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## THE ROLE OF GROWTH HORMONE IN THE ADAPTABILITY OF ATLANTIC SALMON (*SALMO SALAR*) TO SEAWATER

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### ABSTRACT

Boeuf, G., A. Le Roux, A. Severe, P. Prunet, and P. Y. Le Bail. 1990. The role of growth hormone in the adaptability of Atlantic salmon (*Salmo salar*) to seawater, p. 125-131. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

Gill Na<sup>+</sup>,K<sup>+</sup>-ATPase activity of juvenile Atlantic salmon reared in fresh water during three successive years increased from February to April-May, while blood plasma GH levels increased strongly from the beginning of April and thereafter remained high. Direct transfer to full salinity seawater triggered a transitory increase in growth hormone (GH) at any time of year. A cholesterol implant containing ovine growth hormone (oGH) in presmolts (5-6 mo before migration of 0-age fish stimulated gill Na<sup>+</sup>,K<sup>+</sup>-ATPase activity, resulted in high survival in full salinity (35 o/oo) seawater, good osmotic regulation, and subsequent good growth. Implantations of oGH in small parr resulted in 36% survival after 8 wk in seawater, compared with 0% in controls. Possible roles of GH are discussed during parr-smolt transformation.

### RÉSUMÉ

Boeuf, G., A. Le Roux, A. Severe, P. Prunet, and P. Y. Le Bail. 1990. The role of growth hormone in the adaptability of Atlantic salmon (*Salmo salar*) to seawater, p. 125-131. In R. L. Saunders (ed.) Proceedings of Canada-Norway finfish aquaculture workshop, September 11-14, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1761.

L'activité Na<sup>+</sup>,K<sup>+</sup>-ATPasique branchiale de juvéniles de saumon atlantique maintenus en eau douce augmente régulièrement de février à avril-mai durant trois années successives tandis que le niveau circulant d'hormone de croissance (GH) s'élève plus ou moins brusquement à partir de début avril et ensuite se maintient à valeur plus forte. Des transferts directs à pleine salinité (35 o/oo) déclenchent une augmentation transitoire de la GH quelque soit le moment de l'année. Une implantation de GH ovine contenue dans de la poudre de cholestérol compactée a permis chez des pré-smolts de 1ère année (à plus de 5-6 mois de la migration) de stimuler l'activité ATPasique branchiale en eau douce, une excellente survie en eau salée (35 o/oo) après transfert, une bonne osmorégulation et une bonne croissance par la suite. L'implantation de petits parrs avec de la oGH au même moment nous a donné une survie de 36% après 8 semaines en mer comparativement à 0% pour les témoins. Nous discutons les implications possibles de la GH dans la smoltification.

## INTRODUCTION

The physiological, biochemical, and endocrinological changes occurring at smolting have been reviewed by Fontaine (1975), Wedemeyer et al. (1980), Boeuf (1987), and Hoar (1988). Smolts are able to tolerate direct transfer to seawater (35 o/oo) without osmotic disequilibrium, and continue to grow (Parry 1960; Boeuf 1987; Boeuf et al. 1989a). In contrast, juvenile salmon which have either lost or not attained smolt status are unable to adapt, survive, or grow in full strength seawater. Among the hormones involved in the parr-smolt transformation, growth hormone (GH) seems to play a major role. Treatments of fish with mammalian GH demonstrated a possible influence of this hormone on growth (Donaldson et al. 1979), smoltification, and seawater adaptation (Komourdjian et al. 1976; Clarke et al. 1977; Nagahama et al. 1982). With the advent of salmon homologous GH RIAs, Sweeting et al. (1985), Young et al. (1989) for coho salmon (*Oncorhynchus kisutch*), then Boeuf et al. (1989a) and Prunet et al. (1989) for Atlantic salmon (*Salmo salar*), demonstrated the occurrence of a GH surge at the end of smolting of these species in fresh water. After transfer of salmonids from fresh water to seawater, GH increases sharply some hours after transfer (Bolton et al. 1986; Boeuf et al. 1989a).

We studied the changes in circulating concentration of GH in juvenile salmon during smolting and after transfer to seawater, and also tested the effects of GH implants on seawater adaptability and subsequent growth of potential smolts and parr.

## MATERIALS AND METHODS

Fish used in the experiments were of a Norwegian strain of cultured salmon (1986, 1987, and 1988) introduced to the Le Conquet hatchery (Brittany, 48° N). Elorn River fish (natural population of Brittany) were used for the implant studies. The fish were reared in fresh water in Ewos tanks (4 m<sup>2</sup>) under natural photoperiod and temperature. River pH was 6.5-7.0 and temperature changes from 10°C in December to 5°C in February and 15°C in June (see Boeuf et al. 1989a, b for details). From September preceding the smolting year (7 mo old), upper modal group fish (Boeuf et al. 1985) were selected for experiments and maintained at a stocking density of 100/m<sup>2</sup> and fed automatically using dry pellets (SS1 IFREMER). The seawater transfers involved transporting the

fish to the Centre Océanologique de Bretagne in Brest, at least 2 wk before, where they were held in freshwater tanks identical to those used later with seawater. On the day of transfer, the freshwater (FW) supply was stopped, the seawater (SW) supply started, and the fish were in full seawater (34.5-35.6 o/oo) within 1 h.

