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The effect of CO_2, total ammonia nitrogen and pH on growth of juvenile lumpfish (*Cyclopterus lumpus*)

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ABSTRACT

The objective of this study was to examine the effects of CO_2, total ammonia nitrogen (TAN: nitrogen bound as either NH_3 or NH_4^+) and pH on the growth and survival of juvenile lumpfish in a two-month growth study. The results demonstrate the complex interactions of these water quality variables. The specific growth rate (SGR) of the lumpfish was progressively reduced with increasing [CO_2] concentration above 5–10 $mg \cdot L^{-1}$. However, growth may be reduced at even lower [CO_2] and the results provide no clear safe limits under which the growth of lumpfish is unaffected by CO_2 concentration. At the lowest [CO_2] tested (8 $mg \cdot L^{-1}$) the SGR was reduced compared with controls. Moreover, in treatments where the [CO_2] concentration increased to 8 $mg \cdot L^{-1}$ as pH was artificially reduced, the SGR was similarly reduced. These results indicate that lumpfish juveniles are very sensitive to increased [CO_2] concentration in water. The SGR of the lumpfish was progressively reduced as the NH_4^+ concentration increased while maintaining the [NH_3] constant (70–80 $\mu g \cdot l^{-1}$). However, in these treatments the [CO_2] also increased to 8–10 $mg \cdot L^{-1}$ due to changes in pH and this likely contributed to reduced growth. Moreover, due to nitrification in the rearing systems, the nitrite concentration also increased to critical levels (1.5–4.2 $mg \cdot L^{-1}$) that could affect growth. Therefore, the results do not provide clear evidence for effects of increased NH_4^+ on the growth of lumpfish. Taken together, the results of the experiments show that lumpfish are sensitive to perturbations in water quality and provide a benchmark for operational welfare indicators in lumpfish aquaculture.

1. Introduction

Lumpfish (*Cyclopterus lumpus*) are extensively used as a biological delousing control in the culture of farmed Atlantic salmon (*Salmo salar* L.) (Imsland et al., 2014, 2016, 2018, 2021; Powell et al., 2018). Lumpfish have proven to be a viable alternative option for lice control that is not as invasive and more conducive to better welfare of salmon than some other treatments (Imsland et al., 2014, 2016, 2018, 2021; Powell et al., 2018). The species is now reared in land-based facilities to match the high demand of lumpfish for these purposes (Jonassen et al., 2018). Despite commercial trials for lumpfish production starting ten years ago, comparatively little is known about the rearing needs of cleaner fish in land-based production facilities (Jonassen et al., 2018; Powell et al., 2018). Hatcheries often face the challenges of bacterial infections and unstable microbial communities in the water, which can result in fish mortality (Alarcón et al., 2016; Dahle et al., 2020). Recirculating systems, especially, face the challenge to ensure appropriate water quality for the fish (Timmons et al., 2002). However, maintaining the welfare of aquatic organisms in aquaculture is of the utmost importance, both for ethical reasons as well as economic purposes.

At present, juvenile lumpfish are raised in land-based farms using either flow-through or recirculating aquaculture systems (Dahle et al., 2020). Both systems have high investment costs and, therefore, it is of essence to maintain maximum productivity while minimizing running costs. Increased fish density will improve productivity although too high density may reduce growth (Thorarensen and Farrell, 2011; Prabhu et al., 2014). It is also important to minimize net water exchanges to reduce the environmental footprint and production costs (Philis et al.,

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2019; Crouse et al., 2021). However, reduced water exchange with high fish density will impair water quality due to fish respiration with reduced oxygen saturation and accumulation of carbon dioxide (CO_2) and ammonia (NH_3) in the system which in turn may reduce growth and welfare of fish (Piedrahita, 2003; Thorarensen and Farrell, 2011; Skov, 2019).

There is a paucity of information on the effect of water quality on the growth and welfare of lumpfish. Oxygen saturation directly affects the metabolic rate of lumpfish (Remen et al., 2022). Furthermore, Jørgensen et al. (2017) found reduced growth rate at 81% oxygen saturation compared with 96% saturation, however, they did not test higher levels and therefore, it is not entirely clear at which levels oxygen saturation becomes limiting to growth. Moreover, in the experiment of Jørgensen et al. (2017), oxygen saturation was controlled by water exchange and, therefore, other water quality parameters such as CO_2 and NH_3 may also have affected the growth performance. Little is known about the effects of CO_2 and NH_3 on the growth and welfare of lumpfish. In intensive aquaculture systems, where the pH is generally maintained between 7 and 8, the CO_2 produced by the fish is primarily hydrated and converted to bicarbonate through the carbonate equilibrium. However, the excretion of CO_2 into the rearing water will also reduce the pH as bicarbonate and H^+ are produced. Increased levels of CO_2 can reduce growth and affect the welfare of fish (Skov, 2019). It has been suggested that lumpfish show a tolerance to CO_2 and pH values comparable to that of other marine species (Treasurer et al., 2018), however, this remains to be confirmed.

