Aquaculture 290 (2009) 1–8

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Gill observations in Atlantic salmon (*Salmo salar*, L.) during repeated amoebic gill disease (AGD) field exposure and survival challenge

Richard S. Taylor [a,b,*], Warren J. Muller [c], Mathew T. Cook [a], Peter D. Kube [a], Nicholas G. Elliott [a]

[a] CSIRO National Food Futures Flagship, CSIRO Marine and Atmospheric Research, GPO Box 1538, Hobart, Tasmania 7001, Australia
[b] National Centre for Marine Conservation and Resource Sustainability, University of Tasmania, Launceston, Tasmania 7250, Australia
[c] CSIRO National Food Futures Flagship, CSIRO Mathematical and Information Sciences, GPO Box 664, Canberra, ACT 2601, Australia

ARTICLE INFO

Article history:
Received 25 November 2008
Received in revised form 22 January 2009
Accepted 22 January 2009

Keywords:
Survival challenge
Tolerance
Susceptibility
Disease resistance
Survival
Immunity
Gill score
Neoparamoeba

ABSTRACT

Amoebic gill disease (AGD) of Atlantic salmon in Tasmania is proactively treated by freshwater bathing when gross gill assessment ('gill score') indicates a moderate level of disease in a cage population. This generally ensures that few fish are exposed to severe disease symptoms and that few die, but also means that a proportion of the population shows little gross evidence of AGD. Individuals exhibiting few AGD symptoms at bath may be more resistant, or simply reflect an uneven spread of the disease through the population. This study had three main aims, firstly, to determine whether all fish in a cage population eventually require freshwater treatment after first infection; secondly, to ascertain whether there is any evidence of development of resistance to AGD; and thirdly, to see if there is a relationship between the level of proliferative gill reaction to the parasite, assessed by gill score, and time to mortality when the disease is left untreated. These aims were achieved by following gill score trajectories of individual fish through three rounds of natural AGD infection and relating these to the eventual fate of the fish in a natural AGD survival challenge after the second freshwater bath. There was no evidence of complete innate resistance to AGD as each fish eventually required a first freshwater bath. There was no relationship between the rate of first infection and the ultimate survival of each fish. For the second and third exposures, significant differences ($P<0.001$) were observed between the surviving fish and those that died in the challenge. Individual gill scores at the latter measurements were suggestive of development of resistance to AGD. Mortality during a natural summer AGD challenge in an un-bathed population of fish, that had two previous treated exposures to the disease, was 67.7% and gill symptoms at the onset of losses accurately predicted the rate of mortality.

1. Introduction

Atlantic salmon have been farmed in sea cages in Tasmania, Australia since the mid 1980s and production has grown to 23,600 t, worth AU$272 million in 2007 (ABARE, 2008). The most serious disease threat to the industry is amoebic gill disease (AGD), caused by an infection of fish gills by the protozoan ectoparasite *Neoparamoeba perurans*, see Young et al. (2007). AGD is not peculiar to Australia and has been reported to affect several temperate cultured teleost fish species around the world (Kent et al., 1988; Munday et al., 2001; Nowak et al., 2002). It is becoming more prevalent, possibly associated with higher water temperatures (Steinum et al., 2008). Mortality rates of 25–30% were recorded in Tasmanian salmon farms in the mid-1980s (M. Hortle, pers. comm.) and losses of between 12–82% have recently been reported from four Norwegian Atlantic salmon farms that had not previously encountered the disease (Steinum et al., 2008).

