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# LH secretion and ovulation following exposure of Arctic charr to different temperature and photoperiod regimes: Responsiveness of females to a gonadotropin-releasing hormone analogue and a dopamine antagonist

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ABSTRACT

The timing of ovulation and LH plasma levels were investigated in Arctic charr reared at 5 °C and 10 °C, exposed to the ambient photoperiod, or short or long-day photoperiod regimes during the prespawning period. The effectiveness of sGnRHa alone, or sGnRHa combined with a dopamine antagonist, in stimulating LH secretion and inducing ovulation was also investigated. With the natural photoperiod, ovulation occurred spontaneously at 5 °C, but was inhibited at 10 °C. A transition from 10 to 5 °C soon resulted in suppression of the inhibition. At 5 °C, the effectiveness of sGnRHa was similar to that of sGnRHa combined with pimozide in stimulating LH secretion and inducing ovulation. At 10 °C, sGnRHa + pimozide was more effective that sGnRHa alone in stimulating LH secretion and inducing a high rate of ovulation, suggesting that dopamine-induced inhibition of LH secretion could occur naturally in Arctic charr at 10 °C. Exposure of Arctic charr to a long day (LD) photoperiod regime in fall and winter did not completely inhibit ovulation, but markedly delayed it and prolonged the ensuing ovulation period. The LD photoperiod also reduced LH plasma levels in females while they were ovulating, but did not modify the responsiveness of the pituitary to GnRHa stimulation compared to a control group exposed to a short-day (SD) photoperiod. There was an interval of several weeks after a transition from LD to SD before LH secretion and ovulation were stimulated.



## 1. Introduction

It is now well established that environmental factors exert a great influence over the initiation and modulation of reproductive development in salmonids. Since the work of Hoover (1937), many articles have been published concerning the effects of photoperiod manipulations in successfully advancing or delaying the salmonid spawning period (see Bromage et al., 1992, for a synthesis). In the Arctic charr, *Salvelinus alpinus*, as in other salmonids that spawn during fall or early in the winter, exposure to short days from the middle of summer accelerated complete ovary development and facilitated ovulation. Conversely, exposure to long days during the same period delayed spawning by several months (Gillet, 1994). Since the early 1990s, several studies have reported delayed or inhibited ovulation in several salmonid species in response to exposure to unsuitably high temperatures before and during the spawning period: in Arctic charr kept at temperatures above 10 °C (Gillet, 1991), in Atlantic salmon, *Salmo salar*, above 14 °C (Taranger and Hansen, 1993), and in rainbow trout, *Oncorhynchus mykiss*, above 18 °C (Pankhurst et al., 1996).

To date, little is known about the mechanisms by which long days or high temperatures inhibit or delay final maturation and ovulation in Arctic charr. It is generally assumed that the effects of environmental factors are mediated through the neuro-endocrine system, and particularly through the modulation of gonadotropin secretion (Hontela and Peter, 1978; Bon et al., 1999). Environmental cues probably act on specific targets in the brain–pituitary–gonadal axis. In salmonids, as in higher vertebrates, two gonadotropins secreted by pituitary (FSH and LH) are involved in the endocrine control of gonad development and gamete release (Suzuki et al., 1988; Breton et al., 1998; Swanson et al., 2003). It has been demonstrated that FSH (or GTH I) is involved in initiating and stimulating gametogenesis, whereas LH (or GTH II) promotes final maturation and gamete release (Slater et al., 1994; Prat et al., 1996; Breton et al., 1998; Bon et al., 1999; Davies et al., 1999). LH release is regulated by hypothalamic factors (Van Der Kraak et al., 1998). LH release is stimulated by gonadotropin-releasing hormone (GnRH) delivered directly to the pituitary by hypothalamic neurons (Kah et al., 1993; Yu et al., 1997). Several GnRH agonists have been tested and shown to induce an LH surge and ovulation in salmonids (see Mylonas and Zohar, 2001, for a synthesis). LH secretion is influenced not only by stimulatory hypothalamic

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neuro-endocrine factors, but is also under inhibitory hypothalamic control. In many teleosts, including cyprinids and salmonids, dopamine exerts direct inhibitory control on LH release, counteracting the stimulatory effect of GnRH (Peter et al., 1991). The importance of this dopaminergic inhibitory regulation varies between teleost species. In many cyprinids, the inhibitory influence of dopamine is very marked at the end of vitellogenesis. This is why it is useful to give combined treatment with a GnRH agonist and a dopamine antagonist to induce a high rate of ovulation (Peter et al., 1988). In salmonids it is generally assumed that dopamine inhibition has only a minor impact on LH release (Van Der Kraak et al., 1998; Breton et al., 1998; Dufour et al., 2005). The inhibition of LH release by dopamine also varies during the seasonal reproductive cycle. It seems to peak at the end of vitellogenesis, and to decline during the final maturation and gamete release under the control of various environmental cues (Senthilkumaran and Joy, 1995).

In rainbow trout (Pankhurst and Thomas, 1998) and Atlantic salmon (King and Pankhurst, 2004) GnRHa treatments did not suppress the inhibition of ovulation induced by high temperatures. In Arctic charr, a combined treatment with a GnRH agonist and pimozide, a dopamine antagonist, was efficient to induce ovulation in half of the females at 10 °C (Gillet et al., 1996). This could indicate that inhibition of LH release by dopamine occurs in Arctic charr reared at high temperatures during the spawning season. To date, the mechanisms by which exposure to long-day photoperiod (LD) inhibits or delays ovulation have not been investigated in salmonids.

In the present study, we carried out several different experiments in an attempt to identify the mechanisms by which LD or high temperatures inhibit or delay final oocyte maturation and ovulation in Arctic charr. We experimented with the effects of exposure to different temperatures or photoperiods, or to both factors combined, on the regulation of LH secretion and the timing of ovulation in Arctic charr during the prespawning and spawning periods. GnRHa and pimozide treatments were also used to assess the responsiveness of pituitary to GnRH stimulation, and to find out whether dopamine-induced inhibition of LH secretion occurred and whether it was linked to the environmental conditions.