Ammonia is a toxic product of protein catabolism and the primary nitrogenous metabolite produced and excreted into the water by the fish (Thorarensen and Farrell, 2011; Randall and Tsui, 2002). Increased concentration of NH_3 in water can reduce the growth of fish and, at high levels, it can cause mortalities (Thorarensen and Farrell, 2011; Kolarevic et al., 2012; Thorarensen et al., 2018). NH_3 is a weak base reacting with water to produce ammonium (NH_4^+) and hydroxy ion. The total amount of nitrogen as either NH3 or NH_4^+ is referred to as total ammonia nitrogen (TAN). The proportion of TAN present as NH_3 or NH_4^+ depends on the pH, with reduced pH shifting the equilibrium towards NH_4^+. Between pH of 7 and 8, most of the TAN is present as NH_4^+ which has little or no effects on the growth of fish while NH_3 can reduce growth and welfare (Timmons et al., 2002; Thorarensen and Farrell, 2011). The tolerance of fish to NH_3 may vary among species (Randall and Tsui, 2002) and information on the tolerance of lumpfish to NH_3 is lacking.

In commercial aquaculture, the concentrations of CO_2 and NH_3 increase simultaneously while affecting the pH of the rearing water. In turn, the pH affects the carbonate and ammonia equilibrium. In a recent study, Thorarensen et al. (2018) found that the growth of Atlantic cod (*Gadus morhua*) was reduced when exposed to low concentration of CO_2 and NH_3 simultaneously while the same concentrations of these compounds had no effect on growth when applied separately. Moreover, the results of that study indicated that the NH_4^+ concentration may also affect the growth of cod. The objective of the present study was to examine this complex relationship and how it affects the growth and welfare of lumpfish juveniles. Specifically, we tested if NH_4^+ might affect the growth of lumpfish.

2. Materials and methods

2.1. Rearing system

Four identical hybrid systems, where water was partially reused, were built for the experiments (Fig. 1). In each system, the incoming seawater (23‰) and reused water was passed through an aeration column before entering a mixing tank. In treatments where the pH and TAN concentrations were changed, concentrated solutions of acid or TAN were added with dosing pumps (see below for further description). The [CO_2] and [O_2] were adjusted by bubbling the gasses into the mixing tank. From the mixing tank, the water was passed to the four rearing tanks, 110 rearing L each. The outflow water from the tanks was filtered through plastic netting (5 mm opening) to catch uneaten pellets before passing to a reservoir where reused water was pumped to the aeration column. The systems did not include a biofilter, but some nitrification activity was evident, possibly from bacteria on surfaces inside the system. The total volume of each system was approximately 505 L. The inflow of new seawater into each system was 4 L·min^{-1} while the total flow into each tank was (new and recirculated water) was 4·min^{-1}.

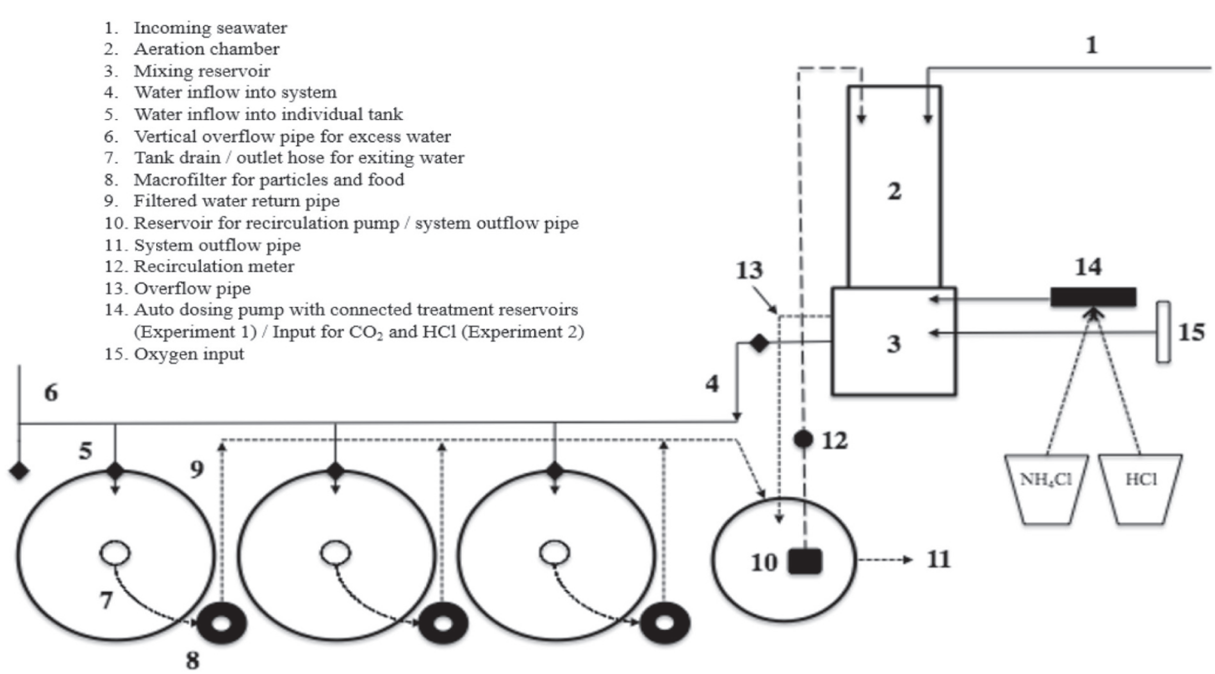

Fig. 1. The design of an individual system. Four of these systems were built next to each other to have four different experimental treatments running simultaneously.

