# Effects of alkalinity on ammonia removal, carbon dioxide stripping, and system pH in semi-commercial scale water recirculating aquaculture systems operated with moving bed bioreactors

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## ARTICLE INFO

## ABSTRACT

When operating water recirculating systems (RAS) with high make-up water flushing rates in locations that have low alkalinity in the raw water, such as Norway, knowledge about the required RAS alkalinity concentration is important. Flushing RAS with make-up water containing low alkalinity washes out valuable base added to the RAS (as bicarbonate, hydroxide, or carbonate), which increases farm operating costs when high alkalinity concentrations are maintained; however, alkalinity must not be so low that it interferes with nitrification or pH stability. For these reasons, a study was designed to evaluate the effects of alkalinity on biofilter performance, and $CO_2$ stripping during cascade aeration, within two replicate semi-commercial scale Atlantic salmon smolt RAS operated with moving bed biological filters. Alkalinity treatments of nominal 10, 70, and 200 mg/L as $CaCO_3$ were maintained using a pH controller and chemical dosing pumps supplying sodium bicarbonate ($NaHCO_3$). Each of the three treatments was replicated three times in each RAS. Both RAS were operated at each treatment level for 2 weeks; water quality sampling was conducted at the end of the second week. A constant feeding of 23 kg/day/RAS was provided every 1–2 h, and continuous lighting, which minimized diurnal fluctuations in water quality. RAS hydraulic retention time and water temperature were 4.3 days and $12.5 \pm 0.5\,^\circ$C, respectively, typical of smolt production RAS in Norway.

It was found that a low nominal alkalinity (10 mg/L as $CaCO_3$) led to a significantly higher steady-state TAN concentration, compared to when 70 or 200 mg/L alkalinity was used. The mean areal nitrification rate was higher at the lowest alkalinity; however, the mean TAN removal efficiency across the MBBR was not significantly affected by alkalinity treatment. The $CO_2$ stripping efficiency showed only a tendency towards higher efficiency at the lowest alkalinity. In contrast, the relative fraction of total inorganic carbon that was removed from the RAS during $CO_2$ stripping was much higher at a low alkalinity (10 mg/L) compared to the higher alkalinities (70 and 200 mg/L as $CaCO_3$). Despite this, when calculating the total loss of inorganic carbon from RAS, it was found that the daily loss was about equal at 10, and 70 mg/L, whereas it was highest at 200 mg/L alkalinity. pH recordings demonstrated that the 10 mg/L alkalinity treatment resulted in the lowest system pH, the largest increase in [H+] across the fish culture tanks, as well as giving little response time in case of alkalinity dosing malfunction. Rapid pH changes under the relatively acidic conditions at 10 mg/L alkalinity may ultimately create fish health issues due to e.g. $CO_2$ or if aluminium or other metals are present. In conclusion, Atlantic salmon smolt producers using soft water make-up sources should aim for 70 mg/L alkalinity considering the relatively low loss of inorganic carbon compared to 200 mg/L alkalinity, and the increased pH stability as well as reduced TAN concentration, compared to lower alkalinity concentrations.

## 1. Introduction

Water recirculating aquaculture systems (RAS) are increasingly used to produce Atlantic salmon smolt (Bergheim et al., 2009; Dalsgaard et al., 2013; Kolarevic et al., 2014). These systems are often intensive, operating with low system flushing rates, high stocking densities, pure oxygen supplementation, biofiltration to remove ammonia, and various forms of aeration to remove dissolved carbon dioxide ($CO_2$). Pure oxygen supplementation is used to support higher feed loads and increased fish production in a given RAS, but this also create conditions where elevated levels of dissolved $CO_2$ can accumulate if inadequate air-to-water contacting is not provided (Summerfelt et al., 2000). Fish can sense and will avoid areas of high dissolved $CO_2$ (Clingerman et al., 2007), when possible. However, chronic exposure to elevated concentrations of dissolved $CO_2$ has been associated with reduced growth (Danley et al., 2005; Fivelstad et al., 2007), reduced condition factor (Fivelstad et al., 1998, 2003a, 2003b), and nephrocalcinosis (Landolt, 1975; Fivelstad et al., 1999; Hosfeld et al., 2008) in salmonids. In addition, free acid produced during nitrification reacts with bicarbonate alkalinity in the water releasing more carbon dioxide than the autotrophic nitrifying bacteria consume (U.S. EPA, 1975). The conversion of total ammonia nitrogen (TAN) to nitrate nitrogen ($NO_3$–N) with nitrifying bacteria consumes approximately 0.15–0.19 kg sodium bicarbonate ($NaHCO_3$) for every 1 kg of feed consumed by the fish (Davidson et al., 2011). And, if this alkalinity loss is not compensated for by supplementation with a base (such as sodium hydroxide or $NaHCO_3$), the alkalinity and pH of the system will decrease (Loyless and Malone, 1997). The loss of alkalinity and the increase of dissolved $CO_2$ are both conditions that reduce the pH of the recirculating water according to acid–base equilibrium of the carbonate system (e.g. Loyless and Malone, 1997; Colt, 2006). As stocking density and system hydraulic retention time in RAS have increased in recent years, application of technologies to control alkalinity, pH, and dissolved $CO_2$ have become significantly more important. Preventing large drops in pH can be critical to prevent solubilizing metals, such as aluminium, because of their toxic effect on fish (Skogheim and Rosseland, 1986; Fivelstad et al., 2003b).

Carbon dioxide is excreted (along with ammonia) through the fish's gills in proportion to its feed and oxygen consumption rate. A considerable amount of dissolved $CO_2$ is also produced in the biofilter (Summerfelt and Sharrer, 2004). Controlling dissolved $CO_2$ from accumulating to detrimental levels is particularly important in fish farms that use intensive water recycling systems. These systems use oxygenation units to create high levels of available dissolved oxygen in the culture tanks, but oxygenation units provide insufficient gas exchange to strip much dissolved $CO_2$. In addition, the concentration of dissolved $CO_2$ produced within the culture tank can be quite large when pure oxygenation is used, with up 8–12 mg/L of dissolved $CO_2$ produced in a single pass at high stocking densities. Dissolved $CO_2$ is stripped from the recirculating water, typically after the biofilter and before the oxygenation process (Summerfelt et al., 2000; Summerfelt and Sharrer, 2004). Dissolved $CO_2$ stripping is based on the principle that the partial pressure of $CO_2$ in air contacted with water is less than the partial pressure of the $CO_2$ dissolved in the water. The dissolved $CO_2$ therefore comes out of solution and is stripped off as a gas. Increasing the volume of air flow that is contacted with the water flow will increase the dissolved $CO_2$ that can be removed.

