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## Effects of GnRH $\alpha$ and pimoziide treatments on the timing of ovulation and on egg quality in Arctic charr (*Salvelinus alpinus*) at 5 and 10°C

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### Abstract

The effectiveness of different gonadotropin releasing hormone analogues (GnRH $\alpha$ ) and/or pimoziide for inducing ovulation in Arctic charr was investigated at two different temperatures : 5°C, a temperature suitable for spontaneous ovulation in Arctic charr and 10°C, a temperature which inhibited ovulation in Arctic charr. At 5°C all the different GnRH analogues tested were able to induce and synchronize ovulation. At 10°C a sustained release preparation of D-tryptophan<sup>6</sup> luteinizing hormone releasing hormone (D-Trp<sup>6</sup>LH-RH) at 20  $\mu\text{g.kg}^{-1}$  and an acute release preparation of D-arginine<sup>6</sup>salmon GnRH (D-Arg<sup>6</sup>sGnRH) at 100  $\mu\text{g.kg}^{-1}$  were able to induce ovulation in 80% of the females within 15 days (versus 2% in controls). Pimoziide alone or in combination with a low dose of D-Arg<sup>6</sup>sGnRH was able to induce some ovulation at 10°C, suggesting that a dopamine inhibition of gonadotropin secretion could occur at 10°C. Egg survival in groups receiving GnRH $\alpha$  at 5°C was comparable to controls (73%) except in the group receiving GnRH $\alpha$  in combination with pimoziide (45%). At 10°C, egg survival was significantly lower than controls at 5°C, except for the group receiving pimoziide alone. At both temperatures, egg survivals of each spawn were negatively correlated with the plasma gonadotropin (GnH2) level of the reproducing females except in groups receiving a sustained release preparation of D-Trp<sup>6</sup>LH-RH.

**Keywords:** Arctic charr, ovulation, egg, temperature, hormone, pimoziide, gonadotropin.

*Effet de traitements au GnRH $\alpha$  et au pimoziide sur le rythme des ovulations et la qualité des oeufs chez l'omble chevalier (Salvelinus alpinus) à 5 et 10°C.*

### Résumé

L'efficacité de différents analogues du GnRH et/ou du pimoziide pour induire l'ovulation chez l'omble chevalier est étudié à deux températures : 5°C, une température propice pour l'ovulation spontanée de l'omble chevalier et 10°C, une température inhibant l'ovulation chez l'omble chevalier. A 5°C tous les différents analogues du GnRH étudiés sont capables d'induire et de synchroniser les ovulations. A 10°C, une préparation à diffusion prolongée du D-Trp<sup>6</sup>LH-RH à 20  $\mu\text{g.kg}^{-1}$  et une forme à diffusion instantanée du D-Arg<sup>6</sup>sGnRH à 100  $\mu\text{g.kg}^{-1}$  sont capables d'induire l'ovulation chez 80 % des femelles en une quinzaine de jours (contre 2 % chez les témoins). Le pimoziide seul ou associé à une faible dose de D-Arg<sup>6</sup>sGnRH est capable d'induire quelques ovulations à 10°C, suggérant qu'une inhibition de type dopaminergique de la sécrétion gonadotrope puisse se produire à 10°C. La survie des oeufs provenant des poissons traités au GnRH $\alpha$  à 5°C est comparable à celle des témoins (73 %), excepté dans le groupe des poissons traités avec du GnRH $\alpha$  associé à du pimoziide (45 %). A 10°C, la survie des oeufs est significativement inférieure à celle des témoins à 5°C, sauf dans le groupe de poissons traités uniquement au pimoziide. Aux deux températures, la survie des oeufs de chaque ponte est corrélée négativement avec la teneur en gonadotropine plasmatique de la femelle génitrice (valeur maximale observée dans les quatre jours suivant le traitement), sauf dans les groupes recevant la préparation à diffusion prolongée du D-Trp<sup>6</sup>LH-RH.

**Mots-clés :** Omble chevalier, ovulation, oeuf, température, hormone, pimoziide, gonadotropine.

## INTRODUCTION

Recently, fish farmers have shown considerable interest in rearing Arctic charr (*Salvelinus alpinus* L.) in cold water. Aquacultural progress with Arctic charr has not been rapid because of the difficulties in producing a sufficient number of eggs of quality (Tabachek and de March, 1991). The Arctic charr can be considered to be a cold tolerant stenothermal species with growth rates of juvenile fish being depressed at temperatures above 15°C (Jobling *et al.*, 1993). However, reproduction success may be more influenced by increases in temperature than growth. Ovulation has been reported to be inhibited at temperature over 10°C (Gillet, 1991). At lower temperatures (7 or 8°C), the spawning period of Arctic charr often extends over several months. However this may be a considerable disadvantage because of the stress of repeatedly handling broodstock (Gillet, 1991 ; Jansen, 1993). Low hatching success of eggs is often an additional problem (de March, 1995).

