

Influence of early thermic and photoperiodic control on growth and smoltification in Atlantic salmon (*Salmo salar*)

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Abstract

Atlantic salmon juveniles were subjected to a precocious (day 104 after first feeding) photoperiodic (P) and thermic (T) control. After rearing the fry at 16°C, constant (16 hours of light and 8 hours of darkness, 16°C) (C) and shortened simulated natural S photoperiod and temperature regimes were used in a factorial experimental design until transfer to seawater. The growth obtained with constant (C) regimes was better than with S regimes. In all cases, we observed very early (day 141, mid-June) a clear segregation of the population into two different subpopulations. Among the conditions, all the differences between the upper and lower modes were growth-dependent. We observed different developmental trends of the gill (Na⁺-K⁺)-ATPase activity: the S regime gave a very progressive increase of the enzymatic activity, while we had a rapid decrease after a first phase of increase with a constant photoperiod. The absolute levels of the gill (Na⁺-K⁺)-ATPase activity were slightly lower than those observed in one year-old smolts. However, they are quite similar to those of upper-mode fish that have a good salinity tolerance. After transfer to seawater, fish subjected to shortened simulated temperature and photoperiod had the lowest mortality and osmotic disturbance (after 48 hours and 3 weeks). Although the freshwater phase generally lasts more than 15-17 months for this species in a natural environment, we concluded that it is possible to obtain a good salinity tolerance after 7-8 months, with shortened simulated natural photoperiod and temperature regimes, in spite of the absence of a real smoltification; these regimes, however, must be applied during the "dynamic" presmolting phase when the growth is intense.

Keywords: Photoperiod, temperature, 0⁺ smolts, Atlantic salmon.

Effet d'un contrôle précoce de la température et de la photopériode sur la croissance et la smoltification du saumon atlantique (Salmo salar).

Résumé

Des juvéniles de saumons atlantiques (*Salmo salar*) ont été soumis précocement (104 jours après la première distribution alimentaire) à un contrôle de la photopériode (P) et de la température (T). Le premier alevinage s'est déroulé à 16°C; puis des régimes de photopériode et température constants (C) (16 heures de jours et 8 heures de nuit, 16°C) et simulés naturels raccourcis (S) ont été étudiés à partir d'un plan expérimental factoriel appliqué jusqu'au transfert en mer. La croissance obtenue est meilleure avec les régimes constants (C) qu'avec les régimes simulés (S). Nous avons obtenu très tôt (jour 141, à la mi-juin), et dans tous les cas, une ségrégation de la population en deux sous-groupes. Les différences enregistrées entre les modes haut et bas sont toutes liées à la croissance. L'activité de la (Na⁺-K⁺)-ATPase branchiale évolue différemment en fonction des cas: le régime à photopériode simulée (S) permet une augmentation progressive de l'activité enzymatique alors que dans le cas où la photopériode est constante une rapide diminution de l'activité fait suite à une phase d'augmentation. Les niveaux absolus de la (Na⁺-K⁺)-ATPase branchiale sont légèrement inférieurs à ceux observés sur des smolts de 1 an. Cependant, ils sont voisins de ceux de juvéniles de saumons appartenant au mode haut d'une population (smolts potentiels) qui ont une bonne tolérance aux salinités élevées.

Après un transfert en mer, les poissons soumis au régime S ont une moindre mortalité, un déséquilibre osmotique plus faible. Bien que la phase de vie en eau douce se déroule généralement en 15-17 mois pour cette espèce, en conditions naturelles, nous concluons qu'il est possible d'obtenir une bonne tolérance à la salinité après 7-8 mois, si l'on applique des régimes de photopériode et température simulés naturels raccourcis et ceci en l'absence d'une réelle smoltification; ces régimes doivent cependant être utilisés durant une phase dynamique de présomltification, lorsque la croissance est forte.

Mots-clés : Photopériode, température, smolts 0⁺, saumon atlantique.

INTRODUCTION

The biological cycle of Atlantic salmon (*Salmo salar*) includes two phases that occur in different environments: river and ocean. At a given time of its river life, the young salmon undergoes deep physiological modifications, including a progressive adaptation to seawater, before initiating its downstream migration, generally occurring in spring. This phenomenon is known as the "smoltification" process, abundantly described in the literature (see review Bœuf, 1992). The duration of the freshwater phase is variable and depends mainly upon the temperature regimes (Hoar, 1988). In the most favourable conditions, this freshwater phase takes 15 to 17 months in wild populations.

The exploitation of the species by aquaculture techniques (cage rearing and ranching operations) has been tremendously increasing over the past ten years, and represents to date a considerable economic value. For obvious economic reasons, special attention has been given to the possibilities of reducing the duration of this freshwater phase in order to reduce the cost of the smolts. These attempts included the use of geothermal effluents to stimulate the growth (Isaksson, 1985); the use, in Chile, of salmon eggs issued from northern hemisphere broodstocks permitted a smoltification to be obtained during the southern hemisphere spring, allowing the transfer of the fish to seawater after a 10-11 month freshwater rearing period (Bœuf and Médina, 1990). From a more general point of view, this objective became a major preoccupation of the industry (Simpson, 1985), stimulating a considerable amount of scientific works. Research approaches included a possible hormonal control of smoltification (Bœuf *et al.*, 1990a), and, more frequently, the effect of environmental parameters on growth and smoltification (Johnston and Saunders, 1981; Saunders *et al.*, 1985; Clarke *et al.*, 1985; McCormick *et al.*, 1987; Stefansson *et al.*, 1988; Villareal *et al.*, 1988; Duston and Saunders, 1990; Saunders and Harmon, 1990; Duston *et al.*, 1991).