## 2. Materials and methods

### 2.1. Source of fish stock and description of rearing procedure

The experiments were conducted in the rearing facilities of the laboratory of National Institute of Agronomic Research (INRA), located on the shores of Lake Geneva (46°N) at Thonon (France). The fish used in the experiments were the offspring of wild Arctic charr from Lake Geneva. Three-year-old females were used for the experiments on temperature and photoperiod. They were reared in 2000-L circular tanks supplied with water pumped from a depth of 51 m in Lake Geneva. Water temperature fluctuated from 5.5 °C at the end of winter, to 9.5 °C at the end of summer. A refrigeration system was used to maintain water temperature at 5 ± 0.5 °C in two tanks all year round. In some tanks, spring water at a constant temperature (11 ± 1 °C) was added to the water from Lake Geneva to maintain the water temperature at 10 ± 0.5 °C. Several groups of fish were maintained under an artificial long-day regime by 60 W 24 V bulbs controlled by time switches. Short-day experiments were carried out in tanks put in a light proof room. Fish were fed 7 h daily with dry pellets at the ration recommended in a published table for rainbow trout, i.e. 0.5–1% of body weight, according to water temperature. The fish weighed between 500 g and 1.5 kg.

### 2.2. Experiment 1: Ovulation rate, pituitary responsiveness to sGnRHa stimulation and effect of a dopamine antagonist at 5 and 10 °C with a natural photoperiod

Two groups of Arctic charr females were acclimatized in tanks at 5 and 10 °C at the end of October under a natural photoperiod. At the beginning of December, when the first ovulations occurred spontaneously at 5 °C, several groups of fish were randomly selected from each tank and assigned to the different treatments (at 10 °C, no ovulated female was detected at this time). In both tanks at 5 and 10 °C, groups of females were intraperitoneally injected with saline (control groups, NaCl solution at 0.7 g/L, 0.5 ml/kg) or with 20 μg/kg of D Arg6 sGnRH, a potent analogue of gonadotropin-releasing hormone in salmonids (sGnRHa) (Peter et al., 1987), purchased from Bachem (Weil am Rhein, Germany). sGnRHa was administered alone or in combination with pimozide (5 mg/kg), a dopamine antagonist purchased from Sigma–Aldrich (Saint Quentin Fallavier, France). Groups of fish were also injected with pimozide alone. Another group was transferred from 10 to 5 °C (Table 1). sGnRHa was dissolved in saline and pimozide was suspended in saline. The injection volume was 0.5 ml/kg body weight. At both temperatures, the different groups of fish were identified by alcian blue spots applied with a dermojet inoculator. Thereafter, the fish were checked several times a week to detect ovulation, and to establish the cumulative percentage of ovulated females in each group. Day 0 was the day when the sGnRHa or saline treatments were administered.

In all the experimental groups, apart from the group transferred from 10 to 5 °C, 13 females were individually tagged (Floy-Tags, Seattle, USA). At time 0 (H0), 2, 6, 12, 24, 48 and 72 h after sGnRHa, pimozide or saline treatment, blood samples were taken from a caudal vessel. After sampling, blood was centrifuged for 10 min at 5000*g*, and plasma samples were collected and kept frozen until the LH plasma concentrations were determined using a specific radio-immunoassays for salmonids (Breton et al., 1998; Govoroun et al., 1998).

### 2.3. Experiment 2: Effectiveness of different photoperiod manipulations in autumn in delaying ovulation in Arctic charr

Fish were reared under the natural photoperiod in a tank supplied with water from Lake Geneva from the end of the preceding spawning season until August. From August, 5 groups of 20 females were transferred into tanks exposed to a long-day photoperiod (LD: 17 h of light daily) on different occasions. One group (group B) was transferred to LD on 24 August, and kept under LD until the end of ovulation. The other four groups were transferred from the natural photoperiod to LD on 8 August (C), 1 September (D), 1

**Table 1**
Treatments, temperatures and numbers of fish in the different experimental groups in experiment 1.

| Experimental group | Temperature | Treatment | Number of fish |
|---|---|---|---|
| 1 | 5 °C | Saline (0.5 ml/kg) | 30 |
| 2 | 5 °C | D Arg6 sGnRH (20 μg/kg) | 20 |
| 3 | 5 °C | D Arg6 sGnRH (20 μg/kg) + pimozide (5 mg/kg) | 20 |
| 4 | 5 °C | Pimozide (5 mg/kg) | 13 |
| 5 | 10 °C | Saline (0.5 ml/kg) | 30 |
| 6 | 10 °C | D Arg6 sGnRH (20 μg/kg) | 20 |
| 7 | 10 °C | D Arg6 sGnRH (20 μg/kg) + pimozide (5 mg/kg) | 20 |
| 8 | 10 °C | Pimozide (5 mg/kg) | 13 |
| 9 | Transfer from 10 to 5 °C on day 0 | Saline (0.5 ml/kg) | 30 |

October (E) and 6 November (F), and all four groups were transferred back into a tank exposed to a natural photoperiod on 16 December (8 h of light daily) (Fig. 1). Fish continuously kept under natural photoperiod formed the control group (A) (8 h of light daily in winter and 16 h in summer). Each group of fish was identified by alcian blue spots produced by a dermojet inoculator. The fish were checked regularly to detect ovulation, and to establish the cumulative percentage of ovulated females in each group. D0 was the day when the first ovulated female was detected in the control group. For each female in each group, the date of ovulation was expressed as the number of days since D0. The mean time to ovulation was calculated for each group as the arithmetic mean of the date of ovulation of the different females. All the tanks were supplied with water pumped at a depth of 51 m. The monthly means of water temperature were shown in Fig. 1.

### 2.4. Experiment 3: Timing of ovulation, plasma LH levels, pituitary responsiveness to sGnRHa stimulation, and the effect of a dopamine antagonist at 5 and 10 °C under short-day or long-day photoperiods

A group of Arctic charr females were reared under long-day photoperiod (LD: 17 h of light daily) from the beginning of September in a tank supplied with water from Lake Geneva. Lake water temperature decreased from 8.5 °C in September to 6 °C in mid-January. On January 23, 2 groups of 60 females were acclimatized to 5 °C and 10 °C, respectively. At both temperatures, 30 females were kept under a long-day photoperiod, and another 30 females were transferred into tanks exposed to a short-day photoperiod (SD: 7 h of light daily) (Table 2). Ten additional females were acclimatized at 5 °C, SD. Twenty females in each group were individually tagged by placing visible implant tags in the adipose eyelid (V.I. tags, Northwest Marine Technology, Shaw Island, USA). Once a week from January 23 (day 0), females were checked to detect ovulation. On each occasion, blood samples were also taken in tagged females for plasma LH measurements. On February 28 some unovulated females in all the four experimental groups were selected to study the responsiveness of pituitary to sGnRHa stimulation: in each group five females were intraperitoneally injected with D Arg6 GnRH (20 μg/kg), five females were injected with sGnRHa combined with pimozide (5 mg/kg), and five control females were injected with saline. Blood samples were taken for plasma LH measurements carried out 0 (H0), 5, 10 and 24 h after the sGnRHa treatments.

