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Effects of (*Margaritifera margaritifera*) glochidial infection on performance of tank-reared Atlantic salmon (*Salmo salar*)

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Abstract

Experimental infection of pearl mussel glochidia on the performance of Atlantic salmon hosts was examined. Infection intensity was 1392±641 SD glochidia per fish six weeks after challenge but declined significantly 15 weeks after infection to means of 50 and 112 fish^{-1} in two trial tanks and, by 6 months numbers were <1 fish^{-1}. This loss was attributed to fungal treatments with a combination of malachite green which was still the authorised treatment during the study in 2001 and formalin. The weight of infected fish was significantly lower than controls at 15 weeks but this was not significant after 1 year. The condition factor of infected and naïve fish was not significantly different. Lactate was measured as a possible indicator of stress in infected fish but there was no significant difference with controls. Fish that had been previously infected with glochidia were re-infected in their second year and comparison made with infection of naïve fish to determine whether glochidial infection elicits an immune response. Although there was no significant difference in glochidial numbers in both groups at around 9000 glochidia host^{-1} 3 weeks after infection, numbers of glochidia in naïve fish did not change to 15 weeks after challenge, whereas there was a significant reduction in previously infected fish to 116 fish^{-1}. Various treatments were used to provoke closure of the glochidial valves. It is concluded that infection of salmon with glochidia levels in the current study had no significant effect on salmon performance, condition and stress as measured by assay of lactate.

Keywords: Pearl mussel; *Margaritifera margaritifera*; Glochidia; Salmonid; *Salmo salar*

1. Introduction

The endangered freshwater pearl mussel, *Margaritifera margaritifera*, has a dispersive parasitic larval (glochidial) stage and large numbers are shed by adult mussels in mid-summer. These attach to the gills of salmonids where they encyst and remain for c. 12 months before excysting and dropping to the river bed where they settle (Hastie and Young, 2001). Compared to other parasitic infections of salmon and trout, glochidiosis is of little economic importance. However, in certain situations, pearl mussels can cause problems for fish farmers (Hastie and Young, 2003). Most studies of the mussel–host relationship have focussed on distribution and

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ecology (Young and Williams, 1984a,b; Hastie and Young, 2001, 2003), effects of previous challenges on glochidial survival (Young et al., 1987), and comparative susceptibility of different fish hosts to infection (Meyers and Millemann, 1977; Bauer, 1987). Relatively little attention has focussed on the impacts of glochidia on the performance and survival of the host (Treasurer and Turnbull, 2000). While some authorities have suggested that glochidiosis has little effect on host growth and survival, others have indicated that high levels of infection in farmed situations may induce mortalities and anglers and fishery managers have implied that pearl mussels may be detrimental to endangered salmonid stocks. The impact of pearl mussel glochidia on their host is difficult to measure and monitor in the field. In the present study the potential impact of infection was assessed by the artificial infection of cultured Atlantic salmon and by measurement of juvenile salmon in replicated tanks with comparison with naive fish. Although immune responses of fish to bacterial and viral infection have been frequently documented, measurement of host immune response to parasitic infection has been limited (Meyers et al., 1980; Bauer and Vogel, 1987). It has been suggested that older fish previously challenged with glochidia have lower infection intensity and, although it has been inferred that fish mount an immune response, this has not been verified (Bauer, 1987; Hastie and Young, 2001). Therefore previously challenged fish were re-infected experimentally, and compared with infection of naïve control fish, to assess if an immune response could be elicited.

2. Methods

2.1. Infection

Uninfected juvenile salmon of 5.6±1.5 SD g mean weight were transferred from a salmon hatchery in Lochaber in June 2001 to an outdoor experimental hatchery. 300 fish were infected by cohabiting with five spawning pearl mussels checked for spawning condition by carefully opening their shell valves with special opening tongs, and checking for the presence of glochidia in the modified gill structures of the female mussels (Young and Williams, 1984a). Salmon were cohabited in a tank overnight with pearl mussels to permit natural infection. The fish were evenly divided between two 1 $m^2 \times 40$ cm glassfibre tanks. Flow rate was 101 min^{-1}, and water temperatures were recorded daily and were in the range 3 to 17 °C during the first year of the infection study. A total of 300 non-infected control fish were maintained in two other adjacent tanks. Fish were fed twice daily to satiation with 2 mm Trouw diet. Mortalities were removed daily and recorded. Fifty fish from each tank were anaesthetised in 20 mg l^{-1} benzocaine and measured two weeks after infection and then at 6, 10, 15, 25, 30 and 36 weeks to fork length mm and weight to 0.01 g. Ten of these fish were killed, five from each tank, by overdose in benzocaine and gills from the left side of the fish excised and examined at ×10 magnification and glochidia counted on the first gill arch. As previous studies have found no significant difference in glochidia numbers between gill arches (Treasurer and Turnbull, 2000) the total number of glochidia per fish was calculated by a multiplier of 8. Glochidia were measured at ×40 magnification laterally across the carapace using an ocular micrometer. Blood samples were collected from the caudal fin from infected and control fish 15 weeks after commencement of the experiment and samples tested for lactate as a measurement of stress. Plasma lactate was determined enzymatically using Sigma Diagnostic kits (Sigma; Proc. no. 735). The condition of salmon was measured as (body mass g 100/length^3). A sample of fish used in the first infection experiment was also examined at the donor hatchery from the main stock 5 months after natural infection in the hatchery. Twenty fish were measured and gills examined as above.

2.2. Re-infection experiment

One year post-infection, the remaining fish in both trial and control groups were infected with glochidia on 15 July 2002 by experimental challenge. Fish were sampled 6 and 12 weeks after infection and numbers of glochidia counted on all gills in 5 fish from each treatment.

2.3. Methods for promoting closure of glochidia

An attempt was made to prevent infection of hatchery reared salmon by glochidia by causing closure of the glochidia before they passed through the hatchery. Collected spat of 0.5 ml volume were introduced to 5 ml capacity six well plates containing test solutions. Spat were assessed for closure of valves, immediately and after exposure to test solutions for 5 min. 10 ml of unchlorinated water were added to the mussel suspension at this point and mussel viability was assessed after 24 h. The treatments were: NaCl at 200, 2000, 5000, 10,000 and 50,000 mg l^{-1}, hydrogen peroxide at 17,500, 35,000, 175,000 and 350,000 mg l^{-1}, formaldehyde at 50, 100, 200, 400, 1000, 2000, and 20,000 mg l^{-1}, and copper sulphate at 250, 500, 5000, 50,000 mg l^{-1}.