Vol. 78: 29–36, 2007
doi: 10.3354/dao01855

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published October 31

Effect of an acute necrotic bacterial gill infection and feed deprivation on the metabolic rate of Atlantic salmon *Salmo salar*

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ABSTRACT: In this study, experiments were conducted to examine the effect of an acute necrotic bacterial gill infection on the metabolic rate (M_{O_2}) of Atlantic salmon *Salmo salar*. Fed and unfed Atlantic salmon smolts were exposed to a high concentration (5×10^{12} CFU ml^{-1}) of the bacteria *Tenacibaculum maritimum*, their routine and maximum metabolic rates ($M_{O_{2rout}}$ and $M_{O_{2max}}$, respectively) were measured, and relative metabolic scope determined. A significant decrease in metabolic scope was found for both fed and unfed infected groups. Fed infected fish had a mean ± standard error of the mean (SEM) decrease of 2.21 ± 0.97 µM O_2 g^{-1} h^{-1}, whilst unfed fish a mean ± SEM decrease of 3.16 ± 1.29 µM O_2 g^{-1} h^{-1}. The decrease in metabolic scope was a result of significantly increased $M_{O_{2rout}}$ of both fed and unfed infected salmon. Fed infected fish had a mean ± SEM increase in $M_{O_{2rout}}$ of 1.86 ± 0.66 µM O_2 g^{-1} h^{-1}, whilst unfed infected fish had a mean ± SEM increase of 2.16 ± 0.72 µM O_2 g^{-1} h^{-1}. Interestingly, all groups maintained $M_{O_{2max}}$ regardless of infection status. Increases in $M_{O_{2rout}}$ corresponded to a significant increase in blood plasma osmolality. A decrease in metabolic scope has implications for how individuals allocate energy; fish with smaller metabolic scope will have less energy to allocate to functions such as growth, reproduction and immune response, which may adversely affect the efficiency of fish growth.

KEY WORDS: *Tenacibaculum maritimum* · Metabolic rate · Atlantic salmon · Feed ration · Oxygen consumption · Bacteria · Exercise

INTRODUCTION

Stressors, including disease, impose a metabolic cost on fish that consists of 2 mechanisms: (1) an energy requirement to manage the disturbances associated with the stressor; and (2) an energy cost related to correcting the associated ionoregulatory imbalance (Barton & Iwama 1991). Measurements of oxygen consumption rates, which are essentially an indirect measurement of metabolic rates (M_{O_2}) (and will be referred to as such), are used to evaluate the amount of energy an organism is using at any given moment (Jobling 1994). M_{O_2} of most animals fluctuates between 2 extremes. The lower limit is typically referred to as 'standard' or 'basal' M_{O_2} ($M_{O_{2basal}}$) and is the M_{O_2} of a quiescent animal in a post-absorptive nutritional state, below which physiological function is impaired (Brett & Groves 1979, Jobling 1994). However, fish ordinarily expend energy above this level, due to activities such as feeding and locomotion, and this lower level is referred to as 'routine' M_{O_2} ($M_{O_{2rout}}$). The upper extreme is referred to as 'active' or 'maximum' M_{O_2} ($M_{O_{2max}}$) and the range through which the aerobic M_{O_2} can vary is referred to as the 'scope for activity' or 'metabolic scope' ($M_{O_{2max}}$ to $M_{O_{2basal}}$) (Fry 1947). Alternatively, metabolic scope can be expressed as 'relative metabolic scope,' which is the difference between $M_{O_{2rout}}$ and $M_{O_{2max}}$ (Wieser 1985).

