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Nile tilapia *(Oreochromis niloticus)* show high tolerance to acute ammonia exposure but lose metabolic scope during prolonged exposure at low concentration

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ABSTRACT

Ammonia is a respiratory gas that is produced during the process of protein deamination. In the unionised form (NH_3), it readily crosses biological membranes and is highly toxic to fish. In the present study we examined the effects of unionized ammonia (UIA), on the resting oxygen consumption (MO_2), ventilation frequency (f_V), heart rate (HR) and heart rate variability (HRV) in Nile tilapia (*Oreochromis niloticus*). Fish were either exposed to progressively increasing UIA concentrations, up to 97 μM over a 5 h period, or to a constant UIA level of 7 μM over a 24 h period. For both treatment groups resting MO_2, HR and f_V were recorded as physiological variables. Relative to the control group, the fish groups exposed to the incremental UIA levels did not exhibit significant changes in their MO_2, HR and f_V at UIA concentrations of 4, 10, 35, or 61 μM compared to control fish. Exposure to 97 μM UIA, however, elicited abrupt and significant downregulations ($p < 0.05$) in all three responses, as MO_2, HR and *fv* decreased by 25, 54 and 76 % respectively, compared to control measurements. Heart rate became increasingly irregular with increasing UIA concentrations, and heart rate variability was significantly increased at 61 and 97 μM UIA. Prolonged exposure elicited significant changes at exposure 7 μM UIA. Standard (SMR) and maximum metabolic rate (MMR) were significantly reduced, as was the corresponding f_V and HR. It is evident from this study that Nile tilapia is tolerant to short term exposure to UIA up to 61 μM but experience a significant metabolic change under conditions of prolonged UIA exposures even at low concentrations.

1. Introduction

Ammonia nitrogen is a metabolic end-product resulting from amino acid deamination and represents more than 70 % of the nitrogenous waste excreted by fish (Dosdat et al., 1996; Zhang et al., 2011). Apart from the nitrogen waste from fish (Zeitoun et al., 2016), the decomposition of uneaten feed and faecal matter within production systems may also contribute to the pool of dissolved ammonia in the culture environment (Fan et al., 2022). Depending on feeding regime, the average Nile tilapia (*Oreochromis niloticus*) excretes between 9.6 and 12.9 mM kg^{-1} d^{-1} of total ammonia nitrogen (Kobayashi et al., 2007; Skov et al., 2017). This is thought to be excreted predominantly as unionized ammonia (UIA) across the gills by diffusion, while a smaller fraction of ionized ammonia (IA) is actively transported via Rh glycoproteins (Nakada et al., 2007). In the absence of nitrification processes or nitrogen sinks, dissolved ammonia accumulates in the aquatic environment. Ammonia in water exists as either ionized ammonium, NH_4^+ (IA), or unionized ammonia, NH_3 (UIA) in an equilibrium that can be written as $NH_3 + H_2O \Leftrightarrow NH_4^+ + OH^-$, with a pKa value of approximately 9.25 at 25 °C (Quijada-Rodriguez et al., 2015). The pKa value is temperature dependent and decreases with increasing temperature. Within the neutral pH range, only 1–2 % of ammonia nitrogen exists in the unionized form, but the fraction of UIA rapidly increases with rising pH (Wilkie, 1997; Quijada-Rodriguez et al., 2015).

Large diel changes in carbon dioxide levels and temperature occur in tropical earthen ponds due to photosynthesis and respiration (Hargreaves and Tucker, 2004). Resultant changes in pH drive large changes in the ammonium speciation in the culture water (Wurts, 2003; Shuvasish and Chavhan, 2017). Depending on the buffering capacity of the water, pH levels in the mornings may be as low as 6 (Makori et al., 2017; Gyamfi et al., 2022), but may increase above pH 9 at midday, when photosynthesis has stripped all free CO_2 from the water. Ammonia excretion rates by Nile tilapia coincide with this, typically peaking some 6 h after feeding (Obirikorang et al., 2020b). During mid-afternoon, digesting fish have high ammonia nitrogen excretion rates, coupled with peak pH and temperature levels of pond water. This combines to

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create conditions in which the UIA fraction reaches 44–53 % (at 30–35 °C and pH 9), which may persist for several hours.

Fish exposed to UIA respond with a range, physiological, and metabolic changes (Franklin and Edward, 2019; Parvathy et al., 2023). Under the most severe conditions, these are manifested as convulsion, loss of equilibrium, irregular swimming, gill haemorrhaging and necrosis, kidney damage, and mortality (Zeitoun et al., 2016). From an aquaculture perspective, it appears also that chronic exposure to ammonia can lead to restricted feeding and poor growth performance (*Dicentrarchus labrax*) (Dosdat et al., 2003).