2.2. Experiment 1 – CO_2 study

2.2.1. Fish and rearing conditions

The fish in the CO_2 study hatched mid-April 2020 at the Stofnfiskur facility (now Benchmark Genetics) in Hafnir, Iceland. On October 6, 2020, approximately 450 fish (~10 g each) were transported to Verið Hólar University College Research Station in Sauðárkrókur, Iceland. The fish were acclimated in three control tanks for one month before the study began. The lumpfish were measured from the tip of the mouth to the base of the tail fin, weighed, and distributed into 12 experimental tanks containing 110 l of water. Each tank was stocked with 37 fish (19.9 ± 0.1 g, mean ± SE). The length and weight of the fish were measured on November 6, December 7, and December 21 of 2020.

Four different treatments (Table 1) were tested in this experiment: 1) Control group (Control), without any adjustments of water quality. 2) Low CO_2 concentration (LCO2), where the fish were reared at 8 $mg \cdot L^{-1}$ with a pH of 7.5. 3). High CO_2 (HCO2), where the fish were exposed to 15 $mg \cdot L^{-1}$ CO_2 and a pH of 7.2. 4). Reduced pH (LpH), where the pH was regulated to similar levels as in treatment HCO2. Since CO_2 concentration and pH are closely connected and both could potentially affect the growth of fish, it was essential to test the effects these two parameters both separately and jointly. The increased CO_2 concentration in the HCO2 treatment caused the pH to drop to 7.23. Therefore, the LpH treatment was set up to test specifically the effect of reduced pH. The average pH in the LpH treatment was 7.26 which caused the CO_2 concentration to increase to similar levels (8 $mg \cdot L^{-1}$) as in the LCO2 treatment.

The concentration of CO_2 in water was increased by injecting CO_2 gas (Linde, Reykjavik) from a pressurized tank into the aeration chamber (Table 1, Fig. 1). The pH was adjusted by administering a 1.08 M solution of HCl with a dosing pump to the mixing reservoir (Fig. 1). With the data collected from the daily measurements, adjustments were made as needed to the dosing pumps to meet experimental parameters. The dosing pumps were calibrated prior to the start of the experiment as well as periodically throughout the study.

2.3. Experiment 2 – NH_4^+ study

2.3.1. Fish and rearing conditions

The lumpfish used in the NH_4^+ experiment was provided by the Marine and Freshwater Research Institute research station in Grindavík, Iceland. On October 31, 2019, 500 fish (mean weight 7 g) were transported to Verið. The fish were acclimated using the same methods detailed for the CO_2 study. After the acclimation period, the fish were weighed and measured. The fish were then distributed among the twelve experimental tanks with 35 fish (13.8 ± 0.2 g mean weight ± SE) in each tank. The length and weight of all fish was measured on December 3, 2019, January 3, 2020, and February 3, 2020.

The fish were exposed to four different treatments (Table 2): Two concentrations of TAN, 18 $mg \cdot L^{-1}$ (TAN18) and 33 $mg \cdot L^{-1}$ (TAN33) while maintaining the NH_3 concentration (73–80 $\mu g \cdot l^{-1}$) similar in both treatments by adjusting the pH to 7.5 and 7.2 respectively. 3) Reduced pH (LpH) tested the effect of reducing pH to 7.2 as in the TAN33 treatment by adding HCl to the rearing water. 4) The control group (Control) was reared in water where neither TAN was added, nor pH reduced with HCl.

The concentration of TAN in the TAN18 and TAN33 treatments was increased by administering stock solutions containing 0.97 M and 1.94 M NH_4Cl, respectively, with programmable dosing pumps to the mixing reservoir (Fig. 1). Similarly, stock solutions containing 0.54 M and 1.08 M HCl were used to lower the pH to fit experimental parameters (for the TAN18 and TAN33 treatments, respectively). The dosing pumps were connected to the reservoirs that were refilled every 2–3 days.

2.4. Feed and feeding

In both experiments the lumpfish were fed BioMar Inicio pellets, size 1.5–2 mm, which contained 47% crude protein, 20% crude fat, and 10% ash. The fish were given food at a daily ration of 2% of body mass provided in four feedings each day (Imsland et al., 2019). The lumpfish showed little interest in feed on the bottom of the tank. To minimize water fouling, excess feed was removed from the tanks after approximately 30 min. Following each growth measurement, the size and amount of the feed pellets were increased to reflect mean biomass and specific growth rate.

