Characterizing changes of dissolved organic matter composition with the use of distinct feeds in recirculating aquaculture systems via high-resolution mass spectrometry

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HIGHLIGHTS

- First LMW-DOM characterization of recirculating aquaculture systems (RAS) by HRMS
- Untargeted HRMS analysis showed molecular alterations of LMW-DOM in RAS.
- Standard feed contributed to CHO, CHOS and lignin/CRAM-like chemical groups in DOM.
- RAS feed contributed to CHNO, CHNOS and unsaturated hydrocarbon chemical groups in DOM.
- The Kendrick plot showed removal of CHNO, CHNOS and halogenated homologous chemicals.

GRAPHICAL ABSTRACT
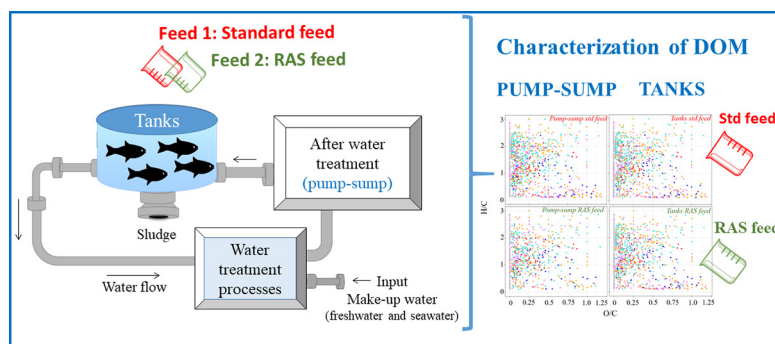
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ABSTRACT

Recirculating aquaculture systems (RAS) are a new alternative to traditional aquaculture approaches, allowing full control over the fish production conditions, while reducing the water demand. The reduction of water exchange leads to an accumulation of dissolved organic matter (DOM) that can have potential effects on water quality, fish welfare and system performance. Despite the growing awareness of DOM in aquaculture, scarce scientific information exists for understanding the composition and transformation of DOM in RAS. In this study, a non-targeted approach using ultra-performance liquid chromatography coupled to a hybrid quadrupole-time of flight mass spectrometer (UPLC-QTOF-MS) was used to characterize compositional changes of low molecular weight (LMW) DOM in RAS, when operated under two different feed types. A total of 1823 chemicals were identified and the majority of those contained a CHON chemical group in their structure. Changes in the composition of LMW-DOM in RAS waters were observed when the standard feed was switched to RAS feed. The DOM with the use of standard feed, consisted mainly of lignin/CRAM-like, CHO and CHOS chemical groups, while the DOM that used RAS feed, was mainly composed by unsaturated hydrocarbon, CHNO and CHNOS chemical groups. The Bray-Curtis dissimilarity cluster demonstrated differences in the composition of DOM from RAS and was associated to the type of feed used. When the RAS feed was used, the Kendrick mass defect plots of $-CH_2-$ homologous units in the pump-sump (after

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the water treatment) showed a high removal capacity for CHNO, CHNOS and halogenated chemicals with high Kendrick mass defect, KMD > 0.7. To our knowledge, this is the first report of LMW-DOM characterization of RAS by high-resolution mass spectrometry (HRMS).

1. Introduction

Fish is a primary source of animal proteins, micronutrients and essential fatty acids whose demand is increasing due to the rapid human population growth (Bogard et al., 2015; Thilsted et al., 2016). The over-exploitation of capture fisheries and the effects of global climate change on aquatic biodiversity, prompts the need for novel production systems to sustain the rise of the global fish demand (Lem et al., 2014; Lipper et al., 2014; Subasinghe, 2017). Recirculating aquaculture systems (RAS) with their innovative low water exchange rates are constantly gaining approval over more traditional approaches in the aquaculture industry (Tidwell and Allan, 2001; Bostock et al., 2010). The RAS recycle the natural input of water through a multi-step water treatment process that reduces the need for new additions of water, while they minimize the concentration of contaminants in the system effluents. Moreover, they increase the biosecurity by preventing the escape of fish (Martins et al., 2010). The ability of RAS to control the water quality as means of optimizing the fish growth, make them superior over traditional aquaculture systems (Timmons and Ebeling, 2007). However, one main drawback of RAS is the accumulation of organic matter (OM), particularly the dissolved organic matter (DOM) (Badiola et al., 2012; Van Rijn, 2013). Fecal waste and feed spill are the two main sources of endogenous production of DOM in RAS (Ackefors and Enell, 1994; Cripps and Bergheim, 2000). The significance of exogenous DOM is dependent on the quality and quantity of makeup water that is used for water exchange (Bilotta and Brazier, 2008). The water exchange rate and the type of the treatment system (e.g. ozone, UV, skimming) determines the extent in which the DOM can accumulate within a system. This accumulation of DOM can promote the growth of opportunistic bacteria, affecting the biofiltering performance (Summerfelt and Sharrer, 2004; García-Ruiz et al., 2018) and hindering the oxidative disinfection processes of the systems (OIE, 2019). Other challenges of DOM accumulation in RAS include the contamination of the fish environment, which further risks the welfare of the fish (Bregnballe, 2015), while the deterioration of water quality increases the need for further water treatment processes, and consequently, leads to higher production costs (Cai and Leung, 2017; Subasinghe, 2017).

Current methods used in RAS for monitoring changes of DOM include biological oxygen demand (BOD), chemical oxygen demand (COD) and total organic carbon (TOC) (Lin et al., 2003; Guerdat et al., 2011; Spiliotopoulou et al., 2018). Nonetheless, the lack of data concerning the DOM composition obtained by those methods, moved research towards two more specialized analytical techniques in RAS that prioritize the DOM fraction content: i) the UV absorbance at 254 nm, which is particularly sensitive to the presence of aromatic fractions of DOM; and ii) the excitation-emission matrices (EEMs) of fluorescence combined with parallel factor analysis (PARAFAC), which can identify different classes of fluorescent DOM (FDOM). The ability of the latter to characterize the fluorescence fraction of DOM, provides a more descriptive approach to monitor OM changes in RAS. Fluorescence EEMs with PARAFAC was used for the characterization of FDOM in freshwater with rainbow trout (*Oncorhynchus mykiss*), and to indicate the presence of humic substances-like (HS-like) chemical groups in fresh and marine water with hybrid tilapia (*Oreochromis aureus x Oreochromis niloticus*) and gilthead seabream (*Sparus aurata)*, respectively (Hambly et al., 2015; Yamin et al., 2017). Moreover, this technique was applied to detect discharged DOM from salmonid land-based aquaculture systems (Nimptsch et al., 2015). However, despite

being a non-destructive and sensitive technique with the ability to characterize FDOM, it lacks in identifying the presence of different chemical groups in other DOM fractions. Thus, it is deemed necessary to apply even better analytical approaches to obtain a more detailed understanding of the overall quality of DOM in RAS and its potential effects on the systems.

