows data of Porter et al. (2001a) on the effects of temperatures of 4°C and 12°C on diel plasma melatonin levels of Atlantic salmon maintained under both long and short daylengths. Under both photoperiod regimes, the highest night-time values were found at the higher temperatures. Similar data have been recorded for melatonin secretion from pineal glands in culture (Zachmann et al., 1991, 1992b; Falcón et al., 1992; Max and Menaker, 1992; Porter et al., unpublished). Max and Menaker (1992) also reported that in addition to enhancing melatonin levels in the dark, higher temperatures also increased the sensitivity of the pineals to light, i.e. the light intensity necessary to reduce melatonin secretion to basal levels in the day was lower at higher temperatures.

There is also evidence from the in vitro pineal studies of Zachmann et al. (1991, 1992b) and Falcón et al. (1992) in white sucker (*Catastomus commersoni*) and pike that under DD, diel temperature cycles can induce rhythmic production of melatonin (see also Ekström and Meissl, 1997). However, it is not known whether such effects also occur in vivo or whether they are due solely to direct Q_{10} effects on the biosynthesis or metabolism of melatonin.

Overall, it is clear that there are temperature effects on melatonin secretion. Whether these are functionally important in the entrainment of reproduction must await studies of the effects of altered temperature on diel and seasonal patterns of melatonin secretion in tropical species, like the carps, where photoperiod is thought not to be such an important influence. Possibly, temperature's effects on melatonin secretion functions more to enhance the differences in melatonin profile (i.e. daylength) over the changing seasons. However, the differences in amplitude of plasma melatonin could act as a cue in their own right.

9.4. Endogenous secretion of melatonin

Although the light–dark (LD) cycle is the principal determinant of the diel pattern of melatonin secretion in the majority of teleosts so far studied, there is substantial evidence for endogenous rhythms of melatonin secretion (see review of Ekström and

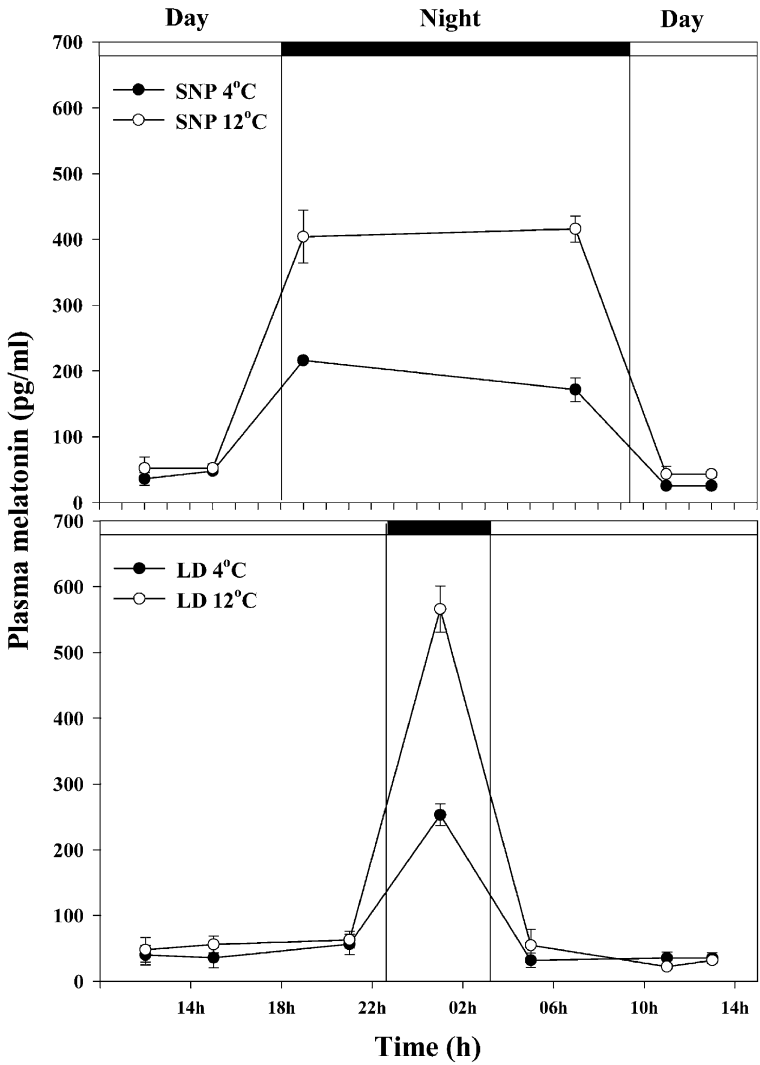


Fig. 8. Diel profiles of plasma melatonin levels (Mean ± SEM; pg/ml) under short (upper) and long (lower) daylengths in Atlantic salmon maintained in either 4°C or 12°C water supplies. In some cases, the error bars are too small to be depicted (after Porter et al., 2001a).

Meissl, 1997; Iigo et al., 1994a, 1997; Bolliet et al., 1994, 1995, 1996; Kezuka et al., 1989a; Okimoto and Stetson, 1999a,b). Much of this evidence derives from in vitro studies. These studies show that under LD cycles, cultured pineals, as expected, release melatonin into the culture media during the dark phase of the photoperiod. However, if the pineals of many of the species, which have been studied are then transferred to DD, they continue to produce melatonin as though they were still under the previous LD

cycle. The period lengths of these rhythmic secretions of melatonin are, however, slightly different from the 24-h LD cycle. In the case of the pike (Falcón et al., 1992), the period length is longer than 24 h whereas it is shorter in the white sucker (Zachmann et al., 1991) and of lengths ranging from 19.5 to 29 h in several poecilid species (Okimoto and Stetson, 1999a,b). Collectively, these data point to the presence in the pineal of a circadian oscillator or clock, which is responsible for controlling melatonin secretion. Normally, this clock is entrained by the LD cycle, but under constant conditions, it 'free runs' with its own endogenous periodicity. Fig. 9 shows an example of circadian periodicity in vitro in the Atlantic halibut (Porter et al., unpublished). Under the LD cycle, melatonin secretion was entrained by photoperiod. However, this rhythmicity continued under DD.

Out of the 30 species included in the review of Ekström and Meissl (1997) and the studies of Iigo et al. (1994a, 1997) and Porter et al. (unpublished; Fig. 9) only four species failed to show any evidence of endogenous rhythmicity and these were all salmonids. The non-endogenous nature of melatonin secretion of the salmonid pineal is also supported by the in vivo study of Randall et al. (1991b) and Randall and Bromage (unpublished) with the rainbow trout. They showed that under both DD and LL, plasma melatonin levels of fish, previously under LD regimes, remained at high and basal levels, respectively, with no signs of any rhythmic changes.

Although the non-salmonid pineals all exhibited endogenous patterns of melatonin secretion in culture, in most cases the amplitude of the secretion became reduced, i.e. dampened with each successive cycle. Although this may have been due to sub-optimal culture conditions, it is more likely that the oscillators are themselves "slaves", i.e. are driven by a master oscillator or clock, which is external to the pineal.

Despite the pineal showing clear evidence of endogenous circadian rhythmicity, it is difficult to see how this endogenicity relates to the circannual rhythm or clock which is thought to control reproductive function (see Section 5). Work by Duston and Bromage (1986) using LD cycles of different length to 24 h, i.e. T-cycles (comprising LD 6:42, LD 6:48 and LD 6:54) showed clearly that spawning was not timed from counts of the numbers of 24-h LD cycles.

9.5. Melatonin, the pineal and reproduction

The clear effects of photoperiod on the timing of reproduction on the one hand and on the diel and seasonal patterns of melatonin on the other provide strong circumstantial evidence that melatonin is the intermediary in these processes. Direct experimental evidence for this involvement is, however, far less convincing.

There are two principal forms of approach which have been utilised with the aim of establishing melatonin's role in controlling reproduction. For practical reasons neither approach is entirely satisfactory in fish. The first approach is removal of the pineal gland or pinealectomy (PNX). This removes the principal if not the only source of the diel patterns of melatonin secretion (Gern et al., 1978b; Kezuka et al., 1992; Porter et al., 1995, 1996). Melatonin is also produced by the retina and probably the digestive tract although, unlike the pineal source, there are not thought to be any day–night differences

