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Effect of long-term pinealectomy on growth and precocious maturation in Atlantic salmon, *Salmo salar* parr

Ian Mayer*

Department of Zoology, Stockholm University, 106 91 Stockholm, Sweden

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Abstract − While the pineal organ in poikilothermic vertebrates consistently influences a number of physiological processes, its precise role, and what controlling signal (hormonal and/or neural) is abolished by its removal, is still uncertain. For this reason, the effect of long-term pinealectomy (PINX) on seasonal growth and reproductive development was investigated in Atlantic salmon, *Salmo salar*, parr. Mean daytime plasma melatonin level in the control, sham-operated and PINX fish was 35 pg·mL^{-1} in all groups, while mean night-time levels were 296, 255 and 25 pg·mL^{-1}, respectively, indicating that PINX abolished the natural nocturnal rise in melatonin. Pinealectomy did not influence the incidence or timing of early sexual maturation in the male parr. However, pinealectomy significantly affected growth, its effect being strongly seasonally dependent. Compared to sham-operated and control fish, pinealectomized fish showed significantly lower specific growth rates (SGRs) during the period of lengthening photoperiod up until the summer solstice. Thereafter, corresponding to the season of decreasing photoperiods, the pinealectomized fish exhibited higher SGRs. These results suggest a functional relationship between the pineal organ and somatic growth in the Atlantic salmon, although what controlling signal from the pineal (melatonin or neural) is involved has yet to be determined.

pineal organ / pinealectomy / melatonin / growth / reproduction / *Salmo salar*

Résumé − Effet à long terme de l'ablation de la glande pinéale sur la croissance et la maturation précoce chez les juvéniles du saumon atlantique, *Salmo salar*. Chez les vertébrés poïkilothermes, la glande pinéale affecte nombre de processus physiologiques, son rôle est encore incertain concernant le contrôle hormonal et/ou neuronal qui serait supprimé lors de son ablation. Pour cette raison, l'effet à long terme d'une pinéalectomie (PINX), sur la croissance saisonnière et la maturation, a été étudié chez les juvéniles du saumon atlantique, *Salmo salar*. Le niveau moyen de mélatonine du plasma, chez les poissons témoins, chez les poissons « opérés » mais sans ablation, et chez les poissons ayant subi l'ablation de la glande pinéale (PINX) est de 35 pg·mL^{-1} pour tous les groupes dans la journée, et respectivement 296, 255 et 25 pg·mL^{-1} la nuit ; indiquant que les saumons PINX ne subissent pas l'augmentation naturelle de mélatonine la nuit. La pinéalectomie n'a pas d'incidence sur la maturation sexuelle et son déclenchement précoce chez les mâles. Cependant, la pinéalectomie affecte la croissance, et son effet est fortement dépendant de la saison. Comparés aux saumons témoins ou simplement opérés, les saumons ayant subi la pinéalectomie ont des taux de croissance spécifique plus faibles (SGR) durant l'allongement de la photopériode jusqu'au solstice d'été. Puis, lors de la réduction de la longueur du jour, les saumons ayant subi l'ablation montrent des taux de croissance plus élevés. Ces résultats laissent supposer une relation entre la glande pinéale et la croissance somatique chez le saumon atlantique, bien que le signal contrôlé (mélatonine ou neuronal) par la glande pinéale n'est pas encore été identifié.

glande pinéale / pinéalectomie / mélatonine / croissance / reproduction / *Salmo salar*

1. INTRODUCTION

In mammals, a functional role of the pineal organ has been strongly implicated in an increasing number of physiological processes (Ebadi et al., 1993). Most conclusively, it is now well established that the primary hormone of the pineal organ, melatonin, mediates photoperiodic information in the control of sea-

* Corresponding author: ian.mayer@zoologi.su.se

sonal reproduction (Tamarkin et al., 1985; Reiter, 1993). However, to date, evidence of a similar role for the pineal organ in non-mammalian vertebrates is far from conclusive (Mayer et al., 1997a, b). While many studies on poikilothermic vertebrates, involving pinealectomy have shown that the pineal organ clearly influences a number of physiological processes, its precise role, and what controlling signal (hormonal and/or neural) is abolished by its removal, is still far from clear.