Colonisation of the gills by this parasite causes proliferative cell change reactions, including severe epithelial hyperplasia, hypertrophy, oedema and interlamellar vesicle formation (Adams and Nowak, 2001, 2003; Adams et al., 2004; Adams and Nowak, 2004a). This can be seen grossly as the formation of white mucoid spots and plaques on the gill surface. Full diagnosis of AGD requires histopathology to confirm the presence of the parasite associated with damaged gill tissue (Adams and Nowak, 2001, 2003). However, the commercial producers utilise a categorical field evaluation of 'gross gill score' (hereafter termed 'gill score') that describes the extent of visible white patches on a scale of 'clear' to 'heavy' (Table 1) to schedule proactive freshwater bath treatments. In advanced infections, this presumptive scoring method is known to have a moderate to good agreement with histopathological diagnosis (Adams et al., 2004) but is less reliable for less severe cases (Clark and Nowak, 1999). Gill score is a gross measure of the degree of host response to the presence of *N. perurans*. The degree of lesion development is known to be in direct proportion to the infective parasite concentration and progression of the infection (Morrison et al., 2004). The primary infective role of *Neoparamoeba* spp. was confirmed by Adams and Nowak, (2004b) who demonstrated that trophozoite settlement occurs only on healthy gill tissue.

* Corresponding author. CSIRO Food Futures Flagship, CSIRO Marine and Atmospheric Research, GPO Box 1538, Hobart, Tasmania 7001, Australia. Tel.: +61 3 62325222; fax: +61 3 62325000.
E-mail address: richard.taylor@csiro.au (R.S. Taylor).

0044-8486/$ – see front matter
doi:10.1016/j.aquaculture.2009.01.030

Table 1
Gross gill score system to estimate the severity of AGD.

Infection level	Gill score	Gross description
Clear	0	No sign of infection and healthy red colour
Very light	1	1 white spot, light scarring or undefined necrotic streaking
Light	2	2–3 spots/small mucus patch
Moderate	3	Established thickened mucus patch or spot groupings up to 20% of gill area
Advanced	4	Established lesions covering up to 50% of gill area
Heavy	5	Extensive lesions covering most of the gill surface

Adapted from Tasmanian Atlantic salmon farming company, Tassal Operations Pty Ltd.

Subsequent lesion development and progression is then dependant upon proliferation and migration of amoeba along the filamental regions. The formation of hyperplastic lesions may be indicative of a fortification strategy adopted by teleosts against gill ectoparasites (Adams, 2003). Larger lesions are characterised by squamous epithelial and mucous cell stratification and are rarely colonised by trophozoites (Adams and Nowak, 2001). Filament regions with fully fused secondary lamellae deny trophozoites the opportunity to exploit the interstitial mucous layer between lamellae. However, lesion margins exhibit concentrated trophozoite attachment. As larger AGD lesions develop, they coalesce and periodically slough away mucus and hyperplastic epithelium containing trophozoites, so the relationship between lesion area and parasite numbers presumably changes. It is conceivable that disparity between lesioned area and parasite mass could occur where the host is able to reduce parasite numbers with little concurrent lesion healing, or that hosts may vary in their proliferative response to the presence of *N. perurans.* However, the extent of AGD lesion generally reflects the degree of *N. perurans* infection. Indeed, preliminary evidence from Young et al. (2008), utilising a PCR technique for the detection of *N. perurans*, demonstrated that the degree of amplification was consistent with the severity of AGD identified by histopathology of six fish from a first infection challenge, suggesting that the gill pathology reflects the degree of parasitism.

Currently the only commercially effective treatment for AGD is freshwater bathing for a minimum of 2 h (Powell et al., 2001). The osmotic effect of bathing removes gill mucus and gill-associated amoeba and promotes a rapid healing of gill lesions (Munday et al., 2001; Clark et al., 2003). Reinfection is primarily due to waterborne trophozoites attaching to healthy gill tissue, but may also occur from low numbers of amoebae remaining upon the gills post bathing (Adams and Nowak, 2004b). Pre-existing proliferative epithelial tissue appear to have an inhibitory effect upon trophozoite attachment (Adams and Nowak, 2004a), but these lesions heal rapidly and are then available for reinfection. The numbers of gill associated amoeba have been shown to return to prebath levels within 10 days (Clark et al., 2003). Lesion formation from reinfection typically begins between 1 and 2 weeks post bath (Adams and Nowak, 2004b). The reiterative process of freshwater bathing adds up to 20% to the cost of production (Munday et al., 2001) through increased farm infrastructure, added labour and operating costs. A typical farm of one million fish uses over 500 Ml of freshwater in a 15–18 month production cycle, bathing each pen approximately 8–12 times (G.Purdon, Tassal, pers. comm.). With freshwater resources limited in Tasmania, bathing is not seen as a long-term solution for the industry.

