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Stimulation of parr–smolt transformation by hormonal treatment in Atlantic salmon (*Salmo salar* L.)

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Abstract

Atlantic salmon reared under natural conditions in a fresh water (FW) hatchery at Le Conquet (Brittany, 48°N) were treated with different hormones in order to trigger or advance the parr–smolt transformation and to improve seawater (SW) adaptability. In the first experiment, juveniles were implanted with ovine growth hormone (oGH) pellets (7 μg/g body weight for pre-smolts, 25 μg/g for parr) and compared with sham-operated and control salmon at different times of the year. Different results were observed following direct exposure to full salinity (35‰) SW depending on the date of treatment. Prior to smoltification (November, December and February), pre-smolts treated with oGH had higher gill Na^+,K^+-ATPase activity in FW, lower plasma osmolarity after SW transfer, and higher growth rate in SW compared with sham and control fish. However, for pre-smolts and smolts treated during April, May or June, there was no difference between treated and untreated fish. At any time of year, oGH-implanted parr survived SW transfer better than sham and control fish. Using a recombinant trout growth hormone (rtGH) at lower doses (0.28 and 1.4 μg/g), a high percentage survival was observed after direct SW transfer for both pre-smolts (100% after 30 days in SW compared with 86% in sham) and parr (65% compared with 0%). rtGH is at least 10 times more efficient than oGH for increasing gill Na^+,K^+-ATPase and salinity tolerance. Treatments with cortisol (8 μg/g in silastic pellets), 3,5,3′-triiodo-L-thyronine (T_3, 20 mg/kg of food during 6 weeks), ovine prolactin (oPRL, 7 μg/g) and oGH (7 μg/g) were used on pre-smolts in October (8 months old). After direct SW exposure, only oGH-treated fish (alone or in combination with other hormones) were able to adapt, survive and grow. Both oPRL and oGH treatment increased gill Na^+,K^+-ATPase activity in fish in FW; however, there was no improvement of SW adaptability in oPRL-implanted salmon.

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1. Introduction

At a specific stage of development, young Atlantic salmon (*Salmo salar*) undergo internal and external transformations (smoltification) which pre-adapt them for survival and growth in the marine environment. As smolts, they begin active downstream migration to the sea. The completion of smoltification generally occurs in the spring. In artificial rearing, it is possible to produce 15-month-old smolts and transfer them to seawater (SW) during their second spring. Many attempts have been made to acclimate 0+-age fish (8–10-month-old pre-smolts) to SW. By changing the external parameters, especially photoperiod and temperature (Saunders et al., 1989; Gaignon and Quemener, 1992), it is possible to produce larger fish that are better able to adapt during their first autumn. This method is used in Norway, Canada, the UK and Chile. Another possibility for producing 0+-age smolts is to use hormonal treatment to induce the parr–smolt transformation earlier.

A few reviews have presented the general patterns of hormonal involvement in smoltification (Fontaine, 1975; Barron, 1986; Hoar, 1988; Boeuf, 1993), and the thyroid, pituitary, and interrenal play important roles. Many attempts have been made to improve SW adaptability using T_4 or T_3, but the results still remain equivocal in salmonids (see review in Boeuf, 1987, 1993). Cortisol has been shown to activate gill Na^+,K^+-ATPase activity and SW adaptability in Pacific and Atlantic salmon (Specker, 1982; Madsen, 1990a; Bisbal and Specker, 1991; McCormick et al., 1991a). Prolactin (PRL) is important in mucus production and FW adaptation, although the pituitary is not necessary for survival of salmonids in FW (reviewed by Hirano et al., 1987). In several species of salmonids, growth hormone (GH) improves SW adaptability (Komourdjian et al., 1976; Clarke et al., 1977; Collie et al., 1989; Boeuf et al., 1990a, b; Madsen, 1990a).

In this study, the possibility of advancing smoltification and improving SW adaptability using hormones in 0+-age fish was examined.

2. Material and methods

Atlantic salmon (*Salmo salar*) came from the hatchery of Le Conquet (Brittany, 48°N) from a strain of the Elorn River. They were reared in FW under natural conditions in Ewos tanks (4 m^2) at a density of 2 kg/m^2. Temperature rose from 6°C in February to 18°C in August. Fish were fed commercial dry pellets using automatic feeders.