In most poikilothermic vertebrates the pineal organ is directly light sensitive and is an integral component of the photoperiod-responding system, which in fishes consists of the retina, pineal organ and endogenous circadian rhythm oscillators (Ekström and Meissl, 1997). The role of the pineal/melatonin in the control of seasonal reproduction has been studied in some depth in fishes. In common with other poikilothermic vertebrates, the results of pinealectomy and/or melatonin administration on reproductive parameters in fishes are often inconsistent, both pro- and antigonadal effects being reported (Gern et al., 1987; Mayer et al., 1997a). The effects of pinealectomy on the timing of spawning has been investigated in fishes, notably in salmonids which in contrast to most other families are short-day breeders, that is, sexual maturation is stimulated by short or shortening photoperiods. Pinealectomy delayed spawning in female rainbow trout, *Oncorhynchus mykiss*, when exposed to a normally stimulatory short photoperiod (Randall et al., 1995), while pinealectomy at the time of the summer solstice resulted in a delay in spawning in 2-year-old rainbow trout (Bromage et al., 1995). Pinealectomy was also found to delay the onset of smoltification in Atlantic salmon, *Salmo salar*, parr (Porter et al., 1998). Overall, these pinealectomy experiments strongly suggest that the pineal organ is involved in conveying photoperiodic information to the reproductive axis in salmonids. However, which component of the pineal signal (hormonal and/or neural) is involved in this process is still not known.

In addition to influencing reproduction, pinealectomy has also been shown to affect a number of other physiological parameters in fishes, including circadian locomotor activity, thermoregulation, and growth and metabolism (de Vlaming, 1975; Zachmann et al., 1992; Ekström and Meissl, 1997). While studies on the influence of the pineal organ on growth and metabolism are scarce, pinealectomy resulted in a season- and photoperiod-dependent reduction in somatic growth in the goldfish, *Carassius auratus* (de Vlaming, 1980).

The inconclusive results from previous studies aimed at elucidating the physiological role of the pineal organ in fishes has been partly due to the fact that the observed effects are strongly influenced by season, reproductive phase and experimental photothermal regime. This highlights the need for further experimental studies, under more controlled conditions, to examine the function of the pineal organ in teleosts. To that end, the role of the pineal organ in the control of seasonal growth and reproduction was investigated in Atlantic salmon parr.

2. MATERIALS AND METHODS

2.1. Fish

One-year-old immature Atlantic salmon parr, reared at the Älvkarleby Salmon Hatchery, Sweden (60°N, 17°E) from a wild brood stock were used. The fish had been raised in 2-m^2 tanks supplied with through-flowing river water, and maintained under simulated natural photoperiod. During their second year of growth a varying proportion of males from this stock always show early sexual ('precocious') maturation.

2.2. Operations

In February 1997, 300 parr were randomly divided into three groups: pinealectomized (PINX), sham-operated (sham) and controls. For the pinealectomy operation, fish were first anaesthetized in 2-phenoxyethanol (0.5 $mL \cdot L^{-1}$, Sigma Chemical Co., St. Louis, USA) and placed on a small operating board held under a binocular microscope. A small opening (5 × 8 mm) was cut in the skull directly above the pineal organ using a hand-held dental drill fitted with a 0.06-mm carbide bit (Dental Grossisten, Stockholm, Sweden). The underlying cartilage layer was cut along three sides, and temporarily lifted up to expose the underlying pineal organ. The pineal stalk was severed at the point of attachment to the diencephalon, and the entire pineal and stalk removed using forceps. The cartilage flap was replaced and the cranial hole plugged with orahesive powder (Sqibb, Middlesex, UK) containing cicatrin antibiotic. Sham operations were performed in the same way as above, except that the pineal organ was not removed. Controls were left intact. At the same time, all fish were weighed (± 0.1 g) and measured (folk length, ± 1 mm), and implanted with a passive integrated transponder (PIT) tag for individual identification.

All fish were transferred to a single 2-m^3 tank supplied with through-flowing river water and maintained under simulated natural photoperiod. During the experimental period, ambient water temperature ranged between 0 and 22 °C. The fish were fed to satiation with commercial salmon pellets (Ewos Ltd., Westfield, Scotland) using an automatic feeder.

