Received: 26 October 2017 | Revised: 17 July 2018 | Accepted: 26 July 2018

DOI: 10.1111/are.13812

ORIGINAL ARTICLE

WILEY Aquaculture Research

The effect of low pH on physiology, stress status and growth performance of turbot (*Psetta maxima* L.) cultured in recirculating aquaculture systems

Vasco C. Mota[1] | Jochem Hop[1] | Luís A. Sampaio[2] | Leon T. N. Heinsbroek[1] | Marc C. J. Verdegem[1] | Ep H. Eding[1] | Johan A. J. Verreth[1]

[1]Aquaculture and Fisheries Group, Wageningen University, Wageningen, The Netherlands

[2]Laboratório de Piscicultura Estuarina e Marinha, Instituto de Oceanografia, Universidade Federal do Rio Grande, Rio Grande-RS, Brazil

Correspondence
Vasco C. Mota, Nofima AS, P.O. Box 6122, NO-9291 Tromsø, Norway.
Email: motvasco@gmail.com

Funding information
Coordenação de Aperfeiçoamento de Pessoal de Nível Superior; European Commission, Grant/Award Number: FP6 CRAFT project no. 032556 (co-funded by the Eur

Abstract

We evaluated the effect of low pH and low and high total ammonia nitrogen (TAN) concentrations on the physiology, stress status and the growth performance of turbot in RAS. Two experiments were conducted. In Experiment 1, turbot (466 g) were grown at control (pH 7.5; TAN ~0.5 mg/L) or low pH and high TAN (pH 5.7; TAN ~50 mg/L) for 55 days. In Experiment 2, turbot (376 g) were grown at control (pH 7.5; TAN ~0.5 mg/L), low pH and low TAN (pH 5.7; TAN ~5 mg/L) or low pH and high TAN (pH 5.7; TAN ~50 mg/L) for 59 days. In Experiment 1, final body weight, feed intake and growth were significantly lower and FCR significantly higher in turbot exposed to low pH and high TAN. In Experiment 2, only growth was significantly lower in turbot exposed to treatment low pH and high TAN as compared to fish in the control treatment and low pH and low TAN. Osmoregulation and stress indicators measured were within normal levels. In conclusion, turbot grew equally well in a water pH of 7.5 or 5.7 provided a low TAN. In contrast, low pH combined with a high TAN impaired turbot performance.

KEYWORDS
aquaculture, fish, NH_4-N, nitrification, total ammonia nitrogen (TAN)

1 | INTRODUCTION

Fish production in recirculating aquaculture systems (RAS) has been gaining momentum with an increasing number of recently built facilities producing various species, ranging from cold seawater Atlantic salmon (*Salmo salar*) to warm freshwater Nile tilapia (*Oreochromis niloticus*) (Dalsgaard et al., 2013; Liu et al., 2016; Martins et al., 2010). Turbot (*Psetta maxima*) production has also significantly increased during the last decade, with several RAS currently in operation (Dalsgaard et al., 2013; Mota, Martins, Eding, Canário & Verreth, 2014; Person-Le Ruyet, 2002). However, turbot production in RAS still needs further improvement; the growth performance usually observed in RAS during the on-growing phase is lower than the potential growth recorded in flow-through systems (Person-Le Ruyet, 2002). This growth reduction was also observed in RAS for other fish species, such as Nile tilapia (Mota, Limbu, Martins, Eding & Verreth, 2015) and European seabass (*Dicentrarchus labrax*) (Deviller et al., 2005). Although several factors can affect fish growth, such as food availability, nutrition and social interactions, the environmental conditions in the culture system are a particularly relevant factor in RAS due to the build-up of substances such as ammonia (Eding, Kamstra, Verreth, Huisman & Klapwijk, 2006), nitrate (Davidson, Good, Williams &

Summerfelt, 2017), carbon dioxide (Stiller et al., 2015) and hormones (Mota, Martins, Eding, Canário & Verreth, 2017) as a consequence of water reuse (Colt, 2006).

Low water pH can potentially improve fish growth by chemical shifting fish metabolites towards less toxic substances and by changing the configuration and activity of the accumulated substances (Ip, Chew, Randall, Patricia & Paul, 2001; Martins, Ochola, Ende, Eding & Verreth, 2009). For example, at low pH, ammonia is mainly present as ammonium which is relatively less toxic. Further, at the RAS level, low pH can improve carbon dioxide removal in trickling biofilters (Eding et al., 2006) and CO_2 degassers in general, by shifting the carbonate system into carbon dioxide. Moreover, low water pH may improve fish performance by decreasing the disease pressure and vulnerability to parasites. This lower pathogen pressure may be one of the factors behind the improved growth and production of European eel (*Anguilla anguilla*) cultured in RAS at a low pH of 4.5–5.5. Low pH conditions have also been used in RAS producing other fish species, such as Dover sole (*Solea solea*), African Catfish (*Clarias gariepinus*) and Nile tilapia (*Oreochromis niloticus)* (Mota et al., 2014). However, several adverse effects have been reported for fish exposed to low environmental pH, such as reduced growth and feed intake (Abbink et al., 2012; Kennedy & Picard, 2012). Moreover, low pH can increase cortisol level, which causes a transient depression on the nonspecific immune activity (Brown, MacLatchy, Hara & Eales, 1990) and can result in plasma acidosis and a concomitant reduction in plasma ions (McDonald, Walker, Wilkes & Wood, 1982). Also, acute acid stress induced acidosis and morphological histopathologies on gill and skin of juvenile cobia (*Rachycentron canadum*) (Rodrigues, Pedron, Romano, Tesser & Sampaio, 2015). When enough acclimation time is provided, the mentioned negative effects seem to be mitigated, Nagae et al. (2001) and van Ginneken, Van Eersel, Balm, Nieveen and Van Den Thillart (1997) show no effect, and D'Cruz, Dockray, Morgan and Wood (1998) and Dockray, Reid and Wood (1996) show even a positive effect on feed intake and growth.

However, little is known about the effects of producing marine species with low pH conditions due to the high buffering capacity of seawater. For instance, Allan and Maguire (1992) found an acceptable growth (<5% growth reduction) of black tiger shrimp (*Penaeus monodon*) with a pH of 5.9. In contrast, Lemarié et al. (2000) observed a 40% growth reduction for seabass cultured at a pH of 5.5. However, this effect might be confounded by a high carbon dioxide concentration. Eshchar, Lahav, Mozes, Peduel and Ron (2006) showed no effect of low pH/high ammonium concentration on seabream (*Sparus auratus*) cultured in a marine RAS. Nevertheless, the latter study only tested pH levels down to 6.8.

Although water pH in RAS is relatively stable, it can drop with (1) alkalinity consumption during nitrification and (2) fish metabolic carbon dioxide excretion. Usually, these two sources of pH drop are counteracted with a carbon dioxide removal unit and with the supply of an alkalinity source like sodium bicarbonate. Decreasing water pH in RAS can have positive effects beyond fish performance; pH drives the ammonia equilibrium in culture water, which has major implications on ammonia toxicity for fish (Ip et al., 2001; Thurston, Russo & Vinogradov, 1981). Low-pH environments increase the NH_4-N fraction from TAN, which is thought to be 300–400 times less toxic for fish than the NH_3-N fraction (Person-Le Ruyet, Galland, Le Roux & Chartois, 1997; Thurston et al., 1981). Managing water pH can be an alternative strategy to maintain NH_3-N at safe conditions to culture fish while TAN accumulates at high levels reducing the biofiltration needs. For instance, Eshchar et al. (2006) showed that intensive flow-through system can operate without biological filtration by changing water pH and still maintain NH_3-N at safe levels for seabream (*Sparus aurata*).

Several studies have investigated the effect of low water pH on several fish species physiology and growth (Abbink et al., 2012; Kennedy & Picard, 2012; Nagae et al., 2001). However, the effect of low pH on physiology, stress status and growth performance of turbot cultured in RAS is unknown. In addition, it is important to differentiate between fish-specific effects of low pH and any confounding effects resulting from operating a low-pH RAS, such as the increase of the NH_4-N fraction from the total ammonia nitrogen (TAN), with the respective decrease of the NH_3-N fraction. This study contributes to this gap of knowledge by testing whether turbot physiology and performance are affected when cultured at low pH, and by distinguishing the effect of low water pH from the effect of high water TAN. Specifically, we evaluated the effect of low pH and low and high TAN concentrations on the physiology, stress status and the growth performance of turbot cultured in RAS.

2 | MATERIALS AND METHODS

2.1 | Experimental design

Two experiments were conducted.

