Gill Na\(^+\)-K\(^+\) ATPase, carbonic anhydrase activities and plasma osmotic and ionic variations during smoltification of Atlantic salmon in the north of Portugal

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Gill Na\(^+\)-K\(^+\) ATPase and carbonic anhydrase activities were measured, on a fortnightly basis, from February to July, in 0+ age Atlantic salmon (Salmo salar), hatched and reared in a freshwater experimental station in Covas, northern Portugal. Plasma osmolarity and ionic composition were also measured. Gill Na\(^+\)-K\(^+\) ATPase activity increased slowly until April (15--19 \(\mu\)moles Pi mg prot\(^{-1}\) h\(^{-1}\)). From April to late May there was a great increase in activity (19--32 \(\mu\)moles Pi mg prot\(^{-1}\) h\(^{-1}\)) followed by a sharp decline in June (15 \(\mu\)moles Pi mg prot\(^{-1}\) h\(^{-1}\)). In contrast, carbonic anhydrase activity decreased significantly from early April to early June (170--70 \(\mu\)moles \(p\)-nitrophenol mg prot\(^{-1}\) h\(^{-1}\)) and increased in late June, suggesting the existence of a compensatory mechanism for the changes in Na\(^+\)-K\(^+\) ATPase activity. Plasma osmolarity and sodium concentration showed lower levels during the period of high ATPase activity. On the other hand, plasma calcium concentrations showed an increase during the same period (3.47--5.98 mM\(^{-1}\) of plasma). A transitory decrease in osmolarity and plasma sodium and chlorine concentrations occurred in March, prior to the surge in Na\(^+\)-K\(^+\) ATPase activity, suggesting that the physiological changes, characteristic of parr-smolt transformation can be a consequence of this loss of freshwater osmoregulatory capacity.

KEYWORDS: Atlantic salmon (Salmo salar L.), Carbonic anhydrase, Na\(^+\)-K\(^+\) ATPase, Plasma osmolarity, Smoltification

INTRODUCTION

Atlantic salmon (Salmo salar) undergo the parr–smolt transformation typical of migratory salmonids. Various morphological, physiological and behavioural changes occur seasonally (usually completed in spring), and are adaptive for downstream migration and seawater entry (Folmar and Dickhoff, 1980). Among these changes are the development of euryhalinity which has been attributed, in part, to an increase in gill Na\(^+\)-K\(^+\) ATPase activity (Folmar and Dickhoff, 1981; Boeuf and Harache, 1982; Boeuf et al., 1985). External environmental factors are important, particularly photoperiod and temperature, to control the onset and development of smoltification in salmonids (Zaugg and Wagner, 1973; Johnston and Saunders, 1981;
Clarke et al., 1985: McCormick et al., 1987). As described by these authors, a maximum response occurs with completion of smoltification in the spring when both daylength and temperature are increasing.

The main purpose of this study was to establish, under the conditions of temperature and photoperiod in the north of Portugal, the appropriate time of the year to transfer young Atlantic salmon to seawater. The rise in gill Na⁺-K⁺ ATPase activity was adopted as a parameter that can give a reasonable indication of the degree of euryhalinity of Atlantic salmon. Plasma ionic and osmotic composition were also measured not only as smolting indicators, but also with the objective of better understanding the mechanisms underlying the development of hyperosmotic ability. Attention was also directed to calcium levels during smolting.

Gill carbonic anhydrase activity was determined in order to test the possible existence of compensatory mechanisms protecting seawater preadapted smolts retained in freshwater.

**MATERIALS AND METHODS**

Atlantic salmon eggs were obtained from Scotland and transported to Portugal in March 1988. They were hatched in Covas (north of Portugal). First feeding occurred at the end of May. The fish were held in round tanks (1.2 m diameter) of 1 000 l capacity, supplied with running freshwater (pH 6.5-7.0) maintained under the natural conditions of photoperiod (42°N) and temperature (8-19°C) represented in Fig. 1. Commercial pellets (dry diets) with high daily feeding frequency were used in all tanks and during the light period according to temperature and fish size as recommended by manufacturer's tables (Ewos, Palencia, Spain). Fish were hand fed.

Samples of gill filaments and plasma were collected from February to June 1989 every 1 or 2 weeks respectively. In each sampling 10 fish were randomly selected from the tanks and anaesthetized (phenoxyethanol 0.5 ml l⁻¹). Individual fork length (FL to 1 mm) and body weight (BW to 0.1 g) were recorded, and the condition factor (CF) calculated (CF = BW.100/FL³). Blood samples were collected from the dorsal aorta in the caudal region using heparinized (lithium heparinate) syringes. Plasma was immediately separated by centrifugation and stored at -20°C for later analysis. Plasma osmolarity was measured using a semi-micro osmometer (Knawer, Massachusetts, USA), plasma sodium and calcium concentrations were measured by atomic absorption spectrophotometry (GBC, Victoria, Australia 903). After lengths and weights had been measured, the entire gill system of individual fish was removed and immediately rinsed in sucrose 0.25 M, pH 7.4, quickly frozen in liquid nitrogen and stored at -80°C.

Na⁺-K⁺ ATPase specific activity expressed as μmoles Pi mg prot⁻¹ h⁻¹, was determined by a method similar to that described by Lasserre et al., (1978). Measurements of inorganic phosphate were determined according to the method of Fiske and Subbarow (1925) and the concentration of proteins by the method of Lowry et al. (1951).

Carbonic anhydrase activity was determined using p-nitrophenyl acetate as a substrate as described by Mashiter and Morgan (1975). The hydrolysis reaction was