

Metabolic effects of amoebic gill disease (AGD) and chloramine-T exposure in seawater-acclimated Atlantic salmon *Salmo salar*

M. J. Leef^{1,2}, J. O. Harris^{1,3}, M. D. Powell^{1,4,*}

¹School of Aquaculture, Tasmanian Aquaculture and Fisheries Institute, University of Tasmania and Cooperative Research Centre for Sustainable Aquaculture of Finfish, University of Tasmania, Locked Bag 1-370, Launceston, Tasmania 7250, Australia

²Present address: National Institute of Water and Atmospheric Research Ltd, PO Box 147, Ruakaka, New Zealand

³Present address: School of Biological Sciences, Faculty of Science and Engineering, Flinders University of South Australia, GPO Box 2100, Adelaide, South Australia 5001, Australia

⁴Present address: Department of Fisheries and Natural Sciences, Bodø University College, 8049 Bodø, Norway

ABSTRACT: Our aim was to determine possible metabolic effects amoebic gill disease (AGD) on Atlantic salmon *Salmo salar*. Standard (R_S) and routine (R_{ROU}) metabolic rates were evaluated by continually measuring oxygen consumption in 2 independent tanks of fish ($18.69 \pm 1.01 \text{ kg m}^{-3}$, mean \pm SE). Active metabolic rate (R_{ACT}) and metabolic scope ($R_{ACT} - R_S$) were assessed using a chasing protocol and determined at 3 time periods: (1) pre-infection, (2) 3 d post-infection, and (3) 2 d post-treatment. On Day 3 of the study, the fish were infected with amoebae isolated from the gills of AGD-affected salmon ($2300 \text{ cells l}^{-1}$). No significant elevations in R_{ACT} or metabolic scope were detected 3 d post-infection and 2 d post-treatment; however, significant elevations in R_S and R_{ROU} were detected 3 d post-infection and 2 d post-treatment. Assessment of R_{ROU} data, especially for the light period, also indicated a rise in oxygen consumption rate over the course of the experiment. Treatment of AGD-affected Atlantic salmon with chloramine-T (CL-T) appeared to briefly mitigate the rise in R_S , as there was a 30% drop (though non-significant) in R_S following treatment. Despite this, R_S continued the upward trend 1 d following treatment. These results suggest that over the course of AGD development, R_S in Atlantic salmon increases. Therefore, considering the physical conditions which constrain R_{ACT} , we expect that metabolic scope would become compromised in fish more heavily affected with AGD. Treatment with CL-T shows promise for mitigating the respiratory effects of AGD and potentially minimising the loss of metabolic scope.

KEY WORDS: Amoebic gill disease · AGD · Oxygen uptake · Metabolic scope · Chloramine-T · Atlantic salmon

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

Amoebic gill disease (AGD) is currently the most significant health issue affecting the production of Atlantic salmon *Salmo salar* (L.) in Tasmania (Munday et al. 1990, 2001). The causative agent *Neoparamoeba* spp. (Dyková et al. 2005) was first described in sea-pen reared coho salmon *Oncorhynchus kisutch* (Walbaum) (Kent et al. 1988, Dyková et al. 2000). Seemingly ubiquitous in nature, *Neoparamoeba* spp. are presumed

responsible for AGD outbreaks in a number of countries including Ireland, USA, Chile, Australia and New Zealand, and also in a number of other fish species including rainbow trout *O. mykiss* (Walbaum) (Munday et al. 1990), turbot *Scophthalmus maximus* (L.) (Dyková et al. 1998) and European seabass *Dicentrarchus labrax* (L.) (Dyková et al. 2000) (reviewed by Munday et al. 2001, Nowak et al. 2002).

Treatment of AGD-affected Atlantic salmon in Tasmania currently involves freshwater bathing; however,

*Corresponding author. Email: mark.powell@hibo.no

the apparent efficacy of freshwater has notably decreased, with a recent study reporting that amoeba numbers returned to pre-bath levels within 10 d (Clark et al. 2003). To date, several chemical additives to freshwater baths have been tested with varying degrees of success (Howard & Carson 1993, Clark & Nowak 1999, Findlay & Munday 2000, Zilberg et al. 2000, Powell & Clark 2003). Chloramine-T (CL-T, Halamid™), a widely used chemotherapeutic and chemoprophylactic treatment for gill diseases in the freshwater aquaculture industry (Thorburn & Moccia 1993) was found to increase freshwater bathing efficacy and reduced amoeba survival (Powell & Clark 2003). Recent studies also suggest that CL-T in seawater is as effective in seawater as in fresh water (Harris et al. 2004, 2005).

Investigations into metabolic rate (determined by measuring oxygen consumption) have proven useful in understanding the energetic costs of smoltification (Maxime 2002), starvation (O'Connor et al. 2000), the response to hypoxia and toxicant exposure (Farrell et al. 1998) and the effects of ploidy and temperature (Altimiras et al. 2002). There are 3 main levels of aerobic metabolism and these include standard, routine and active metabolic rates (Fry 1971). Standard or basal metabolic rate is an approximation of the minimum rate of oxygen consumption for a resting animal, while routine metabolic rate describes the rate of oxygen consumption for a spontaneously active animal (Maxime 2002). Active metabolic rate describes the maximum sustained rate of oxygen consumption for a fish swimming steadily, and therefore determination of both standard and active metabolic rates permits calculation of aerobic metabolic scope (Fry 1971).

