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Egg production in an Arctic charr (*Salvelinus alpinus* L.) brood stock: effects of temperature on the timing of spawning and the quality of eggs

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Abstract

Development of a broodstock of Arctic charr was undertaken in experimental tanks supplied with water pumped from Lake Léman (Geneva) at a depth of 36 metres. Spawners were subjected to different thermal regimes to determine the effects of temperature on spawning time and gamete viability. In the tanks, the ovulation occurred spontaneously at the same time as the 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 reared females was poorer than those of wild fish because the process of overripening was very rapid above 5°C. Provided that spawners were reared at 5°C and examined twice a week to detect ovulation, 80 to 90 percent survival to the eyed stage could be expected for eggs of reared females. 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%).

Keywords : Arctic charr, reproduction, temperature, fertilization, overripening.

Production d'œufs par des géniteurs d'omble chevalier (Salvelinus alpinus *L.*) *en élevage : effets de la température sur la date de fraie et sur la qualité des œufs.*

Résumé

L'élevage de géniteurs d'omble chevalier a été réalisé dans une installation expérimentale recevant de l'eau pompée à 36 mètres de profondeur dans le lac Léman. Différents régimes thermiques ont été expérimenté sur les femelles. Les effets de la température sur la date de ponte et sur la qualité des gamètes ont été évalués. En bassin, l'ovulation se produit spontanément aux dates de pontes habituelles des poissons sauvages du lac. Le rythme des ovulations est ralenti par des températures égales ou supérieurs à 8°C. A partir de 11°C, les ovulations paraissent fortement inhibées. Lorsque la température de l'eau des bassins est supérieure à 5°C au cours des dernières semaines précédant l'ovulation, la qualité des œufs des femelles d'élevage est inférieure à celle des poissons sauvages car la surmaturation des ovules est très rapide au-dessus de 5°C. Dans la mesure où les géniteurs sont élevés à 5°C et examinés deux fois par semaine pour détecter les ovulations, le taux de survie des œufs peut avoisiner 80 à 90 %. Le pourcentage de fécondation des œufs des géniteurs transferrés de 8 à 5°C en décembre est élevé (78 %) mais lorsque le transfert intervient en janvier, la fécondabilité des ovules a tendance à diminuer (63 %).

Mots-clés : Omble chevalier, reproduction, température, fécondation, surmaturation.

INTRODUCTION

The establishment of hatchery broodstocks of Arctic charr would allow the intensification of restocking and the development of aquaculture of a species which has a great potential, as indicated by Gjedrem and Gunnes (1978) and Paspt and Hopky (1983). However, the latter authors have experienced difficulties in obtaining eggs of good quality from reared Arctic charr (Paspt and Hopky, 1984).

We have analysed the performance of a broodstock of Arctic charr of the strain of Lake Geneva. 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 and 1984). But 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 it spawns at the beginning of winter. Consequently thermal requirements are likely to be very low for maturation and spawning in this species. In this experiment, several temperature regimes were investigated for maturation and spawning in reared Arctic charr.

MATERIALS AND METHODS

Source of fish stock

The Arctic charr broodfish originated from eggs collected from wild fish in Lake Geneva. Wild spawners were caught with gillnets layed around spawning grounds. Several successive generations were reared from these eggs at the INRA experimental station in Thonon-les-Bains. Each cohort resulted from mixing the gametes of at least five females and ten males. Each year, 1 000 juveniles were randomly selected. They were reared for three years until they became mature.

Description of rearing procedure

Arctic charr spawners were kept in 4 or 12 m^3 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 (*fig.* 1). A refrigeration system was used to maintain water temperature at 5 ± 1°C in one tank, all year round. Fish were fed with dry pellets (trout commercial food, "Trouvit", protein 42%, lipid 12%) distributed for eight hours daily with automatic feeders. Fish were fed at a ration recommended for rainbow trout. Experimental fish of groups 1 to 9 were randomly selected from the three-year-old spawners (500 fish). Fish weighed between 400 and 1 000 g.

Rearing of spawners under different experimental temperature regimes (table 1)

1) Eighty fish were kept in the water pumped from a depth of 36 metres in 1985-1986 (group 1) and in 1986-1987 (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 July 15th 1986 (group 3) and on September 15th 1987 (group 4).

