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## Research work on Arctic charr (*Salvelinus alpinus*) in France – broodstock management

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### SUMMARY

Development of a broodstock of Arctic charr was undertaken in experimental tanks supplied with water pumped from Lake Geneva at a depth of 36 m. Spawners were subjected to different thermal regimes to determine the effects of temperature on spawning time and gamete viability. In the tanks, ovulation occurred spontaneously at the same time as the spawning of wild fish in the lake. The timing of ovulation was slowed down at 8°C and above. At 11°C, ovulation was inhibited. When the water temperature of the rearing tanks was higher than 5°C for several weeks prior to spawning, the quality of eggs produced by the artificially reared females was poorer than those of wild fish because the process of overripening was very rapid above 5°C. When spawners were reared at 5°C and examined twice a week to detect ovulation, 80–90% survived to the eyed stage. The fertilization rate of eggs produced by spawners transferred from 8 to 5°C in December was high (78%). When the transfer occurred in January, the viability of ova tended to decrease (63%).

The spawning time of Arctic charr was delayed by six weeks when the charrs were subjected to long days (17L/7D) from mid-summer. Ovulation was spread over a period of 2.5 months when fish were maintained under long days until spawning time. In contrast, the termination of the long day conditions in December induced a synchronization of ovulations which occurred over a period of one month. Conditions of long days early in the year followed by short days, advanced the spawning by two months. Twenty month old immature fish maintained under constant long days from October spawned at the beginning of summer. By manipulating the photoperiod and taking into account cold water requirements of spawners, it seems possible to obtain viable eggs from reared Arctic charr all year round.

The effects of different forms of GnRH analogues on the stimulation of gonadotropin secretion and the rate of ovulation were also investigated. At 5°C, injection of GnRH $\alpha$  induced a clear synchronization of ovulation. At 10°C, a salmon GnRH analogue, D-Arg<sup>6</sup>sGnRH $\alpha$  and a biodegradable sustained release form of D-Trp<sup>6</sup>LH-RH $\alpha$  were the most effective in inducing ovulation whereas D-Trp<sup>6</sup>LH-RH $\alpha$  in free form (30  $\mu$ mg/kg) failed to induce ovulation. These results are discussed, along with a comparison of cyclic AMP content in oocyte, stimulation of gonadotropin secretion and egg quality at 5° and 10°C.

**Key words:** Arctic charr, egg, gonadotropins, ovulation, photoperiod, reproduction, spawning, temperature.

### RÉSUMÉ

*Travaux de recherche sur l'omble chevalier en France: Recherche sur la gestion d'un stock de géniteurs en pisciculture*

Nous avons réalisé l'élevage de géniteurs d'omble chevalier dans des bassins alimentés par de l'eau

pompée à 36 m dans le Léman. Les géniteurs sont conditionnés à différentes températures afin de déterminer les effets de ce facteur sur la date de ponte et de viabilité des gamètes. En élevage, l'ovulation se produit spontanément aux dates habituelles de la fraie des ombles sauvages du Léman. Le déroulement des ovulations se ralentit à partir de 8°C et l'ovulation est complètement inhibée à 11°C. Lorsque la température de l'eau est supérieure à 5°C au cours des dernières semaines qui précèdent la ponte, la qualité des oeufs produits par les femelles en élevage est inférieure à celle des oeufs des ombles sauvages, en raison de l'accélération des processus de surmaturation au dessus de 5°C. Lorsque les géniteurs sont élevés à 5°C et contrôlés 2 fois par semaine pour détecter les ovulations, la survie des oeufs peut atteindre 80 à 90% au stade oeillé. Le taux de survie des oeufs produits par les géniteurs transférés de 8 à 5°C en décembre est élevé (78%), mais lorsque le transfert de 8 à 5°C est réalisé en janvier, la viabilité des oeufs a tendance à diminuer (63%).

La ponte de l'omble chevalier est retardée de 6 semaines lorsque les poissons sont conditionnés en jours longs (17L/7N) à partir de la moitié de l'été. La période des ovulations s'étale sur une durée de deux mois et demi lorsque les poissons sont conditionnés en jours longs jusqu'à la date de la fraie, par contre l'arrêt du traitement en jours longs en décembre permet d'obtenir des ovulations synchronisées sur un mois. Le conditionnement des poissons en jours longs à partir du début de l'année, suivi par des jours courts au début de l'été permet d'avancer la date de la durée de la fraie à 2 mois.

Des poissons immatures âgés de 20 mois et conditionnés en jours longs en octobre se reproduisent à partir du début de l'été suivant. Il paraît possible d'obtenir des oeufs viables chez l'omble chevalier en élevage pendant toute l'année par différentes manipulations photopériodiques dans la mesure où les besoins en eau froide des géniteurs sont respectés.

Nous avons étudié les effets de différents analogues du GnRH sur la sécrétion gonadotrope et l'induction de l'ovulation chez l'omble chevalier. A 5°C, l'injection de GnRHa permet de synchroniser efficacement les ovulations. A 10°C, un analogue du GnRH de saumon, le D-Arg<sup>6</sup>GnRHa ainsi qu'une forme retard biodégradable du D-Trp<sup>6</sup>LHRHa font preuve d'une grande efficacité pour induire l'ovulation tandis que l'injection de D-Trp<sup>6</sup>LHRHa en forme libre ne permet pas d'induire l'ovulation. Ce travail inclut des mesures sur l'AMPcycloique intraovocytaire, la gonadotropine plasmatique et la survie des oeufs à 5 et 10°C.

Mots clés: gonadotropine, oeuf, omble chevalier, ovulation, photopériode, reproduction, surmaturation, temperature.

## YFIRLIT

### *Rannsóknir á bleikju í Frakklandi – stjórnun hrygningar*

Kynþroska bleikja var alin í vatni sem dælt var af 36 m dýpi úr Genfarvatni. Hrygningarfiskur var alinn við mismunandi hitastig til þess að ákvarða áhrif hitastigs á hrygningartíma og afföll hrogn. Fiskurinn í kerjunum hrygndi sjálfviljugur á sama tíma og villti fiskurinn í vatninu. Við 8°C og þar yfir seinkaði hrygningu. Við 11°C varð engin hrognalosun. Væri vatnshitinn hærra en 5°C í nokkrar vikur fyrir hrygningu urðu hrognin lélegri en hjá villtum fiski vegna ofþroskunar. Þegar hrygningarfiskur var alinn við 5°C og fylgst með hrognþroska tvisvar í viku, lifðu 80–90% af hrognunum fram að augnhrognastigi. Ef hitastig á hrygningarfiski var lækkað úr 8°C í 5°C í desember var frjóvgunarlutfall hátt, eða 78%. Við hitastigslækkun í janúar jukust afföllin.

Langur dagur (17 tíma dagur/7 tíma nótt) um mitt sumar seinkaði hrygningu um sex vikur. Ef fiskurinn var hafður við langan dag fram að hrygningu dreifðist hrygningin yfir 2,5 mánuði. Ef fiskurinn var settur á stuttan dag í desember, hrygndi allur fiskurinn á aðeins einum mánuði. Sumarlangur dagur snemma árs, sem fylgt er eftir með stuttum degi, flýtir hrygningu um tvo mánuði.

Þegar 20 mánaða gamall ókynþroska fiskur var alinn við langan dag frá því í október, þá hrygndi hann að vori. Það virðist því mögulegt að fá lífvænleg hrogn úr bleikju árið um kring með því að stýra hita og ljósi.

Auk þessa voru athuguð áhrif mismunandi gerða af GnRH eftirlíkinga á framleiðslu kynhormóna og hrygningarhraða. GnRHa-hormónalíkisgjöf við 5°C samhæfði greinilega hrygningu. Við 10°C dugðu laxa-GnRH-líki, D-Arg<sup>6</sup>GnRHa og bundið D-Trp<sup>6</sup>LH-RHa sem leysist hægt úr bindiefni, best

til að framkalla hrygningu. Óbundið D-Trp<sup>6</sup>LH-RHa framkallaði hins vegar ekki hrygningu. Niðurstöður þessara rannsókna eru reifaðar ásamt samanburði á magni hringtengds AMP í hrognafurum, framleiðslu kynhormóna og hrognagæðum við 5°C annars vegar og 10°C hins vegar.

## INTRODUCTION

Arctic charr (*Salvelinus alpinus* L.) has been present in two French lakes, Lake Bourget and Lake Geneva since the end of glaciation. This species was also introduced in many mountain lakes in the Alps, the Pyrenees and the Massif Central. Arctic charr is an important species for both commercial and recreational fishermen. During the second half of this century, catches have dramatically declined in many lakes as a result of eutrophication. This is true in Lake Geneva where important catches were only restored by restocking with underyearlings (Champigneulle *et al.*, 1988). In this context, the establishment of hatchery broodstocks was undertaken to allow the intensification of restocking and more recently to develop the aquaculture of this species which has a great potential, as indicated by Papst and Hopky (1983). However, first trials have highlighted difficulties in obtaining eggs of good quality from reared Arctic charr on French fish farms. We have analysed the performance of a broodstock of Arctic charr of the Lake Geneva strain. Factors likely to influence the spawning time and the quality of eggs were studied by reference to the performance of wild Arctic charr spawners in Lake Geneva, which were taken as controls.

It is now well established that daylength exerts a primary influence over the initiation and modulation of reproductive development in salmonid fish (Hoover, 1937; Breton and Billard, 1977; Bromage *et al.*, 1982, 1984). However, little is known about the influence of temperature on the spawning time in salmonid fish. Arctic charr is the only freshwater fish found in the highest latitudes of the Arctic (Hammar, 1989) and this species spawns at the beginning of winter. Consequently thermal requirements are likely to be very low for maturation and spawning in

this species. Moreover, the effects of photoperiod manipulation on the spawning time of Arctic charr have not yet been investigated. We have subjected Arctic charr broodfish to different temperature and photoperiod regimes, applied at different stages of the reproductive cycle.