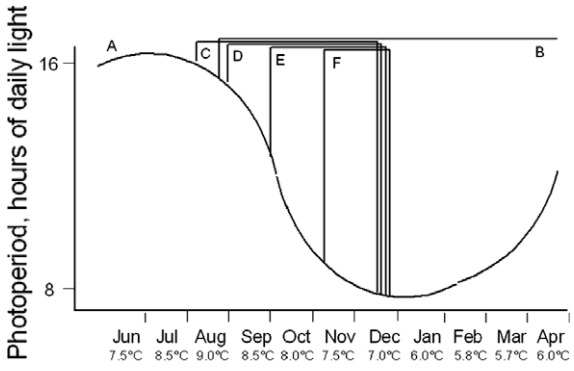


**Fig. 1.** Photoperiod regimes in the different groups of experiment 2. (A) Control fish exposed to natural photoperiod. (B) Long-day photoperiod (17 h of daily light = LD) from 24 August to the end of ovulation. (C) LD from 8 August to 16 December. (D) LD from 1 September to 16 December. (E) LD from 1 October to 16 December. (F) LD from 6 November to 16 December. Monthly means of water temperature were indicated below each month.

**Table 2**
Photoperiod and temperature conditions in the different experimental groups in experiment 3. SD: short day, 7 h of light daily. LD: long day, 17 h of light daily.

| | | | | |
|---|---|---|---|---|
| Before 1 September | All groups: Natural photoperiod, water pumped at a depth of 51 m | | | |
| From September to January 23 | All groups: LD photoperiod:, water pumped at a depth of 51 m | | | |
| From January 23 to the end of experiment | Group I: SD, 5 °C (40 females) | Group II: LD, 5 °C: (30 females) | Group III: SD, 10 °C (30 females) | Group IV: LD, 10 °C (30 females) |

### 2.5. Statistical analyses

Data were analyzed using non-parametric tests: means were compared using the Wilcoxon rank sum test to compare two samples, and the Kruskal–Wallis test to compare several samples. The Friedman test was used to analyze the change in plasma LH levels within each experimental group. The pair-wise comparison of the mean rank of different samples was calculated according to Sprent (1989). Percentages of ovulation in the different groups were compared using $\chi^2$ or Fisher exact probability tests. Analyses were performed using S-plus software.

## 3. Results

### 3.1. Experiment 1: Pituitary responsiveness to sGnRHa stimulation, rate of ovulation and effect of a dopamine antagonist at 5 and 10 °C

#### 3.1.1. Effect of sGnRHa stimulation and pimozide treatment on plasma LH at 5 and 10 °C

At the beginning of the experiment (H0), the plasma LH level was significantly higher in females acclimatized to 5 °C than in those acclimatized to 10 °C: 7.67 ± 0.63 versus 5.01 ± 0.37 ng/ml (Wilcoxon rank sum test, $P < 0.001$). In the control and pimozide-treated groups at both temperatures, (groups 1, 4, 5 and 8) plasma LH levels did not change significantly during the experiment (Table 3). In all the sGnRHa- and sGnRHa + pimozide-treated groups at both 5 and 10 °C, (groups 2, 3, 6 and 7) plasma LH levels increased significantly. At 10 °C, LH levels in groups 6 and 7 significantly decreased from H24 to H48 (Table 3). The plasma LH level peaked at 20.5 ± 3.98 ng/ml in group 2 (H72), at 22.8 ± 4.88 ng/ml in group 3 (H24), at 13.2 ± 1.34 ng/ml in group 6 (H6) and at 18.8 ± 2.14 ng/ml in group 7 (H12). At 10 °C groups 6 and 7 differed significantly at H12 (Kruskal–Wallis test, $P < 0.01$). At 5 °C group 3 never differed from group 2 (Fig. 2 and Table 3).

#### 3.1.2. Effect of sGnRHa stimulation and pimozide treatment on ovulation at 5 and 10 °C

In group 1 (the control group at 5 °C), the cumulative percentage of ovulations increased regularly from day 0 to day 42, half

**Table 3**
Changes in LH plasma levels from H0 to H72. Results of Friedman tests in the experimental groups 1–8, experiment 1. See Table 1 for the treatments in the different groups.

| Experimental group | Result of Friedman test |
|---|---|
| 1 | No significant change between H0 and H72 |
| 2 | LH increased significantly between H0 and H24 ($P < 0.001$) |
| 3 | LH increased significantly between H0 and H24 ($P < 0.001$) |
| 4 | No significant change between H0 and H72 |
| 5 | No significant change between H0 and H72 |
| 6 | LH increased significantly between H0 and H6 ($P < 0.001$) and decreased significantly between H24 and H72 ($P < 0.001$) |
| 7 | LH increased significantly between H0 and H6 ($P < 0.001$) and decreased significantly between H24 and H72 ($P < 0.001$) |
| 8 | No significant change between H0 and H72 |

