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Test the implications of salinity on nitrification kinetics

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Executive summary

A comprehensive study was performed to describe the effects of gradual salinity increase in real, operating RAS with the water and microbiota therein, and with real loadings from fish eating fish feed. Four different salinity increment schemes were applied in triplicate to twelve identical recirculating aquaculture systems and the concomitant changes in nitrogen compounds and water quality parameters were measure daily/weekly for 30 days. In parallel experiments, nitrification kinetics of the biofilters were measured weekly.

Results demonstrate increases in ammonia-concentrations in the systems depending on the salinity increase rate. The salinity range 7 – 13 ppt was the critical range with reduces turn-over. No threshold salinity was proven for the ammonia conversion, demonstrating that hazardous concentrations can be avoided by moderate daily salinity increment and moderate biofilter loading. At higher salinities, >13 ppt, nitrite concentrations started to increase, demonstrating that probably nitrite could be a challenge if salinity is increased too fast or too much.

Water quality parameters (turbidity, microbial activity) were generally transiently improved by increased salinity, although parameters measured all returned to reference values after three weeks.

Introduction

The biological nitrification process is very important for fish farming in recirculating aquaculture systems (RAS). During this microbial two-step process the ammonia excreted by the fish as part of their internal protein and amino acid turnover, is oxidized to nitrite and subsequently nitrate. By the nitrification process, ammonia and nitrite accumulation in the RAS is prevented, keeping concentrations at safe levels in the biofilter is properly dimensioned (Eding *et al*., 2006; Rusten *et al*., 2006).

Traditionally, nitrification is considered to be a two-step process, carried out by ammonia-oxidizing (AOB) and nitrite-oxidizing bacteria (NOB), where the most common bacterial taxa involved are Nitrosomonas sp., and Nitrospira sp. (Hüpeden et al., 2020). However, recent findings indicate a much greater diversity in participating microbes, including Nitrospira sp. bacteria performing complete ammonia oxidation to nitrate (comammox) and ammonia-oxiziding archaea (AOA) (Bartelme et al. 2017).

The excretion of ammonia (Total Ammonia Nitrogen, TAN) depends on several different factors such as fish species, feed composition, and feed conversion ratio (FCR) but the most important factor is the amount of feed consumed per day. Based on these factors, the expected TAN excretion can be calculated (Dalsgaard & Pedersen, 2014) and by that the TAN loading on the biofilter. If the loading exceeds the given turnover capacity in the biofilter/RAS, concentrations will rise. While in the range where turn-over is dependent on substrate concentration (i.e., first order process; Henze *et al*., 2002), increased concentrations will favour increased turn-over but when exceeding some 1 mg TAN or NO_2-N/l the processes are no longer substrate limited and increased loadings result in linearly increased concentrations in the RAS.

In stable operation and conditions, the nitrification process is generally considered relatively stable although particularly NOBs have been described as sensitive to changes (Pedersen et al., 2015). In fluctuating and changing conditions both AOBs and NOBs can be vulnerable. One such changing condition is salinity increase. Recent development in commercial RAS includes saline or brackish water systems (Martins *et al*., 2010, Dalsgaard *et al*., 2013), and for salmonids in particular there can be a need for increasing the salinity to accommodate smoltification and potential sea transfer or to treat e.g., parasite infections (Aihua & Buchmann, 2001). For the industry it is thus imperative to know the potential risks of increasing salinity and at the same time know how to potentially avoid these risks.

Effects of abrupt changes in salinity on the nitrification in biofilters have been previously described (Timmons & Ebeling, 2010; Gonzalez-Silva *et al.*, 2015; Kinyage *et al*., 2019). Navada *et al*. (2019) described effects of rate of salinity increase adding intake (i.e., not RAS) water and synthetic feed solution to a separate biofilter set-up. Most previous studies dealing with effects of increased salinity have thus used pristine water, synthetic loads (organic and NH_4Cl), and some NaCl addition, to biofilters in separate set-ups. The purpose of this experiment was thus to study the effects of salinity increase in real, operating RAS and the microbiota therein, and with real loadings from fish eating fish feed.

Materials & Methods

Experimental set-up

The purpose of the study was to determine the influence of gradually increasing salinity on TAN and nitrite turnover and kinetics in operating freshwater RAS systems with fish fed commercial feed. The trial lasted for 30 days, i.e., day 0-29.

Fish

Juvenile rainbow trout (*Oncorhynchus mykiss*), held in the systems in FW for >10 weeks prior to trial. Trial start average weight 351 g/pcs.

Diet

A commercial diet, Biomar Efico E920 Advance, 4½ mm was used. Every day 100 g feed was given to each tank/system.