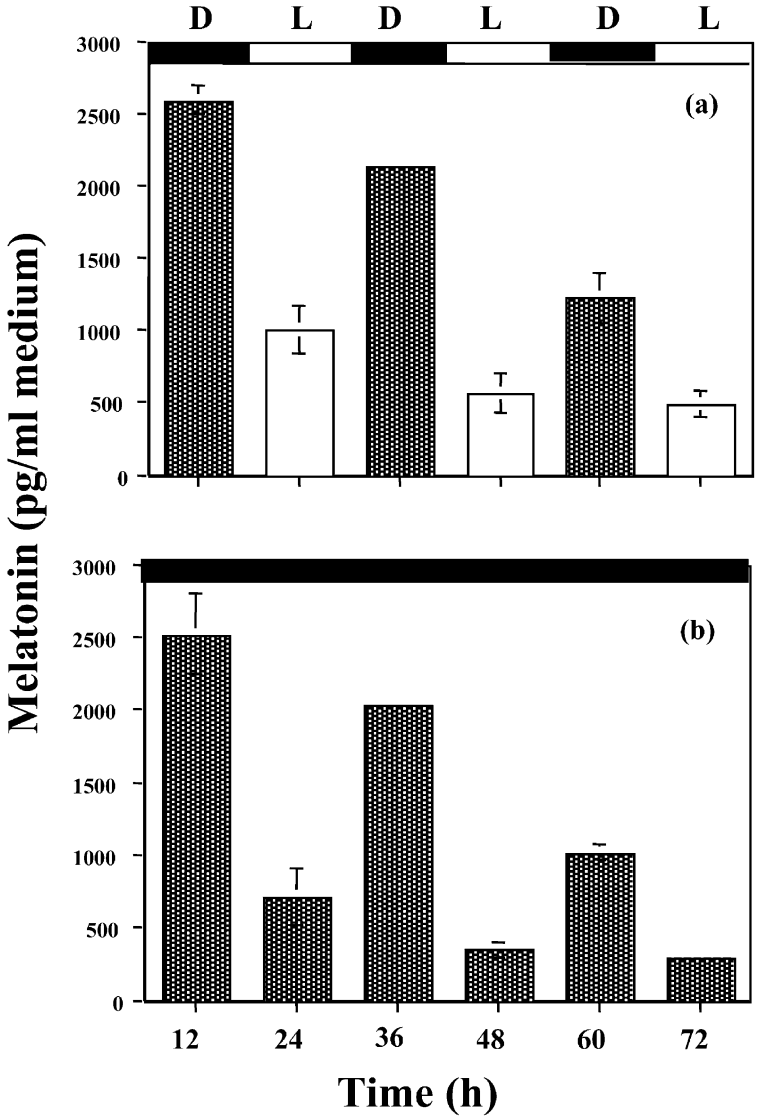


Fig. 9. In vitro secretion of melatonin (Mean ± SEM; pg/ml culture media) from cultured pineal glands of Atlantic halibut maintained under LD12:12 (a) or DD (constant dark; b). The pineals from both groups were removed from fish which had been previously maintained on LD 12:12. In some cases, the error bars are too small to be depicted (from Porter et al., unpublished).

in patterns of secretion from these sources (Gern et al., 1978b; Porter et al., 1995, 1996). Fig. 10 shows the effects of PNX, sham PNX and PNX plus enucleation on plasma melatonin levels in the rainbow trout. Diel melatonin secretion is reduced to day-time values after PNX and there is no increase in melatonin levels during the night. After

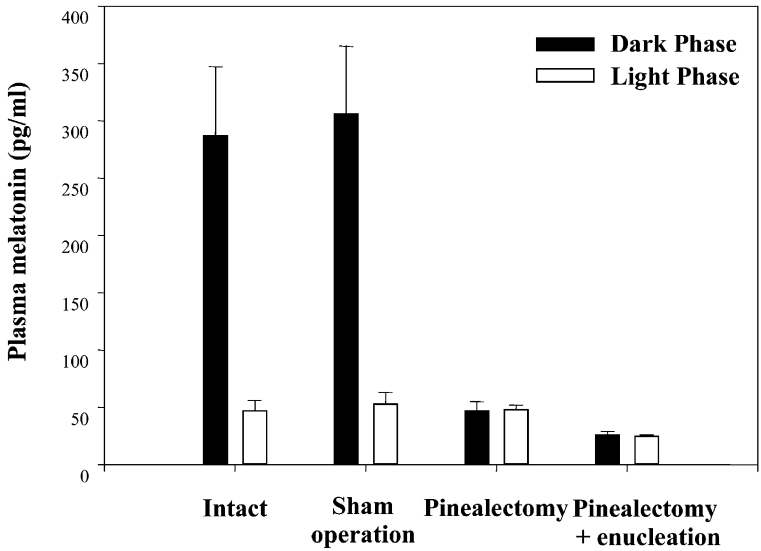


Fig. 10. Mid-light and mid-dark plasma melatonin levels (Mean ± SEM; pg/ml) from intact, sham-operated, pinealectomised and pinealectomised + enucleated rainbow trout. In some cases, the error bars are too small to be depicted (from Porter et al., 1995, 1996).

enucleation, levels are significantly lower than normal day-time values and levels are the same during the day as those at night; possibly this residual melatonin is produced by the alimentary canal (see Ekström and Meissl, 1997). Collectively, these data together with those of Kezuka et al. (1992) indicate that only the melatonin produced by the pineal is influenced by the LD cycle.

Functionally, PNX might be expected to act as though it was continuous light (LL). Possibly more importantly PNX, in addition to removing diel melatonin secretion also severs neural connections between the pineal and the brain. Hence, the PNX procedure is unable to distinguish whether the pineal's undoubted role in the coordination of reproduction is achieved by neural or hormonal mechanisms or a combination of both.

The second approach which has been employed with several vertebrate groups is treatment with exogenous melatonin. Depending on the timing of administration of melatonin, this might be expected to act as though the fish were being exposed to a longer night, i.e. a shorter day. In mammals, three methods have been used to administer melatonin: injection, constant release implant and infusion devices. There are inherent problems with both injections and implants of melatonin, which will be considered later in this section.

Infusion devices are not technically feasible in fish. This is unfortunate because it is this method more than any other which has established melatonin's central role in the control of seasonal breeding in mammals (Bartness et al., 1993). In these studies, pinealectomised hamsters or sheep were maintained under a daylength which in intact animals would not have resulted in seasonal breeding, i.e. short days in hamsters and

long days in sheep. Animals were then infused with diel melatonin levels which simulated those seen normally under long or short daylengths, respectively, i.e. putative stimulatory regimes; other animals were infused with melatonin profiles which would not be expected to be stimulatory. In all experiments, irrespective of the prevailing photoperiod, the duration of the infused melatonin profile, by imitating either long or short days, elicited the expected reproductive response, thus confirming melatonin's role as the intermediary in the transduction of information on daylength to the reproductive axis.

Given that infusion devices are not practicable for fish, workers have been forced to use injections, implants or immersion in solutions of melatonin to administer melatonin. Injections because of the short half-life of melatonin, estimated to be 8–9 min in Atlantic salmon at 4–5°C (Randall et al., unpublished), cause transitory spikes of plasma melatonin and hence do not simulate the normal profile. Implants of melatonin in polymeric prostheses provide long term delivery but only at constant levels. In some mammals, constant high levels do not produce the expected reproductive response (Bartness et al., 1993; Williams and Helliwell, 1993). It would appear that in these species, reproductive development is only elicited if the appropriate melatonin profile is separated by periods of low or basal melatonin secretion. Mayer et al. (1997) failed to find any effects of melatonin on reproduction in the stickleback following daily immersion of fish in solutions of melatonin. However, as sticklebacks are generally too small to provide regular blood samples for diel melatonin measurements, it is not clear from the data provided how the plasma melatonin profile would have been changed by the immersion protocol.

Notwithstanding these practical constraints there are substantial data implicating melatonin in the control and timing of reproductive events in fish (Bromage et al., 1995; Mayer et al., 1997; Ekström and Meissl, 1997). Both melatonin injection and PNX have variously been reported to cause interruptions, atrophy or stimulation of gonad development depending on the times of the year of the treatment and the nature of the reproductive cycle of the fish under investigation (see Garg, 1987; Kezuka et al., 1989b; Bromage et al., 1994, 1995; Randall et al., 1995 and reviews in Mayer et al., 1997; Ekström and Meissl, 1997). These apparently paradoxical effects are to be expected if PNX and melatonin administration are simulating long and short daylengths and if as suggested earlier (Sections 4 and 5) all fish exhibit seasonal variation in response to both long and short days.

10. Transduction of photoperiodic information to the reproductive axis

It is now well established that the clear effects of photoperiod on reproduction are achieved by alterations in timing of the cycles of secretion of the various constituent hormones of the brain–pituitary–gonadal axis. Work in masu salmon (*O. masu*, Amano et al., 1994) and the rainbow trout (Davies et al., 1995, 1999) has shown that the timing of temporal changes in pituitary sGnRH which occur during maturation are significantly altered by photoperiod manipulation. Amano et al. (1995) also showed that the number

of neurones expressing sGnRH mRNA in the preoptic area and the ventral telencephalon were also modified by photoperiodic advancement and delays of spawning compared to that of controls. Although these authors showed clear changes in sGnRH following photoperiodic challenge, Davies et al. (unpublished) were unable to demonstrate any changes in cGnRH-II, which could be related to reproduction.

Amano et al. (1994) and Davies et al. (1999) also reported that the changes in sGnRH were temporally correlated with levels of pituitary and plasma FSH (GtH I) and LH (GtH II) following photoperiodic alterations of maturation, vitellogenesis and spawning. Whether this apparent relationship between sGnRH and FSH is real must await current on-going studies on the relative functions of this and other GnRH species (Holland et al., 1998; Zohar, this volume).

Le Menn et al. (1998) and Bon et al. (1999) also showed that accelerated photoperiods advance the seasonal changes in both FSH and LH. Although the changes in LH following photoperiodic manipulation have previously been widely reported (Bromage et al., 1982a,b; Breton et al., 1985), these data on FSH are new, principally because of earlier problems with assays of this gonadotropic hormone.

Following the changes in GnRH and FSH, there are extensive data describing the seasonal changes in the gonadal steroids: 17β-oestradiol, oestrone, testosterone, 17α hydroxy-progesterone, 17α 20β dihyroprogesterone and vitellogenin following photoperiodic manipulation (Whitehead et al., 1978; Bromage et al., 1982a,b, 1984; Duston and Bromage, 1986, 1987, 1988; Bromage and Cumaranatunga, 1988; Carrillo et al., 1989, 1991, 1993; Taranger, 1993; Taranger et al., 1998; Zanuy et al., 1995; Prat et al., 1999).