2.3. Sampling procedure

On 23 April, and thereafter at near regular monthly intervals, all fish were weighed and measured. At the same time, during the later months and during dissection, note was made of the males' maturity (freely exuded milt). At the end of the experiment, on 3 November 1997 fish were anaesthetized, weighed and measured, and then sampled for blood. For each of the three experimental groups some fish were sampled for

blood during the night (n = ca. 20 per experimental group) or day (n = 10 per experimental group) for the measurement of night-time and daytime plasma melatonin levels, respectively. After severing the caudal peduncle the blood was collected in lithium hepinarized tubes over ice. After centrifugation, the plasma was drawn off, frozen and stored at –70 °C until hormone analysis. Following blood sampling the fish were humanely killed, and the gonads excised and weighed for the determination of the gonadosomatic index (GSI = gonad weight/body weight × 100). All remaining fish that had not been sampled for blood were also weighed and measured and dissected as above during the day.

After each sampling, specific growth rates (SGR, %/day) were calculated for the previous monthly growing period, according to the following formula (Ricker, 1975):

$$SGR = [\ln W_2 - \ln W_1] \times 100/[t_2 - t_1],$$

where W_1 and W_2 are the weights at times t_1 and t_2, respectively. All results were examined for significance by one-way ANOVA followed by post-hoc analyses using Fishers LSD.

2.4. Melatonin measurements

Plasma levels of melatonin were measured by a direct radioimmunoassay (RIA) method based on that of Fraser et al. (1983). Melatonin was measured in duplicate 250-µL plasma samples. The assay utilized sheep melatonin antiserum (batch G/S/704-6483; Stockgrand LTD., Guildford, Surrey, UK), and 3H-melatonin (4 000 rpm per 100 µL; Amersham International Ltd., specific activity, 70–85 Ci·mmol^{-1}). The standard curve (3.9–1 000 pg per 0.5 mL) was prepared using charcoal-stripped pooled plasma collected from Atlantic salmon during the day. The lower limit of detection was 8 pg melatonin·mL^{-1} plasma. An inhibition curve obtained from a serial dilution (1:2) of salmon plasma collected during darkness showed good parallelism with the standard curve (*figure 1*). The intra- and interassay coefficient of variance of a pooled plasma sample containing ca. 300 pg·mL^{-1} was 7.9 and 8.4 %, respectively.

3. RESULTS

3.1. Plasma melatonin levels

Night-time and daytime plasma levels of melatonin from the control, sham and PINX groups are shown in *figure 2*. While all three experimental groups showed similar low daytime levels (ca. 35 pg·mL^{-1}), the characteristic night-time rise in plasma melatonin levels (250–300 pg·mL^{-1}) was only seen in the control and sham groups. In contrast, night-time plasma melatonin levels (ca. 25 pg·mL^{-1}) in the PINX group were similar to the residual daytime levels.

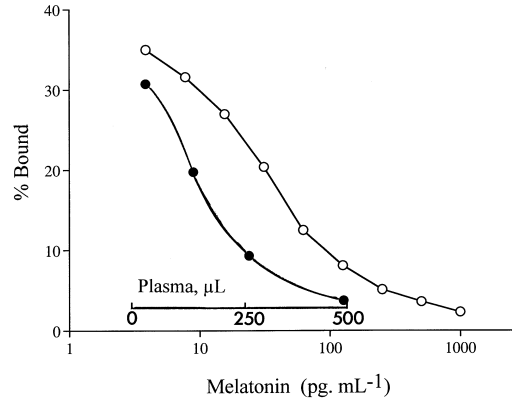

Figure 1. Parallelism of the melatonin standard curve (open circles) and an inhibition curve (solid circles) obtained from a serial dilution (1:2) of pooled Atlantic salmon plasma collected during darkness. All points are the means of duplicate plasma samples.

3.2. Seasonal growth

The initial and final lengths and weights of all fish from the three experimental groups are given in *table I*. At the start of the experiment there were no significant differences between the groups. At the end of the experiment a proportion of males from all groups had undergone early sexual ('precocious') maturation. As these mature males were significantly smaller (both in length and weight, *table I*) than both the immature males and females they were excluded from all growth calculations.