There are only relatively minor differences in oxygen uptake between AGD-affected and naïve Atlantic salmon under normoxic conditions (Powell et al. 2000, Fisk et al. 2002). However, a comprehensive view of the metabolic effects of disease over the course of AGD development is not yet available. Our main objectives were to examine both routine and standard metabolic rate in AGD-affected Atlantic salmon and to assess any changes during the progression of the disease and after bathing with CL-T in seawater. Additionally, active metabolic rates were determined at 3 key periods (pre-infection, post-infection, and post-treatment) and measurements were used to determine the corresponding metabolic scope. We hypothesised that as the infection progressed, standard and routine metabolic rates would increase while active metabolic rate would fall, resulting in a decreased metabolic scope. We did not know whether bathing with CL-T would mitigate or exacerbate this scope reduction.

MATERIALS AND METHODS

Fish, husbandry and maintenance. Atlantic salmon smolts (mean mass \pm SE: 123.01 \pm 8.54 g) obtained from a commercial salmon hatchery (Saltas, Wayatinah, Tasmania, Australia) were acclimated and held in recirculating full strength seawater in a fibreglass Rathbun tank with associated biofilter and swirl separator (4000 l) at a temperature of 15.0 to 16.0°C. Water quality parameters that included temperature, pH, ammonium, nitrite and nitrate concentrations were recorded daily using test kits (Australian Pet Supplies). Fish were weighed to the nearest 0.50 g, measured to the nearest 0.50 cm and maintained on a commercial pellet diet (Atlantic Salmon Grower LE). Feeding was stopped when fish were acclimated to the experimental systems.

Experimental system. Four 300 l plastic respirometer tanks with separate sumps and biofilters were used as experimental tanks. Submersible aquarium pumps (Unistar Power head POW 300-4) were used to recirculate the water from the sump to the tank. Water was delivered to each tank via a 2-way manifold in such a way that it was possible to supply water through an inlet manifold below tank fluid level and make measurements simultaneously. The flow was directed to either an open chamber draining to the tank below water level, or to the main inlet manifold. Lids were made for the tanks to restrict light and prevent fish escapes. This design limited all bubbling of water to the sump and restricted the surface area available for gas transfer to the tank surface only. The drains for the tanks were on the sides at the bases, and vertical pipes with T-pieces at the top were used to regulate depth. This provided a chamber at the top for measurement of outflow water. One tank not stocked with fish acted as a control for determination of background oxygen demand by microorganisms as well as oxygen transfer from the tank to the atmosphere (Fig. 1). The 4 experimental tanks were cleaned and all water was replaced prior to the onset of each experiment. Prior to experimentation, 3 small groups consisting of 15 fish were placed into 3 of the experimental tanks (mean \pm SE: 18.7 \pm 1.0 kg m⁻³). Fish were habituated for ~3 d before the experiment proceeded. Feeding was then discontinued.

Measurement of oxygen consumption rates. Oxyguard stationary probes were calibrated weekly to zero in either water bubbled with nitrogen for 20 min or in an aqueous 2% sodium sulphite solution, and to 100% air saturation in water bubbled with air for 20 min. A period of 4 min was sufficient to obtain a stable signal during calibrations. Each Oxyguard stationary probe was inserted into the top of the outlet pipe so that effluent water flowed continually over the probe. Outlet water oxygen saturation was measured

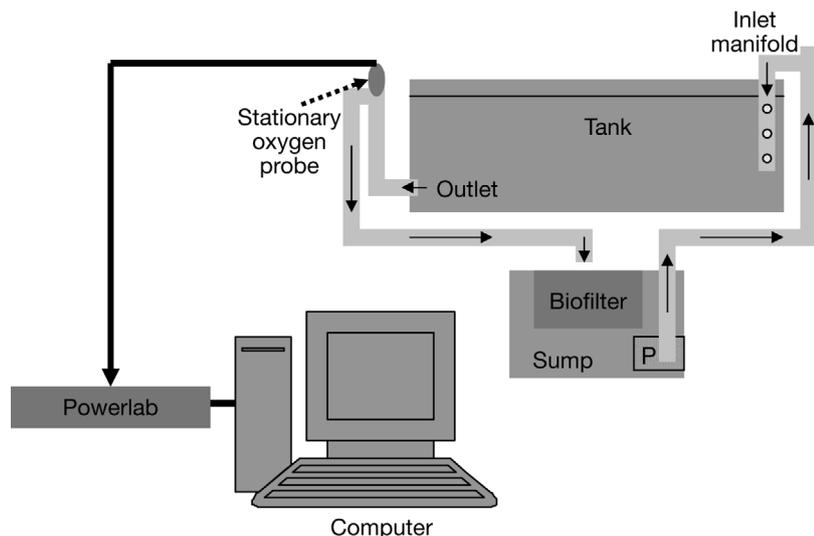


Fig. 1. Schematic of an individual experimental system showing the circular tank, inlet and outlet stations, biofilter, sump, and submersible pump (P). The stationary oxygen probe continually recorded oxygen saturation of outflowing water and was connected to a Powerlab® data acquisition system interfaced to a personal computer

continually with recordings being made every second using a Powerlab®/8SP data acquisition system (AD Instruments) interfaced to a portable personal computer. Air-saturated water in the sump (inlet) of each separate respirometer was measured daily using a handheld Oxyguard Handy Gamma probe similarly calibrated to zero and 100%. Oxygen concentrations within each of the sumps were consistently 100%. Daily checks of pH were made by collecting water in clean glassware from both inlets and outlets and using an Activon (AEP 333) combination electrode connected to a Cameron Instrument BGM 200 blood gas analyser. The probe was calibrated to ± 0.05 pH units using phosphate (pH = 7.00) and borate (pH = 9.28) buffer solutions daily before use (Bruno & Svoronos 1989). Oxygen consumption rates (modified from Forsberg 1996) were calculated from:

$$MO_2 = [(DO_{bi} - DO_{bo}) - (DO_{ti} - DO_{to})] \times F/B$$

where MO_2 is oxygen consumption by fish ($\text{mg O}_2 \text{ kg}^{-1} \text{ min}^{-1}$), DO_{bi} , DO_{bo} , DO_{ti} , DO_{to} are the dissolved oxygen inlet and outlet concentrations (mg l^{-1}) for the control and treatment tanks, respectively, b is before oxygen movement, t is after movement, i is inflow, o is outflow, F is the water flow (l min^{-1}) and B is the biomass (kg) measured at the end of each experiment. Fish oxygen consumption rates (MO_2 : $\text{mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$) were calculated for each hour period of each day.

