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Field study of cyclic hypoxic effects on gene expression in grass shrimp hepatopancreas

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ABSTRACT

Grass shrimp, *Palaemonetes pugio*, are widely used for ecological and toxicological research. They commonly experience cyclic hypoxia in their natural habitats. The response of grass shrimp to laboratory-controlled cyclic hypoxia has been studied in detail, but little is known about how field acclimatized grass shrimp regulate the gene expression and response to cyclic hypoxia. In this study we examined morphometric parameters, relative fecundity and gene expression of grass shrimp collected from two areas in Weeks Bay (Mobile, Alabama). One is a traditionally normoxic location (WBM), and the other is a traditionally cyclic hypoxic location (WC). In the week preceding grass shrimp collection dissolved oxygen (DO) at the field sites was measured continuously. DO was <2 (mg/L DO) and between 2 and 3 (mg/L DO) for 0 and 255 min at WBM, and for 285 and 1035 min at WC, respectively. Weight and length of WBM grass shrimp were significantly greater than weight and length of WC shrimp. WBM shrimp had more eggs than WC shrimp, but the difference was not significant. Shrimp from WC had a significant higher number of parasites than those from WBM. A cDNA microarray was utilized to investigate the changes in gene expression in grass shrimp hepatopancreas. Five genes, previously identified as hypoxia/cyclic hypoxia-responsive genes in laboratory exposure studies, were significantly up-regulated in WC shrimp relative to WBM. A total of 5 genes were significantly down-regulated in the field study. Only one of those genes, vitellogenin, has been previously found in chronic and cyclic hypoxic studies. Up and down-regulation of 7 selected genes was confirmed by qPCR. The overall pattern of gene expression in wild shrimp from cyclic DO sites in Weeks Bay showed only weak correlations with gene expression in shrimp from chronic and cyclic hypoxic laboratory studies. It appears therefore that transcriptome profiles of laboratory acclimated animals are of limited utility for understanding responses in field acclimatized animals that are exposed to a broader array of environmental variables.

1. Introduction

Oxygen content of air is more or less constant. In contrast, the oxygen content of aquatic ecosystems varies markedly daily, seasonally, and spatially. Prolonged, seasonal hypoxia and daily hypoxia/normoxia cycles are common occurrences in estuarine environments. The frequency and spatial extent of these occurrences have increased considerably due to anthropogenically-driven increases of contaminant and nutrient inputs into estuaries (Rabalais et al., 2002). Compared to severe hypoxia or anoxia little is known about the effects of cyclic hypoxia on aquatic species (Li and Brouwer, 2013). Hypoxia to normoxia transitions (ischemia reperfusion) are known to cause cellular injury in mammals due to accumulation of reactive oxygen species (ROS). It is therefore of interest to understand how aquatic organisms are affected by and cope with fluctuating oxygen concentrations in their environment.

Aerobic organisms can produce ROS during oxidative metabolism. Most common ROS including superoxide anion, hydrogen peroxide, and hydroxyl radicals, form as a natural byproduct of cellular metabolism and have important roles in cell signaling, homeostasis, and the activation of transcription factors leading to gene expression (Scandalios, 2002; Guzy et al., 2005). Hypoxia exposure appears to increase cellular ROS production, probably from mitochondrial electron transport complexes. When antioxidant defense mechanisms are overwhelmed, accumulation of ROS can cause significant damage to cell structures and cause molecular damage leading to cell death. The effects of ROS on cell metabolism are well documented in various species, especially in mammals. One area of intense research concerns the mechanism of ROS mediated cellular damage during hypoxia and reoxygenation (ischemia reperfusion injury) (Li and Jackson, 2002). Cells undergo specific changes in antioxidant enzyme activities, mitochondrial function, and membrane transport in response to hypoxia (Yu, 1994; Li and Jackson, 2002; Kietzmann and Gorlach, 2005; Clanton, 2007). Loss of these enzyme activities leads to deleterious cellular and genetic injury during reoxygenation because of the increased ROS production.

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Most organisms in coastal environments are generally well adapted to hypoxia; however, specific adaptations vary depending on the severity and duration of hypoxia. Some mobile organisms can detect and avoid hypoxic waters, such as weakfish *Cynoscion regalis* (Tyler and Targett, 2007). Juvenile weakfish were absent from the preferred habitats whenever DO was <2 mg/L, and returned within 2 h if DO > 2 mg/L. However, such behavior, even though it increases survival, can result in higher predation rate and lower growth rate. The growth rates of juvenile summer flounder *Paralichthys dentatus* and winter flounder *Pseudopleuronectes americanus* were generally reduced as DO decreased when both species were exposed to diel-cycling hypoxia (2–11.0 mg/L DO) in laboratory controlled experiments (Stierhoff et al., 2006). Smith and Able (2003) recorded DO dynamics in marsh salt pools over ten 24 h periods to characterize the responses of five fish species to rapid changes of DO. The greatest DO range of 0–20 mg/L occurred in mid-July. Most species were well adapted to survive these fluctuations. Coiro et al. (2000) measured hypoxic effects on growth of first stage larval marsh grass shrimp, *Palaemonetes vulgaris*, using the patterns of diurnal, semidiurnal, and constant hypoxia. Cyclic exposure (2–8 mg/L DO) resulted in less growth impairment than chronic hypoxia kept at 2 mg/L DO.