In teleosts, UIA is excreted across the branchial epithelium by diffusion, by a downward gradient from blood to water. UIA is subsequently trapped as IA after becoming ionized in the acidic unstirred boundary layer of the gill epithelium (Wilkie, 2002). During conditions of high unionized ammonia, the loss of an outward concentration gradient will impair excretion, or even lead to inward diffusion. Exposing fish to ammonia disturbs cellular metabolism (Smart, 1978) which affects overall function of tissues and organs. Some studies have suggested that ammonia may act on the respiratory system of the fish by affecting oxygen consumption associated with respiratory frequencies and amplitude (Smart, 1976; Zhang and Wood, 2009; Perry and Tzaneva, 2015). High internal levels of ammonia following loading with injections of ammonium salts led to a marked increase in ventilation frequency and amplitude (Zhang and Wood, 2009; Zhang et al., 2011; Eom et al., 2020; Eom and Wood, 2021). There is a consensus that internal ammonia levels can be sensed by the neuroepithelial cells located on the first two gill arches (Zhang et al., 2011), as well as by central chemoreceptors in the brain (Eom and Wood, 2021), which in rainbow trout has been shown to trigger an increase in ventilation to facilitate excretion (McKenzie et al., 1993; Eom and Wood, 2021).

On the other hand, the mechanisms that govern how fish respond to high environmental ammonia (HEA) are not well known. Essentially, it seems unclear whether fish register changes in external ammonia levels or if they sense the resultant increase in internal ammonia levels from inward diffusion. Several studies have shown that HEA leads to hyperventilation in rainbow trout (Smart, 1978) and Atlantic salmon (Fivelstad and Binde, 1994; Knoph, 1996), although these studies were conducted at lethal or near-lethal UIA concentrations. An increased ventilation in response to HEA seems counterintuitive as it is energetically costly, and likely to facilitate further uptake of ammonia (Perry and Tzaneva, 2016). However, the prevailing hypothesis is that following inward diffusion, internal ammonia levels increase, and the same mechanisms as outlined above prevail. This is supported by observations by Zhang et al. (2011), where rainbow trout exposed to 250 μM IA showed an increase in ventilation amplitude, however, this did not occur until 20 min after exposure, supporting the idea that fish are responding to elevations in internal ammonia levels.

To investigate how Nile tilapia (*Oreochromis niloticus*) respond to peak UIA levels in pond production systems, the effects of ammonia on heart rate, gill ventilation frequency (f_V) and oxygen consumption (MO_2), fish were subjected to acutely increasing concentrations of ammonium salts or prolonged exposure to low concentrations, under conditions in which pH levels were allowed to fluctuate or were controlled.

2. Material and methods

2.1. Experimental fish

Nile tilapia (*Oreochromis niloticus*) used for this experiment came from a laboratory stock originally purchased from a commercial supplier as fry (Til-Aqua, Velden, Netherlands). The fish were housed in a common recirculating aquaculture system maintained at a constant temperature of 26 °C and a pH of 7.5. Oxygen saturation was kept above 80 %, and the photoperiod was 12 h light:12 h dark. Fish were hand fed once daily to apparent satiation with a commercial feed (EFICO Cromis 832F, BioMar, France). Prior to experiments, fish were deprived of food for 24 h to allow them to evacuate their gut and minimise any confounding effects of digestion. All fish were weighed for body mass (BM) to the nearest 0.1 g and measured for standard (SL) and total length (TL). All experiments were conducted at 26 °C. All fish were euthanized in an overdose of benzocaine at the end of an experiment. Experimental protocols and procedures used in this study were conducted in accordance with Danish and EU Legislation under permit by the Danish Veterinary and Food Administration (2018–15–0201–01,407).