Experiment 1 compared two treatments, each with three replicated RAS, during a 55-day experimental period: a control treatment (control: pH 7.5; TAN ~0.5 mg/L) and a low-pH and high-TAN treatment ($L_{pH}H_{TAN}$: pH 5.7; TAN ~50 mg/L).

Experiment 2 compared three treatments, each with two replicated RAS, during a 59-day experimental period: a control treatment (control: pH 7.5; TAN ~0.5 mg/L), a low-pH and high-TAN treatment ($L_{pH}H_{TAN}$: pH 5.7; TAN ~50 mg/L) and a low-pH and low-TAN treatment ($L_{pH}L_{TAN}$: pH 5.7; TAN ~5 mg/L).

Note that the TAN concentration in the $L_{pH}H_{TAN}$ treatment of Experiment 1 was let increase (minimum ~2 mg/L – maximum ~100 mg/L), whereas it was kept constant in Experiment 2 (~50 mg/L). The third treatment ($L_{pH}L_{TAN}$) in Experiment 2 was added to distinguish the effect of low pH from the effect of high TAN.

Water pH, in the tested conditions, dropped due to the alkalinity consumption during nitrification, and it was maintained within the target range by adding sodium bicarbonate when necessary. Water TAN levels were monitored daily and, if necessary, ammonium chloride solution was added to keep the levels within the treatment values.

2.2 | Recirculating aquaculture systems

Each RAS (Figure 1) was composed of a circular fish tank (V = 300 L, 0.72 m^2), a sedimentation unit (V = 75 L, hydraulic surface load 150 m^3 m^{-2} day^{-1}), a sump (V = 75 L) with an UV unit (UV-C; 36 W), a water flow control (1.2 – 1.4 m^3/hr), a cooler/heater (TC20, Teco, Italy) and a trickling filter (media: Bio-net, specific surface area 200 m^2/m^3, installed surface area 10.8 m^2; V = 0.054 m^3). Total system water volume was approximately 510 L. For Experiment 2, two RAS were modified by adding an additional loop to keep TAN <10 mg/L at low-pH ($L_{pH}L_{TAN}$) treatment. This loop consisted of a partial bypass in which water was flowing from the sump into a container tank (V = 20 L), then pumped into the bottom section of an up-flow submerged biofilter (media: same as the trickling filter, V = 80 L) and returned from the biofilter top end by gravity back into the sump. To assure a proper water mix, a pump was installed at the top section of the submerged biofilter. The supply of sodium bicarbonate to the container tank was actively controlled using a pH controller pump (Endress-Hauser Liquisys M, Endress+Hauser, Canada). Dissolved oxygen was also continuously supplied just after the container tank. Water exchange rate for Experiment 1 was ~250 and 100 L/kg feed for control treatment and $L_{pH}H_{TAN}$ treatment, respectively, and 500 L/kg feed for all three treatments of Experiment 2.

2.3 | Fish and feeding

All procedures involving animals were carried out in accordance with the Dutch law and were approved by the Animal Experiments Committee of Wageningen University, Wageningen, The Netherlands, with the reference numbers 2008023.b and 2008127.c.

Juvenile turbot were obtained from a commercial turbot farm (GroVisCo B.V., The Netherlands) and transported to the experimental facilities (Wageningen University, The Netherlands). Fish were randomly distributed over the six fish tanks to adapt to the rearing and feeding conditions for 1 week (Experiment 1) and 2 weeks (Experiment 2). During the adaptation period, water quality was maintained as follows: temperature 16–17°C, salinity 14–15 ppt, dissolved oxygen > 7 mg/L, pH 7.2–7.8, TAN < 2 mg/L, NO_2-N < 2.5 mg/L, NO_3-N < 15 mg/L and photoperiod 18L:6D. At the start of Experiment 1, the initial fish number was 138 (23 fish per RAS), mean body weight was 466 g, and mean tank density was approximately 36 kg/m^3 (15 kg/m^2). At the start of Experiment 2, the initial fish number was 150 (25 fish per RAS), mean body weight was 376 g, and mean tank density was 31 kg/m^3 (13 kg/m^2).

Fish were fed twice a day (09:00 and 17:00) until apparent satiation with a commercial dry pellet (9 mm, DAN-EX, Dana feed, Denmark). Feed composition was as follows: 88.0% dry matter, 9.5% ash, 50.6% protein, 18.0% fat, 1.24% phosphorus and gross energy 20.9 kJ/g feed dry matter. Feeding was stopped when three to six pellets remained uneaten. After each meal, uneaten pellets were collected and counted. Weight of individual pellets was determined to calculate feed intake.

2.4 | Water quality measurements

Temperature, salinity, pH and dissolved oxygen were measured once a day (09:00) at Experiment 1 or twice a day (09:00 and 17:00) at

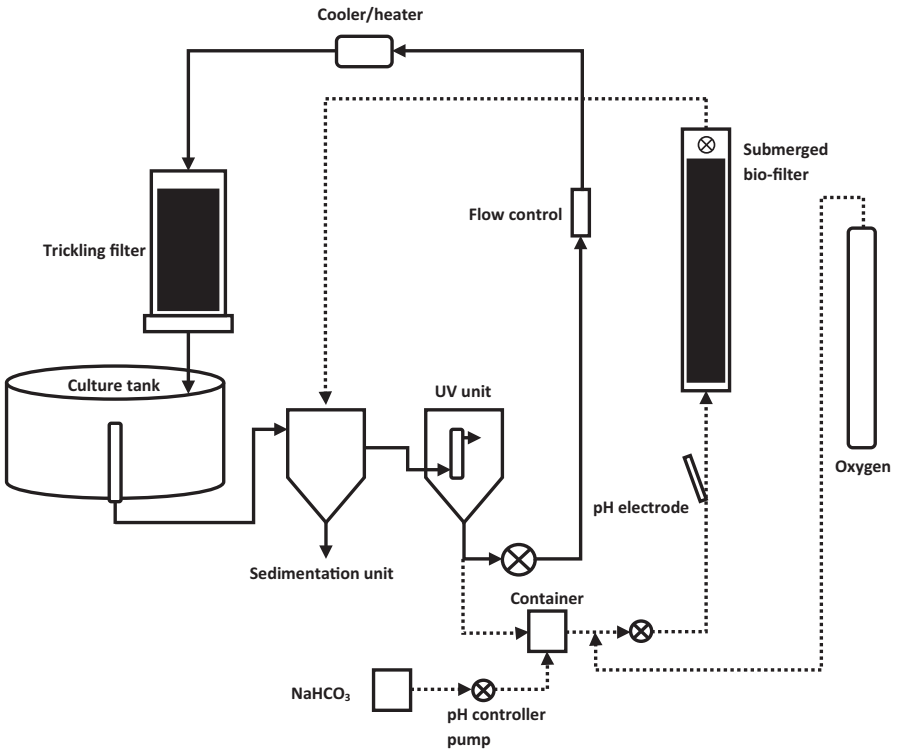

FIGURE 1 Scheme of the recirculating aquaculture systems used to perform the experiments. Dotted lines represent the loop added to the $L_{pH}L_{TAN}$ treatment in Experiment 2

Experiment 2 before feeding in the outlet of the fish tank using portable metres. The analytical water samples for N were analysed with an autoanalyser (SAN Plus, Skalar, The Netherlands) for total ammonia nitrogen (TAN) (Skalar protocol number 155-006 w/r), NO_2-N (Skalar protocol number 467-003) and NO_x-N (Skalar protocol number 461-318). NO_3-N was calculated as NO_x-N–NO_2-N. The NH_3-N and NH_4-N concentrations were calculated from the TAN concentrations as a function of pH, temperature and salinity (Johansson & Wedborg, 1980).

2.5 | Fish, feed and faeces measurements and sampling

At the start and end of both experiments, total fish body weight was determined to the nearest 1.0 g after 24 hr of starvation. Fish were anaesthetized (TMS, Crescent Research Chemicals, USA) prior to handling.

Feed intake was expressed per metabolic body weight and calculated as follows:

$$\text{Relative feeding rate } (\text{RFR}_m, \text{g/kg}^{0.8}/\text{day}) = \text{FI}/(W_{\text{mean}}/1000)^{0.8}$$

where a metabolic weight exponent of 0.8 was used (Dietz, Kroeckel, Schulz & Susenbeth, 2012), FI is the average feed intake per fish per day (in g/fish/day), and W_{mean} is the geometric mean body weight (in g), calculated as follows:

$$W_{\text{mean}}(g) = (W_i \times W_f)^{0.5}$$

where W_i and W_f are the initial and final average individual fish weight (in g) respectively.