Artificial induction and synchronization of spawning has been achieved in trout and salmon using gonadotropin releasing hormone analogues (GnRHa) (Donaldson *et al.*, 1981 ; Crim *et al.*, 1983 ; Breton *et al.*, 1990). Advancement and synchronization of spawning were also obtained in rainbow trout after a combined injection of GnRHa and pimozide (Billard *et al.*, 1984). Sustained administration of GnRHa was also shown to efficiently induce ovulation in salmonids (Breton *et al.*, 1990). In Arctic charr, Jansen (1993) and Haraldsson *et al.* (1993) successfully used GnRHa to induce ovulation at cold temperatures (7 and 5°C, respectively) but spawning induction was not achieved at 10°C. However, winter temperature is often at approximately 10°C in French fish farms

where Arctic charr rearing has been undertaken. Thus, the present work was carried out to study the efficiency of several GnRHa preparations including sustained administration and association with pimozide to induce ovulation at two temperatures, 5 and 10°C. The effects of GnRHa treatments on egg quality and the relation between egg quality and plasma gonadotropin level were also investigated. This paper presents the results obtained over several years of testing different GnRH analogues and combinations with pimozide.

## MATERIAL AND METHODS

The experiments were conducted in 1989-1990-1992-1993 and 1994 at the INRA station at Thonon, France, located on the shore of Lake Geneva. The fish used in the experiments were the offspring of wild Arctic charr of Lake Geneva. These 2 and 3-year-old fish were reared in 4 m<sup>2</sup> circular tanks and fed 8 hours daily with dry pellets at a ration recommended in a published table for rainbow trout, *i.e.* 0.5 to 1.5% of body weight, according to water temperature. The fish were acclimatized at 5°C or 10°C for one month (or slightly more) before the gonadotropin releasing hormone (GnRH) treatments. Each year, all experiments were conducted during the first half of December, when 30% of the females had naturally ovulated at 5°C. Females were anaesthetized in 2-phenoxyethanol (0.3 ml.l<sup>-1</sup>), individually marked with floy tags, weighed to the nearest 1 g and intraperitoneally injected with the different GnRH analogues. The GnRHa treatments and the number of fish used in each experiment are summarized in Table 1.

**Table 1.** – Nature and dosage of the different GnRH analogues tested at 5 and 10°C in Arctic charr. Number of fish in each experiment group (\* sustained release preparation).

Temperature	Treatment	Year and number of fish					Total
		1989	1990	1992	1993	1994	
5°C	Control (saline injected)	10	19	5	8	10	52
	D-Trp <sup>6</sup> LH-RH 30 µg.kg <sup>-1</sup>	10	5				15
	D-Trp <sup>6</sup> LH-RH 20 µg.kg <sup>-1</sup> *	7	11				18
	D-Ala <sup>6</sup> LH-RH 30 µg.kg <sup>-1</sup>		14				14
	D-Arg <sup>6</sup> sGnRH 20 µg.kg <sup>-1</sup>		14	5	8		27
	D-Arg <sup>6</sup> sGnRH 20 µg.kg <sup>-1</sup>						
	+pimozide 5 mg.kg <sup>-1</sup>			5	7	10	22
	Pimozide 5 mg.kg <sup>-1</sup>			5	8		13
	Control (saline injected)	10	16	5	8	10	49
10°C	D-Trp <sup>6</sup> LH-RH 30 µg.kg <sup>-1</sup>	10	6				16
	D-Trp <sup>6</sup> LH-RH 20 µg.kg <sup>-1</sup> *	12	25				37
	D-Arg <sup>6</sup> sGnRH 20 µg.kg <sup>-1</sup>		6	5	8		19
	D-Arg <sup>6</sup> sGnRH 100 µg.kg <sup>-1</sup>		8	5	8		13
	D-Arg <sup>6</sup> sGnRH 20 µg.kg <sup>-1</sup>						
	+pimozide 5mg.kg <sup>-1</sup>			5	8	10	23
	Pimozide 5 mg.kg <sup>-1</sup>			5	8		13

D-Alanine<sup>6</sup> luteinizing hormone releasing hormone (D-Ala<sup>6</sup>LH-RH) and pimoizide were purchased from Sigma (St Louis, MO) and D-Arginine<sup>6</sup> GnRH (D-Arg<sup>6</sup>sGnRH), a salmon GnRH analogue found to be very potent in fish (Peter *et al.*, 1987) from Bachem (Bubendorf, CH-4416, Switzerland). The D-Tryptophan<sup>6</sup>LH-RH (D-Trp<sup>6</sup>LH-RH) used for sustained release was a commercial microencapsulated preparation in a polyglycolic-poly-lactic biodegradable matrix, obtained from Ipsen/Beaufour laboratory (Dreux, 28104 France) and was used as a suspension in a physiological saline solution containing 0.1% Tween 20. The D-Trp<sup>6</sup>LH-RH in acute release preparation was also obtained from the Ipsen/Beaufour laboratory. The sustained release form was intramuscularly delivered whereas other preparations were intraperitoneally injected.