Gill and plasma samples (from 10-25 fish) were taken both in FW and SW according to the methods described in Boeuf et al. (1985, 1989a). Gill Na<sup>+</sup>,K<sup>+</sup>-ATPase activity and blood osmotic pressure were measured as described in Lasserre et al. (1978) and Boeuf et al. (1989a). Plasma GH levels were measured according to the RIA described by Le Bail et al. (in press).

For the GH implant experiments, salmon were selected in the upper mode (presmolts, 129 mm) of the population for the October experiment, and both lower (parr, 89 mm) and upper (presmolts, 134 mm) modes in November. In each experiment, we used three groups of 50 salmon held in the same tank, either untreated (control), or intraperitoneally implanted (Higgs et al. 1975) with a compacted cholesterol powder pellet (sham) or with 250 µg of ovine GH (NIADK-oGH-14) in the same type of pellet. Each group was identified using the scar of the implantation and the presence or absence of adipose fin. Each pellet weighed 8-10 mg and released GH during 3-4 wk (Le Bail, unpubl. data). Fish were transferred to seawater, as previously described, after 11-12 d.

One-way ANOVA, SNK multiple range and Student's t-tests were used to assess the statistical significance in the data.

## RESULTS

### FRESH WATER

In each of the 3 yr of the study, gill microsomal Na<sup>+</sup>,K<sup>+</sup>-ATPase activity rose gradually from February (average length, 130-140 mm) to mid-April (140-165 mm), mid-May (160-190 mm) ( $P < 0.01-0.01$ ), and then decreased. Blood plasma GH was very low in February-March, then increased sharply from the beginning of April, and remained at a high level during May ( $P < 0.001$ ). In 1987, an acute surge occurred in April ( $P < 0.001$ ). In each year of the study, plasma GH remained at higher mean levels after the peaks than before (Fig. 1).

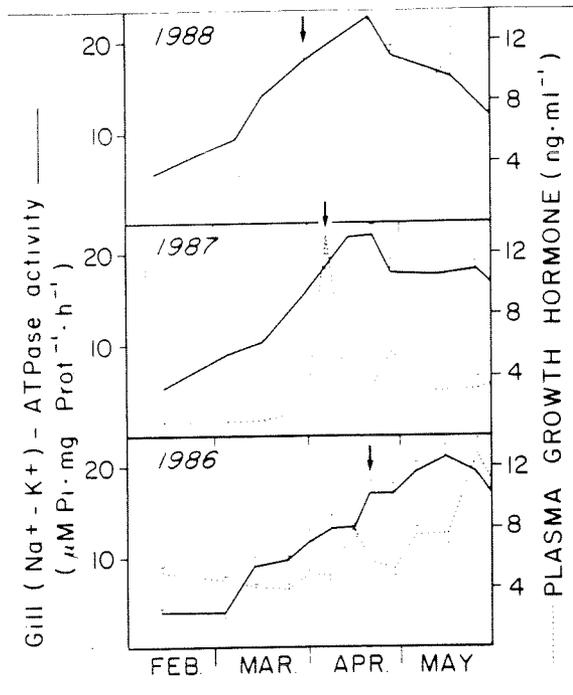


Fig. 1. Gill  $\text{Na}^+\text{,K}^+\text{-ATPase}$  activity and GH changes during the smolting period in freshwater Atlantic salmon reared in the hatchery of Le Conquet in 1986, 1987 and 1988. Data are mean  $\pm$  standard error of the mean for 10-25 fish. The arrows indicate the times of  $T_4$  surges (see Boeuf and Prunet 1985; Prunet et al. 1989; Boeuf et al. 1989a, b).

## SEAWATER

After seawater transfers in presmolting (March), smolting (April), and postsmolting periods (May), plasma GH increased significantly ( $P < 0.001\text{-}0.01$ ) and remained at a high level during 7 d, compared with that in fresh water. After 14 d, there were no significant differences between plasma GH levels in the fish transferred to seawater and freshwater controls (Fig. 2). New results obtained in 1989 confirmed these changes.

