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The Effect of Temperature Extremes on Cardiorespiratory Function in the Grass Shrimp Palaemonetes pugio: Oxygen Limited Thermal Tolerance

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THE EFFECT OF TEMPERATURE EXTREMES ON CARDIORESPIRATORY FUNCTION IN THE GRASS SHRIMP PALAEMONETES PUGIO: OXYGEN LIMITED THERMAL TOLERANCE

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ABSTRACT

The effect of temperature extremes on cardiorespiratory function in the grass shrimp *Palaemonetes pugio*: Oxygen limited thermal tolerance

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Aquatic poikilothermic animals must either cope with or compensate for the mismatch in oxygen supply and demand present at high temperatures. Oxygen limited thermal tolerance explains how aerobic scope is limited by insufficient oxygen supply and sets the performance window in animals. This work explores the effects of temperature on the different components of the oxygen delivery system, in both normoxic and hyperoxic conditions to determine if supplying more oxygen to the system mitigates the effects of temperature effects on the cardiorespiratory system and extends oxygen limited thermal tolerance to higher temperatures. The effect of temperature and oxygen condition was measured on both the whole organism and system level. Critical thermal maxima (CT_{max}) were established in both normoxic and hyperoxic conditions to determine if the mismatch in oxygen supply and demand could be mitigated by supplying more oxygen to the system. CT_{max} was higher in hyperoxia than normoxia indicating failure was not related to a specific temperature. Ventilatory measurements were made through the development of a novel high speed video microscopy method to measure ventilatory volumes in small aquatic crustaceans. Temperature had a significant effect on all ventilatory parameters measured including scaphognathite stroke volume, ventilatory rate, and ventilation

volume. Hypometabolism and hypoventilation were observed in the hyperoxic condition compared to the normoxic condition. This may be a mechanism to save the cost of energy expended for ventilation. Cardiac parameters including heart rate, stroke volume, and cardiac output were also measured using high speed video microscopy. Heart rate exhibited a temperature dependent tachycardia as did increase in cardiac output, but stroke volume was temperature insensitive. There was a significant effect of oxygen condition on stroke volume with a higher stroke volume in hyperoxia. Analysis of pressure data for time in cardiac cycle points to a biomechanical limitation leading to cardiac failure. Cardiac measurements were expanded using integrated micropressure signals and high speed video microscopy to measure intracardiac pressure and cardiac volumes respectively to generate pressure-area loops. The change in pressure (Δ P) is reduced at high temperatures in hyperoxia compared to normoxia leading to a reduction in stroke and cardiac work in hyperoxia. This reduction in cardiac work leads to cardiac failure at higher temperatures in hyperoxia than normoxia. This data also provides further support of a biomechanical limitation of diastolic filling time leading to cardiac failure at high temperatures.

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CHAPTER 1

THE EFFECT OF TEMPERATURE ON THE OXYGEN DELIVERY SYSTEM: OXYGEN LIMITED THERMAL TOLERANCE.

Estuarine organisms live in a dynamic and variable environment. They typically experience wide fluctuations in salinity, dissolved oxygen levels, and temperature both on a diel and seasonal cycle.twenty-four hour period (Thorp and Hoss, 1975; Vernberg and Piyatiratitivorakul, 1998).

These changing conditions represent challenges to which organisms must respond or tolerate to survive.

Organisms maybe be classified into two different groups based on regulation of body temperature. They may be thermoregulators and regulate body temperature metabolically to an internal set point, or they may be thermoconformers with body temperature passively conforming to the environment. If these thermoconforming organisms do not behaviorally regulate body temperature and have a variable body temperature they are termed poikilothermic. If this variation includes a wide thermal tolerance they are additionally termed eurythermal (Cossins and Bowler, 1987; Withers, 1992).

Many aquatic estuarine crustaceans face a particular thermoregulatory challenge. In the aquatic environment, the thermal gradient is the same for both conductive and convective heat exchange, therefore body temperature equals water temperature (Cossins and Bowler, 1987; Withers, 1992). As poikilothermic thermoconformers, their body

temperature passively changes with water temperature, and no metabolic energy is expended on thermoregulation (Withers, 1992).

There are two commonly used methods to test thermal limits of an organism. The first uses a combination of time and temperature to determine the limit (Cossins and Bowler, 1987). One of the more common combinations measures the time at which 50% mortality occurs at a given temperature, LT_{50} (Kivivuori and Lahdes, 1996). Alternatively the temperature at which 50% of the organisms survive for a given time may be used, T_{L50} (Claussen, 1980). Another method raises temperature at a defined rate and determines a critical thermal maximum, CT_{max}, at the onset of specific symptoms of thermal stress such as onset of spasms or loss of righting response (Hutchison, 1961; Lowe and Vance, 1955). Reaching a critical thermal maximum is not necessarily lethal to the organism in the short term, but remaining at that temperature will ultimately lead to death. Different endpoints are used to define these symptoms of thermal stress with debate as to which is more informative (Lutterschmidt and Hutchison, 1997).

These methods have been employed to explore the range of thermal limitation in crustaceans. Thermal death in *Daphnia* was measured using LT_{50} and T_{L50} with varying rates of temperature increase. The mean lethal temperatures ranged from 34.8 to 39.4°C based on the method used with the higher temperatures associated with a shorter exposure time (Kivivuori and Lahdes, 1996). Thermal preference and tolerance were tested in the shrimp, *Palaemonetes kadiakensis* using CT_{max}. CT_{max} ranged from 32.5 to 41.4°C and differed based on the endpoint used to measure thermal response and acclimation temperature. Initial and complete disorientation indicated physiological

stress, while onset of tail fold, end of tail fold, and loss of movement represented the ultimate bio-physio-chemical limitation (Nelson and Hooper, 1982). While a maximal temperature of 41°C was recorded, attempts to acclimate shrimp at to 33°C resulted in death and as such failed (Nelson and Hooper, 1982). Considering this LT_{50} and CT_{max} data, organisms are clearly failing at temperature extremes and it indicates the failure is due to a physiological limitation.

Three paradigms of thermal physiology were proposed by Ege and Krogh (1914) First that the resting metabolic rate will increase with temperature in ectotherms, described above as poikilothermic thermoconformers. Second, that evolutionary and acute responses to temperature are different. The third paradigm, which has since been rejected for marine ectotherms, is that organisms evolved at low temperature show elevated resting metabolic rate compared to eurythermal species measured acutely at the same low temperature (Ege and Krogh, 1914).

The first paradigm of thermal physiology considered metabolic rate. Later research extended this to include the effect of temperature on other biological rates as well. Studies on invertebrates, and crustaceans in particular, focused on temperature effects on the cardiorespiratory system, which includes heart, ventilatory, and oxygen consumption rates (De Wachter and McMahon, 1996b; DeFur and Mangum, 1979; McMahon and Wilkens, 1983).

Most early studies focused on the effect of temperature on heart rate because it is the easiest variable to measure in intact organisms. However, it did not account for the complete physiological function of the cardiovascular system where stroke volume was

not measured. This resulted in an incomplete picture of complete physiological response to increasing temperature (De Wachter and Wilkens, 1996; McMahon and Wilkens, 1983). As of 1996 the effect of temperature on cardiovascular parameters including heart rate, stroke volume, and cardiac output had received little attention in invertebrates (De Wachter and McMahon, 1996b).

Early research across four invertebrate phyla including four crustaceans using intact animals versus isolated cardiorespiratory organs measured an increased heart rate with increased temperature (DeFur and Mangum, 1979). In a study comparing intact versus semi-isolated *Cancer magister* hearts, cardiac parameters of both varied with temperature. Intact hearts exhibited a linear increase in heart rate with temperature, semi-isolated hearts also showed a correlated increase in heart rate with temperature, however at high temperatures the correlation disappeared (De Wachter and Wilkens, 1996). The relationships for stroke volume and cardiac output were also very different for intact and semi-isolated hearts. Stroke volume decreased with increasing temperature for both, but is then maintained at higher temperatures in the intact animals. Cardiac output, which is the product of stroke volume and heart rate, increased in intact animals with the increase really from heart rate. There was a decrease in stroke volume attributed to the decrease in ventricular pressure and time in systole noted in semi-isolated preparation (De Wachter and Wilkens, 1996). In another study, *Cancer magister* exhibited a virtually linear increase in heart rate with temperature as well as increases in ventilation, and oxygen consumption (De Wachter and McMahon, 1996b). In this study stroke volume also changed inversely with temperature. As a group, these studies provided above support the

first paradigm of thermal physiology by not only showing the effect of temperature on metabolism but extending it to other biological rates as well.

The effect of temperature on ventilatory, heart, and oxygen consumption rates can also be described using Q_{10}. A Q_{10} is a ratio of rates over a ten degree Celsius difference and provides a means of showing the temperature sensitivity of a process (Cossins and Bowler, 1987). Most physiological processes, including those discussed above, typically have a Q_{10} of 2 to 3. A ventilatory rate with a Q_{10} of 2 would be expected to double over a ten degree Celsius change. Early research found that Q_{10} tended to decrease as temperature increased (Ege and Krogh, 1914). While Q_{10} may explain temperature sensitivity of a process, its use becomes problematic when comparing animals from very different thermal environments. If we make Q_{10} comparisons relative to each organisms temperature range, rather than focusing on the temperature itself, Q_{10} can be a useful tool (Cossins and Bowler, 1987). While most Q_{10} describe a ratio of rates between a ten degree Celsius range, any temperature range may be used as long as the rate changes significantly between the two temperatures (Cossins and Bowler, 1987).

Species from four invertebrate phyla were used to explore the cardiovascular response to temperature, and it was found that Q_{10} temperature coefficients for heart rate were 2.0-2.5 which is well within the range expected for physiological processes (DeFur and Mangum, 1979). For example, Q_{10} s were calculated for heart and ventilatory rates and oxygen consumption in *Cancer magister.* The Q_{10} for heart rate increased resulting in a negative Q_{10} -temperature relationship. Heart rate continued to increase and Q_{10} to decrease with increasing temperature. The gradual decrease in Q_{10} with values smaller

than 1.8 in the temperature range tested represents a reduction in temperature sensitivity with increasing temperature (De Wachter and McMahon, 1996b). These examples provided above show temperature extremes have an effect at the whole organism level as well as effecting cardiorespiratory parameters, but they do not provide a hypothesis for the cause of the failure. While all the studies show a linear increase in heart rate with temperature, the effect on stroke volume varied.

Three hypotheses for thermal sensitivity have been proposed. These include: one, effects on proteins, two membranes, or three the oxygen delivery system (Portner, 2001). This thermal sensitivity may be related to differences in oxygen supply and demand. A poikilothermic organisms's metabolic rate, and therefore oxygen demand, increases with increasing temperature (McMahon and Wilkens, 1983). The increase in metabolic rate indicates an increased demand for oxygen. At the same time, oxygen solubility in water decreases with increasing temperature. The increased oxygen demand is met with a decreased oxygen supply (Hill et al., 2008).

This mismatch in oxygen supply and demand has led to a hypothesis that seeks to explain the link between oxygen delivery and thermal limitation. The hypothesis of oxygen-limited thermal tolerance unites thermal limitation and adaptation and provides a means of describing the link between physical performance and the environment. This link may also provide a physiological basis to explain the biogeography of an organism. (Portner, 2010; Pörtner, 2002). It may be the oxygen supply and demand that sets the thermal window of the organism. The concept of oxygen-and capacity-limited thermal tolerance explains how aerobic scope, the ability of an organism to increase metabolic

rate on demand, is limited by insufficient oxygen supply at both sides of the thermal window and sets the performance window in animals (Portner, 2010).

Under this hypothesis, organisms fail at temperature extremes due to a breakdown of a component of the oxygen delivery system which maintains the animals oxygen supply. The oxygen delivery system can be broken down into three broad components. First is the ventilatory component which involves moving oxygen from the environment to the respiratory exchange area. The second component is the cardiovascular system, specifically the heart, which propels oxygen-containing hemolymph or blood to the tissues of the body. The third component is at the cellular level providing oxygen to meet aerobic metabolic demands of the cell (McMahon and Wilkens, 1983).

One major tenet of oxygen limited thermal tolerance is a systemic to molecular hierarchy of thermal tolerance, suggesting that most complex level of organization susceptible to thermal extremes is responsible for the failure of the organism (Portner, 2001; Pörtner, 2002)**.** There is a decrease in maximum heat tolerance of organisms as organizational complexity increases. There are examples of prokaryotes that have a maximum heat tolerance greater than 90°C, but that heat tolerance decreases as the phyla become more complex, from simple eukaryotes, to metazoa, and finally metazoan populations (Pörtner, 2002). The rise in metabolic performance with increasing complexity occurs at the expense of greater thermal sensitivity (Pörtner, 2002).

Although multiple systems may be failing, any physiological system that collapses rapidly at the lethal temperature for the whole animal is effectively the cause of death (Somero, 2010). The cardiovascular system is an informative system to investigate

because its function decreases rapidly as CT_{max} is approached (Hochachka and Somero, 2002). The first line of thermal sensitivity being apparent at the highest functional level makes a good case for the integrated function of the ventilatory and cardiovascular components oxygen delivery system as the thermally limited functions (Pörtner, 2002). Factors influencing heart rate also influence ventilatory rate providing another indication of how tightly the two systems are tied together (McMahon, 1999).

Failure at high levels of complexity falls in line with the concept of symmorphosis originally proposed for the mammalian respiratory system. While increased complexity can respond to a higher oxygen demand, there is a cost to maintain that complexity. As such, the components of the system are designed to avoid excess capacity (Hoppeler and Weibel, 1998; Weibel et al., 1991). In this way each part of the system matches demand, but has little room to respond to demands that exceed the usual functional demand (Pörtner, 2002). The principles of organizational complexity and symmorphosis combined with the failures discussed above supports the hypothesis that the first line of thermal sensitivity may be integrated at the ventilatory and cardiovascular systems.

The previous examples of the effect of temperature on ventilatory and cardiovascular parameters do not specifically investigate an oxygen limited thermotolerance, but they begin to provide a basis for it. Additional research specifically exploring oxygen limited thermal tolerance supports the hypothesis of these aspects of the oxygen delivery system setting the thermal limits of organisms.

While the studies discussed earlier investigate the effects of high temperatures on ventilatory and cardiac parameters, they are generally within the organisms temperature range and do not reach CT_{max} (Frederich and Portner, 2000). A switch to anaerobic metabolism at CT_{max} indicates a mismatch between oxygen supply and demand (Frederich and Portner, 2000). For example, in the spider crab, *Maja squinado,* Frederich and Portner, (2000) found that ventilatory rate and P_{O_2} were more susceptible to changes in temperature than heart rate, indicating ventilatory performance as the component of the oxygen delivery system breaking down (Frederich and Portner, 2000).

The effect of oxygen limited thermal tolerance has also been noted at the ecosystem level. In laboratory conditions, eelpout, *Z. viviparous,* and *Pachycara brachycephalum,* and Atlantic cod, *Gadus morhua,* experience limitations in circulatory capacity before ventilatory capacity at increased temperatures (Pörtner and Knust, 2007). With continued warming, both species of fish switch to anaerobic metabolism in fully aerated water (Pörtner and Knust, 2007). Field studies indicate the number of migratory eelpout decreases with warming of the water. Atlantic cod abundance decreased with warming due to both thermal sensitivity of the cod and thermal sensitivity of the copepod fauna that comprise their diet. These field observations support what has been observed in the lab and show how oxygen limited thermotolerance can be seen at the ecosystem level (Pörtner and Knust, 2007).

One of the first investigations of oxygen limited thermal tolerance was performed on goldfish. An increase in oxygen requirement was observed as temperature increased. This led to the postulation that continued demand exceeding supply would lead to failure

of the respiratory and circulatory systems (Weatherley, 1970). The investigation sought to remedy the supply issue by providing what was termed a superabundance of oxygen, which is now referred to as hyperoxia.

The rate of heating was not significant in influencing temperature of death, but mean survival times of goldfish exposed to lethal temperature in oxygen saturated water was 2-5 times greater than aerated water. The upper lethal temperature also increased in oxygen saturated water. These results lend support to the hypothesis of oxygen limited thermal tolerance (Weatherley, 1970). A more recent study also investigated if hyperoxia could alleviate thermal stress in an Antarctic bivalve, *Laturnla elliptica* (Pörtner et al., 2006a). Hyperoxic conditions increased inhalant water and haemolymph P_{O_2} suggesting improved oxygen uptake by the haemolymph to increase aerobic scope (Pörtner et al., 2006a). This indicates that hyperoxia may enhance thermal compensation, or the maintenance of physiological rate and scope in the face of temperature change (Pörtner et al., 2006b).

It is important to note in all of these studies, that not only are these organisms failing, they are failing in fully aerated water. The fact that these organisms are switching to anaerobic metabolism in fully aerated water further points to a mismatch between oxygen supply and demand.

Understanding of oxygen limited thermal tolerance is important because it describes a link between oxygen supply to the tissues and aerobic performance which explains the fitness of an organism in relation to its environment (Portner, 2010) In the face of global climate change, determining biochemical and physiological limits to

thermal adaptation takes on additional significance (Pörtner et al., 2006a). Physiological responses of animals may be very closely related to habitat (Moshiri et al., 1970). Knowledge of thermal limits may be used to determine optimal cultivation temperatures and locations for culture of crustacean species for commercial use. (Díaz et al., 2002; Díaz Herrera et al., 1998). Knowledge of the upper limits of thermal tolerance and a thorough understanding of physiological limitations to whole animal performance can also provide insights essential for understanding a species tolerance for and adaptability to global climate change (Wang and Overgaard, 2007). A full understanding of oxygen limited thermal tolerance requires molecular to ecological integration (Pörtner et al., 2006b).

One example helping to provide this link is in the study of thermal limits in two congeneric species of porcelain crabs (Stillman and Somero, 1996; Stillman, 2002; Stillman, 2004). These species are closely related, occur in discrete vertical zones, and have a wide range of responses to their microhabitat conditions (Stillman, 2002). This makes them ideal study organisms for these thermal comparisons as it allows a more direct comparison between the species. Comparisons between LT_{50}s of a tropical subtidal species, *Petrolisthes cinctipes*, and a temperate intertidal species, *Petrolisthes eriomerus* found a higher LT_{50} for the tropical species (36 to 41°C versus 27 to 35°C) (Stillman, 2002). Q_{10}s for heart rate were similar for both species, however thermal limits were different (Stillman and Somero, 1996). Arrhenius break temperatures (ABT), the temperature at which there is a discontinuity in the slope of the plot of logarithm of heart rate and the inverse of temperature, were calculated for the heart rates of each species.

The ABT temperature for the tropical species was 31.5°C while the ABT for the temperate species was 26.6°C. The habitat of the tropical species, *P. cinctipes,* can be greater than 30°C, which is very close to their ABT (Stillman, 2002).

Thermal acclimation studies on the two crab species showed that acclimation has the most effect on the species from the cooler microhabitats and the lowest effect on species from the hottest microhabitats (Stillman, 2004). This indicates the species from the cooler microhabitats have more room to adjust to increasing temperatures. This result coupled with living in an environment close to their thermal limit leads to the counterintuitive conclusion that the species with the higher heat tolerance is also the most susceptible to global climate change (Stillman, 2004).

This type of research can be applied to other species. Organisms living in environments that are closest to their LT_{50} are most at risk because a small increase in environmental temperature exceeds their LT_{50}. This may lead to changes in population distribution and size depending on organisms ability to adapt, and move. Data can be used to predict these changes and project when and why extinctions occur (Somero, 2010). An understanding of the physiology underlying these distribution patterns can be used to predict future distributions as the global climate changes (Somero, 2010).

While a number of studies have focused on mechanisms of thermal limitation in isolation, Portner (Portner et al., 2006) notes that studies on mechanistic background of performance are scarce. He proposed three mechanisms of thermal sensitivity. These include: effects on proteins, membranes, or the oxygen delivery system. My objective is to investigate the effects of temperature extremes on the oxygen delivery system at the

whole animal level, focusing on responses to acute changes in temperature and how the cardiorespiratory system responds to that increased demand, how far the system can respond, and why it eventually or inevitably fails.

Palaemonetes pugio is an estuarine crustacean exposed to a wide range of temperatures in their native environment, making them ideal organisms to investigate mechanisms of cardiorespiratory regulation using their compensatory responses. They can experience salinity levels of 0-55 ppt, and dissolved oxygen levels of 1.2 kPa-22.7 kPa (9-170 Torr) in a twenty-four hour period. Their temperature tolerance ranges from 5-38°C (Anderson, 1985; Vernberg and Piyatiratitivorakul, 1998).

Chapter 2 presents the development and validation of a new noninvasive method to measure ventilatory parameters in a small aquatic crustacean. Previous methods overestimated ventilation volume and did not measure scaphognathite stroke volume, but assumes the scaphognathite is a fixed volume pump. The scaphognathite chamber is modeled as a conical frustum and ventilatory rate, ventilatory volume, and scaphognathite stroke volume are measured using high-speed video and dimensional analysis using a modification of the method described by Harper & Reiber (1999) for measuring cardiovascular parameters. I validated this method by comparing it with previously described direct methods (McMahon & Wilkens, 1983).

Chapter 3 investigates ventilatory response to temperature in normoxic and hyperoxic conditions. Determination of the effect of temperature on the whole animal is accomplished by determining critical thermal maximum temperatures using loss of righting as a critical endpoint (Nelson and Hooper, 1982). Measurements of the third

component of the oxygen delivery system, aerobic metabolism at the cell, are necessary for comparison with the ventilatory and cardiac parameters. To do so, whole body lactate was measured across a temperature range using an enzymatic method from a commercially available lactate analysis kit. Closed system respirometry was used to measure oxygen consumption using an optical sensing method. These data are also referenced in all following chapters.

Chapter 3 uses the method developed in chapter 2 to measure the response of the ventilatory component of the oxygen delivery system to temperature extremes. Under the hypothesis of oxygen-limited thermotolerance, experiments are conducted at both normoxic (20 kPa, 150 Torr) and hyperoxic (26.6 kPa, 200 Torr) levels to determine if providing the animals more oxygen in their environment mitigates the effects of the failing delivery system. Experiments use animals acclimated to 20°C tested over a temperature range (5-41°C). All rate data is used to calculate Q_{10} relationships. This information is used to inform all following chapters.

Chapter 4 investigates cardiac response to temperature in normoxic and hyperoxic conditions. Cardiac parameters including heart rate, stroke volume, cardiac output, and ejection fraction, are measured using high-speed video and dimensional analysis following the method described by Harper & Reiber (Harper and Reiber, 1999) to identify when the system fails. Rate data is used to calculate Q_{10} relationships.

Chapter 5 uses pressure area loops to measure cardiac energetics including stroke work, pressure, ad time in cardiac cycle to determine the exact mechanisms leading to cardiac failure at temperature extremes. Micropressure and high speed video analysis are

used to generate pressure-area loops using a previously described method (Guadagnoli et al., 2007).

CHAPTER 2

THE USE OF HIGH-SPEED VIDEOMICROSCOPY AS A MEANS TO MEASURE VENTILATORY PARAMETERS IN A SMALL AQUATIC CRUSTACEAN

Abstract

Methods to measure ventilatory parameters in crustaceans are designed for animals larger than *Palaemonetes pugio* and are invasive in nature. Here a novel method using high speed video microscopy is described for measuring ventilatory parameters in small aquatic crustaceans. In addition to allowing measurements in small organisms this method also allows beat to beat measurements of scaphognathite stroke volume. These measurements make it possible to determine if the system acts as a fixed volume pump or if volumes can vary with temperature or beat to beat.

Introduction

The grass shrimp, *Palaemonetes pugio,* is an estuarine species subjected to wide fluctuations in temperature (Anderson, 1985). Persisting in this environment requires a means to tolerate or respond to these fluctuations. Cardiorespiratory responses to temperature have been measured in a number of decapod crustaceans. (DeFur and Mangum, 1979; Frederich and Portner, 2000; Ivleva, 1980; McMahon and Wilkens, 1983)

The ventilatory system of *P. pugio* is similar to that of crayfish and lobster (Withers, 1992). The scaphognathite, also known as the gill bailer, housed in the anterior exhalant chamber, oscillates creating a negative pressure (relative to ambient pressure) in the branchial chamber (Withers, 1992). Water is pulled into the inhalant, or

hypobranchial, chamber, over the walking legs or the posterior and ventral edges of the loosely fitting carapace. The water then circulates over the gills, and out the anterior exhalant chamber (Withers, 1992) (Figure 2.1).

Ventilatory rate is the frequency of scaphognathite oscillations (f_{sc}). Scaphognathite stroke volume is the volume of water per oscillation (V_{sw}), and is the product of these two measurements (V_w) (McMahon and Wilkens, 1983). Ventilatory rate, or scaphognathite frequency, and ventilation volume, the product of ventilatory rate and scaphognathite stroke volume, both increase with increasing temperature (Cumberlidge and Uglow, 1977; Frederich and Portner, 2000; Ivleva, 1980; McMahon et al., 1978).

