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Physiological consequences of chronic exposure of rainbow trout (*Oncorhynchus mykiss*) to suspended solid load in recirculating aquaculture systems

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ABSTRACT

High levels of suspended solids, especially fines, are widely regarded as harmful to fish, particularly in recirculating aquaculture systems (RAS) where accumulation of particles is likely. However, little is known about the consequences of chronic exposure to system-related particles on the stress-levels, well-being and health of fish. In this study, the chronic effects of suspended solids on the physiology and performance of rainbow trout were investigated over the growout period, uncoupled from other potentially confounding water parameters. Compared with fish in a control system with a total suspended solid (TSS) load of 3.9 mg/L, fish in an otherwise comparable treatment system exposed to minimum particle concentrations of more than 30 mg/L exhibited no observable difference in hematological variables (differential leukocyte count, RBC and WBC counts, hematocrit), gill histology, fin condition, or heat shock protein 70 concentrations in gill, liver, skin and head kidney tissues. Slight alterations in feeding behavior and a slight increase in bacterial load on fish and in system water were observed in the treatment RAS, but without any apparent effect on fish performance or health. Furthermore, no significant difference in mortality occurred. The absence of expected effects across a wide range of physiological criteria after long-term exposure suggests that suspended solid levels over 30 mg/L are within the physiological tolerance of this species.

1. Introduction

Recirculating aquaculture systems (RAS) are regarded as an environmentally friendly option for sustainable fish production (Martins et al., 2010) with the potential for reduced environmental impact through facilitating effluent treatment (Piedrahita, 2003). However, despite ongoing modernization, fish production in RAS remains energy- and cost-intensive and its contribution to global production is still small relative to that of flow-through, pond- or cage-based systems (Roque d'orbcastel et al., 2009). Challenges to the economic viability of RAS include high investment costs, the energy-intensive nature of production and difficulties in maintaining system stability leading to imbalances in water parameters, such as high bacterial load (Badiola et al., 2012). Whether maintaining system stability is an advantage or disadvantage of RASs is an ongoing debate. However, looking into systematic surveillance (e.g. Badiola et al., 2012; Martins et al., 2010), RASs turn out to be more vulnerable to system instability compared to open systems.

One possible solution to the energetic cost of RAS is intensification, whereby an increase in stocking densities will reduce energy costs per unit of production (Martins et al., 2005). However, high stocking densities require system stability and optimal water quality to ensure economically competitive and animal-friendly fish production. Animal welfare is becoming a decisive factor in aquaculture production (Ashley, 2007), with increasingly environmentally literate consumers pressing for more responsible methods of food production worldwide.

The management of solids is one of the most difficult technical issues in RAS (Badiola et al., 2012). Within a RAS, suspended solids originate mainly from feces and to a lesser extent from uneaten feed, bacterial material from biofilters, and microfauna (Chen and Malone, 1991; Noble and Summerfelt, 1996; Summerfelt et al., 1999; Wedemeyer, 1996). Solids comprise mostly smooth particles with a density close to that of water (Unger and Brinker, 2013). Particles are known to harm gill structures (Bash et al., 2001; Bilotta and Brazier, 2008; Bruton, 1985; Chapman et al., 1987; Humborstad et al., 2006;

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Wong et al., 2013) and elevate stress levels in fish (Awata et al., 2011; Lake and Hinch, 1999; Sutherland et al., 2008), and standard recommendations commonly quote a safe upper limit of 25 mg/L in rearing water (Alabaster and Lloyd, 1982; Timmons and Ebeling, 2010). While large particles can be easily removed from RAS by mechanical filtration, smaller particles tend to remain in the system and accumulate over time (Becke et al., 2017; Chen et al., 1993; Davidson et al., 2009). These fine particles are widely regarded as especially harmful to fish health and welfare (Chapman et al., 1987; Chen and Malone, 1991). Furthermore, nutrient leaching increases with decreasing particle size (Brinker et al., 2005a; Kvåle et al., 2006) and thus the accumulation of fines presents a high risk of deteriorating water quality. The relatively large surface/volume ratio of fine particles also offers an increased opportunity for bacterial colonization (Berger et al., 1996; Kirchman and Ducklow, 1987). However, the actual effects of accumulating system-related particles in RAS have been insufficiently studied and little is known about the primary effects of these particles on fish health. There is an urgent need for a better understanding of this issue to further optimize RAS performance and fish welfare. A recent study by Becke et al. (2017) uncoupled particle load from potentially interfering water parameters in RAS for the first time and demonstrated against all expectations that in the short term at least, particle loads up to 30 mg/L did not impart any observable negative effects on a wide array of fish health and performance parameters.

There are several studies dealing with effects of suspended sediments from natural environment on fish physiology (e. g. Humborstad et al., 2006; Wong et al., 2013). However, particle effects were only partly decoupled from potentially confounding or debilitating water parameters or chemical contaminants so that the impact of suspended sediment was amplified (Wong et al., 2013). This exemplifies the risk that apparently particle-related effects may be biased by uncontrolled factors such as inappropriate water quality.

