# Effect of $CO_2$ on elemental concentrations in recirculating aquaculture system tanks

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ABSTRACT

High levels of carbon dioxide ($CO_2$) and subsequent changes in water quality parameters in recirculating aquaculture systems (RAS) are known to impair fish health, welfare and growth performance. A three-month trial was conducted in RAS tanks for Atlantic salmon (*Salmo salar*) post-smolt, where the effects of $CO_2$ on various water quality parameters (such as pH, redox potential, conductivity, and toxic elements) in brackish water were investigated. The experimental setup contained 18 fish tanks ($V = 0.5 \, \text{m}^3$) with six $CO_2$ treatments (5, 12, 19, 26, 33 and 40 mg $CO_2$ $L^{-1}$) in triplicate (46 post-smolts tank$^{-1}$). Initial fish body weight was $70 \pm 0.5$ g ($\pm$ SE), final body weight was $232 \pm 11$ g, and the average growth rate was 1.39% d$^{-1}$. Fish were fed continuously (approx. 23 h d$^{-1}$) over satiation (120–140%) during the experiment. Levels of most of the physico-chemical parameters, except for pH (7.65–6.74 for 5–40 mg $CO_2$ $L^{-1}$ treatments) and redox potential (181.73–195.67 mV for 5–40 mg $CO_2$ $L^{-1}$ treatments), did not differ between different $CO_2$ treatments. In total, 56 elements were measured, and 32 of these (such as Mg, S, Ca, Cd, Cu, Fe, Pb) were found to be above the limits of detection. None of the elements (except Fe) demonstrated any significant association with $CO_2$ or any of the physico-chemical variables in the current set-up. Fe concentrations were higher in high $CO_2$ treatments (13.0–13.6 µg L$^{-1}$ for 33–40 mg $CO_2$ $L^{-1}$) compared with the lower ones (8.7–10.1 µg L$^{-1}$ for 5–12 mg $CO_2$ $L^{-1}$), probably due to the differences in pH. Concentrations of dissolved Fe increased over the course of the study, possibly due to the reduced water exchange towards the end of the experiment. Overall, high $CO_2$ levels did not have any significant impact on the physico-chemical properties of water under the conditions of this study. The water exchange rate (39% of system water vol. d$^{-1}$) and unit processes appeared to produce good water quality because all elements measured herein were within safe recommended limits for salmonids.

## 1. Introduction

Aquaculture production has been growing fast over the last decades, and it is estimated that by 2050, aquaculture production will be most likely the main source of fish for human consumption (FAO, 2018). Recirculating aquaculture systems (RASs) are an eco-friendly way of fish farming by producing food while sustaining natural resources. In recent years, RAS has attracted substantial attention due to its distinctive features such as reduced water requirement (Verdegem et al., 2006), reduced waste effluent through the reclamation of nutrients (Piedrahita, 2003), reduced land needs (Martins et al., 2011), flexible location, controlled environment conditions (Liu et al., 2016) and

biosecurity (Zohar et al., 2005).

In RAS, carbon dioxide ($CO_2$) is a crucial parameter to control because long-term exposure to high levels of $CO_2$ can have detrimental consequences for fish, such as reduced growth performance (Smart et al., 1979; Fivelstad et al., 1998; Danley et al., 2005; Mota et al., 2019), hyperventilation (Smith and Jones, 1982), acidosis (Eddy et al., 1977; Ultsch, 1996) and ion mobilization from bones (Storset et al., 1997). Growth reduction appears to be inversely correlated with the $CO_2$ levels; moreover, Mota et al. (2019) have recently reported that the consequences of high $CO_2$ exposure at the post-smolt stage persist onto the later stages of the fish growth. In land-based Atlantic salmon smolt farms, $CO_2$ concentrations generally range from 10 to 25 mg L$^{-1}$ (pH

from 6.0 to 5.5, respectively) (Fivelstad et al., 1999; Terjesen et al., 2013; Gorle et al., 2018). The acceptable level of $CO_2$ for salmon in RAS is debatable; studies have recommended $20 \, \text{mg} \, \text{L}^{-1}$ $CO_2$ as the maximum level for salmonids (reviewed by Thorarensen and Farrell, 2011), whereas $15 \, \text{mg} \, \text{L}^{-1}$ has been provided as a guideline by Norwegian authorities (Norwegian Food Safety Authority, 2004). Nonetheless, levels as low as $10 \, \text{mg} \, \text{L}^{-1}$ have been suggested as a precautionary approach (Fivelstad et al., 1998). However, $CO_2$ can accumulate in intensive production aquaculture systems, exceeding the recommended concentrations (Martens et al., 2006), which can be harmful to fish (Ultsch, 1996; Fivelstad et al., 1999, 2003a).

In addition to $CO_2$ accumulation, the build-up of potentially harmful substances in RAS (Martins et al., 2011; Mota et al., 2014) could present a significant obstacle for species that require very clean water, such as salmonids (Davidson et al., 2011). Accumulating water quality parameters such as feed-related substances (e.g. heavy metals), fish metabolites (e.g. steroids) and system-produced products (e.g. bacteria and bacterial metabolites) are known to harm fish growth performance (Deviller et al., 2005; Mota et al., 2015). Among other accumulating water quality parameters, toxic elements (such as cadmium (Cd), copper (Cu), chromium (Cr), zinc (Zn) and lead (Pb)) are worthy of consideration in RAS. Sources of some toxic elements can be corrosion of pipes and fittings or elements added to the feed as part of the mineral/vitamin premix or from the make-up water (Colt, 2006; Davidson et al., 2009; Martins et al., 2009).

Inorganic elements can be both essential (such as iron (Fe), Cu, nickel (Ni), Zn and manganese (Mn)) and non-essential (such as Cd, Cr and Pb). Essential elements are vital for the normal cellular processes of fish, while non-essential elements can be toxic for fish because they may accumulate in their organs (Canli and Atli, 2003). Living organisms require only trace amounts of essential elements, and an increase in their concentrations can be toxic (Frieden, 1974; Tchounwou et al., 2012). Furthermore, non-essential elements can mimic essential elements and can inhibit several biochemical and physiological functions in fish (Klaassen et al., 2013). Besides, isolated $CO_2$ increase in a RAS can change the chemistry of the RAS water, for example through changes in pH/redox potential. High levels of $CO_2$ reduce pH levels simultaneously in aquatic systems (Fivelstad et al., 1998, 2003b), which encourages the solubility of toxic elements such as aluminium (Al), Cu, Cd and Zn, and increases the potential toxicity of these elements. It is, therefore, important to monitor water quality parameters in RAS for the welfare and health of fish.