A number of methods to determine ventilatory rate and volume have been used previously including direct measurement with the separation of inhalant and exhalant chambers with a rubber hood (Cumberlidge and Uglow, 1977; Larimer, 1961; McMahon and Wilkens, 1977), electromyographic recordings obtained with wires directly implanted in levator muscles (Zinebi et al., 1990), and pressure transducers or impedance electrode and electromagnetic flow meters or impedance (Burggren and McMahon, 1983; McDonald et al., 1977; McDonald et al., 1980; McMahon et al., 1978; Morris and Taylor, 1985)

All of these methods were designed for organisms larger than *P. pugio,* (the crabs *Cancer magister, Carcinus maenas,* and *Cancer productus,* and the crayfish *Oroconectes virilis*, and *Procambarus simulans*) (Cumberlidge and Uglow, 1977; Larimer, 1961; McDonald et al., 1977; McMahon et al., 1978; McMahon and Wilkens, 1977) and cannot

be reliably applied to small aquatic crustaceans. These methods are also all invasive in nature, and are limited in that they do not measure scaphognathite stroke volume. Due to these limitations, ventilatory parameters have not been fully described in *P. pugio* or other small crustaceans.

Noninvasive methods to measure *P. pugio* cardiovascular response to fluctuations in dissolved oxygen levels, particularly hypoxia, have been developed (Guadagnoli et al., 2011; Harper and Reiber, 1999). The objective of this work is to use the existing noninvasive methods for measuring cardiovascular parameters as a framework to create similar methods for measuring ventilatory parameters. High speed video microscopy is employed to measure an index of scaphognathite stroke volume (V_{sw}) through the transparent exoskeleton of *P. pugio*. This volume measurement paired with scaphognathite frequency (f_{sc}) counted directly from the video files is used to calculate ventilation volume (V_w). Most people assume the scaphognathite acts as a fixed volume pump with V_{sw} calculated by dividing V_w by f_{sc} (McMahon and Wilkens, 1983). This method allows beat to beat measurements enabling the determination if the scaphognathite is indeed a fixed-volume pump. These measurements obtained with this new method are further verified by comparison with existing data in larger animals, and direct measurements of ventilation volume.

Materials and Methods

Animal Preparation

Grass shrimp, *Palaemonetes pugio*, were obtained from Gulf Specimen Marine Laboratories Inc (Panacea, FL, USA). Shrimp were maintained in 20L aquaria in aerated

seawater (Coralife) (30 ±2 ppt) at 20˚C, with a 12:12 light:dark cycle for two weeks prior to experimental use. Shrimp were fed marine flakes (Ocean Nutrition Formula Two Flakes) three times a week with food withheld two days before experimentation.

Shrimp were attached to the flattened end of a wooden applicator stick at the dorsal cephalothorax with cyanoacrylate glue. The animal was held in place and positioned within the experimental chamber with a micromanipulator (World Precision Instruments, Sarasota, FL, USA). A high speed video camera (Phantom Miro eX4, Vision Research, Wayne, NJ, USA) was placed over the chamber so video images of the heart and scaphognathite could be captured through the transparent exoskeleton (see methods from (Harper and Reiber, 1999).

Experimental Design

Normoxic (20 kPa, 150 Torr) sea water (30 ±2 ppt) at 20º C was circulated through an experiment chamber and over the animal. Water oxygen partial pressure (P_{O2}) was maintained using a gas mixing system (Cameron Instruments) All animals were initially placed in the experimental chamber and allowed to acclimate for 30 minutes at 20º C, followed by a 30 minute temperature shift (either up or down from the initial 20º C to the next experimental temperature) (10, 20, 30º C) with a 15 minute acclimation period for each experimental treatment. The temperatures were adjusted using a flow through external water jacket and circulating water bath. A minimum of 10 scaphognathite stroke volume and rate recordings were made for each animal at each temperature (n=7).

Video Analysis, Ventilatory Functions

Video images for ventilatory analysis were acquired through the transparent exoskeleton at a rate of 480 Hz using a stereo-microscope (Leica Photo 6) equipped with a Phantom (Miro 4) high speed video camera. Video frames were analyzed using ImageJ (ImageJ, NIH image) to determine area of the anterior exhalant (scaphognathite) chamber using methods based on previous work used to model ventricular volume (Harper & Reiber, 1999). The anterior exhalant chamber was observed from the anterior, posterior, ventral, and dorsal aspects to determine the approximate shape of the chamber. Based on these observations, the anterior exhalant (scaphognathite) chamber is modeled as half of a conical frustum (Figure 2.2). Scaphognathite stroke volume (V_{sw}) determined as follows:

$$V = \frac{\frac{1}{3}\pi h\left(R^2 + Rr + r^2\right)}{2}$$

where R is the radius of base, r is the radius of top, and h is the height. Ventilation volume (V_w) is calculated as product of V_{sw} and f_{sc}**.** This final number was doubled to account for bilateral ventilation. Bilateral ventilation is assumed in all measurements.

Direct Measurement, Ventilation Volume

Measurements of ventilation volume were also determined using a direct method. A piece of latex dental dam was placed around the cephalothorax and fixed to the dorsal side of the animal with cyanoacrylate glue anterior to the walking legs to separate the exhalant and inhalant openings. The animal was then placed in an interlocking chamber with water on the anterior and posterior sides with the dental dam limiting the flow between the chambers to ventilatory flow. The volume of water moved from the posterior to anterior side of the chamber was collected and measured as the ventilation volume.

Statistical Analysis

Statistical analyses were performed in R, with two-way ANOVA used to determine effects of temperature, oxygen condition, and the interaction between temperature and oxygen condition. Pairwise t tests were performed to determine differences between oxygen conditions at each temperature. All values are reported as means ±SE with (n) indicating the number of animals.

Results

Ventilatory rate (f_{sc}) increased significantly with increasing temperature. ($p = < 0.001$) Pairwise comparisons indicated significant differences between all temperature levels. Ventilatory rate increased from 114 ± 10 to 658 ± 32 bpm from 10 to 20° C ($p < 0.0001$) and from 658 ± 32 to 949 ± 52 bpm from 20 to 30° C ($p < 0.0001$) (Figure 2.3).

Scaphognathite stroke volume (V_{sw}) was independent of temperature and did not vary over the tested range ($p = 0.485$). Pairwise comparisons confirmed no significance between temperature levels. Scaphogathite stroke volume was $4.19 \times 10^{-3} \pm 2.8 \times 10^{-4}$ µl/mg at 10° C, $4.61 \times 10^{-3} \pm 4.5 \times 10^{-4}$ µL/mg at 20° C, and $4.54 \times 10^{-3} \pm 3.3 \times 10^{-4}$ µl/mg at 30° C (Figure 2.4).

Ventilation volume (V_w) was calculated as the product of ventilatory rate (f_{sc}) and scaphognathite stroke volume (V_{sw}). Pairwise comparisons found significance between all temperature levels. Ventilation volume increased from 0.514 ± 0.051 to 3.060 ± 0.406 µl/mg/min from 10 to 20° C ($p < 0.0001$) and from 3.060 ± 0.406 to 4.307 ± 0.476 µl/mg/min from 20 to 30° C ($p = 0.026$) (Figure 2.5).

The direct method for measuring ventilatory volume gave a slightly higher value (29.61 ± 1.73 versus 28.65 ± 1.45 ml/30 min) than using the calculated ventilatory volume based on the 20° C average for the animals analyzed using the noninvasive method. This slightly higher value was not significant (p =0.07883) (Figure 2.7).

Discussion

High speed video microscopy provides a unique noninvasive method for approximating ventilation volume (V_w). Previous methods involved covering the animal's mouthparts with a mask and either restraining the animal and measuring the resulting outflow directly, or using an electromagnetic flow meter to detect the outflow (McMahon and Wilkens, 1983). Here scaphognathite frequency (f_{sc}), can be directly and non-invasively observed rather than through measurements of branchial pressure fluctuation or impedance (described McMahon *et al*., 1978) An index of scaphognathite stroke volume (V_{sw}) is measured separately which can account for beat-to-beat changes in stroke volume as opposed to previous methods which involve taking the total ventilation volume and dividing it by the f_{sc} to obtain V_{sw} (McMahon and Wilkens, 1983).

The volumes obtained for animals in control conditions (20° C and normoxia) using this dimensional analysis follow the approximate relationship between stroke volume and body mass previously described (McMahon and Wilkens, 1983) (Figure 2.6). A linear relationship is observed when *P. pugio* ventilation volumes are plotted with those of other decapods crustaceans in relation to body mass. Based on this relationship, this method of high speed video microscopy does appear to be an effective means of estimating ventilation volume in *P. pugio*.

Direct measurements of ventilatory flow are measured as an additional means of verification of the method. These measurements do not statistically differ from those calculated for the same animal based on the average ventilatory rate at 20° C using a paired t-test ($p = 0.07883$). The ventilatory rate for these animals could not be counted directly due to the small size of the animal and the divided chamber creating an obstructed view the anterior exhalant chamber. The fact that these measured volumes also fall within the same range as the calculated volumes adds additional support to the validity of this technique.

Measurements were made at 10, 20 and 30° C. Ventilatory rate (f_{sc}) varied with temperature increasing with increasing temperature, and decreasing with decreasing temperature (McMahon and Wilkens, 1983). These differences observed in these measurements are taken as a further verification that this method can capture changes in minute volume based on the ventilatory rate and scaphognathite stroke volumes.

A variety of observations have been made regarding the scaphognathite, or ventilatory, system. Some have described it as a fixed-volume pump, with V_{sw} being constant at all rates (McDonald et al., 1980; McMahon et al., 1978). Others have suggested that V_{sw} is fixed above certain frequencies, but adjustable at moderate rates (Burggren and McMahon, 1983). It appears that under normoxic conditions the scaphognathite system acts as a fixed volume pump at the moderate temperatures used for this analysis 10, 20 and 30° C (Figure 3). To determine if this holds true for temperature extremes, additional temperatures and rates should be tested.

This high speed video microscopy method is beneficial because it allows beat-to-beat measurement of the V_{sw} and can account for reversals. One disadvantage is that this method assumes bilateral pumping. Further investigations should be made to determine the frequency of unilateral pumping in this animal in addition to exploring ventilatory rates and volumes at an expanded temperature range.

Figures

Figure 2.1 Ventilatory anatomy of *P. pugio*

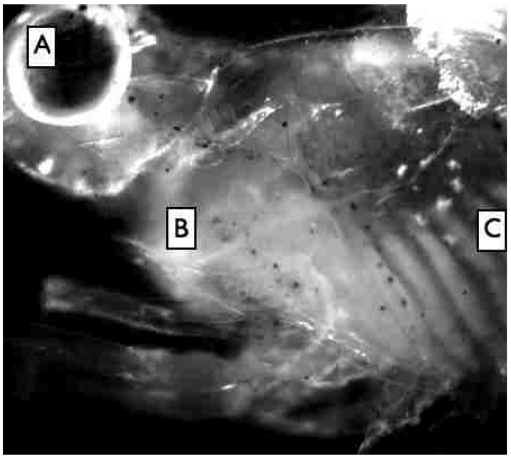

Figure 2.1: Ventilatory anatomy of *P. pugio.* A-eye, B-scaphognathite in anterior exhalant chamber, C-gills in branchial chamber. The direction of water flow is in through the branchial chamber over the gills and out the anterior exhalant chamber.

Figure 2.2 Model of a conical frustum

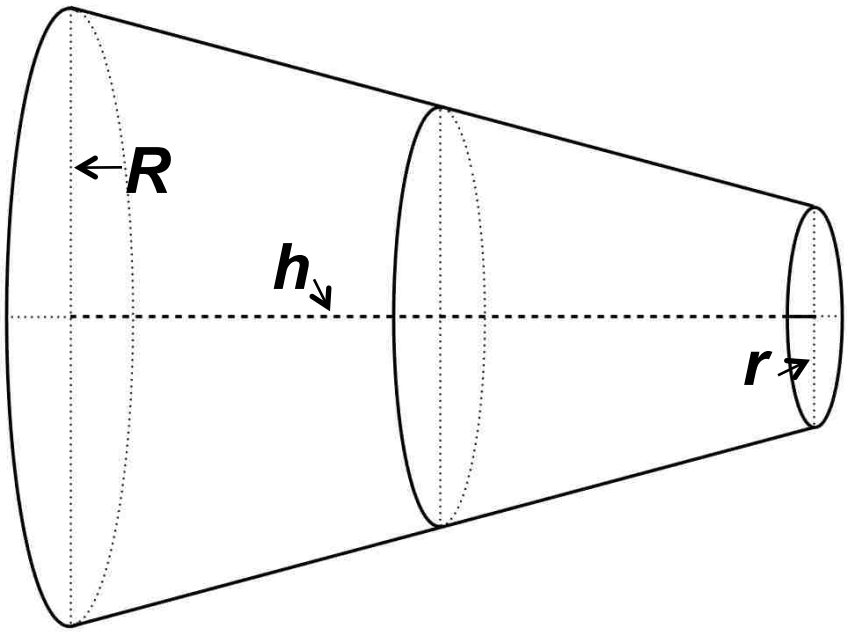

Figure 2.2: The anterior exhalant chamber is modeled as a conical frustum. *R* is the radius of base, *r* is the radius of top, and *h* is the height.

Figure 2.3 *P. pugio* ventilatory rate at varying temperatures

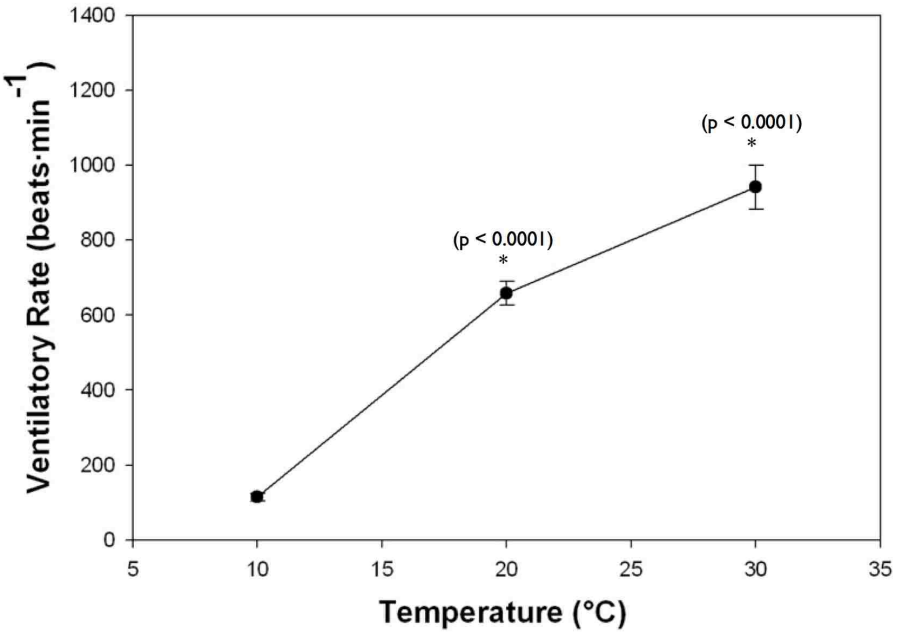

Figure 2.3: *P. pugio* ventilatory rate (f_{sc}) at varying temperatures (n = 7). One-way analysis of variance followed by pairwise t-tests was used to determine differences between values. Stars and p-values indicated significance from previous temperature. Error bars represent S.E.

Figure 2.4 *P. pugio* scaphognathite stroke volume at varying temperatures

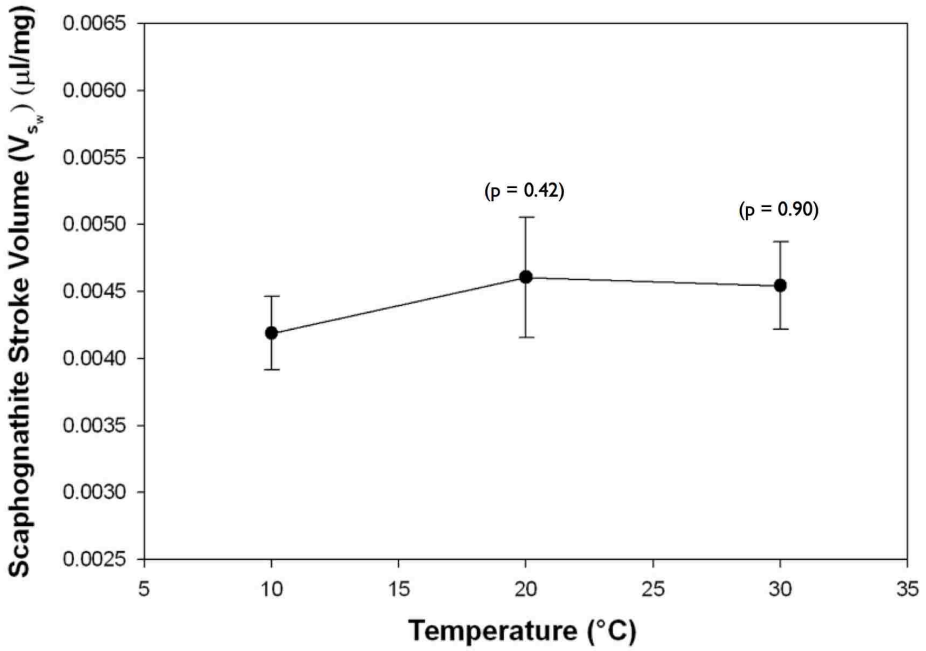

Figure 2.4: *P. pugio* scaphognathite stroke volume (V_{sw}) at varying temperatures (n = 7). One-way analysis of variance followed by pairwise t-tests was used to determine differences between values. Error bars represent S.E.

Figure 2.5 *P. pugio* ventilation volume at varying temperatures

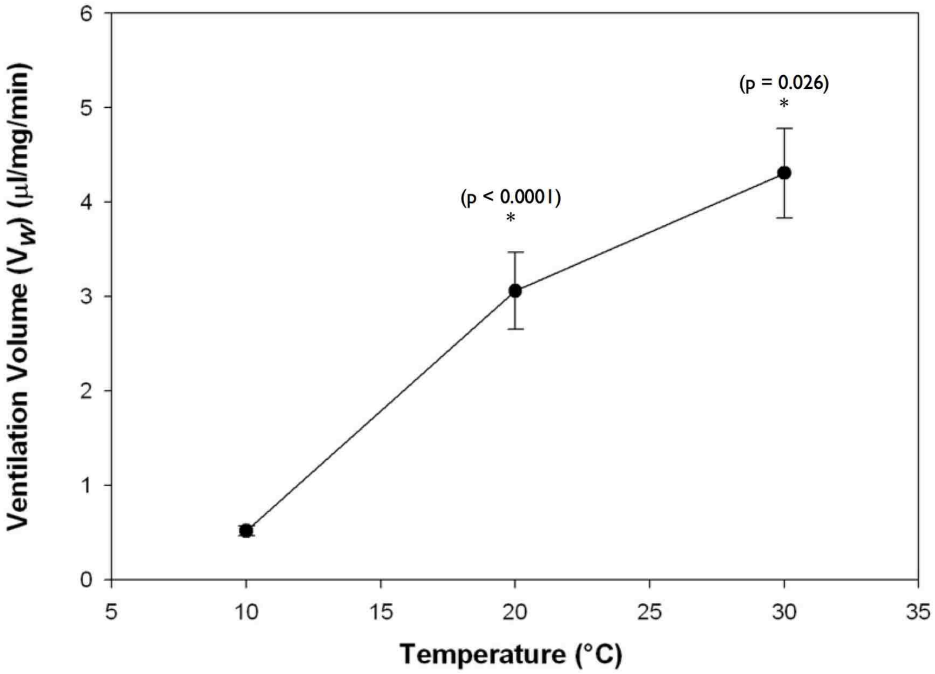

Figure 2.5: *P. pugio* ventilatory volume at varying temperatures (n = 7). One-way analysis of variance followed by pairwise t-tests was used to determine differences between values. Error bars represent S.E.

Figure 2.6 Relationship between scaphognathite stroke volume and body mass

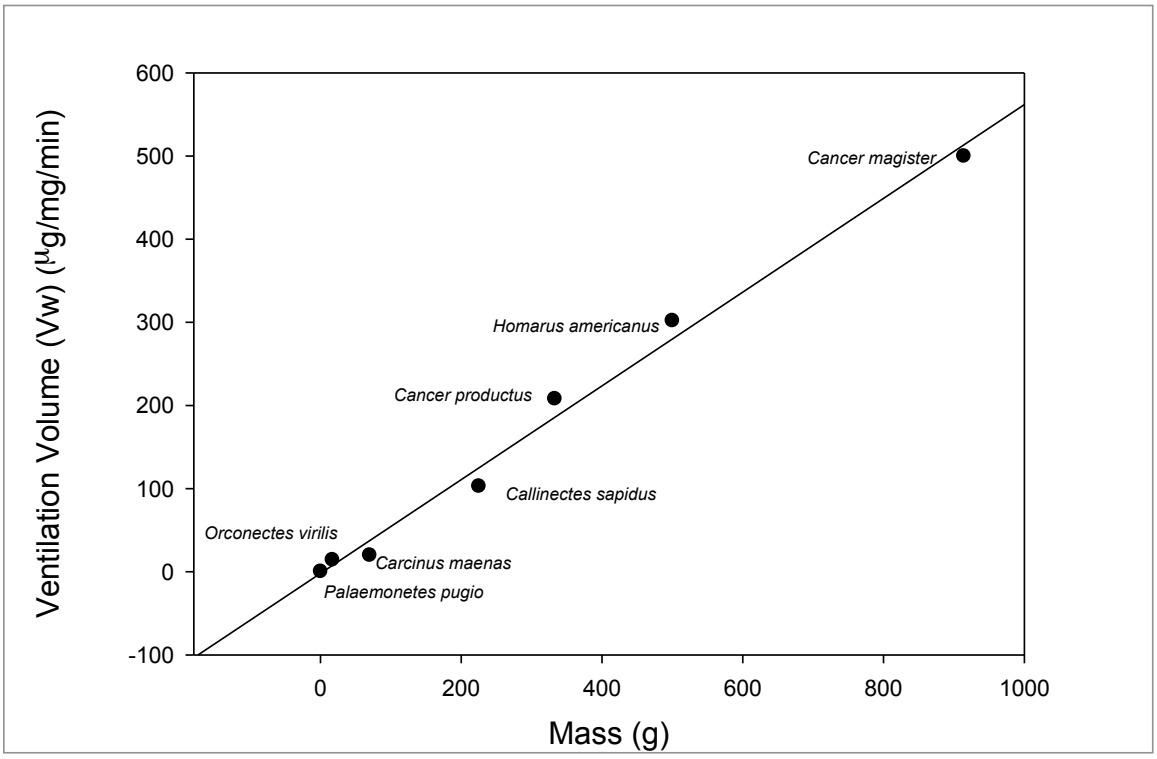

Figure 2.6: Relationship between scaphognathite stroke volume and body mass for seven species of decapod crustaceans including *P. pugio*. (Adapted from *(McMahon and Wilkens, 1983)*)

Figure 2.7 Comparison of direct and calculated methods

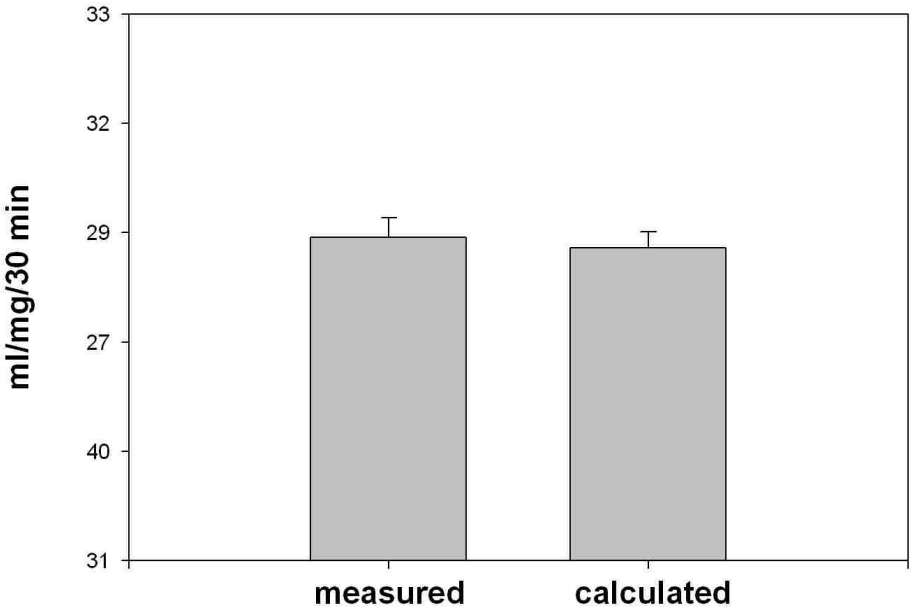

Figure 2.7: Comparison of direct and calculated methods for determining minute volume in *Palaemonetes pugio.* (n = 10) A paired t-test showed no significant difference between the two methods. (p = 0.07883)

CHAPTER 3

THE EFFECT OF TEMPERATURE AND DISSOLVED OXYGEN LEVELS ON VENTILATORY PARAMETERS IN THE GRASS SHRIMP, *Palaemonetes pugio*

Abstract

Aquatic poikilothermic animals must either cope with or compensate for the mismatch in oxygen supply and demand present at high temperatures. Oxygen limited thermal tolerance explains how aerobic scope is limited by insufficient oxygen supply and sets the performance window in animals. Here the ventilatory component of the oxygen delivery system was evaluated in *Palaemonetes pugio* for the effect of temperature and oxygen condition on ventilatory rate, scaphognathite stroke volume, and ventilatory volume. Additionally critical thermal maxima were established in both normoxic and hyperoxic conditions to determine if the mismatch in oxygen supply and demand can be mitigated by supplying more oxygen to the system. CT_{max} was higher in hyperoxia than normoxia indicating failure was not related to a specific temperature. Additionally there was a lack of lactate accumulation until CT_{max} in both normoxia and hyperoxia indicating *P. pugio* is maintains aerobic function until the point of failure. There was a significant effect of temperature on all the ventilatory parameters measured with a temperature dependent increase in ventilatory rate and volume. Hypoventilation and hypometabolism were observed in the hyperoxic condition which may be a mechanism to save the cost of energy expended for ventilation.