2.2. Measurements of heart rate (HR) and ventilation (f_V)

Fish were anaesthetized using a buffered benzocaine solution at a final concentration of 100 mg/L until unresponsive to tactile stimulation. Fish were then placed in a surgical sling, and gills irrigated by pumping water containing 50 mg/L benzocaine via a silicone tube inserted in the mouth. Two ECG electrodes were attached to the fish following the method by Glass et al. (1991). ECG electrodes were fabricated using 21 G needles soldered onto an insulated wire. Needle tips were bent at a 90-degree angle ca. 4 mm from the tip and surface sealed with epoxy glue and heat shrink leaving only the tip exposed. ECG electrodes were inserted anterior and posterior to the heart along the ventral midline and secured with a single superficial suture. Insertion of electrodes and suturing was completed within 2 min and fish were subsequently placed into the experimental chamber for recovery. Electrode leads were externalised via a chimney in the respirometer, and a third reference electrode was placed in the water. The signal from the ECG electrodes was recorded on a PC via a ECG sensor interface and software (Vernier, OR, USA). HR was determined from recordings by counting peaks of the R wave from the first peak and 30 s onwards. The time interval between R waves was measured, and HR variability (HRV) was calculated as the square of the standard deviation of the interval between heartbeats. Ventilation frequency (f_V) was determined over a 30 s period by visual observation and expressed as opercular beats per minutes (bpm).

2.3. Respirometry

Oxygen consumption of fish was determined using computerized intermittent flow-through respirometry using a respirometer with a volume of 11 L. All fish were allowed to acclimate for 24 h to recover from anaesthesia or any surgical procedures. The respirometer was immersed in an outer bath with a volume of 250 L. Oxygen saturation in the outer bath was maintained by continuous aeration. Oxygen saturation levels in the respirometer was measured using a calibrated fibre optic dipping probe inserted in the recirculation loop. Data was collected via an OXY-4 mini O_2 meter (Presens, Germany) to a PC running AutoResp (Loligo Systems, Denmark) which recorded and processed data for each fish. Measurements were performed in loops with a total duration of 8 min. For standard metabolic rate (SMR), the average of 3 consecutive measurements were used, while for maximum metabolic rate (MMR) only the first measurement was used.

2.4. Tolerance threshold for acute UIA exposure

To determine the tolerance levels of Nile tilapia to UIA and determine the experimental concentrations, a pilot experiment was conducted using 5 fish (BM 443 ± 56 g, TL: 28.3 ± 1.7 cm, SL: 23.5 ± 1.4 cm) to determine the UIA tolerance thresholds. Following recovery in the respirometer, MO_2, HR and f_V of the fish were recorded as control measurements. Fish were then exposed to gradually increasing [UIA] at 1 h intervals. Briefly, a 1.5 M stock solution of NH_4Cl was prepared using distilled water, and predetermined volumes were added at hourly intervals. For each experiment and at each UIA concentration, a 10 mL water sample was collected for pH and TAN determination, and the UIA concentration calculated by identifying the fraction of UIA under the

recorded temperature and pH using the ammonia-temperature table, UIA levels obtained were then multiplied by the laboratory results. The resultant UIA concentrations were 4, 10, 35, 61, 97, and 294 μM. No efforts were made to adjust pH, which ranged from 7.6 to 8.6. The experiment was terminated when fish lost equilibrium, and fish were immediately transferred to clean water. All water in the experimental system was replaced between each fish.

2.5. Acute exposure of Nile tilapia to UIA

The final UIA concentrations applied for acute exposure were 0 (control), 4, 10, 35, 61 and 97 μM. Individual fish (BM 497 ± 37 g, TL; 29.9 ± 1.1 cm, SL; 25.1 ± 1.0 cm, $n = 8$) were subjected to hourly increasing UIA levels. After recovery, prior to the onset of the experiment, control values for MO_2, f_V and HR were recorded, and a water sample taken for pH and TAN determination. This procedure was repeated at the end of every hourly interval. Experiments were terminated after 1 h exposure to 97 μM.

2.6. Acute IA exposure

To determine any role of the IA fraction in the observed changes in metabolic rate, HR or f_V during UIA exposure, an additional series of experiments was conducted in which the pH of the experimental water for this stage was maintained constant at 6.5. At pH 6.5 > 99.9 % of TAN is present in ionised form. pH was controlled using a pH controller (AquaMedic, Germany) connected to a peristalic pump (REGLO, Ismatec, USA) that dosed in a 0.01 M solution of hydrochloric acid when pH exceeded the setpoint. Fish (BM 459.0 ± 56.8 g; TL; 28.6 ± 2.1 cm, SL; 24.1 ± 1.4 cm, $n = 6$) were anesthetized, and the protocol followed that for UIA exposure.

