Apparent vascular hypertension associated with Amoebic Gill Disease affected Atlantic salmon (Salmo salar) in Tasmania.

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Abstract
The dorsal aortic (DAP), ventral aortic (VAP) blood pressures and heart rate were measured in Atlantic salmon affected with AGD in a clinical outbreak. DAP and VAP were high compared with published values for other salmonid species. Fish subjected to a 3-h freshwater bath and returned to seawater (the treatment of choice for AGD control) had a significantly lower dorsal aortic pressure, with values comparable to those for other salmonids. Heart rate was not affected by freshwater exposure. These data suggest that there may be a vascular hypertension associated with AGD in Atlantic salmon. Although the source of the hypertension remains to be identified, this finding may help to explain the poor post-stress survival of AGD-affected salmon.

Introduction
Amoebic Gill Disease (AGD) is the most significant disease affecting Atlantic salmon aquaculture in Tasmania. Neoparamoeba pemaquidensis was first identified as the causative agent of AGD in coho salmon (Oncorhynchus kisutch) in 1988 (Kent et al., 1988) and subsequently it was shown to be the cause of AGD in Atlantic salmon in Tasmania (Munday et al., 1990). In addition to Tasmania, AGD outbreaks have been reported in salmonids and non-salmonids in various countries around the world (Nowak et al., 2002). Clinically, AGD manifests grossly as raised white mucoid patches on the gills. Histopathologically, there is extensive multifocal interlamellar hyperplasia, and fusion of the secondary lamellae with the frequent formation of interlamellar vesicles (Adams & Nowak 2002). Coincident with the presence of N. pemaquidensis on the gill is an increase in the number of mucous cells and mucous secretion over the gills.

The primary treatment of AGD is a 3-h freshwater bath (see Parsons et al., 2001 for a review of the process). The effectiveness of freshwater bathing at killing all of the gill amoebae has been recently questioned (Parsons et al., 2001). However, our investigations have revealed that there appeared to be little physiological disadvantage or advantage in exposing sea-reared salmon to fresh water for short periods under these conditions (Powell et al., 2001).

Fish clinically affected by AGD do not suffer from severe respiratory compromise (Powell et al., 2000). However, following an acute exposure to hypoxia (50 % O₂ saturation), the mortality rate was 79% compared with ap-
approximately 10% in non-affected fish (Fisk et al., 2002). Recently we have found that fish with a history of heavy AGD have elongated ventricles with respect to their height or width and hyperplasia/hypertrophy of the compact muscle layer at the expense of the spongiosa (Powell et al., 2002). This suggests the potential association of cardiovascular pathology with AGD. In the present study we measured the dorsal and ventral aortic blood pressures and heart rates of fish clinically affected by AGD prior to and following freshwater exposure.

Materials and Methods

Fish
Amoebic gill disease-affected Atlantic salmon of mass 0.849 kg (± 0.053 kg) and fork length 41.3 cm (± 0.8 cm) (mean ± SE) were obtained from crowds of fish exhibiting a gross clinical infection of AGD awaiting freshwater bathing at a commercial facility. Fish were dip netted from the crowd and transported to a 350 L holding tank in oxygenated seawater at ambient temperature. Fish were held in aerated seawater at ambient temperatures of 18 ± 2°C for up to 24 h prior to surgery and experimentation. Alternatively, salmon (n = 9) were transferred to another 350 L circular tank containing fresh water (the same water used commercially for the freshwater bathing of fish) at 19°C where they were maintained at 200% oxygen saturation for 3 h prior to being returned to the seawater tank and undergoing subsequent surgery.

Surgical procedures.
Fish were anaesthetised with 1:25000 clove oil in chilled oxygenated sea water. Fish were then transferred to a surgical table where the gills were irrigated in the retrograde direction with anaesthetic solution containing 1:100000 clove oil. A heparinised (100 IU.mL⁻¹ ammonium heparin, Sigma Chemical Company, St Louis MO USA), modified Cortland’s saline (Wolf 1963, containing 160 mmol.L⁻¹ NaCl Milligan et al., 1991)-filled PE50 catheter was implanted into the dorsal aorta according to the method by Soivio et al. (1975). Alternatively, the afferent branchial artery of the second left gill arch was occlusively cannulated with PE50 and the cannula held in place with braided silk sutures. Fish were recovered in flowing fresh seawater at ambient temperature (18 ± 2°C) in darkened PVC boxes (50 x 10 x 20 cm) supplied with water at a rate of 10 L.min⁻¹. Following cannulation of the afferent branchial artery it proved difficult to obtain reliable records of ventral aortic pressure in this species. The pressure trace attenuated rapidly. We obtained records from 6 fish that maintained a good pulse pressure, but present only 15 min values because of subsequent attenuation of trace. We decided not to record data from doubly cannulated fish as a routine measure because survival was poor.

Blood pressure measurement.
Immediately upon transfer of the fish to flowing sea water, the catheter was connected to a disposable pressure transducer (DPT-60003, Peter von Berg, Eggharthing, Germany) that was connected to a PowerLab® data acquisition system (AD Instruments, Castle Hill, Australia) interfaced to a portable personal computer. Transducers were calibrated against a column of water. The blood pressure for each fish was measured continuously for the first hour and then for a 10 min period at 2 and 4 h post-surgery. Heart rate was determined from the blood pressure traces triggering from the peak systolic pressure.
The mean dorsal and ventral aortic pressures (DAP and VAP respectively) were averaged for each fish over each 5 min period (first hour) and then over the 10 min recording period at 2 and 4 h. Bathed and non-bathed fish were compared statistically using a 2 factor repeated measures analysis of variance with time and treatment as factors. Specific differences were isolated and analysed with a Bonferroni-corrected test (Glantz 1987).