Non-targeted screening approaches by high resolution mass spectrometry (HRMS) can identify trace concentrations of organic chemicals from the complex DOM mixture and reveal useful information about changes, transformations, and reaction pathways in the DOM composition. Non-targeted screening is a post-measurement processing approach applied to HRMS, which is employed when there is no previous information about the underlying chemicals (Hernández et al., 2005; Krauss et al., 2010; Hernández et al., 2012). The HRMS formula assignment and data treatment emerged from the research domains of natural organic matter (NOM) (Hertkorn et al., 2008; Sleighter and Hatcher, 2008; Remucal et al., 2012; Sleighter et al., 2012), petroleum (Hughey et al., 2001), metabolomics and lipidomics (Cajka and Fiehn, 2016). The characterization of DOM by non-targeted approaches using HRMS, contributed to the evolution of different fields within marine science (Rathgeb et al., 2017) and environmental chemistry, e.g., wastewater (Verkh et al., 2018a) and soil (Brock et al., 2019) characterization. Moreover, non-targeted approaches are used to elucidate the chemical structure of small molecules of emerging contaminants and their transformation products in the broader context of environmental chemistry (Schymanski et al., 2014a), including limnology (Minor et al., 2012) and drinking water treatment technologies (Farré et al., 2019).

The molecular composition of DOM from salmonid aquaculture effluents were previously analysed using ultra high-resolution Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) (Kamjunke et al., 2017). In addition to that, HRMS based on orbitrap and quadrupole time-of-flight (QTOF) instruments were also used for non-targeted analysis of complex mixtures of DOM (Schymanski et al., 2014b; Hawkes et al., 2016; Rathgeb et al., 2017; Simon et al., 2018; Pan et al., 2020). Despite the lower resolution power of those than that of FTICR-MS, they can unravel a significant fraction of DOM and identify low molecular weight (LMW) compounds (Remucal et al., 2012). Furthermore, coupling ultra-performance liquid chromatography (UPLC) to HRMS, can overcome challenges encountered by direct injection (DI), facilitating the fractionation of chemicals and improving the characterization of aquatic DOM (Patriarca et al., 2018). However, still little is known about DOM composition and its transformation within RAS.

With this as background, non-targeted approaches by UPLC-QTOF-MS were employed in this study to elucidate for the first time the composition of LMW compounds in DOM during the production of Atlantic salmon post-smolt in RAS. Two different type of feeds were used, commercial standard and RAS feed for Atlantic salmon, to evaluate the organic loadings in RAS. Samples were also collected after the water treatment (pump-sump) and from the fish rearing tanks, where the feeds were added to the system (Fig. 1), to gain further insights on the effects of the water treatment processes on the composition of LMW in RAS. The composition of LMW-DOM and its fate within the system was assessed by profiling and comparing the number of identified chemicals and their abundances. The alterations in the composition of DOM were further used to assess the water quality and the effectiveness of the water treatment processes in RAS.
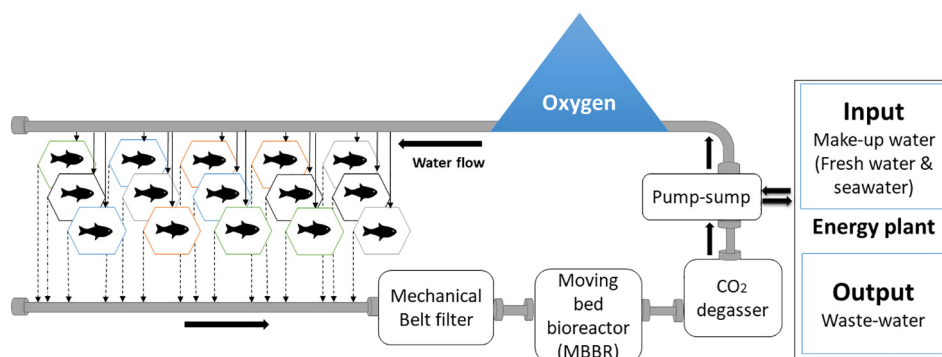
Fig. 1. Experimental set-up of the recirculating aquaculture system (RAS) at the Nofima Centre for Recirculation in Aquaculture (NCRA).

2. Materials and methods

2.1. Experimental system

The experiment was performed at the Nofima Centre for Recirculation in Aquaculture (Terjesen et al., 2013). Atlantic salmon post-smolt was reared in dual drain octagonal tanks (3.3 m³), where the bottom outlet was connected to a swirl separator to collect fecal waste and feed spill. In the system (Fig. 1), the tank effluents passed through a mechanical filter (belt filter, 131 μm screen size), followed by a moving bed bioreactor (MBBR) and a CO_2 degasser. After the pump-sump, the water was re-oxygenated and returned to the tank. Make-up water derived from freshwater and seawater sources to maintain a stable salinity of 12 ng/L. The total volume of the system was approximately 75 m³. To keep the accumulation of OM constant with an increasing feed load during the experiment, the water was exchanged at an average rate of 555 L/kg of feed, corresponding to initially 17% exchange of system volume per day and 30% exchange at the completion of the experiment. Feed and make-up water were adjusted weekly. Hydraulic retention time in the tanks was 30 min, and the tank volume was exchanged twice per hour.

2.1.1. Experimental design

Atlantic salmon (*Salmo salar* L., 1758) with an average weight of 766 g was stocked in 15 octagonal tanks supplied with RAS water. The starting stocking density in the tanks was 30 kg/m³, and the fish were kept at 24 h of light and were fed continuously [~1.4% Body Weight (B.W.)/day (d)]. During the experiment, the oxygen saturation was maintained at > 85% in the tanks. The average temperature in the tanks was set at 15.5 °C and the pH level was adjusted at 7.3 ± 0.1 by addition of sodium bicarbonate.

Two types of commercially available feed for salmon were used: 1) standard aquaculture feed typically used for the grow-out of salmon; and 2) feed optimized for production of salmon in RAS. Although both standard and RAS feed have similar proximate composition and raw materials (Table S1), the RAS feed contains a binder that enlarges the fecal particles, and therefore, prevents the leaching of nutrients and improves the fecal waste removal in the water treatment processes. Thus, the production of suspended solids and organic matter decreases in the system, optimizing waste removal processes and considerably improving water quality (Brinker et al., 2005; Brinker, 2007).

