2013

METABOLISM, HYPOXIA TOLERANCE AND HEAT SHOCK RESPONSE OF AMPHIPODS, EMPHASIZING THE HYPERIID AMPHIPOD PHRONIMA SEDENTARIA

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BY

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A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

BIOLOGICAL SCIENCES

UNIVERSITY OF RHODE ISLAND

2013

DOCTOR OF PHILOSOPHY DISSERTATION

OF

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2013

ABSTRACT

This work investigates the ecophysiology of marine amphipods. Amphipods are an important part of the zooplankton community in the pelagic environment. Amphipods are a food source for a variety of fishes and also have a role in carbon cycling. Little is known about their physiology and how they have adapted to environmental variation.

The Intergovernmental Panel on Climate Change (IPCC) reports that global warming is causing temperatures to rise throughout the world's oceans, a trend that will continue with rising human carbon emissions. As temperature and CO_2 levels increase, oceanic oxygen levels are predicted to decrease, and as a result, oxygen minimum zones will expand. Ocean general circulation models have shown that the detectable decrease in dissolved oxygen concentrations is driven by increasing ocean surface temperatures and enhanced stratification. Low oxygen concentrations and high temperatures affect physiological performance and, consequently, vertical distribution and ecology of marine organisms. Vertically migrating amphipods living in the Eastern Tropical North Pacific currently experience temperature changes of 15 degrees Celsius or more and changes in oxygen concentration from saturation to near anoxia.

Metabolic depression is the reduction in total metabolic rate, including aerobic and anaerobic ATP consumption, to below the basal metabolic rate. This happens in response to environmental stress such as extreme temperatures, desiccation, anoxia and food deprivation. The tolerance of an organism to low oxygen is inversely related to the extent of their metabolic.

Organisms subjected to physiological stress, such as stresses that cause proteins to denature, will respond by producing heat shock proteins (hsps). Hsps act as molecular chaperones and are able to prevent/reduce denaturing of proteins and target proteins that are irreversibly denatured for removal from the cell via the ubiquitin-proteosome pathway. No previous studies have been done on midwater amphipods to see if the temperature gradient they experience during diel vertical migration induces a stress response.

Chapter 1 examines how temperature and oxygen gradients affect the physiology of the amphipod *Phronima sedentaria* by quantifying the aerobic and anaerobic metabolic rates at oxygen levels consistent with those experienced across *Phronima*'s vertical range in tropical regions. Total ATP production (metabolic rate) was compared in specimens subjected to night time surface conditions (oxygenated) and day time conditions (hypoxia).

In Chapter 2 protein concentrations of hsp 70 were measured in specimens subjected to a range of temperatures within and above what they typically experience. Understanding the adaptations of pelagic amphipods to their current environment will help predict the physiological impacts of global warming for amphipods and their predators. One adaptation for living in hypoxia is metabolic depression. Metabolic rates of organisms are affected by a number of variables, particularly by temperature, body mass and ecology. Metabolic rate typically doubles or triples for every 10°C change in body temperature. Routine oxygen consumption rates of most vertically migrating, visually oriented, midwater crustaceans decline with depth primarily due to temperature, but also due to the low light and consequential lack of visual cues which

reduces locomotion needs. Transparent organisms in epipelagic regions would be relieved of this selective pressure because they are hidden from their visual predators. Hyperiid amphipods are the only group of crustaceans that are truly dominated by transparency. The influence of transparency on metabolic rate has not been examined in amphipods.

Chapter 3 sought to determine what environmental and ecological factors influence the rate of metabolism in marine amphipods by examining a broad data set from polar to tropical environments, and including transparent specimens. The data set for this study was obtained from the literature and original data. Recent molecular work allowed us to look at hyperiid metabolism in a phylogenetic context. Understanding patterns of pelagic and deep sea metabolism is important for further understanding of global carbon flux and the consequences of climate change on migration strategies.

ACKNOWLEDGMENTS

This research would not have been possible without my major professor, Dr. Brad Seibel. Brad Steered me in exploring my own interests to create this dissertation. Since I began working in the lab as an undergraduate Brad's mentorship has vastly improved my research and writing abilities, making me a better scientist. Thank you Brad for the many years of guidance. I look forward to your continued mentorship throughout my career.

I would like to thank my committee members, Drs Steve Irvine, Terence Bradley, Karen Wishner and Cheryl Wilga for their helpful comments and advice, which have greatly improved this dissertation. I am especially grateful to Karen Wishner for her expertise in oxygen minimum zones and zooplankton ecology. Karen's insight both at sea and while at URI helped guide my work. Cheryl Wilga has been a resource since I took her comparative vertebrate anatomy course as an undergraduate, thank you for your continued support and advice throughout my career.

I have had the benefit of interaction with many collaborators on my various research trips. I would like to especially thank Drs, Patrick Walsh, Gretchen Hofmann and Steve Haddock for their advice and support. Dr. Sönke Johnson has been especially influential as a resource at sea, and at conferences as well as providing much appreciated career advice.

I would like to thank the University-National Oceanic Laboratory System (UNOLS) for providing logistics and research support for research vessels as well as the captains and crews of the research vessels: Knorr, Steward Johnson, New Horizon, and the Endeavor. This research would not have been possible without them. I would

also like to thank the United States Antarctic program and the support staff at McMurdo station from 2007-2009.

Thank you to the Dr. Niall Howlett for allowing me to use his equipment to conduct my western blots for determination of hsp70 concentrations. His advice during the process was incredibly appreciated. Rebecca Boisvert and Meghan Rego were invaluable in training me to conduct and trouble shoot western blots. Thank you to the remaining members of the Howlett lab for help when I needed it and always making me laugh: Liz Vuono, Paul Azzinaro, Karissa Paquin, and Dave Vierra.

Thank you to my labmates Drs Lloyd Trueblood and Rui Rosa for training me in lab techniques and advising me early on in my graduate career. Thank you to my other Seibel lab mates past and present, Rachel wigton, Abigail Bockus, Trisha Towanda, Stephanie Bush, Jillian Schneider, and Al Nyack. Thank you to the many friends I have made at URI that have been a constant support: Margot Schwalbe, Michele Guidone, Christine Newton, Jamie Rafter, Anabela Maia, and Niels Hobbs.

Finally thank you to my family for their support throughout this process. Especially thank you to my husband Brent, who I met while working in Antarctica while I was doing research. This dissertation would not have been possible without his support. Brent edited many early versions of this dissertation and helped me stay calm when I was stressed, and tolerated my grumpiness when I was low on sleep.

PREFACE

This dissertation is presented in manuscript format in accordance with the guidelines set forth by the Graduate School of the University of Rhode Island. Each chapter is written to stand alone as a separate research question while contributing to the greater body of knowledge ecophysiology of amphipods. Chapter 1 will be submitted to the Marine Ecology Progress Series. Chapter 2 is in preparation for Journal of Comparative Physiology and Biochemistry part A. Chapter 3 is in preparation for Marine Ecology Progress Series.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 2

CHAPTER 3

Publication Status:

Chapter 1
Ecophysiological implications of vertical migration into oxygen minimum zones for the hyperiid amphipod *Phronima sedentaria*

This manuscript with be submitted to the journal Marine Ecology on December 7th, 2013

CHAPTER 1

Ecophysiological implications of vertical migration into oxygen minimum zones for the hyperiid amphipod *Phronima sedentaria*

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Abstract

Phronima sedentaria is a hyperiid amphipod that undergoes a diel vertical migration into a pronounced oxygen minimum zone in the Eastern Tropical North Pacific (ETNP). In this study, oxygen consumption and lactate production were measured in *P. sedentaria* to estimate the aerobic and anaerobic contributions, respectively, to total metabolism under conditions that mimic its day- (1% oxygen, 10°C) and night-time (20% oxygen, 20°C) conditions. When exposed to hypoxia and low temperature, the total metabolism of *P. sedentaria* was depressed by 78% compared to normoxic conditions. The metabolic enzymes citrate synthase (CS) and lactate dehydrogenase (LDH) were also measured as indicators of aerobic and anaerobic metabolism, respectively, and were compared to specimens collected from the California Current and the North Atlantic to assess potential adaptations to low oxygen. LDH activity was not significantly different between regions. Significant differences in CS activity between specimens from different oceans may be due to variation in food availability.

Key words: Metabolic depression, climate change, hypoxia, anaerobic metabolism, oxygen minimum zones, *Phronima,* hyperiid amphipods, zooplankton, lactate

Introduction

In some regions of the oceans, at intermediate depths, biological oxygen use exceeds the rates of oxygen replenishment via the processes of advection and diffusion (Packard et al. 1988) leading to zones of low oxygen. These oxygen minimum zones (OMZs) occur in areas of high primary productivity such as the Eastern Tropical North Pacific, where organic matter from the surface sinks and decays, adding to the oxygen demand at intermediate depths (Levin 2002, Fiedler & Talley 2006). The OMZ in the Eastern Tropical North Pacific (ETNP) is remarkable for both its size and degree of hypoxia (Kamykowski & Zentara 1990). This OMZ extends vertically from 50m to 1200m (Fernández-Álamo & Färber-Lorda 2006). Below 300m oxygen levels vary, but levels can be less than 2 μM (0.15kPa, 0.04ml l^{-1}) (Wishner et al. 2013). The California current has a less severe OMZ, with oxygen levels reaching a minimum of 13.4 μM (0.8kPa, 0.3 ml l^{-1}) (Childress & Seibel 1998).

OMZs are predicted to expand both vertically and horizontally as a result of the changing world climate (Bograd et al. 2008, Stramma et al. 2008, Keeling et al. 2010, Deutsch et al. 2011). Most of the oxygen decrease is attributed to increased stratification, which limits the mixing of oxygenated surface waters with subsurface waters and reduces the subsurface oxygen concentrations (Clark et al. 2013). Increasing global temperatures will warm ocean surface waters, leading to a decrease in oxygen content because oxygen is less soluble in warm water. Oxygen levels influence vertical distribution and ecology of marine animals (Vinogradov et al. 1996, Wishner et al. 2013). The effects on crustacean zooplankton are particularly important

because of the role of zooplankton as a link between marine primary producers and upper trophic levels (Ekau et al. 2010). Understanding how oxygen concentrations affect crustacean physiology is important because expanding OMZs may cause fluctuations in species' vertical and horizontal habitat ranges. Those fluctuations could, in turn, change ecosystem trophic structures due to alterations in predator-prey interactions as well as affecting carbon cycling (Seibel 2011, Doney et al. 2012).

Most studies on hypoxia tolerance of marine animals have been conducted in OMZs where dissolved oxygen levels are relatively higher than in the OMZ of the Eastern Tropical North Pacific. Organisms found in the California Current OMZ are often able to remain aerobic (Childress 1977), so there is little effect of low oxygen on organism distribution. This ability to extract oxygen from hypoxic water is due to a variety of adaptations including: increased ventilation and circulation capacity, high gill surface area, short blood to water diffusion distances, and respiratory proteins with high oxygen affinity and cooperativity (Sanders & Childress 1990, Childress & Seibel 1998). The distribution in such moderate OMZs is dominated by permanent deep-living zooplankton and micronekton throughout the depth range (Vinogradov et al. 1996, Childress & Seibel 1998, Robinson et al. 2010).

At oxygen concentrations less than ~ 10 μM in the ETNP, there is a reduction in biomass at depth. Most organisms either live at the upper or lower OMZ interfaces (zones of steep oxygen gradients), or vertically migrate to more oxygenated waters at night (Vinogradov & Voronina 1962, Wishner et al. 1990, Saltzman & Wishner 1997, Wishner et al. 2013). However, organisms accustomed to variable and transient hypoxia, such as that experienced by diel vertical migrators, will often depress their

total ATP consumption rate to limit the accumulation of harmful anaerobic end products (e.g., H^+) and to conserve fuel stores. For example, the euphausiid *Euphausia eximia* reduces its oxygen consumption rate by more than 50% at 1% oxygen (0.8 kPa), and the contribution from anaerobic pathways was insufficient to make up the energy deficit (Seibel 2011). Thus, total metabolism was depressed. Similarly, metabolic depression has been suggested for the copepods *Gaussia princeps* (Childress 1977) and *Subeucalanus subtenuis* (Cass 2011). Metabolic depression (also known as metabolic suppression) is a common response among marine animals to environmental stressors such as desiccation, food deprivation and low oxygen (Dymowska et al. 2012). The duration of animal survival in anoxia is inversely related to the extent of their metabolic depression (Hand 1998).

Hyperiid amphipods are the third most abundant type of marine zooplankton in the crustacean subphylum, after euphausiids and copepods (Bowman & Gruner 1973, Diebel 1988). The hyperiid amphipod *Phronima sedentaria* (Forskal, 1775), in particular, has a worldwide distribution (Shih 1969, 1991) and is abundant in the pronounced oxygen minimum zone of the Eastern Tropical North Pacific. *P. sedentaria* is a diel vertical migrator, spending nighttime near the surface (0-25 m) and living at depths of 350-600 m during the day (Shih 1969, Childress & Nygaard 1974, Shulenberger 1977). Like most hyperiid amphipods, *P. sedentaria* often lives parasitically on tunicates or siphonophores, using them as a food source and a brood chamber (Madin & Harbison 1977, Laval 1978, Diebel 1992, Gasca & Haddock 2004, Bishop & Geiger 2006). Phronimids eat the internal tissue of their host leaving the remaining gelatinous matrix in a barrel shape (Hirose et al. 2005) that is propelled

through the water with the urosoma (tail) half out the back (Land 1992). Childress and Seibel (1998) suggested that amphipods may be especially tolerant of low oxygen because their gelatinous host provides a substrate that can fuel extended anaerobic metabolism.

This study was conducted to determine whether, and to what extent, *P. sedentaria* depresses metabolism to survive migration into a pronounced oxygen minimum zone and to what extent it relies on anaerobic metabolism. To test this, total metabolic rate was estimated from the accumulation of anaerobic end-products and the rates of oxygen consumption under hypoxia and normoxia. Metabolic enzyme activities were also measured as indicators of the capacity for aerobic and anaerobic metabolic rate in *P. sedentaria* from regions with varying oxygen levels.

Materials and methods

Collection:

Specimens of *Phronima sedentaria* (Forskal, 1775) were collected from the Gulf of California (27ºN 112ºW) in June 2007 and from the Eastern Tropical North Pacific (at the Tehuantepec Bowl, 11°N 98°W and the Costa Rica Dome, 9°N 90°W) in October-November 2007 and December 2008 - January 2009. Specimens were also collected from the North Atlantic, 37º 45N, 71º 24W in September 2011. For all of these locations, specimens were collected in a modified opening-closing Tucker Trawl equipped with a 30 l thermally insulated cod-end (Childress et al. 1978). The net was opened and closed using a MOCNESS- type step motor (Wiebe et al. 1985) and equipped with temperature and pressure sensors. Specimens from the California

Current (between 33 and 34ºN, 118 and 119ºW) were collected in November 2012 using a 505µm mesh bongo net and a 1 m^2 MOCNESS net with 332 µm mesh. A CTD (conductivity, temperature, density) cast was conducted daily at each station to obtain water profile information (Figure 1).

Specimens from each location were used for metabolic rate experiments. Enzyme activities were compared between specimens collected from: the ETNP, a region with a pronounced OMZ; the California Current, where the oxygen levels are higher than in the ETNP; and the North Atlantic, which does not have a strong OMZ.

Only female specimens were used for this study because they were more abundant than males in all locations. A low male to female ratio in hyperiid amphipod populations has been demonstrated in specimens from the North Pacific Central Gyre, with a mean ratio of 1 male to 2 females for 49 distinct species. *P. sedentaria* in the North Pacific Gyre study had a male to female ratio of 2.17 (Shulenberger 1977), but the total number caught was only 19 specimens. The lower abundance of males in the collection for this study with a higher sample size indicates the ratio may be closer to the mean ratio of 0.5 found in other species of hyperiid amphipods.

Total metabolism:

Glycogen stores have been shown to be an important energy store in gammariid crustaceans (Foucreau et al. 2013). Assuming glycogen stores are also used by hyperiids amphipods as substrate during anaerobic metabolism, 1.5 moles of ATP are produced per mole lactate accumulated. 6 moles of ATP are produced per mole O_2 consumed during aerobic metabolism (McDonald et al. 1998). Combining these

components provides a measure of the total ATP produced (total metabolic rate). ATP production in normoxic conditions is considered to be the stable pool of lactate for an organism's function. This stable pool of lactate was subtracted from the total ATP produced in normoxic and hypoxic conditions. Metabolic depression was then calculated from the reduction in total ATP produced when exposed to hypoxic conditions.

Metabolic rate (MO_2):

All respiration experiments were conducted at sea. After collection, specimens found parasitizing tunicates or siphonophores were gently removed from the host before acclimation. For acclimation to laboratory conditions specimens were individually transferred to filtered seawater and allowed to recover for at least 12 hours, ensuring they were in a post-absorptive (starved) state. Filtered (0.2 μm demicap filter, Fisher scientific, USA) and treated (25 μmole l^{-1} each of streptomycin and ampicillin) seawater was poured into a water jacketed gas-equilibration column, which was connected to a temperature controlled circulating water bath (Lauda, Germany). Hypoxic experiments were conducted only in the ETNP. For hypoxic experimental conditions, the water column was bubbled with a certified gas mixture of 1% oxygen (~10 μM, 0.8kPa at 10°C). For normoxic experimental conditions, water was bubbled with 21% oxygen, (balanced with nitrogen) to ensure air saturation. Hypoxic treatments were conducted at 10, 15, and 20 ºC. Normoxia treatments were conducted at 10, 15, 20 and 25ºC in the ETNP and 10, 15 and 20°C in the Gulf of California. Hypoxia at 10ºC is consistent with conditions in the ETNP at ~ 300m

depth, 15ºC is the temperature at intermediate depths of the *Phronima* distribution and 20 ºC is the temperature experienced at night. Surface temperatures can reach 25ºC in this region, so *Phronima* may occasionally experience temperatures that high.

Depending on the size of the organism, either 25 ml glass scintillation vials or glass gas-tight syringes were used as respiration chambers. There was no significant difference in metabolic rate between the chambers used for hypoxic ($T(17)=1.06;p=0.3030$) or normoxic conditions ($T(66)=1.74;p=0.0861$). The chambers were filled with water from the gas equilibration column and a single specimen was immediately placed in the chamber using feather forceps. A blank chamber with no specimen was filled with identically treated water and processed simultaneously to monitor background respiration of microbes. The chambers were sealed (air bubbles were removed) and incubated in a temperature controlled circulating water bath (Lauda, Germany) at 10, 15, 20 or 25°C. All experiments were carried out in darkness. Normoxia experiments were conducted for 5-27 hours. The size and metabolic rate of individuals was used to estimate the duration needed to provide measureable changes in oxygen saturation. Hypoxia experiments were incubated for a shorter duration of 2-6 hours to prevent complete depletion of oxygen in the chambers.

Water was removed from incubation chambers using a 500 microliter syringe (Hamilton, USA). Oxygen concentrations of the water in incubation chambers was measured at the end of the experiment using a Clark-type oxygen electrode (Clark 1956) connected to a Strathkelvin Instruments 782 Oxygen Interface (Strathkelvin Instruments, United Kingdom). The oxygen electrodes were maintained in a thermally jacketed electrode holder (MC100 Microcell, Strathkelvin Instruments, United

Kingdom) attached to the water bath of the appropriate experimental temperature (Marsh & Manahan 1999). The electrode was calibrated prior to measurements using air- and nitrogen-saturated seawater. The oxygen consumption rate of each specimen was calculated by subtracting the final oxygen concentration in the experimental chamber from final concentration in the blank chamber. At the end of incubations, all specimens were immediately blotted dry, frozen in liquid nitrogen, then transferred to a -80ºC freezer. Weights were determined from frozen specimens in the lab for all specimens except for those collected in the Gulf of California. Specimens from the Gulf of California were weighed on a shipboard balance system (Childress & Mickel 1980) and frozen in liquid nitrogen. Metabolic rate was determined per hour incubation per gram body weight for each individual.

A temperature coefficient, or Q_{10} (= $(R_2/R_1)^{((T2-T1)/10)}$, R= oxygen consumption rate, T= temperature), quantifies the factorial change in metabolic rate with 10°C change in temperature and typically falls in the range of 2-3 (Hochachka & Somero 2002). Q_{10} was calculated from the average mass specific routine metabolic rate at each temperature.

L- Lactate measurements:

To determine reliability of handheld lactate meters, measurements of lactate standards were compared using the traditional spectrophotometric method by Gutmann and Wahlefeld (Gutmann & Wahlefeld 1974, Engel & Jones 1978), and the lactate meters Accutrend (Roche Diagnostics Corp., Indianapolis, USA), and Lactate plus (Nova Biomedical, USA). Using the meter instead of the traditional

spectophotometric method reduces cost and duration of sample processing. In the preliminary trials for this study, the Lactate plus meter was not sensitive to lactate values < ~10 μmol g^{-1}. The Accutrend lactate meter provided measurements comparable to the spectrophotometric method. Prepared standard solutions of lactate were used to determine both the Accutrend meter and spectrophotemeter provide reliable and repeatable results. Other studies have also demonstrated that the Accutrend meter is an acceptable alternative to the spectrophotometric method for lactate measurement (Beecham et al. 2006, Pérez et al. 2008).

Lactate was measured in whole organisms from the ETNP. Tissue-specific measurements would miss lactate present in other parts of the body. Determining lactate of the whole organism allows lactate involved in exchange mechanisms, known as lactate shuttles (Brooks et al. 1996), to be accounted for. Measurements were done on the same specimens used for oxygen consumption in order to calculate the total metabolic rate for each individual.

Whole frozen specimens were ground on ice in a prechilled glass tissue homogenizer (Kimble Chase, USA) using a 1:2 or 1:1 dilution with homogenization buffer (465mm NACL, 19mm KCL, 20 mm Tris). The homogenate was centrifuged at 2000 rpm for five minutes at 4°C and the supernatant was removed. L-Lactate concentrations were measured on the Accutrend lactate meter using a 25 μl sample of supernatant. All samples were assayed in triplicate and compared to a lactate standard curve (sodium lactate, L7022, Sigma- Aldrich, MO, USA) which was run daily. The Accutrend lactate meter measures lactate in the homogenate using enzymatic

determination and reflectance photometry at a wavelength of 660nm (Shimojo et al. 1989, Beecham et al. 2006).

Field study:

P. sedentaria samples were collected in two separate trawls, one within and one above the oxygen minimum zone, during the day and night respectively, to assess environmental lactate production for comparison with lab experiments. These trawls were done on January 2, 2009 at station 2 in the ETNP (figure 1). The deep trawl (250-300m depth) was put in the water at 15:20 local time (21:20 GMT) at 09 01.6328º N, 89 59.1241º W. The shallow trawl, (25-50m depth) was put in the water at 22:12 local time (4:12 GMT) at 08 59.4018º N, 90 01.1542º W. Upon net retrieval, 10 specimens of *P. sedentaria* were collected from each trawl and immediately frozen in liquid nitrogen and transferred to a -80ºC freezer. Any specimens found on a tunicate or siphonophore, were gently removed prior to freezing. All specimens were alive and in good condition. The CTD data from that day (Figure 1) indicate that the oxygen concentration at the depth specimens were collected was between 1.6 and 10.6 μM oxygen for the deep trawl and between 48.9 and 195.3 μM oxygen for the shallow trawl. Specimens were weighed in the lab prior to L-Lactate measurement.

*Enzymatic activity***:**

After collection, live specimens were identified and flash frozen in liquid nitrogen at sea. Frozen specimens were shipped back to the University of Rhode Island on dry ice and stored at -80°C. Metabolic enzymes citrate synthase (CS,

Enzyme Commission number (EC) 4.1.3.7) and lactate dehydrogenase (LDH, EC 1.1.1.27) were measured on frozen specimens.

Individual, frozen *P. sedentaria* were hand homogenized on ice in 0.01M Tris buffer, (pH 7.5 at 10ºC) in a prechilled glass tissue homogenizer (Kimble Chase, USA) using a 1/3 dilution for CS and a 1/3-1/15 dilution for LDH (depending on size and activity levels). Homogenate was centrifuged at 4,500 rpm for 10 minutes at 4ºC. Aliquots of supernatant (25 µl) were added to 1 ml cocktail solution in a quartz cuvette. Assays were performed at 20ºC using a Shimadzu spectrophotometer (UV160U, Shimadzu Scientific instruments, Japan) equipped with a water-jacketed cuvette holder connected to a recirculating water bath. Measurements were done within one hour of homogenization in triplicate when possible (some specimens were too small to allow for this). Activities are expressed as µmol of substrate converted to product min^{-1} g^{-1} *P. sedentaria* frozen tissue weight.

The cocktail solution for CS is made of: 0.05 M imidazole buffer, 15 mM Magnesium Chloride solution, 4 mM DTNB (5,5-dithio-bis-2-nitronezoic acid) solution, and 3 mg Acetyl Coenzyme A. 25 µl of 40 mM oxaloacetate solution was added to start the reaction. The background activity was measured before the addition of oxaloacetate and subtracted from the final rate to derive CS activity. The spectrophotometer measures the increase in absorbance at 412 nm, which follows the increase of absorbance as coenzyme A is reduced by DTNB (Bergmeyer et al. 1985).

The LDH cocktail solution is made of: 0.2M Tris buffer (pH 7.2 at 20ºC), 0.15 mM NADH, 100mM KCL, 0.5 mM na-pyruvate; distilled water. The

spectrophotometer records the oxidation of NADH through the decrease in absorbance at 340nm (Bergmeyer et al. 1985).

Statistics:

Statistics were performed using the software SAS version 9.2 (SAS institute inc. USA). One-tailed Students t-tests were used to compare metabolic rates scaled to a common body size. One-way Analysis of Variance (ANOVA) and one-way Analysis of Covariance (ANCOVA)s were used to compare differences between treatments.

Linear regression was used to test the relationship between body mass and metabolic rate. Mass-specific metabolic rate (MO_2) and enzymatic activities typically decline with increasing body mass (M) according to a power equation ($MO_2 = aM^b$), where *a* is a normalization constant and *b* is a scaling coefficient that describes the slope of the relationship. The relationships of metabolism and enzymatic activities versus mass were linearly regressed on a log scale using KaleidaGraph version 4.1 (synergy software, USA) to obtain the power equation.

Results

Total metabolism:

In the species *P. sedentaria* from the ETNP, total metabolism (in ATP equivalents) was depressed by 78% in the hypoxic experimental conditions, consistent with the migration from surface conditions (normoxia, 20ºC) to ~ 300m in the OMZ of the ETNP (10ºC, 1% O_2). Exposure to OMZ conditions (10ºC, 1% O_2) compared to normoxic conditions at the same temperature caused a 35% reduction in total

metabolism. Surface temperature with OMZ oxygen concentrations (20ºC, 1% O_2) resulted in a 64% reduction in metabolism compared to normoxic oxygen concentrations at 20ºC (Figure 2).