Blood samples were taken from a caudal vessel using a heparinized syringe, at the beginning of the treatment and at regular intervals during the four days following GnRH injections, in order to assess the maximum value of plasma gonadotropin level (GtH2) before ovulation. This level was measured by radioimmunoassay according to Breton *et al.* (1978). The detailed effects of different GnRH $\alpha$ s and pimoizide administrations on the stimulation of gonadotropin GtH2 secretion will be developed in a separate paper. In the present work, the relation between egg survival and plasma gonadotropin level before ovulation will be presented.

Fish were checked three times a week after the beginning of the treatments to determine whether they had ovulated. Newly ovulated females were anaesthetized and weighed to the nearest 0.1 g. Ova were collected, drained and weighed to the nearest 0.1 g. About 50 ova were weighed to the nearest 0.1 mg to determine the mean weight per ovum and the relative fecundity of the reproducing females. Ova were fertilized with a mixture of sperm from several males diluted in DIA 532 (Billard, 1977). After water hardening, the eggs were shifted to incubation trays. Eggs from each female were incubated separately at  $6 \pm 1^\circ\text{C}$ . Dead eggs were counted and removed regularly. Survival rates were calculated for each spawn at eyed stage. Only three-year-old females were used to determine egg survival, egg mean weight and relative fecundity because all these parameters could be different between age groups (Bromage and Cumarantunga, 1988).

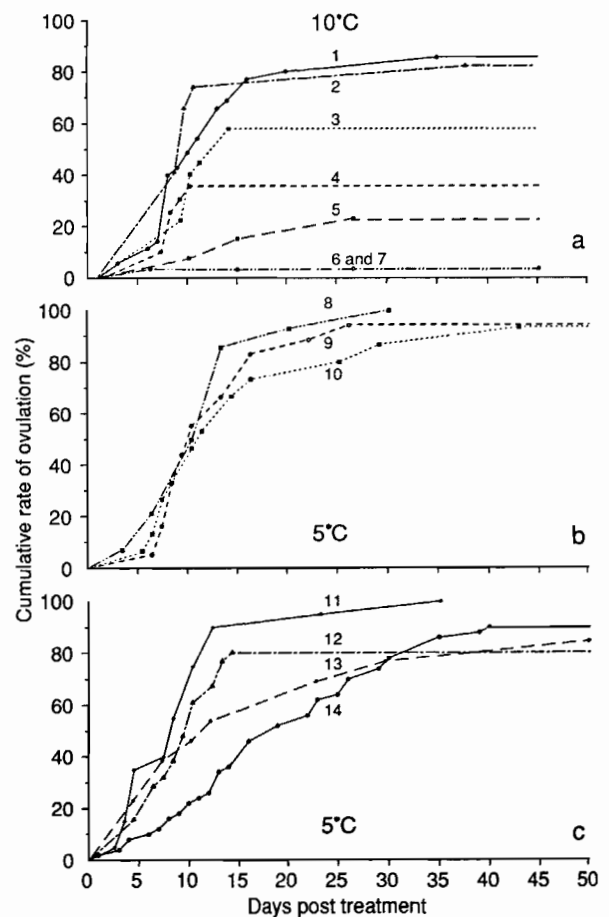
Data were analysed using non parametric tests: the Kruskal-Wallis test to compare ovulation mean time, egg survival, egg mean weight and relative fecundity and the Fisher test to compare the rates of ovulation between the different groups and controls. Results are expressed as mean  $\pm$  S.E. Timing of ovulation did not differ in controls from year to year and the data from different years were therefore pooled. Day 0 was the day when females were injected with GnRH $\alpha$ s. Mean time to ovulation was calculated as the mean of the

number of days from day 0 to individual ovulation for the different females. Cumulative ovulation did not reach 100% in many groups at the end of the experiment. For this reason, mean time to ovulation was calculated from the time to ovulation of the first 80% ovulated females in each group. In groups where cumulative ovulations did not reach 80%, mean time to ovulation was not calculated.