3) Spring water of constant temperature (11 ± 1°C) was used to raise the water temperature of some tanks in winter:

- In December 1987, the water temperature of one tank was maintained at 8°C by mixing spring water and lake water. One group of fish was kept at 8°C until spawning (group 5). Subsequently, 20 unovulated females were transferred into water at 5°C on three different occasions: on December 15th 1987 (group 6), on January 5th 1988 (group 7) and on January 25th 1988 (group 8).
- From the beginning of December until mid-March, 20 spawners were kept in a tank supplied with spring water only (group 9).

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 corresponding females began to ovulate. When the majority of the males were in spawning dress 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 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 fifty 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 in a diluter for artificial fertilization (Billard, 1977), containing milt from several males. Twenty minutes later, eggs were shifted into incubation trays. Eggs from each female were incubated separately at 6 ± 1°C. Dead eggs were counted and removed once a week. Survival rates were calculated when eggs had reached the eyed stage (350 degree-days).

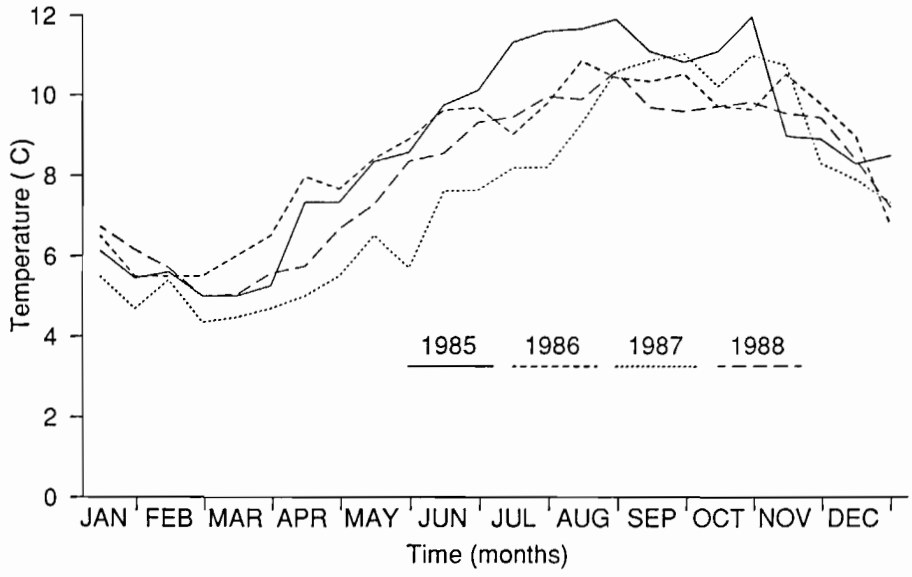

Figure 1. – Yearly variations of water temperature in 1985-1986-1987. Water was pumped from a depth of 36 metres in Lake Geneva.

Table 1. – Summary of the different thermal regimes investigated on ten experimental groups or Arctic charr spawners.

Experimental group	Temperature regime
1	Water pumped from a depth of 36 metres all year round 1985-1986
2	Water pumped from a depth of 36 metres all year round 1986-1987
3	5°C from 15 July 1986 to spawning
4	5°C from 15 September 1987 to spawning
5	8°C from early December 1987 to spawning
6	8°C from early December 1987 to 15 December 1987 then 5°C to spawning
7	8°C from early December 1987 to 5 January 1988 then 5°C to spawning
8	8°C from early Deceber 1987 to 25 January 1988 then 5°C to spawning
9	11°C from early December to the end of March
10	Wild females caught ovulated in the hypolimnion of Lake Geneva (5.5°C)

Wild females from Lake Geneva were caught on natural spawning grounds at an average depth of 60 metres. 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 following the same procedure as for the reared spawners. They composed the group 10.

Study on the ageing of ova

The decrease in ova fertility after ovulation was estimated by fertilizing small quantities of ova from the day where ovulation was first recorded (day 0) and at 2- or 3-day-intervals thereafter until day 10. Each time, about 200 ova were stripped from each female and immediately fertilized. This study was carried out on domestic 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.

Statistical analysis

Means for the different experimental groups (relative fecundity, ova weight and egg survival percentages after are sine transformation) were compared by multiple comparisons of means (Dagneli, 1970). Mean values of egg survival rates shown on *table* 2 for groups 1 and 2 were computed using a two-way analysis of variance for uneven sample sizes as described in Dagneli (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 groups 1 and 2.

