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Changes in some endocrinological and non-specific immunological parameters during seawater exposure in the brown trout

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The influence of autumnal progressive and direct seawater transfers on ionic parameters, plasma growth hormone (GH) and thyroid hormones (TH) and also on the non-specific immune traits phagocytic activity, lysozyme and non-specific cytotoxicity were examined in 45–55 g brown trout (*Salmo trutta*). In both experiments, the seawater transfer induced the same pattern of endogeneous modifications but they were more pronounced and more lasting after the direct seawater transfer than after the progressive one. In seawater-transferred trout, there was a significant transitory increase of the plasma osmolarity, chloride concentration, GH levels and a transient decrease of the TH. The phagocytic activity of the pronephric leucocytes and the lysozyme concentrations were significantly higher in seawater-transferred trout than in controls. Nevertheless, the non-specific cytotoxicity should not be modified after the seawater exposure. Moreover significant positive correlations were observed between plasma GH and chemiluminescence or lysozyme increases. These data support the hypothesis that GH is involved in the salmonids' non-specific immune potential, especially by stimulating the macrophage functions.

Key words: salmonids; *Salmo trutta*; endocrinology; growth hormone; non-specific immunology; phagocytic activity.

INTRODUCTION

Salmonids display a variety of patterns in hypo-osmoregulatory abilities. The anadromous salmonid species develop hypo-osmoregulatory capacities with hormonal modifications during the seasonal process of smolting prior to migration to sea water (SW) (Boeuf, 1993), while in non-migratory forms, such as brown trout *Salmo trutta* Fario, SW tolerance is size-dependent (Dickhoff, 1992). SW transfer induces ionic and osmotic imbalance at first, which is regulated, in a successful transfer, by an increase of the gill (Na-K)-ATPase, secreting excess salts (Boeuf & Harache, 1982, 1984). Hypo-osmoregulatory ability has been reported to be under endocrine control (Dickhoff, 1992). Indeed, both smolting and entry into SW are accompanied by increases in plasma growth hormone (GH) levels (Boeuf *et al.*, 1989; Sakamoto *et al.*, 1990) and cortisol (Franklin *et al.*, 1992). The thyroid hormones (TH) plasma concentrations are unaffected or decrease after SW transfer (Boeuf & Le Bail, 1990).

Furthermore, there is growing evidence of a marked interaction between the endocrinology and immunology of mammals (see for review Kelley, 1989; Weigent & Blalock, 1989). Among other immune influences, pituitary GH has

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been reported to stimulate macrophage functions (Kelley, 1989). Similarly, even if the results are more controversial, TH seem to be active on the immunological potential in mammals (Fabris, 1973) and also in birds, among others by enhancing the natural cell mediated cytotoxicity (Haddad & Marshaly, 1991). In salmonids, relationships between endocrinology and immunology have been observed, especially with cortisol largely described as an immuno-suppressive hormone (Tripp *et al.*, 1987; Pickering *et al.*, 1989). More recently, Kajita *et al.* (1992) reported that GH stimulated the natural cytotoxicity of the rainbow trout *Oncorhynchus mykiss* (Walbaum) spleen, blood and head kidney leucocytes. Possible immune modifications could be considerable when fish are confronted with a new and potentially pathogenic microbiological environment in SW. The non-specific immune process, including phagocytose, lysozyme activity and the non-specific (natural) cytotoxicity constitute a significant step of the immune response and is consequently relevant in fish disease resistance.

The purpose of this study was to investigate in brown trout the effects of progressive and direct SW exposure on physiological osmotic and ionic regulation, on endocrine status of GH and TH and also on non-specific immunological potential: phagocytose, lysozyme concentration and natural cytotoxicity. Furthermore, from the results, this study will try to clarify the relationship between some endocrinological and immunological elements in the resident salmonid, the brown trout.

MATERIAL AND METHODS

FISH AND EXPERIMENTAL PROTOCOL

Female brown trout, 10 months old, weighing 45–55 g were obtained from the SEMII hatchery (Le Drennec, Brittany, France) and transferred to the laboratory 2 weeks before the study began.

Two experiments were conducted. For each experiment 360 fish were divided equally between six 100-l tanks (Ewos). Fish were fed to satiety with a commercial pellet (Trouw, Trouvit 2 or 3 mm). Temperature and photoperiod were natural throughout the study (see later).

During the first experiment, half of the animals (three tanks) were transferred from fresh water (FW) to SW in two steps: they were transferred directly to a salinity of 29 ± 1‰ during 1 week, and then to 35 ± 1‰. The other half of the fish (three tanks) was maintained in FW and used as controls. The experiment began on 26 October and was carried out for 3 months. During this time, the temperature gradually decreased, from 12° C in October to 10° C in November in FW and from 12 to 10·5° C in SW.

The second experiment started on 7 December and was also carried out for 3 months. Half of the fish (three tanks) were transferred directly from FW to SW at 35 ± 1‰. The others were used as controls and maintained in FW. During this experiment, the temperature decreased from 10 to 5° C in FW and from 10 to 7° C in SW.

In both experiments, handling stress was avoided by turning off the FW supply of the tank and turning on the SW supply in the same tank.

SAMPLING

Timetable

The fish were sampled 8 days before (day −8) the SW transfer, just before on day 0 and 1, 2, 4, 7, 21 and 90 days after the SW transfer. On days 0, 1 and 4, four fish were

sampled in each tank to collect the plasma. On days −8, 2, 7, 21 and 90, six fish were sampled per tank both to harvest plasma and immune organs.