Metabolic rate and scope measurements. Routine metabolic rates (R_{ROU}) were based on MO_2 values for spontaneously active groups of salmon. Standard meta-

bolic rates (R_S) for each day of the experiment were estimated as the minimum R_{ROU} measured during the night when fish were relatively motionless while remaining on the bottom of the tank, and interactions between individuals were minimal (Altimiras et al. 2002). In order to measure active metabolic rate (R_{ACT}), a protocol based on Cutts et al. (2002) was used. Fish from each tank were transferred to a 200 l tub in which the water oxygen content was first raised above air saturation (120 to 150%) by injection of pure oxygen. Fish were then agitated into burst swimming performance by chasing them until they were exhausted. Chasing protocols that use energetically inefficient burst swimming in fish (Dickson & Kramer 1971) are thought to be physically and biochemically analogous to exhaustive exercise (Reidy et al. 1995) and are therefore effective in the induction of

R_{ACT} (Cutts et al. 2002). Exhaustion was defined when all the fish refused to swim despite further chasing; this usually occurred within 10 to 12 min. The fish were then returned to their respective tanks and recording of MO_2 was resumed. Due to the lag effect of flow within the respirometer system, maximum MO_2 was measured approximately 20 to 25 min post-exhaustion in order to coincide with the R_{ACT} measurements. Metabolic scope was estimated from $R_{ACT} - R_S$ (Altimiras et al. 2002).

Experimental design and infection protocol. On Day 3 of the experiment, fish from each of the tanks were infected with *Neoparamoeba* spp. at a concentration of 2300 l^{-1} by the addition of amoebae isolated from the gills of fish with clinical AGD using the method of Howard & Carson (1993). Viable amoebae were counted with a haemocytometer using trypan blue as an exclusion stain (Parsons et al. 2001, Roberts & Powell 2003). Unaffected controls were similarly exposed to filtered seawater without the pathogenic amoebae. Confirmation of infection by histological examination of the second left gill arch was made at the end of the experiment on Day 12. All fish were lightly anaesthetised using 0.03 ml l^{-1} clove oil and mean mass and fork length were recorded before the fish were returned to the tank. Six fish from each tank were destructively sampled. The gills of these fish were removed and fixed in seawater/Davidson's fixative overnight, transferred to 70% ethanol for storage and later histological examination. Gill samples were processed (Tissue-Tek II automatic tissue-processing unit), paraffin wax embedded (Shandon Histocentre 2) and $5 \mu\text{m}$ histological sections of the second left gill

arch were cut (Microm microtome) and stained with haematoxylin and eosin (H&E). The total numbers of gill filaments with more than $\frac{3}{4}$ of the filament in clear cross section and the number of filaments with characteristic AGD lesions were recorded (Roberts & Powell 2003) to calculate the percentage of AGD-affected filaments.

On Day 9 of the experiment (6 d post-amoeba addition), fish from each of the tanks were transferred to 3 fully aerated 100 l tubs and treated with 10 mg l^{-1} of CL-T for 1 h after which the fish were returned to their respective tanks and recording of MO_2 was resumed. R_{ROU} measurements were made every day. R_{ACT} measurements were made on 3 separate occasions including Day 1 to represent naïve Atlantic salmon (pre-infection, PRE), Day 6 to represent AGD-affected salmon (AGD), and Day 11 to represent AGD-affected salmon following CL-T treatment (CL-T) in order to assess any differences in metabolic scope following infection and treatment with CL-T. To provide reference points for comparison, a second experimental run was conducted without the addition of amoebae.

Statistical analysis. At the conclusion of this experiment, data from 1 experimental tank was found to be erroneous due to non-functional probes in both experimental runs, and the statistical analysis for each treatment was therefore limited to duplicate tanks. On Day 0, MO_2 data collected for the AGD-affected experiment were lost overnight due to power failure; accordingly, the R_{S} data needed to calculate the pre-infection metabolic scope were those from Day 2. This R_{S} measurement was taken at least 20 h after the R_{ACT} measurement; in this way, any oxygen debt due to the chasing protocol would likely have been repaid (Cutts et al. 2002). On Day 3 of the experiment (amoebae addition), all but 4 h of data for all tanks were again lost due to power failure; data for this day have been omitted from any analyses. Data were also lost for approximately 1 h on Day 11, resulting in the loss of the latter part of the recovery period following the CL-T R_{ACT} measurement. Due to technical difficulties, parts of the control data were unsuitable for analysis and consequently are not presented. Similarly, R_{ACT} data from control fish were found not to be reliable and therefore are not presented. Statistical analysis of R_{ACT} , metabolic scope and recovery was therefore restricted to AGD-affected fish.

Within each treatment group, a 1-way analysis of variance ANOVA followed by a Tukey's post-hoc multiple comparisons

test was used to assess potential differences in R_{S} over each day of the experimental period. R_{ROU} data collected on Day 0 for both treatments were pooled (AGD and controls) to give a pre-experimental value. A 1-way ANOVA followed by a Tukey's post-hoc test was used to assess potential R_{ROU} differences. Analyses of R_{ACT} and metabolic scope were made using paired t -tests to compare Day 6 (AGD) values to measurements for Day 1 (PRE), for Day 6 (AGD) and for Day 11 (CL-T). Recovery data following the PRE and AGD chases were not subjected to statistical analysis; however, a paired t -test was used to assess differences in the time taken for fish to recover. An independent t -test was used to compare the percentage of AGD-affected gill filaments between the 2 experimental tanks. Values throughout the text are expressed as mean \pm SE for a 1 h period. SPSS® (version 10.0, SPSS Science) and Sigma Plot (version 6.0, SPSS Science) were used for all data analyses and presentation. Statistical significance was set at $p < 0.05$.