Fish were transported to the Centre IFREMER of Brest (17 km from Le Conquet) at least 3 weeks before the beginning of each experiment and maintained in the same type of tank (Ewos, 4 m^2). The transfer from FW to SW occurred by turning off the FW supply and turning on SW (salinity 35.5‰; it took about 1 h to change from FW to full salinity SW).

Pre-smolts are fish in the upper modal group (of a length-frequency distribution) and parr are fish in the lower modal group (Thorpe, 1977; Boeuf et al., 1985).

The general conditions of the experiments are described in Tables 1 and 2. For the first experiment, pre-smolts ($n=100$) and parr ($n=50$) were divided into three groups: control fish (untreated); "sham-operated" fish, implanted with a small, compacted cholesterol pellet (10 mg) in the peritoneum; and treated fish implanted with the same type of cholesterol pellet but containing 250 μg of oGH (NIADK-oGH-14; Boeuf et al., 1990a). Growth hormone administered by pellet released the hormone for a period of 2–3 weeks (Fig. 1). In contrast, injection of oGH resulted in hormone elevation for only 2 days. Pellet implantations were used in all subsequent studies. Fish were treated at different times of the year (Table 1); after 12 days they were exposed to SW. Fish from each group were kept in the same tank; groups were distinguished from one another by an adipose fin clip and the implantation scar. During the first month there was no difficulty in recognizing treatment groups. For longer periods, fish were placed in separate tanks.

Table 1
General conditions of the first experiment ($T°$, temperature)

Date of transfer to seawater	FW $T°$ (°C)	SW $T°$ (°C)	Initial		Duration (days)
			weight (g)	length (mm)	
Pre-smolts					
2 May 1988	12	12	51.5±5	174±2	21
7 Nov. 1988	12	12	26.8±0.7	129±1	21
5 Dec. 1988	12	12	30.2±0.3	134±1	54
20 Feb. 1989	10	10	36.6±0.5	144±1	56
19 Apr. 1989	13	12	47.9±0.5	162±1	83
19 Jun. 1989	14	16	70.9±2.1	188±2	101
Parr					
6 Jun. 1988	12	12	20.0±1.5	116±3	101
5 Dec. 1988	12	12	7.9±0.2	89±1	54
20 Feb. 1989	10	10	8.8±0.2	95±1	56
19 Apr. 1989	13	12	12.2±0.2	100±1	83
19 Jun. 1989	14	16	20.3±0.5	117±1	101

Table 2
General conditions of experiments 2 and 3 ($T°$, temperature in °C)

SW transfer	FW $T°$	SW $T°$	Initial weight (g)	Initial length (mm)
26 Oct. 1991	12	12	38.3±1.4	147±2
29 Jan. 1992	10	10	35.2±1.3	141±1

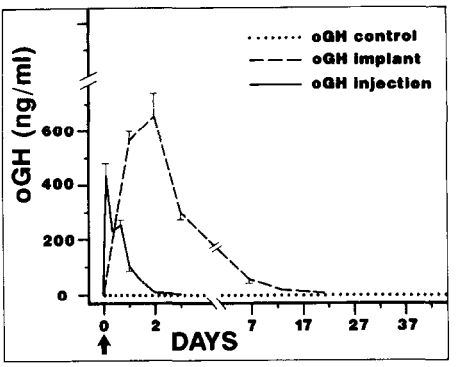

Fig. 1. Plasma levels of oGH in Atlantic salmon juveniles following a single injection or cholesterol implant (for each point, $n=8$). Control fish had undetectable levels (0 ng/ml) of oGH.

The second experiment was conducted under similar conditions. The same type of pellet was used to treat fish with oGH or oPRL (NIADK-PRL-18). T_3 (3,5,3'-triiodo-L-thyronine, sodium salt, Sigma, St. Louis, MO) was dissolved directly in oil and mixed with commercial food pellets (20 mg/kg), prepared weekly and kept frozen until use. Salmon were fed 2% of body weight per day with automatic feeders for 6 weeks in FW before exposure to SW. This treatment resulted in significant increases in circulating T_3 (8–12 ng/ml, Boeuf, unpublished data). Cortisol was delivered by intraperitoneal silastic implants (Medical Grade Elastomere, Dow Corning 382, 1.5 cm length, 3 mm diameter) containing 100 mg of cortisol (8–9 μg/g). This method typically results in maximum steroid release after 3 days (60 ng/ml) and disappearance after 4 weeks. The fish were exposed to SW 12 days following implantation. The efficacy of hormone delivery was estimated according to the methods of Le Bail et al. (1991), Prunet et al. (1985), and Boeuf and Prunet (1985), respectively for GH, PRL and T_3. Combined treatments corresponded to the use of oPRL, T_3, or cortisol in combination with oGH.

