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Characterising organic matter in recirculating aquaculture systems with fluorescence EEM spectroscopy

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ABSTRACT

The potential of recirculating aquaculture systems (RAS) in the aquaculture industry is increasingly being acknowledged. Along with intensified application, the need to better characterise and understand the accumulated dissolved organic matter (DOM) within these systems increases. Mature RASs, stocked with rainbow trout and operated at steady state at four feed loadings, were analysed by dissolved organic carbon (DOC) analysis and fluorescence excitation-emission matrix (EEM) spectroscopy. The fluorescence dataset was then decomposed by PARAFAC analysis using the drEEM toolbox. This revealed that the fluorescence character of the RAS water could be represented by five components, of which four have previously been identified in fresh water, coastal marine water, wetlands and drinking water. The fluorescence components as well as the DOC showed positive correlations with feed loading, however there was considerable variation between the five fluorescence components with respect to the degree of accumulation with feed loading. The five components were found to originate from three sources: the feed; the influent tap water (groundwater); and processes related to the fish and the water treatment system. This paper details the first application of fluorescence EEM spectroscopy to assess DOM in RAS, and highlights the potential applications of this technique within future RAS management strategies.

1. Introduction

Developing and advancing aquaculture technology is critical, as aquaculture is playing an increasingly vital role in sustainable food production (FAO, 2014). Intensive aquaculture systems with up to hundreds of metric tonnes of biomass generate large amounts of faecal matter daily. The organic waste in these systems is linked to the feed input (amount and quality) as well as feed digestibility and utilisation (Dalsgaard and Pedersen, 2011), and measures to remove dissolved and particulate matter are therefore often applied to mitigate negative impacts on water clarity and quality. Recirculating aquaculture systems (RASs) are land-based, closed-loop systems in which fish are grown and harvested. RAS benefits potentially include stable rearing conditions, reduced risk of escapees, options for pathogen control, as well as solids/particulate removal and hence decreased environmental impacts (Martins et al., 2010).

Challenges to RAS include investment and operating costs, as well as the risk of operational failure. These failures may arise from poor water quality conditions and are often associated with disease encounters and/or insufficient pathogen control (Noble and Summerfelt, 1996; Pedersen et al., 2009). Attempts at mitigating such problematic conditions are typically carried out by severe biosecurity measures (Sharrer et al., 2005; Summerfelt et al., 2009) or by chemical treatment often with limited success (Rintamäki-Kinnunen et al., 2005). Despite these challenges, farming RAS at commercial scales is becoming increasingly common with more species being cultured (Dalsgaard et al., 2013) and systems becoming increasingly large. The economic impacts of RAS

Abbreviations: BOD, Biological oxygen demand; C:N, Carbon to nitrogen ratio; CFB, Cumulative feed burden; COD, Chemical oxygen demand; COD:N, COD to nitrogen ratio; DOC, Dissolved organic carbon; DOC:N, DOC to nitrogen ratio; DOM, Dissolved organic matter; EEM, Excitation-emission matrix; FDOM, Fluorescent dissolved organic matter; PARAFAC, Parallel factor analysis; RAS, Recirculating aquaculture system; RU, Raman Units; TOC, Total organic carbon; UV, Ultraviolet; UVA, Ultraviolet A; UVB, Ultraviolet B.

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underperformance and failures are substantial, and minimising these events is important for the advancement of RAS and aquaculture in general. The full potential of RAS is yet to be realised, to become profitable and to accelerate development on land, as current water quality monitoring practices have been shown to be insufficient for reliable, failure-free operation.

The management and mitigation of system failures is at present commonly focussed on maintaining chemical water quality by applying conventional wastewater treatment processes for removal of potentially toxic compounds such as ammonia, as well as by maintaining a balanced microbial community. The effectiveness of these mechanical and biological treatment processes is of utmost importance to RAS viability, and fundamental treatment processes such as particle/solids removal have been shown to be heavily impacted by inherent DOC concentrations (Hem et al., 1994; Summerfelt and Vinci, 2008; Timmons and Ebeling, 2010). For example, Ling and Chen (2005) observed such effects on nitrification efficiency, where an exponential decrease in nitrification performance was recorded with the addition of DOC into the water matrix. Hu et al. (2009) observed negative impacts on nitrification efficiency with increases in carbon to inorganic nitrogen ratios, and a number of studies have recommended that generally elevated C:N ratios are to be avoided (Guerdat et al., 2011; Michaud et al., 2006).

Managing microbial water quality is also an important component of RAS management, as bacteria, parasites and algae can affect fish growth and survival, and ultimately RAS output (Blancheton et al., 2013). Recently, Moestrup et al. (2014) observed instances of fish kills within two Danish marine RAS where the cause was identified to be populations of two dinoflagellate species: *Pfiesteria shumwayae* and *Luciella masanensis*. Like many heterotrophic dinoflagellate species, *Pfiesteria* are reported to thrive in conditions where the water body contains high organic matter content (Burkholder and Glascow, 1997; Glibert et al., 2001; Lewitus et al., 1999), which is often the case in RAS utilising long retention times and insufficient solids/organic matter removal.

