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COMBINED EFFECTS OF DISSOLVED OXYGEN AND TEMPERATURE ON AEROBIC RESPIRATION AND RESPIRATORY RECOVERY RESPONSES OF THE SPIONIFORM POLYCHAETE, *STREBLOSPIO GYNOBRANCHIATA*, IN RELATION TO BODY SIZE

by

Alyssa D. Bennett

A Thesis
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for the Degree of Master of Science

December 2017

COMBINED EFFECTS OF DISSOLVED OXYGEN AND TEMPERATURE ON

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RESPONSES OF THE SPIONIFORM POLYCHAETE,

STREBLOSPIO GYNOBRANCHIATA, IN

RELATION TO BODY SIZE

by Alyssa D. Bennett

December 2017

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ABSTRACT

COMBINED EFFECTS OF DISSOLVED OXYGEN AND TEMPERATURE ON AEROBIC RESPIRATION AND RESPIRATORY RECOVERY RESPONSES OF THE SPIONIFORM POLYCHAETE, *STREBLOSPIO GYNOBRANCHIATA*, IN RELATION TO BODY SIZE

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Elevated surface temperatures exacerbate the threat of hypoxia within coastal ecosystems. These two primary stressors likely interact as they elicit opposing physiological responses from marine organisms. Metabolic depression is typically associated with hypoxia, while metabolic rates increase with temperature. Moreover, physiological effects of combined stressors may not be additive. In light of increasing pressures from hypoxia, elevated ocean temperatures, and other stressors within coastal regions, studies need to examine effects of multiple stressors on physiology of coastal organisms.

Mass-specific aerobic respiration (V_{O2}) was characterized as a proxy for metabolic cost of *Streblospio gynobranchiata*, at combined levels of dissolved oxygen and temperature relative to body size. Also, changes in V_{O2} during acclimation to hypoxia and respiratory recovery following hypoxia exposure were examined. Overall, oxyregulatory abilities were maintained with decreasing dissolved oxygen levels and increasing temperatures except at the highest temperature treatment, indicating the critical temperature was reached within the treatment range. Over a 12 hour period of

hypoxia exposure, this species showed an initial acclimation period, followed by a decreased V_{O2} for the remainder of the exposure. After returning to aerated conditions following acclimation to hypoxia, V_{O2} appeared to increase and decrease in two cycles over a 12 hour period, possibly reflecting energy cycling in terms of ATP usage. V_{O2} peaked at 10 hours, overshooting reference normoxia readings, perhaps indicating an oxygen debt. *Streblospio gynobranchiata* exhibited a high tolerance to these combined stressors, however, further challenges by decreasing oxygen and increasing temperatures may surpass this species' ability to meet energy demands.

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DEDICATION

Last but not least, I would like to thank my family- my parents, brother, and grandparents for supporting me throughout the struggles of graduate school. I also would not have made it without the constant love and support of my best friends and boyfriend. Thank you to everyone who had a hand in my thesis, big or small, and to anyone who had to endure my discussions about polychaetes.

This thesis is in memory of Boots, who was my constant companion through grade school, college, and most of graduate school. You were my best friend and are dearly missed.

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LIST OF ABBREVIATIONS

ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ARH1	Heterogeneous first-order autoregressive
ASW	Artificial saltwater
ATP	Adenosine triphosphate
DO	Dissolved oxygen
GoM	Gulf of Mexico
LMM	Linear mixed model
LSD	Least significant difference
LT_{50}	Lethal time to kill 50%
O_2	Oxygen
ppt	parts per thousand
V_{O2}	Mass-specific respiration rate

CHAPTER I – INTRODUCTION

1.1 Hypoxia

Recent studies have established that oxygen levels are declining globally in marine ecosystems, particularly along coastal margins (Diaz and Rosenberg 2008, Steckbauer et al. 2011). Hypoxia is classically defined as water with an oxygen concentration of 2 mg O_2 l^{-1} or less (Diaz and Rosenberg 2008). Hypoxia is recognized as a major threat to benthic metazoan life and secondary production within coastal ecosystems. Moreover, many organisms may suffer mortality at concentrations higher than the recognized 2 mg O_2 l^{-1} hypoxia threshold (Steckbauer et al. 2011, Vaquer-Sunyer and Duarte 2008). Seasonal hypoxia often occurs in coastal estuaries as bottom oxygen concentrations decrease in the summer to low or hypoxic levels, or even to anoxic levels (Llansó 1991). Eutrophication due to excessive nutrient input has contributed greatly to the expansion of hypoxia globally (Nilsson and Rosenberg 1994). Isolation of surface waters from bottom waters due to stratification causes a decreased diffusion rate of oxygen throughout the water column, and the decomposition of organic matter by bacteria reduces bottom water oxygen concentrations (Rabalias et al. 2002). These two processes are responsible for the development and maintenance of hypoxia and are further exacerbated by salinity, temperature, and basin retention time (Diaz and Rosenberg 1995).

As hypoxic areas, commonly referred to as “dead zones”, grow in number and size, hypoxia has become an increasingly bigger environmental issue (Diaz and Rosenberg 2008). Diaz and Rosenberg (1995) noted that dissolved oxygen constitutes one of the environmental variables that have changed most drastically within a short

period of time. At least 400 areas around the world, making up more than 245,000 km^2, are currently affected by periodic, seasonal, or persistent hypoxia (Steckbauer et al. 2011). Hypoxia and anoxia have occurred naturally through geological time; however, hypoxic events have increased in coastal and estuarine areas due to human contributions (Diaz and Rosenberg 1995). Diaz and Rosenberg (2008) state that "hypoxia and anoxia are among the most widespread deleterious anthropogenic influences on estuarine and marine environments", and on par with other major global environmental issues such as overfishing, harmful algal blooms, and habitat loss. Not only has the number of hypoxic zones increased, but so have the frequency, duration, and severity of oxygen deficiencies increased during the past decade (Vaquer-Sunyer and Duarte 2011). Most of these hypoxic zones occur within 30 km of coastal margins, with the second largest hypoxic zone located along coastal margins in the northern Gulf of Mexico (Rabalais et al. 2002).

Hypoxia in the northern Gulf of Mexico (GoM) is mostly driven by drainage from the Mississippi River (Rabalais et al. 2002). Hypoxia is variable during and between years and is not only found overlying bottom sediments, but also extends up into the water column. Generally within the GoM dead zone occurring off the coast of Louisiana, bottom oxygen levels decline in the spring and summer, eventually resulting in persistent hypoxia in the late summer and fall (Ritter and Montagna 1999). Wind-mixing and intrusion of oxygenated water in the fall usually prevent prolonged hypoxia (Rabalais et al. 2002). Hypoxic events also occur further coastward along the Texas, Mississippi, Alabama, and Florida coasts. Hypoxic events may have occurred in geological time but have increased in the last half century (Rabalais et al. 2002). The increase in hypoxia in the Gulf of Mexico occurred in the 1950's as nitrate from fertilizers became elevated in

the Mississippi River outflow. Other anthropogenic factors contributing to the worsening of oxygen conditions were navigation channelization, flood control, deforestation, and loss of riparian buffer zones (Rabalias et al. 2002). Changing oxygen conditions have led to shifts in phytoplankton composition as well as changes in nutrients and trophic interactions, resulting in shifts of fish stocks with the loss of demersal and displacement of pelagic organisms resulting in a drop in fisheries yield (Steckbauer et al. 2011, Rabalais et al. 2002).

As a major stressor, hypoxia poses many threats to marine ecosystems. Hypoxia may cause changes in biogeochemical cycling of elements and in the distribution of marine organisms. Hypoxia induced mortality of many marine organisms can lead to the loss of fisheries (Steckbauer et al. 2011), thereby affecting the economies of coastal countries (Helly and Levin 2004). Other consequences of "dead zones" are stressed or dying organisms within the sediments, subversion of trophic potential, and the alteration or diminishment of biodiversity, community structure, and ecosystem functioning (Rabalais et al. 2002).

Current research on the consequences of hypoxia to ecosystem function has scratched the surface, but there are still many unanswered questions. It is clear that hypoxia in conjunction with organic enrichment elicits predictable changes in macrobenthic communities (Pearson and Rosenberg 1978), but how such changes might connect to individual organisms through their physiological responses to hypoxia are not well understood. Understanding the physiological effects of hypoxia also grows more challenging when one considers variation in the duration and frequency of exposure along with other contributing environmental factors (Rabalais et al. 2002).

1.2 Climate change

Climate change is another major complicating factor when considering combined effects of physical variables on marine organisms. For example, increasing temperatures may lead to changes in hydrology, biodiversity, and biogeochemical cycles (Vaquer-Sunyer and Duarte 2011). Average surface temperatures have increased globally by 0.6 ± 0.2°C over the past 100 years, and sea level rise and ocean heat content have increased significantly since the 20th century (Houghton et al. 2001). Changes in climate are also influencing weather patterns. Precipitation amounts (0.5-1% per decade), heavy precipitation events (2-4% increase), cloud cover (2% increase), and snow cover and ice extent has changed as well (Houghton et al. 2001). Concentrations of greenhouse gases in the atmosphere will only continue to increase as anthropogenic activities contribute to global climate change, further influencing temperature changes (Matear and Hirst 2003). The projected temperature trend is expected to lead to an increase of 1.8ºC globally by the end of this century (Vaquer-Sunyer and Duarte 2011); and increasing temperatures associated with climate change will also lead to decreased oxygen saturation in oceans around the world (Vaquer-Sunyer and Duarte 2011).

Climate change alone is predicted to lead to a depletion of oceanic oxygen (Diaz and Rosenberg 2008). Because temperatures will rise globally, this will exacerbate hypoxia directly and indirectly. Directly, hypoxic zones are projected to experience a warming of about 2ºC by the end of the century and specifically the Gulf of Mexico may see a 1.5 to 3ºC increase in sea temperature (Figure 1.1, Altieri and Gedan 2015; Biasutti et al. 2012). This increase in temperature exacerbates hypoxia via stratification, decreasing oxygen saturation, and the ability of oxygen to mix within the water column,

initiating hypoxia in other areas (Vaquer-Sunyer and Duarte 2011, Pörtner et al. 2005, Altieri and Gedan 2015). Indirectly, hypoxia will increase with changes in weather patterns such as precipitation increases, which will cause increased runoff. This increased runoff of nutrients will result in algal blooms, which upon death, will cause increased respiration by bacterial decomposition in the water column, directly leading to depleted oxygen concentrations (Justic et al. 1996, Rabalais et al. 2002).

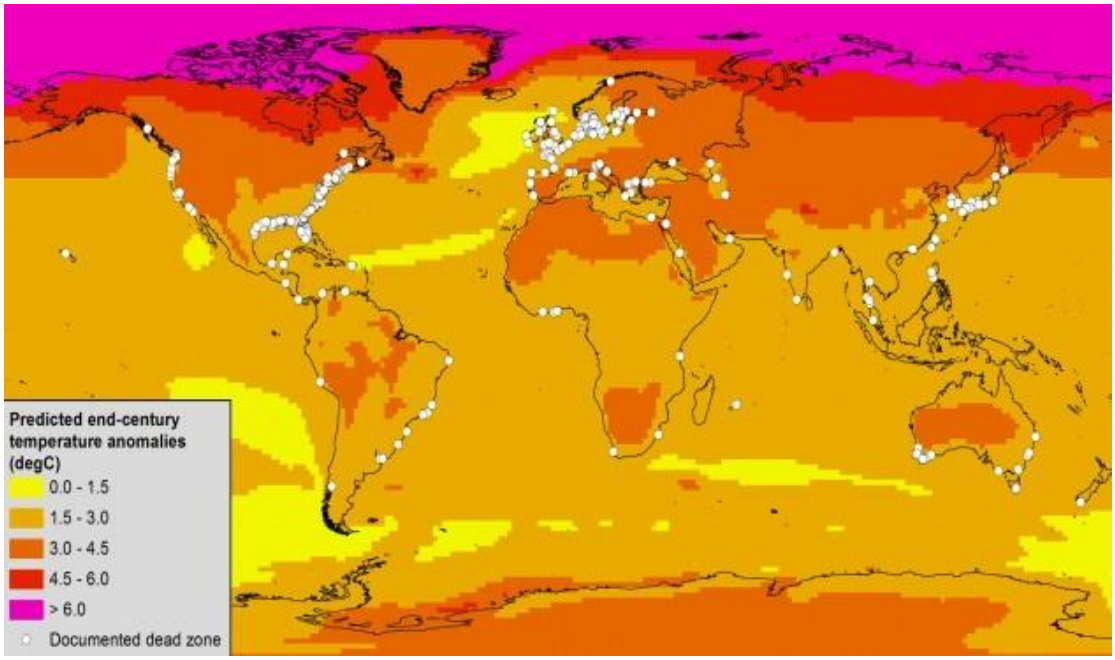

Figure 1.1 Overlapping hypoxic zones and warming ocean temperatures

Map of known dead zones (white dots) and predicted changes in annual air temperature for 2080-2099 versus 1980-1999. From Altieri, A. H., & Gedan, K. B. (2015). Climate change and dead zones. Global change biology, 21(4), 1395-1406.

Hypoxia is only expected to worsen due to warming waters in the future causing a compounding issue of decreased oxygen solubility and increased respiration rates (Leung et al. 2013). Oceans globally have experienced a 4-7% decline in dissolved oxygen concentration due to temperature, as gas solubility decreases with increasing temperature (Matear and Hirst 2003, Weiss 1970). Increasing eutrophication and water temperatures

are expected to cause conditions to worsen within 65% of estuaries in the United States (Figure 1.1, Alteri and Gedan 2015). Climate change also has the potential to cause naturally occurring oxygen minimum zones to spread into coastal waters, directly affecting energy flow and fisheries (Diaz and Rosenberg 2008). For these reasons, hypoxia and warming ocean temperatures associated with climate change may act synergistically to alter conditions within coastal ecosystems. Accordingly, another consequence of climate change is enhanced stress on marine organisms and communities.

1.3 Hypoxia and climate change on the physiology of marine organisms

Decreasing oxygen levels and increasing water temperature are predicted to act synergistically as stressors since (1) oxygen saturation potential declines with temperature; and (2) metabolic demands increase with temperature. Increasing metabolic rates mean increased costs of respiration, enzymatic reactions and protein denaturation, ultimately contributing to higher mortality (Jones 1977; Brown et al. 2004). In light of the combined threat of increased hypoxia and elevated temperatures as potentially synergistic stressors, it is crucial to learn more about how they may elicit ecological changes in the future. Although it is important to understand the synergistic effects of hypoxia and temperature, many previous studies consider the effect of a single stressor at a time.

The effects of hypoxia alone has been examined in many studies, focusing on individual organismal response to community responses. Typically, the first community response of exposure to environmental hypoxia is an increase in respiration rate (Diaz and Rosenberg 1995). Mobile fauna then migrate away and less mobile fauna begin to cease energetically costly processes, usually resulting in metabolic depression, with a reduction of movement and feeding. Triggering of mechanisms to provide more oxygen

or reduce oxygen demand also occurs, including the onset of anaerobic metabolism or acidosis (Pörtner et al. 2005). After some period of hypoxia persistence, mortality begins and reduces the benthic community to only the most tolerant species (Diaz and Rosenberg 1995).

However, the biological response and ability to acclimate to lowered dissolved oxygen varies among species and life stages. For example, median lethal concentrations have been observed from 8.6 mg O_2 l^{-1} for crab zooea to 0.0 mg O_2 l^{-1} for the eastern oyster under 20ºC following standard toxicity testing procedures (Vaquer-Sunyer and Duarte 2008). Mitchell (1914) found that oysters were extremely resistant to lack of oxygen, surviving for up to a week. Bivalves have been noted to have one of the highest tolerances to low dissolved oxygen, followed by deposit feeding polychaetes (Vaquer-Sunyer and Duarte 2008).

Survival, feeding, and reproduction are commonly affected by lowered dissolved oxygen concentrations. Several polychaete species were compared for changes in vital rates under varying levels of hypoxia associated with organic pollution (Reish 1966). *Dorvillea articulata* survived down to the lowest oxygen concentrations tested, 0.65 mg O_2 l^{-1}, but stopped feeding at oxygen levels of 1.0 mg l^{-1}, whereas *Capitella capitata* displayed significant mortality at 1.5 mg l^{-1}; and continued feeding down to 1.65 mg l^{-1} of oxygen. *Neanthes areanaceodentata* displayed a similar pattern, with mortality below 0.90 mg l^{-1}, while feeding ceased at levels lower than 0.95 mg l^{-1}. The more sensitive congener, *Nereis grubei*, only survived at oxygen levels higher than 2.40 mg l^{-1}, and ceased feeding at levels below 2.95 mg l^{-1}. This illustrates how oxygen tolerance varies among species.

There are also a variety of mechanisms used by organisms to cope with low oxygen concentrations. An increase in ventilation rate as oxygen partial pressure declines has been observed in crustaceans, including crayfish, crabs, and shrimp (Wohlgemuth et al. 2000). Wohlgemuth et al. (2000) found that the polychaete, *Arenicola marina,* increased ventilation during moderate hypoxia, but showed a marked decrease in respiration during severe hypoxia. Bivalves have been reported to respond to declining oxygen tensions in two ways: either by increasing ventilation rate or by maintaining a steady ventilation rate while increasing the extraction of oxygen (Kristensen 1983). Mitchell (1914) also found that oyster ventilation was extremely variable under different temperatures. He mainly noted an increase in the rate of oxygen consumption as temperatures increased. Tolerance and adaptations to low dissolved oxygen are influenced by many factors, including life history and other environmental conditions.

Temperature can act as an added physical stress on marine organisms. Temperature typically induces a bi-phasic response: the first phase including an increase in enzymatic activity until a critical threshold is reached, followed by the second phase resulting in a decline in the enzymatic activity, which can result in protein damage and organ failure (Pörtner et al. 2001). Specifically, aerobic respiration is expected to increase with increasing temperature due to enhanced metabolic demands (Alteri and Gedan 2015). Temperature is usually related to an increase in respiration rate due to elevated oxygen requirements, as part of a stress response, or both (Sturdivant et al. 2015). A review of temperature effects on marine benthic organisms reveals a negative relationship between temperature and survival under experimental conditions; LT_{50} (lethal time for 50% of sample) values decline as temperature increases within and sometimes above

natural temperature conditions (Vaquer-Sunyer and Duarte 2011). Also, when organisms are exposed to temperatures near their pejus threshold temperature (i.e., threshold where stress becomes worse), internal hypoxia can occur due to a limited ability to supply enough oxygen to cover oxygen demands at extreme temperatures (Pörtner et al. 2002). This inability to supply enough oxygen can lead to a breakdown of other fundamental organismal functions, and lead to changes in behavior, mobility, feeding, growth, and reproduction. Thus, such effects clearly extend detrimental impacts of increasing temperature to benthic populations and communities.

Synergistic effects of multiple stressors can interact by reinforcing or opposing one another, or combined effects of the factors can cause a critical threshold to be surpassed that would not have been reached by each factor independently (Harley et al. 2006). Due to effects of temperature on oxygen solubility, elevated water temperature engenders conflict between metabolic demands in the face of decreased dissolved oxygen concentration. Consequently, hypoxia tolerance decreases with temperature (Vaquer-Sunyer and Duarte 2011). This can lead to the occurrence of functional hypoxia, which signifies that the organism cannot acquire enough oxygen to meet internal physiological demands at a critical thermal level, despite available ambient oxygen (Pörtner et al. 2005, Farrell & Richards 2009). Thus, it is difficult to understand responses under natural conditions without considering the combined effects of multiple stressors. Responses of marine organisms when faced with multiple stressors and physiological tradeoffs that must occur to ensure survival have been poorly investigated. Moreover, it is important to know how stressors act together to influence the physiology of individuals in order to

understand how populations and communities will respond to such environmental pressures.

1.4 Body size relationships

Many ecological and physiological traits are affected by body size. Body size establishes vital bioenergetic rates used for functional macrobenthic indicators (Schwinghamer 1981, Edgar 1990, Rasmussen 1993). Environmental demands are experienced differently across a range of body sizes and are often expressed by size-dependent shifts in physiological rates (Forbes 1989). These shifts are usually demonstrated by allometric relationships. Allometry describes the change of certain characteristic with changes in size, specifically how traits and body size scale with one another (Shingleton 2010). Traits can include morphological, physiological, and ecological characteristics.

Allometric relationships are described by the power relationship: $Y=aX^b$, where y is the study trait, a is a constant, X is the animal's mass, and b is the scaling exponent. Parameters a and b are used to analyze the allometric relationship. Respiration rate is a physiological trait that has been well studied, specifically in the way that it varies across body size, across a variety of taxa. In relation to respiration, the b value is used to reflect the mechanism of oxygen transport through the body. Von Bertalanffy (1951, 1957) originally proposed three metabolic types related to growth: 1) surface proportional metabolism, b=0.67, 2) weight proportional metabolism, b=1.0, and 3) intermediate surface and weight proportional metabolism, $0.67<b<1$. Since then, other b values have been distinguished which are commonly found across taxa regarding allometric relationships; with b values around 0.75 suggesting fractal branching networks, known as

Klieber's law or the $3/4^{ths}$ rule, while a 0.67 (2/3rds rule) value still suggests a surface area relationship with body size (Shumway 1979, Forbes 1989, Kooijman et al. 2008). However, there are many exceptions to the rules, new and old. The scaling exponents can vary intra- and interspecifically and also may be affected by nutritional state, temperature, season, etc. (Shumway 1979). The scaling parameter *a* is a proportionality constant. For respiration, this allows a comparison of oxygen uptake and determination of metabolic rate and mode of life (Shumway 1979). As function changes with body size, allometric responses of individuals will contribute to population responses like previously discussed, and such responses will likely change in the face of multiple stressors.