2.2. Chemicals and materials

HPLC grade acetonitrile, methanol, hydrochloric acid (HCl) and water were purchased from VWR Chemicals (Trondheim, Norway). Formic acid was purchased from Sigma-Aldrich (Steinheim, Germany). Sterile metal free polypropylene (PP) tubes (VWR Chemicals, Trondheim, Norway) were used for sampling aquaculture waters to analyze dissolved organic carbon (DOC) and turbidity. High-density polyethylene

(HDPE) bottles (VWR Chemicals, Trondheim, Norway) were used for sampling aquaculture waters (to analyze DOM). Polyethersulfone syringe filters (0.45 μm; Whatman™, VWR Chemicals, Trondheim, Norway) were used to filter aquaculture waters and remove the particulate matter from the samples for DOC analysis. Glass microfiber filters (0.7-μm pore size, nominal pore size, GF/F, Whatman™, Sigma-Aldrich, Germany) were applied for the removal of particulate organic matter (POM) before extracting DOM from aquaculture waters. Borosilicate vials (40 mL; EPA screw neck vials with PTFE/Silicone septa) for DOC analysis and DOM sample extracts were obtained from VWR Chemicals (Trondheim, Norway). For DOM extraction, Agilent Bond Elut™ PPL (500 mg, 6 mL; Matriks, Oslo, Norway) SPE cartridges and a peristaltic pump (IPC, Ismatec, Germany) were used. DOM extracts were concentrated with a TurboVap™ LV Automated Evaporation System (Biotage AB, Sweden).

2.3. Water quality measurements

Water quality parameters, including pH, temperature, salinity, oxygen, turbidity and dissolved organic carbon (DOC) were measured in the pump-sump and the three tanks during: a) the standard feed exposure period: days 5, 7 and 10; b) the transition period: day 14; and c) the RAS feed exposure period: days 18, 20 and 25. Temperature, pH and salinity were measured placing the extracted water with a silicon tube in a plastic container with a multi-parametric measuring instrument, WTW Multi 3430 (WTW, Weilheim, Germany). Oxygen was measured inside the pump-sump and the tanks with a Handy Polaris TGP (OxyGuard, Farum, Denmark) portable electrode. Water samples collected in triplicates with 50 mL PP tubes were analysed for turbidity in the laboratory of the facility using a Turbiquant 1500 IR (Merck, Darmstadt, Germany) (APHA, 1999).

Dissolved organic carbon (DOC) was quantified with a Lotix combustion total organic carbon analyzer (Teledyne Tekmar, Mason, USA) (Farmer and Hansell, 2007). Water samples collected in triplicates using PP tubes were filtered with the 0.45 μm polyethersulfone syringe filters. 20 mL-filtered samples were placed in pre-cleaned and pre-combusted borosilicate vials and diluted with deionized water to 40 mL for DOC analysis (Mopper and Qian, 2000).

2.4. Sampling and extraction of DOM

Water samples were collected in triplicates during the three experimental steps (a–c), as described in Section 2.3, using 1 L pre-cleaned HDPE bottles (Tupas et al., 1994). Subsequently, 240 mL of water samples were filtrated using pre-combusted (4 h at 450 °C) GF/F filters. The combustion step contributed towards cleaning and reducing the average pore size of the filters, which increased their efficiency and POM retention capacity (Nayar and Chou, 2003; Gogou and Repeta, 2010).

DOM was isolated by solid-phase extraction (SPE) as described by Dittmar et al. (2008). Briefly, 240 mL of sample were acidified with

HCl to pH ≤ 2 for protonating the organic acids, and hence maximizing their retention on the cartridge sorbents. The Agilent Bond Elut PPL™ SPE cartridges were activated with 6 mL of methanol prior the loading step. Thereafter, 12 mL of 0.01 M HCl were added for the washing step and the cartridges were dried under vacuum for 5 min prior to the DOM elution with 6 mL of methanol. The extracts were stored at −20 °C into the previously cleaned and combusted 40 mL borosilicate vials. Two extraction blanks using 240 mL of Milli-Q water were also prepared for SPE extraction.

DOC recoveries were calculated by dividing the concentration of DOC in the initial fitered water samples by the concentration of DOC in the extract samples. In order to do this, 20 mL of the initial filtered water sample was diluted with 20 mL of deionized water to a total volume of 40 mL followed by DOC measurement (Section 2.3). Sample extracts were dried at 30 °C (and 5 psi) and reconstituted to a volume of 40 mL with deionized water, from which an aliquot was taken for DOC measurements.

2.5. UPLC-QTOF-MS analysis

The UPLC analysis was performed using an ACQUITY UPLC I Class® system connected to a Synapt G2-S Mass spectrometry detector (Waters Corporation, Milford, USA) with negative and positive electrospray ionization sources (ESI+ and ESI-). 200 ng/mL of leucine enkephalin was used as a Lockmass at a flow rate of 10 μL/min to allow correction of exact mass measurements. An Acquity UPLC BEH C_{18} (2.1 mm × 100 mm, 1.7 μm, Waters, Oslo, Norway) chromatographic column was used for reverse-phase separation. Quality control (QC) samples, which contained an aliquot mixture of actual samples, were analysed randomly during the sequence of injections to monitor the stability of the system and the performance of the method. Reagent blanks (RB) were run and subtracted from each sample. Instrumental blanks were run before and after every sample to check for carryover or cross-contamination. 0.1% (v/v) of formic acid was used as additive in both solvents: (A) water and (B) acetonitrile. The injection volume was 5 μL and the flow rate was 0.4 mL/min. The chromatographic gradient was: initial conditions 5% B; 0.02–10.02 min, 5–95% B; 10.02–15.02 min, 95% B; 15.02–15.10 min, 95–5% B; 15.10–18.00 min, 5% B. The capillary voltage was set at −1.50 kV (ESI-) and + 2.50 kV (ESI+), the desolvation flow was fixed at 900 L/h with a desolvation temperature set at 500 °C and the cone voltage set at 40 V. The full scan spectra were acquired within a range of 100 to 1000 m/z.