Introduction

Physiological rates including oxygen consumption, heart, and ventilatory rates are temperature dependent in poikilothermic animals (De Wachter and McMahon, 1996a; DeFur and Mangum, 1979; Frederich and Portner, 2000). These rates increase with increasing temperature (McMahon and Wilkens, 1983). The increased oxygen demand due to the increase in physiological rates can present a problem for aquatic organisms. Oxygen solubility in water decreases with increasing temperature (Hill et al., 2008). This results in a mismatch between oxygen supply and demand with an ever increasing demand for oxygen in the tissues and a decreasing supply in the water.

Investigations into how organisms compensate for this mismatch has led to the development of the hypothesis of oxygen-limited thermal tolerance. The concept of oxygen-and capacity-limited thermal tolerance explains how aerobic scope, the ability to increase metabolism on demand, is limited by insufficient oxygen supply at both sides of the thermal window and sets the performance window in animals (Portner, 2010). The oxygen delivery system can be viewed as being comprised of three broad components. The first is a component to move oxygen from the environment to a respiratory exchange area, or the ventilatory system. The second component is a system to deliver the oxygen from the respiratory exchange area to the tissues of the body, or the cardiovascular system. The third component is the use of that delivered oxygen at the cellular level, or aerobic metabolism of the cell (McMahon and Wilkens, 1983). Based on this hypothesis, a decrease in aerobic scope would be a directly caused by a decreased oxygen supply due

to a failure of the cardiac and ventilatory systems to deliver oxygen (Frederich and Portner, 2000; Pörtner, 2002; Portner et al., 2006).

Palaemonetes pugio is an estuarine decapod crustacean with a distribution range from the Gulf of Mexico to Canada. As an estuarine species, it subjected to wide fluctuations in environmental variables, including salinity, dissolved oxygen, and temperature, and as such has mechanisms in place to respond to these fluctuations. This makes it an ideal organism for the study of oxygen limited thermal tolerance (Anderson, 1985).

The first component of this work investigates the effect of acute temperature change on the ventilatory component of the oxygen delivery system. Ventilatory parameters are measured to determine how these physiological variables adjust in response to temperature stress. Knowledge of the upper limits of thermal tolerance is essential for understanding a species tolerance and adaptability for global climate change (Somero, 2010).

The second component investigates oxygen limited thermal tolerance. If the upper thermal tolerance limits are set by decreased oxygen availability, it should be possible to increase that upper limit by providing more oxygen to the system. Ventilatory parameters are measured in both normoxic and hyperoxic conditions to determine how and if those parameters adjust differently to compensate for the mismatch in oxygen supply and demand.

Materials and Methods

Animal Preparation

Grass shrimp, *Palaemonetes pugio*, were obtained from Gulf Specimen Marine Laboratories Inc (Panacea, FL, USA). Shrimp were maintained in 20L aquaria in aerated seawater (Coralife) (30 ±2 ppt) at 20˚C, with a 12:12 light:dark cycle for two weeks prior to experimental use. Shrimp were fed marine flakes (Ocean Nutrition Formula Two Flakes) three times a week with food withheld two days before experimentation. Shrimp were attached to the flattened end of a wooden applicator stick at the dorsal cephalothorax with cyanoacrylate glue. The animal was held in place and positioned within the experimental chamber with a micromanipulator (World Precision Instruments, Sarasota, FL, USA). A high speed video camera (Phantom Miro eX4, Vision Research, Wayne, NJ, USA) was placed over the chamber so video images of the heart and scaphognathite could be captured through the transparent exoskeleton (see methods from (Harper and Reiber, 1999).

Critical Thermal Maximum

Critical thermal maximum (CT_{max}) was determined by heating individual shrimp at a rate of 0.5 ˚C per minute from a starting point of 20 ˚C in a 250 ml beaker placed in a water bath. (VWR) Water oxygen partial pressures were maintained at either normoxia or hyperoxia using a gas mixing system (Cameron Instruments) with mixed air continuously supplied using an air stone. The animal was continuously observed during the heating process and CT_{max} was defined as loss of righting response.

Oxygen Consumption

Closed-system respirometry was carried out using a noninvasive optical sensing method. Individual shrimp were heated or cooled at a rate of 0.5 ˚C per minute from a starting point of 20 ˚C in a 250 ml beaker placed in a water bath. Water oxygen partial pressures were maintained at either normoxia (20 kPa, 150 Torr) or hyperoxia (26.6 kPa, 200 Torr) using a gas mixing system (Cameron Instruments) with mixed air continuously supplied through an air stone. Upon reaching experimental temperatures (5, 10, 15, 20, 25, 30, 35, 37º C), each animal was held for 15 minutes and placed in a 10-ml chamber containing normoxic (20 kPa, 150 Torr) or hyperoxic (26.6 kPa, 200 Torr) water, and an OxyDot sensor. A fluorescent dye immobilized in a gas permeable hydrophobic polymer was placed inside the chamber in the form of a dot. The dye in the dot absorbs in the blue region and fluoresces in the red region of the spectrum. Oxygen will quench both the emission intensity and lifetime of fluorescent light. This change in emission intensity and lifetime was measured to determine oxygen concentration at five-second intervals using a fiber optic pen. (OxySense 4000B, OxySense Inc.)

Lactate

Individual shrimp were heated at a rate of 0.5 ˚C per minute from a starting point of 20 ˚C in a 250 ml beaker placed in a water bath. Water oxygen partial pressures were maintained at either normoxia (20 kPa, 150 Torr) or hyperoxia (26.6 kPa, 200 Torr) using a gas mixing system (Cameron Instruments) with mixed air continuously supplied through an air stone. Each animal was held at experimental temperature (20, 25, 30, 35, 37º C) for 15 minutes before being flash frozen in liquid nitrogen and held for analysis.

An additional 10 animals per treatment were brought to loss of righting response and were flash frozen in liquid nitrogen and held for analysis. At the time of analysis, whole animals were homogenized in buffer on ice. The protein fraction was precipitated out using TCA and the resulting supernatant was analyzed using a commercially available lactate analysis kit (Pointe Scientific).

Experimental Design

Normoxic (20 kPa, 150 Torr) and hyperoxic (26.6 kPa, 200 Torr) sea water (30 ±2 ppt) at 20º C was placed in an experiment chamber and over the animal. Water oxygen partial pressure (P_{O2}) was maintained using a gas mixing system (Cameron Instruments) All animals were initially placed in the experimental chamber and allowed to acclimate for 30 minutes at 20º C, followed by a 30 minute temperature shift (either up or down from the initial 20º C to the next experimental temperature) (5, 10, 15, 20, 25, 30, 35, 37, 38, 39º C) with a 15 minute acclimation period for each experimental treatment. The temperatures were adjusted using a flow through external water jacket and circulating water bath. A minimum of 10 scaphognathite volume and rate recordings were made for each animal at each treatment (normoxic group n=7; hyperoxic group n=7).

Video Analysis, Ventilatory Functions

Video images for ventilatory analysis were acquired through the transparent exoskeleton at a rate of 240 Hz using a stereo-microscope (Leica Photo 6) equipped with a Phantom (Miro 4) high speed video camera. Video frames were analyzed using ImageJ (ImageJ, NIH image) to determine area of the anterior exhalant (scaphognathite) chamber using methods based on previous work used to model ventricular volume. (Harper &

Reiber, 1999) The anterior exhalant (scaphognathite) chamber is modeled as half of a conical frustum. Scaphognathite stroke volume (V_{sw}) determined as follows:

$$V = \frac{\frac{1}{3}\pi h\left(R^2 + Rr + r^2\right)}{2}$$

where *R* is the radius of base, *r* is the radius of top, and *h* is the height. Ventilation volume (V_W) is calculated as product of V_{SW} and f_{SC}**.** This final number was doubled to account for bilateral ventilation. Bilateral ventilation is assumed in all measurements. A minimum of 10 cycles for each animal at each temperature and environmental treatment

Statistical Analysis

Critical thermal maximum data were analyzed using R*(Team, 2012)*. All other data were analyzed using SAS v9.2 (SAS Institute, Cary, NC) using. Lactate and oxygen consumption were analyzed using two-way analysis of variance and pairwise t-tests. Ventilatory rate, scaphognathite stroke volume, and ventilation volume were analyzed using Proc Mixed. All values are reported as means ±SE with (n) indicating the number of animals.

Results

There was a significantly different critical thermal maximum (CT_{max}) measured at loss of righting response in normoxic (150 Torr) and hyperoxic (200 Torr) conditions. CT_{max} was significantly higher for animals in hyperoxia vs. normoxia (37.5°C normoxia, 38.8°C hyperoxia, n=31, t-test p-value 2.96E-13) (Figure 3.1).

Significant accumulation of lactate compared to baseline levels at 20°C occurred only in hyperoxic animals as they approached or reached CT_{max} with maximum levels

measured at 38°C. Pairwise comparisons of lactate levels for the interaction between temperature and oxygen levels indicated lactate accumulation was significantly higher in the hyperoxic compared to the normoxic condition at 25°C (0.94184 ± 0.1289 mmol/L vs. 0.39996 ± 0.0853 mmol/L, $p < 0.05$) and 30°C (1.21943 ± 0.2293 vs. 0.5725 ±0.7959 mmol/L and $p < 0.05$) and at CT_{max} (1.00508 ± 0.1403 vs. 0.27645 ± 0.0899$p < 0.01$) (Figure 3.2).

There was no significant difference in the interaction between temperature and normoxia or hyperoxia in scaphognathite stroke volume (V_{sw}). There was an effect of temperature with a significant difference in V_{sw} at low vs. high temperatures in both normoxia and hyperoxia (5 and 10°C vs. higher temperatures in hyperoxia and 5°C vs. higher temperatures in normoxia, $p < 0.05$). A greater variation in volumes at increasing temperatures was also observed (Figure 3.3).

Ventilatory rate (f_{sc}) increased linearly with temperature in both hyperoxic and normoxic conditions ($p < 0.01$). All animals in both treatments survived to 35°C, but only three survived 37°C in hyperoxia and five in normoxia. While there is no significant difference in rate for hyperoxic and normoxic animals at low temperatures, a statistically significant hypoventilation is observed in the hyperoxic condition vs. the normoxic condition at at 20°C (419.1895 +31.2895/-30.1634 vs. 576.5693 +36.5988/-35.4727 bpm) and 30°C (684.3706 +54.6064/-52.5106 vs. 890.8139 +62.1528/-60.0571 bpm) (Figure 3.4).

Ventilation volumes (V_w) increased linearly with temperature in both normoxic and hyperoxic conditions ($p < 0.05$). Ventilation volume is the product of ventilatory rate

and scaphognathite stroke volume. Since stroke volume remained constant in both dissolved oxygen conditions, this increase in (V_w) can be attributed to the increase in (f_{sc}). There is no significant difference between the normoxic and hyperoxic conditions, although there is a trend toward significance that corresponds to the significance seen in ventilatory rate (3.5).

Oxygen consumption increased with increasing temperature in both normoxic and hyperoxic conditions ($p < 0.05$). There was also a significant interaction between temperature and oxygen at all temperatures except 15 and 37°C ($p < 0.05$) (See Figure 3.6).

Discussion

Ventilation in aquatic breathers is based on adequate oxygen deivery to the tissues as opposed to acid-base status as seen in air breathing animals (Dejours, 1975). Oxygen and carbon dioxide are equally soluble in air, but in water, oxygen is 28 times less soluble than carbon dioxide (Gannon and Henry, 2004). Aquatic organisms therefore have a high ventilatory drive rate to extract oxygen required from water. Studies have investigated the effect of temperature on ventilation (Cumberlidge and Uglow, 1977; Frederich and Portner, 2000; McMahon et al., 1978) and hyperoxia on ventilation (Dejours et al., 1977), but few have investigated the effects of temperature and hyperoxia on the ventilatory system concomitantly (Portner et al., 2006; Weatherley, 1970).

With oxygen levels determining ventilatory drive, the delivery and availability of oxygen to the tissues may set thermal tolerance in water breathers. The oxygen delivery system can be described as having three main components, a ventilatory component to

extract oxygen from the environment, a pump which delivers that oxygen to the tissues of the body, and finally the metabolic machinery of the cell (McMahon and Wilkens, 1983). Failure or disruption of any one of these components can lead to reduced thermal tolerance as described by the theory of oxygen-limited thermal tolerance (Portner, 2010).

While failure may occur at different levels of organization, the highest level of organization is most sensitive to thermal extremes (Pörtner, 2002). Critical thermal maximum (CT_{max}) measures the effect on the animal a the highest level of individual organization. CT_{max} measured at loss of righting response (Nelson and Hooper, 1982) is evaluated to determine the effect of temperature on *P. pugio* at the highest level of organization, the whole organism. Based on the hypothesis of oxygen limited thermal tolerance, if the CT_{max} is related to a deficiency in the oxygen delivery system, then *P. pugio* should reach CT_{max} at a higher temperature when placed in a hyperoxic environment (Pörtner, 2002). CT_{max} is significantly higher for animals in hyperoxia vs. normoxia (37.5°C normoxia, 38.8°C hyperoxia, n=31, t-test p-value 2.96E-13) The higher CT_{max} observed in hyperoxia supports the hypothesis of oxygen limited thermal tolerance.

Lactate levels are measured to determine the temperature at which anaerobic metabolism begins (De Wachter et al., 1997). The lack of lactate accumulation indicates that the animals are receiving an adequate oxygen supply and not using anaerobic pathways until they reach CT_{max}. The lactate levels measured in *P. pugio* are within the same range as detected in other decapod crustaceans (Morris, 2005; Morris and Oliver, 1999). At the control temperature (20°C) there is no significant difference in lactate levels

between the normoxic and hyperoxic conditions (p=0.6598) This matches previous findings of a lack of association between normoxic and hyperoxic oxygen levels and lactate accumulation (Morris et al., 1988). At CT_{max}, animals in the hyperoxic environment had significantly more lactate accumulation than those in the normoxic environment ($p < 0.05$). A switch to anaerobic metabolism at CT_{max} indicates a mismatch between oxygen supply and demand (Frederich and Portner, 2000). The abrupt increase in lactate levels observed in hyperoxic animals nearing CT_{max} could indicate a rapid failure of the oxygen delivery system. Additionally, these animals are switching to anaerobic respiration in aerated water, indicating failure is due to the limits of the delivery system rather than a lack of oxygen availability in the environment.

The ventilatory system of *P. pugio* is similar to that of crayfish and lobster (Withers, 1992). The scaphognathite, also known as the gill bailer, housed in the anterior exhalant chamber, oscillates creating a negative pressure in the branchial chamber (Withers, 1992). Water is pulled into the inhalant, or hypobranchial, chamber, over the walking legs or the posterior and ventral edges of the loosely fitting carapace. The water then circulates over the gills, and out the anterior exhalant chamber (Withers, 1992).

A variety of observations have been made regarding the scaphognathite system. It has been described as a fixed-volume pump, with V_{sw} being constant at all rates (McDonald et al., 1980; McMahon et al., 1978). An alternative explanation is that V_{sw} is fixed above certain frequencies, but adjustable at moderate rates (Burggren and McMahon, 1983).

These data show the scaphognathite does not act as a fixed pump in *Palaemontes pugio*. While the interactions between temperature and oxygen are not significant there is a significant effect of temperature between 5 and 37°C for both normoxic and hyperoxic conditions ($p < 0.05$). It appears that in normoxia, the scaphognathite system acts as a fixed volume pump at more moderate temperatures, with V_{sw} varying at temperature extremes. While not statistically significant in these data, it appears in hyperoxia V_{sw} is more variable than normoxia at higher temperatures. The apparent difference between ventilation volumes in normoxia and hyperoxia at 20° C can be attributed to both decreased f_{sc} and decreased V_{sw} in hyperoxia. This is in contrast to observations in the crayfish, *Oroconectes virilis*, which show the opposite relationship (Burggren and McMahon, 1983).

Palaemonetes pugio exhibited a temperature dependent increase in ventilatory rate (scaphognathite frequency, f_{sc}) in both normoxia and hyperoxia,($p < 0.05$) however there was a significant hypoventilation at 20 and 30°C in hyperoxia compared to normoxia. ($p < 0.05$) This temperature range represents the animals' functional range, or the range at which the animal is most commonly found (Anderson, 1985).

The decreased ventilatory rate observed in hyperoxia at 20 and 30°C, may represent a mechanism for the animal to save the cost of energy expended for ventilation without adversely effecting the oxygen diffusion gradient (Morris and Taylor, 1985). Hypoventilation has been previously noted in hyperoxia (Dejours and Beekenkamp, 1977). The decreased minute volumes observed in hyperoxia may represent a means for

the animal to save the cost of energy expended for ventilation without adversely effecting the oxygen diffusion gradient (Morris and Taylor, 1985).

Oxygen consumption was measured as a means to determine oxygen delivery at the cellular level. While oxygen consumption was not significantly different between normoxia and hyperoxia at 15 and 37°C, there was a significant difference between the two conditions at all other temperatures ($p < 0.05$), with the hyperoxic condition being hypometabolic compared to the normoxic condition.

Under experimental conditions, the primary physiological parameters contributing to metabolism are cardiac contractions and ventilatory movements. Two cardiac parameters contributing to overall cardiac output are heart rate and cardiac stroke volume. Studies on the effect of temperature on cardiac parameters have shown that while heart rate increases with increasing temperature, the increase is the same in both normoxia and hyperoxia (Mika &Reiber, in prep). Cardiac stroke volume is higher in hyperoxia. It has been suggested that heart rate and cardiac stroke volume are controlled separately, with heart rate responding to changes in primary stimuli, such as temperature, and cardiac stroke volume changing in response to metabolic demand (De Wachter and McMahon, 1996a).Here we observe an increased cardiac stroke volume, but measure an apparently decreased metabolic demand (Mika&Reiber, in prep).

The lack of difference between heart rate in normoxia and hyperoxia leaves the ventilatory movements as the primary contributors to overall metabolism. While there may appear to be a slight trend to increased scaphognathite stroke volume with increasing temperature in hyperoxia (5 and 10°C vs. higher temperatures $p < 0.05$) the volume was

not statistically different from that observed in normoxia. While not statistically significant at all temperatures, there was a trend toward hypoventilation in hyperoxia compared to normoxia. The ventilatory rate in hyperoxia is 77, 86, 78, 90, and 93% of that observed in normoxia at 20, 25, 30, 35, 37°C respectively. The oxygen consumption can be divided by the beats per minute observed at each temperature to get a cost per beat. This can be used as an index of the metabolic cost for ventilation that can be used to compare the two conditions. This index shows that oxygen consumption in hyperoxia was 71, 65, 80, 73% of that observed in normoxia at 20, 25, 30, 35°C degrees respectively. With the assumption that the primary metabolic costs of an animal in these conditions are cardiac and ventilatory movements, and determining that the cardiac movements are the same in hyperoxic and normoxic conditions, the hypoventilation observed may be a direct result of the hypometabolism. Less delivery is required to fuel the tissues. This pattern is reversed at 37 degrees Celsius with the metabolic cost per beat in hyperoxia being 136% of normoxia. This may be attributed to the continuation of cardiac and ventilatory function at higher temperatures in hyperoxia and the failure in normoxia (Mika and Reiber in prep).

Hemolymph oxygen affinity increases as dissolved oxygen content drops. This means that in the hyperoxic condition, ventilation can be reduced without adversely effecting the oxygen diffusion gradient (Mangum, 1983). Additionally, oxygen affinity increases at low temperature, such that at the lower temperatures used here, the affinity is high enough that deoxygenation is observed at the tissue level (Mangum, 1983).

Hypoventilation in hyperoxia is a means by which the animal can reduce energy expenditure (Morris and Taylor, 1985).

The ventilatory patterns observed in *P. pugio* may be a direct consequence of this decreased metabolism rather than regulation of oxygen uptake (Moshiri et al., 1970). In the case of *P. pugio* it may be that response to temperature is a passive response while the response to hyperoxia is a direct consequence of decreased metabolism rather than regulation of oxygen uptake.

Figures

Figure 3.1 *P. pugio* critical thermal maxima in normoxic and hyperoxic conditions

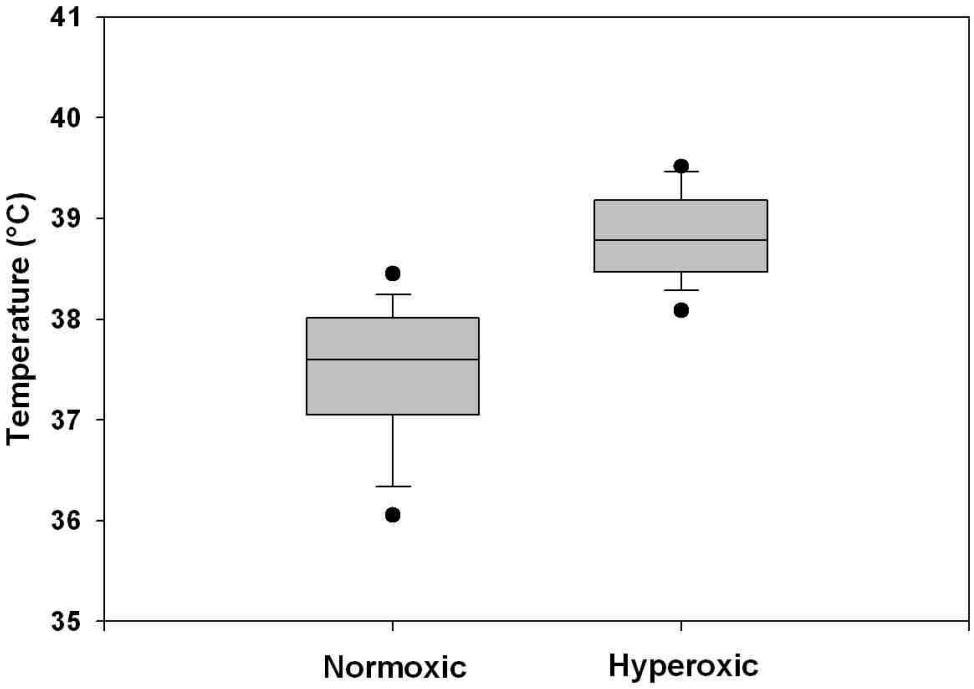

Figure 3.1: Boxplot of *P. pugio* critical thermal maximum (CT_{max}) measured at loss of righting response in normoxic (150 Torr) and hyperoxic (200 Torr) conditions. CT_{max} is significantly higher for animals in hyperoxia vs. normoxia (37.5°C normoxia, 38.8°C hyperoxia, n=31, t-test p-value 2.96E-13) The center line is the median, top and bottom edges of the boxplot represent 75th and 25th percentiles, whiskers 95th and 5th percentiles and the dots individual outliers.