2.5. Water quality measurements

Temperature, pH, oxygen and salinity were measured twice daily, once in the morning and again in the afternoon. This was done to check for changes that might need to be corrected. Oxygen saturation was kept above 85% following welfare recommendations for lumpfish (Hvas et al., 2018; Treasurer et al., 2018; Noble et al., 2019). For both experiments, the water temperature was maintained at the natural incoming seawater temperature, which typically ranges from 7 to 8 °C. In the CO_2 study, water samples were taken weekly to measure TAN, nitrate, nitrite, and alkalinity. CO_2 was measured once daily using a CO_2 portable analyzer (OxyGuard, Farum, Denmark). In the NH_4^+ study, water samples were taken daily to measure the TAN in each individual tank. Nitrate, nitrite, and alkalinity were measured weekly. These measurements were determined using a photometer (Palintest 7500, Gateshead, UK). Based upon TAN, pH, salinity, temperature and alkalinity, the concentration of NH_3, NH_4^+ and CO_2 could be determined by using Microsoft Excel spreadsheets intended for these analyses (Pierrot et al., 2006; American Fisheries Society, 2021).

2.6. Statistical methods

Data analysis was performed using R (R Core Team, 2022). Water quality data was analyzed by comparing the daily means (±SD) of each parameter. Mean size at different times was compared with a one-way mixed model anova using the lme4 package (Bates et al., 2015). The

Table 1
Average (±SD) water quality for each treatment during the CO_2 experiment. CO_2 was measured using a portable CO_2 analyzer (Oxyguard, 2016). TAN, nitrate, nitrite, and alkalinity were measured weekly via photometer (Palintest 7500, Gateshead, UK).

Treatment	Temp. (°C)	Salinity (‰)	Oxygen Sat. (%)	pH	CO_2 ($mg \cdot L^{-1}$)	TAN ($mg \cdot L^{-1}$)	NO_3^- ($mg \cdot L^{-1}$)	NO_2^- ($mg \cdot L^{-1}$)	Alkalinity ($mg \cdot L^{-1}$ $CaCO_3$)
Control (Seawater Only)	7.8 ± 0.1	22.9 ± 0.6	89.7 ± 1.9	7.92 ± 0.04	2.48 ± 1.28	0.15 ± 0.06	6.60 ± 2.69	0.06 ± 0.02	158.61 ± 26.94
Low pH	7.6 ± 0.1	22.9 ± 0.5	87.5 ± 1.9	7.26 ± 0.07	8.19 ± 1.09	0.31 ± 0.21	7.01 ± 3.02	0.07 ± 0.020	138.61 ± 18.78
Low CO_2	7.5 ± 0.7	23.0 ± 0.5	88.1 ± 1.9	7.52 ± 0.12	7.96 ± 1.95	0.21 ± 0.24	7.56 ± 3.10	0.06 ± 0.02	143.61 ± 18.85
High CO_2	7.4 ± 0.1	23.0 ± 0.5	86.8 ± 0.6	7.23 ± 0.10	15.16 ± 3.91	0.13 ± 0.04	8.96 ± 4.08	0.04 ± 0.02	144.17 ± 12.98

Table 2
Average (±SD) water quality (± standard deviation) for each treatment for the duration of the NH_4^+ experiment. CO_2 was calculated based upon the measurements gathered. Ammonia, ammonium, nitrate, nitrite, and alkalinity were measured with a photometer (Palintest 7500, Gateshead, UK).

Treatment	Temp. (°C)	Salinity (‰)	Oxygen Sat. (%)	pH	NH_3 – N ($mg \cdot L^{-1}$)	CO_2 ($mg \cdot L^{-1}$)	NH_4^+ ($mg \cdot L^{-1}$)	NO_3^- ($mg \cdot L^{-1}$)	NO_2^- ($mg \cdot L^{-1}$)	Alkalinity ($mg \cdot L^{-1}$ $CaCO_3$)
Control (Seawater Only)	6.3 ± 0.8	28.5 ± 2.4	93.8 ± 6.3	7.74 ± 0.13	0.006 ± 0.0061	3.66 ± 0.66	0.85 ± 0.05	13.05 ± 1.08	0.04 ± 0.01	151.11 ± 2.42
Low pH	6.3 ± 0.8	28.8 ± 2.9	95.6 ± 6.3	7.31 ± 0.14	0.003 ± 0.0023	8.67 ± 1.59	0.95 ± 0.07	15.54 ± 2.98	0.29 ± 0.28	136.48 ± 7.0
TAN18	6.4 ± 0.8	28.6 ± 2.5	89.8 ± 7.1	7.47 ± 0.09	0.073 ± 0.0221	6.27 ± 1.21	18.12 ± 5.6	16.06 ± 1.86	1.48 ± 0.03	143.89 ± 3.09
TAN33	6.4 ± 0.8	28.6 ± 2.4	92.3 ± 9.5	7.24 ± 0.13	0.080 ± 0.021	10.00 ± 1.39	33.31 ± 8.8	24.94 ± 1.37	4.24 ± 1.42	131.48 ± 6.31

instantaneous growth rate (g) was estimated from the slope of the natural log-transformed weight of the fish over time (Craine et al., 2020):

$$w_t = w_i e^{g \times t}$$

Where w_i is the initial body mass and w_t is the body mass at time t and then specific growth rate (SGR) was calculated as:

$$SGR = 100 \times (e^g - 1)$$

The analysis was performed with the lme4 (Bates et al., 2015) and multcomp packages (Hothorn et al., 2008). A P-value of 0.05 was accepted as the fiducial level of significance.