The acceleration and synchrony of the processes of ovulation is desirable for various hatchery practices. In Arctic charr, identification of ripe females requires frequent broodstock handling owing to the rapidity of the ageing process of ova. Moreover, Arctic charr females require cold water ( $\leq 8^{\circ}\text{C}$ ) for spontaneous ovulation. Pituitary preparations and gonadotropin releasing hormone (GnRH) have increasingly been used to induce ovulation in the salmonids (Jalabert *et al.*, 1978; Sower *et al.*, 1984; Crim *et al.*, 1983; Breton *et al.*, 1990). We have tried to evaluate the use of GnRH analogues to induce and synchronize ovulation in Arctic charr at 5 and 10°C. The latter temperature is similar to the mean temperature of many cold springs in France, it is favourable for growth, but inhibits spontaneous ovulation in Arctic charr.

## MATERIALS AND METHODS

### *Source of fish stock*

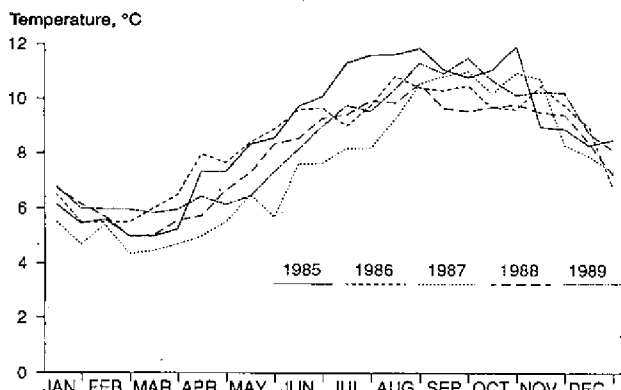
The Arctic charr (*Salvelinus alpinus*) broodfish originated from eggs collected from wild fish in Lake Geneva. Wild spawners were caught in gill nets on the spawning grounds. Several successive generations were reared from these eggs at the INRA Experimental Station in Thonon-les-Bains. Each cohort was produced by pooling the gametes of at least five females and ten males subsequently. Thousand juveniles were randomly selected each year and reared for three years until they became mature.

### Description of rearing procedure

Arctic charr spawners were kept in 4 or 12 m<sup>3</sup> tanks, supplied with water pumped from a depth of 36 m of Lake Geneva. Water temperature fluctuated between 5.5°C in winter and 11°C in summer (Figure 1). A refrigeration system was used to maintain water temperature at 5°C±1°C in one tank, all year round. Fish were fed with dry pellets (trout commercial food, Trouvit, protein 48%, lipid 12%) distributed for eight hours daily with automatic feeders. Fish were fed at a ration recommended for rainbow trout i.e. 0.5 to 1.5% of body weight per day. Experimental fish were randomly taken from the three years old spawners (500 fish) except for group I. The weight of fish 0+, 1+, 2+ in March 1991 are shown in Figure 2.

### Rearing of spawners under different experimental temperature regimes (Table 1)

1. Eighty fish were kept in the water pumped from a depth of 36 m (Lake Geneva water) in 1985–86 (group 1) and in 1986–87 (group 2).
2. Groups of 40 fish were acclimatized at 5°C from summer or autumn until the time of ovulation. They were transferred into cold water on 15 July 1986 (group 3) on 15 September 1987 (group 4), on

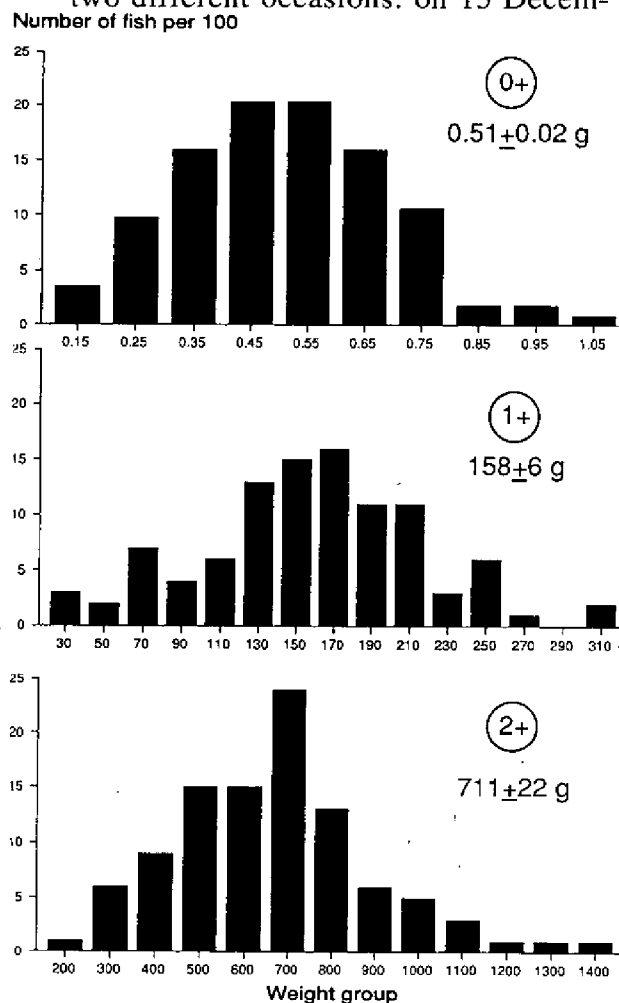


**Figure 1.** Yearly variations of water temperature (1985–89). Water was pumped from a depth of 36 m in Lake Geneva.

1. mynd. Breytingar í vatnshita 1985–1989. Vatnið var tekið af 36 m dýpi í Genfarvatni.

1 September 1989 (group 5) and on 1 October 1990 (group 6, 70 fish).

3. Spring water of constant temperature (11°C±1°C) was used to raise the water temperature of some tanks in winter:
  - From the beginning of autumn, spawners were kept in tanks supplied with spring water: group 7 in winter 1985/86 (20 females), group 8 in 1989/90 (45 females), group 9 in 1990/91 (70 females).
  - On 5 December 1987, 80 females were transferred from 10°C to 8°C (group 10). Subsequently 20 unovulated females were transferred into water at 5°C on two different occasions: on 15 Decem-



**Figure 2.** Weight frequency distribution of 0+, 1+ and 2+ reared Arctic charr on 21 March 1991. 0+ had been fed for two months.

2. mynd. Þyngdardreifing bleikju á 1. (0+), 2. (1+) og 3. (2+) ári 21. mars 1991. Seiði á 1. ári höfðu fengið fóður í 2 mánuði.

**Table 1.** Summary of the different thermal regimes investigated on experimental groups of Arctic charr spawners under natural photoperiod.*I. tafla. Yfirlit yfir eldishita á tilraunahópum af bleikjuhrygnum við náttúrulega daglengd.*

Expt. group	Temperature regime
1	Water pumped from a depth of 36 m all year round 1985–86
2	Water pumped from a depth of 36 m all year round 1986–87
3	5°C from 15 July 1986 to spawning
4	5°C from 15 September 1987 to spawning
5	5°C from 1 September 1989 to spawning
6	5°C from early 1 October 1989 to spawning
7	11°C from autumn until March 1985
8	10°C from 1 September 1989 to January 1990
9	10°C from 1 October 1990 to 29 December 1990 and thereafter 5°C to spawning
10	10°C from autumn until December 1987 – 8°C from early December 1987 to spawning
11	8°C from early December to 15 December 1987 and thereafter 5°C to spawning
12	8°C from early December to 5 January 1988 and thereafter 5°C to spawning
13	8°C from early December to 15 January 1988 and thereafter 5°C to spawning
14	Wild females which had ovulated in the hypolimnion of Lake Geneva (5.5°C)

ber 1987 (group 11) and on 5 January 1988 (group 12). On 25 January 1988 the remaining unovulated females of group 10 were transferred to 5°C water (group 13).

In 1989, 20 fish kept in Lake Geneva water were killed every month from April to December. GSI, expressed as gonadal weight/body weight $\times$ 100, was calculated for females. On 5 December, 10 females of groups 5 and 8 were killed. GSI and oocyte mean weight calculated for every female.

#### *Rearing of spawners under different photoperiod regimes (Table 2)*

Fish were exposed to different light regimes provided by 60 W/24 V bulbs and controlled by time switches. Short-day experiments were carried out in a light-proof room.

1. Constant long days in summer and autumn: Spawners were reared under 17 hours light and 7 hours dark per day (17L/7D). Fish of group B were exposed to artificial light regimes from summer until spawning time. In four other groups, the long day regime was stopped sev-

eral weeks before the presumed spawning time (groups C, D, E and F).

2. Shortened photoperiodic cycle: Spawners were reared under a 17L/7D light regime from 5 January (group G) or 1 April (group H) to 30 June. Subsequently, fish were kept under 7L/17D and transferred to refrigerated water at 5°C before spawning.
3. Constant long days in winter (group I): Twenty month old immature fish were subjected to 17L/7D from the beginning of October to 5 May. Afterwards, they were placed in refrigerated water at 5°C, under a 7L/17D light cycle. Females after first spawning in May or June, were reared under a natural photoperiod.

#### *Detection of ovulation. Fertilization and incubation*

Male fish were included in all experimental groups although the times of the onset of spermiation were not documented. It might be mentioned that in general spermiating males were present in all groups 2–3 weeks before the females began to ovulate. When

**Table 2.** Summary of the different photoperiodic and thermal regimes investigated on experimental groups of Arctic charr spawners.

2. tafla. Yfirlit yfir ljósloftur og hitastig á tilraunahópum með bleikjuhrygnur.