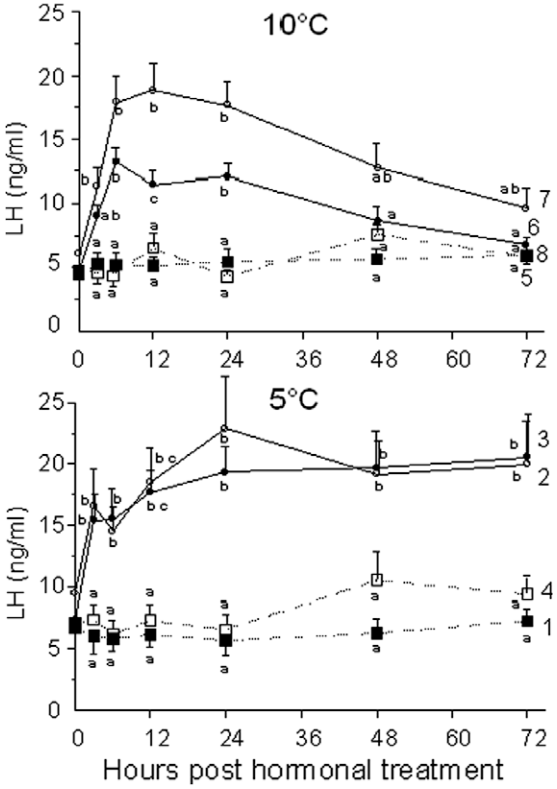


**Fig. 2.** Experiment 1, effects of an injection of sGnRHa (20 μg/kg), pimozide (5 mg/kg) or sGnRHa + pimozide on blood plasma LH levels at 5 and 10 °C. Solid squares: control (saline injected, groups 1 and 5), solid circles: sGnRHa (groups 2 and 6), outlined circles: sGnRHa + pimozide (groups 3 and 7) outlined squares: pimozide (groups 4 and 8). Error bars represent standard error. For each *x*-axis hour, values displaying dissimilar letters are significantly different from each other ($P < 0.05$, Kruskal–Wallis tests).

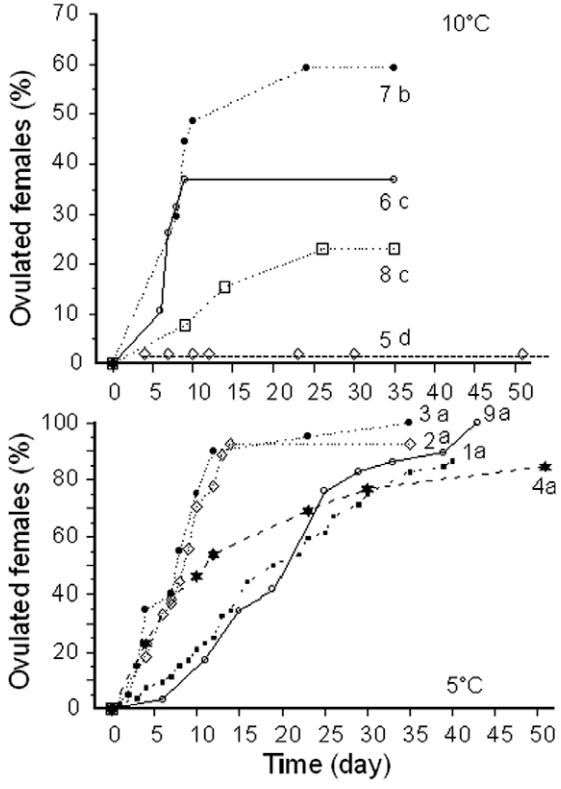


**Fig. 3.** Experiment 1, effects of an injection of sGnRHa (20 μg/kg), pimozide (5 mg/kg) or sGnRHa + pimozide on the timing of ovulation at 5 and 10 °C. Curves represent the cumulative percentage of ovulated females in the different experimental groups: (1) 5 °C, control. (2) 5 °C, sGnRHa. (3) 5 °C, sGnRHa + pimozide. (4) 5 °C, pimozide. (5) 10 °C, control. (6) 10 °C, sGnRHa. (7) 10 °C, sGnRHa + pimozide. (8) 10 °C, pimozide. (9) transfer from 10 to 5 °C at day 0. Values of the final cumulative percentage displaying dissimilar letters are significantly different from each other ($P < 0.05$, $\chi^2$ tests).

of the females having ovulated within 20 days. The same ovulation rate pattern was observed in group 9. In groups 2 and 3 (females treated with sGnRHa alone or combined with pimozide), 90% cumulative ovulation was observed within 12 days (Fig. 3). The final cumulative percentages of ovulations did not differ significantly between the different groups at 5 °C (groups 1, 2, 3, 4 and 9).

At 10 °C, only one female (3%) had ovulated in group 5. Twenty-three percentage in group 8 and 37% in group 6 had ovulated within 9 days. In group 7, 42% of females had ovulated within 9 days, and 59% within 24 days (Fig. 3). The cumulative percentage of ovulations was significantly lower in group 5 than in any of the other groups. The cumulative percentage of ovulations in the group 7 was significantly higher than in group 8.

### *3.2. Experiment 2: Effect of various photoperiod manipulations in fall to delay ovulation in Arctic charr*

In the control group (A), exposed to a natural photoperiod, ovulation started at the beginning of December and ended at the beginning of January. Ovulation occurred later in all the groups acclimatized to LD during the fall. Females transferred to LD at the beginning of August, September, October or November (groups C, D, E and F), and then transferred back to the natural photoperiod in mid-December, ovulated from mid-January to the end of February. Females transferred to LD at the end of August and continuously maintained at LD until ovulation, started to ovulate at the beginning of February and stopped ovulating at the end of April (group B) (Fig. 4). The mean time to ovulation did not differ between groups C, D and E. The values in the other groups differed

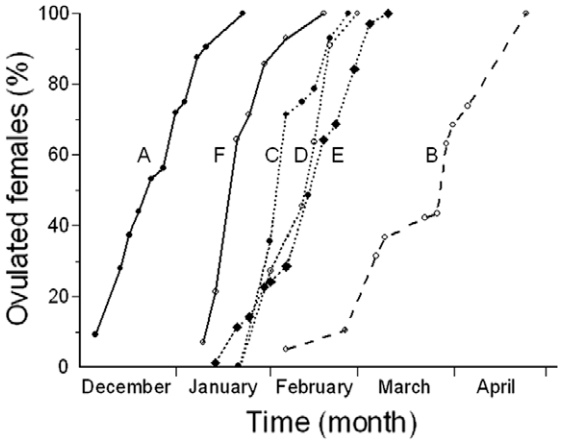


**Fig. 4.** Experiment 2, effects of acclimatization to long days (LD) on the timing of ovulation in Arctic charr. (A) control fish exposed to natural photoperiod, (B) LD (17 h of light daily) from 24 August to ovulation, (C) LD from 8 August to 16 December, (D) LD from 1 September to 16 December, (E) LD from 1 October to 16 December, (F) LD from 6 November to 16 December.

significantly from those in groups C, D and E, and also significantly differed from each other. The females in group F ovulated slightly but significantly earlier than those in groups C, D and E (Kruskal–Wallis test, $P < 0.01$). In group B, the variance of the mean time

**Table 4**
Mean time to ovulation in the different experimental groups in experiment 2. Column with the same letter are not significantly different (Kruskal–Wallis test, $P > 0.05$).

| Experimental group | Group A | Group B | Group C | Group D | Group E | Group F |
|---|---|---|---|---|---|---|
| Mean time to ovulation (day) | 18.4 ± 2.0 a | 98.4 ± 5.3* b | 52.2 ± 1.6 c | 56.2 ± 2.9 c | 59.1 ± 1.8 c | 36.9 ± 2.7 d |

* Variance significantly higher than in group A, $P < 0.005$.

to ovulation was significantly higher than control (Fisher test, $P < 0.005$) (Table 4).