The natural climatic conditions prevailing in France allow a rapid growth (Prouzet and Jézéquel, 1983; Gaignon, 1987) and would make possible the production of accelerated smolts at a moderate cost. It is thus essential in these conditions to obtain a "satisfactory"

smoltification, allowing a precocious transfer to seawater.

The smoltification process is a complex combination of modifications (see review from Bœuf, 1987; Hoar, 1988), depending upon an endogenous rhythm synchronized by variations of several environmental factors (Eriksson and Lundqvist, 1982; Duston and Saunders, 1990). The effects of photoperiod and temperature (Clarke *et al.*, 1981; Johnston and Saunders, 1981) or of photoperiod alone (Clarke *et al.*, 1985; McCormick, 1987; Stefansson, 1989; Bjornsson *et al.*, 1989) have been intensively investigated. This last parameter is sometimes described as a factor allowing an ultimate "accomplishment" of smoltification (Stefansson *et al.*, 1989; Duston, 1990); McCormick (1987), Saunders *et al.* (1989) indicate that the smoltification process can be completely achieved only after an exposure of several months to increasing daylight. Brauer (1982) showed in coho salmon that the growth has an influence on the effects of the photoperiod.

Almost all these studies have been carried out during the normal smoltification period, generally after December (Saunders *et al.*, 1985; McCormick *et al.*, 1987; Stefansson *et al.*, 1989; Duston and Saunders, 1990), and rarely sooner (Saunders *et al.*, 1987; McCormick *et al.*, 1987; Stefansson *et al.*, 1991). Very often they have been using constant photophases, compared to controls using the simulated local photoperiod or a 24 hours continuous daylight. Works being done at earlier stages of the biological cycle were designed to describe the appearance of a bimodal structure of the population during the first autumn, characteristic of the species (Saunders and Henderson, 1988; Villarreal *et al.*, 1988; Stewart *et al.*, 1990; Skilbrei, 1991). The only works concerning the effects of a momentaneous modification of the photophase, using a stable daylight regime in autumn, were carried out by Saunders *et al.* (1989) and more recently by Saunders and Harmon (1990). To our knowledge, no existing papers have been published on the effects of photoperiod on smoltification in completely artificially controlled light conditions since fecundation. The purpose of the present experiment was to study the effect of several photoperiod and temperature regimes, applied at a very early stage on subsequent growth and smoltification.

MATERIAL AND METHODS

Since January 31st 1989, 3075 fry (Norwegian strain from Matre Aquaculture Research Station) were reared in Swedish type tanks (1 × 1 m) at the IFREMER (Institut français pour l'exploitation de la mer) experimental facilities in Brest. During the fry-stage period, the temperature was constant (16 ± 0.5°C) and the photoperiod was a constant 16 L/8 D (light/dark) regime. They were automatically fed on an extruded IFREMER diet (Gaignon, 1987).

The experimental phase began on May 17th, 1989 (day 106 after first feeding). 2500 fish with a mean

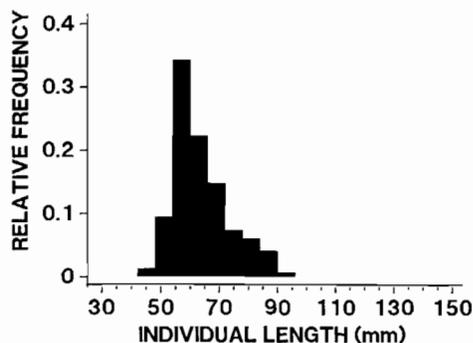


Figure 1. — Length frequency distribution of the fish at the beginning of the experiment (day 106 after first feeding).

weight of 3.49 g (fig 1), issued from the initial population (mortality of 5.8 % during the preliminary phase of the experiment) were randomly and equally distributed in 10 tanks. 2 photoperiodic [*i.e.* one constant (CP): 16 L/8 D and a natural shortened simulated one (SP)] and 2 thermic [*i.e.* one constant (CT) 16°C and a local shortened simulated one (ST)] regimes were applied in a factorial replicated experimental design. Moreover, an additional temperature regime [with a final decrease from August 13th (D195) after an initial constant temperature (16°C)] (CDT) was realized with each photoperiodic regime, without replicate (fig. 2). The illumination with a fluorescent "True lite" tube (20 W) and an incandescent 25 W lamp provides an intensity of 60-80 lux at the surface of the water. No twilight periods were provided. The fluorescent light was switched on 15 minutes after the ignition of the incandescent lamp. The lights were switched off the opposite way. The duration of the fluorescent illumination expressed the day length. The fish were automatically fed on an extruded SSI diet; the pellet-size used is the one defined by Gaignon (1987); the daily feeding rate was based on temperature. The daily feeding duration was equal to the daily photophase.

When the length frequency distribution showed a bimodality, the fish of each tank were graded (July 24th) and fish inferior to the size of 90 mm were discarded. Before grading, 80-100 fish/tank were weighed (to the nearest 0.1 g) and measured (to the nearest 1 mm) every 12-14 days. After grading, the

same procedure was performed on the 25 July, 8 August, 6 September, 25 September (except for CP). After seawater transfer, a total weight and counting were performed. Throughout the experiment, the mortality was recorded daily.

The smoltification was monitored by measurement of the gill (Na⁺-K⁺)-ATPase activity (Bœuf and Harache, 1982), analyzed on 3 fish until August 11th and after, on 4-6 fish sampled every 10 days, using fish with an individual size quite similar to the mean length of those of a given tank. The methods for sampling and analyses were already described by Lasserre *et al.* (1978).

