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MEASUREMENTS OF GILL ($\text{Na}^+ - \text{K}^+$)-ATPASE ACTIVITY AND PLASMA THYROID HORMONES DURING SMOLTIFICATION IN ATLANTIC SALMON (*SALMO SALAR* L.)

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ABSTRACT

Boeuf, G. and Prunet, P., 1985. Measurements of gill ($\text{Na}^+ - \text{K}^+$)-ATPase activity and plasma thyroid hormones during smoltification in Atlantic salmon (*Salmo salar* L.). *Aquaculture*, 45: 111–119.

Gill ($\text{Na}^+ - \text{K}^+$)-ATPase activity, triiodothyronine (T3) and thyroxine (T4) circulating blood plasma levels were monitored in 1983 on yearling Atlantic salmon reared in a hatchery in Brittany. The level of enzyme activity increased between February and April to reach a high plateau between mid-April and mid-May. The same fish presented an acute surge of T4 in mid-April and two small increases of T3 in February and May. The gill enzyme activity and the T4 blood plasma level follow a similar pattern, reaching high values at the same moment. The T4 peak occurs at the new moon and does not seem to be correlated with an increase of temperature. Possible relationships between blood plasma T3, T4 and gill ($\text{Na}^+ - \text{K}^+$)-ATPase activity are discussed.

INTRODUCTION

Great interest, both basic and applied, in the study of smoltification of anadromous salmonids has been shown in recent years. Many scientists throughout the world have investigated different criteria useful for forecasting the most appropriate time to adapt these fish to sea water and more especially in areas where the oceanic salinity is high (Hoar, 1976; Folmar and Dickhoff, 1980; Wedemeyer et al. 1980; Boeuf and Harache, 1982).

Zaugg and McLain (1970) first described the seasonal spring increase of the gill ($\text{Na}^+ - \text{K}^+$)-ATPase activity in freshwater coho salmon prior to the downstream migration, then extended their studies to different Pacific species (Zaugg and McLain, 1972).

Recently, studies on the evolution of circulating blood plasma thyroid hormones have shown an elevation of these compounds at the smolting time (Dickhoff et al., 1978, 1982; Nishikawa et al., 1979; Grau et al., 1981, 1982). Dickhoff et al. (1982) pointed out the value of monitoring the levels of these hormones in Pacific species and proposed further investigation

in different salmonids. Lindahl et al. (1983) and Youngson and Simpson (1984) carried out such studies in Atlantic salmon.

Under natural conditions (35‰ salinity) we have demonstrated (Boeuf et al., 1978; Boeuf and Harache, 1982, 1984) that gill ($\text{Na}^+\text{--K}^+$)-ATPase is a possible predictive physiological criterion for adaptation of migratory species to sea water. Thus we found it of major interest to measure levels of circulating thyroid hormones in the blood plasma and gill ATPase activity during the development of salinity tolerance in Atlantic salmon.

MATERIAL AND METHODS

We studied fish in the hatchery of Le Conquet (20 km from Brest) between December and July of 1983. Eggs were imported from Norway in January 1982 and the Atlantic salmon were reared in tanks supplied by a small river. Inventoried fish (500) were isolated in an Ewos tank (4 m²) in November 1982 and fed on manufactured dry pellets provided at 2 to 6% of body weight per day from automatic feeders. The pH of the water was about 6.5 to 7. The temperature is shown in Fig. 1. The photoperiod was natural (48° N).

Sampling of the gills began in December 1982 and of the blood plasma in January 1983. Blood was collected from the posterior aorta with a heparinized syringe, centrifuged, then stored frozen. Gill filaments were taken from the same fishes, rinsed with 0.25 M sucrose (pH 7.4) and immediately stored in liquid nitrogen. Measurements of gill ($\text{Na}^+\text{--K}^+$) and Mg^{2+} -ATPase activities were obtained according to the methods described by Lasserre et al. (1978) and Boeuf (1979).