1.5 Polychaetes as functional indicators of ecological health

Benthic communities are excellent indicators of estuarine health and ecosystem function due to their sensitivity to organic enrichment and hypoxia, as well as their role in benthic-pelagic coupling (Jørgensen 1996, Dean 2008). Benthic communities have been deemed "the most sensitive parts of the coastal ecosystem to hypoxia" due to their distance from the air-water interface and the inability of resident macrobenthic organisms to escape hypoxic events (Jørgensen 1996, Vanquer-Sunyer and Duarte 2008). The macrobenthic community performs an essential functional service by regulating the transfer of dissolved and particulate matter across the sediment-water interface and mediating nutrient transformations (Hansen and Kristensen 1997, Rakocinski and Zapfe 2005, Surugiu 2005).

The Pearson-Rosenberg (P-R) model is the classic paradigm depicting the response of the benthos to hypoxia as caused by organic enrichment. This model describes: "a shift from a community containing many large, long-lived, burrowing,

equilibrium organisms to a community dominated by small, opportunistic, short-lived surface-dwelling species" (Pearson and Rosenberg 1978, Nilsson and Rosenberg 2000, Rosenberg et al. 2002). This model implies that body size could be correlated to changes in environmental conditions through bioenergetics, thereby serving as a useful indicator of ecological health (Rasmussen 1993). In a review of marine benthic hypoxia, it is stated that seasonal hypoxia is linked to a decrease in abundance of large, long-lived species and an increase in smaller, short-lived species (Diaz and Rosenberg 1995). Mechanistic body-size related responses could characterize an organism's response to environmental stressors such as hypoxia, making the macrobenthos a useful functional indicator of hypoxia (Rasmussen1993; Brey et al. 1996; Rakocinski and Zapfe 2005; Persson and De Roos 2007; Rakocinski 2012).

One benthic taxonomic group, the polychaetes, has been particularly useful as an indicator of ecosystem health (Surugiu 2005). Polychaetes possess many traits that make them useful ecological indicators, including their ease of sampling, high relative abundance, range of reproductive strategies and trophic modes, and responsiveness to pollution (Llansó 1991, Surugiu 2005, Dean 2008). In Dean's (2008) review of the use of polychaetes as indicators of marine pollution, he states, "any long-term changes in the well-being of the benthos should be reflected in the polychaete community."

A polychaete's ability to cope with hypoxia is reflected in its life history traits, and may lead to its classification as tolerant or intolerant. A variety of tolerances to pollution and hypoxia has been observed inter- and intra-specifically in polychaetes because of associated altered metabolic rates and feeding behavior during periods of environmental stress (Nilsson and Rosenberg 1994). Tolerant or opportunistic species are

capable of rapid colonization in disturbed or recently disturbed habitats (Grassle and Grassle 1974), while presence and abundance of non-tolerant, or sensitive species are negatively correlated with increasing organic pollution. Commonly studied tolerant polychaetes include *Capitella capitata, C. minima, Polydora cornuta, Heteromastus filiformis, Lagis koreni, Melinna palmata, Neanthes succinea,* and *Prionospio cirrifera*. Some non-tolerant species include *Perinereis cultrifera, Nereis zonata, Syllis gracilis, Glycera convoluta,* and *Nephtys cirrosa* (Diaz and Rosenberg 1995).

Polychaetes possess different adaptations to abiotic stressors that need to be examined to understand their population responses. More complete information about physiological adaptations on the organismal level will facilitate the parameterization of predictive models for assessing the consequences of declining oxygen levels and climate change (Rakocinski 2012, Rombouts et al. 2013). Information on synergistic effects of multiple stressors relative to body-size of multiple species will help us understand how the benthic community might respond under the continued pressure of an increasing frequency of hypoxia in the face of climate change.

1.6 Model species

Streblospio gynobranchiata is a widespread abundant tube-dwelling polychaete occurring in subtidal estuarine habitats along the Atlantic coast of North and South America, the Gulf of Mexico, and the Mediterranean, Black, and Caspian Seas (Figure 1.2; Rice 1998, Radashevsky and Selifonova 2013). Cinar et al. (2005) described *S. gynobranchiata* as "a key species of polluted soft bottom benthic assemblages." Its name comes from the branchiate structure found on the dorsal setigers of the female, which act as a brood pouch (Radashevsky and Selifonova 2013). This species is very closely related

to its northern sister species, *Streblospio benedicti.* It is morphologically recognized by the presence of brood branchiae, as opposed to brood pouches on *S. benedicti* (Blake and Arnofsky 1999). The life history traits of *S. gynobranchiata* are similar to those of *S. benedicti* in that it is opportunistic, showing rapid colonization of disturbed areas and high reproductive rates (Llansó 1991). However, *S. gynobranchiata* differs in its mode of reproduction. Whereas *S. benedicti* may produce either planktotrophic or lecithotrophic larvae, *S. gynobranchiata* larvae are obligate planktotrophs (Rice 1998). These strategies may reflect differing physical or geographical factors. As *S. gynobranchiata* in the Gulf of Mexico was previously identified as *S. benedicti*, discrepancies or a lack of information about differences between the two species still remains.

Figure 1.2 Adult *Streblospio gynobranchiata*

Photo by Alyssa Bennett

Nevertheless, *S. gynobranchiata* most likely exhibits many of the same traits that make its sibling species a useful model species. The closely related *S. benedicti* is considered to be a pioneering, opportunistic species, often occurring under eutrophication

(Reish 1979). This species may have behavioral and physiological adaptations to seasonal hypoxia and anoxia (Llansó 1991). Ritter and Montagna (1999) found *S. benedicti* to be dominant at three hypoxic stations in Texas Bay. Populations of this species also exhibit expansive growth; its small adult size, short generation time, brooding behavior and high reproductive rate make these worms "dramatic exploiters of newly disturbed areas" (Grassle and Grassle 1974, Llansó 1991). It is also ubiquitous across most of the shallow estuaries, marshes, and mudflats in the United States (Levin 1984). *Streblospio benedicti* can tolerate a wide range of salinities, from 5-30 ppt, but decreases in abundance at low salinities (Ristich et al. 1977). This species also has a broad temperature tolerance, but some Gulf of Mexico populations may be limited by cold water in the winter and spring (Keith and Hulings 1965). *Streblospio benedicti* is also considered to be an indicator species of nutrient pollution (Grassle and Grassle 1974). In a lab study, Levin (1986) observed an increase in body length, segment number, and length per segment, as well as a doubling of brood size, with nutrient (N, P, Si) enrichment. Common behavioral responses observed in *S. benedicti* during hypoxia include the cessation of feeding and reproduction, and inactivity within its tube or stretching out into the water column (Llansó 1991).

Previous studies characterize *S. benedicti* as extremely stress-tolerant (Grassle and Grassle 1974, Pearson and Rosenberg 1978, Levin 1986). At 7% and 14.5% air saturation (0.5 and 1.0 mg l^{-1} O_2), Llansó (1991) did not observe significant mortality of *S. benedicti*, but at anoxia (<2% air saturation), he determined the LT_{50} (lethal time for 50%) for *S. benedicti* to be 43 hours. In a pilot study, I observed that *S. gynobranchiata* has a similar range of tolerance to low oxygen; mortality did not increase after 3 weeks of

exposure at 25°C and 2 mg l^{-1} O_2 (Bennett, pers obsv.). Llansó (1991) also observed that larvae may survive short term hypoxia within the water column, aiding quick re-establishment once normal conditions return. Because of its tolerance of hypoxia, *S. benedicti* and *S. gynobranchiata* are among the most resilient species after disturbance and likely facilitate the recolonization of disturbed benthic areas through their bioturbation activity (Middelburg and Levin 2009). It has been noted that female *S. benedicti* may migrate to recolonize and re-establish new populations after hypoxia, perhaps paving the way for other benthic organisms to re-establish within the sediment and to provide a food source to many larger invertebrates and fish (Llansó 1991).

Due to its capacity to rapidly colonize disturbed areas and its occurrence in shallow marsh habitats, *S. gynobranchiata* in the Gulf of Mexico may occur in areas that undergo periods of intermittent hypoxia and elevated temperature. Ambiguity in the literature relative to physiological and colonization capabilities under hypoxia underscores the need to explore synergistic effects of varying dissolved oxygen (DO) concentrations and temperatures for *S. gynobranchiata*. In this thesis, I will examine the mass-specific respiratory response of the abundant opportunistic polychaete, *Streblospio gynobranchiata*, relative to varying combined levels of dissolved oxygen and temperature. I will also examine changes in the respiratory rate in relation to early acclimation to hypoxia and during recovery from hypoxia. Specifically, this study will examine the physiological response of a tolerant polychaete in terms of aerobic respiration, as a reflection of the cost of maintenance associated with these combined stressors.

1.7 Study objectives

Few studies examine synergistic effects of multiple stressors, therefore, little is known about what will happen as hypoxic zones grow and temperatures increase. Higher temperatures will exacerbate hypoxic effects through both physical and ecological factors. Thus, it is important to understand the physiological tradeoffs that will occur when organisms simultaneously face reduced dissolved oxygen and increased temperatures, which may act in opposition, as reduced dissolved oxygen can induce metabolic depression while increased temperatures are associated with increased metabolic activity.

Currently, the Gulf of Mexico is facing increased threats to fisheries and production due to elevated sea temperatures and worsening hypoxic zones. The Gulf contains the 2nd largest hypoxic zone in the world. Hypoxia is expected to worsen with continued nutrient loading, especially near the Mississippi River outflow (Rabalais 2002). Furthermore, hypoxia is expected to be exacerbated by climate change due to increased nutrient runoff and increased stratification (Justic et al. 1996). Because temperatures will rise globally, hypoxia will be exacerbated with increasing precipitation leading to increased runoff and decreased oxygen levels. Coastal hypoxic zones are also projected to experience a warming of about 2ºC by the end of the century; specifically the Gulf of Mexico may experience increases up to 4ºC (Biasutti et al. 2012). For these reasons, hypoxia and climate change will likely act synergistically to alter conditions within coastal ecosystems.

For my study organism, I have chosen *S. gynobranchiata,* which is a tolerant, opportunistic polychaete with rapid colonization of disturbed areas and high reproductive

rates. As an endemic species of the Gulf of Mexico, it must withstand a wide range of temperatures and is likely to undergo periods of hypoxia. Since this is a benthic, tube-dwelling organism, it has little opportunity to escape hypoxic zones. Little is known about the response of this abundant species to temperature and DO as combined stressors.

Few studies have looked at the synergistic effects of stressors on the physiology of benthic organisms. The overall goal of this study is to gain a better understanding of the metabolic costs of a tolerant polychaete associated with different phases of hypoxia exposure (full acclimation, early acclimation, and recovery). This will also aid to more fully understand the physiological response of *S. gynobranchiata* to low dissolved oxygen and increased temperatures through its respiratory responses relative to body size. The objective for my study is to examine the potentially opposing physiological effects of the combined stressors, increasing temperature and decreasing DO, as well as differences in response to varying degrees of hypoxia exposure.

Accordingly, this thesis will address the following specific objectives:

1. Determine mass-specific aerobic respiration for *S. gynobranchiata* relative to a standard acclimation exposure period under varying combined levels of dissolved oxygen and temperature. Respiration reflects the metabolic rate of organisms and can provide a tool for understanding changes in metabolic costs of these stressors within the naturally fluctuating ecosystem.
2. Determine mass-specific aerobic respiration for *S. gynobranchiata* relative to early acclimation to hypoxia exposure. This objective will provide insights into how this organism can cope with rapidly changing physical conditions,

and the extent to which duration of exposure can elicit varying physiological responses.

3. Assess variation in the capacity of *S. gynobranchiata* to resume characteristic rates of respiration upon return to normoxic conditions after exposure to hypoxic dissolved oxygen levels. The ability to resume normal respiration following exposure to hypoxia will reflect the recovery ability of this species. This response may also provide further insight into anaerobic respiratory responses to hypoxia and temperature. Anaerobic respiration compensates for reduced aerobic

CHAPTER II - INTERACTIVE EFFECTS OF DISSOLVED OXYGEN, TEMPERATURE, AND BODY SIZE ON AEROBIC RESPIRATION OF *STREBLOSPIO GYNOBRANCHIATA*

2.1 Introduction

Determining the metabolic rate of an organism provides a way of quantifying its cost of living (Hulbert and Else 2000). Measuring the rate of oxygen consumption is a proxy for estimating the metabolic rate of an individual (Clarke and Fraser 2004). Thus, understanding how the respiration rate of an organism changes in response to environmental stress can provide insight into associated metabolic costs. As seen for multiple species (Shumway 1983; Nielsen et al., 1995; Hoback and Barnhart 1996; Gamenick et al. 1998; Christensen and Colacino 2000; Linke-Gamenick et al. 2000), a body-size trend in respiration is often evident; small organisms generally exhibit higher mass-specific respiration rates than large organisms under normoxia due to their higher mass-specific metabolic rates. This relationship is an example of allometry, a subfield that concerns how biological traits scale to body size, typically using the power equation: $Y=aX^b$, where y is the trait of interest, a is an intercept at unit body mass, X is the animal's mass, and b is a scaling exponent (Shingleton 2010). The allometric respiration relationship is known to be influenced by temperature and dissolved oxygen supply, as well as other environmental conditions such as food availability (Shumway 1979). This is also affected by the state of the organisms when measured. Metabolic rate is typically measured for individuals at basal or standard rates when the organism is inactive or resting but can also be measured for active individuals. Potential allometric trends in respiration need to be examined relative to combined dissolved oxygen and temperature

levels to determine how ontogenetic shifts vary across treatment levels due to differences in metabolic demand.

Oxygen level directly limits the metabolic rates of individual organisms and is mediated by their adaptations. Organisms are generally classified as oxyregulators or oxyconformers (Herreid 1980). Oxyconformers are metabolically dependent on external DO conditions. Therefore, their respiration rate will also change in the face of changing dissolved oxygen levels. Oxyregulators do not rely on external oxygen levels and can maintain their respiration rates across varying oxygen concentrations (Bridges and Brand 1980, Shumway 1983). However, at some critical oxygen tension, all organisms are forced to become oxyconformers (Willmer et al. 2009). Regulatory ability can vary inter- and intra-specifically due to activity level, adaptations, body size, etc. (Herreid 1980). Due to the general principle that it is more difficult for small organisms to maintain high mass-specific respiration rates than large organisms, small individuals should exhibit oxyconformation whereas large organisms should be relative oxyregulators (Rakocinski 2009).

There is typically a predictable series of metabolic and behavioral responses within the benthic community to hypoxia (Rabalais et al. 2010, Sturdivant et al. 2015). Organisms initially increase respiration, next mobile fauna migrate away, and then sessile fauna decrease normal activity (Sassaman and Mangum 1972, Wannamaker and Rice 2000, Ludsin et al. 2009, Seitz et al. 2009). Hypoxia has been documented to cause degradation of normal functioning of benthic communities including diminished bioturbation activities, as a lack of oxygen can induce metabolic depression- a quiescent state to reduce energy loss (Sturdivant et al. 2015). Bioturbation activity is an infauna-

mediated process that is essential for proper benthic function by maintaining the quality of sediment. During hypoxia, the change in activity of benthic organisms can lead to a breakdown in these essential services, however, some species have been documented to have an ability to maintain normal benthic functioning and show some metabolic plasticity to changing oxygen content (Sturdivant et al. 2015). These organisms that remain active under low oxygen can be considered tolerant and possess features that allow the organism to maintain their metabolic scope in the face of stressors. Those organisms that are not tolerant typically experience mass mortality when dissolved oxygen levels surpass regulatory abilities (Rabalais et al. 2002). Because there are such widespread responses in the face of changing oxygen regimes, it is important to more fully understand associated metabolic costs.

Temperature also has a significant influence on organisms' metabolic rate. Organisms possess a window of thermal tolerance and this directly affects metabolic mechanisms (Clarke and Fraser 2004). It is important to understand more about these thermal limitations as changes in global temperatures will cause shifts in geographical distribution and physiological performance (Pörtner et al. 2005). The general response to increasing temperatures is an increase in metabolic rate, as temperature influences the rate of cellular reactions. However, at a critical temperature, these reactions begin to breakdown. Also, internal hypoxia can be induced by extreme low and high temperatures and can result in a switch to anaerobic metabolism (Pörtner et al. 2005).The loss of aerobic scope at thermal limits is directly related to the inability of the organism to cover the increase in oxygen demand. Therefore, this interaction with decreasing oxygen concentrations due to hypoxia will cause compounding effects. Pörtner et al. (2005) states

"as the availability of ambient oxygen and the thermal sensitivity of animal organisms are closely intertwined, trends of global warming, associated decrements in oxygen availability despite increasing demand…may closely interact to cause large and small-scale shifts in …ecosystem composition and functioning."

Thus, in this study I will examine mass-specific oxygen consumption rates under varying levels of dissolved oxygen and temperature. Mass-specific aerobic respiration in a fully crossed design involving three DO levels and three temperature levels will be measured to represent changes in the metabolic costs associated with these stressors. The objective is to determine mass-specific oxygen consumption of *S. gynobranchiata* under all combined temperature and dissolved oxygen levels. From this data, I will determine base-line respiration rate curves, allometric scaling trends, and oxygen regulation abilities relative to body size. Firstly, I hypothesize that with increasing temperature, there is will be increases in mass-specific respirations rates across all body sizes. As increasing temperatures interact with decreasing dissolved oxygen, responses will also vary interactively, and differ from the effects of low oxygen saturation considered alone. I also hypothesize that smaller body sizes will be more sensitive to dissolved oxygen levels in combination with increasing temperatures compared to larger body sizes, which should maintain the ability to oxyregulate under more extreme levels of dissolved oxygen and temperature. All body sizes will exhibit a stress response at a combination of high temperature and low dissolved oxygen.

2.2 Materials and methods

2.2.1 Specimen collection

Adult *S. gynobranchiata* were collected during low tide in Weeks and Simmons Bayous, MS in April 2014. The top 5 cm of sediment was collected by kick net from sparsely vegetated sand-mud bottom tidal creeks within the bayou and transported immediately to the lab. Sediment containing resident organisms was stored in a 5 gallon aerated aquarium with seawater for up to one week. Adult polychaetes were recovered by gently washing single cups of sediment over a 0.5 mm plastic mesh sieve and retained for laboratory culture.

2.2.2 Culture methods

Methods for the culture of adult *S. gynobranchiata* in the laboratory generally followed procedures developed and used in the culture of *Capitella* sp. in the Grassle laboratory (J. Grassle, pers comm). Briefly, groups of 50 or fewer adult polychaetes were maintained in 4.5” diameter culture dishes with approximately 1 tablespoons of sediment and 2 cm of standing artificial saltwater (ASW). Artificial saltwater was made using Biosea® marine mix aquarium salt and distilled water at a ratio of approximately 2 cups for every 5 liters to achieve 23 ppt. Sediment for cultures was collected from a tidal creek in Davis Bayou, passed through a 1 mm sieve and frozen in 30 ml plastic food safe containers. Before use, sediment was thawed and TetraMin® fish flakes were added at a ratio of 2.5 g of flakes to 54 g of mud. A complete sediment and water change was performed every other week. When cultures were cleaned, the standing water was siphoned to screen for planktonic larvae, and adults were observed for the presence of

eggs within brood pouches. Adult cultures were reared at about 20ºC in complete darkness.

Larval culture methods were developed based on literature on the culture of other spionid polychaetes (Dean and Mazurkiewicz 1975, Schulze et al. 2000) as follows. Gravid *S. gynobranchiata* adults typically release large numbers of trochophore larvae, which were present in the water column of the culture dish; and more eggs and larvae were usually released during cleaning (Figure 2.1A). Whenever larvae were observed, they were immediately transferred to 4.5" fingerbowls filled to ~3 cm with artificial saltwater (ASW). Larvae were kept under natural light conditions at room temperature (~20ºC) and 23 ppt at densities of about 1 larva ml^{-1}, and fed a regular supply of unicellular algae, *T-isochrysis*, *Rhodonomas*, and *Chaetoceros*, obtained from the Thad Cochran Marine Aquaculture Center. Developing larvae were kept alive in standing ASW that was stirred and provided approximately 0.5 ml of the unicellular algae mixture once a day. Larval development was monitored daily using a stereoscope. As larvae were observed to lengthen and approach the bottom to settle, sediment was gently added to the bottom of the culture dish by pipette (Figure 2.1B & C). Small tubes would appear within culture sediment soon after settlement and more sediment enriched with TetraMin® fish flakes was added once no more larvae were observed actively swimming in the water column (Figure 2.1D). This was to avoid excess turbidity in the water column which could cause premature mortality for any remaining planktonic larvae. Juveniles were allowed a period of 4 weeks to grow within adult culture conditions before any further handling.

