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Hypercapnia adversely affects postprandial metabolism in the European eel (*Anguilla anguilla*)

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ABSTRACT

The present study examined the effects of elevated CO_2 partial pressure on the specific dynamic action (SDA) and ammonia excretion in European eel (*Anguilla anguilla*) following forced feeding. Two different hypercapnic scenarios were investigated; one in which pCO_2 oscillated between 20 and 60 mm Hg over 24 hour cycles, and one in which pCO_2 was constant at 60 mm Hg. Since high CO_2 results in low pH with unchanged alkalinity, a normocapnic group at low pH ($pCO_2 \approx 3$ mm Hg, pH = 6.5) was included to investigate possible direct effects of pH. Constant hypercapnia (60 mm Hg) and low pH (pH = 6.5) both significantly increased the duration of the SDA response by 22% and 29%, respectively. Hypercapnia had no effect on standard metabolic rate, while constant or oscillating hypercapnia significantly lowered the maximum metabolic rate compared to controls, causing a significant reduction of the aerobic scope during constant hypercapnia. Under conditions of oscillating pCO_2, the temporal and spatial postprandial increase in ammonia nitrogen excretion was significantly reduced. This group also excreted significantly less ammonia after ingesting a meal. No significant effects on the magnitude or duration of postprandial ammonia excretion were observed at high pCO_2 or low pH/normocapnia. The results demonstrate that despite an exceptional tolerance towards elevated pCO_2 and acidosis, postprandial metabolic processes of the European eel are adversely affected by hypercapnia and low pH.

1. Introduction

Elevated partial pressures of CO_2 (pCO_2) frequently occur in recirculating aquaculture systems (RAS), particularly in intensive systems with high degrees of water re-use and high rearing densities, due to an accumulation of excreted CO_2 (Crocker et al., 2000; Steffensen and Lomholt, 1990). CO_2 readily diffuses across the gill epithelium, resulting in a decline in plasma pH and blood oxygen carrying capacity (Heisler, 1984, 1993). The resulting extra- and intracellular acidosis can be buffered by an increase in bicarbonate (HCO_3^-) ions in exchange for Cl^- ions (Heisler, 1984, 1993). Physiologically, the effects of hypercapnic conditions may manifest as perturbations in acid–base regulation, and respiratory and cardiac dysfunction, while chronic effects may become evident by reduced growth or increased mortality (for reviews see Ishimatsu et al., 2005; Portner et al., 2004). Eels are exceptionally tolerant to both acute and chronic exposure to elevated ambient pCO_2 with no changes in metabolic rate and no elevations of the traditional indicators of stress, such as plasma catecholamine or cortisol (McKenzie et al., 2002, 2003). The tolerance towards hypercapnia has been explained by the ability of eels to regulate intracellular pH despite severe extracellular acidosis (McKenzie et al., 2003), made possible by a tolerance to very low plasma Cl^- levels (Farrell and Lutz, 1975; McKenzie et al., 2003). This enables them to maintain cardiac output despite acidosis and hypoxemia (McKenzie et al., 2002).

In eels, it takes several hours to days before a steady state in acid–base status is re-established after an initial hypercapnic disturbance, (Hyde and Perry, 1989). Also, adjustments in the gene expression of acid–base regulatory ion exchangers differs between short term and long term exposure to hypercapnia in fish gills (Deigweiher et al., 2008). This suggests, that the negative effects of hypercapnia may be more likely to occur under acute or unstable pCO_2 conditions, as has been shown by CruzNeto and Steffensen (1997), who observed that an acute exposure to 25 mm Hg pCO_2 affected the ability to regulate oxygen uptake during hypoxia in *Anguilla anguilla*.