Mean body weights for the three groups at each of the sampling dates are shown in *table II*. By the time of the first sampling date (April) after the operations both the control and sham-operated groups were significantly heavier ($P < 0.001$) than the PINX group. The control and sham groups remained significantly heavier than the PINX group through to July, after

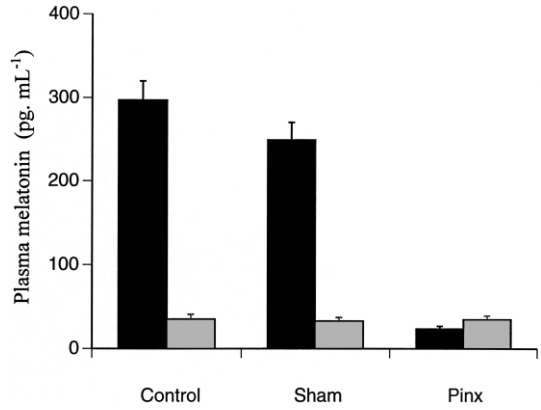

Figure 2. Night-time (dark columns, n = 22) and daytime (light columns, n = 10) plasma melatonin levels in control, sham-operated and pinealectomized (PINX) Atlantic salmon parr .

Table I. Weights and lengths (mean ± SEM) and final gonadsomatic index (GSI) of control, sham-operated and pinealectomized Atlantic salmon parr.

Experimental group	*n*	Weight (g)	Length (cm)	GSI
Initial				
Control	100	14.9 ± 0.2	11.42 ± 0.1	–
Sham-operated	100	15.5 ± 0.2	11.54 ± 0.1	–
PINX	100	15.5 ± 0.1	11.56 ± 0.1	–
Final				
a) Females and immature males				
Control	74	102.3 ± 2.2	20.6 ± 0.2	< 0.1
Sham-operated	82	98.9 ± 2.4	20.4 ± 0.2	< 0.1
PINX	76	100.1 ± 2.6	20.5 ± 0.2	< 0.1
b) Mature males				
Control	13	84.5 ± 3.8	19.4 ± 0.3	7.1 ± 0.3
Sham-operated	12	84.3 ± 4.5	19.3 ± 0.4	7.5 ± 0.3
PINX	8	83.7 ± 6.2	19.1 ± 0.6	6.7 ± 0.6

which no significant differences in mean weight between the groups were observed through to the final sampling in November. No significant differences in weight were observed between the control and sham groups throughout the experiment. Mean lengths (data not shown) showed a similar pattern.

Specific growth rates (SGR, %/day), as calculated for the period between consecutive sampling dates for the three groups are shown in *table III*. In all groups, SGRs were positively correlated to water temperature. The control and sham groups exhibited significantly higher SGRs ($P < 0.01$) than the PINX group until May and June, respectively. Conversely, the PINX group exhibited significantly higher SGRs than the control and sham groups in July and August. From September until the end of the experiment there were no significant differences in SGRs between the three groups. No significant differences in SGRs were ever measured between the control and sham groups.

Table II. Body weights (mean ± SEM) of control, sham-operated and pinealectomized Atlantic salmon parr at all sampling dates*.

Sampling date	Water temperature (°C)	Weight (g)		
		PINX	Sham	Control
20–24 February 1997	0.1	15.5 ± 0.1[a]	15.5 ± 0.2[a]	14.9 ± 0.2[a]
23 April	4.4	15.7 ± 0.2[a]	17.0 ± 0.2[b]	16.6 ± 0.2[b]
23 May	10.6	18.4 ± 0.3[a]	21.1 ± 0.3[b]	20.9 ± 0.3[b]
18 June	16.8	27.0 ± 0.6[a]	32.6 ± 0.6[b]	31.4 ± 0.5[b]
17 July	21.2	53.9 ± 0.9[a]	59.6 ± 1.0[b]	59.5 ± 0.8[b]
20 August	22.1	72.6 ± 1.8[a]	73.6 ± 1.5[a]	75.1 ± 1.6[a]
17 September	13.0	89.7 ± 2.3[a]	89.5 ± 1.9[a]	90.9 ± 1.9[a]
13 October	7.8	97.3 ± 2.6[a]	96.6 ± 2.1[a]	99.8 ± 2.2[a]
3 November	1.6	100.1 ± 2.6[a]	98.9 ± 2.4[a]	102.3 ± 2.2[a]

* Groups within the same row exhibiting different superscripts are significantly different ($P < 0.001$).