System setup

The trial was performed in 12 identical, 0.8 m^3 pilot scale freshwater RAS (Fig. 1) at DTU Aqua in Hirtshals, Denmark. The biofilter elements had only been operating in freshwater until the start of the trial. Four treatments were applied in a triplicate set-up: three control RAS with only FW (60 l/day) added as make-up water (MUW; Ctrl), three RAS with 40 l FW and 20 l seawater (SW, 35 ppt) added/day (Slow), three RAS with 30 l FW and 30 l SW added/day (Med), and three RAS with 20 l FW and 40 l SW added/day (Fast). Each RAS was composed of a 100 L cylindroconical biofilter filled with 40 L RK BioElements (RK BioElements, Denmark) having a specific surface area of 750 m^2/m^3 and operated as a moving bed biofilter with an air flow of 4 L/min; a 200 L pump sump; and a 500 L cylindroconical rearing tank holding the fish and having a metal grid at the bottom to prevent the fish from assessing the bottom cone, which contained a 0.8 L waste collector/settling column (Fig. 1). Two DC Runner 5.2 pumps (Aqua Medic GmbH, Bissendorf, Germany) in the pump sump pumped approximately 1800 L/h to the biofilter and 1300 L/h to the rearing tank, respectively.

Two weeks before the start of the trial biomedia from all biofilters was collected, mixed and re-distributed between the 12 systems.

Based on the water exchange set-up described, the systems increased in salinity at different pace, logarithmically approaching the salinity defined by the FW/SW combination, i.e., 0; 11; 17; and 23 ppt, respectively. Such development in salinity reflects the natural development at given water exchanges ratios in commercial systems.

Sampling, analyses and management routines

Every day at 9:00 am, before any other routine operations, water samples were collected from the pump sump of all 12 systems. The samples were immediately sterile filtered (0.2 µ, Sartorius, Germany) and stored below 5°C in a cooling room for subsequent analysis of TAN, nitrite-N, and (weekly) nitrate-N. Nitrate-N, nitrite-N and ammonium-N were measured by spectrophotometry following ISO 7890-1 (1986), DS 223 DS (1991) and DS 224 (1975), respectively.

Salinity (Hanna Seawater refractometer HI96822), temperature, and pH (Hach multimeter HQ40d) were measured in all sumps and if needed, pH was adjusted using sodium-bicarbonate, targeting the range 7.2 - 7.3 to secure that the nitrification process was not hampered by lack of alkalinity. The oxygen level in all tanks were continuously monitored and was always above 85 % saturation. Bottom cone was then cleaned and the settling column with faecal solids emptied (0.8 l/day). Make-up water (60 l/system/day) was then added and at 10.00 AM feeding started using automatic feeders for 6 hours.

Every week (i.e., day 1, 8, 15, 22, and 29) separate samples were taken from the pump sumps for analysis of alkalinity (titration, Mettler Toledo T50), turbidity (Hach 2100 Qis), and microbial activity. Microbial activity in the water was quantified using the hydrogen peroxide (H_2O_2) decomposition rate assay described in Pedersen et al., (2019), considering the degradation rate constant (k, h-1) as an expression of microbial activity. In short, a 42 ml water sample was placed in a 50 ml centrifuge tube and H_2O_2 was added to the water sample at a final concentration of 10 mg L-1. The decomposition of H_2O_2 was subsequently measured by collecting samples before addition of H_2O_2 (background level), immediately after H_2O_2 addition and every 15 min thereafter for 1 hour. Samples were kept in a water bath at 22°C for the duration of the assay. The degradation rate constant (k, h-1) was then calculated using the data obtained.

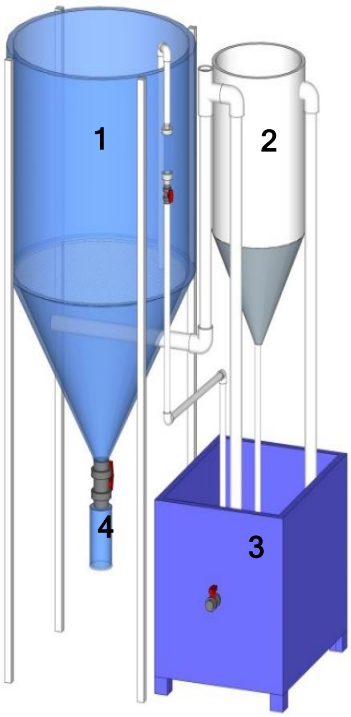

Figure 1. Pilot scale RAS including a: 1) rearing tank; 2) moving bed biofilter; 3) pump sump; and 4) sludge collector Adapted f*rom*: Gregersen, K. J. D. J. *et al.* (2021). Aquacultural Engineering, 95 (2021), 10.1016/j.aquaeng.2021.102195

First-order nitrification kinetics

Low concentration spikes with NH_4Cl were used to evaluate substrate (TAN) limited removal rates (1'-order kinetics). Every week (i.e., day 1, 8, 15, 22, and 29) the 1'-order turnover of TAN was measured by separate spiking experiments. Reactors of 6.4 l were used and 5 l RAS water and 2 l biofilter media from each system (media to be returned to respective system after each trial) was added in a standardized, gentle procedure. At the bottom of each reactor, air was added (0.5 l/min) to keep the biofilter-elements in motion and secure moving bed operation (Fig. 2). Depending on the concentration already present in the specific RAS, NH_4CL was added to reach an initial TAN-concentration around 0.8 mg/l. After spiking, samples were taken from each reactor at time 2, 7, 17, 22, 27, 32, 37,42, 47, 57 min. (day 1, 8) as well as time 67, and 77 min (day 15, 22, 29). For subsequent analyses of TAN, nitrite-N and nitrate-N (t 2 and 77 min only), a volume of 8 ml was sampled from each reactor and filtered using a 0.2 µm sterile filter (Sartorius, Germany) and stored below 4°C for later analysis.