The UPLC-QTOF-MS data was analysed using Masslynx V4.1 and Progenesis QI V2.3 (Waters, Milford, USA). Masslynx V4.1 was used to visually inspect each chromatogram and determine peaks present in the analysed samples, while chemical identification was performed by Progenesis QI V2.3. The data acquired under positive and negative mode was treated in Progenesis QI V2.3 as described by Verkh et al. (2018a). Briefly, positive $[M+H]^+$ and negative $[M-H]^-$ adducts were selected. Then, deconvolution features were grouped into chemicals based on the match between the chromatographic profile and mass spectra. An absolute ion intensity of 500 a.u. and retention time limits from 0.05 min to 15 min under positive mode and from 0.05 to 7 min under negative mode were applied. The molecular formula determination of separated compounds was performed using elemental composition and Chemspider MS/MS library search. Databases available within Chemspider in this experiment were namely, NIST chemistry WebBook, PubChem and KEGG. Molecular formulas were calculated with the following elemental restrictions: $^{12}C_{1-80}$ $^1H_{1-100}$ $^{16}O_{0-20}$ $^{14}N_{0-15}$ $^{32}S_{0-4}$ $^{35}Cl_{0-4}$ $^{79}Br_{0-4}$ using a fragment tolerance of 10 ppm and a ≥ 60% isotopic similarity for elemental composition search. A precursor tolerance of ±5 ppm and a retention time < 0.5 min were established to exclude the detection of duplicate chemical features. The triplicate samples were filtered out using a coefficient of variation (CV) of ≤ 30% to remove the random noise. Chemicals with p-values ≥ 0.05 and m/z > 1000 were removed to avoid false predictions. The selection of the molecular

formula for each identified m/z was performed with the R studio (version 4.0.2). A R studio script was used to filter out the generated empirical formulas matched to a single m/z based on the heuristic rules: H/C < 3.2, O/C < 1.2, N/C < 1.3, and S/C < 0.8 (Kind and Fiehn, 2007); and the smallest absolute mass error (ppm) from a 10% deviation of the highest isotopic similarity (Zhang et al., 2008; Verkh et al., 2018b; Yang et al., 2019).

2.6. Calculations and statistical analysis

For the selected formulas, the double bond equivalent (DBE), the modified aromaticity index (AI_{mod}) values, the double bond equivalency per carbon (DBE/C) and the double bond equivalent minus oxygen (DBE-O) were calculated as described by Kim et al. (2006) and Koch and Dittmar (2006). DBE, DBE/C, DBE-O and AI_{mod} are useful parameters for the characterization of aromaticity and unsaturation of chemical formulas. DBE provides information concerning the number of double bonds and/or rings in a molecule. Despite being used as a rule of thumb, DBE/C and DBE-O parameters are better in providing actual carbon unsaturation values (Cortés-Francisco and Caixach, 2013). AI_{mod} clearly defines condensed aromatic structures by considering the 50% of the oxygen present in the molecule form of carbonyl groups (C=O) (Wagner et al., 2015). These parameters, together with the elemental ratios of H/C, O/C, S/C, N/C, Cl/C and Br/C, were expressed as weight average (wa) values calculated from the intensity of each detected peak (Schmidt et al., 2009). The calculated H/C and O/C ratios of the identified chemicals were represented in Van Krevelen diagrams (Kim et al., 2003), and were classified into seven biochemical classes: unsaturated hydrocarbons (0.01 ≤ O/C ≤ 0.1; 0.75 ≤ H/C ≤ 1.5); lipids (0.01 ≤ O/C ≤ 0.1; 1.5 ≤ H/C ≤ 2.0); proteins (0.1 ≤ O/C ≤ 0.65; 1.5 ≤ H/C ≤ 2.3; N ≥ 1); condensed aromatic structures (0.01 ≤ O/C ≤ 0.65; 0.25 ≤ H/C ≤ 0.75); lignin/carboxyl-rich alicyclic molecules (CRAM)-like (0.1 ≤ O/C ≤ 0.65; 0.75 ≤ H/C ≤ 1.5) with no heteroatoms (only CHO group); carbohydrates (0.65 ≤ O/C ≤ 1.0; 1.5 ≤ H/C ≤ 2.5); and tannins (0.65 ≤ O/C ≤ 0.85; 0.75 ≤ H/C ≤ 1.5) (Mangal et al., 2019). The identification of homologous -CH_2- series using Kendrick mass defect was used to map the removal of organic chemicals in aquaculture waters. The calculation was based on the re-normalization of the exact –CH_2- IUPAC mass (14.01565) to 14.00000. KMD was calculated from the difference between the nominal Kendrick mass (Da) of the molecule and the exact Kendrick mass (Hughey et al., 2001). Bray–Curtis statistical analysis was calculated considering the presence and absence of the identified formulas.

3. Results and discussion

3.1. Water quality

The water quality parameters measured in both the pump-sump and the tanks between the two feed treatments are represented in Table 1. The water quality parameters, including temperature, pH, oxygen and salinity, reflected the commercially relevant conditions used during the production of Atlantic salmon post-smolt (Kroglund et al., 2008; Elliott and Elliott, 2010; Thorarensen and Farrell, 2011).

Significant differences (ANOVA, $p < 0.05$) were observed in turbidity and DOC when the standard feed was exchanged by the RAS feed (Fig. S1), indicating changes of the physicochemical parameters with the type of feed used. Furthermore, the turbidity and DOC parameters were studied with principal component analysis (PCA) using the water quality variables and the samples from the tanks and the pump-sump during the experimental period days (Fig. S2). A negative correlation was observed for turbidity in relation to pH and oxygen. Suspended solids causing turbidity in water absorb heat from the light source, increasing the temperature, which further decreases the oxygen in the aquaculture waters, and consequently, the pH. High loads of salinity correlated negatively with DOC. This can be explained by the high

Table 1
Physico-chemical parameters measured in the pump-sump and the tanks during the experimental period: standard feed use; transition; and RAS feed use.

Water quality parameters	System section	Standard feed			Transition	RAS feed		
		Day 5	Day 7	Day 10	Day 14	Day 18	Day 20	Day 25
Temperature (°C)	Tanks	14.1 ± 0.01	14.7 ± 0.05	15.8 ± 0.01	17.0 ± 0.01	17.3 ± 0.05	15.1 ± 0.01	14.6 ± 0.01
	Pump	14.1 ± 0.01	14.7 ± 0.01	15.7 ± 0.01	16.9 ± 0.01	17.2 ± 0.01	15.1 ± 0.01	14.6 ± 0.01
pH	Tanks	7.21 ± 0.01	7.22 ± 0.01	7.21 ± 0.02	7.11 ± 0.01	7.22 ± 0.02	7.32 ± 0.01	7.40 ± 0.20
	Pump	7.62 ± 0.01	7.61 ± 0.01	7.62 ± 0.01	7.61 ± 0.01	7.73 ± 0.01	7.71 ± 0.01	7.60 ± 0.01
Oxygen (%)	Tanks	90.4 ± 2.58	89.4 ± 3.22	87.0 ± 1.90	87.8 ± 1.00	89.1 ± 1.57	89.3 ± 4.60	94.0 ± 3.03
	Pump	96.8 ± 0.20	97.1 ± 0.50	98.0 ± 0.02	97.6 ± 0.50	98.4 ± 0.10	97.2 ± 0.10	97.8 ± 0.10
Salinity (% SAT)	Tanks	12.4 ± 0.01	12.4 ± 0.01	12.1 ± 0.01	11.5 ± 0.01	11.6 ± 0.01	14.0 ± 0.01	11.5 ± 0.01
	Pump	12.4 ± 0.01	12.4 ± 0.01	12.1 ± 0.01	11.5 ± 0.01	11.2 ± 0.01	14.0 ± 0.01	11.5 ± 0.01
Turbidity (NTU)	Tanks	3.23 ± 0.23	4.91 ± 0.53	4.70 ± 0.63	4.61 ± 0.34	2.62 ± 0.18	3.62 ± 0.53	3.61 ± 0.24
	Pump	3.52 ± 0.22	4.52 ± 0.52	4.51 ± 0.22	5.22 ± 0.37	2.41 ± 0.22	4.32 ± 0.49	3.90 ± 0.58
DOC (ppm)	Tanks	18.8 ± 0.22	18.7 ± 0.22	28.9 ± 1.76	19.9 ± 0.36	24.1 ± 0.48	25.4 ± 0.38	33.7 ± 0.32
	Pump	19.1 ± 0.53	19.2 ± 1.02	29.3 ± 0.19	19.7 ± 0.17	25.0 ± 0.59	25.0 ± 0.10	28.6 ± 1.58

