Comparative Biochemistry and Physiology, Part A 191 (2016) 216–225

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

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

Physiological performance of warm-adapted marine ectotherms: Thermal limits of mitochondrial energy transduction efficiency

Eloy Martinez [a,*,1], Eric Hendricks [b], Michael A. Menze [b], Joseph J. Torres [a]

[a] College of Marine Science, University of South Florida, Saint Petersburg, FL 33701, USA
[b] Department of Biological Science, Eastern Illinois University, Charleston, IL 61920, USA

ARTICLE INFO

Article history:
Received 11 March 2015
Received in revised form 19 June 2015
Accepted 11 August 2015
Available online 20 August 2015

Keywords:
Temperature
Marine
Mitochondria
Teleostei
Lagodon
Micropogonias
Caranx
Eugerres
OXPHOS
Leak

ABSTRACT

Thermal regimes in aquatic systems have profound implications for the physiology of ectotherms. In particular, the effect of elevated temperatures on mitochondrial energy transduction in tropical and subtropical teleosts may have profound consequences on organismal performance and population viability. Upper and lower whole-organism critical temperatures for teleosts suggest that subtropical and tropical species are not susceptible to the warming trends associated with climate change, but sub-lethal effects on energy transduction efficiency and population dynamics remain unclear. The goal of the present study was to compare the thermal sensitivity of processes associated with mitochondrial energy transduction in liver mitochondria from the striped mojarra (*Eugerres plumieri*), the whitemouth croaker (*Micropogonias furnieri*) and the palometa (*Trachinotus goodei*), to those of the subtropical pinfish (*Lagodon rhomboides*) and the blue runner (*Caranx crysos*). Mitochondrial function was assayed at temperatures ranging from 10 to 40 °C and results obtained for both tropical and subtropical species showed a reduction in the energy transduction efficiency of the oxidative phosphorylation (OXPHOS) system in most species studied at temperatures below whole-organism critical temperature thresholds. Our results show a loss of coupling between O_2 consumption and ATP production before the onset of the critical thermal maxima, indicating that elevated temperature may severely impact the yield of ATP production per carbon unit oxidized. As warming trends are projected for tropical regions, increasing water temperatures in tropical estuaries and coral reefs could impact long-term growth and reproductive performance in tropical organisms, which are already close to their upper thermal limit.

1. Introduction

Physiological constraints, thermal tolerance in particular, play an important role in limiting species' habitat selection and range of distribution. Most individuals inhabit environments close to their thermal optimum (Pörtner, 2001, 2002; Somero, 2005). Within the optimal thermal range, biochemical processes, especially enzyme-mediated processes, exhibit a higher performance than at temperatures above or below the thermal optimum. Since teleosts inhabiting tropical estuaries experience high temperatures (25–30 °C) year round, it follows that their thermal optima are higher than those of ecological analogues in subtropical and temperate estuaries and are likely among the highest found in aquatic ectotherms.

Seasonal fluctuations in the temperature of coastal tropical regions are small in comparison to those observed in subtropical estuaries. For example, in the subtropical Tampa Bay estuary (USA), with a mean annual water temperature of 24 °C, water temperatures have been observed to change by up to 15 °C in a matter of weeks (Badylak et al., 2007). In contrast, the smaller tropical estuary of San Juan Bay (Puerto Rico) varies in temperature by less than 6 °C throughout the year, from an annual mean of 28 °C (SJBEP Program Report, 2011)[2]. Although the different thermal regimes experienced by fishes inhabiting subtropical and tropical estuaries are well documented, comparative physiological characteristics of estuarine teleosts from the two different thermal environments are not. Most of our understanding about thermal tolerance in marine tropical regions stems from invertebrate studies, where it has been established that tropical invertebrates live close to their upper thermal limit (Coles et al., 1976; Urban, 1994; Maté, 1997; Stillman and Somero, 2000).