Data points below 0.5 mg TAN/l and above 0.15 mg TAN/l from the spiking experiments were used to assess the substrate dependent removal rates of ammonia. First order degradation kinetics were described using least square linear regression on ln-transformed substrate concentrations versus time as applied in Prehn et al., (2012). Regressions were fitted in the form y = ax + b where y is the ln-transformed substrate concentration in the bulk water, x is the time after spiking, and a (slope) and b are constants. From the slope of the regression line of the ln-transformed data multiplied by the water volume of the reactor and divided by the surface area of the biofilter media used, the first order rate constant k_{1a} (m/d) was calculated (von Ahnen et al., 2015).

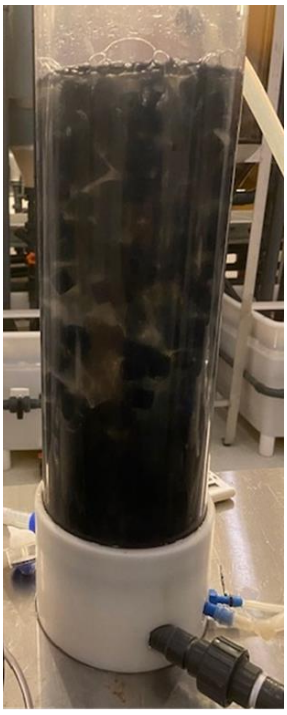

Figure 2. One of the 6.4 l reactors used for spiking events to measure 1'-order nitrification kinetics.

The relative inhibition (%) caused by salinity increases was calculated according to the formula: Relative Inhibition = $[(N_R - N_S)/N_R]* 100\%$, where, N_R and N_S are same-day removal rate at reference (FW) reactor (Ctrl) and reactors with the different salinity levels, respectively, expressed in m/day for k_{1a}.

Results & Discussion

The salinity in the different RAS progressed as expected (Fig. 3) with no or very little difference (±1 ppt, few days) between the triplicates within each code.

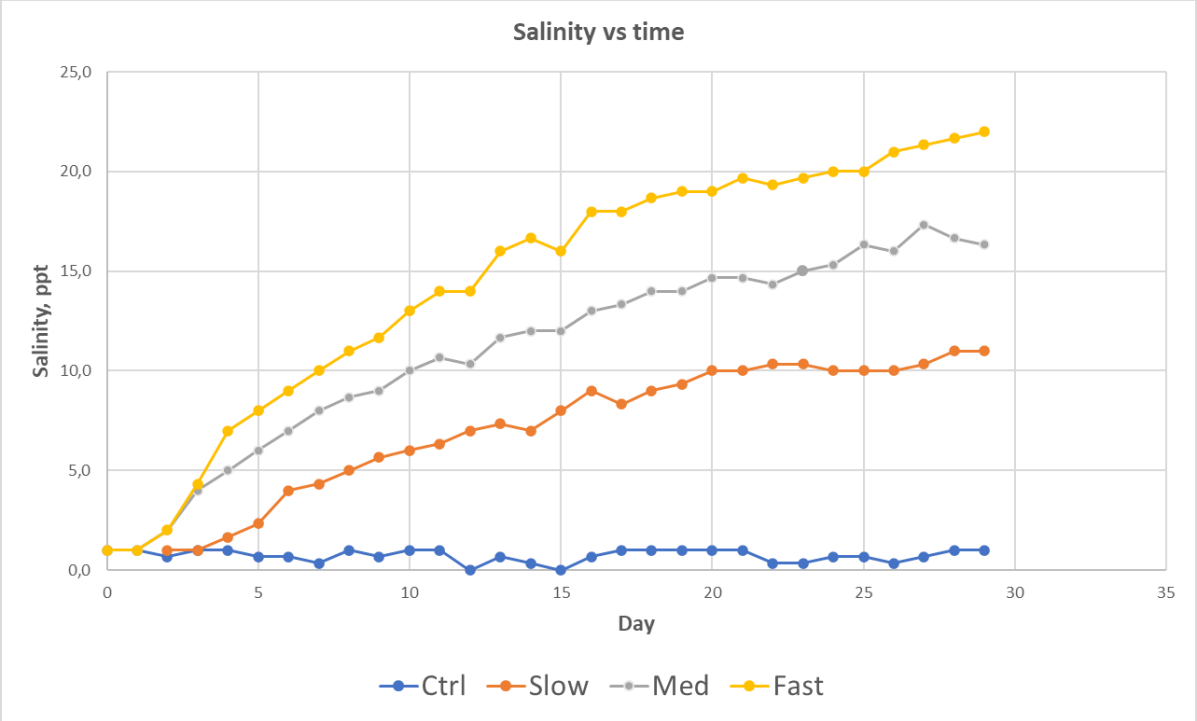

Figure 3. Daily salinity measurements. Each point is an average of the three systems within each code.

