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Effects of salinity and hypoxia-induced hyperventilation on oxygen consumption and cost of osmoregulation in the estuarine red drum (*Sciaenops ocellatus*)

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ABSTRACT

Understanding the physiological responses of fishes to salinity changes and aquatic hypoxia is essential for the conservation of marine species. Salinity changes affect the osmotic gradient across the gill epithelium, while hypoxia increases gill ventilation and the flow of water over the gills. Both processes affect the diffusive movement of ions and water across the gill epithelium, and the rate of active ion transport required for maintaining osmotic homeostasis. Consequently, salinity and hypoxia may affect the energetic cost of osmoregulation, and consequently the energy available for other physiological functions such as migration, growth, and reproduction. Historically, studies have assessed the costs of osmoregulation and ventilation in fishes via standard metabolic rate (SMR); however, few studies have used a multi-stressor approach that fully accounts for the osmorespiratory compromise. Here, we determined the combined effects of salinity and hypoxia on SMR, routine metabolic rate (RMR), and plasma ion concentrations in red drum (*Sciaenops ocellatus*) acclimated to salinities ranging from freshwater to hypersalinity. Surprisingly, there was no significant change in any parameter as a consequence of salinity or hypoxia, including the relatively extreme scenario of combined hypersalinity and hypoxia exposure. We conclude that changes in the osmotic gradient across the gill epithelium and the flow of water over the gills have a negligible effect on the whole animal energy budget of *S. ocellatus*, suggesting that the cost of osmoregulation is a minor component of basal metabolism regardless of oxygenation status.

1. Introduction

Salinity and dissolved oxygen represent two of the major abiotic factors that govern the performance and geographical distribution of teleost fishes in the marine environment (IPCC, 2014). Anthropogenic climate change is causing a rise in the frequency, severity, and duration of salinity changes and hypoxic events in freshwater, coastal, and marine habitats across the globe (Nielsen et al., 2012; Drake et al., 2013; Duggan et al., 2014; Diaz and Rosenberg, 2008; Zhang et al., 2010; McBryan et al., 2013). Understanding the physiological responses of teleost fishes to anthropogenic salinity changes and aquatic hypoxia is essential for sustainable management and conservation of key species.

Teleost fishes maintain the water and ion composition (osmolality) of their extracellular fluids relatively constant at approximately 300 mmol kg^{-1} (~10 ppt) independent of environmental salinity. However, teleost gills have a high permeability to water and ions, and teleost fishes, therefore, experience a net loss of ions at hypo-osmotic water salinities (< 10 ppt), and a net gain of ions at hyper-osmotic salinities (> 10 ppt). Teleosts counteract the diffusive movement of ions through active transport processes in the gills, gastrointestinal tract, and kidneys, which results in net ion uptake or excretion as required. The active transport of ions against a concentration gradient involve the action of ATP-driven pumps (i.e., Na^+/K^+-ATPase) and therefore imposes an energetic 'cost of osmoregulation' on the animal proportional with the rate of compensatory active ion transport (Perry et al., 2003; Evans et al., 2005; Grosell, 2011).

The diffusive movement of ions across the gill epithelium is a function of the concentration gradients of individual ions across the epithelium, the membrane surface area and permeability, and the transepithelial potential. Consequently, the rate of compensatory active ion transport required for maintaining osmotic and ionic homeostasis must vary between salinities, and the baseline (i.e., lowest) cost of osmoregulation must be at the salinity where the requirement for compensatory ion transport is smallest. For euryhaline species, the baseline cost of osmoregulation is hypothesized either at iso-osmotic salinities

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(~10 ppt) because the net movement of ions is minimal, or at common habitat salinities because the osmoregulatory machinery is 'calibrated' to these salinities (review by Ern et al., 2014). For individual species, exposure to salinities different from the salinity for baseline cost of osmoregulation would be expected to increase the requirement for compensatory active ion transport and thus the cost of osmoregulation.

The osmorespiratory compromise is the trade-off between the optimal gill structures for ion regulation and gas exchange (Randall et al., 1972; Nilsson, 1986; Sardella and Brauner, 2007). Aquatic hypoxia causes a decline in the partial pressure gradient of oxygen across the gill epithelium and the diffusive uptake of oxygen from the environment. To maintain oxygen-uptake, hypoxia-exposed fish compensate for the associated arterial hypoxemia by increasing gill ventilation (i.e., hyperventilation) (Farrell and Richards, 2009). Consequently, hypoxia-induced hyperventilation at a given salinity increases the net movement of ions and water across the gill epithelium, which increases the rate of active ion transport required for maintaining osmotic balance, and thus the cost of osmoregulation at that salinity. This increase in osmoregulatory costs has been proposed as a potential energetic constraint for fish that are exposed to chronic respiratory stress – most recently with respect to ocean acidification (Esbaugh et al., 2012; Heuer et al., 2012; Ern and Esbaugh, 2016; Esbaugh et al., 2016; Esbaugh, 2017).

Aerobic scope is the difference between basal metabolism and maximal metabolism, and quantifies the energy available physiological functions such as migration, growth, and reproduction. In species where the cost of osmoregulation is large relative to basal metabolism, basal metabolism would be expected to vary between animals at different salinities (Wood and Marshall, 1994; Boeuf and Payan, 2001). Furthermore, the effect of salinity on basal metabolism would be expected to increase with increasing gill ventilation in hypoxia-exposed animals. In contrast, species where the cost of osmoregulation is small would not be expected to incur significant effects of salinity or hypoxia on basal metabolism. In habitats with salinity changes and aquatic hypoxia, species required to allocate a significant part of their energy budget for osmoregulation are at an energetic disadvantage against species with a lower cost of osmoregulation (Perry et al., 2003). Consequently, species-specific differences in the cost of osmoregulation may play a key role in how salinity changes and aquatic hypoxia affect the energy budget and performance of individual species. The ability to quantify baseline costs of osmoregulation, and determine how it is affected by salinity changes and hypoxia-induced hyperventilation is therefore important when attempting to assess the impact of environmental change on individual species and the structure of aquatic ecosystems.