The salinity tolerance test was performed by a direct seawater transfer (35 ‰), without tank change, at fortnightly intervals for the two replicates. The osmotic pressure and chlorides were analyzed respectively on a Ruebling osmometer and on a radiometer chloridometer, on fish sampled 48 hours and 3 weeks (only in the case of the first replicate transferred) after seawater transfer. These data, added with the gill (Na⁺-K⁺)-ATPase activity, are considered to be a good criterion for seawater adaptation of Atlantic salmon according to previous results (Bœuf and Harache, 1982).

When the length segregations are clearly established, the observed population structures were normalized with the NORMSEP program (Tomlinson, 1970; after Hasselblad, 1966) which determines the mean and the standard deviation of the different subgroups with an iterative method.

The condition factor was calculated with the following expression $K = 100 W/L^3$, where W and L are respectively the individual weight and length of a given fish.

The specific growth rate was calculated with the expression: $1/n (\text{Log } W_f - \text{Log } W_i) \times 100$ where n, W_i and W_f are respectively the number of days, the initial and final weight.

In the following text, a "condition" will define a particular experimental treatment combination given to the fish of one tank and will be characterized by a couple of levels of each of the factors, for example: CP × SP.

Until the beginning of the temperature decrease, for the statistical analysis, we combined the CDT data with those of CT which had the same temperature (*i.e.* until D195 three replicates were used for this regime).

Standard deviations are given with the mean value on the figures. The influence of the different conditions were studied on the bimodal population with a Kruskal-Wallis H test (Sachs, 1984). On the normalized subgroups, the different parameters including the limit threshold between the two subgroups were compared with a two-way ANOVA and Scheffe's multiple range test (Sachs, 1984). This type of analysis is also used, after grading, to compare the influence of the

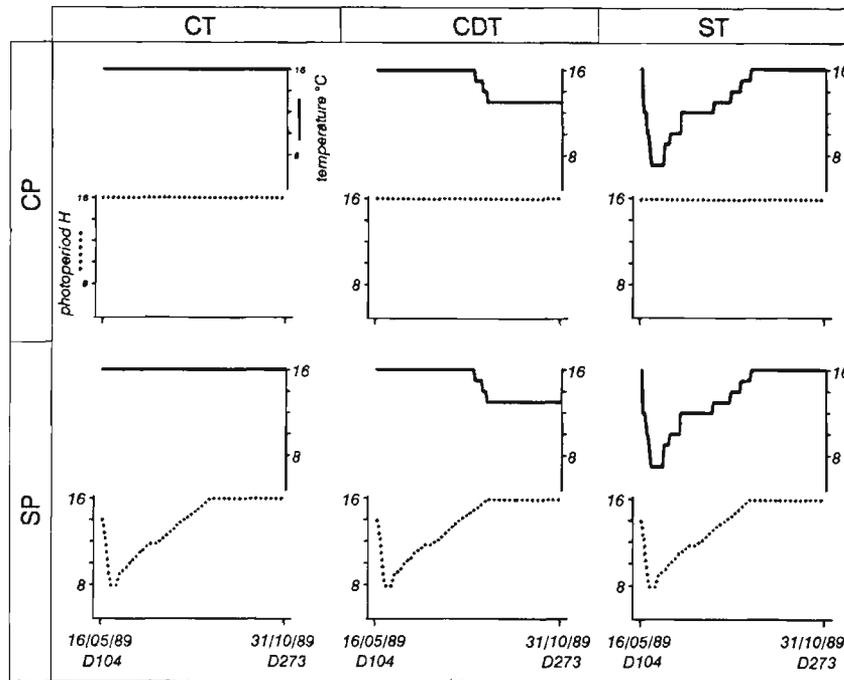


Figure 2. — Temperature (°C) and photoperiod (hours) regimes for the different conditions. T: temperature, P: photoperiod, C: constant, S: simulated, CDT: initial constant temperature.

conditions and regimes on length and weight. A significant level of $p < 0.05$ is used to establish statistically significant differences. For each date, the values of ATPase (expressed in $\mu\text{m Pi mg Prot.}^{-1} \text{h}^{-1}$), osmotic pressure and chlorides were compared with a Kruskal-Wallis H test. The ATPase evolution of each tank is characterized by the linear regression giving the higher correlation coefficient.

RESULTS

Survival, growth, condition factor

During the experiment, the mortality was nil in all tanks until transfer to seawater. From the very beginning of the experiment, the treatments studied affected the instantaneous growth rate: the groups reared under constant regimes (temperature and photoperiod) showed a higher growth rate than those observed with variable regimes during the first 22 days of the experiment (table 1). Then the population, initially showing a unimodal structure (fig. 1), evolved towards a typical bimodal length frequency distribution (fig. 3). Bimodality appeared earlier under a 16°C constant temperature regime [CT and CDT on June 21st, at D141 after first feeding (fig. 3)]. On July 20th (D170), before the first grading, the proportion of fish of the upper mode of the normalised population was

photoperiod-dependent (table 2); however, the limit threshold between the two modes was higher for groups subjected to constant regimes (temperature and photoperiod) (table 2). The growth observed before the first grading (D170, July 20) was different according to the conditions: fish from the groups CPCT showed larger mean sizes and weights than

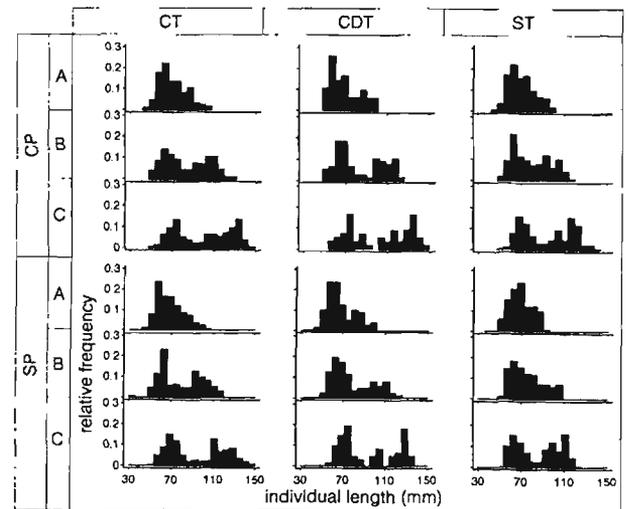


Figure 3. — Length frequency distribution (cumulated replicate) before grading for the different experimental conditions. T: temperature, P: photoperiod, C: constant, S: simulated. For each one of these A: 26 May 1989, B: 21 June 1989, C: 19 July 1989.