RESULTS

Fish sampled prior to transfer into the respirometers and the additions of amoebae were clear of all signs of AGD, and therefore fish were considered AGD-naïve prior to the experiment. As a result of this, AGD and unaffected control fish groups were pooled for statistical analysis at this time point. At the end of the experiment, all AGD fish sampled clearly showed typical

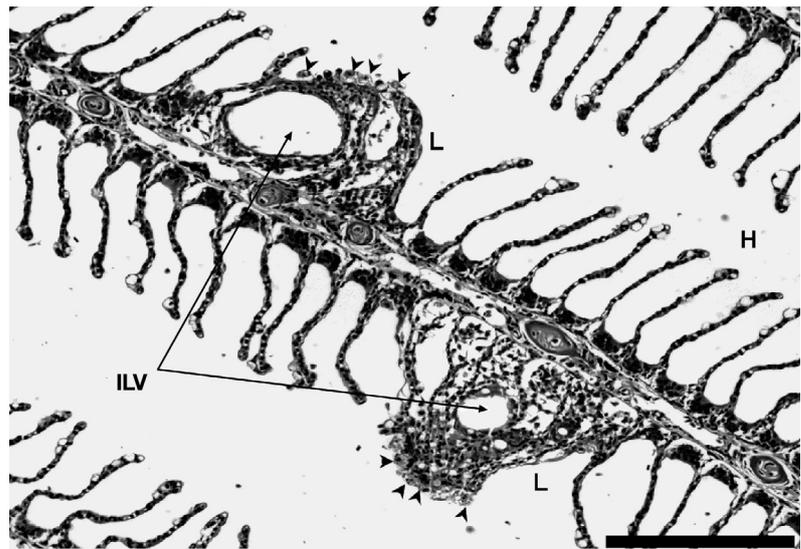


Fig. 2. *Salmo salar*. Histological section of gill tissue showing amoebic gill disease (AGD) pathology including fused secondary lamellae resulting in the typical AGD lesion (L), healthy lamellae (H), amoebae aligned along the peripheral edge of the lesion (arrowheads) and characteristic interlamellar vesicles (ILV) commonly associated with AGD lesions. Haematoxylin and eosin stain; scale bar = 100 μm

AGD pathology based upon histological assessment of gills (Fig. 2). AGD severity (as percentage of affected gill filaments) was found not to be significantly different between tank replicates within the AGD group ($t_{10} = 1.28$, $p = 0.23$), with mean values of 55.95 ± 10.12 and $42.23 \pm 3.63\%$ (range 16 to 84 %). Control fish sampled at the end of the experimental period were clear of AGD pathology symptoms and therefore confirmed as AGD-unaffected.

Within the AGD-affected group, R_S appeared to increase in association with the development of AGD ($F_{9,10} = 6.97$, $p < 0.01$), with significant differences between Days 4 and 5 (1 and 2 d post-amoebae addition) and Days 9 and 11 (day of CL-T treatment and 2 d post-treatment) detected (Fig. 3). Following treatment with CL-T (Day 10), the increase in R_S appeared to attenuate; however, by Day 11 R_S continued its upward rise. In contrast, R_S of control fish was relatively unchanged over the duration of the experimental period with no significant time effects observed ($F_{4,5} = 0.52$, $p = 0.73$).

For the AGD-affected fish, R_{ROU} measurements on Day 2 (prior to amoebae addition), Day 7 (4 post-amoebae addition) and Day 10 (1 d post CL-T bath treatment) also indicated an increase in MO_2 over the

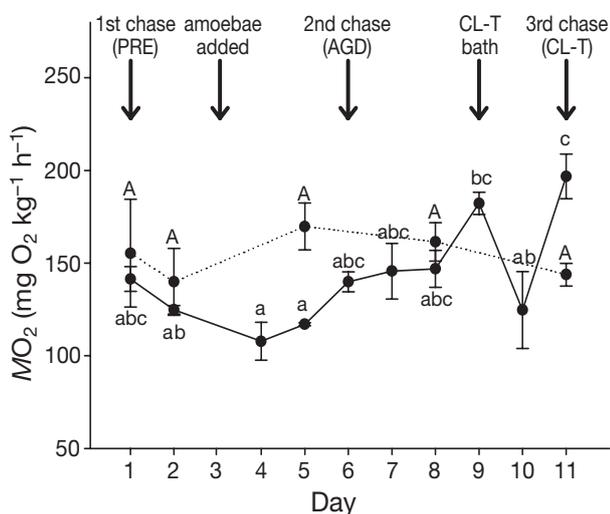


Fig. 3. *Salmo salar*. Standard metabolic rate (R_S) for amoebic gill disease (AGD)-affected (solid line) and AGD-unaffected control (dotted line) assessed as oxygen consumption (MO_2) estimated as the minimum routine metabolic rate (R_{ROU}) measured in each of the 11 d experimental periods. Arrows indicate important events that occurred in the study such as the addition of amoebae (on Day 3) and subsequent treatment with a 10 mg l^{-1} chloramine-T (CL-T) bath (on Day 9). 1st, 2nd and 3rd chase days, termed PRE (pre-infection), AGD and CL-T, refer to days where active metabolic rate (R_{ACT}) and metabolic scope were assessed. Values are mean \pm SE for duplicate tanks ($n = 15$ fish per tank). Days with different letters are significantly different (Tukey's test, $p < 0.05$). For details regarding infection and treatment protocols see main text

course of the experiment, especially during the light period when there was activity within the laboratory (Fig. 4). Over the duration of this experiment, a consistent diel rhythm of R_{ROU} was evident; from the beginning of the light period, MO_2 increased to a plateau then gradually fell to a minimum during the last part of the night. Average R_{ROU} values increased significantly at Day 7 for AGD-affected fish ($p < 0.01$) compared to the Day 1 (PRE) value but for not controls ($p = 0.42$). Following treatment with CL-T however, R_{ROU} values for both AGD-affected and control fish were significantly higher at Day 10 than at Day 0 ($p < 0.01$ and $p < 0.01$, respectively) (Fig. 5).