For the third experiment, recombinant-trout GH supplied by Eurogentec (Belgium) was used at three doses (5, 10 and 50 μg/fish) in a cholesterol pellet. After 12 days the fish were exposed to SW.

Blood and gill tissue were sampled and measured for osmolarity and Na^+,K^+-ATPase activity, respectively, as described in Boeuf et al. (1985, 1989).

One-way ANOVA, Student's t-test, and Cochran's q-test for proportions were used to assess statistical significance of the data.

3. Results

3.1. Experiment 1

Survival of pre-smolts after exposure to SW was very high in April, May and June for all fish; there was no statistical difference in survival and growth be-

tween the three groups of fish (Fig. 2). In November and December, survival after 21 days in SW was higher in oGH-treated fish compared with control and sham-operated salmon ($P<0.001$). oGH-treated fish also grew well in SW (see Fig. 2, 54 days in SW). In February, although there was no statistical difference in survival, oGH-treated fish were significantly larger ($P<0.01$) after 56 days in SW.

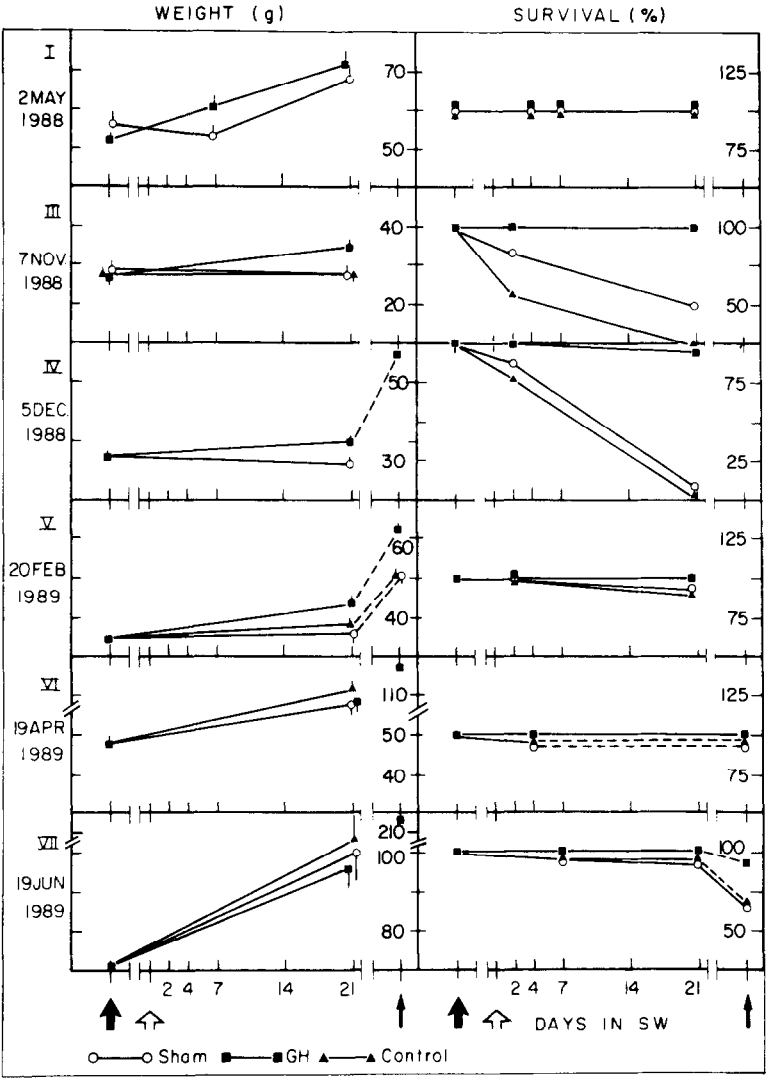

Fig. 2. Survival and growth of oGH-treated (7 μg/g) or control Atlantic salmon pre-smolts reared in FW and after exposure to SW (12 days after treatment). For weight, the expressed results are mean ± standard error ($n=50$ or all surviving fish). The large black arrow indicates the day of implantation, the white arrow the day of SW exposure and the small black arrow the end of the experiment (see Table 1).