Expt. group	Photoperiodic regime	Thermal regime
A	Natural photoperiod all year round 1986–87	Water pumped from a depth of 36 m
B	17L/7D from 16 August 1986 to spawning	Water pumped from a depth of 36 m
C	17L/7D from 16 August 1987 to 15 December 1987	Water pumped from a depth of 36 m
D	17L/7D from 21 June 1988 to 15 December 1988	Water pumped from a depth of 36 m
E	17L/7D from 16 August 1988 to 15 December 1988	Water pumped from a depth of 36 m
F	17L/7D from 16 August 1989 to 15 December 1989	Water pumped from a depth of 36 m
G	17L/7D from 4 January 1990 to June 1990 and thereafter 7L/17D from 1 July to spawning	Water pumped from a depth of 36 m and after 5°C from 1 July to spawning
H	17L/7D from 1 April 1988 to 30 June 1988 and thereafter 7L/17D from 1 July to spawning	Water pumped from a depth of 36 m and thereafter 5°C from 1 Sept. to spawning
I	17L/7D from 1 Oct. 1988 to 5 May 1989 and afterwards 7L/17D from 6 May 1989 to spawning. Afterwards natural photoperiod until the second spawning	5°C from 6 May 1989 to spawning. Subsequently water pumped from a depth of 36 m,

the majority of the males were in spawning condition and colour and gave milt, females were regularly examined twice a week, except for group 1 which was examined once a week. Each ovulated female was anaesthetized in 2-phenoxyethanol (0.3 mg/l) 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 in order to determine the mean weight per ova and the relative fecundity of females (number of ova/kg). Ova were fertilized with a mixture of sperm from several males diluted in DIA 532 (Billard, 1977). After hardening, twenty minutes later, eggs were shifted into incubation trays. Eggs from each female were incubated separately at  $6^{\circ}\text{C}\pm 1^{\circ}\text{C}$ . Dead eggs were counted and removed once a week. Survival rates were calculated when eggs had reached the eyed stage (350 degree-days). Wild females from Lake Geneva were caught on natural spawning grounds at an average depth of 60 m. Catches of ripe females were obtained from the end of November to the beginning of January. Females which were ovulating when caught were fertilized and incubated follow-

ing the same procedure as for the reared spawners. These fish comprised group 14.

#### *Study of the ageing of ova*

The decrease in ova fertility after ovulation was estimated by fertilizing small quantities of ova from the day when ovulation was first recorded (day 1) and at 2 or 3 days-intervals thereafter until day 11. Each time, about 200 ova were stripped from each female and immediately fertilized. This study was carried out on reared females which had ovulated in December in the Lake Geneva water. Water temperature fluctuated between 7 and 8°C (group 2). This was repeated for females kept at 5°C for more than 2 months (group 4), and also for two wild females caught in pre-ovulation stage in December which were kept in a tank supplied with Lake Geneva water.

#### *Induction of spawning at 5° and 10°C*

On 1 September 1989, two groups of 45 females were acclimatized to 5 and 10°C respectively. On 1 October 1990 groups of 70 females were acclimatized to the same temperatures as the previous year. On 4 De-

**Table 3.** Treatments with GnRH analogues<sup>a)</sup>.

3. tafla. Meðferðarhópar í tilraunum með hormónagjöf, GnRH-líki.

Treatment	Number of fish	
	5°C	10°C
<b>1989</b>		
Saline (control)	10	10
D-Trp <sup>6</sup> , Des Gly <sup>10</sup> LH-RHa, 30 µg/kg	10	10
D-Trp <sup>6</sup> , Des Gly <sup>10</sup> LH-RHa <sup>b)</sup> , 20 µg/kg	7	12
<b>1990</b>		
Saline (control)	19	16
D-Arg <sup>6</sup> sGnRHa, 20 µg/kg	14	6
D-Arg <sup>6</sup> sGnRHa, 60 µg/kg		7
D-Ala <sup>6</sup> , Des Gly <sup>10</sup> LH-RHa, 20 µg/kg	14	
D-Trp <sup>6</sup> , Des Gly <sup>10</sup> LH-RHa, 20 µg/kg		6
D-Trp <sup>6</sup> , Des Gly <sup>10</sup> LH-RHa <sup>b)</sup> , 20 µg/kg	6	6
D-Trp <sup>6</sup> , Des Gly <sup>10</sup> LH-RHa <sup>b)</sup> , 30 µg/kg	8	7
D-Trp <sup>6</sup> , Des Gly <sup>10</sup> LH-RHa <sup>b)</sup> , 60 µg/kg		7

- a) The D-Trp<sup>6</sup>LH-RHa used for sustained release was a commercial microencapsulated form, in a polyglycolic-poly-lactic biodegradable matrix, obtained from Ipsen/Beaufour laboratory. It was used as a suspension in a physiological saline solution containing 0.1% Tween 20. D-Trp<sup>6</sup>LH-RHa was also obtained from the Ipsen/Beaufour laboratory. D-Ala<sup>6</sup>LH-RHa was purchased from Sigma (St. Louis MO). D-Arg<sup>6</sup>sGnRH (Salmon GnRH analogue) was synthesized (Breton *et al.*, 1990).
- b) Micro encapsulated slow release form.

cember 1989 and on 5 December 1990, fish were injected with GnRH analogues (Table 3). By that time, 30% of the females had already ovulated at 5°C but none had ovulated at 10°C. Blood samples were carried out at t0 (time of injection), t1 (1 hour later), t2, t3, t4, t6, t9, t24 and t48 (1989) or t0, t2, t5, t8, t24 and t48 (1990), in order to follow the biological activities of these peptides, assessed by their ability to stimulate plasma gonadotropin (GtH) level. This level is measured by radioimmunoassay according to (Breton *et al.*, 1978). Thereafter, fish were checked several times a week to detect ovulation. Intra-follicular cAMP levels have been shown to play a central role in the regulation of trout oocyte sensitivity to the maturation inducing steroid (17 $\alpha$ -hydroxy-20 $\beta$ -dihydroprogesterone) and in the intra-oocyte mechanisms leading to germinal vesicle breakdown (GVBD) (Jalabert and Finet, 1986). We have measured intra-follicular cAMP contents in fish raised at 5° and 10°C, in order to test a

possible involvement of this nucleotide in the blockade of maturation at 10°C. For that purpose, intra-follicular cAMP was measured in 10 fish from each temperature, all slaughtered on 3 December 1989. Measurements were made on posterior mid and anterior part of one ovary after defolliculation according to (Finet *et al.*, 1988). There were 5 replicates per determination. The experiments were carried out with 2 and 3 year old females. Three year old females were selected in experiments on cAMP contents, plasma gonadotropin levels and egg survival.

#### Statistical analysis

Means for the different experimental groups (relative fecundity, ova weight, egg survival percentages of eggs after arc sine transformation and plasma GtH levels) were compared by multiple comparisons of means (Dagnelie, 1970). Mean values of egg survival rates shown in Table 4 for the groups 1 and 2 were computed using a two ways anal-



**Table 4.** Survival rates (%) of spawns at eyed stage (mean±SE) from groups of Arctic charr at different thermal regimes. See Table 1 for details of thermal regimes for the different groups.

4. tafla. Hlutfall (%) lifandi bleikjuhrogn á augnstigi (meðaltal og staðalskekking) eftir mismunandi hitastigsmeðferð á hrygnum. Sjá lýsingu á tilraunahópum í 1. töflu.

Expt. group	November	December	January	February	March	Examination of females
1	4.3±2.2 (8.9°C)	17.4±5.1 (8.4°C)	36.5±5.5 (5.8°C)			Once a week
2	29.6±4.7 (9.8°C)	61.5±5.4 (7.8°C)	83.8±2.2 (5.1°C)			Twice a week
3		80.2±4.8 (5°C)				Twice a week
4		90.6±2.2 (5°C)				Twice a week
5		78.3±2.6 (5°C)				Twice a week
6		81.9±3.2 (5°C)				
7,8,9	No ovulation (11°C)					
9	After transfer at (5°C)		64.3±5.8			Twice a week
10		58.0±6.2 (8°C)				Twice a week
11		78.2±3.3 (5°C)				Twice a week
12			69.3±4.7 (5°C)			Twice a week
13				62.8±7.0 (5°C)		Twice a week
14		78.9±2.5 (5.5°C)				Caught during ovulation

ysis of variance for uneven sample sizes as described in Dagnelie, 1970. The use of relative fecundity assumes that there is no correlation between body weight and relative fecundity. The correlation between relative fecundity and body weight was calculated for the groups 1 and 2.

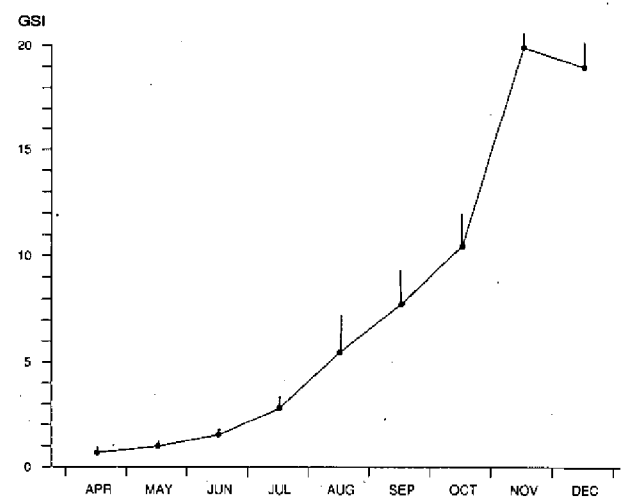
## RESULTS

### Effects of temperature

*Female gonado-somatic index (Figure 3).* Ovaries developed slowly from April to August. In September and October, ovaries grew faster. The development of ovaries was completed in November. Females (group 9) reared at 10°C in autumn have larger oocytes ( $P<0.05$ ) and higher GSI ( $P<0.05$ ) than fish (group 5) reared at 5°C (Table 5).

*Timing of ovulation (Figure 4).* Ovulations in fish reared in water pumped from a depth of 36 m (groups 1 and 2) were first recorded at the end of November when Lake Geneva water temperature started to decrease below 10°C. Hundred percent of the females had spawned by mid-January, when water temperature was close to 5°C. The majority of

the females reared in cold water (groups 3, 4, 5 and 6) ovulated at the same time as fish in groups 1 and 2. However, the first females spawned by mid-October (group 3) and the beginning of November (group 4). Hundred percent of the females had spawned by mid-January. No ovulation was recorded among the fish of group 7 kept at 11°C or the fish of group 9 (10°C) in November and December.