R_{ACT} values for Day 6 (AGD 3 d post-amoebae addition) were not significantly different from Day 1 (PRE) measurements ($t_1 = -1.88$, $p = 0.31$) or between Day 6 (AGD) and Day 11 (CL-T) 2 d after CL-T treatment ($t_1 = -0.83$, $p = 0.56$) (Fig. 6). Although there was a significant rise in R_S over the course of the AGD infection (see Fig. 3), there were no significant changes in metabolic scope between PRE and AGD measurements or between AGD and CL-T measurements. Therefore, metabolic scope appeared not to be compromised in response to disease ($t_1 = -0.73$, $p = 0.60$) or significantly altered following treatment ($t_1 = 1.23$, $p = 0.44$) (Fig. 6).

Recovery as time taken for $R_{ACT} MO_2$ to return to pre-chase levels was not significantly different between the PRE (112 min) and AGD (125 min) periods ($t_1 = 3.0$, $p = 0.20$) (Fig. 7). Data for the period following the CL-T R_{ACT} measurement were incomplete and are not presented.

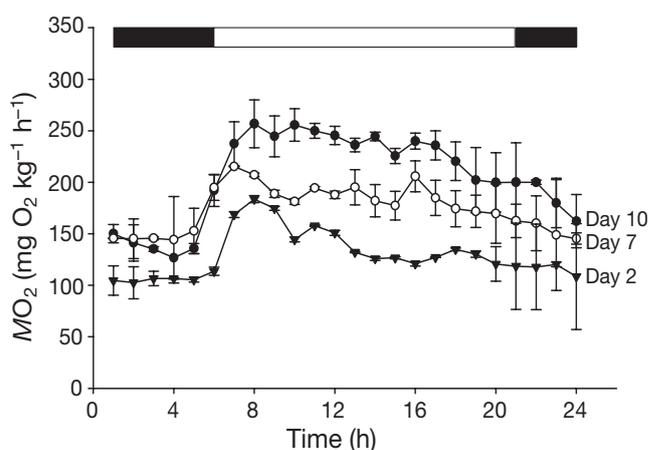


Fig. 4. *Salmo salar*. Routine metabolic rate (R_{ROU}) for amoebic gill disease (AGD)-affected Atlantic salmon assessed as oxygen consumption (MO_2) calculated for each hour over a 24 h period. Time series plots for Day 2 (prior to amoebae addition, \blacktriangledown), Day 7 (4 d post amoebae addition, \circ) and Day 10 (1 d after a 10 mg l^{-1} chloramine-T bath treatment, \bullet), which were important experimental time periods, are displayed. Upper horizontal bar shows light/dark periods. Values are mean \pm SE for duplicate tanks ($n = 15$ per tank). Data were not analysed for statistically significant differences

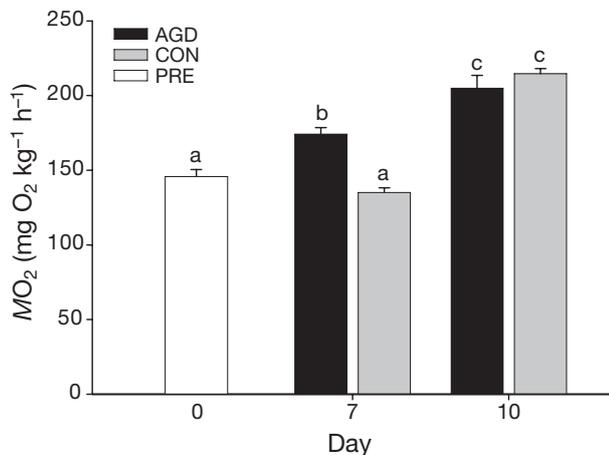


Fig. 5. *Salmo salar*. Mean routine metabolic rate (R_{ROU}) for control (CON) and amoebic gill disease (AGD)-affected fish assessed as oxygen consumption (MO_2 , averaged over a 24 h period). Pre-infection (PRE) experimental values are presented as Day 0; Day 7 data collected 4 d post amoebae addition; Day 10 data collected 1 d after a 10 mg l^{-1} chloramine-T (CL-T) bath treatment. Values are mean + SE for duplicate tanks. Bars with different letters are significantly different (Tukey's test, $p < 0.05$)

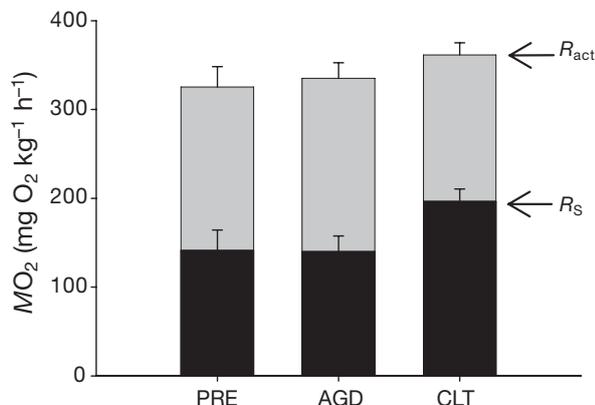


Fig. 6. *Salmo salar*. Standard (R_S , black bars) and active (R_{ACT} , grey bars) metabolic rates as oxygen consumption (MO_2) for fish pre-infection (PRE), 3 d post amoebae addition (amoebic gill disease, AGD) and 2 d after a 10 mg l^{-1} chloramine-T (CL-T) bath treatment. Area between R_S and R_{ACT} measurements bars represents metabolic scope estimated from $R_{ACT} - R_S$. Values are mean + 1 SE for duplicate tanks. There were no significant differences in R_{ACT} or metabolic scope among AGD, CL-T and PRE ($p > 0.05$). For details of R_{ACT} , infection and CL-T treatment protocols see main text