Figure 3.2 Lactate levels at varying temperatures in normoic and hyperoxic conditions

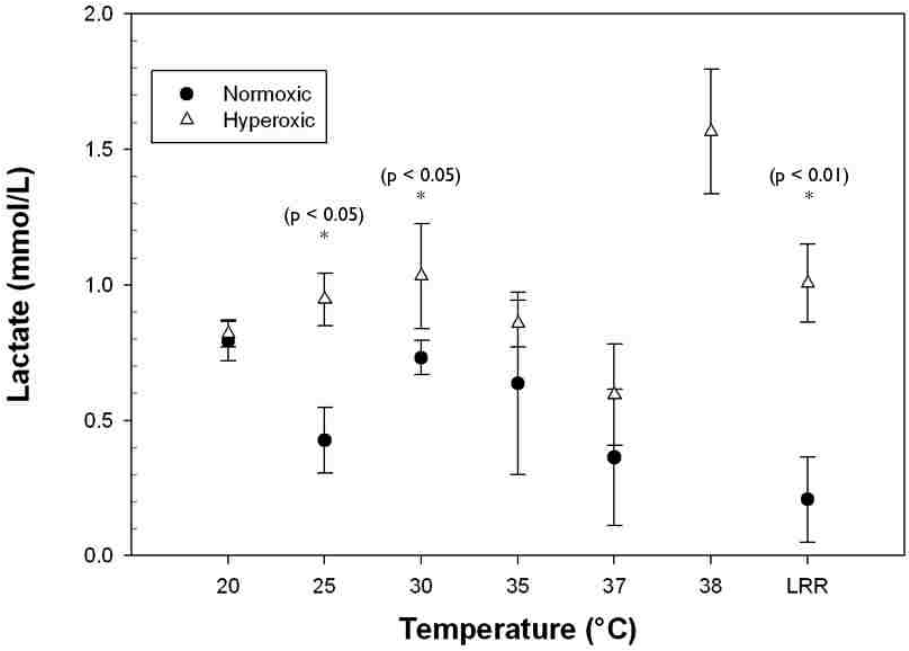

Figure 3.2: Lactate levels measured at varying temperatures in normoxic and hyperoxic conditions (n = 10). Two-way analysis of variance followed by pairwise t-tests was used to determine differences between values.

Figure 3.3 *P. pugio* scaphognathite stroke volume at varying temperatures in normoxic and hyperoxic conditions

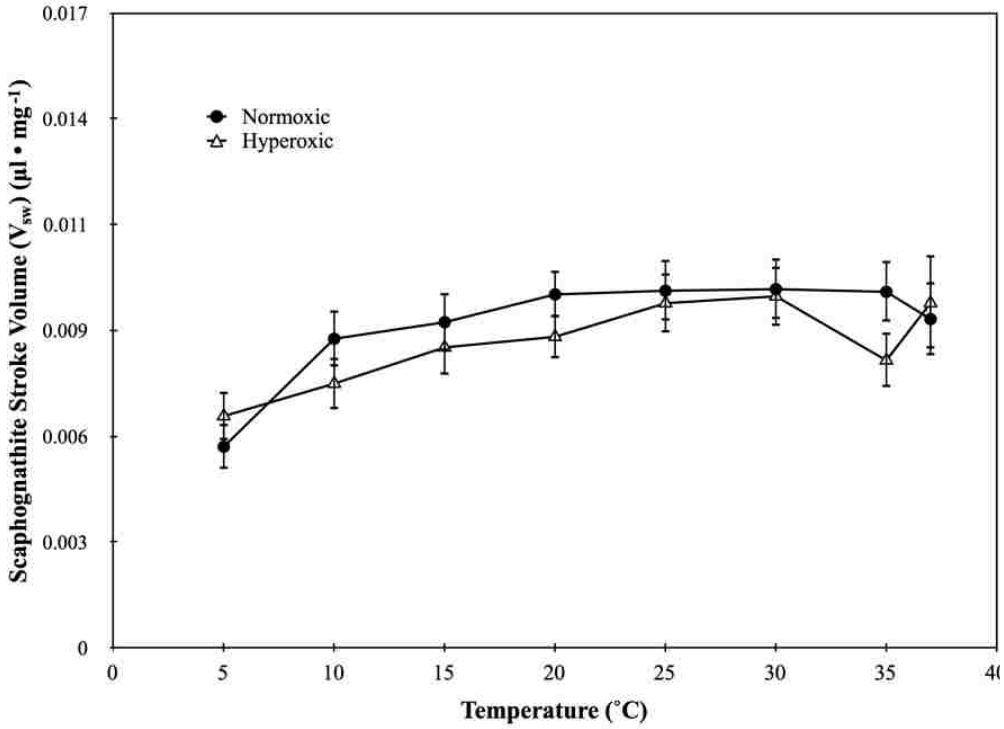

Figure 3.3: *P pugio* scaphognathite stroke volume (V_{SW}) at varying temperatures (n = 10 5-35°C, n = 5 normoxia 37°C and n = 3 at 37°C). Repeated measures linear mixed effects analysis followed by pairwise t-tests was used to determine differences between values. No significant difference in the interaction between oxygen and temperature was detected. Error bars represent S.E.

Figure 3.4 *P. pugio* ventilatory rate at varying temperatures in normoxic and hyperoxic conditions

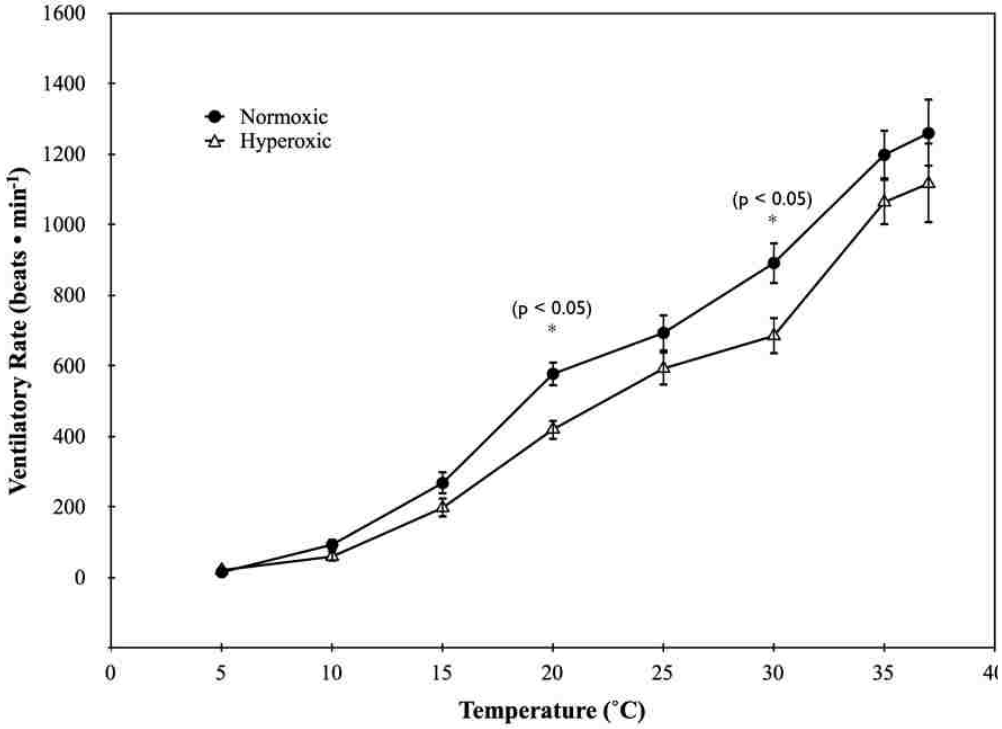

Figure 3.4: *P. pugio* ventilatory rate (f_{SC}) at varying temperatures (n = 10 5-35°C, n = 5 normoxia 37°C and n = 3 at 37°C). Repeated measures linear mixed effects analysis followed by pairwise t-tests was used to determine differences between values. Stars and p-values indicate a significant difference in the interaction between oxygen and temperature. Error bars represent S.E.

Figure 3.5 *P. pugio* ventilation volume at varying temperatures in normoxic and hyperoxic conditions

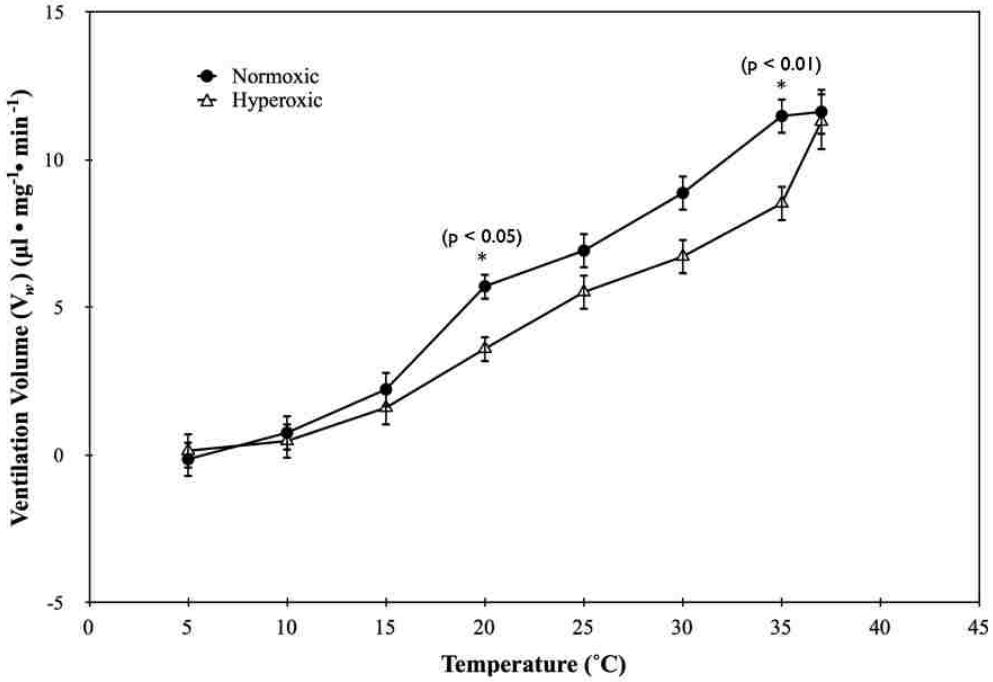

Figure 3.5: *P pugio* ventilation volume (V_W) at varying temperatures (n = 10 5-35°C, n = 5 normoxia 37°C and n = 3 at 37°C). Repeated measures linear mixed effects analysis followed by pairwise t-tests was used to determine differences between values. No significant difference in the interaction between oxygen and temperature was detected. Error bars represent S.E.

Figure 3.6 Oxygen consumption at varying temperatures in normoxic and hyperoxic conditions

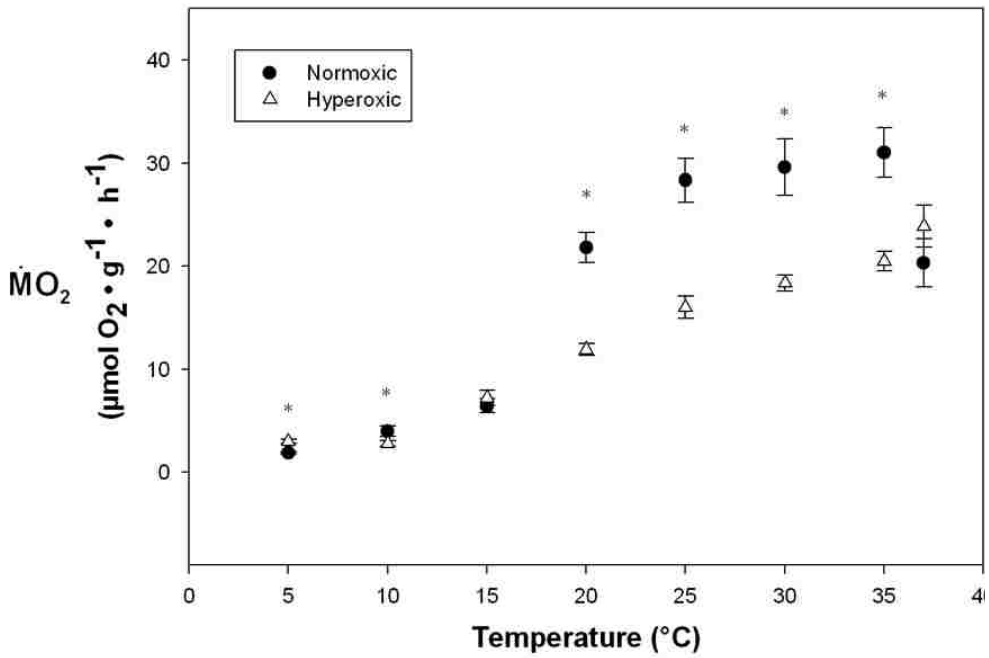

Figure 3.6: Oxygen consumption at varying temperatures (n = 10 5-37°C) Stars indicate a significant difference in the interaction between oxygen and temperature ($p < 0.05$) Error bars represent S.E.

CHAPTER 4

THE EFFECT OF TEMPERATURE AND DISSOLVED OXYGEN LEVELS ON CARDIOVASCULAR PARAMETERS IN THE GRASS SHRIMP, *Palaemonetes pugio*.

Abstract

Metabolic rate increases with increasing temperature in poikilothermic animals, to maintain aerobic metabolism, internal oxygen delivery rates must also increase. Additionally aquatic animals must deal with a mismatch in oxygen supply and delivery due to decreased oxygen solubility at high temperatures and the increased demand with metabolic rate. Cardiac parameters including heart rate, stroke volume, cardiac output, and ejection fraction were determined over a temperature range (5-39º C) in normoxic and hyperoxic conditions in *Palaemonetes pugio* to determine when and how the system failed. There is a temperature dependent tachycardia and a similar increase in cardiac output, but stroke volume is temperature insensitive. There was a significant effect of oxygen condition on stroke volume with a higher stroke volume in hyperoxia. These effects on heart rate and stroke volume support neural control of heart rate and metabolic control of stroke volume. Cardiac function is maintained to CT_{max} in both normoxic and hyperoxic conditions. Analysis of pressure data for time in cardiac cycle points to a biomechanical limitation leading to cardiac failure.

Introduction

Over time many species have evolved a number of physiological, biochemical and/or molecular mechanisms to acclimate or adapt to environmental temperature

fluctuations experienced in aquatic environments (Florey and Kriebel, 1974; Ivleva, 1980; Karlsson et al., 2011; McDonald et al., 1977). Decapod crustaceans are among the organisms which have been investigated for the effects of temperature with a specific focus on temperature effects on the cardiovascular system (DeFur and Mangum, 1979; Frederich and Portner, 2000; Hilton et al., 2010; Jury and Watson, 2000). There are a number of intrinsic and extrinsic factors controlling heart rate in these crustaceans (McMahon, 1999). Intrinsically the neurogenic heart is controlled by a cardiac ganglion embedded in the cardiac muscle (Cooke, 2002). Extrinsic factors include two pair of cardioacceleratory and one pair of cardioinhibitory nerves that innervate both the cardiac ganglion and the heart muscle itself (McMahon, 1999) and a pericardial organ secreting neurohormones (McMahon and Wilkens, 1983; Wilkens and McMahon, 1992). Any effect of temperature on cardiovascular parameters is in addition to the complex interaction of these factors. The cardiovascular system of these crustaceans can be highly sensitive to temperature extremes and may be the limiting physiological system that establishes the critical thermal maximum temperature which can establish a defined crustacean species habitat range (Stillman, 2002; Stillman, 2004).

In poikilothermic animals, as temperature increases, metabolic demand increases (Hill et al., 2008). At the same time, oxygen solubility decreases with increasing temperature (Hill et al., 2008). This observation has led to the hypothesis of oxygen limited thermal tolerance as an explanation for cardiac failure at temperature extremes, especially in marine organisms (Pörtner, 2002). Oxygen limited thermal tolerance

explains the limitation of aerobic scope, or the ability to increase metabolism on demand, at temperature extremes (Portner, 2010).

Cardiovascular responses to temperature vary among species depending on the mechanisms employed to maintain oxygen delivery to the tissues and include, changes in heart rate (f_H), stroke volume (V_s), and hemolymph flow, etc. Typically, crayfish, crabs and lobsters, exhibit a temperature-induced tachycardia with a concomitant decrease in V_s to maintain cardiac output (V_b) at a constant level (De Wachter and McMahon, 1996a; De Wachter and McMahon, 1996b).

This study investigates the mechanisms by which temperature dependent cardiac failure occurs, or why these animals cannot compensate for extreme temperatures and as such do not survive. There are three broad areas of focus for cardiac failure related to the intrinsic and extrinsic control of the heart; the cardiac pacemaker (cardiac ganglion), myocardial contractile mechanisms, and cardiac physiological function (McMahon and Wilkens, 1983), with the last as the focus of this chapter.

Heart rate and/or stroke volume may be altered to maintain cardiac output at a level which matches oxygen supply with demand. A number of the previous studies on cardiac parameters used an isolated or *in vitro* heart preparation. With the removal of other influences, including the acceleratory and inhibitory neurons and neurohormonal control from the pericardial organ, the isolated preparation shows the intrinsic pumping performance of the heart. This does not necessarily represent how the heart responds *in vivo* as demonstrated in a study comparing the effect of temperature on isolated heart and whole animal preparations (De Wachter and McMahon, 1996b). The whole animal

preparation shows a temperature dependent tachycardia while in the isolated preparation heart rate appears to be temperature insensitive. This indicates the temperature dependence of heart rate is only partly related to control of the cardiac ganglion and the cardioacceleratory nerves and pericardial organ play a role as well. The isolated preparation does not fully represent the whole animal response (De Wachter and McMahon, 1996b).

In the current study, whole animal preparations were used and in addition to changes in f_H, V_s, V_b, and ejection fraction, time phases of the cardiac cycle are analyzed to establish how the heart responds to changing temperature by adjusting contractile phase mechanisms allowing the heart to beat faster and thus increase oxygen delivery to the tissues.

Materials and Methods

Animal Preparation

Grass shrimp, *Palaemonetes pugio*, were obtained from Gulf Specimen Marine Laboratories Inc. (Panacea, FL, USA). Shrimp were maintained in 20L aquaria in aerated seawater (Coralife) (30 ±2 ppt) at 20˚C, with a 12:12 light:dark cycle for two weeks prior to experimental use. Shrimp were fed marine flakes (Ocean Nutrition Formula Two Flakes) three times a week with food withheld two days before experimentation. Shrimp were attached to the flattened end of a wooden applicator stick at the dorsal cephalothorax with cyanoacrylate glue. The animal was held in place and positioned within the experimental chamber with a micromanipulator (World Precision Instruments, Sarasota, FL, USA). A high speed video camera (Phantom Miro eX4, Vision Research,

Wayne, NJ, USA) was placed over the chamber so video images of the heart and could be captured through the transparent exoskeleton (see methods from (Harper and Reiber, 1999).

Experimental Design

Normoxic (20 kPa, 150 Torr) and hyperoxic (26.6 kPa, 200 Torr) sea water (30 ±2 ppt) at 20º C was placed in an experiment chamber and over the animal. Water oxygen partial pressure (P_{O2}) was maintained using a gas mixing system (Cameron Instruments) All animals were initially placed in the experimental chamber and allowed to acclimate for 30 minutes at 20º C, followed by a 30 minute temperature shift (either up or down from the initial 20º C to the next experimental temperature) (5, 10, 15, 20, 25, 30, 35, 37, 38, 39º C) with a 15 minute acclimation period for each experimental treatment. The temperatures were adjusted using a flow through external water jacket and circulating water bath. A minimum of 10 cardiac cycle recordings were made for each animal at each treatment (normoxic group n=10; hyperoxic group n=7).

Video Analysis, Cardiac Functions

Video images for cardiac analysis were acquired through the transparent exoskeleton at a rate of 240 Hz using a stereo-microscope (Leica Photo 6) equipped with a Phantom (Miro 4) high speed video camera. Video frames were analyzed using ImageJ (ImageJ, NIH image) to determine area of the lumen of the heart using methods based on previous work (Harper & Reiber, 1999). The heart was modeled as a trapezoid. Cardiac stroke volume (V_s) is determined as follows:

$$V_s = w(0.5h(b + a))$$

where w is the width, h is the height, a is the base length, and b is top length. Height, base length, and top length are measured, and width of the heart was determined to be 0.64h (systole) and 0.67h (diastole) (Harper and Reiber, 1999).

Cardiac output (V_b) is calculated as product of f_h and V_s. A minimum of 10 cycles for each animal at each temperature and environmental treatment were measured

Statistical Methods

Heart rate was analyzed using a permutation based ANOVA with a Bray-Curtis distance metric is used to assess significance. Pairwise comparisons were made using permutation based post hoc tests with Bonferroni adjusted p-values presented. These analyses were all done using 'distlm' software *(Anderson, 2004)*. Stroke volume, cardiac output, and ejection fraction were analyzed using mixed effects analysis of variance to assess significance. Tukey tests are used for pairwise comparisons. These analyses were done in SAS v9.2 (SAS Institute, Cary NC) using Proc Mixed. To meet assumptions of the analysis, stroke volume and cardiac output were square-root transformed. Back-transformed least-squares means and standard errors are reported for these variables. Minute volume and ejection fraction were not transformed, and as such least-squares means and standard errors are reported unchanged.

Results

There was a significant effect of temperature on heart rate ($p < 0.05$). *P. pugio* exhibited a temperature dependent tachycardia in both normoxic and hyperoxic conditions. All animals survive to 35°C. There was no significant difference between treatments until 37°C (normoxia n=8 468.875 ± 39.445 bpm, hyperoxia n=7 535.714 ±

20.006 bpm; $p < 0.05$) and 39 °C (normoxia n=4 284.000 ± 97.935 bpm, hyperoxia n=4 625.750 ± 20.006; $p < 0.05$) (Figure 4.1).

While stroke volume (V_s) was significantly higher in hyperoxic than normoxic conditions at most of the temperatures measured (5°C 132.947 + 14.904/-14.112 vs. 226.324 +22.51/-21.450 µl/min; 15°C 126.799 +14.141/-13.394 vs. 204.654 + 21.439/-20.371 µl/min; 20°C 136.242 +11.768/-11.281 vs. 223.766 +18.001/-17.305 µl/ min; 30°C 131.416 +14.389/-13.641 vs. 202.657 +21.337/-20.269 µl/min; 35°C 130.070 +14.317/-13.570 µl/min; $p < 0.05$), there was no significant difference within either condition (Figure 4.2).

Cardiac output (V_b), as the product of heart rate and stroke volume, exhibited a significant increase with increasing temperature in both normoxic and hyperoxic conditions, as well as a significant difference between normoxia and hyperoxia at 20, 35, 37 and 39º C (20º C 33.79 + 3.31/-3.15 vs. 53.60 +4.97/-4.75 ml/min; 35º C 71.03 +6.17/-5.91 vs. 110.20 +9.17/-8.81 ml/min; 37º C normoxia n=8 72.15 +6.68/-6.39 vs. hyperoxia n=7 103.57 +8.90/-8.53 ml/min; 39º C normoxia n=4 36.64 +6.21/-5.73 ml/ min vs. hyperoxia n=4 117.14 +11.40/-10.88; $p < 0.05$) (Figure 4.3).

Ejection fraction (calculated as stroke volume/end diastolic volume) was insensitive to both temperature and oxygen treatment with no significant effects between or within treatments (Figure 4.4).

Discussion

Grass shrimp tolerate a wide range of seasonal temperature variations of between 2-38°C with optimal temperature conditions being close to 25°C (Vernberg and

Piyatiratitivorakul, 1998). As is the case for many crustaceans, the cardiovascular system is the first physiological system to fail at extreme temperature (Stillman, 2002) and as such may be one of the primary systems determining the animals critical thermal maximum (CT_{max}). Based on the hypothesis of oxygen limited thermal tolerance, failure of the cardiovascular system at extreme temperatures may be attributed to a breakdown in the oxygen delivery system (Pörtner, 2002). If CT_{max} is influenced by oxygen limitation, then theoretically CT_{max} should increase in a hyperoxic environment. Previous data collected in our lab indicate critical thermal maximum increases in a hyperoxic environment, with a CT_{max} of 37.5°C in normoxia, and 38.8°C hyperoxia (Mika and Reiber, in prep).

Here this examination was extended to investigate the effect of temperature and oxygen condition on the cardiovascular component of the oxygen delivery system. A temperature dependent tachycardia was observed in both normoxic and hyperoxic conditions ($p < 0.05$), a pattern also observed in other decapod crustaceans (De Wachter and McMahon, 1996b; DeFur and Mangum, 1979; Worden et al., 2006) A break was observed between normoxia and hyperoxia at 37°C, a temperature approaching the normoxic CT_{max}, with heart rate continuing to increase in hyperoxia but decrease in normoxia ($p < 0.05$). This pattern is even more pronounced at 39°C, with a very sharp decline in heart rate in normoxia and a continued increase in hyperoxia ($p <0.05$). Maintenance of heart rate in the hyperoxic environment beyond normoxic CT_{max} supports the hypothesis of oxygen limited thermal tolerance, as cardiovascular function is maintained once the oxygen limitation is mitigated (Pörtner et al., 2006a).

In addition to temperature dependent tachycardia, heart rate also became more variable as the animals approach CT_{max}. This increase in variability has also been observed in the freshwater shrimp *Palaemonetes antennarius* (Ungherese et al., 2008). The variability may be a sign of the decline of regulatory mechanisms leading to failure of the animal at increased temperature.