**Figure 3.** Monthly changes in gonado-somatic-index (GSI) of reared Arctic charr females.  
3. mynd. Breytingar á hlutfalli þunga kynkirtla og heildarþunga (GSI) á bleikjuhrygnum í eldi.

**Table 5.** Effects of temperature on the development of ovaries in autumn. Fish were reared at 10° and 5°C from 1 September 1989. Fish were slaughtered on 5 December 1989.

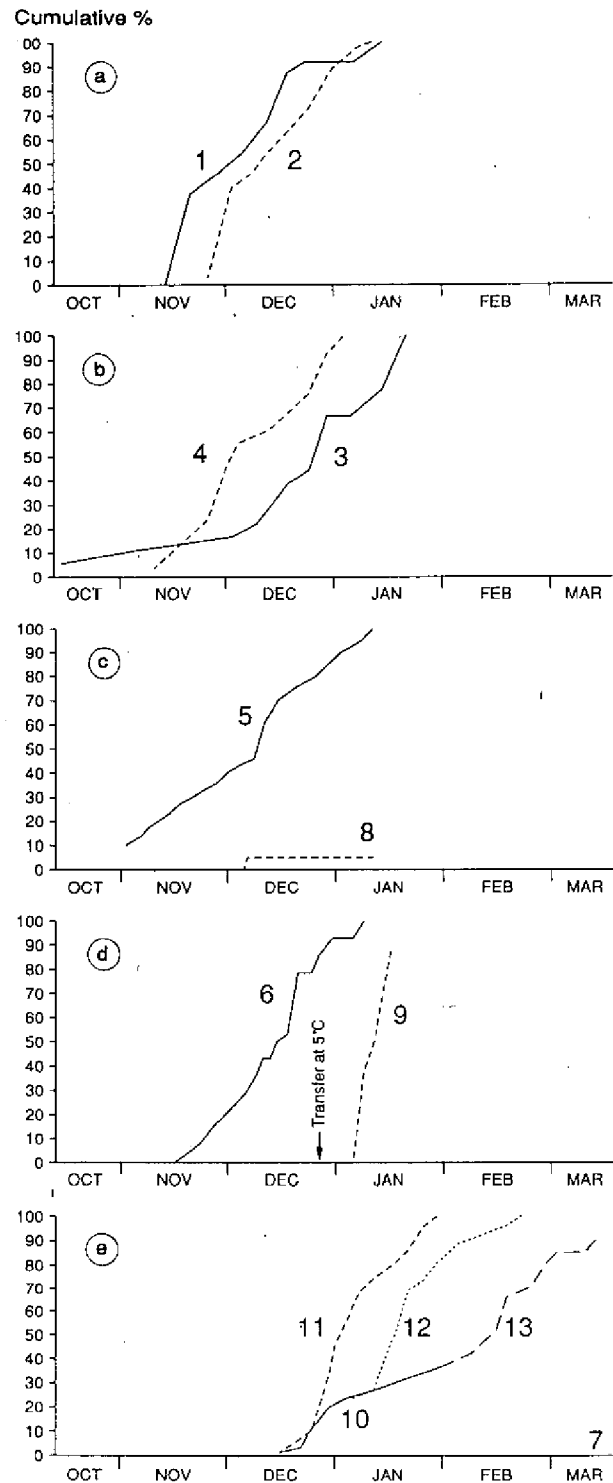
5. tafla. Áhrif hitastigs á hrognþroska að hausti. Fiskur alinn við 10°C og 5°C frá 1. september 1989 og slátrað 5. desember 1989.

	5°C	10°C
GSI	14.9±1.1	17.7±1.1
Mean weight of an oocyte, mg	25.6±1.7	38.0±1.9

In group 8 one female ovulated in early December. Fish kept at 8°C started to ovulate in mid December. Only 37% of the females had spawned at the end of January (group 10). The whole population ovulated within one month following the transfer into cold water at 5°C (groups 11 and 12). In group 13, only 80% of the females had ovulated by the end of the experiment in March. In group 10, 85% of the females ovulated within 15 days after transfer to 5°C on 29 December.

**Figure 4.** Profiles of cumulated percentages of ovulated females in different temperature treatments; (a) fish reared in water pumped from Lake Geneva (groups 1 and 2); (b) fish reared at 5°C from 15 July 1986 (group 3) and 15 September 1987 (group 4); (c) fish reared at 5°C (group 5) and at 10°C (group 8) from 1 September 1989; (d) fish reared to 5°C (group 6) and at 10°C (group 9) from 1 October 1990. On 29 December 1990, fish of group 9 were transferred to 5°C; (e) fish reared at 11°C (group 7) and 8°C (group 10) from early December until the ovulation (group 10), or transferred at 5°C on 25 December (group 11), 5 January (group 12) and 25 January (group 13).

4. mynd. Safnhlutfall hrygna sem höfðu losað hrogn frá október til mars við mismunandi eldshita; (a) vatn úr Genfarvatni 1985–1986 (hópur 1) og 1986–1987 (hópur 2); (b) eldshiti 5°C frá 15. júlí 1986 (hópur 3) og frá 15. september 1987 (hópur 4); (c) eldshiti 5°C (hópur 5) og 10°C (hópur 8) frá 1. september 1989; (d) eldshiti 5°C (hópur 6) og 10°C (hópur 9) frá 1. október 1990. Hópur 9 fluttur í 5°C 29. desember 1990; (e) eldshiti 11°C (hópur 7) og 8°C (hópur 10) frá desember 1987 til hrygningar eða flutt í 5°C 25. desember 1987 (hópur 11), 5. janúar 1988 (hópur 12) og 25. janúar 1988 (hópur 13).



**Table 6.** Ova mean weight and relative fecundity of Arctic charr (mean±SE). See Table 1 for details of the thermal regimes of the different experimental groups. Number of fish in brackets. *6. tafla. Meðalþungi hrognna og hlutfallsleg frjósemi (hrognafjöldi/kg) bleikjuhrygna (meðaltal og staðalskekka) eftir hitastigsmeðferð. Sjá lýsingu á tilraunahópum í 1. töflu. Fjöldi fiska í hóp innan sviga.*

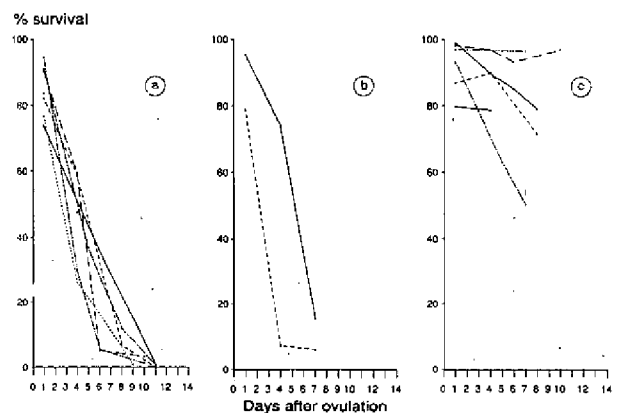
Expt. group	Weight of one ovum mg	Relative fecundity number of eggs/kg
Group 1 (25) (Lake Geneva water)	51.7±1.5	3886±231
Group 2 (37) (Lake Geneva water)	57.9±1.9	3525±153
Group 4 (21) (5°C)	46.9±2.0	4393±327
Group 5 (12) (5°C)	37.0±1.7	4583±153
Group 6 (35) (5°C)	39.0±0.9	4509±153
Group 10 (15) (8°C)		4093±304
Group 11 (19) (8→5°C)		4606±260
Group 12 (20) (8→5°C)		3927±195
Group 13 (10) (8→5°C)		3591±311
Group 14 (21) (Wild fish)	55.0±2.4	3581±281

*Quality of eggs (Tables 5 and 6).* The viability of eggs from females of groups 1 and 2, reared in Lake Geneva water, significantly increased ( $P<0.01$ ) between November and January in relation to the decrease of monthly water temperature. The viability of eggs from females checked twice a week (group 2), was significantly higher ( $P<0.01$ ) than that of females checked only once a week (group 1). Females kept at 5°C for several months produced ova of comparable viability to that of wild females (groups 3, 4, 5, 6 and 14). Relative fecundity did not change significantly with weight:  $r=0.09$  in group 1 and  $r=0.11$  in group 2. The relative fecundity of

reared females of experimental groups was comparable with that of wild fish. The mean ova weight of fish reared in cold water (groups 4, 5 and 6) was significantly lower ( $P<0.05$ ) than that of wild fish (group 14; Table 6).

Females kept at 8°C (group 10) produced eggs of lower ( $P<0.05$ ) quality than those of wild females (group 14). When fish were transferred into water at 5°C before ovulation (groups 11, 12 and 13), the quality of eggs was improved in comparison with group 10 ( $P<0.05$ ). But the viability of eggs decreased from group 11 to group 13 with the length of acclimatization at 8°C (groups 11, 12 and 13).

*Patterns of overripening of eggs (Figure 5).* The process of overripening for ova of group 2, at 8°C, was very rapid. It was the same for ova of wild females, kept at 8°C after capture. In most fish kept in cold water (5°C; group 4), the viability of ova was maintained at high and constant values for more than a week.