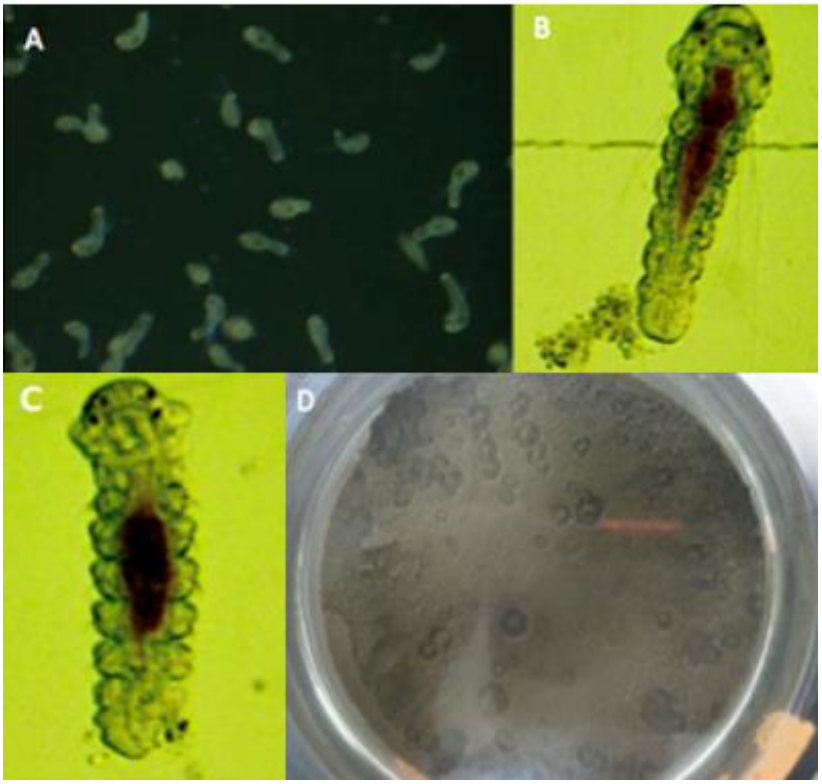

Figure 2.1 Stages of *Streblospio gynobranchiata* larval development

A) Newly released trochophore larvae B) 7-setiger larvae C) 11-setiger larvae D) Culture dish with tube formations from newly settled juveniles. Photos by Alyssa Bennett.

2.2.3 Experimental conditions

Individuals of various body sizes were exposed to treatments of varying combinations of percent oxygen saturation and temperature (Table 2.1). Dissolved oxygen levels within each treatment were high (100% saturation), medium (60% saturation), or low (20% saturation). Percent saturation was used to characterize levels to keep oxygen treatments consistent across all temperatures. Use of percent saturation standardized all treatments to normoxia at every temperature. Actual oxygen concentrations (mg/l) representing percent saturation levels are presented in Table 2.1. The specific dissolved oxygen concentrations representing low, medium, and high levels

were chosen to represent those most likely elicit different physiological responses by the organisms. A parallel study indicated that there was little significant difference in the respiratory rate of the polychaete *Capitella teleta* across a small range of moderate dissolved oxygen saturation levels (50 and 70%) (Gillam 2016).

Three target treatment temperatures (15, 25, and 35ºC) were chosen to encompass current natural seasonal conditions (15 and 25ºC), as well as the potential future warm extreme (35ºC) in the northern Gulf of Mexico where the model species occurs. At three National Oceanic and Atmospheric Association (NOAA) Tides & Currents stations in 2014, daily multiple readings of ocean surface temperature in 3 to 8 feet of water sometimes exceeded 32.2ºC (CO-OPS 2014). At the Pascagoula NOAA Lab station, the surface water temperature reached over 31.7ºC in June and July 2014, and peaked at 33.8ºC in August 2014. The water temperature at the Dauphin Island station reached 32.5ºC in July and exceeded 32.8ºC in August 2014. Given these observed high temperatures, the heat content of water within tidal creeks, bayous and marshes in the northern Gulf of Mexico could easily exceed 33ºC. Thus, considering climate change trends, 35ºC is a feasible water temperature facing many species in the future.

Table 2.1

Experimental treatment levels

Target temperature (ºC)	**Measured temperature (ºC)**	**Target % O_2 saturation**	**Measured % O_2 saturation**	**Measured dissolved oxygen (mg/L)**	**N**
15	16.6 ± 1.20	20	21.4 ± 3.21	1.83 ± 0.27	8
		60	61.4 ± 2.91	5.26 ± 0.20	11

		100	100.2 ± 1.68	8.41 ± 0.33	12
25	25.0 ± 0.71	20	21.3 ± 2.90	1.67 ± 0.93	15
		60	60.6 ± 1.85	4.38 ± 0.14	12
		100	101.7 ± 5.08	7.35 ± 0.35	16
35	33.7 ± 1.09	20	21.0 ± 2.49	1.33 ± 0.20	8
		60	63.7 ± 5.28	3.96 ± 0.32	4
		100	100.1 ± 1.98	6.34 ± 0.18	10

Treatment levels of temperature and dissolved oxygen for respiration measurements (±) 1 standard deviation and sample size (N= number of measurements over experimental periods).

2.2.4 Experimental setup

Individual polychaetes were placed in 5 cm diameter glass petri dishes containing mud and ASW within a sealed air chamber (BioSpherix ©) into which nitrogen gas was pumped and regulated at a predetermined rate (Proox© Model 110 O_2 regulator) to maintain the dissolved oxygen treatment level across the air-water interface of specimen containers. These sealed chambers were located within a Precision© Low Temperature Incubator (Model 815) to maintain the appropriate temperature treatment. Before the addition of study organisms, the chamber was acclimated for 24 hours at the desired dissolved oxygen and temperature to ensure that the water-gas interface had reached equilibrium and dissolved oxygen had stabilized at the treatment level. Dissolved oxygen and temperature of the treatment water were checked daily using a handheld optical dissolved oxygen meter (YSI ProODO® Digital Professional Series). Polychaetes were acclimated for 24 hours to a combination of 20%, 60%, or 100% dissolved oxygen and 15, 25 or 35ºC within the sealed air chambers positioned within the incubator before

measuring their respiration. The 24-hour exposure time was chosen to adequately ensure acclimation to the experimental conditions in order to allow for physiological adjustments in the test organism (Bridges & Brand 1980, Pedersen 1991).

The FireStingO2 (2 channel) oxygen sensing meter was used to measure oxygen consumption for individual polychaetes, following procedures recommended by Dr. Amy Maas (pers. comm.) of Woods Hole (WHOI), who has been using the FireStingO2 meter with pteropods. An oxygen sensor foil was secured on the inside of a syringe using silicone marine sealant and a corresponding adapter was glued to the outside of the syringe. Syringes were chosen as the chamber apparatus because they allow the volume to be adjusted for small volumes of treatment water, such as the 2 ml needed to accurately measure oxygen depletion by these very small polychaetes. Sensors were calibrated to 0% and 100% saturated water. Preliminary trials showed that 2 ml of treatment water was enough to measure oxygen decline without the individual depleting or changing the oxygen content far from the assigned treatment level during the measurement period. Also, the use of syringes ensures that all extraneous oxygen is occluded. During oxygen consumption measurement, the syringe was filled with treatment water and the polychaete was transferred by pipette from its dish in the hypoxia chamber, placed in a triangular mesh pouch and then into the syringe. The mesh pouch was used to mimic the feeling of a tube and therefore decrease the movement of the worm by allowing it to feel less exposed while inside the syringe. The volume of water was then adjusted, any remaining air bubbles were removed, and a stopcock on the end of the syringe was closed to seal off the syringe. The bare fiber-optic oxygen probe, secured to the syringe by the adapter, was then attached to the oxygen meter and the decline in

oxygen concentration was recorded continuously via the Oxygen Logger software™. Syringe chambers were placed in a Boekel Grant Optima™ Model GD100 circulating water bath kept at the constant temperature of the treatment being tested. The syringes were secured in the waterbath, but allowed to float on the surface in the current to help circulate the water within the syringe, thereby reducing the possibility of local depletions of oxygen around the individual. The decline in oxygen concentration over a 40-minute period was recorded to determine each individual's oxygen consumption (V_{O2}). The last 10 minutes of the 40-minute period provided the definitive V_{O2} rate to avoid any noise due to sensor acclimation to the treatment water and temperature. A reference measurement was recorded for each channel of the instrument to account for any background oxygen consumption by bacteria, or other sources of drift, etc. All DO consumption measurements were recorded in complete darkness (c.f. Shumway 1979, Bridges and Brand 1980, Kristensen 1989, Sagasti et al. 2001).

2.2.5 Analysis

The focus of this study was to determine variations in allometric physiological responses of *S. gynobranchiata* under multiple combined levels of dissolved oxygen and temperature. Thus, the analytical approach will focus on defining size-scaling response curves with respect to temperature and DO combinations.

Oxygen concentration over time during each individual trial was recorded in a Microsoft Excel spreadsheet using Oxygen Logger software™. Oxygen uptake rate was calculated as the slope of the regression line of oxygen concentration against time (Vismann and Hagermann 1996; Linke-Gamenick et al. 2000; Christensen et al. 2011). The slope for each individual V_{O2} (mg O_2 g^{-1} h^{-1}) calculation is defined as: d(DO)/dt=

(DeclineO_2-Drift) . Calculation of the mass-specific respiration rate, V_{O2} (mg O_2 g^{-1} h^{-1}), was determined using the expression:

$$\mathbf{V_{O2} = (d(DO)/dt)*(Vr-Va)/m}$$

V_{O2} = instantaneous oxygen consumption rate (mg O_2/mg/h);

d(DO)/dt = rate of decrease of DO (mg O_2 L^{-1} h^{-1});

Vr = respirometer volume (L);

Va = volume of experimental animal (L); and

m = animal mass (g).

Individual V_{O2} values were plotted against mass and fit with power curve regressions (i.e., $V_{O2} = aW^b$) using the nonlinear fitting (nls) procedure in R (version 3.3.2.Ink). The *a* and *b* parameters were determined by log transforming the data and plotting log-V_{O2} versus log-mass using the lm function in R which yields the coefficients for the power equation.

The entire respiration data set was analyzed as a two-way ANCOVA, with logV_{O2} as the response and log-mass as the covariate in SPSS (version 18), in order to examine combined effects of DO and temperature on respiration rates. Because ANCOVA slopes relative to mass were nonparallel when both DO and temperature factors were included (i.e., DO × Temp × mass - F = 2.803; P = 0.012; DO × mass - F = 6.074; P = 0.003), comparisons were made separately across the three temperature levels for each of the three DO levels using univariate ANCOVA in order to identify changes in respiration rate that occurred as a result of the change in temperature. The equality of error variances assumption was examined using Levene's tests, for which only the complete two-way ANCOVA model was heterogeneous.

A stepwise procedure was followed to determine the necessary sequence and outcomes of statistical tests within each level of DO (Figure 2.2). These tests were run in order to determine if respiration rates differed between the temperature treatments and if there was a difference in body size responses between temperature treatments. First an ANCOVA was run to examine whether slopes were parallel by testing for the interaction between temperature and mass within a given DO level. If a non-significant interaction indicated that slopes were parallel across all three temperature levels, the ANCOVA was rerun without the interaction term to further test for the differences in temperature treatment elevations (height of linearized relationships) within a given DO level, which were used to determine if respiration rate was sustained at different levels under different temperatures. Pending a significant overall difference among temperature levels, pairwise differences in V_{O2} elevations were determined between temperature levels using LSD tests. Slopes were not parallel if the interaction between temperature and mass within a given DO level was significant, leading to subsequent pairwise comparisons of slopes using custom lmatrix commands to identify which temperature × mass slopes differed. Finally, ANCOVA was used to find temperature-related differences within remaining temperature subsets (i.e., same slopes after removing data for temperature levels with different slopes) for which slopes were homogeneous within given DO levels.

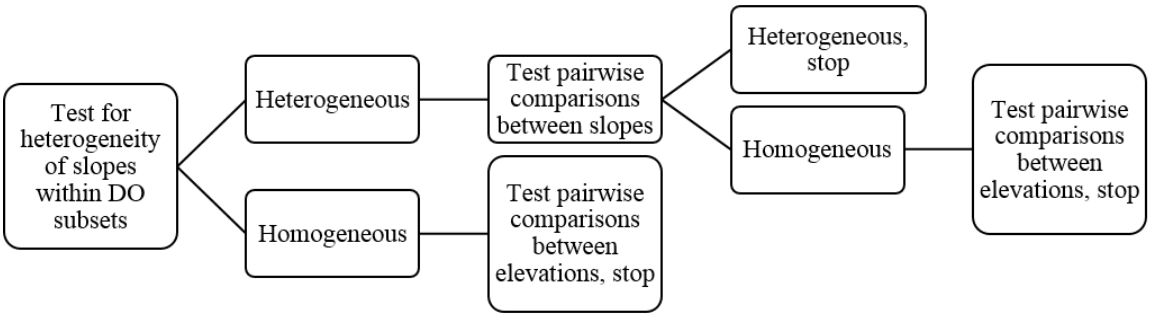

Figure 2.2 Flowchart for stepwise testing procedure

Flowchart outlining the procedure for testing slopes and elevations across temperature levels within separate DO levels.

2.3 Results

2.3.1 The effect of dissolved oxygen and temperature on aerobic respiration

Respiration rates and body mass were measured from 241 individual polychaetes ranging in size from 0.11 to 2.36 mg. Respiration rates varied from 0.06 to 12.825 mg O_2 g^{-1} hr^{-1}. Approximately 88% mortality was observed during acclimation of individuals at 35ºC and low DO, 38% mortality at 35ºC and medium DO, and 43% mortality at 35ºC and high DO, explaining the smaller sample sizes at those treatment combinations (Table 2.2). All other treatments had low levels of mortality from 0 to 3 individuals. The R^2 values determined from the non-linear model regression fit of mass-specific respiration and mass ranged from 0.67 to 0.74; there was a significant relationship between body mass and respiration rate for 8 of the 9 treatments, with the remaining high temperature-low DO treatment having too little data (N=2) to determine the relationship (Table 2.2). Regressions showed that smaller individuals maintained higher respiration rates than larger individuals across all treatments (Table 2.2, Figure 2.3). As smaller organisms typically have a larger surface area to volume ratios and higher energy demands for growth than larger organisms, this ontogenetic trend is commonly observed for many species.

Table 2.2

Relationships between mass-specific respiration rates and body size

Temp	DO	N	R^2	p-value	% mortality
15ºC	20%	39	0.6914	<0.001	7%
	60%	32	0.6671	<0.001	3%
	100%	37	0.6702	<0.001	0%
25ºC	20%	33	0.7287	<0.001	6%
	60%	42	0.7285	<0.001	0%
	100%	33	0.6664	<0.001	3%
35ºC	20%	2	NA	NA	88%
	60%	10	0.7438	0.0013	38%
	100%	13	0.7426	<0.001	43%

Sample size (N), R^2 values, and percent mortality for each experimental treatment. P-values for the nonlinear model regression fit of mass-specific respiration rate and mass.

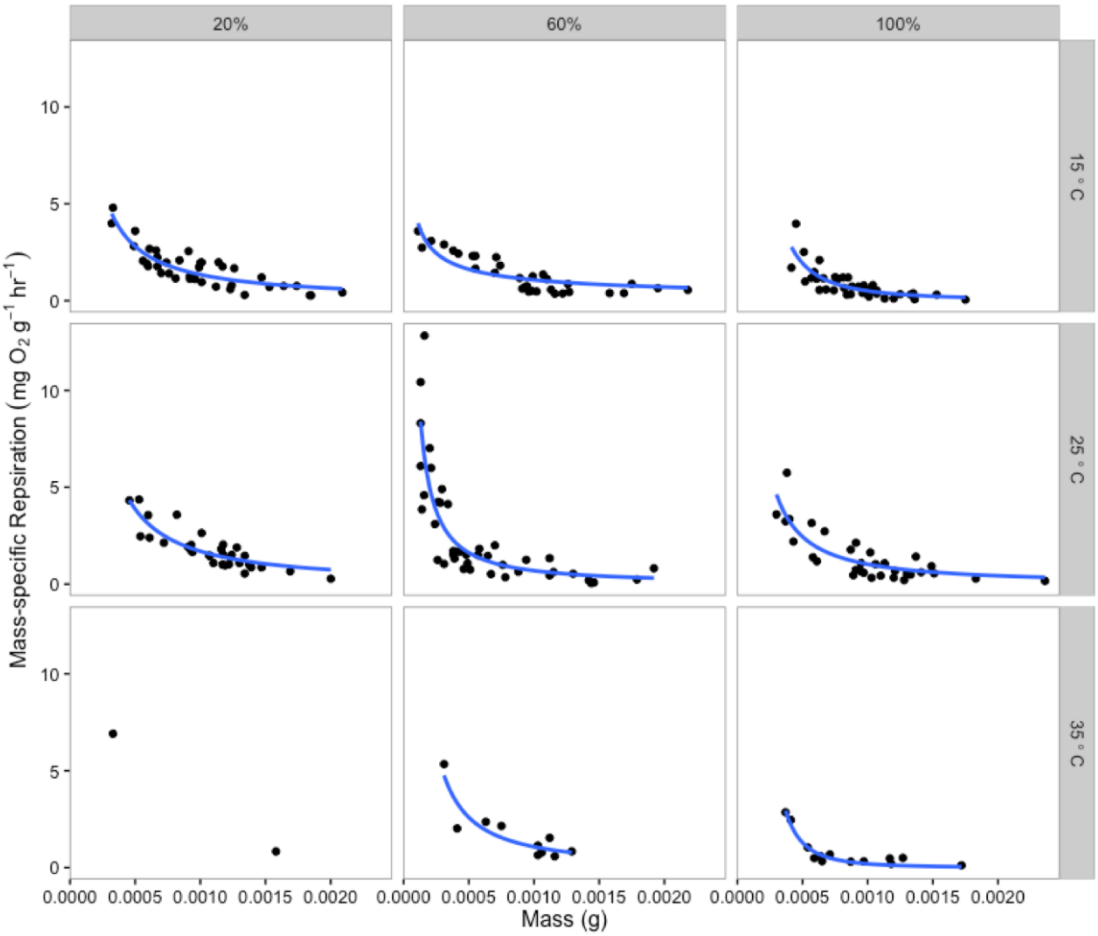

Figure 2.3 Relationships between mass-specific respiration rates and body size

Mass-specific respiration for Streblospio gynobranchiata *at three dissolved oxygen saturations crossed with three temperature regimes.*

A two-way ANCOVA with mass as the covariate on the linearized data of V_{O2} and mass showed a significant effect of all variables on respiration rate: mass, DO, and temperature (Table 2.3). DO was significant as shown by main effect (P= <0.001) and also through the interaction effects with both temperature and mass (P= 0.002, 0.003). Temperature was significant through the interaction effects (P= 0.002, 0.008), which underscores the synergistic effects of temperature with dissolved oxygen. After removing the non-significant mass × temperature interaction term, the interactions between DO and

mass as well as the three-way interaction between DO, temperature, and mass remained significant ($p = 0.003, 0.012$), establishing heterogeneity of the slopes (Table 2.4). When considering partial effect sizes (partial Eta squared or η^2) for model terms, which represent proportions of the variance in the dependent variable after factoring out the effect of the covariate (Green and Salkind 2003), the observed cumulative effect for terms including DO was substantially greater than that for temperature in this study. This was inferred by cumulative η^2 values of 0.254 vs. 0.142, respectively, for all terms involving said factors within the analysis (Table 2.4). Conventional cut-off values for small, medium and large effects are 0.01, 0.06 and 0.14, respectively. Therefore, both DO and temperature have large effects on respiration rate, with DO related terms having around double the effect of temperature. When all nine treatments were tested together, there was a significant heterogeneity of variance, however, the F value of 3.883 was not egregious.

Table 2.3

Effects of mass, DO, and temperature on mass-specific respiration rates

Factor	**Sum of squares**	**Df**	**Mean square**	**F**	**Sig.**
log(Mass)	12.391	1	12.391	273.579	<0.001
DO	0.651	2	0.326	7.189	0.001
Temperature	0.005	2	0.003	0.059	0.942
Temperature * DO	0.775	4	0.194	4.279	0.002
log(Mass) * DO	0.550	2	0.275	6.074	0.003

log(Mass) * Temperature	0.002	2	0.001	0.018	0.982
log(Mass) * Temperature *DO	0.637	4	0.159	3.515	0.008

Results of two way ANCOVA, including non-significant terms. Sig.= P-value.

Table 2.4

Effects of mass, DO, and temperature on respiration rates, significant terms only

Factor	Sum of squares	Df	Mean square	F	Sig.	Partial eta squared
log(Mass)	12.391	1	12.391	273.579	<0.001	0.551
DO	0.651	2	0.326	7.189	0.001	0.061
Temperature	0.005	2	0.003	0.059	0.942	0.001
Temperature * DO	0.775	4	0.194	4.279	0.002	0.071
log(Mass) * DO	0.55	2	0.275	6.074	0.003	0.052
log(Mass) * Temperature *DO	0.762	6	0.127	2.803	0.012	0.07

Results of the two way ANCOVA after the removal of non-significant terms, including partial effect sizes (partial eta squared). Sig.= P-value.

Because a conventional ANCOVA could not tease out the differences due to each factor, the data was partitioned into the three respective dissolved oxygen levels in order to determine which slopes differed among the temperature treatments within each level of DO (Figure 2.4). After partitioning, Levene's tests for variance heterogeneity were non-significant among temperature levels for all three DO subsets (20% DO: F= 2.766; P = 0.070; 60%DO: F=1.934; P=0.151; 100% DO: F=0.698; P= 0.501). Following the flowchart testing procedure (i.e., Figure 2.2), slopes were determined to be parallel across temperature levels for the 20% DO dataset; the interaction between temperature level and

mass was found to be non-significant (F= 0.298, P= 0.743) (Table 2.5). Thus the 20% DO dataset was rerun without the mass interaction term, for which the temperature factor was statistically significant (P= 0.005). Subsequent LSD tests showed that elevations differed significantly between 15ºC and 25ºC levels (P= 0.001), but did not differ between 35ºC and either 15ºC or 25ºC levels (P= 0.321, 0.921). The adjusted estimated marginal mean was higher at 25ºC than 15ºC, indicating that worms maintained a higher mass-specific respiration rate at 25ºC than at 15ºC (Figure 2.5A). This result makes sense since metabolism is typically elevated at higher temperatures. The lack of a significant difference in slopes or elevations between 15ºC and 35ºC levels at 20% DO is likely due to a lack of power, due to a low number of observations (N=2) for the 35ºC treatment at 20% DO.