Maintaining adequate alkalinity concentrations has been reported to be critical for sustaining nitrification, i.e., the wastewater literature reports that 40–80 mg/L (as $CaCO_3$) is the minimum alkalinity required to support nitrification (Paz, 1984; Biesterfeld et al., 2003). Villaverde et al. (1997) reported a linear increase in nitrification efficiency of 13% per unit pH increase from pH

5.0 to 8.5. Rusten et al. (2006) found that the nitrification rate dropped to only half of the original rate when alkalinity dropped from approximately 115 mg/L as $CaCO_3$ (pH 7.3) to 57 mg/L (pH 6.7) in a bench-scale experiment performed using biofilm carriers collected from a turbot farm's moving bed biological reactor (MBBR). Moreover, Colt (2006) warns that the nitrification process slows down at low pH and Chen et al. (2006) recommend maintaining an alkalinity of 200 mg/L as $CaCO_3$ to support nitrification when water exchange rate is minimal. RAS operated at suboptimal alkalinity could theoretically encounter larger pH swings, higher concentrations of TAN and $NO_2$–N if nitrification efficiency drops, and microbial community instability (Mydland et al., 2010), which may be harmful to the fish. However, the consequences of operating a RAS without adequate alkalinity have been little studied, particularly for systems used to produce Atlantic salmon. This is a species which is sensitive to elevated concentrations of nitrite nitrogen without concurrent chloride adjustments (Gutierrez et al., 2011), to relatively low levels of $NH_3$–N (Kolarevic et al., 2013), and $CO_2$ (Fivelstad, 2013). Research is needed to determine if maintaining an alkalinity of 80–200 mg/L as $CaCO_3$ is really beneficial, because operating at high alkalinity levels will increase the cost of supplementation with base. Interestingly, high nitrification rates at low pH and alkalinity have been reported previously in laboratory scale reactor experiments (Tarre and Green, 2004). When operating RAS with high make-up water flushing rates in locations such as Norway that have low alkalinity in the raw water (Kristensen et al., 2009), knowledge about required RAS alkalinity will be particularly important. Furthermore, since it has recently been proposed that larger tank scales increase performance in salmon (Espmark et al., 2013), we wanted to study effects of alkalinity in larger scale RAS. For these reasons, a study was designed to evaluate the effects of alkalinity on $CO_2$ stripping during cascade aeration, plus biofilter performance within salmon smolt semi-commercial scale RAS operated with moving bed biological filters. The goal of the study was to test the following hypotheses, that increasing alkalinity concentrations from 10 to 200 mg/L in a RAS will (1) stabilize system pH, (2) decrease $NO_2$–N and TAN concentrations and variability, and (3) increase TAN removal efficiency and removal rate across the MBBR. Finally, we hypothesize that (4) a higher alkalinity will decrease $CO_2$ removal efficiency, and increase total inorganic carbon (TIC) removal, across forced-ventilation cascade degassers and thus lead to elevated costs associated with bicarbonate dosing.

## 2. Materials and methods

The studies were conducted at the Nofima Centre for Recirculation in Aquaculture at Sunndalsøra, Norway, described in Terjesen et al. (2013).

### 2.1. Experimental treatments

Alkalinity of the recirculating water was maintained at three treatment levels, i.e., at nominal 10, 70, and 200 mg/L as $CaCO_3$, using an online pH electrode (Sensorex 8000CD-pH with solution ground and amplifier, Golden Grove, USA) located in the sump at the base of the $CO_2$ stripping column. Each pH probe was equipped with an automatic cleaning system; a water jet programmed to flush the probe each tenth minute (Storvik Aqua, Sunndalsøra, Norway). This automatic cleaning was found to be clearly necessary to maintain stable pH during the 14 days of each treatment replicate (Kolarević et al., 2011). The pH probes were calibrated using Merck two-point buffers (Merck, Darmnstadt, Germany), each week, right after a treatment period ended, and after seven days into each treatment. A dedicated pH controller (Model WPD 320, Walchem, Holliston, MA) was used to control a chemical dosing pump (Model

EHE, Iwaki, Holliston, MA) supplying sodium bicarbonate (NaHCO$_3$) to maintain a pH set-point in each RAS. Ground well water at Nofima in Sunndalsøra contains around 6–20 mg/L as CaCO$_3$ of alkalinity, depending on season and the pump well in use (Terjesen et al., 2013).

Thus, a pH control system was used to control the treatment alkalinity because, according to acid–base equilibrium in freshwater with low ammonia levels, water pH is an approximate measure of the alkalinity when the water temperature and dissolved CO$_2$ concentration remain constant (Summerfelt et al., 2001). Changes in dissolved CO$_2$ do not affect alkalinity, per the definition, but do affect the water pH. Thus, stripping dissolved CO$_2$ increases the pH of water as it decreases the total inorganic carbon concentration, but it does not change the alkalinity concentration. Maintaining a constant dissolved CO$_2$ concentration required maintaining a constant CO$_2$ production rate and removal rate for a given alkalinity treatment. A relatively constant CO$_2$ production rate was achieved by maintaining a continuous photoperiod for the fish, constant daily feed rate of 23 kg/day/RAS, and feeding every 1–2 h, 24 h a day. Continuous lighting and feeding 24 h daily have been found to minimize diurnal fluctuations in water quality, i.e., the change in TAN, CO$_2$, O$_2$, and TSS, concentrations across the culture tank (Davidson et al., 2009; Kolarevic et al., 2009). Water temperature was logged each fifth minute in the CO$_2$-degasser sump, and was maintained at 12.8 ± 0.4 °C (SD) (RAS1) and 12.7 ± 0.4 °C (RAS2) throughout the study.

Each treatment was replicated three times in two replicate RAS, i.e., six quasi-replicates were provided for each treatment (Table 1). Both RAS were operated at each treatment level for 2 weeks; water quality sampling was conducted at the end of each second week. The experimental design, both in terms of replicate systems, and length of periods, was necessary to make this study at a semi-commercial scale possible. The study lasted 20 weeks, because the first treatment tested had to be repeated due to pH instabilities during the start of the trial (Table 1).