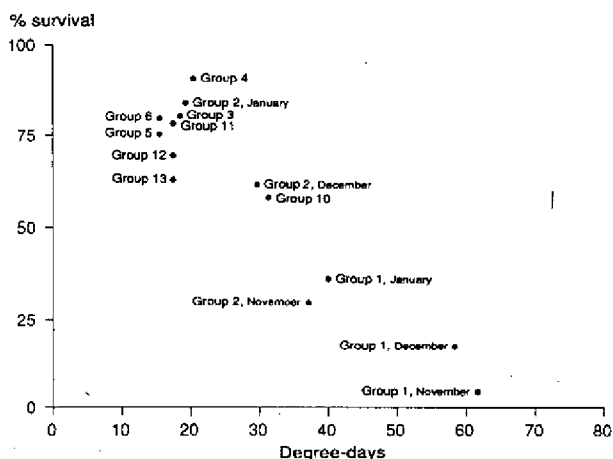


**Figure 5.** Effects of overripening on survival of eggs at eyed stage; (a) reared females of group 2, December 1987, 8°C; (b) wild females caught unovulated and kept at 8°C, December 1987; (c) reared females of group 4, December 1987, 5°C. Each curve represents data from one female: *5. mynd. Áhrif afþroskunar á hrognadauða fram að augnstigi; (a) hrygnur í eldi við 8°C (hópur 2, desember 1987); (b) villtar hrygnur, veiddar fyrir hrygningu og haldið við 8°C (desember 1987); (c) hrygnur í eldi við 5°C (hópur 4, desember 1987). Hver lína samsvarar einni hrygnu.*

Figure 6 summarizes the survival rates of eggs in all groups of reared females, except groups held at 10°C which did not ovulate. It can be observed that by increasing the number of degree-days between checks, the average egg quality decreased, except for groups 11, 12 and 13. The viability of eggs decreased from group 11 to 13 with the length of acclimatization at 8°C, though the number of degree-days between two checks remained constant.

### Effects of photoperiod

**Timing of ovulation (Figures 7, 8 and 9).** Fish which were maintained under long days from mid-summer started to ovulate in mid-January i.e. two months after the controls (group A). Continuous exposure to long days until spawning led to ovulations occurring over three months (group B) whereas termination of the long day regime in mid-December induced a synchronization of the ovulations within a few weeks (groups C, D, E and F). Fish reared under a shortened photoperiodic cycle started to ovulate at the

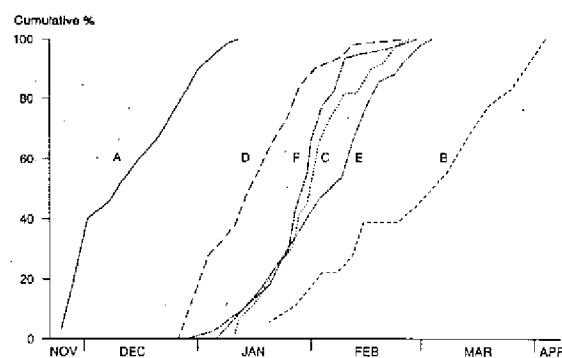


**Figure 6.** Mean egg survival at eyed stage for the different experimental groups in relation to the number of degree-days between sequential checks of females. See Table 1 for details of thermal regimes for the different groups.

6. mynd. Meðalhrognadauði fram að augnstigi í mismunandi tilraunahópum eftir fjölda gráðudaga milli athugana á hrygningarástandi. Sjá texta við 1. mynd um meðferð tilraunahópa.

end of August (group G) or in the beginning of October (group H). In both the groups, ovulations occurred within one month. Immature fish (30 month old) subjected to long days in winter (group I) started to ovulate at the end of May i.e. 6 months before the controls. Ovulations occurred over 5 months. Subsequently, these fish ovulated again 8 months later, at the end of winter.

**Quality of eggs (Table 7).** The survival rates of eggs in all the experimental groups did not differ from wild females. The mean weight of the ova were significantly ( $P < 0.05$ ) lower in females of the groups G and I than in



**Figure 7.** Profiles of cumulated percentages of ovulated females. A: Fish reared under natural photoperiod winter 1986–87. B: Fish exposed to long days (17L/7D) from 16 August 1986 to spawning. C: Fish exposed to long days (17L/7D) from 16 August 1987 to 15 December 1987. D: Fish exposed to long days (17L/7D) from 21 June 1988 to 15 December 1988. E: Fish exposed to long days (17L/7D) from 16 August 1988 to 15 December 1988. F: Fish exposed to long days (17L/7D) from 16 August 1989 to 15 December 1989.

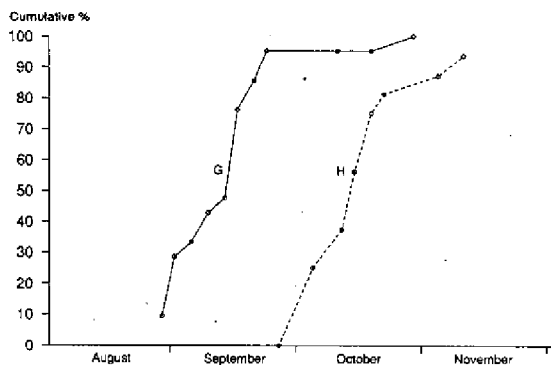
7. mynd. Safnhlutfall hrygna sem höfðu losað hrogn frá nóvember til apríl við mismunandi ljóslotumeðferð. A: Náttúruleg daglengd 1986–1987. B: Langur dagur (17 klst. dagur/7 klst. nótt) frá 16. ágúst 1987 til hrygningar. C: Langur dagur frá 16. ágúst 1987 til 15. desember 1987. D: Langur dagur frá 21. júní 1988 til 15. desember 1988. E: Langur dagur frá 16. ágúst 1988 til 15. desember 1988. F: Langur dagur frá 16. ágúst 1989 til 15. desember 1989.

**Table 7.** Survival rates of eggs at eyed stage, relative fecundity and ova mean (mean±SE) weight in the different groups of Arctic charr. See Table 2 for details of photoperiodic regimes for the different groups.

7. tafla. Hlutfall (%) lifandi hrognna á augnstigi, hlutfallsleg frjósemi (hrognafjöldi/kg) og meðalþyngd hrognna (meðalal og staðalskekkja) eftir ljóslotumeðferð á bleikjuhrygnum. Sjá lýsingu á tilraunahópum í 2. töflu.

Expt. group	Survival rate of eggs	Relative fecundity, number of eggs/kg	Weight of one ovum, mg
A		3525±153	57.9±1.9
C	67.49±4.02	2993±100	53.64±1.72
D	72.18±3.30	3162±123	51.79±1.96
F	62.96±3.38	3040±98	56.96±1.67
G	73.39±4.17	5563±256	39.7±1.28
H	79.42±3.78	3788±285	50.56±2.85
I (First spawning)	71.18±3.83	3990±183	44.02±1.84
I (Second spawning)	92.71±3.53	3382±359	46.67±2.67

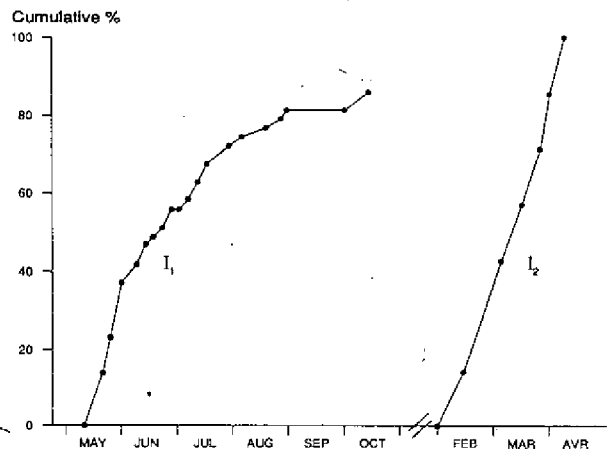
controls (A) and in wild females. Relative fecundity was significantly ( $P<0.05$ ) higher in females of the group G than in controls (A) and in wild females.



**Figure 8.** Profiles of cumulated percentages of ovulated females. Fish exposed to a shortened photoperiodic cycle. G: 17L/7D from 4 January 1990 to 30 June 1990 then 7L/17D from 1 July to spawning. H: 17L/7D from 1 April 1988 to 30 June 1988 then 7L/17D from 1 July to spawning. 8. mynd. Safnhlutfall hrygna sem losuðu hrogn frá ágúst til nóvember. Tilraunahópar voru aldrei við stutta daglengd. G: Langur dagur (17 klst. dagur/7 klst. nótt) frá 4. janúar 1990 til 30. júní 1990 og stuttur dagur (7 klst. dagur/17 klst. nótt) frá 1. júlí til hrygningar. H: Langur dagur frá 1. apríl 1988 til 30. júní 1988 og stuttur dagur (7 klst. dagur/17 klst. nótt) frá 1. júlí til hrygningar.

#### Induction of spawning at 5 and 10°C

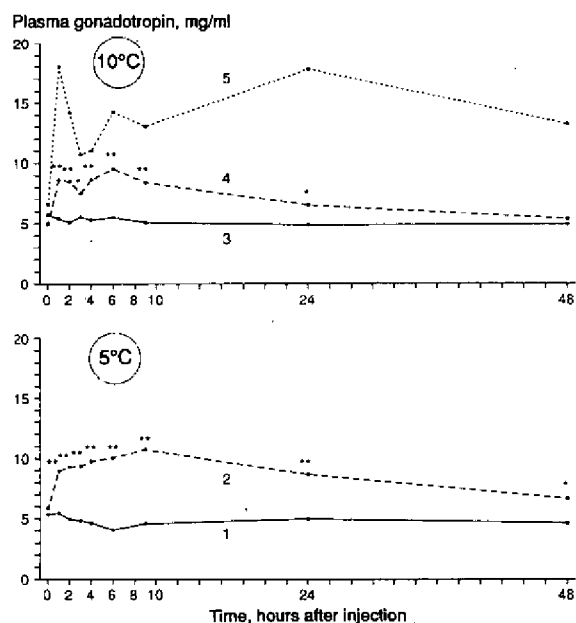
Plasma gonadotropin and cAMP content of oocyte (Figures 10, 11 and 12). 1989. At 5°C and 10°C, treatments with D-Trp<sup>6</sup>LH-



**Figure 9.** Profiles of cumulated percentages of ovulated females. I<sub>1</sub>: Fish exposed to long days (17L/7D) from 1 October 1988 to 15 May 1989 then 7L/17D from 16 May 1989 to spawning. I<sub>2</sub>: Second spawning of the same fish, kept in natural photoperiod condition after first spawning in May and June.