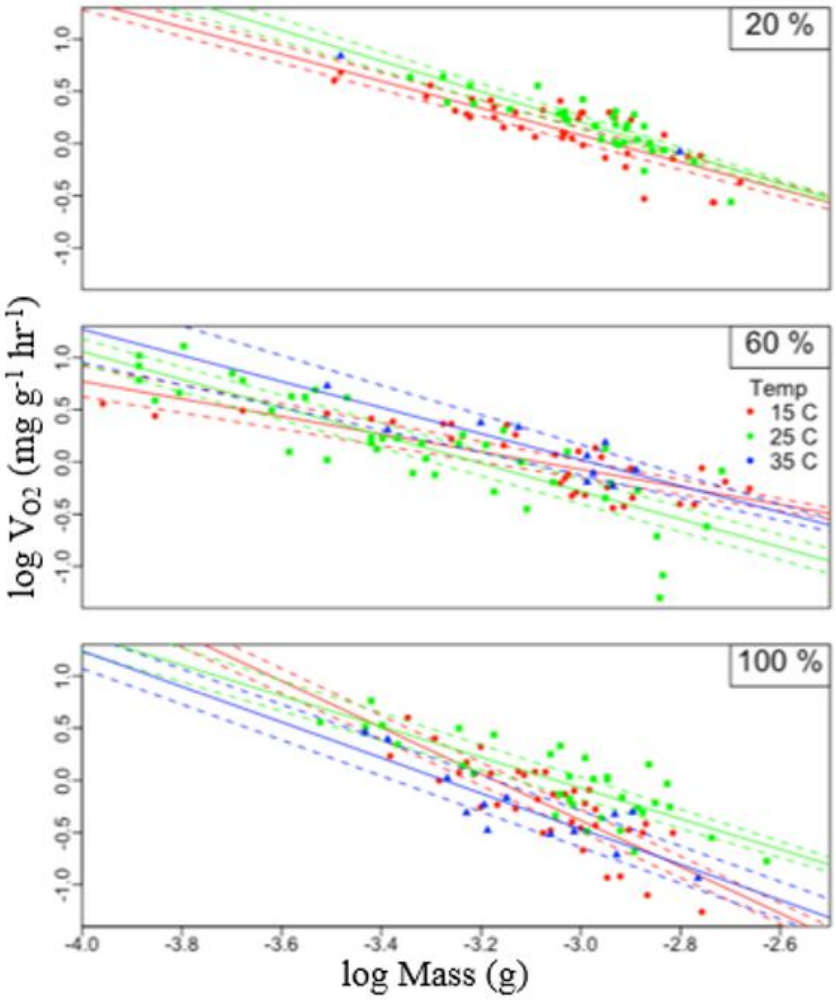

Figure 2.4 Linearized relationship between mass-specific respiration rate and mass

Linearized mass-specific respiration rates with 95% confidence intervals of each temperature treatment within each dissolved oxygen level.

Table 2.5

The effects of temperature on respiration rates and body size responses

DO		**20%**			**60%**			**100%**	
Test sig.	Interaction term	Slope	Elevation	Interaction term	Slope	Elevation	Interaction term	Slope	Elevation
All temp	NS			*			NS		
15 v. 25ºC		NA	**		**	NA		NA	***
25 v. 35ºC		NA	NS		NS	**		NA	***
15 v. 35ºC		NA	NS		NS	.		NA	NS

*Results of univariate analysis of variance (ANOVAs) comprising pairwise comparisons of slopes and elevations of mass-specific respiration rate regressions for the three DO levels. NS= non-significant, NA= not tested, *= <0.05, **= <0.01, ***= <0.001, . =marginally non-significant.*

At 60% dissolved oxygen, the temperature-mass interaction term was significant (Table 2.5, F=3.983, P= 0.023), indicating the slopes were not parallel, and therefore, it was necessary to test for pairwise differences between the slopes at each temperature. The only significant in-slopes difference was between the 15ºC and 25ºC levels (Table 2.5; P= 0.006). Thus, respiration rates differed across body sizes between these two temperature levels at 60% DO saturation. Respiration rates for smaller individuals appeared to increase much more rapidly at 25ºC than 15ºC, indicating elevated metabolic rates for smaller worms at higher temperatures. Because the slopes did not differ significantly between 35ºC and either 15ºC or 25ºC levels, the elevations were also tested and found to be significantly different between 25ºC and 35ºC (P =0.004), while marginally non-significant between 15ºC and 35ºC (P = 0.059) (Table 2.5, Figure 2.5B). Again, significance may have been precluded by the small sample size at 35ºC (i.e., N= 10). This shows that respiration was maintained at higher rates overall at 35ºC than at 25ºC, and most likely than at 15ºC.

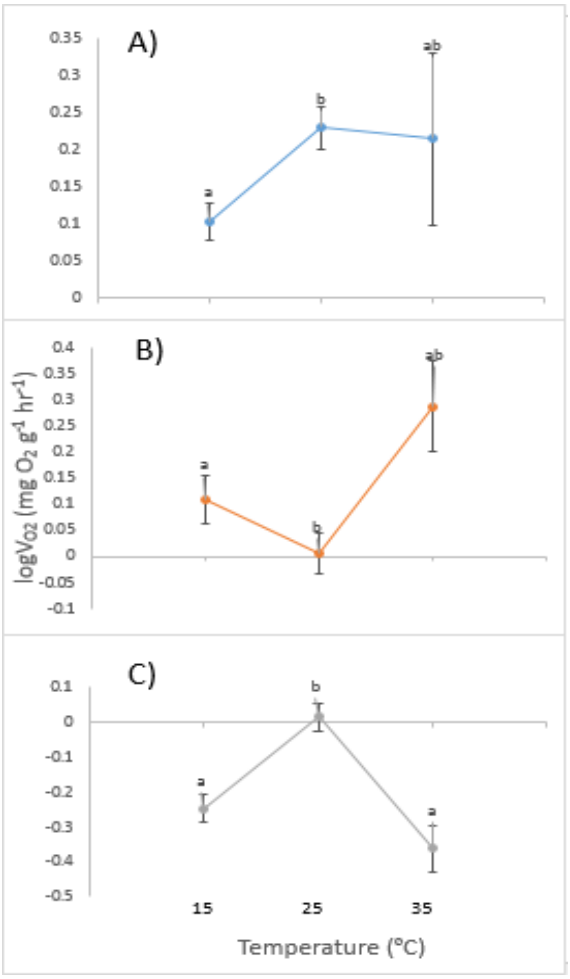

Figure 2.5 Estimated marginal means of logged mass-specific respiration rates

Estimated marginal means ± one standard error of logged mass-specific respiration rates at each temperature level for the A) 20% DO subset, B) 60% DO subset, C) 100% DO subset.

At 100% dissolved oxygen, the opposite pattern was evident across temperatures compared to that for the 60% saturation level; respiration was highest at the intermediate temperature (Figure 2.5C). As the interaction term was not significant (Table 2.5, F= 2.827, P= 0.065), pairwise comparisons of the slopes were not necessary. However, elevations were significantly different between 15ºC and 25ºC levels (P = <0.001) and between 25ºC and 35ºC levels (P = <0.001). Respiration rates were higher at 25ºC than at 15ºC, but decreased between 25ºC to 35ºC (Figure 2.5C).

2.3.2 Dissolved oxygen and temperature on metabolic scaling

Allometric scaling of the relationship between oxygen consumption and body size, quantified as exponents from the log-log linear regressions of oxygen consumption versus weight, varied among each experimental treatments. Mass-specific scaling exponents *b'* ranged from -1.227 to -0.251 (Table 2.6). Accordingly, exponent (*b)* values of corresponding oxygen consumption rate (OCR) curves (where $b = b' + 1$) mostly fell within previously reported ranges for metabolic scaling (Shumway 1979, Hulbert and Else 2000, Glazier 2005, 2006), ranging from 0.53 to 0.74, however, a few fell well below these typical values (Table 2.6, Figure 2.6A). Under 20% DO, *b* values were highest at 15ºC and lowest at 25ºC with values close to 0.75 and 0.67 at 15ºC and 35ºC, respectively. At 60% DO, *b* values increased with temperature and fell around the 2/3rd and 3/4th rule at 25ºC and 35ºC, respectively. However, at 100% DO, all *b* values fell well below the commonly identified scaling values from literature (Table 2.6, Figure 2.6A). There was also higher variability in the *b* values under full saturation (Table 2.6). The *a* values were small but consistent with literature values for sedentary organisms. At 25ºC, *a* values tended to increase as dissolved oxygen decreased, supporting the idea that respiration rates increased with decreases in oxygen saturation (Table 2.7, Figure 2.6B). No obvious temperature-related trend was apparent in the scaling exponent; however, for the most part, the constant *a* apparently increased with increasing temperature. Indeed, statistical comparisons of elevations within DO levels supported the conclusion that respiration rates increased with temperatures.

Table 2.6

Scaling exponents (*b'* and *b*)

	20%		60%		100%	
	b'	b	b'	b	b'	b
15ºC	-0.294	0.706 ± 0.142	-0.844	0.156 ± 0.109	-1.227	-0.227 ± 0 .264
25ºC	-0.469	0.530 ± 0.161	-0.354	0.661 ± 0.129	-0.473	0.542 ± 0.181
35ºC	-0.349	0.651	-0.251	0.749 ± 0.260	-0.705	0.295 ± 0.303

Mass-specific scaling exponent b' and corresponding oxygen consumption scaling exponents b (b=b'+1), ± one standard error, obtained from the linear form of the mass-specific relationship (log V_{O2}= log a+ b logW) relating V_{O2} to body size for all 9 treatments. Note: b' and b values at 35ºC and 20% DO from only 2 individuals.

Table 2.7

Scaling constant *a* values

	20%	60%	100%
15ºC	0.00016 ± 0.3692	0.00249 ± 0.4590	0.00086 ± 0.1252
25ºC	0.00006 ± 0.3300	0.00005 ± 0.3717	0.00004 ± 0.2692
35ºC	0.00014	0.00018 ± 0.1567	0.000003 ± 0.1140

Scaling constant a ± one standard error calculated as the inverse log of the value obtained from the linear form of the mass-specific relationship (log V_{O2}= log a+ b logW) relating V_{O2} to body size for all 9 treatments. Note: a value at 35ºC and 20% DO from only 2 individuals.

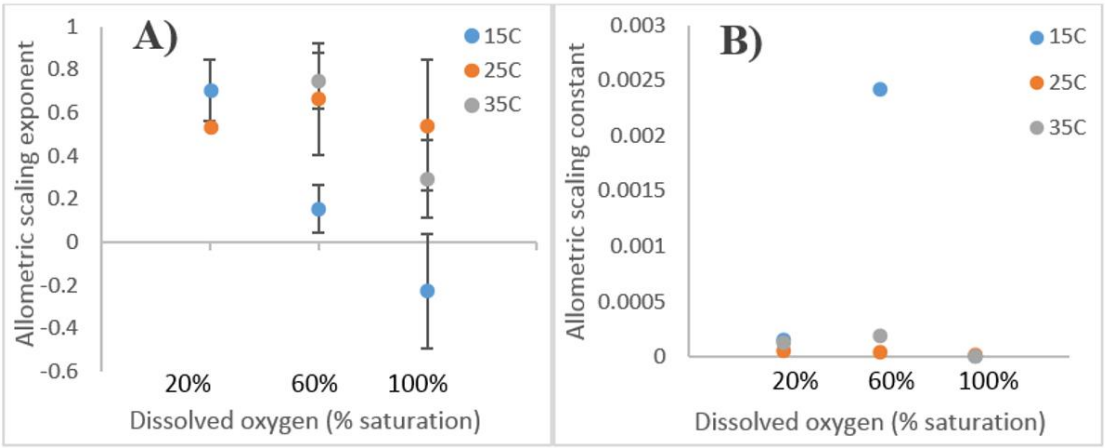

Figure 2.6 Allometric parameters (*b* and *a*)

A) Allometric scaling exponent b (b=b'+1) *and B) constant* a *(of* $V_{O2} = aW^b$*) for three temperatures crossed with three dissolved oxygen saturations.*

2.4 Discussion

2.4.1 The effect of dissolved oxygen and temperature on aerobic respiration rate

The effects of dissolved oxygen and temperature were found to have varying influences on respiration rate, which were interwoven and complicated. Herreid (1980) states that regulation of oxygen consumption is dependent on many factors, including species, physiological state of the individual, temperature, and salinity. While interaction effects of dissolved oxygen, temperature, and mass were present in this study, the effect of dissolved oxygen on the change in respiration rate was significant (Table 2.8). This main effect of dissolved oxygen on respiration rate supports the fact that available oxygen is a major influence on metabolic rates. The interaction with mass is expected due to the fact that, as previously stated, respiration varies allometrically with mass. Teasing out these interaction effects is challenging, as metabolic rates depend both on the mass of the organism and temperature in a nonlinear manner. However, by focusing on the effects of temperature differences within dissolved oxygen levels, insights into respiration patterns were obtained.

When comparing the three temperature treatments within each dissolved oxygen treatment, it was expected that respiration rates would increase with temperature due to the fact that temperature increases kinetic energy at the cellular level. This trend was supported by the slope comparisons determined by the univariate ANCOVA with either a higher slope or larger increase in elevation with higher temperatures seen across all temperatures at 20% DO, from 25ºC to 35ºC at 60% DO, and from 15ºC to 25ºC at 100%

DO (Table 2.8). Again, the exception of an increase in respiration rate at 35ºC may have been influenced by mortality, as the parameter estimates were only measured from survivors.

Under each dissolved oxygen level, there was a significant increase in the elevations from 15ºC to 25ºC (20 and 100% DO), and in some cases from 25ºC to 35ºC (60% DO), indicating that respiration rates were maintained at higher rates with higher temperatures (Table 2.8). This effect of temperature on metabolic rate is a well-known reaction as metabolic rates rise with increases in temperature. As temperatures increase, mitochondrial respiration is accelerated (Heise et al. 2006). Resting metabolic rate co-varies with environmental temperature because higher temperatures induce higher kinetic energy at the cellular level and this directly leads to increased metabolic rates (Clarke and Fraser 2004). Therefore, the cost of maintenance as reflected by resting metabolism increases at higher temperatures because more ATP is required as cellular processes speed up due to higher kinetic energy. The succinct explanation for increases in metabolic rate with temperature as stated by Clarke and Fraser (2004): "an organism living at a higher temperature has no option but to synthesize more ATP and consume more oxygen." This same trend has been observed for other benthic organisms. Oxygen uptake was observed to increase for *A. succinea* with a change in temperature from 25ºC to 30ºC (Sturdivant et al. 2015).

The lack of a significant increase in respiration rate at 35ºC in this study could have been due to small sample sizes as a result of high mortality occurring during acclimation. Conversely, mortality itself likely signified a critical thermal limit was reached for this organism. Because mortality occurred under all DO treatments at 35ºC,

even under full oxygenation, internal or functional hypoxia may have been responsible and lead to the increased mortality at the two lower dissolved oxygen treatments. Indeed, there was a significant decrease in the elevations between 25ºC and 35ºC at 100%, which supports the interpretation that there was a breakdown of the ability to maintain respiration rate referred to as metabolic depression. Shumway (1983) suggests that the effects of temperature on metabolic rate are greater within an organisms' natural temperature range to which they are adapted, than to more extreme temperatures at which the organisms can no longer compensate for the increased metabolic demands. The rate of turnover for enzymes is highly tuned to the temperature at which the organism is adapted and any change in that stability can have consequences on those rates (Clarke and Fraser 2004). Because normal metabolic processes are adapted to operate at a specific temperature, any change can impact the energetic costs associated with cellular processes that are required to survive (Clarke and Fraser 2004). This may have been observed at the highest treatment temperature in this study, as 35º C was a projection of the changing climate and not a temperature that this organism currently encounters.

Table 2.8 Summary of hypotheses, statistical tests, and results

Hypothesis	**Test**	**Results**
Increasing temperature will cause increased respiration rate.	Test of the heterogeneity of slopes- pairwise comparison of elevations	Respiration rates significantly increased from 15 to 25ºC (under 20% and 100% DO) and from 25 to 35ºC (under 60% DO).
Temperature will interact with low dissolved oxygen to vary respiration rates.	Two-way ANCOVA	Significant interaction effect of dissolved oxygen and temperature on respiration rate.

Smaller body sizes will oxyconform, larger will oxyregulate under low dissolved oxygen.	Test of the heterogeneity of slopes- pairwise comparison of slopes	Smaller sizes oxyregulated under all treatments and even increased respiration rate from 15 to 25ºC under 60% DO. Larger specimen maintained similar respiration rates across all treatments.
All body sizes will exhibit a stress response at a combination of high temperature and low dissolved oxygen.	Test of the heterogeneity of slopes	A stress response of decreased respiration and mortality was observed at 35ºC under all DO's. However, oxyregulation was maintained across all body sizes under all other treatments.

Changes in respiration rates with temperature varied with oxygen regimes in a way that suggests differences in oxyregulation capacity, because oxyconformation was noted at 35ºC; whereas, oxyregulation was maintained at all other temperatures. At 20% DO, the elevation of the respiration rates increased from 15º to 25ºC, but neither were significantly different from rates at 35ºC. The opposite trend was seen at 60% DO, where respiration rates decreased from 15º to 25ºC and appeared to increase again at 35ºC, although this increase was not statistically significant. The trend at 100% was similar to that seen at 20% DO, with the respiration rates increasing from 15º to 25ºC and then decreasing from 25ºC to 35ºC. These differences in direction of the change in respiration mainly occurred at 25ºC across the three oxygen regimes. It appears that temperature induced respiration rates to increase with a change from 15º to 25ºC only under the lowest and highest oxygen concentrations, while respiration rates actually decreased from 15º to 25ºC at 60% DO. This could indicate that *Streblospio gynobranchiata* was physiologically unchallenged at 25ºC under 60% DO, as a low respiration rate indicated

low energy expenditure. This might reflect that *S. gynobranchiata* is better adapted to lower than normoxic conditions, as this species is generally associated with organically polluted and hypoxic areas. Many organisms survive easily on oxygen saturations above 50%, therefore, it is not surprising that 60% dissolved oxygen would not induce much change in respiration rate (Llansó 1991). In a review on marine benthic hypoxia, respiration of benthic organisms did not increase until oxygen saturation fell to 50-60% in the field (Diaz and Rosenberg 1995). Moreover, this reversal in pattern at 60% DO is supported by the largest sample size for all treatments (N=42).

Respiration rate appeared to increase as oxygen concentration decreased. At 15ºC and 35ºC, mean V_{O2} rates were negatively correlated with DO levels. However, at 25ºC, mean respiration rate increased from 100% to 60% DO but then lowered slightly at 20% DO. This increase in respiration rate associated with a decrease in dissolved oxygen is consistent with hyper-regulation. Diaz and Rosenberg (1995) noted that the first response of the benthos to declining oxygen was to increase respiration rates. This was also seen in a study on *A. succinea*, in which the oxygen uptake rate increased under hypoxia (Sturdivant et al. 2015).

Respiration rates increase with lower oxygen concentrations until some critical oxygen level is reached (P_{crit}), at which point most organisms can no longer maintain the ability to oxyregulate, and become oxyconformers (Herreid 1980). Critical oxygen levels are also known to vary ecologically and phylogenetically. Most notably, P_{crit} levels increase with temperature. In the current study, cool and moderate temperatures did not induce oxyconformity or mortality as seen across all dissolved oxygen levels at 15 and 25ºC. This suggests that P_{crit} was not reached and therefore falls below 20% DO, which

was the lowest oxygen concentration tested. Conversely, oxyconformity and mortality were observed at 35ºC, suggesting that P_{crit} was much higher under temperature stress. This is typical, as P_{crit} is usually lower at low temperatures and increases with temperature (Rakocinski and Gillam 2017). A study on *Capitella teleta* followed the same general trend as *S. gynobranchiata*. At 15ºC and 20ºC, *C. teleta* maintained oxyregulation and did not reach its critical oxygen level, while at its highest temperature regime (25ºC) it began to oxyconform (Rakocinski and Gillam 2017). This switch from oxyregulation to oxyconformity seen in both *S. gynobranchiata* and *C. teleta* at high temperatures exemplifies the plasticity of their respiratory strategies. Similarly, many other tolerant benthic organisms have low critical oxygen concentration values. For example, *Alitta succinea* exhibited oxygen-independent respiration rates (i.e., oxyregulation) between 30-100% oxygen saturation; respiration increased below 30% DO, and then reached its critical saturation between 10-16% DO during experiments at 25º and 30ºC (Sturdivant et al. 2015). *Capitella sp. M*. is able to regulate oxygen consumption down to 10% oxygen concentration at 16ºC in the laboratory, and likewise, so is *Cirriformia tentaculata* (Gamenick et al. 1998, Bestwick et al. 1989). The capacity of *S. gynobranchiata* to tolerate low oxygen levels under low and moderate temperatures is certainly comparable to other tolerant species previously studied.

A multitude of adaptations allow aquatic organisms to oxyregulate in the face environmental variation. Adaptations can be influenced by acclimation. Acclimation of respiration rate to lowered oxygen concentrations can result in an enhanced capacity to oxyregulate, especially when faced with these stressors again (Bayne and Livingstone 1977). In *Mytulis edulis*, gradual increases in respiration rates with exposure time are

attributed to acclimation to the exposure. The inclusion of a 24 hour acclimation period in this study may have influenced the higher respiration rates seen at lower oxygen concentrations, as longer acclimation periods seemed to increase oxyregulatory abilities of *S. gynobranchiata*. Other adaptations could also have influenced the oxyregulatory abilities of the study species. Perhaps the lack of observed oxyconformity at 15ºC and 25ºC was due to the fact that *S. gynobranchiata* is a small organism that can more efficiently meet their metabolic demands than larger organisms due to their large surface area to volume. Hypoxia-tolerant polychaetes can also have other life history traits that make them tolerant such as specialized respiration organs and blood pigments (Diaz and Rosenberg 1995). As an organism with a small adult body size, *S. gynobranchiata* must maintain a higher overall metabolic rate in general but is also able to maintain or exceed that rate in the face of hypoxia at low and moderate temperatures. Considering that *Streblospio gynobranchiata* represents a narrow range of small body sizes, all sizes were able to regulate oxygen consumption under low and moderate temperatures, either by maintaining rates or hyper-regulating. Due to their relatively high surface areas, small organisms typically have no difficulty obtaining oxygen until very low oxygen partial pressures are reached (Herried 1980).