### 3.3. Experiment 3: Plasma LH levels, timing of ovulation, pituitary responsiveness to sGnRHa stimulation, and the effect of a dopamine antagonist at 5 and 10 °C under short-day or long-day photoperiods

#### 3.3.1. Plasma LH levels and ovulation rates

On January 23, at the beginning on the experiment, plasma LH levels were very low in all the groups. All four experimental groups had previously been exposed to an LD photoperiod since early September, and LH levels did not differ between the different groups. In groups III and IV (fish transferred to 10 °C), plasma LH levels did not significantly change until the end of the experiment whatever the photoperiod regime (Table 5). In fish transferred to 5 °C (groups I and II) plasma LH increased significantly. LH increase was significantly higher in group I (SD photoperiod) than in group II (LD photoperiod) (Fig. 5 and Table 5). In groups I and II, the increase in plasma LH was related to the number of ovulated females.

At the end of the experiment, 58% of the females had ovulated in group I. The percentage of females which had ovulated was significantly lower in group II, 25% (Fisher exact probably test, $P < 0.05$). In group acclimatized to 10 °C, no female ovulated in any photoperiod regime (Fig. 6).

**Table 5**
Changes in LH plasma levels from January 23 to February 20. Results of Friedman tests in the experimental groups I–VI, experiment 3. See Table 2 for the treatments in the different groups.

| Experimental group | Result of Friedman test |
|---|---|
| I | LH increased significantly from the third blood sampling on February 6 ($P < 0.001$) |
| II | LH increased significantly from the fourth blood sampling on February 13 ($P < 0.01$) |
| III | No significant change from January 23 to February 20 |
| VI | No significant change from January 23to February 20 |

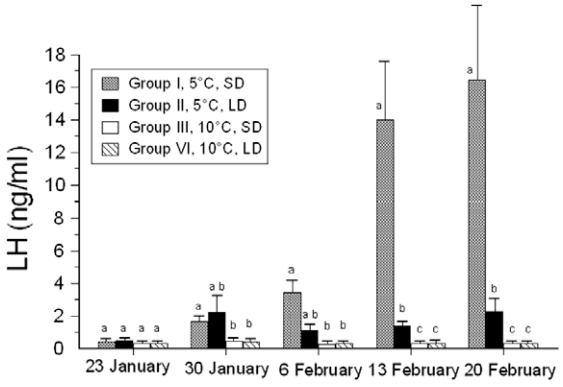


**Fig. 5.** Experiment 3, effects of acclimatization at 5 and 10 °C to short days (SD, 7 h of light daily) or long days (LD, 17 h of light daily) on blood plasma LH levels. All the fish were reared under LD from September to 23 January in water pumped from Lake Geneva at 51 m. Error bars represent standard error. For each *x*-axis date, values displaying dissimilar letters are significantly different from each other ($P < 0.05$, Kruskal–Wallis tests).

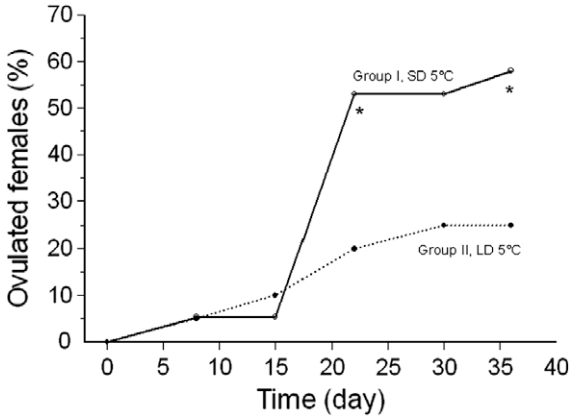


**Fig. 6.** Experiment 3, effects of acclimatization at 5 and 10 °C to short days (SD, 7 h of light daily) or long days (LD, 17 h of light daily) on the timing of ovulation. No females ovulated at 10 °C, whatever the photoperiod. All the fish were reared under LD from September to 23 January in water pumped from Lake Geneva at 51 m. *$P < 0.05$ between group I and II for the same date (Fisher exact probability tests).

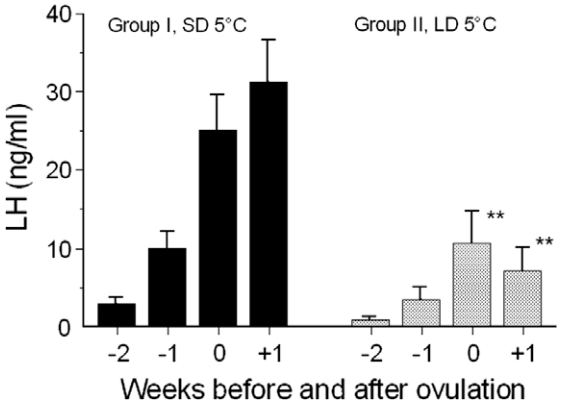


**Fig. 7.** Experiment 3, LH plasma levels during the weeks that preceded and followed ovulation at 5 °C with the SD or LD photoperiod. Error bars represent standard error. **LH level significantly lower than in group I for the same week, $P < 0.01$ (Wilcoxon rank sum tests).

Plasma LH levels during the week of ovulation and the week following ovulation were significantly higher in ovulating females exposed to SD that in those exposed to LD (Fig. 7).