9. mynd. Safnhlutfall hrygna sem losuðu hrogn í tilraunahóp I. Langur dagur (17 klst. dagur/7 klst. nótt) frá 1. október 1988 til 15. maí 1989 og síðan stuttur dagur (7 klst. dagur/17 klst. nótt) til hrygningar. Náttúruleg ljóslota eftir 1. hrygningu. 1: Fyrsta hrygning. 2: Önnur hrygning.

RHa induced a significant increase ( $P<0.05$ ) in plasma gonadotropin levels. The increments became significant as early as one hour after injection ( $9.32\pm 2.18$  vs  $5.42\pm 1.23$  ng/ml at  $5^{\circ}\text{C}$  and  $8.58\pm 3.35$  vs  $5.36\pm 1.97$  ng/ml at  $10^{\circ}\text{C}$ ). Maximum gonadotropin levels were observed 6 hours after injection at  $10^{\circ}\text{C}$  and 9 hours after injection at  $5^{\circ}\text{C}$ . At  $5^{\circ}\text{C}$ , plasma gonadotropin levels remained elevated for at least 24 hours whereas at  $10^{\circ}\text{C}$  plasma gonadotropin levels decreased more rapidly between 9 and 24 hours. Plasma gonadotropin was markedly elevated in one control female which ovulated spontaneously on 8 December.

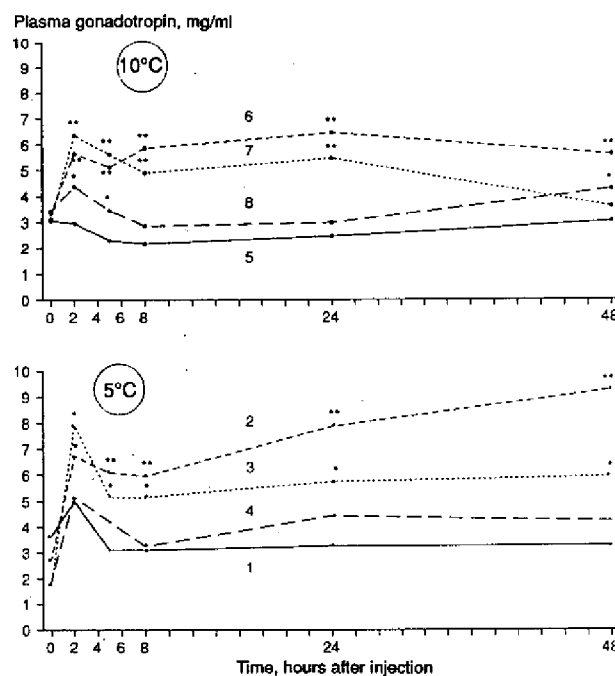


**Figure 10.** Plasma gonadotropin levels in Arctic charr in 1989. 1: Control females at  $5^{\circ}\text{C}$ . 2: Females injected with  $30\ \mu\text{g}/\text{kg}$  body weight of D-Trp<sup>6</sup>LH-RHa at  $5^{\circ}\text{C}$ . 3: Control females at  $10^{\circ}\text{C}$ . 4: Females injected with  $30\ \mu\text{g}/\text{kg}$  body weight of D-Trp<sup>6</sup>LH-RHa at  $10^{\circ}\text{C}$ . 5: Individual female at  $10^{\circ}\text{C}$  in control which has ovulated on 8 December 1989 (án hormónagjafar). \* $P<0.05$  (control/treated fish); \*\* $P<0.01$ .

10. mynd. Yfirkynehormón í blóðvökva úr bleikju 1989 eftir hormónagjöf og hitastigi. 1 og 3: Viðmiðunarahópar. 2 og 4:  $30\ \mu\text{g}/\text{kg}$  D-Trp<sup>6</sup>LH-RHa. 5: Ein hrygna í viðmiðunarahóp sem losaði hrogn 8. desember 1989 (án hormónagjafar). \* $P<0,05$  (viðmiðun/hormónamedferð); \*\* $P<0,01$ .

High levels of cAMP were observed in the oocytes of the females reared at  $10^{\circ}\text{C}$  in autumn. At  $5^{\circ}\text{C}$ , cAMP levels were markedly lower ( $P<0.001$ ).

1990. At  $5^{\circ}\text{C}$ , treatments with D-Arg<sup>6</sup>s-GnRHa and D-Ala<sup>6</sup>LH-RHa induced an increase of plasma gonadotropin levels ( $9.2\pm 2.5$  and  $7.9\pm 2.5$  respectively vs  $3.2\pm 1.6$  ng/ml in controls;  $P<0.05$ ) which was maintained for

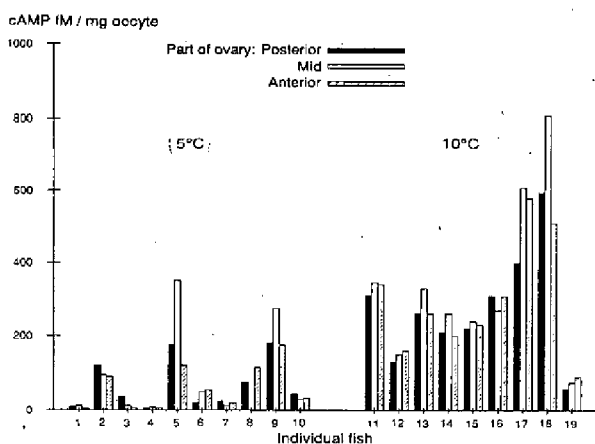


**Figure 11.** Plasma gonadotropin levels in Arctic charr in 1990. 1: Control females at  $5^{\circ}\text{C}$ . 2: Females injected with  $20\ \mu\text{g}/\text{kg}$  body weight of D-Arg<sup>6</sup>sGnRHa at  $5^{\circ}\text{C}$ . 3: Females injected with  $20\ \mu\text{g}/\text{kg}$  body weight of D-Ala<sup>6</sup>LH-RHa at  $5^{\circ}\text{C}$ . 4: Females injected with  $20\ \mu\text{g}/\text{kg}$  body weight of D-Trp<sup>6</sup>LH-RHa (slow release form) at  $5^{\circ}\text{C}$ . 5: Control females at  $10^{\circ}\text{C}$ . 6: Females injected with  $20\ \mu\text{g}/\text{kg}$  body weight of D-Arg<sup>6</sup>sGnRHa at  $10^{\circ}\text{C}$ . 7: Females injected with  $20\ \mu\text{g}/\text{kg}$  body weight of D-Trp<sup>6</sup>LH-RHa at  $10^{\circ}\text{C}$ . 8: Females injected with  $20\ \mu\text{g}/\text{kg}$  body weight of D-Trp<sup>6</sup>LH-RHa (slow release form) at  $10^{\circ}\text{C}$ . \* $P<0.05$  (control/treated fish); \*\* $P<0.01$ .

11. mynd. Yfirkynehormón í blóðvökva bleikju 1990 eftir hormónagjöf og hitastigi. 1 og 5: Viðmiðunarahópar. 2 og 6:  $20\ \mu\text{g}/\text{kg}$  D-Arg<sup>6</sup>sGnRHa. 3:  $20\ \mu\text{g}/\text{kg}$  D-Ala<sup>6</sup>LH-RHa. 4 og 7:  $20\ \mu\text{g}/\text{kg}$  D-Trp<sup>6</sup>LH-RHa. 8:  $20\ \mu\text{g}/\text{kg}$  D-Trp<sup>6</sup>LH-RHa (torleyst). \* $P<0,05$  (viðmiðun/hormónamedferð); \*\* $P<0,01$ .

48 hours whereas plasma gonadotropin of fish which were injected with D-Trp<sup>6</sup>LH-RHa (slow release form) did not differ from the controls. Maximum gonadotropin levels were observed 2 hours after injection of D-Ala<sup>6</sup>LH-RHa and 48 hours after injection of D-Arg<sup>6</sup>s-GnRHa. At 10°C, plasma gonadotropin was raised in the treated fish, except in the fish treated with the slow release form of D-Trp<sup>6</sup>LH-RHa. Maximum gonadotropin levels were observed 2 hours after injection of D-Trp<sup>6</sup>LH-RHa (6.3±1.5 ng/ml) and 24 hours after injection of D-Arg<sup>6</sup>s-GnRH (6.4±1.5 ng/ml). Forty-eight hours after D-Arg<sup>6</sup>sGnRHa injections, plasma gonadotropin levels were significantly lower ( $P<0.05$ ) at 10°C than at 5°C (5.5±1.7 vs 9.2±2.5 ng/ml).

*Effects of GnRH analogues on ovulation timing (Figures 13 and 14). 1989.* At 5°C, 100% of the fish treated with D-Trp<sup>6</sup>LH-RHa (slow release form) ovulated within 21 days, compared to only 75% of the fish over 38 days in the controls. An acute injection of D-Trp<sup>6</sup>LH-RHa (free form 30 µg/kg) induced a rapid increase in the rate of ovulation: 60% of the fish ovulated within 14 days. Thereafter, the rate of ovulation increased slowly. It reached

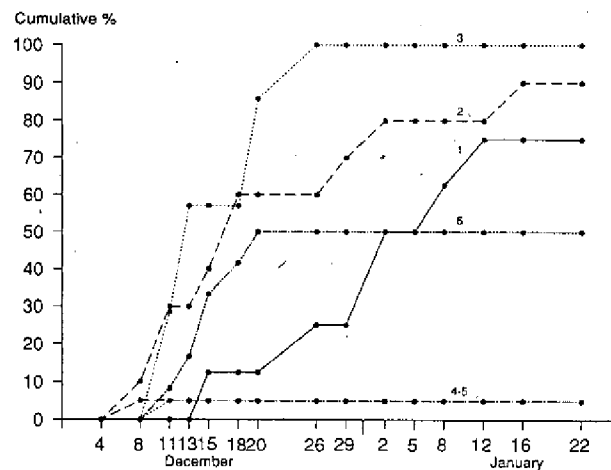


**Figure 12.** cAMP content in oocyte of Arctic charr reared at 5 and 10°C for two months. Fish were slaughtered on 3 December.