### 2.2. Water recirculating systems

Two RAS were used in this study (Fig. 1). Each RAS contained 56.2 m$^3$ of total water volume, including ten 3.3 m$^3$ culture tanks, a belt filter (Model SFK 400 with 60 μm belt sieve opening, Salsnes, Namsos, Norway), three centrifugal pumps (1.5 kW/pump, ITT Flygt, Trondheim, Norway) to lift 0.75 m$^3$/min each, at 6 m head, from a sump following the belt filter, a moving bed bioreactor (MBBR), a forced ventilated cascade aeration column for stripping CO$_2$ from the water flowing by gravity out of the MBBR, a pump sump below the degasser of 1.9 m$^3$ volume, three centrifugal pumps (3 kW/pump, ITT Flygt), to lift a nominal flow of 0.75 m$^3$/min each against 12–13 m head from a sump at the base of the stripping column, and a down flow bubble contactor (AquaOptima, Trondheim, Norway) to add pure oxygen gas to the water just before it entered each culture tank. Each MBBR contained three 7.0 m$^3$ chambers that each contained 3.5 m$^3$ of media (Biofilm Chip P, KrügerKaldnes, Sandefjord, Norway) with a specific surface area of 900 m$^2$/m$^3$ (manufacturers statement).

To ensure that the waste load on the MBBR approached maximal capacity at a relevant culture tank water quality for Atlantic salmon smolt (0.2–0.7 mg/L TAN) (Dalsgaard et al., 2013; Terjesen et al., 2013), data from Terjesen et al. (2013) was used to calculate the required media area in the present study. These authors found that the capacity of all three chambers combined in the RAS1 (or RAS2) MBBR was 61 kg feed/day, calculated to equal 2083 g TAN produced/day, at 50% feed protein, to maintain maximal 0.7 mg/L TAN in the return flow from the culture tanks, at 14 °C and 1.9 m$^3$/min flow. In the present study a reduced feed load of 23 kg/day/RAS, was used. Based on Terjesen et al. (2013) this feed load

(47% protein in feed, see below) should result in 700 g TAN/day/RAS released to the culture tank water (nitrogen retention of 53% for Atlantic salmon parr, Grisdale-Helland and Helland, 1997). At the temperature of 12.8 °C used in the present study, this TAN production would require one-third of the total MBBR area (i.e. 3140 m$^2$), giving a 68% TAN removal to 0.22 mg/L in the effluent, and require a 1.1 m$^3$/min system flow rate. Thus, the recirculating water at this flow rate was only pumped through one of the three MBBR chambers (the last) per RAS during the present study (Fig. 1).

The CO$_2$ stripping column used contained 1.6 m depth of random packing (2.4 m$^3$ of Nor-Pac rings 5 cm diam., Jaeger Environmental Products, Houston, TX, USA) and used a fan to ventilate 10 volumes of air for every one volume of water passing counter-current through the column. The down flow bubble contactors were connected to one control circuit and online O$_2$ probe (Model DO6441, Sensorex) per culture tank. The oxygen controller was used to maintain dissolved oxygen in the culture tanks at between 85% and 90% of saturation. Although these RAS are equipped with systems for ozonation, the recirculating water was not ozonated during the study, to ensure that any nitrite accumulation due to the alkalinity treatments could occur freely, without being oxidized by ozone.

Water flow rate through each MBBR and make-up water flow rate were measured using magnetic flow meters (Sitrans FM Magflo, Siemens, Munich, Germany) with continuous data logging (each fifth minute). Water flow through each MBBR (RAS1: 1147 ± 8 l/min, RAS2; 1148 ± 7 l/min) and make-up water flow (RAS1: 9 ± 0 l/min, RAS2; 9 ± 0 l/min) were held constant throughout the study, and was similar between the RAS. Thus, 99.2% of the flow was reused each pass through the RAS, and the daily system water exchange of each RAS was 23%, i.e. the mean hydraulic retention time within each RAS was estimated to be 4.3 day. The mean hydraulic retention time (HRT) through the 3.3 m$^3$ fish culture tanks, 7 m$^3$ MBBR chamber, and the 1.9 m$^3$ sump below the CO$_2$-degasser were 29 min, 5.3 min, and 1.9 min, respectively. Note, pH was continuously monitored immediately after the cascade column in the sump for dosing sodium bicarbonate and controlling pH/alkalinity. The approximately 1.9 min HRT within the sump allowed the dehydration of carbonic acid, i.e., the rate limiting step with a 22 s half-life, and reallocation of bicarbonate and carbonate (both nearly instantaneous) to return to acid–base equilibrium (Kern, 1960; Grace and Piedrahita, 1994) and achieve a stable pH immediately following CO$_2$ stripping.

The biofilters had been connected to tanks with feeding salmon parr for a least six months prior to the beginning of the study to thoroughly establish the biofilters with bacteria. Ten weeks prior to the experiment, all pipes were cleaned, and the CO$_2$ degasser media taken out and thoroughly cleaned. The biofilter media in each RAS were taken out, mixed between RAS1/2 to ensure similar biofilm and MBBR microbiota, and then returned in equal volume (3.5 m$^3$) to the RASs. Subsequently, each RAS was stocked with similar biomass, and maintained on equal feed load (see below) until start of the trial. All routine work on both RAS was conducted equally, and at approximately the same time each day. In addition, all daily flushing/scrubbing of sediments (i.e., the belt filter) and flushing of tanks was done after water quality sampling had been completed for the day.

### 2.3. Atlantic salmon

Atlantic salmon parr of SalmoBreed strain (SalmoBreed AS, Bergen, Norway) was used. The fish were stocked into five 3.3 m$^3$ culture tanks in each RAS seven weeks before initiating data collection, leaving five tanks with water circulating at same rate as in the other tanks, but with no fish. To avoid too high fish density, right after sampling period 8, half the number of fish of all five tanks were moved into the five previously empty tanks. At the start of the

**Table 1**
Experimental treatment schedule. Both RAS were operated at each nominal treatment level for 2 weeks and each treatment was replicated three times in each of the two replicate RAS.

| Last treatment day | Treatment period | Nominal RAS#1 alkalinity (as CaCO$_3$) | Nominal RAS#2 alkalinity (as CaCO$_3$) |
|---|---|---|---|
| 12/28/2010[a] | 1[a] | 10 | 70 |
| 1/11/2011 | 2 | 200 | 10 |
| 1/25/2011 | 3 | 10 | 200 |
| 2/8/2011 | 4 | 200 | 10 |
| 2/22/2011 | 5 | 70 | 70 |
| 3/8/2011 | 6 | 10 | 200 |
| 3/22/2011 | 7 | 70 | 70 |
| 4/5/2011 | 8 | 200 | 200 |
| 4/19/2011 | 9 | 70 | 10 |
| 5/3/2011 | 10 | 10 | 70 |

[a] Treatment period 1 (10 and 70 mg/L alkalinity) was repeated after the final treatment, due to pH instabilities at the start of the study.

experiment (start of period 1) each of five tanks per RAS contained 3935 salmon parr of initial size of 61.1 ± 1.6 g (SD).