Not surprisingly, given the clear effects of photoperiod on this endocrine cascade and also on pineal function and melatonin, there is considerable interest in identifying the link between these processes. Studies in several mammals using iodomelatonin have shown highly specific binding in the suprachiasmatic nucleus (SCN) and the pars tuberalis, both areas which are involved in the control of reproductive function (Williams and Helliwell, 1993). Similar high affinity binding sites have also been demonstrated in fish (Martinoli et al., 1991; Ekström and Vanecek, 1992; Davies et al., 1994; Pang et al., 1994; Molina-Borja et al., 1994; Iigo et al., 1994b; Falcón et al., 1996; Gaildrat et al., 1998). However, these high affinity receptors are far more widely dispersed in the brains of fish, albeit with concentrations in the pretectal area, the optic tectum, the thalamus and cerebellum, i.e. areas principally concerned with the handling of visual information. Very low levels of binding have been found in the pituitary or the areas of the brain known to contain GnRH neurones. Following the cloning of the first melatonin receptor by Ebisawa et al. (1994), Mazurais et al. (1999) developed riboprobes for two melatonin receptor subtypes: Mel 1a and Mel 1b (see reviews by Reppert et al., 1996; Reppert, 1997) and by in situ hybridisation compared their distribution with that of iodomelatonin binding in adjacent sections of brain. Both techniques gave similar distributions of receptor localisation, although the molecular approach provided much more detailed information on the cells, which were expressing melatonin receptors. Consistently, the highest signal was detected in the stratum periventriculare of the optic tectum and the posterior pretectal nucleus.

The study of Mazurais et al. (1999) also failed to show any melatonin receptor expression in the pituitary and only weak expression in the areas of the brain known to

contain GnRH neurones or dopaminergic neurones. Hence, any effects of melatonin on the GnRH neurones must be an indirect one via interneurones. It is possibly of interest here to mention that GABA neurones, which are known to influence GnRH cells, are also found in the pretectum and optic tectum.

Although these studies suggest how melatonin might affect reproductive function, an important question which remains is how these processes might interact with the underlying endogenous circadian and circannual processes of pineal and reproductive function. Recently, various clock genes (e.g. frequency—*frq*, period—*per* and timeless —*tim*) have been identified in *Neurospora*, *Drosophila* and several mammals (see Dunlap, 1998, for review). All of these genes are thought to be involved with the generation of circadian periodicity. Clock genes appear to function by transcribing clock proteins, which gradually build up and exert a progressively increasing negative feedback on the interaction of the transcriptional activators of clock gene expression. Hence, this system is self-limiting with a circadian periodicity. Using riboprobes for *Per 1* and mel 1b, Brierley et al. (1999) showed similar patterns of distribution of the two genes in the optic tectum and cerebellum of rainbow trout and cod. Whether these genes interact to control aspects of circadian function and how they might control longer-term circannual processes is not known.

11. Application of photoperiod techniques to aquaculture

Initially, the principal area in which knowledge of the effects of photoperiod on reproduction was used by aquaculture was the manipulation of spawning time. The ability to control spawning has enabled supplies of seed (eggs and/or fry) to be made available to grow-out farms for periods of the year outwith the normal spawning season of the fish in question (Bromage, 1995). By exploiting such supplies of seed, grow-out farms are better able to meet the market requirements for year-round production of market-sized fish. In practice, this has required the development of methods to both advance and delay spawning, ideally to any time in the year.

Arising from this knowledge base, more recently photoperiod techniques have been used by grow-out farms specifically to delay or inhibit reproductive development. This capability has been particularly helpful to grow-out farms for, by inhibiting or delaying maturation until after the fish have reached their marketable size, producers have been able to avoid the reductions in growth and marked deteriorations in flesh quality and appearance which inevitably accompany maturation in many farmed fish.

Given that the procedures and underlying photoperiodic mechanisms are the same, whether they are designed to produce out-of-season seed or to avoid maturational change, both will be considered together in the following text. This will include consideration of the procedures used on-land in covered enclosures (tanks, ponds, raceways, etc.) as well as the more recently developed methods for use with cages in the sea or in fresh water or with uncovered enclosures on-land. These descriptions should be read in conjunction with the background information regarding the principles and mechanisms involved in the photoperiodic response detailed in the earlier sections of this review.

As mentioned above, the first uses of photoperiod manipulation on farms were to adjust spawning time in order to produce out-of-season supplies of seed. Generally, this involved the installation of light-proof covers over broodstock enclosures and the provision of artificial lighting controlled by automatic timeclocks. For most farms, heavy duty polythene or butyl linings over a simple metal, plastic pipe or wooden framework provides a cheap and effective method of blacking-out broodstock enclosures. Total blackout is recommended as at many times in the year, the artificial light will be different from that of the ambient.

Tungsten or fluorescent sources of illumination can be used, preferably with a spectrum as close as possible to that of natural light. They should provide intensities of at least 100 lux at the water's surface in all areas of the enclosures. Intensities less than 20 lux should be avoided as they may lead to inconsistent results. Although the progressive increases and decreases in light intensity which are seen during the twilight periods of dawn and dusk are not required for the photoperiodic response, they do reduce the stress which can be induced by sudden abrupt changes in light. Dimming devices can be easily fitted to tungsten lights. Fluorescent lights may require a second system of less bright lights to be installed, which are switched on shortly before and after the main system and, hence, provide the necessary twilight periods.

Design of appropriate photoperiod regimes for the manipulation of spawning of a particular species of fish first requires information on the times of the year when maturation begins and spawning occurs. Preferably, these data should be gleaned from local stocks of the fish of related strain who have been maintained under ambient daylength for that latitude and the same range of seasonal water temperatures. Correlation of the different stages of maturation with the different phases of the annual light cycle provides the basis for the design of all forms of light manipulation. Thus, salmonids which generally spawn in Autumn or Winter under a decreasing daylength begin maturation up to a year earlier under a short but increasing photoperiod. Consequently, advancements in spawning are achieved by bringing forward the exposure to long and then short days. Conversely, delaying the onset of long days results in later spawnings. By contrast, flatfish which spawn in Spring or early Summer commence maturation the previous autumn and here spawning can be advanced or delayed respectively by earlier or later exposures to short and then longer daylengths.

Generally, in the first instance, it is best for farms to use out-of-phase seasonal light cycles (see Section 2), at least until the response of the fish to photoperiod and the quality of the gametes has been clearly established. For similar reasons, farms are advised initially to only attempt modest alterations in spawning time. Some farms progressively advance or delay spawnings over successive years and once the degree of advancement or delay is achieved they maintain the stock on out-of-phase 12-month seasonal light cycles. Once the "track record" and performance of the stock have been established, then portions of the seasonally-changing light cycles can be substituted with constant photoperiods (see Section 3), which are easier to apply and use on commercial farms.

In general, advancements and delays of spawning lead to smaller and larger eggs, respectively, at least as far as the salmonids are concerned (Bromage and Cumaranatunga, 1988). However, the quality of the gametes has been shown to be no different from

those derived from broodstock under ambient condition (Bromage et al., 1992, 1993a,b, 1994).

Although from the foregoing account it is clear that photoperiod can easily be manipulated in land-based enclosures with full black-out and artificial lighting, such controls are difficult to install on some tanks or ponds and are of course impossible for sea or freshwater cage systems where it is thought much of the future growth in aquaculture is likely to occur. Accordingly, there has been continuing strong interest by industry in the development of procedures, which can be used with uncovered tanks or cages whether at sea or in fresh water. As a result of this commercial incentive, Duston and Bromage in the mid-80s initiated a series of studies on the seasonal effects of 2-month periods of artificial continuous light (LL) on the spawning of rainbow trout maintained in uncovered tanks, i.e. without blackout. This work was completed by Randall and Bromage and subsequently published in Randall et al. (1991c, 1998, 2001). Although the tanks were in a partly open building and hence were shaded from full sunlight, the fish were subject to seasonal changes in ambient daylength, albeit at subdued intensity, in addition to the 2-month periods of artificial LL. Results were encouraging for they showed that spawning could be both advanced or delayed by exposure to LL early in the year or after the Summer Solstice, respectively. This was an important development because it offered farmers the ability to photoperiodically manipulate maturation without blackout. Probably, more significantly it offered the possibility of the procedures being used on fish in sea cages, in which much of the future growth in aquaculture was likely to occur.

Unfortunately, follow-up studies by Davies and Bromage (unpublished) on trout in totally uncovered tanks exposed to full sunlight gave conflicting results. This was thought to be due to the substantially greater differences in light intensity supplied by the artificial lights and full ambient daylight in the outside tanks, compared to the artificial light and subdued natural daylight to which the fish were exposed in the partly covered tanks described in the earlier work of Duston et al.

Subsequently, pioneering work by Hansen et al. in Norway working with sea cages exposed to full daylight showed that the maturation of Atlantic salmon could be altered by exposure to continuous light (LL) provided the artificial lights were of sufficient brightness (Endal et al., 1991; Taranger et al., 1991; Hansen et al., 1992). Initially 6×1000 W above-cage mounted, halogen sources were used on 12–15 m^2 sea cages; latterly 4×400 W metal halide lights have proved equally effective. Recently, Porter et al. (1999a,b, 2001a) showed that the effectiveness of the additional lighting in influencing maturation was related to the reductions in night-time melatonin levels (Fig. 11). The importance of achieving high light intensities has subsequently led to the use of submersible lights as substantial portions of the light from above-cage lights are reflected at the water's surface (Porter and Bromage, unpublished).

