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Differences in metabolic response to *Loma salmonae* infection in juvenile rainbow trout *Oncorhynchus mykiss* and brook trout *Salvelinus fontinalis*

Mark D. Powell[1,*], David J. Speare[2], Joanne Daley[2], Jan Lovy[2]

[1]School of Aquaculture, Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, Locked Bag 1370, Launceston, Tasmania 7250, Australia

[2]Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown, Prince Edward Island C1A 4P3, Canada

ABSTRACT: Routine and post-exercise metabolic rates were measured for juvenile rainbow trout *Oncorhynchus mykiss* and brook trout *Salvelinus fontinalis* infected with the microsporidium gill parasite *Loma salmonae* under laboratory conditions. Rainbow trout increased routine and post-exercise metabolic rate in response to infection compared with controls. Brook trout, on the other hand, lowered routine metabolic rate without effecting post-exercise metabolic rate compared to controls. The result of these 2 different strategies may either reflect defense of metabolic scope or a difference in the rate of recovery of the excess post-exercise oxygen consumption between the 2 species in response to the same infection.

KEY WORDS: *Loma salmonae* · Metabolic rate · Microsporidian · Oxygen consumption · Salmonid

INTRODUCTION

Microsporidian gill disease caused by *Loma salmonae* is of major economic significance to salmonid husbandry in hatcheries (Hauck 1984) and marine net-pens (Kent et al. 1989, Speare et al. 1989), where it forms xenomas of hypertrophied cells visible as white cysts on the gills. On the basis of PCR-based detection, it is suggested that in rainbow trout at 15°C the parasite enters the host through the gut, quickly migrates to cardiovascular tissues, and then migrates to the gills where characteristic xenomas are formed (Speare et al. 1998, Sanchez et al. 2000, 2001a,b). Of particular interest is that the growth rate of the fish is suppressed almost at the onset of xenoma resolution, when xenomas are replaced by a multifocal branchitis (Speare et al. 1998).

Although metabolic rates have been measured for most salmonid species, little information exists with regard to the effects of disease on this parameter. Fisk et al. (2002) noted that in Atlantic salmon *Salmo salar* L. there was no difference in routine metabolic rates between fish affected by amoebic gill disease and control fish. However, under hypoxia, infected individuals had higher oxygen consumption rates, indicating a possible increase in metabolic scope. The aim of the present study was to examine routine and maximal metabolic rate in *Loma salmonae*-infected freshwater rainbow and brook trout.

MATERIALS AND METHODS

Juvenile rainbow trout *Oncorhynchus mykiss* (mean wt 34.4 ± 1.7 g SE, mean fork length 15.4 ± 0.2 cm SE) and brook trout *Salvelinus fontinalis* (mean wt 33.4 ± 2.4 g SE, mean fork length 15.2 ± 0.3 cm SE) were obtained from Cardigan Fish hatchery, Cardigan,

*Email: mark.powell@utas.edu.au

Prince Edward Island, and acclimated at 15°C for several months in the aquarium facility of the Atlantic Veterinary College, in Charlottetown.

A total of 150 fishes were distributed between 3 flow-through tanks, and individuals in 2 of the tanks were infected with 2×10^6 *Loma salmonae* spores by feeding with infected gill tissue. The third tank contained uninfected fishes (control). The *L. salmonae* spores fed to the fishes had been isolated previously from infected rainbow trout or brook trout. The brook trout *Salvelinus*-variant of *L. salmonae* was derived from that described by Sanchez et al. (2001b), whereby the sequential pathology is the same as that for the *Oncorhynchus*-variant used in rainbow trout. This standard method of infection challenge with *L. salmonae* results in 100% of the fishes becoming infected within 6 wk (Speare et al. 1998). Water in all tanks was maintained at 15°C and 95% air-saturation. The fishes were fed daily (equivalent of 2% of their body weight) on a commercial extruded-pellet diet (HiPro 3.0 GR, Corey Feed Mills).