Growth was expressed per metabolic body weight and calculated as follows:

$$\text{Relative growth rate } (\text{RGR}_m, \text{g/kg}^{0.8}/\text{d}) = [\text{GR}/(W_{\text{mean}}/1000)^{0.8}]/t$$

where GR is the average weight gain per fish per day (g/fish/d) and t is the experimental duration in days.

Thermal growth coefficient (TGC) was calculated as follows:

$$\text{TGC} = 1000 \times (W_f^{1/3} - W_i^{1/3})/(T \times \Delta t)$$

where T is the water temperature in °C and Δt is the number of days between time T_1 and T_2.

Feed conversion ratio (FCR) was calculated as follows:

$$\text{FCR}(\text{DM g/g}) = \text{FI}_{\text{DM}}/(W_f - W_i)$$

where FI_{DM} is the total feed intake in dry matter per fish over the experimental period.

For fish body composition analysis, five fish in total were randomly sampled from the total population and analysed at the beginning of the experiment (Day 0) and thirty fish (5 fish/tank) were analysed at the end of Experiment 2 (Day 59). Fish were killed with an anaesthetic overdose (TMS) and frozen at −20°C. For feed composition analysis, a sample of the feed used during experiments was collected and stored refrigerated. For nutrient digestibility measurements, faeces were collected from the third week onwards (Experiment 2) during a 72-hr period each week. Faecal collection was performed with a glass bottle attached to a sedimentation unit (Figure 1), covered with ice, and faeces collected twice a day and immediately frozen at −20°C. Fish samples were grounded and homogenized in a mincing machine (Model TW-R 70, FEUMA Gastromaschinen GmbH, Germany). Faeces and feed samples were homogenized and, for some analysis, freeze-dried and grounded with a centrifugal grinding mill (Retsch/Brinkmann ZM 100/w 1.1 mm sieve; Verder NV, The Netherlands). Proximate composition of fish, feed and faeces was determined according to the ISO standard analysis for determination of dry matter (DM; ISO 6496, 1983), crude ash (ISO 5984, 1978), acid insoluble ash (AIA; ISO 5985, 1981), crude fat (ISO 6492, 1999), crude protein (ISO 5983, 1997, crude protein = Kjeldahl-N × 6.25), energy (ISO 831,1998) and phosphate (Autoanalyzer, Skalar protocol number 503-011). Briefly, dry matter was determined by drying the samples for 4 hr at 103°C, ash content was determined by ashing the samples for 4 hr at 550°C, and AIA was determined by boiling the ash residual in 0.1N H_2SO_4 for 15 min, filtered over ash-free filter paper, and re-ashed for 4 hr at 550°C. Protein content was determined as N × 6.25 following the Kjeldahl procedure after acid digestion, fat content was determined by the Soxhlet extraction with petroleum ether for 4 h, gross energy was determined by bomb calorimetry (IKA-C-7000; IKA Analysentechnik, Germany), and total phosphate was determined by spectophotometric analysis of orthophophate (SAN autoanalyser) after digestion of the ashed sample.

The apparent digestibility coefficient (%) was calculated as follows:

$$\text{ADC } (\%\text{ of nutrient}) = (1 - (\text{AIA}_{\text{diet}}/\text{AIA}_{\text{faeces}} \times \text{Nutrient}_{\text{faeces}}/\text{Nutrient}_{\text{diet}})) \times 100\%$$

The maintenance energy requirement was calculated as follows:

$$\text{Maintenance energy requirement } (\text{kJ/kg}^{0.8}/\text{d})\text{DE}_m(\text{kJ/kg}^{0.8}/\text{d}) = \text{DE}_I - 1/k_{gDE} \times \text{RE}$$

where DE_I is energy intake (kJ fish^{-1} day^{-1}) and RE is retained energy (kJ/fish/d).

2.6 | Blood parameters

At the end of the Experiment 2, ten fish per tank were caught, anaesthetized (TMS), and killed with a blow on the head. Blood (2 ml) was collected from caudal blood vessels using a hypodermic syringe previously flushed with heparin (Leo, 5000 IE/ml). Whole blood was immediately used for heamatocrit determination by filling two microcapillary tubes and centrifugation at 3,000 g for 10 min. The remaining blood was centrifuged at 3,000 g for 10 min at 4°C, and the plasma stored in Eppendorf tubes and frozen (−20°C) for further analysis. The following parameters were measured from blood plasma samples: osmolality (by the test-time freezing-point depression method on a micro osmometer, Advanced Instruments,

Netherlands), chloride concentration (Jenway PCL M3 chloride meter, Jenway Limited, England), TAN (Ammonia Assay Kit, Sigma-Aldrich, USA), cortisol (Enzyme-Linked Immunosorbent Assay Kit, Neogen Corporation, USA), glucose (GOD-POP method, Spinreact, Spain) and lactate (using the principle of conversion of lactic acid into pyruvate and hydrogen peroxide by lactate oxidase, Lactate Reagent 735-10; Trinity Biotech, Ireland).

2.7 | Statistics

Statistical analyses were performed with IBM SPSS Statistics V24 (IBM, Corp., USA). The experimental unit considered was the fish tank (n = 3, Experiment 1, or n = 2, Experiment 2) for all parameters with the exception of blood parameters where fish were considered the experimental unit (n = 10, Experiment 2). Percentage data were transformed using arcsin $[\sqrt{(x+1)}]$ prior to statistical analysis. Analysis of water quality and fish performance was conducted using a t test (Experiment 1) or one-way ANOVA (Experiment 2), to test for overall differences among treatments. Analyses of blood parameters were conducted using analysis of covariance (ANCOVA) using final fish weight as a covariate (Packard & Boardman, 1999). Homogeneity of variances was previously tested using Levene's test. When significant, ANOVAs and ANCOVAs were followed by a Tukey HSD test to identify differences among treatments. Feed intake was analysed by repeated-measures t test (Experiment 1) or repeated-measures ANOVA (Experiment 2) to compare the treatment (pH/TAN) and time (experimental weeks) effect through the two experimental periods. Sphericity was tested using Mauchly's test, and when this assumption was violated, the Greenhouse-Geisser correction was applied. Pairwise comparisons were used to identify differences among treatments. A significance level (α) of 0.05 was used. Data are expressed as mean ± standard deviation (*SD*).

3 | RESULTS

3.1 | Water quality

Water quality parameters measured during the two experiments are summarized in Table 1 and Figure 2. Dissolved oxygen was significantly lower in the control treatment than in the $L_{pH}H_{TAN}$ treatment in Experiment 1, 7.0 ± 0.3 and 7.3 ± 0.3 mg/L respectively ($p < 0.05$). Temperature and salinity (Table 1) were maintained within the target range and were similar among treatments in both experiments. Water pH was similar for both experiments, with a nearly constant pH of 7.5 in the control treatment, and 5.7 in the $L_{pH}L_{TAN}$ and $L_{pH}H_{TAN}$ treatments after the first week (Figure 2). Because the NH_4-N fraction comprised close to 100% of the TAN concentration in all treatments of both experiments, only TAN results are described here. The average TAN concentrations were similar between the same treatments of both experiments (Table 1); however, TAN continuously increased during Experiment 1 for $L_{pH}H_{TAN}$ (min 2.7–max. 101.4 mg/L of TAN), whereas in Experiment 2, TAN remained constant after the first week (min 41.1–max. 53.9 mg/L of TAN) (Figure 2). The NH_3-N concentrations remained low (<0.016 mg/L)

TABLE 1 Summary of water quality parameters measured at the fish tank effluent for experiments 1 and 2

Parameters	Control	$L_{pH}H_{TAN}$	$L_{pH}L_{TAN}$	*p*-value
Experiment 1[1]				
Temperature (°C)	17.7 ± 0.6	17.4 ± 0.7		0.20
Salinity (ppt)	15.3 ± 0.3	15.4 ± 0.2		0.37
Dissolved oxygen (mg/L)	7.0 ± 0.3	7.3 ± 0.3		**0.02**
pH	7.48 ± 0.02	5.78 ± 0.02		**<0.01**
TAN (mg/L)[2]	0.3 ± 0.0 (0.2–0.4)	48.7 ± 2.3 (2.7–101.4)		**<0.01**
NH_3-N (μg/L)	3.0 ± 0.0	9.0 ± 1.4		**<0.01**
NO_2-N (mg/L)	0.30 ± 0.05	0.09 ± 0.01		**<0.01**
NO_3-N (mg/L)	142.1 ± 3.9	125.9 ± 1.8		**<0.01**
Experiment 2[3]				
Temperature (°C)	17.5 ± 0.0	17.4 ± 0.1	17.7 ± 0.1	0.21
Salinity (ppt)	15.2 ± 0.0	15.2 ± 0.3	14.9 ± 0.0	0.22
Dissolved oxygen (mg/L)	7.7 ± 0.2	8.0 ± 0.0	7.9 ± 0.2	0.20
pH[4]	7.44 ± 0.04[a]	5.73 ± 0.09[b]	5.75 ± 0.06[b]	**<0.01**
TAN (mg/L)[2, 4]	0.5 ± 0.0[a] (0.4–0.6)	48.2 ± 0.6[b] (41.1–53.9)	5.4 ± 3.0[c] (3.5–8.0)	**<0.01**
NH_3-N (μg/L)[4]	4.0 ± 0.3[a]	7.8 ± 0.4[b]	1.0 ± 0.6[c]	**<0.01**
NO_2-N (mg/L)	0.59 ± 0.06[a]	0.15 ± 0.01[b]	0.15 ± 0.03[b]	**<0.01**
NO_3-N (mg/L)	106.3 ± 4.3[a]	90.0 ± 1.5[b]	62.2 ± 1.0[c]	**<0.01**