DISCUSSION

Although previous investigations of metabolic rate in AGD-affected Atlantic salmon have been made, this is the first study to incorporate continual measurement of oxygen uptake rates (MO_2) to assess resting (R_S), routine (R_{ROU}) and active (R_{ACT}) metabolic rates, with subsequent estimation of metabolic scope over the course

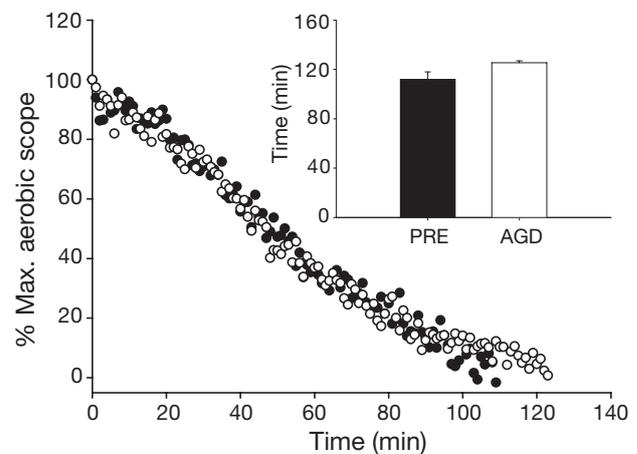


Fig. 7. *Salmo salar*. Normalised post-exercise recovery rate calculated as: $(\text{recovery } MO_2 - R_{ROU}) / (R_{ACT} - R_{ROU}) \times 100$ of fish pre-infection (PRE: ●) and 3 d after amoebae addition (amoebic gill disease, AGD: ○), where MO_2 is oxygen uptake rates, R_{ROU} is routine metabolic rates, and R_{ACT} is active metabolic rates. Inset shows time taken for MO_2 to return to R_{ROU} levels. There were no significant differences between PRE and AGD recovery times following exhaustive chasing used to induce R_{ACT} ($p < 0.05$). Chloramine-T (CL-T) recovery not shown

of a *Neoparamoeba* spp. infection and following a known effective treatment of AGD.

Unlike the unaffected control fish, there was a clear upward trend in R_S in the AGD-affected fish following addition of amoebae to the systems. This suggests an association between increasing R_S and AGD development, as opposed to a simple acclimation effect of fish to the experimental systems. In agreement with previous results, the apparent increase in R_S did not appear to occur until 2 d after amoebae exposure. Prior investigations into the respiratory pathogenesis of AGD in Atlantic salmon have found that minor but significant respiratory effects occur approximately 48 h after amoebae exposure (Leef et al. 2005). In our study R_S values on Days 9 and 11, (6 and 8 d after amoebae exposure, respectively), were significantly higher than on Days 4 and 5 (1 and 2 d after amoebae exposure, respectively). Additionally, the onset of characteristic respiratory acidosis previously reported for Atlantic salmon clinically affected by AGD (Powell et al. 2000) did not appear until 7 d after amoebae exposure when fish had >50% of filaments AGD-affected (Leef et al. 2005). Although AGD severity was not assessed on Day 9, it is unlikely that severity would have been significantly different from that at the end of the study (6 d after exposure) because fish were treated with CL-T on Day 9 and the progression of AGD would have been halted. The significant elevation of R_S on Days 9 and 11 (6 and 8 d after amoebae exposure) may therefore have occurred in association

with the development of a respiratory disturbance such as acidosis.

Previous studies have shown that CL-T is effective at killing *Neoparamoeba* spp. (Powell & Clark 2003) and its addition to seawater can produce efficacy levels similar to that found in freshwater treatment (Harris et al. 2004). In this study, treatment with CL-T at 10 mg l⁻¹ appeared to briefly mitigate the rise in R_S , as there was an approximately 30% drop (not statistically significant) in R_S following treatment. CL-T has been widely used in the treatment of gill diseases in the freshwater aquaculture industry (Thorburn & Moccia 1993). In rainbow trout *Oncorhynchus mykiss*, acute CL-T exposure at 9 mg l⁻¹ in fresh water induces both respiratory and acid-base disturbances that may be directly related to hyperventilation (Powell & Perry 1997) and an increase in branchial mucus production due to the irritant effect on the gills (Powell & Perry 1996). MO_2 also increases following CL-T exposure at 9 mg l⁻¹ (Powell & Perry 1999); therefore, the brief rise of R_S following CL-T exposure in our study was not expected.

Absolute values obtained for R_S , and R_{ROU} prior to amoebae addition were also comparable to those of other salmonids (Evans 1990, Kieffer et al. 1998, Altimiras et al. 2002, Maxime 2002). While assessments of R_S and R_{ROU} were made using commonly practiced methods, assessment of R_{ACT} in the present study was made using a chasing protocol similar to that of Cutts et al. (2002). Due to the experimental system, R_{ACT} measurements were taken approximately 20 to 25 min after fish were returned to the respirometer tanks following the chasing protocol. This time delay occurred as the change in measured oxygen concentration in the outflowing water would have lagged behind the corresponding change in activity due to a wash-out effect within the chamber (Cutts et al. 2002). Within our study, R_{ACT} values were in relatively close agreement with the R_{ACT} of approximately 380 mg O₂ kg⁻¹ h⁻¹ reported for triploid brown trout *Salmo trutta* subjected to a similar chasing protocol (Altimiras et al. 2002).