Life history traits of *S. gynobranchiata* such as its small adult body size, short life-span, and rapid reproduction rates have been attributed to its tolerance during and after disturbance. This species has been noted to rapidly recolonize areas after disturbance and to dominate areas subject to frequent hypoxia due to their opportunistic life history traits (See Chapter I, Llansó 1991, Ritter and Montagna 1999, Grassle and Grassle 1974). The tolerance of *S. gynobranchiata* is supported by the observation of this

species in many hypoxic areas, including by its dominance at three hypoxic stations in Texas (Ritter and Montagna 1999). Under experimental conditions, *S. gynobranchiata's* sister species, *S. benedicti*, tolerated hypoxia for 2 weeks with no significant mortality (Llansó 1991). From the present study, it appears that *S. gynobranchiata* exhibits the capacity for independent respiration up to relatively low critical oxygen levels at low and moderate temperatures, thus demonstrating considerable physiological plasticity. The idea that opportunistic species can oxyregulate is supported by a similar study on *A. succinea*, where it was noted that this species can maintain activity even under low oxygen conditions (Sturdivant et al. 2015). It was also noted that this species may help maintain benthic function during hypoxia and aid in the recovery of the benthic community from effects of environmental stressors. In all of these cases, successful oxyregulation under low oxygen concentrations suggests tolerant organisms maintain aerobiosis for as long as possible in order to delay switching to less efficient or more energetically costly methods of meeting metabolic demands (Paterson and Thorne 1995). This strategy is employed by organisms that are frequently exposed to low dissolved oxygen. Because of *S. gynobranchiata's* position in shallow, muddy areas that may frequently be exposed during tides, it may be subject to intermittent hypoxia.

In some instances, the hypoxia-temperature interaction is manifested as metabolic depression or oxyconformation (Pörtner et al. 2005). This has been observed primarily for organisms that must withstand low tides and extreme temperatures. These organisms may switch to anaerobic metabolism to save energy during low oxygen events. During this study, no signs of metabolic depression occurred until the highest temperature treatment. A review by Diaz and Rosenberg found that for many benthic organisms,

metabolic depression did not occur until around 14% oxygen saturation (Diaz and Rosenberg 1995), but as stated earlier, the P_{crit} thresholds decrease at higher temperatures and some oxyconformity was observed at the highest temperature. It is clear that *S. gynobranchiata* possesses physiological adaptations to sustain aerobiosis for some time in the face of oxygen limitation.

Elevated respiration rates with decreasing oxygen content are a known response strategy of benthic infauna (Wannamaker and Rice 2000). In this study, changes in respiration rates were more dynamic for smaller *S. gynobranchiata* than for larger worms across the DO treatments. This could reflect an ontogenetic trend in oxyregulation ability under low dissolved oxygen. Generally, mass-specific respiration of large organisms is less dependent on oxygen supply than smaller organisms (Shumway 1979). This same trend was seen for *Alitta succinea*; mass-specific oxygen consumption rates were higher for smaller organisms during hypoxia (Sturdivant et al. 2015). All sizes of *S. gynobranchiata* exhibited some degree of oxyregulation, at times hyper-regulation, implying some tolerance across the full body range. However, by oxyregulating more than large organisms, small specimen were incurring relatively greater costs, and thus may not be as able to meet demands at even lower dissolved oxygen.

2.4.2 Dissolved oxygen and temperature on metabolic scaling

Although *S. gynobranchiata* does not exhibit a wide range of body sizes, allometric changes in respiration rates were observed. For each treatment combination (except 35ºC at 20% DO for which mortality was too high), smaller worms exhibited significantly higher mass-specific respiration rates. This is expected, as smaller organisms respire relatively more per gram than larger organisms (Shumway 1979,

Glazier 2006). This is at least partly due to greater energetic demands as well as greater capacity to meet those demands afforded by a higher surface area to volume ratios for smaller organisms (Glazier 2006). Such metabolic scaling has also been linked to the high metabolic costs associated with greater growth rates during early development. Allometric scaling exponents are used to determine how certain traits change with body size. For example, the mass-specific allometric scaling exponent, *b*, (i.e., $b = b' + 1$; where b' is the mass-specific exponent) describes how respiration rates change with body size. Two common metabolic scaling theories purport *b* exponents of 0.67, the 2/3rd rule, or 0.75, the 3/4th power law, depending on mechanisms involving nutrient and oxygen transport systems (Glazier 2005, 2006, White et al. 2011). In this study, the scaling exponent *b* ranged fairly widely, from 0.156 to 0.749 (excluding an outlier at -0.227), depending on DO and temperature combinations. In a comprehensive study on metabolic rates of several polychaetes, *b* values ranged from 0.41 to 0.79 (Shumway 1979), and for a variety of invertebrates the scaling exponent varied from -1.20 to 2.01 (Glazier 2005). For the present study, *b* values for *S. gynobranchiata* for most of the treatment combinations varied somewhere between the common 2/3rd and 3/4th scaling rules. Following the 2/3rd rule (i.e., $b = 0.67$) implies that the metabolic rate is dependent on its surface to volume ratio (Glazier 2005). The 2/3rd rule is widely accepted because processes such as food absorption, respiratory gas exchange, and waste elimination are surface area dependent, thus limiting the ability for metabolism to scale isometrically with body size (Hulbert and Else 2000, Glazier 2005). This makes sense for small, soft bodied organisms such as polychaetes, which obtain much of their oxygen through their skin. Under hypoxia, all of the *b* values fell around the 2/3rd rule. The organisms were

mostly relying on surface area properties to obtain oxygen. Under 60% DO, *b* was extremely low at 15ºC. However, *b* was 0.66 at 25ºC, and 0.75 at 35ºC. At 100% DO, all *b* values fell below the 2/3rd rule. The allometric scaling values also exhibited higher variability at 100% DO, perhaps partially reflecting that under full oxygenation, individuals can undertake optional metabolic processes, for example related to reproduction, protein synthesis, etc. The wide range of *b* values could have been affected by variation in the small size range, consisting of both immature and mature specimens. In a study by Shumway (1979), it was noted that *b* values can be difficult to determine across narrow weight ranges, especially given that specific values can be affected by nutrition state, temperature, season, etc. Allometric scaling "rules" are controversial; *b* values usually range from 0.67 to 0.9 over the major animal groups (Agutter and Wheatley 2004). Patterson's (1992) review on metabolic rates in aquatic algae and invertebrates conversely found *b* values ranging from 0.3 to 1.2, in accordance with the delivery of nutrients by diffusion through surfaces of organisms. It seems that data from the present study roughly follows the metabolic scaling rules; however, the causes of variation are harder to determine.

In the relationship between metabolic rate and mass, described by the equation $Y=aX^b$, *a* can be used to compare oxygen consumption across organisms of similar sizes (Shumway 1979). The constant values were all extremely small, which compares favorably with small values found for other sedentary organisms (Shumway 1979). In the present study, *a* values often increased as dissolved oxygen decreased, confirming that respiration rate also increased with low dissolved oxygen. However, *a* also typically increased up to a point (i.e., at 35ºC), signifying an increase in respiration rates at higher

temperatures indicative of an increase in metabolic activity, followed by metabolic depression near its thermal limit. Such changes in metabolic scaling exponents as temperatures increase and dissolved oxygen decreases denote commensurate changes in the cost of maintenance, or living.

2.4.3 Potential adaptations to stressors

Aquatic organisms exhibit many possible physiological and behavioral adaptations for counteracting effects of low oxygen, including functional hypoxia due to temperature, increased surface area, ventilation, or circulation, reduced activity, or the use of efficient respiratory pigments, as well as anaerobic respiration. It is unclear exactly which adaptations *S. gynobranchiata* employs to deal with hypoxia, but surface area seems to play a large role in oxygen exchange for this small, elongated organism as is suggested by those allometric scaling *b* values around 0.67 (Clarke and Fraser 2004). This species also has a pair of external banded gills, which increases the surface area and efficiency of oxygen absorption. *Streblospio gynobranchiata* may spread these branchiae outside of their tube onto the sediment surface or into the water column to obtain more oxygen, similarly to many other polychaetes. For example *Cirriformia tentaculata* (Bestwick et al. 1989) uses this adaptation. This adaptation was also noted for *S. gynobranchiata's* sister species, *S. benedicti*, which was observed exhibiting this behavior during experimental exposure to 7% air saturation (Llansó 1991).

The oxyregulatory abilities of *S. gynobranchiata* observed in this study may be influenced by certain physiological adaptations. *Streblospio gynobranchiata* may possess efficient red blood cells with high oxygen binding affinities or alternative blood pigments. It has not been published which pigments this species possesses, but the

observation of red pigment through their palps and gills suggests the presence of hemoglobin (S. Rice, pers. comm.). However, some darker fluid which sometimes appears throughout the body may indicate the presence of more than one respiratory pigment (pers. obsv.). Polychaetes commonly possess hemoglobin, myoglobin, and chlorocruorin. *C. tentaculata* possess erythrocruorin which has a high affinity for oxygen and protects against excess loss of oxygen (Bestwick et al. 1989). Anaerobic pathways may also be induced by hypoxia, and the presence of enzymes indicative of anaerobic metabolism will be quantified for exposed specimens at a later time. It is believed that *S. gynobranchiata* uses the "exploitative" method of anaerobic metabolism, which produces alanine, succinate, and other volatile fatty acids; thus, providing more energy than pathways that form lactate for enduring longer periods of hypoxia (Hockachka and Somero 1973). These exploitative pathways also result in less accumulation of acidic end-products.

Besides physical and physiological changes, behavior may also change in response to reduced oxygen. In the present study, darkness during experiments precluded observation of the specimens' behavior. However, during acclimation specimens formed tubes within the available sediment and remained within them for the duration of the period. The only specimens observed on the sediment surface were dead individuals that had vacated their tubes during acclimation to the 35ºC temperature treatments. Ventilation may have been occurring within the tubes, but it was not observed or measured. Ventilation behaviors have been noted for many polychaetes, which may contract their bodies to pull oxygenated waters into their tubes (Kristensen 1983, Bestwick et al. 1989, Dales 1958, Leung et al. 2013). Once specimens were removed

from the sediment and prepared for oxygen consumption measurements, they were allowed a period to adjust after handling, and oxygen consumption measurements included only the last 10 minutes of the 40 minute recording period in order to preclude any changes in respiration rate due to excessive movement as the worms adjusted. This also allowed the oxygen probe to stabilize, although oxygen consumption measurements typically always exhibited a downward trend. Most of the worms appeared fairly inactive during respiration measurement, and mesh pouches were meant to approximate a protected environment, such as a tube. Some random head swinging was observed, which may have moved oxygenated water over their gills. General inactivity could also signify behavioral changes due to hypoxia, as many organisms enter quiescent states to conserve energy during low oxygen exposure (Sagasti et al. 2001). Indeed, it has been noted that *Streblospio benedicti* is mostly inactive during experimental exposure to hypoxia (Llansó 1991). Further observations during acclimation to hypoxia might elucidate behavioral mechanisms employed to facilitate aerobiosis and survival.

The habitat in which species occur may also mitigate the effects of oxygen stress. In a study on *Nereis spp*., there was a correlation between regulatory ability and habitat, where organisms inhabiting muddy sediment tended to regulate better than those in highly oxygenated sand (Kristensen 1983). *Streblospio gynobranchiata* occurs mainly inshore in marsh tidal creeks within highly organic, low oxygenated muddy sediments. Diaz and Rosenberg (1995) also noted that tolerance was boosted by high quantities of organic matter within the sediment. This may be due to the fact that organisms found in highly organic sediments can mitigate the metabolic-tradeoff in increasing respiration when under oxygen stress by being able to continuously obtain energy from this nearly

unlimited resource (Sturdivant et al. 2015, Childress and Seibel 1998). The oxyregulatory ability exhibited by *Streblospio gynobranchiata* across a wide range of oxygen saturation levels may have been influenced by this worm being adapted to muddy sediment under frequently low oxygen concentrations.

2.5 Summary

Mass-specific respiration rates changed with temperature as well as with dissolved oxygen for *Streblospio gynobranchiata*, a common and abundant opportunistic polychaete characteristic of nearshore estuarine habitats frequently subject to environmental hypoxia and high temperatures. Moreover, a critical temperature threshold was observed at 35ºC, marked by high mortality during acclimation and possibly, metabolic depression. Similar findings are widely reported throughout the literature, although there is considerable variation between and within many species. A body sized trend was observed, in that smaller organisms tended to drive the trend of increased respiration rates, while large specimens better regulated oxygen consumption. Observed increases in V_{O2} at higher temperatures and lower dissolved oxygen levels also comported with expected metabolic scaling values, as seen with the scaling exponent *b*. Many physiological and behavioral traits may account for *S. gynobranchiata's* ability to maintain aerobiosis during oxygen stress, including physiological adjustments to surface area or blood pigment efficiency and behavioral changes such as ventilation or inactivity.

In relation to dissolved oxygen and temperature, it appeared that dissolved oxygen had a major influence over the metabolic rate of *S. gynobranchiata*, as found through the highly significant effect noted in the two-way ANCOVA. However, when comparing the effect of temperature on respiration rate within the partitioned DO levels, a significant

effect was also noted. This further exemplifies the fact that stressors should be considered together and specific responses may be due to the combination of such stressors. Overall, *S. gynobranchiata* tend to maintain oxyregulatory abilities under low oxygen conditions, except when near their thermal limit at 35º C, exemplifying their opportunistic life history traits and general tolerance.

CHAPTER III –AEROBIC RESPIRATION OF *STREBLOSPIO GYNOBRANCHIATA* DURING ACCLIMATION TO HYPOXIA

3.1 Introduction

The duration of exposure to hypoxia has significant effects on the level of impact on benthic communities and may entail different physiological adaptive responses within and among organisms (Willmer et al. 2009). The variable responses of aquatic organisms to oxygen stress are a direct result of the severity and duration of exposure to low oxygen (Diaz et al. 1992). Diaz et al. (1992) stated that because of the "complexity and rapidity" of changes in environmental oxygen concentrations, it is necessary to further test the effects of hypoxia on living resources and their responses through field and laboratory tests. The first response to hypoxia typically includes maintaining oxygen delivery, whether that entails an increase in respiration rate, change in oxygen binding affinity, or other adaptations (Wu 2002). The second response usually includes the conservation of energy by metabolic depression or down regulation of other metabolic processes. Prolonged hypoxia will eventually induce anaerobic metabolism. Any of these responses can lead to reductions in growth, feeding, reproduction, and overall fitness (Wu 2002). The severity and duration of exposure to reduced dissolved oxygen will directly affect the probability of mortality, especially in organisms that cannot employ avoidance behaviors.

Both physiological and biochemical responses are different during short-term hypoxia than during prolonged exposure (Wu 2002). In nature, hypoxia can occur during short periods, diurnally or tidally; but can also occur over a long durations, eliciting different adaptations (Burnett and Stickle 2001). Within the first 12 hours of exposure to hypoxia, many organismal responses could occur as a result of acclimation to the changing

conditions, possibly including complete shutdown of respiratory capabilities, or the switch to anaerobic respiration. These early responses can have cascading effects on later costs associated with extended hypoxia exposure. Organisms found in tidal habitats, which must endure periods of low to no oxygen during low tide, may employ different adaptations to maintain oxyregulation (Wells et al. 1980). For example, polychaetes adapted to tidally influenced habitats regulate oxygen consumption by ventilating their burrows, increasing circulation of internal fluids, and increasing the concentration of hemoglobin in the blood (Wells et al. 1980). These organisms also have gills and blood vessels designed for large surface area to increase transport and diffusion of oxygen into the body from the surrounding environment. All of these adaptations were noted in the terebellid worm, *Terebella haplochaeta*, after only 2 hours of experimental low tide. Also, *T. haplochaeta* became an oxyconformer below 35% oxygen saturation at 20ºC, indicating that organisms well adapted to low oxygen environments can be negatively affected over a short period of exposure. Similar adaptations may occur in organisms that face other low oxygen situations that do not involve daily exposure to low oxygen. How many other types of organisms respond to short or long term hypoxia is not well known.

Many experimental studies on the effects of hypoxia exposure duration have focused on fishes. Metabolic depression occurred in rainbow trout after 24 hours of exposure to a P_{O2} of 80 torr (~19% DO), and fish entered anaerobic metabolism after further reduction in P_{O2} (Boutilier et al. 1988). Over a 90 minute period of exposure to hypoxia, sturgeon employed a series of adaptive responses (Burggren and Randall 1978). V_{O2} decreased within the first 10 minutes, but then stabilized and returned to normoxic levels after 30 to 45 minutes of exposure. This shows that there can be a wide range of responses occurring

minutes to hours after hypoxia exposure, in addition to other longer-term effects that occur after several hours and days of exposure.

The overall ecological effect of hypoxia also depends on the duration of exposure events. Prolonged hypoxia exposure generally results in mass mortalities in the benthos (Wu 2002). Consequently, major shifts in species composition may occur in relation to hypoxia duration. Changes in benthic fauna can also have cascading trophic effects on fish populations (Rabalias 2002, Wu 2002). Hypoxia tends to favor small benthic organisms with short life cycles, and many polychaete species are extremely tolerant to oxygen levels below 1 mg O_2 $l.^{-1}$ (Diaz and Rosenberg 1995). Therefore, it is important to understand the tolerance of abundant polychaetes in areas commonly affected by hypoxia to determine what level of damage will occur. Types of organisms that are able to persist during periods of hypoxia can help maintain the functional integrity of the benthic environment, promote the re-establishment of sensitive species, and provide prey for local fishes, thereby mitigating the impacts of hypoxia (Sturdivant 2015). Information on the individual metabolic responses of tolerant benthic organisms over a 12 hour period of exposure to hypoxia (20% oxygen saturation) under normal temperature (i.e., 25ºC) should provide insights into the capacity of tolerant organisms to acclimate to these stressful conditions. It was hypothesized that mass-specific respiration rates would vary with exposure time under hypoxia. Also, respiration rates may vary from immediate exposure to later exposure due to acclimation responses.

3.2 Materials and methods

3.2.1 Collection and culture

Streblospio gynobranchiata were collected, maintained and cultured in the laboratory following methods detailed in Chapter II.

3.2.2 Experimental setup

Oxygen consumption rates were measured for individual polychaetes after 3, 6, 9, and 12 hours of exposure to hypoxia (20% dissolved oxygen), along with a corresponding baseline oxygen consumption measurement at 0 hours under normoxia (100% DO). Individuals from cultures were placed in glass petri dishes with sediment inside the incubator (Precision© Low Temperature Incubator Model 815) for acclimation to 25ºC at 100% dissolved oxygen 24 hours prior to exposure to hypoxia. Each individual was labelled in order to consistently measure them on the same oxygen meter channel for comparison of respiration rates over time. After the 24-hour acclimation at 100% DO, individuals were transferred into respirometry syringes and a baseline respiration measurement was obtained along with a corresponding background measurement. Respiration under normoxia was measured for an hour using the FireStingO2 meter, as described in Chapter II. Immediately following this baseline measurement, individuals were placed into petri dishes and exposed to hypoxic water. The dishes were then transferred to the Biospherix© sealed air chamber set at 20% dissolved oxygen saturation for 3 hours. At the 3-hour mark, the respiration rate was measured for each individual for an hour. After each successive 3-hour measurement, polychaetes were returned to their petri dishes within the sealed air chamber and exposed to hypoxia for 3 more times for 3 hours, followed by respiration measurements for an hour (i.e., at 3, 6, 9,and 12 hrs). After

oxygen consumption was recorded at the 12-hour mark, each individual was weighed. A corresponding background measurement was made an hour before each 3-hour period of exposure to account for any bacterial respiration within the treatment water.

3.2.3 Analysis

The decline in oxygen during each repeated measurement period, recorded using Oxygen Logger software™ and stored in a Microsoft Excel spreadsheet, was used to quantify oxygen uptake as the slope of the regression of oxygen concentration versus time (Vismann and Hagermann 1996; Linke-Gamenick et al. 2000; Christensen et al. 2011). The slope for each individual V_{O2} (mg O_2 mg^{-1} h^{-1}) estimate was defined as: d(DO)/dt= (Decline O_2-Drift) . Calculation of the mass-specific respiration rate, V_{O2} (mg O_2 g^{-1} h^{-1}), was determined as:

$$\mathbf{V_{O2} = (d(DO)/dt)*(Vr-Va)/m}$$

V_{O2} = instantaneous mass-specific oxygen consumption rate (mg O_2 $mg^{-1}h^{-1}$);

d(DO)/dt = rate of decrease of DO (mg O_2 L^{-1} h^{-1});

Vr = respirometer volume (L);

Va = volume of experimental animal (L); and

m = animal mass (g).

Using a linear mixed model (LMM) approach in SPSS (version 18), a repeated measures ANCOVA was performed using time as the repeated factor and heterogeneous first order autoregressive covariance structure (ARH1) to determine differences in respiration rates over 12 hours of exposure to hypoxia. Individuals served as subjects and mass served as a covariate. The time factor encompassed the four 3-hour periods as well as the initial baseline measurement as a reference. In addition, time, mass, and the time

by mass interaction served as fixed factors in the model, and an intercept was included. Several contrasts tested *a priori* hypotheses relative to differences across time: (1) the reference normoxia reading (0 hr) versus the mean for the first hypoxic period (3 hr); (2) the reference versus the mean for the latter three hypoxic periods (6, 9, and 12 hr); (3) the first hypoxic period versus the mean for the latter three hypoxic periods; and (4) the mean of the second (6 hr) and third (9 hr) hypoxic periods versus the fourth (12 hr) hypoxic period.