The cost of osmoregulation has been investigated in a large number of teleost species with estimates ranging from virtually zero to one-third of basal metabolism, indicating that the cost of osmoregulation may be highly species-specific (reviewed by Ern et al., 2014). The majority of these studies are based on salinity-induced changes in basal metabolism (i.e., standard metabolic rate, SMR), and rely on the assumption that only the cost of osmoregulation is affected by salinity. However, salinity exposures may also affect cardiovascular (Olson and Hoagland, 2008; Pedersen et al., 2014) and gastrointestinal (Gallaugher et al., 2001; Seth et al., 2011) functions, as well as the level of activity during measurements (Steffensen et al., 1984; Little and Finger, 1990; Morgan and Iwama, 1996). It can therefore not be excluded that estimates of osmoregulatory cost based solely on salinity-induced changes in oxygen consumption are influenced by changes in both activity and the energy demand of physiological functions other than those directly involved in osmoregulation. Furthermore, salinity exposure has been shown to induce metabolic suppression at tissue-level in some species (Sardella and Brauner, 2008). Such a response could potentially balance out a salinity-induced increase in cost of osmoregulation, resulting in no detectible difference in SMR. Consequently, the cost of osmoregulation, and its potential role in shaping the response of fishes to climate changes, remain a topic of debate (Ern et al., 2014; IPCC, 2014).

Red drum (*Sciaenops ocellatus*) is a euryhaline teleost that can tolerate a broad range of salinities (0–60 ppt), making it an ideal candidate for osmoregulatory studies. Here we determine the ventilatory response of *S. ocellatus* to hypoxia exposure, and determine the effects of hyperventilation on SMR, routine metabolic rate (RMR), and plasma ion concentrations in fish at 35 ppt (control) and after 2 weeks acclimation to 0, 10 and 60 ppt salinities. In combination, these measurements allow us: 1) to estimate the cost of osmoregulation in *S. ocellatus* at salinities ranging from freshwater to hypersalinity; and 2) to assess additional metabolic costs when exposed to a respiratory stress that exacerbates the osmorespiratory compromise. These data will provide a thorough assessment of the energetic and homeostatic challenges associated with osmoregulation in a wide range of relevant environmental conditions.

2. Materials and methods

2.1. Experimental animals

All experiments were carried out under the auspices of the University of Texas at Austin Institutional Animal Care and Use Committee. Red drum (*Sciaenops ocellatus*) were raised at the Marine Science Institute (Port Aransas, Texas, USA) at 22 ± 1 °C and salinity of 35 ppt. Animals were fed to satiation on dry feed daily, and fasted for 48 h prior to measurements. The water temperature was maintained at 24 ± 0.1 °C during all measurements. A total of 72 fish were used in this study: 32 for oxygen consumption, 32 for gill ventilation, and 8 for blood parameters. Individual fish were used only once in the experiments described below (i.e., no fish were tested more than once). Animals were euthanized using MS-222 (250 mg/L buffered with $NaHCO_3$) followed by spinal transection at the termination of gill ventilation and cannulation experiments.

2.2. Salinity and hypoxia exposures

Salinities were generated by mixing Instant Ocean Sea Salt (Instant Ocean, Spectrum Brands, Blacksburg, United States) with dechlorinated tap water. Two weeks prior to the onset of experimentation the fish were randomly divided into 4 groups and transferred from the 35 ppt holding tanks into 4 acclimation tanks kept at 0 ppt, 10 ppt, 35 ppt, and 60 ppt, respectively. For 10 ppt and 35 ppt exposure groups, the fish were transferred directly from 35 ppt to 10 ppt and 35 ppt, respectively. For 0 ppt and 60 ppt exposure groups, the fish were first transferred from 35 ppt to 10 ppt and 50 ppt, respectively. After 24 h, the fish were transferred again from 10 ppt to 0 ppt, and from 35 ppt to 60 ppt. Acclimation salinities were maintained throughout experimentation. Hypoxia exposures were generated by bubbling a mixture of air and N_2. Water oxygen tension (pO_2) was measured using optical oxygen sensors (PreSens, Regensburg, Germany), and regulated using Witrox 1 oxygen meters and DAQ-M instruments (Loligo Systems, Tjele, Denmark). Two pO_2 exposure levels were used in this study; normoxia (100% air saturation) and hypoxia (50% air saturation, 75 mmHg).

2.3. Gill ventilation

Gill ventilation in normoxia and hypoxia was measured in 8 fish at 0 ppt (body mass = 197 ± 6 g), 10 ppt (body mass = 179 ± 4 g), 35 ppt (body mass = 180 ± 5 g) and 60 ppt (body mass = 184 ± 6 g), respectively, using a total of 32 fish. Opercular displacements were assessed as indices of ventilation using a Model 2991 impedance converter (UFI, Morro bay, California, USA) that detected and quantified the changes in impedance between the pairs of brass plates attached to the opercula (Peyraud and Ferret–Bouin, 1960). The change in impedance was recorded using a PowerLab data acquisition system (ADInstruments, Colorado Springs, USA) at 100 Hz and analyzed with LabChart software (ADInstruments, Colorado Springs, USA). A typical