Biometric parameters

Each sampled fish was measured from the head to the tail-fork and weighed to calculate the condition factor according to the following formula:

$$K=[P(\mathrm{g})/L^3(\mathrm{mm})] \times 10^5.$$

Blood collection

Each fish, starved for 24 h, was killed rapidly by a blow to the head and the blood was collected immediately from the caudal vessels by means of a heparinized Vacutainer. Plasma samples were prepared by centrifugation at 2000 *g* for 15 min at 4° C and frozen at −20° C in aliquots until analysis. In each type of water, there were 12 samples on days 0, 1 and 4, and 18 samples on days −8, 2, 7, 21 and 90.

Gill samples

On days 0 and 21, the gill filaments were removed by dissection, rinsed with 0·25 M sucrose, pH 7·4, and stored rapidly in liquid nitrogen.

Cell preparation procedure of the immune organs

The head kidney and/or the spleen of six fish, both controls and those transferred to SW, were removed by dissection and used immediately. The cell suspension of each organ was prepared by grinding tissue through a metallic filter (Collector Belco—100 μm). The cells, which were suspended in Hank's balanced salt solution without phenol red (HBSS) or in RPMI 1640 Dutch modification, according to the studied immunological parameter, were adjusted to the appropriate concentration of viable cells employing a Thoma's haemocytometer by Trypan blue exclusion.

ANALYTICAL PROCEDURE

Plasma parameters

Ionic and osmotic parameters. Plasma osmolarity was measured with an Advanced Instrument osmometer (mosmol l^{-1}) and plasma Cl^-, by argentimetric titration with a Radiometer, model CMT 10 (mequiv. l^{-1}).

Endocrinal parameters. Plasma concentrations of tri-iodothyronine (T_3) and thyroxine (T_4) were determined by radioimmunoassay (Boeuf & Prunet, 1985). Plasma growth hormone (GH) levels were measured by specific homologous radioimmunoassay (Le Bail *et al.*, 1991).

Lysozyme assay. Plasma lysozyme activity was assayed according to the method described by Muona & Soivio (1992) with modifications. Briefly, the assay is based on the decrease in optical density at 450 nm of a *Micrococcus lysideikticus* suspension after incubation for 15 min with the plasma. For each assay a standard curve was made with lyophilized hen egg white lysozyme (Sigma L-6876). The results are expressed as μg ml^{-1} equivalent of hen egg white lysozyme activity.

Branchial (Na^+-K^+)-ATPase. Gill (Na^+-K^+)-ATPase was determined according to the method used by Lasserre *et al.* (1978).

Cellular immunological parameters

Phagocytic activity measured by chemiluminescence. Chemiluminescence (CL) assay was performed according to the method described by Scott & Klesius (1981) modified as Obach & Baudin Laurencin (1992) using opsonized zymosan adjusted to 5 mg ml^{-1}.

Chemiluminescence was measured at 20° C every 5 min for 20 min and then, every 10 min up to 60 min on a 1250 LKB luminometer. Only the maximal values of CL observed during this time were used as results.

Natural cytotoxicity assay. Lymphocyte isolation procedure: the head kidney and splenic cells, suspended in RPMI 1640 supplemented with antibiotics (penicillin 450 U ml^{-1} and streptomycin 75 mg ml^{-1}) and 2 nM of 2-mercapto-ethanol, were layered on top of a 3 ml Ficoll-plaque (Histopaque-1077, Sigma) and centrifuged at 600 *g* for 30 min at 20° C. The lymphocytes were collected at the RPMI/Ficoll interface and washed twice. The final lymphocyte suspension was adjusted to $5 \cdot 10^6$ viable lymphocytes ml^{-1} in RPMI 1640 supplemented with 2 mM L-glutamine and 10% foetal calf serum.

Cytotoxicity assay: the lymphocytes were used as effector cells and a rainbow trout cell line (RTH, rainbow trout hepatoma, Fryer *et al.*, 1981) were used as target cells. The same volumes of the target cells ($5 \cdot 10^4$ cells ml^{-1}) and effector cells ($5 \cdot 10^6$ cells ml^{-1}) were added to culture tubes. For each fish organ, the tubes were duplicated. After centrifugation (200 *g*, 5 min at 20° C), the tubes were incubated 18 h at 22° C in an airtight gas box containing 5% CO_2. Triplicated control tubes without effector cells were prepared at each assay. The percent of cytotoxicity was calculated using the following method:

$$\% \text{ cytotoxicity} = [(A - B)/A] \times 100 \text{ (Angelidis, 1987)}$$

where A is the number of viable RTH cells in controls and B is the number of viable RTH cells in tubes with effectors cells. The number of the viable cells was determined by Trypan blue exclusion employing a Mallassez's haematocytometer.

STATISTICAL ANALYSIS

Concerning the osmolarity, chloride concentration, plasma TH levels and lysozyme concentration, data were subjected both to Student's test to compare FW and SW trout on the same day, and to one-way analysis of variance (ANOVA) together with Newman–Keuls multiple comparison procedure to assess the significance of a change during the experiment for a given environmental medium.

Plasma GH concentrations and cellular immunological parameters did not follow a normal distribution so the results were subjected to two non-parametric tests, the Mann–Whitney test, which compares FW and the SW transferred trout at the same time and the Kruskall–Wallis test followed by the Dunn test to assess the significance of a change throughout the experiment for a given treatment.

$(Na^+\text{-}K^+)$-ATPase activities and condition factor were submitted to the Student's test to compare both fish.

Linear regressions were used to correlate plasma GH levels after log transformation with chemiluminescence and lysozyme concentration.

RESULTS

BIOMETRIC PARAMETERS

Condition factors (Fig. 1) decreased transitorily after the progressive and direct SW transfers. These decreases which were significant ($P<0 \cdot 05$ Student's test) on day 1, 2 and 4 after progressive transfer, were more pronounced and more lasting, until day 7 after direct transfer. Moreover, condition factors of the SW transferred trout were significantly lower than those of the controls 90 days after transfer.