reactivity of nitrifying and heterotrophic bacteria, which remove efficiently organic carbon and ammonium in the saline environment (Yang et al., 2018).

3.2. Elemental composition of LMW-DOM in RAS waters

Water samples from both system locations (the pump-sump and the tanks) collected on the days with maximum DOC load (day 10 with standard feed and day 25 with RAS feed) were extracted to study the effect of feed type on DOM composition. Recovery of DOM from the extracted samples (30.3 ± 2.90%) was relatively low. This was attributed to the structural and compositional variance of DOM from RAS waters (Li et al., 2016). A total of 1823 elemental compositions (chemicals) were detected in RAS waters (943 for ESI- and 880 for ESI+). From those, 1153 and 1081 compositions were identified in day 10 (when standard feed was used) in the pump-sump and the tanks, respectively, while 825 and 1055 compositions were identified in day 25 (when RAS feed was used) in the pump-sump and the tanks, respectively (Table 2).

The large number of identified formulas were displayed using the Van Krevelen diagrams (Fig. 2). These plots with the atomic ratio of H/C in the y-axis and O/C in the x-axis provided information concerning the type of chemical groups and classes found in DOM based on their H/C and O/C characteristic ratios (Kim et al., 2003). The Van Krevelen diagrams showed a large diversity of chemicals in each sample with a wide range of H/C and O/C ratios, which can indicate the presence of DOM pools from different sources. Such diversity can be attributed to the fish pellets of different characteristics and nutrient compositions. Thus, this can be further related to different responses from the microbial communities, which can alter the actual DOM composition of the aquaculture samples (Karle et al., 2007). Seasonal variations in the input water also alters the DOM composition and concentration (Benner and Opsahl, 2001; Kristensen et al., 2009; Seifert et al., 2016). Within the system, the differences in the composition of the microbial communities (Karle et al., 2007), the changes in the physico-chemical parameters (Benner and Opsahl, 2001; Roth et al., 2015) and the water treatment processes can also lead to DOM transformation and increase the diversity of DOM compounds. The nature of the inlet water (Kristensen et al., 2009; Seifert et al., 2016), in addition to the contribution of microbial and photodegradation processes for

the removal of the labile DOM, can be sources of ubiquitous composition (Wagner et al., 2015). Despite showing differences in their DOM composition, the samples also showed a common set of molecules that indicate concomitant sources of DOM. The molecular formulas identified in DOM (from RAS waters) were represented in the Van Krevelen diagrams and were classified into six chemical groups according to their elemental composition (Fig. 2). For both feed and sample locations, the CHON group of chemicals had the highest number of assigned molecular formulas followed by the halogenated and CHNOS group of chemicals. It is noteworthy that *N*-containing chemicals in aquaculture waters are found in proteins, dissolved primary amines (DPA) and urea. These compounds that are released from fish feed and feces can increase the microbial activity, while elevating the discharge of dissolved organic nitrogen into the system (Burford and Williams, 2001; Hudson et al., 2008). Formation and degradation of proteins as well as the presence of ammonia produced by fish excretions and food pellets can be another source of CHON and CHONS chemical groups in RAS (Sayer and Davenport, 1987; Schoonen and Xu, 2001; Fernandez-Jover et al., 2007; Valle et al., 2018). The presence of the CHNOS group of chemicals can also indicate anthropogenic origins (Kamjunke et al., 2017) and incorporation of sulphur in the CHNO group during the reaction with hydrogen sulphide (H_2S) under anaerobic conditions (Vairavamurthy and Mopper, 1987; Perlinger et al., 2002; Heitmann and Blodau, 2006; Gonsior et al., 2011). Chlorinated and brominated chemicals can be naturally occurring in nature, but they can also be released by anthropogenic activity as important environmental contaminants (e.g., chlorinated and brominated flame retardants) (Gribble, 1994, 2000; Ballschmiter, 2003). The ingredients and additives of fish feed, and their inappropriate handling and storage can be a potential source of those contaminants in the system (Carro et al., 2005; Fink-Gremmels, 2012). The lowest number of assigned formulas were for CHO containing chemicals followed by those containing CHOS. Low numbers of the CHO group of chemicals denote bacterial biomass production (Jonsson et al., 2007) and their rapid chromatographic elution as highly hydrophilic chemicals (due to the use of reversed-phase chromatography in analysis). Incorporation of sulphur in the CHO group can also take place under similar conditions as those for the CHNO group of chemicals, forming CHOS group derivatives,

Table 2
Characteristics of LMW-DOM based on the intensity weight average (wa) values in the water after the water treatment (pump-sump) and in the tanks with standard feed at day 10 and with RAS feed at day 25.

