



# Freshwater treatment of amoebic gill disease and sea-lice in seawater salmon production: Considerations of water chemistry and fish welfare in Norway



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## ABSTRACT

Amoebic gill disease (AGD) and sea lice are two of the most significant disease issues that the Norwegian Atlantic salmon aquaculture industry is facing. Although both diseases respond to various extents, to freshwater treatment, the chemistry, interactions, and efficacy of treatment can be variable. These variations can have significant impacts upon the success and failure of treatment and costs to the production cycle. Although it is known that soft freshwater is most effective in bathing of Atlantic salmon with AGD and that most of the freshwaters in Norway fall into the soft category, the low alkalinity and buffering capacity of such waters may impact on the pH and metal toxicity of the water source in use. Similarly dissolved organic carbon can be beneficial in treatment, although sequestration of metal ions can be reversed as the water pH drops due to high densities of fish and accumulations of carbon dioxide. Alternative treatments such as the use of oxidative disinfectants such as hydrogen peroxide used for AGD and sea lice control may have potential although the interactions in seawater with organic loads and dissolved organic carbon are unclear. Similarly the use of oxidative disinfectants in freshwater will depend upon the water chemistry and interactions with treatment chemicals, fish, and water organic content. The logistics of treating large biomasses of Atlantic salmon on marine farms are challenging. The use of well boats offers potential although maintaining water quality during treatments is essential for both AGD and sea lice treatments to optimize fish welfare and treatment efficacy.

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## 1. Introduction

Since 2010, there has been a sharp increase in the number of amoebic gill disease (AGD) outbreaks in Western Europe with Ireland, Scotland (including the Shetland Isles), and the western coast of Norway being affected. In Norway outbreaks (positively diagnosed cases) increased from 5 in 2012 to 56 in 2013 to 70 in 2014 with a progressive advance northwards of the outbreaks in successive years. AGD is caused by the amoeba *Paramoeba perurans*, first identified as the cosmopolitan agent of AGD by Young et al. (2007). Prior to this, *P. perurans* had been identified as a potential pathogen in Norwegian salmon, found coincidentally with other causes of gill disease (Steinum et al., 2008), a feature often noted in Irish outbreaks (Bermingham and Mulchay, 2006). The pathology and immunology associated with (*Neo*) *Paramoeba* sp. had been well described and subject to several reviews (Nowak et al., 2013; Powell et al., 2008). In short, infection of the gill with *P. perurans* leads to a hyperplastic epithelial response of the gill accompanied by mixed inflammatory and immunological responses (the literature is often conflicting see reports by Bridle et al., 2006; Morrison et al., 2006; Pennacchi et al., 2014; Nowak et al., 2013) and an acute systemic hypertension occurring in Atlantic salmon (Leef et al., 2005a, 2007). The primary treatments for AGD are in the form of freshwater baths although in Europe some success using hydrogen peroxide bath treatments have been reported (Rodger, 2014).

The treatment of Atlantic salmon using large-scale baths for the control of parasites is not a new concept. The use of freshwater bath treatments for AGD has been in place since the disease became established in Australia in the mid-1980s although application for the control of salmon lice (*Lepeophtheirus salmonis*) on a commercial scale is relative new. Recently, the use of short-term bath treatments of Atlantic salmon during the marine phase of the production cycle has increased in Norway. For example, the use of hydrogen peroxide alone has tripled from 2538 tonnes in 2012 to 8262 tonnes in 2013 ([www.fhi.no](http://www.fhi.no)). Although there may be several reasons why this increase has occurred, one of the reasons is a reduction in the trigger level of lice number enforced by the authorities. The increased occurrence of amoebic gill disease (AGD) and infections with resistant/multi-resistant strains of sea-lice has caused this development. If this treatment strategy is to be developed and applied in the industry, a number of issues concerning water quality on fish welfare and treatment efficiency need to be addressed, and knowledge-gaps identified.

Subjecting seawater (SW, hyperosmotic) adapted teleost fish to a procedure combining abrupt transfer to a hypo-osmotic freshwater (FW) environment at high fish densities, crowding and handling is a procedure likely to cause a degree of stress in the fish. Maintaining fish in these conditions also causes metabolite accumulation (carbon dioxide/ammonia/ammonium) in the water with subsequent water quality changes that may further aggravate this stress. Thus, a fundamental knowledge about the effects of FW treatment on stress and physiology alone, and combined with water quality changes is needed to ensure fish welfare and optimal treatment effect. Treatment efficacy may also be influenced by the chemical composition of freshwater used. This review aims to summarize current knowledge on the subject.