## IMPLANTATIONS

In each experiment, the control fish either did not survive transfer to seawater or, if they survived, they did not grow well. In contrast, GH implanted presmolts showed good adaptation to seawater, survival, and growth. Gill  $\text{Na}^+\text{,K}^+\text{-ATPase}$  activity doubled in 11-12 d ( $P < 0.01\text{-}0.001$ ) compared with controls. GH implanted fish maintained normal osmotic pressure. Implanted parr had much better survival than control fish and had good growth.

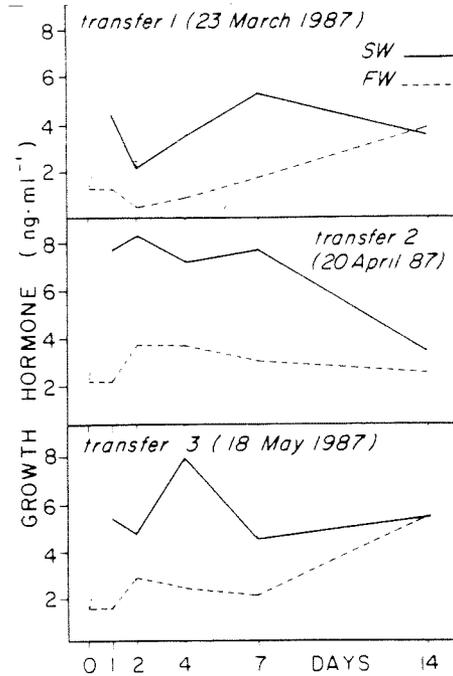


Fig. 2. Changes in blood plasma growth hormone levels after direct transfer of presmolts, smolts, and postsmolts of Atlantic salmon from fresh water to seawater at three dates. Data points show mean  $\pm$  standard error of the mean for 10 fish.

For both parts of the population (implanted parr and presmolts), no mortality occurred between 21 and 54 d in seawater.

## DISCUSSION

Few studies have been published on the role of GH during salmonid smoltification (Sweeting et al. 1985; Young et al. 1989; Boeuf et al. 1989a). In Atlantic salmon completing smoltification, GH peaks occur coincidentally with increases in  $T_3$  (Boeuf et al. 1989a; Prunet et al. 1989). In fish in short rivers, the first GH increase appears before, during, or after the  $T_4$  surge, 2-3 wk before the maximum gill ATPase level. In comparison, salmon from a long river (Loire-Allier in France, more than 1000 km), showed an increase of plasma GH much earlier in the year (February) and remained high until April (Boeuf et al., unpubl. data). These results suggest that in addition to having a role in osmoregulation, GH may also be involved in migration behavior.

Donaldson et al. (1979) reviewed the clear effects of mammalian GH on growth of salmonids and fish in general and, in addition, this hormone is also very active during smolting, an intense growth phase in salmonid life. In addition to its growth stimulating effect, GH appears to play other roles during smolting: following transfer to seawater, there are significant increases in plasma GH levels, suggesting that this hormone is involved in osmoregulation. These increases are independent of season. GH injections or implants (Richman and Zaugg 1987) (Table 1, 2) are more effective than thyroid hormone (TH) treatments (by injections, in the food, in water...) (Miwa and Inui 1985; Saunders et al. 1985; Boeuf, unpubl. data) in stimulating gill ATPase activity, and improving seawater adaptability (Komourdjian et al. 1976; Miwa and Inui 1985). Miwa and Inui (1985) specified that GH and  $T_3$  work synergistically to promote adaptation to

seawater. GH is also very active on peripheral deiodination of  $T_4$  to  $T_3$  (De Luze and Leloup 1984) and it may be that this phenomenon is of fundamental importance during parr-smolt transformation.

In the November experiment (Table 2), the effects of oGH implantation are very clear; treated fish showed a stimulation of gill ATPase activity, control of osmotic disequilibrium after direct full salinity seawater contact, and grew well in seawater. The idea of using GH treatment to stimulate seawater adaptability is not new but previous studies required long series of injections which were very stressful and did not allow the fish to feed normally. However, by injections of GH, Komourdjian et al. (1976) and Clarke et al. (1977) obtained a better adaptation to low salinity seawater (29 or 30 o/oo) than in controls or sham injected salmon. More recently, bGH implants stimu-

Table 1. Results (mean  $\pm$  standard error of the mean for eight fish) of three batches of Atlantic salmon presmolts after direct transfer to seawater (35 o/oo), 12 d after implantation (or not) with ovine growth hormone (oGH) on October 26 (E1). Weight and fork length are expressed in g and mm, plasma osmotic pressure in milliosmoles per liter and gill ATPase activity in micromoles of inorganic phosphate  $\cdot$  mg of protein $^{-1} \cdot$  h $^{-1}$  of incubation at 37°C. Fish were directly transferred to seawater (35 o/oo, 12°C) on November 7, 1988. Implants were of powdered cholesterol with (experimental) or without (sham) oGH.