Table 2. – Survival rates of spawns at eyed stage (mean+S.E., %) from several groups of Arctic charr. See table 1 for details of thermal regimes for the different groups.

Experimental group		November	December	January	Mean interval between two examinations of females (days)
1	Temperature	8.91°C	8.4°C	5.8°C	6.9
	Ova viability	4.3 ± 2.2	17.4 ± 5.1	36.2 ± 5.5	
2	Temperature	9.8°C	7.8°C	5.1°C	3.8
	Ova viability	29.6 ± 4.7	61.5 ± 5.4	83.8 ± 2.2	
3	Temperature		5°C		3.7
	Ova viability		80.2 ± 4.8		
4	Temperature		5°C		4.8
	Ova viability		90.6 ± 2.2		
10	Temperature		5.5°C		caught ovulating
	Ova viability		78.9 ± 2.5		

RESULTS

Timing of ovulation (*fig.* 2)

Fish reared in water pumped at a depth of 36 metres. Ovulation in groups 1 and 2 were first recorded at the end of November when Lake Geneva water temperature started to decrease. In mid-January, 100% of the females had spawned when water temperature was close to 5°C.

Fish reared in cold water (5°C). The majority of the females of groups 3 and 4 ovulated at the same time as fish of groups 1 and 2. However, the first female spawned in mid-October (group 3) or at the beginning of November (group 4). 100% of the females had spawned at the beginning of January.

Fish kept at 8°C and transferred to 5°C (groups 5, 6, 7, 8). Fish kept at 8°C started to ovulate in mid December. Only 37 percent of the females had spawned by the end of January (group 5). The whole population ovulated within one month following the transfer into cold water at 5°C (groups 6, 7). In group 8, only 80% of the females had ovulated at the end of the experiment in March.

Fish kept at 11°C. No ovulation was detected among the twenty fish of group 9 between December and March.

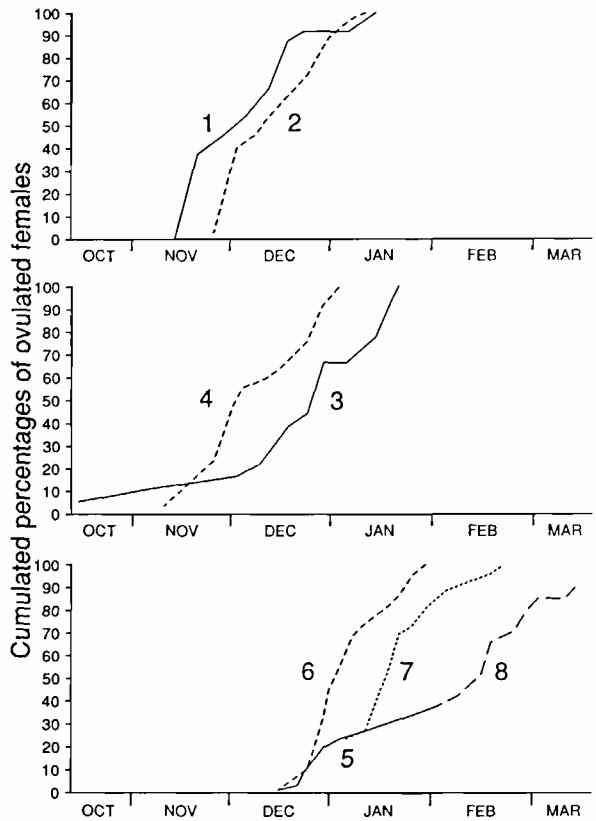

Figure 2. – Profiles of cumulated percentages of ovulated females. *Top:* Fish reared in water pumped from Lake Geneva (groups 1 and 2). *Middle:* Fish reared at 5°C from 15 July 1986 (group 3) and 15 September 1987 (group 4). *Bottom:* Fish reared at 8°C until the ovulation (group 5) or transferred at 5°C on 25 December (group 6), 5 January (group 7) and 25 January (group 8).