M_{O_2} measurements are highly sensitive and can vary due to a number of factors, including fish size (Hunt von Herbing & White, 2002), nutritional history of the fish (Jobling 1981, Carter & Brafield 1991, Shoemaker

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et al. 2003), prior stresses (Lankford et al. 2005), and environmental conditions, such as temperature and salinity (Schurmann & Steffensen 1997, Morgan & Iwama 1998). Recent studies have begun to highlight the link between infection status and M_{O_2}, suggesting that M_{O_2} measurements might be an effective tool for quantifying the impact of disease on fish (Wagner et al. 2003, Powell et al. 2005a, Tierney et al. 2005, Wagner et al. 2005). However, the impact of gill diseases on the M_{O_2} of Atlantic salmon has had little attention. A recent study that examined the effect of amoebic gill disease on Atlantic salmon found that salmon, in response to the disease, had significantly elevated $M_{O_{2rout}}$ whilst being able to maintain $M_{O_{2max}}$ despite a perceived reduction in gill surface area (authors' unpubl. data). However, little information exists with regard to the effect that bacterial infections, particularly necrotic bacterial gill infections, have on M_{O_2} and metabolic scope.

Tenacibaculum maritimum (formerly *Flexibacter maritimus*) causes a skin infection resulting in ulcerative dermatitis, although gill infections with a necrotizing branchitis are not unusual, with experimental infections progressing similarly to natural infections (Handlinger et al. 1997). This disease affects a number of wild and cultured species including Atlantic salmon *Salmo salar*, greenback flounder *Rhombosolea taprina*, striped trumpeter *Latris lineata*, red sea bream *Pagrus major*, black seabream *Acanthopagrus schegeli*, Japanese flounder *Paralichthys olivaceus* and rock bream *Oplegnathus fasciatus* (Baxa et al. 1986, Wakabayashi et al. 1986, Handlinger et al. 1997). Atlantic salmon experimentally infected with *T. maritimum* were unable to maintain homeostatic regulation of blood plasma osmolality, resulting in significant increases in blood ion concentration (Powell et al. 2004). In marine teleosts, osmoregulation represents a large proportion of the energy expenditure associated with $M_{O_{2rout}}$ (Jobling 1994); thus, any disturbance due to disease may significantly increase the $M_{O_{2rout}}$ of a quiescent fish.

The $M_{O_{2rout}}$ of Atlantic salmon has been shown to decrease significantly during periods of feed deprivation (O'Connor et al. 2000). The reduction in $M_{O_{2rout}}$ may be due to changes in the biochemical composition of the organs, relative organs weights, changes in protein turnover rates, and reduced immune function (Jobling 1994, Lim & Klesius 2003). Withholding feed has also been shown to be a successful strategy for disease management for some diseases (Shoemaker et al. 2003). For example, feed-deprived Atlantic salmon had a lower cumulative mortality of 2% compared to 36% mortality in fed animals when experimentally challenged with a pathogenic bacteria *Vibrio salmonicida* (Damsgard et al. 1998). Conversely, feed deprivation has been shown to increase mortality in channel catfish *Ictalurus punctatus* challenged with the bacteria *Edwardsiella ictaluri* (Lim & Klesius 2003) and *Flavobacterium columnare* (Shoemaker et al. 2003).

The objective of the present study was to determine the combined effects of an acute necrotic bacterial gill infection, using *Tenacibaculum maritimum* as a model, and nutritional deprivation on $M_{O_{2rout}}$ and $M_{O_{2max}}$ and subsequent metabolic scope.

MATERIALS AND METHODS

Fish husbandry. Atlantic salmon smolts with a mean ± standard error of the mean (SEM) mass of 68.4 g ± 1.7 g and a mean ± SEM fork length of 22.0 ± 1.1 cm were obtained from the Saltas freshwater commercial hatchery in Wayatinah, Tasmania, Australia, and acclimated over 2 wk to full strength seawater (35‰, 1 µm filtered) at 16°C (±1°C) in a rectangular fibreglass 4000 l Rathbun tank. Previous experiments conducted at this facility have shown that this is a sufficient amount of time to ensure a majority of the fish smolt correctly.