A further important finding of these studies in sea cages was that the timing of the exposure to LL affected the number of fish which subsequently underwent maturation (Taranger et al., 1991, 1998, 1999a; Hansen et al., 1992; Porter et al., 1999a,b). The earlier the exposure, the fewer fish matured as grilse. Similar reductions in maturity had also been reported earlier by Duston and Bromage (1987) working with trout in covered tanks and under totally artificial photperiods. This effect is illustrated in Fig. 4. As

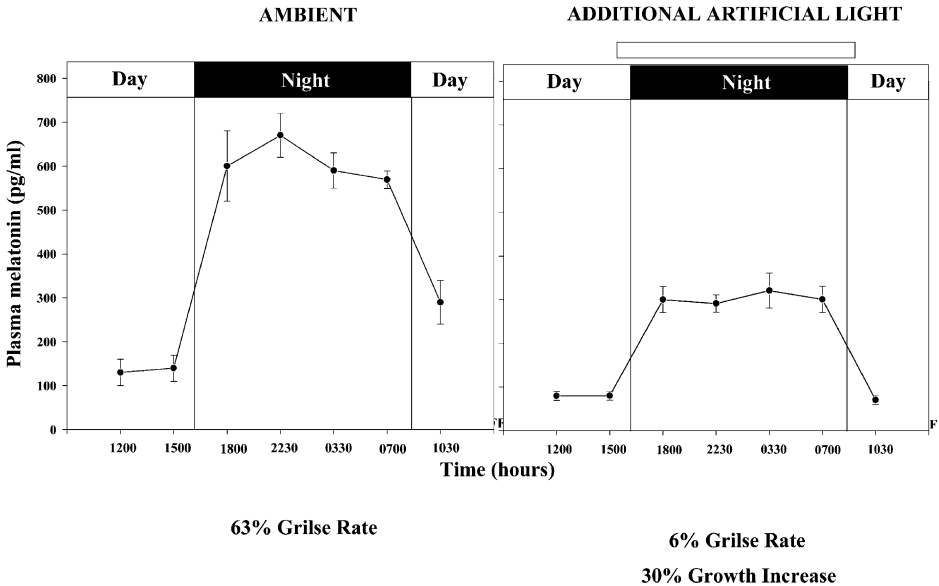


Fig. 11. Diel profiles of plasma melatonin levels (Mean ± SEM; pg/ml) of Atlantic salmon maintained in sea cages under ambient daylength (left) and ambient + additional night-time illumination (right). The duration of the night-time illumination is shown as the open bar. Melatonin levels and grilse rates were reduced and growth increased by the additional light (from Porter et al., 1999a).

discussed in Section 8, these authors proposed that the decision to mature was a gated rhythm and that progress through this 'gate' to maturity was dependent on the fish being of sufficient size or energetic status. These authors suggested that the fish exposed to the most stimulatory photoperiodic advance had not achieved the necessary size and hence were unable to mature during that reproductive cycle. Similar reasons were also proposed by Hansen et al. (1992) to explain the reductions in grilsing, which followed the earliest exposures to LL. Up to 57% reductions in grilsing have been achieved with those methods and in addition these fish also exhibit up to 30% increased growth and appetite (Taranger et al., 1991, 1998, 1999a; Hansen et al., 1992; Porter et al., 1999a).

At present, sea cage lighting techniques are more commonly being used widely to reduce maturity levels and increase growth in Atlantic salmon (Taranger et al., 1995; Porter et al., 1999a,b). Preliminary trials are also underway with sea bass and sea bream (Porter and Bromage, unpublished) and cod (Karlsen et al., 1999; Porter et al., 2001a,b). Similar techniques are also being commonly used in the production of 0 + smolts in freshwater cages (Porter et al., 1999c). At present cage lighting is not being employed by farms to produce out-of-season eggs although recently Futter et al. (unpublished) delayed maturity in a commercial broodstock of rainbow trout using floating lights in circular ponds. The aim being to produce later eggs. Delayed maturity and hence, improved growth and flesh quality have also been achieved in Pacific salmon by exposing fish in cages to extended light after the summer solstice (Clarke, 1990).

It should be emphasised that measurements of melatonin provide an invaluable tool for the further development of techniques of light manipulation in uncovered tanks or cages. Not only do these measurements enable us to judge how the ambient and artificial light is perceived by fish (Porter et al., 1999a,b,c, 2001a,b), but they also provide information on how daylength may differentially affect reproduction, growth and other internal rhythms.

12. Overall conclusions

Current evidence indicates that photoperiod is the principal proximate cue in many fish and that this entrains an endogenous rhythm which in turn controls reproduction. This information is thought to be transmitted to the reproductive axis by diel and seasonal changes in melatonin. It is probably that temperature, nutritional status or size together with other factors act in a permissive way, possibly by gating mechanisms, to enable maturation to proceed (Fig. 12). Further understanding of the interaction of photoperiod with these mechanisms should enable farmers to produce the all-year-round supplies of fish of consistent size and quality that markets require.

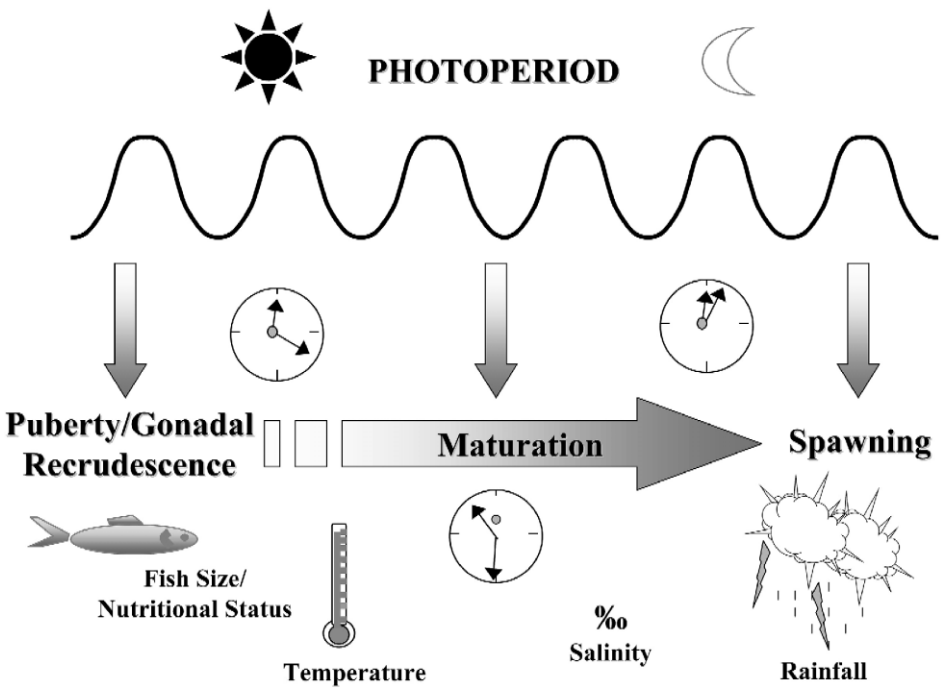


Fig. 12. Diagrammatic representation of the entrainment by photoperiod of the endogenous processes or clocks which time reproduction. The other environmental factors probably act in a permissive manner to ‘gate’ these endogenous processes.

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References

Allison, L., 1951. Delays of spawning of Eastern brook trout by means of artificially prolonged light intervals. Prog. Fish-Cult. 13, 111–116.

Alvarino, J.M., Randall, C.F., Bromage, N., 1993a. Effect of skeleton photoperiods on melatonin secretion in the rainbow trout *Oncorhynchus mykiss* (Walbaum). Aquacult. Fish. Manage. 24, 157–162.

Alvarino, J.M., Randall, C.F., Bromage, N., 1993b. Pattern of melatonin secretion in the rainbow trout exposed to light pulses of different duration and intensity. Actas IV. Congr. Nac. Acuicult., 191–196.

Amano, M., Okumoto, N., Kitamura, S., Ikuta, K., Suzuki, Y., Aida, K., 1994. Salmon gonadotropin-releasing-hormone and gonadotropin are involved in precocious maturation induced by photoperiod manipulation in underyearling male masu salmon, *Oncorhynchus masou.* Gen. Comp. Endocrinol. 95, 368–373.

Amano, M., Hyodo, S., Kitamura, S., Ikuta, K., Suzuki, Y., Urano, A., Auda, K., 1995. Short photoperiod accelerates preoptic and ventral telencephalic salmon GnRH synthesis and precocious maturation in underyearling male masu salmon. Gen. Comp. Endocrinol. 99, 22–27.

Arnold, C.R., 1988. Controlled year-round spawning of red drum, *Sciaenops ocellatus* in captivity. Contrib. Mar. Sci. 30, 65–70 (Supplement).

Baggerman, B., 1980. Photoperiodic and endogenous control of the annual reproductive cycle in teleost fishes. In: Ali, M.A. (Ed.), Environmental Physiology of Fishes. Plenum Press, New York, pp. 553–568.

Bartness, T., Goldman, B., 1989. Mammalian pineal melatonin: a clock for all seasons. Experientia 45, 939–945.

Bartness, T.J., Powers, J.B., Hastings, M.H., Bittman, E.L., Goldman, B.D., 1993. The timed infusion paradigm for melatonin delivery: what has it taught us about the melatonin signal, its reception and photoperiodic control of seasonal responses? J. Pineal Res. 15, 161–190.

Beacham, T.D., Murray, C.B., 1993. Acceleration of maturity of pink salmon (*Oncorhynchus gorbuscha*) using photoperiod control. Aquacultur 109, 315–325.

Billard, R., 1981–1982. The reproductive cycle in teleost fish. Estratto dallo Rivista Italiana Piscicultura e Ittiopatologia, pp. 1–32.

Björnsson, B.T., Halldorsson, O., Haux, C., Norberg, B., Brown, C., 1998. Photoperiod control of sexual maturation of the Atlantic halibut (*Hippoglossus hippoglossus*): plasma thyroid hormone and calcium levels. Aquaculture 166, 117–140.

Bolliet, V., Bégay, V., Ravault, J.-P., Ali, M.A., Collin, J.-P., Falcón, J., 1994. Multiple circadian oscillators in the photosensitive pike pineal gland: a study using organ and cell culture. J. Pineal Res. 16, 77–84.

Bolliet, V., Falcón, J., Ali, M.A., 1995. Regulation of melatonin secretion by light in the isolated pineal organ of the white sucker (*Catostomus commersoni*). J. Neuroendocrinol. 7, 535–542.

Bolliet, V., Ali, M.A., Lapointe, F.-J., Falcón, J., 1996. Rhythmic melatonin secretion in different teleost species: an in vitro study. J. Comp. Physiol. B 165, 677–683.