[1]Values are given as mean ± *SD*, n = 3. [2]TAN values are given as mean ± *SD* and (minimum and maximum). [3]Values are given as mean ± *SD*, n = 2. Values with different superscripts in the same line and p-values in bold indicate significant differences among groups ($p < 0.05$). [4]Mean excludes the first week measurements

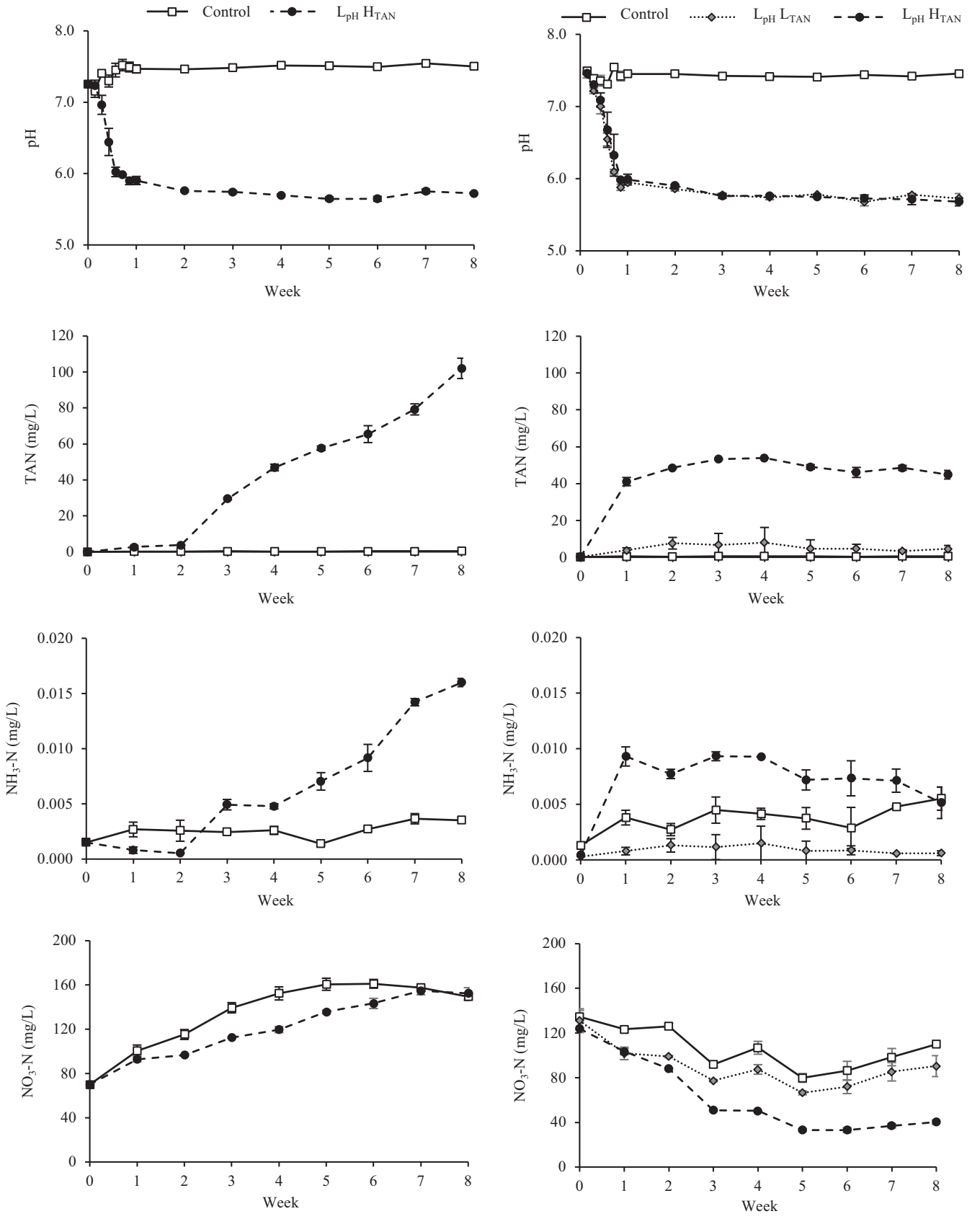

FIGURE 2 Water quality parameters, pH, TAN, NH_3-N and NO_3-N, in fish tank outlets during experiments 1 and 2. Values are presented as mean ± *SD*. *n* = 3 (Experiment 1) and *n* = 2 (Experiment 2) for all parameters

for all treatments. Nitrite (NO_3-N) increased up to 160 mg/L during the first 7 weeks in Experiment 1 and remained under 150 mg/L thereafter by daily adjusting water exchange rates (Figure 2). In Experiment 2, the daily water exchange rate was fixed at 500 L/kg feed for all three treatments, which lead to different NO_3-N mean concentrations (control: 106.3 ± 4.3, $L_{pH}H_{TAN}$: 90.0 ± 1.5 and $L_{pH}L_{TAN}$: 62.2 ± 1.0 mg/L).

3.2 | Growth performance and survival

Fish were healthy during both experiments, with high turbot survival (> 95%) and no significant differences among treatments (Table 2). In Experiment 1, final body weight, feed intake and growth were significantly lower ($p < 0.05$) and FCR significantly higher ($p = 0.03$) in turbot exposed to low pH and high TAN ($L_{pH}H_{TAN}$) as compared to fish in the control treatment. In Experiment 2, no differences were observed among treatments for the various performance parameters with the exception of growth that was significantly lower ($p = 0.03$) in turbot exposed to low pH and high TAN ($L_{pH}H_{TAN}$) as compared to turbot only exposed to low pH ($L_{pH}L_{TAN}$) or to control conditions.

TABLE 2 Summary of performance parameters measured in juvenile turbot exposed to high pH (*control*), low pH ($L_{pH}L_{TAN}$) and low pH together with high TAN ($L_{pH}H_{TAN}$)

Parameters	Control	$L_{pH}H_{TAN}$	$L_{pH}L_{TAN}$	*p*-value
Experiment 1[1]				
Survival (%)	97 ± 3	95 ± 0		0.32
IBW (g/fish)	458 ± 14	475 ± 13		0.23
FBW (g/fish)	689 ± 10	627 ± 22		**0.01**
Feed intake ($g/kg^{0.8}/d$)[1]	4.81 ± 0.31	3.81 ± 0.33		**0.02**
RGR ($g/kg^{0.8}/d$)	6.78 ± 0.57	4.59 ± 0.50		**0.01**
TGC	1.15 ± 0.08	0.79 ± 0.08		**<0.01**
FCR (DM g/g)	0.71 ± 0.03	0.83 ± 0.06		**0.03**
Experiment 2[3]				
Survival (%)	98 ± 3	98 ± 3	98 ± 3	1.00
IBW (g/fish)	378 ± 6	373 ± 3	375 ± 4	0.57
FBW (g/fish)	613 ± 23	550 ± 16	601 ± 1	0.06
Feed intake ($g/kg^{0.8}/d$)[2]	5.47 ± 0.11	4.41 ± 0.13	4.80 ± 0.51	0.09
RGR ($g/kg^{0.8}/d$)	7.24 ± 0.36[a]	5.75 ± 0.36[b]	7.06 ± 0.11[a]	**0.03**
TGC	1.22 ± 0.05[a]	0.97 ± 0.04[b]	1.17 ± 0.01[a]	**0.03**
FCR (DM g/g)	0.76 ± 0.02	0.77 ± 0.03	0.68 ± 0.08	0.33

Notes. IBW: initial body weight; FBW: final body weight; RGR: relative growth rate; TGC: thermal growth coefficient; FCR: feed conversion ratio
[1]Values are given as mean ± *SD*. $n = 3$ for all parameters. [2]Feed is expressed in dry matter and feed spillage is subtracted. [3]Values are given as mean ± *SD*. $n = 2$ for all parameters. Values with different superscripts in the same line and *p*-values in bold indicate significant differences among groups ($p < 0.05$).