IONIC AND OSMOTIC PARAMETERS

The plasma osmolarity of the trout in FW was between 300 and 320 mosmol l^{-1}, for a chloride concentration between 120 and 140 mequiv. l^{-1} (Fig. 2). The

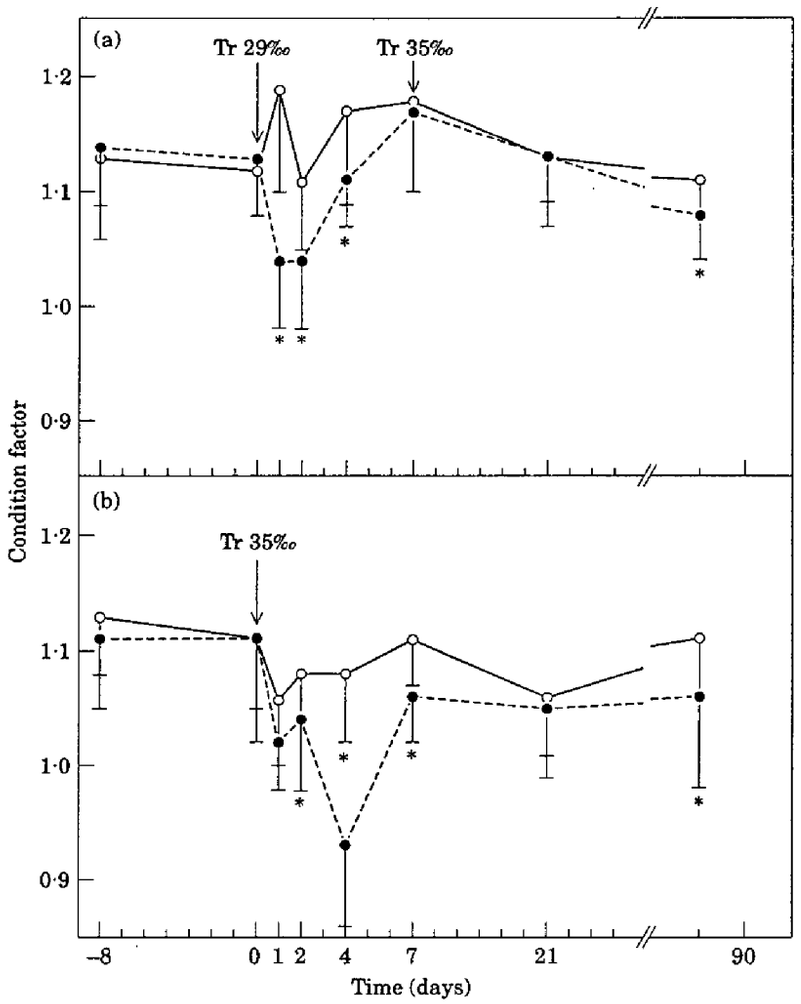

FIG. 1. Condition factor in freshwater-transferred (○) and (a) progressive and (b) direct seawater-transferred trout (●). Each point represents the mean ± S.E. of 12 or 18 fish. *, Significantly different at 5% level with the Student's test from corresponding control values.

SW transfer provoked a transient osmotic imbalance depending on the transfer, progressive or direct.

On the first day after the progressive SW transfer, plasma osmolarity and chloride levels increased significantly ($P<0{\cdot}05$, ANOVA and Student's test), 30% above FW values. Levels then decreased but stayed significantly higher than in controls 2 and 4 days after the transfer.

In direct SW transfer, plasma osmolarity and chloride levels rose significantly ($P<0{\cdot}05$, ANOVA and Student's test) from day 1 until day 4, 50% above FW values, before declining slightly. The osmotic balance was restored 21 days after transfer.

SW transfers, both progressive and direct resulted in a significant increase ($P<0{\cdot}05$, Student's test) of the branchial (Na^+-K^+)-ATPase (Table I).