## RESULTS

### Timing of ovulation (Fig. 1)

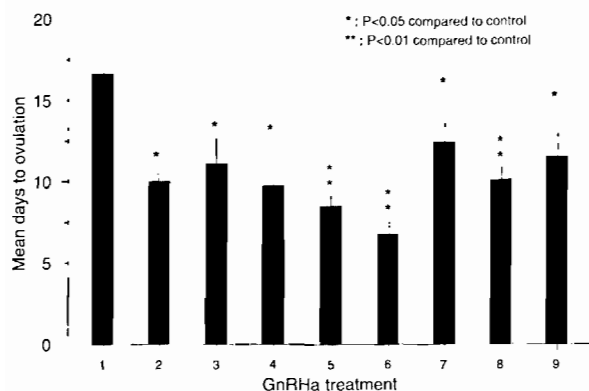
At  $5^\circ\text{C}$  the cumulative percent of ovulation increased regularly in the controls from day 0 to day 40, reaching 90% at the end of the experiment



**Figure 1.** — Cumulative percentages of ovulated Arctic charr over a 50-day period following GnRH $\alpha$  treatments: (1)  $10^\circ\text{C}$ , sustained release form of D-Trp<sup>6</sup>LH-RH; (2)  $10^\circ\text{C}$  D-Arg<sup>6</sup>sGnRH,  $100 \mu\text{g}\cdot\text{kg}^{-1}$ ; (3)  $10^\circ\text{C}$  D-Arg<sup>6</sup>sGnRH,  $20 \mu\text{g}\cdot\text{kg}^{-1}$  + pimoizide; (4)  $10^\circ\text{C}$  D-Arg<sup>6</sup>sGnRH,  $20 \mu\text{g}\cdot\text{kg}^{-1}$ ; (5)  $10^\circ\text{C}$  pimoizide; (6)  $10^\circ\text{C}$  D-Trp<sup>6</sup>LH-RH in free form; (7)  $10^\circ\text{C}$  controls (saline injected); (8)  $5^\circ\text{C}$  D-Ala<sup>6</sup>LH-RH; (9)  $5^\circ\text{C}$ , sustained release form of D-Trp<sup>6</sup>LH-RH; (10)  $5^\circ\text{C}$  D-Trp<sup>6</sup>LH-RH in free form; (11)  $5^\circ\text{C}$  D-Arg<sup>6</sup>sGnRH  $20 \mu\text{g}\cdot\text{kg}^{-1}$  + pimoizide; (12)  $5^\circ\text{C}$  D-Arg<sup>6</sup>sGnRH,  $20 \mu\text{g}\cdot\text{kg}^{-1}$ ; (13)  $5^\circ\text{C}$  pimoizide; (14)  $5^\circ\text{C}$  controls (saline injected).

(Fig. 1c, curve 14). In all the GnRH treated groups at 5°C, the rate of ovulation increased more rapidly than in controls within 5 to 10 days after the beginning of the treatment: 50% of ovulation was reached within 10 days whereas it needed 20 days to obtain the same result in the controls (Fig. 1b, c). At this temperature, pimoziide alone also induced more rapid ovulations than controls (50% on day 12), and seemed to potentiate the effect of D-Arg<sup>6</sup>sGnRH when injected together with this analogue. After 50 days, this combined treatment induced 100% ovulation as well as D-Ala<sup>6</sup>LH-RH (fig. 1b, c, curves 8 and 11). For all the GnRH<sub>a</sub> treated groups there was an initial sharp increase of the rate of ovulation either followed by a plateau, as in group 12 (D-Arg<sup>6</sup>sGnRH) or a gradual increase like the controls. Fish receiving GnRH<sub>a</sub> and/or pimoziide all had a significantly lower mean time to ovulation than the 5°C control (Fig. 2). Two weeks after the beginning of GnRH<sub>a</sub> treatments, the ovulation rates were significantly higher in fish receiving the different GnRH<sub>a</sub> preparations than in controls, except in the group receiving the acute release preparation of D-Trp<sup>6</sup>LH-RH: within two weeks, more than 80% of the females had already ovulated in groups receiving D-Arg<sup>6</sup>sGnRH, D-Ala<sup>6</sup>LH-RH and D-Trp<sup>6</sup>LH-RH in sustained release preparation. At the same time, ovulation rate was 75% in fish receiving the acute release preparation of D-Trp<sup>6</sup>LH-RH, 50% in fish receiving pimoziide alone and 36% in controls.

At 10°C, only one female out of 49 ovulated in controls (Fig. 1a, curve 7). The same result was obtained in fish receiving the acute treatment by D-Trp<sup>6</sup>LH-RH. In all the other groups the rate of ovulation followed a same initial increase as in fish reared at 5°C, but the maximum values obtained were more dispersed, and never reached 100% at the



**Figure 2.** – The effect of GnRH<sub>a</sub> and pimoziide on mean time to ovulation in Arctic charr: (1) 5°C controls; (2) 5°C sustained release form of D-Trp<sup>6</sup>LH-RH; (3) 5°C D-Trp<sup>6</sup>LH-RH in free form; (4) 5°C D-Ala<sup>6</sup>LH-RH; (5) 5°C D-Arg<sup>6</sup>sGnRH, 20 µg.kg<sup>-1</sup>; (6) 5°C D-Arg<sup>6</sup>sGnRH + pimoziide; (7) 5°C pimoziide; (8) 10°C sustained release form of D-LH-RH; (9) 10°C D-Arg<sup>6</sup>sGnRH, 100 µg.kg<sup>-1</sup>. \* :  $p < 0.05$  compared to 5°C control ; \*\* :  $p < 0.01$  compared to 5°C control.