Table 1. *Oncorhynchus mykiss* and *Salvelinus fontinalis*. Range of oxygen tensions (mm Hg) in respirometers during oxygen consumption measurements of uninfected (Control) and *Loma salmonea*-infected (Infected) fishes

	Rainbow trout		Brook trout	
	Control	Infected	Control	Infected
Routine				
Start	157.1–152.0	158.4–149.8	155.3–149.8	156.2–150.5
End	148.3–127.0	140.1–113.3	134.7–98.0	151.3–129.0
Post-exercise				
Start	159.7–155.5	159.2–152.4	152.2–142.9	160.8–150.0
End	27.9–98.6	126.5–94.6	109.0–91.5	123.4–105.5

After 4 wk post-inoculation, the fishes were starved for 24 h prior to being randomly selected and placed for 24 h into individual, black, acrylic respirometer chambers (internal volume approximately 1 l) supplied with the same flow-through freshwater as that supplying the tanks. Food was withheld from fish whilst they were in the respirometer chambers. A total of 9 rainbow trout and 8 brook trout were tested from each treatment (infected or uninfected controls). Control and infected individuals of each species were tested on the same day.

To determine oxygen uptake, the water flow to each chamber was stopped and the chamber sealed. A 1 ml water sample was removed and injected into a thermostatically controlled oxygen electrode (1302 electrode, Strathkelvin instruments) calibrated against a 2% $NaSO_3$ solution (zero) and air-saturated freshwater (~158 mm Hg). The electrode was connected to a Strathkelvin Instruments model 782 O_2 meter. A second water sample was removed after 15 min and remeasured, after which the water flow was restored to the respirometer chambers. Oxygen consumption rate, MO_2, was calculated as below:

$$\frac{[(PO_{2i} - PO_{2e}) \times \alpha] \times V}{t \times M}$$

modified from Cech (1990)

where PO_{2i} and PO_{2e} are the initial and final water oxygen tensions respectively (mm Hg), α is the molar oxygen solubility in water (after Cameron 1986) in mmol O_2 l^{-1} mm Hg^{-1}, V is the respirometer chamber volume (l), t is the measurement time (15 min) and M is the mass of the fish (g). Removal of the water sample enables a small amount of air to enter the chamber. However, at water oxygen tensions used in this study (Table 1), oxygen transfer is negligible (M. D. Powell unpubl.) and within the margin of error acceptable for measurements using static respirometry. However, all measurements were corrected for the oxygen transfer rate from the air to the water.

The same fishes were then individually removed from their respective respirometer chambers and 'chased' (i.e. forced into continual swimming) according to the method of Altimiras et al. (2002). Briefly, each fish was placed in a 10 l bucket of water (15°C) to which oxygen was added to maintain 120% air saturation (measured by YSI 550 dissolved oxygen meter: Yellow Springs Instruments), and was then chased in the bucket using a blunt stick or by hand to ensure that it swam continuously for 10 min. The fish was then immediately returned to the respirometer chamber (transfer time <15 s), which was sealed; oxygen measurements were then made as described above. All fishes were subsequently removed from the respirometers. Rainbow trout were euthanased with an overdose of benzocaine (>60 mg l^{-1}), their gills removed, and *Loma salmonae* xenomas on the first gill arch were counted using a dissecting microscope. The remaining gill arches were fixed in 10% neutral buffered formalin and processed for histological examination. Brook trout were anaesthetised with benzocaine (60 mg l^{-1}) and non-lethally screened, and their gills scored for xenomas infection as clear (0), light (1), medium (2) or heavy (3) under a dissecting microscope (similar to the method of Ramsay et al. 2003). We killed 3 *L. salmonae*-infected brook trout with an overdose of benzocaine anaesthetic, and their gills were dissected and fixed in 10% neutral buffered formalin and processed for histological examination.

Routine (non-chased) and post-exercise (chased) oxygen consumption rates were compared for each

species using a 2-factor repeated-measures analysis of variance with control/infected and routine/post-exercise measurement as fixed factors. Where differences were detected, Bonferroni post-hoc comparisons were used to identify differences in means; p values <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

After 4 wk post-inoculation, rainbow trout contained 1.8 ± 0.8 (mean ± SE) xenomas per arch compared to zero in the controls. Although this level of infection is low compared with that reported for other studies (Becker et al. 2002), 4 wk post-infection represented a point at which the infection was increasing rapidly. Some rainbow trout were also tested at Week 7 post-infection with identical results (M. D. Powell unpubl.). Similarly infected brook trout scored an average of 2.0 (± 0.4 SE) compared with controls that had no xenomas. There were no differences in the presentation of xenomas on the gills of rainbow trout or brook trout (Fig. 1).