Blood plasma concentrations of triiodothyronine (T3) and thyroxine

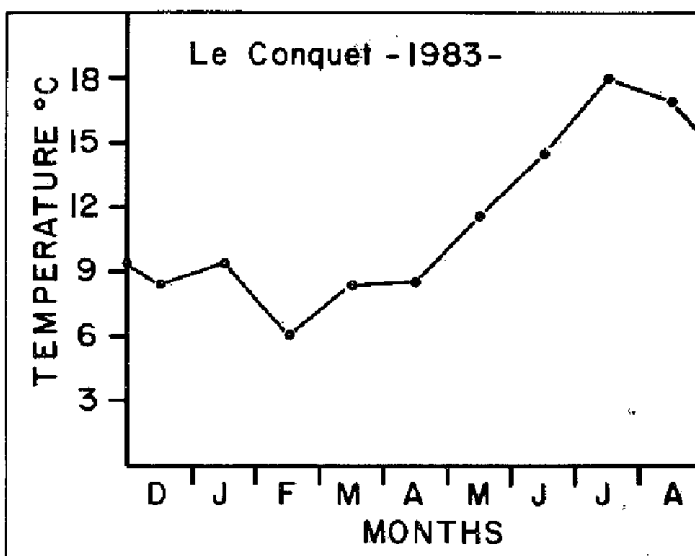


Fig. 1. Temperature of the hatchery water.

(T4) were determined by radio-immunoassay according to the method of Chopra (1978), modified by McKenzie et al. (1978). Standard purified hormones (Sigma) were diluted in barbital buffer (pH 8.6) in a range of 0.3 to 40–80 $\text{ng}\cdot\text{ml}^{-1}$ (ten points) for each hormone. We use in this standard 0.5 $\text{g}\cdot\text{l}^{-1}$ of bovine γ -globulin (Cohn, Fraction II, Sigma) and thimerosal (merthiolate, 10 $\text{mg}\cdot\text{l}^{-1}$). Antisera were purchased from Denmark (Kruse, 1976); ^{125}I -labeled hormones (specific activity: T3, 20 μCi ; T4, 50 $\mu\text{Ci}\cdot\text{ml}^{-1}$) were obtained from Amersham France. Each tube contained 225 μl as follows: 150 μl of radiolabeled hormone (in barbital buffer pH 8.6 and 1 $\text{mg}\cdot\text{ml}^{-1}$ of 8-anilino-1-naphthalene-sulfonic acid, sodium salt); 25 μl of antiserum at the dilution obtained from the titration curve ($0.75\cdot 10^{-3}$ for T3; $0.15\cdot 10^{-3}$ for T4); and 50 μl of the unknown plasma (dilution at 10^{-1}).