Based on this, the present study sought evidence of chronic effects of accumulating particles on performance and physiology of rainbow trout over a whole growout period, uncoupled from potentially confounding or debilitating water parameters. The experimental design was chosen such that the elevated solid fraction in the treatment RAS comprised mainly fine particles to increase the potential for damage. We hypothesized that chronic exposure to particle loads exceeding the recommended value of 25 mg/L would result in physiological and health effects expressed as changes in hematology, gill histology, fin condition and stress protein expression. We further hypothesized that a chronic increase in suspended solids within the RAS would increase bacterial load, creating adverse environmental conditions that would ultimately impair survival and growth performance of rainbow trout during their growout stage.

2. Materials and methods

2.1. Husbandry

In an eighteen-week exposure trial, the effects of high suspended solid load on the physiology and performance of rainbow trout in two replicate RASs (each with a volume of 6 m^3) were evaluated. The experimental systems both comprised 10 green circular fiberglass tanks, each with a capacity of 330 L (Fig. 1 A). Water exchange was reduced to a minimum and only limited to water loss due to backwashing of drum filter and evaporation. The study was performed with all-female rainbow trout (Störk strain) to exclude sex-related effects and thereby minimize variation. Approximately 600 rainbow trout were held in each system (60 fish per tank), with an average initial weight of 86.6 ± 12.0 g (control group) and 86.5 ± 10.7 g (treatment group) and maximum stocking densities of 68.4 ± 2.6 kg m^{-3} (control) and 65.2 ± 2.3 kg m^{-3} (treatment). The control RAS was operated under regular conditions, while the particle load of the treatment RAS was artificially increased by collecting backwash water from the drum filter into a tank and re-injecting it at regular intervals into the water buffer of the system (Fig. 1 B) using a mud pump (Wilo-EMU KS 8 ES, Dortmund, Germany). Under this process, larger particles were fragmented by shear forces (McMillan et al., 2003). The rate of flow of backwash water into the system was kept constant by the flow out of the biofilter. Thus, particles originated from feces and to a lower extend from uneaten feed. No artificial particles were added. In both systems, the drum filter (HDF801-1H, Hydrotech, Vellinge, Sweden) was equipped with a 100 µm gauze. The photoperiod was fixed at 12L:12D (Lumilux daylight lamps provided around 140 lx at the water surface between 0700 h and 1900 h) with a sigmoidal transition period of 30 min. The fish were fed restrictively by hand, six days a week (Sunday to Friday) using a commercial feed (Biomar EFICO Enviro 921, Aarhus C, Denmark; diet composition: see Becke et al., 2017) starting at 1.6 % of body weight at the beginning of the trial and declining to 1.2 % by the end. Bacterial growth in both systems was controlled with UV irradiation of the system water (Barrier L20, Wallace & Tiernan, Günzburg, Germany; UV dose: 40 mJ/cm^2 flow volume: 6600 L/h, lamp wattage: 80 W, measurement range UV sensor: 200 W/m^2). Fish were put into the RAS three weeks before the beginning of the experiment to ensure acclimatization to the new environment. Experiments were conducted according to the German Animal Welfare Act (TierSchG) and approved by Referat Tierschutz of Regierungspräsidium Tübingen (AZ 35/9185.81-7).

2.2. Water parameters

Similar water parameters (Table 1) were maintained in both systems, within limits known to preclude impacts on fish health or performance during the whole growout period. Thus, potential effects of particle accumulation were isolated from all measured water parameters which were considered being most relevant for fish health (Timmons and Ebeling, 2010). To avoid a decline in water quality, the biofilters deployed were over-dimensioned and sufficient to remove the waste associated with 4.5 kg feed/day, well in excess of the maximum 2.7 kg/day supplied in the present study. pH was measured daily (pH 320 with electrode Sentix41, WTW, Weilheim, Germany) in the outflow of the tanks and adjusted to approx. 7.4 by the addition of sodium hydrogen carbonate. NH_4-N concentration was measured in both RAS every 45 min using an automatic device (AMTAX SC, Hach, Germany) to ensure continuity of monitoring (Fig. 2). Carbon dioxide concentrations were determined up to thrice weekly in the fish tanks using a Portable Dissolved CO_2 Analyser (OxyGuard, Farum, Denmark).

Further tests were carried out three times a week to determine levels of NH_4-N, NO_2-N and $N0_3$-N in water from the connecting tubes of each system, using the Hach (Germany) analysis kits LCK 304: 0.2–2.5 mg/L; LCK 341: 0,05–2 mg/L; and LCK 339: 1–6 mg/L respectively. Oxygen concentrations (Oxygen Probes, OxyGuard, Farum, Denmark) and temperature (Temperature Probes, Oxyguard, Farum, Denmark) were monitored continuously at the outlets of two tanks in each system. Turbidity was measured in parallel with total suspended solids (TSS) using a turbidity meter (PCE-TUM 20, PCE Instruments, Germany).

2.3. Total suspended solids

The concentration of total suspended solids was determined according to method 2540 D of the American Public Health Association (APHA, 1998), with the exception that 0.45 µm cellulose-acetate filters (diameter: 50 mm, 11106–50-N, Sartorius AG, Göttingen, Germany) were used instead of glass-fiber filters. Prior to use, the filters were pretreated in boiling distilled water for 2 h, dried at 103 °C, and weighed (0.1 mg). Water samples were collected using a tube at a water depth of ca. 30 cm from five tanks in each system, then mixed in equal parts to create a representative sample for each system. Samples were collected in the early morning, before feeding, in order to represent the daily minimum solid loads (best case scenario). Thus, it was ensured