Table 1. — Growth rate (mean in %/day) during the first 22 days of the experiment (T=temperature, P=photoperiod, C=constant, S=simulated). For each condition and each factor () the same letter represents a homogeneous group. NS: non significant. (1): data combined with initial constant temperature (CDT). (2): ANOVA gives significant differences but it is impossible to classify the regimes with Scheffe's multiple range test.

Growth rate (%)	Constant temperature CT (1)	Simulated temperature ST	Photoperiod P (2)	
Simulated photoperiod SP	2.69 a ± 0.20	1.76 b ± 0.01	2.32 ± 0.25	<i>p</i> < 0.05
Simulated photoperiod SP	2.09 ab ± 0.09	1.57 b ± 0.21	1.88 ± 0.15	
Temperature T	2.39 (a) ± 0.16	1.66 (b) ± 0.10	Interaction NS	
	<i>p</i> < 0.01			

Table 2. — Influence of thermic (T) and photoperiodic (P) regimes (C: constant, S: simulated) on length frequency distribution parameters before grading. The mean ± standard errors are given. For each factor and parameter, the same letter represents a homogeneous group. (1) and (2): see table 1.

Parameter	CT (1)	ST	CP	SP
Percentage of the normalized upper mode subgroup (UM) (see text for explanation)	53.5 ± 2.0	54.5 ± 0.5	56.4 a ± 1.2	51.4 b ± 1.3
	NS		<i>p</i> < 0.05	
Percentage of fish > 90 mm after grading	47.3 ± 2.5	43.2 ± 2.1	49.8 ± 1.8	41.6 ± 1.5
	NS		<i>p</i> < 0.01 (2)	
Limit threshold between normalized LM (lower mode) and UM subgroups (mm)	91.1 a ± 1.6	84.8 b ± 2.0	91.4 ± 1.7	85.7 ± 2.0
	<i>p</i> < 0.01		<i>p</i> < 0.05 (2)	
Lower subgroup length (mm)	72.5 a ± 0.85	68.9 b ± 1.65	73.2 a ± 0.81	68.9 b ± 1.16
	<i>p</i> < 0.01		<i>p</i> < 0.001	
Upper subgroup length (mm)	121.3 a ± 1.55	108.7 b ± 2.93	119.6 a ± 2.83	112.9 b ± 3.80
	<i>p</i> < 0.001		<i>p</i> < 0.01	

those of CPST and SPCT (with the exception of the mean weight of CPST fish), being themselves larger than those of SPST (table 3). In the same way, the condition factor was higher for the CPCT group than for CPST and SPCT, being themselves higher than those of SPST (table 3). Moreover, the mean lengths of each mode were higher for constant thermic and photoperiodic regimes (CP > SP, CT > ST) (table 2).

After grading, the growth of the remaining fish (figs. 4 and 5) varied according to the various regimes of the experiment (table 4): on September 7th (D219) the mean weights, lengths and condition factor of the CP fish were higher than those of groups exposed to SP. The mean weight and length of fish submitted to

Table 3. — Length, weight and condition factor (mean ± standard error) for each condition (cumulated replicate) before grading. (1) data combined with CDT. The same letter represent a homogeneous group for each parameter (statistical analysis: Kurskall-Wallis H test).

Parameter	Condition factor	
Length (mm)	CPCT (1)	102.72 ± 2.13 a
	CPST	94.96 ± 1.99 b
	SPCT (1)	94.83 ± 1.90 b
	SPST	86.49 ± 1.67 c
Weight (g)	CPCT (1)	17.32 ± 0.96 a
	CPST	12.94 ± 0.76 ab
	SPCT (1)	13.18 ± 0.73 b
	SPST	9.19 ± 0.49 c
Condition factor	CPCT (1)	1.30 ± 0.010 a
	CPST	1.24 ± 0.009 b
	SPCT (1)	1.23 ± 0.008 b
	SPST	1.19 ± 0.008 c

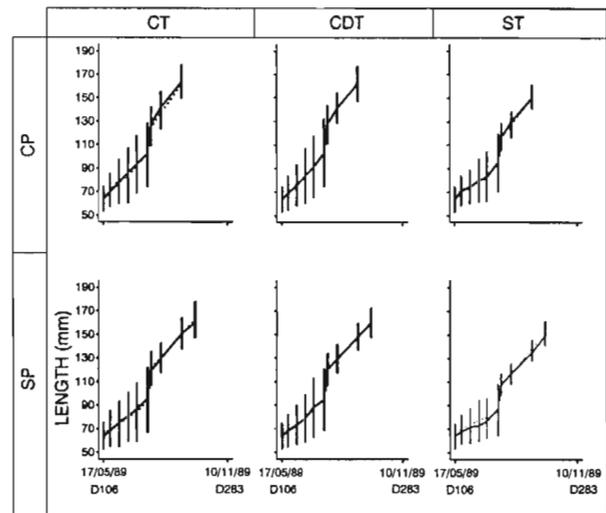


Figure 4. — Length (mm) of salmon subjected to different conditions. T: temperature, P: photoperiod, C: constant, S: simulated. For CPCT, CPST, SPCT, SPST, the two curves show the replicate tanks.

