Comparative Biochemistry and Physiology, Part A 181 (2015) 87–99

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Hypo-osmotic stress-induced physiological and ion-osmoregulatory responses in European sea bass (*Dicentrarchus labrax*) are modulated differentially by nutritional status

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ARTICLE INFO

Article history:
Received 5 November 2014
Received in revised form 26 November 2014
Accepted 27 November 2014
Available online 5 December 2014

Keywords:
Salinity acclimation
Fasting
Osmoregulation
Na^+/K^+-ATPase
$Na^+/K^+/2Cl^-$
Ammonia excretion

ABSTRACT

We investigated the impact of nutritional status on the physiological, metabolic and ion-osmoregulatory performance of European sea bass (*Dicentrarchus labrax*) when acclimated to seawater (32 ppt), brackish water (20 and 10 ppt) and hyposaline water (2.5 ppt) for 2 weeks. Following acclimation to different salinities, fish were either fed or fasted (unfed for 14 days). Plasma osmolality, $[Na^+]$, $[Cl^-]$ and muscle water content were severely altered in fasted fish acclimated to 10 and 2.5 ppt in comparison to normal seawater-acclimated fish, suggesting ion regulation and acid–base balance disturbances. In contrast to feed-deprived fish, fed fish were able to avoid osmotic perturbation more effectively. This was accompanied by an increase in Na^+/K^+-ATPase expression and activity, transitory activation of H^+-ATPase (only at 2.5 ppt) and down-regulation of $Na^+/K^+/2Cl^-$ gene expression. Ammonia excretion rate was inhibited to a larger extent in fasted fish acclimated to low salinities while fed fish were able to excrete efficiently. Consequently, the build-up of ammonia in the plasma of fed fish was relatively lower. Energy stores, especially glycogen and lipid, dropped in the fasted fish at low salinities and progression towards the anaerobic metabolic pathway became evident by an increase in plasma lactate level. Overall, the results indicate no osmotic stress in both feeding treatments within the salinity range of 32 to 20 ppt. However, at lower salinities (10–2.5 ppt) feed deprivation tends to reduce physiological, metabolic, ion-osmo-regulatory and molecular compensatory mechanisms and thus limits the fish's abilities to adapt to a hypo-osmotic environment.

1. Introduction

Salinity is a limiting factor for many marine organisms. Owing to global warming, glaciers and ice caps have been rapidly disappearing, and more frequent intense rainfall events occur. Consequently, the salinity gradient of some marine ecosystems such as enclosed bays, estuaries and the inshore waters has gradually reduced over the last few decades (Wong et al., 1999; Freeland and Whitney, 2000; Pierce et al., 2012). Moreover, euryhaline teleosts, including diadromous and non-diadromous fish, often encounter osmotic challenges at different stages of their life cycle. The shift in the ambient salinity can alter overall fitness of fish with a severe challenge to ion-osmoregulation (Kelly and Woo, 1999; Roessig et al., 2004; Yan et al., 2004). A high plasticity of the ion-transporting ability is thus necessary to cope with a wide range of environmental salinities. Ion and acid–base balance in fish is mainly mediated by the gills, kidneys and intestines. Among them, the gills, which are exposed to external environments, are the major organ for balancing ion movement between the internal milieu and the external environment. Mitochondrion-rich cells (MRCs or chloride cells) present in the gill epithelium are the main site for active ion uptake and secretion, and these MRCs can modulate ion transport mechanisms to ensure intracellular homeostasis during osmotic challenges. When transferring marine teleosts to salinities below the iso-osmotic point, Na^+ and Cl^- transport across the gill epithelia switches from excretion to uptake (Marshall and Grosell, 2005). These ion transport mechanisms in marine teleosts are coordinated by ion channels, cotransporters (e.g., $Na^+/K^+/2Cl^-$, Na^+/Cl^-) and energy dependent ion transport enzymes like the Na^+/K^+-ATPase (NKA) (Hwang et al., 2011; Hiroi and McCormick, 2012). NKA is present on the basolateral membrane of branchial epithelium that actively transports Na^+ out and K^+ into the animal cells. It plays a key role in maintaining intracellular homeostasis by providing a driving force for many ion-transporting systems in the gills (Marshall and Bryson, 1998; Hirose et al., 2003; Lin et al., 2004a,b; Hwang and Lee, 2007). In teleosts and in diadromous species, NKA activity, abundance and its expression are often suggested as indicators of the osmoregulatory ability (Jensen et al., 1998; Marshall, 2002; Evans et al., 2005; Giffard-Mena et al., 2008). NKA generates a low intracellular Na^+ gradient; thereby inducing the transport of Na^+, K^+ and Cl^- into the cell through the presence of a basolateral $Na^+/K^+/2Cl^-$ cotransporter (NKCC), apical Na^+/Cl^- cotransporter (NCC) (Hiroi et al., 2008; Hsu et al., 2014) and an apically located Cl^- channel

