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# GILL DISEASE ASSOCIATED WITH *PARAMOEBA*, IN SEA REARED ATLANTIC SALMON IN IRELAND

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*Abstract*

Gill disease associated with amoebic infection, in sea reared Atlantic salmon in Ireland, is reported. Affected fish were in their first year at sea, whereas adjacent stocks of previous year classes were apparently unaffected. Amoebae were identified as *Paramoeba* sp., according to their morphology and serological reaction.

*Case history*

During September-November of 1995, sea reared Atlantic salmon (*Salmo salar*) in Ireland were affected by gill disease associated with infection by a *Paramoeba* sp. All affected fish were of stocks transferred to sea water in the spring of 1995, whereas adjacent stocks of previous year classes were apparently unaffected. Fish from a total of 10 sites were found to have gill pathology and associated amoebae. However, in 5 of these sites there was only a low degree of gill pathology and a low number of amoebae. Fish from the other affected sites showed moderate to high levels of gill pathology and amoebae. Only 3 sites experienced significant mortalities, and in these cases there had been previous episodes of unrelated diseases, which may have compromised the condition of the fish. There is some question as to whether the amoebae were secondary to a degree of gill damage already present, as in some cases focal gill hyperplasia and fusion were found approximately one month prior to any observable amoebic infection. There was no evidence of bacterial gill infections, algal blooms or other factors which may have accounted for this previous gill pathology. Water temperatures varied from approximately 17°C at the beginning of September, to 12°C at the end of November. However, temperatures in the previous July and August had been up to 21°C. Both inshore and offshore sites were affected by the amoebae. Rainfall had been very low throughout the year, and therefore, water in all sites was probably at full salinity.

*Clinical signs and diagnosis*

Affected fish appeared lethargic and congregated at the surface and edges of the cage, and showed signs of respiratory distress (flared opercula, "coughing"). Grossly, the gills showed high levels of mucous, together with eroded filament edges and grey / brown areas of apparently necrotic tissue. The characteristic patches of dense mucous, considered pathognomic in Tasmanian Atlantic salmon affected with amoebic gill disease (AGD) (Alexander 1991), were not seen. Histopathology of the gills showed hyperplasia and fusion of secondary lamellae, and focal necrosis. Amoebae were present, showing a rounded nucleus with central nucleolus and an adjacent parasome. In gills with advanced hyperplasia and fusion, the amoebae were often enclosed between fused lamellae (Fig. 1), with some evidence of them penetrating below the epithelial layer. Amoebae in 'wet preparations' of fresh gill material showed numerous pseudopodia and exhibited a very slow amoeboid movement. Amoebae were isolated and maintained for periods of up to 3 weeks using agar plates seeded with bacteria (Roubal *et al.* 1989). The morphology of the observed amoebae was consistent with *Paramoeba* sp. described from AGD of farmed Atlantic salmon in Tasmania, Australia (Munday *et*

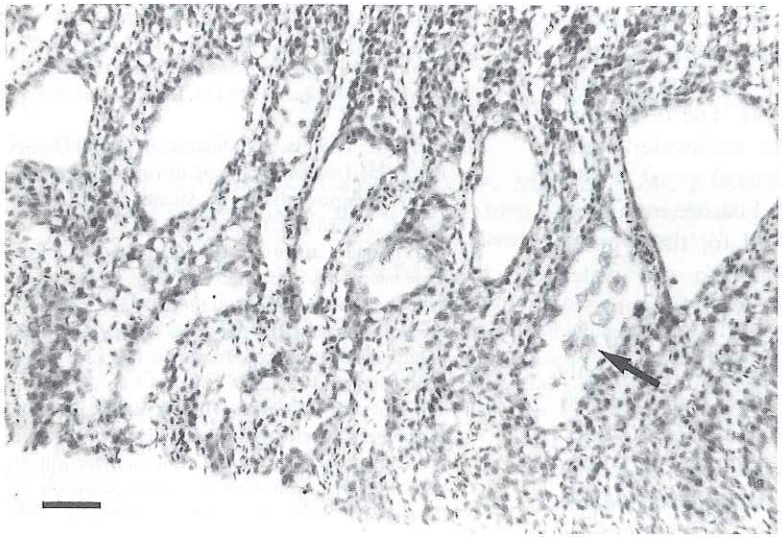

Fig. 1. Section of gill, showing advanced epithelial hyperplasia and fusion (H & E). Note fusion of secondary lamellae, forming "cysts", with enclosed paramoebae (arrow). Bar = 50 µm.