Bon, E., Breton, B., Govoroun, M.S., Le Menn, F., 1999. Effects of accelerated photoperiod regimes on the reproductive cycle of the female rainbow trout: II-Seasonal variations of plasma gonadotropins (GTH I and GTH II) levels correlated with ovarian follicle growth and egg size. Fish Physiol. Biol. 20, 143–154.

Borg, B., Peute, J., Reschke, M., van den Hurk, R., 1986. Effects of photoperiod and temperature on testes, renal epithelium, and pituitary gonadotropic cells of the threespine stickleback, *Gasterosteus aculeatus* L. Can. J. Zool. 65, 14–19.

Breton, B., Zohar, Y., Billard, R., 1985. Photoperiod and gonadotropin control of the reproductive cycle in the female rainbow trout. In: Loft, B., Holmes, W.N. (Eds.), Current Trends in Comparative Endocrinology. Hong Kong Univ. Press, Hong Kong, pp. 1243–1246.

Brierley, I., Mazurais, D., Drew, J., Morgan, P.J., Ross, A.W., Anglade, I., Randall, C., Bromage, N., Kah, O., Williams, L., 1999. Melatonin receptor and peri gene expression in the teleost fish brain. In: Taranger, G.L., Norberg, B., Stefansson, S., Hansen, T., Kjesbu, O., Andersson, E. (Eds.), Proceedings of VIth International Symposium on Reproductive Physiology of Fish, Bergen 4–9 July 99, p. 341.

Bromage, N.R., 1987. The advancement of puberty or time of first-spawning in female rainbow trout (*Salmo gairdneri*) maintained on altered-season light cycles. Proceedings of IIIrd International Symposium on Reproductive Physiology of Fish, St John's Canada, August, p. 303.

Bromage, N., 1995. Broodstock management and seed quality-general considerations. In: Bromage, N., Roberts, R.J. (Eds.), Broodstock Management and Egg and Larval Quality. Blackwell, Oxford, pp. 1–24.

Bromage, N., Cumaranatunga, R., 1988. Egg production in the rainbow trout. Rec. Adv. Aquacult. 3, 63–138.

Bromage, N., Duston, J., 1986. The control of spawning in the rainbow trout using photoperiod techniques. Rep. Inst. Fres. Res. Drottn. 63, 26–35.

Bromage, N.R., Jones, J., 1991. The effects of seasonal alterations in ration on fecundity and maturation in rainbow trout (*Onchorynchus mykiss*). In: Scott, A.P., Sumpter, J., Kime, D., Roffe, M. (Eds.), Procedings of IV International Symposium on Reproductive Physiology of Fish. Fish Symp. 91, p. 162, Sheffield.

Bromage, N.R., Whitehead, C., Breton, B., 1982a. Relationships between serum levels of gonadotropin, oestradiol-17β and vitellogenin in the control of ovarian development in the rainbow trout. II. Effects of alterations in environmental photoperiod. Gen. Comp. Endocrinol. 47, 366–376.

Bromage, N.R., Whitehead, C., Elliot, J., Breton, B., Matty, A., 1982b. Investigations into the importance of daylength on the photoperiodic control of reproduction in the female rainbow trout. In: Richter, C., Goos, H.Th. (Eds.), Reprod. Physiol. Fish. Pudoc Press, Wageningen, pp. 233–236.

Bromage, N.R., Elliott, J.A., Springate, J.R.C., Whitehead, C., 1984. The effects of constant photoperiods on the timing of spawning in the rainbow trout. Aquaculture 43, 213–223.

Bromage, N., Duston, J., Randall, C., Brook, A., Thrush, M., Carrillo, M., Zanuy, S., 1990. Photoperiodic control of teleost reproduction. Prog. Comp. Endocrinol. 342, 620–626.

Bromage, N., Jones, J., Randall, C., thrush, M., Davies, B., Springate, J., Duston, J., Barker, G., 1992. Broodstock management, fecundity, egg quality and the timing of egg production in the rainbow trout. Aquaculture 100, 141–166.

Bromage, N.R., Randall, C.R., Thrush, M., Duston, J., 1993a. The control of spawning in salmonids. In: Roberts, R.J., Muir, J. (Eds.), Recent Advances in Aquaculture, vol. 4, Blackwell, Oxford, pp. 55–65.

Bromage, N., Randall, C., Davies, B., Thrush, M., Duston, J., Carrillo, M., Zanuy, S., 1993b. Photoperiodism and the control of reproduction in farmed fish. In: Lahlou, B., Vitiello, P. (Eds.), Aquaculture: Fundamental and Applied Research, vol. 43, American Geophysical Union: Coastal and Estuarine Studies Series, Washington, D.C., pp. 81–102.

Bromage, N.R., Randall, C.F., Davies, B., Porter, M., 1994. Photoperiod, melatonin and finfish reproduction. In High Performance Fish. Am. Fish. Soc., 137–142.

Bromage, N.R., Randall, C.F., Porter, M.J.R., 1995. How do photoperiod, the pineal gland, melatonin and circannual rhythms interact to coordinate seasonal reproduction in salmonids? In: Goetz, F., Thomas, P. (Eds.), Reproductive Physiology of Fish V Symposium. University of Texas Publications, Austin, USA, pp. 164–166.

Brook, A., Bromage, N., 1988. Photoperiod: the principal environmental cue for reproduction in the dace. J. Interdiscip. Cycle Res. 19, 165–166.

Brown, N.P., Bromage, N.R., Shields, R.J., 1995. The effect of spawning temperature on egg viability in the Atlantic halibut (*Hippoglossus hippoglossus*). In: Goetz, F., Thomas, P. (Eds.), Reproductive Physiology of Fish V Symposium. University of Texas Publications, Austin, USA, p. 181.

Burton, M., 1991. A physiological basis for non-annual spawning in winter flounder. In: Scott, A.P., Sumpter, J., Kime, D. (Eds.), Proceedings of IV International Symposium on Reproductive Physiology of Fish. Fish Symp. 91, p. 164, Sheffield.

Bye, V., 1984. The role of environmental factors in the timing of reproductive cycles. In: Potts, G., Wootten, R. (Eds.), Fish Reproduction: Strategies and Tactics. Academic Press, London, pp. 187–205.

Carlson, A.R., Hale, J.G., 1973. Early maturation of brook trout in the laboratory. Prog. Fish Cult. 35, 150–153.

Carrillo, M., Bromage, N., Zanuy, S., Prat, F., Serrano, R., 1989. The effects of modifications in photoperiod on spawning time, ovarian development, and egg quality in the sea bass. Aquaculture 81, 351–365.

Carrillo, M., Bromage, N., Zanuy, S., Serrano, R., Ramos, J., 1991. Egg quality and fecundity in sea bass (*Dicentraxchus labrax*) and the effects of photoperiodically induced advances and delays of spawning time. In: Scott, A.P., Sumpter, J., Kime, D., Roffe, M. (Eds.), Proceedings of IVth International Symposium on Reproductive Physiology of Fish, Norwich, UK, pp. 259–261.

Carrillo, M., Zanuy, S., Prat, F., Serrano, R., Bromage, N.R., 1993. Environmental induction of spawning in sea bass. In: Roberts, R.J., Muir, J. (Eds.), Recent Advances in Aquaculture, vol. 4, Blackwell, Oxford, pp. 43–54.

Clarke, C., 1990. Light control lengthens growout season. Fish Farm News. The Aquaculture Journal of the Pacific North West 3, 26.

Corson, B., 1955. The use of artificially controlled light to induce early spawning of brook trout. Prog. Fish Cult. 17, 99–102.

Davies, B., Bromage, N.R., 1991. The effects of fluctuating seasonal and constant temperatures on the photoperiodic advancement of reproduction in female rainbow trout. In: Scott, A.P., Sumpter, J., Kime, D., Roffe, M. (Eds.), Proceedings of IV International Symposium on Reproductive Physiology of Fish. Fish Symp. 91, pp. 154–157.

Davies, P.R., Hanyu, I., Furukawa, K., Nomura, M., 1986. Effect of temperature and photoperiod on sexual maturation and spawning of the common carp: II. Under conditions of low temperature. Aquaculture 52, 51–58.

Davies, B., Randall, C.F., Bromage, N.R., 1991. Absolute daylength and the entrainment of an endogenous clock controlling reproduction in the female rainbow trout. In: Scott, A.P., Sumpter, J., Kime, D., Roffe, M. (Eds.), Proceedings of IV International Symposium of Reproductive Physiology of Fish. Fish Symp. 91, p. 166.

Davies, B., Hannah, L.T., Randall, C.F., Bromage, N.R., Williams, L.M., 1994. Central melatonin binding sites in rainbow trout (*Oncorhynchus mykiss*). Gen. Comp. Endocrinol. 96, 19–26.

Davies, B., Swanson, P., Bromage, N.R., 1995. The effects of photoperiod and temperature on serum Gth I, Gth II and the timing of maturation in the female rainbow trout. In: Goetz, F., Thomas, P. (Eds.), Reproductive Physiology of Fish V Symposium. University of Texas Publications, Austin, USA, p. 185.

Davies, B., Bromage, N., Swanson, P., 1999. The brain–pituitary–gonadal axis of female rainbow trout *Oncorhynchus mykiss*: Effects of photoperiod manipulation. Gen. Comp. Endocrinol. 115, 155–166.

de Vlaming, V.L., 1972. Environmental control of teleost reproductive cycles; a brief review. J. Fish Biol. 4, 131–140.

Duncan, N.J., Selkirk, C., Porter, M., Magwood, S., Bromage, N., 1999. The effect of altered photoperiods on maturation of male and female Atlantic salmon (*Salmo salar*), observations of different responses and mechanisms. In: Taranger, G.L., Norberg, B., Stefansson, S., Hansen, T., Kjesbu, O., Andersson, E. (Eds.), Proceedings of IVth International Symposium on Reproductive Physiology of Fish, Bergen 4–9 July 99, p. 344.