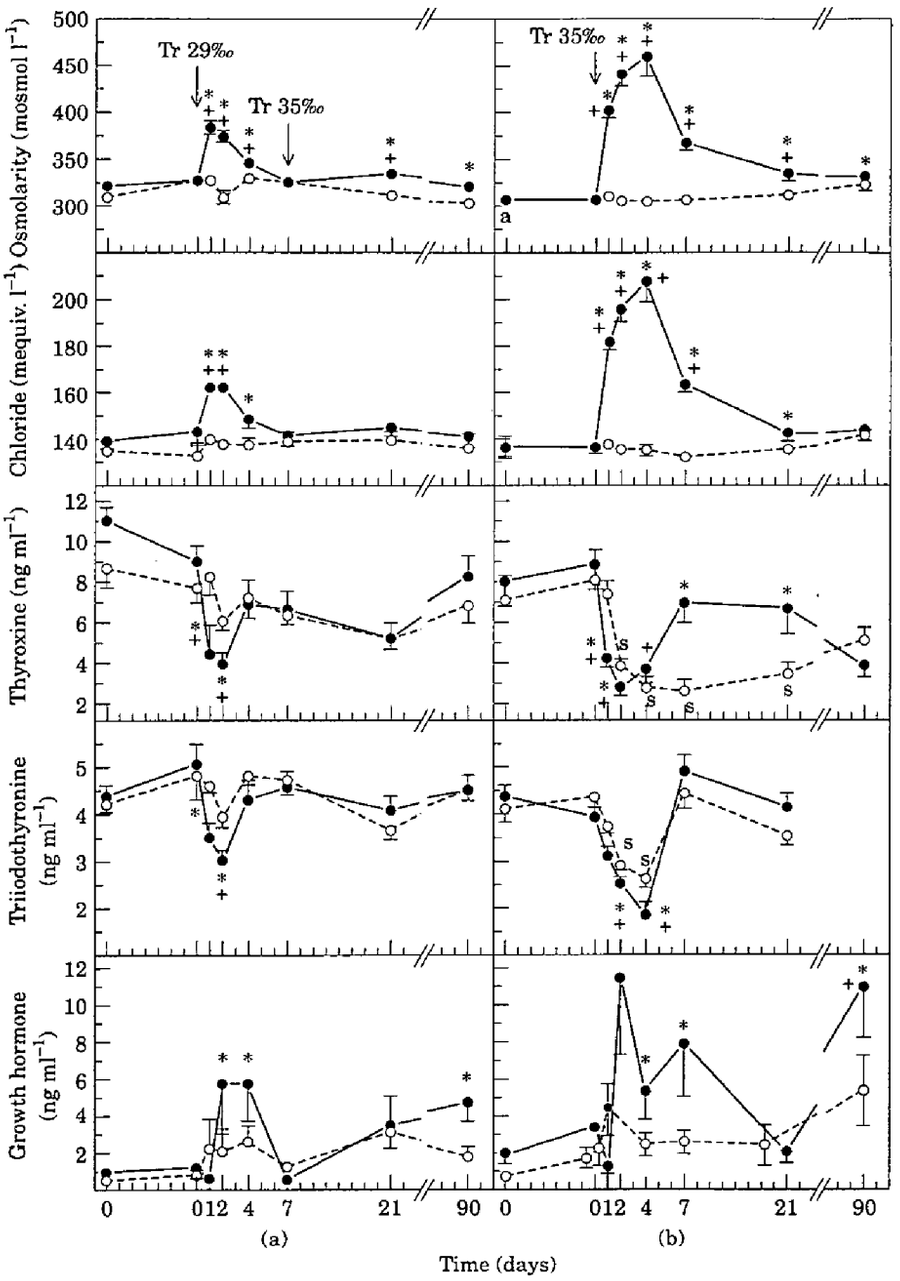

FIG. 2. Plasma osmolarity, chloride concentration, thyroid hormone and growth hormone (GH) in freshwater-transferred (○) and in (a) progressive and (b) direct seawater- (SW) transferred trout (●). Each point represents the mean ± S.E. of 12 or 18 fish. *, Significantly different from the corresponding control values at 5% level. Student's test for the all except the GH for which is the Mann–Whitney test. +, Significantly different from the pre-SW values at 5% level. ANOVA Newman–Keuls for all except for the GH for which is the Kruskall–Wallis test followed by the Dunn test. s, Significantly different from the pre-SW time values at 5% level. ANOVA Newman–Keuls.

TABLE I. Mean ± standard error of gill (Na^+-K^+)-ATPase activity in freshwater- and progressive or direct seawater-transferred trout

	Day 0		Day 21	
	FW	SW	FW	SW
Progressive transfer	4·00 ± 2·39	4·72 ± 1·95	3·87 ± 2·06	29·84 ± 2·62
Student	NS		S	
Direct transfer	3·62 ± 1·40	3·50 ± 1·17	3·90 ± 0·73	19·39 ± 1·63
Student	NS		S	

S or NS, Significance at 5% level using Student's test between the seawater-transferred trout and the corresponding controls.

ENDOCRINOLOGY

Thyroid hormones (TH)

During the first 24 h of the SW transfer, a transitory drop appeared in plasma concentration of the TH (Fig. 2).

T_3 circulating levels of SW transferred fish decreased slightly but significantly ($P<0·05$, ANOVA and Student's test), 1 ($P<0·05$ Student's test only) and 2 days after the progressive transfer and 2 and 4 days after the direct one. Then, T_3 levels rose until reaching the level of plasma concentrations of the FW fish.

Moreover, there was a transient and significant drop ($P<0·05$, ANOVA and Student's test) in the T_4 levels 1 and 2 days after progressive and direct transfers remaining until day 4 ($P<0·05$, ANOVA) after direct transfer (Fig. 2). However, during the second experiment, TH plasma concentrations of controls decreased significantly ($P<0·05$, ANOVA) on days 2 and 4 for T_3, and on days 2 until 21 for T_4.

Growth hormone (GH)

The transfer to a hyperosmotic environment induced a transitory increase of the GH plasma levels which was more marked and more lasting after direct transfer (Fig. 2). GH concentrations rose from day 2 after the progressive transfer and remained significantly ($P<0·05$, Mann–Whitney test) higher than the controls until 4 days after transfer. For the direct transfer, GH levels increased on day 2 to 11 ng ml^{-1} and even if they decreased, they remained significantly higher ($P<0·05$, Mann–Whitney test) than the FW trout on days 4 and 7. Then, the circulating levels of GH decreased markedly 7 days after progressive transfer and 21 days after direct transfer. Three months after the SW transfers, in both experiments, plasma GH concentrations of the transferred fish were significantly higher ($P<0·05$, Mann–Whitney test) than those of the controls.