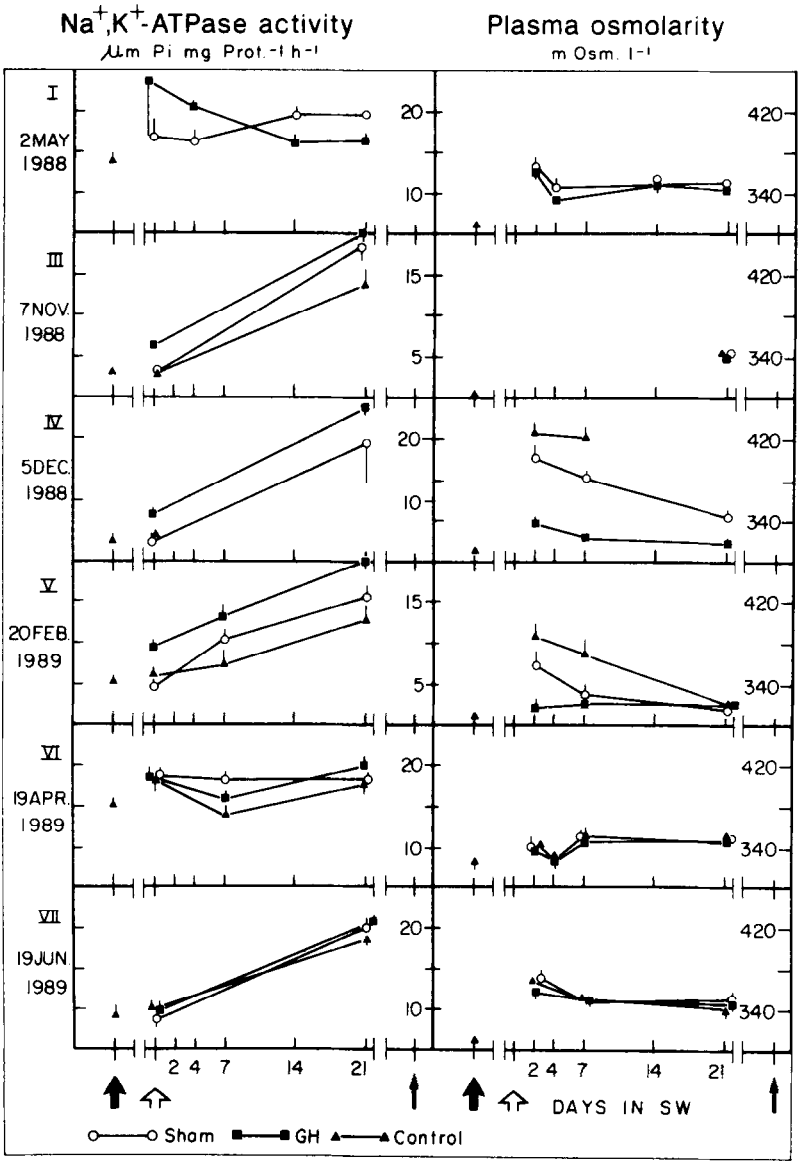

Fig. 3. Gill Na^+,K^+-ATPase activity (μmol P_i·mg protein^{-1} h^{-1}) and plasma osmolarity in oGH-treated (7 μg/g) or control Atlantic salmon pre-smolts reared in FW and after exposure to SW (12 days after treatment). The results are mean ± standard error ($n = 10$). The large black arrow indicates the day of implantation, the white arrow the day of the SW transfer and the small black arrow the end of the experiment (see Table 1).