Several studies have investigated the effects of raised $CO_2$ levels on fish growth performance and welfare (Ultsch, 1996; Fivelstad et al., 1999, 2003a, 2003b), however, not enough is known about the impact of raised $CO_2$ levels on different aspects of the water quality in RAS. The first objective of this study was to investigate the consequences of a wide range of $CO_2$ concentrations ($5–40 \, \text{mg} \, \text{L}^{-1}$) on various water quality parameters, such as pH, redox potential, conductivity and elemental concentrations in Atlantic salmon in RAS tanks. Several inorganic elements, such as heavy metals, are shown elsewhere (Martins et al., 2011; Mota et al., 2015) to accumulate in systems that reuse water. Whether these elements are present in an Atlantic salmon post-smolt RAS is unknown. Therefore, the second objective of this study was to characterise and quantify several inorganic elements ($N = 52$) in RAS tanks which could be used as a reference for future studies.

## 2. Material and methods

### 2.1. Experimental design and set-up

The detailed experimental design was reported by Mota et al. (2019). Briefly, 18 tanks ($V = 0.5 \, \text{m}^3$) were used as experimental units in a monofactorial design with six $CO_2$ treatments ($CO_2$ concentrations) in triplicate: 5, 12, 19, 26, 33 and $40 \, \text{mg} \, \text{L}^{-1}$ of $CO_2$ in 12 ppt salinity (Fig. 1). The total RAS water volume was $79 \, \text{m}^3$, water flow was

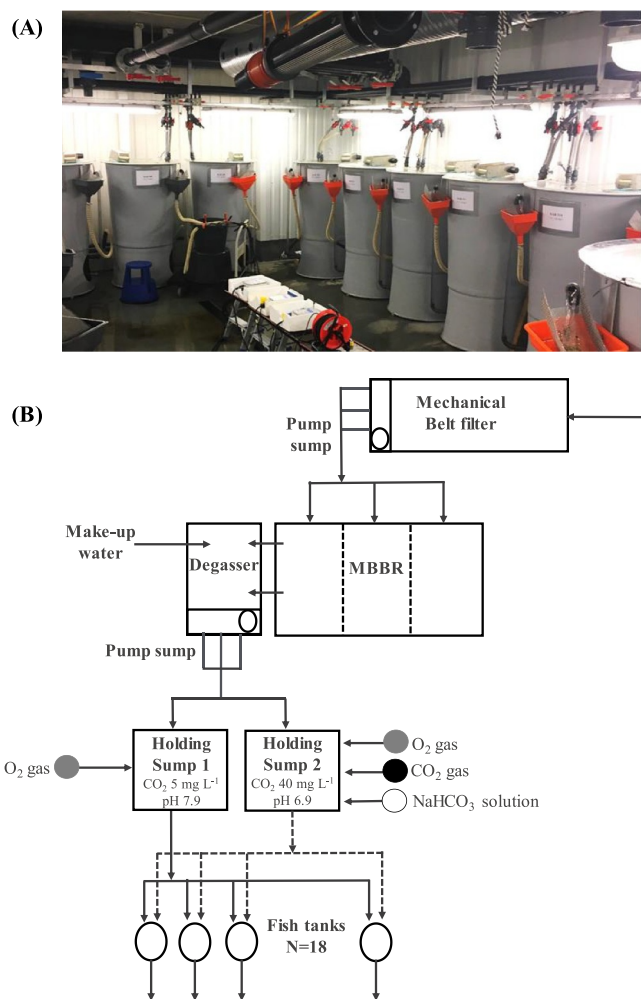**Fig. 1.** Overview of experimental design. (A) Fish tanks used for the experiment and (B) scheme of the experimental system used.

$11 \, \text{L} \, \text{min}^{-1}$ and the water exchange rate was approx. 39% of system water vol. $\text{d}^{-1}$ (Supp. Table 1). The system hydraulic retention time (HRT) was approximately 2.8 days, while HRT for the experimental fish tanks was 45 min. The make-up water (12 ppt salinity) was added from the top of the degasser column (Fig. 1).

The different $CO_2$ concentrations in each fish tank were obtained from the specific mix of two water inlets (Fig. 1): holding sump 1 ($CO_2 = 5 \, \text{mg} \, \text{L}^{-1}$) and holding sump 2 ($CO_2 = 40 \, \text{mg} \, \text{L}^{-1}$). Each holding sump ($V = 2.2 \, \text{m}^3$ each) had pure oxygen gas added through an oxygenator and a submerged pump to ensure good mixing of the water. Holding sump 2 had $CO_2$ gas added through an oxygenator from a pressurized $CO_2$-gas bottle. Acidification action of $CO_2$ in holding sump 2 was stabilized to pH 6.9 using $NaHCO_3$ solution ($50–75 \, \text{g} \, \text{L}^{-1}$). In the treatment tanks (5 to $40 \, \text{mg} \, CO_2 \, \text{L}^{-1}$), no $NaHCO_3$ solution was added and the decrease in pH levels was solely due to the $CO_2$ concentrations.

At the start of this experiment, each tank contained 46 Atlantic salmon post-smolt weighing $71 \, \text{g}$ ($\pm 0.5 \, \text{g}$). During the experiment, fish were fed continuously (approx. $23 \, \text{h} \, \text{d}^{-1}$) over satiation (120–140%) with a commercial diet (3–4 mm, Nutra Olympic, Skretting, Norway) using an automatic belt feeder. Final fish body weight was $232 \, \text{g}$ ($\pm 11 \, \text{g}$) and the average growth was $1.39\% \, \text{d}^{-1}$.

### 2.2. Water quality in fish tanks

Water quality in fish tanks was maintained within the recommendations for Atlantic salmon post-smolt (Thorarensen and

**Table 1**

General water quality parameters (mean values ± SE) measured during the experiment.

| Variables | Value | Method of analysis and sampling |
|---|---|---|
| Dissolved oxygen (% saturation) | 93.33 ± 0.42 | DO Handy Polaris, OxyGuard, Denmark |
| Temperature (°C) | 12.7 ± 0.02 | DO Handy Polaris, OxyGuard, Denmark |
| pH | See Fig. 2 | pH-electrode Sentix 980, WTW, Germany |
| Conductivity (mS cm$^{-1}$) | See Fig. 2 | IDS conductivity, TetraCon 925, WTW, Germany |
| Redox Potential (mV) | See Fig. 2 | WTW Multi-Parameters 3430 |
| Total ammonia nitrogen (TAN) (mg L$^{-1}$) | 0.13 ± 0.01 | Method HI 93700, C203 2008 photometer, Hanna Instruments, Quebec, Canada (U.S.EPA, 1983) |
| Turbidity (NTU) | 7.65 ± 0.26 | Turbiquant 1500 IR, Merck, Darmstadt, Germany (APHA, 1999) |
| Nitrite nitrogen (NO$_2$-N) (mg L$^{-1}$) | 0.030 ± 0.003 | Method HI 93707, C203 2008 photometer, Hanna Instruments, Quebec, Canada (U.S.EPA, 1983) |
| Nitrate nitrogen (NO$_3$-N) (mg L$^{-1}$) | 16.85 ± 0.15 | Method 350.1, Automated analyzer, Flow Solution IV, OI Analytical, College Station, TX, USA |
| Total Alkalinity (CaCO$_3$ mg L$^{-1}$) | 139.67 ± 7.72 | Method 8203, Digital titrator, Hach, USA. |
| Total suspended solids (TSS) (mg L$^{-1}$) | 9.78 ± 0.29 | Method 2540 D (TSS dried at 103–105 °C) (APHA, 2005) |

Farrell, 2011) (see Table 1 for details). On-line temperature, pH, O$_2$ saturation (%) and system flow measurements were recorded every 5 min as described by Mota et al. (2019). The CO$_2$ concentration was continuously monitored (every 1 min) by a CO$_2$ sensor (OxyGuard, Denmark) connected to an analogue unit (Pacific, OxyGuard, Denmark).