2.7. Prolonged exposure of UIA on standard metabolic rate (SMR) and maximum metabolic rate (MMR) of Nile tilapia (O. niloticus)

To measure the effect of UIA on SMR and MMR of *O. niloticus*, fish (BM 437 ± 47 g, TL; 28.5 ± 1.3 cm, SL; 23.7 ± 1.3 cm, $n = 8$) were exposed for 24 h to 7 μM - UIA. Following recovery and acclimation, control values for SMR, HR, and f_V were recorded. To avoid damage to ECG leads, MMR of the fish was determined following a 5 min air exposure protocol as described by Khan et al. (2018). Fish were returned to the respirometer, and MO_2, HR, and f_V were measured during the first measurement loop. Fish were allowed to recover for 12 h, the recirculation in the experimental tank was closed and the required volume of NH_4Cl stock solution was pipetted into the experimental water every hour until a UIA concentration of 7 μM was obtained. After 24 h exposure, the SMR, f_V, HR were measured, and the MMR procedure was repeated. Metabolic scope (MS) was defined as MMR less SMR for each individual fish, and scope for HR and f_V were determined in a similar manner for each individual fish.

2.8. Statistics and calculations

All results are presented as mean ± standard error of the mean. All data were checked for normality and homogeneity of variance using the Shapiro-Wilk test and the Brown-Forsythe equal variance test, respectively. The effect of acute exposure of UIA on MO_2, f_V and HR and HRV of *O. niloticus* among the treatments were analysed with Kruskal-Wallis (One Way Analysis of Variance on Ranks followed by Dunnś post-test). Paired T- test was used to analyse pre- and post-exposure data from the prolonged UIA exposure for SMR, MMR, f_V, HR and MS of the fish. Results were considered significant at $p \leq 0.05$. All statistical analyses and graphs were done using SigmaPlot (v.14.4. Systat Software Inc, San Jose, CA, USA).

3. Results

3.1. Effects of acute UIA exposure on MO_2, f_V and HR

Acute exposure to UIA caused loss of equilibrium (LOE) at 294 μM within 30–40 min. No fish showed LOE at UIA concentrations of 97 μM or less over the 5 h period. Water pH ranged from 7.6 to 8.6. In response to acute UIA exposure, metabolic rate of Nile tilapia showed a tendency to decrease with increasing UIA but was only significantly reduced only at 97 μM UIA. Here, the MO_2 of the fish had decreased by approximately 25 % compared to control conditions (Fig. 1A). HR and f_V of Nile tilapia both showed a decreasing trend in response to increasing UIA, with significant reductions at or above 61 μM (Fig. 1B), where HR decreased by 49–54 %, while f_V decreased by 65–76 %. HRV showed a progressive increase as UIA levels increased progressively and became significantly different from control conditions at 61 and 97 μM -UIA (Table 1).

3.2. Effects of prolonged exposure of UIA on standard metabolic rate (SMR) and maximum metabolic rate (MMR) of O. niloticus

Exposure to 7μM-UIA for 24 h caused a significant decrease in SMR (17 %, Fig. 2A), HR (30 %, Fig. 2B), and f_V (22 %, Fig. 2C). Similarly, MMR decreased by 39 %, as did f_V (27 %) and HR (18 %) during MMR. Consequently, there was a significant loss in MS (51 %, Fig. 2D) compared to control fish. Scope for HR and f_V decreased by 14 % and 39 %, respectively, but were not significant (Fig. 2E-F).

3.3. Effects of increasing NH_4^+ at constant pH (6.5) on the metabolic rate of O. niloticus

Exposure to increasing concentrations of IA at pH 6.5, metabolic rate of *O. niloticus* was not significantly affected between the treatments (Table 2). Although, f_V and HR showed tendencies to decrease at the highest [IA] no significant differences were observed.

4. Discussion

4.1. Acute UIA exposure on MO_2, f_V and HR

In the present study, the effects of acute exposure to increasing levels of unionised ammonia (UIA) on the respiratory system in Nile tilapia were assessed by observation on changes in heart rate, ventilation frequency and oxygen consumption. Following exposure to UIA, tilapia displayed a general trend to decrease MO_2, HR and *fv*, that became statistically significant at 97 μM. An increased influx of UIA would initiate ammonia detoxification mechanisms as well as increased ventilation, presumably at an energetic cost. For example, rainbow trout exposed to lethal UIA concentrations (43 μM) respond with significant increases in oxygen consumption (>3-fold), ventilation frequency (2-fold), and heart rate (1.5-fold) (Smart, 1978). In contrast, Atlantic salmon (*Salmo salar*) exposed to 17–24 μM UIA only showed slight (<10 %), albeit significant, increases in ventilation (Knoph, 1996). Increased ventilation has not been considered to be driven by an increase in the requirement to excrete ammonia, as baseline ventilation should satisfy this. Instead, Randall and Ip (2006) proposed that changes in ventilation are triggered by the neuroepithelial cells (NECs) located on the filaments of the gill arches (Dunel-Erb et al., 1982) as increasing levels of ionised ammonia depolarise the potassium channels of the NECs. Lang et al. (1987) also showed significant increases in ventilation frequency at 18 μM UIA. Ventilation appears to be modulated primarily by internal ammonia sensing in the brain and at the NECs (Eom and Wood, 2021).