Q_{10}s were calculated as a reference for the temperature sensitivity of heart rate (Table 4.1). Heart rates in hyperoxia and normoxia showed no significant difference between treatments until 37°C, and as such the Q_{10} values are similar to that temperature as well. Q_{10}s for heart rate would be expected to fall in the physiological range of 2.0-2.5 (DeFur and Mangum, 1979). Previous research suggests that as temperature increases Q_{10} tends to decrease in progressive manner (Krogh, 1914). *P. pugio* follows this pattern and falls below 2.0 at 30°C. While a Q_{10} of less than 1.5 may indicate a reduction in temperature sensitivity (De Wachter and McMahon, 1996a), the fact that it remained above 1.0 indicates that heart rate was still sensitive to increasing temperature across the full range in hyperoxia and only falls below 1.0 at CT_{max} in normoxia.

Stroke volume (V_s) did not vary with temperature in *P. pugio* which is in distinct contrast from the decrease in stroke volume with temperature noted in *Homarus americanus,* and *Cancer magister* (De Wachter and McMahon, 1996a; De Wachter and McMahon, 1996b; De Wachter and Wilkens, 1996; Worden et al., 2006). This indicates stroke volume is temperature independent in *P. pugio*. This lack of a change in stroke volume with tachycardia does follow a similar pattern observed following injection of branchial oxygen in *Procambarus clarkii* (Reiber, 1997). In the current study, there was a

significant interaction between temperature and oxygen condition with a higher stroke volume in hyperoxia compared to normoxia across most of the temperature range tested. The increased stroke volume in hyperoxia is attributed to both a higher end diastolic volume and end systolic volume compared to normoxia (Figure 4.5).

Studies of cardiac performance in *Cancer magister* suggest that heart rate and stroke volume are controlled separately with heart rate responding to changes in primary stimuli such as temperature and stroke volume changing in response to variation in metabolic demand (De Wachter and McMahon, 1996a). Results from the Reiber lab follow this pattern, with heart rate responding to temperature and stroke volume changing in response to metabolic demand in hypoxia (Guadagnoli et al., 2011). Factors influencing heart rate also influence ventilatory rate showing how tightly the two systems are tied together (McMahon, 1999). In *P. pugio*, both heart and ventilatory rate increase with increasing temperature (Mika and Reiber, in prep). The control centers for each of these organs are neurally mediated with the cardiac ganglion controlling heart rate (Cooke, 2002) and a central pattern generator for ventilatory rate (McMahon and Wilkens, 1983), although peripheral oxygen sensors may also play a role in adjusting ventilatory rates with changes in oxygen tension (Kusakabe et al., 1991; Wilkens et al., 1989).

The increase in heart rate and maintenance of stroke volume does not lead to a maintenance of cardiac output over the temperature range as described in other species (De Wachter and McMahon, 1996b; Worden et al., 2006), but an increase in cardiac output with increasing temperature and oxygen conditions. The significant increase in

cardiac output with temperature is attributed to the heart rate component of cardiac output, while the significant difference between oxygen conditions is attributed to stroke volume. This pattern has also been described in studies of the effect of exercise on cardiac output. With exhaustive exercise, circulating oxygen levels are depleted and cardiac output increases due to an increase in stroke volume. During low exercise, circulating oxygen levels do not decrease and increases in cardiac output are due to and increase in heart rate (McMahon, 1999). These results suggest heart rate is neurally mediated while stroke volume is influenced by oxygen sensors responding to hemolymph oxygen content or other neurohormonal input (McMahon, 1999).

The increase in cardiac output with temperature in *P. pugio* falls in line with the hypothesis of oxygen limited thermal tolerance. The higher cardiac output in the hyperoxic condition delivers more oxygen to the tissues which in turn supports a higher CT_{max} in hyperoxia.

Maintenance of cardiovascular variables, including heart rate, stroke volume and cardiac output to CT_{max} indicates *P. pugio* copes with acute temperature stress to the point of CT_{max}. Maintenance of cardiac output beyond 37°C in the hyperoxic condition indicates cardiovascular failure is not due to the 37°C temperature, but that another mechanism may be responsible.

Ejection fraction does not vary over the temperature range or by treatment. This indicates that a cardiomyopathy is not responsible for failure at extreme temperatures. If the cardiac muscle were damaged, ejection fraction would change at CT_{max} which isn't the case. Based on these data, it is not likely that impairment of the cardiac muscle itself

leads to cardiac failure. While ejection fraction is a useful measure to indicate contractility, its calculation depends on end diastolic volume according to Starling's law which brings in the extrinsic variable of preload. Future independent measures of contractility are required to determine if it is responsible for higher thermal tolerance in the hyperoxic condition (McMahon and Wilkens, 1983).

To determine if the increased heart rate is contributing to cardiac failure, previously collected pressure data was analyzed to determine time in cardiac cycle, providing insight into the biomechanics of heart beat. Analysis of pressure data to investigate the individual aspects of heart rate indicated that the increase in heart rate is a direct function of a decrease in time in diastole, with the time in systole fixed across heart rates while time in diastole decreases asymptotically until it is functionally flat (Figure 4.6). With the variables of stroke volume and time in systole being fixed, the maximal heart rate, and thus oxygen delivery rate, is dependent on cardiac filling time. At the point where diastole or the cardiac filling rate approaches zero the animal can no longer maintain cardiac output and as such meet metabolic demands. Diastolic filling time then becomes the limiting factor.

Future studies in the lab will involve a continuation of these pressure measurements to further analyze time in cardiac cycle to determine the effect of rate on diastolic filling time. This will be coupled with video to create pressure-area loops which can be used to measure stroke and cardiac work as well as contractility independent of preload and afterload.

These results provide important insights into how high environmental temperature effects cardiac function. The maintenance of cardiovascular parameters in hyperoxia beyond normoxic CT_{max} supports the hypothesis of oxygen limited thermal tolerance implicating reduced cardiac output and therefore oxygen delivery in cardiac failure.

Figures

Figure 4.1 *P. pugio* heart rate at varying temperatures in normoxic and hyperoxic conditions

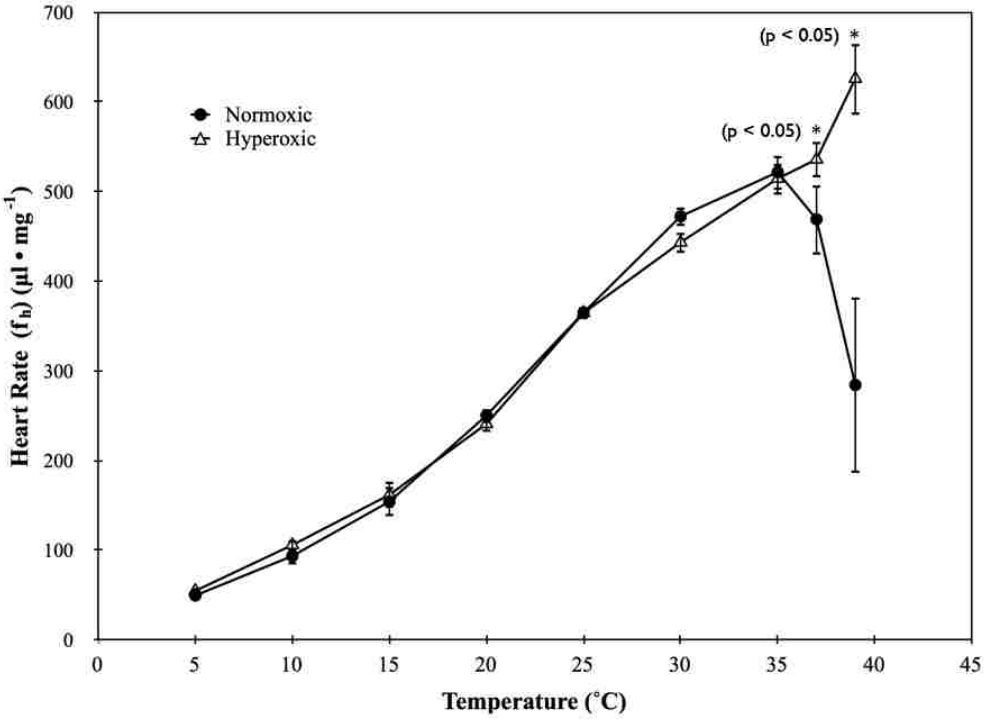

Figure 4.1: *P pugio* heart rate (f_H) at varying temperatures (normoxia n = 10 5-35°C, hyperoxia n=7 5-35°C; normoxia n=8 37°C, hyperoxia n=7 37°C; normoxia and hyperoxia n=4 39°C). Permutation based ANOVA with a Bray-Curtis distance metric was used to assess significance. Pairwise comparisons were made using permutation based post hoc tests with Bonferroni adjusted p-values presented. No significant difference in the interaction between oxygen and temperature was detected from 5-35°C. A significant interaction between oxygen and temperature was observed at 37 and 39°C. Error bars represent S.E.

Figure 4.2 *P. pugio* stroke volume at varying temperatures in normoxic and hyperoxic conditions

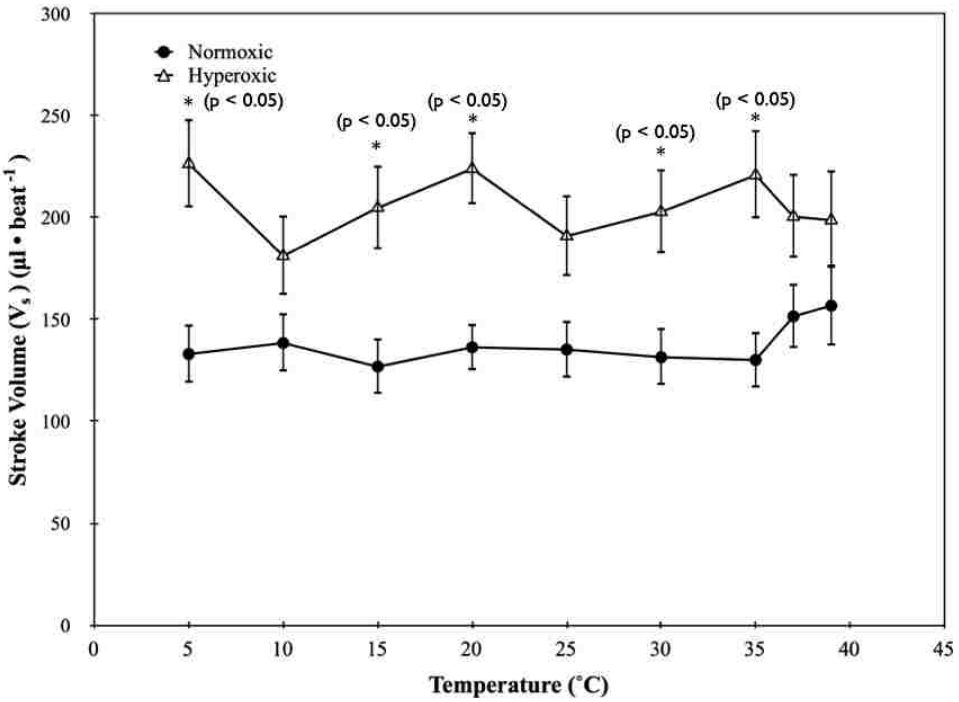

Figure 4.2: *P pugio* stroke volume (V_s) at varying temperatures (normoxia n = 10 5-35°C, hyperoxia n=7 5-35°C; normoxia n=8 37°C, hyperoxia n=7 37°C; normoxia and hyperoxia n=4 39°C). Mixed effects analysis of variance was used to assess significance. Tukey tests are used for pairwise comparisons. No significance of temperature was noted within either normoxia or hyperoxia. A significant interaction between oxygen and temperature was observed at 5, 15, 20, 30, and 35°C. Error bars represent S.E.

Figure 4.3 *P. pugio* cardiac output at varying temperatures in normoxic and hyperoxic conditions

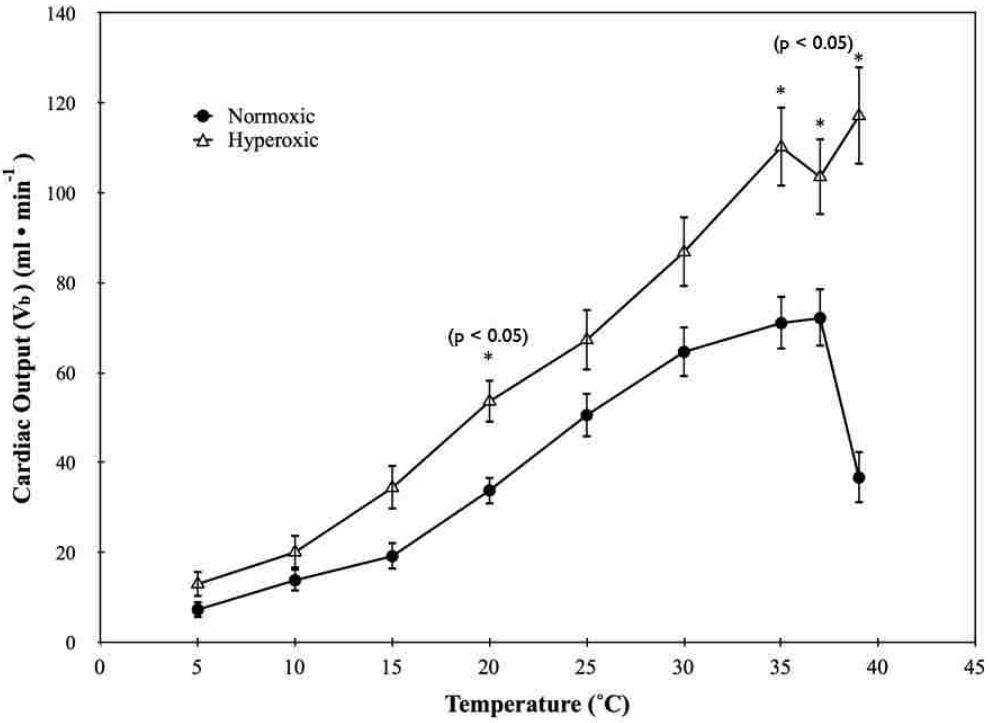

Figure 4.3: *P pugio* cardiac output (V_b) at varying temperatures (normoxia n = 10 5-35°C, hyperoxia n=7 5-35°C; normoxia n=8 37°C, hyperoxia n=7 37°C; normoxia and hyperoxia n=4 39°C). Mixed effects analysis of variance was used to assess significance. Tukey tests were used for pairwise comparisons. Temperature has a significant effect in both normoxia and hyperoxia ($p < 0.05$). A significant interaction between oxygen and temperature was observed at 20, 30, 35, and 39°C. Error bars represent S.E.

Figure 4.4 Ejection fraction at varying temperatures in normoxic and hyperoxic conditions

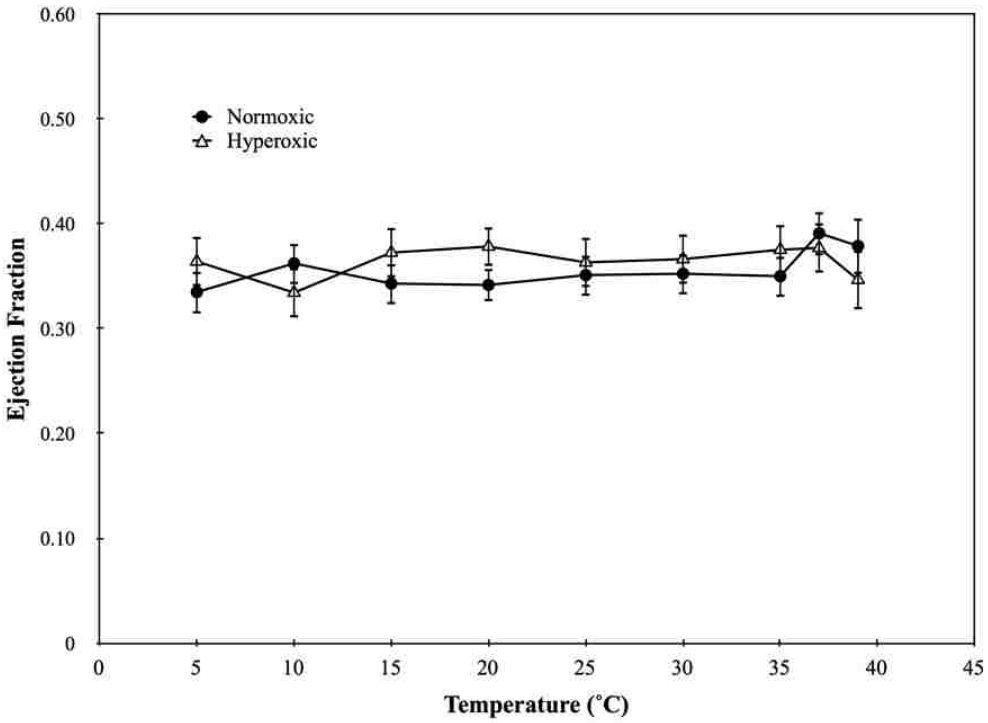

Figure 4.4: Ejection fraction calculated as stroke volume/end diastolic volume at varying temperatures (normoxia n = 10 5-35°C, hyperoxia n=7 5-35°C; normoxia n=8 37°C, hyperoxia n=7 37°C; normoxia and hyperoxia n=4 39°C). Mixed effects analysis of variance were used to assess significance. Tukey tests were used for pairwise comparisons. No significance of temperature was noted within either normoxia or hyperoxia. No significant difference in the interaction between oxygen and temperature was noted at any temperature tested. Error bars represent S.E.

Table 4.1 Q10 for heart rate in normoxia and hyperoxia

Temperature Range	Normoxia Q_{10}	Hyperoxia Q_{10}
5-10°C	3.59	3.76
10-15°C	2.72	2.34
15-20°C	2.64	2.22
20-25°C	2.13	2.29
25-30°C	1.68	1.48
30-35°C	1.22	1.35
35-37°C	0.59	1.23
37-39°C	0.08	2.17

Table 4.1: Q_{10}s for heart rate calculated across the temperature range in both normoxia and hyperoxia.

Figure 4.5 Comparison figure of *P pugio* end diastolic (EDV) and end systolic (ESV) volumes at varying temperatures and oxygen condition

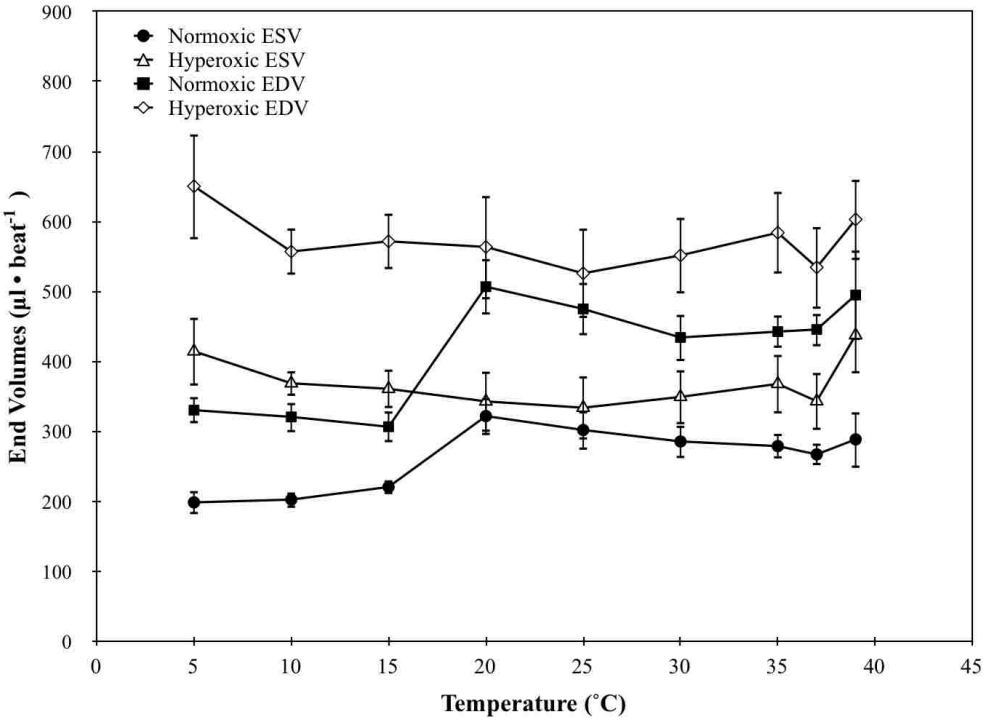

Figure 4.5: Comparison figure of *P pugio* end diastolic (EDV) and end systolic (ESV) volumes at varying temperatures and oxygen conditions (normoxia n = 10 5-35°C, hyperoxia n=7 5-35°C; normoxia n=8 37°C, hyperoxia n=7 37°C; normoxia and hyperoxia n=4 39°C).

Figure 4.6 Survey of time in cardiac cycle vs. heart rate in *P. pugio.*

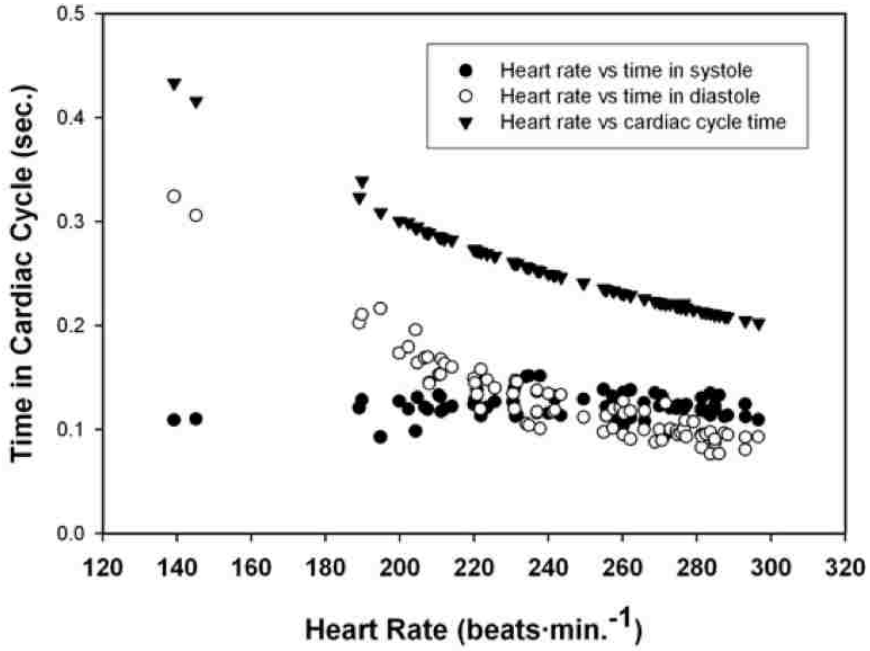

Figure 4.6: Survey of time in cardiac cycle vs. heart rate in *P. pugio.*

CHAPTER 5

CHANGES IN CARDIAC PERFORMANCE WITH TEMPERATURE AND DISSOLVED OXYGEN LEVELS ON CARDIOVASCULAR PARAMETERS IN THE GRASS SHRIMP, *Palaemonetes pugio*.

Abstract

Poikilothermic animals experience a mismatch in oxygen supply and demand with increasing temperature. This has led to the hypothesis of oxygen limited thermal tolerance with limitation in aerobic scope and limitations of the oxygen delivery systems setting critical thermal maxima. Oxygen limited thermal tolerance explains why organisms fail at high temperatures, but does not describe a mechanism for failure. Thermal tolerance may be related to a failure of the cardiovascular system at high temperatures in decapod crustaceans. This work investigates mechanisms of cardiac failure at high temperatures in the decapod crustacean, *Palaemonetes pugio. P. pugio* could respond to the oxygen supply and demand issue by either increasing supply through increased cardiac output, or through decreased demand through reduced cardiac work. This work used integrated micropressure signals and high speed video microscopy to measure intracardiac pressure and cardiac volumes respectively to generate pressure-area loops. The high speed video data provides data on heart rate, stroke volume, and cardiac output. Pressure-area loops were generated to provide estimates of stroke work, cardiac work, and myocardial oxygen consumption. All experiments were performed in both normoxic and hyperoxic conditions to determine how the system responds to reduced oxygen availability at extreme temperatures and if that response is altered in a

hyperoxic environment. Here cardiac output was maintained to CT_{max} in both normoxic and hyperoxic conditions, but there was no significant difference between them. The change in pressure (Δ P) was reduced at high temperatures in hyperoxia compared to normoxia leading to a reduction in stroke and cardiac work in hyperoxia. This reduction in cardiac work led to cardiac failure at higher temperatures in hyperoxia than normoxia.

Introduction

Poikilothermic animals experience a mismatch in oxygen supply versus demand with increasing temperature (Hill et al., 2008). This mismatch has led to the hypothesis of oxygen limited thermal tolerance with failure of the animal at temperature extremes due to a limitation in aerobic scope and limitations of the oxygen delivery systems setting critical thermal maxima (Portner, 2010). Decapod crustaceans are among the species that exhibit this temperature dependent failure. The cardiovascular system of these species has been implicated as the physiological system establishing thermal limits (Stillman, 2002).