While fish with light AGD may be capable of maintaining oxygen uptake and defending R_S and R_{ROU} , there is evidence to suggest that in some teleosts a reduction in functional gill area causes a reduction in R_{ACT} , potentially reducing metabolic scope (Duthie & Hughes 1987, Davison et al. 1990). Indeed, despite measures to mitigate morphological changes within the gill, fish heavily affected with AGD show significantly reduced MO_2 under hypoxic conditions (Fisk et al. 2002). The lack of difference between PRE (prior to infection) and AGD (4 d post-infection) R_{ACT} measurements and recovery data may reflect a low level of infection at 4 d after infection. Additionally, the final measurement of R_{ACT} , taken when fish were more heavily affected with AGD, may be confounded by the

use of the CL-T treatment. Therefore, we were unable to accurately assess the effects of severe AGD on R_{ACT} . However, considering the increase in R_S that was evident over the course of the infection, we suspect that R_{ACT} would have been elevated to defend metabolic scope, as in rainbow trout with the microsporidian gill parasite *Loma salmonae* (Powell et al. 2005). In juvenile Atlantic salmon, Cutts et al. (2002) noted that variation in R_{ACT} was less than that of R_S , suggesting that there must be constraints on the maximum scope for activity. Indeed, R_{ACT} must be constrained by the maximum rate that oxygen can be transported to respiring tissues (Kaufman 1990). Conversely the costs of maintenance are under no such pressure (Cutts et al. 2002).

As hypothesised, our study clearly indicated that over the course of an experimental outbreak of AGD, R_S and R_{ROU} increase. Considering this, a decrease in metabolic scope would have been expected; however, over the duration of the experiment there was no significant changes in R_{ACT} . It is possible that the absence of fish with advanced AGD prior to treatment precluded significant effects on R_{ACT} . Therefore in light of the physical conditions that constrain R_{ACT} , we expect that metabolic scope would in turn become compromised in untreated AGD-affected fish. The implications of a restricted metabolic scope suggest that fish heavily affected with AGD are limited in their ability to deal with stressors (e.g. normal husbandry practices, including bathing, net changes, cage towing, and also changing environmental parameters, e.g. abnormally high summer temperatures, low oxygen availability and changes in salinity). In this study, treatment of AGD with CL-T shows promise in AGD treatment as a potential moderator of the proposed metabolic effects.

Acknowledgements. The authors thank M. Attard for help with fish husbandry, the University of Tasmania AGD research group for technical support, and B. Nowak and P. Montague for advice on the manuscript. This work formed part of a project of Aquafin CRC, and received funds from the Australian Government's CRCs Program, the Fisheries R&D Corporation and other CRC participants.

LITERATURE CITED

- Altimiras J, Axelsson M, Claireaux C, Lefrancois C, Mercier C, Farrell AP (2002) Cardiorespiratory status of triploid brown trout during swimming at two acclimation temperatures. *J Fish Biol* 60:102–116
- Bruno OI, Svoronos PDN (1989) CRC handbook for chemical analysis. CRC Press, Boca Raton, FL
- Clark A, Nowak BF (1999) Field investigation of amoebic gill disease in Atlantic salmon, *Salmo salar* L., in Tasmania. *J Fish Dis* 22:433–443
- Clark G, Powell M, Nowak B (2003) Effects of commercial freshwater bathing on reinfection of Atlantic salmon, *Salmo salar*, with amoebic gill disease. *Aquaculture* 219:135–142