After treatment with oGH (Fig. 3), gill Na^+,K^+-ATPase activity had increased in February, November, and December in FW fish just before exposure to SW. In December and February, the osmotic imbalance of salmon exposed to

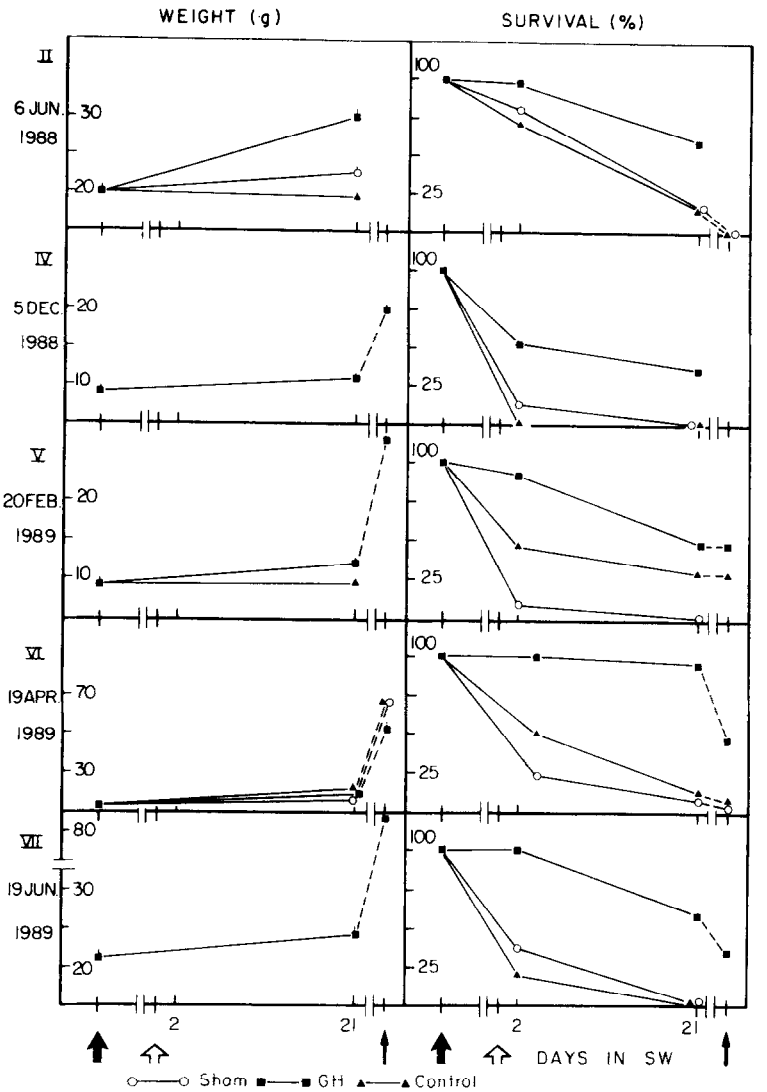

Fig. 4. Survival and growth of oGH-treated (25 μg/g) or control Atlantic salmon parr reared in FW and after exposure to SW (12 days after treatment). For weight, the results are mean ± standard error (n=all surviving fish). The large black arrow indicates the day of implantation, the white arrow the day of SW exposure and the small black arrow the end of the experiment (see Table 1).

SW was less in oGH-treated fish. In April, May and June, there were no statistical differences between the three groups for the gill enzyme activity and plasma osmolarity; all fish had high gill Na^+,K^+-ATPase activity in FW and low plasma osmolarity after SW exposure.

When treated with oGH, parr were always better able to survive and grow during 21 days following SW transfer than control fish (Fig. 4). Survival of control