Temperature, pH, oxygen and salinity were measured inside the fish tanks due to operational reasons, while other water quality parameters were measured at each fish tank effluent. Samples for elemental analysis were collected in clean plastic bottles from each fish tank effluent on weeks 0, 2, 5, 9, 10, 11 and 12.

### 2.3. Elemental analysis

The water samples were filtered with 0.45 μm polyethersulfone membrane filters (VWR International, Norway) into polypropylene (PP) vials (VWR International, Norway) immediately after collection. After filtration, HNO$_3$ (5 M) was added to a final concentration of 0.1 M HNO$_3$ for the dissolution of elements in each sample. Samples were then stored at 4 °C until elemental analysis was performed.

Elemental composition was determined using a Thermo Scientific ELEMENT 2 high-resolution inductively coupled plasma mass spectrometer (ICP-MS, Thermo Fisher Scientific, USA) (Sørmo et al., 2011; Halbach et al., 2017). To assess possible contamination during sample preparation, blank samples of HNO$_3$ (prepared in-house using Milestone SubPUR sub-boiling distillation apparatus) and water (18.2 MΩ, MilliQ) were prepared using the same procedure as for the samples. Results were corrected for reagent blank values. The instrument was calibrated using 0.6 M HNO$_3$ solutions of matrix-matched multi-element standards and these standards were run after every 10 samples. A calibration curve was generated using five different concentrations made from multi-element standards. Method detection limits (MDL) were based either on three times the standard deviation of the blanks or on the instrument detection limits (IDLs). The IDLs were estimated from the subsequent analysis of solutions containing decreasing concentrations of the element. Finally, concentrations resulting in a relative standard deviation of < 25% ($n = 3$ scans) were selected as IDLs with baseline corrections applied for these values.

### 2.4. Data and statistical analysis

Data were tested for normality and homogeneity of variances (Shapiro–Wilk test and Levene test, respectively). Significant changes in elemental concentrations over the course of the study were determined using the Wilcoxon signed-rank test. Significant differences in physico-chemical parameters and elemental concentrations among different CO$_2$ treatments were determined using a Kruskal–Wallis test (with Mann–Whitney $U$ test for pairwise comparison). A two-way analysis of variance (ANOVA) (with Tukey post hoc test) was performed to test the impact of CO$_2$ and pH on Fe concentration in different CO$_2$ treatments. Spearman's correlation was performed to determine the correlation

between elements and physico-chemical parameters. Statistical analysis was performed with SPSS® V25 (IBM Corp., Armonk, NY, USA). All statistically significant differences quoted at $p < 0.05$. Standard error (SE) is presented as a measure of variability for all samples.

## 3. Results

### 3.1. Water quality

General water quality parameters measured in this study are presented in Table 1. Physico-chemical parameters (except redox potential and conductivity) measured during the course of this study were published by Mota et al. (2019). Most of the water quality parameters (except pH and redox potential) did not differ between different CO$_2$ treatments (see Mota et al., 2019 for details). Differences in pH and redox potential among CO$_2$ treatments were generally related to CO$_2$ concentrations (Fig. 2). Higher CO$_2$ concentration had lower pH ($p < 0.001$) and higher redox potential ($p < 0.001$) (Fig. 2). Redox potential ($\rho = 0.529$ at $p < 0.001$) was positively, while pH ($\rho = -0.943$ at $p < 0.001$) was negatively correlated with CO$_2$ concentrations.

### 3.2. Elemental concentrations

Of the 56 elements (including metals and metalloids) analysed, 32 were above the limits of detection. Elemental concentrations ranged from ng L$^{-1}$ to mg L$^{-1}$ (Table 2). Concentrations of 10 elements, namely, antimony (Sb), Cd, cerium (Ce), cesium (Cs), cobalt (Co), lanthanum (La), neodymium (Nd), Pb, scandium (Sc) and tungsten (W) were detected at < 1 μg L$^{-1}$ level. In contrast, 14 elements, namely, Al, arsenic (As), barium (Ba), Cu, Cr, Fe, lithium (Li), Mn, molybdenum (Mo), Ni, rubidium (Rb), uranium (U), vanadium (V) and Zn were measured at < 1 mg L$^{-1}$ level (Table 2). Among the eight macroelements measured in fish tank water, magnesium (Mg, 398.7 ± 0.9 mg L$^{-1}$) had the highest mean concentration ($p < 0.001$) followed by sulfur (S, 302.4 ± 0.9 mg L$^{-1}$), calcium (Ca, 136.4 ± 0.4 mg L$^{-1}$), potassium (K, 121.20 ± 0.38 mg L$^{-1}$), silicon (Si, 2.09 ± 0.02 mg L$^{-1}$), strontium (Sr, 2.42 ± 0.01 mg L$^{-1}$), boron (B, 1.390 mg L$^{-1}$ ± 0.004) and phosphorus (P, 0.76 mg L$^{-1}$ ± 0.01) in the decreasing order (as expected for seawater).

