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Elevated nitrate levels affect the energy metabolism of pikeperch (*Sander lucioperca*) in RAS

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ABSTRACT

The increasing demand for pikeperch (*Sander lucioperca)* from recirculating aquaculture systems (RAS) has raised the need for detailed knowledge on water quality parameters. Nitrate thresholds are of interest as nitrate accumulates in RAS and influences fish physiology. The trial was conducted in a recirculating aquaculture respirometer with pikeperch (average body weight (BW) 367 ± 1.1 g) successively reared at three different nitrate-nitrogen (NO_3–N) concentrations (N30: 30 mg L^{-1}, N120: 120 mg L^{-1} and N240: 240 mg L^{-1}) and compared to a control group reared at the lowest possible nitrate-nitrogen concentration (N0: 5 mg L^{-1}). Pikeperch were fed once per day with a commercial diet at three different feeding levels corresponding to 0.3% BW, 0.6% BW and 0.9% BW in triplicates for 8 days at either nitrate-nitrogen concentration before fasting for additional 3 days. Oxygen consumption and ammonia excretion were measured for 22-h in fed and fasting pikeperch to examine the influence of nitrate on energy metabolism. Metabolisable energy, retained energy and digestible energy requirements for maintenance (DE_m) as well as the efficiency for energy utilisation (k_g) and relative protein utilisation for energy metabolism (as ammonia quotient (AQ)) were determined. Specific dynamic action (SDA) was calculated to estimate the amount of energy spend on ingestion, digestion, absorption and assimilation of feed at different nitrate levels. SDA was significantly increased in the N240 treatment. Results of DE_m showed a significant difference between N30 and N120 with DE_m of N120 being about 73% higher than DE_m of N30. The efficiency k_g was significantly decreased between N240 and both N120 and N30 by 10% and 11% respectively. Increased values in AQ in the feed depleted fish in the N240 treatment indicates that fasting pikeperch in the high nitrate treatment had to use body protein to fuel an average of 46 ± 7% of their energy metabolism. The results of this trial show that pikeperch tolerate NO_3–N concentrations of up to 240 mg L^{-1} but energy requirements are most favourable at concentrations of 30 mg L^{-1} NO_3–N.

1. Introduction

Percid fish are promising candidates for intensive aquaculture practices due to their relatively fast growth and high filet yield as well as flesh quality (Mathis et al., 2003; Zakęś et al., 2012). The increasing demand of pikeperch (*Sander lucioperca)* from recirculating aquaculture systems (RAS) has raised the need for detailed knowledge on water quality requirements for optimal growth (Dalsgaard et al., 2013; Hermelink et al., 2013; Schram et al., 2013). Relatively little is known on the life-stage specific requirements and thresholds, e.g. in respect to dissolved nitrogenous compounds as a water quality parameter of high relevance. Our current knowledge in this field is frequently based on standard toxicity tests conducted over a short period of time using various fish species (Colt, 2006; Timmons and Ebeling, 2007). This information only provides an approximation considering nitrate thresholds for pikeperch under full production cycle RAS conditions.

Metabolic protein breakdown leads to the accumulation of nitrogenous end products under low water exchange rates in RAS production (Ip et al., 2001; van Rijn, 2007). Nitrogenous end products are mostly excreted as ammonia and urea via fish gills (Evans et al., 2005). In RAS, aerobic biofilters oxidize these compounds to nitrite (NO_2^-) and subsequently to nitrate (Colt et al., 2006; Pierce et al., 1993).

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Nitrate accumulates in RAS and achieving low concentrations imply increased costs either due to increased water exchange or by use of a denitrification unit (Austin, 1988; Müller-Belecke et al., 2013; Pierce et al., 1993). Understanding the effects of nitrate and possible interactions with pikeperch performance in RAS is crucial to reduce costs and increase productivity.

Acute and chronic adverse effects of nitrate have been reported for several fish species. Chronic effects on production performance were described at 125 mg NO_3–N L^{-1} for marine fish whereas freshwater fish tolerated up to 500 mg NO_3–N L^{-1} with LC 50 values exceeding 1000 mg NO_3–N L^{-1} (Camargo et al., 2005; Colt, 2006; Monsees et al., 2016; Timmons and Ebeling, 2007; van Bussel et al., 2012; van Rijn, 2007). Increased nitrate concentrations were found to affect the osmoregulatory ability, cause methemoglobinemia and have the potential to disrupt endocrine function in different freshwater fish species (Hamlin et al., 2008; Hrubec et al., 1996; Tilak et al., 2007). Nitrate levels of 60–100 mg NO_3–N L^{-1} were assumed to be the reason for deformities, abnormal swimming behaviour and general health problems in studies conducted on rainbow trout (*Oncorhynchus mykiss)* and common carp (*Cyprinus carpio)* in low water exchange RAS (Davidson et al., 2014; Davidson et al., 2011; Martins et al., 2009b).

Research conducted on juvenile pikeperch (27 g initial weight) found no negative effect of nitrate levels of up to 350 mg NO_3–N L^{-1} (Schram et al., 2013) while a similar study indicates that NO_3–N levels above 68 mg L^{-1} (Müller-Belecke et al., 2013) result in reduced feed intake in subadult pikeperch. This supports Martins et al. (2009a) hypothesis, that the sensitivity of a given species in respect to water quality parameters in RAS increases with increasing body size and age. This was empirically confirmed for Nile tilapia (*Oreochromis niloticus)* and fathead minnow (*Pimephales promelas)* (Atwood et al., 2001; Palachek and Tomasso, 1984). It is therefore crucial to evaluate water quality criteria specifically for discrete life stages.