- Cutts CJ, Metcalfe NB, Taylor AC (2002) Juvenile Atlantic salmon (*Salmo salar*) with relatively high standard metabolic rates have small metabolic scopes. *Funct Ecol* 16: 73–78
- Davison W, Graig EF, Carey PW (1990) Oxygen uptake in the Antarctic teleost *Pagothenia borchgreinki*. Limitations imposed by X-cell gill disease. *Fish Physiol Biochem* 8: 69–77
- Dickson IW, Kramer RH (1971) Factors influencing scope for activity and active and standard metabolism of rainbow trout (*Salmo gairdneri*). *J Fish Res Board Can* 28:587–596
- Duthie GC, Hughes GM (1987) The effects of reduced gill area and hyperoxia on the oxygen consumption and swimming speed of rainbow trout. *J Exp Biol* 127:349–354
- Dyková I, Figueras A, Novoa B, Casal JF (1998) *Paramoeba* spp., an agent of amoebic gill disease of turbot *Scophthalmus maximus*. *Dis Aquat Org* 33:137–141
- Dyková I, Figueras A, Peric Z (2000) *Neoparamoeba* Page, 1987: light and electron microscopic observations on six strains of different origin. *Dis Aquat Org* 43:217–223
- Dyková I, Nowak BF, Crosbie PBB, Fiala I, Pecková H, Adams MB, Machááková B, Dvořáková H (2005) *Neoparamoeba branchiphila* n. sp., and related species of the genus *Neoparamoeba* Page, 1987: morphological and molecular characterization of selected strains. *J Fish Dis* 28:9–64
- Evans DO (1990) Metabolic thermal compensation by rainbow trout: effects on standard metabolic rate and potential usable power. *Trans Am Fish Soc* 119:585–600
- Farrell AP, Gamperl AK, Birtwell IK (1998) Prolonged swimming, recovery and repeat swimming performance of mature sockeye salmon *Oncorhynchus nerka* exposed to moderate hypoxia and pentachlorophenol. *J Exp Biol* 201: 2183–2193
- Findlay VL, Munday BL (2000) The immunomodulatory effects of levamisole on the nonspecific immune system of Atlantic salmon, *Salmo salar*. *J Fish Dis* 23:369–378
- Fisk DM, Powell MD, Nowak BF (2002) The effects of amoebic gill disease and hypoxia on survival and metabolic rate of Atlantic salmon (*Salmo salar*). *Bull Eur Assoc Fish Pathol* 22:190–194
- Forsberg OI (1996) The impact of varying feeding regimes on oxygen consumption and excretion of carbon dioxide and nitrogen in post-smolt Atlantic salmon (*Salmo salar* L.). *Aquac Res* 28:101–113
- Fry FEJ (1971) The effect of environmental factors on the physiology of fish. In: Hoar WS, Randall DJ (eds) *Fish physiology*. Academic Press, New York, p 1–98
- Harris JO, Powell MD, Attard M, Green TJ (2004) Efficacy of chloramine-T as a treatment for amoebic gill disease (AGD) in marine Atlantic salmon (*Salmo salar* L.). *Aquac Res* 35:1448–1456
- Harris JO, Powell MD, Attard MG, DeHayr L (2005) Clinical assessment of chloramine-T and freshwater as treatments for the control of gill amoebae in Atlantic salmon, *Salmo salar* L. *Aquac Res* 36:776–784
- Howard T, Carson J (1993) Verification of *Paramoeba* species are consistently associated with gill damage in fish affected with amoebic gill disease. In: Valentine P (ed) *Proceedings of the Saltas research and development review seminar*. Salmon Enterprises of Tasmania, Dover, p 69–80
- Kaufman R (1990) Respiratory costs of swimming in larval and juvenile cyprinids. *J Exp Biol* 150:343–366
- Kent ML, Sawyer TK, Hedrick RP (1988) *Paramoeba pemaquidensis* (Sarcocystigophora: Paramoebidae) infestation of the gills of coho salmon *Oncorhynchus kisutch* reared in sea water. *Dis Aquat Org* 5:163–169
- Kieffer JD, Alsop D, Wood CM (1998) A respirometric analysis of fuel use during aerobic swimming at different temperatures in rainbow trout (*Oncorhynchus mykiss*). *J Exp Biol* 201:3123–3133
- Leef MJ, Harris JO, Powell MD (2005) Respiratory pathogenesis of amoebic gill disease (AGD) in experimentally infected Atlantic salmon (*Salmo salar*). *Dis Aquat Org* 66: 205–213
- Maxime V (2002) Effects of transfer to sea water on standard and routine metabolic rates in smolting Atlantic salmon at different stages of seawater adaptability. *J Fish Biol* 61: 1423–1432
- Munday BL, Foster CK, Roubal FR, Lester FGJ (1990) Paramoebic gill infection and associated pathology of Atlantic salmon, *Salmo salar* and rainbow trout, *Salmo gairdneri*, in Tasmania. In: Perkins FO, Cheng TC (eds) *Pathology in marine science*. Academic Press, San Diego, CA, p 215–222
- Munday BL, Zilberg D, Findlay V (2001) Gill disease of marine fish caused by infection with *Neoparamoeba pemaquidensis*. *J Fish Dis* 24:497–507
- Nowak BF, Carson J, Powell MD, Dykova I (2002) Amoebic gill disease in the marine environment. *Bull Eur Assoc Fish Pathol* 22:144–147
- O'Connor KI, Taylor AC, Metcalfe NB (2000) The stability of standard metabolic rate during a period of food deprivation in juvenile Atlantic salmon. *J Fish Biol* 57: 41–51
- Parsons H, Nowak B, Fisk D, Powell M (2001) Effectiveness of commercial freshwater bathing as a treatment against amoebic gill disease in Atlantic salmon. *Aquaculture* 195: 205–210
- Powell MD, Clark GA (2003) *In vitro* survival and the effect of water chemistry and oxidative chemical treatments on isolated gill amoebae from AGD affected Atlantic salmon. *Aquaculture* 220:135–144
- Powell MD, Perry SF (1996) Respiratory and acid-base disturbances in rainbow trout (*Oncorhynchus mykiss*) blood during exposure to chloramine T, paratoluenesulphonamide, and hypochlorite. *Can J Fish Aquat Sci* 53: 701–708
- Powell MD, Perry SF (1997) Respiratory and acid-base disturbances in rainbow trout blood during exposure to chloramine-T under hypoxia and hyperoxia. *J Fish Biol* 50: 418–428
- Powell MD, Perry SF (1999) Cardio-respiratory effects of chloramine-T exposure in rainbow trout. *Exp Biol Online* 4:5
- Powell MD, Fisk D, Nowak BF (2000) Effects of graded hypoxia on Atlantic salmon infected with amoebic gill disease. *J Fish Biol* 57:1047–1057
- Powell MD, Speare DJ, Daley J, Lovy J (2005) Differences in metabolic response to *Loma salmonae* infection in juvenile rainbow trout *Oncorhynchus mykiss* and brook trout *Salvelinus fontinalis*. *Dis Aquat Org* 67:233–237
- Reidy SP, Nelson JA, Tang Y, Kerr SR (1995) Post exercise metabolic rate in Atlantic cod and its dependence upon the method of exhaustion. *J Fish Biol* 47:377–387
- Roberts SD, Powell MD (2003) Comparative ionic flux and gill mucous cell histochemistry: effects of salinity and disease status in Atlantic salmon (*Salmo salar* L.). *Comp Biochem Physiol A* 134:525–537
- Thorburn MA, Moccia RD (1993) Use of chemotherapeutics on trout farms in Ontario. *J Aquat Anim Health* 5:85–91
- Zilberg D, Findlay VL, Girling P, Munday BL (2000) Effects of treatment with levamisole and glucans on mortality rates in Atlantic salmon (*Salmo salar* L.) suffering from amoebic gill disease. *Bull Eur Assoc Fish Pathol* 20:15–19