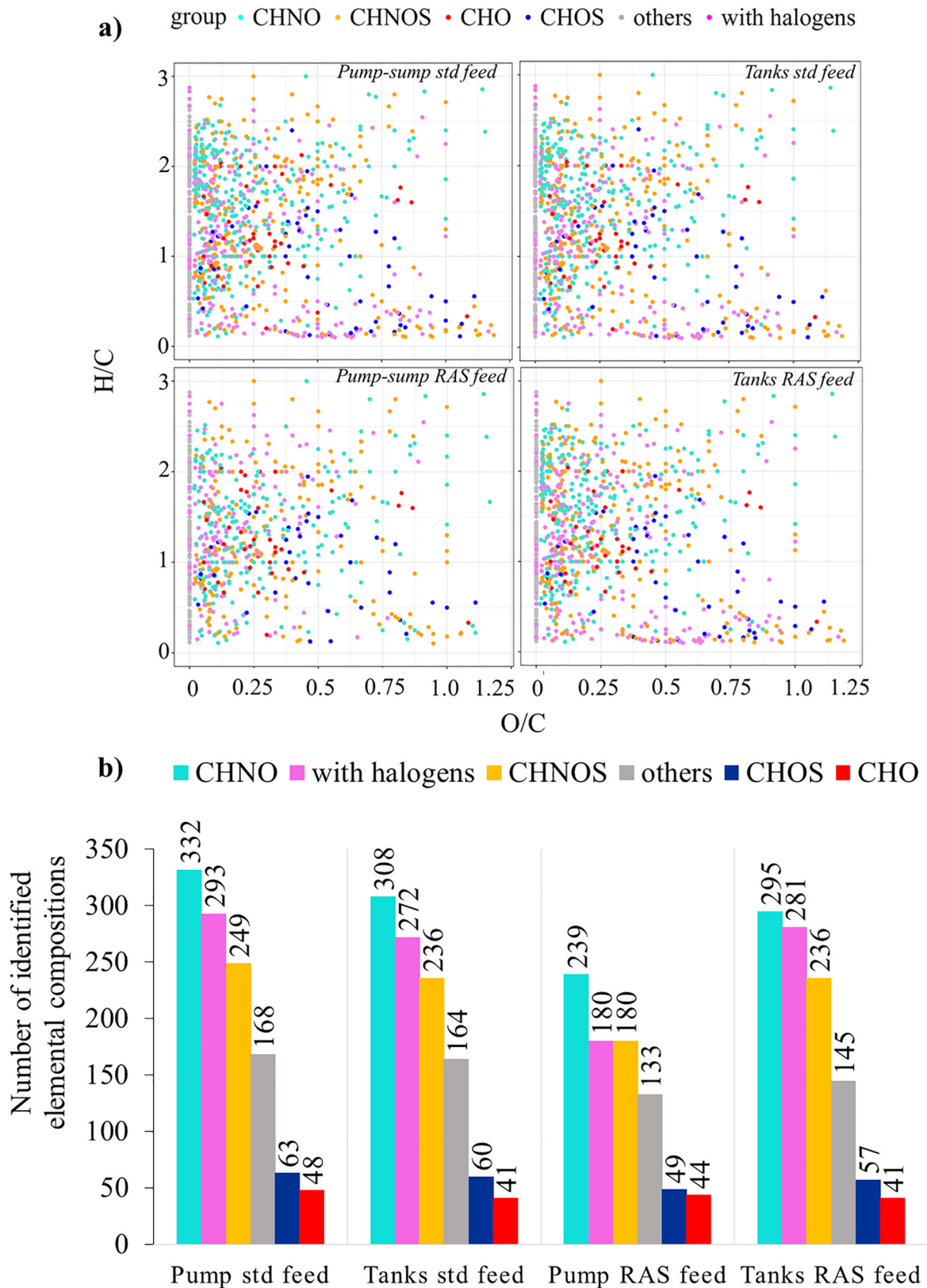|	Identified formulas	H/C$_{wa}$	O/C$_{wa}$	N/C$_{wa}$	S/C$_{wa}$	Cl/C$_{wa}$	Br/C$_{wa}$	m/z$_{wa}$	DBE/C$_{wa}$	DBE-O$_{wa}$	AI$_{wa}$
Pump std	1153	1.14	0.25	0.27	0.08	0.03	0.00	446.48	0.63	7.22	0.53
Tanks std	1081	1.13	0.27	0.26	0.09	0.03	0.00	447.05	0.63	6.83	0.55
Pump RAS	825	1.24	0.22	0.31	0.05	0.01	0.00	390.87	0.60	7.15	0.50
Tanks RAS	1055	1.08	0.23	0.28	0.08	0.04	0.00	468.13	0.66	8.35	0.57

Fig. 2. Comparison of LMW-DOM in the water after the water treatment processes (pump-sump) and in the tanks with standard feed at day 10 and with RAS based feed at day 25: a) Van Krevelen diagrams are depicted for the groups: CHNO, CHNOS, CHO, CHOS, others, and with halogens; and b) Number of identified elemental compositions are shown based on the chemical groups.

which can be also attributed to anthropogenic surfactants and compounds associated with seafood deterioration (Grigorakis et al., 2003; Kamjunke et al., 2019).

Further details provided by the Van Krevelen diagrams, which are related to the molecular characteristics of DOM from RAS water samples is presented in Table 2. In the Van Krevelen diagrams, a shift to higher H/C

ratios was observed in the pump-sump samples with RAS feed compared to the other samples. This indicated a higher aliphatic character in their chemical profile, which in turn was confirmed by the lowest values (expressed as wa) of DBE/C_{wa} (0.60) and AI_{mod} (0.50). In contrast, the decrease of the H/C ratios in the remaining samples showed a higher aromatic character in their chemical profile having the higher values for DBE/C_{wa}, $DBE-O_{wa}$ and AI_{mod}. The ratios of N/C_{wa} decreased from the pump-sump to the tanks. It is noteworthy that nitrogen was found in higher proportion in water samples with RAS feed (than those with standard feed), which can be further attributed to the type of feed used and its effect on the nitrification capacity of the water treatment processes (Eva, 2017). The ratios of S/C_{wa}, Cl/C_{wa} and Br/C_{wa} were consistent among the samples, except for the water samples from the pump-sump with RAS feed. The uncommon composition of DOM found in the pump-sump

samples can be attributed to the transformation of DOM during the water treatment processes in the presence of RAS feed.