### 3.3. Effect of CO$_2$ on elemental concentrations

CO$_2$ concentration did not seem to have any impact on elemental concentrations because none of the elements detected (except Fe) in the water varied significantly among treatments under the conditions of this study (Table 2). In addition, none of the elements (except Fe) showed any significant correlation with CO$_2$ concentrations. Levels of all the elements in the fish tanks (with different CO$_2$ treatments) were almost the same as the levels measured in the inlets from the holding tanks (Table 2). However, within each treatment, some of the elements
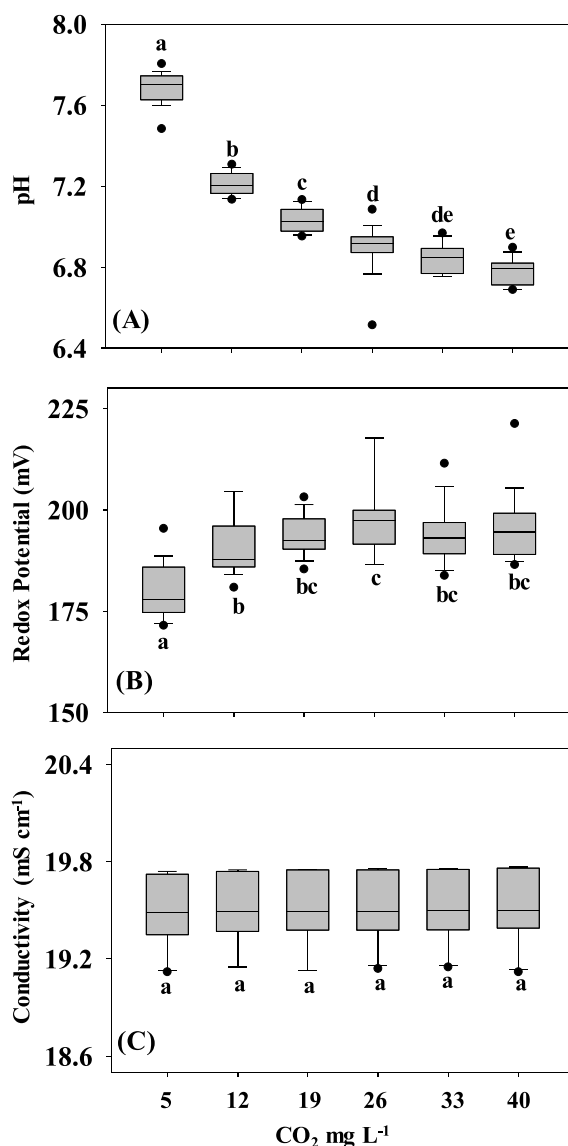
**Fig. 2.** Impact of $CO_2$ concentration on (A) pH, (B) redox potential (mV), and (C) conductivity (mS cm$^{-1}$). Boxes represent the median and 25–75 percentiles and bars represent minima and maxima. Significant differences ($p < 0.05$) among $CO_2$ treatments are shown by different letter codes (there is no significant difference between data with the same letter code).

showed a small but significant increase in concentrations ($p < 0.05$) over the course of the study (Table 3). Concentrations of Cd, Cu, Fe, Ni and V increased significantly ($p < 0.05$) in almost all $CO_2$ treatments (Table 3). In contrast to this, concentrations of Ce, Cs, W and Si decreased significantly ($p < 0.05$) towards the end of the experiment (Table 3).

### 3.4. The behaviour of dissolved Fe

The concentration of Fe was negatively correlated with pH ($\rho = -0.459$ at $p < 0.001$) and positively correlated with $CO_2$ ($\rho = 0.618$ at $p < 0.001$) and conductivity ($\rho = 0.449$ at $p < 0.001$). Fe concentrations were significantly different ($p < 0.001$) among different $CO_2$ treatments and increased significantly over the period of three months ($p < 0.001$) (Table 2, Fig. 3). Within each treatment, Fe concentration increased significantly ($p < 0.001$) towards the end of the experiment (Table 2, Fig. 3). Fe was correlated ($p < 0.05$) with some essential elements such as P, Co, Mo, V, Zn, Sr, Si, Ca and Li

(Table 2). Fe was also positively correlated ($p < 0.05$) with some highly toxic elements such as As, Cd and Cu (Table 2).

## 4. Discussion

The current study was a part of a three-month trial (Mota et al., 2019), which was conducted at the Nofima Centre for Recirculation in Aquaculture, Sunndalsøra, Norway (Terjesen et al., 2013). Mota et al. (2019) studied the impact of $CO_2$ concentrations on the growth performance, welfare and health of Atlantic salmon (*Salmo salar*), whereas this study aimed to investigate differences in water quality conditions in fish tanks with different $CO_2$ concentrations at short HRT. A single water line (make-up tank) was used to supply water to all experimental tanks. This set-up provided the same basal water to all fish tanks and kept the variation in physico-chemical parameters among the replicates to a minimum. In this study, all fish tanks (receiving different $CO_2$ concentrations) were part of the same system; hence the chemical effects of $CO_2$ on different water quality parameters were not studied at RAS level. Therefore, in future, studies are needed to evaluate the impact of $CO_2$ concentrations on water quality at system level.

Water quality is an important factor to consider in RAS because poor water quality can increase fish susceptibility to diseases. High levels of $CO_2$ not only affect the fish directly but also indirectly by changing the physico-chemical properties of water, such as pH and chemistry of toxic elements in an aquatic system (Fivelstad et al., 1998, 2003b). The toxicity of $CO_2$ to fish is greater at lower pH levels ($<$ pH 6.0), as is evident from freshwater studies (Alabaster and Lloyd, 1982). Low pH is reported to be toxic for salmonids (Randall and Lin, 1993); however, the pH levels in this study (6.8 to 7.7) were within the recommended pH levels for salmonids (Poxton and Allouse, 1982; Thorarensen and Farrell, 2011). There was a difference of ~1 pH unit between lowest (5 mg L$^{-1}$) and highest (40 mg L$^{-1}$) $CO_2$ treatments. An important element to consider in relation to $CO_2$ toxicity is alkalinity because the extent of the pH decrease after $CO_2$ accumulation in fish tanks is determined by alkalinity (Fivelstad, 2013). Recently, Good et al. (2018) highlighted the importance of alkalinity in buffering the pH and reducing the harmful effects of high $CO_2$ in RAS, such as elemental toxicity. In the present study, alkalinity was moderately high at around 140 mg L$^{-1}$ as $CaCO_3$ but was enough to keep the treatments within the pH range 6.8 to 7.7. Freshwater sources used in Norwegian RAS usually have low alkalinity, generally in the range of 1.5 to 10 mg L$^{-1}$ as $CaCO_3$ (Kristensen et al., 2009; Fivelstad, 2013; Good et al., 2018). Hence, the use of brackish water and efficient pH control through alkalinity dosing as used herein could be essential measures towards ensuring low elemental toxicity in post-smolt RAS with high $CO_2$ levels.

High $CO_2$ levels did not seem to have any significant impact on the elemental composition of the water in the current set-up because concentrations of elements (except Fe) neither varied among different $CO_2$ treatments nor did they show any significant correlation with $CO_2$. In addition, none of the elements (except Fe) correlated with pH, which further confirmed the independence of the elemental composition of water to $CO_2$. There was a small yet significant temporal increase in the concentration of some elements (like Co, Cu, Cr, Fe, Zn, P, Sr) within each treatment. When increases in the concentrations of these elements were compared with the make-up water (holding sumps 1 and 2), the levels were comparable in almost all cases. This was mainly because the water was changed more often in the first half of the experiment (Sup. Table 1); therefore, the increase in the concentrations of these elements could be due to the low water exchange towards the end of the experiment. In addition, fish feeds are often supplemented with minerals (such as Zn, Fe, Cu, Mn and P) and some trace elements (FAO, 2018). The increase in the concentrations of elements observed towards the end of the experiment could partly be attributed to the feed residues and to the excretion from fish in water. However, other feed minerals like selenium (Se) did not accumulate in the water.