The development in system salinity over time observed fits the theoretical expected by using the formula: $Salinity_{day\ x} = (1 - e^{(-60*x)/800}) *$ salinity of make-up water mix (23.3; 17.5; and 11.7, respectively) with 60 (l) representing the amount of make-up water/day and 800 (l) the system volume.

Such a progression in salinity represents real life RAS where you normally add a given amount of make-up water/day with a given salinity. In a system, salinity thus logarithmically approaches the salinity of the make-up water over time. To our knowledge, this experiment is the first description of the effects of "natural" progressive salinity increase in operating systems. Others have demonstrated effects of abrupt (Kinyage *et al*. 2019) or shorter term linear salinity increases (Navada *et al*., 2019) on biofilter kinetics only in separate, lab. set-ups often using non-RAS water. In this experiment, continuously operating RAS were used, with biofilters operating under normal conditions, normal RAS water, normal microbiota and a fixed loading from fish given fish feed.

Effect of salinity on TAN and nitrite concentrations in RAS

TAN

The TAN-concentrations in the 12 systems developed as shown in fig. 4a + b.

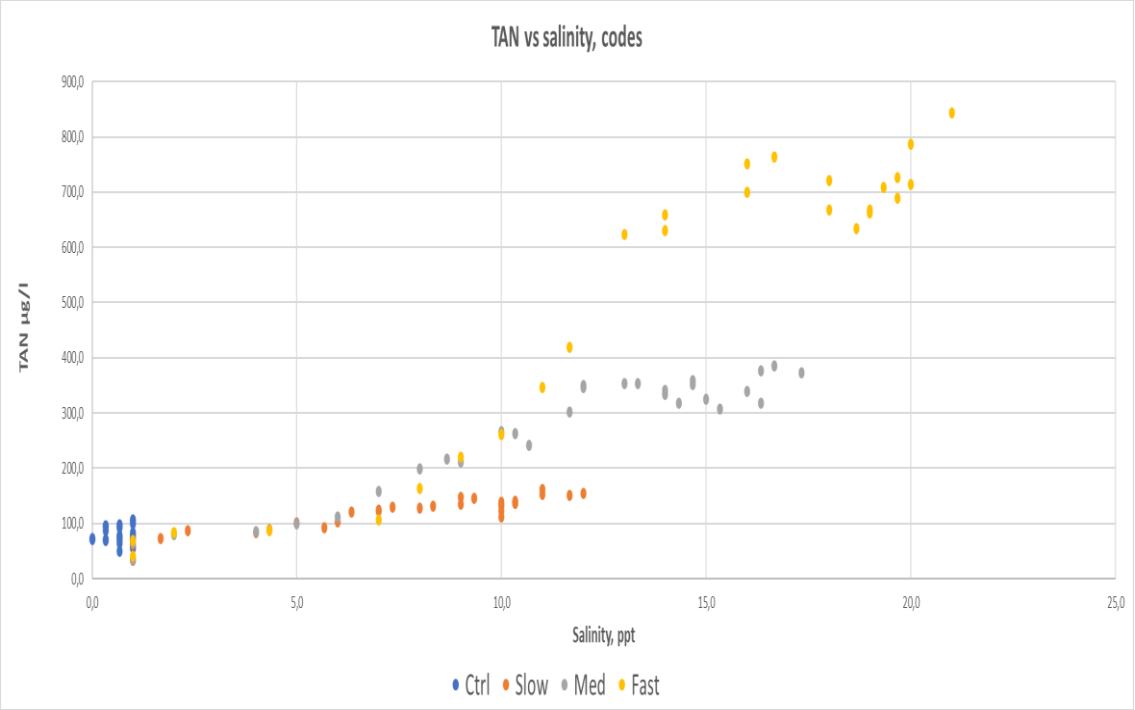

Figure 4a. Development in TAN-concentrations depicted against salinity. Each point is an average of triplicate RAS.

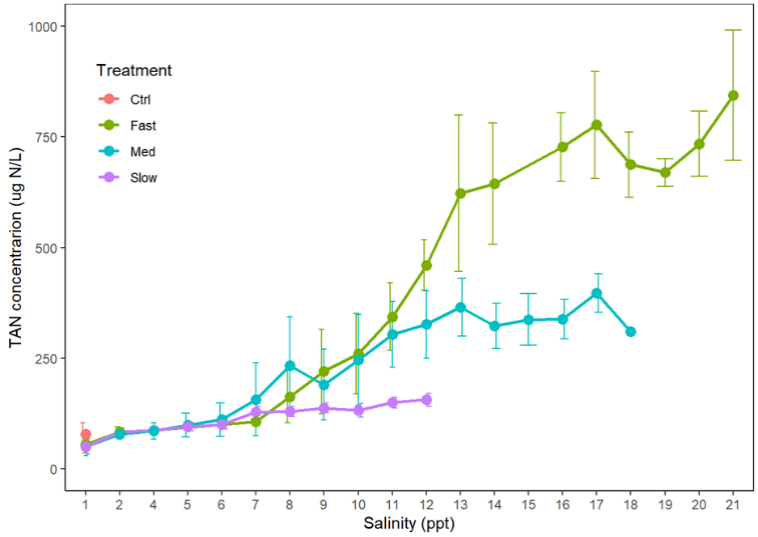

Figure 4b. Development in TAN-concentrations depicted against salinity with standard deviation (n=3).