All experiments were conducted in a separate temperature-controlled room (17°C) that housed 4 individual 400 l recirculation systems. Each system consisted of a 200 l conical bottom tank and a 200 l sump, which contained a biofilter and mesh to remove solids and catch uneaten feed. One hundred salmon (25 per tank) were removed from a holding tank and anesthetized using clove oil (0.003%, 90% active ingredient eugenol, Langford Sales and Marketing). A 29-gauge needle was used to make a sub-dermal injection of Alcian blue dye into one of 6 defined positions on the abdomen of the fish to enable individual identification, with 16 fish tagged for each tank (O'Connor et al. 2000). All fish were fed for 1 wk in order to establish a feeding response, after which fish in 2 tanks were fed twice daily to apparent satiation for a further 2 wk, whilst the fish in the remaining 2 tanks had their feed withheld. Water quality was measured daily throughout the experiment, dissolved oxygen remained above 95% saturation, total ammonia levels peaked at 2 mg l^{-1} 5 d after transfer from the holding tank to the experimental system and was below 0.5 mg l^{-1} at the beginning of the first M_{O_2} measurement.

M_{O_2} measurements. Pre-inoculation $M_{O_{2rout}}$ and $M_{O_{2max}}$ were sampled following the 2 wk feeding or withholding period, and post-inoculation M_{O_2} was taken 30 h after inoculation with bacteria (see 'Inoculation' below). Food was withheld for 24 h prior to M_{O_2} measurements to ensure that fish were in a post-absorptive state to avoid confounding results with specific dynamic action (Jobling 1994). Fish were placed into respirometry boxes 16 h prior to measurement. The respirometry boxes were

connected to 2 separate 150 l recirculation systems, with 9 boxes per system; inlet water into the respirometry boxes was 100% air saturated.

A thermostatically controlled oxygen electrode (1302 Electrode, Strathkelvin Instruments) connected to a Strathkelvin Instruments model 782 O_2 meter was calibrated using a 2% $NaSO_3$ (zero) solution and air saturated seawater (155 mm Hg), prior to the M_{O_2} measurements. To determine $M_{O_{2rout}}$, water and airflow to the boxes was halted, and a 3 ml sample of water removed from the box and injected into the oxygen electrode. After 10 min, the water in the box was mixed by pumping the 3 ml syringe 10 times in order to obtain a homogenous water mixture, after which a final water sample was taken and the oxygen content re-measured using the electrode (Powell et al. 2005a).

$M_{O_{2max}}$ was measured using protocols similar to those found in Cutts et al. (2002). Briefly, individual salmon were removed from respirometry boxes and placed into a 50 l cylindrical container that contained hyperoxic seawater (120% air saturation, 17°C), and the fish were chased by hand to exhaustion (10 min). Fish were immediately returned to the 1 l boxes and their oxygen consumption rate measured (as above). M_{O_2} was calculated using the following formula:

$$M_{O_2} = \frac{[(pO_{2i} - pO_{2e}) \times \alpha] \times V}{T \times M} \quad (1)$$

where pO_{2i} and pO_{2e} are the initial and final oxygen partial pressures respectively (mm Hg), α is the molar O_2 solubility in water (µM O_2 l^{-1} mm Hg^{-1}), V is the respirometer box volume (l), T is the time between the initial and the final oxygen measurements (s) and M is the mass (g) of the fish (Cameron 1986, Cech 1990). Where there was not a complete seal between the air-water interface and the oxygen transfer rates were found to be 0.183 mm Hg over a 10 min period, the results were corrected accordingly. $M_{O_{2max}}$ was measured immediately after net ammonia excretion rates were determined (see below). $M_{O_{2rout}}$ and $M_{O_{2max}}$ and net ammonia excretion rates were re-measured (as described above) 30 h post-inoculation.

Net ammonia excretion rates. Net ammonia excretion rate measurements were performed between the $M_{O_{2rout}}$ and $M_{O_{2max}}$ determinations. Water flow to the respirometer boxes was stopped and a 3 ml water sample was extracted and immediately frozen at –20°C. After 1 h a second water sample was taken, frozen and water flow was resumed. Water samples were analysed using methods described by Verdouw et al. (1978). Excretion rates were calculated using the following formula:

$$NH_4^+{}_{flux} = \frac{([NH_4^+]_{final} - [NH_4^+]_{initial}) \times V}{M} \quad (2)$$

where $[NH_4^+]$ is the concentration of ammonia (µmol l^{-1}), V is the volume (l) of the respirometry box and M is the mass of the fish (g). Air was supplied throughout the experiment with dissolved oxygen levels remaining above 95% saturation for the duration of the experiment.