**Table 2.** – Egg survival in 3-year-old Arctic charr in the different experimental groups.

Temperature	Treatment	Survival at eyed stage (%)
5°C	Control	73.6 ± 2.7
	D-Ala <sup>6</sup> LH-RH	76.5 ± 5.6
	D-Trp <sup>6</sup> LH-RH	72.6 ± 7.2
	D-Trp <sup>6</sup> LH-RH sustained release	71.0 ± 6.2
	D-Arg <sup>6</sup> sGnRH 20 µg.kg <sup>-1</sup>	69.4 ± 4.5
	D-Arg <sup>6</sup> sGnRH+Pimoziide	45.0 ± 6.4 *
	Pimoziide	62.5 ± 5.8
10°C	Control	05.0 (1 female)
	D-Trp <sup>6</sup> LH-RH	25.8 (1 female)
	D-Trp <sup>6</sup> LH-RH sustained release	44.4 ± 5.6 *
	D-Arg <sup>6</sup> sGnRH 20 µg.kg <sup>-1</sup>	43.4 ± 14.0 *
	D-Arg <sup>6</sup> sGnRH 100 µg.kg <sup>-1</sup>	39.1 ± 10.0 *
	D-Arg <sup>6</sup> sGnRH+Pimoziide	34.8 ± 5.7 *
Pimoziide	72.9 ± 6.4	

\*  $p < 0.05$  compared to the control 5°C (Kruskal and Wallis test).

end of the experiment. The sustained release form of D-Trp<sup>6</sup>LH-RH and the D-Arg<sup>6</sup>sGnRH at a dose of 100 µg.kg<sup>-1</sup> were the most potent, inducing 80% ovulation on day 15 (Fig. 1a, curves 1 and 2). The D-Arg<sup>6</sup>sGnRH at a lower dose was less efficient (37% ovulation, Fig. 1a, curve 3). Its action was potentiated by pimoziide to a greater extent than at 5°C (51% ovulation, Fig. 1a, curve 4). Pimoziide alone also had an effect, inducing 3 ovulations out of 13 females (Fig. 1a, curve 5).

### Egg quality

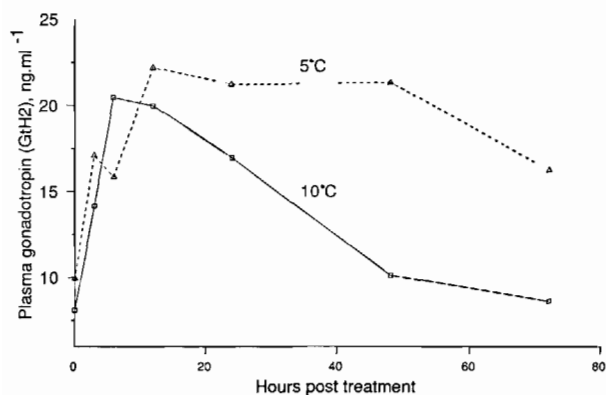
Table 2 summarizes egg survival rates for the different treatments. At 5°C, fish injected with D-Arg<sup>6</sup>sGnRH+pimoziide had significantly lower egg survival rates than the 5°C control. At 5°C, other experimental groups did not differ significantly from the controls. At 10°C, all the fish had significantly lower egg survivals than controls at 5°C, except for the fish receiving pimoziide alone which did not differ from the 5°C control.

Egg mean weight did not significantly differ at 5 and 10°C whatever the experimental group. Also, there was no significant difference in relative fecundity between groups, although there was a tendency towards a decrease in fecundity in all the groups receiving pimoziide (Table 3).

The maximum values of GtH2 plasma levels were generally obtained between 8 and 24 hours after treatment at both temperatures. Egg survivals were negatively correlated with maximum GtH2 plasma levels of the reproducing female, except for groups receiving the sustained release preparation of D-Trp<sup>6</sup>LH-RH (Fig. 3 and 4).

**Table 3.** – Quantity, gonadosomatic index and weight of ova produced by Arctic charr in the different experimental groups (mean  $\pm$  SE). The different groups did not differ significantly for the three parameters, Kruskal-Wallis test (\* sustained release form).