One mechanism that has been proposed as causative for increased ventilation is a reduction in the blood oxygen carrying capacity. This is primarily based on the observations of Brockway (1950) that CO_2 and metabolic waste starts to accumulate with increasing levels of UIA, eventually leading to an 85 % reduction in blood oxygen content. Sousa

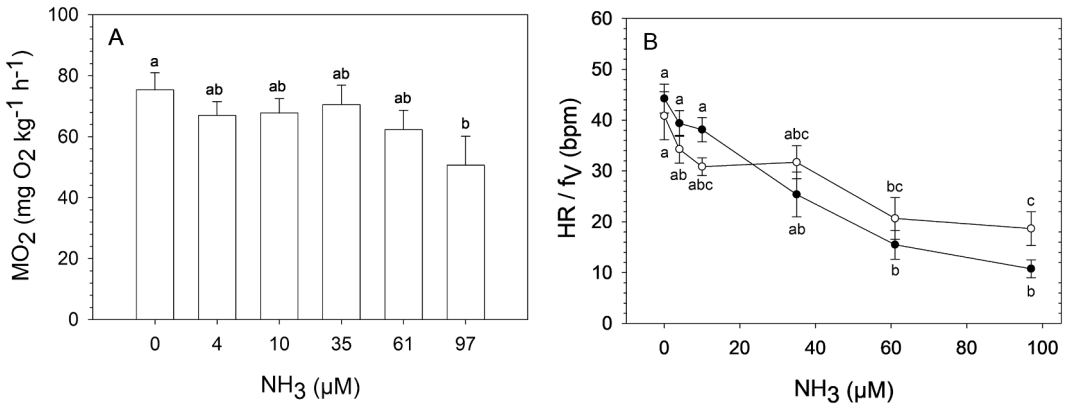

Fig. 1. Effect of acute and progressively increasing UIA exposure on (A) resting oxygen consumption rates (MO_2), and (B) ventilation frequency (f_V, filled circles) and heart rate (HR, open circles) in Nile tilapia. MO_2 showed a tendency to decrease with increasing UIA concentrations and became significantly depressed at 97 μM compared to control conditions. Progressive and concentration dependent decreases were observed for both f_V and HR, which became significant at 61 and 97 μM. Different superscripts indicate significant differences between treatments. All values are presented as mean ± SE (n = 8).

Table 1
Progressively increasing exposure of Nile tilapia to unionised ammonia caused dose-dependent reductions in heart rate (HR). The mean heart rate variability (HRV) increased up to 7-fold, indicative of a loss in control of electrical conductance. All values are presented as mean ± SE (n = 8).

		HRV (s)		
UIA (μM)	HR (bpm)	HRV_{mean}	HRV_{min}	HRV_{max}
0	40.86 ± 12.48	0.35 ± 0.21	0.09	0.69
4	34.29 ± 7.25	0.50 ± 0.30	0.22	0.98
10	30.86 ± 4.60	0.63 ± 0.40	0.08	1.17
35	31.71 ± 8.60	1.15 ± 0.89	0.21	2.83
61	20.67 ± 10.01	2.54 ± 2.17	0.80	5.60
97	18.67 ± 8.07	1.46 ± 0.25	1.18	1.59

and Meade (1977) followed up on this with a series of studies on the effects of UIA on the oxy/deoxy states of hemoglobin and concluded that the enzymatic stimulation of glycolysis caused by suppression of aerobic metabolism through suppression of the citric acid cycle (TCA) caused a pH drop that eventually caused a simultaneous Bohr shift and Root effect. Inhibition of the TCA cycle in rainbow trout by UIA was demonstrated by Arillo et al. (1981) showing that brain mitochondrial ATP and NADH levels are gradually depleted by exposure to increasing UIA concentrations via changes in glutamate dehydrogenase activity and loss of α-ketoglutarate. This loss in capacity for aerobic energy production is accompanied by an increase in lactate levels.