Viability of eggs (*tables* 2, 3, 4)

The variability of eggs from females of groups 1 and 2, reared in Lake Geneva water, significantly improved ($p < 0.01$) 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 and 10, *table* 2). Relative fecundity did not change significantly with weight: $r = 0.09$ in group 1 and $r = 0.11$ in group 2. Relative fecundity of reared females of experimental groups 1, 2 and 4 were comparable with wild fish. The mean ova weight of fish reared in cold water (group 4) was signficantly lower ($p < 0.05$) than that of wild fish (group 10, *table* 3).

Table 3. – Ova mean weight and relative fecundity of Arctic charr (mean + S.E.). See table 1 for details of the thermal regimes of the different experimental groups. Number of fish in brackets.

Experimental 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 10 (21) (wild fish)	55.0 ± 2.4	3581 ± 281

Females kept at 8°C (group 5) produced eggs of lower ($p < 0.05$) variability than those of wild females (group 10). When fish were transferred into water at 5°C before ovulation (groups 6, 7, 8), the quality of eggs was improved in comparison with group 5 ($p < 0.05$). But the viability of eggs decreased from group 6 to group 8 with the length of acclimatization at 8°C. In the same way, the relative fecundity significantly ($p < 0.05$) decreased from group 6 to group 8 (*table* 4).

Patterns of overripening of eggs (*fig.* 3, 4, 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 catching. 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 6 summarizes the survival rates of eggs in all groups of reared females, except group 9 (no ovulation). The X-axis shows the mean number of degree-days between two checks. The Y-axis shows the mean values of egg survival rates for the different groups. It can be observed that by increasing the number of degree-days between two checks, one decreased the average egg quality, except for groups 6, 7 and 8. The viability of eggs decreased from group 6 to group 8 with the length of acclimatization at 8°C, though the number of degree-days between two checks remained constant.

Table 4. – Survival rates of spawns at eyed stage and relative fecundity (mean + S.E.) for several experimental groups of Arctic charr. See table 1 for details of thermal regimes of different groups. Relative fecundity is expressed in number of eggs/kg. All the females were examined twice a week to detect the ovulations.

Experimental group	Survival rate of eggs (%)	Relative fecundity
Group 5 (8°C)	58.0 ± 6.2	4093 ± 304
Group 6 (8 → 5°C on 15 December)	78.2 ± 3.3	4606 ± 260
Group 7 (8 → 5°C on 5 January)	69.3 ± 4.7	3927 ± 195
Group 8 (8 → 5°C on 25 January)	62.8 ± 7.0	3591 ± 311

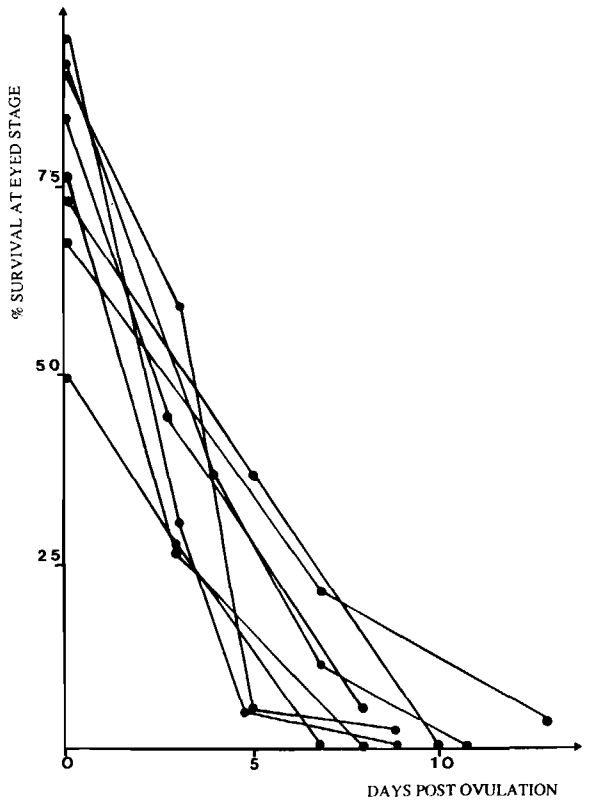

Figure 3. – Effects of overripening on survival of eggs at eyed stage. Reared females of group 2, December 1987, 8°C. Each curve represents data for one female.