A select number of studies have determined the critical thermal-tolerance windows in tropical fishes to assess potential effects of climate change on tropical marine teleosts (Mora and Ospina, 2001, 2002; Rajaguru and Ramachandran, 2001; Ospina and Mora, 2004; Eme and Bennett, 2009; Eme et al., 2011). Based on the wide thermal window

* Corresponding author. Tel.: +1 787 239 6004; fax: +1 804 828 1622.
E-mail address: emartinez4@vcu.edu (E. Martinez).
[1] Present address: Center for Environmental Studies, Virginia Commonwealth University, Richmond, VA 23284, USA.

[2] http://estuario.org/index.php/annual-reports

http://dx.doi.org/10.1016/j.cbpa.2015.08.008
1095-6433/

of tolerance in various estuarine species, those authors have suggested that tropical species may be better poised to survive long-term warming trends associated with climate change than previously thought (Eme et al., 2011). In the present study, we provide evidence that sub-lethal effects of temperature at the mitochondrial level are evident, and potentially significant.

Our current understanding of whole-organism thermal tolerance relies heavily on critical, rather than sub-lethal analyses of organismal performance as a function of temperature. The influence of environmental change on mitochondrial energy transduction efficiency and resulting effects on whole-organism physiological performance are poorly resolved. Studies of teleost mitochondria indicate that substrate flux and oxygen consumption rates poorly estimate energy balance and flow in organisms whose body temperature regularly fluctuates (Weinstein and Somero, 1998; Hardewig et al., 1999; Pörtner et al., 1999; Hilton et al., 2010; Mark et al., 2012; Martinez et al., 2013). Since energy production relies on the efficiency of mitochondrial ATP production, a detailed analysis of mitochondrial performance is likely to be a more accurate indicator of temperature effects on whole-organism physiological performance than the critical thermal maximum (Weinstein and Somero, 1998; Pörtner at. al., 1999; Martinez et al., 2013).

Although the tolerance window of some estuarine fishes is beyond any temperature found in their natural habitat (Mora and Ospina, 2001, 2002; Eme and Bennett, 2009), the long-term implications of gradual changes in temperature on physiological performance and survival are unknown. In particular, the effects of thermal heterogeneity on mitochondrial performance are yet to be determined. Based on previous studies on terrestrial systems, thermal heterogeneity of habitats favor an organism's ability to adapt to changes in their thermal regime (Deutsch et al., 2008; Tewksbury et al., 2008; Huey et al., 2009). If we extend this to the marine milieu, it is possible that tropical organisms experiencing stable but high temperatures, such as teleosts associated with coral reefs and estuaries, could be particularly challenged by increasing habitat temperatures as they shift to a warmer sub-optimal range.

The goal of this study was to employ a series of estuarine teleosts as tropical and subtropical study systems to compare the thermal sensitivity of mitochondrial energy transduction. To achieve our goal, this study examines the oxidative phosphorylation (OXPHOS) system in liver mitochondria from the striped mojarra (*Eugerres plumieri*), the whitemouth croaker (*Micropogonias furnieri*) and the palometa (*Trachinotus goodei*), and compares them to the subtropical pinfish (*Lagodon rhomboides*) and the blue runner (*Caranx crysos*). Mitochondrial function was assayed at various temperatures, and the thermal sensitivity of mitochondrial complex I (NADH:ubiquinone reductase) and complex II (succinate dehydrogenase) activity was determined.

2. Methodology

2.1. Chemicals

All chemicals for respiration measurements were purchased from Sigma-Aldrich (St. Louis, MO) or Fisher Scientific (Fair Lawn, NJ). Water for solution preparation was purified with a Milli-Q Reagent Water System (Billerica, MA) to an electrical resistance of 18 mΩ.

2.2. Study systems

Subtropical specimens were collected during the fall (October) in the southern portion of Tampa Bay, Florida using hook and line. Water temperature at the collection site was 27.9 °C. After collection, all specimens were transported in aerated 19 L containers to the aquarium facility of the University of South Florida, College of Marine Science. Specimens were transferred to holding tanks equipped with a flow-through water system for at least two weeks prior to analysis, and fed pathogen-free frozen mysid shrimps every 48 h. Holding tanks consisted of three 570 L fiberglass rectangular tanks, and specimens were held at low densities (less than 10 individuals per tank) at any given time. Temperature was controlled (28 ± 2.0 °C), and nutrients were monitored biweekly.