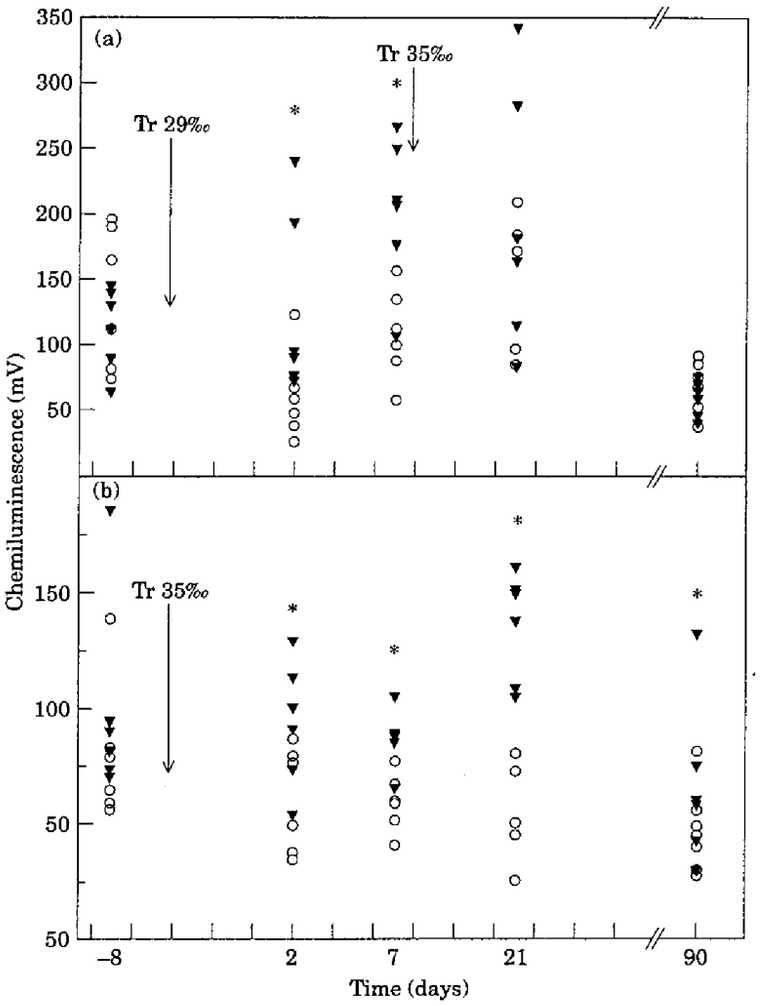

FIG. 3. Maximal individual values of the chemiluminescence of the head kidney leucocytes in freshwater-transferred (○) and (a) progressive or (b) direct seawater- (SW) transferred trout (▼). *, Significantly different from the corresponding control values at 5% level. Mann–Whitney test.

FW trout GH concentrations increased weakly and not significantly from day 1 until day 4 in the first experiment and on day 2 in the second.

IMMUNOLOGICAL PARAMETERS

Phagocytic activity measured by chemiluminescence

A large variation in the maximal individual values of the CL response was observed between fish in the same sample and between the days of experimentation, which was inherent to the technique. However, the statistical comparison of the maximal individual values by the Mann–Whitney test showed that the CL of the SW transferred fish was significantly higher ($P<0{\cdot}05$) than that of the controls 2 and 7 days after the progressive transfer and also 2, 7, 21 and 90 days after the direct one.

Natural cytotoxicity

The intensity of the response varied widely between fish belonging to the same tank and between the tanks. In both experiments and organs, the lysis percentages of the RTH cells were similar, between 15 and 30% (Table II).

The SW transfer did not modify the non-specific cytotoxicity. Only the cytotoxicity of the splenic lymphocytes of the direct transferred trout appeared significantly higher ($P<0{\cdot}05$, Mann–Whitney test) than in the controls on day 21.

Plasma lysozyme concentration

In the first experiment, the plasma lysozyme concentrations were not determined until day 4 after the SW transfer because of a technical problem.

In both SW transfers, the plasma lysozyme concentration was higher in the transferred trout than in the controls (Fig. 4). The lysozyme concentrations of the SW transferred fish were significantly higher ($P<0{\cdot}05$, Student's test) than the controls 7, 21 and 90 days after progressive transfer and 1, 4, 7, 21 and 90 days after direct transfer. Maximal concentrations were observed on day 21 in the first experiment and on day 4 in the second. Whatever the SW transfer, the lysozyme concentrations in the SW transferred trout increased significantly ($P<0{\cdot}05$, ANOVA), on day 21 for the progressive transfer and from day 4 until day 21 for the direct transfer.

LINEAR REGRESSIONS

The linear regressions between the individual values of GH and CL or lysozyme concentrations were carried out only when both the GH and the CL or lysozyme concentrations were significantly higher in SW transferred trout than in controls. Then, as a result a significant ($P<0{\cdot}05$) positive correlation was found between the GH (log-transformed) and the CL individual values on day 2 after the progressive transfer [Fig. 5(a)] and on days 2 and 7 after the direct one [Fig. 5(b), (c)]. Another significant ($P<0{\cdot}05$) positive correlation was observed between plasma GH (log-transformed) and lysozyme concentrations on days 4 and 7 after the direct transfer [Fig. 5(d), (e)].

DISCUSSION

Boeuf & Harache (1982, 1984) and Almendras *et al.* (1993), observed a serious osmotic imbalance during the SW transfer of resident brown trout. The intensity of this disequilibrium depends on the method of SW transfer; it was less marked during progressive transfer, than during direct transfer. Therefore, a progressive SW transfer improves the 'quality' of the adaptation to the marine environment.

Branchial (Na-K)-ATPase activity is often used as an index of the hypo-osmoregulatory status, particularly for the smolting species (Dickhoff, 1992), and it is normally higher in SW than in FW teleosts. In the present study, the (Na-K)-ATPase activities of between 3 and 4 μmol Pi mg protein^{-1} h^{-1} detected in FW fish were comparable with the observations made in sedentary species, about 5 μmol Pi mg protein^{-1} h^{-1} (Boeuf & Harache, 1984). The significant increase in the (Na-K)-ATPase activity observed 3 weeks after each transfer allowed the fish to establish electrolytic homeostasis after SW entry.