12. mynd. cAMP í hrognum bleikjuhrygna sem aldar voru við 5 og 10°C í tvo mánuði. Slátrað 5. desember.

90% at the end of the experiment. At 10°C, one female ovulated in the control. The same results were obtained in fish treated with D-Trp<sup>6</sup>LH-RHa (free-form) whereas 50% of the fish treated with D-Trp<sup>6</sup>LH-RHa (slow release form) ovulated within 15 days. Thereafter the rate of ovulation did not increase in the latter group.

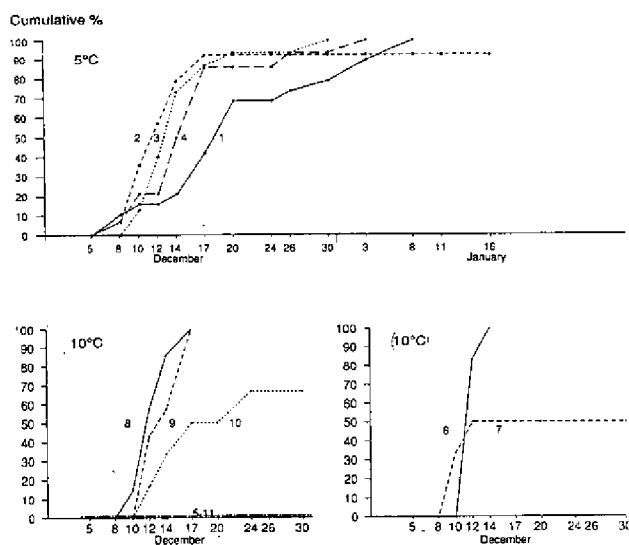
**1990.** Ovulation was markedly advanced in all the groups injected with GnRHa at 5°C. An injection of D-Arg<sup>6</sup>sGnRHa (20 µg/kg) induced a rapid increase in the rate of ovulation: 90% of the females ovulated within 9 days at 5°C. The rate of ovulation reached 50% within 7 days at 10°C in fish injected with D-Arg<sup>6</sup>sGnRHa (20 µg/kg) whereas it reached 100% within 9 days in fish injected with 60 µg/kg.



**Figure 13.** Profile of cumulated percentages of ovulated females in 1989/1990. 1: Control females at 5°C. 2: Females injected with 30 µg/kg of D-Trp<sup>6</sup>LH-RHa at 5°C. 3: Females injected with 20 µg/kg of D-Trp<sup>6</sup>LH-RHa (slow release form) at 5°C. 4: Control female at 10°C. 5: Females injected with 30 µg/kg of D-Trp<sup>6</sup>LH-RHa at 10°C. 6: Females injected with 20 µg/kg of D-Trp<sup>6</sup>LH-RHa (slow release form) at 10°C.

13. mynd. Safnhlutfall hrygna sem losuðu hrogn 1989–1990 eftir hormónameðferð og hitastigi. 1: Viðmiðunarahópur við 5°C. 2: 30 µg/kg D-Trp<sup>6</sup>LH-RHa við 5°C. 3: 20 µg/kg D-Trp<sup>6</sup>LH-RHa (torleyst) við 5°C. 4: Viðmiðunarahópur við 10°C. 5: 30 µg/kg of D-Trp<sup>6</sup>LH-RHa við 10°C. 6: 20 µg/kg of D-Trp<sup>6</sup>LH-RHa (torleyst) við 10°C.

Hundred percent of fish injected with D-Trp<sup>6</sup>LH-RHa (free-form) ovulated at 5°C whereas the same treatment failed to induced ovulation at 10°C. The slow release form of D-Trp<sup>6</sup>LH-RHa induced a 100% rate of ovulation within 12 days at the latter temperature



**Figure 14.** Profile of cumulated percentages of ovulated females in 1990/1991. 1: Control females at 5°C. 2: Females injected with 20 µg/kg of D-Arg<sup>6</sup>sGnRH at 5°C. 3: Females injected with 20 µg/kg of D-Trp<sup>6</sup>LH-RHa (slow release form) at 5°C. 4: Females injected with 20 µg/kg of D-Ala<sup>6</sup>LH-RHa at 5°C. 5: Control females at 10°C. 6: Females injected with 60 µg/kg of D-Arg<sup>6</sup>sGnRH at 10°C. 7: Females injected with 30 µg/kg of D-Arg<sup>6</sup>sGnRH at 10°C. 8: Females injected with 60 µg/kg of D-Trp<sup>6</sup>LH-RHa (slow release form) at 10°C. 9: Females injected with 30 µg/kg of D-Trp<sup>6</sup>LH-RHa (slow release form) at 10°C. 10: Females injected with 20 µg/kg of D-Trp<sup>6</sup>LH-RHa (slow release form) at 10°C. 11: Females injected with 20 µg/kg of D-Trp<sup>6</sup>LH-RHa at 10°C.

14. mynd. Safnhlutfall hrygna sem losuðu hrogn 1990–1991 eftir hormónameðferð og hitastigi. 1 og 5: Viðmiðunarhópar. 2: 20 µg/kg D-Arg<sup>6</sup>sGnRH við 5°C. 3 og 10: 20 µg/kg D-Trp<sup>6</sup>LH-RHa (torleyst) við 5°C. 4: 20 µg/kg D-Ala<sup>6</sup>LH-RHa við 5°C. 6: 60 µg/kg D-Arg<sup>6</sup>sGnRH við 10°C. 7: 30 µg/kg D-Arg<sup>6</sup>sGnRH við 10°C. 8: 60 µg/kg D-Trp<sup>6</sup>LH-RHa (torleyst) við 10°C. 9: 30 µg/kg D-Trp<sup>6</sup>LH-RHa (torleyst) við 10°C. 10: 20 µg/kg D-Trp<sup>6</sup>LH-RHa (torleyst) við 10°C. 11: 20 µg/kg D-Trp<sup>6</sup>LH-RHa við 10°C.

in fish treated with 60 or 30 µg/kg whereas only a 67% rate of ovulation was recorded within 21 days in fish treated with 20 µg/kg. The rate of ovulation reached 100% within 35 days in the controls at 5°C whereas no ovulation was recorded in the controls at 10°C.

*Effects of spawning induction on the viability of eggs (Table 8).* The data for egg quality are recorded in Table 8. Egg survival of the fish reared at 5°C was around 75% in both the treated fish and the controls, except in the fish treated with 20 µg/kg of D-Trp<sup>6</sup>LH-RHa in 1990 (52%). At 10°C, egg survival exhibited a wide range of variation, and the viability was lower than 50% in four groups out of six.

## DISCUSSION

### *Effects of temperature on Arctic charr reproduction*

*Timing of spawning.* The ovulation of Arctic charr reared in tanks supplied with water pumped from 36 m in Lake Geneva occurred spontaneously at the same time as for the wild spawners in the same lake. Spawning in the lake occurs at depths of 60–80 m (Dussart, 1952). Differences in hydrostatic pressure and conditions of captivity did not appear to alter this phenomenon significantly.

A temperature threshold lower than 11°C appeared necessary to trigger a spontaneous ovulation. This result has never been described in other salmonid species to the authors knowledge. This phenomenon is probably linked to the Arctic origin of the charr which is the only freshwater fish able to colonize the lakes located in high latitudes (Hammar, 1989). Fish kept at 8°C (group 10) exhibited a delay in ovulation compared with fish acclimatized at 5°C (groups 3, 4, 5 and 6). Subsequent transfer of fish from 8 to 5°C stimulated and synchronized the ovulations (groups 11, 12 and 13). The same result was obtained when the fish were transferred from 10 to 5°C (group 9) at the end of



**Table 8.** Effects of GnRH induction of spawning on egg viability (survival at eyed stage, mean±SE).  
8. tafla. Áhrif hormónagjafa á hlutfall (%) lifandi bleikjuhroga á augnstigi (meðaltal og staðalskekkja).

Treatment	Temperature	
	5°C	10°C
<b>1989–1990</b>		
Control	71.2±8.8	5.0 (1 fish)
D-Trp <sup>6</sup> LH-RH, 30 µg/kg	72.6±7.2	No ovulation
D-Trp <sup>6</sup> LH-RH <sup>a</sup> , 30 µg/kg	76.7±7.6	50.3±13.3
<b>1990–1991</b>		
Control	76.4±6.8	No ovulation
D-Ala <sup>6</sup> LH-RH, 20 µg/kg	76.5±5.6	
D-Arg <sup>6</sup> sGnRH, 20 µg/kg	73.0±3.6	39.8±20.2
D-Arg <sup>6</sup> sGnRH, 60 µg/kg		40.6±12.6
D-Trp <sup>6</sup> LH-RH <sup>a</sup> , 20 µg/kg	51.68±13.8	34.6±8.2
D-Trp <sup>6</sup> LH-RH <sup>a</sup> , 30 µg/kg	81.0±7.1	71.8±6.2
D-Trp <sup>6</sup> LH-RH <sup>a</sup> , 60 µg/kg		36.2±12.4

a) Micro encapsulated slow release form.

December. Thus, it appears that a cold shock markedly synchronizes ovulations in Arctic charr. Requirements for cold water during spawning could be linked to temperature thresholds required for development of embryos. Indeed Arctic charr embryos require water temperatures lower than 8°C (Jungwirth and Winkler, 1984). Low temperature requirements are likely to be related to the phase of spawning only, since fish acclimatized at 5°C from the beginning of summer (group 3) did not ovulate earlier than fish transferred to water at the same temperature at the end of summer (groups 4, 5 and 6).