CT and CDT did not differ significantly, but were superior to ST; differences were observed among the condition factors: the condition factor for CT (1.286) was higher than the one for CDT (1.247), but they were both similar to ST (1.264) (table 4).

During the transfer to seawater, biometric samplings were realized only for the SP fish. The mean weights and lengths of SPST were lower (*p* < 0.001) than those of SPCT and SPCDT that were similar [during the previous sampling on September 6th, the mean weights and lengths of SPCT and SPCDT were also similar (*p* = 0.170)]. The condition factors of the various conditions were not significantly different (*p* = 0.157).

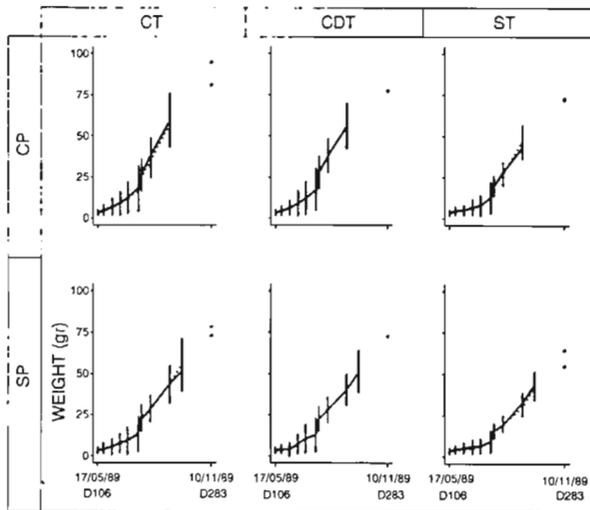


Figure 5. — Weight (g) of salmon subjected to different conditions. T: temperature, P: photoperiod, C: constant, S: simulated. The points represent the mean weight (g) of each batch after transfer to seawater. For CPCT, CPST, SPCT, SPST, the two curves (and points) show the replicate tanks.

Table 4. — Length (mm), weight (g) and condition factor (mean \pm standard error) of the upper mode (cumulated replicate) before transfer to seawater (Sept. 7th). See explanations table 1.

Length (mm)	CT	CDT	ST	P
CP	162.9 a +1.1	162.0 a +1.9	150.6 b +0.8	157.0 a +0.8
SP	150.8 a +1.2	148.3 b +1.3	136.3 c +0.7	144.3 b +0.7
$p < 0.001$				
T	156.8 (a) +1.0	154.3 (a) +1.3	143.9 (b) +0.7	interaction NS
$p < 0.001$				
Weight (g)	CT	CDT	ST	P
CP	58.6 a +1.4	56.2 a +1.8	45.2 b +0.7	51.9 a +0.8
SP	43.7 b +1.1	40.4 b +1.1	31.2 c +0.5	37.8 b +0.6
$p < 0.001$				
T	51.1 (a) +1.0	47.3 (a) +1.2	38.6 (b) +0.6	interaction NS
$p < 0.001$				
Condition factor	CT	CDT	ST	P
CP	1.334 a +0.012	1.292 ab +0.018	1.308 a +0.007	1.310 a +0.006
SP	1.247 bc +0.006	1.220 c +0.014	1.222 c +0.008	1.226 b +0.005
$p < 0.001$				
T	1.286 (a) +0.007	1.247 (b) +0.012	1.264 (ab) +0.006	interaction NS
$p < 0.01$				

Smoltification

The gill ($\text{Na}^+ - \text{K}^+$)-ATPase activity changed during the experiment and, for all the cases, values higher than $10 \mu\text{m Pi mg Prot.}^{-1} \text{h}^{-1}$ have been observed. However, the evolution was different according to the

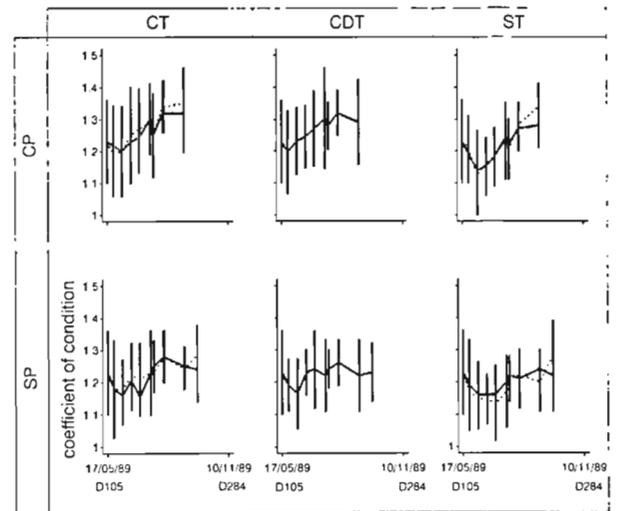


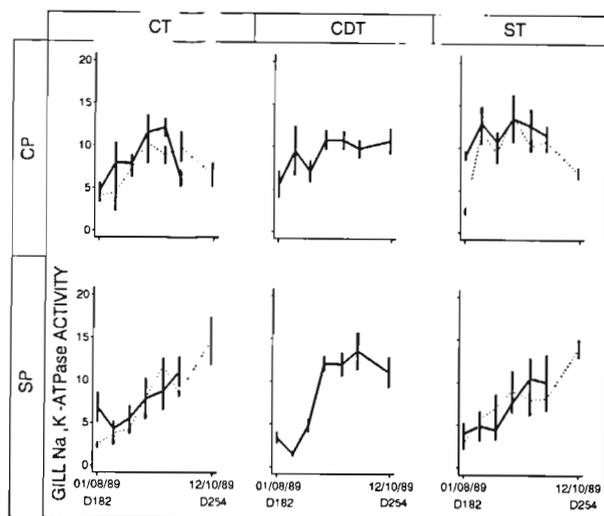
Figure 6. — Condition factors for the different conditions. T: temperature, P: photoperiod, C: constant, S: simulated. For CPCT, CPST, SPCT, SPST, the two curves show the replicate tanks.