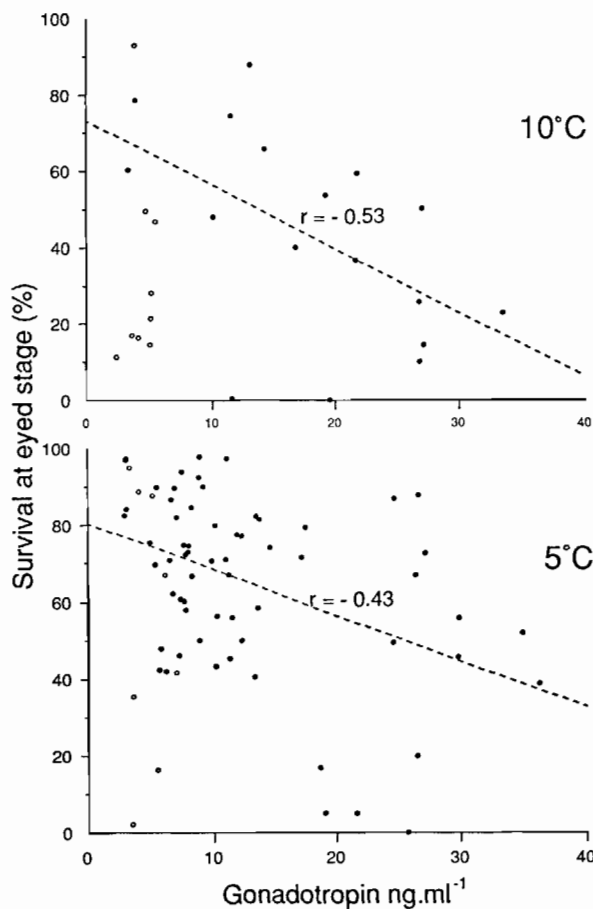
Temperature	Treatment	GSI	Mean ova weight (mg)	Relative fecundity (egg.kg $^{-1}$ )
5°C	Control (saline injected)	14.7 $\pm$ 0.7	43.8 $\pm$ 1.7	3 504 $\pm$ 206
	D-Trp $^6$ LH-RH 20 $\mu$ g.kg $^{-1}$ *	14.9 $\pm$ 1.0	33.2 $\pm$ 2.5	4 678 $\pm$ 344
	D-Trp $^6$ LH-RH 30 $\mu$ g.kg $^{-1}$	16.4 $\pm$ 1.5	38.2 $\pm$ 2.7	4 384 $\pm$ 400
	D-Ala $^6$ LH-RH 30 $\mu$ g.kg $^{-1}$	14.8 $\pm$ 0.9	36.1 $\pm$ 0.9	4 103 $\pm$ 224
	D-Arg $^6$ sGnRH 20 $\mu$ g.kg $^{-1}$	14.6 $\pm$ 0.9	45.8 $\pm$ 2.3	3 288 $\pm$ 257
	D-Arg $^6$ sGnRH 20 $\mu$ g.kg $^{-1}$ +pimozide 5 mg.kg $^{-1}$	12.0 $\pm$ 1.1	46.1 $\pm$ 2.9	2 664 $\pm$ 223
	Pimozide 5 mg.kg $^{-1}$	11.6 $\pm$ 0.9	47.5 $\pm$ 2.0	2 423 $\pm$ 151
10°C	D-Trp $^6$ LH-RH 20 $\mu$ g.kg $^{-1}$ *	14.8 $\pm$ 0.6	40.8 $\pm$ 1.9	3 763 $\pm$ 211
	D-Arg $^6$ sGnRH 20 $\mu$ g.kg $^{-1}$	14.0 $\pm$ 1.2	42.3 $\pm$ 2.9	3 467 $\pm$ 300
	D-Arg $^6$ sGnRH 100 $\mu$ g.kg $^{-1}$	15.5 $\pm$ 1.8	38.8 $\pm$ 2.8	4 152 $\pm$ 552
	D-Arg $^6$ sGnRH 20 $\mu$ g.kg $^{-1}$ +pimozide 5mg.kg $^{-1}$	12.6 $\pm$ 1.1	49.9 $\pm$ 2.8	2 601 $\pm$ 255
	Pimozide 5 mg.kg $^{-1}$	13.7 $\pm$ 2.0	50.8 $\pm$ 2.7	2 530 $\pm$ 247



**Figure 3.** – Example of stimulation of gonadotropin secretion after a GnRH $\alpha$  treatment: GtH2 plasma levels after an injection of D-Arg $^6$ sGnRH in combination with pimozide at 5 and 10°C in 1993. 5 females at each temperature.

## DISCUSSION

The results of the present study clearly indicate that GnRH $\alpha$  is effective for inducing ovulation in Arctic charr. At 5°C the effectiveness of GnRH $\alpha$  for inducing and synchronizing ovulation in Arctic charr was in agreement with the results of previous studies in Arctic charr (Janson, 1993; Haraldsson *et al.*, 1993) and in other salmonids: the coho salmon (Donaldson *et al.*, 1981), the rainbow trout (Breton *et al.*, 1990) and the brown trout (Mylonas *et al.*, 1993). In addition to previous results (Gillet, 1991), this study demonstrates that the exposure of Arctic charr to 10°C or above can lead to the almost complete inhibition of ovulation. It appears that both sustained or acute release modes of GnRH $\alpha$  administration were able to induce ovulation at 10°C. However, the efficiency of the treatment greatly varied with the nature, form and dosage of GnRH $\alpha$ . The acute release form of D-Trp $^6$ LH-RH was ineffective for inducing ovulation. At 10°C, only a sustained release form of LH-RH or a very high dosage of a GnRH analogue (D-Arg $^6$ sGnRH) at



**Figure 4.** – Correlation between plasma gonadotropin level (GtH2) of each reproducing female and egg survival of her spawn. Plasma gonadotropin level was assessed by the maximum value observed during the four days following GnRH treatment. Solid circle : value for the 3-year-old females except for fish receiving the sustained release preparation of D-Trp $^6$ LH-RH. Open circle : value for fish receiving the sustained release preparation of D-Trp $^6$ LH-RH. These values were excluded from the calculation of correlation.