Metabolic rate:

The temperature dependence of metabolism, or Q_{10} ($Q_{10}= (R_2/R_1)^{(10/(T2-T1)}$, R= oxygen consumption rate, T= temperature) for specimens from the ETNP was 1.75 between 10 and 25 °C. The Q_{10} was 1.83 between 10 and 20°C, and 1.26 between 15 and 20°C. For specimens from the Gulf of California, the Q_{10} was 1.79 between 10 and 20°C and 1.90 between 15 and 20°C. Using the Q_{10} values, metabolic rates were normalized to 20°C for comparison. The average oxygen consumption for *P. sedentaria* normalized to 20°C in normoxia was 3.65 ±0.26 μmol O_2 $g^{-1}hr^{-1}$, and 1.87±0.73 μmol O_2 $g^{-1}hr^{-1}$ in hypoxia. In the ETNP, MO_2 was significantly related to body mass according to $MO_2=0.3268*M^{-0.543}$ and $MO_2=2.4572*M^{-0.208}$ for hypoxic and normoxic treatments respectively (Figures 3 and 4). The slopes of regression lines for hypoxic and normoxic linear regressions were significantly different (ANCOVA $f(2,55)= 34.53$; $p<0.0001$). Metabolic rates were scaled to a common weight of 0.25g using the above regression equations. Hypoxia had a significant effect on metabolic rate (t-test: $t(56)=8.23$; $p<0.0001$). Mean metabolic rate for specimens normalized to 20°C and 0.25g was 0.842±0.120 μmol $g^{-1}hr^{-1}$ in hypoxia, and 3.44±0.23 μmol O_2 $g^{-1}hr^{-1}$ in normoxia (Figure 5).

The average oxygen consumption for specimens from the Gulf of California normalized to 20°C was 2.99±0.155 μmol O_2 $g^{-1}hr^{-1}$. MO_2 was significantly related to

body mass according to: $MO_2=1.9071M^{-0.25}$. The average rate for specimens from the North Atlantic normalized to 20°C was 6.34±0.94 μmol O_2 $g^{-1}hr^{-1}$, and the regression equation relating body mass to metabolic rate is: $MO_2= 3.92M^{-0.263}$ (Figure 5). Slopes of the regression lines are not significantly different (ANCOVA: F(5,87)=-0.21; p<0.8103). There is a significant difference in MO2 in normoxic conditions between the ETNP, Gulf of California and the North Atlantic (ANCOVA: F(3,89)=21.88;P<.0001, Figure 5, Table 1).

L-Lactate:

The concentrations of L-lactate in whole organism samples of *P. sedentaria* after approximately five hours of exposure to ~1% oxygen or normoxia levels at different temperatures are presented in Figure 6. Total L-lactate concentrations in whole organisms were significantly higher (T(34)=-4.76; p<0.0001) in hypoxic (10.49±1.82 μmol g^{-1}, n=15), compared to normoxic (2.85±0.40 μmol g^{-1}, n=21) treated specimens. There was no significant effect of temperature on lactate accumulation in normoxic conditions. Lactate accumulation was significantly higher at higher temperatures for hypoxic conditions (ANOVA, f(2,11)= 4.92; p<0.0297, Figure 6). Lactate accumulation in hypoxia was an average of 4.51±1.23 umol g^{-1} at 10° C, 8.71±1.24 umol g^{-1} at 16°, and 17.15±4.75 umol g^{-1} at 20°C.

Field study:

There was no significant difference in lactate accumulation for specimens collected in the shallow trawl versus the deep trawl (t-test: t(19)=-1.52; p=0.1461,

Figure 7). Based on CTD data from the day of collection (ETNP station 2, Figure 1), specimens from the deep trawl were collected at oxygen concentrations between 1.581 and 10.637 μM, close to and below *P. sedentaria*'s critical partial pressure ($P_{crit,}$ the oxygen partial pressure at which an organism's aerobic metabolic rate can no longer be maintained, Seibel, 2011) of 28 μM at 10°C (Childress 1975). The shallow trawl collected specimens at oxygen concentrations above the P_{crit}, between 48.9 and 195.3 μM. Specimens from the deep trawl had an average L- lactate accumulation of 22.56 ± 1.38 μmol g^{-1} (n=10). Shallow trawl specimens had an average L- lactate accumulation of 26.019 ± 1.78 μmol g^{-1} (n=11). CTD data from that same day and station recorded that the oxygen levels for the deep trawl were between 1.6 and 10.6 μM oxygen and the shallow trawl were between 48.9 and 195.3 μM oxygen (Figure 1).

Field caught specimens of *P. sedentaria* had significantly higher accumulation of lactate than any of the specimens used in laboratory experiments (t-test: $t(55)=-11.47$, $p<0.001$), and a significantly higher lactate accumulation than specimens for normoxia treatment experiments (t-test: $t(40)=-17.30$; $p<0.0001$, figure 7). Specimens from the two trawls had a combined average lactate accumulation of 24.29±1.58 μmol g^{-1}. Specimens used in normoxia experiments in the lab had an average lactate accumulation of 3.60±0.67 μmol g^{-1}.

Enzymatic activity:

For whole specimens from the ETNP, CS activity was an average of 1.11± 0.07 units g^{-1} (range in mass 0.07-0.47 g). CS activities were plotted on a log axis to

obtain the regression equation $CS=1.3609x^{0.157}$ (Figure 8A). Specimens from the North Atlantic had a regression equation $CS=1.1204x^{-0.328}$ and an average activity of 2.23 ± 0.27 units g^{-1} (span in activity from 1.02-3.23 units g^{-1} , range in mass 0.058-0.497 g). The regression equation for specimens from the California Current was $CS=0.811x^{-0.214}$. Specimens from the California Current had an average activity of 1.37±0.1 units g^{-1} (range 0.88-2.94 units per g, size range, 0.04-0.39 g). The slopes of the linear regressions for each collection location were significantly different (ANCOVA: $F(5,47)=14.4$, $p=<0.0001$) (Figure 8A). Enzyme activities were then scaled to a common weight of 0.15g (using the regression equations in Figure 5A) to eliminate weight as a factor in the comparison; regressions could not be compared due to differences in slopes. There was a significant effect of location on scaled CS activity, (one-way ANOVA between subjects design, $F(2,50)=30.23$; $p<0.0001$). Mean scaled CS activity was 1.05±0.06, 1.25±0.07 and 2.133±0.16 units g^{-1} CS for the ETNP, California Current and North Atlantic respectively (Figure 9A). Tukey's Honestly Significant Difference (HSD) test showed that specimens from the North Atlantic had significantly higher CS activity than specimens from the ETNP and California current (Figure 9A; $p<0.05$). There were no significant differences between the ETNP and California Current.

In ETNP specimens, LDH activities scaled positively with body mass, with a regression equation of $LDH =49.073x^{0.727}$ (Figure 8B). LDH activity was an average of 19.002±2.09 units g^{-1} (range in mass from 0.07-0.47g). LDH activity for specimens from the North Atlantic was an average of 9.96±1.73 units g^{-1} (range from 4.79-20.28 units g^{-1}, range in size from 0.058-0.497g) and the regression equation was

LDH=7.3406$x^{-0.108}$. LDH activity for the California Current was an average of 9.89±1.06 units g^{-1} (range 5.21-22.3 units per g, range in size, 0.04-0.39 g) and the regression equation for the California Current was LDH= 24.63$x^{0.443.}$ The slopes of the regressions were significantly different (one way ANCOVA: f(5,45) 6.08, p<0.0002, Figure 8B). Enzyme activity was then scaled to a common weight of 0.15g using the regression equations from Figure 5B. There was no significant effect of location on LDH activity (one-way ANOVA between subjects design: f(2,48)=2.17; p<0.1251). Mean scaled LDH activity was 13.87±1.47 units g^{-1} for the ETNP, 11.08±1.67 units g^{-1} in the California Current and 9.89±1.67 units g^{-1} in the North Atlantic (figure 9B).

Discussion

Total metabolism:

When *Phronima sedentaria* was exposed to conditions matching those to which they are exposed during their daytime migrations into the OMZ, total metabolism was depressed by 78% relative to normoxic conditions at surface temperatures (Figure 2). Anaerobic metabolism, estimated from lactate accumulation, did increase in hypoxic conditions, but was not enough to compensate for the decrease in aerobic ATP production during hypoxic exposure. Hypoxic conditions alone reduced total metabolism by 35% compared to normoxia at the same temperature.

In pronounced OMZs, where oxygen concentrations are commonly below 5% of air saturation (1% O_2, ~15 μM), metabolic depression is anticipated to be a widespread mechanism allowing energy conservation during daytime forays into

hypoxia (Seibel 2011). Two other vertical migrators found in the ETNP exhibit metabolic depression under the same conditions to which *P. sedentaria* was subjected (1% O_2 at 10ºC): Humboldt squid and euphausiid *Euphausia eximia.* Humboldt squid, *Dosidicus gigas,* reduced total metabolism by 82%; decreased routine metabolic rate from 8.9 to 1.6 umol O_2 $g^{-1}hr^{-1}$ and increased mantle octopine production from 0.5 to 5.24 umol g^{-1} at 10ºC (Rosa & Seibel 2010). *Euphausia eximia* exhibits a 45% reduction in total metabolism (Seibel 2011). Studies conducted in the ETNP have demonstrated metabolic rate depression in response to hypoxia in the copepod *S. subtenuis* (Cass, 2011; exposed to 3% oxygen at 17°C), and three species of pteropod ((Maas et al. 2012) reduced respiration rate 35-50% under 1% oxygen at 11°C), but the anaerobic contribution to total metabolism was not measured on these organisms.

Metabolic depression is often accomplished by a decrease in energetically costly activities. Depression below resting metabolic rate would include reduction of bodily activities such as movement, feeding, digestion, heart rate and ventilation (Storey & Storey 1990). Further metabolic depression below basal metabolic rate can be accomplished by a combination of decrease in protein synthesis, reduced transcription/translation, or diminished ion transport (Storey & Storey 2004). *Meganyctiphanes norvegica*, a species of krill from Osloford, Norway, have a lower swimming speed in water with lower oxygen content (Klevjera & Kaartvedta 2011). The krill species *Euphausia mucronata* slowed swimming and decreased oxygen consumption slightly when subjected to oxygen concentrations between 0.564 and 4.794 μM, (equivalent to a partial pressure between 0.203 kPa and 1.72 kPa) and stopped swimming below 0.564 μM (Teal & Carey 1967). Other marine diel

migrators have low locomotor activity during the day as well (Jaffe et al. 1999, Svetlichny et al. 2000), even in oxygenated conditions (Hiroki 1988). The pelagic shrimp (*Sergestes similis)* from the North Pacific Ocean is a diel migrator that exhibits similar swimming speeds during the day and at night and actively swims for downward migration. However, this species remains primarily above the oxygen minimum zone (Cowles 2001), indicating that low daytime activity may be more common in crustaceans adapted to migrate into OMZs. Swimming activity monitored in the Cowles study (2001) was always in a downward pattern, regardless of depth. Therefore, Cowles results may be a response to the lights from the ROV used to observe the shrimp and not a reflection of true day and night swimming behaviors. One study demonstrated that *Phronima sedentaria* will swim actively only at low light levels (below 3 cd m^{-2}), and suggested that this is a mechanism to remain at a constant light level (isolume) and therefore maintain the desired depth in the water column (Land 1992). Thus, it is not possible to conclude definitively that low oxygen is driving the reduced locomotion at depth in OMZs. Regardless, reduced activity in response to low light at depth represents an adaptation that facilitates survival in low oxygen regions.

P. sedentaria feeds more readily at night. Passage of salps through the gut of *Phronima* at night required, on average, 4 hrs 46 min and during the day more than 14 hours (Diebel 1988). This suggests that *P. sedentaria* may be able to decrease metabolism by reducing feeding and digestion rates at depth. In addition to reduction of feeding, digestion and movement, *P. sedentaria* is able to regulate biochemical pathways to accomplish metabolic rate depression. This is evident because the current

study eliminated feeding and digestion as factors by the long acclimation period, and movement was minimized by keeping specimens in darkness. Therefore, metabolic depression exhibited by hypoxia-treated specimens compared to the control specimens must have been accomplished by the shutdown of cellular processes. The arrest of cellular processes as potential mechanisms for rate reduction has not yet been examined in hyperiid amphipods, but may include reduced protein synthesis, reduced transcription/translation or ion transport (reviews by: Hand, 1998; Storey and Storey, 2004).

In the OMZ of the California current, some migrating crustaceans are able to regulate their routine metabolism down to the lowest oxygen level they experience during the day, and therefore remain aerobic. These species have very low critical partial pressures (P_{crit}), at which anaerobic metabolic pathways are upregulated (Pörtner & Grieshaber 1993, Seibel 2011). At oxygen concentrations below the P_{crit}, anaerobic pathways may be used as a supplement to oxidative phosporylation for ATP production. The crab *Pleuroncodes planipes* is an example of a pelagic crustacean that is able to remain aerobic when migrating into the OMZ of the California Current. The P_{crit} of *P. planipes* decreases with temperature, allowing it to have a very low P_{crit} of 0.26 kPa (3.53 μM) at 10°C when migrating into the OMZ (Quetin & Childress 1976). *P. planipes* is more abundant in the ETNP, which has lower oxygen levels than the California Current. In the ETNP, *P. planipes'* low P_{crit} at 10°C is most likely sufficient to remain aerobic in the lowest oxygen exposure. The lophogastrid *Gnathophausia ingens* is a permanent resident of the California Current OMZ that is able to remain aerobic. This species has a large gill surface area compared to

crustaceans living at higher oxygen partial pressures, as well as the ability to efficiently ventilate the gills, and a high ventilatory volume in low oxygen (Childress 1971). Euphausiids in OMZs have enlarged gill surface areas to increase oxygen uptake from the water so they can continue using aerobic metabolism in addition to supplementing it with anaerobic metabolism. At least one species, *Euphausia mucronata*, actively swims and feeds while in the OMZ. This is based on equal probability of finding fed animals from day and night collection, and finding plant material in the guts of surface specimens and animal material in those collected at depth (Antezana 2002).

The copepod *Gaussia princeps* cannot remain aerobic at the lowest oxygen concentrations experienced in its vertical distribution near California, but it can tolerate hypoxic conditions (0.2 ml l^{-1}, 8.93μM) for approximately 12 hours, presumably by using anaerobic metabolism and a lower metabolic rate during the day at depth (Childress 1977). In more pronounced OMZs, such as the one in the ETNP, it is uncommon for organisms to remain fully aerobic at depth because the oxygen levels are below the P_{crit} for most species. Seibel (2011) postulated a hypoxic threshold (~0.8 kPa), below which further enhancement of oxygen extraction capacity is constrained. It is not known if *Phronima* from the sampled locations have different adaptations for enhanced oxygen extraction from the water. Hyperiid amphipods that have been examined do not have oxygen binding pigments to enhance oxygen extraction from the water (Spicer & Morritt 1995). The reported mean P_{crit} for *P. sedentaria* is 2.11 kPa (28 μM at 10°C) which was determined from two specimens from the California Current (Childress 1975). For this study, specimens from the

ETNP were able to survive 6 hours at 0.8 kPa at 10°C (13.4 μM) but accumulated 4.51±1.23 μmol g^{-1} lactate. Assuming the P_{crit} is the same for the ETNP as the California Current, *Phronima sedentaria* is adapted to survive below its critical partial pressure by depressing total metabolic rate and increasing anaerobic metabolism.

Metabolic rate:

Thermal effects on respiration are often quantified by the Q_{10}, the factorial rise in a rate process for a 10°C increase in temperature (Hochachka & Somero 2002). The Q_{10} for respiration is often between 2 and 3. Outside an organism's normal temperature range, the Q_{10} may be elevated (Hochachka and Somero, 2002). Hence, the Q_{10}s of 1.26-1.9 reported in this study imply that *P. sedentaria* is probably within its normal physiological temperature range when vertically migrating. The mean oxygen consumption rate for *P. sedentaria* when compared between regions and normalized to the same temperature is significantly different between the ETNP, Gulf of California and North Atlantic (Figure 5). The average rate for the ETNP is approximately 20% higher than the Gulf of California. As shown in Table 1, the rates for the ETNP and California Current both fall within the range of most literature values. The sample size for the North Atlantic is small (4 total). Future work in the North Atlantic on a larger sample size would clarify if rates are higher in this region, The difference in oxygen consumption rate between the ETNP and North Atlantic is relatively small and may also be due to differences in regional productivity at the time of collection. The variation in metabolic rate could be due to differences in food availability in the regions when the studies were conducted, or local adaptations.

Bishop and Geiger (2006) reported a mean MO_2 of 13.07 μmol $g^{-1}hr^{-1}$ at 20 °C. The size range overlapped with the present study (range ~0.04-.45g). This value is 10 times higher than literature values and the rates from the current study (Table 1). We suspect these rates are exaggerated by stress as their specimens were only acclimated to laboratory conditions for one hour before measurements were started, which is probably not adequate time to allow for gut clearance or for animals to become accustomed to respirometry chambers. Also, it was not mentioned if experiments were conducted in darkness. These differences in methodology may result in rates for the Bahamas specimens to be elevated relative to the rates reported here. Two specimens from the current study in the North Atlantic were not used in the analysis because they were both brooding females and had very high rates, 18.67 and 9.74 μmol $g^{-1}hr^{-1}$ at 20 °C. The higher of the two was very active in the chamber, had been used for photographs prior to incubation, and is representative of an extremely stressed organism. Bishop and Granger (2006) concluded that the metabolic rate of *Phronima* is not lower than other pelagic crustaceans or pelagic amphipods. However, they compared *Phronima*'s rate to intertidal gammarid amphipods and various epipelagic shrimp species. They did not compare it to any pelagic amphipod species or to the results from Childress 1975.

In fact, the rates of oxygen consumption are close to the relatively low rates of many mesopelagic dwelling organisms. Respiratory rates in some midwater groups decrease with increasing depth of occurrence (Childress 1971, 1975). Childress (1975) examined the respiratory rates of some midwater crustaceans at temperatures characteristic of their minimum depth of occurrence near southern California.

Excluding *P. sedentaria*, the range of rate for epipelagic species (Minimum depth of occurrence, MDO, 0-100m) was 17.32-3.47 μmol $g^{-1}hr^{-1}$. The range in rate for mesopelagic (MDO 400-900m) species in the same study was 2.4-0.924 μmol O_2 $g^{-1}hr^{-1}$. *P. sedentaria* from Childress's study at 10°C had a respiration rate of 1.69 μmol O_2 $g^{-1}hr^{-1}$, which falls in the range of mesopelagic specimens from that region despite the fact that its minimum depth of occurrence is shallow (25 m). Low metabolic rates in mesopelagic zooplankton are hypothesized to be related to the decreasing selection for locomotory capacity because low light levels limit predator-prey interactions among visually oriented organisms (Childress 1995, Seibel & Drazen 2007). The low rate in *Phronima* may be related to its transparency, as this limits their visibility to predators and prey even in well-lit surface waters. Cephalopods, being highly visual predators, exhibit a decline in oxygen consumption with increasing minimum habitat depth similar to the crustaceans. However, squids from the family Cranchiidae have low metabolic rates despite occupying shallow water for at least part of their life history. It has been suggested that transparency relieves them from selective pressures on locomotion and metabolism associated with predator-prey interactions (Seibel & Carlini 2001). *Phronima* is highly transparent (Johnsen 2001), as is the salp barrel they are housed in. In fact, hyperiid amphipods are the only group of pelagic arthropods that are truly dominated by transparent forms (Johnsen 2001).

L-Lactate:

In laboratory experiments, whole specimens of *P. sedentaria* exposed to 1% oxygen concentrations (0.8 kPa, 14 μM at 10°C) had a significantly higher

accumulation of lactate (4.51±1.23 μmol g^{-1}) than specimens exposed to normoxic conditions (3.07±0.58 μmol g^{-1}, Figure 6). Increasing temperature significantly elevates the amount of lactate accumulated in hypoxic exposed specimens, but did not have a significant effect on normoxic exposed specimens (Figure 6). The lactate accumulation in hypoxic conditions is lower than reported concentrations for other crustaceans considered to be relatively hypoxia intolerant. The intertidal prawn *Palaemon elegans* subjected to slightly more hypoxic conditions (0.66 kPa, 8.96 uM at 10°C) for a similar duration accumulated a higher amount of lactate in the whole body (13.1±0.25 μmol g^{-1}, (Taylor & Spicer 1987)). Lactate accumulation in normoxic conditions was similar to this study: 3.4 μmol g^{-1}. *Palaemon elegans* experiences hypoxia in high shore tide pools but is not able to survive environmental anoxia (Taylor and Spicer, 1987). The nordic krill, *Meganyctiphanes norvegica,* is a diel migrator that has poor anaerobic capacity but occasionally encounters hypoxia when there is poor bottom water exchange in the Nordic fjords. In these conditions, oxygen concentrations at their daytime depth is close to or below their P_{crit} of 4-5 kPa at 8°C. Prolonged exposure (18hr) at 6 kPa PO_2 resulted in haemolymph lactate concentrations of 9.91±1.68 mmol l^{-1}. There was 100% mortality at 1.8kPa. 18 hours exposure to oxygen of 14.9 kPa, well above their $P_{crit,}$, led to 3.01±1.05 mmol l^{-1} lactate (Spicer et al. 1999). Although these lactate values are for haemolymph and not whole organisms, 9 mmol l^{-1} is higher than *P. sedentaria* whole body values (Figure 6) when subjected to oxygen concentrations below its own P_{crit} of 28 μM (2.11 kPa at 10°C; Childress 1975). The low levels of lactate accumulated during hypoxic

exposure in *P. sedentaria* are possible because total metabolism is depressed, an ability that many of these other species apparently lack.

It has been proposed that the scope for total lactate production may be correlated with the duration of periods of environmental exposure to hypoxia or anoxia (Pritchard & Eddy 1979). The prawns *P. elegans* and *P. serratus* have a low capacity for lactate accumulation, indicating they cannot survive long periods of hypoxia. Immediately after death, maximum lactate concentrations in tissue are 16.7 and 9.6 μmol g^{-1} for *P. elegans* and *P. serratus* respectively (Taylor & Spicer 1987). Anoxia tolerant crustaceans have been found to have much higher maximum levels of lactate. For example, burrowing shrimp species *Upogebia pugettenisis* and *Callianassa californiensis* exposed to 12 hours of anoxia had levels of 22.1±5.6 and 11.3±0.6 respectively, with maximum levels of 60 μmol g^{-1} lactate for *Upogebia* and 20 μmol g^{-1} lactate for *Callianasse. Callianasse* can survive up to 60 hours of anoxic condition (Zebe 1982). Live *P. sedentaria* specimens frozen directly from the trawl had an average lactate accumulation of 24.29±1.58 μmol g^{-1}, with the highest being 34.76 μmol g^{-1} (Figure 7). This value suggests a high capacity for lactate accumulation, similar to intertidal species. The laboratory *P. sedentaria* exposed to 0.8 kPa water had an average accumulation of 4.51±1.23 μmol g^{-1} lactate, which is much lower than the highest levels from organisms collected in the trawl (mean 24.29±1.58 μmol g^{-1}, Figure 7). The relatively low levels of lactate accumulated after exposure to oxygen partial pressures below their P_{crit} is consistent with the idea that metabolism is depressed and the requirement for anaerobic metabolism is minimized. The relatively high capacity for lactate accumulation, as evidenced by the trawl caught

specimens, may be related to locomotory and metabolic activity above the routine level such as that required for predator-prey interactions or the migration in low oxygen itself.

There was no significant difference in lactate accumulation for specimens collected in the shallow versus deep trawl (Figure 7). This may be due to the stresses of capture in the cod end of the net including: crowding, containment, temperature, and pressure changes, among others. This possibility would also explain the higher lactate concentrations in organisms frozen directly from the trawl compared to those acclimated in the laboratory for experiments. This finding indicates the importance of allowing specimens to acclimate to laboratory conditions before conducting physiology experiments.

Enzymatic activity:

The metabolic enzyme CS is an indicator of aerobic potential and LDH is an indicator of anaerobic glycolytic potential. Both of these enzymes have been previously measured in *Phronima* specimens from Exumas Sound, Bahamas (Bishop & Geiger 2006) where there is not an oxygen minimum zone. The average CS activity of *P. sedentaria* from the Bahamas was 3.00±1.90 (mean size ~ .25g, range ~0.04-.45g). The CS activity of *Phronima* in the Bahamas is higher than specimens from all three locations used in this study (mean 1.57 units g^{-1}, Figure 8B). This difference may be an artifact of the size distribution of the specimens used by Bishop and Geiger, for which we have only the range. The size ranges for their study and ours overlapped but if the distribution is skewed toward large or small specimens, the mean enzymatic

activity will be similarly skewed. Their mean CS activity falls within the range of values reported here.

In the North Atlantic, the average CS activity scaled for a 0.15g organism using the measured scaling coefficient of -0.10845, is 2.133±0.16 units g^{-1}. The California Current average activity for the same size is 1.25±0.07 units g^{-1} and the ETNP enzyme activity is an average of 1.05±0.06 units g^{-1}. Specimens from the North Atlantic had a significantly higher CS activity than the other two locations (one- way ANOVA between subjects design, $F(2,50)=30.23$; $p<0.0001$).; Figure 8A). Nutritional status contributes to differences in metabolic enzyme activities in copepods, with activity decreasing in unfed specimens (Clarke & Walsh 1993). Similarly, CS activity in the hepatopancrease of two deep sea crabs was significantly lower after one month of food deprivation, although activity in muscle tissue was not affected (Company et al. 2008). CS activity in the North Atlantic was 0.68 units g^{-1} higher than those measured in the ETNP and California Current. The higher aerobic capacity is consistent with the higher average metabolic rate in the North Atlantic than the other locations (Figure 6). This variation could be due to differences in food availability in the regions when the studies were conducted.

Gonzalez and Quiñones (2002) hypothesized that LDH activity would be elevated in organisms adapted to low oxygen environments. Evidence in the literature for increased LDH activity in organisms, particularly crustaceans, adapted to hypoxia is mixed. A study comparing enzymatic activities of different copepods species found that epipelagic copepods have a lower LDH activity, and are therefore less reliant on glycolytic energy sources than mesopelagic and bathypelagic copepods. The meso-

and bathypelagic species may use glycolysis as an energy source for burst swimming in low oxygen (Thuesen et al. 1998). LDH activities were distinct for each copepod species ranging from 0.086 to 70.027 units g^{-1}. Thuesen et al. hypothesize that survival in low oxygen is influenced by buffering ability and substrate stores and that LDH is primarily for burst swimming (Thuesen et al. 1998).