DISCUSSION

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 in the wild spawners from Lake Geneva. Spawning in the lake occurs at a depth between 60 to 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. As far as I know, this fact has never been described in other salmonid fish. This phenomenon is probably linked

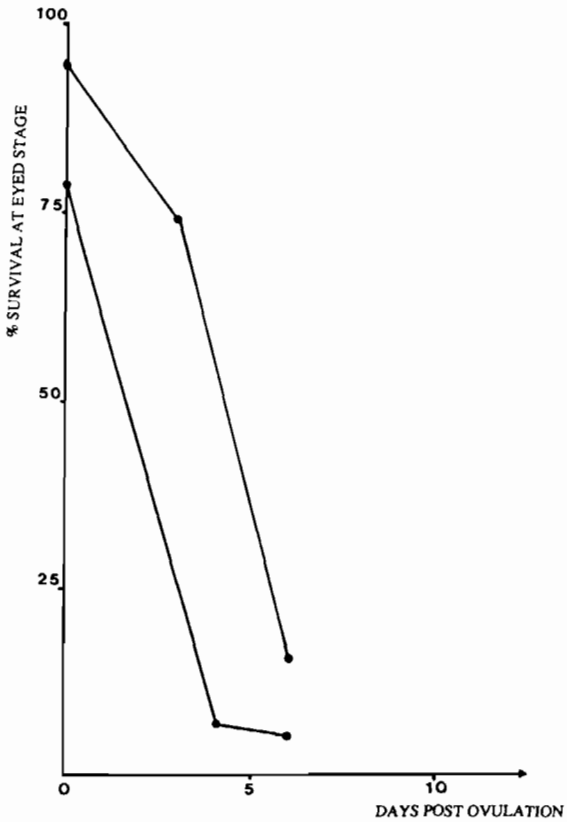

Figure 4. – Effects of overripening on survival of eggs at eyed stage. Wild females caught unovulated and kept at 8°C, December 1987. Each curve represents data for one female.

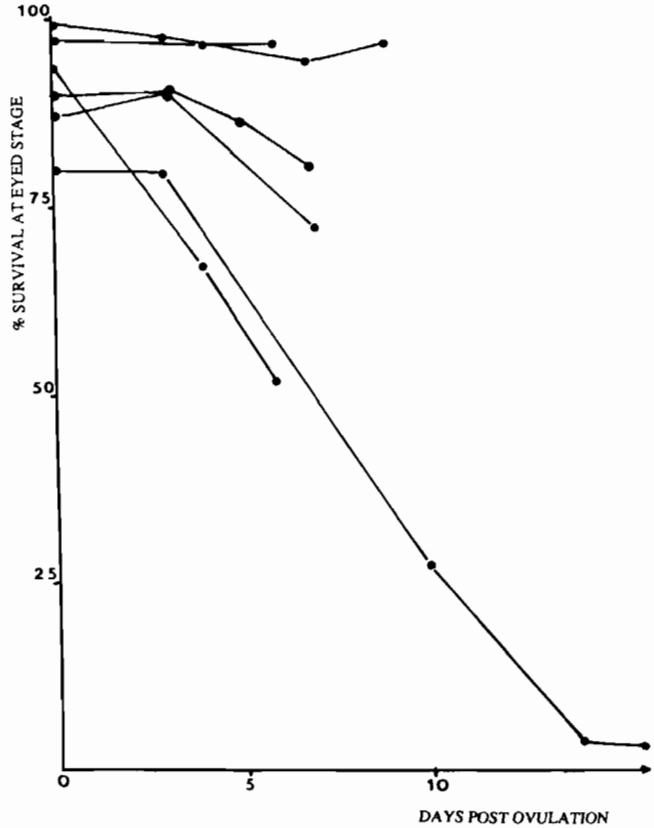

Figure 5. – Effects of overripening on survival of eggs at eyed stage. Reared females of group 4, December 1987, 5°C. Each curve represents data for one female.

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 5) delayed ovulation in comparison with fish acclimatized at 5°C (groups 3 and 4). Subsequent transfer of fish from 8 to 5°C stimulated and synchronized the ovulations (groups 6, 7, 8). Requirements for cold water during spawning can 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 related to the phase of spawning only, since fish acclimatized at 5°C from the beginning of summer (group 3) do not ovulate earlier than fish transferred to water at the same temperature at the end of summer (group 4).

