Estuarine, Coastal and Shelf Science 91 (2011) 102–109

Contents lists available at ScienceDirect

Estuarine, Coastal and Shelf Science

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

Salinity-related variation in gene expression in wild populations of the black-chinned tilapia from various West African coastal marine, estuarine and freshwater habitats

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ARTICLE INFO

Article history:
Received 26 April 2010
Accepted 14 October 2010
Available online 29 October 2010

Keywords:
fish
estuary
cytochrome c oxydase-1
NADH dehydrogenase
Na^+–K^+-ATPase
voltage-dependent anion channel
Sarotherodon melanotheron

ABSTRACT

This study measured the relative expression of the genes coding for Na^+, K^+-ATPase 1α(NAKA), voltage-dependent anion channel (VDAC), cytochrome c oxidase-1 (COX), and NADH dehydrogenase (NDH), in gills of six wild populations of a West African tilapia species, acclimatised to a range of seasonal (rainy or dry) salinities in coastal, estuarine and freshwater sites. Previous laboratory experiments have demonstrated that these genes, involved in active ion transport, oxidative phosphorylation, and intra-cellular ATP transport, are relatively over-expressed in gill tissues of this species acclimated to high salinity. Positive correlations between relative expression and ambient salinity were found for all genes in the wild populations (Spearman rank correlation, $p < 0.05$), although for some genes these were only significant in either the rainy season or dry season. Most significantly, however, relative expression was positively correlated amongst the four genes, indicating that they are functionally interrelated in adaptation of *Sarotherodon melanotheron* to salinity variations in its natural environment. In the rainy season, when salinity was unstable and ranged between zero and 37 psu across the sites, overall mean expression of the genes was higher than in the dry season, which may have reflected more variable particularly sudden fluctuations in salinity and poorer overall water quality. In the dry season, when the salinity is more stable but ranged between zero and 100 psu across the sites, NAKA, NDH and VDAC expression revealed U-shaped relationships with lowest relative expression at salinities approaching seawater, between 25 and 45 psu. Although it is not simple to establish direct relationship between gene expression levels and energy requirement for osmoregulation, these results may indicate that costs of adaptation to salinity are lowest in seawater, the natural environment of this species. While *S. melanotheron* can colonise environments with extremely high salinities, up to 100 psu, this was related to high relative expression for all genes studied, indicating that this imposes increased energy demand for osmotic homeostasis in gill tissues. This study is the first to demonstrate, in fish and in wild populations, that expression of NAKA, VDAC, NDH and COX are interrelated in gill tissues, and are involved in long-term acclimatisation to a salinity range between 0 and 100 psu.

1. Introduction

The black-chinned tilapia *Sarotherodon melanotheron* (family Cichlidae) is a marine euryhaline teleost that is widely distributed in West African coastal, estuarine and lagoon ecosystems. It is a very hardy species and is particularly notable for its ability to tolerate a wide range of environmental salinities (Philippart and Ruwet, 1982; Campbell et al., 1986; Ouattara et al., 2003; Lemarié et al., 2004). The species is found in marine coastal waters, but also in freshwater habitats, where salinity is constant throughout the year (Falk et al., 2003). It also, however, maintains populations in estuaries, such as those of the Saloum and the Gambia rivers, which exhibit extreme variations in salinity (Albaret et al., 2004; Panfili et al., 2006), ranging from freshwater to extremely hypersaline water (up to 130 psu) and where seasonal variations in salinity can be considerable. Relatively little is known about mechanisms of salinity adaptation in this species, in particular the molecular responses which may underlie its exceptional euryhalinity.

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doi:10.1016/j.ecss.2010.10.015

We recently investigated molecular responses of the black-chinned tilapia to salinity extremes under experimental conditions (Tine et al., 2008). Fish were acclimated to either freshwater (0 psu) or hypersaline water (70 psu) for 45 days, and two suppression subtractive hybridisation (SSH) libraries were constructed. One comprised the genes relatively over-expressed at 0 psu while the other comprised those over-expressed at 70 psu. Interestingly, the partial cDNA of genes coding for the Na^+–K^+-ATPase 1α(NAKA) pump (GenBank accession no. ES881735), the Voltage-dependent Anion Channel (VDAC) (GenBank accession no. ES881863), cytochrome c oxdase-1 (COX) (GenBank accession no. ES881722), and NADH dehydrogenase (NDH) (GenBank accession no. ES881798), were isolated from the hypersaline library. Accordingly, we proposed that these genes must be implicated in acclimation to hyper-osmotic conditions in *S. melanotheron*.