As shown in Figure 4a+b TAN-concentrations increased with salinity although at different pace and to different levels depending on the rates of salinity increase. The freshwater (Ctrl) systems remained low (< 0.1 mg TAN/l) during the full experiment whereas the others all started to increase a little when passing a salinity of 6 ppt. Interestingly, this increase was not a result of passing a certain salinity "threshold". Rather, there seemed to be gradually increasing effect of increased salinity in the approximate range 7 – 13 ppt after which TAN-concentrations levelled out. Previous studies applying abrupt salinity changes (Gonzalez-Silva *et al*., 2016; Kinyage *et al*., 2019; Navada *et al*., 2019) indicates the same salinity range as critical for 0'-order (substrate-unlimited) TAN-turnover.

Even more interesting is the difference in resulting TAN-concentration in the different RAS groups depending on the salinity increase rate. While Fast reached some 420 µg/l at 12 ppt (d 9) and Med reached 300-350 µg/l (d 13-15), Slow only reached 155 µg/l (d 28-29) at the same salinity demonstrating the importance of increase rate for continuous TAN-conversion in operating systems. A statistical treatment (two-way Anova) of the data supports this by revealing significant effect of salinity and of treatment and an important, significant interaction between the two from 9 ppt onwards.

In this trial, 100 g feed/60 l MUW was applied, corresponding to a system feed loading of 1.67 kg/ m3 MUW (or 600 l MUW/kg feed applied), which is quite intensive. Biofilter loading was 100 g/d on 40 l of biofilter media, corresponding to 2.5 kg feed/d/m^3 media or 3.3 g/d/m^2 surface area which is moderate loading. It is expected that a higher biofilter loading could have resulted in higher TAN-concentrations than observed in the present study. As long as the system is still in the 1'-order concentration range (normally considered to be below 1.0 mg TAN/l) the turn-over is supported by an increase in TAN-concentration whereas higher concentrations does not further support the turn-over.

According to Dalsgaard & Pedersen (2011) 100 g of the specific feed applied will result in a production of some 3.5 g TAN/d at given conditions. In the RAS used, this corresponds to an increase in TAN-concentration of 4.4 mg/l/d given no TAN-turnover. Despite the turnover being hampered by increased salinity it is never-the-less obvious that a significant part of the daily TAN-production is still being nitrified by the biofilters.

Whether the increased salinity (mainly) induces/necessitates a shift in the ammonia-oxidising bacteria (AOB) composition or it (mainly) induces/necessitates an adaptation to salinity of the existing FW-bacteria is not well known. Recent studies are not consistent and since only few investigate operating biofilters in operating RAS this question is still debated. The different response to salinity increase rate and the limited time-span for it seems to indicate that perhaps adaptation is the main process taking place. More studies are, however, needed to illuminate this interesting question.

Nitrite-N

The nitrite-N concentrations in the 12 systems developed as shown in fig. 5.