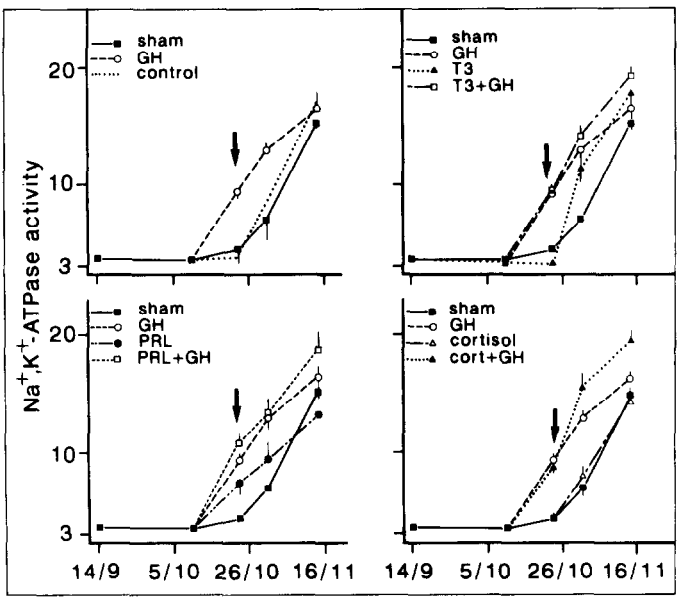

Fig. 5. Gill Na^+,K^+-ATPase activity (μmol P_i·mg protein^{-1} h^{-1}) in control and hormone-treated Atlantic salmon pre-smolts reared in FW and after exposure to SW. The results are mean ± standard error ($n=10$). The black arrow indicates the day of SW exposure.

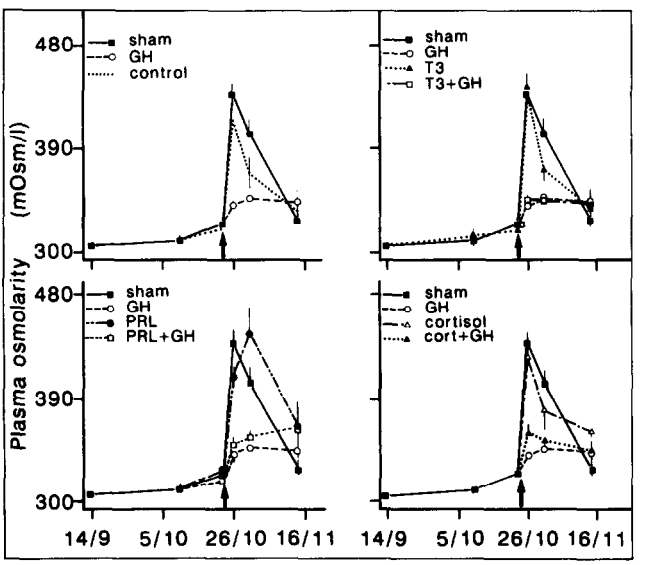

Fig. 6. Plasma osmolarity in control and hormone-treated Atlantic salmon pre-smolts reared in FW and after exposure to SW. The results are mean ± standard error ($n=10$). The black arrow indicates the day of SW exposure.

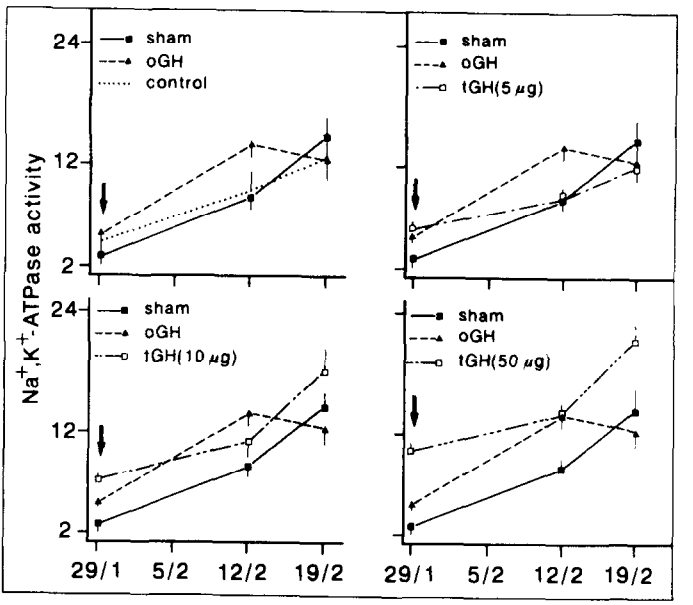

Fig. 7. Gill Na^+,K^+-ATPase activity (μmol P_i·mg protein^{-1}·h^{-1}) in control, oGH (250 μg/pellet) and rtGH-treated (3 doses) Atlantic salmon pre-smolts reared in FW and after exposure to SW. The results are mean ± standard error ($n=10$). The black arrow indicates the day of SW exposure.