3. Results

3.1. Water quality – CO_2 study

In the CO_2 experiment, temperature, salinity, and oxygen remained relatively stable across treatments as indicated by the low coefficient of variation (standard deviation <2% of grand mean) (Table 1). The overall TAN levels were low and remained <0.5 $mg \cdot L^{-1}$ across all treatments. Nitrites and nitrates were also lower in the CO_2 experiment when compared to the NH_4^+ study (Table 2).

3.2. Water quality – NH_4^+ study

Temperature and salinity remained stable across all treatments for the duration of the NH_4^+ experiment (Table 2). Oxygen saturation (89.8%) was at the recommended levels for lumpfish production (80–90% O_2) (Noble et al., 2019; Treasurer et al., 2018). The NH_4^+ concentrations were 18.12 ± 5.61 $mg \cdot L^{-1}$ and 33.31 ± 8.77 $mg \cdot L^{-1}$ for TAN18 and TAN33 treatments respectively (mean ± SD, Table 2). The NH_3 was 73 $\mu g \cdot l^{-1}$ in the TAN18 and 80 $\mu g \cdot l^{-1}$ in the TAN33 system. Compared with the control and low pH treatments, the NO_2^- concentrations in the TAN18 and TAN33 treatments were elevated to 1.48 $mg \cdot L^{-1}$ and 4.24 $mg \cdot L^{-1}$ respectively (Table 2). The NO_3^- was also 55–91% higher in the TAN33 treatment than in all other treatments.

3.3. Growth and mortality – CO_2 study

The average mortality rate was 2% and not significantly ($P = 0.99$) different among treatment groups. Increased CO_2 concentration reduced growth rate. The final weight of the HCO2 group was significantly ($P < 0.001$, Fig. 2a) lower than in all other groups. The SGR in the control group was significantly higher than in all other treatments and the SGR of the HCO2 treatment was significantly ($P < 0.01$, Fig. 2b) lower than in the other groups.

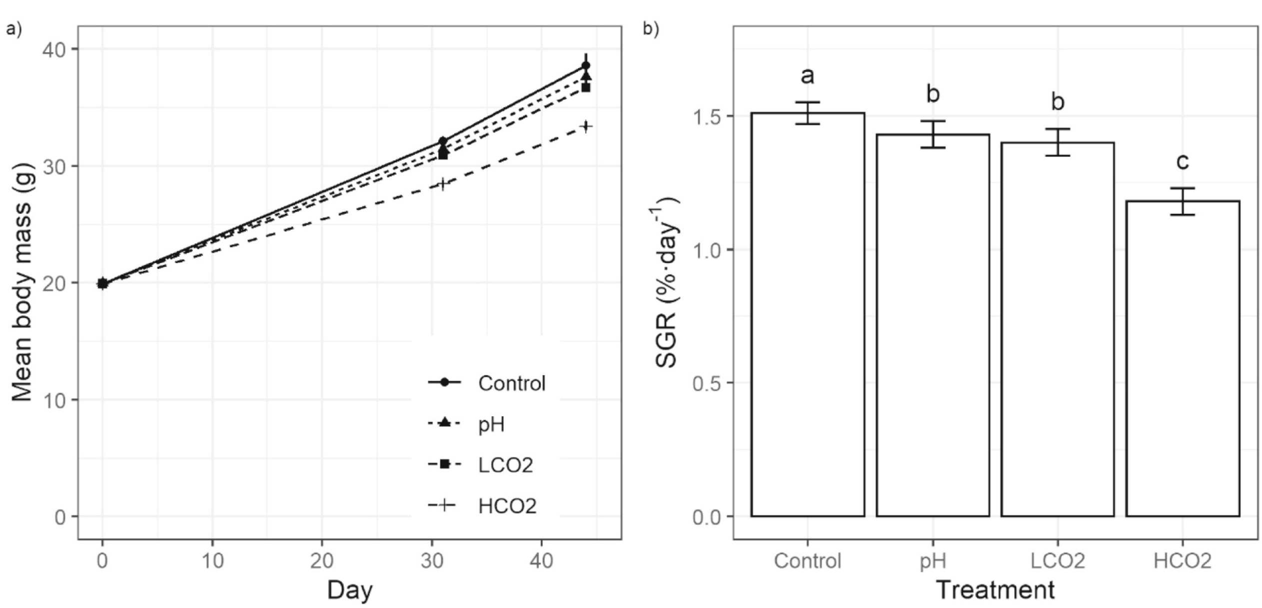

Fig. 2. The growth of lumpfish exposed to different concentrations of CO_2 and reduced pH (Experiment 1). a) Mean body mass. b) Specific growth rate. Vertical lines show standard error of the mean and significant differences in SGR are identified with different letters. Information about significant differences in mean body mass is given in text.