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http://dx.doi.org/10.1016/j.cbpa.2014.11.024
1095-6433/

homologous to the human cystic fibrosis transmembrane conductance regulator (CFTR) (Hwang et al., 2011; Hiroi and McCormick, 2012). NKCC seems to play a central role in the maintenance of the electrolyte content, transepithelial ion and water movement in polarised cells (Russell, 2000; Cutler and Cramb, 2001). As such, its pattern of expression at different osmoregulatory sites dictates the adaptation of teleosts to osmotic challenge (Lorin-Nebel et al., 2006).

The capacity of euryhaline fish to adapt to varying environmental salinities depends on the activation of ion transport process. The synthesis and operation of ion transport proteins impose energetic regulatory costs for active uptake or secretion of ions. Therefore, a sufficient and timely energy supply is a prerequisite for the operation of ion/osmoregulatory mechanisms in fish gills to cope with a wide range of environmental salinities through a series of coordinated physiological processes that facilitate switching between hyperosmotic and hyposmotic environment (or vice versa) (Boeuf and Payan, 2001; Chang et al., 2007; Tseng and Hwang, 2008; Evans, 2010). The energy requirement of the branchial cell is reported to be maintained by oxidation of glucose obtained from the circulation (Mommsen et al., 1985). In this context, the quantification of energy stores (e.g., glycogen, lipid and protein) in tissues would offer a better understanding of metabolic fuel supply to the ion-osmoregulatory system. Consequently, nutritional status of fish can have a pronounced impact on performance, and determine the competency to adapt to changing environments.

Periods of nutrient deprivation is a natural phenomenon in wild populations of fish especially during reproduction and migration and also occurs regularly for the cultured fish. It has already been demonstrated that energy deficiency due to feed deprivation can adversely affect numerous physiological systems such as energy supply, metabolism, ionic balance and endocrine functions (Gaylord et al., 2001; Small et al., 2002; Peterson and Small, 2004; Small, 2005; Small and Peterson, 2005; Bucking and Wood, 2006a,b). The majority of the research has investigated the impact of salinity stress alone on the adaptive responses in fish (Jensen et al., 1998; Lin et al., 2003; Lorin-Nebel et al., 2006; Giffard-Mena et al., 2008); the assessment of such responses when fish are subjected to additional ecologically relevant stressful conditions is rather scarce. However, some work has been done looking at the interaction of starvation with the salinity changes in marine fish but they were mainly focused on survival (Woo and Murat, 1981; Rodríguez et al., 2005). Therefore, the present experiment was designed to study the metabolic, physiological and ion-osmoregulatory responses in fish, which occur as compensatory mechanisms to deal with salinity variations in combination with feed deprivation.

The fish gill is also the site of excretion of excess nitrogen in the form of ammonia, and models suggest that ion regulation is also linked to the ammonia excretion pathways (Wilkie, 1997, 2002; Wright and Wood, 2009). Basolateral transporters such as NKA and NKCC are primarily associated with ion transport, but their importance in ammonia excretion has also been implicated since similarities in the hydration radius of K^+ and NH_4^+ allow substitution at transport sites (Wilkie, 1997; Randall et al., 1999; Alam and Frankel, 2006). Although the bulk of ammonia transport is thought to be through NH_3 diffusion either directly or through Rhesus proteins, intracellular NH_4^+ can be extruded across the apical membrane, presumably via a Na^+/H^+ antiporter, with NH_4^+ substituting for the H^+ (Wilkie, 1997). In this scenario, it can be presumed that an alteration in ion- and osmoregulation during individual and or combined effect of salinity stress and starvation may also hamper internal ammonia homeostasis.