3.3. Compositional changes of LMW-DOM in RAS samples with two types of fish feed

To gain a better insight on the availability and transformation of DOM between the water samples in RAS with standard and RAS feed, the differences on the average normalized abundance of the six chemical groups and major biochemical classes were represented in Fig. 3. In Fig. 3(a), abundant peaks were ascribed to the CHOS (13.5 to 27.4%) and CHO (11.9 to 25.6%) group of chemicals. The presence of electronegative groups such as hydroxyls, carboxylic acids and thionyls in the compound structure can make them more acidic increasing their ionization
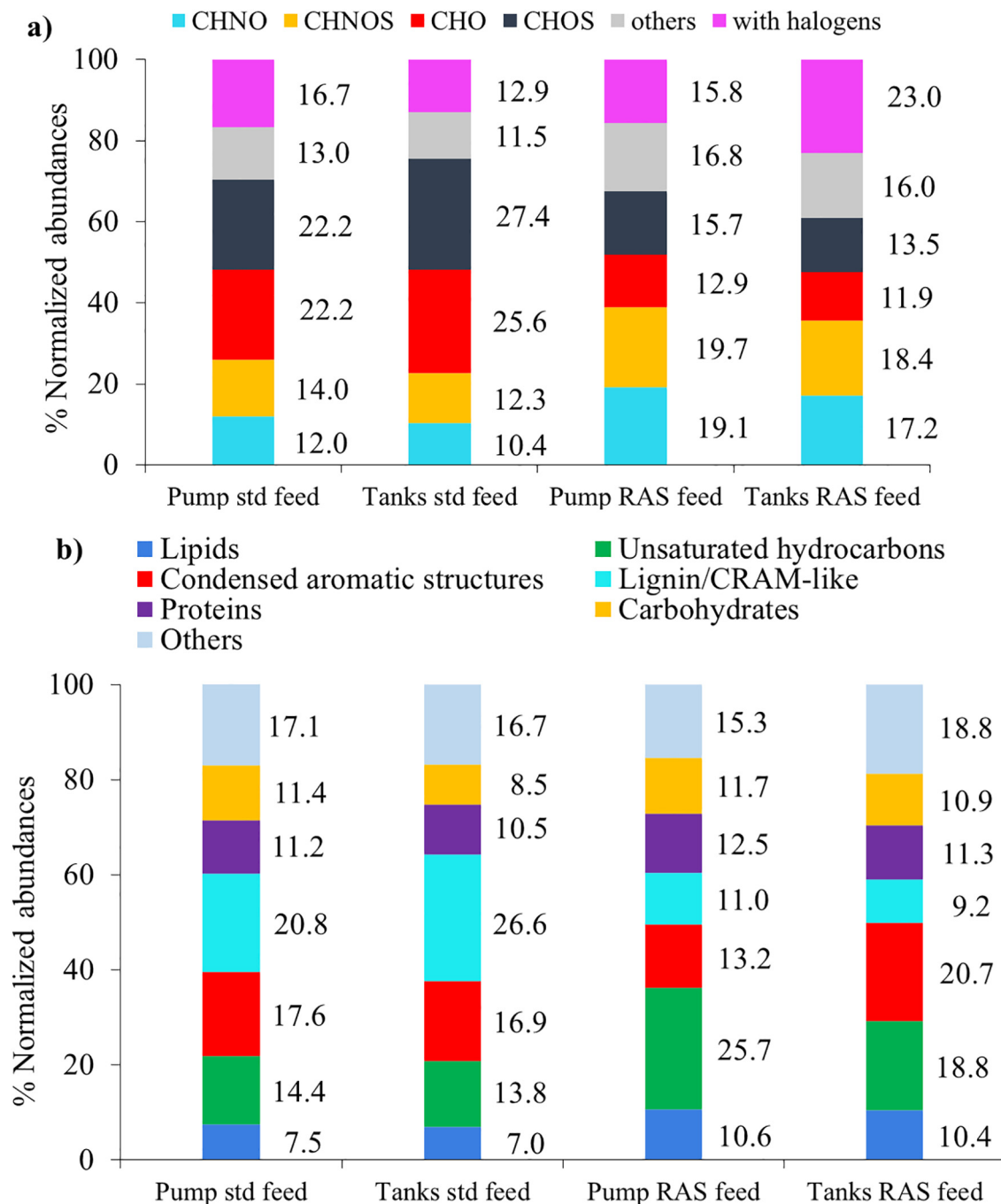

Fig. 3. Comparison of the normalized abundances of LMW-DOM (in percentages) in the water after the water treatment processes (pump-sump) and in the tanks with standard feed at day 10 and with RAS feed at day 25: a) Groups: CHNO, CHNOS, CHO, CHOS, others, and with halogens; and b) Major biochemical classes.

efficiencies under ESI (−), and consequently, their abundances in the samples (Schug and McNair, 2003). Although N-containing chemicals are easily ionizable under positive ESI (+), they can be highly suppressed under ESI (−) by mixtures of compounds with high ionization efficiencies, even more so when sulfonic acids are present in high abundance (Thurman et al., 2001; Gonsior et al., 2011). Under ESI (−), high abundant m/z peaks in the CHOS group were identified as O3S and O5S species in the samples with standard feed (Fig. S3), which can be associated to sulfonic acids, while explaining the decrease in abundances of the CHNO (10.4 to 19.1%) and CHNOS (12.3 to 19.7%) groups (Gonsior et al., 2011). The abundance of each biochemical class, based on its H/C and O/C ratios, differed between the water samples that contained a different type of feed (Fig. 3(b)). Even though in the Van Krevelen diagrams, the main portion of the identified groups of chemicals was found in the proteinic region (0.1 ≤ O/C ≤ 0.65; 1.5 ≤ H/C ≤ 2.3; N ≥ 1), differences were observed in their abundances (Fig. 3(a)). The transition from standard to RAS feed, resulted in an increase of lipids and unsaturated hydrocarbons by 3.30 and 8.13%, respectively, and a decrease of lignin/CRAM-like chemicals by 14%. Carbohydrates, condensed aromatic structures and proteins displayed similar abundances for both two feeds and all sample locations. Low percentages of carbohydrates can be associated with the high proportion of hydrophilic saccharides in DOM, which are not extractable by PPL columns (Dittmar et al., 2008), but also with the low ionization efficiency of the minor recoverable fraction of carbohydrates under ESI (−) (Raeke et al., 2016). Lignin/CRAM-like chemicals were the most abundant class found in the water with standard feed, while unsaturated hydrocarbons were dominant in the water with RAS feed. Despite the similar composition of nutrients in standard and RAS feed pellets, the DOM composition in RAS waters was evidently different depending on the type of feed used; this was visualized by the Bray-Curtis dissimilarity diagram, which was based on the presence and absence of m/z peaks in the samples (Fig. S4).

3.4. Removal of LMW-DOM after the water treatment processes

The transformation of LMW-DOM after the water treatment processes was studied through the exploration of homologous –CH_2- series in chemical compounds using KMD. Although no differences were encountered in the transformation of LMW-DOM in water with standard feed (Fig. S5), the removal of LMW-DOM was observed when RAS feed was used (Fig. 4). From the tanks to the pump-sump water, a high proportion of CHNO, CHNOS and halogenated chemicals with m/z > 600 were removed (Figs. 2(b) and 4). This observation was also noticed by a decrease in the m/z_{wa} value from the tanks to the pump-sump, when RAS based feed was used (Table 2). The removal of compounds with KMD > 0.7 can indicate an increased reduction of hydrogen-rich chemicals, which can further indicate less oxidation. The DOM transformation during the water treatment processes is shown in Fig. S6, where those chemicals that were removed, remaining and produced in the tanks and pump-sump with RAS feed were plotted in Van Krevelen H/C vs O/C and H/C vs m/z diagrams. Similar trends were found for the groups of CHNO, CHNOS and halogenated chemicals, where a high proportion of the removed chemicals were distributed in between the regions of 1.5–2.5 H/C, O/C < 0.6 and m/z > 600. By contrast, the produced chemicals were shifted to lower H/C ratios (1.0–2.0) and to lower masses (m/z < 600). The majority of the remaining groups of CHNO and halogenated chemicals were found distributed in between the ratios of 0.5 to 2.0 H/C and O/C < 0.5, while the CHNOS group chemicals were distributed broadly between the ratios of H/C and higher O/C (<0.8). These combined Van Krevelen and KMD results suggested that the type of feed used, not only altered the LMW-DOM composition, but also had an effect on its removal after the water treatment processes (pump-sump).