The broad clinical definition of AGD is gill lesions in the presence of attached amoeba trophozoites. Therefore, resistance to AGD may include elements of both host control of the proliferative response and immune response to the parasite. Evidence of a level of innate immunity to AGD was provided by Bridle et al. (2005), who noted that a subpopulation of naïve fish exposed to a severe AGD infection were able to resist becoming heavily infected and furthermore survive the challenge. Evidence that fish with AGD that are bathed and then become reinfected appear more resistant to this subsequent infection compared to naïve fish and that resistance may increase with repeated exposures was presented by Findlay et al. (1995) and Vincent et al. (2006). This is suggestive of the acquisition of some type of response. Development of resistance to AGD may be associated with stimulation or activation of the nonspecific immune system (Findlay and Munday, 1998). Indeed, (Bridle et al. (2003) demonstrated that immunostimulants could enhance the inflammatory response and increase survival to AGD challenge. There is empirical evidence for a humoral antibody response with anti-*Neoparamoeba* antibodies measured in the serum of Atlantic salmon and rainbow trout (Findlay et al., 1995). However, they do not appear to elicit any specific protection. Gross et al. (2004) demonstrated that all fish develop a modest serum antibody activity to *Neoparamoeba* spp. when sent to sea and subjected to natural AGD infection and commercial bathing, but there was no evidence of protection. Similarly, Vincent et al. (2006) found serum antibodies in 50% of fish that had previously been exposed to *Neoparamoeba* spp. To date there has been no systematic recording of tagged individuals over the production cycle to determine their response following multiple baths and reinfection events. Furthermore, the current laboratory-based challenge system developed to measure the immune response, and to trial AGD vaccines, relies upon survivability as the measure. This is an acute trial that is usually only run through first infection with limited capacity to simulate bathing and reinfection.

Currently there has been no definitive work linking AGD survival to gill score, the link between the measure used experimentally and the measure used practically is not known. The purpose of this study was to examine differences between individuals in their time to first bath (i.e. to determine if there is any innate resistance to AGD in the population), to track a cohort of tagged fish subjected to continual natural infection and observe fluctuations in gill score over a period of seven months as an indicator of AGD. Finally we wanted to determine whether gill score is a good predictor of survival if fish are left untreated.

2. Materials and methods

2.1. Time to first bath trial

Mixed-sex Atlantic salmon spawned in 2006 were intramuscularly tagged with Passive Integrated Transponder (PIT) tags at the Salmon Enterprises of Tasmania Pty Ltd (SALTAS) Wayatinah hatchery in early June 2007. The fish were held in the hatchery for a further six weeks under lights (22 L:2D) at ambient temperature. Once smoltified, 1830 fish (average weight 173 g ± 53) were transferred to a 10 × 10 × 8 m (800 m^3) marine fish pen in a commercially stocked lease at Tassal Operations Pty. Ltd., Dover, Tasmania on 31st July 2007 and fed commercial pellets *ad libitum.*

Following marine input, the development of AGD was monitored by fortnightly gill inspection of commercial standard subsamples (gill score of 40 randomly sampled fish) until 51 days post stocking, at which stage the remaining 1787 fish were gill scored by two experienced operators using a scale from 0 to 5 (Table 1). All fish of gill score 2 and above were bathed in soft riverine water (carbonate hardness and general hardness <20 mg/l, pH 7.1) for a minimum of 2 h and returned to the main 10 m net, while fish of low gill score (0 to 1) were returned to a 5 × 5 × 5 m (125 m^3) net suspended inside the main net. The fish in the 5 m net were reassessed on a weekly basis and any individuals of gill score 2 or above were removed, bathed and returned to the main net.

2.2. Repeat AGD exposure and survival challenge

In a separate experiment utilising fish from the 2005 spawned cohort, 1504 mixed-sex PIT tagged Atlantic salmon (average weight 228 g ± 47) and one thousand untagged adipose clipped fish (167 g ± 38)