Concentrations of almost all elements (except Ca, K and Mg) studied

**Table 2**
Average concentrations (mean ± SE) of elements measured for different $CO_2$ treatments. Elemental concentration for water from holding tanks ($CO_2$ concentrations for holding sump 1 and holding sump 2 are 5 and 40 mg L$^{-1}$ respectively) are given on the right side of table for comparison. Significant differences ($p < 0.05$) among $CO_2$ treatments are shown by different letter codes (there is no significant difference between data with same letter code). ρ shows Spearman's rho correlation coefficient between Fe and other elements in different $CO_2$ treatments (5, 12, 19, 26, 33 and 40 mg $CO_2$ L$^{-1}$), and values in bold are significant at *$p < 0.05$, **$p < 0.01$ or ***$p < 0.001$. *n* represents the number of samples used for analyses.

| | CO₂ Treatments (n = 18 for each treatment) | | | | | | ρ (n = 108) | Holding Sum 1 (n = 5) | Holding Sum 2 (n = 5) |
|---|---|---|---|---|---|---|---|---|---|
| | 5 mg L⁻¹ | 12 mg L⁻¹ | 19 mg L⁻¹ | 26 mg L⁻¹ | 33 mg L⁻¹ | 40 mg L⁻¹ | | | |
| | *Concentrations ng L⁻¹(ppt)* | | | | | | | *Concentrations ng L⁻¹(ppt)* | |
| Antimony | 70.56 ± 5.02 | 72.39 ± 3.35 | 72.17 ± 3.16 | 71.44 ± 3.36 | 75.61 ± 3.60 | 71.94 ± 3.71 | 0.087 | 85.2 ± 1.12 | 68.80 ± 2.44 |
| Cadmium | 24.11 ±1.53 | 23.89 ±1.55 | 25.56 ± 1.26 | 24.28 ± 1.58 | 24.14 ± 1.51 | 24.89 ± 1.82 | **0.576**\*\* | 23.21 ± 2.73 | 24.61 ± 3.14 |
| Cerium | 15.32 ±3.19 | 14.13 ±1.07 | 19.07 ±3.77 | 13.93 ± 1.00 | 17.63 ± 3.50 | 14.83 ± 1.56 | -0.139 | 11.78 ± 1.10 | 11.34 ± 0.75 |
| Cesium | 100.39 ±1.27 | 98.89 ± 1.29 | 101.11 ± 1.05 | 98.56 ± 1.23 | 100.40 ± 1.64 | 99.28 ± 1.05 | -0.111 | 94.80 ± 2.37 | 100.80 ± 2.44 |
| Cobalt | 54.83 ± 2.95 | 53.56 ±2.86 | 56.56 ±2.64 | 53.28 ± 3.05 | 51.47 ± 2.59 | 53.94 ± 2.91 | **0.510**\*\* | 56.80 ± 5.39 | 58.61 ± 4.76 |
| Lanthanum | 47.83 ± 3.08 | 46.61 ±2.88 | 51.22 ±3.47 | 46.61 ± 2.84 | 47.97 ± 2.98 | 47.61 ± 3.02 | -0.184 | 49.20 ± 4.75 | 44.41 ± 1.50 |
| Neodymium | 20.33 ± 0.90 | 18.61 ±0.88 | 18.61 ±0.77 | 19.83 ± 1.10 | 19.97 ± 1.01 | 18.56 ± 0.94 | -0.099 | 20.20 ± 0.86 | 16.40 ± 1.12 |
| Lead | 4.08 ± 1.32 | 2.62 ± 0.35 | 3.36 ± 0.48 | 2.57 ± 0.31 | 4.91 ± 1.41 | 3.72 ± 0.69 | 0.044 | 3.77 ± 0.45 | 2.84 ± 0.22 |
| Scandium | 23.76 ± 3.82 | 17.83 ±2.63 | 24.44 ±3.27 | 21.06 ± 2.72 | 21.69 ± 2.35 | 21.39 ± 1.93 | 0.091 | 26.27 ± 6.32 | 30.02 ± 5.20 |
| Tungsten | 10.06 ± 2.66 | 9.06±2.46 | 9.02 ±2.63 | 9.19 ± 2.14 | 8.91 ± 2.53 | 10.06 ± 2.55 | **-0.491**\*\* | 4.50 ± 1.47 | 5.38 ± 1.88 |
| | *Concentrations µg L⁻¹ (ppb)* | | | | | | | *Concentrations µg L⁻¹ (ppb)* | |
| Aluminium | 2.17 ± 0.51 | 1.90 ± 0.42 | 1.96 ± 0.42 | 2.69 ±0.69 | 2.57 ± 0.54 | 3.64 ± 1.04 | -0.029 | 1.28 ± 0.18 | 1.20 ± 0.22 |
| Arsenic | 0.92 ± 0.04 | 0.98 ± 0.05 | 0.98 ± 0.04 | 0.96 ± 0.05 | 0.97 ± 0.05 | 0.96 ± 0.04 | **0.594**\*\* | 1.10 ± 0.10 | 1.10 ± 0.13 |
| Barium | 6.22 ± 0.24 | 6.32 ± 0.24 | 6.17 ± 0.24 | 6.39 ± 0.28 | 6.27 ± 0.25 | 6.23 ± 0.27 | **-0.295**\*\* | 5.64 ± 0.08 | 5.94 ± 0.17 |
| Copper | 1.94 ± 0.06 | 1.95 ± 0.05 | 1.95 ± 0.05 | 1.94 ± 0.06 | 1.93 ± 0.06 | 1.96 ± 0.06 | **0.425**\*\* | 1.87 ± 0.11 | 1.94 ± 0.24 |
| Chromium | 0.34 ± 0.01 | 0.35 ± 0.02 | 0.35 ± 0.01 | 0.35 ± 0.01 | 2.26 ± 1.93 | 0.37 ± 0.04 | **0.251**\*\* | 0.35 ± 0.04 | 0.38 ± 0.04 |
| Iron | **8.71 ± 0.64**[a] | 10.14 ± 0.50[ab] | 11.39 ± 0.40[bc] | 12.06 ± 0.50[bcd] | 13.00 ± 0.49[cd] | 13.58 ± 0.21[d] | | 9.57 ± 0.94 | 14.57 ± 0.43 |
| Lithium | 50.47 ± 0.22 | 50.87 ± 0.28 | 50.58 ± 0.28 | 49.98 ± 0.26 | 50.22 ± 0.32 | 50.14 ± 0.27 | **-0.275**\* | 50.6 ± 0.50 | 51.06 ± 0.26 |
| Manganese | 0.89 ± 0.03 | 0.88 ± 0.03 | 0.90 ± 0.03 | 0.88 ±0.03 | 0.89 ± 0.02 | 0.89 ± 0.03 | 0.154 | 0.93 ± 0.02 | 0.85 ± 0.04 |
| Molybdenum | 4.63 ± 0.06 | 4.67 ± 0.07 | 4.62 ± 0.10 | 4.60 ± 0.05 | 4.55 ± 0.05 | 4.65 ± 0.05 | **0.314**\*\* | 4.57 ± 0.21 | 4.64 ± 0.14 |
| Nickel | 0.46 ± 0.02 | 0.47 ± 0.02 | 0.46 ± 0.02 | 0.46 ± 0.02 | 0.48 ± 0.03 | 0.46 ± 0.02 | **0.381**\*\* | 0.48 ± 0.027 | 0.49 ± 0.02 |
| Rubidium | 38.27 ± 0.19 | 38.69 ± 0.16 | 38.81 ± 0.53 | 38.39 ± 0.19 | 38.14 ± 0.22 | 38.46 ± 0.19 | 0.155 | 38.22 ± 0.46 | 38.50 ± 0.47 |
| Uranium | 0.98 ± 0.02 | 0.98 ±0.02 | 0.98 ± 0.02 | 0.97 ± 0.02 | 0.97 ± 0.02 | 0.95 ± 0.02 | -0.147 | 0.97 ± 0.02 | 0.95 ± 0.02 |
| Vanadium | 1.07 ± 0.03 | 1.05 ± 0.03 | 1.05 ± 0.03 | 1.05 ± 0.03 | 1.01 ± 0.03 | 1.02 ± 0.03 | **0.393**\*\* | 1.08 ± 0.06 | 1.06 ± 0.07 |
| Zinc | 7.01 ± 0.56 | 7.18 ± 0.40 | 7.49 ± 0.33 | 7.56 ± 0.51 | 7.53 ± 0.59 | 7.75 ± 0.57 | **0.660**\*\* | 7.28 ± 0.66 | 8.23 ± 0.89 |
| | *Concentrations mg L⁻¹ (ppm)* | | | | | | | *Concentrations mg L⁻¹ (ppm)* | |
| Boron | 1.40 ± 0.01 | 1.41 ± 0.01 | 1.40 ± 0.01 | 1.38 ± 0.01 | 1.39 ± 0.01 | 1.38 ± 0.01 | **-0.439**\*\* | 1.39 ± 0.02 | 1.340 ±14.4 |
| Calcium | 136.50 ± 0.98 | 136.60 ± 0.86 | 137.50 ± 1.43 | 136.50 ± 0.96 | 135.90 ± 0.73 | 135.20 ± 1.00 | **-0.362**\*\* | 135.60 ± 2.24 | 136.70 ± 2.81 |
| Magnesium | 398.00 ± 1.20 | 399.40 ±1.61 | 402.80 ± 3.90 | 3970.70 ± 1.98 | 397.31 ± 2.04 | 396.91 ± 1.73 | 0.072 | 399.91 ± 3.72 | 404.70 ± 3.97 |
| Phosphorus | 0.76 ± 0.03 | 0.77 ±0.03 | 0.77 ± 0.03 | 0.76 ± 0.03 | 0.76 ± 0.03 | 0.76 ± 0.03 | **0.500**\*\* | 0.77 ± 0.06 | 0.78 ± 0.06 |
| Potassium | 121.20 ± 0.70 | 121.16 ± 0.60 | 122.21 ± 1.56 | 120.85 ± 0.80 | 121.60 ± 0.77 | 120.30 ± 0.81 | -0.017 | 120.91 ± 1.99 | 122.80 ± 0.53 |
| Silicon | 2.08 ±0.06 | 2.10 ± 0.06 | 2.10 ± 0.05 | 2.08 ± 0.06 | 2.07 ± 0.06 | 2.09 ± 0.06 | **0.214**\* | 2.08 ± 0.15 | 2.08 ± 0.14 |
| Strontium | 2.41 ± 0.01 | 2.43 ± 0.01 | 2.50 ± 0.02 | 2.42 ± 0.02 | 2.40 ±0.01 | 2.42 ± 0.01 | **0.188**\*\* | 2.43 ± 0.03 | 2.46 ± 0.02 |
| Sulfur | 300.60 ± 1.43 | 303.80 ± 1.80 | 305.70 ± 3.10 | 303.1 ± 2.2 | 300.70 ± 1.7 | 300.60 ± 1.76 | **0.298**\*\* | 302.80 ± 5.6 | 304.80 ± 4.3 |