3.4. Growth and mortality – NH_4^+ study

The overall mortality rate was 0.5% and not significantly ($p = 0.99$) different among treatment groups. The final body-mass of the NH_4^+ 33 group was lower ($P < 0.01$) than in the Control and LpH groups (Fig. 3a). There was no significant difference in the final weight of other groups (Fig. 3a). The SGR of the TAN33 group was significantly ($P < 0.001$) lower than in the Control and LpH groups, but not significantly ($P = 0.16$) different from the TAN18 group (Fig. 3b). Moreover, the SGR in the LpH group was higher than the TAN33 group ($P < 0.05$) groups (Fig. 3b).

4. Discussion

To our knowledge, this is the first study to examine the effects of pH, TAN and CO_2, on the growth of lumpfish juveniles. The first experiment addressed the effects of low and moderate concentrations of CO_2, the maximum concentration (15 $mg \cdot L^{-1}$) being near the suggested safe limits for salmonids (Thorarensen and Farrell, 2011; Bergheim and Fivelstad, 2014). The growth of the HCO2 fish that were exposed to 15 $mg \cdot L^{-1}$ of CO_2 was significantly reduced compared with all other groups (Fig. 2a, Fig. 4). The growth of the LCO2 group was also reduced compared with the Control group (Fig. 2b). Furthermore, a similar reduction in growth was observed in both experiments in the groups where the pH was reduced, and the CO_2 concentration was near 8 $mg \cdot L^{-1}$ (Figs. 2-3.) These results show consistently that the growth, and possibly the welfare of lumpfish juveniles, is reduced at a CO_2 concentration of 8 $mg \cdot L^{-1}$ (Figs. 2b, 3b). Studies on other species, such as Atlantic cod (Moran and Støttrup, 2011; Thorarensen et al., 2018) and Atlantic salmon (Khan et al., 2018; Mota et al., 2020) also shown a small reduction in growth when exposed to a concentration of 8–10 $mg \cdot L^{-1}$ CO_2 although recommended levels for aquaculture fish are 10–15 $mg \cdot L^{-1}$ CO_2. (Bergheim and Fivelstad, 2014; Thorarensen and Farrell, 2011). However, there are some indications that the fish acclimated to the high [CO_2] since the growth trajectories of the LpH, LCO2 and HCO2 groups diverged less from that of the Control group during days 31–62 compared with days 1–30 (Fig. 2a). Exposure of fish to increased [CO_2] results in acidosis which is corrected through acclimation by increasing the [HCO_3^-] (Brauner et al., 2019). The acclimation may result in the long-term effects of [CO_2] near 8 $mg \cdot L^{-1}$ during the entire production period being minimal.

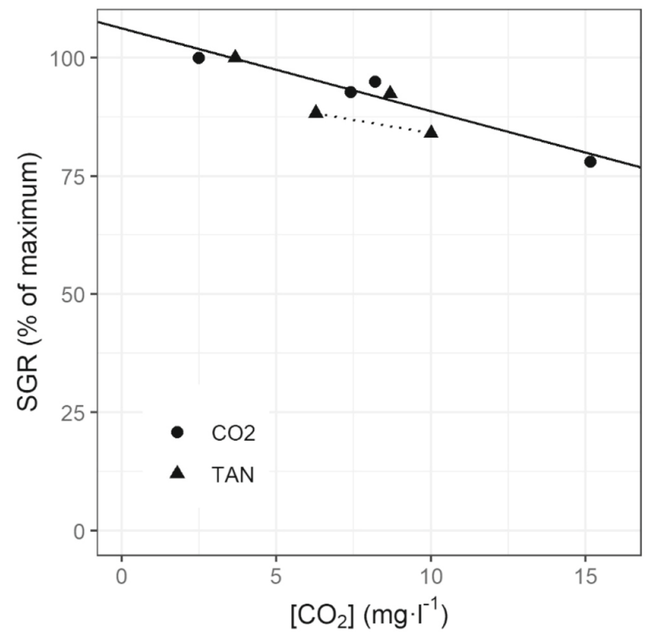

Fig. 4. The growth rate of lumpfish expressed as the % of maximum (the mean for the control groups) in each experiment. The solid regression line includes all results from experiment 1 where fish were exposed to different levels of CO_2 and the control group and low pH group in experiment 2. In all these treatments TAN was below 1 $mg \cdot L^{-1}$. The broken line connects the two groups exposed to higher TAN concentration.