The salmon parr were fed commercial diets (EWOS Micro, Bergen, Norway) with 3 mm pellet sizes throughout the study. When changing feed lots (i.e. different shipments of same feed type), samples were taken and analyzed for proximate composition, according to Terjesen et al. (2013), to ensure that minor feed changes did not influence the system loads. The average proximal chemical composition (% w/w of feed "as is") was 94.0 ± 0.7 (SD) dry matter, 46.9 ± 0.8 crude protein, 22.4 ± 0.5 fat, and 8.3 ± 0.1 ash.

Prediction of feed intake by the salmon smolt was estimated according to fish size and water temperature using algorithms provided by the feed supplier. The day after beginning each treatment, the average weight and number of individual fish per tank were recorded in order to quantify the biomass of fish in each tank. Based on these inputs, the number of fish that had to be removed from
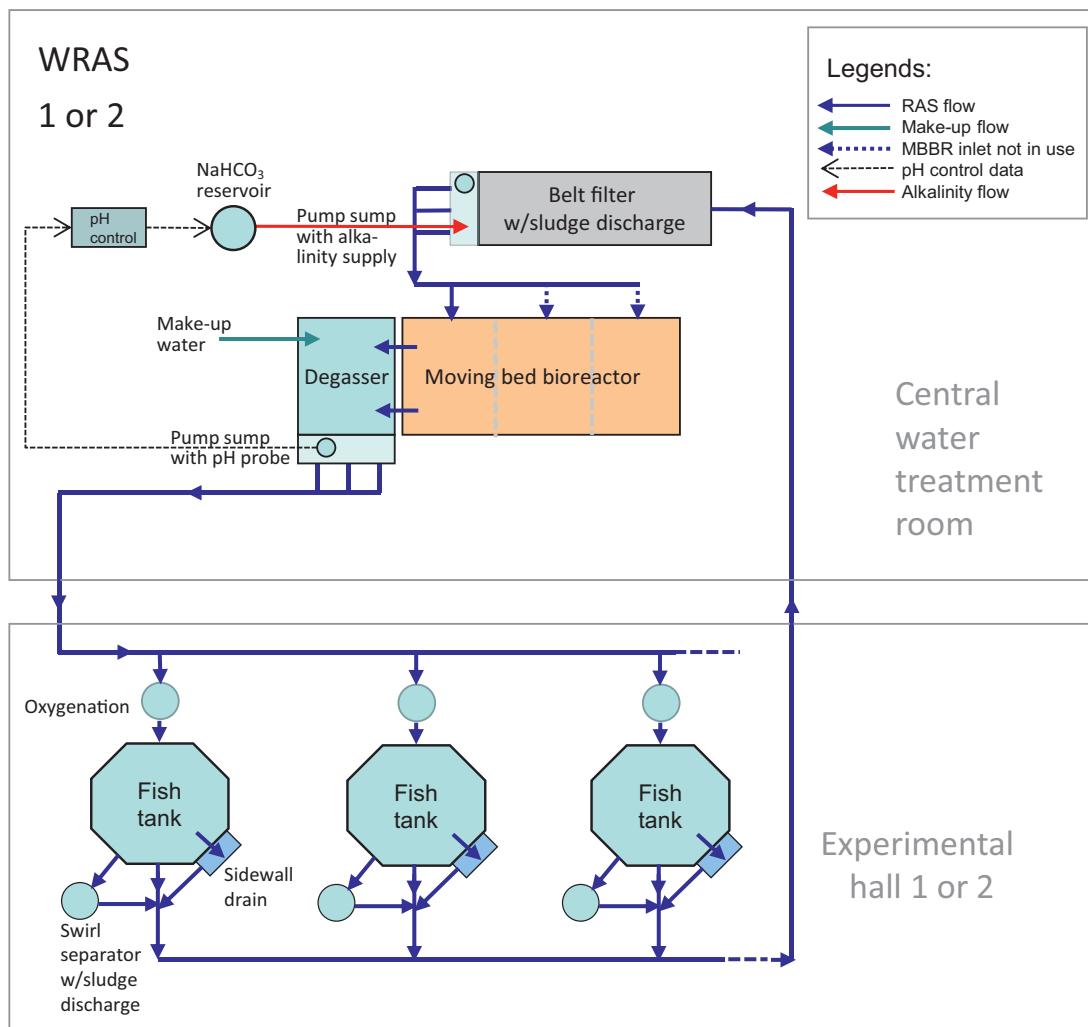


**Fig. 1.** Process flow drawing of the two RAS used in this study. Only three fish tanks are shown, out of the 10 culture tanks that were used in each RAS during this study. Refer to section 2.2 for description of components. Note that the moving bed bioreactor contains three chambers, but flow was only added at the head of the chamber closest to the degasser during this study.

**Table 2**

Mean pH and alkalinity (±s.e.) measured in the sump below the $CO_2$ stripping column for the three nominal alkalinity treatments, i.e., 10, 70, and 200 mg/L. Values represent analyses of samples collected each 14th day of each treatment ($n = 6$ per treatment).

| Nominal alkalinity (as $CaCO_3$) | RAS #1 alkalinity (as $CaCO_3$) | RAS #2 alkalinity (as $CaCO_3$) | RAS #1 pH | RAS #2 pH |
|---|---|---|---|---|
| 10 mg/L | 11 ± 1 mg/L | 9 ± 1 mg/L | 6.66 ± 0.13 | 6.43 ± 0.01 |
| 70 mg/L | 76 ± 5 mg/L | 83 ± 6 mg/L | 7.73 ± 0.04 | 7.52 ± 0.04 |
| 200 mg/L | 188 ± 17 mg/L | 207 ± 29 mg/L | 8.12 ± 0.03 | 7.90 ± 0.04 |

each system to maintain the 23 kg/day/RAS feed rate was calculated. The fish biomass was adjusted accordingly that same day.