**Table 3**
Changes in elemental concentrations (Concentration first half of exp. - Concentration second half of exp., mean ± SE). Significant differences are represented by * ($p < 0.05$). Number of samples for $CO_2$ treatment is 6 and 12, whereas for holding sump 1 and 2 is 1 and 4 for first and second half of experiment respectively.

| | CO₂ Treatments | | | | | | Hold. Sum 1 | Hold. Sum 2 |
|---|---|---|---|---|---|---|---|---|
| | 5 mg L⁻¹ | 12 mg L⁻¹ | 19 mg L⁻¹ | 26 mg L⁻¹ | 33 mg L⁻¹ | 40 mg L⁻¹ | | |
| | *Concentrations ng L⁻¹(ppt)* | | | | | | *Concentrations ng L⁻¹(ppt)* | |
| Cadmium | 11.57 ± 1.88* | 11.66 ± 1.38* | 8.25 ± 2.26* | 10.58 ± 1.71* | 9.00 ± 2.06 | 11.55 ± 2.36* | 12.65 | 14.05 |
| Cerium | -12.40 ± 9.07 | -5.10 ± 2.36 | -20.94 ± 9.19* | -2.28 ± 2.03* | -13.34 ± 10.26 | -4.01 ± 3.84* | 0.36 | -0.86 |
| Cesium | -0.39 ± 2.78* | -1.19 ± 2.60* | -3.69 ± 2.41 | 0.57 ± 3.11 | -5.59 ± 4.27 | -4.41 ± 1.53* | 4.86 | 6.00 |
| Cobalt | 21.41 ± 3.71* | 15.34 ± 5.29 | 11.24 ± 4.65 | 20.41 ± 4.65* | 13.64 ± 3.68* | 10.32 ± 5.20 | 13.74 | 2.14 |
| Chromium | 48.46 ± 20.12* | 27.72 ± 29.62 | 43.91 ± 27.50 | 45.85 ± 20.17* | 32.68 ± 18.20* | 73.73 ± 55.19 | 75.27 | 103.99 |
| Tungsten | -18.71 ± 4.97* | -14.94 ± 5.20* | -17.10 ± 4.81* | -13.68 ± 4.08* | -15.49 ± 5.93* | -16.10 ± 5.31* | -6.78 | -8.76 |
| | *Concentrations µg L⁻¹ (ppb)* | | | | | | *Concentrations µg L⁻¹ (ppb)* | |
| Copper | 0.34 ± 0.08* | 0.33 ± 0.06* | 0.24 ± 0.09* | 0.39 ± 0.06* | 0.34 ± 0.07* | 0.34 ± 0.07 | 0.24 | 0.26 |
| Iron | 4.78 ± 0.53* | 4.03 ± 0.58* | 2.96 ± 0.45* | 3.44 ± 0.67* | 2.71 ± 0.48* | 2.54 ± 0.58* | 4.17 | 1.65 |
| Nickel | 0.10 ± 0.02* | 0.14 ± 0.02* | 0.07 ± 0.03* | 0.09 ± 0.03* | 0.14 ± 0.05* | 0.13 ± 0.03* | 0.13 | 0.09 |
| Vanadium | 0.22 ± 0.03* | 0.24 ± 0.03* | 0.23 ± 0.02* | 0.25 ± 0.02* | 0.24 ± 0.03* | 0.24 ± 0.03* | 0.24 | 0.30 |
| Zinc | 2.87± 0.50* | 2.46 ± 0.57* | 1.38 ± 0.68 | 3.38 ± 0.57* | 2.63 ± 0.91 | 3.21 ± 0.67* | 2.77 | 3.30 |
| | *Concentrations mg L⁻¹ (ppm)* | | | | | | *Concentrations mg L⁻¹ (ppm)* | |
| Phosphorus | 0.16 ± 0.04* | 0.17 ± 0.04* | 0.16 ± 0.03 | 0.16 ± 0.04* | 0.16 ± 0.04* | 0.16 ± 0.04 | 0.15 | 0.16 |
| Silicon | -0.06 ± 0.10 | -0.03 ± 0.09* | -0.06 ± 0.09* | -0.03 ± 0.09* | -0.06 ± 0.10* | -0.04 ± 0.10* | 0.02 | 0.02 |
| Strontium | 0.06 ± 0.03 | 0.08 ± 0.03* | 0.06 ± 0.04 | 0.09 ± 0.02* | 0.06 ± 0.02 | 0.05 ± 0.02 | 0.04 | 0.05 |