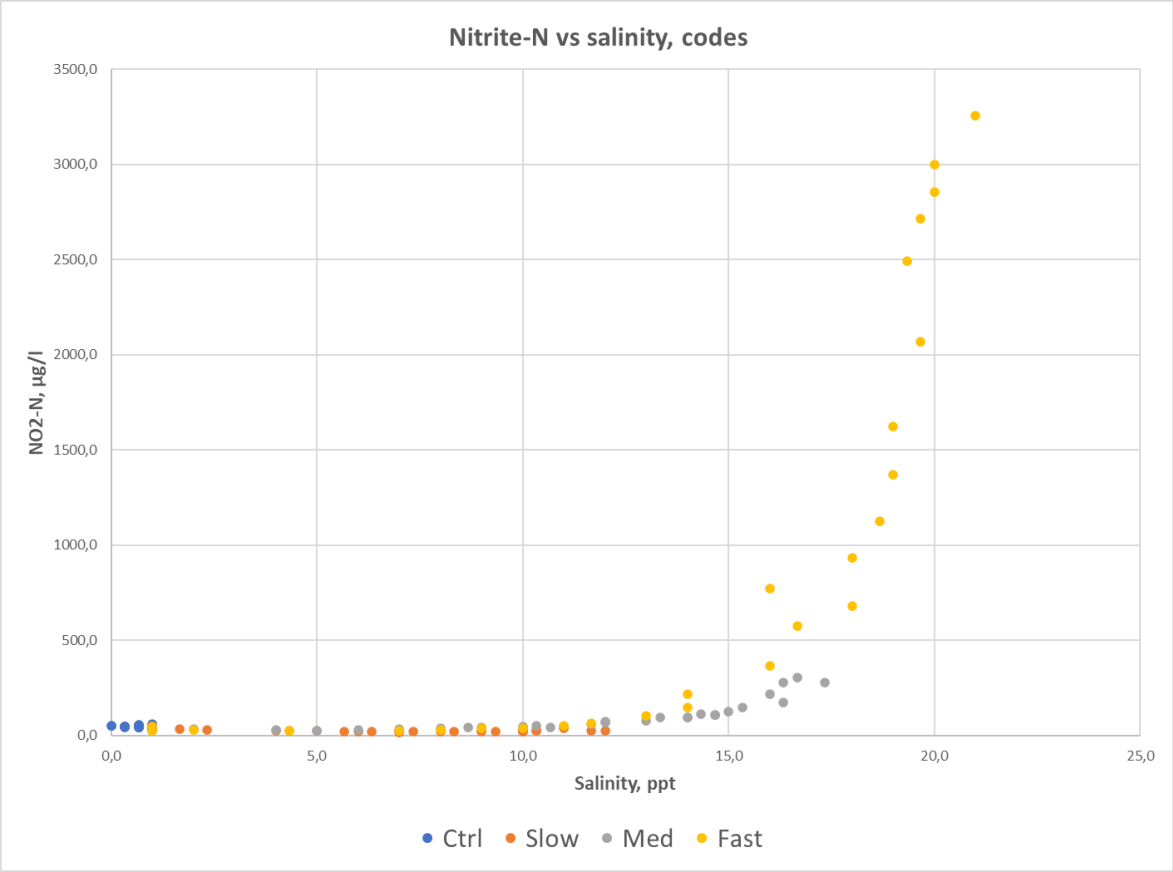

Figure 5. Development in NO_2-N-concentrations depicted against salinity. Each point is an average of triplicate RAS.

As shown in Figure 5, NO_2-N-concentrations increased with salinity when passing some 12 ppt. Perhaps at a different pace and to different levels depending on the rates of salinity increase but this is difficult to evaluate given the salinities reached in the different systems. The freshwater (Ctrl) systems remained low (< 0.055 mg NO_2-N/l; avg 0.048 mg NO_2-N/l) and Slow even lower (avg. 0.026 mg NO_2-N/l) during the full experiment, whereas Med started to increase above 0.075 mg NO_2-N/l when passing a salinity of 13 ppt (d 16) ending around 0.3 mg NO_2-N/l at 17 ppt (d 29). Fast exceeded 0.1 mg NO_2-N/l when reaching 13 ppt (d 10) and increased rapidly to 3.0 mg NO_2-N/l at 20 ppt (d 25). At day 26 the experiment was terminated for the three Fast RAS and some 50% of the system volume was replaced.

The effect of increased salinity observed in this study is in line with previous studies (Kinyage *et al*., 2019) although the effect in this study occurs at a higher salinity (+13 ppt). It is interesting, that the effect on the nitrite-oxidizing bacteria (NOB) occurs more abrupt and at higher salinity than the AOBs. The reason for this is not known but could indicate a different succession/adaptation process for the NOBs.

Pedersen *et al*., (2015) have previously described the NOBs to be somewhat more sensitive to changes than the AOBs and obviously at least the Fast salinity increase had a severe effect on nitrite oxidation. Although salinity (i.e., chloride) generally counteracts the negative effect of elevated nitrite concentrations, it is evident that nitrite, rather than ammonia, is a severe threat during increased salinity in operating RAS. Unfortunately, the current study cannot tell whether (or to which extend) the rate of salinity increase influences or moderates the effect on the nitrite oxidation process.

Nitrate-N

The nitrate-N concentrations in the 12 systems were quite constant throughout the experiment and did not change between codes as shown in Fig. 6.

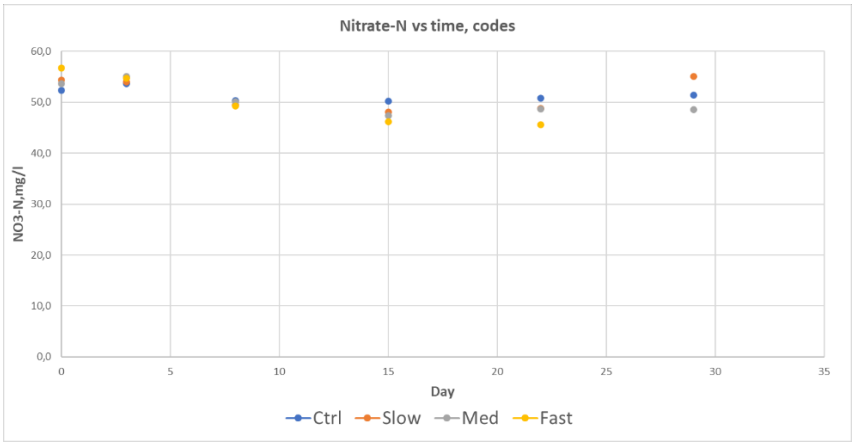

Figure 6. Nitrate-N concentrations over time. Each point is an average of triplicate RAS.

Figure 6 illustrates the recirculation intensity of the systems with nitrate-N concentrations above 50 mg NO_3-N/l. No obvious difference can be observed between the treatments, underlining that despite hampered processes the vast majority of ammonia is still converted to nitrate.

Effect of salinity on first-order nitrification kinetics

TAN – conversion

Fig. 7 illustrates typical results from the spiking experiments to determine the 1'-order TAN conversion in the individual systems.