The hypothesis of oxygen limited thermal tolerance presents an explanation for cardiac failure at temperature extremes, but it doesn't describe a physiological mechanism responsible for cardiac failure. There are two possible mechanisms to compensate for cardiac failure. An organism could either deliver more oxygen to the most metabolically active systems with the highest demand like the ventilatory and cardiovascular systems. In terms of cardiovascular response this would be accomplished through an increase in cardiac output. An increase in cardiac output could be accomplished through an increase in heart rate or stroke volume. Conversely an organism

could reduce oxygen demand through a reduction in cardiac work by reducing heart rate, stroke work, or vascular resistance, thus minimizing the effects of oxygen limitation. In both of these scenarios it might be possible to extend critical thermal maxima.

The cardiovascular system in *P. pugio* and decapod crustaceans in general consists of a single neurogenic ventricle, a distribution system of seven arterial valves leading to seven arteries for delivery and sinuses and ostia for return to the ventricle, and hemolymph as the oxygen carrying fluid (McLaughlin, 1983). Oxygen delivery requires coordination of all components of this system and failure of the cardiovascular system could be related to any of these components.

The cardiovascular response of decapod crustaceans to increased temperature varies among species. Typically, heart rate (f_H) increases with increasing temperature (DeFur and Mangum, 1979). Stroke volume may either decrease to maintain cardiac output (De Wachter and McMahon, 1996b) or increase to increase cardiac output (Mika and Reiber, in prep). The reduction in stroke volume with increased heart rate may be a result of a biomechanical limitation in diastolic filling time (Mika and Reiber, in prep).

Changes in pressure in the distribution system may also affect oxygen delivery. Redistribution of hemolymph flow to different arterial systems has been measured in response to hypoxia (Guadagnoli and Reiber, 2005). Additionally contractile elements may be present in the arterial system beyond the cardioarterial valves which could result in vascular change (Wilkens et al., 2008).

While many studies have examined alterations in independent factors during temperature exposure, the data thus far have not been integrated to determine whether

these changes benefit the animal by reducing cardiac work and determining if cardiac failure is the result of limits to heart performance or limits to delivery capability. Here in addition to changes in f_H, V_s, cardiac output (V_b) and ejection fraction, intra-cardiac pressure was also measured to determine time in cardiac cycle and generate pressure-area loops to measure cardiac energetics. The hypotheses of increased cardiac output, reduced cardiac work, and reduced diastolic filling time leading to cardiac failure at temperature extremes were tested. In addition, experiments were run in both normoxic and hyperoxic environments to determine if limitations in thermal tolerance are due to a breakdown of the cardiovascular component of the oxygen delivery system.

Materials and Methods

Animal Preparation

Grass shrimp, *Palaemonetes pugio*, were obtained from Gulf Specimen Marine Laboratories Inc. (Panacea, FL, USA). Shrimp were maintained in 20L aquaria in aerated seawater (Coralife) (30 ±2 ppt) at 20˚C, with a 12:12 light:dark cycle for two weeks prior to experimental use. Shrimp were fed marine flakes (Ocean Nutrition Formula Two Flakes) three times a week with food withheld two days before experimentation. Shrimp were attached to the flattened end of a wooden applicator stick at the dorsal cephalothorax with cyanoacrylate glue. The animal was held in place and positioned within the experimental chamber with a micromanipulator (World Precision Instruments, Sarasota, FL, USA). A high speed video camera (Phantom Miro eX4, Vision Research, Wayne, NJ, USA) was placed over the chamber so video images of the heart and could be

captured through the transparent exoskeleton (see methods from (Harper and Reiber, 1999).

Experimental Design

Normoxic (20 kPa, 150 Torr) and hyperoxic (26.6 kPa, 200 Torr) sea water (30 ±2 ppt) at 20º C was placed in an experiment chamber and over the animal. Water oxygen partial pressure (P_{O2}) was maintained using a gas mixing system (Cameron Instruments) All animals were initially placed in the experimental chamber and allowed to acclimate for 30 minutes at 20º C, followed by a 30 minute temperature shift (either up or down from the initial 20º C to the experimental temperature of either 5, 10, 15, 20, 25, 30, 35, 37, 38º C with a 15 minute acclimation period for each experimental treatment. The temperatures were adjusted using a flow through external water jacket and circulating water bath. A minimum of three 7 second readings are collected for each animal at each treatment from which a minimum of 5-10 cardiac cycles are analyzed (normoxic group n=7; hyperoxic group n=7).

Intraventricular Pressure

Intraventricular pressure was measured using a servo-null pressure system (model 900A, WPI) and synchronized with digital video data using a data acquisition device (USB-6341 X Series DAQ , National Instruments) at a rate of 600 Hz. A glass micro-pipette (2-5 mm diameter tip filled with 3M NaCl) was inserted through the soft dorsal arthrodial membrane at the junction of the thorax and abdomen and advanced into the ventricle using a micromanipulator (World Precision Instruments). The servo-null pressure system measures resistance at the tip of the pipette and exerts a counter pressure

in response to any change in pressure at the tip. Intraventricular pressure was calculated as the difference between the measured pressure within the ventricle and the zero-pressure recorded when the tip was placed in the experimental chamber at a level adjacent to the heart (Guadagnoli et al., 2007; Tobita and Keller, 2000).

Video Analysis, Cardiac Functions

Video images for cardiac analysis including pressure-area (P-A) loops were acquired through the transparent exoskeleton at a rate of 600 Hz using a stereo-microscope (Leica Photo 6) equipped with a Phantom (Miro 4) high speed video camera. High speed video collection was synchronized with pressure data using a data acquisition device (USB-6341 X Series DAQ, National Instruments, Austin, TX). Video files were analyzed using imageJ (NIH) to determine maximum and minimum cardiac areas representing end diastolic and end systolic volumes respectively. These data were used in a customized LabView (National Instruments, Austin, TX) program to determine frame by frame pixel changes at the leading edge of the ventricle to measure ventricular area. Pressure and area data were integrated to form pressure-area loops using the LabScribe Pressure Volume module (iWorx, Dover, NH).

Stroke volume was determined by modeling the heart as a trapezoid. Cardiac stroke volume (V_S) is determined as(Harper and Reiber, 1999) follows:

$$V_s = w(0.5h(b + a))$$

where w is the width, h is the height, a is the base length, and b is top length. Height, base length, and top length are measured, and width of the heart was determined to be $0.64h$ (systole) and $0.67h$ (diastole) (Harper and Reiber, 1999).

Cardiac output (V_b) was calculated as product of f_H and V_s. over a minimum of 5 to 10 cycles for each animal at each temperature and environmental treatment.

Statistical Methods

Two way ANOVA was used to determine effects of temperature, oxygen condition, and the interaction between temperature and oxygen condition. Pairwise t tests were performed to determine differences between oxygen conditions at each temperature.

Results

There was a significant effect of temperature on heart rate ($p < 0.05$). *P. pugio* exhibit a temperature dependent tachycardia. There is no difference between the normoxic and hyperoxic conditions with the exception of 37°C where heart rate was significantly higher under hyperoxia (562.275714 ± 38.10509 vs. 635.805143 ± 22.10141) (Figure 5.1).

There was no significant effect of temperature on stroke volume (V_s) in both the normoxic and hyperoxic conditions. Stroke volume was significantly higher in normoxic than hyperoxic conditions at 30°C, there were no significant differences at any of the other temperatures tested (109.04882 ± 18.98390 vs. 74.65973 ± 12.99723 µl/min; $p < 0.05$) (Figure 5.2).

When cardiac output (V_b) is calculated as the product f_H and V_s, no significant effect of temperature or oxygen condition was observed within or between treatments (Figure 5.3). Similarly, ejection fraction (calculated as stroke volume/end diastolic volume) was insensitive to both temperature and oxygen treatment with no significant effects between or within treatments (Figure 5.4).

Minimum pressure (P_{min}) was significantly lower in hyperoxic conditions than normoxic conditions at 5 and 25°C (0.191286 ± 0.33293 vs. -15.032714 ± 1.80443 mmHg $p < 0.001$; -28.719571 ± 2.90256 vs. -38.733714 ± 2.11417 mmHg $p < 0.05$), but there were no significant differences at any of the other temperatures tested (Figure 5.5).

Maximum pressure (P_{max}) was significantly lower in hyperoxic conditions than normoxic condtions at 5°C (16.287286 ± 1.12928 vs. 0.58000 ± 0.26900 mmHg), but there were no significant differences at any of the other temepratures tested (Figure 5.6).

There was a significant effect of temperature on ΔP in both normoxic and hyperoxic conditions ($p < 0.05$). There was a significant difference between normoxic and hyperoxic conditions at 37° C (14.936398 ± 1.89500 vs. 9.420881 ± 1.19587 mmHg $p < 0.01$). ΔP approaches significance between normoxic and hyperoxic conditions at 10, 20, and 30° C (10° C 20.226551 ± 2.56752 vs. 14.922379 ± 1.89422 mmHg; 20° C 31.470674 ± 3.99482 vs. 23.208480 ± 2.94604 mmHg; 30° C 18.941495 ± 2.40439 vs. 14.137591 ± 1.79460 mmHg) (Figure 5.7).

There was a significant effect of temperature on *dp/dt* in both normoxic and hyperoxic conditions ($p < 0.05$), but no significant difference between conditions. (Figure 5.8).

There was a significant effect of temperature on stroke work in both normoxic and hyperoxic conditions ($p < 0.05$). Stroke work was significantly lower in hyperoxic conditions than normoxic conditions at 5° C (0.05696 ± 0.01842 vs. 0.023 ± 0.00744 mJoules $p < 0.05$) and 37° C (0.02154 ± 0.00697 vs. 0.00491 ± 0.00159 mJoules $p < 0.001$) (Figure 5.9).

There was a significant effect of temperature on cardiac work ($p < 0.05$). There was a significant difference between normoxic and hyperoxic conditions at 37° C (11.939048 ± 3.92338 vs. 3.112978 ± 1.02298 mJoules/min $p < 0.001$) (Figure 5.10).

Discussion

Grass shrimp tolerate seasonal temperature variations between 2-38°C and have been shown to have a thermal maximum of between 37-39°C. As is the case for many crustaceans the heart is the first physiological system to fail at extreme temperature (Stillman, 2002) and as such may be one of the primary systems determining the animals critical thermal maximum (CT_{max}). Critical thermal maximum experiments were run in normoxia and hyperoxia to determine if failure is due to a breakdown in the oxygen delivery system. Increased CT_{max} observed in hyperoxia compared to normoxia suggests that this may be the case (Mika and Reiber, in prep).

Pressure-area (P-A) loops provide the same information as pressure-volume loops and have been generated to describe cardiovascular dynamics in several different species including *P. pugio* (Guadagnoli et al., 2007; Guadagnoli et al., 2011; Keller et al., 1991). Video and pressure data were collected synchronously both to create P-A loops to determine changes in cardiac energetics and analysis of cardiac and pressure parameters.

As is the case with many decapod crustaceans, *P. pugio* exhibit a temperature dependent tachycardia in both normoxic and hyperoxic conditions (DeFur and Mangum, 1979) with no significant difference in heart rate (f_H)between the conditions until 37° C. At this temperature f_H in normoxia falls while it continues to increase in hyperoxia until a decrease at 38°C. This decrease correlates with the critical maximum temperatures

previously described for both with a CT_{max} of 37.5°C in normoxia, and 38.8°C hyperoxia(Mika and Reiber, in prep).

Stroke volume (V_s) may either decrease with temperature (De Wachter and McMahon, 1996b; De Wachter and Wilkens, 1996; Worden et al., 2006) or be temperature insensitive (Mika and Reiber, in prep). Here V_s was temperature insensitive matching previous research in *P. pugio* studies. In previous studies, there was a significant difference in V_s stroke volume between the normoxic and hyperoxic conditions with a significantly lower V_s in hyperoxia (Mika and Reiber, in prep). In this study there was no significant difference between the oxygen conditions except at 30°C with stroke volume higher in the normoxic condition. At 37°C the volumes are nearly identical. Even though the volumes are identical, there is more oxygen per hemolymph volume in the hyperoxic condition. Maintaining V_s in hyperoxia still provides more oxygen for delivery than the normoxic condition, providing a potential solution for increasing oxygen limited thermal tolerance.

Once cardiac output (V_b) was calculated as the product of f_H and V_s, the significances observed in f_H and V_s alone were no longer present. There may be a variety of V_b responses to temperature. It may decrease with increasing temperature (De Wachter and McMahon, 1996b), increase with increasing temperature (De Wachter and Wilkens, 1996), or it may be temperature insensitive (Worden et al., 2006). In *P. pugio,* there was a significant effect of temperature with V_b increasing with increasing temperature. No significant difference was observed between the two oxygen treatments. With the maintenance of stroke volume, increases in cardiac output can be attributed to increased

heart rate. There was a trend for decreased V_b at 37°C in normoxia and an increase in hyperoxia. Normoxic animals reached CT_{max} and failed at 37.5°C while hyperoxic animals maintained function to a CT_{max} of 38.8°C (Mika and Reiber, in prep). As with V_s, although there was no significant difference between oxygen conditions, more oxygen per volume was delivered in hyperoxia. Additionally oxygen consumption is decreased in the hyperoxic condition (Mika and Reiber, in prep). At 37°C, not only is more oxygen being delivered in hyperoxia, less oxygen is required. Thus t other components of the cardiovascular system must be responsible for cardiac failure.

Intraventricular pressures have been previously measured in other decapod crustaceans (Belman, 1975; Reiber, 1994), however few studies exist measured pressures in an animal as small as *P. pugio.* The mean pressures measured here at 20°C are in the same range as those previously measured in *P. pugio* (Guadagnoli et al., 2011). Negative pressures were also measured which also corresponds to previous measurements in *P. pugio* (Guadagnoli et al., 2011). There were few significant differences between normoxic and hyperoxic conditions in maximum and minimum pressures, with a significant difference at 5°C in P_{max} and 5 and 25°C in P_{min}. Maximum and minimum pressures were reduced in hyperoxia compared to normoxia. While the individual values may not be significant at each temperature, this does lead to an observable difference in the total change in pressure through the cardiac cycle. When the difference is calculated to measure ΔP, significance differences between treatments becomes apparent. There was a temperature dependent increase in ΔP from 5-25°C. At 30°C there was a significant drop in ΔP ($p < 0.001$) back to 10°C levels that was maintained through the 35 and 37°C

measurements. At 37°C there was a significant difference between the normoxic and hyperoxic conditions, with the hyperoxic ΔP being much lower than the normoxic ΔP This drop in pressure could potentially allow for an increase in passive filling of the ventricle although there was not a significant difference in end diastolic volume. The decrease in ΔP also contributes to a reduction in stroke work.

dp/dt is a measure of contractility and myocardial oxygen consumption (Sagawa et al., 1988). There was a temperature dependent increase in *dp/dt* with it being significantly higher from 20-37°C than the lower temperatures with a peak at 25°C and decreases at higher temperatures, although this difference is not statistically significant. The increase in *dp/dt* indicates an increase in myocardial oxygen consumption which would be associated with increasing f_H. This also corresponds to the temperature dependent increase in oxygen consumption previously measured in *P. pugio* (Mika and Reiber, in prep). The fall in *dp/dt* after 25°C may indicate the heart is beginning to fail, however we don't know if its an actual fall in myocardial oxygen consumption or if cardiac function is compromised with increased temperature. Based on whole animal oxygen consumption and maintenance and even increase in cardiac functions beyond 25°C, a change in metabolic response is favored over the beginnings of failure of the cardiac muscle.

Pressure-Area (P-A) loops were generated to assess cardiac energetics. The area enclosed by the loop indicates stroke work (Sagawa et al., 1988) (Figure 5.11). There was a temperature dependent decrease in stroke work with increasing temperature with a significant decrease compared to colder temperatures in normoxia from 30-37^o C, and in

hyperoxia from 25-37^{o} C with the exception of 30^{o} C. There was also a significant reduction in stroke work in hyperoxia at 37^{o} C compared to normoxia. With no significant differences in V_s the reduction in stroke work was primarily due to the reduction in ΔP at higher temperatures and specifically 37^{o} C in hyperoxia. This is similar to results seen in hypoxia where the reduction in stroke work is due to reduced pressure as there is no change in stroke volume (Guadagnoli et al., 2011). Stroke work in both normoxia and hyperoxia increases at CT_{max}. This may represent a last effort to meet oxygen demand.

Cardiac work, as the product of heart rate and stroke work, was also reduced in hyperoxia compared to normoxia at 37°C despite the higher heart rate in hyperoxia. As with stroke work this leaves reduced ΔP responsible for the overall reduction in work.

One hypothesis for cardiac failure at high temperatures and high heart rates was a biomechanical limitation in diastolic filling time. Stroke volume is temperature insensitive and does not change. Previous measurements of time in cardiac cycle using pressure measurements found that time is systole is similarly fixed (Mika and Reiber, in prep). Unfortunately as heart rates reach 400 beats per minute, diastolic filling time cannot be distinguished as a subset of time in diastole on the pressure files, making direct measurements of diastolic filling time this particular method impractical for determining if this type of biomechanical limitation is responsible for cardiac failure at high heart rates. Although diastolic filling time could not be measured directly, maintenance of heart rate beyond normoxic CT_{max} in hyperoxia indicates nervous function failure, or cardiac ganglion failure, is not implicated for cardiac failure. As heart rate increases, stroke

volume is maintained. These measurements lend further support to the hypothesis of a biomechanical limitation to diastolic filling time. This pattern corresponds to observations of exercise in humans where tachycardia places a similar limitation on diastolic filling time (Higginbotham et al., 1986). In humans cardiac output may be maintained through an increase in contractility leads to decreased end systolic volume and a maintained stroke volume (Warburton et al., 2002). In *P. pugio*, *dp/dt* increased between 37 and 38°C in hyperoxia, however no increase in ejection fraction was observed.

P. pugio may also increase cardiac output to meet oxygen demand. Cardiac output shows a temperature dependent increase in hyperoxia, however, it is not significantly different from the increase in normoxia. While these outputs are the same, the oxygen available for delivery through the hemolymph by volume is higher in the hyperoxic condition which may contribute to the increased CT_{max}.

Finally there was a decrease in cardiac and stroke work at 37°C. This reduction in oxygen demand allows the reduced supply to meet metabolic requirements of the animal at high temperature. In hyperoxic conditions, *P. pugio* tolerate temperatures above normoxic CT_{max} through a combination of factors all related to alleviating the mismatch in oxygen supply and delivery, supporting the overall hypothesis of oxygen limited thermal tolerance.

Figure 5.1 *P. pugio* heart rate at varying temperatures in normoxic and hyperoxic conditions

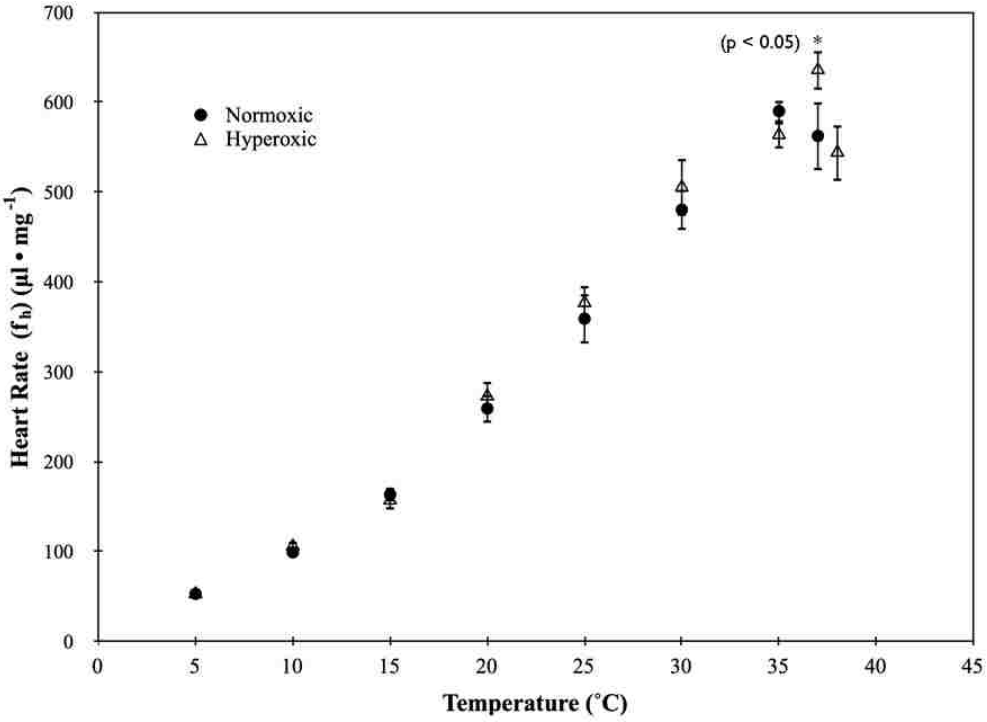

Figure 5.1: *P. pugio* heart rate (f_H) at varying temperatures (normoxia n = 7 5-37°C, hyperoxia n=7 5-38°C). Two-way ANOVA is used to assess significance. Pairwise t tests are used to compare normoxic and hyperoxic conditions. No significant difference in the interaction between oxygen and temperature is detected from 5-35°C. A significant interaction between oxygen and temperature is observed at 37 °C ($p < 0.05$). Error bars represent S.E.

Figure 5.2 *P. pugio* stroke volume at varying temperatures in normoxic and hyperoxic conditions

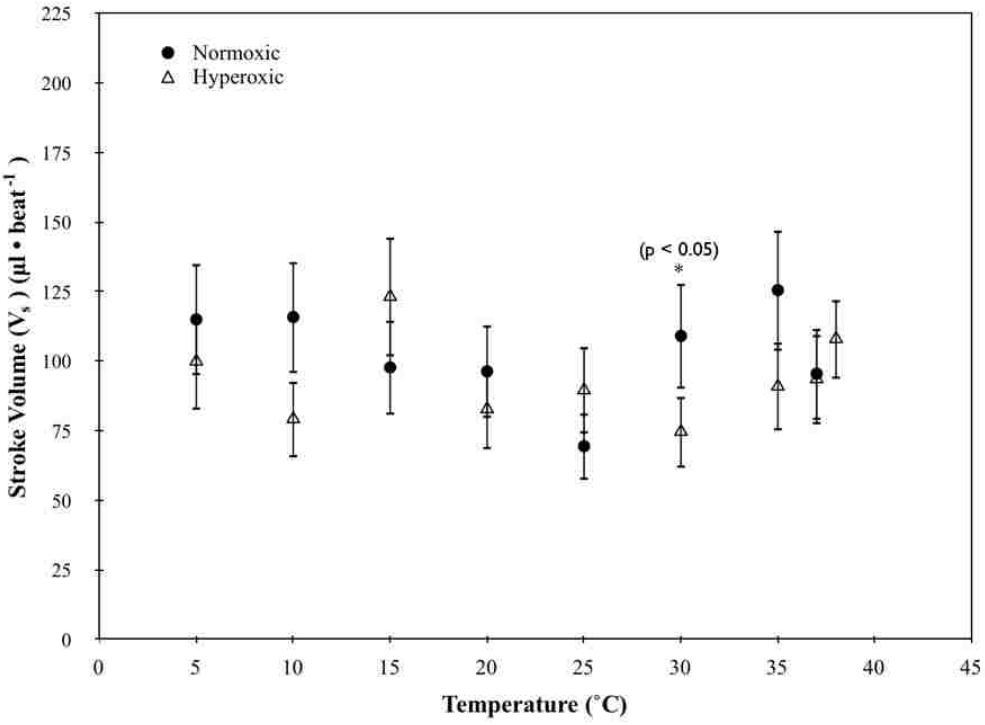

Figure 5.2: *P. pugio* stroke volume (V_s) at varying temperatures (normoxia n = 7 5-37°C, hyperoxia n=7 5-38°C). Two-way ANOVA is used to assess significance. Pairwise t tests are used to compare normoxic and hyperoxic conditions. No significance of temperature is noted within either normoxia or hyperoxia. No significant difference in the interaction between oxygen and temperature is detected across the temperature range with the exception of 30°C. Error bars represent S.E.