Negative sign (−) in front of concentration levels represents a decrease in concentration.
Elemental concentrations measured from holding sump 1 and 2 were not included in the statistical analysis due to limited number of samples.
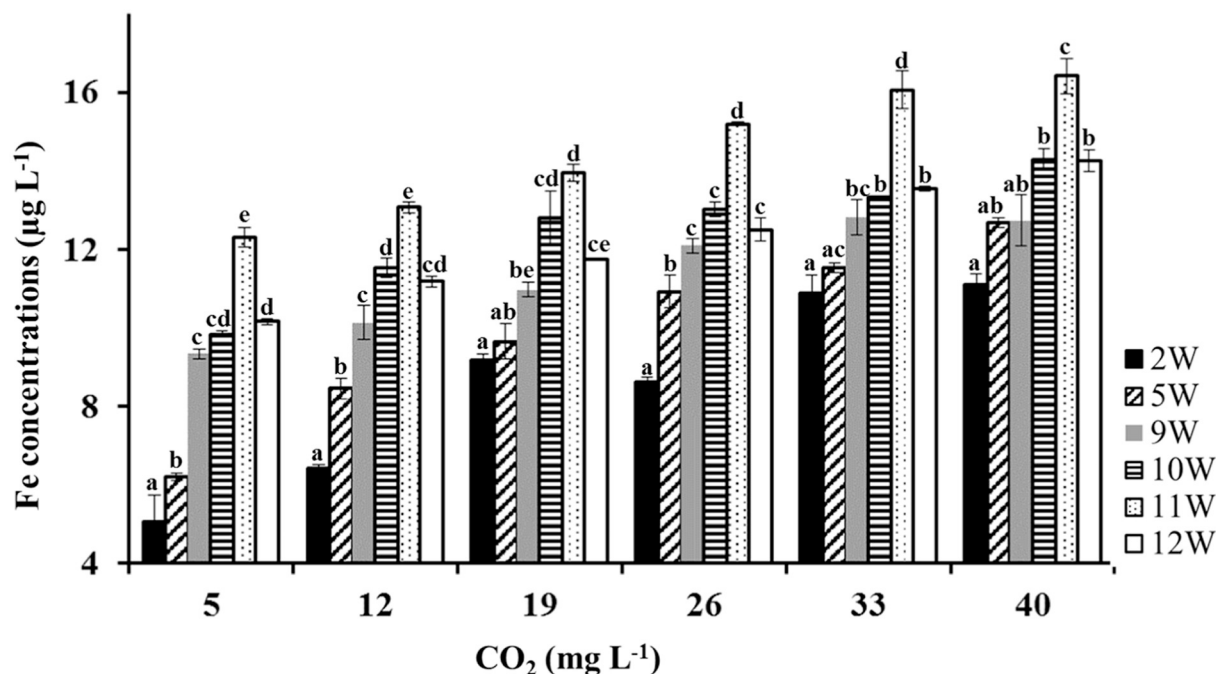
**Fig. 3.** Impact of $CO_2$ (subsequent pH drop) on dissolved Fe concentrations ($\mu g\,L^{-1}$) in fish tanks during the 12-week period. Significant differences ($p < 0.05$) among different sampling times for each $CO_2$ treatments are shown by different letter codes (there is no significant difference between data with the same letter code). Code: 2 W = sampling at 2 weeks, 5 W = sampling at 5 weeks, 9 W = sampling at 9 weeks, 10 W = sampling at 10 weeks and 11 W = sampling at 11 weeks and 12 W = sampling at 12 weeks.

**Table 4**

Comparison of elemental concentration (minimum – maximum, mg $L^{-1}$) measured in this study with maximum recommended concentrations for salmonid cultures as reported in literature (Davidson et al., 2009, 2011 references therein). BDL represent below detection limit. Detection limits ($\mu g\,L^{-1}$) for each element are given in the column on the right side of table.

| Elements | Concentrations (min. – max.) (mg $L^{-1}$) | Recommended limits (mg $L^{-1}$) | Detection limits ($\mu g\,L^{-1}$) |
|---|---|---|---|
| Aluminum | 0.00 - 0.015 | 0.01 - 1.00 | 0.2 |
| Arsenic | 0.001 - 0.0014 | 0.05 - 0.40 | 0.025 |
| Barium | 0.005 - 0.009 | <5 | 0.013 |
| Beryllium | BDL | 0.01 1.10 | 0.01 |
| Boron | 1.31 - 1.49 | <5 | 0.08 |
| Cadmium | 0.00001 - 0.00004 | 0.0003 - 0.0700 | 0.02 |
| Calcium | 126.35 - 154.68 | 4 - 160+ | 2.0 |
| Chromium | BDL | 0.03 - 0.10 | 0.005 |
| Cobalt | 0.00003 - 0.00008 | 0.010 - 0.05 | 0.004 |
| Copper | 0.0016 - 0.0024 | 0.006 - 0.070 | 0.03 |
| Iron | 0.004 - 0.017 | 0.1 - 1.1 | 0.02 |
| Lead | BDL | 0.01 - 4.0 | 0.002 |
| Magnesium | 377.69 - 458.77 | 15–28+ | 0.10 |
| Manganese | 0.0006 - 0.0011 | 0.05 - 1.00 | 0.006 |
| Mercury | BDL | 0.0001 - 0.0020 | 0.001 |
| Molybdenum | 0.004 - 0.006 | 8+ | 0.02 |
| Nickel | 0.0003 - 0.0008 | 0.01 - 0.40 | 0.015 |
| Phosphorous | 0.632 - 0.992 | 3+ | 0.4 |
| Potassium | 111.29 - 142.55 | 5 - 10+ | 5.0 |
| Selenium | BDL | 0.005 - 0.020 | 0.15 |
| Silver | BDL | <0.003 | 0.02 |
| Sulfur | 281.63 - 345.06 | NA | 10.0 |
| Uranium | 0.0008 - 0.0011 | <0.1 | 0.0003 |
| Vanadium | 0.0008 - 0.0013 | 0.1 | 0.0003 |
| Zinc | 0.0038 - 0.015 | 0.005 - 0.269 | 0.025 |