Dunlap, J.C., 1998. Common threads in eukaryotic circadian systems. Curr. Opin. Genet. Dev. 8, 400–406.

Duston, J., Bromage, N.R., 1986. Photoperiodic mechanisms and rhythms of reproduction in the female rainbow trout. Fish Physiol. Biochem. 2, 35–51.

Duston, J., Bromage, N.R., 1987. Constant photoperiod regimes and the entrainment of reproduction in the female rainbow trout (*Salmo gairdneri*). Gen. Comp. Endocrinol. 65, 373–384.

Duston, J., Bromage, N., 1988. The entrainment and gating of the endogenous circannual rhythm of reproduction in the female rainbow trout. J. Comp. Physiol. A. 164, 259–268.

Duston, J., Bromage, N., 1991. Circannual rhythms of gonadal maturation in female rainbow trout (*Oncorhynchus mykiss*). J. Biol. Rhythms 6, 49–53.

Duston, J., Saunders, R.L., 1999. Effect of winter food deprivation on growth and sexual maturity of Atlantic salmon (*Salmo salar*) in seawater. Can. J. Fish. Aquat. Sci. 56, 201–207.

Ebisawa, T., Karne, S., Lerner, M.R., Reppert, S.M., 1994. Expression cloning of a high-affinity melatonion receptor from *Xenopus* dermal melanophores. Proc. Natl. Acad. Sci. U. S. A. 91, 6133–6137.

Ekström, P., Meissl, H., 1997. The pineal organ of teleost fishes. Rev. Fish Biol. Fish. 7, 199–284.

Ekström, P., Vanecek, J., 1992. Localization of 2-[^{125}I]-melatonin binding sites in the brain of the Atlantic salmon *Salmo salar* L. Neuroendocrinoly 55, 529–537.

Endal, H.P., Hansen, T., Stefansson, S.O., Taranger, G.L., 1991. Different light regimes affect growth and sexual maturation in Atlantic salmon postsmolts. In: Scott, A.P., Sumpter, J.P., Kime, D.E., Rolfe, M.S. (Eds.), Proceedings of IV International Symposium on Reproductive Physiology of Fish, p. 167.

Eriksson, L., Lundqvist, H., 1980. Photoperiod entrains ripening by its different effect in salmon. Naturwissenschaften 67, 202–203.

Falcón, J., Guerlotté, J.F., Voisin, P., Collin, J.P., 1987. Rhythmic melatonin biosynthesis in a photoreceptive pineal organ: a study in pike. Neuroendocrinoly 45, 479–486.

Falcón, J., Brun-Marmillon, J., Claustrat, B., Collin, J.-P., 1989. Regulation of melatonin secretion in a photoreceptive pineal organ: an in vitro study in the pike. J. Neurosci. 9, 1943–1950.

Falcón, J., Thibault, C., Bégay, V., Zachmann, A., Collin, J.-P., 1992. Regulation of the rhythmic melatonin secretion by fish pineal photoreceptor cells. In: Ali, M.A. (Ed.), Rhythms in Fishes. Plenum, New York, pp. 167–198.

Falcón, J., Molina-Birja, M., Collin, J.P., Oaknin, S., 1996. Age-related changes in 2-[^{125}I]-iodomelatonin binding sites in the brain of the sea bream (*Sparus aurata*, L.). Fish Physiol. Biochem. 15, 401–411.

Follett, B., 1984. Birds. 4th edn. In: Lamming, G. (Ed.), Marshall's Physiology of Reproduction, vol. l, Livingstone, pp. 283–350.

Futter, C.W., Roed, A.J., Porter, M.J.R., Randall, C.F., Bromage, N.R., 1999. The effects of differential light intensities on the diel rhythm of melatonin release in rainbow trout. In: Taranger, G.L., Norberg, B., Stefansson, S., Hansen, T., Kjesbu, O., Andersson, E. (Eds.), Proceedings of VIth International Symposium on Reproductive Physiology of Fish, Bergen 4–9 July 99, p. 340.

Gaildrat, P., Ron, B., Falcón, J., 1998. Daily and circadian variations in 2-[^{125}I]-iodomelatonin binding sites in the pike brain (*Esox lucius*). J. Neuroendocrin. 10, 511–517.

Garg, S.K., 1987. Seasonal effects of pinealectomy on testicular recrudescence and secretory activity of seminal vesicles in the catfish *Heteropneustes fossilis* (Bloch). J. Fish Biol. 30, 377–387.

Gern, W.A., Owens, D.W., Ralph, C.L., 1978a. Plasma melatonin in the trout: day-night change demonstrated by radioimmunoassay. Gen. Comp. Endocrinol. 34, 453–458.

Gern, W.A., Owens, D.W., Ralph, C.L., 1978b. Persistence of the nycthemeral rhythm of melatonin secretion in pinealectimized and optic tract-sectioned trout (*Salmo gairdneri*). J. Exp. Zool. 205, 371–376.

Gillet, C., Rideau, I., Breton, B., 1998. Effects of acclimitization to long days at the end of the reproductive cycle on the timing of ovulation and on gonadotropin secretion in Arctic charr (*Salvelinus alpinus*). Bull. Fr. Peche Piscic. 350–51, 241–253.

Girin, M., Devauchelle, N., 1978. Decalage de la periode de reproduction par raccourcissement des cycles photoperiodique et thermique chez des poissons marin. Ann. Biol. Anim., Biochim., Biophys. 18, 1059–1066.

Gwinner, E., 1986. Circannual Rhythms. Springer-Verlag, Berlin, 154 pp.

Haddy, J.A., Pankhurst, N.W., 2000. The effects of salinity on reproductive development, plasma steroid levels, fertilisation and egg survival in black bream *Acanthopagrus butcheri*. Aquaculture 188, 115–131.

Hansen, T., Stefansson, S.O., Taranger, G.L., 1992. Growth and sexual maturation in Atlantic salmon, *Salmo salar* L., reared in sea cages at two different light regimes. Aquacult. Fish. Manage. 23, 275–280.

Hazard, T.P., Eddy, R.E., 1951. Modification of the sexual cycle in brook trout (*Salvelinus fontinalis*) by control of light. Trans. Am. Fish Soc. 80, 158–162.

Henderson, N., 1963. Influence of light and temperature on the reproductive cycle of the eastern brook trout. J. Fish. Res. Board Can. 20, 859–897.

Holland, M.C.H., Gothilf, Y., Meiri, I., King, J.A., Okuzawa, K., Elizur, A., Zohar, Y., 1998. Levels of the native forms of GnRH in the pituitary of the gilthead seabream, *Sparus aurata*, at several characteristic stages of the gonadal cycle. Gen. Comp. Endocrinol. 112, 394–405.

Hoover, E.E., 1937. Experimental modification of the sexual cycle in trout by control of light. Science 86, 425–426.

Hoover, E., Hubbard, H., 1937. Modification of the sexual cycle in trout by control of light. Copeia 1937 (4), 206–210.

Horvath, L., 1986. Carp oogenesis and the environment. In: Billard, R., Marcel, J. (Eds.), Aquaculture of Cyprinids. INRA, Paris, pp. 110–117.

Iigo, M., Kezuka, H., Suzuki, T., Tabata, M., Aida, K., 1994a. Melatonin signal transduction in the goldfish, *Carrassius auratus*. Neurosci. Biobehav. Rev. 18, 563–569.

Iigo, M., Kobayashi, M., Ohtani-Kaneko, R., Hara, M., Hattori, A., Suzuki, T., Aida, K., 1994b. Characteristics, day–night changes, subcellular distribution and localization of melatonin binding sites in the goldfish brain. Brain Res. (Amsterdam) 644, 213–220.

Iigo, M., Hara, M., Ohtani-Kaneko, R., Hirata, K., Tabata, M., Aida, K., 1997. Photic and circadian regulations of melatonin rhythms in fishes. Biol. Signals 6, 225–232.

Imsland, A.K., Folkvord, A., Jónsdóttir, Ó.D.B., Stefansson, S.O., 1997. Effects of exposure to extended photoperiods during the first winter on long-term growth and age at first maturity in turbot (*Scophthalmus maximus*). Aquaculture 159, 125–141.

Jobling, M., Johnsen, H.K., Pettersen, G.W., Henderson, R.J., 1995. Effect of temperature on reproductive development in Arctic charr, *Salvelinus alpinus* (L.). J. Therm. Biol. 20, 157–165.

Jones, J., Bromage, N.R., 1987. The influence of ration size in the reproductive performance of female rainbow trout (*Salmo gairdneri*). Proceedings of IIIrd International Symposium on Reproductive Physiology of Fish, Aug 1988, p. 202.

Karlsen, Ø., Taranger, G.L., Dahle, R., Norberg, B., 1999. Effects of exercise and continuous light on early sexual maturation in farmed Atlantic cod (*Gadus morhua* L.). In: Taranger, G.L., Norberg, B., Stefansson, S., Hansen, T., Kjesbu, O., Andersson, E. (Eds.), Proceedings of VIth International Symposium on Reproductive Physiology of Fish, Bergen 4–9 July, pp. 328–330.

Kezuka, H., Furukawa, K., Aida, K., Hanyu, I., 1988. Daily cycles in plasma melatonin levels under long and short photoperiod in the common carp, *Cyprinus carpio*. Gen. Comp. Endocrinol. 72, 296–302.

Kezuka, H., Aida, K., Hanyu, I., 1989a. Melatonin secretion from goldfish pineal gland in organ culture. Gen. Comp. Endocrinol. 75, 217–221.