TABLE II. Lysis percentage (mean ± standard error) of the RTH cells by pronephric or splenic non-specific cytotoxic cells of freshwater- and the progressive or direct seawater-transferred trout

	Day −8		Day 2		Day 7		Day 21		Day 90	
	FW	SW	FW	SW	FW	SW	FW	SW	FW	SW
Head kidney:										
	$n=5$	$n=6$	$n=5$	$n=6$	$n=6$	$n=6$	$n=6$	$n=5$	$n=6$	$n=6$
Progressive transfer	26·6 ± 5·4	21·0 ± 2·2	19·2 ± 2·6	20·3 ± 4·9	26·8 ± 1·4	26·5 ± 2·4	21·8 ± 2·1	28·2 ± 3·4	22·6 ± 2·0	21·0 ± 2·4
Dunn	a	A	a	A	a	A	a	A	a	A
Mann–Whitney	NS		NS		NS		NS		NS	
	$n=6$	$n=6$	$n=6$	$n=5$	$n=6$	$n=5$	$n=6$	$n=6$	$n=6$	$n=6$
Direct transfer	22·9 ± 6·6	19·7 ± 2·4	17·9 ± 2·9	15·4 ± 1·3	18·2 ± 3·0	19·5 ± 2·3	16·5 ± 3·3	17·5 ± 2·8	25·3 ± 9·5	27·1 ± 5·1
Dunn	A	a	A	a	A	a	A	a	A	a
Mann–Whitney	NS		NS		NS		NS		NS	
Spleen:										
	$n=6$	$n=5$	$n=4$	$n=4$	$n=5$	$n=5$	$n=6$	$n=6$	$n=6$	$n=6$
Progressive transfer	19·2 ± 3·2	25·6 ± 2·9	27·5 ± 5·8	30·6 ± 4·3	18·9 ± 3·1	23·7 ± 1·9	21·3 ± 2·7	25·4 ± 2·3	20·4 ± 1·2	18·1 ± 2·4
Dunn	A	a	A	a	A	a	A	a	A	a
Mann–Whitney	NS		NS		NS		NS		NS	
	$n=6$	$n=6$	$n=6$	$n=4$	$n=6$	$n=5$	$n=6$	$n=6$	$n=6$	$n=6$
Direct transfer	19·9 ± 2·8	20·4 ± 1·8	23·1 ± 7·3	15·6 ± 3·4	14·4 ± 1·8	17·6 ± 3·5	16·7 ± 2·3	23·7 ± 2·9	24·6 ± 2·6	26·9 ± 1·9
Dunn	a	AB	a	A	a	AB	a	AB	a	B
Mann–Whitney	NS		NS		NS		S		NS	

n, Number of sampled fish.

Letters represent the significance at 5% level given by the Kruskall–Wallis test followed by the Dunn test, capital letters are used for the seawater-transferred trout and lower case for the controls. Columns with different letters are significantly different.

S or NS, Significance at 5% level with the Mann–Whitney test between seawater-transferred trout and the corresponding controls.

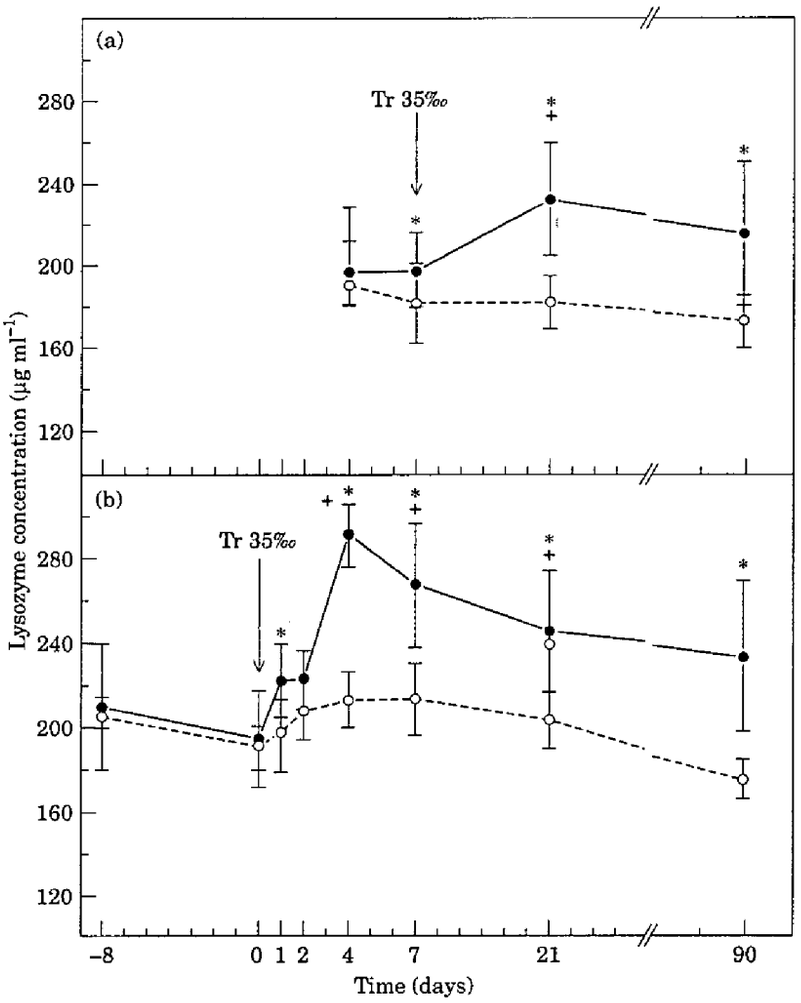

FIG. 4. Plasma lysozyme activity (equivalent to μg ml^{-1} of hen egg white lysozyme) of freshwater-transferred (○) and (a) progressive or (b) direct seawater- (SW) adapted trout (●). *, Significantly different from the corresponding control values at 5% level by the Student's test. +, Significantly different from the pre-SW values at 5% level by ANOVA Newman–Keuls.