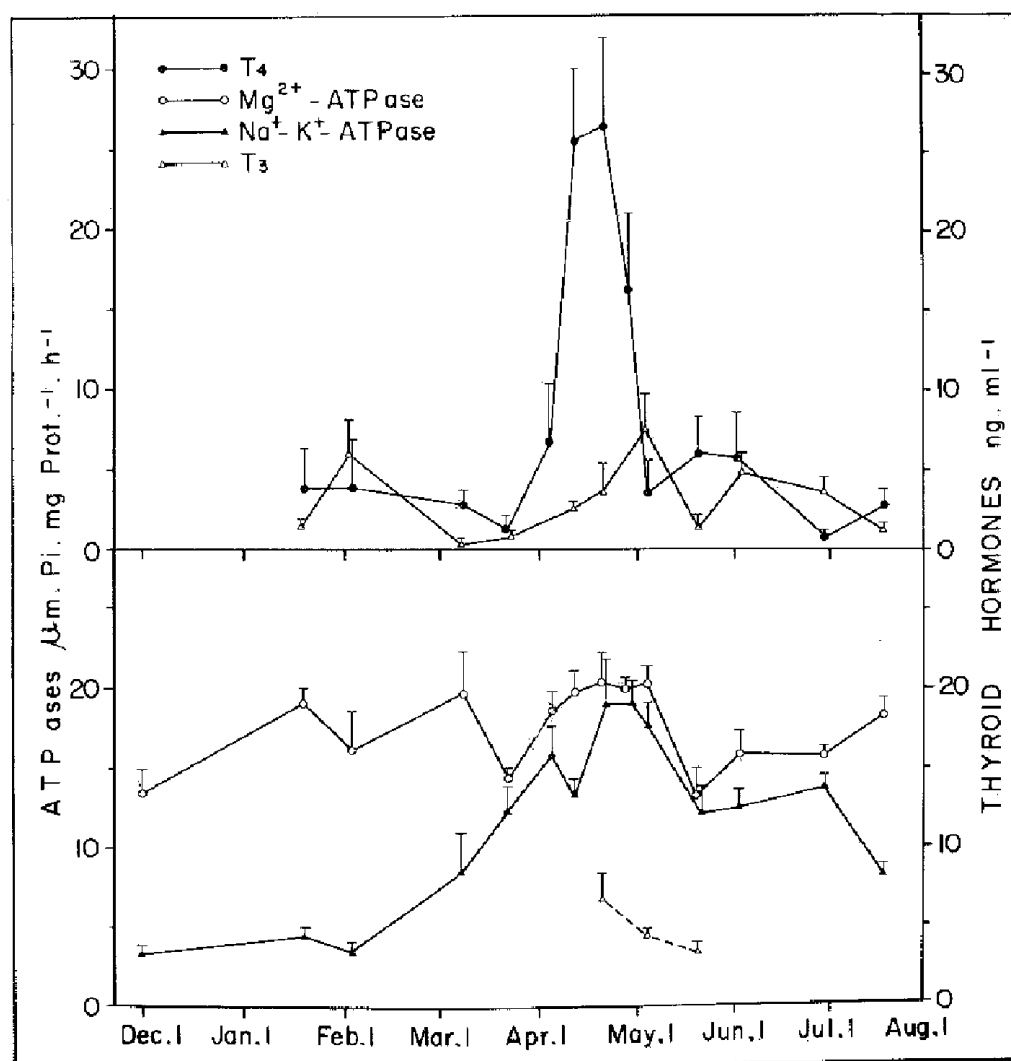


Fig. 2. Evolution of blood plasma triiodothyronine (T3), thyroxine (T4) gill ($\text{Na}^{+}\text{-K}^{+}$)- and Mg^{2+} -ATPase in Atlantic salmon reared in fresh water. Values linked by dotted lines correspond to the pre-smolts. Vertical bars correspond to \pm SEM.

Assay tubes were capped and incubated for 2 h at 37°C followed by at least 1 h at 4°C. Antibody was then precipitated by addition of 0.5 ml of cold 17% polyethylene glycol followed by thorough mixing. After 30 min at 4°C the precipitate was centrifuged at 5000 rpm for 30 min at 4°C. The supernatant was decanted by inverting the tubes. The pellet was counted in a LKB gamma counter for 1 min per tube. The standard curve was constructed and unknowns calculated by the computer of the counter (spline function). The intraassay coefficient of variation was equal to 3–7%. At each sampling we used between 10 and 25 fish. All samples of the same experiment were measured in the same assay. Results are expressed as mean \pm standard error of mean.

RESULTS

Measurement of gill (Na⁺-K⁺)-ATPase level (Fig. 2).

In the winter of 1983, the temperature was rather low (Fig. 1). The gill (Na⁺-K⁺)-ATPase activity remained low until mid-March and then increased to reach a plateau in early April. This high level was maintained until mid-May and decreased slowly to a low level in July. During the same period, the temperature rose from 12°C to 18°C. Between March and June it was possible to separate the population into subgroups, one showing a rise of

TABLE I

Gill (Na⁺-K⁺)-ATPase activity ($\mu\text{m Pi} \cdot \text{mg prot.}^{-1} \cdot \text{h}^{-1}$) in relation to the size of the salmon. We chose 11 $\mu\text{m Pi} \cdot \text{mg prot.}^{-1} \cdot \text{h}^{-1}$ according to our method of measurement to separate the two size levels. It is thus possible to present the limit size between smolts and pre-smolts (mean \pm SEM)