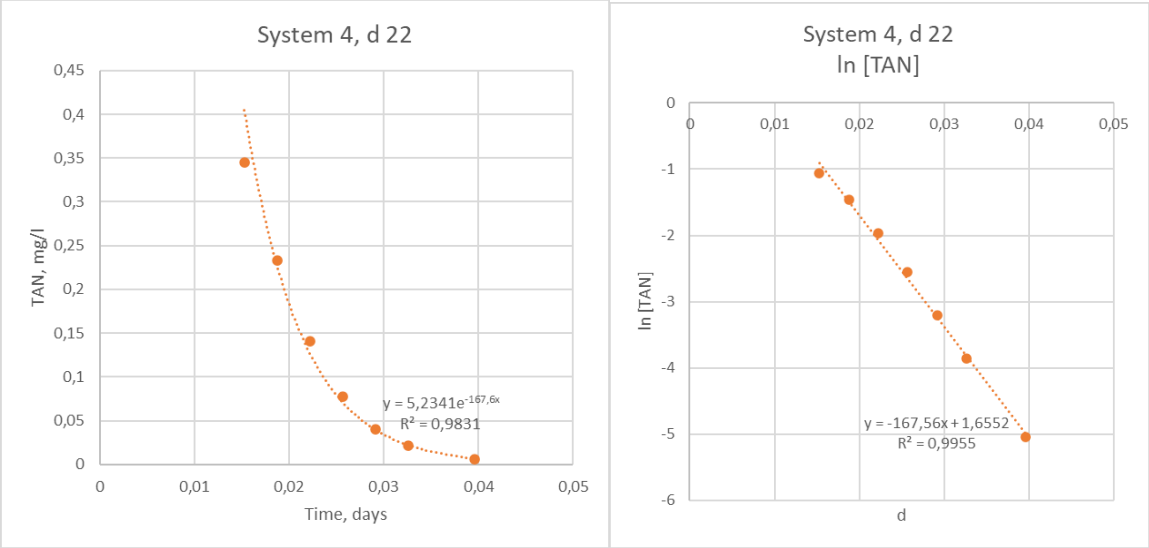

Figure 7. An example of TAN-concentration vs time in the reactors following spiking. Only TAN-concentrations between 0.5 and 0.015 mg/l are included. The y-axis on the left is TAN-concentrations (mg/l) while they have been ln-converted on the figure right. The first order rate constant, k_{1a}, are calculated by using the exponent in the formula $y = a*e^{-k1a}$ or the slope in the ln-transformed data, in this case 167.6, multiplied by water volume (5 l or 0.005 m^3) and divided by the surface area of the bio elements used (1.5 m^2). Here, k1a = 0.56 m/d.

The observed 1'-order rate constants for TAN-conversion are shown in Table 1.

Table 1. First-order rate constants, k_{1a}, (m/d), describing the TAN-conversion kinetics for all systems (average of triplicate treatments) measured every week of the trial.

Day		K_{1a} (m/d) average	Inhibition vs Ctrl (%)	Salinity ppt
1	Ctrl	**0,37**		1
	Slow	**0,45**		1
	Med	**0,41**		1
	Fast	**0,44**		1
8	Ctrl	**0,47**		1
	Slow	**0,36**	**23**	6
	Med	**0,19**	**59**	9
	Fast	**0,17**	**63**	11
15	Ctrl	**0,56**		0
	Slow	**0,42**	**25**	8
	Med	**0,26**	**54**	12
	Fast	**0,20**	**64**	16
22	Ctrl	**0,61**		0
	Slow	**0,52**	**16**	10
	Med	**0,27**	**57**	14
	Fast	**0,16**	**74**	19
29	Ctrl	**0,65**		1
	Slow	**0,52**	**20**	12

	Med	0,24	63	16
	Fast	Not in	-	22

Table 1 illustrates the effect of salinity increase (and rate thereof) on the first-order TAN-conversion. As can be seen from the table, all salinities (>6 ppt) caused some kind of inhibition of the first-order TAN-conversion, increasing with increasing salinity. It should also be noted that the inhibition occurring in the Slow group is much less than Med and Fast at equivalent salinities.

Reduced k_{1a} values means that the velocity of TAN conversion is reduced. However, in the first order concentration area (below some 1.0 mg TAN/l) where the process is substrate (i.e., TAN) limited, the concomitant increased TAN concentration supports increased turnover at the higher concentration. This fits nicely with the observations made at system level where systems with reduced k_{1a} have increased TAN-concentrations, still not entering the 0'-order (substrate independent) concentration area, though. As mentioned, higher biofilter loadings might have pushed the TAN concentration further up.

Ammonia conversion in system water

To test for potential TAN conversion in the water phase, 5 l of system water (without biofilter media) was tested in reactors on day 15. After relevant spiking with NH_4Cl samples were taken at time 2, 32, and 62 min and subsequently analysed for TAN and nitrite-N. Results are shown in Table 2.

Table 2. Results from reactor experiments using 5 l RAS water only (no bio-elements). Water TAN turnover corresponds to 2-4 % of the turn-over achieved with bio-elements over 1 hour.