Kezuka, H., Kobayashi, M., Aida, K., Hanyu, I., 1989b. Effects of photoperiod and pinealectomy on the gonadotropin surge and ovulation in goldfish *Carassius auratus*. Nippon Suisan Gakkaishi 55, 2099–2103.

Kezuka, H., Iigo, M., Furukawa, K., Aida, K., Hanyu, I., 1992. Effects of photoperiod, pinealectomy and ophthalmectomy on circulating melatonin rhythms in the goldfish, *Carassius auratus*. Zool. Sci. 9, 1047–1053.

Kissil, G.W., Lupatsch, I., Elizur, A., Zohar, Y., 2000. Long photoperiod delayed spawning and resulting increased somatic growth in gilthead seabream (*Sparus aurata*). Aquaculture (in press).

Le Menn, F., Bon, E., Govourun, M., Breton, B., 1998. Effect of shortened photoperiod on the annual blood levels of GTH1 and GTH 2 in rainbow trout. Ann. N.Y. Acad. Sci. 839, 427–430.

MacQuarrie, D.W., Markert, J., Vanstone, W.E., 1978. Photoperiod induced off-season spawning of coho salmon (*Oncorhynchus kisutch*). Ann. Biol. Anim., Biochim., Biophys. 18, 1051–1058.

MacQuarrie, D., Vanstone, W., Markert, J., 1979. Photoperiod off-season spawning of pink salmon. Aquaculture 18, 289–302.

Magwood, S.J., Bromage, N., Duncan, N.J., Porter, M., 1999. The influence of salinity on reproductive success in female Atlantic salmon (*Salmo salar*) grilse. In: Taranger, G.L., Norberg, B., Stefansson, S., Hansen, T., Kjesbu, O., Andersson, E. (Eds.), Proceedings of VIth International Symposium on Reproductive Physiology of Fish, Bergen 4–9 July 99, p. 346.

Martinoli, M.G., Williams, L.M., Kah, O., Titchener, L.T., Pelletier, G., 1991. Distribution of central melatonin binding sites in the goldfish (*Carassius auratus*). Mol. Cell. Neurosci. 2, 78–85.

Max, M., Menaker, M., 1992. Regulation of melatonin production by light, darkness and temperature in the trout pineal. J. Comp. Physiol. 170A, 479–489.

Mayer, I., Bornestaf, C., Borg, B., 1997. Melatonin in non-mammalian vertebrates. Physiological role in reproduction? Comp. Physiol. Biochem. 118A, 515–531.

Mazurais, D., Brierley, I., Anglade, I., Drew, J., Randall, C., Bromage, N., Michel, D., Kah, O., Williams, L.M., 1999. Central melatonin receptors in the rainbow trout: comparative distribution of ligand binding and gene expression. J. Comp. Neurol. 409, 313–324.

McCarty, C.E., Chamberlain, G.W., Miget, R.J., Haby, M.G., 1990. Design and operation of a photoperiod/temperature spawning system for red drum. Red Drum Aquacult. 1990, 44–45.

McCormick, S.D., Naiman, R.J., 1984. Some determinanats of maturation in brook trout, *Salvelinus fontinalis*. Aquaculturee 43, 269–278.

Molina-Borja, M., Falcón, J., Urquiola, E., Oaknin, S., 1994. Characterization of 2-[^{125}I]-iodomelatonin binding sites in the brain, intestine and gonad of the gilthead sea bream (*Sparus aurata*). Pfluegers Arch. 427 (Suppl. 1) R5.

Morehead, D.T., Ritar, A.J., Pankhurst, N.W., 2000. Effect of consecutive 9- or 12-month photothermal cycles and handling on sex steroid levels, oocyte development and reproductive performance, in female striped trumpeter *Latris lineata* (Latrididae). Aquaculture 189, 293–305.

Morrison, J., Smith, C., 1986. Altering the spawning cycle of the rainbow trout by manipulating water temperature. Prog. Fish. Cult. 48, 52–54.

Munro, A.D., 1990. Tropical freshwater fish. In: Munro, A., Scott, A., Lam, T. (Eds.), Reproductive Seasonality in Teleosts: Environmental Influences. CRC Press, Florida, pp. 145–239.

Nakari, T., Soivio, A., Pesonen, S., 1988. The ovarian development and spawning time of *Salmo gairdneri* R. reared in advanced and delayed annual photoperiod cycles at naturally fluctuating water temperature in Finland. Ann. Zool. Fenn. 25, 335–340.

Okimoto, D.K., Stetson, M.H., 1999a. Properties of the melatonin-generating system of the sailfin molly, *Poecilia velifera*. Gen. Comp. Endocrinol. 114, 293–303.

Okimoto, D.K., Stetson, M.H., 1999b. Presence of an intrapineal circadian oscillator in the teleostean family Poecilidae. Gen. Comp. Endocrinol. 114, 304–312.

Pang, C.S., Ali, M.A., Reddy, P.K., Leatherland, J.F., Brown, G.M., Pang, S.F., 1994. 2-[^{125}I]-iodomelatonin binding in the brain of four salmonids. Biol. Signals 3, 230–238.

Pankhurst, N.W., Purser, G.J., Van Der Kraak, G., Thomas, P.M., Forteath, G.N.R., 1996. Effect of holding temperature on ovulation, egg fertility, plasma levels of reproductive hormones and in vitro ovarian steroidogenesis in the rainbow trout *Oncorhynchus mykiss*. Aquaculture 146, 277–290.

Poncin, P., 1989. Effects of different photoperiods on the reproduction of the barbel, *Barbus barbus* (L.), reared at constant temperature. J. Fish. Biol. 35, 395–400.

Poncin, P., 1991. Environmental and endocrine control of barbel (*Barbus barbus*) reproduction. In: Scott, A.P., Sumpter, J., Kime, D., Roffe, M. (Eds.), Proceedings IV International Symposium on Reproductive Physiology of Fish. Fish Symp. 91, pp. 148–150, Sheffield.

Porter, M.J.R., Randall, C.F., Bromage, N., 1995. The effect of pineal removal and enucleation on circulating melatonin levels in Atlantic salmon parr (1995). In: Goetz, F., Thomas, P. (Eds.), Reproductive Physiology of Fish V Symposium. University of Texas Publications, Austin, USA, p. 75.

Porter, M.J.R., Randall, C.F., Bromage, N.R., 1996. The effect of pineal removal on circulating melatonin levels in Atlantic salmon parr. J. Fish Biol. 48, 1011–1013.

Porter, M., Duncan, N., Mitchell, D., Bromage, N., 1999a. The use of cage lighting to reduce plasma melatonin in Atlantic salmon (*Salmo salar*) and its effects on the inhibition of grilsing. Aquaculture 176, 237–244.

Porter, M.J.R., Duncan, N., Roed, A.J., Oppedal, F., Taranger, G.L., Bromage, N.R., 1999b. Differential effects of light intensity on growth, maturation and plasma melatonin in Atlantic salmon and its importance in aquaculture. In: Taranger, G.L., Norberg, B., Stefansson, S., Hansen, T., Kjesbu, O., Andersson, E. (Eds.), Proceedings of VI International Symposium on Reproductive Physiology of Fish, Bergen 4–9 July 99, pp. 321–324.

Porter, M., Duncan, N., Bromage, N., 1999c. Light-controlled smolts for year-round 3–4 kg salmon. Fish Farmer 22, 10–11.

Porter, M.J.R., Duncan, N., Stefansson, S.O., Bromage, N.R., 2001a. Temperature, light intensity and plasma melatonin levels in juvenile Atlantic salmon. J. Fish Biol. 58, 431–438.

Porter, M.J.R., Stefansson, S., Nyhammer, G., Karlsen, Ø., Norberg, B., Bromage, N.R., 2001b. Environmental influences on melatonin secretion in Atlantic cod (*Gadus morhua* L.) and their relevance to commercial culture. Fish Physiol. Biochem. (in press).

Prat, F., Zanuy, S., Carrillo, M., Bromage, N., 1999. Effects of long term constant short and long photoperiod regimes on the spawning performance and sex steroid levels of both sexes of sea bass (*Dicentrachus labrax* L.). J. Fish Biol. 54, 125–137.

Randall, C.F., Bromage, N., 1998. Photoperiodic history determines the reproductive response of rainbow trout to changes in daylength. J. Comp. Physiol. 183, 651–660.

Randall, C., Duston, J., Bromage, N., 1987. Photoperiodic history and the entrainment of the annual cycle of reproduction in the female rainbow trout. Proceedings of IIIrd International Symposium on Reproductive Physiology of Fish, St. John's Canada, p. 31.

Randall, C.F., Bromage, N.R., Thrush, M.A., Davies, B., Symes, J., 1991a. Photoperiodism and melatonin rhythms in salmonid fish. In: Scott, A.P., Sumpter, J., Kime, D., Roffe, M. (Eds.), Proceedings of IV International Symposium on Reproductive Physiology of Fish. Fish Symp. 91, pp. 136–138.

Randall, C., Thrush, M., Bromage, N., 1991b. Absence of an endogenous component regulating melatonin secretion in the rainbow trout. Adv. Pineal Res. 5, 279–281.

Randall, C., Bromage, N., Symes, J., 1991c. Short periods of continuous light can both advance and delay spawning in the rainbow trout. In: Scott, A.P., Sumpter, J., Kime, D., Roffe, M. (Eds.), Proceedings of IV International Symposium on Reproductive Physiology of Fish. Fish Symp. 91, p. 171.

Randall, C.F., Bromage, N., Thorpe, J., Miles, M., Muir, J., 1995a. Melatonin rhythms in Atlantic salmon (*Salmo salar*) maintained under natural and out-of-phase seasonal photoperiods. Gen. Comp. Endocrinol. 98, 73–87.