Several authors reported that (Na-K)-ATPase increase observed after SW transfer is accompanied by endocrine changes, among others, an elevation of plasma GH as observed Boeuf *et al.* (1989) and Collie *et al.* (1989) in anadromous salmonids and Sakamoto *et al.* (1990) in rainbow trout. In brown trout, the plasma GH levels were modified significantly by environmental salinity. Furthermore, adaptation to a hyperosmotic environment with stimulation of the gill (Na-K)-ATPase in non-smolting species has been observed after administration of GH (Bolton *et al.*, 1987; Almendras *et al.*, 1993). This GH action on the gill (Na-K)-ATPase might be mediated by GH specific receptors, already shown by Fryer & Bern (1979) and Sakamoto & Hirano (1991) in salmonid gills.

After this transitory increase GH concentrations returned to a level close to the initial FW concentration. This is consistent with the results obtained by

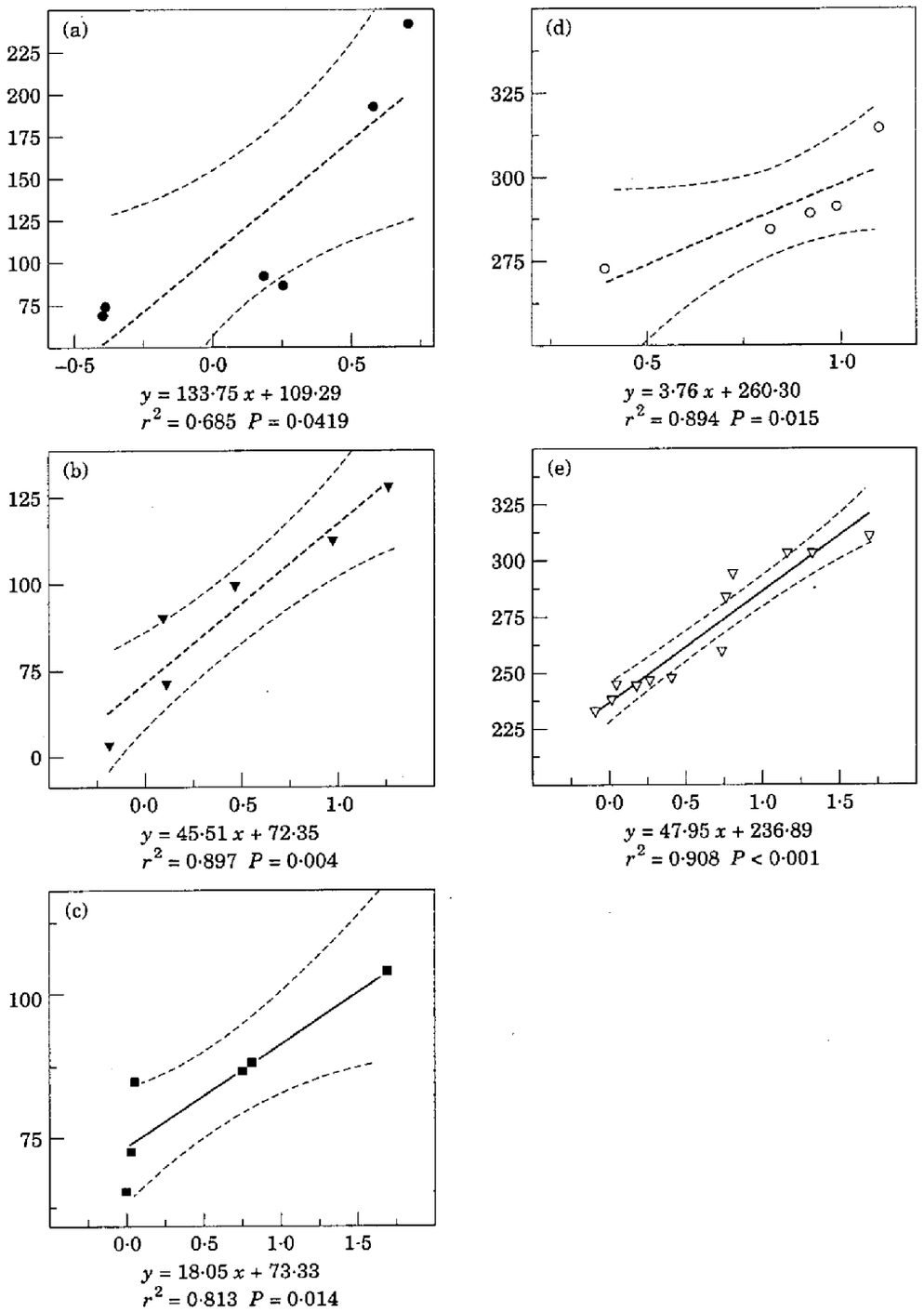

FIG. 5. Linear regressions between the log of GH concentration and the chemiluminescence. (a) On day 2 after progressive transfer (●); (b) on day 2 after direct transfer (▼); (c) on day 7 after direct transfer (■). Linear regressions between the log of GH concentration and the lysozyme (d) on day 4 after the direct transfer (○) and (e) on day 7 after the direct transfer (▽). (a)–(c) y=Chemiluminescence (mV); x=log Growth Hormone (ng ml^{-1}). (d), (e) y=Lysozyme (μg ml^{-1}); x=log Growth Hormone (ng ml^{-1}).

Sakamoto *et al.* (1990) and Sakamoto & Hirano (1991) after rainbow trout transfer to 75 or 80% sea water. According to these authors, two major factors, regulation of the GH secretion from the pituitary by feedback controls and increased in the plasma metabolic clearance may regulate the plasma GH levels.