Inoculation. Cultures of *Tenacibaculum maritimum* strain 00/3280 were obtained from cultures held at the Tasmanian Aquaculture and Fisheries Institute (Fish Health Unit, Department of Primary Industries and Water, Tasmania, Australia). Isolates were originally isolated from farmed trout raised in seawater and identified using a 16S ribosomal RNA (rRNA) PCR primer set specific for *T. maritimum*. Cultures were subsequently stored at –80°C on MicroBank beads (Pro-Lab Diagnostics) for later use (Powell et al. 2004). Briefly, 200 ml of Shieh's medium formulated with seawater mineral salts buffer (MSB) was inoculated with *T. maritimum* and gently agitated at 20°C for 48 h. The suspension was centrifuged at 2500 relative centrifugal force (RCF), the pellet washed twice and resuspended in 15 ml of sterile seawater, and the bacterial concentration determined by serial dilution. Following the pre-inoculation $M_{O_{2max}}$ measurement, a 200 µl suspension of *T. maritimum* (5×10^{12} cells ml^{-1}) was applied evenly over all of the 8 gill arches of the anaesthetised Atlantic salmon (Powell et al. 2004, 2005b). The 00/3280 strain of *T. maritimum* has been shown to be highly pathogenic to Atlantic salmon at high concentrations in a previous study (Powell et al. 2004). Furthermore, in Powell et al. (2004, 2005b) the infection remained primarily as a gill disease and did not manifest as a skin lesion. As the aim of this particular trial was to use an acute necrotic gill infection as a model for examining the effects on M_{O_2}, it was deemed appropriate to use a high bacterial concentration similar to that previously described (Powell et al. 2004). Furthermore, this allowed for a relative comparison with regard to the physiological effect of infection on the fish with previous studies conducted (Powell et al. 2004, 2005b). Control fish received a saline solution and all fish were returned to their respective tanks. After 30 h, $M_{O_{2rout}}$, $M_{O_{2max}}$ and net ammonia excretion rates were re-measured (post-inoculation samples) as described above.

Following the post-inoculation M_{O_2} measurements, fish were given a lethal overdose of clove oil (0.005%), weighed and fork length measured. Blood samples (3 ml) were taken via a caudal puncture and were centrifuged at 8000 RCF for 2 min using a Spinwin MC-01 (Tarsons Products) and the plasma was decanted and frozen (–20°C). Blood plasma osmolality was measured using a Wescor Vapro 5520 vapour pressure osmometer (Helena Laboratories). Additionally, a sterile plastic loop was used to sample mucus

from the gills and plated on Shieh's marine agar to determine the presence of *Tenacibaculum maritimum*. Cultures were incubated at 20°C for 36 h and colonies were confirmed as *T. maritimum* by colony colour and shape (Wakabayashi et al. 1986, Powell et al. 2005b). This was considered sufficient as the experiment was following protocols used in Powell et al. (2005b), in which colony colour and shape were used primarily as a means of identifying *T. maritimum*. Furthermore, the aim of this particular experiment was to determine the effect of gill necrosis on M_{O_2} rather than examining the specific effects of *T. maritimum* per se; therefore, identification past colony shape and colour was deemed unnecessary. The entire gill was excised and rinsed gently in seawater and placed into saltwater Davidson's fixative for 24 h, then into 70% ethanol for histology (Nowak & Adams 2003). The entire stomach from the end of the oesophagus to the beginning of the pyloric caeca was removed and placed in 10% buffered formalin. Twenty-four h following fixation, the internal organs were transferred into 70% ethanol. The second left anterior gill arch and a thin slice (approximately 3 mm) of a centre section of stomach was removed, dehydrated, embedded in paraffin wax, sectioned at 5 µm and stained with haematoxylin and eosin (H&E). Gill sections were then viewed at 100× magnification for signs of focal branchial lamellar necrosis and associated bacterial mats. Stomach cross-sections were viewed for signs of feed deprivation, such as a thinning of the longitudinal and circular muscle layer and necrosis of the mucosal epithelium.