Treatment	Salinity d 19 (ppt)	Decrease in TAN conc. (µg NH4-N/l) in 1 hour	Decrease in NO_2-N conc. (µg NO2-N/l) in 1 hour
Ctrl – t2			
Ctrl – t62	0	30,8	-13,6
Slow – t2			
Slow – t62	8	53,3	-11,3
Med – t2			
Med - t62	12	13,6	-10,7
Fast – t2			
Fast – t62	16	7,3	-14,8

As can be seen from Table 2 the TAN turnover in the water phase, although existing, is very low. The removal observed corresponds to some 2-4 % of the TAN removal achieved in an hour with bio-elements in the reactor. Seemingly, the salinity has almost identical effect on water turn-over as on biofilter. The oxygenation of TAN results in a certain increase in nitrite, although not 1:1. This indicates, that a little nitrite-conversion is also occurring in the water phase, although not fully matching the formation from TAN oxygenation.

It can be noted as a side note that, using the Ctrl result, the total TAN conversion in all system water (800 l) constitutes some 20% of the total system conversion while the biofilter converts some 80 %.

Effects of salinity increase on water quality parameters

Temperature in the 12 RAS was 16.4°C ± 0,39 throughout the experiment with no differences between treatments.

The turbidity in the systems is shown in Table 3.

Table 3. Turbidity (fnu) in the systems in relation to treatment and time. Values are averages of triplicate RAS. * indicates stat. significant effects.

Treatment	**d 8** (p<0.007)	**d 15*** (p<0,001)	**d 22**	**d 29**
Ctrl	9,78	9,87	8,35	6,25
Slow	5,45	4,63	8,43	9,76
Med	6,25	4,65	8,43	9,76
Fast	6,92	5,71	7,34	NA

A transient (day 8 and 15) improvement (i.e., reduction) in turbidity can be observed as a consequence of a salinity increase, although not related to the magnitude of it. A reduction in turbidity is related to less particles in the water. This could be related to salinity increase killing some groups of free-living bacteria and/or it could be related to agglomeration of smaller particles at increased salinity. From day 22 onwards, there is no statistical difference between treatments, indicating that bacteria and/or particles are again increasing in the saline systems.

The H_2O_2 degradation rate (k) is shown in Table 4.

Table 4. Microbial activity (k, h^{-1}) in the water quantified by the hydrogen peroxide (H_2O_2) decomposition rate assay described in (Pedersen et al., 2019) considering the degradation rate constant (k, h-1) as an expression of microbial activity. Values are averages of triplicate RAS. * indicates stat. significant effects.

Treatment	**d 8** (p<0.072)	**d 15*** (p<0.01)	**d 22**	**d 29**
Ctrl	1,68	1,50	1,52	1,54
Slow	0,73	0,51	1,17	1,63
Med	0,72	0,55	1,09	2,04
Fast	0,70	0,50	1,23	NA

For microbial activity a transient (day 8 and 15) improvement (i.e., reduction) in microbial activity can as well be observed as a consequence of a salinity increase, although not related to the magnitude of it. This reduction is probably related to salinity increase killing some groups of free-living bacteria/microbes. From day 22 onwards, there is no statistical difference between treatments, indicating that bacteria/microbes are again increasing in the water of the saline systems.

The alkalinity was above 1.5 mmol/l in the FW systems (Ctrl) and above 2.0 mmol/l in all saline systems, thus supposedly not hampering the nitrification process.

Fish performance is shown in Table 5. As can be observed, there is a negative effect of increasing salinity, particularly in Fast. Whether this is an effect of water quality changes, of fish reactions, or merely an effect of increased energy expenditure for osmoregulation is not known.

Table 5. Fish growth performance parameters during the trial (d0 - d29).
Values are averages of triplicate RAS.

Treatment	Avg. fish weight (g) d 29	FCR	SGR
Ctrl	443	0.88	0,78
Slow	436	0.92	0,75
Med	437	0.97	0,73
Fast	406	1.15	0,63

Take-home messages for the industry

- Ammonia accumulation is a risk when gradually increasing salinity in a RAS, but severe TAN concentrations can be avoided by moderate loading on the biofilters and spending the appropriate time on increasing the salinity
- The longer the time, the less the implications on TAN conversion (i.e., no “threshold” salinity)
- Keeping the TAN concentration within the 1’-order range provides opportunity for positive feed-back regulation. In this range, higher TAN-concentrations favours increased turn-over.
- The negative effect of increased salinity on TAN conversion is starting around 7 ppt
- Although the process is hampered, most of the TAN is still converted
- Nitrite accumulation might be a greater risk than TAN accumulation. Although salinity is generally considered to counteract the negative effects of nitrite on fish, still there is a risk that the concentration can become harmful
- The negative effect of increased salinity on nitrite conversion is starting around 13 ppt
- Probably there is less implications on nitrite conversion the slower the salinity increases although not really demonstrated in this study. It is this unknown whether a “threshold” salinity exist for nitrite oxidation
- Increasing salinity just a little generates transient improvements in water quality parameters like turbidity and microbial activity

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