The pinfish, *L. rhomboides*, is a demersal estuarine species commonly associated with vegetated bottom hard structures and the brackish water surrounding mangroves (Robins and Ray, 1999). *L. rhomboides*' diet consists of vegetation as well as small mollusks, polychaetes, and juvenile fishes (Montgomery and Targett, 1992; Robins and Ray, 1999). The blue runner, *C. crysos*, is a schooling pelagic predator found throughout the coastal subtropical Atlantic. Despite its active pelagic habit, the species is mainly found schooling in shallow (0–100 m) water; it is most frequently observed in the estuarine pelagial where it feeds on small fishes, shrimp and other invertebrates (Cervigón et al., 1992).

Tropical specimens were collected during the winter season (December) in neighboring waters of the Punta Santiago Estuary area in Humacao, Puerto Rico. Specimens were collected using a 20-meter long seine net, and later transported in aerated 19 L containers to a 190 L holding tank at the University of Puerto Rico, Humacao Campus. Water temperature at the collecting site was 27.8 °C. Specimens were held for less than 72 h in artificial seawater at habitat salinity and aquarium room temperature (25.0 ± 2.0 °C) prior to experiments.

Tropical species included the striped mojarra, *E. plumieri*, the whitemouth croaker, *M. furnieri*, and the palometa, *T. goodei*. The striped mojarra is often found in tropical estuaries, primarily over soft bottom. It is commonplace in Caribbean estuaries with a distribution that extends to subtropical regions. The mojarra's diet comprises infaunal species of crustaceans, bivalves, and detritus (Bussing, 1998). The whitemouth croaker is commonly found over the sandy bottom of estuaries where it feeds upon crustaceans, mollusks and fishes (Isaac, 1988). The palometa is an active pelagic species frequently found in tropical estuaries. Analogous to the subtropical *C. crysos*, *T. goodei* is also a schooling species that feeds primarily on crustaceans and fishes (Cervigón and Los Roques, 1991).

2.3. Isolation of liver mitochondria

Fresh livers were excised and processed according to Martinez et al. (2013). Briefly, liver tissue from one or more individuals (~1.0 g of liver tissue) was minced in an ice-cold petri dish, then homogenized in 8 mL of a sucrose-based isolation medium (250 mM Sucrose, 1 mM EGTA, 10 mM K_2PO_4, 1% BSA, pH = 7.4, 20 °C) using an ice-cold Dounce homogenizer (Kontes, Vineland, NJ). Five passes with a loose fitting pestle were followed by two passes with a tight fitting pestle. Homogenate was transferred to 1.5 mL centrifuge tubes and centrifuged at 650 *g* for 10 min at 4 °C to remove cellular debris and undisrupted tissue. The supernatant was collected and again centrifuged at 9600 *g* for 15 min at 4 °C to sediment the mitochondrial fraction. Pellets were washed with isolation medium, resuspended, and twice consecutively recollected by centrifugation at 9600 *g* for 15 min at 4 °C. The final pellet was suspended in 300–500 μL of isolation medium and stored on ice until assayed.

2.4. Mitochondrial respiration

To assess the thermal sensitivity of mitochondrial respiration, high-resolution respirometry systems were employed. Those systems comprised two 2.0 mL water-jacketed respirometric chambers (DW-1, Hansatech Instruments, Norfolk, England) equipped with Clark-type polarographic oxygen electrodes (C-1, Hansatech Instruments, Norfolk, England). Chamber temperature was controlled using a circulating, refrigerated water bath (E200, Lauda-Königshofen, Germany). Electrodes were calibrated in air- and nitrogen-saturated respiration medium (500 μL — see below) at each assay temperature. Respiration