Avoidance is not always possible and animals must rely on physiological mechanisms to take up as much oxygen as possible from ambient environment or switch to anaerobic metabolic pathways to supply energy (Martinez-Cruz et al., 2012), or both. Many crustaceans including grass shrimp *Palaemonetes pugio* appear to adapt to cyclic hypoxia quite well. However, the details of how these animals regulate the metabolic and physiological changes are still not clear. Generally the cellular antioxidant defense systems can maintain oxygen radical induced damage at low level (Zenteno-Savin et al., 2006). Some enzymes of these systems have been identified in crustaceans, such as superoxide dismutase (CuZnSOD and MnSOD in the cytosol, and MnSOD in mitochondria) (Brouwer et al., 1997, 2003; de Oliveira et al., 2005; Garcia-Triana et al., 2010), catalase (Trasvina-Arenas et al., 2013), glutathione peroxidase (GPx) and GSH reductase (Jiang et al., 2009; Li and Brouwer, 2009b), and metal-binding proteins including metallothionein (English and Storey, 2003; Brouwer et al., 2007; Li and Brouwer, 2013).

The emerging field of toxicogenomics provides tools to monitor regulation of many genes simultaneously and illustrate the mechanisms that underlie hypoxic and cyclic/hypoxic effects on living tissues of various organisms and may help to identify gene expression profiles that may serve as biomarkers. Brouwer et al. (2007) examined the gene response of grass shrimp exposed to severe (1.5 ppm DO) and moderate (2.5 ppm DO) chronic hypoxia under laboratory condition using custom cDNA macroarray containing 78 clones from a hypoxia responsive suppression subtractive hybridization cDNA library. The same cDNA macroarray was also used to assess grass shrimp response to cyclic hypoxia (1.5–8 ppm DO) in laboratory (Brown-Peterson et al., 2008). A comparative study using the same macroarray was conducted to evaluate the effects of cyclic hypoxia on grass shrimp in laboratory and caged field conditions (Brown-Peterson et al., 2011).

We have expanded on the limited studies above by examining gene expression profiles of grass shrimp in laboratory exposures of both chronic (Li and Brouwer, 2009b) and cyclic (Li and Brouwer, 2013) hypoxia using custom cDNA microarray printed with 661 annotated transcripts obtained from multiple EST (expressed sequence tag) libraries (Li and Brouwer, 2009a). Expression of different genes was significantly regulated depending on the exposure length and type, and sampling time. In the present study, we evaluated the gene expression in wild-caught grass shrimp from a cyclic hypoxic field site to determine if potential biomarkers identified in laboratory exposures can be used in field studies as well. Specifically, the gene expression pattern of wild-caught shrimp will be compared to previous results from laboratory controlled hypoxic exposures.

2. Materials and methods

2.1. Collection of field samples

Grass shrimp were collected on September 11, 2006 at 10–11:00 h from two sites (Fig. 1) in Weeks Bay (Mobile, AL, USA). One is a traditionally normoxic location, Weeks Bay Mouth (WBM, 30°22.663 N, 87°50.253 W), and the other is a traditionally cyclic hypoxic location, Weeks Creek (WC, 30°22.251 N, 87°49.949 W). Twenty grass shrimp were collected by hand-held dip net from each location, and the thorax/hepatopancreas was removed and immediately stored in 1 mL RNALater (Ambion Inc., Austin, TX, USA) for subsequent RNA extraction and microarray analysis.

Shrimp were collected again on September 13, 2006 at 9–10:00 h from the same locations in Weeks Bay. Twenty grass shrimp were collected by hand-held dip net from each location, placed into buckets of aerated water, and transported to the local laboratory at the Weeks Bay National Estuarine Research Reserve System site for egg counts and dissection. The thorax/hepatopancreas of 20 shrimp from each location was removed and stored in 1 mL RNALater (Ambion Inc.) for subsequent RNA extraction and microarray analysis.

DO, temperature, depth, and salinity were measured every 15 min at both sites continuously during September 6–13 using a YSI Model 600XLM data sonde. Additionally, water quality data were taken during each sample collection using a YSI Model 600XLM data sonde. DO, temperature, pH, depth, and salinity were recorded at the surface and bottom at abovementioned sites prior to collection of animals.

A brief description of the methods used in this study is given in Sections 2.2 to 2.8. For further detail please refer to Li and Brouwer (2009b).

2.2. Isolation and quantification of total RNA

Total RNA was isolated from grass shrimp hepatopancreas using Stat-60 (Tel-Test, Friendswood, TX, USA). After precipitation, RNA was DNase-treated and stored in RNA Storage Solution (Ambion Inc.). RNA was quantified using a NanoDrop Spectrophotometer (ND-1000, NanoDrop Technologies, Wilmington, DE, USA), and quality was assessed on a 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA,

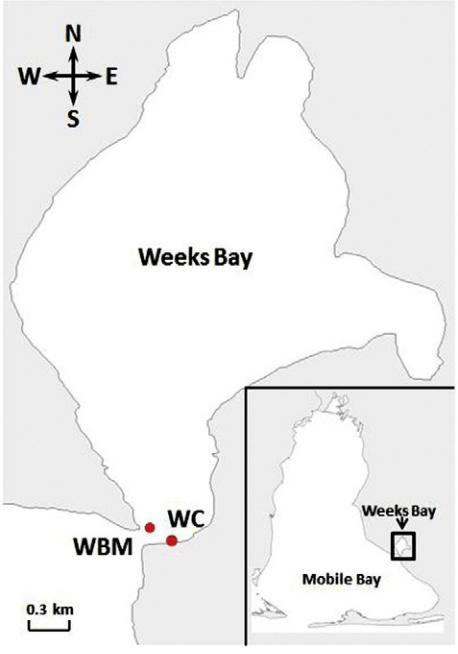

Fig. 1. Grass shrimp collection sites in Weeks Bay located near Mobile Bay's eastern shore. Weeks Creek (WC), cyclic hypoxic site, and Weeks Bay Mouth (WBM), normoxic location.