Statistical analyses. The study was completed in triplicate to obtain statistically significant numbers of fish, so there were 19 fed infected, 8 unfed infected, 11 fed uninfected and 16 unfed uninfected fish in total. Statistical analyses were conducted using the statistical package SPSS for Windows (Version 11.5). A 2-tailed *t*-test was used to determine whether there was a significant difference in change in weight between the fed treatments (infected and uninfected) and the unfed groups. A paired sample *t*-test was used to determine the difference between $M_{O_{2rout}}$ pre- and post-inoculation for each individual group. A similar analysis was used for $M_{O_{2max}}$ and metabolic scope. A 1-way analysis of variance (ANOVA) was used to determine differences in blood plasma osmolality among the 4 groups and significant differences were investigated using a Tukey's post-hoc test. For the ammonia flux, paired sample *t*-tests were used to determine difference between the treatments pre- and post-inoculation. Furthermore, two 1-way ANOVAs were used to determine whether there was a significant difference between the 4 treatments pre-inoculation and post-inoculation.

RESULTS

Tenacibaculum maritimum putatively diagnosed by colony shape and colour was recovered from all salmon that were exposed to the bacteria and no cultures developed from samples collected from control fish. Infection with *T. maritimum* presented itself as yellowish mucoid patches at the filament tips. Histologically, there was branchial epithelial necrosis, with overlying bacterial mats. Histological examination of the sections of stomach revealed that both fed and unfed groups had signs of feed deprivation that included necrosis of the mucosal epithelium and a general thinning of the longitudinal and circular muscle layers, this was more prevalent in the unfed treatments. Furthermore, fed infected fish in the M_{O2} study lost on average 5.90 ± 1.65 g (mean ± SEM) of their initial mass, whilst unfed infected fish lost 14.6 ± 1.33 g (mean ± SEM). For the uninfected treatments the fed group lost an average of 5.93 ± 1.65 g (mean ± SEM) and the unfed group lost an average of 8.61 ± 2.56 g (mean ± SEM). There was no statistical difference between the change in weight of the fed uninfected and fed infected fish ($p = 0.993$) and the unfed infected and unfed uninfected fish ($p = 0.127$). However, the unfed infected fish lost significantly more weight than the fed infected fish ($p = 0.003$) as did the unfed uninfected fish when compared to the fed uninfected fish ($p = 0.432$).

Mortalities occurred in all groups, with a majority of the mortality occurring immediately after the initial transfer from the seawater acclimation tanks to the experimental tanks; this was attributed to fish in poor condition that had failed to correctly smolt. However, no mortalities in fish were recorded the week prior to the initial M_{O_2} measurement.

Metabolic scope and M_{O_2}

Metabolic scope decreased significantly for fed infected fish from pre- to post-inoculation; the reduction represented an overall decrease of 40% from pre-inoculation levels ($p = 0.036$, Fig. 1). Also, metabolic scope decreased significantly in the unfed infected group with an overall reduction of 49% from pre-inoculation levels ($p = 0.04$, Fig. 1). Uninfected groups had no significant change in metabolic scope.

$M_{O_{2rout}}$ increased significantly from pre- to post-inoculation levels in infected salmon that were fed and unfed ($p = 0.01$ and 0.02, respectively, Table 1). Fed infected fish had an average increase in $M_{O_{2rout}}$ (mean ± SEM) of 1.86 ± 0.66 µM O_2 g^{-1} h^{-1} whilst unfed infected fish had an average increase (mean ± SEM) of 2.16 ± 0.72 µM O_2 g^{-1} h^{-1}. $M_{O_{2max}}$ increased significantly for unfed uninfected fish ($p = 0.01$, Table 1) with