In Europe, aquaculture production of European eel takes place in RAS under intensive conditions (Dalsgaard et al., 2013), and is generally characterized by high rearing densities reaching some 300 kg m^{-2}. In such conditions, an accumulation of excreted CO_2 can occur (Steffensen and Lomholt, 1990). Complete removal of excess CO_2 is not prioritized and the pH value of the water in eel farms is typically maintained from below 6.0 to 5.0. The extent and frequency of hypercapnic conditions will also depend on feeding schedules due to changes in general activity levels and metabolism associated with feeding events (Owen et al., 1998). Feeding once or twice per day will likely cause pCO_2 to fluctuate on a diurnal basis, while shorter intervals between meals or a continuous feeding regime may result in pCO_2 levels constantly elevated. The implications of elevated pCO_2 on feeding and growth in fish have been studied

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in a few species only, but the results indicate that the effect depends on fish species, fish size, salinity and temperature (Ishimatsu et al., 2008). Severe chronic hypercapnia ultimately reduces growth in Atlantic salmon parr (*Salmo salar*) (Fivelstad et al., 2007) and in juvenile white sturgeon (*Acipenser transmontanus*) (Crocker and Cech, 1996; Crocker et al., 2000). Reduced feed intake has been reported in Sea bass (*Dicentrarchus labrax*) (Cecchini et al., 2001) and in spotted wolfish (*Anarhichas minor*) (Foss et al., 2003). Furthermore, preliminary data from a study performed under conditions similar to the present ones, demonstrates a reduction in specific growth rate of 42% and 56% in *A. anguilla* when exposed to a pCO_2 of 60 mm Hg or a pCO_2 oscillating between 20 and 60 mm Hg, respectively (P.B. Pedersen, unpublished).

The postprandial increase in metabolic rate (SDA) that occurs after feeding represents the cumulative energy expenditure from ingesting and digesting a meal, and the subsequent absorption, assimilation and deposition of nutrients, with protein synthesis constituting the largest part of the SDA response (Beamish and Trippel, 1990; Brown and Cameron, 1991; Jobling, 1981). Environmental factors like temperature and dissolved oxygen can affect the SDA response in fish (Jordan and Steffensen, 2007; Jourdan-Pineau et al., 2010; McCue, 2006; Secor, 2009; Zhang et al., 2010), but to our knowledge, the effect of hypercapnia on SDA has so far not been studied in teleosts. The postprandial rise in oxygen consumption is accompanied by an increase in excretion of ammonia, the main dissolved nitrogenous waste product in freshwater fishes (Wood, 2001). The postprandial ammonia excretion is affected by several factors including species, temperature and body size (Leung et al., 1999); ration size (Leung et al., 1999; Owen et al., 1998); protein intake (Engin and Carter, 2001); and amino acid composition (Larsen et al., 2012). A few studies have demonstrated that environmental hypercapnia can affect protein metabolism in fish, causing a shift towards increased protein catabolism and reduced anabolism. An increased endogenous ammonia production and concomitant excretion was observed in carp (*Cyprinus carpio*) (Claiborne and Heisler, 1986), while an 80% decrease in the hepatic protein synthesis rate was observed in two Antarctic species, *Pachycara brachycephalum* and *Lepidonotothen kempi* (Langenbuch and Portner, 2003). From an aquaculture perspective, this is an undesired effect, since it reduces protein retention and might lead to deterioration in water quality.

The aim of the present work was to study postprandial oxygen consumption and ammonia excretion in the European eel at elevated pCO_2, a typical condition in intensive recirculating aquaculture systems. Two scenarios of hypercapnia were chosen to mimic potential effects of different feeding regimes. In one treatment ($Osc{\cdot}CO_2$), oscillating pCO_2 levels (20–60 mm Hg) were chosen to mimic the results of one single daily feeding event, while the second treatment ($Hi{\cdot}CO_2$) having a high but constant pCO_2 (60 mm Hg) mimicked the results of a continuous feeding regime. To determine whether observed effects were caused by elevated pCO_2 levels or by the resultant reduction in pH, a third treatment (Lo·pH) was included. Here, a normocapnic environment was maintained, but pH was lowered to the same level as in $Hi{\cdot}CO_2$ by the addition of diluted HCl. Treatments were compared to a normocapnic control condition ($pCO_2 \approx 3$ mm Hg, pH = 7.8). The a priori hypothesis was that hypercapnia would suppress the postprandial peak in oxygen consumption rate (MO_2) reflective of a decreased protein synthesis rate, and possibly prolong the duration of the postprandial state as observed in hypoxic cod (Jordan and Steffensen, 2007). The potential negative effect of hypercapnia was hypothesized to be exacerbated at oscillating pCO_2 levels, owing to the added stress of the disequilibria in acid–base status and a preliminary observation of reduced growth.

