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The effects of *Lepeophtheirus salmonis* infections on the stress response and immunological status of Atlantic salmon (*Salmo salar*)

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Abstract

This study was conducted to determine the effects of a high level of infection of the parasitic copepod *L. salmonis* on the stress response and immunological status of Atlantic salmon. An initial low-level initial infection was carried out 14 d prior to a second infection in which twice as many parasites were introduced. Plasma cortisol and prostaglandin E_2 (PGE_2) levels were monitored concurrent to the expression of six immune-related genes over five sample times (9, 21, 26, 33 and 40 days post initial infection, dpii). The mean lice counts on the infected fish increased significantly from the first infection (16.3 ± 1.89 at 9 dpii) to the second (142.8 ± 12.8 at 26 dpii). Plasma cortisol levels increased significantly at 26, 33 and 40 dpii in infected fish compared to controls. Plasma PGE_2 levels were significantly higher in infected fish at 9, 33 and 40 dpii, when compared to controls. At 9 dpii, expression of interleukin-1β (IL-1β), tumour necrosis factor-α (TNFα)-like cytokine, major histocompatibility class II (MH II), transforming growth factor-β (TGFβ)-like cytokine and cyclooxygenase-2 genes were increased in infected fish compared to controls. The expression of most of these genes returned to control levels at 21 dpii when the highest expression of the MH class I gene was observed in infected fish (significantly higher than controls). Major histocompatibility class I gene expression remained higher in infected fish at 26 and 33 dpii compared to controls and this was observed for the TNFα-like gene. By 33 dpii, MH class II and TGFβ-like genes had higher expression in infected fish compared to controls. Interleukin-1β and TNFα-like gene were the only genes that showed significantly higher expression in infected fish compared to controls at 40 dpii, while MH class I gene expression was significantly depressed in infected fish at this time. The expression of nearly all immune-related genes studied here increased following initial infection with *L. salmonis*, however, immunological stimulation did not reduce parasite numbers or protect against re-infection.

Keywords: *Lepeophtheirus salmonis*; Gene expression; Cytokines; COX-2; Inflammation; Prostaglandin E_2; Atlantic salmon; Infection

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1. Introduction

Molecular characterisation of pathogenic organisms and their fish hosts has become a common approach for studying host–parasite relationships. Recently, several papers have been published on parasitic ciliates, flagellates and monogeneans and their interactions with their host species [1–4]. While these types of studies exist for terrestrial parasitic arthropods [5], the literature is limited with respect to economically important arthropod parasites of fish, such as the sea louse, *Lepeophtheirus salmonis*.

The ectoparasitic copepod *L. salmonis* becomes infective towards salmonid hosts (genera *Salmo*, *Salvelinus* and *Oncorhynchus*) upon reaching its free-swimming copepodid stage. Following infection, the parasite undergoes a series of moults passing through four attached chalimus stages (I–IV) and two mobile pre-adult stages (I–II) prior to becoming an adult. *Lepeophtheirus salmonis* infections on susceptible hosts (i.e. Atlantic salmon, *Salmo salar*) are notable in that no significant inflammatory response is elicited despite its feeding on host mucus, tissues and blood [6]. However, a well-developed inflammatory response is associated with resistance to infection in coho salmon (*Oncorhynchus kisutch*) [6]. Suppression of this response, by the administration of cortisol, results in the loss of resistance to infection [7]. It has recently been proposed that immunomodulation of the host in the absence of stress (i.e. high levels of cortisol) is responsible for the lack of response of Atlantic salmon to *L. salmonis* [8,9]. The identification of trypsin and PGE_2 in the secretions of *L. salmonis* has given validity to this hypothesis [8,10,11]. Prostaglandin E_2 is a vasodilatory compound that inhibits several salmonid immune-related genes including interleukin-1β (IL-1β), cyclooxygenase-2 (COX-2), and major histocompatibility (MH) class I and II [12]. Prostaglandin E_2 may be elevated in Atlantic salmon infected with *L. salmonis* [13].

Due to its importance to salmonid aquaculture, numerous studies on the biology, ecology and the host–parasite relationship have been published on *L. salmonis* [9,14,15]. There are a few studies that have used functional assays, such as macrophage respiratory burst and phagocytosis, to examine the effects of *L. salmonis* infection on the host. With respect to effects on immune-related gene expression there are limited data. Fast et al. [12] examined the effects of PGE_2, a component of *L. salmonis* secretions, on immune-related gene expression on the Atlantic salmon head kidney cell line (SHK-1). It was demonstrated, in the absence of a stress response, that PGE_2, at physiologically meaningful levels, was able to inhibit expression of immune-related genes: IL-1β, COX-2 and MH class I and II. Therefore, PGE_2 could be used by *L. salmonis* not only to increase blood to the feeding site but also to prevent leukocyte recruitment and presentation of parasitic antigens to T lymphocytes. The effects of a single, low-level infection (8–15 lice/fish) on constitutive and inducible immune-related gene expression in Atlantic salmon has also been examined [16]. In that study, increases were observed in MH class II and IL-1β expression and decreases in MH class I gene expression and attributed to a change in lymphatic tissue cell populations in response to parasitic infection. There are no data currently, however, on the effects of repeat infections or at higher levels of infection on salmonid immune functions.

The purpose of this study was to examine the effects of high levels of infection on the stress response and expression of immune-related genes of Atlantic salmon. As it is uncommon for heavy infections to result from a single pulse of copepodids, the high level of *L. salmonis* infections, in this study, were obtained by two infections separated by a period of 2 weeks.

2. Materials and methods

2.1. Fish

Post-smolt Atlantic salmon (Saint John River strain) were maintained in 1000 L tanks containing 300 L of seawater (SW). Fish were initially obtained from a freshwater hatchery following smoltification. Tanks were supplied with flow-through SW at 11–13 °C and maintained under a 12-h light:12-h dark photoperiod. Fish were separated into two populations (one uninfected, one infected) each with 50 fish. The average weights of the fish ranged from 281.9 g to 371.3 g over the course of the study.

2.2. Copepodid production

Ovigerous *L. salmonis* were collected from recently harvested fish at salmon processing plants in New Brunswick, Canada. They were transported back to the laboratory on ice (ca. 10–15 °C), where their eggstrings were removed