There is a paucity of information on the minimum oxygen saturation required for maximum growth of lumpfish. Jørgensen et al. (2017) found reduced growth at oxygen saturation of 81% compared with 96% saturation. However, the latter was the highest oxygen saturation tested and, therefore, the critical oxygen saturation for growth may be even higher. In the present study, the oxygen saturation was 87–96% and set

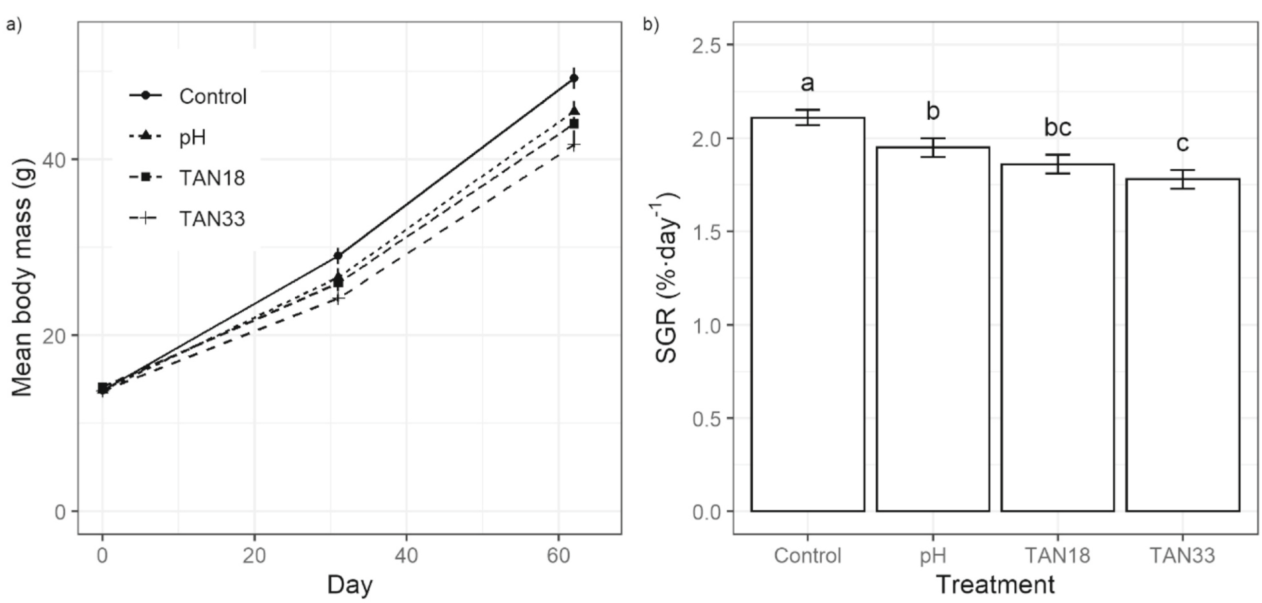

Fig. 3. The growth of lumpfish exposed to different concentrations of TAN and reduced pH (Experiment 2). a) Mean body mass. b) Specific growth rate. Vertical lines show standard error of the mean and significant differences in SGR are identified with different letters. Information about significant differences in mean body mass is given in text.

with reference to welfare recommendation for the species (Noble et al., 2019; Treasurer et al., 2018) and, therefore, higher oxygen levels might have contributed to even better growth. However, within each experiment the differences in mean oxygen saturation were small (2–3%) and unlikely to have contributed to differences in growth between treatment groups. Nonetheless, the effects of oxygen saturation on the growth of lumpfish merits further study. The growth of fish is contingent on their aerobic scope (the difference between standard and maximum metabolic rate) being large enough to support the metabolic cost of digestion and assimilation (Wang et al., 2009). The minimum oxygen saturation required for the maximum metabolic rate (and aerobic scope) of lumpfish varies with temperature and fish size, increasing with increasing temperature and decreasing with increased size of fish (Remen et al., 2022). Therefore, it is likely that the critical oxygen saturation for growth follows a similar pattern. Moreover, increased CO_2 concentrations can reduce the aerobic scope of fish, and this could affect growth performance through similar pathways as hypoxia (Skov, 2019). However, results from Hosfeld et al. (2008) suggest that increased oxygen saturation does not ameliorate the effects of increased CO_2 concentrations on the growth of Atlantic salmon parr.

The growth rate of the lumpfish in the present experiment decreased linearly with increasing CO_2 concentration (Fig. 4). Similar linear reduction in growth rate without a clear breaking point or an identifiable critical concentration over which the growth rate is reduced have also been reported for Atlantic salmon smolts and postsmolts (Khan et al., 2018; Mota et al., 2020). However, this may vary between life stages since CO_2 concentrations of 10–15 $mg{\cdot}L^{-1}$ do not appear to affect the growth of Atlantic salmon parr (Fivelstad et al., 2015). Taken together, these results suggest that the sensitivity of lumpfish to CO_2 is similar to that of Atlantic salmon smolts and Atlantic cod. Moreover, lumpfish are likely to encounter conditions in their natural habitat where the fish are affected by increased CO2 levels, for example in the demersal zone (Kennedy et al., 2016).