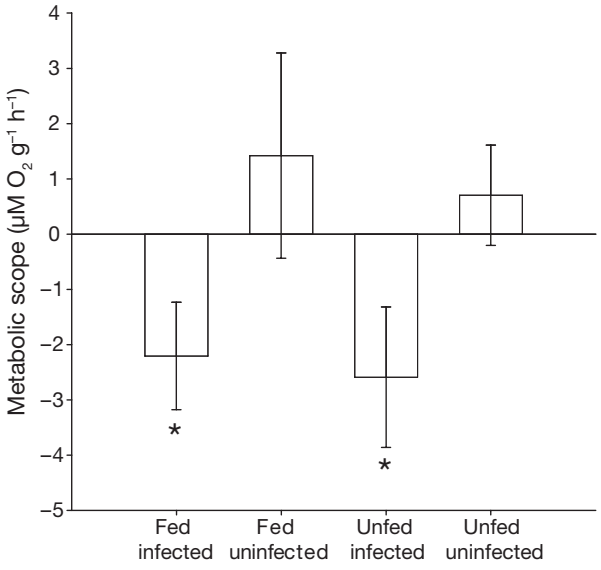

Fig. 1. *Salmo salar.* Mean (±SEM) change in metabolic scope from pre- to post-inoculation of Atlantic salmon exposed to *Tenacibaculum maritimum*. *Significant change ($p < 0.05$) in metabolic scope from pre- to post-inoculation

an average increase (mean ± SEM) of 2.51 ± 0.61 μM O_2 g^{-1} h^{-1}, whilst all other treatments maintained $M_{O_{2max}}$ between pre- and post-inoculation.

Blood plasma osmolality and net ammonia excretion rates

Both infected fed and unfed salmon had significantly higher blood plasma osmolality than uninfected treatments ($p < 0.001$, Fig. 2A). There was no significant change in net ammonia excretion rates from pre- to post-inoculation for any of the groups. However, a 1-way ANOVA showed that there was a significant difference amongst the groups pre-inoculation, with fed infected fish having an average net ammonia excretion rate significantly higher than that of the unfed uninfected treatment ($p = 0.03$, Fig. 2B).

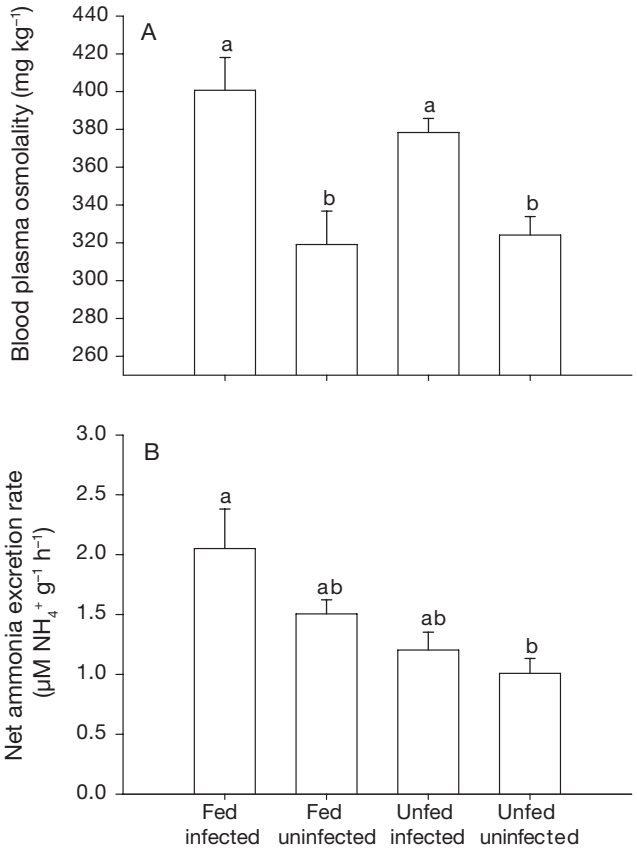

Fig. 2. *Salmo salar.* (A) Mean (±SEM) blood plasma osmolality of fed and unfed Atlantic salmon exposed to *Tenacibaculum maritimum*. (B) Mean (±SEM) net ammonia excretion rates of Atlantic salmon pre- inoculation. Different superscripts indicate a significant difference among treatments

DISCUSSION

This study was conducted to test the hypothesis that exposing the gill tissue of Atlantic salmon to *Tenacibaculum maritimum* would result in necrosis of the gill tissue, which would represent a significant additional energetic cost and that this cost could be quantified through changes in M_{O_2}. It was expected that