*al.* 1990, Roubal *et al.* 1989). Serological examination of the organism was conducted by an indirect immunofluorescent antibody test (IFAT), using rabbit antiserum to amoeba known to colonise the gills of fish affected by AGD (Howard and Carson 1993). Wax sections of affected gills were de-waxed and immunostained, using antisera specific to *Paramoeba* sp. (strain PA-016), *Flabellula* spp. (FLB-004), *Platyamoeba plurinucleolus* (UQ-1) and a *Vanella / Platyamoeba* mix of species (MP-1). Fluorescent bodies consistent in size and shape to cells of *Paramoeba* sp., were detected in the gill sections; the amoebae were detected in close association with damaged gill tissue. No fluorescent bodies were detected with antisera to the other genera of gill associated amoebae.

*Treatment*

Treatment with formalin in sea water baths (200 ppm, for 45-60 min.) was attempted at 2 sites. In one case there was a reduction in the number of amoebae on the gills and a fall in mortalities following treatment, whereas in the other case there was no apparent effect on either numbers of amoebae or the mortality rate.

*Discussion*

AGD is a significant problem in sea reared Atlantic salmon and rainbow trout (*Oncorhyncus mykiss*) in Tasmania (Findlay *et al.* 1995, Munday *et al.* 1990, Roubal *et al.* 1989), and has also occurred in coho salmon (*O. kisutch*) in western USA (Kent *et al.* 1988) and in chinook (*O. tshawytscha*) in New Zealand (Findlay *et al.* 1995). In Europe, the disease has occurred in sea-reared Atlantic salmon, rainbow and brown trout (*S. trutta*) in France (Findlay *et al.* 1995). Amoebae in gill sections from affected Atlantic salmon in France have been shown to give a positive reaction with antisera specific to *Paramoeba* sp. (Howard and Carson 1994). Although at least 6 different genera of amoebae have been identified from cases of AGD in Tasmania, *Paramoeba* sp. has been most consistently associated with disease outbreaks and intimately associated with gill damage characteristic of AGD (Howard and Carson 1992, 1993). Amoebae associated with AGD in western USA have been identified as *Paramoeba pemaquidensis* (Kent *et al.* 1988). In Tasmania the disease is associated with increasing water temperatures between 12 - 20°C, and full or near-full strength sea water, although the initiation of the disease is not fully understood (Roubal *et al.* 1989). In Tasmania, as in Ireland, focal gill changes due to unknown causes have been noted prior to amoebic colonisa-

tion (Nowak and Munday 1994). *Paramoeba* sp. are highly susceptible to freshwater and are inactivated within 2 hours (Howard and Carson 1994). The disease in Tasmania is treated with freshwater baths (Munday *et al.* 1990, Roubal *et al.* 1989) which is highly effective. Fish are immersed rapidly in freshwater, held for three hours and then transferred back to sea water (Alexander 1991). Baths with formalin or hydrogen peroxide have also been tried but with limited success (Cameron 1994), although *Paramoeba* sp. is inactivated by 100 ppm of hydrogen peroxide within 30 minutes *in vitro* (Howard and Carson 1994). Atlantic salmon, naturally or experimentally exposed to *Paramoeba* sp., show some resistance to re infection (Findlay *et al.* 1995). In the farm situation, this resistance is an important factor in the management of the disease and may enable a reduction in the number of freshwater baths required during disease outbreaks. In the cases from Ireland, fish at sea for more than one summer were apparently not susceptible to amoebic infection. This may have been due to previous exposure, although amoebae had not been identified in stocks in previous years. The occurrences in 1995 in Ireland were associated with the unusually high temperatures and low rainfall of that year, which may represent important disease initiating factors.

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