The present study focused on the impact of four nitrate levels commonly found in RAS on adult pikeperch (from 367.2 ± 1.1 g to 448.3 ± 28.9 g body weight). The main objective was to evaluate the effect on energy metabolism efficiency and energy requirements above maintenance by using automated group respirometry. This method allows to quantify the energy budget and metabolic fuel use on a short term basis without sacrificing the fish. The study was based on measurements of routine metabolism (normal daily activity including feeding and spontaneous swimming) as this is considered to best represent the metabolism in RAS production (MacIsaac et al., 1997). We hypothesized that increased levels of nitrate have an impact on both the energy metabolism efficiency as well as the maintenance requirements of pikeperch.

2. Material and methods

2.1. Fish

The trial was conducted at Gesellschaft für marine Aquakultur (GMA) Büsum, Germany. Pikeperch (*Sander lucioperca)* were obtained from Fischzucht Rietschen (Rietschen, Germany) and accustomed to the respirometer system for one week before the experiment. Animal husbandry was in accordance to the EU Directive 2010/63/EU for animal experiments.

2.2. Respirometer system

The recirculating aquaculture respirometry system (RARS) is described in detail by Stiller et al. (2013) and consists of 10 tanks (250 L volume each), a trickling biofilter, a particle filter and a sedimentation unit. The system is purpose build for longterm respirometry studies of groups of fish. The tanks have a separate in and outflow and are continuously supplied with recirculating freshwater at 300 L h^{-1}. They are completely closed with water entering in the middle of the tank and exiting through an overflow pipe at the top of the tank. Feeding can be maintained through the overflow pipe with an additional funnel. Each tank has a separate outflow at the bottom that can be used to collect uneaten feed and faeces. A circulation pump in the tanks ensures that water is mixed sufficiently and that all uneaten feed and faeces congregates above the separate outflow. Oxygen concentration (oxygen optode 4330, Serial No. 1557, Aandera data instruments, Bergen, Norway), temperature (compensation thermometer Type 201,085), pH (pHNBS intermediate junction electrode, Ionode IJ44, TPS Pty Ltd., Brisbane, Australia), dissolved carbon dioxide (purpose built flow through headspace analyser, MK-2 pCO2 / Fast Analyser (SubCtech GmbH, Kiel, Germany) with non-dispersive infrared detection, LI-840A (LI-COR Biosciences, Nebraska, United States)) and total ammonia-nitrogen (TAN, loop flow orthophtalaldehyde fluorometric autoanalyser, μMac, SYSTEA S.p.A., Anagni, Italy) were measured online every 12 min in each tank over a course of 22-h. One tank was kept empty of fish and used as a reference. In this tank, walls were cleaned manually on a daily basis to avoid any effect of biofilm build-up on oxygen consumption.

2.3. Experimental design

The experiment was designed as a restricted ration experiment with three feeding levels in parallel triplicates that were subsequently exposed to four nominal nitrate-nitrogen concentrations (N0: 0 mg L^{-1}, N30: 30 mg L^{-1}, N120: 120 mg L^{-1}, N240: 240 mg L^{-1}) over a period of 11 days each (total duration of experiment 55 days). Fish were slowly acclimatised to each nitrate-nitrogen concentration for 7 days and the 22-h measurements were conducted on the eighth day. The measurements were repeated after three days of feed depletion in order to evaluate the effect of feed intake on energy metabolism. The experiment was concluded with an additional period of N0 nitrate concentration to assess any irreversible influence of the increased nitrate concentrations.

The tanks were stocked with 6 individuals per tank (367.2 g ± 1.1 g live body weight). Fish were weighed individually at the start (day 0) and end (day 56) of the experiment to assess live body weight on measurement days.

Fish were fed once per day with a single portion of a commercial diet (ALLER Metabolica, 6 mm, ALLER Aqua, Christiansfeld, Denmark, macro nutrient profile (measured): moisture 7%, crude protein 50%, crude fat 15%, ash 8%, nitrogen free extractives 20%, energy 21.26 g MJ kg^{-1}) with three replicate tanks per feeding level. The feeding levels were calculated on basis of metabolic body weight (MBW) and corresponded to a daily feed intake (DFI) of 0.3, 0.6 and 0.9% bodyweight (1.25, 2.00 and 2.75% MBW). Faeces were removed before feeding through the separate bottom outflow and uneaten pellets were removed from the tanks and counted 20 min after feeding. The average weight of one dry pellet was determined prior to the start of the experiment and multiplied with the number of uneaten pellets to calculate the actual feed intake.

The experimental procedure was repeated for each subsequent period of altered nitrate concentration. Nitrate concentrations in the whole system were increased genuinely over three days by mixing sodium nitrate ($NaNO_3$) and potassium chloride (KCL) (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) in tap water at a weight-ratio of 27:1 thus similar to the ratio found in natural seawater (Romano and Zeng, 2009; Romano and Zeng, 2007). Actually realized nitrate concentrations were measured daily as nitrate-nitrogen (NO_3–N) with a pre-dosed photometric test (LCK 340, Hach Lange GmbH, Düsseldorf, Germany).

2.4. Energy metabolism efficiency and maintenance requirements

All measurements were normalised to an individual fish. Calculations were based on the metabolic body weight (MBW) to compensate for growth during the experiment, using the common