Figure 5.3 *P. pugio* cardiac output at varying temperatures in normoxic and hyperoxic conditions

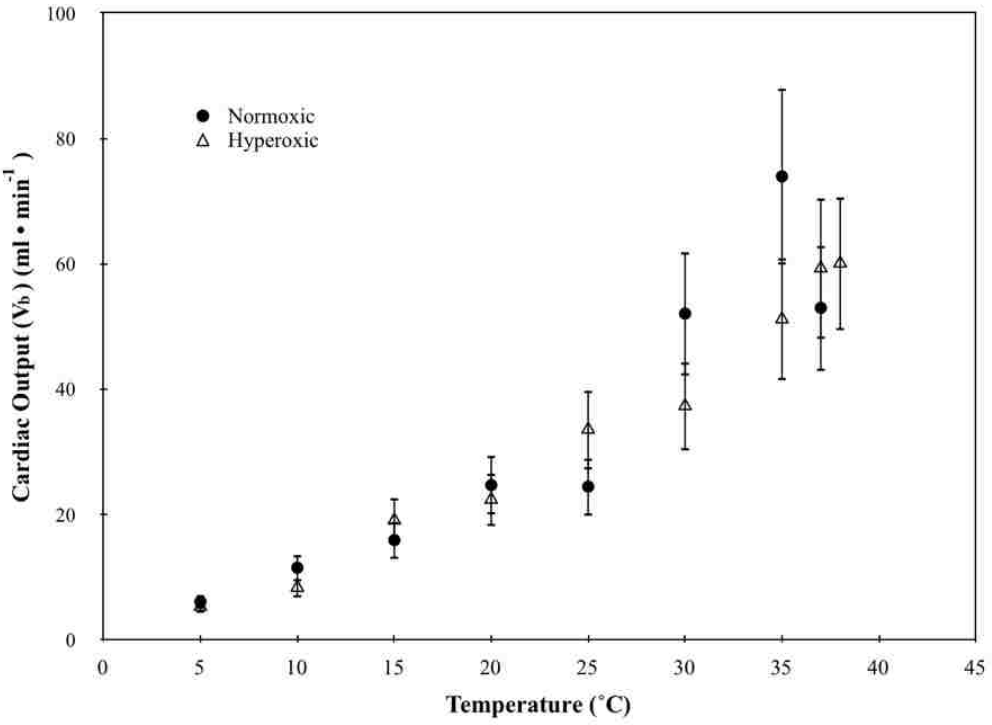

Figure 5.3: *P. pugio* cardiac output (V_b) at varying temperatures (normoxia n = 7 5-37°C, hyperoxia n=7 5-38°C). Two-way ANOVA is used to assess significance. Pairwise t tests are used to compare normoxic and hyperoxic conditions. Temperature has a significant effect in both normoxia and hyperoxia ($p < 0.05$). No significant difference in the interaction between oxygen and temperature is noted at any temperature tested. Error bars represent S.E.

Figure 5.4 Ejection fraction at varying temperatures in normoxic and hyperoxic conditions

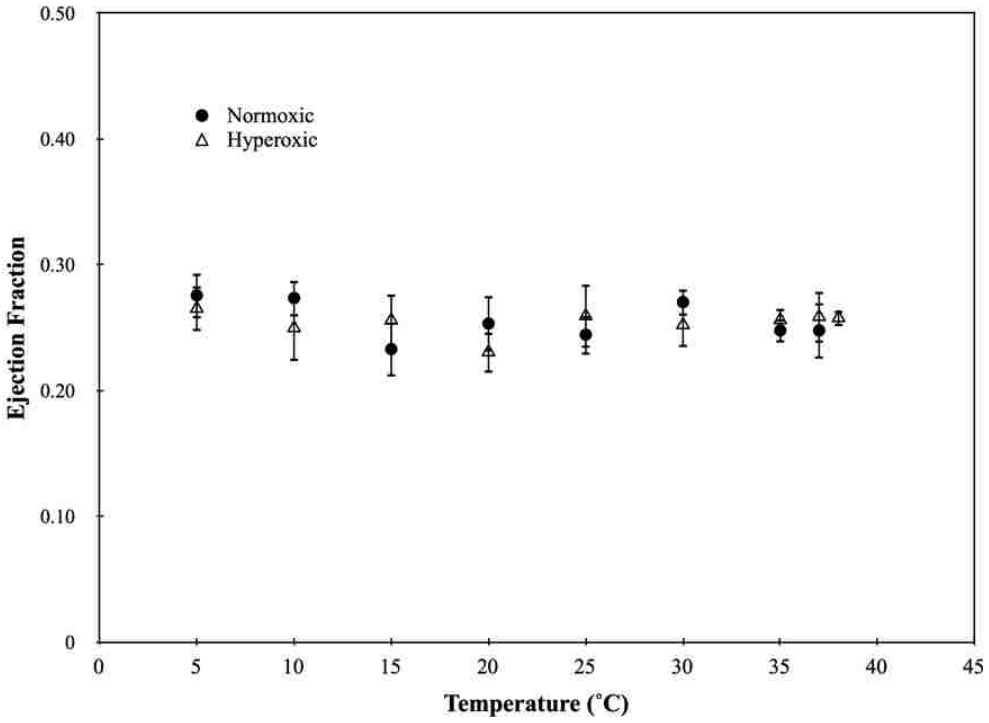

Figure 5.4: *P pugio* ejection fraction at varying temperatures (normoxia n = 7 5-37°C, hyperoxia n=7 5-38°C). Two-way ANOVA is used to assess significance. Pairwise t tests are used to compare normoxic and hyperoxic conditions. No significance of temperature is noted within either normoxia or hyperoxia. No significant difference in the interaction between oxygen and temperature is noted at any temperature tested. Error bars represent S.E.

Figure 5.5 *P. pugio* minimum pressure at varying temperatures in normoxic and hyperoxic conditions

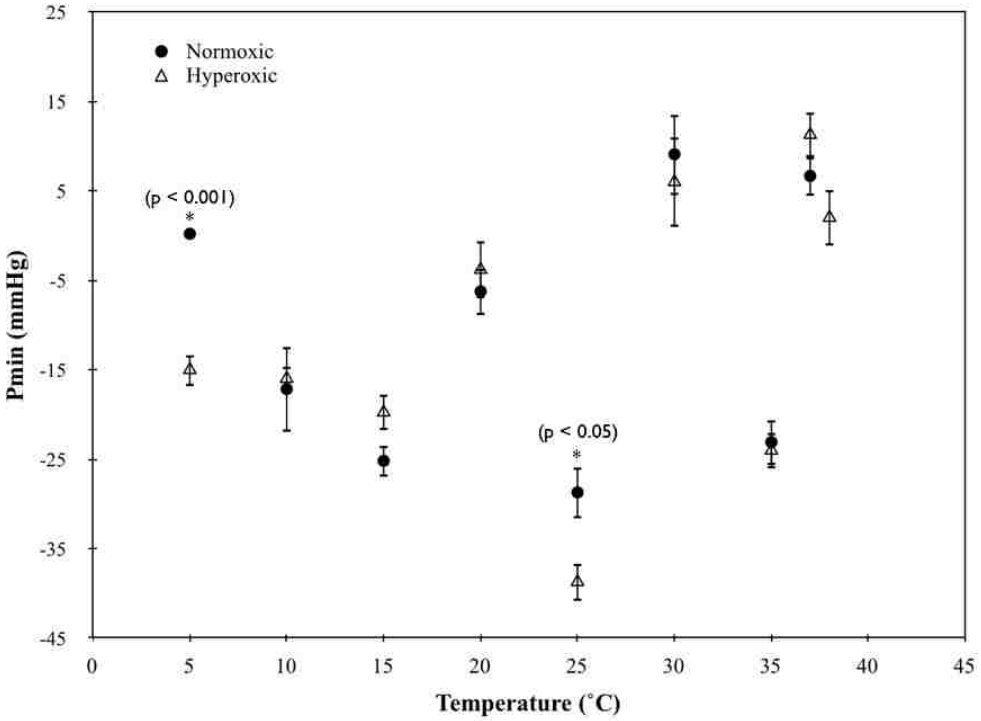

Figure 5.5: *P. pugio* minimum pressure (P_{min}) at varying temperatures (normoxia n = 7 5-37°C, hyperoxia n=7 5-38°C). Two-way ANOVA is used to assess significance. Pairwise t tests are used to compare normoxic and hyperoxic conditions. A significant interaction between oxygen and temperature is observed at 5°C ($p < 0.001$) and 25°C ($p < 0.05$). Error bars represent S.E

Figure 5.6 *P. pugio* maximum pressure at varying temperatures in normoxic and hyperoxic conditions

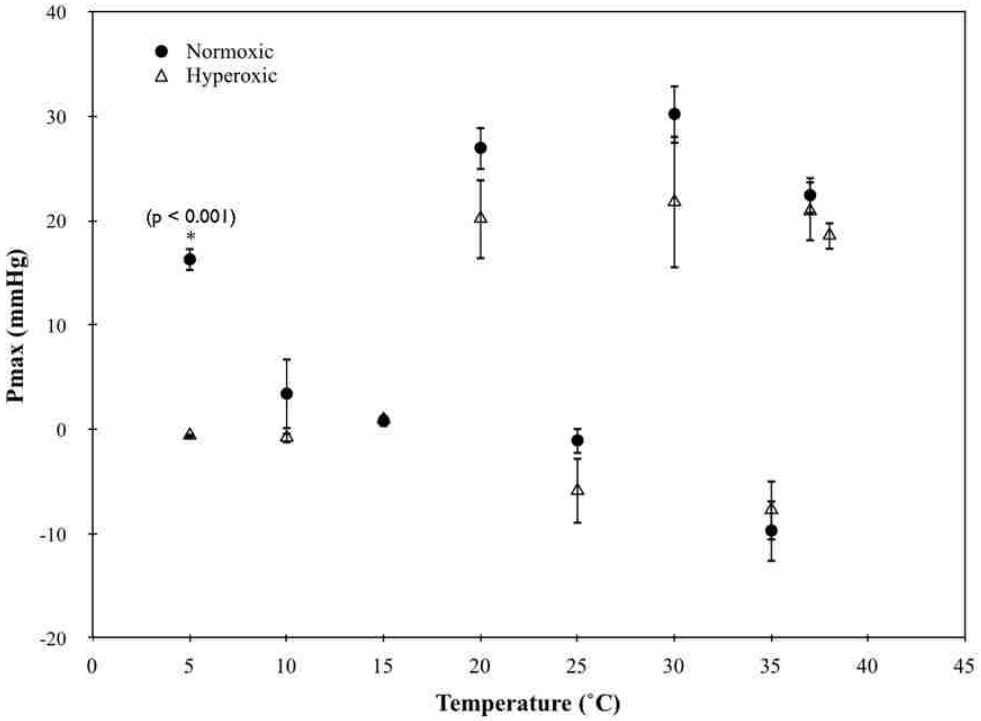

Figure 5.6: *P. pugio* maximum pressure (P_{max}) at varying temperatures (normoxia n = 7 5-37°C, hyperoxia n=7 5-38°C). Two-way ANOVA is used to assess significance. Pairwise t tests are used to compare normoxic and hyperoxic conditions. A significant interaction between oxygen and temperature is observed at 5°C ($p < 0.001$). Error bars represent S.E.

Figure 5.7 *P. pugio* Δ*P* at varying temperatures in normoxic and hyperoxic conditi

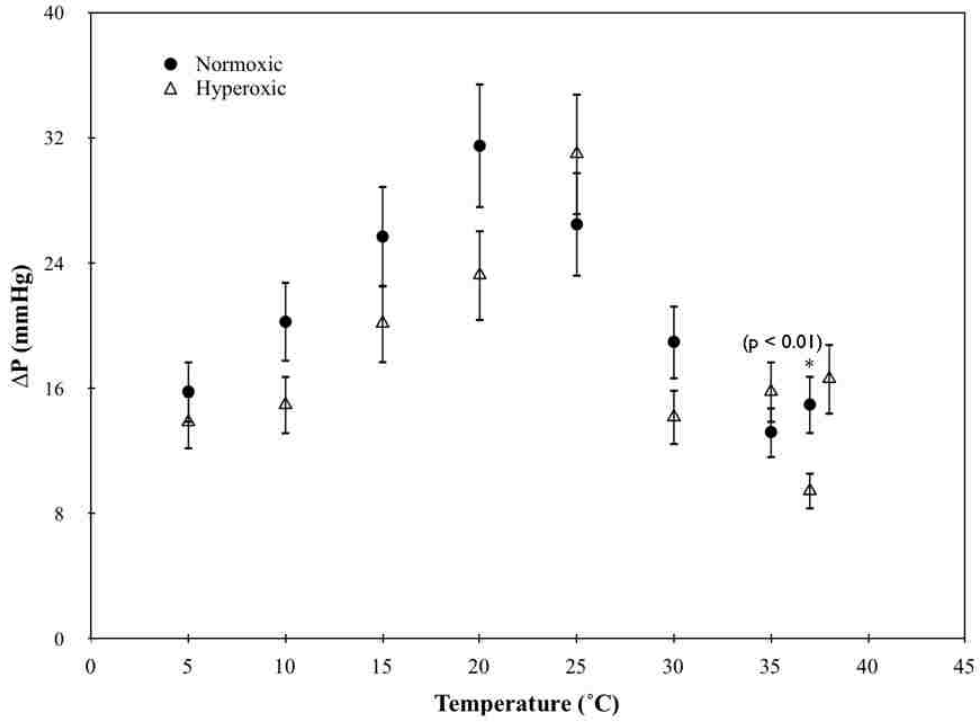

Figure 5.7: *P. pugio* Δ*P* at varying temperatures (normoxia n = 7 5-37°C, hyperoxia n=7 5-38°C). Two-way ANOVA is used to assess significance. Pairwise t tests are used to compare normoxic and hyperoxic conditions. Temperature has a significant effect in both normoxia and hyperoxia ($p < 0.05$). A significant interaction between oxygen and temperature is observed at 37 °C ($p < 0.001$) Error bars represent S.E.

Figure 5.8 *P. pugio dp/dt* at varying temperatures in normoxic and hyperoxic conditions

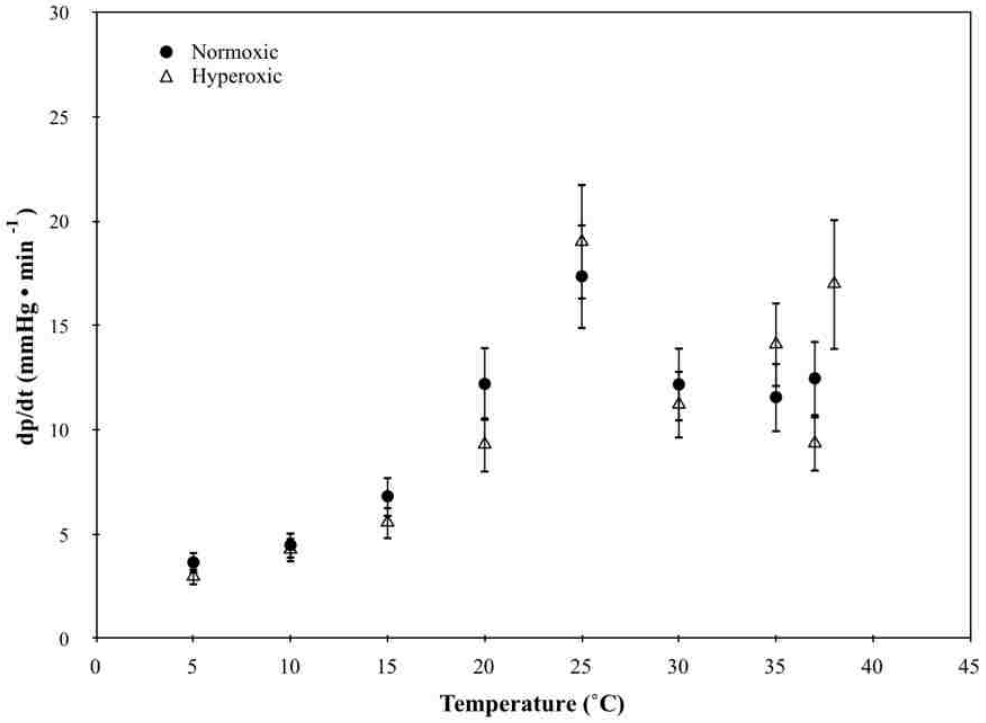

Figure 5.8: *P. pugio dp/dt* at varying temperatures (normoxia n = 7 5-37°C, hyperoxia n=7 5-38°C). Two-way ANOVA is used to assess significance. Pairwise t tests are used to compare normoxic and hyperoxic conditions. Temperature has a significant effect in both normoxia and hyperoxia ($p < 0.05$). Error bars represent S.E.

Figure 5.9 *P. pugio* stroke work at varying temperatures in normoxic and hyperoxic conditions

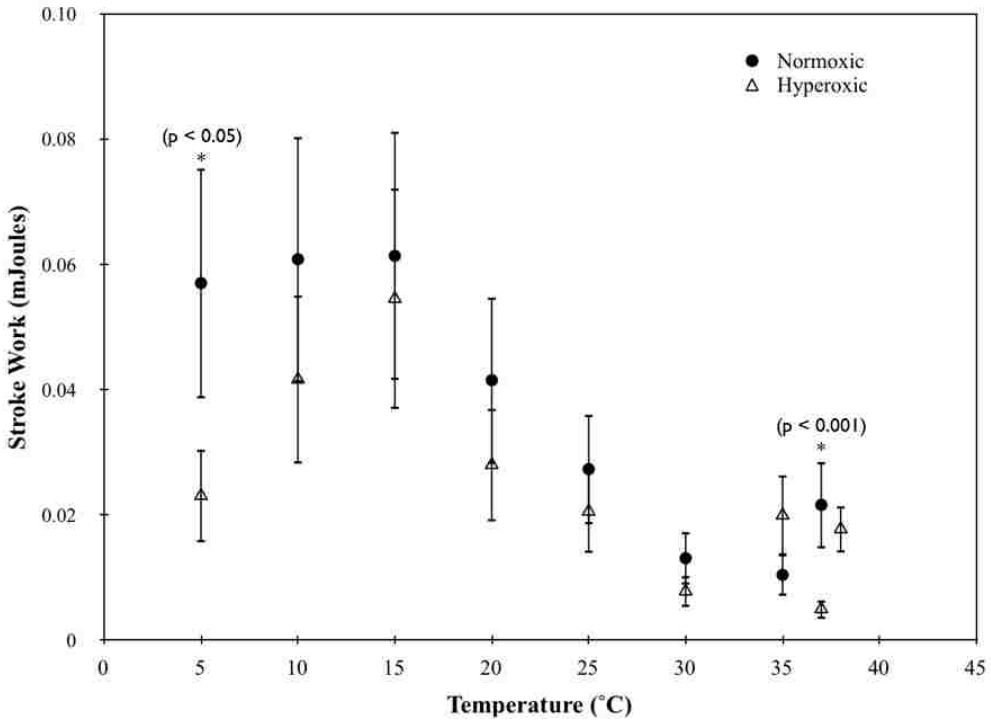

Figure 5.9: *P. pugio* stroke work at varying temperatures (normoxia n = 7 5-37°C, hyperoxia n=7 5-38°C). Two-way ANOVA is used to assess significance. Pairwise t tests are used to compare normoxic and hyperoxic conditions. Temperature has a significant effect in both normoxia and hyperoxia ($p < 0.05$). A significant interaction between oxygen and temperature is observed at 5°C ($p < 0.05$) and 37 °C ($p < 0.001$) Error bars represent S.E.

Figure 5.10 *P. pugio* cardiac work at varying temperatures in normoxic and hyperoxic conditions

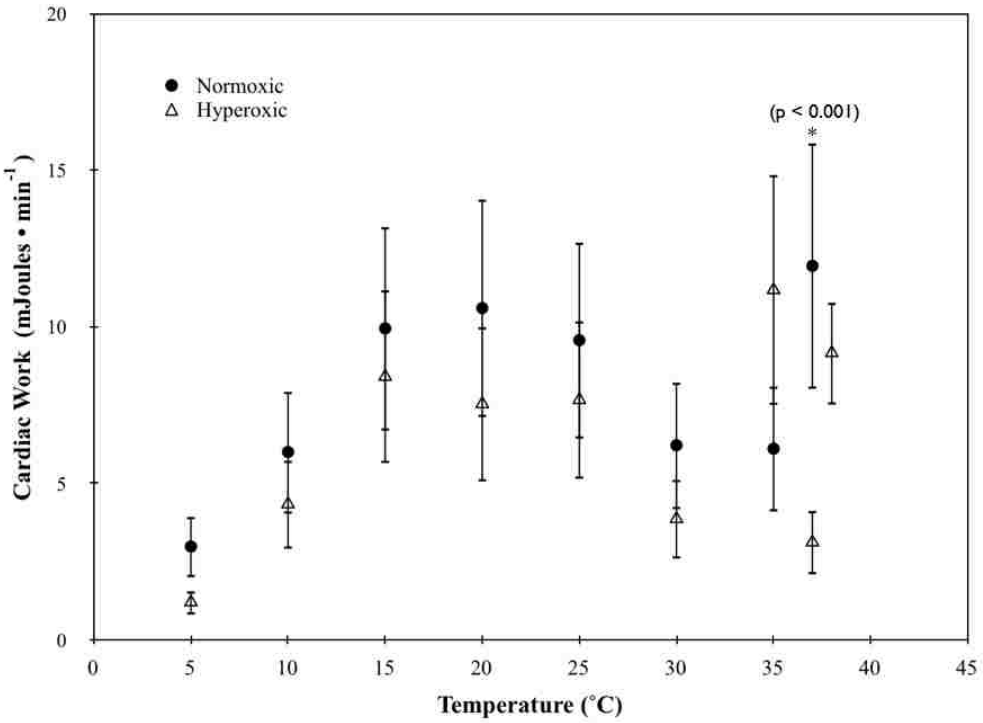

Figure 5.10: *P. pugio* cardiac work at varying temperatures (normoxia n = 7 5-37°C, hyperoxia n=7 5-38°C). Two-way ANOVA is used to assess significance. Pairwise t tests are used to compare normoxic and hyperoxic conditions. Temperature has a significant effect in both normoxia and hyperoxia ($p < 0.05$). A significant interaction between oxygen and temperature is observed at 37 °C ($p < 0.001$) Error bars represent S.E.

Figure 5.11 Pressure-Area loop at 20°C and normoxia

Figure 5.11: Pressure-Area loop at 20°C and normoxia. Volume measured in μL is on the x-axis and pressure measured in mmHg is on the y-axis.

CHAPTER 6

CONCLUSIONS AND FUTURE DIRECTIONS

The results of this dissertation extend the knowledge on crustacean cardiorespiratory response to temperature extremes, and provides support for the hypothesis of oxygen limited thermal tolerance described by Portner (Portner, 2001; Portner, 2010). The results also support the need for global climate change research on marine organisms, first by providing data that indicates how close *P. pugio* is living to its thermal limits (Stillman, 2004) and second by explaining how future changes in environmental temperature could effect cardiorespiratory physiology and influence its potential range. This represents the first time all of these cardiorespiratory parameters have been measured in a crustacean this small.

To show cardiorespiratory failure at temperature extremes is due to oxygen limited thermal tolerance the different components of the oxygen delivery system were individually evaluated to determine the effect of temperature on those components. Individual chapters focused on components of the oxygen delivery system, and explore the effects of temperature and oxygen condition on each of them.

Chapter 2 described a novel and effective method developed to measure ventilatory parameters in a small aquatic crustacean using high speed video microscopy. Previous methods were designed for larger animals and did not allow for beat to beat measurements of the scaphognathite. Here beat to beat measurements are possible and permit a more complete evaluation of not just ventilatory rate but ventilatory volume as well. This is a method is not limited to *P. pugio*, but can also be applied with other

organisms such as *Daphnia* expanding the research capabilities on the organisms of this scale.

Chapter 3 described the first test of the hypothesis of oxygen limited thermal tolerance was first tested. Testing CT_{max} in a hyperoxic environment where more oxygen was supplied to the delivery system and comparing it to a normoxic CT_{max} showed that animals are able to survive to a higher temperature in a hyperoxic environment. This is the first line of support oxygen limited thermal tolerance. Based on this result, all future experiments were conducted in both normoxic and hyperoxic conditions.

The method developed in Chapter 2 made it possible to collect scaphognathite stroke volume which combined with ventilatory rate provided ventilatory volume. There was a very clear effect of temperature on ventilatory rate, ventilatory volume, and scaphognathite stroke volume. Here it became clear that the scaphognathite does not act as a fixed volume pump as previously described. Two of the most interesting results were hypoventilation and hypometabolism in the hyperoxic condition versus the normoxic condition. This may be an energy saving mechanism to reduce overall work. Additionally, the lack of lactate accumulation until CT_{max} indicates *P. pugio* is maintaining aerobic function right up to failure.

The work in Chapter 4 tested basic cardiac variables across a temperature range in normoxic and hyperoxic conditions to determine the effect of temperature and oxygen condition on the cardiac component of the oxygen delivery system. There was a significant effect of temperature on both heart rate and cardiac output, but stroke volume is temperature insensitive. Unlike heart rate, there was a significant effect of oxygen

condition on stroke volume with a higher stroke volume in hyperoxia. These effects support the neural control of heart rate and stroke volume responding to changes in metabolic demand. As with measurements of ventilatory parameters, cardiac function is maintained to CT_{max} for each oxygen condition. Ejection fraction did not vary over the temperature range or by treatment, indicating a cardiomyopathy is not responsible for failure at temperature extremes. Previously collected pressure data was analyzed to determine times in cardiac cycle to determine if a biomechanical limitation might contribute to cardiac failure. The results support a biomechanical reduction in diastolic filling time leading to cardiac failure.