conditions (fig. 7). With a constant photoperiod (CP), the evolution was best expressed by a second step model. A progressive photoperiod after a short "winter season" (SP) gave a very progressive evolution of the ($\text{Na}^+ - \text{K}^+$)-ATPase. A linear model gave the best adjustment in this case. The final decrease of temperature to 13°C (CDT) was the only thermic condition that tended to modify the enzymatic activity: for the constant photoperiod the plateau remained for a longer time.

However, at any date, only the CPST was different from the other conditions: at the time of the first sampling (August 1st, D182), the replicates were heterogeneous; for both the following points, the ($\text{Na}^+ - \text{K}^+$)-ATPase levels were higher than those of all the other conditions (fig. 7).

Transfer to seawater

The first transfers with a 35‰ salinity occurred on September 26th (D238) and October 11th (D253) of the first year of rearing for respectively the first and second replicate. Even if the gill ($\text{Na}^+ - \text{K}^+$)-ATPase activities were similar for all conditions, after 48 hours the first transfer gave significant differences for osmotic pressures and chlorides: there was a photoperiodic influence and the SPST showed a less osmotic disturbance than CPCT (table 5). 3 weeks after the transfer to seawater, the osmotic pressures and chlorides were similar for all the conditions and regimes.



$$\text{CPCT: } Y = 2.922 + 0.305 X - 0.003 X^2 \quad (R^2 = 0.856);$$

$$Y = 3.736 + 0.464 X - 0.077 X^2 \quad (R^2 = 0.649)$$

$$\text{CPST: } Y = 9.325 + 0.226 X - 0.004 X^2 \quad (R^2 = 0.536);$$

$$Y = 4.393 + 0.405 X - 0.005 X^2 \quad (R^2 = 0.644)$$

$$\text{SPCT: } Y = 4.642 + 0.104 X \quad (R^2 = 65.2);$$

$$Y = 1.896 + 0.175 X \quad (R^2 = 89.9)$$

$$\text{SPST: } Y = 0.146 + 3.106 X \quad (R^2 = 87.1);$$

$$Y = 0.131 + 3.551 X \quad (R^2 = 86.3)$$

Figure 7. — Changes in gill ($\text{Na}^+\text{-K}^+$)-ATPase activity for the different conditions. T: temperature, P: photoperiod, C: constant, S: simulated. For CPCT, CPST, SPCT, SPST, the two curves show the replicate tanks. The regressive equations describe the enzymatic activity (see text for explanations).

No mortality was observed during the first 15 days in seawater; after 7 weeks, the mortality for CPCT, CPST, SPCT and SPST conditions were respectively 5.3; 15.5; 4.2; 1.3 % of the initial number of transferred fish.

No mortality was observed during one month after the other batches had been transferred to seawater a fortnight later.

For each tank, the growth in salt water was quite similar to that previously observed in freshwater, after the grading (fig. 5) and not different between the conditions and regimes (table 6).

DISCUSSION

Although some of the groups were reared with a high constant temperature of 16°C, no disease or mortality appeared. The final mean weight observed in this case was higher than that obtained under the colder ST treatment (minimum: 8°C), but no different from that obtained under the CDT treatment which had a lower 13°C constant temperature during the final phase of the experiment. These results suggest that under our rearing conditions and with the food used, the maximum growth rate would be between

Table 5. — Gill ($\text{Na}^+\text{-K}^+$)-ATPase (with the mean weight of the analyzed fish) before transfer to seawater, osmotic pressure and plasma chloride after 48 hours in salt water (35‰ salinity). T: temperature; P: photoperiod, C: constant, S: simulated. The mean \pm standard error are given. For each condition and each factor () the same letter represents a homogeneous group.

Gill ATPase (21st Sept.) (sampled fishweight)	CT	CDT	ST	P
CP	8.1 \pm 1.1 a (62.9 \pm 3.0)	9.8 \pm 1.0 a (63.1 \pm 4.3)	11.1 \pm 0.7 a (5.5 \pm 1.1)	9.8 \pm 0.6
SP	9.7 \pm 0.9 a (49.5 \pm 2.4)	13.6 \pm 2 a (49.9 \pm 1.3)	9.1 \pm 1.6 a (39.3 \pm 0.8)	10.6 \pm 0.9
T	8.9 \pm 0.7	11.7 \pm 1.2	10.2 \pm 0.8	interaction NS
	NS			
Plasma chloride (28th Sept.) mmol.l ⁻¹	CT	ST	P	
CP	152 a \pm 7	147 ab \pm 4	149 (a) \pm 4	p < 0.01
SP	141 ab \pm 4	129 b \pm 1	135 (b) \pm 3	
T	147 (a) \pm 4	138 (a) \pm 4	interaction NS	
	NS			
Osmotic pressure (28th Sept.) mOsm.l ⁻¹	CT	ST	P	
CP	386 a \pm 15.62	374 ab \pm 6.73	380 (a) \pm 8	p < 0.01
SP	360 ab \pm 8.92	333 b \pm 3.41	346 (b) \pm 6	
T	373 \pm 9	354 \pm 8	interaction NS	
	NS			

Table 6. — Growth rate (mean in %/day \pm standard error) during the first 17 days after transfer to seawater. T: temperature, P: photoperiod, C: constant, S: simulated.