Table III. Specific growth rates (SGR, %/day) of control, sham-operated and pinealectomized Atlantic salmon parr. SGRs (mean ± SEM) are calculated for the period between two successive sampling dates*.

Sampling date	SGR (%/day)		
	PINX	Sham	Control
20–24 February 1997	–	–	–
Operated			
23 April	0.02 ± 0.01[a]	0.15 ± 0.01[b]	0.18 ± 0.01[b]
23 May	0.53 ± 0.02[a]	0.72 ± 0.02[b]	0.77 ± 0.03[b]
18 June	1.48 ± 0.03[a]	1.67 ± 0.04[b]	1.57 ± 0.04[a,b]
17 July	2.30 ± 0.04[a]	2.01 ± 0.04 [b]	2.13 ± 0.05[b]
20 August	0.93 ± 0.02[a]	0.66 ± 0.02[b]	0.73 ± 0.02[b]
17 September	0.76 ± 0.02[a]	0.70 ± 0.02[a]	0.68 ± 0.02[a]
13 October	0.31 ± 0.02[a]	0.29 ± 0.02[a]	0.36 ± 0.02[a]
3 November	0.14 ± 0.01[a]	0.11 ± 0.01[a]	0.12 ± 0.01[a]

* Groups within the same row exhibiting different superscripts are significantly different ($P < 0.01$).

3.3. Reproductive development

The GSI values are shown in *table I*. The proportion of males in each of the three groups that showed early sexual maturation are shown in *figure 3*. In male parr, sexual maturation is an all-or-nothing event, either the males (like all the females) remain immature with thread-like testes (GSI < 0.1), or they mature (GSI > 5.0). The proportion of males that matured was not significantly different (Yates corrected Chi-square) between the three experimental groups: 18, 25 and 27 % in the PINX, control and sham groups, respectively.

All early maturing males started to exude running milt, following gentle abdominal pressure, at the same time, in mid-September. While minor temporal differences in the timing of maturation between the three groups could have occurred, these were not detected due to the time between sampling dates. However, the

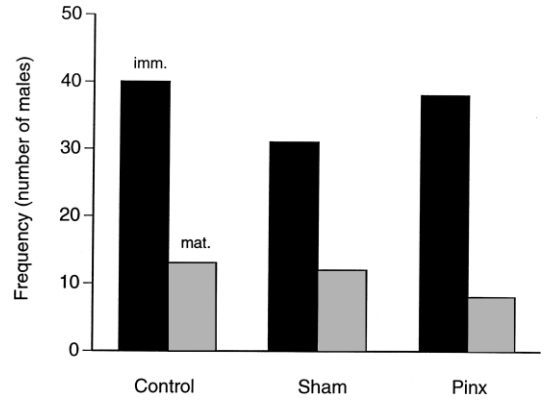

Figure 3. Proportion of immature (dark columns) and mature (light columns) males in control, sham-operated and pinealectomized Atlantic salmon parr sampled in November.

fact that all maturing males started to exude milt in September suggests that there were no major differences in the timing of maturation between the groups.

4. DISCUSSION

Results from the plasma melatonin measurements confirmed two points. First, as the night-time rise in melatonin levels was not evident in the PINX group, this clearly indicated that the surgical operations resulted in functional pinealectomy. Second, the similarity in the day and night melatonin levels in the PINX fish indicates that the normal nocturnal rise in plasma melatonin is the result of pineal rather than extra-pineal (retina and possibly gastrointestinal tract) melatonin production.

In fishes, it is now well established that physiological rhythms, including seasonal reproduction and growth, are strongly influenced by photoperiod (Bromage et al., 1993; Forsberg, 1995). As the pineal organ forms an integral component of the photoperiod-responding system in fishes, it seems probable that its removal would disrupt the timing of seasonal physiological rhythms, especially in light of the fact that it is now believed that the endogenous circadian oscillator in most fishes is located in the pineal itself (Ekström and Meissl, 1997). Indeed, it is now well established that PINX in fishes influences seasonal rhythms, although the effects are inconsistent (Mayer et al., 1997a), and what controlling signal from the pineal (hormone or neural) is abolished by its removal remains unclear. Further, the observed effects of PINX often differ according to gender, reproductive phase and the photothermal regime.

