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## Cloning and expression of TNF- $\alpha$ , IL-1 $\beta$ and COX-2 in an anadromous and landlocked strain of Atlantic salmon (*Salmo salar* L.) during the smolting period<sup>☆</sup>

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### Abstract

The parr–smolt transformation involves complex modulation of immune parameters, affecting both cell populations and humoral factors. The expression of cytokines was studied in salmon cells and tissues during this period using an anadromous and a landlocked freshwater resident dwarf strain of Atlantic salmon (*Salmo salar* L.). The constitutive activity of three immunoregulatory genes encoding the cytokines tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-1 $\beta$  (IL-1 $\beta$ ) and the cyclo-oxygenase (COX) isoform COX-2 was investigated in head kidney, spleen and gill tissue from healthy, unvaccinated fish by real-time PCR. The TNF- $\alpha$  gene was generally lower expressed than COX-2 and IL-1 $\beta$ 1, which were approximately expressed at equal levels and constitutive expression was seen for COX-2 and IL-1 $\beta$ 1 in all tissues examined and at all sampling dates. The expression of all three genes in head kidney and spleen tissue seemed to be highest at the sampling in May for both strains around the time of seawater transfer suggesting an influence of smolting related hormones on cytokine expression. The gill tissue experienced the highest expression of IL-1 $\beta$ 1 and COX-2 at all sampling dates indicating that this organ is immunologically important.

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**Keywords:** Cytokine; Interleukin 1; Cyclo-oxygenase; Tumour necrosis factor- $\alpha$ ; Anadromous; Atlantic salmon; Smolting

<sup>☆</sup> The cDNA sequences of salmon TNF- $\alpha$  and COX-2 reported in this paper have been submitted to the EMBL database under the accession numbers AY848945 and AY848944, respectively. The cDNA sequence for IL-1 $\beta$  was obtained from Dr P.M. Cupit, pers. comm. and is identical to accession number AY617117.

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## 1. Introduction

The smoltification of juvenile anadromous salmonids involves morphological, physiological and behavioural changes of the fish from a freshwater-adapted to a salt water-adapted form. Increased activity is seen in most endocrine tissues during smoltification, including the thyroid gland, inter-renal cells, prolactin cells, pancreatic islets, stannius corpuscles and the caudal neurosecretory system [1]. Major hormones that are known to have immunomodulatory effects in fish are the corticosteroid hormone cortisol, the thyroid hormones T3 and T4, prolactin and growth hormone. Among the immunological parameters that are known to be affected during the smoltification are changes in leukocyte numbers, serum protein concentrations, immunoglobulin levels and an increased susceptibility to disease [2–5]. The mechanisms that underlie this altered immune status remain unknown. At present, no data exists on the constitutive expression of cytokine genes during smoltification. The aim of the study was, therefore, to investigate the impact of the smoltification on the expression of the cytokines interleukin-1 $\beta$  (IL-1 $\beta$ ), tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) and the prostaglandin H synthase gene cyclo-oxygenase-2 (COX-2). IL-1 $\beta$  and TNF- $\alpha$  are key mediators of the inflammatory response [6,7]. Both genes have recently been cloned and characterised from rainbow trout (*Oncorhynchus mykiss*) and common carp (*Cyprinus carpio*) and their expression is induced by bacterial lipopolysaccharide [8–11].

Although the neuroendocrine and immune systems were originally thought to be independent of each other there is now sufficient evidence to believe that there is a significant level of bi-directional communication between them. This communication is important for the maintenance of homeostasis and is enabled by the expression of hormone and cytokine receptors as well as their ligands by the cells and tissues of both systems. One consequence of this ‘cross talk’ is the negative effect of smoltification on salmonid immune system. Cortisol, the major product of the teleost hypothalamus–pituitary–interrenal-axis is highly expressed during smoltification [1]. Verburg-van Kemenade et al. [12] have observed that B lymphocytes, especially circulating B lymphocytes, are easily triggered to enter a cortisol-induced apoptosis. This probably accounts for previous observations that both lymphocyte proliferation and in vitro antibody responses are impaired after the administration of cortisol to fish [13]. In rainbow trout, LPS induced expression of IL-1 $\beta$  has been shown to be diminished by relatively high doses (300 ng/ml) of cortisol [14]. The influence of COX-2 within the immune system is via synthesis of prostaglandins (PG) that have a variety of functions, but are important in all stages of inflammation [15]. The roles of PGs in the inflammatory response of mammals include vasodilation and increased vascular permeability by interaction with histamine and bradykinin, and down regulation of leukocyte functions by reduction of the respiratory burst, lymphocyte proliferation and antibody production [16].

To investigate smolt-related changes, we used two different populations of juvenile Atlantic salmon from southern Norway; an ordinary anadromous strain Vosso and a landlocked strain Blege which is a freshwater resident dwarf salmon that has been isolated from the sea for approximately 9000 years due to elevation of the land post-glaciation. Earlier studies describe that the isolation of the Blege salmon to some degree may have resulted in lost key elements of the smoltification associated with marine life [17]. As a juvenile in the river, the Blege salmon looks like a usual parr, but becomes silvery as smolts at the time around migration into the lake [18]. Later studies have described the relict Blege strain as a “semi-smolt”. The strain had poor seawater-tolerance and high mortality after seawater entrance. Morphologically, they showed only moderate silvering and a slight increase in condition factor, and significantly lower Na<sup>+</sup>, K<sup>+</sup>-ATPase activity, compared to anadromous control fishes during the normal smoltification period [17]. We examined the expression of the cytokines TNF- $\alpha$ , IL-1 $\beta$  and COX-2 in the primary lymphoid organs head kidney and spleen since these comprise central parts of the immune system in fish. In addition, the gills were examined since evidences exist that this organ has immune functions [9,19] and the gill tissue is known to undergo histological changes during smoltification.

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