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Effects of fasting and re-alimentation on gill and intestinal morphology and indicators of osmoregulatory capacity in genetically selected sea bass (*Dicentrarchus labrax*) populations with contrasting tolerance to fasting

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ABSTRACT

Fasting and refeeding occur naturally in predators but this is largely ignored when dealing with farmed fish. Therefore, the effects of 3-week fasting and re-alimentation (2.5% of the individual body mass) were investigated using two genetically selected populations (F2 generation) of 250 g juvenile sea bass (*Dicentrarchus labrax* L.). Blood osmolarity, gill and intestinal morphology and expression of the sodium pump (Na^+, K^+-ATPase, NKA) were studied on two phenotypes showing different degrees of body mass loss during food deprivation: one group losing body mass rapidly during fasting (F+) and the other one limiting body mass loss during the same period (F−).

Blood osmotic pressure significantly decreases due to re-alimentation in both groups, but this is compensated in the F+ group. In this group, gill ionocytes are smaller and less numerous, but a significantly higher NKA gene expression is noted in the gills in comparison to the F− individuals 48 and 72 h after re-alimentation, and also in the posterior intestine 72 h after re-alimentation. This most probably occurs to compensate for a higher salt intake during nutrient absorption in comparison to the F− group. Furthermore, refed F− fish absorb more lipids along the proximal anterior intestine, and take longer to digest than the F+ group, and show enterocyte vacuolization in the posterior intestine.

Therefore, the two selected populations have different postprandial digestive strategies: the F− fish optimize feed efficiency first at the cost of optimal hydromineral adjustment, while the F+ group invests in osmoregulatory performance at the expense of digestive physiology.

Statement of relevance: Our paper is highly relevant to the general field of commercial aquaculture. There is an increasing number of research articles dealing with fasting and refeeding in commercial fish and how to improve fish nutrition based on these physiological data and genetic selection.

1. Introduction

Organisms withstand environmental stress by maintaining homeostatis through physiological and biochemical regulations. This demands trade-offs in the use of metabolic energy. In response to environmentally forced energetic trade-offs, populations adapt and reallocate metabolic energy depending on their genotypic variance (Applebaum et al., 2014). In Teleost fish for example, the regulation of ion and water competes with digestive physiology (Taylor et al., 2010; Wood et al., 2010). At different salinities, some species modify their energetic substrate as well as the energy used during nutrient absorption (Gracia-López et al., 2006). The nutritional status of the fish (fasting/fed) can also directly affect the metabolic response to salinity acclimation (Kültz and Jürss, 1991; Polakof et al., 2006). Furthermore, diet manipulation such as the addition of NaCl to the food can result in greater feeding efficiency and mass gain when fish are reared in freshwater, and lead to a better adaptability during transfer from fresh to saltwater (Al-Amoudi, 1987; Cnaani et al., 2012, 2010; Fontaínhas-Fernandes et al., 2000; Gatlin et al., 1992; Harpaz et al., 2005; Salman and Eddy, 1990).

Different mechanisms of salt and water movement in the gills, gut, kidney and through the skin (i.e. larval stages) maintain stability in the internal medium of teleosts (Varsamos et al., 2005; Whitehead, 2010). In the gills, specialized ionocytes (mitochondria-rich cells or chloride cells) play a major role in ion and water exchanges (Evans et al., 1999; Hiroi and McCormick, 2012; Lorin-Nebel et al., 2006).

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Similarly, the fish intestine not only regulates nutrient absorption but also ion and water exchanges (Grosell, 2010; Whittamore, 2011). It is essential for the teleost to couple high drinking rates with intestinal fluid absorption in order to compensate for the loss of water to the hyperosmotic environment. Although salt intake due to feeding in seawater does not exceed daily intake from drinking (Dabrowski, 1986; Shehadeh and Gordon, 1969), such an acute intake induces a diet-dependent challenge (Grosell and Taylor, 2007; Taylor et al., 2007). Despite the desalinization occurring in the esophagus and fluid dilution in the stomach, the luminal fluid that enters the anterior intestine remains slightly hyperosmotic in comparison to the body fluids (approximately 400–475 mOsm.kg^{-1}). It then becomes isoosmotic along the anterior intestine (Marshall and Grosell, 2006). Water absorption occurs mainly in the proximal and middle part of the anterior intestine and is primarily associated with NaCl cotransport through a 'solute-linked' water transport process occurring in the lateral spaces between adjacent cells of the intestinal mucosa (Larsen et al., 2009; Thiagarajah and Verkman, 2006). Different ions are then secreted back into the gut, creating an excreted luminal fluid that is isoosmotic to the body fluids but has a different ionic concentration. The final role of intestinal function in osmoregulation occurs in the rectum with solute-coupled water absorption (Gregorio et al., 2013).

As seen in various Vertebrates, the intestine is one of the most costly tissues to maintain (Cant et al., 1996), functionally linking energy intake and energy used. It is one of the most plastic organs which can be severely atrophied during fasting and rapidly reactivated at refeeding, even after prolonged fasting (Ali et al., 2003; Blier et al., 2007; Gas and Noailliac-Depeyre, 1976; Krogdahl and Bakke-McKellep, 2005; Lignot, 2012; McCue, 2010; Zaldúa and Naya, 2014). Teleost fish show accelerated growth ('compensatory growth') due to the enhanced feed intake, nitrogen production and feed conversion efficiency after refeeding (Ali et al., 2003; Won and Borski, 2013). Hyperphagia may not be the only factor explaining accelerated growth rate during refeeding. Optimizing resource allocation between structural, storage and gonadal growth, maintenance and energy-consuming activities such as foraging and predator avoidance could also play a role (Ali et al., 2003).