3.3 Results

Respiration rates appeared to vary greatly among the twenty subjects during 12 hours of hypoxia exposure (Figure 3.1). The body sizes of subjects ranged 5-fold, from 0.42 to 2.22 mg. The autoregressive heterogeneous variance structure fit the model well, as shown by significant Wald Z values for every time period (all $Z > 2.9$; $P < 0.005$), as well as a significant Wald Z value for the ARH1 rho parameter ($Z = 2.072$; $P = 0.038$). Thus, time points that were closer tended to be more similar, and variance was heterogeneous across time points. This reflects the dependence in sequential respiration measurements from individual worms.

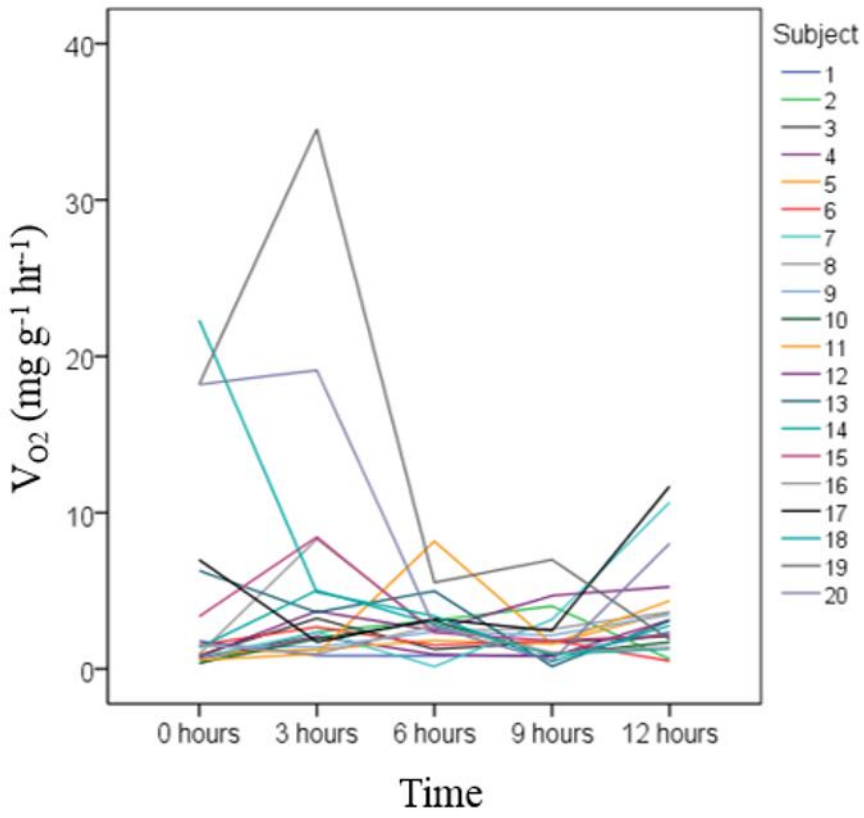

Figure 3.1 Mass-specific respiration rates during hypoxia acclimation

Individual mass-specific respiration rates for all 20 test organisms over 12 hours of exposure to hypoxia, including the reference normoxia measurement at 0 hours.

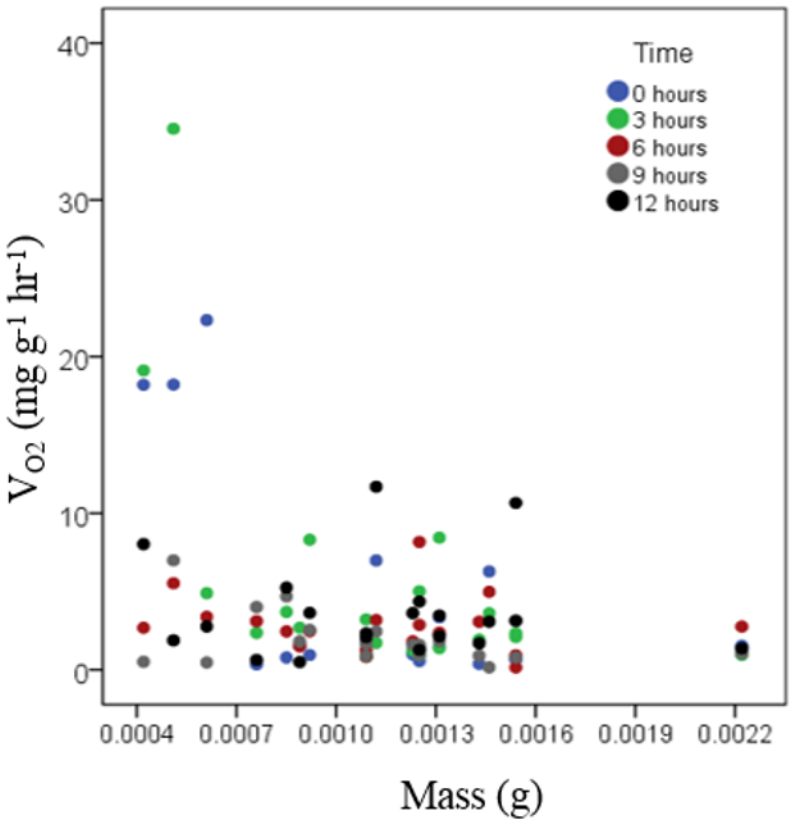

Figure 3.2 Relationship of V_{O2} and mass during acclimation to hypoxia

Individual mass-specific respiration rates across the range of body sizes during exposure to 12 hours of hypoxia, including reference normoxia readings at 0 hours.

The repeated measures ANCOVA showed that time (F= 4.443; P = 0.005), mass (F= 14.678; P = 0.001), and the time by mass interaction (F=3.588; P = 0.014) all had significant effects on the respiration rate. Mass-specific respiration rates were elevated at 0 and 3 hours, which were all represented by small worms, less than 0.00075 g (Figures 3.1 and 3.2). However, after 6 hours of exposure to hypoxia, mass-specific respiration rates of small individuals had fallen to similar levels as larger individuals (Figure 3.3). It appears that body size mediated differences were muted from 6 to 12 hours of hypoxia

exposure. Furthermore, there was an observed pattern of decreasing variance in respiration across the time periods and body size (Figures 3.2 and 3.4).

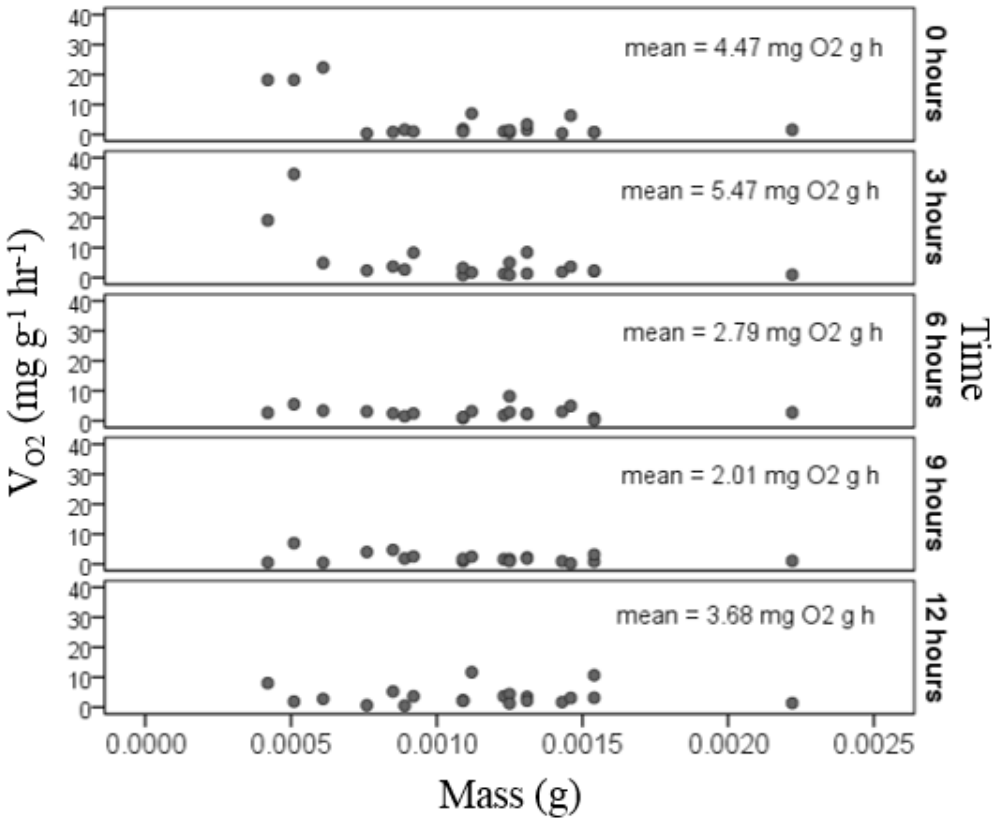

Figure 3.3 V_{O2} for the twenty test subjects during each time period

Individual mass-specific respiration rate of 20 test organisms divided into the 5 measured time increments with mean respiration rate at each level.

Based on the contrasts used to test the custom hypotheses, there was no difference in mean respiration rates between the reference normoxic (0 hrs) and initial hypoxic period (i.e., at 3 hrs) (t = -0.46; P= 0.649; Table 3.1, Figure 3.4). However, the mean respiration rate for the reference was significantly higher than the aggregate mean of the latter three exposure periods, at 6, 9, and 12 hours (t = 3.173; P = 0.004; Table 3.1), indicating a sustained decrease in respiration rate during the latter three periods compared

to the initial normoxic rate. This suggests that a period of metabolic depression occurred as compared to metabolic rates under normoxia. Mean respiration between the first hypoxic period (3 hrs) and the mean for the aggregate latter three hypoxic periods also differed significantly (t = 3.225; P = 0.004; Table 3.1), again reflecting a sustained decrease during the latter three periods compared to the measurement following only three hours of exposure to hypoxia. This suggests there was early oxyregulation at 3 hours. Finally, mean respiration rates for the 2nd and 3rd hypoxic periods (6 and 9 hrs) did not differ from that for last hypoxic period (t = -0.215; P = 0.832; Table 3.1), showing there was no effective difference in respiration rates after 3 hours of exposure to hypoxia, suggesting respiration rate remained depressed throughout the last 6 hours (from 6 to 12 hrs) of the exposure experiment (Figure 3.4).

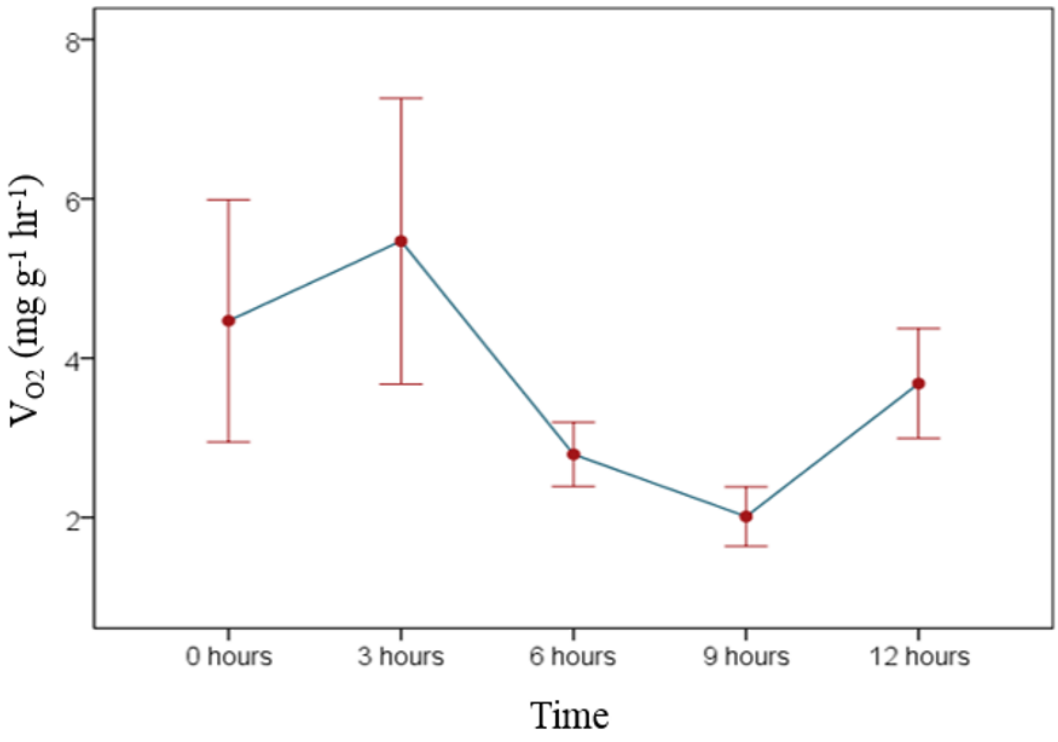

Figure 3.4 Mean V_{O2} during acclimation to hypoxia

Mean mass-specific respiration rates ± one standard error over 12 hours of exposure to hypoxia, including the reference normoxia reading at 0 hours.

Table 3.1

Differences in respiration rates during acclimation to hypoxia

Contrasts tested	**Sig. value**
0 v. 3 hr	0.649
0 v. 6, 9, 12 hr	0.004
3 v. 6, 9, 12 hr	0.004
6, 9 v. 12 hr	0.832

Significance values of the contrasts used to test a priori *hypotheses to find differences in respiration rate over the acclimation time.*

3.4 Discussion

Over the 12 hour period of exposure to hypoxia, two main aerobic responses were noted. Initially, the respiration rate did not differ from rates under normoxia within the first 3 hours of exposure to hypoxia, although the mean respiration was notably elevated. This will be referred to as Phase I. Respiration rate then decreased for the remainder of the hypoxia exposure period and will be called Phase II, as illustrated by significantly lower mean respiration rates over the latter three time periods. In Phase I, the lack of an initial change in respiration from the reference reading to the first exposure (3 hr) to hypoxia may reflect the maintenance of oxyregulation, or there could have been a delayed response before specimens recognized the change in oxygen concentration from 100% to 20% saturation. This suggests that there may be some lag in the implementation of the proper "machinery" used to combat the reduction in oxygen concentration. Similarly, *Arenicola marina*, a polychaete species adapted to low oxygen environments, showed no change in oxygen consumption after 2 hours of anaerobic conditions (Dales

1958). The subsequent decrease in *S. gynobranchiata*'s respiration rates seen in Phase II from 6 hours of hypoxia exposure and for the remainder of the experiment presumably reflects a reduction in energetic costs, and could signify a form of metabolic depression. Such down regulation in metabolism is known to be induced as an initial response to hypoxia in order to mitigate differences in oxygen supply and ATP demand (Ali et al. 2012). On the cellular level, it has been found that a common first response to hypoxia is the release of reactive oxygen species by the mitochondria to induce a suite of factors to combat low oxygen. This may even result in the onset of anaerobic metabolism (Ali et al. 2012). Oxyconformity to decreasing dissolved oxygen during the early onset of hypoxia may also be an immediate stress response due to the shock of the quickly changing oxygen regime. The present experiment was performed at a non-stressful temperature level, 25ºC, therefore this response could be expected for this species during the early stages of the onset of hypoxia in nature.

In Chapter II, a general trend of increased respiration rates at low dissolved oxygen levels after 24 hours of acclimation to hypoxia was noted. In contrast, for this exposure experiment, respiration rates were reduced during the latter six hours of exposure, which seems contradictory. However, when comparing the reference values in this experiment (24 hrs of acclimation at 25ºC and 100% DO) to the data collected under the same conditions in Chapter II, it was noted that the V_{O2} rates agreed across the two experiments. Since there was agreement across the reference rates of the two experiments, it can be postulated that the increase in respiration seen under hypoxia at 24 hours could be extended to the understanding of changes in respiration rate over time and can be considered Phase III in *S. gynobranchiata's* hypoxia response. Therefore, the

lower respiration rate in Phase II seen from 6 to 12 hours of exposure to hypoxia is likely a product of the shorter acclimation time. This might reflect effects of early stress as the organism acclimates to the new conditions; whereas, by 24 hours (Phase III) specimens are fully acclimated and thus able to increase oxygen consumption as a result of regaining their oxyregulatory capabilities. Lower respiration during Phase II (6-12 hrs of hypoxia exposure) also may indicate some delay in feedback systems which switch on to combat exposure to low oxygen. By the time Phase III (24 hrs) is reached, biochemical pathways may be fully induced to mitigate decreased oxygen availability allowing a return of oxyregulation, as opposed to unresponsive pathways as early as 12 hours after initial exposure, which induced some form of metabolic depression.

In a similar study on the mussel, *Mytilus edulis*, there was a positive relationship between mass-specific oxygen consumption and exposure time, as was seen here from Phase II to Phase III (12 to 24 hrs of acclimation to hypoxia); higher V_{O2} was observed after long term exposure consisting of 1 to 5 days (Bayne and Livingstone 1977). This suggests that mussels exhibited the capacity to sustain oxyregulation after acclimating to low dissolved oxygen. Acclimation to hypoxia actually enhanced the ability of this mussel to regulate oxygen consumption when challenged with further declines in oxygen tension. The authors also saw the accumulation of metabolites suggesting the use of an anaerobic pathway during acclimation to reduced oxygen concentrations. This further suggests this anaerobic pathway was used as a response to maintain energy gain while V_{O2} was depressed earlier. The authors maintained that exposure to low oxygen tensions resulted in the use of auxiliary adaptations that can increase oxygen utilization efficiency, such as increased heart rhythm and ventilation rate as well as the oxidation of anaerobic

substrates and end products. This was determined by the accumulation and subsequent decline of substrates found in anaerobic pathways during acclimation. In another study, the polychaete, *Hydriodes elegans*, showed a similar trend of an immediate decrease in respiration rate, followed by a slow increase in respiration rate with increasing exposure time when exposed to low oxygen at 2 or 4 mg O_2 l^{-1} for 4 days (Leung et al. 2013). Therefore, when incorporating responses seen in Chapter II and III, a general exposure response involving an initial short acclimation period with no change in respiration rate (Phase I) followed by a decrease in respiration rate in the near term (Phase II) and an eventual increase over extended periods of exposure to low oxygen (Phase III) is supported in the literature.

It is also important to note that an ontogenetic difference in the response was apparent. Small specimens had the highest respiration rates under normoxia, as well as initially, at 3 hours of hypoxia exposure. However, after 6 hours of exposure to hypoxia, mass-specific respiration rates of small individuals had fallen to similar levels shown by larger individuals. Although the small organisms typically maintain higher mass-specific metabolic rates than larger individuals under normoxia, hypoxia apparently induced the depression of mass-specific respiration rates differentially with respect to body size.

The lowered aerobic respiratory rate exhibited by *S. gynobranchiata* in response to early acclimation of hypoxia could have significant ecological impacts through the implied variability of the metabolic rates of these organisms. In some cases, many organisms enter a period of metabolic depression in the face of environmental stress and no longer meet their energetic demand, resulting in mortality. However, for some organisms, entering a state of oxyconformity or metabolic depression is response

allowing for mechanisms such as anaerobic metabolism to take place and mitigate energetic losses to allow the resumption of oxyregulation during extended periods of hypoxia. Consequences on benthic communities may therefore, be less impactful if some tolerant species are left behind to continue vital community services (Sturdivant et al. 2015). This change in community structure in the short term may also allow for the quick recovery of hypoxia impacted areas as it aids the successional transition of benthic organisms upon the return of oxygenated conditions (Rabalais et al. 2002).

3.5 Summary

When exposed to hypoxia for 12 hours, *S. gynobranchiata* exhibited 2 general phases. Phase I: an early acclimation response to the new oxygen regime signified initially by no change in respiration rate. Phase II: rates subsequently decreased and remained significantly lower for the remainder of the duration of exposure to hypoxia, likely representing an initial adaptive stress response to the changed conditions, in order to conserve energy. Tying these results into the results from Chapter II, a third phase can be inferred in which *S. gynobranchiata* is able to regain oxyregulatory abilities through an acclimation response involving biochemical, physiological, or behavioral adjustments at some point between 12 and 24 hours of exposure (Phase II and III). The period of metabolic depression observed from 6 to 12 hours of hypoxia exposure may be a vital period in which the organism is able to reduce energetic costs and store energy to withstand longer periods of hypoxia stress. The ability for *S. gynobranchiata* to resume maintenance of oxygen consumption regulation exemplifies its respiratory plasticity and tolerance to drastically changing environmental conditions.

CHAPTER IV – RECOVERY OF AEROBIC RESPIRATION BY *STREBLOSPIO GYNOBRANCHIATA* AFTER EXPOSURE TO HYPOXIA

4.1 Introduction

Recovery of respiration after a hypoxic event refers to the return of an organism to pre-anoxic metabolic conditions (Ellington 1983). As thus far discussed, hypoxia places extra demands on an organism's ability to obtain energy and maintain normal metabolic functioning, but this ability varies greatly among and within species. Therefore, the ability to return to normal metabolic conditions, i.e. metabolic activity characteristic of an animal under no environmental stress, after such an exposure may also widely vary inter- and intra-specifically. There are several commonly recognized categories of aerobic responses during recovery from hypoxia. Some aquatic invertebrates show depressed muscle oxygen consumption, while others show no change in respiration rate, and others show low to supernormal levels of oxygen consumption when recovering from exposure to hypoxia (Figure 4.1, Herreid 1980).