	1 Dec. 1982.	19 Jan. 1983	3 Feb. 1983	8 Mar. 1983	22 Mar. 1983	5 Apr. 1983
Mean of low levels of enzymatic activity	3.3 ± 0.6	4.5 ± 0.6	3.6 ± 0.5	9.1 ± 1.1	—	—
Limit size (mm)	—	—	—	—	<126	<129
Mean of high levels of enzymatic activity	3.3 ± 0.6	4.5 ± 0.6	3.6 ± 0.5	14.3 ± 0.6	11.3 ± 1.6	16.0 ± 1.6
Mean size of sampled fish (mm)	(F) 98.2 ± 1.5	(F) 111.3 ± 2.5	(F) 114.4 ± 2.3	(F) 118.5 ± 1.5	(S) 129.5 ± 1.8	(S) 139.8 ± 3.4

(F): whole population. (S): only smolts.

(Na⁺-K⁺)-ATPase activity (>11 $\mu\text{m Pi} \cdot \text{mg protein}^{-1} \cdot \text{h}^{-1}$) and the other staying at a low level. It is thus possible to determine a limiting size between populations of smolts and pre-smolts (Table I).

Measurement of gill Mg²⁺-ATPase activity

Throughout the experiment the activity of this enzyme remained between 10 and 20 $\mu\text{m Pi} \cdot \text{mg prot.}^{-1} \cdot \text{h}^{-1}$ (Fig. 2).

Change in plasma thyroxine and triiodothyronine during the parr-smolt transformation (Fig. 2).

From January to March the plasma thyroxine level remained low. In April it rose abruptly to reach a peak of 24–26 $\text{ng} \cdot \text{ml}^{-1}$ in mid-April; within a further 2 weeks thyroxine decreased to the basal level. The T4 peak coincided with the new moon (13 April). The changes in triiodothyronine level were much smaller: two small increases were detected, one in early February and the other in early May when the T4 level was decreasing.

DISCUSSION

Since the first report of Hoar (1939), subsequently confirmed by Fontaine

12 Apr. 1983	21 Apr. 1983	28 Apr. 1983	3 May 1983	19 May 1983	2 June 1983	30 June 1983	18 July 1983
—	6.8 ±1.6	—	4.5 ±0.5	3.4 ±0.6	—	—	9.3 ±0.6
<110	<113	<126	<125	<145	<150	<165	—
13.2 ±0.9	19.0 ±2.8	19.1 ±1.4	17.6 ±1.5	12.2 ±1.8	12.5 ±1.1	13.9 ±0.8	9.3 ±0.6
(S) 119.6 ±3.6	(S) 141.3 ±5.8	(S) 140.0 ±2.2	(S) 132.7 ±4.3	(S) 151.7 ±1.5	(S) 160.4 ±4.3	(S) 174.5 ±3.6	(F) 170.0 ±3.2

et al. (1952) and Leloup and Fontaine (1960), evidence for a role of the thyroid system in salmonid smoltification has accumulated (reviewed by Fontaine, 1975; Hoar, 1976; Folmar and Dickhoff, 1980). The use of a sensitive radio-immunoassay of thyroid hormones allowed us to demonstrate the existence of a blood plasma thyroxine peak during the smolting of Atlantic salmon. This result is in agreement with previous reports on Pacific salmon species (Dickhoff et al., 1978, 1982; Nishikawa et al., 1979; Grau et al., 1981, 1982). The smolting stage in Atlantic salmon was characterized by monitoring the gill ($\text{Na}^+ - \text{K}^+$)-ATP-ase activity level, a criterion which was demonstrated to be valuable under natural conditions, i.e. 35‰ salinity, and temperature ranging from 7 to 15°C in spring (Boeuf et al., 1978, 1982; Boeuf and Harache, 1982, 1984). Therefore, it is interesting to see that the thyroxine surge appears during a period when the level of gill enzyme activity is high and when the adaptability of the fish to sea water is maximum (Boeuf et al., 1978). Different authors have already described the increase of this ATPase activity in Atlantic salmon (McCartney, 1976; Saunders and Henderson, 1978; Johnston and Saunders, 1981; Boeuf et al., 1985).

