EFFECTS OF DIEL-CYCLING HYPOXIA ON THE CARDIAC ACTIVITY AND GROWTH OF *ARGOPECTEN IRRADIANS*

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Samuel Jonathan Gurr

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Samuel Jonathan Gurr

We, the thesis committee for the above candidate for the Master of Science in Marine Science degree, hereby recommend acceptance of this thesis.

Nils Volkenborn, Ph.D.
Advisor
School of Marine and Atmospheric Science
Stony Brook University

Christopher J. Gobler, Ph.D.
Co-Advisor, Second Reader
School of Marine and Atmospheric Science
Stony Brook University

Robert Cerrato, Ph.D.
Committee Advisor, Third Reader
School of Marine and Atmospheric Science
Stony Brook University

This thesis is accepted by the Graduate School

Charles Tabor
Dean of Graduate School

Thesis

Abstract of the Thesis

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ABSTRACT

Bottom water oxygen concentrations in coastal environments can oscillate between fully oxygenated and hypoxic conditions on a daily basis. How benthic organisms deal with such drastic changes in oxygen availability is not well understood. Specifically, we do not know the magnitude, duration, and frequency at which diel-cycling hypoxia conditions become harmful. Here we have used non-invasive infrared sensors to measure the cardiac activity of the Atlantic bay scallops, *Argopecten irradians*, in response to diel-cycling hypoxia *in-situ* over one-month periods as well as in the laboratory in controlled incubations using animals conditioned to contrasting field conditions. In the field, heartbeat rates at a well-oxygenated site were relatively stable while scallops deployed at two sites with pronounced diel-cycling hypoxia were elevated and more variable. Maximal heartbeat rates during diel-cycling hypoxia were commonly recorded around dawn when oxygen concentrations fell to 5 mg L^{-1} indicating a sub-lethal response to dissolved oxygen (DO) concentrations higher than what is typically defined as hypoxia. Laboratory incubations confirmed the tight link between DO and cardiac activity. An increase of heartbeat rate in response to an initial decline from fully oxygenated conditions was indicative of a regulatory response in which cardiac activity was enhanced presumably to maintain oxygen supply. At DO below 3 mg L^{-1} heartbeat rates declined reaching a state of acardia during anoxia, suggesting a conformer response to severe hypoxia. *In-situ* and laboratory data was integrated into a

novel conceptual model to characterize four phases that interpret cardiac and respiratory activity in diel-cycling hypoxia. Heartbeat frequency was a suitable proxy for respiration under normoxia, but scallops were unable to compensate for reduced oxygen availability by increasing heartbeat rates below hypoxic thresholds. Pre-conditioning to diel-cycling hypoxia did not affect survival or cardiac activity in anoxic and severe hypoxic treatments. However, *A. irradians* pre-conditioned to diel-cycling hypoxia were less responsive to normoxia with heightened effort to maintain vital aerobic functions after long-term exposure to severe hypoxia. Survival after 12-14 hours of anoxia and mortality after 23-32 hours of anoxia convey physiological limitations unaffected by acclimation. We speculate that repetitive exposure to periods of DO oscillations with exposure below 5.0 mg L^{-1} in the field can cause sub-lethal effects to *A. irradians* affecting fitness, growth, and reproductive success.

DEDICATION PAGE

I dedicate this Master's thesis to my mother and father. Their encouragement to pursue my interests and explore the Long Island Sound shoreline throughout my growing years has driven me to become the scientist I am today.