The results of the second experiment also confirm the sensitivity of lumpfish to poor water quality. The SGR decreased progressively with increased concentration of TAN and NH_4^+ (Fig. 3b). However, other water quality parameters, such as pH, CO_2, and nitrite, may also have contributed to reduced growth in the groups treated with increased NH_4^+. These effects will now be discussed in turn. As mentioned above, the SGR of the LpH group in this experiment was reduced compared with the Control group, likely due the elevated CO_2 concentration (Table 2, Figs. 3b, Fig. 4). The CO_2 concentration was also increased in the TAN18 (6 $mg{\cdot}L^{-1}$) and the TAN33 (10 $mg{\cdot}L^{-1}$) treatments due to reduced pH which may have contributed to reduced growth of these groups (Fig. 3b, Fig. 4). However, the SGR of the TAN18 and TAN33 groups is lower than expected based on CO_2 concentration (Fig. 4) so it is likely that other factors may also have contributed to the reduce growth. The NH_3 concentration was similar (70–80 $\mu g{\cdot}l^{-1}$ NH_3-N) in the TAN 18 and TAN33 treatments (Table 2) and above the recommended levels (3–12 $\mu g{\cdot}l^{-1}$ NH3-N) for salmonid aquaculture (Timmons et al., 2002; Bergheim and Fivelstad, 2014). However, the recommended levels for the maximum NH_3 concentration in aquaculture are primarily based on short term studies that do not account for the ability of fishes to acclimate to higher concentrations over time. Thus, salmonids can acclimate and maintain long-term growth at 30–60 $\mu g{\cdot}l^{-1}$ NH_3-N (Kolarevic et al., 2012; Becke et al., 2019). After an acclimation period of one month, spotted wolffish (*Anarhichas minor*) grew equally well at 170 $\mu g{\cdot}l^{-1}$ NH3-N as control fish (Foss et al., 2003). Similarly, Atlantic cod appear to acclimate to similar ammonia concentrations as the salmonids although growth rate is reduced at $>$100 $\mu g{\cdot}l^{-1}$ (Foss et al., 2004; Remen et al., 2008; Thorarensen et al., 2018). Therefore, increased [NH_3] in the present experiment may have had little effect on the growth of the fish.

Both nitrite (NO_2^-) and nitrate (NO_3^-) were increased in the TAN18 and TAN33 treatments and more so in the latter (Table 2). The increased TAN concentration in these treatments may have stimulated growth of nitrifying bacteria in pipes, aerators and on the tank surface (Fig. 1). The oxidation of ammonia increases the concentration of NO_2^- and NO_3^- while reducing alkalinity and all these effects are evident in the TAN18 and TAN33 treatments (Table 2). The concentration of NO_3^- was below the maximum recommended level of $<$20 $mg{\cdot}L^{-1}$ (Camargo et al., 2005). However, the concentration of NO_2^- both in the TAN33 group (4.2 $mg{\cdot}L^{-1}$) and the TAN18 group (1.5 $mg{\cdot}L^{-1}$), was above the recommended levels in salmon aquaculture (0.5 $mg{\cdot}L^{-1}$) (Bergheim and Fivelstad, 2014). Atlantic salmon parr show slightly reduced growth at 2–5 $mg{\cdot}L^{-1}$ NO_2^- (Gutirrez et al., 2019). Similarly, Atlantic cod juveniles show reduced growth at all NO_2^- concentration ≥ 1 $mg{\cdot}L^{-1}$ (Siikavuopio and Sæther, 2006). Therefore, it is likely that the increased NO_2^- concentration in the TAN18 and TAN33 groups may have contributed to the reduced growth of lumpfish. NO_2 is generally less toxic to saltwater fish than freshwater fish, although exposure could lead to serious symptoms of nitrite toxicity such as sluggishness, gill hypertrophy, and methaemoglobinemia (Eddy and Williams, 1987; Jensen, 2003). While elevated NO_2^- can cause mortality, only one fish died in the TAN33 tanks suggesting that the NO_2^- exposure was well below the lethal limits. Nevertheless, it is possible that the increased NO_2^- concentration may have contributed to the suppressed the growth of fish in the TAN18 and TAN33 treatments. One of the objectives of this study was to test the hypothesis that high concentrations of NH_4^+ affect the growth of the lumpfish. However, due to the complex changes in water quality in the TAN18 and TAN33 groups described above it is not possible to conclude about potential effects of NH_4^+ on the growth of the lumpfish based on this study.

5. Conclusion

It is concluded that lumpfish show similar sensitivity to water quality as do salmonids and Atlantic cod. The growth of lumpfish is progressively reduced as CO_2 concentration increases above 5–10 $mg{\cdot}L^{-1}$. However, the growth of lumpfish may be reduced at even lower CO_2 concentrations since we found no clear breaking point over which the growth of the fish was reduced. Therefore, the results of this study do not provide safe limits for CO_2 concentration in lumpfish aquaculture. In land-based aquaculture several water quality variables, such as oxygen, pH, CO_2, and NH_3/NH_4^+, change simultaneously and show complex interactions. Therefore, it may be difficult to clearly identify the effects of a single parameter. One of the objectives of the present experiment was to test the hypothesis that high concentrations of NH_4^+ may affect the growth of lumpfish. We found little evidence in support of the hypothesis although this effect cannot be excluded.

CRediT authorship contribution statement

Amber Christina Monroe: Investigation, Visualization, Writing – original draft. **Albert Kjartan Dagbjartarson Imsland:** Conceptualization, Funding acquisition, Project administration, Writing – review & editing. **Helgi Thorarensen:** Conceptualization, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

There is no conflict of interest in relation to this study.

Data availability

Data will be made available on request.

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