The European sea bass (*Dicentrarchus labrax* L.) is a marine teleost, is widely distributed throughout the Europe and extensively used for aquaculture and is therefore of great commercial and ecological importance. It is euryhaline at all developmental stages and in the wild, they move seasonally between the open sea and hyposaline environment such as lagoons/estuaries. This requires a modification in the ion-osmoregulatory response (Barnabé et al., 1976; Kelley, 1988). Therefore, in the present study, we used juveniles of European sea bass as a test organism to examine how this species manipulates its various biological processes in order to retain body homeostasis when confronted with different stressors such as salinity and feed deprivation at the same time. Overall, we hypothesised that sea bass would be more affected in hypo-osmotic environments, that feeding would improve the capacity of sea bass to retain their ionic balance and ammonia homeostasis and that it would provide the necessary energy for different compensatory responses, thus facilitating acclimation to lower salinities.

To achieve our goals, we assessed (i) osmoregulatory ability by measuring ion concentration in plasma, activity and expression of branchial NKA, NKCC gene expression, H^+-ATPase activity and muscle water content; (ii) energy budgets—glycogen, lipid and protein reserves were quantified as indicators of energy use; (iii) ammonia and urea homeostasis, by determining ammonia and urea accumulation in plasma and their excretion rate, as an indicator of nitrogen metabolism and excretion; and (iv) plasma lactate as an indicator of anaerobic metabolism.

2. Materials and methods

2.1. Experimental system and animals

European sea bass (*Dicentrarchus labrax*) juveniles (14–18 g) were obtained from Ecloserie Marine (Gravelines, France). Fish were kept at the University of Antwerp in tanks (1000 L), filled with artificial seawater (Meersalz Professional Salt, 32 ppt salt) for at least a month. Thereafter, a total of 240 fish were distributed into eight 200 L tanks ($n = 30$ per tank; 32 ppt) equipped with a recirculating water supply in a climate chamber where temperature was adjusted at 17 ± 1 °C, and photoperiod was set at 12 h light and 12 h dark. Water quality was ensured through an additional bio-filter containing wadding, activated charcoal and lava stones. Fish were acclimated to the above-mentioned constant salinity, temperature and photoperiod for 3 weeks prior to the experiment and were fed with commercial pellets (Skretting, Boxmeer, The Netherlands) at a rate of 2% of their wet body weight/day.

After 3 weeks, fish were progressively exposed to three experimental salinities: 20 ppt (~500 mOsm/kg, pH 8.17; 2 tanks), 10 ppt (~249 mOsm/kg, pH 8.10; 2 tanks) and 2.5 ppt (~69 mOsm/Kg, pH 7.87; 2 tanks). Changes in salinity were progressed by reducing the salinity by 5% each 3 days until the desired salinity was reached. Fish maintained at normal seawater salinity 32 ppt (~800 mOsm/Kg, pH 8.25; 2 tanks) were designated as control group. Each experimental group and the control were acclimatised at the desired salinity for 2 weeks and were fed daily at a rate of 2% on their wet body weight. Experimental salinities were adjusted by diluting artificial seawater with filtered freshwater, and salinity was measured using a hand-held refractometer.

After being acclimatised at the respective salinities for 2 weeks, feeding was withheld for 14 days in one of the respective tank for each of the salinity group while feeding (2% bodyweight/day) was continued in the respective parallel tank. Water was exchanged by 40–60% twice a week to remove uneaten food and minimise the build-up of nitrates and nitrites. The salinity was tested and controlled daily by adding clean water (of the appropriate salinity) to compensate for the loss of water by evaporation. All animal experiments were approved by the local ethics committee, University of Antwerp, and conducted according to the guidelines of the Federation of European Laboratory Animal Science Associations.

2.2. Experimental protocol and excretion measurement

After 14 days of feeding or fasting, 16 fish (8 fed and 8 fasted) were transferred from their respective salinity-acclimated tank to individual 8 L glass aquaria (water volume set to 5 L) with the salinity matching