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1. INTRODUCTION

1.1 Hypoxia in coastal environments

In coastal ecosystems hypoxia is a common physicochemical environmental stressor with an increasing global occurrence in frequency and severity due to nutrient loading and eutrophic conditions (Diaz and Rosenberg 2008). Periods of hypoxia or low dissolved (DO) develop when oxygen demand outweighs supply. Hypoxia intensifies seasonally from the effects of elevated temperature, light intensity, stratification and eutrophication (Diaz 2001; Howarth et al. 2011; Wallace et al. 2014). Large quantities of oxygen are consumed through bacterial degradation of algae resulting in hypoxic conditions within bottom waters that can last weeks to months. In shallow water environments, photosynthetic activity during the day and community respiration during night can lead to extremely variable oxygen availability (D'Avanzo and Kremer 1994). Diel-cycling hypoxia seasonally intensifies in summer and co-occurs with acidification among coastal environments (Wallace et al. 2014). Coupled stressors pose dynamic stress oscillations on seasonal as well as on daily time scales to organisms of commercial and ecological importance (Tyler et al. 2009; Baumann et al. 2014; Gobler and Baumann 2016; Gobler et al. 2017).

1.2 Effects of hypoxia on benthic invertebrates

Ecological issues and alterations of community structure associated with hypoxia are undisputed and result in mass mortalities, metabolic alterations, and escape responses of benthic and pelagic fauna (Howell and Simpson 1994; Diaz and Rosenberg 1995; Burnett and Stickle 2001). Such conditions may constitute a significant burden for benthic organisms affecting physiology, growth, and reproductive success (Baker and Mann 1992; Thomas et al. 2007; Cheung et al. 2008; Wu 2009; Gobler et al. 2014; Steckbauer et al. 2015; Keppel et al. 2016; Gobler et al. 2017), but sub-lethal effects of diel-cycling hypoxia on benthic organisms are not well understood. Importantly, many biological response variables (such as growth rates, development, mortality, etc.) integrate over relatively long time scales that limit the ability to understand and identify effects of dynamic environmental stressors and thresholds at which conditions become harmful.

1.3 Cardiac activity as response variable

Cardiac activity is a widely used proxy of whole animal metabolism (Helm and Trueman 1967) and can be measured with high temporal resolution to reveal immediate metabolic responses to changes in the environment (Burnett et al. 2013; Chapperon et al. 2016). Heartbeat rates of invertebrates exposed to environmental stress have been extensively studied (DeFur and Mangum 1979; Gainey and Shumway 1988; Braby and Somero 2006), but past efforts with minimally invasive techniques were mostly constrained to laboratory use (Aagaard et al. 1991; Rovero et al. 1999; Xing et al. 2016; Seabra et al. 2016). Maximum heartbeat rate at upper critical temperature (T_{cII}) and Arrhenius breakpoint temperature (ABT) identify thermal windows of optimal aerobic performance and acute physiological limitations for marine invertebrates (Fredrich and Pörtner 2000; Pörtner 2012; Xing et al. 2016; Chapperon et al. 2016; Tagliarolo and McQuaid 2016). For example, the European spider crab *Maja squinado* reaches maximum cardiac activity at 31.5°C (T_{cII}) followed by an increase in products of anaerobic metabolism at 33°C when heartbeat rates decline (Fredrich and Pörtner 2000). This provides evidence that heartbeat rate is closely linked with scope of aerobic performance and metabolic transitions. Parallel measurements of heartbeat and respiration rates as a function of temperature (Defur and Magnum 1979) indicate a strong relationship between both physiological parameters, but few studies have addressed the cardiac response of marine invertebrates to dynamically changing oxygen availability (Pörtner and Grieshaber 1993; Grieshaber et al. 1994; Aguirre-Velarde et al. 2016). Some marine invertebrates exhibit a short cardiac upswing under the initial DO decline from full oxygen saturation and drastically decrease heartbeat rate under hypoxia and anoxia exposure (Brand and Roberts 1973; Nicholson 2002). An abnormal decline, elevation, and cease of heartbeat rate are referred to as bradycardia, tachycardia, and acardia, respectively. Slight tachycardia during DO decline followed by bradycardia under severe oxygen limited conditions is evidence of an attempt to maintain aerobic function and transition to anaerobic metabolism (Bayne 1971; DeFur and Pease 1988). Here we have used non-invasive heartbeat sensors in an ecophysiological approach to measure cardiac activity *in-situ* during diel-cycling hypoxia and in the laboratory under hypoxia and anoxic simulations to increase an understanding of immediate physiological responses to stress conditions.