Randall, C.F., Porter, M.J.R., Bromage, N.R., 1995b. Preliminary observations on the effects of melatonin implants and pinealectomy on the timing of reproduction in rainbow trout. In: Goetz, F., Thomas, P. (Eds.), Reproductive Physiology of Fish V Symposium. University of Texas Publications, Austin, USA, p. 196.

Randall, C.F., Bromage, N.R., Duston, J., Symes, J., 1998. Photoperiod induced phase-shifts of the endogenous clock controlling reproduction in the rainbow trout: a circannual phase–response curve. J. Reprod. Fertil. 112, 399–405.

Randall, C.F., Bromage, N.R., Porter, M.J.R., Gardener, J., Auchinachie, N.A., 1999. Circannual rhythms of reproduction in rainbow trout. In: Taranger, G.L., Norberg, B., Stefansson, S., Hansen, T., Kjesbu, O., Andersson, E. (Eds.), Proceedings of VIth International Symposium on Reproductive Physiology of Fish, Bergen 4–9 July 99, pp. 325–327.

Randall, C.F., Bromage, N., Duston, J., Symes, J., 2001. The effects of short periods of continuous light on the timing of reproduction in the rainbow trout, *Oncorhynchus mykiss*. Aquaculture (in press).

Reppert, S.M., 1997. Melatonin receptors: molecular biology of a new family of G protein-coupled receptors. J. Biol. Rhythms 12 (6), 528–531.

Reppert, S.M., Weaver, D.R., Godson, C., 1996. Melatonin receptors step into the light: cloning and classification of sub-types. Trends Pharmacol. Sci. 17, 100–102.

Rowe, D.K., Thorpe, J.E., 1990. Suppression of maturation in male Atlantic salmon (*Salmo salar* L.) parr by reduction in feeding and growth during spring months. Aquaculture 86, 291–313.

Rowe, D.K., Thorpe, J.E., Shanks, A.M., 1991. Role of fat stores in the maturation of male Atlantic salmon (*Salmo salar* L.) parr. Can. J. Fish. Aquat. Sci. 48, 405–413.

Sánchez-Vázquez, F.J., Iigo, I., Madrid, J.A., Zamora, S., Tabata, M., 1997. Daily cycles in plasma and ocular melatonin in demand-fed sea bass, *Dicentrachus labrax* L. J. Comp. Physiol. B 167, 409–415.

Smith, P., Bromage, N.R., Shields, R., Gamble, J., Gillespie, M., Dye, J., Young, C., Bruce, M., 1991. Photoperiod controls spawning time in the Atlantic halibut (*Hippoglossus hippoglossus*). In: Scott, A.P., Sumpter, J., Kime, D., Roffe, M. (Eds.), Proceedings of IV International Symposium on Reproductive Physiology of Fish. Fish Symp. 91, p. 172.

Sundararaj, B., Vasal, S., 1976. Photoperiod and temperature control in the regulation of reproduction in the female catfish. J. Fish. Res. Board Can. 33, 959–973.

Sundararaj, B., Vasal, S., Halberg, F., 1982. Circannual rhythmic ovarian recrudescence in the catfish. Adv. Biosci. 41, 319–337.

Takashima, F., Yamada, Y., 1984. Control of maturation in masu salmon by manipulation of photoperiod. Aquaculture 43, 243–257.

Tamaru, C.S., Lee, C.-S., Kelley, C.D., Miyamoto, G., Moriwake, A., 1994. Oocyte growth in the striped mullet *Mugil cephalus* L. maturing at different salinities. J. World Aquacult. Soc. 25, 109–115.

Taranger, G.L., 1993. Sexual maturation in Atlantic salmon, *Salmo salar* L.; aspects of environmental and hormonal control. Dr Scient. Thesis, University of Bergen, 1993.

Taranger, G.L., Hansen, T., 1993. Ovulation and egg survival following exposure of Atlantic salmon, *Salmo salar* L., broodstock to different water temperatures. Aquacult. Fish. Manage. 24, 191–196.

Taranger, G.-L., Haux, C., Walther, B., Stefansson, S., Björnsson, B.Th., Hansen, T., 1991. Photoperiodic control of growth, incidence of sexual maturation and ovulation in adult Atlantic salmon. In: Scott, A.P., Sumpter, J., Kime, D., Roffe, M. (Eds.), Proceedings of IV International Symposium on Reproductive Physiology of Fish. Fish Symp. 91, pp. 143–147, Sheffield.

Taranger, G.L., Daae, H., Jørgensen, K.O., Hansen, T., 1995. Effects of continuous light on growth and sexual maturation in sea water reared Atlantic salmon, *Salmo salar* L. In: Goetz, F.W., Thomas, P. (Eds.), Proceedings of the 5th International Symposium on the Reproductive Physiology of Fish. University of Texas, Austin, USA, 2–8 July, p. 200.

Taranger, G.L., Haux, C., Stefansson, S.O., Björnsson, B.Th., Walther, B.Th., Hansen, T., 1998. Abrupt changes in photoperiod affect age at maturity, timing of ovulation and plasma testosterone and oestradiol-17β profiles in Atlantic salmon, *Salmo salar*. Aquaculture 162, 85–98.

Taranger, G.L., Haux, C., Hansen, T., Stefansson, S.O., Björnsson, B.J., Walther, B.Th., Kryvi, H., 1999a. Mechanisms underlying photoperiodic effects on age at sexual maturity in Atlantic salmon, *Salmo salar*. Aquaculture 177, 47–60.

Taranger, G.L., Stefansson, S.O., Oppedal, F., Andersson, E., Hansen, T., Norberg, B., 1999b. Photoperiod and temperature affects gonadal development and spawning time in Atlantic salmon (*Salmo salar* L.). In: Taranger, G.L., Norberg, B., Stefansson, S., Hansen, T., Kjesbu, O., Andersson, E. (Eds.), Proceedings of VIth International Symposium on Reproductive Physiology of Fish, Bergen 4–9 July, p. 345.

Tate, A.E., Helfrich, L.A. Off-season spawning of sunshine bass (*Morone chrysops* × *M.saxatilis*) Exposed to 6- or 9-month phase-shifted photothermal cycles. Aquacult. (in press).

Taylor, M.H., 1986. Environmental and endocrine influences on reproduction of *Fundulus heteroclitus*. Am. Zool. 26, 159–171.

Thomas, P., C.R., Holt, G.J., 1995. Red drum and other sciaenids. In: Bromage, N.R., Roberts, R.J. (Eds.), Broodstock Management and Egg and Larval Quality. Blackwell, Oxford, pp. 118–137.

Thorpe, J.E., 1986. Age at first maturity in Atlantic salmon, *Salmo salar*: freshwater period influences and conflicts with smolting. In: Meerburg, D.J. (Ed.), Salmonid age at maturity, Can. Spec. Publ. Fish Aquat. Sci. vol. 89, pp. 7–14.

Thorpe, J.E., 1989. Developmental variation in salmonid populations. J. Fish. Biol. 35 (Supplement A) 295–303.

Thorpe, J.E., Talbot, C., Miles, M.S., Keay, D.S., 1990. Control of maturation in cultured Atlantic salmon, *Salmo salar*, in pumped sea water tanks, by restricting food intake. Aquaculture 86, 315–326.

Watanabe, W.O., Ellis, E.P., Elis, S.C., Feeley, M.W., 1998. Progress in controlled maturation and spawning of summer flounder *Paralichthys dentatus* broodstock. J. World Aqua. Soc. 29, 393–404.

Webb-Brewer, M.A.H., van Eenennaam, J.P., Doroshov, S.I., Moberg, G.P., 1999. Preliminary observations on the effects of holding temperature on reproductive performance of female white sturgeon, *Acipenser transmontanus* Richardson. Aquaculture 176, 315–329.

Whitehead, C., Bromage, N., 1980. Effects of constant long and short day photoperiods on the reproductive physiology and spawning of the rainbow trout. J. Endocrinol. 87, 6–7.

Whitehead, C., Bromage, N., Forster, J., Matty, A., 1978. The effects of alterations in photoperiod on ovarian development and spawning time in the rainbow trout. Ann. Biol. Anim., Biochim., Biophys. 18, 1035–1053.

Whitehead, C., Bromage, N., Breton, B., 1983. Changes in serum levels of gonadotropin, oestradiol-17β and vitellogenin during the first and subsequent reproductive cycles of female rainbow trout. Aquaculture 34, 317–326.

Williams, L.M., Helliwell, R.J.A., 1993. Melatonin and seasonality in the sheep. Anim. Reprod. Sci. 33, 159–182.

Zachmann, A., Knijff, S.C.M., Bolliet, V., Ali, M.A., 1991. Effects of temperature cycles and photoperiod on rhythmic melatonin secretion from the pineal organ of a teleost (*Catostomus commersoni*) in vitro. Neuroendocrinol. Lett. 13, 325–330.

Zachmann, A., Knijff, S.C.M., Alin, M.A., Anctil, M., 1992a. Effects of photoperiod and different intensities of light exposure on melatonin levels in the blood, pineal organ, and retina of the brook trout (*Salvelinus fontinalis* Mitchill). Can. J. Zool. 70, 25–29.

Zachmann, A., Falcón, Knijff, S.C.M., Bolliet, V., Ali, M.A., 1992b. Effects of photoperiod and temperature on rhythmic melatonin secretion from the pineal organ of the white sucker (*Catostomus commersoni*) in vitro. Gen. Comp. Endocrinol. 86, 26–33.

Zanuy, S., Carrillo, M., 1984. La salinité: un moyen pour retarder la ponte du bar. In: Barnabe, G., Billard, R. (Eds.), L'Aquaculture du Bar et des Sparides. INRA, Paris, pp. 73–80.

Zanuy, S., Prat, F., Carrillo, M., Bromage, N., 1995. Effects of constant photoperiod on spawning and plasma 17β-oestradiol levels of sea bass (*Dicentrachus labrax*). Aquat. Liv. Res. 8, 147–152.