3.5. Benefits of UPLC-QTOF-MS in RAS

This study showed the great potential of non-targeted approaches using UPLC-QTOF-MS to qualitatively and semi-quantitatively determine a wide range of elemental compositions in LMW-DOM. Although it is not a quantitative analysis, this approach detected important changes in various LMW-DOM samples from different system locations and treatments. Moreover, it can provide the possibility of tracking a wide range of organic compounds using different libraries available in the Progenesis QI V2.3 software. Thus, the applicability of this technique
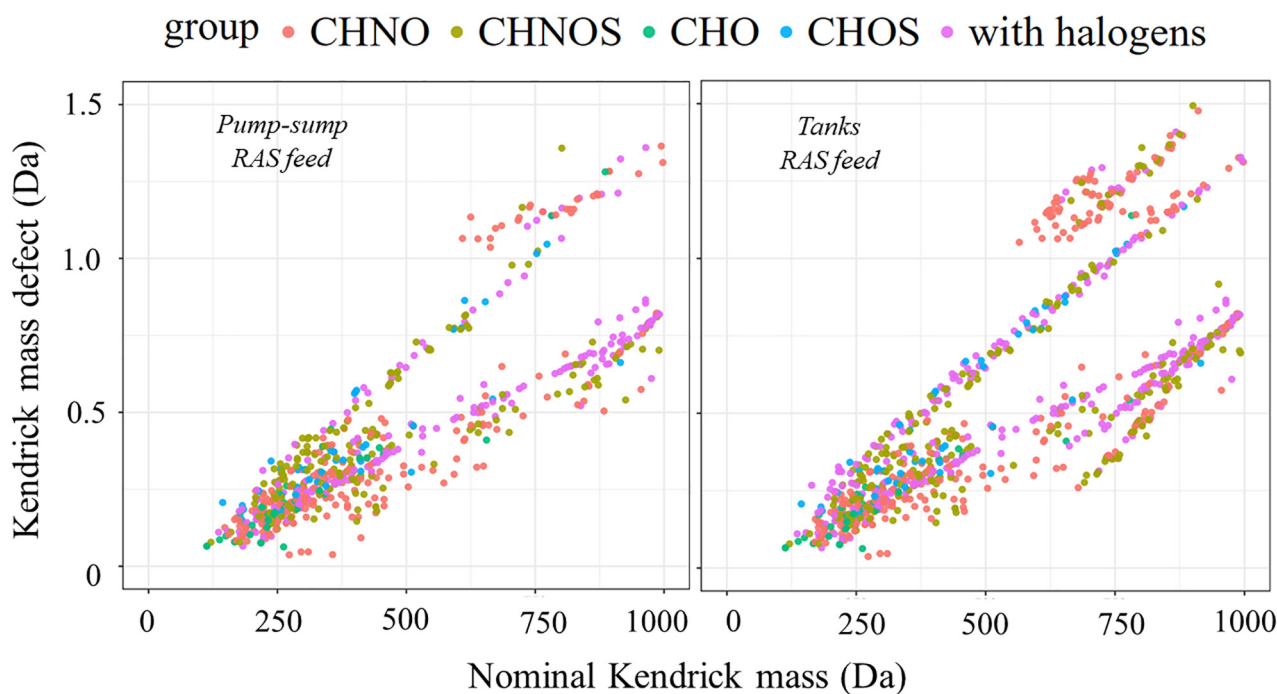

Fig. 4. Kendrick mass defect vs nominal Kendrick mass for the –CH_2- homologous series in the CHNO, CHNOS, CHO, CHOS and halogenated groups found in the water after the water treatment processes (pump-sump) and in the tanks exposed to RAS feed at day 25.

in aquaculture, especially in RAS, can give insights about the chemical composition of DOM, helping the operators to create the best conditions for fish welfare and system performance.

4. Conclusions

The non-targeted approach using UPLC-QTOF-MS revealed changes in the composition of LMW-DOM within RAS, but also between the two types of feed. In total, 1823 elemental compositions were identified and classified into various chemical groups, including CHNO, CHNOS, CHO, CHOS and halogens (Cl-, Br-containing chemicals). From those, the CHNO group of chemicals was found to be the largest. The Van Krevelen diagrams revealed that the chemicals found in water, demonstrated different origins (due to the variation in the H/C and O/C ratios) when investigated under two different types of feed. It was observed that the LMW-DOM fraction of the water was dominated by unsaturated hydrocarbons when the RAS feed was used, whereas lignin/CRAM-like chemicals became dominant when the standard feed was used. Although both experimental feeds were similar in their proximate composition, different abundances of chemical groups indicated changes in the LMW-DOM composition when the one feed type was replaced by the other. The most abundant chemicals in the water with standard feed were containing the groups of CHO and CHOS, while those in the water with RAS feed were containing in higher abundance the groups of CHNO and CHNOS. The molecular compositional change of the LMW-DOM in the water from the tanks compared to that in the water which was returned to the tanks after the water treatment processes (pump-sump), clearly reflected the removal of CHNO, CHNOS and halogenated chemical groups through the water treatment processes (when the RAS feed was used). This study shows that LC-QTOF-MS is a high-resolution alternative that can be used for non-target screening of DOM in RAS waters. The ability of this technique to track DOM changes at a molecular level make it useful in the successful operation of RAS. Future research on DOM transformation under different water treatment processes, salinity, fish species and treatment types could be performed with this technique, contributing to the improvement of fish productivity and welfare at a profitable cost.

CRediT authorship contribution statement

Patricia Aguilar-Alarcon: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing - original draft, Writing - review & editing. **Susana Villa Gonzalez:** Methodology, Resources, Software, Writing - review & editing. **Mads Adrian Simonsen:** Methodology, Data curation, Formal analysis, Writing - review & editing. **Ana R. Borrero-Santiago:** Investigation, Methodology, Supervision, Writing - review & editing. **Josep Sanchis:** Resources, Writing - review & editing. **Andre Meriac:** Conceptualization, Resources, Writing - review & editing. **Jelena Kolarevic:** Conceptualization, Resources, Writing - review & editing. **Alexandros G. Asimakopoulos:** Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing - review & editing. **Øyvind Mikkelsen:** Funding acquisition, Project administration, Resources, Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.142326.

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