Ultimately, the reduction in metabolic rate observed in the present study may have been induced by this decrease in aerobic energy production. In essence, it is quite similar to the response of Nile tilapia to aquatic hypoxia (Bergstedt et al., 2021) which is also a decrease in metabolic rate.The decline in MO_2 could also be based on the assumption that when the concentration of UIA in water increases to more than 21 μM, the oxygen content of the blood of the fish decreases by about 85 % of its normal value, due to combinations of decreased oxygen carrying capacity and reductions in haematocrit and haemoglobin concentration of (Brockway, 1950).

Water breathing fish typically show a hyperventilatory response to increasing UIA levels (Zhang et al., 2011; Parvarthy et al., 2023). However, in the present study, exposing Nile tilapia to increasing UIA resulted in a downregulation in f_V, with significant reductions occurring at or above 61 μM. The hypoventilation observed in the current study could be a coping mechanism employed by the fish to minimize further ammonia uptake across the gills and also provide the fish with time for behavioural avoidance (Eom and Wood, 2021). Also, the ventilatory inhibition observed in the present study could possibly be overwhelmed by the ventilatory stimulation elicited by gradually rising internal ammonia levels in the fish. Generally, the results of this study revealed that, the trend with decreasing MO_2, fv and HR are more or less instantaneous which might indicate that Nile tilapia sense external ammonia levels at exposure.

Data on HR and fv showed Nile tilapia group exposed to UIA showed cardiorespiratory synchrony, an interaction that certainly contributed to the strong correlation of the HR and fv as recorded in the present experiment. Following increasing UIA, fish developed bradycardia accompanied by a general reduction in the total cardiac output and higher HRV. The decrease in HR and increase in HRV of Nile tilapia in this study could be a characteristic of the cardiovascular stress response suggested by Bessemer et al. (2015) and is related to an increase in the parasympathetic activity (Laborde et al., 2017). Activation of the parasympathetic system in fishes leads to a decrease in heart rate and an increase in HRV (Berntson et al., 1997). This is achieved by release of acetylcholine from the vagus nerve that stimulates muscarinic acetylcholine receptors, that respond by increasing cell membrane K^+ conductance (Sakmann et al., 1983). It is well known that in some tissues, due to ionic similarities, NH_4^+ can compete with K^+ in the transportation through K^+ selective channels (Ip and Chew, 2010). If high environmental UIA levels lead to an accumulation of IA in Nile tilapia, this could perceivably be part of the cause of the observed decrease in HR and increase in HRV observed with increasing UIA concentration. The increased HRV could also be a direct effect of oxygen deficiency on the cardiac muscle of the fish (Fritsche and Burggren, 1996) and consequently slowing of heart rate while making efforts of increasing ventilation by vagal activity (Egginton et al., 2006).

Ammonia exposure is known to impair certain neuromotor function through depolarization, affecting response times (McKenzie et al., 2009) and locomotor ability (Randall and Wicks, 2000), which could extend to inhibition of the ability to perform cardiac work. This probably led to increased HRV of the ammonia exposed fish groups as firing becomes irregular. Lastly, a possible explanation to reduction in HR could also be increase in cardiac cholinergic signal triggered by ammonia at exposure which led to regulatory range for heart beat adjustments of the fish. According to Vornanen (2017), cholinergic activity increases under a decline of oxygen which results to a depression in HR of the fish as noticed in this study. The significant reduction of HR in the present study could also be explained by the findings of Burleson and Milsom (1995) and Burleson (2009), that at a low detection of oxygen levels in the environment, acetylcholine is released from the vagus nerve onto the cardiac pacemaker cells leading to the reduction in heart rate of the fish.