### 2.4. Water quality monitoring

Water quality samples were collected after 14 days into each treatment replicate. Each sampling event was done at the same time each 14th day, starting at 08:00 and completed in two hours, at 10:00 AM. Water samples for TAN (total ammonia nitrogen), $NO_2$–N (nitrite nitrogen), $NO_3$–N (nitrate nitrogen), TSS (total suspended solids), $CO_2$, alkalinity, pH and TIC (total inorganic carbon) were collected before and after the MBBR. In addition, $CO_2$, alkalinity, pH and TIC were also sampled after the $CO_2$ stripping column, i.e., in the sump below the $CO_2$ stripping column. Handheld meters were used for pH determination (Hach HQ40D with PHC10101 electrodes, Hach Lange, Düsseldorf, Germany), and these were two-point calibrated using NBS buffers each day of use. In some cases, dissolved $CO_2$ was also estimated using an Oxyguard portable $CO_2$ analyzer (Oxyguard, Birkerød, Denmark). Alkalinity was measured by titration according to Standard Methods (APHA, 2005), using a HACH Digital Titrator Model 16900 (Hach, Loveland, Colorado, USA), and a Orion 720Aplus pH meter. TAN, $NO_2$–N, and $NO_3$–N samples were analyzed using an autoanalyzer (Flow Solution IV, OI Analytical, College Station, TX, USA), according to U.S. E.P.A Method 350.1 (U.S. EPA, 1983) for TAN and U.S. E.P.A Method 353.2 (U.S. EPA, 1983) for $NO_3$–N and $NO_2$–N. TIC was analyzed on fresh samples kept on ice, collected with siphons and without air-bubbles into glass flasks with tapered stoppers, according to method 6/93 Rev. B (Perstorp Analytical, Perstorp, Sweden); further details are provided by Terjesen et al. (2013). TIC was also used to calculate dissolved $CO_2$, using pH, and temperature measured at the same time and location as when collecting water samples for TIC analysis. The carbonate system constants in Summerfelt et al. (2001) were used in the calculations.

### 2.5. Statistical analyses

Data are presented as the treatment means ± s.e. unless otherwise noted. SPSS (Chigaco, IL, USA) syntax written for randomized designs was used to assign alkalinity treatments randomly to experimental period and to RAS #, with the constraint that no treatment was allowed to follow directly after a similar treatment, in the same RAS (Table 1). Prior to statistical analyses all percentage data were arcsine square root transformed. Analyses on the effects of alkalinity were done using one-way ANOVAs in SPSS. If significant ($p < 0.05$), Tukey's post hoc tests were subsequently applied to determine between-treatment significant differences.

## 3. Results and discussion

The pH controller maintained relatively constant alkalinity for each treatment replicate (i.e., for 10, 70, and 200 mg/L) throughout the study (Table 2). For example, to maintain the low dose alkalinity treatment, the pH was controlled at 6.66 ± 0.13 and 6.43 ± 0.01 in the sump below the $CO_2$ stripping column in RAS #1 and RAS #2, respectively, while alkalinity in the same location averaged 11 ± 1 mg/L and 9 ± 1 mg/L as $CaCO_3$, respectively. The medium and

high dose alkalinity treatments were maintained similarly using pH control (Table 2).

Significant differences were found between the quasi-steady state TAN concentration measured between alkalinity concentrations at the MBBR inlet, MBBR outlet, and degasser sump (Table 3). The mean TAN concentration entering the MBBR was reduced from 0.65 ± 0.08 mg/L at the nominal 10 mg/L alkalinity treatment compared to 0.43 ± 0.04 mg/L and 0.39 ± 0.05 mg/L of TAN at the nominal 70 mg/L and 200 mg/L alkalinity treatments, respectively. The mean TAN concentration exiting the MBBR dropped from 0.39 ± 0.06 mg/L at the 10 mg/L as $CaCO_3$ alkalinity treatment compared to 0.22 ± 0.03 mg/L and 0.23 ± 0.04 mg/L of TAN at the 70 mg/L and 200 mg/L as $CaCO_3$ alkalinity treatments. These results suggest that the MBBR was able to maintain lower TAN concentrations when alkalinity concentrations entering the MBBR were maintained at nominal 70 or 200 mg/L as $CaCO_3$ compared to a sustained alkalinity of only 10 mg/L as $CaCO_3$ alkalinity. No differences in steady state TAN concentration were distinguished between alkalinity treatments of 70 mg/L and 200 mg/L as $CaCO_3$. Hence, our hypothesis that increased alkalinity reduces TAN concentrations in RAS for Atlantic salmon smolts was supported by the results. In RAS for Atlantic salmon smolts, a relatively low TAN concentration is maintained, even as low as 0.2 mg/L (Dalsgaard et al., 2013). Hence in such RAS, the TAN substrate concentration will not be saturating. It must therefore be noted that in RAS operated for other fish species, at higher intensities and much higher steady-state TAN levels, the alkalinity requirement may be different from that found in the present study.

Regarding nitrite concentrations, unlike for TAN, no significant differences were observed between treatments, which averaged 0.42–0.58 mg/L, and were quite variable (Table 3). In the same type of RAS, we have earlier shown that although TAN and $CO_2$ removal capacity was higher than anticipated using common dimensioning rules, nitrite removal did not meet specifications because higher than 0.1 mg/L $NO_2$–N was observed. In a study comparing fixed bed and moving bed reactors Suhr and Pedersen (2010) found no differences between the bioreactor systems in accumulation of $NO_2$–N. The finding that the experimental treatments did not affect nitrite in the present study, suggest that in this set-up, nitrite removal was not limited by alkalinity. The mean $NO_3$–N concentration exiting the MBBR ranged from 40 to 42 mg/L for the three treatments. No significant change could be distinguished either in $NO_3$–N concentration across the MBBR, which indicates that equal TAN conversion to $NO_3$–N occurred for the three treatments as was intended with the use of equal fish feed loading throughout the study.

The mean areal nitrification rate across the MBBR ranged from 0.09 to 0.14 g/d/m² (Table 4). There were significant differences between the mean areal nitrification rates across the MBBR, i.e., 0.14 ± 0.02 g/d/m² and 0.09 ± 0.02 g/d/m², calculated at the alkalinity treatments of 10 mg/L and 200 mg/L as $CaCO_3$, respectively. No differences were suggested in TAN removal efficiency across the MBBR in a single pass, with removal efficiencies ranging from 41 to 50% removal for each treatment (Table 4). Interestingly, these results suggest that the mean areal nitrification rates were significantly higher at a nominal alkalinity of 10 mg/L as $CaCO_3$ compared to 200 mg/L as $CaCO_3$, which is opposite of our hypothesis. Compared to previous studies, the areal nitrification rates reported here

**Table 3**

TAN concentration (±s.e.) measured at the MBBR inlet, MBBR outlet, and degasser sump, plus $NO_2$–N concentration measured at the MBBR outlet, at the low (10 mg/L), medium (70 mg/L), and high (200 mg/L) alkalinity concentrations. Values represent analyses of samples collected each 14th day of each treatment ($n$ = 6 per treatment).