Table 1. *Salmo salar.* Mean (±SEM) routine ($M_{O_{2rout}}$) and maximum ($M_{O_{2max}}$) metabolic rates (μM O_2 g^{-1} h^{-1}) pre- and post-inoculation for fed and unfed Atlantic salmon. *Significant difference between pre- and post-inoculation levels

		Fed		Unfed	
		Infected	Uninfected	Infected	Uninfected
$M_{O_{2rout}}$	Pre-inoculation	5.00 ± 0.35	6.16 ± 0.55	5.32 ± 0.74	4.32 ± 0.58
	Post-inoculation	6.86 ± 0.54*	5.31 ± 1.10	7.48 ± 0.69*[a]	5.78 ± 0.38
$M_{O_{2max}}$	Pre-inoculation	10.53 ± 0.53	11.32 ± 0.74	11.08 ± 1.26	8.37 ± 0.43
	Post-inoculation	10.19 ± 0.61	11.89 ± 1.07	10.08 ± 0.84	10.63 ± 0.47*

[a]One outlier removed due to incomplete water sample

infected salmon would spend more energy maintaining osmotic homeostasis as a result of the damage to the respiratory and ionoregulatory epithelium of the gills (Powell et al. 2004). The results showed that the variation in 2 consecutive M_{O_2} measurements on the same individual is small and stable enough to allow comparisons between infected and uninfected fish, thus providing a valuable tool for assessing fish health. The major outcome of this study was that necrosis induced by exposure to *T. maritimum* significantly affected metabolic scope and $M_{O_{2rout}}$, although infected fish were able to maintain $M_{O_{2max}}$.

The metabolic scope of Atlantic salmon exposed to *Tenacibaculum maritimum* was 40 to 49% (fed and unfed, respectively) of that of control fish, suggesting that fish with a necrotic bacterial gill infection have reduced capacity to perform work (Lankford et al. 2005). Furthermore, prior nutritional status did not appear to significantly affect the level of reduction in metabolic scope. To date, limited research has been published examining the effect of bacterial gill infections on the M_{O_2} of fish. Of the studies that have focussed primarily on disease and metabolic scope, rainbow trout infected with the parasitic haemoflagellate *Cryptobia salmositica* showed a 44% reduction in metabolic scope (Kumaraguru et al. 1995). Alternatively, rainbow trout infected with *Loma salmonae* successfully maintained metabolic scope by increasing $M_{O_{2max}}$, whilst brook trout in the same study increased metabolic scope by decreasing $M_{O_{2rout}}$ (Powell et al. 2005a). A decrease in metabolic scope has implications for energy allocation by individuals; fish with smaller metabolic scope will inevitably have less energy to allocate to functions such as the replenishment of energy stores, growth, reproduction and immune response (Cutts et al. 2002, Lankford et al. 2005).

The decreases in metabolic scope observed in the current study were due to an increase in $M_{O_{2rout}}$ for the fed and unfed infected treatments. There are a number of possible explanations for the observed increase in $M_{O_{2rout}}$ in infected fish, with the most likely cause being that the additional energy was spent to maintain osmotic homeostasis. This hypothesis was supported by the increase in blood plasma osmolality seen in infected fish. The increase in blood plasma osmolality supports the results found by Powell et al. (2004), in which Atlantic salmon infected by the same strain of *Tenacibaculum maritimum* had an increase in blood plasma osmolality. It is possible that the increase in $M_{O_{2rout}}$ was due to an up-regulation of Na^+, K^+-ATPase as well as other osmoregulatory organs. Na^+,K^+-ATPase is the primary enzyme responsible for maintaining osmotic homeostasis; thus, any shift in the osmotic equilibrium may result in a significant up regulation of this particular enzyme (Jobling 1994). However, enzyme activity was not specifically measured in the present study. It is also reasonable to assume that exposure to *T. maritimum* and the subsequent necrosis elicited a stress response in the fish. Atlantic salmon exposed to *T. maritimum* have significantly elevated plasma lactate levels pre-mortem (Powell et al. 2004) and similar results were found for Chinook salmon *Oncorhynchus tshawytscha* exposed to *Renibacterium salmoninarum*, which had significantly increased levels of cortisol and lactate, indicating a stress response to infection (Mesa et al. 2000). Increased lactate and cortisol levels in fish have been shown to directly correlate to increases in M_{O_2} (Davis & Schreck 1997). The effect on $M_{O_{2rout}}$ of the transfer of fish from fresh to salt water 4 wk prior to the first M_{O_2} measurement is suspected to be minimal, as previous studies have shown only a moderate effect on $M_{O_{2rout}}$ (± 5 to 6%) immediately after transfer (Maxime 2002).