A species of scorpaenid fish, *Sebastolobus alascanus*, from the California Current OMZ had higher LDH activity (183±73 units g^{-1}) following acclimatization to hypoxia than those held for three months in normoxic laboratory conditions (89±28 units g^{-1}; Yang et al. 1992). High LDH activities in some medusae was hypothesized to help sustain swimming during vertical migration and also promote hypoxia tolerance when migrating through OMZs (Thuesen et al. 2005). In the Humboldt current system off South America, where there is a permanent subsurface oxygen minimum zone, the euphausiid, *Euphasia mucronata*, has a specific LDH activity two orders of magnitude higher than the copepod, *Calanus chilensis*, from the same region that remains in oxygenated waters (Gonzalez & Quiñones 2002). The LDH activity of a 0.25g *E. mucronata* is 12.98 units g^{-1}, using the regression equation from Gonzalez and Quiñones (2002). *C. chilensis* is a non-migrator that remains in oxygenated waters and is much smaller in maximum body size than the vertically migrating *E. mucronata*. Given that *C. chilensis* and *E. mucronata* are not only different taxa, but also ecologically distinct, this comparison does little to answer the question at hand. To test the hypothesis of elevated LDH activity relating to survival in hypoxia, the same, or closely related species should be compared from regions with and without

OMZs. This type of comparison would avoid confusion from variation in ecology and life history.

The LDH activity of *P. sedentaria* from the Bahamas measured at 20º C was 3.00±2.00 units g^{-1} (mean size ~ 0.25g, range ~0.04- 0.45 g, Bishop & Geiger 2006), which is lower than activities for all locations in this study (Figure 9B). Similar to the difference in CS activity between the present study and Bishop and Geiger, the lower LDH value may be an artifact of the size distribution of the specimens, or variation in nutritional status. *P. sedentaria* is expected to use anaerobic glycolysis for burst swimming as well as metabolic demand while migrating into regions of low oxygen. Anaerobic glycolysis may be an important strategy for burst swimming when manoeuvring the salp barrel they live in (Bishop and Geiger 2006). In the current study, *P. sedentaria* mean scaled LDH activity for a 0.15 g organism measured at 20º C was not significantly different between specimens collected from regions with oxygen minimum zones versus the oxygenated Atlantic Ocean (figure 9B). This study adds to the growing support that LDH activity is not related to survival in low oxygen environments.

Body size in relation to oxygen availability:

Chapelle and Peck (1999; 2004) propose that the concentration of oxygen in the water limits the maximum potential size in aquatic amphipods. Because oxygen solubility increases at cold temperatures, this finding may explain polar and deep-sea gigantism, and the increase in body mass with latitude among interspecific, but closely related species (Blackburn et al. 2008).

The conclusions of Chapelle and Peck 1999 were questioned by Spicer and Gaston who argued that oxygen partial pressure, not its concentration, would determine the restrictions on size (Spicer & Gaston 1999). They propose that the oxygen partial pressure gradient across the gills is what drives the movement of oxygen. Oxygen partial pressure does not change with latitude, but it does change with depth. Spicer and Gaston (1999) postulated that temperature is the more likely factor for the correlation of latitude and size than oxygen concentration or partial pressure. At the lower latitudes, the influence of increasing temperature on body size may be explained by oxygen limitation from reduced dissolved oxygen in water and increased respiratory rate (Atkinson 1995). In the pelagic environment, some groups of zooplankton show specific adaptations of the circulatory system to enhance oxygen extraction and overcome the limitations of oxygen partial pressure (Childress & Seibel 1998). These circulatory adjustments allow organisms to obtain the oxygen they need, allowing routine metabolism to continue and therefore not impacting body size. Oxygen does not become limiting until a P_{crit} has been reached. Virtually all marine organisms can regulate their metabolism to at least 68 μM oxygen at 5°C (5kPa). This critical level will increase with temperature (Seibel 2011). If oxygen is not limiting size in low oxygen environments, it will not limit body size in well oxygenated conditions such as the Antarctic where oxygen concentrations are ~300μM or the deep sea.

Previous work has suggested that broad theories of temperature-size relationships should incorporate multiple factors assessed in a taxon dependant way (Angilletta et al. 2004). Similarly, theories on oxygen-size relationships need to

consist of multivariate factors including ecology, life history, and physiology, among other factors. Recent work has proposed a more multivariate approaching by a measure of oxygen supply, known as the Oxygen Supply Index (OSI), that combines oxygen solubility and partial pressure with gas diffusion rates (Verberk et al. 2011). Moving forward, this new approach may achieve a better understanding of body size patterns for some taxa, but it has yet to be tested along gradients of oxygen found in OMZs.

The current study did not set out to address the ongoing discussion of how oxygen concentration, partial pressure or a combination of the two drives patterns in body size of aquatic ectotherms. However, if oxygen concentration is the limiting factor in maximum body size, then this trend would also be seen across the gradients of the water column such as in OMZs. We examined a single species of amphipod from four different locations, each with varying oxygen concentrations; from a severe OMZ in the ETNP to no OMZ in the North Atlantic. Due to this range, we felt it relevant to address the ongoing debate by addressing the size range of specimens from our collection. As noted previously *Phronima* has not been examined for adaptations for enhanced oxygen extraction from the water. Hyperiid amphipods that have been examined do not have oxygen binding pigments to enhance oxygen extraction from the water (Spicer & Morritt 1995).

Reported literature values for maximum size are in length (reported maximum size of female *P. sedentaria* is 42 mm (Vinogradov et al. 1996)). Length was not measured in this study because specimens were frozen in liquid nitrogen at the end of experiments. Length measurement and microscope light needed for a digital

measurement had the potential to increase stress on the specimens which could elevated lactate levels. Some of the specimens collected at each location were brooding eggs, or had juveniles on the inside of the barrel they were removed from. This indicates that the size range sampled includes size at maturity. In the ETNP, mean size was 0.249 ± 0.018 g and range was 0.0068- 0.562g, n=79. In the Gulf of California, mean size was 0.261±0.025g, range 0.037-0.474g, n=27. In the California Current, mean size was 0.123±0.007, from 0.039-0.393g, n=21. North Atlantic specimens mean size was 0.224 ± 0.042g from 0.058-0.497g, n=8. When the 95% largest organisms from each location were compared for weight, location was not a significant factor (one-way ANOVA $f(3,5)=4.31;p<0.0748$). Oxygen concentration decreases with depth in regions with OMZs. The ETNP has the most pronounced OMZ with the lowest levels of oxygen and the North Atlantic, which has no OMZ, has the highest oxygen levels (Figure 1). The lack of a significant difference in maximum size between these locations indicates environmental oxygen concentration does not limit maximum size in this species of amphipod.

Significance:

The expansion of hypoxic zones due to global climate change may cause changes in zooplankton distribution which has ecological implications including: altered species composition of an area, changes in prey availability, prey size or predation risk (Ekau et al. 2010), and/ or changes in trophic dynamics due to shifts in predator-prey interactions (Taylor & Rand 2003, Kodama et al. 2006, Ekau et al. 2010, Ikeda 2012, Wishner et al. 2013).

Climate change is causing an increase in surface water temperature and decrease in oxygen concentrations (Keeling et al. 2010) which will have important impacts on zooplankton physiology, ecology, and vertical distribution as well as carbon cycling in the region (Vinogradov & Voronina 1962, Somero 2002, Seibel 2011). Anaerobic metabolism and metabolic depression are not sustainable for long periods of time due to substrate limitations and end product accumulation. Organisms must return to oxygenated surface waters for part of the night to burn off accumulated end products. The combination of increasing temperature and decreasing oxygen supply will compress the habitable nighttime depth range of vertically migrating species (Seibel 2011, Wishner et al. 2013).

In the southern California Current region, a > 60% decline in some mesopelagic fishes is likely due to the decline of midwater oxygen levels. The aggregation of mesoplagic micronekton in the hypoxic boundary layer of the OMZ in the California Current suggests that they descend as deeply as possible to avoid visual predators while avoiding the effects of hypoxia. The shoaling of the OMZ may increase the vulnerability of these diel migrators by forcing them into better-lit waters during the day, enhancing the chance of predation from visually oriented predators (Koslow et al. 2011). Expanding OMZs would similarly effect zooplankton diel migrators that track oxygen levels, or are constrained by temperature, forcing them into shallower well-lit waters during the day and subsequent increased predation (Wishner et al. 2013).

Diel migrators that are not able to alter daytime depths will be exposed to lower oxygen for a greater time and distance. In the ETNP, the daytime biomass peak

at 200-300m, associated with diel vertical migration and located at the upper oxycline or OMZ core, was present at the same depth at two locations, despite different oxygen concentrations between the locations (Wishner et al. 2013). Nordic krill, *Meganyctiphanes norvegica,* is an example of a crustacean that is not specifically adapted to maintain oxygen uptake or capacity for anaerobic metabolism, but still vertically migrates into hypoxia (oxygen concentrations equivalent to their critical partial pressure of 4-6 kPa). Their migration rhythm must be very strong, and not overridden by low oxygen stress, since these krill enter potentially lethal conditions presumably to avoid visual predators (Spicer et al. 1999). As OMZs expand, some species of zooplankton may not be able to modify this migration rhythm. The distance to travel and duration in low oxygen could be beyond their physiological abilities, which could compromise their long term existence in regions with expanding OMZs (Wishner et al. 2013).

Oceanic transport of carbon is known as the biological carbon pump. Diel migrating zooplankton play a significant role in this interaction (Ducklow et al. 2001). Zooplankton consume phytoplankton near the ocean surface at night and migrate down during the day where they metabolize ingested food, release carbon in the forms of dissolved organic carbon (DOC), sinking faecal material, and CO_2, therefore transporting carbon to depth (Longhurst et al. 1990, Ducklow et al. 2001). Respiration and metabolic activity are among the most important components of carbon flux (Burd et al. 2010). To depress metabolism, *Phronima* will decrease feeding, digestion and respiration. This depression will result in a reduction of faecal pellet production and CO_2 excretion at depth, leading to an overall decrease in the species' contribution to

carbon flux. If metabolic depression is common to vertically migrating zooplankton, the decreased carbon input at depth would reduce the efficiency of the biological carbon pump in regions with pronounced OMZs (Seibel 2011). Burd and colleagues (2010) have noted a problem of imbalances in estimates of organic carbon sources (biogeochemical) and sinks (ecological) below the photic zone. An overestimate of metabolic activity at depth is one potential reason for this imbalance (Burd et al. 2010). Metabolic depression may be one of the reasons for the overestimate of zooplankton contribution to carbon sources at depth since they are reducing their respiration and feeding rates while in the OMZ.

Remineralisation of particulate organic carbon sets the concentration of deep ocean nutrients, which are then returned to the surface via upwelling, providing a feedback loop for the strength of primary productivity. If the carbon pump is reduced, this remineralisation will also decrease (Buesseler et al. 2007). Reduction in carbon transport to the deep sea would lead to amplification of the positive feedback on climate change and reduce total anthropogenic carbon sequestration in the ocean (Sarmiento et al. 1998, Buesseler et al. 2007).

Conclusions:

In the ETNP, the species *P. sedentaria* is adapted for diel exposure to critical oxygen partial pressures by depressing metabolism while migrating into the OMZ. LDH activity of *P. sedentaria* did not increase with decreasing environmental oxygen concentrations. This indicates that the anaerobic enzyme LDH is not used to increase anaerobic potential for *P. sedentaria* to survive migration into hypoxic conditions. As

global warming continues, oxygen minimum zones may expand and *Phronima sedentaria* may change daytime depth to avoid hypoxic waters. This could affect predator-prey interactions in the region as well as carbon cycling (Seibel 2011).

Acknowledgments

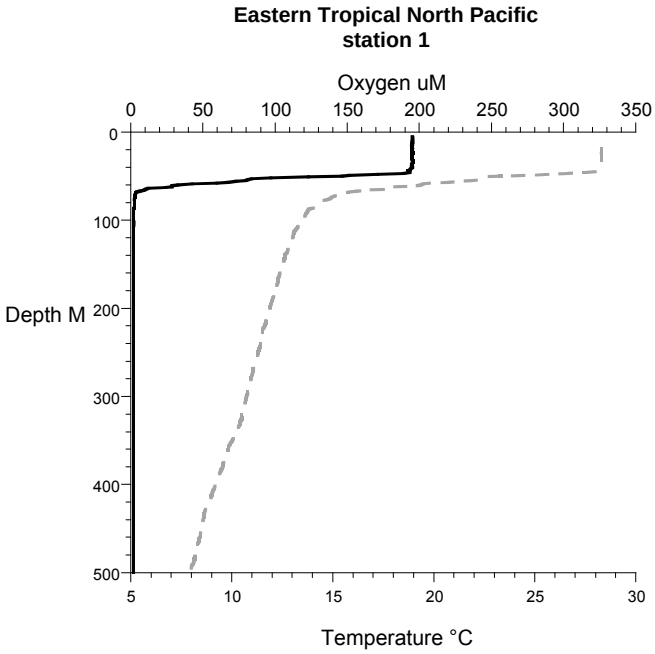
Thanks to Kendra Daly for organizing the ETNP cruises. Thanks to Karen Wishner for helpful comments and suggestions to improve this manuscript, as well as insightful discussions. This research would not have been possible without the Captains and crews of the R/V Knorr, R/V New Horizon, R/V Endeavor and R/V Steward Johnson. Thanks also to Rui Rosa, Trisha Towanda, Jillian Schneider, Christine Cass, Lloyd Trueblood, Stephanie Bush, Amy Maas and Al Nyack for assistance in net deployment for specimen collection. The Bongo net used for specimen collection during the California Current cruise was loaned to L. Elder from the Pelagic Invertebrates Collection of Scripps Institute of Oceanography. Thanks to Mark Ohman and Shonna Dovel for assistance with bongo net loan and deployment logistics.

Funding

This work was funded by the following National Science Foundation grants: In the ETNP OCE-0526502 to Karen Wishner and Brad Seibel, and OCE-0526545 to Kendra Daly. In the North Atlantic OCE-0852160 and in the Gulf of California OCE-0526493, both to Brad Seibel. Collection in the California Current was done by Leanne Elder as a participant in the 2012 University-National Oceanographic

Laboratory System (UNOLS) chief scientist training cruise, which was funded by National Science Foundation grant OCE-1041068 to Clare Reimers.

1A



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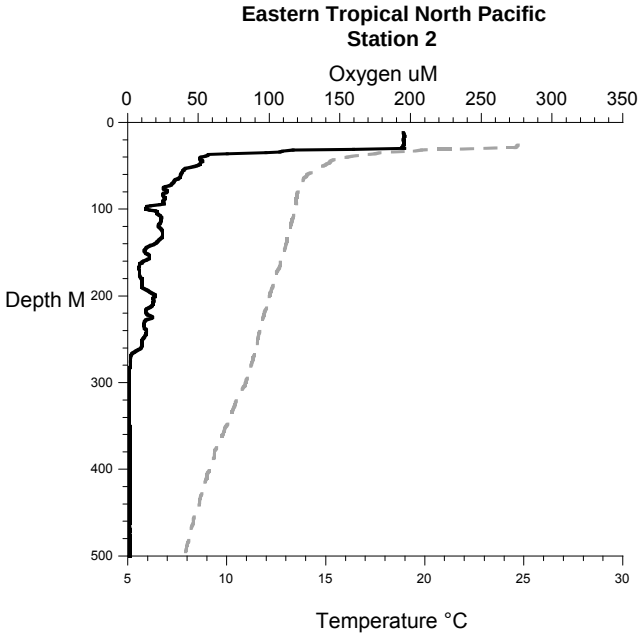

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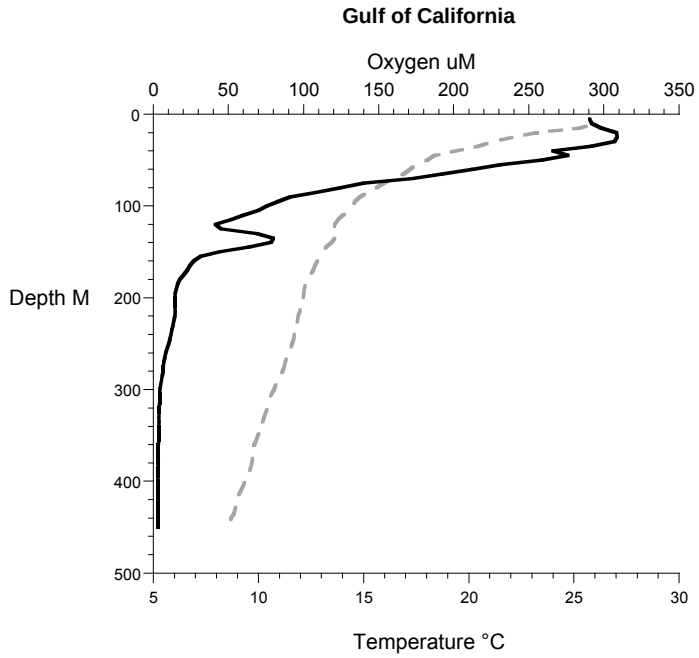
Gulf of California
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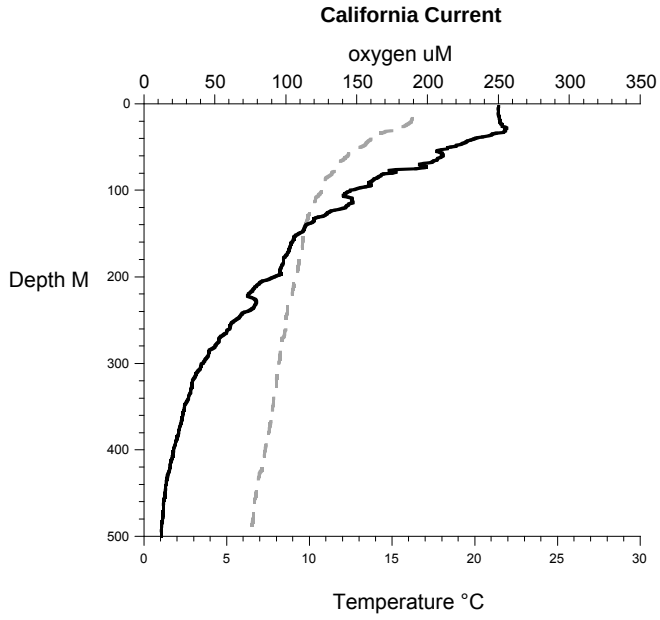
California Current
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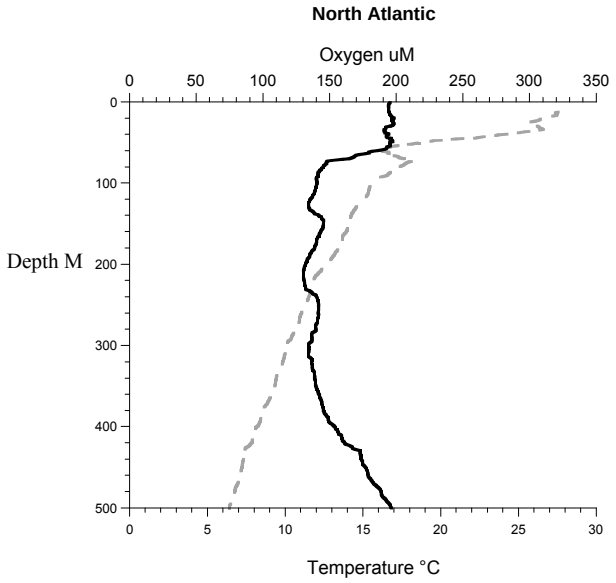


Figure 1: Representative water profiles of the top 500 meters for all study locations. Data collected with shipboard CTDs (conductivity, temperature, density), temperature -grey dashed line, and oxygen-black line.
A: Eastern Tropical North Pacific Station 1, the Tehuantepec Bowl, 11°N 98°W.
B: Eastern Tropical North Pacific station 2, the Costa Rica Dome 8.5ºN 90ºW, January 2, 2009
C: Gulf of California 27°14N 111°29W, June 2007
D: North Atlantic 39º58N, 67º59W, September 25, 2011
E: California Current 33º44N, 118º46W November 11, 2012

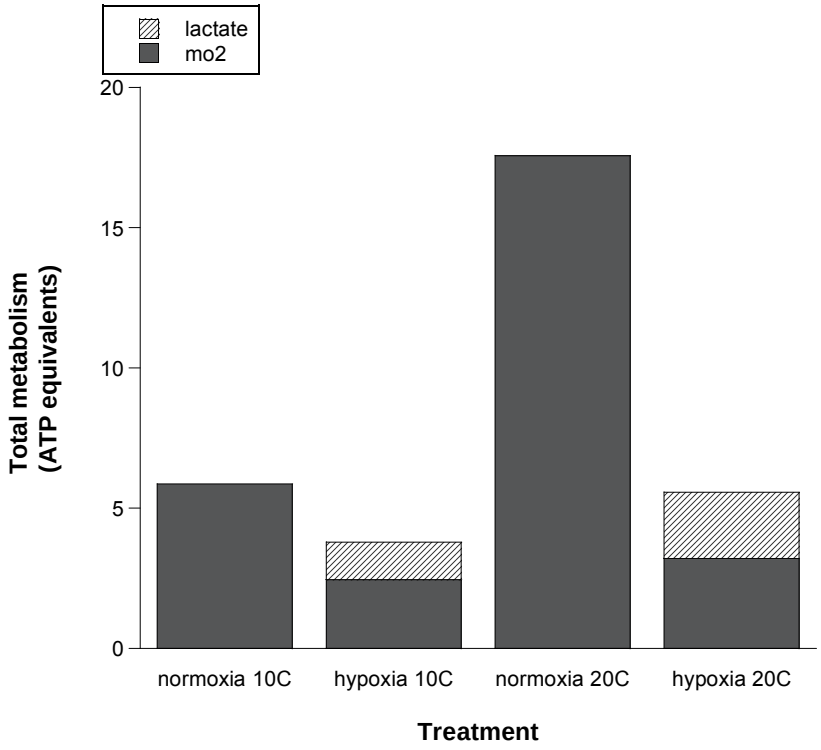


Figure 2 : Total metabolism of *P. sedentaria*. Striped: ATP produced from anaerobic metabolism, L- Lactate µmol g^{-1}. Grey: ATP produced from aerobic metabolism, µmole Oxygen g^{-1} hr^{-1}. At 10ºC the combine aerobic and anaerobic ATP production is reduced by 35% in hypoxic compared to normoxic conditions. At 20 ºC total ATP production is reduced by 64% in hypoxic conditions. The migration from normoxic, 20ºC conditions to 1% 0_2, 10ºC results in a 78% reduction in total metabolism.

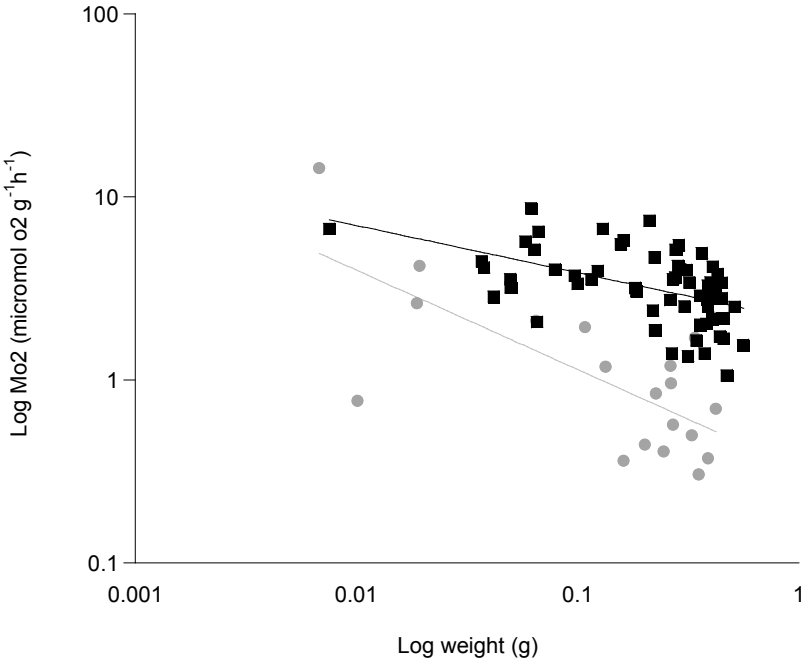


Figure 3: Routine oxygen consumption rates (MO_2) for *Phronima sedentaria,* from the Eastern Tropical North Pacific, reported in micromoles per gram frozen weight per hour on a log scale. MO_2 was significantly related to frozen weight for hypoxic (open circles) and normoxic (black squares) treatments. All MO_2s were normalized to 20ºC for comparison and are reported on a log scale. Linear regression equation for hypoxia: $MO2=0.3268M^{(-0.543)}$, $R^2=0.58$, normoxia: $MO_2=2.4572M^{(-0.2079)}$ $R^2=0.21$. n=19 hypoxia, n=39 normoxia. Slopes of the scaling curves are significantly different (ANCOVA ($f(2,55)= 34.53$; $p<0.0001$), therefore metabolic rates were scaled to a common weight for further comparison (figure 5).

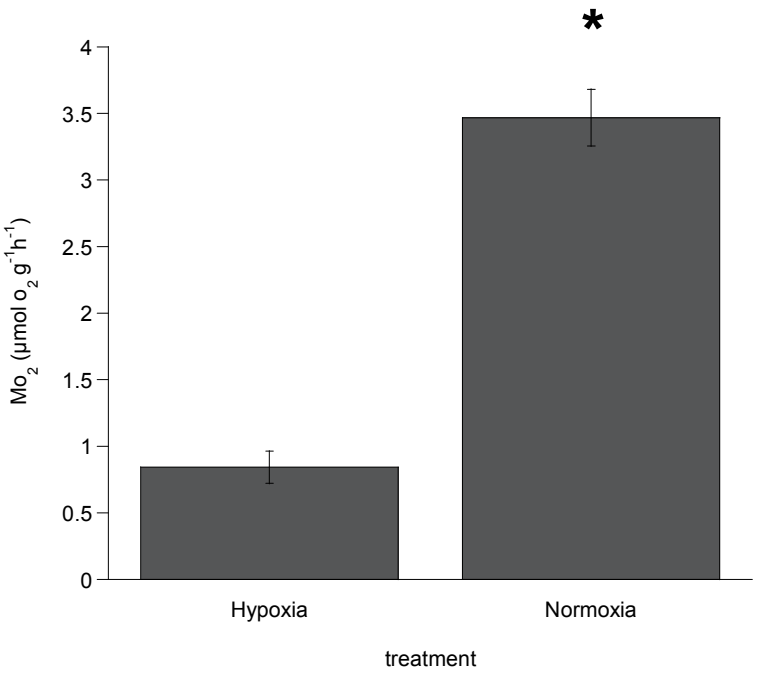


Figure 4: Routine oxygen consumption rates (MO_2) for *Phronima sedentaria*, reported in micromoles per gram frozen weight per hour. Values are mean ±se for specimens normalized to 20°C and 0.25g using the regression equations from figure 4. Metabolic rate was significantly reduced in hypoxic conditions, (t-test: $t(56)=8.23$; $p<0.0001$). N=19 for hypoxia, n=39 for normoxia.