Monitoring the level of enzyme activity during the smolting period allows, in our experiment, the separation of the fish population into two parts, one being smolt, the other only pre-smolt (see Table I and Lassere et al., 1978). However, such analysis cannot be made in all cases using only the level of T4; although variations of both T4 and ATPase show a similar and synchronized general pattern with an apparent relationship, it is not always possible to correlate each T4 level with ATPase activity as some fish present a high level of thyroxine associated with a low gill enzyme activity and others a low level of T4 and high gill enzyme activity. However, for the same salmon, when the level of T4 is very high, the level of ATPase activity is always high, also in our experiment.

Folmar and Dickhoff (1981) studied simultaneously the gill ($\text{Na}^+ - \text{K}^+$)-ATPase activity and blood plasma T3 and T4 levels in coho salmon, and found concurrent peaks for ATPase activity and T4 in the spring. They concluded that the successful transition of coho salmon into sea water was related to the successful completion of an endogenous thyroid hormone surge prior to seawater entry. Dickhoff et al. (1982), confirmed these conclusions and indicated that the information on plasma thyroid hormone levels could be used in determining the optimal time to release fish. In comparing our results with those reported in this last paper, we observe many similarities (one T4 surge, earlier in Brittany; two small increases of T3, one at the same moment in February—March, the second one earlier in France). Considering the greater affinity of T3 than of T4 for salmon hepatic nuclear receptors (Darling et al., 1982), measurements of their plasmatic levels must be made for a good understanding of mechanisms (Dickhoff and Darling, 1983). The relative constancy of the T3 level in the blood plasma could be explained by modifications of proportions of circulating T4 (Fok and Eales, 1984).

The increase of gill enzyme activity occurred concurrently with the first T3 increase and the level was already high at the start of the T4 surge. The decrease occurred at the same moment as the second T3 increase. In any case, it is not easy today to specify the exact relationships between these levels of thyroid hormones and the gill (Na⁺-K⁺)-ATPase activity. Both smolting and ambient salinity (Folmar and Dickhoff, 1981) have an effect on circulating thyroid hormones (Folmar and Dickhoff, 1979; Dickhoff et al., 1982), and the results presented here establish a relationship. In the eel, Leloup et al. (1981) have also shown a stimulation of T4 secretion and T3-T4 conversion after transfer from fresh water to sea water. On the other hand, direct involvement of these hormones in ionic and osmotic regulation in fish is presently debated: Knoepfel et al. (1982) demonstrated an influence of the integrity of the thyroid on (Na⁺) balance in *Fundulus heteroclitus*, but Milne and Leatherland (1980) found no involvement of thyroid hormones in osmotic regulation of rainbow trout.

Grau et al. (1981, 1982) demonstrated the relationship between thyroxine surge and lunar phases in Pacific species. It seems here that the Atlantic salmon is able to react in the same way. However, this observation has to be confirmed. In Atlantic salmon, Youngson and Simpson (1984) did not find an elevation of T4 in the blood plasma of captive fish in spring in Scotland whereas they found it in wild, downstream migrating salmon. They correlated the level of T4 with stream discharge. Our results are different in the hatchery. Lindahl et al. (1983) have already shown in Sweden a T4 surge under specific conditions (very cold winter and a rapid increase of temperature in spring) in 25-26-month-old fish of 133-180 mm, occurring in late May and related to water temperature. They were not able to correlate the silvery aspect to the T4 level. In Brittany, river temperatures remain mild (6-8°C) during the winter and the T4 peak occurs at the beginning of the thermal increase (9°C).

The level of gill Mg²⁺-ATPase activity remained more or less constant throughout the experiment, and so it cannot be related to the parr-smolt transformation.

Our results confirmed the involvement of thyroid hormones in Atlantic salmon smoltification. However, further investigations are to be undertaken to clarify the exact role of thyroid hormones in osmoregulation and smoltification. This is a very complicated phenomenon and several parameters, not necessarily linked, are changing concurrently.

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