#### 3.3.2. Pituitary responsiveness to sGnRHa stimulation and effect of a dopamine antagonist at 5 and 10 °C under short-day or long-day photoperiods

In control groups at all the temperatures and photoperiods tested, plasma LH levels did not increase significantly from H0 to H24 (Friedman tests, $P > 0.05$). In all the sGnRHa and sGnRHa + pimozide-treated groups, whatever the temperature and photoperiod, plasma LH levels increased significantly from H0 to H24 (Friedman tests, $P < 0.01$). At H24 in group III, plasma LH levels were significantly higher in the sGnRHa + pimozide-treated females than in the sGnRHa-treated females. At H24 in the other three groups, no significant difference could be detected between the sGnRHa and sGnRHa + pimozide-treated females (Fig. 8). During the 2 weeks following the treatment, ovulated females were detected in the 5 °C sGnRHa-treated groups, at both photoperiods, whereas no ovulation was observed in the

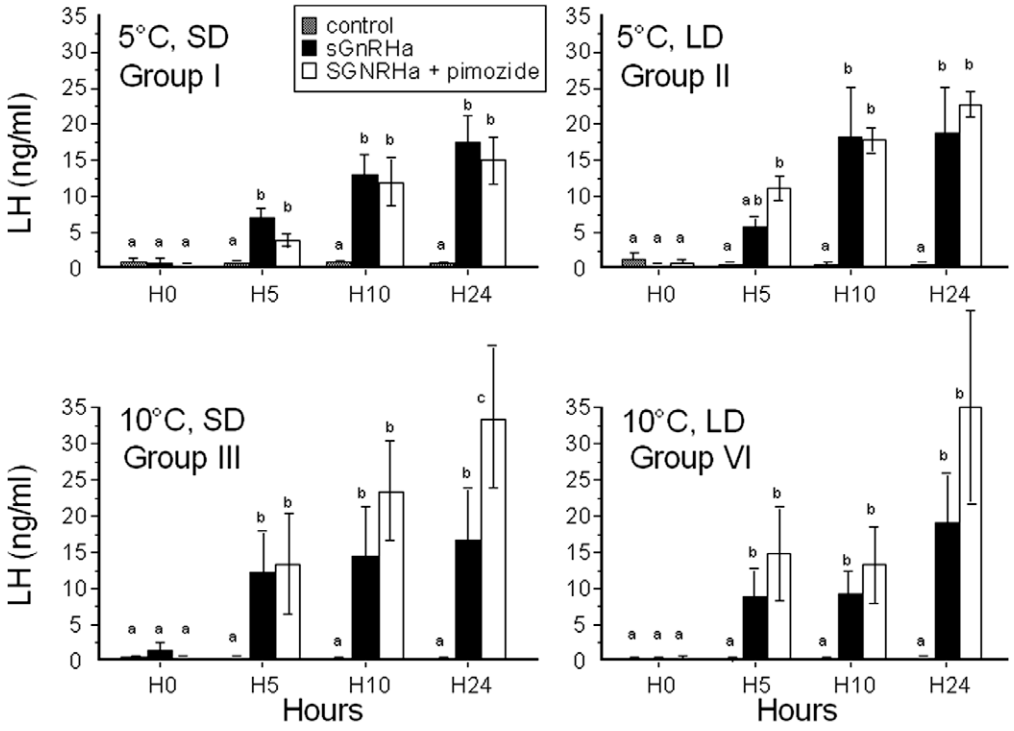


**Fig. 8.** Experiment 3, effects of an injection of sGnRHa (20 μg/kg), or sGnRHa + pimozide (5 mg/kg) on blood plasma LH levels at 5 and 10 °C with the SD or LD photoperiod. Error bars represent standard error. In each group and for each *x*-axis hour, values displaying dissimilar letters are significantly different from each other ($P < 0.05$, Kruskal–Wallis tests).

**Table 6**
Number of ovulated females in response to a sGnRHa treatment or a treatment with sGnRHa + pimozide at 5 and 10 °C under SD or LD photoperiods, experiment 3.

| Temperature | 5 °C | | 10 °C | |
|---|---|---|---|---|
| Photoperiod | SD (Group I) | LD (Group I)I | SD (Group III) | LD (Group VI) |
| Control<br>Total five females | 1 | 0 | 0 | 0 |
| GnRH, 20 μg $kg^{-1}$<br>Total five females | 3 | 2 | 0 | 0 |
| GnRH, 20 μg $kg^{-1}$ + pimozide 5 mg $kg^{-1}$<br>Total five females | 3 | 2 | 3 | 0 |

sGnRHa-treated groups at 10 °C (Fisher exact probably test, $P < 0.05$). In the sGnRHa + pimozide-treated groups exposed to the SD photoperiod, the same number of ovulated females (3) was detected at 5 and 10 °C. In groups subjected to the same treatment under the LD photoperiod no ovulation was detected at 10 °C versus two ovulated females at 5 °C (Table 6).

## 4. Discussion

Consistent with the results of our previous studies (Gillet, 1991; Gillet et al., 1996), we found that ovulation in Arctic charr was almost completely inhibited by a high temperature (10 °C) during the spawning period (data shown in Fig. 3). The results were also consistent with those reported in the other salmonids which have been investigated so far: the rainbow trout (Pankhurst et al., 1996) and the Atlantic salmon (Taranger and Hansen, 1993; King and Pankhurst, 2004). As in the Atlantic salmon, the inhibitory effect of high temperatures on ovulation was suppressed by transferring the fish into cold water (King and Pankhurst, 2004). Exposure of Arctic charr to a long-day photoperiod regime in fall and winter did not completely abolish ovulation, but markedly delayed it and extended the ovulation period of the different females (data shown in Fig. 4). These observations are consistent with findings of previous investigations (Gillet, 1994) and with published studies on the rainbow trout (Bourlier and Billard, 1984; Bromage et al., 1984). In the present work, both high temperatures and a long-day photoperiod reduced LH secretion in female Arctic charr, but the underlying mechanisms by which high temperature and long-day photoperiod inhibit LH secretion could differ to some extent.