*Viability of eggs.* The poor viability of eggs produced by reared Arctic charr spawners has already been reported (Paspt and Hopky, 1984; Davaine, personal communication). In all cases, spawners were kept at temperature higher than 6°C during reproduction. In groups 1 and 2 reared in water pumped from 36 m the viability of ova became poorer with higher mean temperatures, whereas the viability of eggs produced by fish reared at 8°C (group 10) remained low until January. Moreover, the viability of ova from reared spawners kept at 5°C for more than a month was quite comparable to that of wild females in the

lake. These results highlight the importance of temperature in controlling the quality of eggs. Our results are in agreement with those of Krieger and Olson (1988) who showed that spawning of Arctic charr of the Nauyuk Lake strain (Canadian arctic area) was more successful amongst broodstock that had been raised in cold (6.5°C) rather than warm water (8–17°C). Wild fish in Lake Geneva, which spawn below the thermocline, are usually found in water close to 5°C. In mountain lakes and in northern regions, Arctic charr may spawn in shallow water (Johnson, 1980). It is likely that such differences in behaviour are linked to the cooling of surface waters during autumn.

One effect produced by high temperatures was to accelerate the ageing processes or overripening of the ova. The decrease in ova viability was slower at 5°C than at higher temperatures. The same phenomenon has already been described for two other salmonid species, the rainbow trout and the brown trout (Billard and Gillet, 1981). However, for the rainbow trout, the acceleration of the ageing processes was only observed above 10°C. In trout, ova at 10°C high fertilization rates are maintained for 10 days after ovulation, after where ova viability decreased

slowly over the following 8 to 10 days (Escaffre *et al.*, 1977; Springate *et al.*, 1984). In Arctic charr, this pattern was only observed at 5°C. At higher temperatures, the viability of ova decreased after only 3 days. It is likely that the accelerated rate of ageing of ova at temperatures higher than 5°C was linked to the delay observed in the timing of ovulation of Arctic charr exposed to the same temperatures during spawning. In another species, *Cyprinus carpio* L., Sjafei, 1985 has shown that temperature disturbances during the late phases of maturation, both delayed ovulation and shortened the phase of maximum viability, as in Arctic charr at 8°C.

Females of groups 4, 5 and 6, kept at 5°C from the beginning of autumn had smaller ova than wild fish or fish of group 1. These fish had completed the major part of their gametogenesis in water at 8°C or above. In summer and in the beginning of autumn, growth and gametogenesis of Arctic charr in natural environment take place at temperatures higher than 5°C. In rainbow trout, Breton and Billard (1977) reported favourable effects of warm temperatures on gametogenesis. However, Arctic charr in which spawning was delayed, due to their acclimation to 8°C, produced eggs of good quality when they were transferred into cold water (5°C) on 15 December, one or two weeks prior to ovulation (group 11). When the transfer of unovulated females occurred later, in January (groups 12 and 13), the viability of ova tended to decrease. Further investigations would be required to determine what is the optimal decrease of water temperature in autumn for Arctic charr spawning.

#### *Effect of photoperiod on Arctic charr reproduction*

Arctic charr kept under a normal thermoperiodic regime and subjected to long days (17L/7D) from mid-summer showed a delay in spawning. The mid-period of ovulation occurred about six weeks after that of the

control group kept under normal photoperiod. This result is in agreement with those for other salmonid species: rainbow trout (Bourlier and Billard, 1984; Bromage *et al.*, 1984), brook trout (Henderson, 1963), masu salmon (Takashima and Yamada, 1984). Ovulation occurred over a period of 2.5 months when the fish were subjected to long days until the spawning time. In contrast, termination of the long day regime in December induced a synchronization of the ovulations over just one month. According to Bromage *et al.* (1984) seasonally-changing cues, i.e. long days then short days appear to be necessary to synchronize ovulation in rainbow trout. As far as Arctic charr is concerned the short days may act only a few weeks prior to the onset of the ovulation.

When the beginning of the long day regime was imposed at the summer solstice, fish (group D) did not spawn later than fish in groups subjected to long days from 15 August. Long days at the beginning of summer did thus not seem to affect gametogenesis.

A shortened photoperiodic cycle induced an advancement in spawning by two months, as in other salmonid fish that have been investigated so far. This result could be explained by the stimulatory effects of long days on the onset of gametogenesis and of short days on the completion of gametogenesis as this has been reported in rainbow trout (Bromage *et al.*, 1984). Thus the influence of daylength over the initiation and modulation of the reproductive development in Arctic charr appears similar to that in rainbow trout (see Bromage and Duston, 1986 for more detailed discussion).

Two year old immature fish subjected to long days in winter showed an advancement in spawning of six months. Skarphédinsson *et al.* (1985) reported that long photoperiods also enhanced early sexual development in rainbow trout. Moreover, McCormick and J. Naiman (1984) reported that size was an important determinant of maturation in brook

trout, a species close to Arctic charr. One may hypothesize that the Arctic charr (group I) had not reached the necessary physiological condition required to trigger the gonadal development during their second spring. Subsequently, during their second autumn, a large proportion of the fish were able to start reproductive development, provided that they were subjected to a long day photoperiod regime. Normally, long days do not occur before the following summer. When the fish were subjected to long days in October, the first ovulations were recorded 8 months later, i.e. in May. Subsequently ovulations occurred over a period of 5 months. One may hypothesize that females which spawned in summer and autumn were not receptive to long days in October. Subsequently, they became receptive in winter or early spring. After spawning, the spent females were transferred to an outside tank, under natural photoperiod in June. At this time, fish were subjected to long days which induced the initiation of a new ovarian development. The following reproductive cycle lasted 8 months, as in the case of a shortened photoperiodic cycle.

The viability of eggs produced by the delayed females did not differ from that of wild females. The possibility of delaying spawning by 1.5 months allowed the ovulation to occur under a more favorable temperature range (5–6°C) than is usual in later autumn (7–8°C). When the water was cooled to 5°C, the viability of eggs produced by reared females did not differ from that of wild females, irrespective of the season. However, advanced females (groups G, H, I) produced smaller and greater numbers of eggs than wild fish (group 14) or females under natural photoperiod (group A). One may hypothesize that the weight of fish increases at a slower rate during a shortened reproductive cycle than in control. In brown trout, a slow growth rate leads to the production of smaller and more numerous eggs compared to normally growing fish (Billard and De Fremont, 1980).

#### *Induction of spawning at 5 and 10°C*

The lack of spontaneous ovulation at 10°C in Arctic charr could be explained by several hypotheses: Either there is no activation of the hypothalamo-pituitary axis to induce a surge in gonadotropin secretion, or the ovary is not responsive to gonadotropin stimulation. Plasma gonadotropin level did not differ in controls maintained at 5 and 10°C. Although the responses differed slightly, injection of GnRHa significantly increased the gonadotropin secretion at both temperatures. The effectiveness of GnRHa injection in stimulating the gonadotropin secretion has been reported for other salmonids: rainbow trout (Weill *et al.*, 1978), brown trout (Crim *et al.*, 1983) and Atlantic salmon (Crim and Glebe, 1984). In Arctic charr reared at 10°C, the stimulatory action of a given GnRHa appeared lower and less prolonged than at 5°C. Thus the blockade of the pituitary responsiveness to GnRH is apparently not the reason of the lack of spontaneous ovulation. Another possibility not studied in this work could be a lack of GnRH release either from preoptic GnRH neurosecretory cells or from pituitary GnRH endings as demonstrated in the brown trout (Breton *et al.*, 1986) and the roach (Breton *et al.*, 1988). The slow release form of D-Trp<sup>6</sup>LH-RHa did not induce an increase of plasma gonadotropin levels, this is in contrast with its ability to induce ovulation. Possibly the slow release of GnRH from the polylactic polyglycolic matrix, induced a discrete but continuous and prolonged increase in gonadotropin secretion at both 10 and 5°C leading to ovulation in both cases.

In the rainbow trout, there is a negative correlation between oocyte cAMP concentration and the median effective dose of 17 $\alpha$ -hydroxy-20 $\beta$ -dihydroprogesterone for induction of GVBD (germinal vesicle breakdown, i.e. resumption of meiosis and final oocyte maturation) (Jalabert and Finet, 1986). Thus in the present work the high cAMP oocyte concentrations at 10°C may have blocked the action of the maturation inducing ster-

oid, whose production would need a prolonged gonadotropin stimulation. Consequently ovulation is induced at this temperature only by the slow release form of D-Trp<sup>6</sup>LH-RHa and GnRHa whose action was prolonged for at least 48 hours. These results would also mean that the decrease in oocyte cAMP levels is a preliminary condition to hormone action for inducing maturation. Whether this decrease is linked to an action of gonadotropin or 17 $\alpha$ -hydroxy-20 $\beta$ -dihydroprogesterone can not be concluded from these studies.

At 5°C, all treatments with GnRHa induced a synchronization of ovulation compared to the control group, as has been reported in Atlantic salmon (Crim and Glebe, 1984), coho salmon (Sower *et al.*, 1984) and rainbow and brown trout (Billard *et al.*, 1984). Treatment with D-Arg<sup>6</sup>sGnRH was the most effective method of synchronizing ovulation. Over 80% of the treated fish spawned within four days. This was obviously related to the increased efficiency of D-Arg<sup>6</sup>sGnRH in stimulating gonadotropin secretion than all the other GnRHa which were tested.

The viability of eggs did not differ between experimental groups and controls at 5°C. At 10°C, in spite of an examination of females twice a week to prevent overripening, many egg batches were of poor quality. Low viability of eggs has been reported in Atlantic salmon when ovulation was induced by LH-RHa treatment in September (Crim and Glebe, 1984). These authors suggested that LH-RHa treatment was carried out at a too early stage of reproductive development. One may hypothesize that the same constraints may apply to the Arctic charr females reared at 10°C. Moreover, the short duration of the increased levels of plasma gonadotropin at 10°C after LH-RHa injection could affect the viability of eggs. In trout, plasma gonadotropin begins to rise a few days prior to ovulation and remains high until removal of the eggs (Billard *et al.*, 1978; Fostier *et al.*, 1978; Breton *et al.*,

1983). Further studies would be required to determine the range of temperature in which treatment with GnRHa could be undertaken and to determine the role of high temperatures on the decrease of egg viability.

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