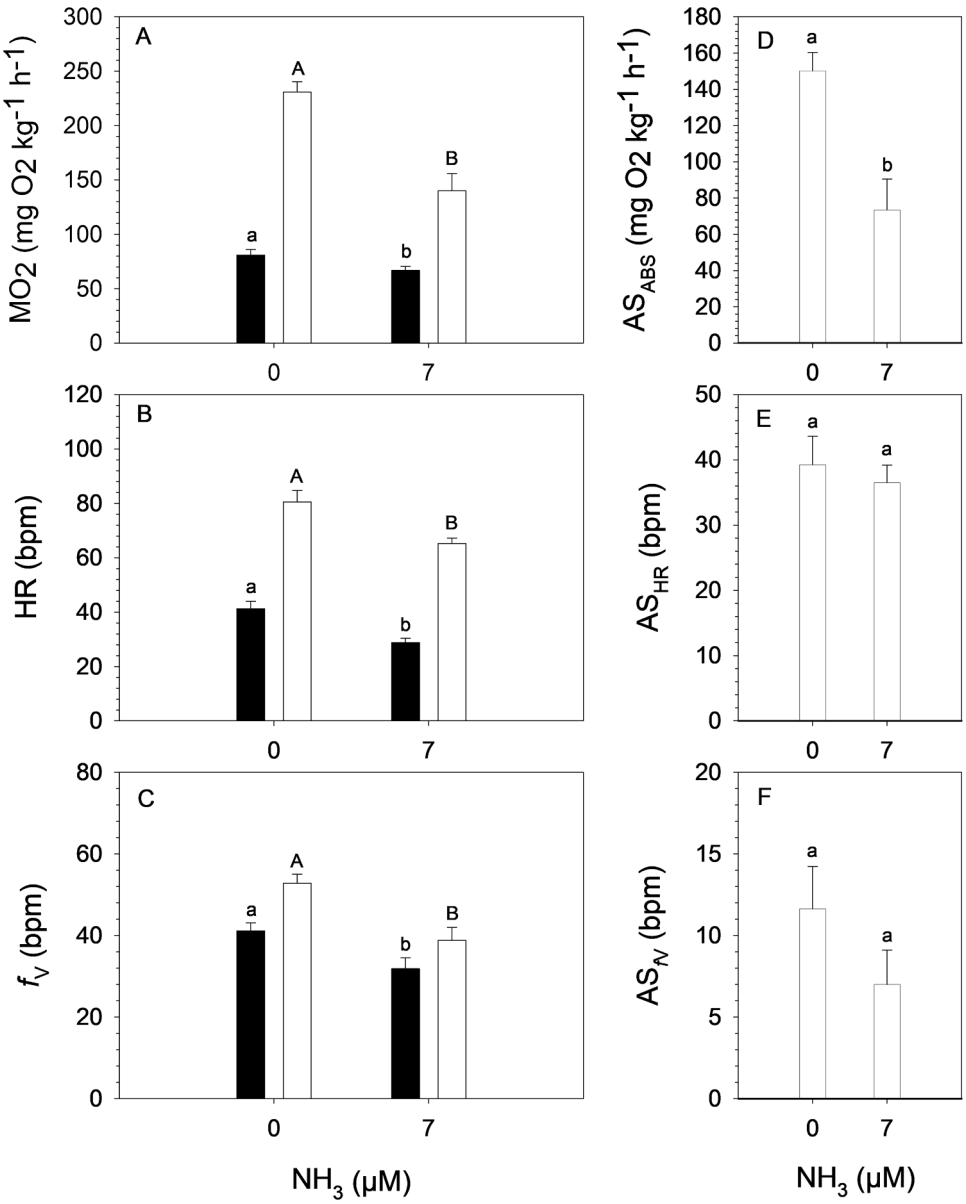

Fig. 2. Oxygen consumption rates (MO_2) in Nile tilapia following 24 h exposure to 7μM unionised ammonia showed significant decreases in both resting (black bars) and maximum oxygen uptake rates (open bars) (A), as well as decreased heart rate (HR) (B), and ventilation frequency (f_V) (C). The resultant loss in absolute aerobic scope (AS_{ABS}) was nearly 50 % (D), while scope for heart rate (AS_{HR}, E) and ventilation frequency (AS_{fV}, F) were not significantly affected. Different superscripts indicate significant differences between measurements obtained during exposure to 0 or 7μM, and conditions of resting and maximum oxygen uptake rates. All values are presented as mean ± SE ($n = 8$).

Table 2
Exposure to progressively increasing concentrations of ionised ammonia IA at a constant pH of 6.5 did not affect metabolic rate (MO_2), ventilation frequency (f_V), or heart rate in Nile tilapia. All values are presented as mean ± SE ($n = 6$).