100  $\mu\text{g}\cdot\text{kg}^{-1}$ ) were able to induce similar rates of ovulation than those obtained at 5°C. These results possibly indicate that the blockade of ovulation at 10°C was partially due to a central inhibition and that the removal of this inhibition required either a prolonged stimulation of GtH2 (sustained release form of GnRH, Breton *et al.*, 1990) or a large acute release of gonadotropin (high dosage of GnRH). An overall comparison of the results obtained at 5°C and 10°C clearly demonstrated that the induction of ovulation at 10°C required higher dosages of GnRH than at 5°C. At 10°C, a dopamine receptor antagonist (pimozide) potentiated the action of D-Arg<sup>6</sup>sGnRH as already reported in other species and especially in cyprinids (Peter *et al.*, 1986). At 10°C, pimozide alone induced 23% of ovulation in Arctic charr which implies that a dopamine inhibition of gonadotropin GtH2 secretion could block the ovulation in Arctic charr. At 5°C, Arctic charr did not require a combined treatment using GnRH and pimozide, possibly because the dopamine inhibition of gonadotropin secretion was less effective than at 10°C.

There was no apparent correlation between a reduced mean time to ovulation and the final cumulative rates of ovulation. Especially at 5°C, treatments which induced 100% ovulation were not those which most diminished the mean time to ovulation. Thus in the choice of a treatment, the nature of the GnRH analogue and its dosage should be considered in relation to the objective, that is either to induce a rapid synchronization of ovulation or to obtain 100% ovulation over the longer term.

Egg survival rates in Arctic charr at 5°C were comparable to those obtained in previous studies of GnRH induced ovulation in other salmonids (Crim *et al.*, 1983; Fitzpatrick *et al.*, 1984; Breton *et al.*, 1990). However, the mean survival percentage of eggs produced by fish injected with D-Arg<sup>6</sup>sGnRH in combination with pimozide was significantly lower than in controls. An adverse effect of pimozide in combination with GnRH on egg survival has already been reported in rainbow trout (Billard *et al.*, 1984) who hypothesized that pimozide could have a deleterious effect on oocytes. They also suggested that the poor egg quality could be due to a high gonadotropin level before ovulation. In our experiments, survival of eggs produced by females injected with pimozide alone did not differ significantly from controls at either 5 or 10°C. At the latter temperature, the mean survival percentage of eggs was higher in females treated with pimozide

alone than in all the groups treated with GnRH, suggesting that pimozide did not damage directly the eggs. The occurrence of a negative correlation between plasma GtH2 level and egg survival seems to confirm Billard's hypothesis of the adverse effect of high GtH2 levels on egg quality. As suggested by Mylonas *et al.* (1993), GnRH treatments could lead to a slight asynchrony between the process of meiotic maturation regulated by the maturation inducing steroid and the process of ovulation regulated by prostaglandins. Very high levels of GtH2 could enhance the asynchrony between the two processes. This emphasizes the risk of overstimulation of GtH2 secretion and the need to determine for each species the appropriate GnRH dosage that gives good rates of ovulation combined to the production of good egg qualities. Plasma GtH2 level was certainly not the only factor involved in the control of egg quality because egg survival varied widely in groups treated with the sustained release form of D-Trp<sup>6</sup>LH-RH while GtH2 levels were always very low.

Egg survival was not uniformly reduced in all ovulating females in the experimental groups which had a mean survival percentage significantly lower than controls. In all experimental groups, survival rates as high as those achieved by control fish were also obtained for some females injected with GnRH. The explanation for the difference in egg survival among females treated with GnRH might be found either in the different responsiveness of females to GnRH injection (*i.e.* the difference of plasma GtH2 levels after treatment) or in the difference of maturation stage of females at the time of treatment. In coho salmon (Fitzpatrick *et al.*, 1984) and in Atlantic salmon (Crim and Glebe, 1984), the occurrence of females with low fertility was higher for fish injected with GnRH that ovulated early in season. In our work, most females injected with GnRH at 10°C produced eggs of poor quality. This low fertility at 10°C may be caused by a lack of maturation of females due to the absence of spontaneous ovulation at this temperature.