Turbot growth was 32% and 21% lower in $L_{pH}H_{TAN}$ compared to fish in control treatments of experiments 1 and 2 respectively. Figure 3 shows that feed intake in the $L_{pH}H_{TAN}$ treatment remained significantly lower ($p < 0.05$) than in the control treatment on the Experiment 1 third week onwards, whereas feed intake levels in all three treatments similarly increased through Experiment 2 ($p = 0.08$).

3.3 | Energy mass balance and digestibility

Apparent digestibility coefficients and retention efficiency were similar among experimental treatments in Experiment 2 ($p > 0.05$; Table 3). No energy balance differences were observed among experimental treatments for Experiment 2 (Table 4), apart from retained energy that was significantly lower ($p < 0.05$) in $L_{pH}H_{TAN}$ (28.2 ± 0.6 kJ^{-1} $fish^{-1}$ day^{-1}) as compared to the control treatment (36.4 ± 2.4 kJ^{-1} $fish^{-1}$ day^{-1}).

3.4 | Blood parameters

Blood physiology parameters measured at the end of Experiment 2 are shown in Table 5. Haematocrit, cortisol and glucose levels were

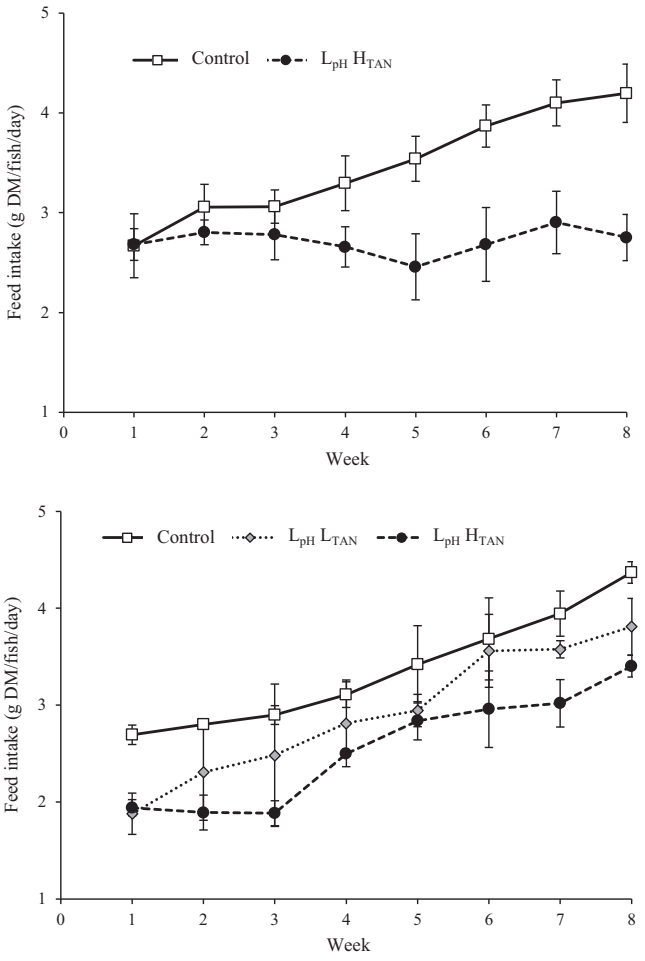

FIGURE 3 Feed intake during experiments 1 and 2. Values are presented as mean ± *SD*. $n = 3$ (Experiment 1) and $n = 2$ (Experiment 2) for all parameters

TABLE 3 Apparent digestibility coefficients, excretion and retention efficiency of turbot exposed to high pH (control), low pH ($L_{pH}L_{T\text{-}N}$) and low pH together with high TAN ($L_{pH}H_{TAN}$) for 59 days (Experiment 2)

	Control	$L_{pH}H_{TAN}$	$L_{pH}L_{TAN}$	*p*-value
Apparent digestibility coefficients (%)[a]				
Dry matter	83.1 ± 3.1	76.9 ± 1.8	72.7 ± 5.0	0.11
Nitrogen	92.0 ± 1.2	86.4 ± 2.6	84.1 ± 4.6	0.09
Phosphorus	39.4 ± 4.6	44.7 ± 5.3	31.8 ± 6.6	0.16
Energy	87.1 ± 2.3	81.2 ± 2.4	78.1 ± 4.7	0.09
Retention efficiency (% intake)				
Nitrogen	43.7 ± 1.9	45.4 ± 3.1	48.7 ± 5.4	0.50
Phosphorus	41.4 ± 2.6	42.1 ± 2.3	45.9 ± 4.9	0.47
Energy	50.5 ± 1.2	50.8 ± 1.0	49.9 ± 2.4	0.87

Notes. Values are given as mean ± *SD*. $n = 2$ for all parameters [a]After correction for salt in the settled faeces.

not affected ($p > 0.05$) by the different experimental treatments. Osmolality and chloride were significantly lower ($p < 0.05$) in turbot exposed to $L_{pH}L_{TAN}$ compared to specimens exposed to low pH in combination with high TAN ($L_{pH}H_{TAN}$). TAN blood concentration was significantly higher ($p < 0.03$) in $L_{pH}H_{TAN}$ (5.93 ± 4.19 μg/L) compared to $L_{pH}L_{TAN}$ (3.09 ± 1.74 μg/L) and similar to fish in the control treatment (3.61 ± 3.11 μg/L). Lactate blood levels were significantly lower ($p < 0.01$) in turbot exposed to low pH ($L_{pH}L_{TAN}$ and $L_{pH}H_{TAN}$) as compared to control.

4 | DISCUSSION

This study showed similar turbot survival and performance cultured in a low pH of 5.7 ($L_{pH}L_{TAN}$) or high pH of 7.5 (control) provided a low-TAN RAS environment. Nutrient digestibility and retention, energy balance, maintenance requirements, stress status and ion regulation were also not affected by exposure to low pH conditions. On the other hand, juvenile turbot raised in low pH combined with a high TAN ($L_{pH}H_{TAN}$) in a RAS environment showed reduced growth and feed intake, coupled to poorer FCR.

The acclimation capacity to low-pH environments seems to be species-specific, where some species exhibit increased growth and no ionoregulatory disturbance, such as rainbow trout (Dockray, Morgan, Reid & Wood, 1998; Dockray et al., 1996), while others are extremely sensitive and cannot acclimate, such as juvenile Pacific sockeye salmon (*Oncorhynchus nerka*) (Kennedy & Picard, 2012). Low-pH environment has been associated with high mortality (Abbink et al., 2012), reduced feed intake and growth (Kennedy & Picard, 2012), increased ion permeability of the gill integumental epithelium (McDonald et al., 1982; Wendelaar Bonga, 1997), significant misbalances of sodium and chloride ions (Butler, Day & Namba, 1992) and disturbances in haematology, fluid volume

TABLE 4 Energy balance and maintenance requirement of turbot exposed to high pH (control), low pH ($L_{pH}L_{TAN}$) and low pH together with high TAN ($L_{pH}H_{TAN}$) for 59 days (Experiment 2)

	Control	$L_{pH}H_{TAN}$	$L_{pH}L_{TAN}$	*p*-value
Energy balance (kJ^{-1} $fish^{-1}$ day^{-1})				
Energy intake	72.2 ± 3.0	55.4 ± 2.4	62.7 ± 6.9	0.08
Faecal energy	9.4 ± 0.4	7.2 ± 0.3	8.2 ± 0.9	0.08
Branchial and urinary energy	3.5 ± 0.0	2.6 ± 0.1	2.8 ± 0.6	0.16
Heat energy	22.0 ± 1.5	17.6 ± 1.5	20.6 ± 3.4	0.18
Retained energy	36.4 ± 2.4^{a}	28.2 ± 0.6^{b}	31.2 ± 2.0^{ab}	**<0.05**
Maintenance energy requirement ($kJ/kg^{0.8}/d$)	19.4 ± 1.9	15.1 ± 2.0	18.1 ± 5.7	0.56

Notes. Values are given as mean ± *SD*. $n = 2$ for all parameters. Values with different superscripts in the same line and *p*-values in bold indicate significant differences among groups ($p < 0.05$).