		No manipulation (control)	Implanted "sham"	Implanted oGH (250 $\mu$ g)
Starting FW Oct. 26 1988	Weight	28.3 $\pm$ 0.8	27.7 $\pm$ 0.8	26.8 $\pm$ 0.7
	Length	130 $\pm$ 1	129 $\pm$ 1	129 $\pm$ 1
Day of transfer	Gill Na $^+$ ,K $^+$ -ATPase activity	3.1 $\pm$ 0.5	3.1 $\pm$ 0.2	6.4 $\pm$ 0.4
48 h seawater	Mortality	35%	20%	0%
21 d seawater	Mortality	75%	50%	0%
	Weight	27.1 $\pm$ 2.2 -4.3%	27.9 $\pm$ 1.3 +0.72%	34.0 $\pm$ 1.6 +26.9%
	Length	133 $\pm$ 3 +2.3%	133 $\pm$ 2 +3.1%	143 $\pm$ 2 +10.9%
	Osmotic pressure	345 $\pm$ 6	341 $\pm$ 4	340 $\pm$ 4
	Gill ATPase activity	14.2 $\pm$ 1.8	17.7 $\pm$ 1.5	20.4 $\pm$ 1.0

Table 2. Results (mean  $\pm$  standard error of the mean for eight fish) of three batches of Atlantic salmon presmolts and parr implanted (or not) on November 24 (E2). Fish were directly transferred to seawater (35 o/oo, 12°C) on December 5, 1988. Implants were of powdered cholesterol with (experiment) or without (sham) oGH.

		No manipulation (control)	Implanted "sham"	Implanted oGH (250 $\mu$ g)
Starting FW 12°C Nov. 24, 1988	Weight of presmolts	30.9 $\pm$ 0.4	30.8 $\pm$ 0.4	30.2 $\pm$ 0.3
	Length of presmolts	134 $\pm$ 0.4	135 $\pm$ 0.5	134 $\pm$ 0.4
	Weight of parr	8.1 $\pm$ 0.2	7.9 $\pm$ 0.2	7.9 $\pm$ 0.2
	Length of parr	89 $\pm$ 0.7	88 $\pm$ 0.5	89 $\pm$ 0.7
Day of transfer presmolts	Gill Na <sup>+</sup> ,K <sup>+</sup> -ATPase activity	3.6 $\pm$ 0.7	4.1 $\pm$ 0.6	7.1 $\pm$ 0.9
48 h in seawater	Mortality	24%	8%	0%
	Osmotic pressure	427 $\pm$ 10	405 $\pm$ 18	338 $\pm$ 6
7 d in seawater	Osmotic pressure	423 $\pm$ 8	383 $\pm$ 10	326 $\pm$ 3
21 d in seawater	Mortality	100%	93%	6%
	Weight	-	29.5 $\pm$ 1.5 -4.2%	34.0 $\pm$ 0.6 +12.6%
	Length	-	134 $\pm$ 2 -0.7%	147 $\pm$ 1 +9.7%
	Osmotic pressure	-	343 $\pm$ 8	318 $\pm$ 4
	ATPase activity	-	19.4 $\pm$ 7.9	25.0 $\pm$ 1.0
	54 d in seawater	Weight	-	-
Length		-	-	158 $\pm$ 1.7 +17.9%
Parr	Mortality 48 h	100%	88%	48%
21 d in seawater	Mortality	100%	100%	64%
	Weight	-	-	11.1 $\pm$ 1.1 +40.5%
	Length	-	-	100 $\pm$ 3 +12.4%
54 d in seawater	Weight	-	-	20.2 $\pm$ 2.7 +155.7%
	Length	-	-	119 $\pm$ 6.1 +33.7%

lated growth (Down et al. 1988) and increased gill ATPase activity (Richman and Zaugg 1987) but did not promote seawater adaptability. From our results, presmolts and parr responded to GH and adapted to high salinity (35 o/oo). Transfer of control fish to salt water, as already described (Parry 1960; Boeuf et al. 1989a), showed either high mortalities or poor growth.

In conclusion, oGH implants appear to trigger reactions in the fish which would normally develop only 5-6 mo later during smolting. They caused development of smolt characteristics, including stimulation of the gill ATPase activity, salinity tolerance, osmotic adaptation, and growth in high salinity. From the results, we cannot exclude a slight role of the cholesterol powder, this compound serving as, perhaps, a precursor of corticosteroids, which are also active in seawater adaptability (Hoar 1988).

These data show that GH plays a major role in the parr-smolt transformation phenomenon and seawater adaptation. Smolting salmonids develop, in fresh water, most of the systems they need for survival and growth in salt water; migration or seawater transfer may act only as a final stimulus to complete these processes. GH appears to facilitate some of these transformations, not only in terms of growth, but also in several changes occurring at this time.

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