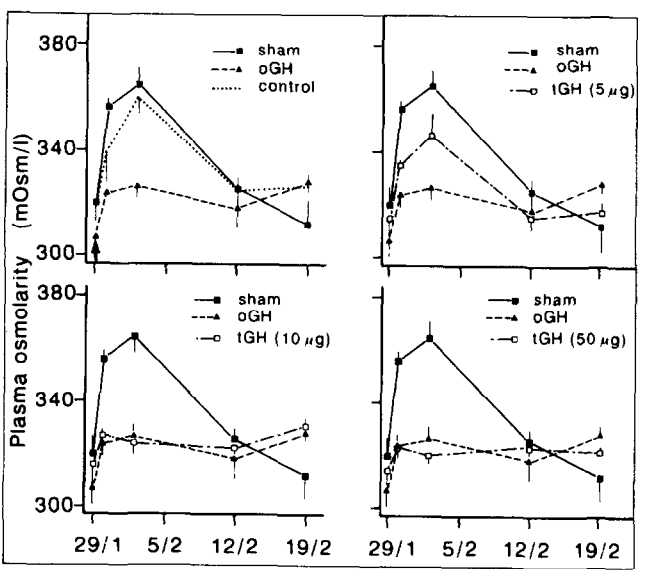

Fig. 8. Plasma osmolarity in control, oGH (250 μg/pellet) and rtGH-treated (3 doses) Atlantic salmon pre-smolts reared in FW and after exposure to SW. The results are mean ± standard error ($n=10$). The black arrow indicates the day of SW exposure.

Table 3
Survival of the different groups in experiments 2 and 3 (in % after 30 days in SW)

Experiment 2								
Control	Sham	oGH	T_3	T_3/oGH	oPRL	oPRL/oGH	Cor	Cor/oGH
81.6	73.6	98.9	89.0	100	75.2	94.3	87.1	91.7

Experiment 3					
Control	Sham	oGH	rt GH 5 μg	rtGH 10 μg	rtGH 50 μg
89.0	85.8	100	95.0	100	100

and sham-operated salmon was 0–20% after 21 days in SW. After 3 weeks in SW,oGH-treated fish continued to grow, but survival was variable after long periods in SW, 45% after the 20 February transfer (56 days), 44% after the 19 April transfer (86 days), and 35% after the 19 June transfer (101 days).

3.2. Experiment 2

Treatment with cortisol or T_3 did not modify gill Na^+,K^+-ATPase activity of fish in FW (Fig. 5). After exposure to SW, the levels in all groups increased. After treatment with oPRL or oGH, enzyme activity increased in fish in FW. Treatment of pre-smolts with oGH (alone or in combination with another hormone) resulted in a significant increase in gill enzyme activity ($P<0.01$ compared with sham-operated salmon).

After SW exposure, control-, cortisol-, oPRL- and T_3-treated fish suffered an acute osmotic disequilibrium; surviving fish needed at least 3 weeks to return to normal levels for fish in SW (Fig. 6). Salmon treated with oGH alone or in combination showed only a small osmotic imbalance after exposure to SW.

3.3. Experiment 3

Doses of rtGH of 0.28 and 1.4 μg/g body weight (10 or 50 μg/pellet) led to greater increases in gill Na^+,K^+-ATPase activity than 250 μg/pellet oGH ($P<0.01$, Fig. 7). Although enzyme activity was higher, SW adaptability was identical to that of oGH-treated fish. After exposure to SW (Fig. 8), control and sham-operated salmon had high plasma osmolarity, while rtGH-treated fish (10–50 μg/pellet) and oGH-treated (250 μg/pellet) pre-smolts had only a 8–10% increase in plasma osmolarity. Therefore, rtGH appears more active than oGH, acting at lower doses. General data for survival for the last two experiments are expressed in Table 3.

4. Discussion

From the first experiment it is evident that the responsiveness of Atlantic salmon to oGH treatment is variable, depending on the time of the year and the physio-

logical status of the fish. Gill microsomal Na^+,K^+-ATPase activity and salinity tolerance increase in smolts between January and May (Saunders and Henderson, 1978; Boeuf et al., 1985). A clear response to oGH treatment was observed in pre-smolts in winter and early spring. Similarly, oGH treatment of parr was always effective in increasing gill Na^+,K^+-ATPase activity. However, no response was obtained in April, May, and June (smolts) when gill Na^+,K^+-ATPase activity and salinity tolerance were high in controls, as a result of natural developmental changes. Some of this variability may be due to the natural completion of smoltification during which plasma GH levels are elevated (Boeuf et al., 1989) and the fish are able to adapt and grow in seawater. Sheridan (1989) has shown that the response of metabolic parameters of smolts to exogenous hormone treatments is lower than that of parr; the reduced sensitivity of lipid metabolism in smolts may be due to their progressed state of lipid depletion.