2. Materials and methods

2.1. Fish and holding conditions

European eel were obtained from a commercial farm (Stensgaard Åleopdræt, Randbøl, Denmark) and transported to the holding facility at the Technical University of Denmark, National Institute of Aquatic Resources, Section for Aquaculture, Hirtshals, Denmark. Fish were evenly distributed into 4 separate 330 L tanks (approx. 20 eels per tank) at a density of approx. 1.2 kg m^{-3}. Upon arrival, all eels received a 24 hour mebendazole bath treatment (1 mg L^{-1} Vermox, Janssen Pharmaceuticals Inc., Belgium) to rid them of any infestations with *Pseudodactylogyrus* spp. (Buchmann and Bjerregaard, 1990). Water was continuously recirculated (40 L min^{-1}, Eheim 1260) through a submerged biofilter (BIO-BLOK®, 150 m^2 m^{-3}, EXPO-NET A/S, Denmark) connected to each tank and 20% of the water volume was exchanged daily by fresh tap water (pH 7.76 ± 0.11, $pCO_2 \approx 3.2$, alkalinity 3.8 mEq L^{-1}). Temperature was maintained at 23 ± 1 °C by aquarium heaters controlled by thermostats (T Controller 2001C, Aqua Medic,

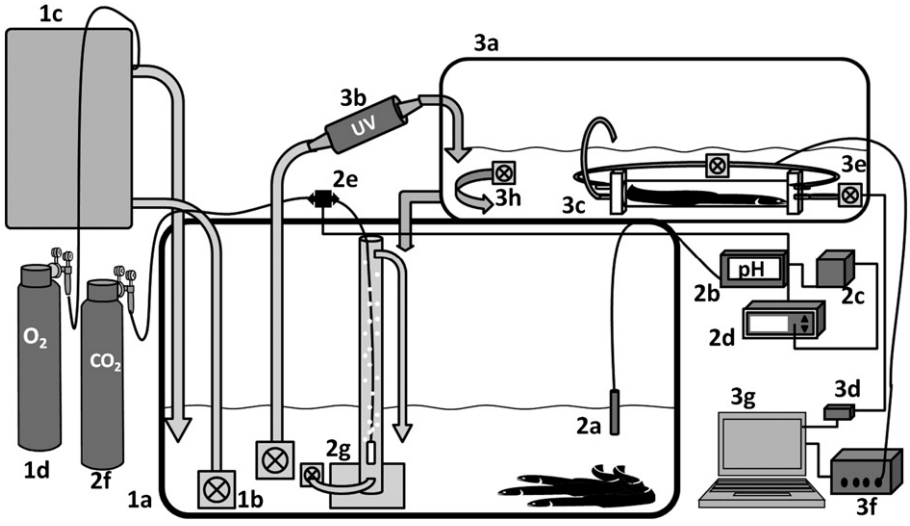

Fig. 1. A schematic illustration of the experimental setup. Holding conditions. 1a: Holding tank, 1b: Biofilter pump, 1c: Biofilter, 1d: Oxygen tank. CO_2 control. 2a: pH probe, 2b: pH meter, 2c: Galvanic isolation amplifier, 2d: Programmable instrument, 2e: Solenoid valve, 2f: CO_2 gas, 2 g: CO_2 mixing column with pump. Respirometry. 3a: Respirometer holding tank, 3b: Water supply to respirometer tank via UV sterilizer, 3c: Respirometer with recirculation loop (NB only 1 of 4 depicted), 3d: AD converter, 3e: Flush pump, 3f: Fiber optic O_2 sensor, 3g: Laptop PC, 3h: Circulation pump. Arrows indicate flow of water. This schematic illustrates the setup for the $Hi{\cdot}pCO_2$ experiment and a few modifications were applied to the $Osc{\cdot}pCO_2$ and Lo·pH setups. See Section 2 for further details.