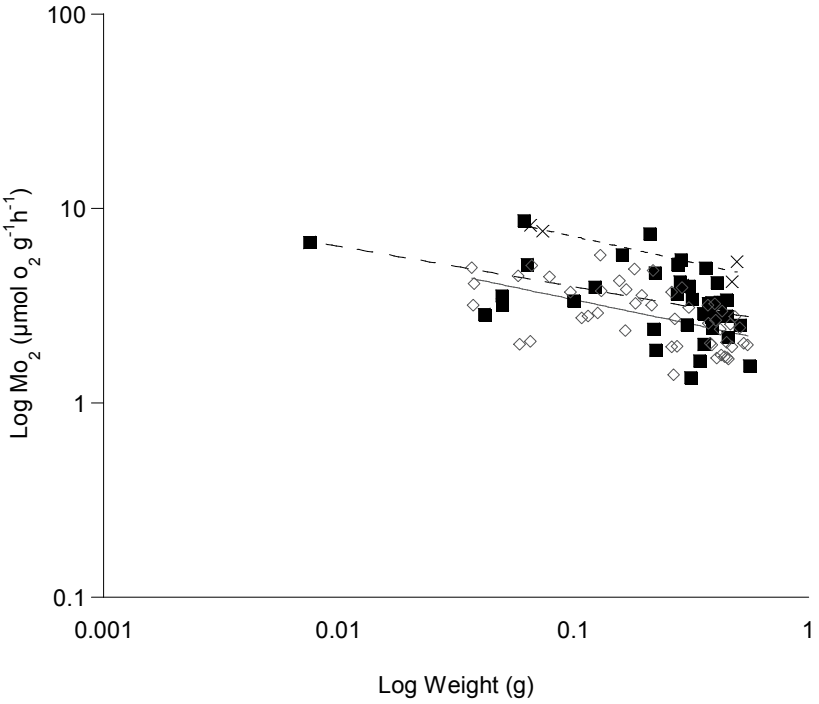


Figure 5: Routine oxygen consumption rates (Mo_2) in normoxic conditions normalized to 20°C, reported on a log scale. for the ETNP (black squares), Gulf of California (grey diamonds with no fill) and North Atlantic (x). ETNP data is also shown in figure 4. N= 49 for the Gulf of California, regression equation $Mo_2=1.907M^{(-0.25)}$, $R^2=0.29$. For the North Atlantic N=4, regression equation $Mo2=3.92M^{(-0.263)}$, $R^2=0.93$. Slopes of the regression lines are not significantly different (ANCOVA: $F(5,87)=-0.21$;$p<0.8103$). Mo_2 is significantly different between regions, (ANCOVA: $F(3,89)=21.88$; $p<0.0001$. The mean Mo_2 for each region is shown in table 1.

Mo_2 ($\mu mol\ o_2\ g^{-1}hr^{-1}$)	Location	Reference
3.65±0.26	Eastern Tropical North Pacific	This study
2.99±0.16	Gulf of California	This study
6.34±0.94	North Atlantic	This Study
2.13	California Current	(Childress, 1975)
2.68	Western Subarctic Pacific	(Ikeda, 2012)
3.65	Mediterranean Sea	(Mayzaud et al. 2005)
13.7	Central Atlantic	(Bishop and Granger, 2006)

Table 1: Average routine metabolic rate (Mo_2) by location for *Phronima sedentaria*. Rates were normalized to a common temperature of 20°C using Q_{10}s from this study when possible, or assuming a Q_{10} of 2. There is a significant different in average Mo_2 between the ETNP, Gulf of California and the North Atlantic: figure 6, ANCOVA: $F(3,89)=21.88$; $p<.0001$.

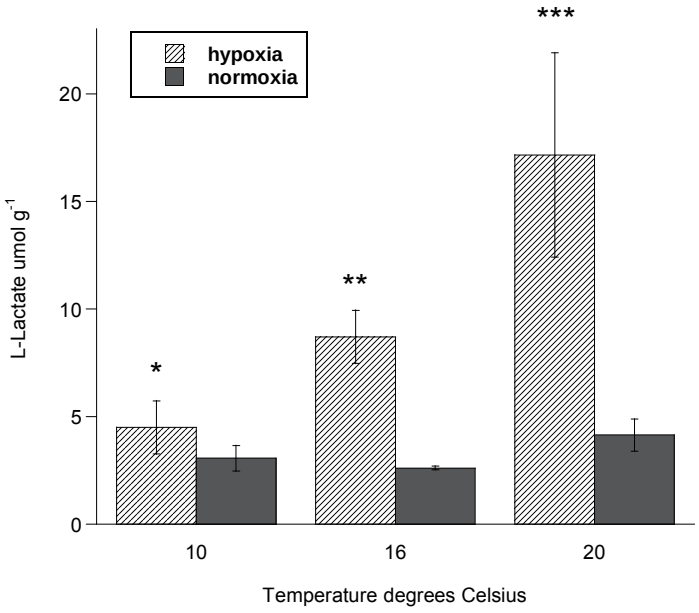


Figure 6: L- Lactate accumulation in whole specimens of *Phronima sedentaria* from the Eastern Tropical North Pacific. Striped: hypoxic, Dark grey: normoxic. Lactate accumulation was significantly higher at higher temperatures for hypoxic conditions (ANOVA, $f(2,11)= 4.92$; $p<0.0297$). There was no significant effect of temperature on lactate accumulation in normoxic conditions. For 10°C n= 5 in normoxia and 3 in hypoxia, for 16°C n=10 in normoxia and 9 in hypoxia, for 20°C n=8 in normoxia and 4 in hypoxia. All values shown are means ±SE.

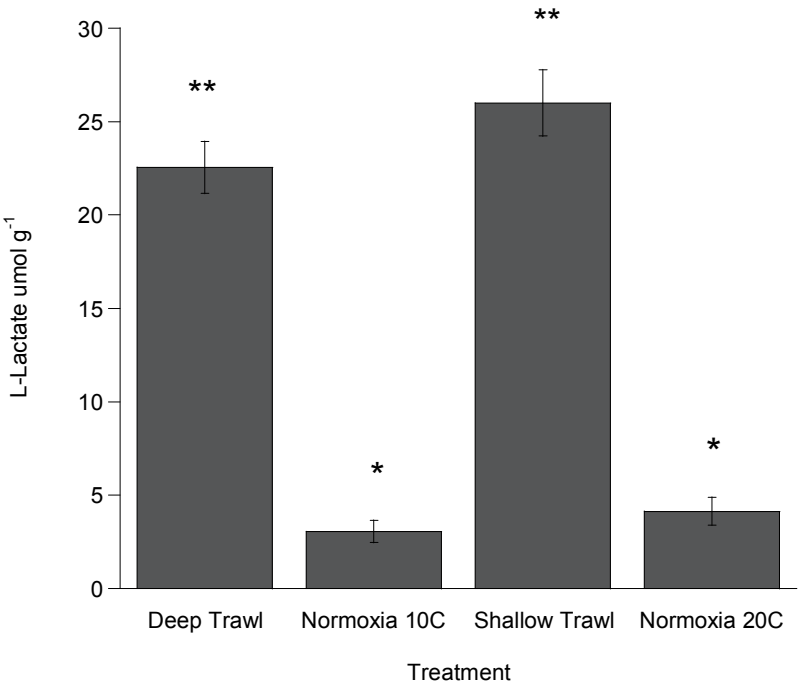
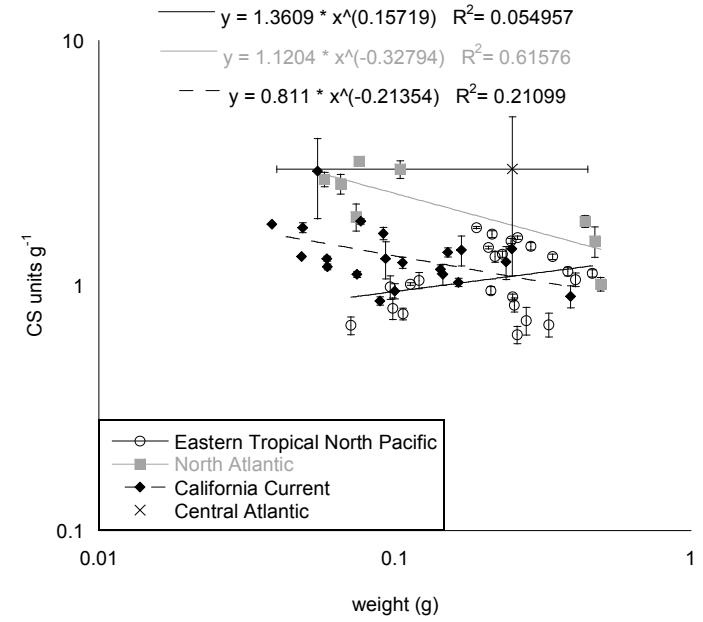
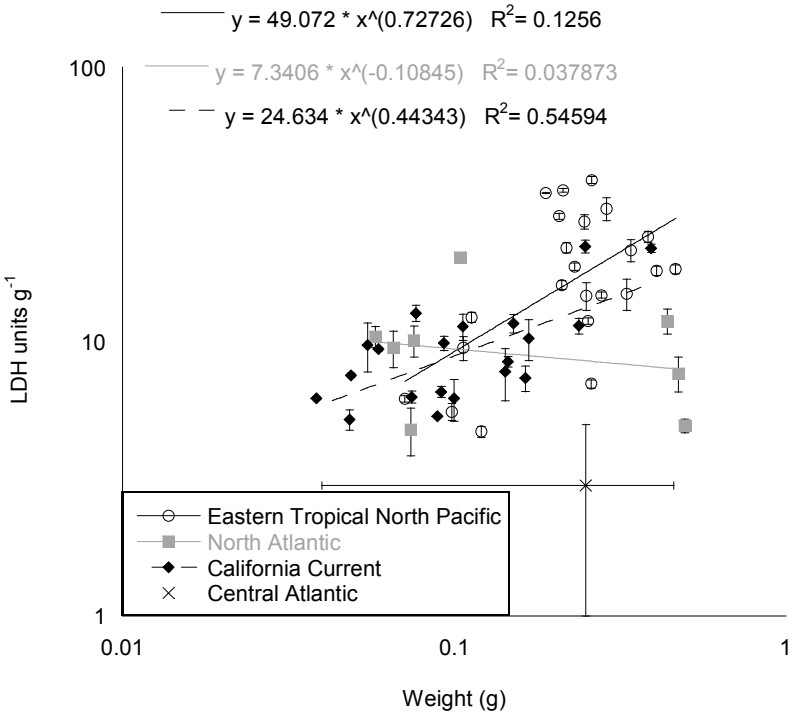


Figure 7: Lactate accumulation in specimens of *Phronima sedentaria* collected directly from deep (250-300m) and shallow (25-50m) trawls compared to experimental organisms subjected to normoxia at 10 and 20 °C. There is no significant difference between the deep and shallow trawls (t-test: t(19)=-1.52; p=0.1461). Oxygen concentration at the depth the specimens were collected at was between 1.581 and 10.637 μM oxygen for the deep trawl and between 48.852 and 195.333 μM oxygen for the shallow trawl. All values are mean ± se. This indicated organisms frozen directly after collection in the field (trawl samples) were more stressed than organisms allowed to acclimate to laboratory conditions (normoxia samples). Specimens from the trawls had a significantly higher lactate accumulation than specimens acclimated to the lab and used for normoxia experiments (t-test: t(40)=-17.30;p<0.0001). The average lactate accumulation for the two trawls was 24.29±1.58 μmol g^{-1}. The average lactate accumulation for normoxia experiments in the lab was 3.60±0.67 μmol g^{-1}.



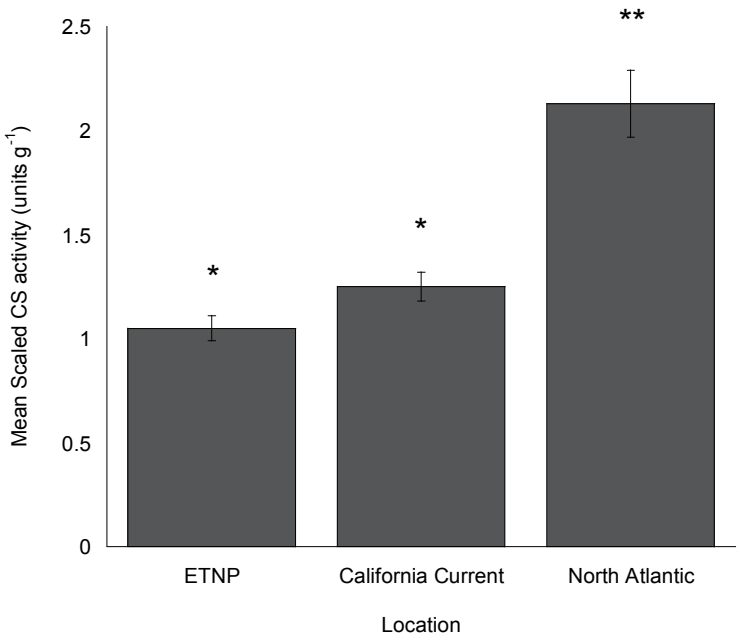
8A



8B

Figure 8A: Mass specific activity Citrate synthase (CS) and 8B: Mass specific activity Lactate dehydrogenase (LDH) in whole specimens of *Phronima sedentaria* shown on a log scale. N=25 for CS from the ETNP, N=23 for LDH Eastern Tropical North

Pacific, N=8 for CS and LDH from the North Atlantic, N=21 CS for the California current, 20 LDH California current. Regression equations are shown on the graphs. CS is an indicator of aerobic potential and LDH is an indicator on anaerobic potential. Location has a significant effect on LDH activity (ANCOVA: $f(5,38)= 4.40$; $P<0.003$), error bars represent standard deviation. The x in both plots represents the mean activity level from a previous measurement done in the Bahamas, in the Central Atlantic by Bishop and Geiger 2006, the x axis error bar represents the size range for that study, the y error bar represents the range in activity for their study.



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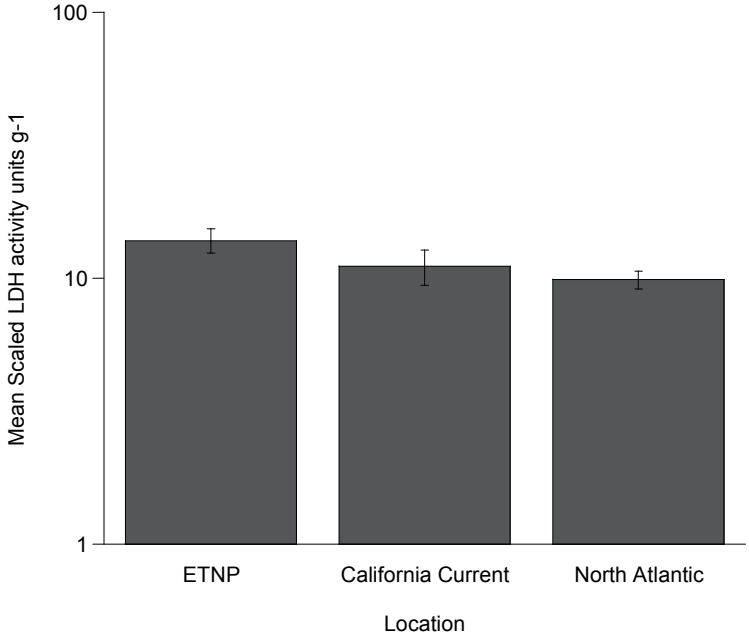

9B

Figure 9: Scaled enzyme activities in units per gram compared between *Phronima sedentaria* specimens from the Eastern Tropical North pacific (ETNP), California Current and North Atlantic. Values represent the mean activity scaled to a common mass of 0.15g ±se. A: CS activity was significantly higher in the North Atlantic than the other two locations (One- way ANOVA between subjects design, $F(2,50)=30.23$; $p<0.0001$). B: LDH activity was not significantly different between the three locations (one-way ANOVA between subjects design: $f(2,48)=2.17$; $p<0.1251$). * indicated significant difference ($P<0.05$)

References cited:

Angilletta MJ, Steury TD, Sears MW (2004) Temperature, growth rate, and body size in ectotherms: Fitting pieces of a life-history puzzle. Integrative and Comparative Biology 44:498-509

Antezana T (2002) Adaptive behaviour of euphausia mucronata in relation to the oxygen minimum layer of the humboldt current. Oceanography of the eastern Pacific 2:29-40

Atkinson D (1995) Effects of temperature on the size of aquatic ectotherms: Exceptions to the general rule. Journal of Thermal Biology 20:61-74

Beecham RV, Small BC, Minchew CD (2006) Using portable lactate and glucose meters for catfish research: Acceptable alternatives to established laboratory methods? North American Journal of Aquaculture 68:291-295

Bergmeyer HU, Bergmeyer J, Grabl M (1985) Methods of enzymatic analysis. Verlag Chemie, Weinheim Germany VIII

Bishop RE, Geiger SP (2006) Phronima energetics: Is there a bonus to the barrel? Crustaceana 79:1059-1070
Blackburn TM, Gaston KJ, Loder N (2008) Geographic gradients in body size: A clarification of bergmann's rule. Diversity and Distributions 5:165-174
Bograd SJ, Castro CG, Di Lorenzo E, Palacios DM, Bailey H, Gilly W, Chavez FP (2008) Oxygen declines and the shoaling of the hypoxic boundary in the california current. Geophysical Research Letters 35:L12607
Bowman TE, Gruner H-E (1973) The families and genera of hyperiidea (crustacea: Amphipoda), Vol. Smithsonian Institution Press
Brooks GA, Fahey TD, White TP (1996) Exercise physiology: Human bioenergetics and its applications, Vol. Mayfield publishing company
Buesseler KO, Lamborg CH, Boyd PW, Lam PJand others (2007) Revisiting carbon flux through the ocean's twilight zone. Science 316:567-570
Burd AB, Hansell DA, Steinberg DK, Anderson TRand others (2010) Assessing the apparent imbalance between geochemical and biochemical indicators of meso-and bathypelagic biological activity: What the@ $♯! Is wrong with present calculations of carbon budgets? Deep Sea Research Part II: Topical Studies in Oceanography 57:1557-1571
Cass CJ (2011) A comparative study of eucalanoid copepods residing in different oxygen environments in the eastern tropical north pacific: An emphasis on physiology and biochemistry. University of South Florida, Tampla, FL
Chapelle G, Peck LS (1999) Polar gigantism dictated by oxygen availability. Nature 399:114-115
Chapelle G, Peck LS (2004) Amphipod crustacean size spectra: New insights in the relationship between size and oxygen. Oikos 106:167
Childress JJ (1971) Respiratory adaptations to the oxygen minimum layer in the bathypelagic mysid *gnathophausia ingens*. The Biological Bulletin 141:109-121
Childress JJ (1975) The respiratory rates of midwater crustaceans as a function of depth of occurrence and relation to the oxygen minimum layer off southern california. Comparative Biochemistry and Physiology Part A: Physiology 50:787-799
Childress JJ (1977) Effects of pressure, temperature and oxygen on the oxygen consumption rate of the midwater copepod gaussia princeps. Marine Biology 39:19-24
Childress JJ (1995) Are there physiological and biochemical adaptations of metabolism in deep-sea animals? Trends in Ecology and Evolution 10:30-36
Childress JJ, Barnes AT, Quetin LB, Robison B (1978) Thermally protecting cod ends for the recovery of living deep-sea animals. Deep-Sea Research 25:419-422
Childress JJ, Mickel TJ (1980) A motion compensated shipboard precision balance system. Deep-Sea Research 27:965-970
Childress JJ, Nygaard M (1974) Chemical composition and buoyancy of midwater crustaceans as function of depth of occurrence off southern california. Marine Biology 27:225-238
Childress JJ, Seibel BA (1998) Life at stable low oxygen levels: Adaptations of animals to oceanic oxygen minimum layers. The Journal of Experimental Biology 201:1223-1232
Clark LC (1956) Monitor and control of blood and tissue oxygen tensions. Transactions - American Society for Artificial Internal Organs 2:41-48
Clark TD, Sandblom E, Jutfelt F (2013) Aerobic scope measurements of fishes in an era of climate change: Respirometry, relevance and recommendations. The Journal of experimental biology 216:2771-2782

Clarke ME, Walsh PJ (1993) Effect of nutritional status on citrate synthase activity in acartia tonsa and temora longicornis. Limnology and Oceanography:414-418

Company JB, Thuesen EV, Childress JJ, Rotllant G, Zal F (2008) Effects of food deprivation on enzymatic activities of the mediterranean deep-sea crab, geryon longipes a. Milne-edwards, 1882 and the pacific hydrothermal vent crab, bythograea thermydron williams, 1980 (decapoda, brachyura). Crustaceana 81:67-85

Cowles DL (2001) Swimming speed and metabolic rate during routine swimming and simulated diel vertical migration of sergestes similis in the laboratory. Pacific Science 55:215-226

Deutsch C, Brix H, Ito T, Frenzel H, Thompson LA (2011) Climate-forced variability of ocean hypoxia. Science 333:336-339

Diebel CE (1988) Observations on the anatomy and behavior of *phronima sedentaria* (forskal) (amphipod: Hyperiidea). Journal of Crustacean Biology 8:79-90

Diebel CE (1992) Arrangement and external morphology of sensilla on the dorsal surface of three genera of hyperiid amphipods (phronima, lycaea, and vibilia). Journal of Crustacean Biology:714-728

Doney SC, Ruckelshaus M, Duffy JE, Barry JPand others (2012) Climate change impacts on marine ecosystems. Marine Science 4:11-37

Ducklow HW, Steinberg DK, Buesseler KO (2001) Upper ocean carbon export and the biological pump. Oceanography 14:50-58

Dymowska AK, Manfredi T, Rosenthal JJC, Seibel BA (2012) Temperature compensation of aerobic capacity and performance in the antarctic pteropod, clione antarctica, compared with its northern congener, c. Limacina. The Journal of Experimental Biology 215:3370-3378

Ekau W, Auel H, Pörtner H, Gilbert D (2010) Impacts of hypoxia on the structure and processes in pelagic communities(zooplankton, macro-invertebrates and fish). Biogeosciences 7:1669-1699

Engel PC, Jones JB (1978) Causes and elimination of erratic blanks in enzymatic metabolite assays involving the use of nad+ in alkaline hydrazine buffers: Improved conditions for the assay of l-glutamate, l-lactate, and other metabolites. Analytical Biochemistry 88:475-484

Fernández-Álamo MA, Färber-Lorda J (2006) Zooplankton and the oceanography of the eastern tropical pacific: A review. Progress in Oceanography 69:318-359

Fiedler PC, Talley LD (2006) Hydrography of the eastern tropical pacific: A review. Progress in Oceanography 69:143-180

Foucreau N, Cottin D, Piscart C, Hervant F (2013) Physiological and metabolic responses to rising temperature in *gammarus pulex* (crustacea) populations living under continental or mediterranean climates. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology

Gasca R, Haddock SHD (2004) Associations between gelatinous zooplankton and hyperiid amphipods (crustacea: Peracarida) in the gulf of california. Hydrobiologia 530:529-535

Gonzalez RR, Quiñones RA (2002) Ldh activity in euphausia mucronata and calanus chilensis: Implications for vertical migration behaviour. Journal of Plankton Research 24:1349-1356

Gutmann I, Wahlefeld AW (1974) L-(+)-lactate determination with lactate dehydrogenase and nad. Methods of Enzymatic Analysis 3:1464-1468

Hand SC (1998) Quiescence in artemia franciscana embryos: Reversible arrest of metabolism and gene expression at low oxygen levels. Journal of Experimental Biology 201:1233
Hiroki M (1988) Relation between diel vertical migration and locomotor activity of a marine hyperiidean amphipod, themisto japonica (bovallius). Journal of Crustacean Biology 8:48-52
Hirose E, Aoki MN, Nishikawa J (2005) Still alive? Fine structure of the barrels made by phronima (crustacea: Amphipoda). Journal of the Marine Biological Association of the United Kingdom 85:1435-1439
Hochachka PW, Somero GN (2002) Biochemical adaptation: Mechanism and process in physiological evolution, Vol. Oxford University Press, USA
Ikeda T (2012) Metabolism and chemical composition of zooplankton from 500 to 5,000 m depth of the western subarctic pacific ocean. Journal of Oceanography 68:641-649
Jaffe JS, Ohman MD, Robertis AD (1999) Sonar estimates of daytime activity levels of euphausia pacifica in saanich inlet. Canadian Journal of Fisheries and Aquatic Sciences 56:2000-2010
Johnsen S (2001) Hidden in plain sight: The ecology and physiology of organismal transparency. The Biological Bulletin 201:301-318
Kamykowski D, Zentara S-J (1990) Hypoxia in the world ocean as recorded in the historical data set. Deep Sea Research Part A Oceanographic Research Papers 37:1861-1874
Keeling RF, Körtzinger A, Gruber N (2010) Ocean deoxygenation in a warming world. Annual Review of Marine Science 2:199-229
Klevjera TA, Kaartvedta S (2011) Krill (meganyctiphanes norvegica) swim faster at night. Limnology and Oceanography 56:765-774
Kodama K, Horiguchi T, Kume G, Nagayama Sand others (2006) Effects of hypoxia on early life history of the stomatopod oratosquilla oratoria in a coastal sea. Marine Ecology Progress Series 324:197-206
Koslow J, Goericke R, Lara-Lopez A, Watson W (2011) Impact of declining intermediate-water oxygen on deepwater fishes in the california current. Marine Ecology Progress Series 436:207-218
Land MF (1992) Locomotion and visual behaviour of mid-water crustaceans. Journal of the Marine Biological Association of the United Kingdom 72:41-60
Laval P (1978) The barrel of the pelagic amphipod *phronima sedentaria* (forsk.) (crustaces: Hyperiidea). Journal of Experimental Marine Biology and Ecology 33:187-211
Levin L (2002) Deep-ocean life where oxygen is scarce oxygen-deprived zones are common and might become more so with climate change. Here life hangs on, with some unusual adaptations. American Scientist 90:436-444
Longhurst AR, Bedo AW, Harrison WG, Head EJH, Sameoto DD (1990) Vertical flux of respiratory carbon by oceanic diel migrant biota. Deep Sea Research Part A Oceanographic Research Papers 37:685-694
Maas AE, Wishner KF, Seibel BA (2012) Metabolic suppression in thecosomatous pteropods as an effect of low temperature and hypoxia in the eastern tropical north pacific. Marine Biology 159:1955-1967
Madin LP, Harbison GR (1977) The associations of amphipoda hyperiidea with gelatinous zooplankton--i. Associations with salpidae. Deep Sea Research 24:449-456, IN441-IN444, 457-463
Marsh AG, Manahan D (1999) A method for accurate measurements of the respiration rates of marine invertabrate embryos and larvae. Marine Ecology Progress Series 184:1-10