Growth rate (%)	CT	ST	P	
CD	0.63 a \pm 0.14	0.73 a \pm 0.07	0.68 \pm 0.07	NS
SP	0.73 a \pm 0.02	0.68 a \pm 0.21	0.71 \pm 0.08	
T	0.68 \pm 0.06	0.71 \pm 0.09	interaction NS	
	NS			

13 and 16°C, as generally found for the Atlantic salmon (Foda and Henderson, 1977; McCauley and Casselman, 1980), but slightly below the temperature (16°C) proposed by Knutsson and Grav (1976). The natural shortened simulated photoperiod regime (SP) had a negative influence on growth (initial growth rate, mean length and weight before grading, upper and lower group mean length, final length and weight)

compared to the constant photoperiod regime (CP). In the latter case, illumination duration was higher than in the other case and authorized the best growth as obtained by Clarke *et al.* (1981) on coho salmon or McCormick *et al.*, 1987; Villarreal *et al.*, 1988, and Bjornsson *et al.*, 1989 on Atlantic salmon: an extended day length can stimulate the pituitary axis and, subsequently, growth (Komoudjian *et al.*, 1989). However, this author, working with a continuous artificial daylight, observed in his own experimental condition that the influence does not persist more than 3 months. In the same way, McCormick *et al.* (1987) showed that the weights obtained after a complete freshwater cycle in a natural photoperiodic regime or with continuous daylight are similar. In these 2 cases, it is possible that the lack of alternating light/dark during a long period leads to a negative effect unlike in our situation where alternance exists. Generally, the relative influence of temperature and photoperiod is difficult to establish (Clarke *et al.*, 1981). However, in our experiment, the weight and length observed in the two conditions SPCT and CPST [*i.e.* "constant regime of one factor (P or T) × simulated shortened regime of the other factor (T or P)"] were similar, and there was an intermediate value between the cases where the factor time-courses were the same (CPCT and SPST). This suggests, in spite of the temperature differences (and including the smoltification phase that represents an important part of the total rearing period), that the photoperiod could have a significant influence on the growth, possibly of the same magnitude as temperature.

As generally observed in the first autumn of the biological cycle of Atlantic salmon by several authors (Thorpe, 1977; Bailey *et al.*, 1980; Kristinsson *et al.*, 1985; Skilbrei, 1988; Stefansson *et al.*, 1989), a heterogeneous individual growth appeared in all our experimental groups and led to a bimodal structure of the populations even when the photoperiod and temperature were constant. This phenomenon results from an acceleration of the individual growth (Kristinsson *et al.*, 1985) when the fish reach a given size (Skilbrei, 1991) dependent on their genetic origin (Skilbrei, 1988) and under the control of environmental factors like photoperiod (Stewart *et al.*, 1990; Skilbrei, 1991). During our experiment, the fish reached a size sufficient for initiating this process very early (from June of the first year of rearing), much earlier than generally observed in Scotland, Norway, Canada by the above mentioned authors or in natural conditions in France (Gaignon, 1987).

The lowest ST growth rate led to a delayed length frequency segregation (*fig. 3 B*), with no effect on the upper subgroup percentages which were similar for CT and ST. This confirms the recent findings of Skilbrei (1991) who showed that temperature has no effect on length frequency distribution. Conversely, the percentages of the upper subgroup were higher for the constant photoperiodic regime (CP) than for

the simulated one as always observed by the authors studying the influence of photoperiodic regimes: an extension of received daylight would allow more fish to reach the initial size at which the fish enter the upper modal subgroup (Saunders *et al.*, 1987; Villarreal *et al.*, 1988; Saunders *et al.*, 1989; Stefansson *et al.*, 1989; Stewart *et al.*, 1990). However, in our case, an extended period of individual increased growth rate would not exist for CP: the length frequency segregation appeared at the same time for CP and SP. The lack of individual branding does not allow one to know if the initial size at which the growth rate increases is similar for all the conditions, nor if the growth rate for a particular length frequency is different according to the regimes (Stewart, 1990).

The upper-mode fish initiate their smoltification during their first year of life and this confirms that, even in our experimental situation, these fish are potential smolts as observed on the normal production cycle of 15-17 months (Thorpe, 1977; Thorpe *et al.*, 1980; Kristinsson *et al.*, 1985; Bœuf *et al.*, 1985).

The smoltification began very early: in all the cases there was an important increase of gill ($\text{Na}^+\text{-K}^+$)-ATPase before mid September of the first year of rearing (or after less than 230 days or 9 months of feeding); the condition CPST (constant photoperiod) gave a high level of enzymatic activity during the first fortnight of August (D 200 or 7 months). Hoar (1988) after Eriksson and Lundqvist (1982) concluded that, when there is no variation of the environmental factors (constant temperature: 11°C; day length: 12 hours), change in the body's silvery coloration, darkening of the fin margins, variation of the condition factor and of the growth rate show the existence of an endogenous rhythm with a 10-month period. The external factors are considered, in natural conditions, as a "brake" on this rhythm and in controlled conditions as a "zeitgeber" of the development. In our experiment, the absence of photoperiod variations (CPCT condition for example) does not prevent the smoltification that starts after 7 months of feeding. The growth rates obtained here with more favourable temperature and photoperiod than the ones by Eriksson and Lundqvist are probably the reason for precocious smoltification. This shows the importance of the external factors and, among these, the level of temperature, without thermic variation, on all the process leading to the "first" smoltification.