TABLE 5 Blood physiology of fish exposure to high pH (control), low pH ($L_{pH}L_{TAN}$) and low pH together with high TAN ($L_{pH}H_{TAN}$) for 59 days (Experiment 2)

	Control	$L_{pH}H_{TAN}$	$L_{pH}L_{TAN}$	*p*-value
Haematocrit (%)	15.97 ± 4.54	17.21 ± 4.95	18.32 ± 5.61	0.70
Osmolality ($mOsm/Kg/H_2O$)	319 ± 4^{ab}	320 ± 4^{b}	316 ± 3^{a}	**0.04**
Chloride (mmol/L)	132 ± 5^{a}	139 ± 4^{b}	131 ± 3^{a}	**<0.01**
TAN (μg/L)	3.61 ± 3.11^{ab}	5.93 ± 4.19^{b}	3.09 ± 1.74^{a}	**0.03**
Cortisol (ng/ml)	0.62 ± 0.37	1.21 ± 2.35	2.78 ± 6.12	0.37
Glucose (mmol/L)	2.25 ± 1.09	1.92 ± 0.47	2.01 ± 0.65	0.07
Lactate (mmol/L)	0.26 ± 0.14^{a}	0.15 ± 0.13^{b}	0.06 ± 0.05^{b}	**<0.01**

Notes. Values are given as mean ± *SD*. $n = 10$ for all parameters. Values with different superscripts in the same line and *p*-values in bold indicate significant differences among groups ($p < 0.05$).

distribution and circulatory function (Milligan & Wood, 1982). The majority of these studies addressed the effect of low pH in the context of acidification of freshwater ecosystems, where other variables resulting from a low-pH environment, such as ammonium (NH_4-N) (Eshchar et al., 2006) and aluminium (Brown et al., 1990; Fivelstad et al., 2003) toxicity likely affected fish performance. Likewise, in seawater acidification studies, the conversion of carbonates to carbon dioxide may result in high dissolved carbon dioxide environments and thus a factor contributing to reduced fish performance in low-pH environments (Abbink et al., 2012; Stiller et al., 2015). Moreover, the rate of acidification and the absence of additional stressors seems to be an important factor driving the capacity to withstand long-term exposure to low environmental pH, as shown for Mozambique tilapia (*Oreochromis mossambicus*) (van Ginneken et al., 1997). In the current study, several measures were taken to avoid confounding factors: (a) water pH took approximately one week to decrease from the control level (7.5) to the low-pH treatment level (5.7); (b) despite dissolved carbon dioxide was not measured, it was likely within optimal levels for turbot (<26 mg/L) (Stiller et al., 2015) due to the low fish densities (max. = 53 kg/m^3), low hydraulic retention time in fish tanks (<40 min), and high efficiency of trickling filters in degassing carbon dioxide (Eding et al., 2006); and iii) low NH_4-N in Experiment 2 by introducing a loop with a submerged biofilter to remove TAN. The absence of confounding factors that are known to affect fish performance can explain the similar feed intake, FCR and growth performance here observed for fish exposed solely to low pH ($L_{pH}L_{TAN}$) and to those grown at a pH of 7.5 (control). Moreover, fish growth rates in control and $L_{pH}L_{TAN}$ treatments are comparable to growth rates obtained in other studies (Li, Liu & Blancheton, 2013; Mallekh, Lagardere, Anras & Lafaye, 1998). No major signs of disturbed or impaired physiological status were found, haematocrit and chloride were not affected, and plasma osmolarity was within the normal levels even though it significantly differed among experimental treatments (van Ham et al., 2003). Glucose and cortisol plasma levels did not differ and were within the considered nonstress levels (van Ham et al., 2003). Moreover, despite lactate plasma differed among treatments, the measured levels were lower (<0.3 mmol/L) than previous studies with stressed turbot (>2–5 mmol/L) (van Ham et al., 2003). Altogether, these results show that juvenile turbot can adapt and cope with low environmental pH provided that a slow acidification rate, low carbon dioxide and low NH_4-N environment are present.

Total ammonia nitrogen (TAN) is the sum of two ammonia fractions, ammonia (NH_3-N) and ammonium (NH_4-N). Although the effect of high TAN concentrations on fish growth performance is largely unknown, growth reduction caused by high NH_3-N is usually expected for several marine fish species, such as turbot (Alderson, 1979; Foss, Imsland, Roth, Schram & Stefansson, 2007, 2009; Person-Le Ruyet et al., 1997; Skøtt Rasmussen & Korsgaard, 1996), Dover sole (Alderson, 1979), European seabass (Dosdat et al., 2003; Lemarié et al., 2004), Atlantic cod (Foss, Siikavuopio, Sæther & Evensen, 2004), cobia (Rodrigues, Schwarz, Delbos & Sampaio, 2007) and Atlantic salmon (Kolarevic et al., 2013). The threshold level of NH_3-N below which little or no effect on turbot growth is recorded ranges from 0.1 mg/L (Alderson, 1979) to 0.5 mg/L (Person-Le Ruyet et al., 1997). In our study, despite the high TAN (up to 100 mg/L), NH_3-N levels were below 0.02 mg/L, which is notably lower than the levels shown above to affect turbot performance. Therefore, it is likely that the growth differences here observed between the control and $L_{pH}H_{TAN}$ treatments are related to the high NH_4-N concentrations. Although few studies tested the effect of chronic NH_4-N exposure on fish growth, it is expected that NH_4-N is considerably less toxic (300–400 times) to fish than NH_3-N (Person-Le Ruyet, Boeuf, Infante, Helgason & Le Roux, 1998; Person-Le Ruyet et al., 1997; Thurston et al., 1981). Indeed, a previous study exposed seabream fingerlings to high TAN and low pH and observed no adverse effects on growth and cortisol levels (Eshchar et al., 2006). However, the high-TAN treatment of the latter study was relatively low (5.4 ± 1.2 mg/L), which is similar to the low-TAN treatment of the current experiment ($L_{pH}L_{TAN}$: 5.4 ± 3.0 mg/L) and notably lower than the high-TAN treatments here used ($L_{pH}H_{TAN}$ 48.7 ± 2.3 mg/L and 48.2 ± 0.6 mg/L, respectively for experiments 1 and 2). It can therefore be concluded that high TAN concentrations affect fish growth, whereas extremely high TAN concentrations also affect feed intake and FCR.

Fish growth performance observed in Experiment 2 was superior to that of Experiment 1. This can be related to nitrate (NO_3-N) toxicity, which affects growth at concentrations equal or higher than 125 mg/L (van Bussel, Schroeder, Wuertz & Schulz, 2012). In Experiment 1, both treatments (control and $L_{pH}H_{TAN}$) were exposed to NO_3-N concentrations higher than 125 mg/L (Figure 2), whereas in Experiment 2, this threshold was only crossed in the first two weeks. This might explain the higher growth observed in Control 2 than in Control 1 and in $L_{pH}H_{TAN}$ 1 than in $L_{pH}H_{TAN}$ 2. However, only NO_3-N concentrations higher than 250 mg/L impact fish survival (van Bussel et al., 2012), which explains the high survival observed in both experiments.

Finally, it is important to note that water pH has large impacts on the biofilter performance, particularly on the nitrification rate, which is indirectly responsible for water TAN concentrations. In general, pH can exert two main effects on nitrifying bacteria. First, activation–deactivation of nitrification can occur because the pH optima for nitrification is around a pH of 8.0 (Grunditz & Dalhammar, 2001; Villaverde, Garcia-Encina & Fdz-Polanco, 1997), and it can completely cease at pH 5.5 (Forster, 1974). Second, a nutritional effect can also be observed because low pH shifts the carbonate system towards free carbon dioxide. Bearing in mind the water pH used in our study (5.7), more than 80% of total carbonate is carbon dioxide and <20% is bicarbonate, this carbon dioxide is continuously degassed in the trickling filters thus depleting the water from carbonates (Eding et al., 2006). The effect of bicarbonate limitation is more pronounced than that of low pH (Tarre & Green, 2004) and was likely responsible for the high TAN accumulation observed in $L_{pH}H_{TAN}$ treatment in Experiment 1. The additional loop added to

$L_{pH}L_{TAN}$ treatment, where a partial flow was enriched with bicarbonate and oxygen before going through a submerged biofilter, was a solution to maintain the overall RAS pH at 5.7 and still provide optimal conditions for nitrification to occur. Ultimately, the design used in our experiment demonstrates that it is possible to run a RAS at low pH without the accumulation of toxic fish metabolites (carbon dioxide and NH_4-N). The effect of low pH in RAS together with a higher availability of nutrients, namely TAN, on bacteria populations dynamics was not addressed in our study but could be interesting to study in further detail.

Juvenile turbot grew and survived equally well in a RAS with water pH of 7.5 and 5.7 over seven weeks provided a low-TAN environment, and no major effects were noted in energy mass balance, digestibility and blood parameters. This finding shows the feasibility to adopt a new production strategy to improve turbot performance in RAS. Further research should aim to determine the maximum noneffect concentration of ammonium (NH_4-N).