Mayzaud P, Boutoute M, Gasparini S, Mousseau L, Lefevre D (2005) Respiration in marine zooplankton: The other side of the coin: Co 2 production. Limnology and Oceanography:291-298

McDonald DG, McFarlane WJ, Milligan CL (1998) Anaerobic capacity and swim performance of juvenile salmonids. Canadian Journal of Fisheries and Aquatic Sciences 55:1198-1207

Packard TT, Minas HJ, Coste B, Martinez Rand others (1988) Formation of the alboran oxygen minimum zone. Deep Sea Research Part A Oceanographic Research Papers 35:1111-1118

Pérez EH, Dawood H, Chetty U, Esterhuizen TM, Bizaare M (2008) Validation of the accutrend® lactate meter for hyperlactatemia screening during antiretroviral therapy in a resource-poor setting. International Journal of Infectious Diseases 12:553-556

Pörtner HO, Grieshaber MK (1993) Critical p02(s) in oxyconforming and oxyregulation animals: Gas exchange, metabolic rate and the mode of energy production. In: The vertebrate gas transport cascade: Adaptations to environment and mode of life p330-357

Pritchard A, Eddy S (1979) Lactate formation in callianassa californiensis and upogebia pugettensis (crustacea: Thalassinidea). Marine Biology 50:249-253

Quetin LB, Childress JJ (1976) Respiratory adaptations of pleuroncodes planipes to its environment off baja california. Marine Biology 38:327-334

Robinson C, Steinberg DK, Anderson TR, Arístegui Jand others (2010) Mesopelagic zone ecology and biogeochemistry–a synthesis. Deep Sea Research Part II: Topical Studies in Oceanography 57:1504-1518

Rosa R, Seibel BA (2010) Metabolic physiology of the humboldt squid, *dosidicus gigas*: Implications for vertical migration in a pronounced oxygen minimum zone. Progress in Oceanography 86:72-80

Saltzman J, Wishner KF (1997) Zooplankton ecology in the eastern tropical pacific oxygen minimum zone above a seamount: 1. General trends. Deep Sea Research Part I: Oceanographic Research Papers 44:907-930

Sanders N, Childress J (1990) Adaptations to the deep-sea oxygen minimum layer: Oxygen binding by the hemocyanin of the bathypelagic mysid, gnathophausia ingens dohrn. The Biological Bulletin 178:286-294

Sarmiento JL, Hughes TMC, Stouffer RJ, Manabe S (1998) Simulated response of the ocean carbon cycle to anthropogenic climate warming. Nature 393:245-249

Seibel BA (2011) Critical oxygen levels and metabolic suppression in oceanic oxygen minimum zones. The Journal of Experimental Biology 214:m326-336

Seibel BA, Carlini DB (2001) Metabolism of pelagic cephalopods as a function of habitat depth: A reanalysis using phylogenetically independent contrasts. Biological Bulletin, Marine Biological Laboratory, Woods Hole 201:1-5

Seibel BA, Drazen JC (2007) The rate of metabolism in marine animals: Environmental constraints, ecological demands and energetic opportunities. Philosophical Transactions of the Royal Society B: Biological Sciences 362:2061

Shih C-T (1969) The systematics and biology of the family phronimidae (crustacea: Amphipoda). Dana Reports no 74:1-100

Shih C-T (1991) Description of two new species of phronima latreille, 1802 (amphipoda: Hyperiidea) with a key to all species of the genus. Journal of Crustacean Biology:322-335

Shimojo N, Naka K, Nakajima C, Yoshikawa C, Okuda K, Okada K (1989) Test-strip method for measuring lactate in whole blood. Clinical chemistry 35:1992
Shulenberger E (1977) Hyperiid amphipods from the zooplankton community of the north pacific central gyre. Marine Biology 42:375-385
Somero GN (2002) Thermal physiology and vertical zonation of intertidal animals: Optima, limits, and costs of living. Integrative and Comparative Biology 42:780-789
Spicer JI, Gaston KJ (1999) Amphipod gigantism dictated by oxygen availability? Ecology Letters 2:397-403
Spicer JI, Morritt D (1995) Oxygen carriage by the haemolymph of hyperiid amphipods. JMBA-Journal of the Marine Biological Association of the United Kingdom 75:997-998
Spicer JI, Thomasson MA, Strömberg JO (1999) Possessing a poor anaerobic capacity does not prevent the diet vertical migration of nordic krill meganyctiphanes norvegica into hypoxic waters. Marine ecology Progress series(Halstenbek) 185:181-187
Storey KB, Storey JM (1990) Metabolic rate depression and biochemical adaptation in anaerobiosis, hibernation and estivation. Quarterly Review of Biology:145-174
Storey KB, Storey JM (2004) Metabolic rate depression in animals: Transcriptional and translational controls. Biological Reviews 79:207-233
Stramma L, Johnson GC, Sprintall J, Mohrholz V (2008) Expanding oxygen-minimum zones in the tropical oceans. Science 320:655
Svetlichny L, Hubareva E, Erkan F, Gucu A (2000) Physiological and behavioral aspects of calanus euxinus females (copepoda: Calanoida) during vertical migration across temperature and oxygen gradients. Marine Biology 137:963-971
Taylor AC, Spicer JI (1987) Metabolic responses of the prawns palaemon elegans and p. Serratus (crustacea: Decapoda) to acute hypoxia and anoxia. Marine Biology 95:521-530
Taylor JC, Rand PS (2003) Spatial overlap and distribution of anchovies (anchoa spp.) and copepods in a shallow stratified estuary. Aquatic Living Resources 16:191-196
Teal JM, Carey FG (1967) Respiration of a euphausiid from the oxygen minimum layer. Limnology and Oceanography 12:548-550
Thuesen EV, McCullough KD, Childress JJ (2005) Metabolic enzyme activities in swimming muscle of medusae: Is the scaling of glycolytic activity related to oxygen availability? Journal of the Marine Biological Association of the UK 85:603-611
Thuesen EV, Miller CB, Childress JJ (1998) Ecophysiological interpretation of oxygen consumption rates and enzymatic activities of deep-sea copepods. Marine Ecology Progress Series 168:95-107
Verberk WC, Bilton DT, Calosi P, Spicer JI (2011) Oxygen supply in aquatic ectotherms: Partial pressure and solubility together explain biodiversity and size patterns. Ecology 92:1565-1572
Vinogradov M, Voronina N (1962) Influence of the oxygen deficit on the distribution of plankton in the arabian sea Deep Sea Research and Oceanographic Abstracts. Elsevier, p 523-530
Vinogradov ME, Volkov AF, Semenova TN, Siegel-Causey D (1996) Hyperiid amphipods (amphipoda, hyperiidea) of the world oceans, Vol. Science Publications Incorporated, Lebanon, USA
Wiebe P, Morton A, Bradley A, Backus Rand others (1985) New development in the mocness, an apparatus for sampling zooplankton and micronekton. Marine Biology 87:313-323
Wishner K, Levin L, Gowing M, Mullineaux L (1990) Involvement of the oxygen minimum in benthic zonation on a deep seamount. Nature 346:57-59

Wishner KF, Outram DM, Seibel BA, Daly KL, Williams RL (2013) Zooplankton in the eastern tropical north pacific: Boundary effects of oxygen minimum zone expansion. Deep Sea Research Part I: Oceanographic Research Papers 79:122-140

Yang TH, Lai NC, Graham JB, Somero GN (1992) Respiratory, blood, and heart enzymatic adaptations of sebastolobus alascanus (scorpaenidae; teleostei) to the oxygen minimum zone: A comparative study. The Biological Bulletin 183:490-499

Zebe E (1982) Anaerobic metabolism in *upogebia pugettensis* and *callianassa californiensis*(crustacea, thalassinidea). Comparative Biochemistry and Physiology Part B: Comparative Biochemistry 72:613-617

Publication Status

Chapter 2: The Stress response to naturally occurring temperature flux for the vertically migrating hyperiid amphipod Phronima sedentaria

This manuscript will be submitted to the Journal of Comparative Physiology and Biochemistry part A

CHAPTER 2:

The Stress response to naturally occurring temperature flux for the vertically migrating hyperiid amphipod Phronima sedentaria

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Abstract

The hyperiid amphipod *Phronima sedentaria* experiences a temperature change of 15°C during diel migration in the Eastern Tropical North Pacific (ETNP). The aim of this study was to determine if the natural temperature gradient experienced by *Phronima sedentaria* results in a thermal stress response. Specimens were acclimated to their night time temperatures (23°C) and subsequently subjected to a range of temperatures within and above what they typically experience. In the ETNP *P. sedentaria* tolerates its normal migration to the surface, but is close to an upper temperature limit and approaching a maximum duration of exposure. 23°C is stressful for *Phronima sedentaria*, but is somewhat tolerated via heat-shock response at longer exposure times. An escalation in hsp 70 concentrations occurred at 29°C, which coincided with a 50% mortality rate and a significant increase in anaerobic metabolism (measured as L-lactate accumulation) under oxygenated conditions. Understanding the adaptations of pelagic amphipods to their current environment will help predict the physiological impacts of global warming for amphipods and their predators.

Introduction

Phronima sedentaria is an abundant species of hyperiid amphipod, rumored to have inspired the design of the monster in the 'Alien' movies. Its enormous eyes and claws belie its small size. *Phronima* parasitizes pelagic tunicates, turning their barrel-shaped bodies into a brood chamber (Harbison et al., 1977; Laval, 1978; Diebel, 1988). While most pelagic species of amphipods "hitch-hike" on gelatinous zooplankton that serve as physical and metabolic substrate (Harbison et al. 1977, Madin & Harbison 1977, Gasca & Haddock 2004), the relationship between *Phronima* and its parasitized host is unique in that the host is transformed by the parasite (Land 1992). Phronimids eat the internal tissue of their siphonphore or tunicate host leaving the remaining gelatinous matrix in a barrel shape (Hirose et al. 2005) that is propelled through the water with the urosoma (tail) half out the back (Land 1992).

Phronima sedentaria (*Forskål, 1775)* is found throughout the world oceans and, like many zooplankton, is a diel vertical migrator, spending the day in deeper colder waters and nighttime foraging near the surface (Shih 1969, Shulenberger 1977, Vinogradov et al. 1996, Voznesensky et al. 2004). *P. sedentaria* may encounter a temperature change of 15°C during its diel vertical migrations, experiencing surface temperatures approaching 30°C in some regions. Such wide temperature variation within the natural range of a species can be stressful (Hofmann & Somero 1995). Furthermore, the maximum habitat temperatures of many warm-adapted organisms (such as those found in the tropics) are near their maximum thermal limits. Additional increases in temperature due to climate change may not be tolerated by such organisms (Somero 2010).

Oceanic temperatures have increased over the past century as a likely result of anthropogenic carbon dioxide emissions (Trenberth et al. 2007). Increasing environmental temperatures are predicted to affect the physiological performance, and consequently the vertical distribution and ecology of marine organisms (Saltzman & Wishner 1997, Somero 2002, Seibel 2011, Doney et al. 2012). Organisms subjected to thermal stress typically respond by expressing heat shock proteins (hsps) (DuBeau et al. 1998) and may become oxygen-limited resulting in the upregulation of anaerobic metabolism (Pörtner, 2002). Hsps act as molecular chaperones that are able to prevent/reduce denaturing of proteins and target those that are irreversibly denatured for removal from the cell via the ubiquitin-proteosome pathway. Hsp 70 is one of the most highly conserved heat shock proteins, especially noted for its role in recovery from thermal stress (reviewed by (Feder & Hofmann 1999).

We have quantified the critical temperature for a tropical population of *Phronima sedentaria* from the Eastern Tropical North Pacific. The expression of hsp 70 and the production of the anaerobic end product, lactate, were quantified at temperatures spanning the range experienced by *Phronima sedentaria* (*Forskål, 1775)* across their vertical distribution. We tested the hypothesis that the highest temperatures experienced within the natural range can induce a stress response that would result in an increase in synthesis in heat shock protein 70, and a shift to anaerobic metabolism.

Materials and methods

Collection:

Phronima sedentaria (Figure 1) were collected in the Eastern Tropical North Pacific (ETNP) at the Costa Rica Dome (8.5°N; 90°W; Station 2 Figure 2) in January 2009 aboard the R/V Knorr (Woods Hole Oceanographic Institute). Collection was done using a tucker trawl with a thermally insulated cod end (Childress et al. 1978). Specimens were identified according to published taxonomic keys (Shih 1991, Vinogradov et al. 1996). Physical vouchers to confirm the identification were preserved in formaldehyde and housed in the Seibel lab at the University of Rhode Island.

Specimens were collected from two separate trawls on January 1st and 2nd 2009 in discrete tows between the depths of 250 and 300m with a speed of 1.5- 2 knots. The first trawl was opened at depth at 1509 local time (2109 GMT) at 09 º 10.4370 N, 89 º 56.5019 W and closed at 1539 (2139 GMT). The second tow was opened at depth at 1525 local time (2125 GMT) at 09 º 01.6328 N, 89 º 59.1241 W and closed at 1614 local time (2214 GMT). CTD data from the same day show that the ambient temperature where these specimens were collected was approximately 12° Celsius. Sightings from blue water SCUBA diving, and other trawls has shown that this species can be found near the surface at night in water at temperatures of 23-25° Celsius (personal observations).

Thermal stress:

Specimens were sorted immediately after retrieval and identified quickly under a microscope to reduce stress. Specimens were placed in chilled filtered seawater until experimentation. The specimens in good condition (intact with no injuries) were separated into containers with 0.2 micron filtered sea water and exposed to their approximate nighttime temperature (23°C) for 3, 9 or 24 hours before raising or lowering the experimental temperature. This acclimation of 3, 9 or 24 hours allowed enough time for specimens to recover from any trawl-related stress as indicated by lactate measurements (Elder & Seibel In Prep). Passage of salps through the gut of *Phronima* at night requires, on average, 4 hrs 46 min (Diebel 1988). The acclimation to night time temperature followed by experimental duration was enough time for gut clearance in specimens of *Phronima*, ensuring that further analysis did not include genetic material from prey.

Exposure to subsequent experimental temperatures was accomplished using an aluminum thermal gradient block (Henkel & Hofmann 2008). This block consisted of a piece of aluminum with holes drilled through each end and taped with brass fittings to accommodate heating and chilling lines. The heating and chilling lines were connected to temperature controlled water baths (Lauda, Germany). Water then flowed directly against the aluminum for optimal thermal transfer. Evenly spaced wells were drilled in the top of the block in rows of four to allow up to four replicated experiments at each temperature. Prior to experiments the wells were filled with fresh water and allowed to come to temperature.

The specimens of *P. sedentaria* were placed in an open scintillation vial (25ml volume) with 0.2 micron filtered seawater at 23°C and then put in a well of the thermal block where the water was allowed to come to temperature. The vials took ~15 minutes to get to the desired temperature. Experiments were run for five hours at temperatures: 10, 15, 20, 23, 25 and 29 ± 1 °C. Table 1 outlines the number of individuals for each treatment. During the experiment the thermal block was loosely covered by black plastic bags to block light. Oxygen level of water in experimental vials was checked using a Clark-type oxygen electrode (1302 Strathkelvin Instruments, United Kingdom;(Clark 1956)) to make sure water remained above the published critical oxygen partial pressure of 2.11 kPa (28 μM at 10°C)(Childress 1975). Specimens were then taken out of the vial with feather forceps and blotted dry before being immersed in liquid nitrogen and stored at -80 degrees Celsius. Approximately one half of the specimens used for experiments were frozen at 0100 local time. The remaining experiments were ended at 1300 local time.

Lactate:

Individual whole frozen specimens were ground on ice in a prechilled glass tissue homogenizer (Kimble Chase, USA) using a 1/3 dilution with grinding buffer, 465mm NACL, 19mm KCL, 20 mm Tris, 1mM EDTA, containing a 1 x protease inhibitor cocktail (Sigma p2714) and 0.1% detergent (IGEPAL Sigma 18896). The homogenate was centrifuged at 2000 rpm for five minutes at 4°C and the supernatant removed. L-lactate concentrations were measured on the Accutrend lactate meter using a 25 μl sample of supernatant. All samples were assayed in duplicate and

compared to a lactate standard curve (sodium lactate, L7022, Sigma- Aldrich, MO, USA) which was run daily. Remaining supernatant was flash frozen in liquid nitrogen and stored at -80 until needed for western blots.

Western blots:

Lysate was thawed on ice and centrifuged at 13400 rpm for 2 minutes. Protein concentration was determined using the Pierce BCA protein assay (Bio-Rad, USA). Thirty µg total protein of each sample lysate was mixed with 1/3 lysate volume of 4x NuPAGE LDS buffer containing 10% β-mercaptoethanol and then boiled for 10 minutes at 95°C. Lysate was loaded on to 4-12% bis tris gels (Invitrogen). Heat shocked HeLa cells (Enzo, USA, ADI-LYC-HL101) were added as a control between gels to compare relative intensities of samples to. Proteins were electrophoresed at 120V for 15 minutes, and 150V for approximately 2 hours in 1X MOPS running buffer. Gels were soaked in transfer buffer (5.82g Tris, 2.93g Glycine, 2x 940 µl 20% SDS, 100mL Methanol, q.s. to 1000ml with deionized water) for 20 minutes and electroblotted (Bio Rad, Trans-blot 170-3940) for 30 minutes at 25 volts onto a polyvinylidene difluoride (PVDF) membranes (Fisher IPVH00010). The membrane was washed in 10X TBST (TBS: 400g NaCl, 10g KCl, 150g Tris then qs to 5L of DI water. With 5mL tween into 4.5L of DI water, pH of 7.4)
twice for 10 minutes, and then blocked in 5% milk powder diluted in TBST for one hour at room temperature. This was followed by 3 five minute TBST washes. The membrane was then incubated in a 1:1,000 dilution of HSP 70 antiserum (Stressgen SPA-822) overnight at 4 °C. After washing, the secondary antibody (anti-mouse

Igc:HRP-Linked, GE Healthcare Biosciences NA931) was added for one hour at room temperature.

Immunoreactive proteins were then visualized with Chemiluminecent substrate Western lightening (Perkin Elmer, NEL102001EA) for 2 minutes. Following a one minute exposure, on kodak biomax XAR film (Sigma, F5388-50EA) the film was developed and HSP 70 expression was determined semi-quantitatively using Image J software.

Statistics:

Statistics were performed using the software SAS version 9.3 (SAS institute inc. USA). One-way Analysis of Variance (ANOVA), with between subjects design were conducted to compare differences in lactate accumulation or hsp 70 concentration between treatments.

Results:

At the time of collection surface temperatures of the ETNP were between 23 and 25°C. The maximum surface temperature recorded in the ETNP during this cruise was 27°C. Based on published distribution for *Phronima sedentaria*, temperatures at the deepest range of daily migrations are between 8 and 10°C. This indicates *Phronima sedentaria* may experience a temperature change of 13-17°C in the ETNP during diel migration in the ETNP (Figure 3).

There was no significant difference in mortality for 3, 9 or 24 h exposure to nighttime temperature (23°C). Mortality data for those exposure times is combined

for subsequent analyses. There was no mortality of specimens between 10 and 20°C. At 23°C 13% of specimens died (1 out of 7) and at 25°C 30% of specimens died (2 out of 8); Table 1, Figure 4). The most significant mortality occurred at 29°C, at which temperature 50% of the experimental specimens died (4 out of 8 total specimens at that temperature; Table 1, Figure 4). Dead organisms had a significantly higher accumulation of lactate, and so are not included further.

There was no significant difference in hsp 70 concentration or lactate accumulation between specimens frozen at 0100 local time and specimens frozen at 1300 local time. No further evaluation of diel rhythms was conducted.

No significant differences in lactate accumulation were observed between specimens exposed to the night time temperature of 23°C for 3, 9 or 24 hours (ANOVA: $f((2,50)=2.35$; $p<0.1062$). Lactate data for the three exposure times are combined for further analyses (Figure 5). Exposure to 29°C resulted in a significant increase in lactate accumulation relative to all other temperatures (Figure 5; one way ANOVA, $F(5,15)=8.26$; $p=0.0025$). At 29°C the average L-lactate production in live specimens was 20.5 ±4.52 μmol g^{-1}. For all other temperatures (10-25°C) there was no significant difference in lactate accumulation. The average lactate accumulation after five hour exposure to 10, 15, 20, 23 or 25 °C was 2.89±0.797 μmol g^{-1}. A previous study on *P. sedentaria* found that specimens frozen immediately after collection had very high levels of lactate (≥20 μmol g^{-1}) indicating use of anaerobic metabolism in oxygenated conditions, which is thought to be a result of capture stress (Elder & Seibel In Prep). The low values measured here at temperatures below 29°C indicated that acclimation time was sufficient to recover from collection stress.

For western blot analysis using an antibody for hsp 70, one band occurred at approximately 70kDa (Figure 6). Dead specimens were not used for western blot analysis. Samples subjected to 23 and 25° C after the 9 hour incubation at 23°C were combined and designated 24°C. Specimens acclimated to 23°C for only 3 hours had significantly lower hsp70 levels than either 9 or 24 hour acclimated specimens (ANOVA: $F(2,47)=7.82$; $p<0.0012$; Figure 7). There was no difference in hsp70 expression between the 9 and 24 h exposures at 10-20°C. For specimens in the 24 h initial exposure, hsp70 levels were elevated at 29°C compared to lower temperatures for that exposure duration (Figure 7, ANOVA: $F(5,24)=2.57$, $P<0.0535$). Elevated temperature (29°C) did not induce hsp70 expression in individuals pretreated for only 3 hours at 23°C (Figure 7).

Discussion:

For this study we assessed mortality, lactate and hsp 70 accumulations in specimens exposed to night time temperature (23°C) for varying durations to determine if temperatures at the surface induce a stress response. During daily migrations *Phronima sedentaria* experiences a temperature change of ~15°C (Figure 3) with sustained upper temperatures near 23°C at night. Phronima migrates between the surface and 200-350m in during diel migration (Shulenberger 1977, Shih 1991). This temperature change when migrating through the thermocline would be rapid, with a change of up 10°C degrees across 50m (Figure 3). The prediction for this study was that the temperatures routinely experienced by *P. sedentaria* within its natural range would induce a stress response. If this stress response occurred, it would result

in a shift to anaerobic metabolism due to oxygen-limitation (discussed below; Pörtner, 2002) which can be measured by a increase in lactate production under oxygenated conditions. In addition a stress response would result in an increase in hsp 70 concentrations. In contrast to this prediction, we found that both lactate and hsp70 levels were consistent across a temperature range of 10-25°C. There was an increase in both lactate and hsp70 occurred at 29°C, a temperature not experienced during our expedition (December) in the Eastern North Tropical Pacific. However, we further postulated that exposure time could be an important factor in the expression of a stress response. In support of this latter hypothesis, we demonstrated a modest elevation in hsp70 expression in specimens that were pre-exposed to 23°C for 9 or 24 hours relative to those pre-exposed for only 3 hours (Fig. 7). The 13% mortality at 23°C and 30% mortality at 25°C (Figure 4) indicates some amount of stress at night time temperatures. In all acclimation treatments, including 3-hour specimens, subsequent exposure to 25°C for five hours did not result in significant hsp70 expression. The less than 30% mortality and lack of an increase in hsp 70 suggests that *Phronima* is somewhat tolerant of nighttime temperatures for at least 8 hours, equivalent to its nightly exposure duration before returning to cooler depths.

Pörtner (2002) has suggested that upper critical temperatures are related to a mismatch between oxygen supply and demand. This is supported by an elevation in lactate at 29°C. However, lactate levels did not increase at temperatures below 29°C at any exposure duration. This suggests that the heat-shock response in the 9 and 24-hour pre-exposures is independent of oxygen stress. In addition temperatures below 23°C did not result in a reduced amount of lactate production or hsp70 concentrations

(figure 5 and 7), indicating that the low lactate levels measured were a true "basal" level. Total mortality was 13% at 23 and 25°C and there was no mortality at temperatures below 23°C (Figure 4). This suggests that the modest heat-shock response at temperatures below 29°C was successful at protecting the organism from detrimental effects of thermal stress.

At 29°C *P. sedentaria* had a significant increase in lactate production (Figure 5), a significant increase in hsp 70 concentration (Figure 7), and a 50% mortality rate (Figure 4). This indicates that the critical temperature range for *Phronima sedentaria* in the ETNP is between 26 and 29°C, which is slightly higher than the ambient surface temperatures during our expedition.

The increase in lactate production at 29°C represents the onset of anaerobic metabolism. At their critical temperature, organisms experience a failure of ventilatory or circulatory systems to meet elevated oxygen demand, which results in reduced aerobic scope and a transition to anaerobic metabolism under oxygenated conditions. This loss of system function is thought to reflect the earliest level of thermal stress and is known as oxygen and capacity limited thermal tolerance (Pörtner 2010). Our measurements suggest that thermal stress begins earlier than this critical or "pejus" temperature but that protective mechanisms are effective, at least for short periods of time. Although we did not test heart or ventilatory function directly, the onset of anaerobic metabolism in aerobic conditions indicates this mismatch in oxygen supply, which is caused in part by the inability to deliver enough oxygen to the body (Somero 2005). Survival beyond critical temperature leads to a decline in

performance and is time limited due to low ATP yield from anaerobic glycolysis (Pörtner 2002, Pörtner 2010).

The pejus range (Latin for 'turning worse') is the range when organisms are past optimum conditions but can still survive with reduced aerobic activity (Jost et al. 2012). During the pejus range, there is an increase in ventilation rate with temperature to compensate for increasing oxygen demand with temperature. At the upper pejus temperature ventilation rate becomes relatively constant and Po_2 begins to decrease (Frederich & Pörtner 2000). Oxygen supply to tissues and overall aerobic scope, is suspected to be linked to fitness and functioning at the ecosystem level (Pörtner 2010), although this relationship has yet to be conclusively determined (Clark et al. 2013).