For both species tested, routine oxygen consumption rates of controls were equivalent to those measured in brown trout at similar temperatures by Altimiras et al. (2002). In our study, post-exercise oxygen consumption rates were higher than those for pre-exercise (routine) (rainbow trout: $F_{8,35}$ 114.9, p < 0.0001; brook trout: $F_{7,31}$ 88.2, p < 0.0001) (Fig. 2). Both the pre-exercise (routine) and post-exercise oxygen consumption of rainbow trout was higher in *Loma salmonae*-infected fishes compared with uninfected controls ($F_{8,35}$ 69.18, p < 0.0001). However, in brook trout the pre-exercise (routine) oxygen consumption rate of *L. salmonae*-infected fish was lower than that of controls, and post-exercise oxygen consumption rates were similar between control and *L. salmonae*-infected fish ($F_{7,31}$ 5.63, p = 0.0494) (Fig. 2).

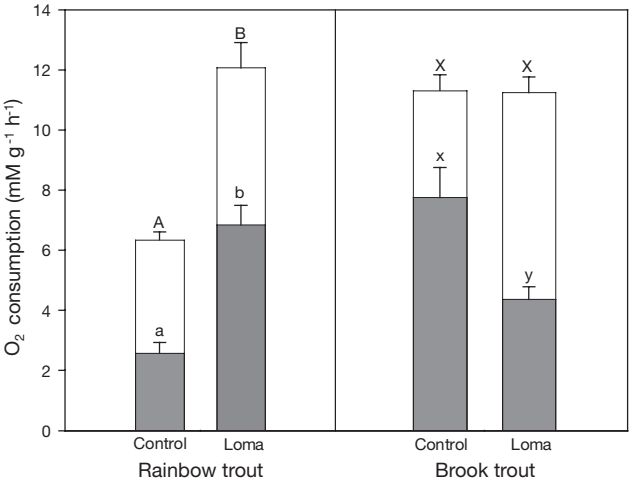

Fig. 2. *Oncorhynchus mykiss* and *Salvelinus fontinalis*. Mean (+SE) routine and maximal oxygen consumption rates for control and 4 wk *Loma salmonae*-infected (Loma) rainbow trout (n = 9) and brook trout (n = 8). Different letters indicate significant differences between routine (shaded) and maximal (open) rates at p > 0.05

The routine and post-exercise oxygen consumption rates of rainbow trout were regressed against xenoma number to determine whether the level of infection influenced the rate of oxygen consumption. A weak, albeit non-significant, relationship was found for both oxygen consumption rates (Fig. 3). It was not possible

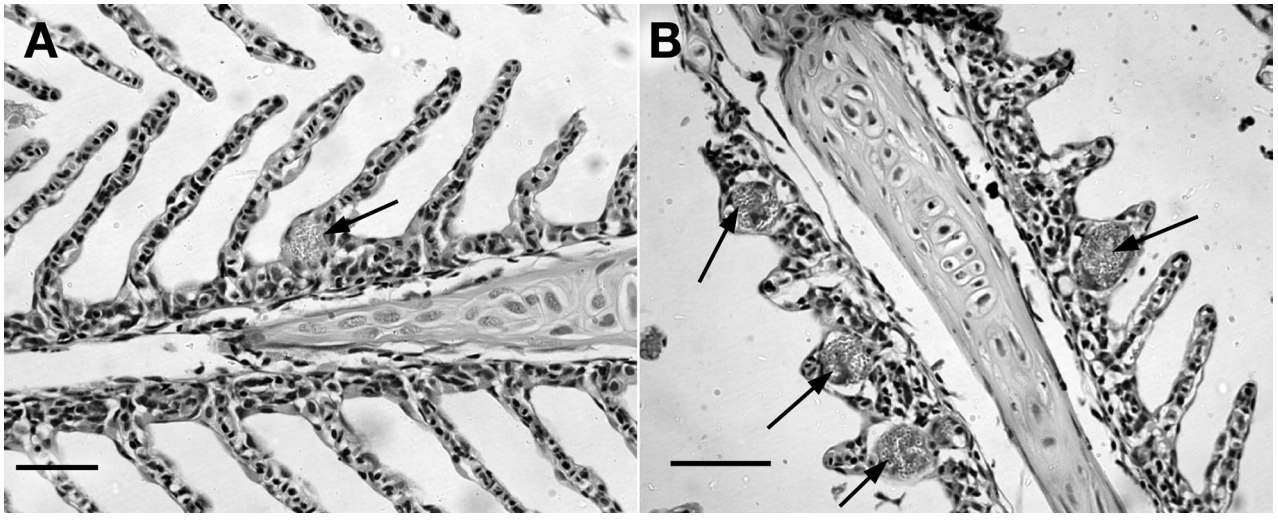

Fig. 1. (A) *Oncorhynchus mykiss*; (B) *Salvelinus fontinalis*. Gill xenomas (arrowed) after 4 wk infection with *Loma salmonae*. (H&E Stain; scale bars = 50 µm)