This suggests that the efficient and successful management of a RAS is heavily reliant on controlling the accumulation and quality of dissolved organic matter (DOM) in the system. It follows that implementing more adequate monitoring and control of the organic content of RAS waters could therefore serve to detect deviations in treatment efficiency, and as a preventative measure for RAS failure and subsequent mortality events.

Current RAS monitoring methods typically include the analysis of bulk organic matter in the water, including particulate matter, and inorganic nitrogen species such as ammonia, nitrate and nitrite. Additionally, gross indicators such as chemical oxygen demand (COD) and biological oxygen demand (BOD) can be applied, however, analysis of BOD typically requires 5–7 days which introduces an inherent delay in response to events. The organic matter within RASs is derived from a range of sources, each varying in relative importance with time, and so bulk organic matter measurements alone (e.g. DOC or absorbance at 254 nm, A_{254}) may not provide an adequate estimate of the extent to which organic matter character may be fluctuating and potentially influencing RAS performance.

The fraction of DOM that is fluorescent, FDOM, and its spectral characteristics of, have been successfully utilised as quantitative and qualitative measures of DOM across a range of natural and engineered systems including, lakes and rivers (Baker, 2001, 2002; Yamashita et al., 2010), wastewater (Bridgeman et al., 2013; Hambly et al., 2010a; Reynolds and Ahmad, 1997), drinking water (Shutova et al., 2014; Stedmon et al., 2011) and marine systems (Coble, 1996; Coble et al., 1990). Different components of FDOM have been shown to be tracers of DOM sub-fractions, including biorefractory, labile and photochemically-active components (Stedmon and Cory, 2014). Fluorescence excitation-emission matrix (EEM) spectroscopy has emerged as the state-of-the-art in FDOM analysis and has become increasingly common as an analytical tool for water sciences (Coble et al., 2014). EEMs are generated by recording emission spectra over a range of excitation wavelengths and combining them to form a detailed contour map of the fluorescent properties of organic matter in a water sample. Multivariate data analysis techniques, in particular parallel factor analysis (PARAFAC), have proven their abilities to reliably decompose EEMs into independently varying fluorescent components, allowing more accurate identification of independent FDOM components (Murphy et al., 2013). As such, the ability of fluorescence spectroscopy to successfully characterise aquatic systems has become increasingly evident. Fluorescence analysis is a growing research area within natural and engineered water systems, and individual components of FDOM have been shown to correlate well with BOD (Hudson et al., 2008; Reynolds and Ahmad, 1997), COD (Bridgeman et al., 2013; Lee and Ahn, 2004), ammonia, nitrates and phosphates (Baker and Inverarity, 2004), as well as microbiological indicators such as total coliforms and *E. coli* (Cumberland et al., 2012). These are important parameters within RAS, though currently unable to be reliably monitored online and analysed within a reactive timeframe. Fluorescence spectroscopy, however, is a fast, sensitive and non-destructive analysis technique, and hence shows great potential in its application to real-time monitoring of RAS and aquaculture in general. More specifically, these properties could enable fluorescence analysis to become a valuable, real-time monitoring tool to optimise RAS management.

This study provides the first application of fluorescence EEM spectroscopy and PARAFAC analysis to characterise DOM in aquaculture systems, more specifically within a RAS. Whether changes in FDOM are consistent across all types of RAS are yet to be determined, however this study evaluates the application of the EEM-PARAFAC technique itself. The aim was to test if the technique can identify characteristic organic matter fractions from the complex organic RAS matrix, and to therefore outline the potential of using fluorescence as a sensitive monitoring parameter of RAS water.

2. Materials and methods

2.1. Experimental design

Four identical 1700 L freshwater RASs (Fig. 1) were stocked with juvenile rainbow trout (*Oncorhynchus mykiss*) with an average weight of 131 ± 6 g and brought to steady state conditions (in terms of nitrate equilibrium) over 2 months.

Four daily feed inputs were applied within the study (125, 250, 375 or 500 g). The daily water renewal of each RAS was 80 L, corresponding to relative water renewal rates of between 160 and 640 L/kg feed, and the cumulative feed burden (CFB) ranged from 1.6 to 6.3 kg feed per m^3 water renewal. The stocking density was adjusted to the feed given; initial biomasses were 14, 28, 42 and 56 kg/m3 at the four levels. As a consequence of the experimental design with fixed daily feed allocation the corresponding feeding ratio decreased from 1.8% to 1.1% of fish biomass per day during the experimental period due to biomass gain. An experimental fish feed consisting of 44% protein and 30% lipid (Pedersen et al., 2012) (Table 1) was utilised and allocated daily from 9:00 to 15:00 h by belt feeders. Forty litres of RAS water was drained daily from the swirl separator in each RAS, and replaced with 80 L of non-chlorinated tap water. The 40 L excess was discharged by overflow and evaporation. This corresponded to a water exchange