GH naturally increases during smolting in Atlantic salmon (Boeuf et al., 1989; Prunet et al., 1989; Boeuf and Le Bail, 1990), and this hormone plays a major role during SW adaptation of salmonids (Bolton et al., 1987; Boeuf et al., 1990b; Sakamoto et al., 1990, 1991). To explain this action of GH on osmoregulation and smoltification, different hypotheses have been proposed, including the "growth effect" (increased SW tolerance as a result of greater body size), T_4–T_3 conversion, cortisol activation, or a more direct effect, possibly mediated by IGF-I (McCormick et al., 1991b). It is also possible that GH could play a direct role through GH receptors of the gill (Sakamoto and Hirano, 1991). This study showed that parr or pre-smolts respond more effectively than smolts to exogenous GH treatment.

Although both ovine and recombinant trout GH were efficient in increasing gill Na^+,K^+-ATPase activity and SW adaptation, no effect of T_3 or cortisol was seen in this study. For T_3, many attempts have been made to improve SW adaptability, and although a few authors have obtained positive effects, most have not (see Boeuf, 1987, 1993). There are also conflicting results regarding the effect of cortisol on salinity tolerance of salmonids. In Pacific species (Richman and Zaugg, 1987; McCormick and Bern, 1989; Madsen, 1990b), cortisol stimulated gill Na^+,K^+-ATPase activity and improved SW adaptability. The same patterns were found in brown trout (Madsen, 1990a). In Atlantic salmon, Langdon et al. (1984) were unable to demonstrate an effect of cortisol on gill Na^+,K^+-ATPase activity. In the present work, using a silastic implant, no response was found to cortisol. However, Bisbal and Specker (1991), using implants with a maximum release of 160–170 ng/ml, found significant effects of cortisol on gill Na^+,K^+-ATPase activity. As with GH, differences in responsiveness to cortisol may occur seasonally or according to the developmental status of the fish (McCormick et al., 1991a).

Previous studies have demonstrated a role for prolactin in adaptation of salmonids to FW (Prunet and Boeuf, 1985; Hirano et al., 1987; Sakamoto et al., 1991). In the present study, prolactin treatment resulted in increased gill Na^+,K^+-ATPase activity but not (as might be expected from high enzyme levels) improved osmoregulation in SW. The oPRL-treated salmon had high osmotic disturbances in saltwater; oGH co-implantation presented this imbalance. Madsen

and Bern (1992) have reported on antagonism between PRL and GH in SW adaptation of brown trout and coho salmon.

In addition to establishing that mammalian GH stimulates SW adaption, the present study shows that rtGH is also effective at relatively low doses. The dose–response effect of rtGH was clear (Fig. 8) indicating a minimum effective dose of 0.28 μg/g. Previous studies (Boeuf, unpublished data) indicate that the minimum effective dose of oGH is 5–7 μg/g, showing that rtGH is at least 10 times more efficient than oGH in vivo. In vitro studies of rainbow trout GH receptors indicate that rtGH is bound with an affinity 30-fold higher than that for oGH (Yao et al., 1991).

In conclusion, GH treatment increases gill Na^+,K^+-ATPase activity and SW adaptability in Atlantic salmon, as in other salmonid species. The smolt is characterized by a high level of gill, Na^+,K^+-ATPase enzyme activity in FW (prior to downstream migration) and the capacity to acclimate to SW with minimal osmotic disturbance and to survive and grow in SW. GH implantation (with both mammalian and recombinant-fish hormone) seems to "mimic" the parr–smolt transformation in pre-smolts. Following GH-treatment, fish which are larger than 25 g are able to survive and grow in 35‰ salinity 6 months prior to natural smolting. The SW adaptability and growth of small parr (8–10 g fish) can also be improved by GH treatment, though not to the same extent seen in larger fish.

5. Acknowledgements

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