GH concentrations observed in trout 90 days after transfer, were significantly higher than in FW trout. In SW trout, the condition factor was significantly lower than in FW trout, possibly because food intake was reduced as noted by Fauré (1991) in other experiments. Furthermore, Sumpter *et al.* (1991) established that starvation induced GH level increases in rainbow trout.

After SW transfers, plasma T_3 and T_4 were temporarily and significantly decreased. The weak drop observed in the present study is consistent with the results obtained by Boeuf *et al.* (1989) in Atlantic salmon *Salmo salar* L., after autumn transfer and Lebel & Leloup (1992) in brown trout. After this decrease, plasma TH levels reached concentrations close to the initial FW levels, probably due to the activation of the thyroid through a variety of feedback loops.

In FW fish TH and GH levels followed those of the SW transferred fish but the changes were not significant compared to the pre-SW transfer time, except for TH during the second experiment. The frequent coming and going in the stock farming hall due to the numerous sampling during the first days after the transfers might have induced a chronic stress causing a weak rise of GH levels and a drop in TH concentrations (Pickering, 1993). During the second experiment, the FW temperatures fell to 5° C which might be a reason for the significant drop observed in the T_4 concentration in FW fish until day 21 (Eales & Shostak, 1986).

The endocrinological modifications observed during SW adaptation of brown trout in this study, particularly in GH, could have influenced the brown trout immunological potential, as demonstrated by several studies in mammals (Berczi, 1989; Kelley, 1989; Weigent & Blalock, 1989).

In non-specific immunity, especially in phagocytic cells, the chemiluminescence of the pronephric leucocytes was higher in SW transferred trout whatever the method of transfer, progressive or direct. However, this increase was significant regardless of the day of sampling after direct transfer, and only on days 2 and 7 after the progressive one. Thus, some similarities appear between the increases of the phagocytic activity and those of the plasma GH levels. Indeed, both of them were higher and more lasting after direct transfer and a positive correlation has been observed between both these parameters. These results were in agreement with the observation of Sakai *et al.* (1991) who noticed that injection of recombinant chum salmon *Oncorhynchus keta* (Walbaum) GH enhanced the CL of the pronephric leucocytes of rainbow trout. Furthermore, this phenomenon seems to corroborate the observations made in mammals by Edwards *et al.* (1988) who noted, after *in vivo* and *in vitro* treatment with native or recombinant porcine GH, an increase of superoxide anion production during the phagocytose by rat zymosan-stimulated macrophages. According to Kelley (1989), GH could be ' a newly defined macrophage activiting factor '.

The plasma lysozyme, which is probably mainly produced by the phagocytic cells (Murray & Fletcher, 1976), was also higher in SW transferred trout than in controls. As for CL, this increase was more pronounced after direct transfer and a significant positive correlation existed between plasma levels of GH and

lysozyme 4 and 7 days after direct transfer. This result might be due to the GH which could affect the brown trout phagocytic cells enhancing the release of lysozyme. Indeed, in humans, GH injections enhanced the lysozomal activity of the polymorphonuclear leucocytes of both hypopituitary dwarfs and normal subjects (Rovensky *et al.*, 1985).

In the present study, an additional non-specific immunological function was studied, the natural cytotoxicity effected by the non-specific cells (NCC). In mammals, Saxena *et al.* (1982) with mice, and Davila *et al.* (1987) with rats, have demonstrated that GH enhances the natural cytotoxicity of peripheral blood or splenic cells. Haddad & Mashaly (1991) had the same results in chickens. More recently, Kajita *et al.* (1992) increased the natural cytotoxicity of rainbow trout spleen, kidney and blood leucocytes after a chum salmon recombinant GH injection. In the present study, the control lysis percentages of the RTH cells, between 15–30%, seemed higher than the 14–15% observed by Kajita *et al.* (1992). The different target cells used, RTH in this study and a mouse cell line (P-815) by Kajita *et al.* (1992), might explain this difference. However, the splenic leucocyte natural cytotoxicity was significantly higher in the direct transferred trout than in the controls, on day 21 only. Therefore, it seems that the natural cytotoxicity was not affected by the SW transfers despite the GH level changes. However, unlike Kajita *et al.* (1992), who injected recombinant GH (0·1 ml of a solution at 10 $\mu g\ ml^{-1}$), the GH level modifications observed in the present study were endogeneous and probably weaker than those observed by these authors, which could explain the lack of natural cytotoxicity stimulation. On the other hand, it is possible that another phenomenon could mask an eventual stimulation induced by GH, a temporary drop in TH concentrations. Indeed, some studies have shown that the administration of TH enhanced natural cytotoxicity. Sharma *et al.* (1982) reported that an exogeneous administration of T_4 increased both *in vitro* and *in vivo* the splenic and peritoneal natural killer (NK) cell activity in some mice, depending on the strain. Haddad & Mashaly (1991) observed that T_3 could act with GH to increase chicken natural cell-mediated cytotoxicity.

Other authors have found that high levels of TH, either endogeneous (Grave's disease) or exogeneous (injection in mice and humans) reduced the activity of the NK cells (Papic *et al.*, 1987; Stein-Streilein *et al.*, 1987). Further studies should be carried out to determine the effect of the TH on natural salmonid cytotoxicity.

In conclusion, SW transfer induced endocrinological and immunological modifications in resident brown trout. Some similarities and also positive correlations were observed between GH, phagocytose and plasma lysozyme concentration increases which suggested that among the endocrine changes, GH plays a significant part on the immune competences especially by enhancing the macrophage functions. Further studies are now being conducted on brown trout, in FW and during SW adaptation, to determine the direct effects of GH administration *in vivo* on the immune potential. The results will be presented in a future paper.

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