IA (μM)	UIA (μM)	MO_2 (mg O_2 kg^{-1} h^{-1})	f_V (bpm)	HR (bpm)
0	0	94.8 ± 2.3	49.5 ± 1.7	44.7 ± 4.0
273	0.4	92.2 ± 6.5	45.3 ± 2.9	41.3 ± 3.2
358	0.6	100.8 ± 4.3	45.2 ± 2.9	38.7 ± 3.3
988	1.8	85.9 ± 3.7	42.3 ± 2.0	35.8 ± 3.3
1687	3.1	82.6 ± 3.3	45.0 ± 3.2	41.3 ± 2.7
3008	5.4	90.6 ± 6.1	42.2 ± 3.7	37.7 ± 1.7

4.2. *Prolonged exposure of UIA on standard metabolic rate (SMR) and maximum metabolic rate (MMR) of O. niloticus*

At the early stage of exposing fish to an environmental stressor, behavioural adjustments of reducing muscular and other activity like changes in cardio-respiratory function facilitates metabolic savings of the fish. During 24 h UIA exposure, this experiment revealed non-recovery of metabolic rate of *O. niloticus* from the control fish group. Ventilation in water contributes substantially to SMR (Steffensen and Lomholt, 1983) and its reduction enables metabolic rate suppression. Ammonia exposure significantly affected SMR and MMR of the fish. In this study, Nile tilapia responded to ammonia toxicity by an overall reduction in MO_2, both in terms of SMR and MMR with a simultaneous reduction in f_V and HR, although it is difficult to disentangle cause and effect. The reduction in SMR could be a self-protective mechanism, essentially just a side effect of decreased MO_2 to avoid UIA uptake at

exposure. This physiological adaptation of the fish to reduce SMR under a challenging environmental condition could also prolong the time that the organism can survive under unfavourable conditions, as has been demonstrated during prolonged hypoxia (Bergstedt et al., 2021). MMR reduction could also indicate a loss of fitness caused by a malfunction of the oxygen convection system (cardiac pumping capacity) possibly coupled with reduced diffusion (lamellae thickening, decreased diffusion coefficient).

Maximizing MS positively affects fish biological performance because it reduces situations requiring energetic prioritisation between competing physiological processes. On the contrary, fish with reduced MS according to Lefrançois and Claireaux (2003), are likely to experience impaired performance like swimming. The reduced performances can also have a pronounced effect on respiratory metabolism and the ability of the cardiorespiratory system to meet the O_2 requirement of the fish (McKenzie et al., 2017). In the present study, prolonged exposure of Nile tilapia to even 7 μM-UIA caused a loss of MS to about 51 % which could possibly be caused by a loss in cardiac and ventilatory scope as observed.

4.3. Increasing NH_4^+ at constant pH (6.5) on the metabolic rate of O. niloticus

The findings of this study showed that at a pH of 6.5, NH_4^+ has no acute effect on Nile tilapia at exposure. Data revealed that metabolic rate of *O. niloticus* and its associated parameters were not significantly affected between the treatments in this experiment. These results could be explained that tilapia gills are not permeable to IA, in agreement with the fact that gills of freshwater teleost are relatively impermeable to cations (Evans, 1984). Since NH_4^+ has a hydrated ionic radius that is slightly larger than Na^+ and approximately the same as K^+ (Knepper et al., 1989), it is unlikely that appreciable passive NH_4^+ diffusion takes place under typical freshwater conditions.

This study provides important knowledge on the times that UIA is expected to increase in pond systems during Nile tilapia production. As observed in this experiment, at fluctuating pH which is most likely to occur in pond system, there is tendency of high fraction UIA during the afternoons where pH is high. This could lead to increased fraction of UIA and in early mornings where pH is usually low the fraction of UIA is reduced to cause any significant physiological effect like a change metabolic rate and ventilation of the cultured fish.

5. Conclusion

The present study demonstrated that Nile tilapia tolerate of up to 61 μM UIA for several hours without significant physiological effects, but at UIA concentrations ≥91 μM there was a loss of metabolic rate and its associated changes. At higher UIA exposure, oxygen consumption, heart rate and ventilation frequency of Nile tilapia is reduced. Acute exposure to UIA caused loss of equilibrium at 294 μM. High levels of IA appear to have no short-term effects. Prolonged exposure (24 h) at low UIA concentration (7 μM), metabolic rate of Nile tilapia was reduced to facilitate metabolic suppression. Such a reduction in metabolic rate will probably affect feeding and growth performance by fish in production systems but should be determined experimentally. Particularly earthen ponds in which high pH levels are experienced are likely to have high UIA concentrations. Pond management strategies should include avoiding TAN buildup and aeration, possibly combined in a simple in-pond biofiltration unit.

CRediT authorship contribution statement

Stephen Gyamfi: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Regina Esi Edziyie:** Validation, Formal analysis, Writing – review & editing. **Kwasi Adu Obirikorang:** Data curation, Writing – original draft, Writing – review & editing, Supervision. **Daniel Adjei-Boateng:** Writing – review & editing, Writing – original draft, Project administration, Funding acquisition, Conceptualization. **Peter Vilhelm Skov:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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