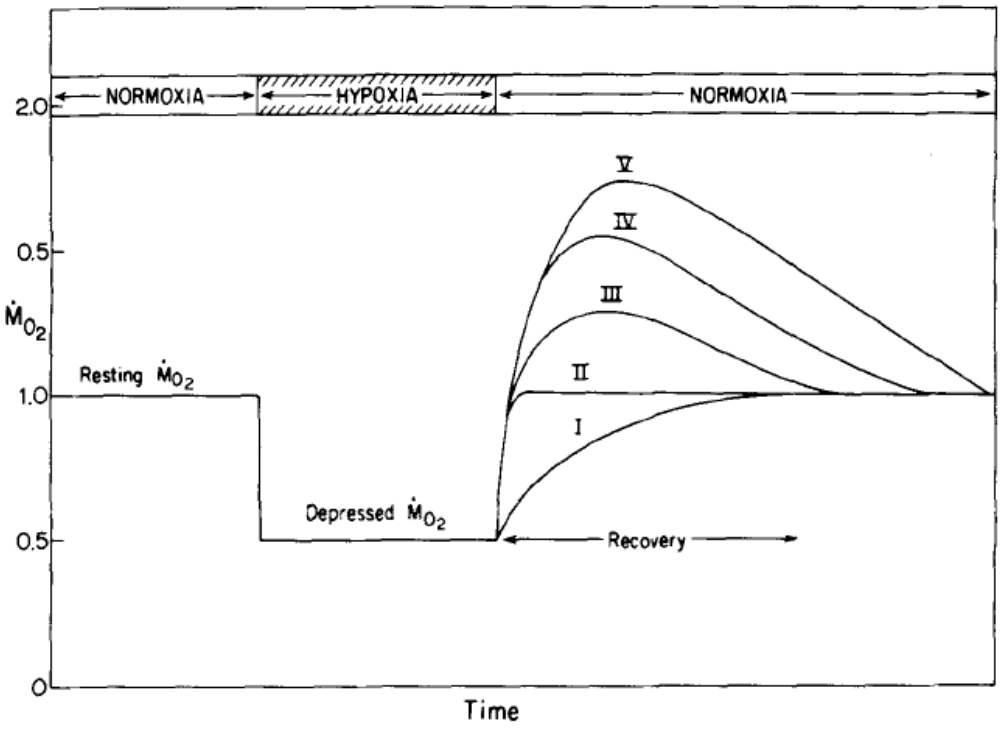

Figure 4.1 Common responses of oxygen consumption after hypoxia

Five common responses of oxygen consumption after a period of hypoxia. Curve I, negative oxygen debt. Curve II, no O2 debt. Curve III, subnormal O2 debt. Curve IV, normal O2 debt. Curve V, supernormal O2 debt. From Herreid, C. F. (1980). Hypoxia in invertebrates. *Comparative Biochemistry and Physiology Part A: Physiology*, *67*(3), 311-320.

This period of respiratory overshoot is known as an 'oxygen debt', and is defined by the organism entering a period of supernormal oxygen consumption following the restoration of oxygenated conditions (Bridges and Brand 1980, Ellington 1983). Oxygen debt may be an important part of metabolic recovery following anaerobic metabolism in many invertebrates (Ellington 1983). Supernormal oxygen consumption has been reported in a wide variety of invertebrates and is linked with an organism's need to increase energy for anaerobic end product disposal and resaturation of the body tissues (Bridges and Brand 1980).

Levels of oxygen consumption in many crustaceans, including marsh crabs, spiny lobster, ghost shrimp, and marine crayfish, show marked spikes after restoration to normoxia (Teal and Carey 1967, Nimura and Inoue 1969, Thompson and Pritchard 1969, McMahon and Wilkens 1972, Taylor et al. 1977, Bridges and Brand 1980). In addition, Bayne and Livingstone (1977) found *Mytilus edulis* L. exhibits higher oxygen consumption than normal after hypoxia. The isopod, *Saduria entomon*, showed an 8 hr period of recovery marked by a peak in oxygen consumption of approximately 0.57 mg O_2 g^{-1}; whereas its V_{O2} under normoxia was typically 0.109 mg O_2 g^{-1} (Vismann and Hagerman 1996). In the same study, these authors also found that the process of lactate oxidation accounted for almost half of the oxygen debt.

In most cases, the degree of oxygen consumption overshoot is dependent on the length and severity of hypoxia (Bridges and Brand 1980). The expression of oxygen debt may provide insights into biochemical and physiological requirements in response to hypoxic conditions (Bayne and Livingstone 1977). Although the relationship between oxygen debt and end product accumulation is undefined, quantifying any change in respiratory rate can provide insights into what recovery adaptations are available to an organism exposed to hypoxic conditions (Ellington 1983). Here, I will determine changes in mass-specific respiration rates for 12 hours following 24 hours of exposure to hypoxia (20% DO) at 25ºC. It is hypothesized that upon return to normoxia, *S. gynobranchiata* may enter a period of oxygen debt before returning to normal respiratory rates.

4.2 Materials and methods

4.2.1 Collection and culture

S. gynobranchiata were collected and kept in the laboratory in the same manner as described in Chapter II.

4.2.2 Experimental setup

A baseline respiration measurement was collected for twenty individual under normoxia initially after acclimation to 100% DO saturation for 24 hours within the incubator at 25ºC. Baselines were determined by measuring oxygen consumption for each individual in the same manner as previously described. Specimens were kept in 5 cm glass petri dishes with sediment and ASW. Containers were labeled in order to repeatedly measure individuals on the same oxygen meter channel for comparing respiration rates over time. After baseline measurements, individuals were acclimated to 24 hours of hypoxia (20% DO saturation) within a BioSpherix© chamber in the incubator at 25ºC, again in petri dishes with sediment and treatment ASW. Following acclimation, respiration rate under hypoxia (20% DO) was measured before placing specimens back into their labelled petri dishes filled with normoxic ASW and replaced in the incubator at 25ºC (following Bayne and Livingstone 1977, Shumway 1981, Hervant at al. 1995). During recovery, the oxygen consumption rate of individuals was measured for an hour every 2 hours for 12 hours, starting from the time of water replacement. Each respiration measurement was preceded with a corresponding background measurement to account for any bacterial respiration or drift. Fairly frequent respiration measurements during the recovery period were possible because there was little risk of changing the treatment DO at 100% DO saturation.

4.2.3 Analysis

The decline in oxygen during each repeated measurement period was recorded using Oxygen Logger software™ and stored in a Microsoft Excel spreadsheet, and used to quantify oxygen uptake as the slope of the regression of oxygen concentration versus time (Vismann and Hagermann 1996; Linke-Gamenick et al. 2000; Christensen et al. 2011). The slope for each individual V_{O2} (mg O_2 g^{-1} h^{-1}) estimate was defined as: d(DO)/dt= (DeclineO_2-Drift) . Calculation of the mass-specific respiration rate, V_{O2} (mg O_2 g^{-1} h^{-1}), was be determined as:

$$\mathbf{VO_2 = (d(DO)/dt)*(Vr-Va)/m}$$

V_{O2} = instantaneous oxygen consumption rate (mg O_2 g^{-1} h^{-1});

d(DO)/dt = rate of decrease of DO (mg O_2 L^{-1} h^{-1});

Vr = respirometer volume (L);

Va = volume of experimental animal (L); and

m = animal mass (g).

Using a linear mixed model (LMM) approach in SPSS (version 18), a repeated measures ANCOVA was performed using time as the repeated factor and a heterogeneous first order autoregressive covariance structure (ARH1) to determine differences in respiration rate over the 12 hour recovery period from hypoxia. Individuals served as subjects and mass served as a covariate. The time factor encompassed the six 2-hour periods, as well as normoxia (-24 hr) and hypoxia (0 hr) baseline measurements as a reference. In addition, time, mass, and the time by mass interaction served as fixed factors in the model, and an intercept was included. Eight contrasts tested *a priori* hypotheses relative to differences in respiration across time: (1) the hypoxia reference

point (0 hr) versus the mean for the normoxic (-24 hr) reference point; (2) the normoxic reference point versus the aggregate mean for the six recovery periods (2-12 hr); (3) the normoxic reference point versus the aggregate mean for the earliest two recovery periods (2 and 4hr); (4) the normoxic reference point versus the aggregate mean for the middle two recovery periods (6 and 8 hr); (5) the normoxic reference point versus the aggregate mean for the last two recovery periods (10 and 12 hr); (6) the aggregate mean for the earliest two recovery periods versus the aggregate mean for the middle two recovery periods; (7) the aggregate mean for the middle two recovery periods versus the aggregate mean for the last two recovery periods; and (8) the aggregate mean for the earliest two recovery periods versus the aggregate mean for the last two recovery periods.

4.3 Results

A longitudinal chart (Figure 4.2) illustrates time course respiration measurements for the twenty subjects while recovering from 24 hours of hypoxia. The body sizes of subjects ranged roughly 6-fold, from 0.31 g to 2.01 mg. The autoregressive heterogeneous variance structure fit the model well, as shown by significant Wald Z values for every time period (all $Z > 2.9$; $P < 0.008$), as well as a significant Wald Z value for the ARH1 rho parameter ($Z = 2.502$; $P = 0.012$). Thus, time points that were closer tended to be more similar and variance was heterogeneous across time points.

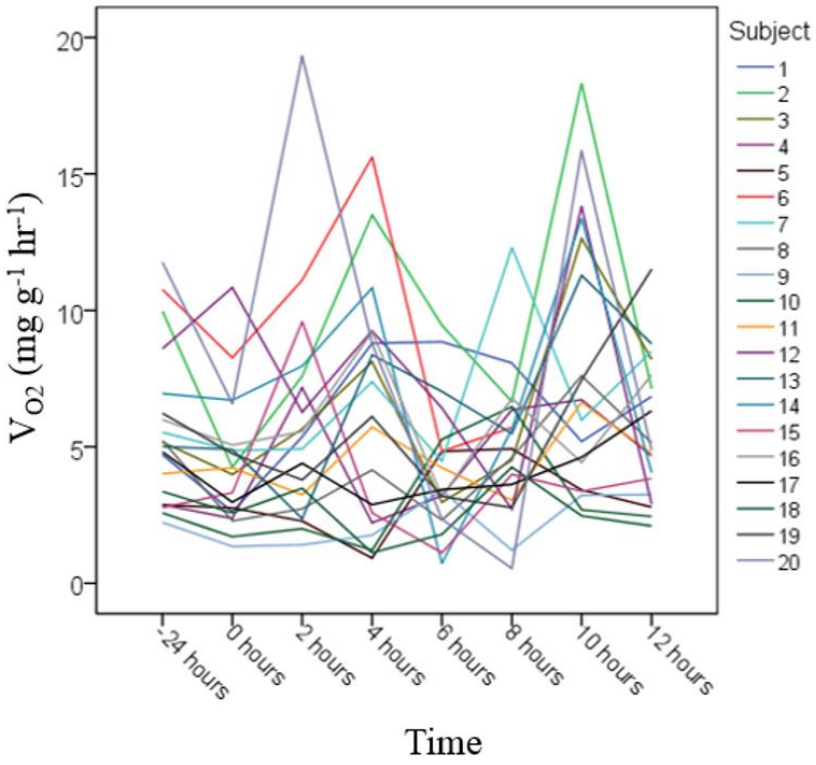

Figure 4.2 V_{O2} before, during, and after hypoxia exposure

Longitudinal chart showing variation in mass-specific respiration rates for 20 test organisms during 12 hours of recovery from hypoxia exposure. Respiration at -24 hours illustrates the reference normoxia reading for each specimen, and respiration at 0 hours represents the measurement after acclimation to hypoxia. All subsequent time periods are during recovery.

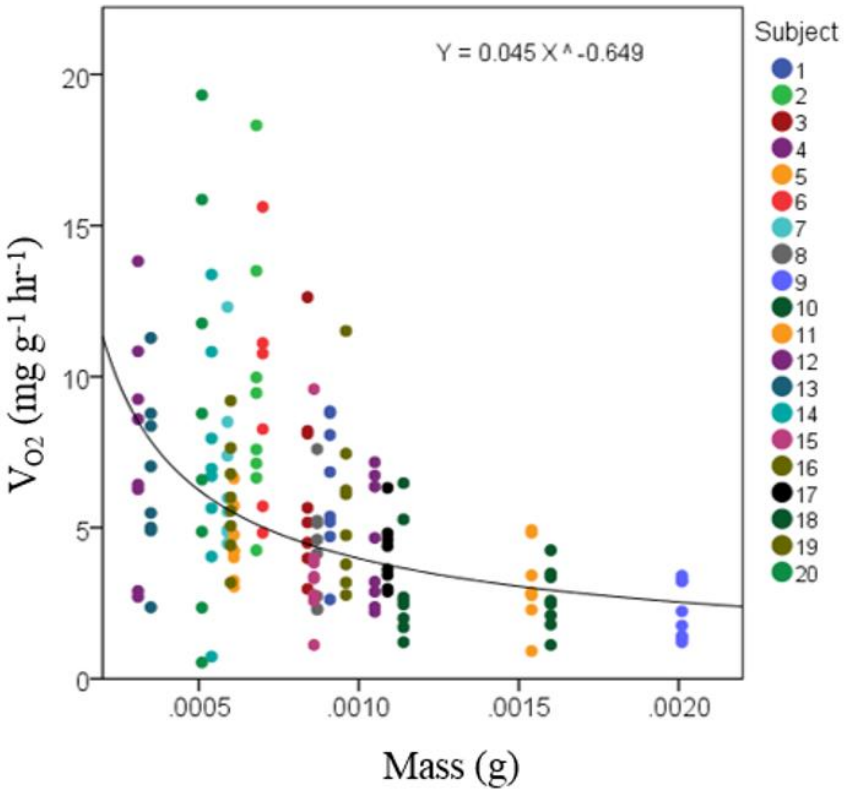

Figure 4.3 Relationship of V_{O2} and mass before, during, and after hypoxia

Mass-specific respiration rates from all 8 time increments against body sizes of the 20 individual test organisms.

The repeated measures ANCOVA showed that time (F= 4.311; P= 0.002), mass (F= 33.397; P < 0.0005), and the time by mass interaction (F = 2.454; p= 0.040) all had significant effects on the respiration rate. Distinct size-related decreasing heterogeneity in V_{O2} across time, in addition to decreasing mean V_{O2} with body size is illustrated by the negative exponential power curve of V_{O2} versus body size (Figure 4.3). Thus, a negative relationship between mass-specific respiration rate and mass was noted, as well as a clear trend that variance in respiration rate over time decreased progressively for larger subjects. This may mean that smaller organisms have to put more energy into recovery.

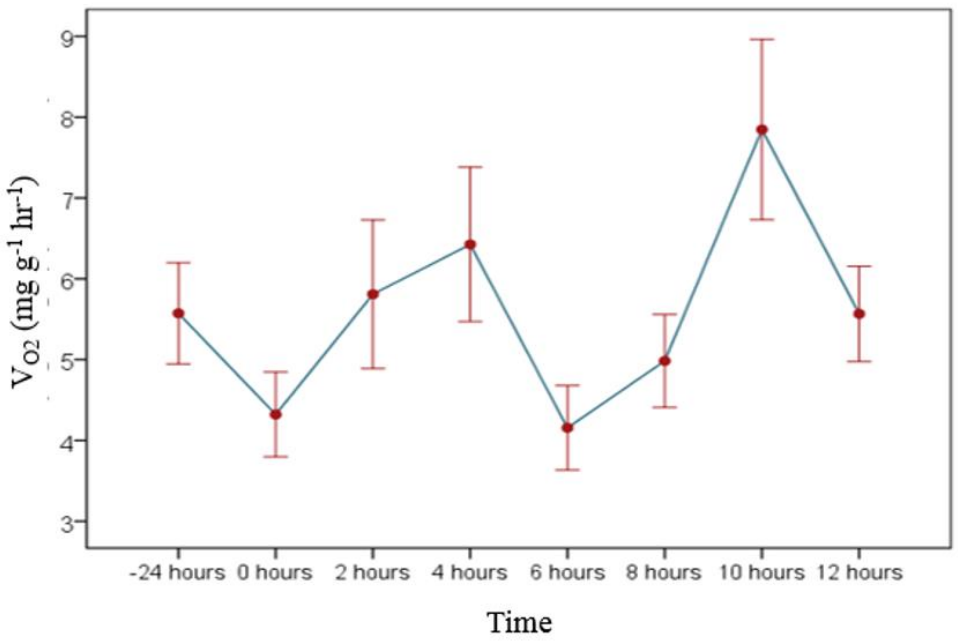

Figure 4.4 Mean V_{O2} before, during, and after hypoxia exposure

Mean mass-specific respiration rates ± one standard error of S. gynobranchiata *prior to (-24 hr), during (0 hr), and after exposure to hypoxia (12 hr).*

Table 4.1

Differences in respiration rates before, during, and after exposure to hypoxia

Contrasts tested	**Sig. value**
-24 v. 0 hr	0.277
0 v. 2-12 hr	0.189
0 v. 2, 4 hr	0.032
0 v. 6, 8 hr	0.124
0 v. 10, 12 hr	0.062
2, 4 v. 6, 8 hr	0.002
6, 8, v. 10, 12 hr	0.002
2, 4 v. 10, 12 hr	0.970

Significance values of the contrasts used to test a priori *hypotheses to find differences in respiration rate over the recovery time.*

A pattern of temporal cycling of respiration over the recovery period was revealed by the 2 subsequent periods of increasing and decreasing mean V_{O2} over time (Figure 4.4); as corroborated by the 8 *a priori* contrasts involving the custom hypotheses (Table 4.1). Alternating directions of difference were apparent in respiration levels over the recovery periods. First, there was no difference in the estimated mean respiration rates between reference normoxia and reference hypoxia time points (t = 1.110; P= 0.277; Table 4.1). Neither was there a difference between the reference hypoxia time point and the aggregate mean respiration for all subsequent recovery time periods (t = -1.328; P = 0.189; Table 4.1). However, there was a significant difference between the reference hypoxia time point and the aggregate mean for the two early recovery periods (2 and 4 hrs) (t = -2.198; P = 0.032; Table 4.1), but no difference between the reference hypoxia time point and the aggregate mean for the two middle recovery periods(i.e., 6 and 8 hrs) (t = 1.566; P = 0.124; Table 4.1), and a marginally non-significant difference between the reference hypoxia time point the aggregate mean for the two late recovery periods (10 and 12 hrs) (t = -1.932; P = 0.062; Table 4.1). In addition, there were significant differences between the aggregate mean respiration rates representing early and mid-recovery periods (t = 3.287; P = 0.002; Table 4.1) as well as between mid and late recovery periods (t = -3.271; P = 0.002; Table 4.1). However, the aggregate mean respiration rates for the early recovery periods (2 and 4 hrs) did not differ from that for the late recovery period (10 and 12 hrs) (t = 0.038; P = 0.970; Table 4.1), further supporting the temporal cycling interpretation. These alternating temporal differences in respiration rates may indicate fluctuating internal demands for oxygen. This is further

illustrated by the variation in means seen in Figure 4.5. It is also important to note that the reference normoxia and hypoxia respiration rates measured for this experiment are in close agreement with V_{O2} measured under the same conditions (100% and 20% DO at 25ºC), exhibiting the same allometric pattern and similar respiration values as those from Chapter II. Therefore, the changes seen during the recovery period vary from what is considered "normal" baseline respiration values for *S. gynobranchiata*. However, a longer time period of respiration rates need to be measured during recovery from hypoxia to confirm and further elucidate this temporal cycling pattern.

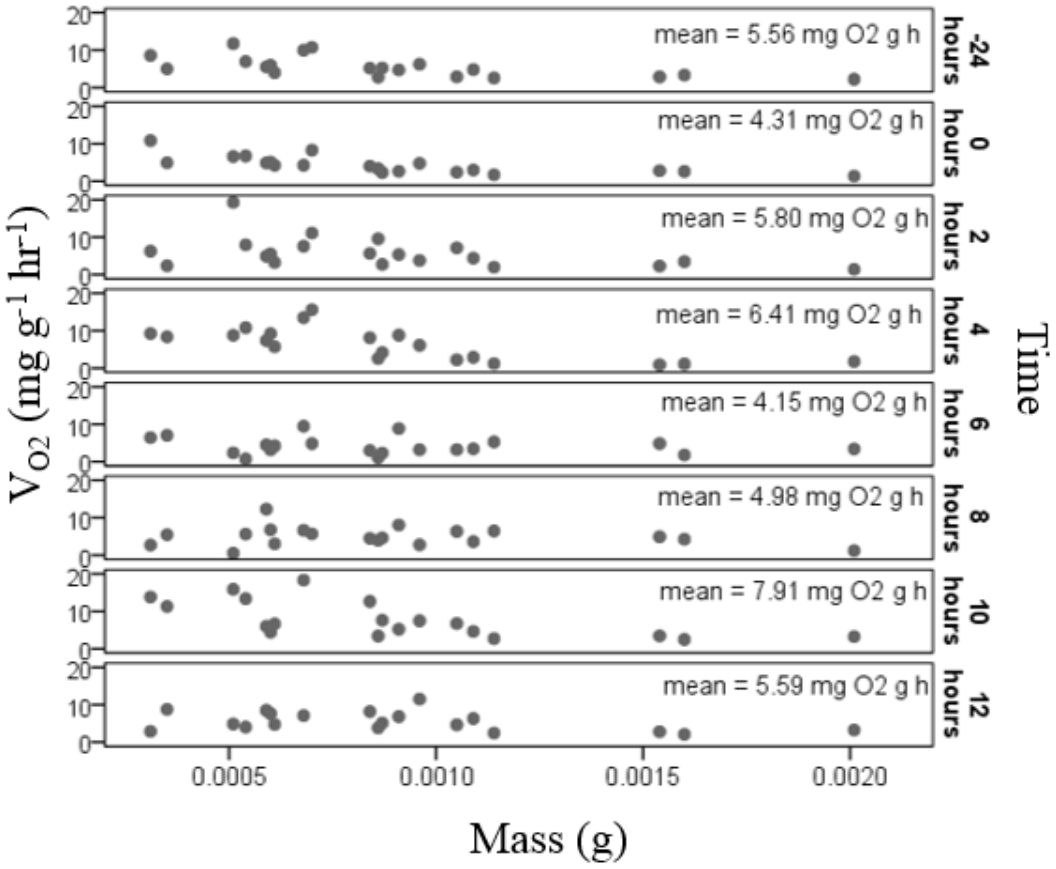

Figure 4.5 V_{O2} for the twenty test subjects during each time period

Mass-specific respiration rates for individual S. gynobranchiata across the 8 time periods.