## 2. Norwegian freshwater quality

### 2.1. Chemistry of natural water sources

Norwegian surface waters are characterized by being of low alkalinity and soft, i.e. having a low bicarbonate buffering capacity and

consequently low  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  content (Henriksen et al., 1989; Kristensen et al., 2009; Skjelkvåle et al., 2005) (Fig. 1). High precipitation rates and low evaporation due to the temperate climate, combined with acidic and weathering-resistant bedrock give rise to this chemical composition of the surface waters. Water pH is therefore also naturally low in many sites (Fig. 1), with additional reductions caused by acidification in the southern and south-western regions (Skjelkvåle et al., 2005). Two major concerns arise from a low buffering capacity and/or pH, namely a strong further pH decrease when  $\text{CO}_2$  accumulates in the water and an increased gill permeability caused by low  $\text{Ca}^{2+}$  saturation of ion channels in the gills (Evans et al., 2005). The first may cause mobilization of metal ions (if present) (Fivelstad et al., 2003), while the latter results in increased susceptibility to metals (Leivestad et al., 1980). Low pH increases the efflux of  $\text{Na}^+$  and  $\text{Cl}^-$  across the gill surface due to an osmotic gradient of about  $350 \text{ mOsm L}^{-1}$  between the fish and the freshwater environment (Fig. 1). This problem is exacerbated by  $\text{H}^+$  ions competing for gill binding sites with  $\text{Ca}^{2+}$  (Pagenkopf, 1983; Wilson, 2012). Additionally, metals such as Al may be mobilized to gill reactive forms (<http://www.hydroearth-syst-sci-discuss.net/4/3317/2007/hessd-4-3317-2007.pdf>).

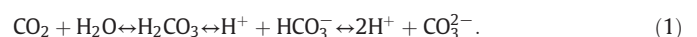
Total organic carbon (TOC) levels are, in general, relatively high in Norwegian water sources with a high degree of variability (Fig. 2). Fulvic acids in TOC of humic origin contribute to the low water pH in Norway (Lydersen et al., 2002), and also contribute to transport of associated metals. Metals bound to humic substances are generally less bioavailable than low molecular weight metal species (also denoted free metal ions), and elevated TOC may thus serve to protect fish from harmful effects of metals provided that remobilization is not enhanced by decreased pH (Andren et al., 2006; Rosseland and Staurnes, 1994), and/or increased ionic strength (Bjerknes et al., 2003; Teien et al., 2008) in the water.

Water chemistry is important in the health and physiological integrity of Atlantic salmon when stressed by other pathogens such as sea lice with the main focus studied to date being acidification of freshwater and its associated implication with the mobility of toxic metal ion species such as  $\text{Al}^{3+}$  (Finstad et al., 2007, 2012) (Fig. 3). In particular the episodic and fluctuating effects of acidified freshwater enhances the stress effects and reduced survival of post-smolts infected with sea lice (Finstad et al., 2012). Thus, not all freshwater sources can be deemed suitable or optimal for the treatment of Atlantic salmon in a parasite control regime.

### 2.2. Metabolite accumulation: effects on water chemistry

Dissolved oxygen levels in the treatment water must be maintained by addition of oxygen gas, and it is vital to maintain levels above 80% saturation to ensure no compromise of ventilation rate or oxygen uptake (Powell et al., 2000). In practice, oxygen levels are typically maintained at over 120%. In the following discussion on metabolites, adequate oxygenation is assumed.

Carbon dioxide ( $\text{CO}_2$ ) is generated as the end product of aerobic metabolism in a theoretical molar ratio of 1–0.7 to consumed oxygen. In practice, and in an aquaculture setting, about 1.1 mg  $\text{CO}_2$  is produced for each mg  $\text{O}_2$  consumed (Fivelstad and Binde, 1994). The solubility of  $\text{CO}_2$  in water and body fluids is very high due to reaction with water and generation of  $\text{HCO}_3^-$  and  $\text{H}^+$  (the bicarbonate buffering system). Reactions of the bicarbonate system are described (simplified) below (Eq. (1))



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