The European sea bass *Dicentrarchus labrax* is a eurythermic (5–28 °C) and euryhaline (0 ppt-hypersaline condition) coastal species that migrates, as juveniles and adults, between the open sea and brackish coastal lagoons (and even freshwater estuaries) for feeding. Therefore, this study was designed in order to test the energetic trade-off between digestive processes and osmoregulatory functions in two selected populations of juvenile sea bass with either high (F+) or low (F−) mass loss during fasting (Dupont-Prinet et al., 2010). We aimed to identify morphological and functional differences at the branchial and intestinal levels during fasting and throughout the postprandial period.

2. Materials and methods

2.1. First and second generations of selective breeding (Fig. 1)

Selective breeding of the first and second generations of sea bass was carried out at IFREMER (Station Expérimentale d'Aquaculture, Palavas-les-Flots, France) between May and July 2011.

The first generation of selected sea bass was bred according to Dupont-Prinet et al. (2010). At 68 days post fertilization, sea bass were transferred to a 5 m^3 fiberglass tank supplied with bio-filtered aerated seawater at 20 °C, and were fed ad libitum (self-feeder) with a standard commercial diet (Neogrower, Le Gouessant, France) containing 45% protein and 17% lipids. At day 306 post fertilization, 2000 fish were individually tagged by inserting a PIT-tag® (Passive Integrated Transponder, AEG-Id, Ulm, Germany) horizontally just behind the head to prevent any change of position subsequent to implantation. Fish were then reared for an additional period of four weeks following a standard rearing protocol (Chatain, 1994) before being anesthetized (2-phenoxy-ethanol, 0.4 ml.l^{-1}, Aquaveto). They were individually identified using a PIT-Tag reader, weighed to the nearest 0.1 g and their fork length was measured to the nearest mm. They were then submitted to two successive cycles of three-week food deprivation three-week ad libitum refeeding by self-feeder, with body mass and fork length measured at the end of each deprivation and refeeding period. Individuals representing 25% of the highest and lowest mass loss during fasting (labeled F+ and F−, respectively) and with the 25% highest and lowest weight gain following refeeding (labeled WG+ and WG−, respectively) were identified, as described in Grima et al. (2010a, 2010b).

For the second generation, individuals selected for extreme performance to starvation resistance (F+/F−) were crossed in order to obtain fertilized eggs, each group being a mix of WG+ and WG− individuals (20 males F+ × 5 females F+ and 20 males F− × 5 females F−). Newly-hatched larvae and young juveniles were maintained and 2000 of these fish were individually tagged at day 221 post fertilization. After an additional period of four weeks, they were anesthetized, individually identified, weighed and measured before the fasting and re-alimentation experiment.

2.2. Fasting and re-alimentation experiments

Fifty F+ and 50 F− fish were transferred to five 3 m^3 tanks (10 F+ and 10 F− in each tank) supplied with bio-filtered seawater at 20 °C with a salinity of 37 g.l^{-1}, under a 12 h:12 h light:dark photoperiod. Fish were then submitted to two successive cycles of 3-week feed deprivations and 3-week ad libitum refeeding by self-feeder (Neogrower n°5: protein 43%, lipids 20%, ash 5.6%, fiber 3%).

At the completion of the second 3-week fasting period, anesthetized fish were either sacrificed (2-phenoxy-ethanol, 500 mg.l^{-1}, Aquaveto) for blood, branchial and intestinal tissue sampling or force-fed (2.5% body mass). Re-alimented fish were then left in their tanks for 4, 24, 48 or 72 h after force feeding and then sacrificed. Blood samples were taken in heparinized syringes for blood osmotic pressure determination and measured with a micro-osmometer (Model 3300, Advanced Instruments, Needham Heights, MA, USA). Tissue samples (second gill arches, proximal and distal segments of the anterior intestine, posterior intestine) were directly transferred into either TRIzol® (ThermoFisher, 15596026), Bouin or glutaraldehyde fixatives.

Selective breeding and animal experimentation operated at the IFREMER facilities were authorized by French animal experimentation regulations (C-34-192-6). Technical staff and scientists conducted animal experiments in accordance with good animal practice under individual authorizations from the DDPP (Direction Départementale de la Protection des Populations - Hérault).

2.3. Light microscopy and NKA immunolabeling

For light and fluorescent microscopy, samples of the gills, anterior intestine (proximal and distal segments) and posterior intestine (rectum) were fixed in Bouin's solution for 48 h, then washed in 70% alcohol and dehydrated in an ascending series of ethanol and finally processed for embedding in Paraplast X-TRA® (Sigma-Aldrich, P3808). Then, 4 μm sections were cut on a Leitz Wetzlar microtome, collected on glass slides and either stained using the classical Masson's trichrome staining protocol (Martoja and Martoja-Pierson, 1967) or directly used for immunolabeling.

Prior to NKA immunolabeling, sections were immersed in Histoclear (Histological Clearing Agent, Agar, R1345) for 10 min (2 × 5 min) for dewaxing, placed in butanol for 5 min then hydrated through a graded series of ethanol. Slides were rinsed in a solution of 10 mM phosphate-buffered saline (PBS), 150 mM NaCl and 0.01% Tween-20, pH 7.3, for 10 min then treated with 50 mmol NH4Cl in (PBS), pH 7.3, for 5 min to mask free aldehyde groups of the fixative. The sections were incubated for 10 min with a blocking solution (BS) containing 1% bovine serum albumin (BSA) and 0.1% gelatin in PBS. They were then left for 2 h at room temperature in a humidity chamber where sections were covered