The role of the pineal organ in the photoperiodic control of seasonal reproduction has been widely studied in fishes (Gern et al., 1987; Ekström and Meissl, 1997; Mayer et al., 1997a). While inconsistent, PINX in long-day breeding species usually resulted in pro- and antigonadal effects when fish were kept under long and short photoperiods, respectively. The effects were generally the reverse in short-day breeding species. However, in the present study long-term PINX influenced neither the incidence or timing of sexual maturation in male Atlantic salmon parr. This is in contrast to other studies on salmonids (Popek et al., 1992; Randall et al., 1995). As these latter studies looked at maturation in adult female rainbow trout a degree of caution needs to be applied when comparing with precocious maturation in male salmon parr. However, in light of the present results it cannot be ruled out that the timing of physiological rhythms, including seasonal reproduction, can be synchronized by neurally transmitted photoperiod signals from the retina alone in some species.

The lack of a physiological effect of the pineal/melatonin in the control of reproduction in fishes is difficult to comprehend since in fishes as in higher vertebrates melatonin has been demonstrated to influence reproduction by exerting an effect at the hypothalamic level. For example, it has been demonstrated that melatonin administration can influence gonadotropinsecretion in mature Atlantic croaker, *Micropogonis undulatus*, both indirectly via the hypothalamus and directly at the pituitary level (Khan and Thomas, 1996). This result suggests that a complex interaction exists between melatonin and gonadotropin-releasing hormone in the modulation of pituitary gonadotropin release in the control of reproduction.

Few studies have looked at the role of the pineal on growth in fishes. Nevertheless PINX resulted in a season- and photoperiod-dependent reduction of somatic growth in the goldfish (de Vlaming, 1980). These observations indicate a functional relationship between the pineal organ and growth in this long-day breeding species, with PINX resulting in a decrease in linear growth in fish exposed to short but not long photoperiods. Similarly, the results of the present study also indicated a strong season- and photoperiod-dependent effect of PINX in juvenile Atlantic salmon. Essentially, PINX resulted in a significant decrease in SGRs during the season of increasing photoperiod until the summer solstice, to such an extent that by mid-June the mean body weights of the sham and control fish were 21 and 16 % greater, respectively, than those of the PINX fish. Conversely, after the summer solstice, a period corresponding to a decreasing photoperiod, the pinealectomized fish showed significantly higher SGRs, to the extent that by the end of the study there were no differences in mean body weight between the groups.

Although the relationship between the pineal/melatonin and vertebrate growth remains ambiguous, melatonin administration elevates plasma GH levels (John et al., 1990; Valcavi et al., 1993), and in mammals at least, it has been shown that melatonin stimulates insulin-like growth factor-I secretion in vitro (Schaeffer and Sirotkin, 1997). While similar studies on fishes are lacking, no significant changes in plasma GH levels were observed in Atlantic salmon parr following prolonged exposure (via the water) to physiological levels of melatonin. In fishes, it seems more likely that the pineal influences growth indirectly by affecting various metabolic processes or perhaps appetite (Ekström and Meissl, 1997). Alternatively, it has been suggested that the pineal, via its primary hormone melatonin, can influence somatic growth through its effect on feeding behaviour.

The present results only illustrate how much remains unknown of the functional role of the pineal organ in fishes, as in other poikilothermic vertebrates. Its involvement in the photoperiodic control of seasonal rhythms, and more so how it integrates with the other components of the photoperiod-responding system has still to be firmly established. The present results only confirm earlier conclusions that the pineal/melatonin does not play an all important role in the photoperiodic control of seasonal reproduction in fishes. In contrast, the present results suggest that the

pineal organ influences growth in fishes, and is seasonal- (photoperiod-) dependent. Again in this respect, what controlling signal from the pineal (melatonin or neural) is involved remains unclear. A better understanding in the relationship between the pineal/melatonin and seasonal growth rhythms, and in a broader sense feeding behaviour, would be beneficial both from a scientific and a practical point of view.

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