| Nominal alkalinity (as $CaCO_3$) | TAN MBBR inlet (mg/L) | TAN MBBR outlet (mg/L) | TAN Degasser sump (mg/L) | $NO_2$–N MBBR outlet (mg/L) |
|---|---|---|---|---|
| 10 mg/L | 0.65 ± 0.08[b] | 0.39 ± 0.06[b] | 0.37 ± 0.06[b] | 0.42 ± 0.13[a] |
| 70 mg/L | 0.43 ± 0.04[ab] | 0.22 ± 0.03[a] | 0.19 ± 0.03[a] | 0.58 ± 0.12[a] |
| 200 mg/L | 0.39 ± 0.05[a] | 0.23 ± 0.04[ab] | 0.20 ± 0.03[a] | 0.52 ± 0.24[a] |

[a,b] Treatment means not sharing a common letter are significantly different ($p < 0.05$).

were similar to the areal rates measured across the same or larger MBBRs in the same facility, except in this other study all three MBBR chambers were utilized (Terjesen et al., 2013). Pfeiffer and Wills (2011) reported an areal nitrification rate approximately twice that measured in the present study; however, in that study the temperature was higher at 24–25 °C. Two factors may explain the higher areal removal rate at lower alkalinity. First, we should note that the TAN production rate should be the same for all treatments because the same amount of feed was consumed daily throughout the study. Thus, the difference in the MBBR areal nitrification rates between treatments could be explained by TAN removal on surfaces outside of the MBBR, i.e., nitrification could have occurred in biofilms that had formed on pipe, sump, tank and $CO_2$ stripping column packing surfaces. The higher alkalinity treatment (200 mg/L) may have supported more nitrification activity outside of the MBBR than the low alkalinity treatment, which would explain the lower areal nitrification rate within the MBBR at the higher alkalinity. This hypothesis tends to be supported by the higher TAN concentrations maintained during the lower alkalinity conditions, possibly because less TAN was removed on surfaces outside of the MBBR under low alkalinity conditions. A second explanation is also based on the inlet TAN concentration that was higher at the 10 mg/L nominal alkalinity; earlier nitrification kinetic studies demonstrates that increased substrate concentration result in higher removal rates (Chen et al., 2006; Rusten et al., 2006), as indeed found in the present study. However, at the lowest alkalinity treatment, 10 mg/L, the MBBR outlet concentration was higher (Table 3), i.e. at this low alkalinity level the same low outlet TAN concentration was not maintained. This is indicated also by the unchanged removal efficiency (Table 4), and the effect likely led to the higher steady-state TAN level at 10 mg/L, as discussed above. The results invite to a more detailed investigation about the removal rates at different inlet concentrations, at the three alkalinity levels. Possibly, at above 70 mg/L alkalinity, the removal rate at lower inlet concentrations are higher, than at low alkalinity, considering our findings of an unchanged efficiency despite a significantly lower inlet TAN concentration. The present areal nitrification rates were comparable to the rates reported by Rusten et al. (2006). However, as shown in Fig. 8B of Terjesen et al. (2013), the measured removal rates in the same type of RAS as in the present study, were higher at the lowest effluent TAN concentrations, than could be predicted from Rusten et al. (2006). In conclusion, future studies should investigate the hypothesis that this MBBR is particularly efficient at very low inlet TAN concentrations, but that at such low substrate concentrations alkalinity should be at or above 70 mg/L as $CaCO_3$.

The dissolved $CO_2$ concentration entering the degasser did not differ significantly between alkalinity treatments, and averaged 6.8 ± 0.7 mg/L (calculated from TIC), and 7 ± 0 mg/L (measured with probe). The absolute removal of TIC (Table 5) was greater at the highest alkalinity (1.65 mg/L TIC removed) than at the two lower alkalinity treatments (0.97 and 0.93 mg/L TIC removed). This is supported by the findings of Colt et al. (2012), that the differences between true $CO_2$ removal (equal to the decrease in TIC) and the apparent $CO_2$ removal (equal to the decrease in $CO_2$ concentration after re-equilibration) are small for low alkalinities in freshwater but are larger in high alkalinities. A tendency ($p = 0.11$) was

**Table 4**

The mean areal nitrification rate (±s.e.), and mean TAN removal efficiency (±s.e.) across the MBBR for the low (10 mg/L), medium (70 mg/L), and high (200 mg/L) alkalinity treatments. Values represent analyses of samples collected each 14th day of each treatment ($n$ = 6 per treatment).

| Nominal alkalinity (as $CaCO_3$) | Areal nitrification rate[a] (g/d/m$^2$) | TAN removal efficiency (%) |
|---|---|---|
| 10 mg/L | 0.14 ± 0.02[B] | 41 ± 5 |
| 70 mg/L | 0.11 ± 0.01[AB] | 50 ± 3 |
| 200 mg/L | 0.09 ± 0.02[A] | 43 ± 3 |

[A,B] Treatment means not sharing a common letter are significantly different ($p < 0.05$).

[a] Areal nitrification rate was calculated by multiplying the flow rate of recirculating water passing through the biofilter with the change (i.e., inlet minus outlet) in the combined TAN plus $NO_2$–N concentration across the biofilter; this product was then divided by the total estimated surface area of media in the biofilter.

observed however, suggesting a higher $CO_2$ stripping efficiency at the 10 mg/L nominal alkalinity treatment when measured with probe (Fig. 2), but this was not found when $CO_2$ concentration was calculated from the TIC concentration, pH, and temperature data. The mean $CO_2$ removal efficiencies ranged from 54 to 63% across the 1.6 m tall forced-ventilated aeration columns. This is quite effective considering that the mean $CO_2$ inlet concentration was under 7 mg/L. In comparison, Moran (2010) reports that $CO_2$ stripping efficiency averaged 75–77% across a 1.65 m packing depth (similar to the present study) at an inlet concentration of 10 mg/L (slightly higher than the present study) in freshwater when measured with a $CO_2$ probe. The results on the mean removal efficiency are comparable to earlier studies in this RAS which was designed to avoid
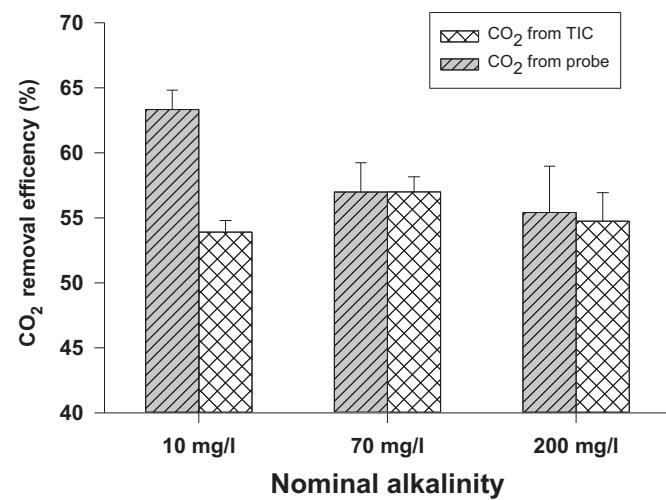