Lactate accumulation at 29° C in this study (20.5 ±4.52 μmol g^{-1}, Figure 5) is similar to the lactate level of 17.15±4.75 μmol g^{-1} in the same species subjected five hours of environmental hypoxia levels (1% oxygen) at the intermediate temperature of 20°C. Lactate concentrations at 25°C and below were comparable to levels in the previous study when exposed to normoxic conditions 2.85±0.40 μmol g^{-1} (Elder & Seibel In Prep). This indicates that specimens were experiencing tissue level hypoxia at 29°C despite the oxygenated conditions of the water they are in. This tissue level hypoxia could be due in part to failure of ventilatory or circulatory systems, but factors other than oxygen transport can also be thermally limited and potentially cause decline in performance that leads to tissue hypoxia (Clark et al. 2013).

A critical temperature of approximately 30°C is found in several other crustacean species. The spider crab *Maja squinado* from Roscoff France has a critical temperature close to 30°C, which was indicated by accumulation of anaerobic end

products succinate and lactate. This coincided with very low arterial PO_2 values (Frederich & Pörtner 2000). The critical temperature range at which anaerobic metabolism begins in the intertidal crabs *Carcinus maenas* and *Cancer irroratus* is 34°C and 30°C, respectively. Interestingly, hsp70 was not detected in either of these crabs, but it may be due to the experimental design, which included a rapid rate of temperature increase (Jost et al. 2012). Our results suggest a 14-30 hour lag in the onset of hsp70 expression following exposure to stressful temperatures.

In addition to anaerobic metabolism, survival beyond critical temperatures is supported by protection of proteins and membranes by heat shock proteins and antioxadative defense (Pörtner & Knust 2007, Pörtner 2010). Hsp 70 is a biochemical indicator for the degree of protein unfolding in a cell and therefore an indirect indicator of protein damage (Hofmann 2005).

The majority of studies on heat shock response in ectothermic invertebrates have focused on intertidal organisms, especially mussels. A theme from these studies is the plasticity of hsp expression, where past thermal history has an impact on induction temperature (Hofmann et al. 2002, Hofmann 2005). In the intertidal, thermal history can vary with season and tide level. In temperate regions of the pelagic realm, seasonal changes can have an effect on surface temperatures. In the tropics however, temperature profiles of the water column are relatively stable (Fernández-Álamo & Färber-Lorda 2006). In both temperature and tropical waters vertical migators are the organisms that will experience drastic temperature changes during their transit between surface and deeper waters. The lack of a full stress

response in *Phronima sedentaria* at 23°C indicates that this species is adapted to the current, relatively constant, surface temperatures of the region.

Vertically migrating calanoid copepods (*Calanus finmarchicus*) from the temperate waters of the Gulf of Maine demonstrated a heat shock response when exposed to high environmental temperatures (Voznesensky et al. 2004). Following exposure to their maximum summer habitat temperature (18-20°C), *C. finmarchicus* had an increase in hsp 70 mRNA expression when compared to specimens kept at 8°C. After 30 minutes at 20°C and after 48 hours at 18°C, specimens exhibited a ~ 4 fold increase in hsp 70 expression (Voznesensky et al. 2004). The heat shock response in these vertically migrating copepods may increase survival by allowing them to tolerate high temperatures while at the surface before migrating down to deep waters with more optimal temperatures (Voznesensky et al. 2004). The specimens of *Phronima* examined here were acclimated to their winter temperatures. Summer temperatures may reach 30°C (Pennington et al. 2006).

Induction of hsp70 by heat shock has been shown in several studies on freshwater gammarid amphipods, primarily from Lake Baikal in Russia (Bedulina et al. 2010, Shatilina et al. 2011, Bedulina et al. 2013). High constitutive levels of hsp 70 are thought to provide a general protective mechanism against heat shock, and possibly other stresses, in freshwater amphipods (Bedulina et al. 2013). There was a stronger hsp response in intertidal amphipods from a variable habitat (sublittoral) versus a less variable habitat (supra-littoral) (Bedulina et al. 2010). This may indicate that the heat-shock response is critical for tolerating natural temperature fluctuations, even below critical extremes.

Rythmic pre-synthesis of hsps to prepare for potential heat stress, such as prior to low tide, has not been found in rocky intertidal organisms (Hofmann et al. 2002). The dependable timing of diel migration compared to the variability of low tide levels, suggest that vertical migrators would be more likely to have an anticipatory increase in hsp production than intertidal organisms. For this study half the organisms were frozen at 1am, and half were frozen at 1pm. At 1 am diel migrators would have been at the surface for a few hours, while at 1pm they would have arrived at depth several hours prior. If *P. sedentaria* were producing hsp in anticipation of vertical migration, one would expect lower levels of hsp in the group subjected to the same temperature frozen at 1pm compared to the group frozen at 1am. However, there was no significant difference in the hsp concentrations or level of mortality between the two freezing times.

Implications/conclusions

23°C is stressful for *Phronima sedentaria*, but is somewhat tolerated via heat-shock response at longer exposure times. Five hour recovery at lower temperature does not result in reduced hsp concentrations. An escalation in hsp 70 concentrations occurred at 29°C (Figure 7) , which coincided with a 50% mortality rate (Figure 4) and a significant increase in anaerobic metabolism under oxygenated conditions (Figure 5). In the ETNP *P. sedentaria* tolerates its normal migration to the surface, but is close to an upper temperature limit and approaching a maximum duration of exposure. Though *P. sedentaria* experiences a large temperature fluctuation during vertical migration, the consistency of the surface temperatures has allowed *P.*

sedentaria to adapt to the change, which can be seen by the lack of stress response at surface temperatures of 23-25°C.

Upper thermal tolerance limits are correlated with the maximum habitat temperatures in intertidal organisms (Stillman & Somero 2000). Although limited exposures to current maximum temperatures of the region do not induce a full stress response, the critical temperature of 29°C may be reached in summer and, due to global warming (Deser et al. 2010), during future winters. The Eastern Tropical Pacific warms by approximately 0.8-1.0°C per century (Deser et al. 2010). If organisms are already close to their critical temperatures, global warming will cause some species to be over their thermal limits and may quickly affect their biogeographic range. Increasing temperature and decreasing oxygen supply will compress the night time habitat of vertically migrating species (Seibel 2011, Elder & Seibel In Prep). This change will have important impacts on zooplankton physiology, ecology, and vertical distribution as well as carbon cycling (Vinogradov and Voronina 1962; Seibel 2011; Somero 2002).

Acknowledgments:

Thanks to Gretchen Hofmann for numerous insightful discussions. The Hofmann Lab provided the thermal block design which was developed by Tim Crombie and Sean Place. The URI equipment development lab fabricated the thermal gradient block. This work would not have been possible without the Captain and Crew of the R/V Knorr. A special thanks to Dr. Niall Howlett, and the members of the Howlett lab,

especially Rebecca Boisvert, for use of equipment, assistance with western blot methods and troubleshooting.

Funding:

National Science Foundation grants: OCE-0526545 to Kendra Daly and OCE-0526502 to Karen Wishner and Brad Seibel. Additional funding was provided by The Crustacean Society Graduate Student Fellowship to Leanne Elder and the University of Rhode Island enhancement of graduate research grant, also to Leanne Elder.

Figures:

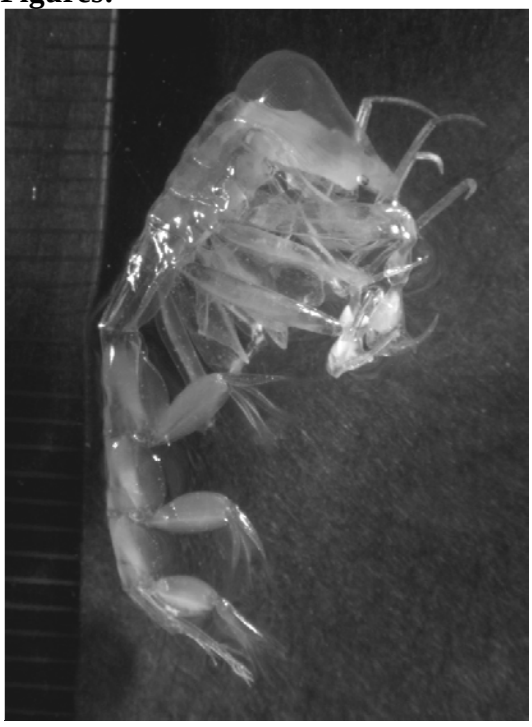

Figure 1: *Phronima sedentari* photo taken by L. Elder.

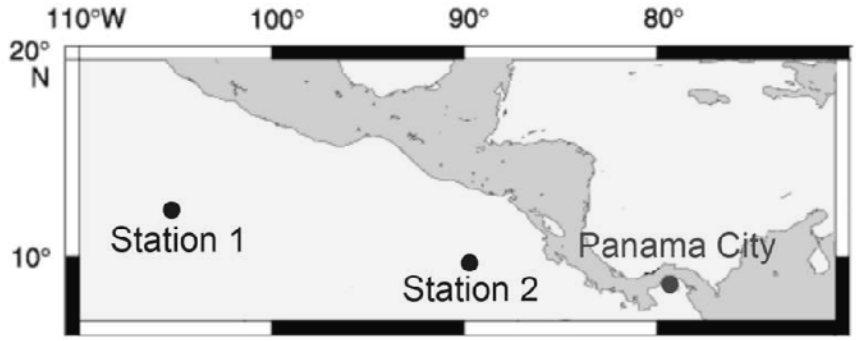

Figure 2: Map of stations for Eastern Tropical North Pacific (ETNP) during collection aboard the R/V Knorr in 2008. Specimens for these experiments were collected at station 2, the Costa Rica Dome (8.5°N, 90°W) using a tucker trawl.

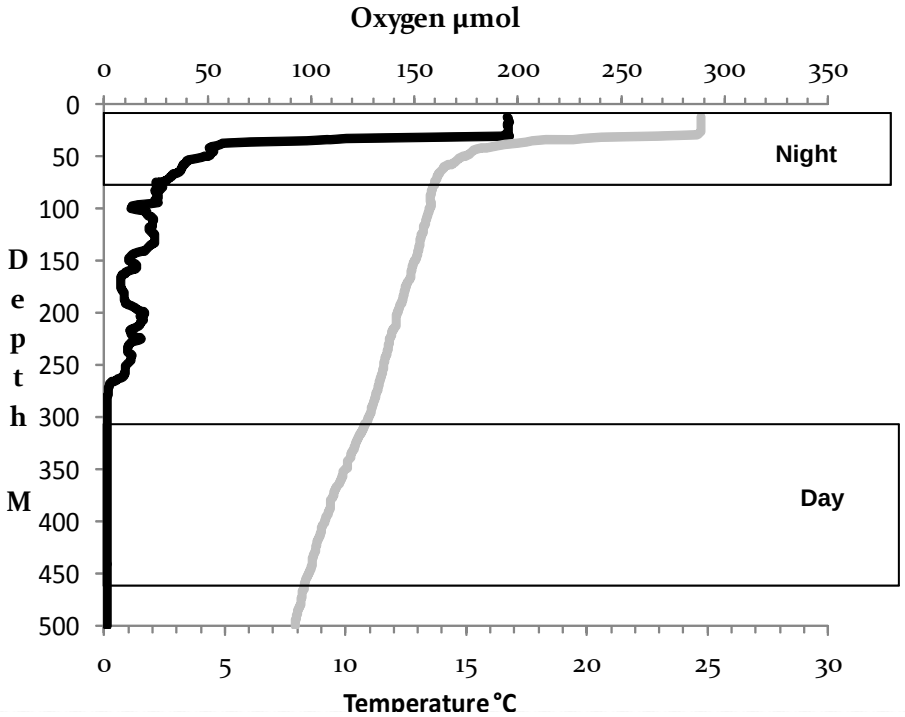

Figure 3: The Costa Rica Dome (Station 2) CTD profile of oxygen (black line) and temperature (grey line). Boxes represent approximate day and night time distributions of *Phronima sedentaria* based on published distributions (Shih, 1991; Shulenberger, 1977).

Duration of exposure to 23°C	Five hour follow-up exposure temperature °C	n	n dead at end
3 hours	10	4	
	15	4	
	20	4	
	23	4	
	29	4	3
9 hour	10	4	
	15	4	
	20	4	
	23	3	1
	25	3	2
	29	1	
24 hour	10	3	
	15	3	
	20	3	
	25	3	
	29	3	1

Table 1: Thermal stress experimental setup for initial duration of acclimation to night time temperature of 23°C for 3, 9 or 24 hours and a follow-up five hour exposure to the designated follow-up temperature. n is number of individuals kept at those conditions. n deceased at end is the number deceased at the end of each experiment.

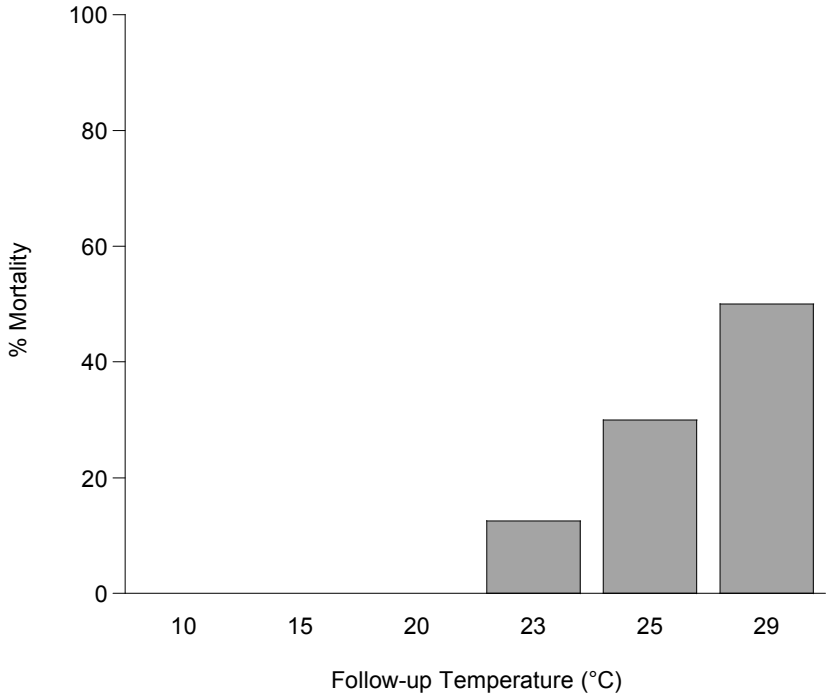

Figure 4: Percent mortality of *Phronima sedentaria* after acclimation to night time temperature of 23C and exposure to the follow-up temperature listed. Temperatures with no bar had no mortality. There was a 50% mortality rate at 29°C.

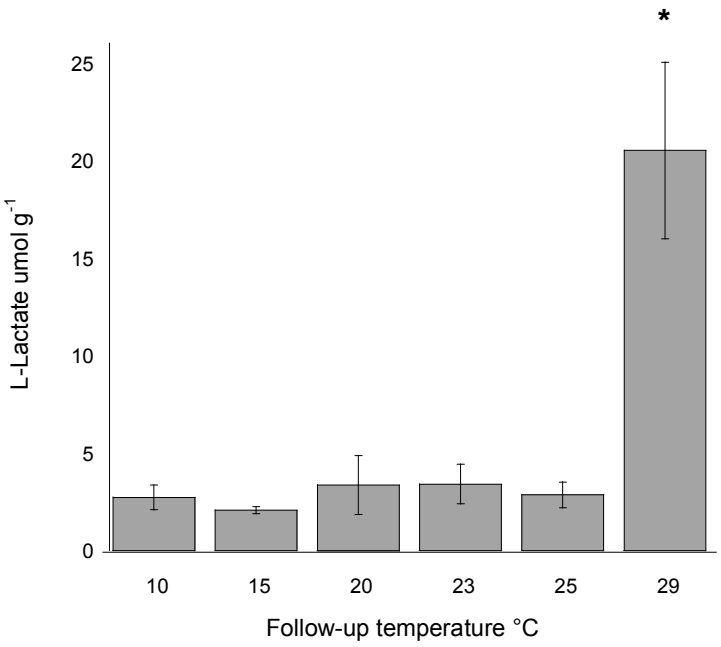

Figure 5: There was no significant difference in lactate accumulation between the different night time exposure durations (ANOVA: f(2,50)=2.35; p<0.106). Therefore accumulation of lactate in µmol g^{-1} was averaged among the specimens acclimated to night time temperature (23°C) and then incubated for five hours at the designated follow-up temperatures. All values are mean ±se. There was a significantly higher accumulation of lactate at 29°C (one way ANOVA, F(5,15)=8.26; p=0.0025).

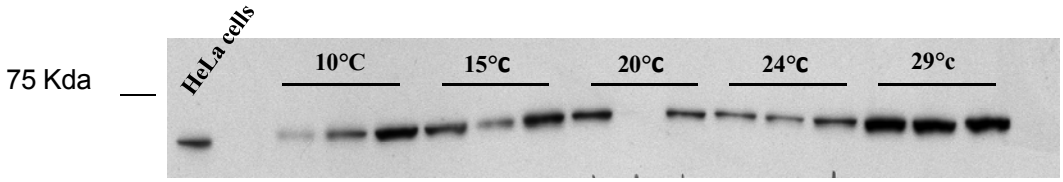

Figure 6:
Representative Western blot analysis of levels of hsp 70 in *Phronima sedentaria* relative to control (HELA cells first lane on the left). The marker from the protein ladder at 75 Kda is also marked in the figure, to show that the band is at 70 Kda. This gel is the samples for 24 hours at 23°C and then incubated at the follow-up temperatures. The last three lanes on the right are samples that have been kept at 29°C. These three lanes had significantly higher relative intensity than the other samples, indicating significantly higher hsp 70 concentration.

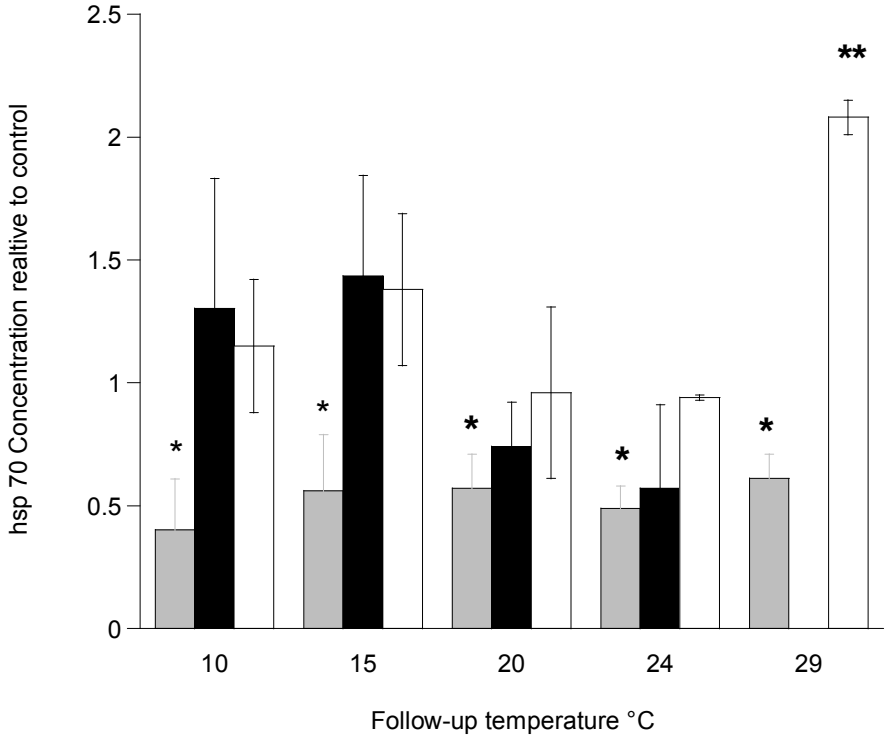

Figure 7:
Mean hsp 70 concentration ±se for specimens exposed to night time temperature of 23°C for 3(gray), 9 (black) or 24 hours (white) followed by exposure to the designated temperatures. * indicates Hsp 70 concentration at 3 hours was significantly lower than 9 and 24 hour: ANOVA: $f(2,47)=7.82$; $p<0.0012$. ** indicates there was a nearly significant increase in hsp 70 concentration in organisms acclimated to 23°C for 24 hours before a five hour incubation at 29°C (ANOVA: $F(5,24)=2.57$, $P<0.0535$). Hsp 70 was not quantified for specimens that were dead at the end of the incubation.

References Cited:

Bedulina, D., Evgen'ev, M., Timofeyev, M., Protopopova, M., Garbuz, D., Pavlichenko, V., Luckenbach, T., Shatilina, Z., Axenov-Gribanov, D., Gurkov, A., 2013. Expression patterns and organization of the hsp70 genes correlate with thermotolerance in two congener endemic amphipod species (Eulimnogammarus cyaneus and E. verrucosus) from Lake Baikal. Molecular ecology.

Bedulina, D., Zimmer, M., Timofeyev, M., 2010. Sub-littoral and supra-littoral amphipods respond differently to acute thermal stress. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 155, 413-418.

Childress, J.J., 1975. The respiratory rates of midwater crustaceans as a function of depth of occurrence and relation to the oxygen minimum layer off southern California. Comparative Biochemistry and Physiology Part A: Physiology 50, 787-799.

Childress, J.J., Barnes, A.T., Quetin, L.B., Robison, B., 1978. Thermally protecting cod ends for the recovery of living deep-sea animals. Deep-Sea Research 25, 419-422.

Clark, L.C., 1956. Monitor and control of blood and tissue oxygen tensions. Transactions - American Society for Artificial Internal Organs 2, 41-48.

Clark, T.D., Sandblom, E., Jutfelt, F., 2013. Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. The Journal of experimental biology 216, 2771-2782.

Deser, C., Phillips, A.S., Alexander, M.A., 2010. Twentieth century tropical sea surface temperature trends revisited. Geophysical Research Letters 37.

Doney, S.C., Ruckelshaus, M., Duffy, J.E., Barry, J.P., Chan, F., English, C.A., Galindo, H.M., Grebmeier, J.M., Hollowed, A.B., Knowlton, N., 2012. Climate change impacts on marine ecosystems. Marine Science 4.

DuBeau, S.F., Pan, F., Tremblay, G.C., Bradley, T.M., 1998. Thermal shock of salmon in vivo induces the heat shock protein hsp 70 and confers protection against osmotic shock. Aquaculture 168, 311-323.

Elder, L.E., Seibel, B.A., In Prep. Ecophysiological implications of vertical migration into oxygen minimum zones for the hyperiid amphipod Phronima sedentaria. Marine Ecology Progress Series.

Feder, M.E., Hofmann, G.E., 1999. HEAT-SHOCK PROTEINS, MOLECULAR CHAPERONES, AND THE STRESS RESPONSE: Evolutionary and Ecological Physiology. Annual Reviews in Physiology 61, 243-282.

Fernández-Álamo, M.A., Färber-Lorda, J., 2006. Zooplankton and the oceanography of the eastern tropical Pacific: A review. Progress in Oceanography 69, 318-359.

Frederich, M., Pörtner, 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.

Gasca, R., Haddock, S.H.D., 2004. Associations between gelatinous zooplankton and hyperiid amphipods (Crustacea: Peracarida) in the Gulf of California. Hydrobiologia 530, 529-535.

Harbison, G., Biggs, D., Madin, L., 1977. The associations of Amphipoda Hyperiidea with gelatinous zooplankton—II. Associations with Cnidaria, Ctenophora and Radiolaria. Deep Sea Research 24, 465-488.

Henkel, S.K., Hofmann, G.E., 2008. Thermal ecophysiology of gametophytes cultured from invasive< i> Undaria pinnatifida</i>(Harvey) Suringar in coastal California harbors. Journal of Experimental Marine Biology and Ecology 367, 164-173.

Hofmann, G.E., 2005. Patterns of Hsp gene expression in ectothermic marine organisms on small to large biogeographic scales. Integrative and Comparative Biology 45, 247-255.

Hofmann, G.E., Buckley, B.A., Place, S.P., Zippay, M.L., 2002. Molecular Chaperones in Ectothermic Marine Animals: Biochemical Function and Gene Expression. Soc Integ Comp Biol, 808-814.

Hofmann, G.E., Somero, G.N., 1995. Evidence for protein damage at environmental temperature: seasonal changes in levels of ubiquitin conjugates and hsp70 in the intertidal mussel *Mytilus Trossulus*. The Journal of Experimental Biology 198, 1509-1518.

Jost, J.A., Podolski, S.M., Frederich, M., 2012. Enhancing thermal tolerance by eliminating the pejus range: a comparative study with three decapod crustaceans. Mar Ecol Prog Ser 444, 263-274.

Land, M.F., 1992. Locomotion and visual behaviour of mid-water crustaceans. Journal of the Marine Biological Association of the United Kingdom 72, 41-60.

Madin, L.P., Harbison, G.R., 1977. The associations of Amphipoda Hyperiidea with gelatinous zooplankton--I. Associations with Salpidae. Deep Sea Research 24, 449-456, IN441-IN444, 457-463.

Pennington, J.T., Mahoney, K.L., Kuwahara, V.S., Kolber, D.D., Calienes, R., Chavez, F.P., 2006. Primary production in the eastern tropical Pacific: A review. Progress in Oceanography 69, 285-317.

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., 2010. Oxygen-and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. The Journal of experimental biology 213, 881-893.

Pörtner, H.O., Knust, R., 2007. Climate change affects marine fishes through the oxygen limitation of thermal tolerance. Science 315, 95-97.

Saltzman, J., Wishner, K.F., 1997. Zooplankton ecology in the eastern tropical Pacific oxygen minimum zone above a seamount: 1. General trends. Deep Sea Research Part I: Oceanographic Research Papers 44, 907-930.

Seibel, B.A., 2011. Critical oxygen levels and metabolic suppression in oceanic oxygen minimum zones. The Journal of Experimental Biology 214, m326-336.

Shatilina, Z.M., Wolfgang Riss, H., Protopopova, M.V., Trippe, M., Meyer, E.I., Pavlichenko, V.V., Bedulina, D.S., Axenov-Gribanov, D.V., Timofeyev, M.A., 2011. The role of the heat shock proteins (HSP70 and sHSP) in the thermotolerance of freshwater amphipods from contrasting habitats. Journal of Thermal Biology 36, 142-149.

Shih, C.-t., 1969. The systematics and biology of the family Phronimidae (Crustacea: Amphipoda). Høst.

Shih, C.-T., 1991. Description of two new species of Phronima Latreille, 1802 (Amphipoda: Hyperiidea) with a key to all species of the genus. Journal of Crustacean Biology, 322-335.

Shulenberger, E., 1977. Hyperiid amphipods from the zooplankton community of the North Pacific central gyre. Marine Biology 42, 375-385.

Somero, G.N., 2002. Thermal physiology and vertical zonation of intertidal animals: optima, limits, and costs of living. Integrative and Comparative Biology 42, 780-789.