The lack of spontaneous ovulation at 10 °C could be explained by two hypotheses: either there could be no activation of the hypothalamo-pituitary axis to induce a surge in LH secretion or the ovaries may not respond to LH stimulation. The possibility that failure to ovulate at 10 °C was due to a lack of vitellogenesis can be ruled out because female Arctic charr reared at 10 °C for three months have ovaries and oocytes of normal size at the end of fall (Gillet and Breton, 1992). At the beginning of experiment 1, plasma LH was significantly higher in the 52 females acclimatized to 5 °C than to 10 °C. There has been little investigation of LH secretion relative to temperature in salmonid species. However, no difference in plasma LH levels were observed in rainbow trout reared at 9, 12, 15, 18 and 21 °C during the prespawning period (Pankhurst et al., 1996), whereas in Atlantic salmon, King and Pankhurst (2004) observed that plasma 17α,20β-dihydroxy-4-pregnen-3-one (17α,20βOHP), the maturation-inducing steroid, reached higher levels in females kept at 6 °C than at 11 °C and above all than at 16 °C. 17α,20βOHP is produced in the ovaries of maturing females in response to a LH stimulation, which could mean that plasma LH levels were higher in maturing female Atlantic salmon at 6 °C than at 16 °C. In contrast, in female goldfish with mature ovaries (*Carassius auratus*), a spring spawner unlike Arctic charr, raising the temperature stimulated LH secretion (Gillet et al., 1977, 1978; Hontela and Peter, 1978).

In Arctic charr reared at 5 °C, injections of sGnRHa induced an increase of plasma LH levels as in the other salmonid species inves-

tigated so far. LH stimulation persisted for at least 3 days (data shown in Fig. 2), which was longer than has been observed in other salmonids (Breton et al., 1990; Mylonas and Zohar, 2001). The accelerated decrease observed at 10 °C, between 24 and 48 h after the sGnRHa treatment could be due to a more rapid hormone clearance and ovarian uptake than at 5 °C, as has been reported in goldfish (Cook and Peter, 1980a,b). In experiment 1, maintaining female Arctic charr at 10 °C reduced the responsiveness of their pituitaries to sGnRHa stimulation compared to females kept at 5 °C (data shown in Fig. 2). In experiment 3, there was no difference between pituitary sensitivity to sGnRHa stimulation at 5 and 10 °C under the SD photoperiod (data shown in Fig. 8), but the number of tested females was smaller in experiment 3 than in experiment 1: 5 versus 13 individuals. Moreover all the fish in experiment 3 had been subjected to a rearing period under an LD photoperiod from September to mid-January which could have modified their pituitary sensitivity to sGnRHa stimulation compared to those in experiment 1, that had been reared under a natural photoperiod. To date, the effects of a GnRHa treatment carried out at different temperatures on LH secretion have not been studied in other salmonids, but several authors have reported the effects of GnRHa treatment at different temperatures on 17α,20βOHP. In rainbow trout (Pankhurst and Thomas, 1998) as in Atlantic salmon (King and Pankhurst, 2004) GnRHa treatment of maturing females was more effective at low than at high temperature in stimulating 17α,20βOHP secretion. But it was not possible to determine if the pituitary responsiveness to GnRHa stimulation and LH plasma levels were higher at the lower temperature in both species or/and if the responsiveness of ovaries to LH stimulation was reduced by high temperatures.

At 5 °C, treatment with a dopamine antagonist, pimozide, did not affect the sensitivity of the pituitaries to sGnRHa stimulation, as had already been reported in the rainbow trout (Breton et al., 1998), whereas in fish reared at 10 °C, pimozide significantly enhanced the effect of sGnRH stimulation on LH secretion (data shown in Fig. 2). This implies that in female Arctic charr reared at 10 °C dopamine inhibited LH secretion at the end of vitellogenesis. It could be surmized that this phenomenon had never been previously observed in salmonid species because dopamine antagonists had not previously been tested at high temperatures. Marked inhibition of LH secretion by dopamine has been reported at the end of vitellogenesis in cyprinid species (Peter et al., 1991), and in an Indian catfish, *Heteropneustes fossilis* (Senthilkumaran and Joy, 1995) both of which spawn in spring when the water temperature is rising. In the second species, high and rising temperatures suppressed the inhibition of LH secretion by dopamine. This suggests that decreasing and low temperatures in the fall may suppress the dopaminergic inhibition of LH secretion in salmonids, which could partially explain why Arctic charr, Atlantic salmon and rainbow trout all fail to ovulate if they are kept at unsuitably high temperatures during the spawning period. The abolition of dopaminergic inhibition at low temperatures could also explain how falling temperatures constitute an environmental cue that triggers ovulation and spawning activity in salmonid species (Heggberget, 1988). However, the inhibitory effect of high temperatures on the hypothalamo-pituitary axis via the inhibition of LH secretion by dopamine does not exclude the possibility that temperature could also affect the responsiveness of the ovaries to LH stimulation. Pankhurst and Thomas (1998) and King and Pankhurst (2004) have suggested that high temperatures both impair pituitary responsiveness to GnRHa stimulation and limit 20β-hydroxysteroid dehydrogenase activity in the ovary. This may contribute to the lack of 17α,20βOHP production observed in rainbow trout and Atlantic salmon at high temperatures. In Arctic charr, a transfer from 10 to 5 °C immediately stimulates 17α,20βOHP secretion both *in vivo* and *in vitro*, suggesting that high temperatures could also limit 20β-hydroxysteroid dehydrogenase activity (Breton and Gillet, unpublished data).

The ovulation rates at 5 and 10 °C found in experiment 1 were consistent with the data on LH secretion (data shown in Fig. 3). At 5 °C, sGnRHa treatment accelerated ovulation rates within 12 days as has previously been reported in Arctic charr (Gillet et al., 1996) and in other salmonid (Donaldson et al., 1981; Breton et al., 1990). At 5 °C, combining sGnRHa with pimozide did not improve the effectiveness of the treatment. At 10 °C, pimozide alone was able to induce some ovulation, suggesting that at high temperatures the abolition of dopamine inhibition facilitated ovulation, even without GnRHa stimulation. The suppression of ovulation by dopamine antagonists at 10 °C was confirmed by the higher rate of ovulation in the response to sGnRHa + pimozide compared to sGnRHa alone. In rainbow trout (Pankhurst and Thomas, 1998), and in Atlantic salmon (King and Pankhurst, 2004), kept at high temperatures, a single GnRHa treatment did not induce ovulation. However, in Atlantic salmon treatment with a sustained-release form of GnRHa (biodegradable microspheres) was able to induce 75% ovulation at high temperature (Vikingstad et al., 2008), and in rainbow trout, repeated GnRHa treatments also induced ovulation (Pankhurst and Thomas, 1998). Similar observations had previously been reported in Arctic charr (Gillet et al., 1996). Further studies will be required to find out whether prolonged or repeated GnRHa stimulation could suppress the inhibition of LH secretion by dopamine.