**Fig. 2.** Mean (± s.e.) $CO_2$ stripping efficiency recorded across the forced ventilated cascade aeration columns at the three alkalinity treatments, i.e., 10 mg/L, 70 mg/L, and 200 mg/L as $CaCO_3$. Note that the white hatched bars and grey hatched bars represent stripping efficiency where $CO_2$ concentrations were calculated from TIC measurements or probe measurements, respectively. Values represent analyses of samples collected each 14th day of each treatment ($n$ = 6 per treatment). Stripping efficiency was calculated from the difference in the column inlet and outlet concentrations, divided by the inlet concentration, and multiplied by 100.

**Table 5**

Total inorganic carbon (TIC) loss at the three alkalinity treatments, divided into loss through nitrification, degasser, gain from make-up water, and loss through the overflow in the degasser sump.

| Component | Treatment (mg/L as $CaCO_3$) | | |
|---|---|---|---|
| | 10 | 70 | 200 |
| TIC use in nitrification (C, g/min)[a] | 0.3 | 0.2 | 0.2 |
| TIC inlet degasser (mg/L C) | 2.5 | 21.0 | 41.3 |
| TIC removal (%) | 38.3 | 4.4 | 4.0 |
| TIC removal (mg/L C) | 0.97 | 0.93 | 1.65 |
| System flow rate (l/min) | 1150 | 1147 | 1146 |
| TIC loss degasser (C, g/min) | 1.1 | 1.1 | 1.9 |
| Make-up flow rate (l/min) | 9.0 | 9.0 | 9.0 |
| TIC make-up water (mg/L C)[b] | 4.1 | 4.1 | 4.1 |
| TIC from make-up (C, g/min) | 0.0 | 0.0 | 0.0 |
| TIC degasser sump (mg/L C) | 1.6 | 20.0 | 39.7 |
| TIC loss sump overflow (C, g/min) | 0.0 | 0.2 | 0.4 |
| Total TIC loss (C, g/min) | 1.4 | 1.4 | 2.4 |

[a] Calculated using TAN removal for the three treatments (Table 3), system flow rate, and an alkalinity consumption of 7.07 g $CaCO_3$ per g TAN removed in nitrification (Chen et al., 2006).

[b] The make-up water TIC concentration refers to measurements on samples collected after period 10.

$CO_2$ levels above 10 mg/L (Terjesen et al., 2013), concentrations that may have adverse effects on Atlantic salmon performance, health and welfare (Fivelstad, 2013).

Results from the present study did not support our fourth hypothesis, i.e., that increased alkalinity would decrease $CO_2$ removal efficiency. We had assumed that dissolved $CO_2$ removal efficiency would decrease across the cascade column at high alkalinities because the $CO_2$ concentration at the outlet of the column would be partly replenished by a shift in acid–base equilibrium from this large pool of carbonate. However, Colt et al. (2012) suggests that in freshwater, there is no practical difference (i.e., <1% difference) between the apparent $CO_2$ removal rate and the true $CO_2$ removal rate when the alkalinity is 100 mg/L as $CaCO_3$ (2 meq/L) or less. They note that the difference in apparent $CO_2$ removal rate and the true $CO_2$ removal rate would be less than or equal to 5% when the alkalinity is 200 mg/L as $CaCO_3$ (4 meq/L). Thus, for practical purposes, $CO_2$ stripping is not impacted by acid–base equilibrium at the higher alkalinity concentrations tested. From the perspective of a steady-state mass balance, this suggests that the concentration of $CO_2$ accumulating in identical RAS that are receiving the same feed rate and are operated at the same water flow rate would have the same steady-state $CO_2$ concentration exiting the culture tank (and exiting the biofilter), because $CO_2$ production and removal are not impacted by alkalinity under the alkalinity range typically used in RAS. Thus, dissolved $CO_2$ stripping efficiency is predicted to be a fixed property of a degassing unit, irrespective of alkalinity under freshwater conditions, when alkalinity is ≤200 mg/L as $CaCO_3$.

The TIC concentration increased with alkalinity, and was significantly different ($p < 0.001$) between all treatments, as would be expected. In the degasser inlet, TIC averaged 2.5 ± 0.8 mg/L at 10 mg/L nominal alkalinity, 21.0 ± 1.3 mg/L at 70 mg/L alkalinity, and 41.3 ± 5.1 mg/L at 200 mg/L nominal alkalinity. At the lowest alkalinity treatment, nominal 10 mg/L, the relative $CO_2$-fraction in TIC increased because the pH was also reduced (Table 2). This difference in TIC composition influenced the fate of the carbon when passing the $CO_2$ degasser. When the RAS water entered the $CO_2$ stripping column at 10 mg/L alkalinity, as much as 38% of the system carbon was removed. In contrast, at the two higher alkalinities only 4% of the TIC was lost when passing the degasser (Fig. 3).

Over all treatments, TIC removal from the RAS averaged only 0.2–0.3 g/min due to nitrification and 1.1–1.9 g/min due to $CO_2$ stripping (Table 5). We also estimate that TIC removal due to the nitrification within the $CO_2$ stripping column (probably <10% of nitrification occurring in the MBBR based on surface area and

concentration), would amount to only 0.02–0.03 g/min. Thus, loss of TIC to nitrification in the $CO_2$ stripping column is negligible in practice compared to the loss due to $CO_2$ stripping.