Somero, G.N., 2005. Linking biogeography to physiology: evolutionary and acclimatory adjustments of thermal limits. Frontiers in Zoology 2, 1.

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.H., Somero, G.N., 2000. A comparative analysis of the upper thermal tolerance limits of eastern Pacific porcelain crabs, genus Petrolisthes: influences of latitude, vertical zonation, acclimation, and phylogeny. Physiological and Biochemical Zoology 73, 200-208.

Trenberth, K.E., Jones, P.D., Ambenje, P., Bojariu, R., Easterling, D., Klein Tank, A., Parker, D., Rahimzadeh, F., Renwick, J.A., Rusticucci, M., 2007. Observations: Surface and Atmospheric Climate Change. Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. S. Solomon, D. Qin, M. Manning, et al. Cambridge, United Kingdom and New York, NY, USA., Cambridge University Press.

Vinogradov, M.E., Volkov, A.F., Semenova, T.N., Siegel-Causey, D., 1996. Hyperiid amphipods (Amphipoda, Hyperiidea) of the world oceans. Science Publications Incorporated, Lebanon, USA.

Voznesensky, M., Lenz, P.H., Spanings-Pierrot, C., Towle, D.W., 2004. Genomic approaches to detecting thermal stress in Calanus finmarchicus (Copepoda: Calanoida). Journal of Experimental Marine Biology and Ecology 311, 37-46.

Publication status

Chapter 3:
Effect of ecology, habitat, phylogeny and environmental conditions on rates of metabolism in diverse marine amphipods

This manuscript will be submitted to the journal Marine Ecology Progress Series.

CHAPTER 3

Effect of ecology, habitat, phylogeny and environmental conditions on rates of metabolism in diverse marine amphipods

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Abstract:

This study sought to determine what environmental and ecological factors influence the rate of metabolism in marine amphipods by examining a broad data set from polar to tropical environments, and including transparent specimens. The data set for this study was obtained from the literature and original data. Recent molecular work allowed us to look at hyperiid metabolism in a phylogenetic context. Understanding patterns of pelagic and deep sea metabolism is important for further understanding of global carbon flux and the consequences of climate change on migration strategies. hyperiid amphipods from the mesopelagic clade Physosomata had significantly lower metabolic rates than species in the epipelagic clade Physocephalata. Transparent species also had significantly lower metabolic rates than nontransparent species. The lower rates in mesopelagic and transparent amphipods support the visual interactions hypothesis: decreasing selection for locomotory capacity limits predator-prey interactions among visually oriented organisms and results in lower metabolic rates. Lower rates of metabolism in benthic and mesopelagic gammarids may also be attributed to the visual interactions hypothesis.

Introduction:

Amphipods are a highly diverse group of peracaridan crustacean. Peracaridans (which include amphipods, mysids, cumaceans, isopods and tanaids) bear eggs in a thoracic brood pouch, where juveniles hatch, rather than having true larval stages (Bousfield 1973, Dick et al. 1998). The synapomorphic character uniting amphipods is the arrangement of the three pairs of posterior appendages known as the uropods (Browne et al. 2007) into a fan-like structure. Pelagic amphipods, though low in relative abundance compared to copepods and euphausiids, are an important part of the epi- and meso-pelagic communities. Hyperiidae and gammaridae are two suborders of amphipods that are found in the pelagic environment.

Gammarids comprise nearly 85 percent of all amphipod species, and are primarily benthic (though there are some pelagic species). Gammarids can be found in almost every environment that has at least some moisture, including: marine, brackish and fresh water, sediment burrows and tubes, caves, as well as humid terrestrial environments (Bousfield 1973). Hyperiids are the younger suborder that evolved from gammarid amphipods (Bousfield 1973, Martin & Davis 2001). Recent phylogenetic work indicates hyperiids are polyphyletic, with two independent radiations from gammarids: the Physocephalata and the Physosomata (Browne et al. 2007, Hurt et al. 2013). Hyperiids are exclusively marine, primarily found in the open ocean, although there are a few coastal species (Bowman & Gruner 1973). They are commensally or parasitically associated with gelatinous zooplankton at some time in their lives (Harbison et al. 1977, Laval 1980, Gasca & Haddock 2004). Females are

thought to spend the majority of their lives on the host, while male hyperiids may be largely free swimming, spending time on gelatinous zooplankton as a food source during development and as the place to locate females for reproduction (Madin & Harbison 1977). Hyperiid amphipods are known for their large, image forming eyes, with many having double eyes, which may be used for downward scanning of objects that reflect light from above (Land 1992), although eyes of some deep sea members of the clade Physosomata are reduced or absent (Bowman & Gruner 1973). Hyperiid amphipods are dominated by transparent forms (Johnsen 2001). They are also a food source for larger predators and contribute to carbon flux and other biogeochemical cycling via their strategy of diel vertical migration (Vinogradov et al. 2004, Yamada et al. 2004, Ikeda 2013).

Diel migrating zooplankton play a significant role as a link between marine primary producers and upper trophic levels (Ducklow et al. 2001, Ekau et al. 2010). Zooplankton consume phytoplankton near the ocean surface at night and migrate down during the day where they metabolize ingested food, release carbon in the forms of dissolved organic carbon (DOC), sinking faecal material, and CO_2, therefore transporting carbon to depth (Longhurst et al. 1990, Ducklow et al. 2001). Estimates of the contribution of migrating zooplankton to this "biological Pump" depend on accurate assessment of the rates of metabolism of species involved. Respiration and metabolic activity are among the most important components of carbon flux (Burd et al. 2010). Burd and colleagues have noted a problem of imbalances in estimates of organic carbon sources and sinks below the photic zone that may result from imprescise or inaccurate metabolic rates used in calculations (Burd et al. 2010).

Metabolism can vary intarspecifically with temperature (Donnelly & Torres 1988), body mass (Glazier 2005, 2006), as well as with environmental differences such as oxygen levels (Seibel 2011, Elder & Seibel In Prep) and interspecifically due to ecological habits and phylogenetic influences (Seibel & Carlini 2001). Metabolic rates in visually-orienting pelagic organisms, including crustaceans, are documented to decline with depth (Seibel & Drazen 2007), primarily due to reduced demand for energy for predator-prey interactions in the light-limited deep sea (Childress & Mickel 1985, Seibel & Drazen 2007). Transparency may relieve the demand for strong swimming abilities and high metabolism in such organisms (Seibel & Carlini 2001, Elder & Seibel In Prep).

Torres et al. (1994) noted that Antarctic pelagic crustaceans (when normalized to the same mass and temperature) have similar or lower metabolic rates compared to temperate species, suggesting no metabolic temperature compensation. Torres et al (1994) also noted that the representation of amphipods within the micronektonic crustacea community decreases with increasing sea surface temperature. Due to the abundance of amphipods in colder environments, previous work on their metabolism has primarily been conducted in polar coastal waters. Metabolic rates of pelagic crustaceans, including both gammarid and hyperiid amphipods, decrease with increasing depth of occurrence in the Antarctic (Ikeda 1988, Torres et al. 1994). A recent analysis found that metabolic rates of pelagic amphipods decrease with increasing depth of collection in all regions (Ikeda 2013); however, depth of collection is not always ecologically relevant and therefore it is difficult to interpret this result. Rates of hyperiid amphipods were higher on average than rates of gammarids in the

Antarctic, partially due to the smaller body size of hyperiids in that region (Torres et al. 1994) or to their association with the benthos, providing greater opportunities for refuge from predators.

This study aimed to determine what environmental and ecological factors influence the rate of metabolism in marine amphipods by examining a broad data set from polar to tropical environments and including transparent specimens. Specifically, we tested the effect of temperature, oxygen, body mass, habitat (benthic versus pelagic) and transparency on the metabolic rates of diverse amphipods across a latitudinal and depth range. The data for this study was obtained from the literature and original data. Recent molecular work (Hurt et al. 2013) allowed us to look at hyperiid metabolism in a phylogenetic context. Understanding patterns of pelagic and deep sea metabolism is important for further understanding of global carbon flux and the consequences of climate change on migration strategies.

Methods

Collection:

Collection locations and methods are listed in table 1. For most locations specimens were collected with a Mother Tucker Trawl, a modified opening-closing Tucker Trawl equipped with a 30 l thermally insulated cod-end (Childress et al. 1978). The net was opened and closed using a MOCNESS- type step motor (Wiebe et al. 1985) and equipped with temperature and pressure sensors. Immediately after the codend reached the surface, the contents were placed in a large container full of chilled seawater. At McMurdo station, Antarctica, specimens were collected by hand

along shore. The species *Abyssochromeni* was also collected using a bait bag filled with dead fish and left overnight in a hole on the sea ice just south of McMurdo station. For all collection methods, upon retrieval, specimens were individually transferred to filtered seawater and allowed to recover for at least 12 hours, ensuring they were in a post-absorptive (starved) state. Specimens were identified using published keys (Vinogradov et al. 1996) and representatives of each species were preserved in 4% formaldehyde, or 70% ethanol to confirm identification.

Metabolic rate (MO_2):

All respiration experiments were conducted at sea or, in the case of Antarctica, at the lab in McMurdo station. Depending on the size of the organism, either 25 ml or 50ml glass gas-tight syringes were used as respiration chambers. The chambers were filled with filtered (0.2 μm demicap filter, Fisher scientific, USA) and treated (25 μM liter $^{-1}$ each of streptomycin and ampicillin) seawater, and a single specimen was immediately placed in the chamber using feather forceps. A blank chamber with no specimen was filled with identically treated water and processed simultaneously to monitor background respiration of microbes. The chambers were sealed and incubated in a temperature controlled circulating water bath (Lauda, Germany). All experiments were carried out in darkness. Experiments were conducted for 5-27 hours. The size, volume and metabolic rate of individuals determined the duration needed to provide sufficient time for measureable changes in oxygen saturation.

Water was removed from incubation chambers using a 500 microliter syringe (Hamilton, USA). Oxygen concentrations of the water in incubation chambers were

measured at the end of the experiment using a Clark-type oxygen electrode (Clark 1956) connected to a Strathkelvin Instruments 782 Oxygen Interface (Strathkelvin Instruments, United Kingdom). The oxygen electrodes were maintained in a thermally jacketed electrode holder (MC100 Microcell, Strathkelvin Instruments, United Kingdom) attached to a water bath at the appropriate experimental temperature (Marsh & Manahan 1999). The electrode was calibrated prior to measurements using air- and nitrogen-saturated seawater. The oxygen consumption rate of each specimen was calculated by subtracting the final oxygen concentration in the experimental chamber from final concentration in the blank chamber. At the end of incubations, all specimens were immediately blotted dry, frozen in liquid nitrogen, then transferred to a -80ºC freezer. Specimens were weighed on a motion compensated shipboard balance system (Childress & Mickel 1980) and frozen in liquid nitrogen. Metabolic rate was determined per hour incubation per gram body weight for each individual.

A temperature coefficient, or Q_{10} (= $(R_2/R_1)^{((T2-T1)/10)}$, R= oxygen consumption rate, T= temperature), quantifies the factorial change in metabolic rate with 10°C change in temperature and typically falls in the range of 2-3 (Hochachka & Somero 2002). Q_{10} was calculated from the average mass specific metabolic rate at each temperature. Mean metabolic rates of hyperiid amphipod species were normalized to the same temperature (10°C) and mass (0.25g) using measured Q_{10} and scaling coefficient and plotted as a function of the minimum depth of occurrence (MDO) (Figure 2). Routine metabolic rate data was summarized from our studies and those in the literature.

Rates from Literature:

Oxygen consumption values were adjusted to 10°C, which was chosen because it was the approximate median temperature for the range of data collected. Published temperature coefficients were used where available, or we assumed a Q_{10} of 2. For comparison between regions and across depth gradients, species rates were adjusted to a common body size of 0.25g using published scaling coefficients, the coefficient derived from the present study for data original to this study, or assuming a scaling coefficient of -0.25 for mass specific metabolism. Quarter-power scaling is not universal (Glazier 2006) but is commonly used when scaling coefficients are not available because of its prevalence; thus we considered it a safe assumption (Schmidt-Nielson 1984). Mean oxygen consumption rates were used for some species because published papers often do not give all the data for individual specimens but give mean values and size range.

Literature values of oxygen consumption were used only if they met the following requirements: Measurements were made in the absence of food, in darkness, and at temperatures within the natural environmental range for the particular species. All rates in the present study are presented as oxygen consumed per unit wet body mass. Thus, only studies providing wet mass, or the data necessary to calculate wet mass (e.g. dry mass and % water), were included.

Statistics:

Statistics were performed using the software SAS version 9.3 (SAS institute inc. USA). One-way ANOVAs were used to compare differences in metabolic rates

between groups. Linear regressions were also used to compare the relationship of metabolic rate with mass or minimum depth of occurrence. Mass-specific metabolic rate (MO_2) and enzymatic activities typically decline with increasing body mass (M) according to a power equation ($MO_2 = aM^b$), where *a* is a normalization constant and *b* is a scaling coefficient that describes the slope of the relationship. For linear regression against habitat depth, species were assigned a minimum depth of occurrence (MDO), the depth below which 90% of the individuals of a given species have been captured (Childress & Nygaard 1974, Childress 1975). However, due to the uncertainty of the species' vertical distributions, we also used a one-way ANOVA to assess differences in metabolic scaling curves for species believed to live above or below a particular depth. For pelagic species, a depth of 100m was used, since the majority of the decline in metabolism occurs in the upper 100 m in other visually-orienting pelagic taxa. For benthic species, a depth of 500 m was used following the protocol of Torres et al. (1994). Benthic organisms typically display little or no decline in metabolic rate with depth (Seibel & Drazen 2007).

A linear regression was used to compare the relationship of metabolic rate and environmental temperature in different species of the hyperiid amphipod *Themisto*. A one tailed t-test was also used to compare the mean metabolic rate of cold water (less than 1°C) versus warmer water (5°C or above) species of *Themisto*.

Results:

Oxygen consumption rates of eight species of hyperiid amphipod and two species of gammarid amphipods were measured (Table 2) from six study locations

(Table 1). Representative study specimens are pictured in Figure 1. MO_2 values for all species of hyperiid amphipods combined declined significantly with body mass ($MO_2=1.1074M^{-0.38}$, $r^2=0.54$; Figure 2). Regression equations for individual species are also provided in the figure legend. Metabolic rates of 25 additional hyperiid and 7 gammarid amphipod species were taken from the literature and used with data original to this study for the remaining analysis (Tables 3 and 4).

Mass- and temperature-normalized metabolic rates declined significantly with increasing depth of occurrence according to the regression equation $MO_2=1.074^{-0.38}$ ($p<0.0087$, Figure 3). The previously published regression equation for decline in MO_2 with increasing depth of occurrence in pelagic crustaceans (Childress 1975) visually appears to be outside of the 95% confidence limits for the regression equation for hyperiid amphipods below 150m (Figure 3).

Only five species of hyperiid amphipods from the literature and this study had a minimum depth of occurrence below 150 meters (Table 3). All these specimens are in the clade Physosomata. Species with a minimum depth of occurrence deeper than 150 meters had a mean metabolic rate of 1.54±0.67 µmol O_2 $g^{-1}hr^{-1}$ (n=5), which is significantly lower than the mean metabolic rate of 7.27±0.85 µmol O_2 $g^{-1}hr^{-1}$ (n=28) for species with a minimum depth of occurrence above 100 meters (ANOVA: $F(1,31)=7.46$, $P< 0.0087$, Figure 4).

Deep living pelagic gammarids (500m and below) had an average metabolic rate of 3.18 µmol O_2 $g^{-1}hr^{-1}$ when normalized to 10°C and 0.25g. Epipelagic gammarids from 100m and above had an average metabolic rate of 6.16 µmol O_2 $g^{-1}hr^{-1}$ (Table 4).

For this study there were 7 transparent species of hyperiid amphipod from the clade Physocephalata, and one species from the clade Physosomata (Table 3). When normalized to a common temperature of 10°C, MO_2 for transparent species of hyperiid amphipod had a significantly lower mean metabolic rate (3.74±0.88 μmol O_2 $g^{-1}hr^{-1}$, n=8) than non-transparent species in the clade Physocephalata (mean rate 12.00±1.38 μmol O_2 $g^{-1}hr^{-1}$, n=20), ANOVA f(1,26)=13.16, P<0.0012 (Figure 5). When normalized to the same temperature, all hyperiids from the clade Physocephalata (including transparent species) had a significantly higher MO_2 than hyperiids from the deep-living clade Physosomata (ANOVA: f(1,31)=10.14, p<0.0033, Figure 5).

Metabolic rates were available for five species in the genus *Themisto* from five different locations (Table 3). *T. japonica* has been studied at two locations. The environmental temperature ranged from -0.1°C in the Barents Sea (North of Russia, connects to the Arctic Ocean) to 10°C in the North Atlantic. Metabolic rates of these specimens were compared to see if rates from polar species were higher than species from warmer climates once normalized to temperature (10°C) and scaled to a body mass of 0.25g. Metabolic rates were not significantly related to environmental temperature, according to the regression equation MO_2=5.63-0.048x, R^2=.09, Figure 6. MO_2 is not significantly different between specimens from cold water regions (less than 1°C) and specimens from regions with temperature of 5°C or higher (t-test: t(4)=-1.84; P<0.1617).

Rates of gammarid amphipods for the species *Epimeriid* sp. and *Abyssochromeni plebs* (pictured Figure 1G) were adjusted to 10°C. In both species, the MO_2 scales significantly with body mass (Figure 7). The scaling relationship

published by Seibel and Drazen (2007) for benthic crustaceans ($MO2=1.9031m^{-0.27}$) is also plotted (Figure 7). This work on benthic crustaceans included gammarid amphipods from tropical to polar environments. Neither the slopes nor the elevations of the regressions for the two Antarctic species was significantly different than the regression line from Seibel and Drazen (2007, Figure 7). However there is a substantial interspecific variation ampng the species studied by Seibel and Drazen (2007). It may be worth noting that *Epimeriid sp*. falls entirely above the regression line.

Discussion:

This study increases the number of amphipod species for which metabolic rates are available by nearly 50% and more than doubles the number of pelagic species measured. The mean metabolic rates for marine hyperiid amphipods were lowest in the deep-living Physosomata, with a mean metabolic rate of 1.54±0.67 μmol O_2 $g^{-1}hr^{-1}$ and highest in the shallow-living Physocephalata with and mean metabolic rate of 7.27±0.85 μmol O_2 $g^{-1}hr^{-1}$ (Figure 5). This 3-4-fold variation in metabolism is attributable to physical, morphological, ecological, and phylogenetic differences between species, as well as size differences within and between species. Specifically, habitat depth, temperature, transparency, and body mass are determinants of metabolism, while their relative association with the benthos or latitude had little effect on metabolism. Within the Physocephalata metabolic rates of transparent species had lower metabolic rates (mean of 3.74±0.88 μmol O_2 $g^{-1}hr^{-1}$) than non-transparent species (mean12.00±1.38 μmol O_2 $g^{-1}hr^{-1}$) (Figure 5). This difference in

metabolic rate may be attributed to the reduced susceptibility to visual predation and therefore less reliance on strong locomotion in transparent species. Transparency is achieved, in part, by increased water content of tissues that further reduces wet-mass specific MO_2.

Habitat Depth

Respiratory rates in many midwater groups decrease with increasing habitat depth (Childress 1971, 1975, Seibel & Drazen 2007). Low metabolic rates in mesopelagic zooplankton are hypothesized to be related to the decreasing selection for locomotory capacity because low light levels limit predator-prey interactions among visually oriented organisms (Cowles et al. 1991, Childress 1995, Seibel & Drazen 2007). Visually-limited groups (those without image-forming eyes or those found in constant darkness) have low, but variable, metabolic rates at all depths (Seibel & Drazen 2007). Childress (1975) examined the respiratory rates of midwater crustaceans at temperatures characteristic of their depth of occurrence near southern California. In Childress 1975 the range of metabolic rates for epipelagic species (MDO 0-100m) was 17.32-3.47 μmol $g^{-1}hr^{-1}$. The range in rates for mesopelagic (MDO 150-900m) species in the same Childress (1975) study was 2.4-0.924 μmol O_2 $g^{-1}hr^{-1}$. Childress' study included one gammarid and two hyperiid amphipods. Data from those specimens were included in the present study (Table 3).

Studies on the vertical distribution of hyperiid amphipods are scarce, but most species in the clade Physocephalata have a minimum depth of occurrence of 0-50m, well within the epipelagic zone (Table 3). The hyperiid amphipod clade Physosomata

is generally found in the mesopelagic and bathypelagic zones (Vinogradov et al. 1996, Hurt et al. 2013). Eyes are often small, inconspicuous or absent in the Physosomata (Bowman & Gruner 1973, Vinogradov et al. 1996). In the mesopelagic, vision is used to see dim, downwelling daylight and bioluminescence, which is often point-source flashes not relevant for protracted predator-prey interactions (Warrant & Locket 2004). In this study, Physosomata with inconspicuous or absent eyes are represented by the genera *Lanceola*, *Scina*, and *Chuleola*. The eyes of *Scina crassicornis* function like other mesopelagic zooplankton, with a spectral sensitivity and response latency that allows them to capture the maximum amount of light in the mesopelagic zone (Cohen & Frank 2007). *Megalanceola* spp. have eyes that are narrow, kidney shaped and relatively large (Vinogradov et al. 1996). All of these Physosomata genera have significantly lower metabolic rates than hyperiids that have a minimum depth of occurrence above 100m (Figure 3). The rate for these species (1.54 ± 0.67 μmol O_2 $g^{-1}hr^{-1}$) is within the range of rates for other mesopelagic crustaceans examined by Childress (1975). The mean for epipelagic non-transparent hyperiids in this study (12.00 ± 1.38 μmol O_2 $g^{-1}hr^{-1}$) is within the range of other epipelagic crustaceans. The only other Physosomata species in this study was *Paraphronima* spp. (Figure 1 E), which, along with *Cystosoma* spp., was only recently assigned to this clade (Hurt et al. 2013). *Paraphronima* spp. is a vertical migrator with a much shallower minimum depth of occurrence than the other Physosomata represented but does reach depths of 500m or deeper during the day (Brusca 1967, Vinogradov et al. 1996). Since *Paraphronima* spp. spends some time in the photic zone, the low metabolic rate of this

species may be due to its nearly complete transparency (see below), more than its presence in the dimly-lit mesopelagic zone.

Deep living gammarids had lower rates (1.25 μmol O_2 $g^{-1}hr^{-1}$) than shallow living gammarids (4.01 μmol O_2 $g^{-1}hr^{-1}$) at 0.5°C (Torres et al. 1994). Data from the Torres study is included in the present study (Table 4). Data collected for this study had a similar trend; deep living gammarids (500m and below) had an average metabolic rate of 3.18 μmol O_2 $g^{-1}hr^{-1}$ when normalized to 10°C and 0.25g. Epipelagic gammarids, from 100m and above had an average metabolic rate of 6.16 μmol O_2 $g^{-1}hr^{-1}$. Torres et al (1994) noted that this difference in metabolic rate may not be due to reduced activity levels, since the ratios of maximum to minimum rates are not significantly different between shallow and deep living specimens. Deep living species of micronekton crustaceans are able to alter their activity levels and metabolic rates equivalently to shallow species (Torres et al. 1994). Their respiratory rate is lower probably because their body composition is different (reduced protein and lipid levels, higher water levels (Childress & Nygaard 1974) than epipelagic amphipods.

Some studies examine metabolic rates as a function of depth of capture rather than minimum depth of occurrence. In these studies organisms are captured in net tow where the net remains open between the deepest depth and the surface. So epipelagic organisms are also captured in tows that start in the mesopelagic because the tow collects continuously from the mesopelagic up to the surface. The mean metabolic rate of all individuals from a fixed collection depth interval is plotted against the middle of the depth interval. As noted by Childress et al. (2008), this

method results in the same species represented in more than one depth interval. For example Ikeda (2013) reports the species *Phronima sedentaria* with both deep and shallow capture depths, 2 m and 750 m. This deeper depth is not ecologically significant since *Phronima sedentaria* is not found below 600m (Shih 1969, Shulenberger 1977). Also because *Phronima sedentaria* is a vertical migrator, the minimum depth of occurrence is a more accurate depiction of the ecological pressures relevant to metabolic demand and visual predator-prey interactions. Comparing this epipelagic species to deep sea species that do not spend time in the photic zone does not test the visual interactions hypotheses (Childress et al. 2008). In addition, this misleading depth range results in inaccurate estimates of the contribution of these organisms to carbon flux.

Transparency

Several species of epipelagic amphipods had rates that were low relative to other epipelagic crustacean species. *Phronima sedentaria* (Figure 1D), like *Paraphronima* spp., was in the range of mesopelagic specimens from Childress's study (1975) on midwater crustaceans, despite the fact that its minimum depth of occurrence is shallow (25 m). Elder and Seibel (in prep.) hypothesized that the low rate in *P. sedentaria* is related to its transparency, which should limit its visibility to predators and prey even in well-lit surface waters. Similarly, squids from the family Cranchiidae have low metabolic rates despite occupying shallow water for at least part of their life history. It was suggested that transparency relieves them from selective pressures on locomotion and metabolism associated with predator-prey interaction

(Seibel and Carlini, 2001). Other Cephalopods, being highly visual predators, exhibit a decline in oxygen consumption with increasing minimum habitat depth similar to the crustaceans (Seibel et al. 1997). Transparency is one of the few means of camouflage from visual predators available to oceanic organisms. The distribution of transparency in primarily photic aquatic environments overlaps with the habitat of visual predators (Johnsen 2001), providing evidence that this is an adaptation to avoid visual predators.

The families of Hyperiidae which are semitransparent or absolutely transparent included in this study are Oxycephalidae, Paraphronimidae, and Phronimidae (Bowman & Gruner 1973, Vinogradov et al. 1996). Mean metabolic rates adjusted to 10°C were compared between transparent and non-transparent hyperiid amphipods that have a minimum depth of occurrence above 100 meters. Transparent amphipod species had a significantly lower mean metabolic rate (3.74±0.88 μmol O_2 $g^{-1}hr^{-1}$) than non-transparent species (mean rate 12.00±1.38 μmol O_2 $g^{-1}hr^{-1}$), when normalized to 10°C ($P<0.0012$; Figure 5). Metabolic rates ranged from 8.86 μmol O_2 $g^{-1}hr^{-1}$ at 10°C in *Cranocaplalus scleroticus* (Figure 1B), a species of Oxycephalidea, to 1.52 μmol O_2 $g^{-1}hr^{-1}$ at 10°C in *Paraphronima* spp. (Table 3, Figure 5). Transparent hyperiids in the Physosomata are 'globular' in shape, mainly bathypelagic, and have weakly developed muscles resulting in weak swimming abilities (Bowman & Gruner 1973). *Paraphronima* is the only transparent species of Physosomata for which metabolic rates have been measured, but in line with the low activity of Physosomata, the metabolic rate is lower than any of the transparent Physocephalata. Transparent members of Physocephalata, including many Oxychechalidae (Figure 1B and H for examples), are slender and elongate (Bowman & Gruner 1973), improving their

swimming efficiency due to their streamline shape. All transparent species from the family Oxycephalidae had higher metabolic rates than the other transparent amphipods species. This is most likely due to their reported strong swimming abilities and relatively active behavior.