Following the 4 h pressure recording, the fish were killed by an overdose of anaesthetic (1:10000 clove oil in seawater). Fork length and body mass were measured. A smear was made from the second left gill arch for an indirect fluorescent antibody test (IFAT) (Douglas-Helders et al. 2001) to determine the presence of *N. pemaquidensis*. The gills and heart were dissected and placed into seawater Davidson’s solution for subsequent histological examination.

Gills were embedded in paraffin and sectioned at 5 mm and stained with haematoxylin and eosin. The number of filaments in a given section with AGD lesions, presence of *N. pemaquidensis* and extent of hyperplasia was evaluated.

**Results**

There was a significant difference in the DAP of unbathed fish compared to bathed fish at 1, 2 and 4 h following surgery (Fig. 1.). However, there was no difference in the heart rate of either bathed or unbathed fish following surgery (Fig. 1.). The measurement of ventral aortic pressure was difficult because the pressure reading would often decline to levels below that of dorsal aortic pressure over the 4 h of the experiment. However, it was possible to obtain simultaneous recordings from 6 fish at 15 min of recovery yielding a mean (± SE) DAP of 43.3 ± 1.9 cm H₂O and VAP of 60.6 ± 3.7 cm H₂O with a ratio of DAP:VAP of 0.73 ± 0.03.

There were no differences in the prevalence of AGD between the bathed and non-bathed groups based upon IFAT (37.5% and 36.4 % positive n = 8 and n = 9 respectively) or the number of lesioned filaments per section (0.03 ± 0.01 and 0.03 ± 0.01 respectively).

**Discussion**

The significant difference in the DAP but not heart rate between bathed and unbathed fish suggested that the effect of elevated blood pressure was likely due to differences in vascular resistance rather than blood flow. However, without measurements of cardiac blood flow this cannot be confirmed. Our fish were held at the upper limit of their thermal tolerance range and therefore comparisons with fish held in water closer to their preferred temperature must be treated with caution. Dorsal aortic blood pressures in resting freshwater species of *Oncorhynchus*, which are the closest relatives of *Salmo* for which data are available, range from 27 to 42 cm water (Kiceniuk & Jones, 1977; Wood & Shelton, 1980; Fritsche et al., 1992; Thorarensen et al., 1993; Le Mével et al., 1998). Values in our bathed fish fell within this range. Altimiras et al. (2002) recorded a value of 32 cm water for DAP in *S. trutta*. Values in our untreated fish exceeded 44 cm water, even at 4 h post-surgery. These values are higher than any recorded in teleost fish apart from members of the Scombridae (Bushnell et al., 1992) which
supports the contention that the salmon were hypertensive. The ratio of DAP/VAP is in the same range for healthy rainbow trout (Farrell, 1993) which in turn would suggest that increased branchial vascular resistance was unlikely, rather that increased blood pressures were as a consequence of an increase in systemic vascular resistance.

Heart rates in *S. salar* at 4 h were high, averaging c. 85 to 90 b.p.m. This is considerably higher than the range of 16 to 36 bpm recorded in four members of this species by telemetry as they ascended a river in their spawning migration in October and November in Scotland (Altimiras *et al.*, 1996). We presume water temperatures for these four fish were considerably lower than those experienced by those in Tasmania. Our experimental animals were held in boxes and might be expected to be considerably more stressed than free-swimming animals, but as noted above, the DAP of the treated fish was in the range of those recorded in *O. mykiss* and *O. tshawytscha* held under similar conditions.

It is unlikely that the hypertension observed is as a direct consequence of surgery since bathed and unbathed fish produced different responses. Both groups of fish were handled in an identical manner except for bathing. If the osmotic effects of bathing are considered, it would be reasonable to expect bathed fish to be hypertensive due to osmotic uptake of water and the subsequent increased blood volume. During the bathing, fish were exposed to 200% hyperoxia for 3 h and hyperoxia did not affect blood pressure or vascular resistances in freshwater rainbow trout, *O. mykiss* (Powell unpublished). The fact that blood pressure recordings were stable at 4 h post-surgery suggests that recovery from surgery was not a major influence in the difference between the two groups tested.

The prevalence of AGD among the fish used in this experiment was quite low with a low incidence of AGD-like lesions on the gill filaments even though fish were being bathed commercially based upon gross clinical signs of raised white mucoid patches on the gills. Similarly there were no differences in the number of IFAT-positive fish between the bathed and unbathed group. This suggests that the severity of AGD was low in the fish used in this study. In other studies under-
taken at the same time on the same cohort of fish, the total number of amoebae on the gills was also quite low (Powell & Clark 2002). Since freshwater bathing has been recently shown to be of limited efficacy (Parsons et al., 2001; Clark 2002) it is possible that amoebae numbers were reduced on the gill but this reduction was not detectable with the IFAT technique.

There may be an association between AGD and a vascular hypertension in cultured Atlantic salmon, and freshwater bathing may relieve this hypertension. Whether the hypertension is directly due to AGD via the production of an exotoxin (or similar factor) by the *N. pemaquidensis* or whether it is an incidental finding in AGD-compromised salmon remains to be investigated.

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**References**