To date, there has been less investigation of the inhibition or delay of ovulation by LD photoperiod during the prespawning period than of the inhibition of ovulation by high temperatures in salmonids. In several other species as the cod, *Gadus morhua* (Hansen et al., 2001) and the Eurasian perch, *Perca fluviatilis*, (Migaud et al., 2004), LD photoperiod regimes inhibited reproduction, LD photoperiod being applied all year round, and this would have inhibited the onset of ovary development. This does not occur in salmonid (Scott et al., 1984). In experiment 2, in groups C, D and E, LD was equally effective in delaying ovulation, and in group F it was only slightly less effective (data shown in Fig. 4 and Table 4). In groups C, D and E, stopping the LD treatment in mid-December resulted in suppression of the inhibition of ovulation about a month later, since the fish ovulated during the last 2 weeks of January and the first 2 weeks of February, in accordance with previous findings (Gillet, 1994). Moreover the ovulation period in these three groups was as long as in the control group, but was twice as long in group B. These observations could mean that suppression of the inhibitory effect of LD on ovulation in experiment 2 would require exposure to SD (the natural photoperiod in December) for about one month. In Arctic charr, it takes longer to suppress the inhibitory effect of LD on ovulation than that of high temperatures, since a transfer from 10 to 5 °C triggered ovulation a few days later (Gillet, 1991 and results of the present work, Fig. 3), as this has been also reported in Atlantic salmon (King and Pankhurst, 2004). The time taken to produce an inhibitory effect on ovulation after a transfer to a high temperature has not been studied in Arctic charr, but in Atlantic salmon, it took about a week (Taranger and Hansen, 1993). In the present work, the time required to reach the maximum inhibitory effect after a transfer to LD was probably more than 40 days, because after ceasing LD treatment on 16 December, the females in group F (LD treatment started on 6 November and lasted for 40 days) started to ovulate slightly but significantly earlier than those in groups C, D and E. In the last three groups, the LD treatments were equally effective even though they were applied for 130, 107 and 77 days, respectively. Moreover LD treatment was started from August (group C), September (group D) or October (group E) and the females were at different stages of ovary development (Gillet, 1995). The similarity of effectiveness of the LD treatments in groups C, D

and E in delaying ovulation could be explained in several ways. The length of LD treatment could exceed the minimum length to be efficient, but we cannot rule out the possibility that a sensitive period occurred during a specific step during the prespawning period in November or early in December. The sensitive period must have occurred after the summer solstice, because LD treatments started before this date resulted in earlier spawning (Bromage et al., 1984; Scott et al., 1984; Gillet, 1994). In experiment 3, ovulation started at the beginning of February in females kept under LD and acclimatized to 5 °C (data shown in Fig. 6), which was consistent with the data for group B in experiment 2. At 5 °C, after a transfer from LD to SD, half of the females had ovulated within 20 days. In this group, the females were transferred to SD later than in groups C, D and E in experiment 2 (on 23 January versus 16 December). It can be surmized that some of the females under SD at 5 °C which ovulated at the beginning of February in experiment 3, would have ovulated even if they had been kept under LD, because exposure to LD delayed but did not completely inhibit ovulation. This could explain why the interval between the transfer to SD and the onset of ovulation was shorter in experiment 3 than in experiment 2.

At the beginning of experiment 3, on 23 January, levels of plasma LH were low in all the females that had previously been reared under LD (data shown in Fig. 5). A transfer to SD and 5 °C resulted in an increase in plasma LH after 2 weeks, which was consistent with the timing of ovulation in this group. After being transferred to 10 °C, the plasma LH did not increase, and in agreement with findings of experiment 1, none of the females ovulated whatever the photoperiod regime. At 5 °C, LH levels were significantly lower in females exposed to LD than to SD during the week when ovulation occurred and the following week (data shown in Fig. 7). This difference could be related to the lower rate of ovulation with LD than SD, and to the extended ovulation period in females ovulating under LD. The responsiveness of the pituitaries to sGnRHa stimulation was apparently not modified by exposure to LD (data shown in Fig. 8). After sGnRHa and sGnRHa + pimozide treatments, the ovulation rates of the females in experiment 3 exposed to SD both at 5 and 10 °C were consistent with the findings of experiment 1. No ovulation was observed in females exposed to LD at 10 °C and treated by GnRHa + pimozide, despite the stimulation of LH secretion, versus 60% ovulation at 10 °C with SD (data shown in Table 6), suggesting that the inhibition of ovulation by high temperatures could be reinforced by exposure to the LD photoperiod. In the present study, we did not detect any inhibition of LH secretion by dopamine in females exposed to the LD photoperiod at the end of February. However, we cannot exclude the possibility that dopamine inhibition occurred earlier when no female exposed to LD had started to ovulate (during December and January). To date, endocrinological studies in salmonids involving photoperiod manipulation have concerned fish exposed to accelerated photoperiod regimes carried out to advance spawning (Bon et al., 1999; Davies et al., 1999), and no information is available about the effect of LD exposure during the spawning period. However the photoperiodic response was mediating by changes in feedback sensitivity between steroid hormones and gonadotropins in a non-salmonid species, the three-spine stickleback, *Gasterosteus aculeatus*, (Hellqvist et al., 2008) and in the ewe (Rosa and Bryant, 2003). The effects of LD photoperiod on LH plasma levels in experiment 3 were compatible with a change in feedback sensitivity between LD and SD photoperiod. The long time observed between a photoperiod manipulation and its effects on the reproductive cycle of the Arctic charr could be explained by the time taken to set up new nerve connections in hypothalamus and to change feedback sensitivity as this has been described in another vertebrate (Malpaux et al., 1996; Rosa and Bryant, 2003).

## 5. Conclusion

Both high temperatures and LD photoperiod reduced LH secretion in prespawning Arctic charr females. At 10 °C, the dopamine inhibitory effect on LH secretion was strong and the blockade of dopamine inhibition potentiated sGnRHa-induced LH secretion. No inhibitory effect of dopamine on LH secretion could be detected in fish exposed to LD photoperiod. The inhibitory effects of high temperatures and LD photoperiod on ovulation were suppressed by transferring the fish into cold water or under SD photoperiod but, it took longer to suppress the inhibitory effect of LD photoperiod on ovulation than that of high temperatures.

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