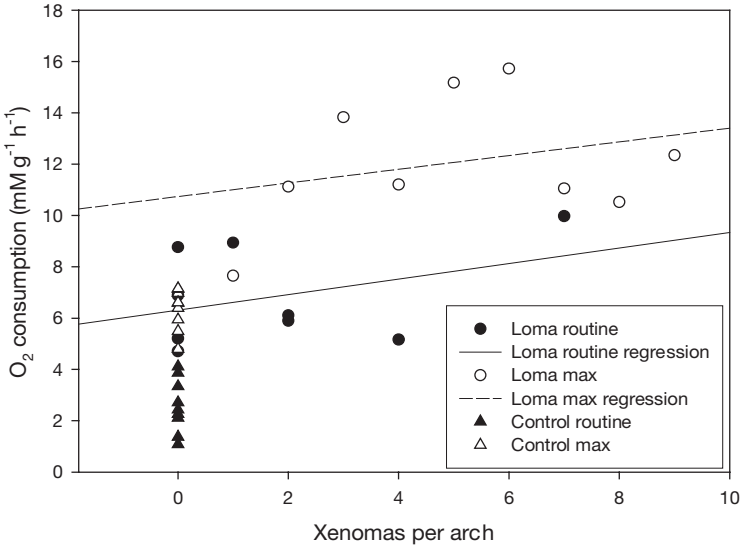

Fig. 3. *Oncorhynchus mykiss.* Scatter-plot of oxygen consumption rate versus number of xenomas per gill arch for control and *Loma salmonae*-infected fish (Loma). Regression lines represent routine (continuous line: slope = 0.397, $r^2 = 0.142$) and post-exercise (dashed line: slope = 0.303, $r^2 = 0.143$) oxygen consumption rates for *L. salmonae*-infected fish

to perform the same assessment for brook trout owing to the non-parametric assessment of xenoma numbers (infection level). This suggested that there may be differences in the oxygen consumption rate response (metabolic rate) of rainbow trout and brook trout to *Loma salmonae* infection. In rainbow trout, routine metabolic rate was increased in infected fish compared with controls (Fig. 2), whereas in brook trout there was a decrease in routine metabolic rate. Although maximal metabolic rates (post-exercise) were significantly higher than routine rates for both species, in rainbow trout the maximal rate for infected fish was higher than that for controls of the same species. In fact, the maximal rate for control fish was the same as the routine rate for the infected group. However, in brook trout, the maximal metabolic rates were identical for both control and infected fish.

Following exercise, oxygen consumption rate is at its maximal value (MO_{2max}); however, this is in effect an instantaneous rate, with the recovery from exercise resulting in excessive post-exercise oxygen consumption (EPOC) (Lee et al. 2003). In our study it was not possible to distinguish between the EPOC and MO_{2max}. However, Lee (2002, cited in Lee et al. 2003) claimed that both the EPOC and the rate of recovery from exercise are proportional to MO_{2max}. From our results it would therefore appear that rainbow trout infected with *Loma salmonae* recover from exercise at a similar rate as uninfected fish (assuming that the difference between routine and post-exercise oxygen consumption rates equates with EPOC). If the EPOC is proportional to MO_{2max}, and we assume that $MO_{2routine}$ is a proxy for standard metabolic rate $MO_{2standard}$ (an instantaneous rate of the lowest measured oxygen consumption within a 24 h period, and not possible to determine in this study), then it may indicate that the metabolic scope (i.e. the difference between standard and maximal metabolic rate [Cech 1990]) is defended in *L. salmonae*-infected trout even though the $MO_{2routine}$ is elevated (Fig. 2). Moreover, the level of infection had no effect on the difference between routine and post-exercise oxygen consumption rates (Fig. 3). Brook trout, on the other hand, appear to use a different metabolic strategy. Routine oxygen consumption decreased in fish with *L. salmonae* infection, and post-exercise (maximum) oxygen consumption remained the same as that of controls. This suggested that either the metabolic cope was effectively increased or else the EPOC was greater than in controls. Either way, the response to a similar infection differed between the 2 species.

This indicates that the maximal oxygen requirements of the 2 species may differ, depending upon whether they are infected or not, and upon the level of stress (exercise) to which they are subjected.

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