Viability of eggs

The poor viability of eggs produced by reared Arctic charr spawners has already been reported (Paspt and Hopky, 1984; Davaine, pers. comm.). In all cases, spawners were kept at temperature higher than 6°C during reproduction. In groups 1 and 2, the higher the mean monthly temperature was, the lower the viability of ova from fish reared in water pumped at 36 m. On the other hand, 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 Kreiger and Olson (1988). According to these authors, spawning of Arctic charr of the Nanyuk lake strain (Canadian Arctic area) has been more successful with broodstock that have been raised in cold water (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 near 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 related to overripeness of 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 fish, the rainbow trout and the brown trout (Billard and Gillet, 1981). However, for the rainbow

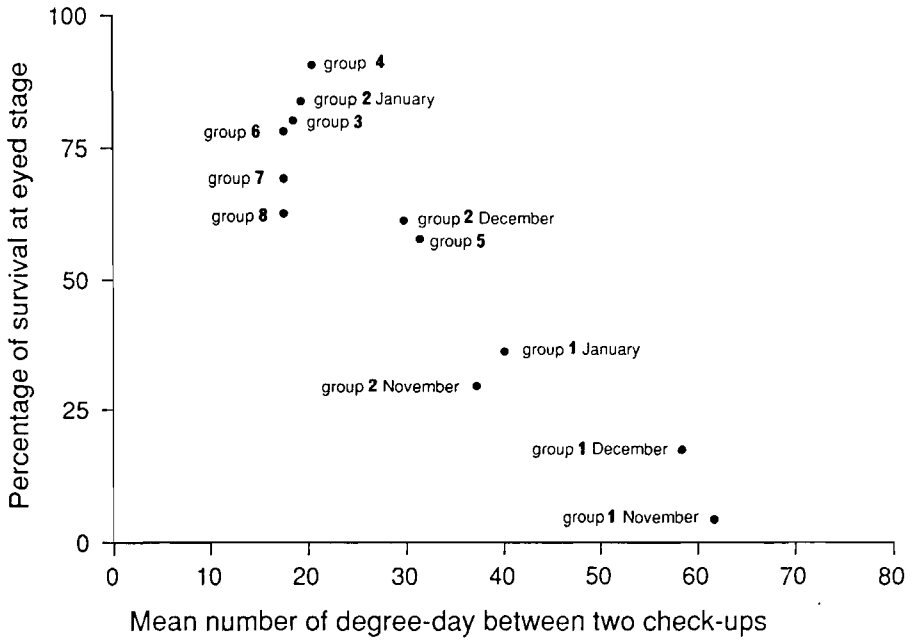

Figure 6. – Changes in the average egg survival at eyed stage for the different experimental groups in relation to the number of degree-days between two checks of females. See table I for details of thermal regimes for the different groups.

trout, the acceleration of ageing processes was only observed above 10°C. In trout, ova kept the maximum ability to be fertilized for 10 days after ovulation, at least, at 10°C, then, ova viability decreased slowly during 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 as soon after the third day of observation. 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 the ovulation and shortened the phase of maximum viability, like in Arctic charr at 8°C. One might hypothesize that the disturbances which lengthen the duration of the phase of ovulation, induce a decrease in the ability of ova to remain viable after ovulation.

Arctic charr in which spawning was delayed, due to their acclimatation to 8°C, produced eggs of good quality when they were transferred into cold water (5°C) on December 15th, one or two weeks prior to ovulation (group 6). When the transfer of unovulated females occurred later, in January (groups 7 and 8), both viability of ova and relative fecundity tended to decrease. This fact suggest that the prolonged maintenance of females with well developed ovaries at 8°C could induce partialy a trend to retention of oocytes or an atresia of some oocytes. Atresia in maturing ovaries has already been reported in brook trout, a species closely related to Arctic charr (Henderson, 1963). But in brook trout, this phenomenon does not become important in natural conditions.

The females of group 4, kept at 5°C from September 15th had smaller ova than wild fish or fish of group 1. These latter had achieved the largest part of their gametogenesis in water at 8°C or above. In summer and the beginning of autumn, the achievement of growth and gametogenesis of Arctic charr take place at temperatures higher than 5°C in natural environment. In another salmonid fish, the rainbow trout, Breton and Billard (1977) have reported favourable effects of warm temperatures on gametogenesis. We assume that cold water was only necessary for Arctic charr during the last weeks before spawning.

Acknowledgements

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