4.4 Discussion

Documented patterns in oxygen consumption following exposure to hypoxia are incredibly variable in the literature. In one study on mussels, four different responses were observed after recovery from exposure to 2 to 5 hours of ~1.2 mg/L O_2 (Bayne and Livingstone 1977). One of the responses involved a repayment of an oxygen debt by increasing oxygen consumption to higher than normoxic levels upon a return to normoxia following exposure to hypoxia. Another response involved no change in respiration rate at normoxia, either before or after hypoxia exposure. The other two responses involved much lower V_{O2} following hypoxia; with one of those responses remaining at low levels for much longer than the other. The mussels showing no difference in V_{O2} either before or after hypoxia were held under conditions similar to organisms in this study, as characterized by a relatively normal temperature regime (i.e., 25º C). In the present study, the recovery period for *S. gynobranchiata* following 24 hours of hypoxia exposure was unlike any of the mussel recovery treatments. The *a priori* contrasts showed no difference in respiration rates between the periods of acclimation to normoxia and after 24 hours of acclimation to hypoxia. Furthermore, there was no difference in mean oxygen consumption between the normoxic reference measurements versus the aggregate mean for all six recovery periods. However, a pattern of temporal cycling in oxygen consumption was apparent, as characterized by an elevated respiration rate at around 2 and 4 hours after a return to normoxic conditions, as well as depressed oxygen consumption at around 6 and 8 hours of recovery, followed by another increase at around 10 hours. A visibly higher respiration rate at 10 hours relative to normoxia and hypoxia may suggest possible repayment of an oxygen debt following 10 hours of recovery. In

further support, respiration rates increased above hypoxic rates during both early (2 and 4 hrs) and late (10 and 12 hrs) periods of recovery, perhaps suggesting two periods of oxygen repayment.

A major part of the metabolic recovery process following hypoxia exposure is thought to entail recharging the ATP pools. This process seemingly occurs very quickly in invertebrates, with ATP levels returning to normal within 15 minutes to an hour (Ellington 1983). However, the temporal cycling respiration pattern exhibited by *S. gynobranchiata* over the recovery period may be indicative of corresponding fluctuations in ATP demand either related or unrelated to oxygen debt. Metabolism, as reflected by oxygen consumption, is subject to feedback control involving the production of ATP as triggered by low ATP concentrations due to utilization. This feedback induces the synthesis of more ATP followed by decreased rates of ATP synthesis as concentrations increase (Clarke and Fraser 2004). Thus, instead of repayment of an oxygen debt by *S. gynobranchiata*, this species might return immediately to the natural cycling of ATP production and utilization. Further measurements of oxygen consumption under normoxia over a longer period, together with direct measurements of metabolites associated with ATP cycling during recovery, would be required to truly understand the role of ATP cycling during recovery in *S. gynobranchiata*.

Another part of the metabolic recovery process following hypoxia exposure involves the disposal of anaerobic end-products. Oxygen debt is usually associated with the accumulation of anaerobic end products (Bayne and Livingstone 1977). Anaerobic pathways are incredibly complex, producing various end products which accumulate in multiple tissues, thus resulting in extremely variable recovery responses to hypoxia. In

two marine crustaceans, oxygen debt as indicated by elevated respiration occurred after exposure to hypoxia; and the level of oxygen debt related positively with duration of exposure time (Bridges and Brand 1980). Additionally, recovery times varied from 4 to 20 hours, as measured by a return to normal lactate levels. The isopod, *Saduria entomon*, also displayed an elevated oxygen consumption overshoot after 1 hour of recovery from hypoxia, and did not return to normal respiration levels for 8 hours (Vismann and Hagerman 1996). The visible overshoot in respiration noted at 10 hours in the present study may specify the amount of time required before *S. gynobranchiata* could dispose of end-products that may have been accumulated during hypoxia exposure.

Respiratory overshoot after exposure and acclimation to low oxygen levels has also been noted for various other crustacean groups, including barnacles, crawfish, mud shrimp and lobster (Bridges and Brand 1980). But most previous studies have focused on bivalves and crustaceans, which are structurally and functionally different from soft-bodied polychaetes. In annelids, excretion of anaerobic end products into the surrounding medium is common and can occur fairly quickly (Ellington 1983). The polychaetes, *A. marina* and *Euzonus mucronata*, as well as the leech, *Hirudo medicinalis*, excreted large amounts of succinate and proprionate directly into the medium (Ellington 1983). In another study, *Arenicola marina* apparently did not incur an oxygen debt after exposure to anoxia, because it was able to store enough oxygen in its blood to sustain it for a period of time (Dales 1958). In most cases, repayment of the oxygen debt is needed to reoxidize the production of lactic acid, but many polychaete species do not use the lactate pathway during anaerobic respiration (Dales 1958). In the case of *A. marina*, it was concluded that an oxygen debt was not incurred because an anaerobic pathway involving

products other than lactic acid was being used, as evidenced by the lack of accumulations of lactate or pyruvate. For *S. gynobranchiata*, the lack of accumulation of anaerobic end-products that were instead immediately excreted into the water upon return to normoxia might account for the lack of a pronounced oxygen debt.

In contrast, the lack of an oxygen debt for *S. gynobranchiata* might reflect the lack of induction of anaerobic metabolism and thus, no end product accumulation, under the exposure conditions employed in this study. As *S. gynobranchiata* maintains aerobic respiration during hypoxia at 20% DO saturation, metabolic rates were not reduced to the extent that much end product accumulation likely occurred; instead, a recovery period involving supernormal oxygen consumption might have been unnecessary (Herreid 1980). However, it is more likely in the present study that this species was using anaerobic pathways with less toxic by-products that could be quickly excreted. Indeed, former hypoxia exposures on *S. gynobranchiata* have revealed the activity of various dehydrogenases involved in anaerobic metabolism after acclimation to the nine treatment combinations of DO and temperature (i.e., Chapter II) in a separate study (pers. obs.).

The duration of the recovery process may also be influenced by life history of the organism, including the need to recover from diurnal tidal air exposure or evade predators. Both of these activities would require quick metabolic recovery from low oxygen conditions. Because *S. gynobranchiata* may be subject to quick changes in oxygen concentration, this species may have adaptations promoting the quick recovery of normal metabolic function, including the quick release of toxic anaerobic by-products, the use of ventilation behavior, increased surface area to more efficiently obtain oxygen, or the use of blood pigments with high oxygen binding affinities.

4.5 Summary

During recovery from exposure to hypoxia, wide fluctuations in mass-specific respiration rates involving apparent cycling processes occurred over the subsequent 12 hour period. There was an indication of a slight overshoot in respiration rate after 10 hours of recovery, perhaps signifying some repayment of an oxygen debt. However, two periods of alternating high and low respiration during recovery is most likely explained by a return to normal cycling of synthesis and use of ATP. Smaller specimens showed more variation in their recovery respiratory response than large organisms, suggesting that smaller *S. gynobranchiata* incur more energetic costs during hypoxia which must be 'paid back' during recovery. Marine invertebrates possess varied adaptations to low oxygen, and polychaetes are apparently well adapted to quickly return to normal respiration following hypoxia, for example, by excreting anaerobic end products in real time to avoid their accumulation. However, this and other specific mechanisms need further testing.

CHAPTER V – CONCLUSIONS

5.1 Interactive effects of dissolved oxygen, temperature, and body size on aerobic respiration of *Streblospio gynobranchiata*

Generally, respiration rates increased with temperature and decreasing oxygen saturation; however, they were depressed at the highest temperature at the thermal limit. The study species has been classified as very tolerant (See chapter I, Cinar et al. 2005, Reish 1979, Grassle and Grassle 1974, Llansó 1991), and did not generally reach critical oxygen thresholds, except at the maximum temperature level (35ºC). They also appeared to hyper-regulate under oxygen stress when exposed to more natural temperatures (15ºC and 25ºC). This was especially apparent for smaller body sizes, and could indicate some physiological basis of size selection for tolerance or survival, with smaller organisms' hyper-regulating more than larger individuals under hypoxia. Nevertheless, all sizes were able to oxyregulate under experimental conditions at natural temperatures, but not under the unnaturally high temperature treatment. Greater hyper-regulation by small organisms compared to large ones under oxygen stress implied relatively greater energetic costs for them. Thus, as DO stress increases, the ability to meet that challenge widens for small organisms. This illustrates the joint effects of increasing temperature under low oxygen concentrations. Maintaining respiration in the face of decreasing oxygen availability along with increasing temperature imposes potentially unsustainable costs for many organisms. Continued increases in oceanic temperatures to levels outside of the thermal envelope (Pörtner et al. 2005, Rabalais et al. 2002) together with declines in oxygen availability may exceed the abilities of many benthic organisms to maintain normal

metabolic function. Sustained altered conditions will result in shifts in communities and diminished benthic ecological functions (Rabalias et al. 2002).

Further information on the respiratory capacity of *S. gynobranchiata* could elucidate the full extent of its tolerance in terms of physiological thresholds related to combined levels of oxygen concentrations and temperatures. In the present study, variation in respiration curves illustrated sensitive responses to jointly changing environmental conditions. This observed variation also agrees with much of the literature calling for cautious application of universal metabolic scaling rules, and for a more full understanding of metabolic scaling under different environmental conditions (Brown et al. 2004, White et al. 2011, Glazier 2005).

5.2 Aerobic respiration of *Streblospio gynobranchiata* during acclimation to hypoxia

Upon the introduction of hypoxic water, acclimation by *S. gynobranchiata* over 12 hours showed an initial period of 3 hours with no change in respiration rate, followed by a reduction in respiration rate from 6 to 12 hours. Many organisms reduce metabolism as an immediate stress response to changing environmental conditions. *S. gynobranchiata* likely uses this response as a way to conserve energy to wait out the period of low oxygen. As seen in Chapter II, longer periods of exposure (24 hrs) resulted in increased respiration, or hyper-regulation. This illustrates the tolerance of *S. gynobranchiata* in terms of its ability to regain regulation of respiration rates after initial acclimation to hypoxia. At some point between 12 and 24 hours of exposure to hypoxia, it seems that this species is able to adapt to the lowered oxygen concentration and regain the ability to oxyregulate. As supported by personal observation, *S. gynobranchiata* can withstand long periods of hypoxia at 20% saturation and 25ºC for greater than 3 weeks. Llansó (1991)

also illustrated the same degree of tolerance in the closely related *S. benedicti*. This tolerance and ability to maintain metabolism in the face of such stressors may have ecological implications for benthic areas subject to hypoxia. *Streblospio gynobranchiata* and other tolerant species may be able to withstand significant periods of hypoxia and therefore help maintain critical ecosystem functions supporting benthic-pelagic coupling, such as bioturbation, nutrient regeneration and continued secondary production (Sturdivant et al. 2015).

5.3 Recovery of aerobic respiration of *Streblospio gynobranchiata* after exposure to hypoxia

Streblospio gynobranchiata did not show much indication of an oxygen debt upon return to normoxia following exposure to hypoxia. Because the mean peak respiration rate after 10 hours of recovery did not differ significantly from respiration under hypoxia, it may not have represented a true overshoot. Instead, the two fluctuating periods of increase and subsequent decrease in respiration during the 12 hour recovery period may have reflected the natural cycling of ATP gain and usage. There are many reasons why this species may not incur an oxygen debt, including no accumulation of end products due to the lack of anaerobic metabolism or direct real-time excretion of end products into its surroundings through dermal osmosis. The fact that this species can readily return to normal metabolic functioning following exposure to hypoxia further underscores why this species is so tolerant, and why it can survive and thrive in areas that may frequently be affected by hypoxia.

5.4 Overall significance and implications

This study of the aerobic respiration responses of *S. gynobranchiata* to multiple combined levels of dissolved oxygen and temperature, as well as its acclimation and recovery response from hypoxia, will contribute to the development of a mechanistic indicator approach. Information on an individual level will enable further understanding of the macrobenthic population responses to these two stressors (Rakocinski 2009). This information can be synthesized within a hypoxia mass balance model to predict changes in biomass-size distributions that might occur under varying levels of stressors. Given the tight coupling of the benthic and pelagic zones, this knowledge could be used to scale up the effects of hypoxia and changing temperatures on individuals to project effects on trophic transfer from the benthos to important fishery species. This would be an effective ecological indicator because it will involve scaling up of changes in vital rates of individual organisms to population-level responses.

APPENDIX A – Permission letters

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Alyssa Bennett

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To: Herreid, Clyde <Herreid@buffalo.edu>
Sent: Tue, Oct 17, 2017 5:55 pm
Subject: Permission to use figure in thesis

Hello Dr. Herreid,

I am seeking permission to use Figure 5 from your publication "Hypoxia in invertebrates" from *Comparative Biochemistry and Physiology Part A: Physiology*, 67(3), 311-320 in my master's thesis at the University of Southern Mississippi. This image will aid in illustrating various recovery responses of invertebrates to hypoxia and connect to my attempt to determine the presence of an oxygen debt after exposure to hypoxia in a benthic polychaete.

Thanks for your attention,

Alyssa Bennett, Graduate Assistant
Benthic Ecology Laboratory
School of Ocean Science and Technology
The University of Southern Mississippi, Gulf Coast Research Lab
703 East Beach Dr.
Ocean Springs, MS 39564
email: alyssa.bennett@usm.edu

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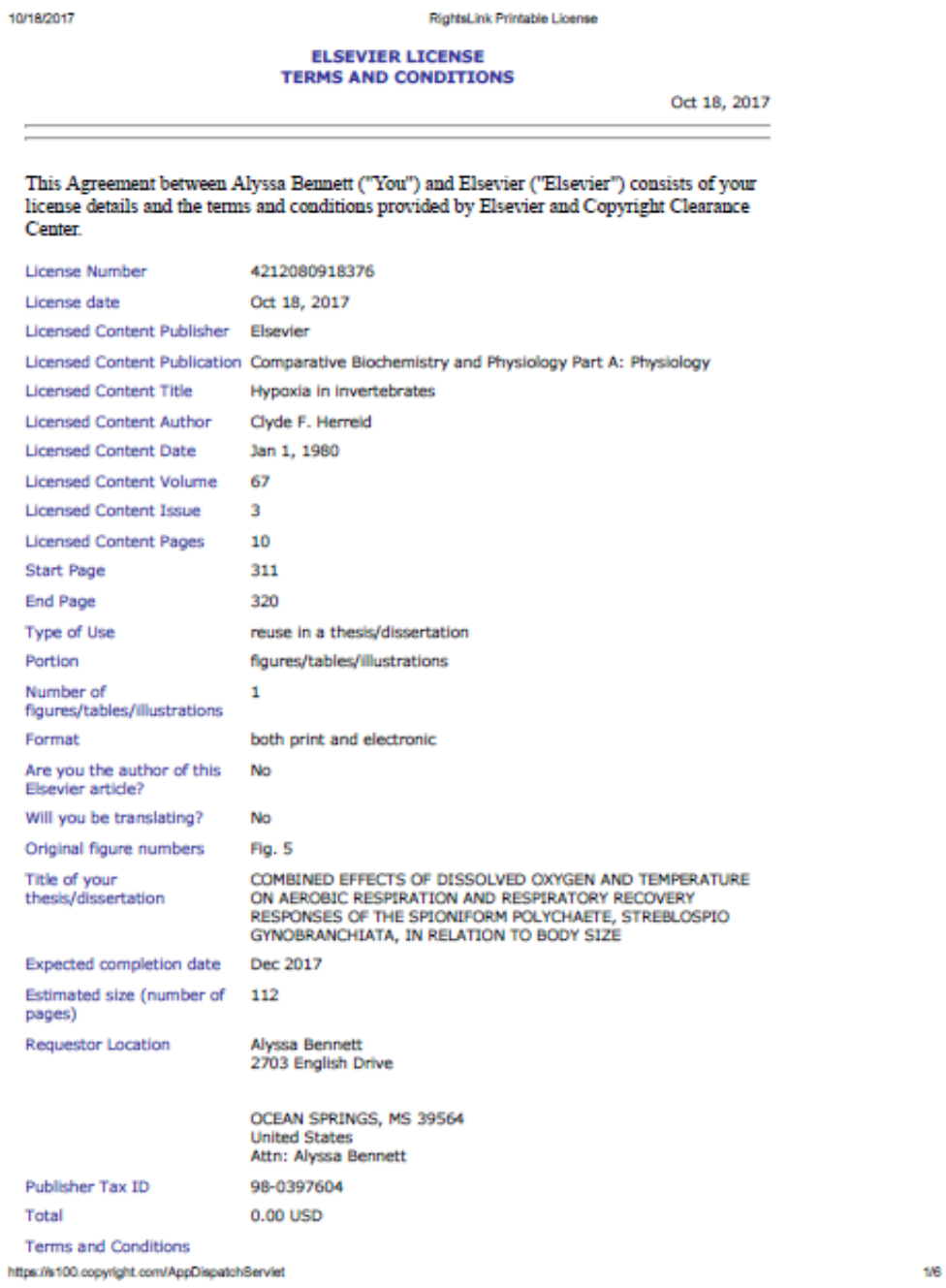

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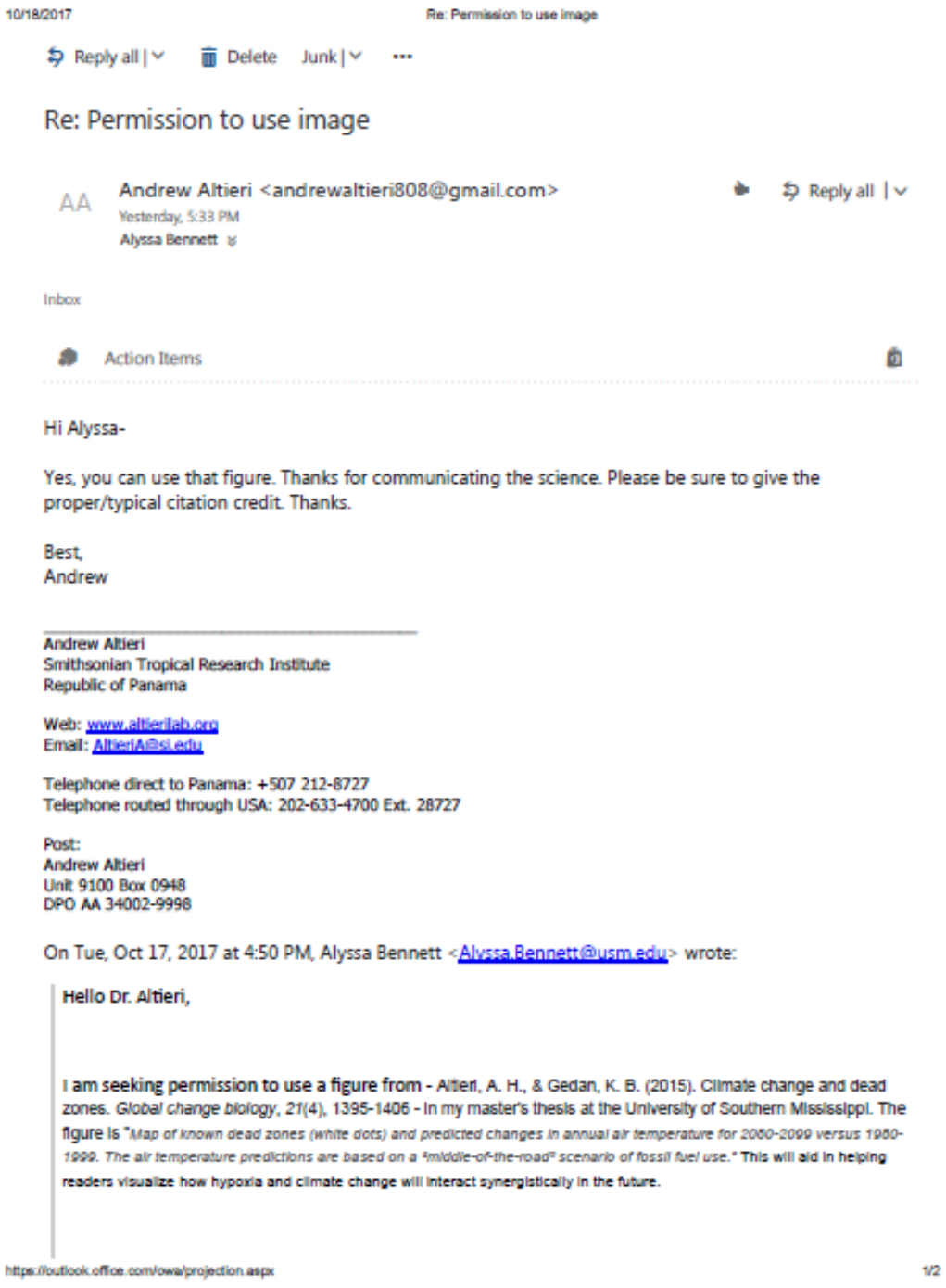

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On Tue, Oct 17, 2017 at 4:50 PM, Alyssa Bennett <Alyssa.Bennett@usm.edu> wrote:

> Hello Dr. Altieri,
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