$M_{O_{2max}}$ for infected Atlantic salmon did not change significantly from pre- to post-inoculation levels, suggesting that the increased costs associated with exposure to *Tenacibaculum maritimum* were not compensated by an increase in $M_{O_{2max}}$. The results concur with those of Powell et al. (2005a), which found no net change in $M_{O_{2max}}$ associated with disease in brook trout infected with *Loma salmonae*, but did find compensatory increases in rainbow trout infected with *L. salmonae*. Necrosis due to exposure to *T. maritimum* effectively reduces the available surface area for respiration (Powell et al. 2004). There is evidence suggesting that in some fish species a reduction in functional gill area is linked to a reduction in $M_{O_{2max}}$ (Duthie & Hughes 1987, Schurmann & Steffensen 1997). By maintaining $M_{O_{2max}}$ despite a reduction in functional gill surface area, salmon in the current study may have employed compensatory mechanisms during infection, such as an increased functional surface area of the gills, increasing the permeability of the gill to ions and increased cardiac output (Booth 1979, Gonzalez & McDonald 1994, Powell et al. 2000). Indeed, salmon infected with *T. maritimum* do not show signs of hypoxemia or a reduced capacity for the transport of oxygen (Powell et al. 2005b). However, as the cardiorespiratory system in salmonids is diffusion-limited, rather than perfusion-limited, any compensatory mechanisms employed must be constrained by the maximum rate that oxygen can be transported through the gill tissue (Powell & Perry 1999, Gallaugher et al. 2001, Cutts et al. 2002).

Net ammonia flux was shown to be unaffected by disease, which confirmed previous findings (Powell et al. 2004). There was a significant difference between the fed infected and unfed uninfected groups, no significant differences were found for the remaining groups. It was expected that a significant difference

would be found between all fed and unfed groups; however, it is possible that the 24 h starvation period prior to measurement that was required for the M_{O2} measurements may have been enough to mitigate the effects of prior feeding. Furthermore, although the fed treatments were supplied with food twice daily to apparent satiation, there was a net decrease in weight over the duration of the experiment, suggesting that some of the fish failed to resume feeding upon transfer into the experimental systems. However, the reduction in weight was minimal and some of this weight loss could be due to the fish not being fed for a total of 48 h prior to sampling. Furthermore, the unfed treatments lost significantly more weight than the fed treatments, thus making the comparisons between fed and unfed treatments valid.

The key finding in the present study was an observed decline in the maximum metabolic scope for activity in Atlantic salmon exposed to *Tenacibaculum maritimum.* This was associated with an increase in plasma osmolality, resulting from branchial epithelial necrosis associated with the disease. Interestingly, despite a high degree of necrosis found in the gill tissue, fish maintained $M_{O_{2max}}$. Reduction in metabolic scope has a number of biological implications, the most important being a reduced ability to allocate energy to more desirable outcomes, such as growth and reproduction, which will have profound implications for efficiency in aquaculture.

Acknowledgements. The authors thank J. Carson for the supply of *Tenacibaculum maritimum*, J. Ransome and M. Barney and the UTAS AGD research group for technical support. This work formed part of a project of Aquafin CRC, and received funds from the Australian Government's CRC Program, the Fisheries R&D Corporation and other CRC participants.

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Editorial responsibility: David Bruno, Aberdeen, UK

Submitted: October 30, 2006; Accepted: July 24, 2007
Proofs received from author(s): October 24, 2007