The RAS pH was significantly lower at an alkalinity of 10 mg/L than at the two higher alkalinity treatments (Table 2). It was also observed that pH oscillations were more pronounced at the lowest alkalinity, since little buffer capacity existed and the dosing controller therefore produced more under- and overshooting of the set-point. Furthermore, we found a significant inverse linear correlation ($r^2 = 0.96$, $p < 0.001$, $n = 20$, data $\log_{10}$ transformed) between alkalinity and the difference between degasser sump [$H^+$] and MBBR inlet [$H^+$]. Thus, as expected the $H^+$ concentration increased more through the culture tanks at lower alkalinity. When switching alkalinity treatments, the rate of the pH decline accelerated from 200 mg/L alkalinity, to 70 mg/L, and was especially rapid down to a pH typical of the 10 mg/L treatment (Fig. 4). This trend is analogous to the situation that will occur during alkalinity dosing malfunction. Hence, in a RAS operated at very low alkalinity, the time available for replacing the dosing equipment, before adverse effects on the fish occur from high $CO_2$, will be considerably less than when operating at 70 or 200 mg/L alkalinity.
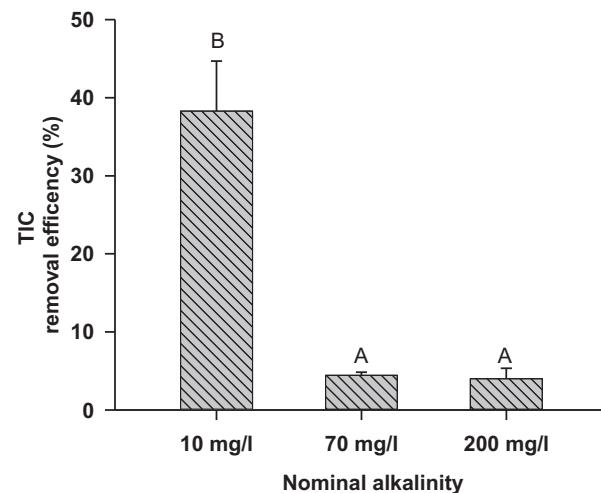
**Fig. 3.** Mean (± s.e.) of the relative amount of total inorganic carbon (TIC) removed across the forced ventilated cascade aeration columns at the three nominal alkalinity treatments, i.e., 10 mg/L, 70 mg/L, and 200 mg/L as $CaCO_3$. Values represent analyses of samples collected each 14th day of each treatment ($n = 6$ per treatment). Treatment means not sharing a common letter are significantly different ($p < 0.05$).
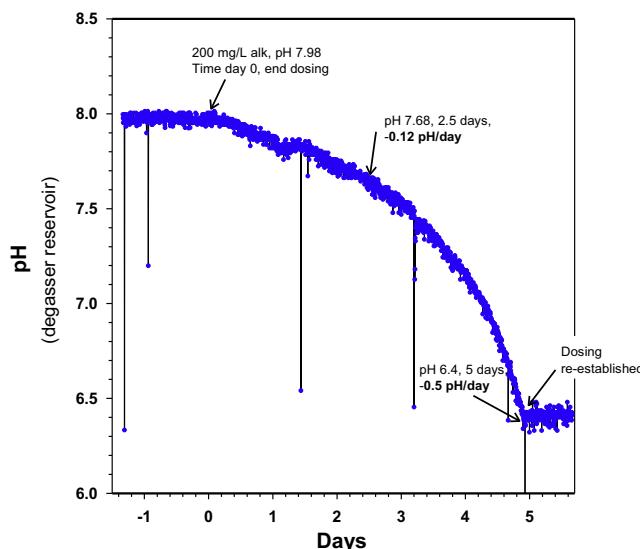
**Fig. 4.** Effect of stopping alkalinity dosing on RAS pH, at a constant feed loading to the system. Note the accelerating decline in pH with time. Alkalinity dosing was re-established at pH 6.4. The pH values of 7.98, 7.68, and 6.4 were typical of alkalinities of nominally 200 mg/L, 70 mg/L, and 10 mg/L as $CaCO_3$, respectively.

Alkalinity dosing constitutes a cost for the RAS farmer. This is especially evident when using soft make-up water sources that are low in alkalinity, which is the case at many locations in Norway (Kristensen et al., 2009) but not in e.g., Eastern U.S (Davidson et al., 2011). When the make-up water source is low in alkalinity, dosing of e.g., bicarbonate must be used. The decision on the controlled alkalinity concentration must be based on effects on the fish, nitrification, degassing, pH stability and loss of carbon through the RAS loop. An inorganic carbon budget was made for the three experimental treatments in the present study (Table 5), incorporating components that differed between treatments. The most significant component was the loss of TIC through the degasser. The 200 mg/L alkalinity treatment showed the highest absolute loss through the degasser, as well as for total inorganic carbon loss out of the system (including TIC lost to water flushing; Table 5). Although only 4% of the TIC entering the degasser is removed in the 200 mg/L alkalinity treatment, the considerably higher TIC concentration in this treatment made the removal significant.

In conclusion, Atlantic salmon smolt producers using soft water make-up sources, and wishing to keep a relatively low steady-state TAN and $CO_2$ concentration in line with salmon tolerances, should aim for 70 mg/L alkalinity considering the relatively low loss of carbon compared to 200 mg/L alkalinity, and the increased pH stability as well as reduced TAN concentration, compared to when using 10 mg/L alkalinity. In the present study, the experimental design precluded studying effects of alkalinity on salmon smolt performance, physiology, and welfare, and such investigations should therefore be undertaken in future studies.

## 4. Conclusions

This study was conducted in semi-commercial scale RAS, at typical water qualities found in Atlantic salmon smolt production facilities. At these conditions, it was found that a low alkalinity (10 mg/L as $CaCO_3$) led to a significantly higher steady-state TAN concentration, compared when 70 or 200 mg/L alkalinity was used. The mean areal nitrification rate was higher at the lowest alkalinity; however, the mean TAN removal efficiency across the MBBR was not significantly affected by alkalinity treatment. The $CO_2$ stripping efficiency showed only a tendency towards higher

efficiency at the lowest alkalinity, but differences were not significant. Thus, dissolved $CO_2$ stripping efficiency across a degassing unit is independent of alkalinity under freshwater conditions, when alkalinity is $\leq 200$ mg/L as $CaCO_3$. In contrast, the relative fraction of total inorganic carbon that was removed from the RAS during $CO_2$ stripping was much higher at a low alkalinity (10 mg/L) compared to the higher alkalinities (70 and 200 mg/L as $CaCO_3$). However, when calculating the total absolute loss of inorganic carbon from RAS, it was found that the daily loss was about equal at 10, and 70 mg/L, whereas it was highest at 200 mg/L alkalinity. pH recordings demonstrated that the 10 mg/L alkalinity treatment resulted in the lowest system pH, the largest increase in [$H^+$] across the fish culture tanks, as well as giving little response time in case of alkalinity dosing malfunction. Rapid pH changes under the relatively acidic conditions at 10 mg/L alkalinity may ultimately create fish health issues due to $CO_2$ or if aluminium or other metals are present. In conclusion, Atlantic salmon smolt producers using soft water make-up sources should aim for 70 mg/L alkalinity considering the relatively low loss of inorganic carbon compared to 200 mg/L alkalinity, and the increased pH stability as well as reduced TAN concentration, compared to when using 10 mg/L alkalinity.

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