This is certainly the case for NAKA, a membrane protein which maintains ion gradients required for cell homeostasis and whose activity in the gills is related to either active ion secretion in hyper-osmotic conditions or active uptake in hypo-osmotic conditions (Morgan and Iwama, 1999; Boeuf and Payan, 2001). The VDAC is a pore protein on the outer mitochondrial membrane (Lemasters and Holmuhamedov, 2006) and is a common pathway for ATP/ADP exchanges between mitochondria and the consumption sites, including the ion pumps (Colombini, 2004). Thus, the VDAC channel may contribute to adjustments of metabolite flux in response to salinity variations. The COX and NDH are not, however, directly related to ion transport at all, being components, respectively, of complexes I and IV of the mitochondrial respiratory transport chain. As such they contribute to the oxidative phosphorylation which provides ATP for all metabolic activities (Kadenbach, 2003). It has been demonstrated in fishes that environmental salinity variations induce changes in mitochondrial metabolism (Suresh and Jayaramani, 1983; Sébert et al., 1997), which are essentially intended to meet the increased energy demand associated with ion transporter activity, and with the synthesis of new salt transporting proteins or hormones involved in osmoregulatory processes (Stanton et al., 2006).

These genes were identified in laboratory acclimation experiments. The objective of the current study was to investigate whether they show variations in expression in wild populations of *S. melanotheron*, that were seasonally acclimatized to the range of environmental salinities which prevailed in various West African coastal marine, estuarine and freshwater habitats. We investigated the hypothesis that their relative expression would be correlated with salinity, and also correlated amongst themselves. We sampled in both the rainy and the dry season, because the estuarine environments are known to vary significantly in their salinity regimes between these two seasons.

2. Material and methods

2.1. Sampling of natural populations

Black-chinned tilapia *S. melanotheron* were collected in 2006, at the end of the dry season (May) and in the middle of the rainy season (October). Six sampling sites were considered, five of which (Guiers Lake, Hann Bay, Missirah, Foundiougne, and Kaolack) are located in Senegal and one (Balingho) in Gambia (Fig. 1). Guiers Lake and Hann Bay were taken as reference freshwater and seawater sites, respectively, in which there was no seasonal variation in salinity. Fish were also collected at three locations of the Saloum estuary (Kaolack, Foundiougne, and Missirah) and in one location of the Gambia estuary (Balingho), sites which are known to exhibit large variations in salinity between rainy and dry seasons. For each location, the salinity and temperature were measured *in situ* with a refractometer (ATAGO) and a thermometer, respectively. Fish were captured by castnet. To limit fish stress and prevent variability due to manipulation, only five fish were sampled from each castnet thrown. Fish were quickly removed from castnets and anesthetised in 2-phenoxyethanol (2.5 ml l^{-1}) before measures of fork length (FL, in mm) and total mass (W, in g). Fish were then killed by rapid decapitation and sex as well as gonad maturity stage recorded according to Legendre et al. (1989). Because gene expression could conceivably be influenced by differences in developmental or sexual stage, only size classes between 120 and 160 mm fork length with sexual stage 1 or 2 were selected for the analyses. Stage 1 corresponds to immature individuals for female and male whereas stage 2 corresponds to beginning of maturation and developing testicles for female and, male respectively (Panfili et al., 2004b). Gills were extracted from these individuals and stored in *RNA later* (Ambion) at 4 °C for 24 h and then at −20 °C until processing.

2.2. RNA extraction and real-time PCR analysis

Total RNA was extracted from the gills preserved in RNA Later (Ambion, USA) with TRIZOL® reagent (Gibco BRL, Gaithersburg, MD, USA) method, according to the manufacturer's instructions.

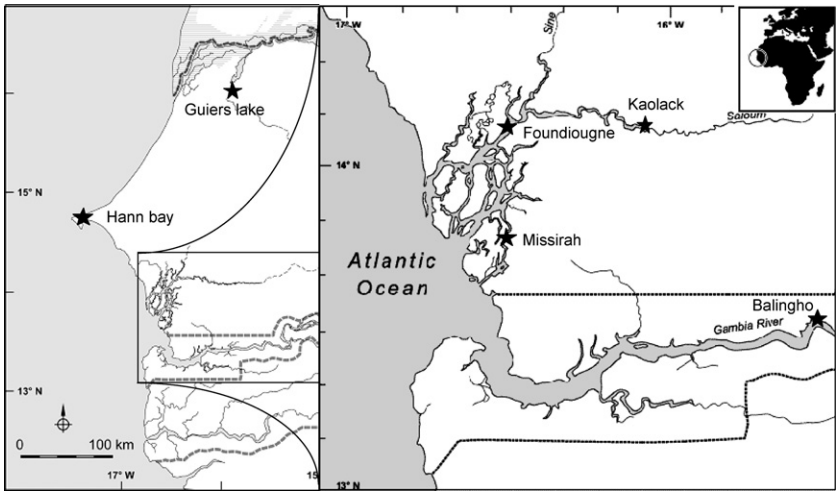

Fig. 1. Sampling locations (black star) of the black-chinned tilapia *Sarotherodon melanotheron* in Saloum and Gambia estuaries. Fish were collected in 2006, in rainy season when salinity only varied between 0 and 37 psu amongst sites, and in the dry season when extremely hypersaline conditions were observed in some estuarine sites, up to 100 psu.