ACKNOWLEDGMENTS

This research was funded by the FP6 CRAFT Project No. 032556 (cofunded by the European Commission). L.A. Sampaio was supported by CAPES. The authors wish to thank Menno ter Veld, Ronald Booms and Tino Leffering for help during fish sampling and laboratorial analysis and Miguel C. Leal for helpful comments to improve the manuscript.

ORCID

Vasco C. Mota http://orcid.org/0000-0002-9718-6440

REFERENCES

Abbink, W., Garcia, A. B., Roques, J. A., Partridge, G. J., Kloet, K., & Schneider, O. (2012). The effect of temperature and pH on the growth and physiological response of juvenile yellowtail kingfish *Seriola lalandi* in recirculating aquaculture systems. *Aquaculture*, *330*, 130–135. https://doi.org/10.1016/j.aquaculture.2011.11.043

Alderson, R. (1979). The effect of ammonia on the growth of juvenile Dover sole, *Solea solea* (L.) and turbot, *Scophthalmus maximus* (L.). *Aquaculture*, *17*, 291–309. https://doi.org/10.1016/0044-8486(79)90085-1

Allan, G. L., & Maguire, G. B. (1992). Effects of pH and salinity on survival, growth and osmoregulation in *Penaeus monodon* Fabricius. *Aquaculture*, *107*, 33–47. https://doi.org/10.1016/0044-8486(92)90048-P

Brown, S. B., MacLatchy, D., Hara, T. J., & Eales, J. G. (1990). Effects of low ambient pH and aluminum on plasma kinetics of cortisol, T3, and T4 in rainbow trout (*Oncorhynchus mykiss*). *Canadian Journal of Zoology*, *68*, 1537–1543. https://doi.org/10.1139/z90-227

van Bussel, C. G., Schroeder, J. P., Wuertz, S., & Schulz, C. (2012). The chronic effect of nitrate on production performance and health status of juvenile turbot (*Psetta maxima*). *Aquaculture*, *326*, 163–167. https://doi.org/10.1016/j.aquaculture.2011.11.019

Butler, P. J., Day, N., & Namba, K. (1992). Interactive effects of seasonal temperature and low pH on resting oxygen uptake and swimming performance of adult brown trout *Salmo Trutta*. *Journal of Experimental Biology*, *165*, 195–212.

Colt, J. (2006). Water quality requirements for reuse systems. *Aquacultural Engineering*, *34*, 143–156. https://doi.org/10.1016/j.aquaeng.2005.08.011

Dalsgaard, J., Lund, I., Thorarinsdottir, R., Drengstig, A., Arvonen, K., & Pedersen, P. B. (2013). Farming different species in RAS in Nordic countries: Current status and future perspectives. *Aquacultural Engineering*, *53*, 2–13. https://doi.org/10.1016/j.aquaeng.2012.11.008

Davidson, J., Good, C., Williams, C., & Summerfelt, S. T. (2017). Evaluating the chronic effects of nitrate on the health and performance of post-smolt Atlantic salmon *Salmo salar* in freshwater recirculation aquaculture systems. *Aquacultural Engineering*, *79*, 1–8. https://doi.org/10.1016/j.aquaeng.2017.08.003

D'Cruz, Leela M., Dockray, Jacqueline J., Morgan, Ian J., & Wood, Chris M. (1998). Physiological effects of sublethal acid exposure in juvenile rainbow trout on a limited or unlimited ration during a simulated global warming scenario. *Physiological Zoology*, *71*, 359–376. https://doi.org/10.1086/515421

Deviller, G., Palluel, O., Aliaume, C., Asanthi, H., Sanchez, W., Franco Nava, M. A., … Casellas, C. (2005). Impact assessment of various rearing systems on fish health using multibiomarker response and metal accumulation. *Ecotoxicology and Environmental Safety*, *61*, 89–97. https://doi.org/10.1016/j.ecoenv.2004.07.011

Dietz, C., Kroeckel, S., Schulz, C., & Susenbeth, A. (2012). Energy requirement for maintenance and efficiency of energy utilization for growth in juvenile turbot (*Psetta maxima*, L.): The effect of strain and replacement of dietary fish meal by wheat gluten. *Aquaculture*, *358*, 98–107. https://doi.org/10.1016/j.aquaculture.2012.06.028

Dockray, J. J., Morgan, I. J., Reid, S. D., & Wood, C. M. (1998). Responses of juvenile rainbow trout, under food limitation, to chronic low pH and elevated summer temperatures, alone and in combination. *Journal of Fish Biology*, *52*, 62–82. https://doi.org/10.1111/j.1095-8649.1998.tb01553.x

Dockray, J. J., Reid, S. D., & Wood, C. M. (1996). Effects of elevated summer temperatures and reduced pH on metabolism and growth of juvenile trout (*Oncorhynchus mykiss*) on unlimited ration. *Canadian Journal of Fisheries and Aquatic Sciences*, *53*, 2752–2763. https://doi.org/10.1139/f96-232

Dosdat, A., Ruyet, J. P.-L., Covès, D., Dutto, G., Gasset, E., Roux, A. L., & Lemarié, G. (2003). Effect of chronic exposure to ammonia on growth, food utilisation and metabolism of the European sea bass (*Dicentrarchus labrax*). *Aquatic Living Resources*, *16*, 509–520. https://doi.org/10.1016/j.aquliv.2003.08.001

Eding, E. H., Kamstra, A., Verreth, J. A. J., Huisman, E. A., & Klapwijk, A. (2006). Design and operation of nitrifying trickling filters in recirculating aquaculture: A review. *Aquacultural Engineering*, *34*, 234–260. https://doi.org/10.1016/j.aquaeng.2005.09.007

Eshchar, M., Lahav, O., Mozes, N., Peduel, A., & Ron, B. (2006). Intensive fish culture at high ammonium and low pH. *Aquaculture*, *255*, 301–313. https://doi.org/10.1016/j.aquaculture.2005.11.034

Fivelstad, S., Waagbø, R., Zeitz, S. F., Hosfeld, A. C. D., Olsen, A. B., & Stefansson, S. (2003). A major water quality problem in smolt farms: Combined effects of carbon dioxide, reduced pH and aluminium on Atlantic salmon (*Salmo salar* L.) smolts: Physiology and growth. *Aquaculture*, *215*, 339–357. https://doi.org/10.1016/S0044-8486(02)00197-7

Forster, J. (1974). Studies on nitrification in marine biological filters. *Aquaculture*, *4*, 387–397. https://doi.org/10.1016/0044-8486(74)90067-2

Foss, A., Imsland, A. K., Roth, B., Schram, E., & Stefansson, S. O. (2007). Interactive effects of oxygen saturation and ammonia on growth and blood physiology in juvenile turbot. *Aquaculture*, *271*, 244–251. https://doi.org/10.1016/j.aquaculture.2007.06.025

Foss, A., Imsland, A. K., Roth, B., Schram, E., & Stefansson, S. O. (2009). Effects of chronic and periodic exposure to ammonia on growth and blood physiology in juvenile turbot (*Scophthalmus maximus*). *Aquaculture*, *296*, 45–50. https://doi.org/10.1016/j.aquaculture.2009.07.013

Foss, A., Siikavuopio, S. I., Sæther, B.-S., & Evensen, T. H. (2004). Effect of chronic ammonia exposure on growth in juvenile Atlantic cod. *Aquaculture*, *237*, 179–189. https://doi.org/10.1016/j.aquaculture.2004.03.013

van Ginneken, V. J. T., Van Eersel, R., Balm, P., Nieveen, M., & Van Den Thillart, G. (1997). Tilapia are able to withstand long-term exposure to low environmental pH, judged by their energy status, ionic balance and plasma cortisol. *Journal of Fish Biology*, *51*, 795–806. https://doi.org/10.1111/j.1095-8649.1997.tb02000.x

Grunditz, C., & Dalhammar, G. (2001). Development of nitrification inhibition assays using pure cultures of *Nitrosomonas* and *Nitrobacter*. *Water Research*, *35*, 433–440. https://doi.org/10.1016/S0043-1354(00)00312-2

van Ham, E. H., Van Anholt, R. D., Kruitwagen, G., Imsland, A. K., Foss, A., Sveinsbø, B. O., … Wendelaar Bonga, S. E. (2003). Environment affects stress in exercised turbot. *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology*, *136*, 525–538.

Ip, Y. K., Chew, S. F., & Randall, D. J., (2001). Ammonia toxicity, tolerance, and excretion. *Fish Physiology*, *20*, 109–148.