here were considerably below the recommended limits for salmonids (Davidson et al., 2009 and references therein, Table 4). It is important to note that the recommended levels used to compare the elemental levels in this study are from freshwater studies, whereas brackish water was used for the current study. This explains the higher level of macroelements (such as Mg, S, and K) observed in this study. Further, safe levels of such elements in brackish water would normally be higher than in freshwater. It should also be noted that only dissolved concentrations of elements were measured herein. It is likely that some of the elements were additionally present in the system as particles (hydroxides, carbonates etc.) or bound to particulate organic matter and, therefore, were not measured. However, toxic elements in the bound phase are less harmful to fish compared with their free forms.

Fe is an essential element involved in oxygen transport, lipid oxidation and cellular respiration, however, excessive dietary Fe is known to have lethal effects on fish health (Salte et al., 1994). In this study, Fe was the only element that was significantly different among $CO_2$ treatments. Concentrations of Fe were directly related to the $CO_2$ concentrations; higher concentrations of Fe were measured in water with high $CO_2$ concentrations. When the concentrations of Fe in fish tanks supplied with 5 and 40 mg $CO_2\,L^{-1}$ were compared with the water inlets (holding sump 1 and holding sump 2 containing 5 and 40 mg $CO_2$ $L^{-1}$, respectively), their levels were almost the same. It appeared that concentrations of Fe measured in different $CO_2$ treatments were just the result of mixing waters from these two water inlets (see Section 2.1 for details) and no other processes affected Fe concentrations in fish tanks with different $CO_2$ concentrations. In addition, Fe content was also tested in $NaHCO_3$ buffer that was used for adjusting the pH, and was found to be in a negligible amount. The strong association of Fe with pH indicates that Fe in the make-up water was potentially bound to organic matter or particles and was released in water when the pH dropped.

In water, Fe may be present in different forms, ranging from simple divalent ($Fe^{2+}$) and trivalent ($Fe^{3+}$) ions, complexes (such as $OH^-$, $SO_4^{2-}$, $Cl^-$) or bound to organic (humic) or inorganic (clay) colloids and particles. The speciation of Fe is dependent on the pH and redox potential. Under acidic conditions (pH < 7) $Fe^{2+}$ is the dominant species (as $Fe(OH)_2$), whereas $Fe^{3+}$ dominates (as $Fe(OH)_3$) under alkaline conditions (pH > 7). $Fe^{2+}$ is the most toxic species of iron to marine organisms (Vuori, 1995), therefore, Fe uptake in a high $CO_2$ environment (low pH) can be harmful to fish because of the greater

proportion of $Fe^{2+}$ compared with the other chemical species of Fe. Other toxic elements like Cd, Co, As, Cu, Mn, Mo and Zn are known to exhibit speciation behaviour similar to Fe (Stumm and Morgan, 2012). This is corroborated by the positive correlation of these elements with Fe. However, Fe concentration was below the recommended levels for salmonids, therefore, the risk of compromising fish welfare was quite low in this case.

Water exchange rate plays a very important role in RAS because low exchange rate RAS can accumulate potentially harmful levels of toxic elements (Martins et al., 2009, 2010, 2011), which can affect the fish health (Davidson et al., 2009; Martins et al., 2009). The concentrations of trace elements (As, Al, Cd, Cr, Cu, Mn, Ni, Pb and Zn) measured in this study were generally lower than those reported by other RAS studies (freshwater) conducted at comparable exchange rates (Martins et al., 2009, 2010, 2011). It should be noted that the make-up flowrate in this study was higher (39% of system water vol. $d^{-1}$) than in a more intensive RAS due to the need for cooling to the low temperature required for optimal fish welfare of Atlantic salmon (Terjesen et al., 2013). This suggests that elements may accumulate to higher levels in commercial RAS facilities, which typically use lower water exchange rates than this study. The current study provides strong evidence that in brackish water RAS operated at high exchange rates, harmful substances, specifically toxic elements, do not accumulate to a level that can compromise fish welfare.

In recirculating systems, water treatment processes and culture tank hydraulics are of key importance for providing safe water quality for fish (Gorle et al., 2018). RAS systems operating with shorter ($< 50$ min) tank HRTs tend to have better water quality due to the efficient flushing of waste metabolites (Summerfelt et al., 2016; Gorle et al., 2018). Although the tank HRT (45 min) was too brief to allow significant accumulation of toxic elements in fish tanks in this study, still, it was long enough to support chemical changes in fish tank water. Chemical changes such as elemental speciation (van Leeuwen et al., 2005), complexation with ligands (Lin et al., 1995) and release from ligand complexes/sediments (Li et al., 2013) take place in a very short period of time (minutes or less). A significant increase in concentrations of dissolved Fe in response to pH drop (high $CO_2$ treatments) measured here is reflective of this. Solubility subsequently affects the bioavailability of toxic elements, and bioavailable forms of toxic elements are readily consumed (within minutes) by micro- and macrobiota in aquatic systems (van Leeuwen et al., 2005).

This is the first ever study where chemical effects of $CO_2$ exposure on parameters other than pH and $CO_2$ itself are investigated in water recirculating system. Our results indicate that $CO_2$ does not have a significant impact on the physico-chemical properties of water under the conditions of our study, and shows that the concentrations of dissolved elements (except Fe) are independent of the $CO_2$ levels. Although the levels of dissolved Fe correlated with $CO_2$ levels (due to pH change), nevertheless Fe concentrations did not increase to the levels that could pose a detrimental effect on fish. Furthermore, the present study investigated the concentrations of several toxic elements that to our knowledge have not been reported by other studies for Atlantic salmon brackish water RAS. It is well known that fish can accumulate toxic elements thousands or millions of times greater than the water they live in, therefore, detailed elemental analysis of fish is also required to confirm the toxicity of current levels of elements on fish.

## Author contributions

Ø.M., V.C.M. and B.F.T. designed the project. G.R.B and V.C.M. conducted the experiments. S.N.A. analysed the data, and S.N.A., S.N., V.C.M and Ø.M. wrote the paper.

## Declaration of Competing Interest

Authors certify that there is no conflict of interest with any financial or nonfinancial organisation regarding the subject matter or materials discussed in this manuscript.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2019.734254.

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