In conclusion, this work demonstrates that GnRH can synchronize ovulation at 5°C in the Arctic charr without loss of relative fecundity and egg quality. However, at 10°C GnRH can induce ovulation but with a loss of egg quality. Beside the central inhibitory mechanism removed by GnRH, there is possibly other levels of inhibition at this temperature, especially at the follicular level, whose removal is also necessary in good synchrony with the central level to obtain eggs with high fertilization rates.

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## REFERENCES

- Billard R. 1977. Utilisation d'un système Tris-glycocolle pour tamponner le dilueur d'insémination pour truite. *Bull. Fr. Piscic.* **264**, 102-112.
- Billard R., P. Reinaud, M.G. Hollebecq, B. Breton 1984. Advancement and synchronisation of spawning in *Salmo gairdneri* and *S. trutta* following administration of LHRH-A combined or not with pimozide. *Aquaculture* **43**, 57-66.
- Breton B., P. Prunet, P. Reinaud 1978. Sexual differences in Salmon gonadotropin. *Ann. Biol. Anim. Biochim. Biophys.* **18**, 754-765.
- Breton B., C. Weil, E. Sambroni, Y. Zohar 1990. Effects of acute versus sustained administration of GnRH on GtH release and ovulation in the rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* **91**, 373-383.
- Bromage N., R. Cumarantunga 1988. Egg production in the rainbow trout. In: Recent Advances in Aquaculture, Vol. 3. J.M. Muir, R.J. Roberts eds. Croom Helm, London, 64-138.
- Crim L. W., B. D. Glebe 1984. Advancement and synchrony of ovulation in Atlantic salmon with pelleted LHRH analog. *Aquaculture* **43**, 47-56.
- Crim L. W., A. M. Sutterlin, D. M. C. Evans, C. Weil 1983. Accelerated ovulation by pelleted LHRH analogue treatment of spring-spawning rainbow trout (*Salmo gairdneri*) held at low temperature. *Aquaculture* **35**, 229-307.
- Donaldson E. M., G. A. Hunter, H. M. Dye 1981. Induced ovulation in coho salmon (*Oncorhynchus kisutch*). II. Preliminary study of the use of LH-RH and two high potency LH-RH analogues. *Aquaculture* **26**, 129-141.
- Fitzpatrick M. S., B. K. Suzumoto, C. B. Schreck, D. Oberbillig 1984. Luteinizing hormone-releasing hormone analogue induces precocious ovulation in adult coho salmon (*Oncorhynchus kisutch*). *Aquaculture* **43**, 67-73.
- Gillet C. 1991. Egg production in an Arctic charr (*Salvelinus alpinus* L.) brood stock: effects of temperature on the timing of spawning and the quality of eggs. *Aquat. Living Resour.* **4**, 109-116.
- Haraldsson H., T. Sveinsson 1993. Effects of LHRH $\alpha$  treatments upon the timing of ovulation and upon egg and offspring quality in Arctic charr, *Salvelinus alpinus* (L.). *Aquac. Fish. Manag.* **24**, 145-150.
- Jansen M. E. 1993. Induction of ovulation in Arctic charr held under unchanging temperature and photoperiod. *Progress. Fish-Cult.* **55**, 32-34.
- Jobling M., E. H. Jorgensen, A. M. Arnesen, E. Ringo 1993. Feeding growth and environmental requirements of Arctic charr. A review of aquaculture potential. *Aquac. Int.* **1**, 20-46.
- de March B. G. 1995. Effects of incubation temperature on the hatching success of Arctic charr eggs. *Progress. Fish-Cult.* **57**, 132-136.
- Mylonas C. C., J. M. Hinshaw, C. V. Sullivan 1993. GnRH $\alpha$  induced ovulation of brown trout (*Salmo trutta*) and its effects on egg quality. *Aquaculture* **160**, 379-392.
- Peter R. E., J. P. Chang, C. S. Nahorniak, R. J. Omeljanuik, M. Sokolowska, S. H. Shih, R. Billard 1986. Interactions of catecholamines and GnRH in regulation of gonadotropin secretion in teleost fish. *Rec. Prog. Hormone Res.* **42**, 513-548.
- Peter R. E., M. Sokolowska, C. S. Nahorniak 1987. Comparison of (D-Arg<sup>6</sup>, Trp<sup>7</sup>, Lcu<sup>8</sup>, Pro<sup>9</sup> Net)-luteinizing hormone-releasing hormone (sGnRh-A), and (D Ala<sup>6</sup>, Pro<sup>9</sup> Net)-luteinizing hormone-releasing hormone (LHRH-A), in combination with pimozide, in stimulating gonadotropin release and ovulation in the goldfish, *Carassius auratus*. *Can. J. Zool.* **65**, 987-991.
- Tabachek J. I., B. G. de March 1991. Research priorities for the culture of Arctic charr *Salvelinus alpinus* (L.) in Atlantic Canada. *Can. Industry Rep. Fish. Aquat. Sci.* **211**.