Johansson, O., & Wedborg, M. (1980). The ammonia-ammonium equilibrium in seawater at temperatures between 5 and 25 C. *Journal of Solution Chemistry*, *9*, 37–44. https://doi.org/10.1007/BF00650135

Kennedy, C. J., & Picard, C. (2012). Chronic low pH exposure affects the seawater readiness of juvenile Pacific sockeye salmon. *Fish Physiology and Biochemistry*, *38*, 1131–1143. https://doi.org/10.1007/s10695-011-9599-4

Kolarevic, J., Selset, R., Felip, O., Good, C., Snekvik, K., Takle, H., … Terjesen, B. F. (2013). Influence of long term ammonia exposure on Atlantic salmon (*Salmo salar* L.) parr growth and welfare. *Aquaculture Research*, *44*, 1649–1664.

Lemarié, G., Dosdat, A., Covès, D., Dutto, G., Gasset, E., & Person-Le Ruyet, J. (2004). Effect of chronic ammonia exposure on growth of European seabass (*Dicentrarchus labrax*) juveniles. *Aquaculture*, *229*, 479–491. https://doi.org/10.1016/S0044-8486(03)00392-2

Lemarié, G., Dutto, G., Le Roux, A., Lemoalle, J., Maxime, V., & Person-Le Ruyet, J. (2000). *Long-term effects of pH and carbon dioxide on growth and feed efficiency in European seabass*. In: *AQUA 2000*. Nice, France: European Aquaculture Society Special Publication.

Li, X., Liu, Y., & Blancheton, J.-P. (2013). Effect of stocking density on performances of juvenile turbot (*Scophthalmus maximus*) in recirculating aquaculture systems. *Chinese Journal of Oceanology and Limnology*, *31*, 514–522. https://doi.org/10.1007/s00343-013-2205-0

Liu, Y., Rosten, T. W., Henriksen, K., Hognes, E. S., Summerfelt, S., & Vinci, B. (2016). Comparative economic performance and carbon footprint of two farming models for producing Atlantic salmon (*Salmo salar*): Land-based closed containment system in freshwater and open net pen in seawater. *Aquacultural Engineering*, *71*, 1–12. https://doi.org/10.1016/j.aquaeng.2016.01.001

Mallekh, R., Lagardere, J., Anras, M. B., & Lafaye, J. (1998). Variability in appetite of turbot, *Scophthalmus maximus* under intensive rearing conditions: The role of environmental factors. *Aquaculture*, *165*, 123–138. https://doi.org/10.1016/S0044-8486(98)00244-0

Martins, C. I. M., Eding, E. H., Verdegem, M. C. J., Heinsbroek, L. T. N., Schneider, O., Blancheton, J. P., … Verreth, J. A. J. (2010). New developments in recirculating aquaculture systems in Europe: A perspective on environmental sustainability. *Aquacultural Engineering*, *43*, 83–93. https://doi.org/10.1016/j.aquaeng.2010.09.002

Martins, C. I. M., Ochola, D., Ende, S. S. W., Eding, E. H., & Verreth, J. A. J. (2009). Is growth retardation present in Nile tilapia *Oreochromis niloticus* cultured in low water exchange recirculating aquaculture systems? *Aquaculture*, *298*, 43–50. https://doi.org/10.1016/j.aquaculture.2009.09.030

McDonald, D., Walker, R., Wilkes, P., & Wood, C. (1982). H+ excretion in the marine teleost *Parophrys vetulus*. *Journal of Experimental Biology*, *98*, 403–414.

Milligan, C. L., & Wood, C. M. (1982). Disturbances in haematology, fluid volume distribution and circulatory function associated with low environmental pH in the rainbow trout, *Salmo gairdneri*. *Journal of Experimental Biology*, *99*, 397–415.

Mota, V. C., Limbu, P., Martins, C. I., Eding, E. H., & Verreth, J. A. (2015). The effect of nearly-closed RAS on the feed intake and growth of Nile tilapia (*Oreochromis niloticus*), African catfish (*Clarias gariepinus*) and European eel (*Anguilla anguilla*). *Aquacultural Engineering*, *68*, 1–5. https://doi.org/10.1016/j.aquaeng.2015.06.002

Mota, V. C., Martins, C. I., Eding, E. H., Canário, A. V., & Verreth, J. A. (2014). Steroids accumulate in the rearing water of commercial recirculating aquaculture systems. *Aquacultural Engineering*, *62*, 9–16. https://doi.org/10.1016/j.aquaeng.2014.07.004

Mota, V. C., Martins, C. I., Eding, E. H., Canário, A. V., & Verreth, J. A. (2017). Water cortisol and testosterone in Nile tilapia (*Oreochromis niloticus*) recirculating aquaculture systems. *Aquaculture*, *468*, 255–261. https://doi.org/10.1016/j.aquaculture.2016.10.023

Nagae, M., Ogawa, K., Kawahara, A., Yamaguchi, M., Nishimura, T., & Ito, F. (2001). Effect of acidification stress on endocrine and immune functions in carp, *Cyprinus Carpio*. *Water, Air, & Soil Pollution*, *130*, 893–898. https://doi.org/10.1023/A:1013855501445

Packard, G. C., & Boardman, T. J. (1999). The use of percentages and size-specific indices to normalize physiological data for variation in body size: Wasted time, wasted effort? *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology*, *122*, 37–44. https://doi.org/10.1016/S1095-6433(98)10170-8

Person-Le Ruyet, J. (2002). Turbot (*Scophthalmus maximus*) grow-out in Europe: Practices, results and prospects. *Turkish Journal of Fisheries and Aquatic Sciences*, *2*, 29–39.

Person-Le Ruyet, J., Boeuf, G., Infante, J. Z., Helgason, S., & Le Roux, A. (1998). Short-term physiological changes in turbot and seabream juveniles exposed to exogenous ammonia. *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology*, *119*, 511–518. https://doi.org/10.1016/S1095-6433(97)00458-3

Person-Le Ruyet, J., Galland, R., Le Roux, A., & Chartois, H. (1997). Chronic ammonia toxicity in juvenile turbot (*Scophthalmus maximus*). *Aquaculture*, *154*, 155–171. https://doi.org/10.1016/S0044-8486(97)00052-5

Rodrigues, R. V., Pedron, J. D. S., Romano, L. A., Tesser, M. B., & Sampaio, L. A. (2015). Acute responses of juvenile cobia *Rachycentron canadum* (Linnaeus 1766) to acid stress. *Aquaculture Research*, *46*, 1241–1247. https://doi.org/10.1111/are.12282

Rodrigues, R. V., Schwarz, M. H., Delbos, B. C., & Sampaio, L. A. (2007). Acute toxicity and sublethal effects of ammonia and nitrite for juvenile cobia *Rachycentron canadum*. *Aquaculture*, *271*, 553–557. https://doi.org/10.1016/j.aquaculture.2007.06.009

Skøtt Rasmussen, R., & Korsgaard, B. (1996). The effect of external ammonia on growth and food utilization of juvenile turbot (*Scophthalmus maximus* L.). *Journal of Experimental Marine Biology and Ecology*, *205*, 35–48. https://doi.org/10.1016/S0022-0981(96)02601-9

Stiller, K. T., Vanselow, K. H., Moran, D., Bojens, G., Voigt, W., Meyer, S., & Schulz, C. (2015). The effect of carbon dioxide on growth and metabolism in juvenile turbot *Scophthalmus maximus* L. *Aquaculture*, *444*, 143–150. https://doi.org/10.1016/j.aquaculture.2015.04.001

Tarre, S., & Green, M. (2004). High-rate nitrification at low pH in suspended-and attached-biomass reactors. *Applied and Environmental Microbiology*, *70*, 6481–6487. https://doi.org/10.1128/AEM.70.11.6481-6487.2004

Thurston, R. V., Russo, R. C., & Vinogradov, G. A. (1981). Ammonia toxicity to fishes. Effect of pH on the toxicity of the Un-ionized ammonia species. *American Chemical Society*, *15*, 837–840.

Villaverde, S., Garcia-Encina, P., & Fdz-Polanco, F. (1997). Influence of pH over nitrifying biofilm activity in submerged biofilters. *Water Research*, *31*, 1180–1186. https://doi.org/10.1016/S0043-1354(96)00376-4

Wendelaar Bonga, S. E. (1997). The stress response in fish. *Physiological Review*, *77*, 591–625. https://doi.org/10.1152/physrev.1997.77.3.591

How to cite this article: Mota VC, Hop J, Sampaio LA, et al. The effect of low pH on physiology, stress status and growth performance of turbot (*Psetta maxima* L.) cultured in recirculating aquaculture systems. *Aquac Res*. 2018;49:3456–3467. https://doi.org/10.1111/are.13812