The gill ($\text{Na}^+\text{-K}^+$)-ATPase activity evolution is photoperiod-dependent. A progressive increase of the day length has a progressive "driving" effect, giving here a linear answer on Atlantic salmon as already shown by Clarke *et al.* (1985) and Clarke *et al.* (1981) on Pacific salmon. The increase of the enzymatic activity with a constant photoperiod, also obtained by Duston and Saunders (1990*b*), shows that an increase of the day length is not necessary to initiate the smoltification. The activation can result from the larger size and so from the best growth rate of these

fish. A size threshold, as existing before leading to the bimodal structure of the population, might occur under some favourable external parameters. As suggested by Saunders *et al.* (1989), one can think that the temperature has a positive influence, but these authors consider that 10°C is a "high" temperature which can be beneficial. Furthermore, as already mentioned by Johnston and Saunders (1981), the temperature used (16°C) does not seem to be a limiting factor as estimated by Duston and Saunders (1990 *a, b*): in this last case, the thermic variations probably have a more negative effect than the absolute levels. On the other hand, a final decrease of the temperature (13°C) has a positive influence on the extension of the high level of the (Na⁺-K⁺)-ATPase activity as already showed on Atlantic salmon (Johnston and Saunders, 1981), steelhead trout and coho salmon (in Wedemeyer *et al.*, 1981). The highest temperatures do not tend to advance the physiological process as observed by Johnston and Saunders (1981).

In all the tanks, the maximum gill (Na⁺-K⁺)-ATPase activity of the fish was about 12-15 μm Pi mg Prot.⁻¹ h⁻¹. These values are generally observed on the upper-mode fish showing a good adaptive capacity to high salinity (Bœuf, 1987) but never reached by lower-mode fish having a low tolerance to high salinity. However, these values are slightly lower than the absolute values registered on 1⁺ smolts reared in natural conditions (Bœuf *et al.*, 1985) – with the same analytical methods. This suggests that the physiological process ending the smoltification state is not completely achieved. The lack of decreased condition factors (*fig. 6*) also confirms this point (adapted from Farmer *et al.*, 1978; McCormick *et al.*, 1987), even if we may admit that the high temperatures tend to favour an increase of the condition factor (Clarke *et al.*, 1981; Johnston and Saunders, 1981) and that the lack of normal winter duration does not allow a sufficient decrease in the weight growth rate compared with the length growth rate (Bjornsson *et al.*, 1989).

These absolute levels cannot be connected either with the long-term mortality or with the differences between the osmotic disturbance observed after transfer into the high salinity salt water for each condition. The lower osmotic disturbance is registered with the simulated photoperiod (SP). In the same way, Bjornsson *et al.* (1989) and Clarke *et al.* (1989) showed the beneficial influence of the presence of short day lengths. Moreover, the lack of significant difference between the two thermic conditions of the simulated photoperiod (*table 8*) masks physiological differences: there was no important osmotic modification (333 mOsm.l⁻¹) for SPST in comparison with that of SPCT (360 mOsm.l⁻¹). We suggest, as already show by Johnston and Saunders (1981) in different experimental conditions from ours, that the progressive increase of photoperiod and temperature, permitting a "progressive" smoltification, favours, in our case, the high-salinity tolerance, even if we may

consider that, according to the usual criterion, smoltification is not completely achieved. Bœuf *et al.* (1990) also obtained excellent survival and growth after a transfer to seawater following a growth hormone stimulation with low level of gill (Na⁺-K⁺)-ATPase activity (6.4) but increasing very quickly (×2 in 12 days). The discordance between the level of the enzymatic activity and the short-term high-salinity tolerance has also been recently observed by Saunders and Harmon (1990) with a precocious photoperiodic control. The large size of the fish (Johnston and Saunders, 1981; Duston and Saunders, 1990; Saunders and Harmon, 1990) and the high growth rate (Ewing *et al.*, 1980) may have eased the acquisition of the euryhalinity status without, however, permitting a high level of gill ATPase activity.

After three weeks in 35‰ salt water, the osmotic pressure and the growth of the fish were "normal" and confirmed that smoltification had been obtained after 7-8 months of rearing. These results are not concordant with the actual knowledge of many authors, *i.e.* it is necessary to have a natural increasing photoperiod over several months, permitting an "accomplished" smoltification and then a high-salinity tolerance and marine growth capacities (Isaksson, 1985; Saunders *et al.*, 1985; McCormick *et al.*, 1987; Stefansson *et al.*, 1989; Saunders *et al.*, 1985). However, the growth obtained during the first weeks of the marine phase does not seem to be dependent on the experimental conditions: as the large size of the fish could facilitate the high-salinity tolerance, it could have an influence on the growth after transfer to seawater.

In conclusion,

– The Atlantic salmon freshwater phase duration is essentially dependent on the growth and therefore on the level of the environmental factors (temperature). Its duration can vary, even with constant level of the external factors. The triggering of the smoltification is probably linked to the size and the growth rate at a given time.

– In these conditions, it is possible to obtain Atlantic salmon smolts with a good high-salinity tolerance after a short freshwater phase (7 to 8 months), quite different from the duration of the natural biological cycle.

– Under rearing conditions, when the production factor levels are very far from those observed in natural conditions, the absolute levels of the usual smoltification criteria may be discussed: the necessity of an accomplished smoltification can be compensated by a "smoltification and growth dynamics".

In spite of the interest of these results for Atlantic salmon smolt production, we must keep in mind the practical constraints for the transfer of such fish to seawater (artificial rearing and natural conditions are out of phase), and the possible effect on the long-term performances (growth and precocious maturation), which have not been assessed.

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