Possible responses to the mismatch in oxygen supply and demand include an increase in oxygen supply through increased cardiac output, a reduction in oxygen demand through reduced stroke and cardiac work, or alternatively cardiac failure could be due to a biomechanical limitation in diastolic filling time. The research reported in Chapter 5 integrates micropressure and high speed video signals to measure intracardiac pressure and cardiac volumes respectively to generate pressure area loops which provide estimates of stroke and cardiac work. There was no significant difference in cardiac output between normoxic and hyperoxic conditions, although cardiac output was maintained to CT_{max} in both. The change in pressure (Δ P) was reduced at high temperatures in hyperoxia compared to normoxia leading to a reduction in stroke and cardiac work in hyperoxia. This reduction in cardiac work leads to cardiac failure at higher temperatures in hyperoxia than normoxia. Unfortunately it was not possible to

distinguish diastolic filling time from time in diastole at heart rates above 400 beats per minute which made analysis of that biomechanical limitation impossible.

These results taken together establish a clear case of oxygen limited thermal tolerance in *Palaemonetes pugio* and describe a mechanism for cardiorespiratory failure with both hypoventilation and reduced contributing to failure at a higher temperature in hyperoxia.

Further studies can be extended to include both hypoxia and temperature to replicate other conditions that may be experienced by *P. pugio* over its habitat range. We have data on temperature (described in Chapters 3, 4, and 5) and hypoxia (Guadagnoli et al., 2011) separately, but combining them will provide a more complete understanding of the interactions possible.

With the reduction of stroke and cardiac work attributed to a reduced change in intracardiac pressure, further micropressure studies are warranted to gain full understanding of peripheral pressures and their effects on cardiovascular function. With the methods developed here it will be possible to describe the hemodynamics of *P. pugio* as has been done previously in crayfish (Reiber, 1994).

Pressure-area loops can be used to describe more of the biomechanics of cardiovascular function. Tachycardia and bradycardia can be neurohormonally induced and the mechanisms observed can be compared to observed in hypoxia and temperature. This can provide a more comprehensive understanding of the roles and interactions between the cardiac ganglion, cardioacceleratory and inhibitory nerves and the pericardial organ (Wilkens et al., 1996).

Another avenue to test is data showing a single pressure peak almost doubled in amplitude for two contractions observed on high speed video. Double peaks have been observed before, but these were double systoles for a single volume which resulted in increased stroke volume and therefore cardiac output (De Wachter and Wilkens, 1996). The data can be analyzed to determine how and if the single pressure changes stroke volume and cardiac output.

The data provided here support the hypothesis of oxygen limited thermal tolerance and extend it to small organisms providing a more comprehensive picture of the potential effects of global climate change on the ecosystem as a whole. The new ventilatory method can be applied to other small organisms to or larval stages of organisms to understand the effects of temperature extremes on cardiorespiratory development.

These data also suggest and explain a mechanism for the decline in cardiac function not previously described. A biomechanical limitation to diastolic filing time is implicated while systematically eliminating other possible mechanisms including neural function decline of the cardiac ganglion. This ties in nicely with similar limitations to diastolic filling time measured in humans, and shows the pattern extends from invertebrate to mammalian cardiovascular physiology. Further analysis of pressure profiles and pressure-area loops will provide a complete picture of the interactions between mechanisms of cardiorespiratory decline at temperature extremes.

Literature Cited

Anderson, G. (1985). Species Profiles: Life Histories and Environmental Requirements of Coastal Fishes and Invertebrates (Gulf of Mexico) Grass Shrimp U.S. Fish and Wildlife Service vol. 82, pp. 19.

Anderson, M. J. (2004). DISTLM v.5: a FORTRAN computer program to calculate a distance-basedmultivariate analysis for a linear model. Department of Statistics, University of Auckland, New Zealand.

Belman, B. W. (1975). Some aspects of the circulatory physiology of the spiny lobster *Panulirus interruptus*. *Marine Biology* **29**, 295-305.

Burggren, W. W. and McMahon, B. R. (1983). An Analysis of Scaphognathite Pumping Performance in the Crayfish Orconectes virilis: Compensatory Changes to Acute and Chronic Hypoxic Exposure. *Physiological Zoology* **56**, 309-318.

Claussen, D. L. (1980). Thermal acclimation in the crayfish, Orconectes rusticus and O. virilis. *Comparative Biochemistry and Physiology Part A: Physiology* **66**, 377-384.

Cooke, I. M. (2002). Reliable, Responsive Pacemaking and Pattern Generation With Minimal Cell Numbers: the Crustacean Cardiac Ganglion. *The Biological Bulletin* **202**, 108-136.

Cossins, A. R. and Bowler, K. (1987). The Temperature Biology of Animals. London: Chapman and Hall.

Cumberlidge, N. and Uglow, R. F. (1977). Size, Temperature, and Scaphognathite Frequency-Dependent Variations of Ventilation Volumes in Carcinus-Maenas (L). *Journal of Experimental Marine Biology and Ecology* **30**, 85-93.

De Wachter, B. and McMahon, B. (1996a). Haemolymph flow distribution, cardiac performance and ventilation during moderate walking activity in Cancer magister (Dana) (Decapoda, Crustacea). *J Exp Biol* **199** 627-633.

De Wachter, B. and McMahon, B. R. (1996b). Temperature effects on heart performance and regional hemolymph flow in the crab Cancer magister. *Comparative Biochemistry and Physiology Part A: Physiology* **114**, 27-33.

De Wachter, B., Sartoris, F., Ouml and Rtner, H. (1997). The anaerobic endproduct lactate has a behavioural and metabolic signalling function in the shore crab. *Journal of Experimental Biology* **200**, 1015-24.

De Wachter, B. and Wilkens, J. L. (1996). Comparison of Temperature Effects on Heart Performance of the Dungeness Crab, Cancer magister, in vitro and in vivo. *The Biological Bulletin* **190**, 385-395.

DeFur, P. L. and Mangum, C. P. (1979). The effects of environmental variables on the heart rates of invertebrates. *Comparative Biochemistry and Physiology Part A: Physiology* **62**, 283-294.

Dejours, P. (1975). Principles of Comparative Respiratory Physiology. North-Holland, Amsterdam: Elsevier Science Ltd.

Dejours, P. and Beekenkamp, H. (1977). Crayfish respiration as a function of water oxygenation. *Respiration Physiology* **- 30**, 241- 251.

Dejours, P., Toulmond, A. and Truchot, J. P. (1977). The effect of hyperoxia on the breathing of marine fishes. *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology* **- 58**, 409- 411.

Díaz, F., Sierra, E., Denisse Re, A. and Rodríguez, L. (2002). Behavioural thermoregulation and critical thermal limits of Macrobrachium acanthurus (Wiegman). *Journal of Thermal Biology* **27**, 423-428.

Díaz Herrera, F., Sierra Uribe, E., Fernando Bückle Ramirez, L. and Garrido Mora, A. (1998). Critical thermal maxima and minima of Macrobrachium rosenbergii (Decapoda: Palaemonidae). *Journal of Thermal Biology* **23**, 381-385.

Ege, R. and Krogh, A. (1914). On the Relation between the Temperature and the Respiratory Exchange in Fishes. *Internationale Revue der gesamten Hydrobiologie und Hydrographie* **7**, 48-55.

Florey, E. and Kriebel, M. E. (1974). The effects of temperature, anoxia and sensory stimulation on the heart rate of unrestrained crabs. *Comparative Biochemistry and Physiology Part A: Physiology* **48**, 285-300.

Frederich, M. and Portner, H. O. (2000). Oxygen limitation of thermal tolerance defined by cardiac and ventilatory performance in spider crab, Maja squinado. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology* **279**, R1531-R1538.

Gannon, A. T. and Henry, R. P. (2004). Oxygen and carbon dioxide sensitivity of ventilation in amphibious crabs, Cardisoma guanhumi, breathing air and water.

Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology **138**, 111-117.

Guadagnoli, J. A. and Reiber, C. L. (2005). Changes in cardiac output and hemolymph flow during hypoxic exposure in gravid grass shrimp, *Palaemonetes pugio*. *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology* **175**, 313-322.

Guadagnoli, J. A., Tobita, K. and Reiber, C. L. (2007). Assessment of the pressure–volume relationship of the single ventricle of the grass shrimp, Palaemonetes pugio. *Journal of Experimental Biology* **210**, 2192-2198.

Guadagnoli, J. A., Tobita, K. and Reiber, C. L. (2011). Changes in cardiac performance during hypoxic exposure in the grass shrimp Palaemonetes pugio. *The Journal of Experimental Biology* **214**, 3906-3914.

Harper, S. L. and Reiber, C. (1999). Influence of hypoxia on cardiac functions in the grass shrimp (*Palamonetes pugio* Holthuis). *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology* **124**, 569- 573.

Higginbotham, M. B., Morris, K. G., Williams, R. S., McHale, P. A., Coleman, R. E. and Cobb, F. R. (1986). Regulation of stroke volume during submaximal and maximal upright exercise in normal man. *Circulation Research* **58**, 281-91.

Hill, R. W., Wyse, G. A. and Anderson, M. (2008). Animal Physiology. Sunderland, Massachusetts: Sinauer Associates, Inc.

Hilton, Z., Clements, K. D. and Hickey, A. J. R. (2010). Temperature sensitivity of cardiac mitochondria in intertidal and subtidal triplefin fishes. *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology* **180**, 979-990.

Hochachka, P. W. and Somero, G. N. (2002). Biochemical Adaptation: Mechanism and Process in Physiological Evolution. New York, NY: Oxford University Press.

Hoppeler, H. and Weibel, E. R. (1998). Limits for oxygen and substrate transport in mammals. *Journal of Experimental Biology* **201**, 1051-1064.

Hutchison, V. H. (1961). Critical Thermal Maxima in Salamanders. *Physiological Zoology* **34**, 92-125.

Ivleva, I. V. (1980). The Dependence of Crustacean Respiration Rate on Body-Mass and Habitat Temperature. *Internationale Revue Der Gesamten Hydrobiologie* **65**, 1-47.

Jury, S. and Watson, W. (2000). Thermosensitivity of the lobster, Homarus americanus, as determined by cardiac assay. *The Biological Bulletin* **199**, 257-264.

Karlsson, A., Heier, L. S., Rosseland, B. O., Salbu, B. and Kiessling, A. (2011). Changes in arterial PO2, physiological blood parameters and intracellular antioxidants in free-swimming Atlantic cod (Gadus morhua) exposed to varying levels of hyperoxia. *Fish Physiology and Biochemistry* **37**, 249-258.

Keller, B. B., Hu, N., Serrino, P. J. and Clark, E. B. (1991). Ventricular pressure-area loop characteristics in the stage 16-24 chick embryo. *Circulation Research* **68**, 226-231.

Kivivuori, L. A. and Lahdes, E. O. (1996). How to measure the thermal death of Daphnia? A comparison of different heat tests and effects of heat injury. *Journal of Thermal Biology* **21**, 305-311.

Krogh, A. (1914). The quantitative relation between temperature and standard metabolism in animals. *Int. Z. Phys. Chem. Biol.* **1**, 491-508.

Kusakabe, T., Ishii, K. and Ishii, K. (1991). Dense granule-containing cells in the wall of the branchio-cardiac veins of a fresh water crayfish (*Astacus leptodactylus*). *Anatomy and Embryology* **183**, 553-557.

Larimer, J. L. (1961). Measurement of Ventilation Volume in Decapod Crustacea. *Physiological Zoology* **34**, 158-166.

Lowe, C. H. and Vance, V. J. (1955). Acclimation of the Critical Thermal Maximum of the Reptile Urosaurus ornatus. *Science* **122**, 73-74.

Lutterschmidt, W. I. and Hutchison, V. H. (1997). The critical thermal maximum: history and critique. *Canadian Journal of Zoology* **75**, 1561-1574.

Mangum, C. P. (1983). Oxygen transport in the blood. In *The Biology of Crustacea*, vol. 5 (ed. L. Mantel), pp. 373-429. New York: Academic Press.

McDonald, D. G., McMahon, B. R. and Wood, C. M. (1977). Patterns of Heart and Scaphognathite Activity in Crab Cancer Magister. *Journal of Experimental Zoology* **202**, 33-43.

McDonald, D. G., Wood, C. M. and McMahon, B. R. (1980). Ventilation and oxygen consumption in the Dungeness crab, Cancer magister. *Journal of Experimental Zoology* **213**, 123-136.

McLaughlin, P. A. (1983). Internal anatomy and physiological regulation. In *The Biology of Crustacea*, vol. 5 (ed. L. Mantel), pp. 1-53: New York.

McMahon, B., Sinclair, F., Hassall, C. D., deFur, P. L. and Wilkes, P. R. H. (1978). Ventilation and control of acid-base status during temperature acclimation in the crab,Cancer magister. *Journal of Comparative Physiology* **128**, 109-116.

McMahon, B. R. (1999). Intrinsic and extrinsic influences on cardiac rhythms in crustaceans. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **124**, 539-547.

McMahon, B. R. and Wilkens, J. L. (1977). Periodic Respiratory and Circulatory Performance in Red Rock Crab Cancer-Productus. *Journal of Experimental Zoology* **202**, 363-374.

McMahon, B. R. and Wilkens, J. L. (1983). Ventilation, perfusion and oxygen uptake. In *The Biology of Crustacea*, vol. 5 (ed. L. Mantel), pp. 289-372. New York: Academic Press.

Morris, S. (2005). Respiratory and acid–base responses during migration and to exercise by the terrestrial crab Discoplax (Cardisoma) hirtipes, with regard to season, humidity and behaviour. *Journal of Experimental Biology* **208**, 4333-4343.

Morris, S. and Oliver, S. (1999). Circulatory, respiratory and metabolic response to emersion and low temperature of Jasus edwardsii: simulation studies of commercial shipping methods. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **122**, 299-308.

Morris, S. and Taylor, A. C. (1985). The Respiratory Response of the Intertidal Prawn Palaemon-Elegans (Rathke) to Hypoxia and Hyperoxia. *Comparative Biochemistry and Physiology a-Physiology* **81**, 633-639.

Morris, S., Taylor, A. C. and Bridges, C. R. (1988). Response of haemolymph oxygen affinity to simultaneous salinity and oxygen stress in the intertidal prawn, *Palaemon elegans* (Rathke). *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology* **90A**, 31-39.

Moshiri, G. A., Goldman, C. R., Gordon, L. G. and Mull, D. R. (1970). The Effects of Variations in Oxygen Tension on Certain Aspects of Respiratory Metabolism in Pacifastacus leniusculus (Dana) (Crustacea: Decapoda). *Physiological Zoology* **43**, 23-29.

Nelson, D. H. and Hooper, D. K. (1982). Thermal tolerance and preference of the freshwater shrimp Palaemonetes kadiakensis. *Journal of Thermal Biology* **7**, 183-187.

Pörtner, H., Peck, L. and Hirse, T. (2006a). Hyperoxia alleviates thermal stress in the Antarctic bivalve, Laternula elliptica: evidence for oxygen limited thermal tolerance. *Polar Biology* **29**, 688-693.

Portner, H. O. (2001). Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. *Naturwissenschaften* **88**, 137-146.

Portner, H. O. (2010). Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. *Journal of Experimental Biology* **213**, 881-893.

Pörtner, H. O. (2002). Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal

tolerance in animals. *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology* **132**, 739-761.

Pörtner, H. O., Bennett, A. F., Francisco Bozinovic, Clarke, A., Lardies, M. A., Lucassen, M., Pelster, B., Fritz Schiemer and Jonathon H. Stillman. (2006b). Trade−Offs in Thermal Adaptation: The Need for a Molecular to Ecological Integration. *Physiological and Biochemical Zoology* **79**, 295-313.

Pörtner, H. O. and Knust, R. (2007). Climate Change Affects Marine Fishes Through the Oxygen Limitation of Thermal Tolerance. *Science* **315**, 95-97.

Portner, H. O., Peck, L. S. and Hirse, T. (2006). Hyperoxia alleviates thermal stress in the Antarctic bivalve, Laternula elliptica: evidence for oxygen limited thermal tolerance. *Polar Biology* **29**, 688-693.

Reiber, C. L. (1994). Hemodynamics of the crayfish *Procambarus clarkii*. *Physiological Zoology* **67**, 449-467.

Reiber, C. L. (1997). Oxygen Sensitivity in the Crayfish Procambarus clarkii: Peripheral O2 Receptors and Their Effect on Cardiorespiratory Functions. *Journal of Crustacean Biology* **17**, 197-206.

Sagawa, K., Maughan, L., Suga, H. and Sunagawa, K. (1988). Cardiac contraction and the pressure-volume relationship. New York: Oxford University Press.

Somero, G. N. (2010). The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. *Journal of Experimental Biology* **213**, 912-920.

Stillman, J. and Somero, G. (1996). Adaptation to temperature stress and aerial exposure in congeneric species of intertidal porcelain crabs (genus Petrolisthes): correlation of physiology, biochemistry and morphology with vertical distribution. *Journal of Experimental Biology* **199**, 1845-1855.

Stillman, J. H. (2002). Causes and Consequences of Thermal Tolerance Limits in Rocky Intertidal Porcelain Crabs, Genus Petrolisthes. *Integrative and Comparative Biology* **42**, 790-796.

Stillman, J. H. (2004). A comparative analysis of plasticity of thermal limits in porcelain crabs across latitudinal and intertidal zone clines. *International Congress Series* **1275**, 267-274.

Team, R. C. (2012). R: A Language and Environment for Statistical Computing. Vienna, Austria.

Thorp, J. H. and Hoss, D. E. (1975). Effects of salinity and cyclic temperature on survival of two sympatric species of grass shrimp (Palaemonetes), and their relationship to natural distributions. *Journal of Experimental Marine Biology and Ecology* **18**, 19-28.

Tobita, K. and Keller, B. B. (2000). Maturation of end-systolic stress-strain relations in chick embryonic myocardium. *American Journal of Physiology - Heart and Circulatory Physiology* **279**, H216-H224.

Ungherese, G., Boddi, V. and Ugolini, A. (2008). Eco-physiology of Palaemonetes antennarius (Crustacea, Decapoda): the influence of temperature and salinity on cardiac frequency. *Physiological Entomology* **33**, 155-161.

Vernberg, F. J. and Piyatiratitivorakul, S. (1998). Effects of salinity and temperature on the bioenergetics of adult stages of the grass shrimp (Palaemonetes pugio Holthuis) from the North Inlet Estuary, South Carolina. *Estuaries* **21**, 176-193.

Wang, T. and Overgaard, J. (2007). The Heartbreak of Adapting to Global Warming. *Science* **315**, 49-50.

Warburton, D., Haykowsky, M., Quinney, H., Blackmore, D., Teo, K. and Humen, D. (2002). Myocardial response to incremental exercise in endurance-trained athletes: influence of heart rate, contractility and the Frank-Starling effect. *Experimental Physiology* **87**, 613-622.

Weatherley, A. H. (1970). Effects of superabundant oxygen on thermal tolerance of goldfish *The Biological Bulletin* **139**, 229-238.

Weibel, E. R., Taylor, C. R. and Hoppeler, H. (1991). The concept of symmorphosis: a testable hypothesis of structure-function relationship. *Proceedings of the National Academy of Sciences* **88**, 10357-10361.

Wilkens, J. L., Cavey, M. J., Shovkivska, I., Zhang, M. L. and Keurs, H. E. D. J. t. (2008). Elasticity, unexpected contractility and the identification of actin and myosin in lobster arteries. *Journal of Experimental Biology* **211**, 766-772.

Wilkens, J. L., Kuramoto, T. and McMahon, B. R. (1996). The effects of six pericardial hormones and hypoxia on the semi-isolated heart and sternal arterial valve of the lobster Homarus americanus. *Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology* **114**, 57-65.

Wilkens, J. L. and McMahon, B. R. (1992). Intrinsic-Properties and Extrinsic Neurohormonal Control of Crab Cardiac Hemodynamics. *Experientia* **48**, 827-834.

Wilkens, J. L., Young, R. E. and Dicaprio, R. A. (1989). Responses of the Isolated Crab Ventilatory Central Pattern Generators to Variations in Oxygen-Tension. *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology* **159**, 29-36.

Withers, P. C. (1992). Comparative Animal Physiology. United States: Brooks/ Cole--Thompson Learning.

Worden, M. K., Clark, C. M., Conaway, M. and Qadri, S. A. (2006). Temperature dependence of cardiac performance in the lobster Homarus americanus. *Journal of Experimental Biology* **209**, 1024-1034.

Zinebi, H., Simmers, J. and Truchot, J. P. (1990). A Peripheral Arterial O2-Sensitive Pathway to the Respiratory Oscillator of the Shore Crab Carcinus Maenas. *Journal of Experimental Biology* **148**, 181-199.

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Summer 2006 at Harry Allen to Chuck Lenzie	Clearance surveys for desert tortoise (*Gopherus agassizii*)
Summer 2006	Construction Monitor Harry Allen to Chuck Lenzie
Summer 2004, 2005 —	Survey for desert tortoise (*Gopherus agassizii*) and sensitive plants Searchlight to River Road—ZOI; Lincoln County ZOI; Thunderbird—presence absence surveys
Summer 2000, 2001, 2002	Field assistant in project investigating prey base differences and reproductive output of flammulated owls (*Otus flammeolus*) in northern Utah
Summer 1997, 1998, 1999 Utah	Internal surveys for bats in abandoned mines throughout
Summer 1997, 1998, 1999	Internal and exit surveys for Townsend's big-eared bats (*Corynorhinus townsenii*) at Logan Cave, UT
July 1997	Anabat night survey for spotted bats (*Euderma maculatum*) in Huntington Canyon, UT
December 1995	Survey breeding habitat for cavity-nesting birds in Nebraska National Forest

PRESENTATIONS:

Teresa L. Mika, Carl L. Reiber. 2013. Cardiac response to temperature in hyperoxia and normoxia in the grass shrimp, *Palaemonetes pugio*. Society of Integrative and Comparative Biology Annual Meeting: San Francisco, CA.

Teresa L. Mika, Carl L. Reiber. 2012. Cardiac performance across temperature extremes in the grass shrimp, *Palaemonetes pugio*. Society of Integrative and Comparative Biology Annual Meeting: Charleston, SC.

Teresa L. Mika, Carl L. Reiber. 2011. Ventilatory response to temperature extremes in the grass shrimp, *Palaemonetes pugio*. Society of Integrative and Comparative Biology Annual Meeting: Salt Lake City, UT.

Teresa L. Mika, Carl L. Reiber. 2010. Effect of Temperature on the Cardio-respiratory System in Grass Shrimp (*Palaemonetes pugio*). APS Intersociety Meeting: Global Change and Global Science: Comparative Physiology in a Changing World: Westminster, CO.

Teresa L. Mika, Carl L. Reiber. 2009. Physiological limitations to cardiovascular function in thermally stressed grass shrimp (*Palaemonetes pugio*). Society of Integrative and Comparative Biology Annual Meeting: Boston, MA.

Carl L. Reiber, Teresa L. Mika 2008. Cardiac function in response to exercise and changing temperature: matching oxygen delivery mechanisms to changing metabolic demands using a poikilothermic (Crustacean) model. APS Intersociety Meeting: The Integrative Biology of Exercise V: Hilton Head, SC

Teresa L. Mika, Carl L. Reiber. 2008. Diastolic filling time limits cardiac output in thermally stressed grass shrimp (*Palaemonetes pugio*): Physiological limits to temperature extremes. *BIOS* Symposium: UNLV.

Teresa L. Mika, Carl L. Reiber. 2008. Diastolic filling time limits cardiac output in thermally stressed grass shrimp (*Palaemonetes pugio*): Physiological limits to temperature extremes. *FASEB J.* 2008 22:lb106

Teresa L. Mika, Carl L. Reiber. 2008. Diastolic filling time limits cardiac output in thermally stressed grass shrimp (*Palaemonetes pugio*): Physiological limits to temperature extremes. Society of Integrative and Comparative Biology Annual Meeting: San Antonio, TX.

Carl L. Reiber and Teresa Mika. 2007. Cardiac temperature sensitivity in the grass shrimp (*Palaemonetes pugio*): Physiological limits to temperature extremes. Society of Integrative and Comparative Biology Annual Meeting: Phoenix, AZ.

Carl L. Reiber, Jutta Guadagnoli, Teresa Mika. 2006. Cardiac Performance During Extreme Temperature and Hypoxic Exposure in Shrimp. American Physiological Society Comparative Physiology Fall Meetings entitled “Integrating Diversity”: Virginia Beach, VA.

PROFESSIONAL SOCIETIES:

Society for Integrative and Comparative Biology

PROFESSIONAL SERVICE AND OUTREACH:

President of Biology Graduate Student Association (BIOS) 2005