The only two species of transparent hyperiid amphipods that have been previously studied for metabolic rate are *Phronima sedentaria* (Childress 1975, Mayzaud et al. 2005, Ikeda 2012) and a single specimen of *Oxychephalus clausi* (Ikeda & McKinnon 2012). Rates for *Phronima sedentaria* were comparable with the data reported from our previous work (Elder & Seibel In Prep). *Oxychephalus clausi* from the coral sea had an average rate of 5.5 μmol O_2 $g^{-1}hr^{-1}$ (Ikeda & McKinnon 2012). *Oxychephalus clausi* has been described as a predatory species that can rapidly devour salps, has a greater developed pleon and urosome (tail region), and can accelerate for burst swimming (Harbison et al. 1977). The well developed pleon and urosome would assist *Oxychephalus clausi* in achieving faster swimming speeds than species with more reduced structures. This is in line with the other activity levels of the other species of Oxychephalidae. The ecological reasons selecting for this more active mode in Oxychephalidae compared to other hyperiids is not known.

Temperature and Metabolic Cold Adaptation

Metabolic cold adaptation is the outdated concept that polar ectotherms have compensated for the depressing effect of temperature on metabolic rate (Clarke 1980). Metabolic rates represent the sum of numerous energetic expenditures, so elevating metabolic rate and the amount of ATP required is energetically costly. It has been

noted that there is no selective advantage in arbitrarily increasing metabolic rate in order to achieve compensation for temperature (Clarke 1993). Therefore metabolic cold adaptation as a concept is largely abandoned. However, polar species are able to adjust or compensate some physiological processes (e.g. enzyme-mediated reactions (Kawall et al. 2002)) for living at low temperatures so that they attain greater activity levels than similar organisms from a warmer habitat acclimated to the same low temperature (Crockett & Sidell 1990). There is an advantage to increasing metabolic rate to a level consistent with that required for ecologically relevant activity levels (Seibel et al. 2007). For example, in pelagic pteropods, active predator-prey interactions select for high maintenance of wing-beat frequencies, the cost of which is reflected in the whole-animal metabolic rate. Polar and temperate species need to swim at similar rates to capture prey, despite differences in habitat temperature, so energy consumption in support of swimming is similar across a latitudinal range (Seibel et al. 2007).

Previous work on amphipods has found no support for metabolic cold adaptation. Torres et al (1994) compared rates of micronekton from studies in the Antarctic, temperate and tropical studies across a depth range. The mass and temperature corrected regressions for Antarctic representatives are either below or similar to regressions from lower latitude locations (Torres et al. 1994). Cowles et al. (1991) also found a lack of support for metabolic cold adaptation when comparing crustaceans from California and Hawaii. Decline in metabolism with depth in isothermal waters support the conclusion that this decline is related to relaxation of pressure from visual predators, not from temperature (Torres et al. 1994).

To examine if temperature compensation of metabolism is present in hyperiids, the genus *Themisto* was compared. For this genus there are five species from five different locations, with two representatives of one species (Figure 1C pictures *Themisto abyssorum*). The hyperiids in the genus *Themisto* are numerous in abundance and high in biomass in the world oceans and are primarily found in high latitude seas (Yamada et al. 2004). *Themisto* are predominantly carnivores and are well studied because of their abundance and importance as an intermediate between primary producers and higher trophic levels (reviewed by (Auel & Werner 2003). For this comparison, three species of *Themisto* were from regions with environmental temperatures of 0.5°C or below, two were from the western subarctic pacific where their habitat temperature is 5°C (Yamada & Ikeda 2003), and one species was collected from the North Atlantic Ocean where habitat temperatures were 10°C (Table 3). When normalized to the same temperature and body mass, the metabolic rates of these species were not significantly different (Figure 6). This indicates that metabolic cold adaptation is not present in this genus. If the species from the 0.5°C waters had a significantly higher rate than the species from the 10 and 5°C waters, than it would indicate metabolic cold compensation.

Two Antarctic gammarid amphipods from this work, *Abysssochromene plebs* (Figure 1G) and *Epimmeriid sp*. were normalized to 10°C and compared to the regression equation for benthic crustaceans from Seibel and Drazen (2007), also normalized to 10°C (Figure 7). That regression includes an extensive review of benthic amphipods from polar to tropical and temperate waters. There was no evidence of metabolic cold adaptation in the polar organisms compared to crustaceans

from other latitudes (Seibel & Drazen 2007). The rates of gammarid amphipods from Antarctica in this study were not significantly different than the Seibel and Drazen (2007) regression, further substantiating the lack of evidence for metabolic cold adaptation in crustaceans.

Ecology

Classification and phylogenetic confusion in hyperiids results from convergent evolution of traits correlated with their pelagic life history and parasitic relationship with gelatinous zooplankton (Hurt et al. 2013). They are known descendants of gammarid amphipods, which are predominantly benthic. Hyperiids are exclusively pelagic, but are commonly thought of as living a benthic-like existence because they live on gelatinous zooplankton (Laval 1980). Juveniles are deposited on the host from the brood pouch of the female. Juveniles are unable to swim on their own and therefore would be unlikely to encounter a host on their own. Females do not produce large numbers of offspring (Gasca & Haddock 2004), as many broadcast spawning pelagic species do to increase chance of progeny survival. This indicates that the gelatinous host acts as a secondary brood pouch for further development of the larva (Laval 1980). The importance of gelatinous hosts for males of pelagic amphipods is not clear. There have been far fewer observations of males on hosts (Harbison et al. 1977), but as previously noted it has not yet been fully examined if males are more independent from hosts than females (Gasca & Haddock 2004).

The time hyperiids spend on substrate (hosts) does not appear to be equivalent to living an entirely benthic lifestyle, because of their relatively high metabolic rates

compared to benthic gammarids and to behavioral observations. The degree of dependence on hosts varies according to species (Ohtsuka et al. 2009). Many species of amphipods have been noted to be strong swimmers and relatively active, especially shallow living ones, and have been observed without a gelatinous host. Pelagic amphipods remain in the water column by either active swimming or a combination of active swimming and resting on floating substrates (gelatinous zooplankton)(Ikeda 2013). Some species, such as *Themisto* occur in swarms in gatherings of up to 38 individuals have been observed from submersibles, though the reason for this behavior is unknown (Vinogradov 1999). Based on gut content analysis *Themisto pacifica* and *Cyphocaris challengeri* preyed on copepods, amphipods, ostracods and cladocerans (Haro-Garay 2004). Examination of the mouth parts of *T. pacifica* determined they can feed on gelatinous tissue and hardier organisms that possess an exoskeleton. This indicates that *T. pacifico* has raptorial capacity. Strong palps and sharp incisors on *C. challengeri* indicate they are predatory by nature, and are able to eat exoskeleton covered small planktonic invertebrates. These morphological conclusions were supported by gut content analysis. *C. challengeri* is an active exclusively carnivorous predator; *T. pacifica* is a more passive predator relying on microphagy/canivory (Haro-Garay 2004).

Some pelagic gammarid amphipods are also adapted to associate with gelatinous zooplankton (Vader 1972). Epipelagic gammarid amphipods have lower metabolic rates than epipelagic hyperiids. Epipelagic gammarids from this study had a metabolic rate of 6.16 μmol O_2 $g^{-1}hr^{-1}$, while epipelagic hyperiids had an average rate of 12.92 μmol O_2 $g^{-1}hr^{-1}$. Torres at al. (2004) also found gammarids had a lower rate

than hyperiids, but offered no explanation for this. Few observations are available for pelagic gammarid amphipods. Epipelagic gammarids may differ in ecology from epipelagic hyperiids.

Benthic gammarids have a lower metabolic rate than nontransparent pelagic hyperiids and gammarids. Their lower rate may be explained by the lack of energy expenditure for vertical migrations or finding a host. Epipelagic amphipods (Vinogradov et al. 1996) are known to vertically migrate on a diel cycle. While they may be traveling on their gelatinous host, their high activity levels indicated they likely spend some time swimming for this migration. Rather than remaining immobile on the gelatinous host, adult hyperiids wander from host to host, and use them as a platform for attaching other prey (Laval 1980). Transparent species have a lower metabolic rate than other mesopelagic species because of the relief from pressure for locomotory performance to avoid visual predators discussed above. Pelagic deep sea hyperiids have the lowest rates of any amphipods. These species are known to have a less hydrodynamic shape (Bowman & Gruner 1973) than gammarids and epipelagic amphipods, as well as visual systems that function well for a lifestyle of sitting and waiting for prey when they are not on gelatinous zooplankton (discussed above in habitat depth section). Deep sea hyperiid amphipod species do not conduct large energy costly vertical migrations on a diel cycle; this also reduces their selection for strong swimming abilities.

Respiration rates of amphipods are similar to euphausiids (krill) but greater than copepods (Ikeda 2013). Euphausiids, which are entirely pelagic, are considered active swimmers with well-developed eyes. Copepods lack image-forming eyes and

are documented to spend less time swimming, alternating between a swimming mode and a cleaning mode (Schmitt et al. 2006).

Many amphipods are negatively buoyant. *Phronima* has a density of ~1.045, which makes it negatively buoyant relative to seawater (1.024; Davenport 1994, Tsukamoto et al. 2009). *Phronima* specimens have increased buoyancy (1.030) when in their salp barrel (Tsukamoto et al. 2009). *Streetsia sp*. has a high specific gravity of 1.146, which is greater than all other crustacean zooplankton examined (Tsukamoto et al. 2009). Most crustacean zooplankton are somewhat negatively buoyant. Copepods have a density around 1.060 and the euphausiid examined has a density of 1.092 (Tsukamoto et al. 2009). Most hyperiid amphipods have a high water content (68 to 93% of wet mass Ikeda 2013) compared to euphausiids (65-78%; Ikeda 2012), However some amphipods also have high ash content relative to copepods and euphausiids (mean 25% of dry mass) due to their robust exoskeletons (Ikeda 2013). This high ash content would decrease buoyancy. Negative buoyancy may result in amphipods actively swimming to maintain position in the water column unless "hitch-hiking" on gelatinous zooplankton, though no direct studies have been. Amphipods may use the host jellies as a resting place and refuge between forays, and a food source at times. The species that approach neutral buoyancy are likely to spend less energy swimming than negatively buoyant species. However, previous work found there was no significant relationship between relative buoyancy and overall respiratory rate (Childress & Nygaard 1974). Relative buoyancy in pelagic crustaceans decreases with increasing depth of occurrence (Childress & Nygaard 1974), but no specimens from the clade Physosomata were included. This clade may be closer to neutral

buoyancy because their globular appearance indicates a high water content. Also they are known to be more inactive than pelagic species.

Conclusions

This study demonstrates that the differences in amphipod metabolism are associated with physical, morphological, ecological, and phylogenetic differences between species. The species in the hyperiid amphipod clade Physosomata had significantly lower metabolic rates than species in the clade Physocephalata. The clade Physocephalata is typically more streamline in morphology, and has a minimum depth of occurrence in the epipelagic zone. Transparent species are found in both Physosomata and Physocephalata, although metabolic rate was only available for one species of Physosomata (Table 3). The lower rates in mesopelagic and transparent amphipods support the visual interactions hypothesis: decreasing selection for locomotory capacity (by transparency or remaining below the photic zone during the day) limits predator-prey interactions among visually oriented organisms and results in lower metabolic rates (Childress & Mickel 1985, Cowles et al. 1991, Seibel & Drazen 2007).

Lower rates of metabolism in benthic and mesopelagic gammarids may also be attributed to the visual interactions hypothesis. Further research needs to be done to determine the reasons for lower metabolic rate in epipelagic gammarids compared to epipelagic hyperiids. This study also adds to the evidence that polar amphipods do not exhibit temperature compensation of metabolism.

Acknowledgments:

Thanks to the captains and crews of the five different research vessels involved in this study. Thanks also to the support staff at McMurdo station. Thanks to Jonathan Cohen for assistance in collection and identification of *Abyssochromeni plebs*. Thank you to Steve Haddock for inviting L. Elder on the R/V Western Flyer to conduct some of this work. Thanks to the science parties on each of the cruises and at McMurdo for assistance in specimen collection.

Funding:

This work was funded by the following National Science Foundation grants: In the ETNP OCE-0526502 to Karen Wishner and Brad Seibel, and OCE-0526545 to Kendra Daly. In the Antarctic, ANT-0538479 to Brad Seibel and Victoria Fabry. In the Gulf of California OCE-0526493 and in the North Atlantic OCE-0852160 both to Brad Seibel.

References:

Auel H, Werner I (2003) Feeding, respiration and life history of the hyperiid amphipod *themisto libellula* in the arctic marginal ice zone of the greenland sea. Journal of Experimental Marine Biology and Ecology 296:183-197

Bousfield EL (1973) Shallow-water gammaridean amphipoda of new england, Vol. Comstock Pub. Associates Ithaca, New York

Bowman TE, Gruner H-E (1973) The families and genera of hyperiidea (crustacea: Amphipoda), Vol. Smithsonian Institution Press

Browne WE, Haddock SHD, Martindale MQ (2007) Phylogenetic analysis of lineage relationships among hyperiid amphipods as revealed by examination of the mitochondrial gene, cytochrome oxidase i (coi). Integrative and Comparative Biology 47:815

Brusca GJ (1967) The ecology of pelagic amphipoda, i. Species accounts, vertical zonation and migration of amphipoda from the waters off southern california.

Burd AB, Hansell DA, Steinberg DK, Anderson TRand others (2010) Assessing the apparent imbalance between geochemical and biochemical indicators of meso-and

bathypelagic biological activity: What the@ $♯! Is wrong with present calculations of carbon budgets? Deep Sea Research Part II: Topical Studies in Oceanography 57:1557-1571
Childress JJ (1971) Respiratory adaptations to the oxygen minimum layer in the bathypelagic mysid *gnathophausia ingens*. The Biological Bulletin 141:109-121
Childress JJ (1975) The respiratory rates of midwater crustaceans as a function of depth of occurrence and relation to the oxygen minimum layer off southern california. Comparative Biochemistry and Physiology Part A: Physiology 50:787-799
Childress JJ (1995) Are there physiological and biochemical adaptations of metabolism in deep-sea animals? Trends in Ecology and Evolution 10:30-36
Childress JJ, Barnes AT, Quetin LB, Robison B (1978) Thermally protecting cod ends for the recovery of living deep-sea animals. Deep-Sea Research 25:419-422
Childress JJ, Mickel TJ (1980) A motion compensated shipboard precision balance system. Deep-Sea Research 27:965-970
Childress JJ, Mickel TJ (1985) Metabolic rates of animals from the hydrothermal vents and other deep-sea habitats. Bulletin of the Biological Society of Washington:249-260
Childress JJ, Nygaard M (1974) Chemical composition and buoyancy of midwater crustaceans as function of depth of occurrence off southern california. Marine Biology 27:225-238
Childress JJ, Seibel BA, Thuesen EV (2008) N-specific metabolic data are not relevant to the 'visual interactions' hypothesis concerning the depth-related declines in metabolic rates: Comment on ikeda et al.(2006). Mar Ecol Prog Ser 373:187-191
Clark LC (1956) Monitor and control of blood and tissue oxygen tensions. Transactions - American Society for Artificial Internal Organs 2:41-48
Clarke A (1980) A reappraisal of the concept of metabolic cold adaptation in polar marine invertebrates. Biological Journal of the Linnean Society 14:77-92
Clarke A (1993) Seasonal acclimatization and latitudinal compensation in metabolism: Do they exist? Functional Ecology 7:139-149
Cohen JH, Frank TM (2007) Vision in the hyperiid amphipod scina crassicornis. JMBA-Journal of the Marine Biological Association of the United Kingdom 87:1201-1206
Cowles D, Childress J, Wells M (1991) Metabolic rates of midwater crustaceans as a function of depth of occurrence off the hawaiian islands: Food availability as a selective factor? Marine Biology 110:75-83
Crockett EL, Sidell BD (1990) Some pathways of energy metabolism are cold adapted in antarctic fishes. Physiological Zoology:472-488
Davenport J (1994) Observations on the locomotion and buoyancy of phronima sedentaria (forskål, 1775)(crustacea: Amphipoda: Hyperiidea). Journal of Natural History 28:787-793
Dick JTA, Faloon SE, Elwood RW (1998) Active brood care in an amphipod: Influences of embryonic development, temperature and oxygen. Animal Behaviour 56:663-672
Donnelly J, Torres J (1988) Oxygen consumption of midwater fishes and crustaceans from the eastern gulf of mexico. Marine Biology 97:483-494
Ducklow HW, Steinberg DK, Buesseler KO (2001) Upper ocean carbon export and the biological pump. Oceanography 14:50-58
Ekau W, Auel H, Pörtner H, Gilbert D (2010) Impacts of hypoxia on the structure and processes in pelagic communities(zooplankton, macro-invertebrates and fish). Biogeosciences 7:1669-1699

Elder LE, Seibel BA (In Prep) Ecophysiological implications of vertical migration into oxygen minimum zones for the hyperiid amphipod phronima sedentaria. Marine Ecology Progress Series

Gasca R, Haddock SHD (2004) Associations between gelatinous zooplankton and hyperiid amphipods (crustacea: Peracarida) in the gulf of california. Hydrobiologia 530:529-535

Glazier DS (2005) Beyond the '3/4-power law': Variation in the intra-and interspecific scaling of metabolic rate in animals. Biological Reviews 80:611-662

Glazier DS (2006) The 3/4-power law is not universal: Evolution of isometric, ontogenetic metabolic scaling in pelagic animals. BioScience 56:325-332

Harbison G, Biggs D, Madin L (1977) The associations of amphipoda hyperiidea with gelatinous zooplankton—ii. Associations with cnidaria, ctenophora and radiolaria. Deep Sea Research 24:465-488

Haro-Garay MJ (2004) Diet and functional morphology of two planktonic amphipods fomr the strait of georgia, british columbia, parathemisto pacifica, (stebbing, 1888) and cyphocaris challengeri (stebbingi, 1888). Crustaceana 76:1291-1312

Hochachka PW, Somero GN (2002) Biochemical adaptation: Mechanism and process in physiological evolution, Vol. Oxford University Press, USA

Hurt C, Haddock SH, Browne WE (2013) Molecular phylogenetic evidence for the reorganization of the hyperiid amphipods, a diverse group of pelagic crustaceans. Molecular phylogenetics and evolution 67:28-37

Ikeda T (1988) Metabolism and chemical composition of crustaceans from the antarctic mesopelagic zone. Deep Sea Research Part A Oceanographic Research Papers 35:1991-2002

Ikeda T (2012) Metabolism and chemical composition of zooplankton from 500 to 5,000 m depth of the western subarctic pacific ocean. Journal of Oceanography 68:641-649

Ikeda T (2013) Metabolism and chemical composition of marine pelagic amphipods: Synthesis toward a global bathymetric model. Journal of Oceanography:1-17

Ikeda T, McKinnon AD (2012) Metabolism and chemical composition of zooplankton and hyperbenthos from the great barrier reef waters, north queensland, australia. Plankton and Benthos Research 7:8-19

Johnsen S (2001) Hidden in plain sight: The ecology and physiology of organismal transparency. The Biological Bulletin 201:301-318

Kawall H, Torres J, Sidell B, Somero G (2002) Metabolic cold adaptation in antarctic fishes: Evidence from enzymatic activities of brain. Marine biology 140:279-286

Land MF (1992) Locomotion and visual behaviour of mid-water crustaceans. Journal of the Marine Biological Association of the United Kingdom 72:41-60

Laval P (1980) Hyperiid amphipods as crustacean parasitoids associated with gelatinous zooplankton. Oceanogr mar Biol ann Rev 18:11-56

Longhurst AR, Bedo AW, Harrison WG, Head EJH, Sameoto DD (1990) Vertical flux of respiratory carbon by oceanic diel migrant biota. Deep Sea Research Part A Oceanographic Research Papers 37:685-694

Madin L, Harbison G (1977) The associations of amphipoda hyperiidea with gelatinous zooplankton—i. Associations with salpidae. Deep Sea Research 24:449-463

Marsh AG, Manahan D (1999) A method for accurate measurements of the respiration rates of marine invertabrate embryos and larvae. Marine Ecology Progress Series 184:1-10

Martin JW, Davis GE (2001) An updated classification of the recent crustacea.

Mayzaud P, Boutoute M, Gasparini S, Mousseau L, Lefevre D (2005) Respiration in marine zooplankton: The other side of the coin: Co 2 production. Limnology and Oceanography:291-298
Ohtsuka S, Koike K, Lindsay D, Nishikawa Jand others (2009) Symbionts of marine medusae and ctenophores. Plankton and Benthos Research 4:1-13
Schmidt-Nielson K (1984) Scaling: Why is animal size so important? Cambridge Univ Press, Cambridge, UK
Schmitt FG, Seuront L, Hwang J-S, Souissi S, Tseng L-C (2006) Scaling of swimming sequences in copepod behavior: Data analysis and simulation. Physica A: Statistical Mechanics and its Applications 364:287-296
Seibel BA (2011) Critical oxygen levels and metabolic suppression in oceanic oxygen minimum zones. The Journal of Experimental Biology 214:m326-336
Seibel BA, Carlini DB (2001) Metabolism of pelagic cephalopods as a function of habitat depth: A reanalysis using phylogenetically independent contrasts. Biological Bulletin, Marine Biological Laboratory, Woods Hole 201:1-5
Seibel BA, Drazen JC (2007) The rate of metabolism in marine animals: Environmental constraints, ecological demands and energetic opportunities. Philosophical Transactions of the Royal Society B: Biological Sciences 362:2061
Seibel BA, Dymowska A, Rosenthal J (2007) Metabolic temperature compensation and coevolution of locomotory performance in pteropod molluscs. Integrative and Comparative Biology
Seibel BA, Thuesen EV, Childress JJ, Gorodezky LA (1997) Decline in pelagic cephalopod metabolism with habitat depth reflects differences in locomotory efficiency. The Biological Bulletin 192:262-278
Shih C-T (1969) The systematics and biology of the family phronimidae (crustacea: Amphipoda). Dana Reports no 74:1-100
Shulenberger E (1977) Hyperiid amphipods from the zooplankton community of the north pacific central gyre. Marine Biology 42:375-385
Torres JJ, Aarset A, Donnelly J, Hopkins TL, Lancraft T, Ainley D (1994) Metabolism of antarctic micronektonic crustacea as a function of depth of occurrence and season. Marine Ecology-Progress Series 113:207
Tsukamoto K, Yamada Y, Okamura A, Kaneko Tand others (2009) Positive buoyancy in eel leptocephali: An adaptation for life in the ocean surface layer. Marine biology 156:835-846
Vader W (1972) Associations between gammarid and caprellid amphipods and medusae. Sarsia 50:51-56
Vinogradov G (1999) Deep-sea near-bottom swarms of pelagic amphipods themisto: Observations from submersibles. SARSIA-BERGEN- 84:465-468
Vinogradov G, Hernandez F, Tejera E (2004) Pelagic amphipods from the cape verde islands (tfmcbm/98 cruise, macaronesia 2000-project). VIERAEA 32:7-27
Vinogradov ME, Volkov AF, Semenova TN, Siegel-Causey D (1996) Hyperiid amphipods (amphipoda, hyperiidea) of the world oceans, Vol. Science Publications Incorporated, Lebanon, USA
Warrant EJ, Locket NA (2004) Vision in the deep sea. Biological Reviews 79:671-712
Wiebe P, Morton A, Bradley A, Backus Rand others (1985) New development in the mocness, an apparatus for sampling zooplankton and micronekton. Marine Biology 87:313-323

Yamada Y, Ikeda T (2003) Metabolism and chemical composition of four pelagic amphipods in the oyashio region, western subarctic pacific ocean. Marine Ecology Progress Series 253:233-241

Yamada Y, Ikeda T, Tsuda A (2004) Comparative life-history study on sympatric hyperiid amphipods (themisto pacifica and t. Japonica) in the oyashio region, western north pacific. Marine Biology 145:515-527

Tables and Figures:

Date	Location	Coordinates	Vessel/ station	Collection method
June 2007	Gulf of California	between 27° N 112° W and 111° W	R/V New Horizon	mother tucker trawl
July 2007	Pacific Ocean off the Coast of California	between 36°N 122°W and 35°N 123° W	R/V Western Flyer	tucker trawl
Oct-Nov 2007	Eastern Tropical North Pacific	Costa Rica Dome, 9°N 90° W and Tehuantepec Bowl, 11° N 98° W	R/V Seward Johnson	mother tucker trawl
Dec 2008-Jan 2009	Eastern Tropical North Pacific (ETNP)	Costa Rica Dome, 9°N 90° W and Tehuantepec Bowl, 11° N 98° W	R/V Knorr	mother tucker trawl
Jan-Feb 2008	Ross Sea, Antarctica	~ 162° to 171° E, ~77° to 78° S	McMurdo Station	ice hole or dippers near shore
Sept 2011	North Atlantic	between 37° N 71°W and 39°N 67° W	R/V Endeavor	mother tucker trawl

Table 1: Dates, locations and methods for collection of experimental organisms original to this study.

Table 2:

Species	Group	Collection location	n	Mean wet Mass (g)	Mass range	Average Mo2 umol g-1 hr-1	Temp °C	Q_{10}	Corrected to 10°C	Average Mo_2 at 10°C
Hyperiella dilatica	Hyperiid	Ross Sea Antarctica	12	0.015	0.005-0.0869	3.79	-2	2	9.3	
			7	0.0054	0.007-0.0031	8.22	2	2	14.31	11.81
Themisto abyssorum	Hyperiid	North Atlantic	8	0.0715	0.037-0.107	6.58	10		6.58	6.58
Vibilia sp. 1	Hyperiid	ETNP	4	0.015	0.012-0.022	9.19	10		9.19	
			9	0.017	0.015-0.019	18.44	26	3.05	6.05	7.62
vibilia sp. 2	Hyperiid	ETNP	12	0.0059	0.0014-0.0098	8.24	10		8.24	
			18	0.0069	0.0012-0.0143	21.89	20	2.66	8.7	8.47
Phronimella elongata	Hyperiid	ETNP	5	0.021	0.006-0.059	2.5	10		2.5	
Cranoceplalus sclerotious	Hyperiid	ETNP	8	0.004	0.0016-0.0099	8.85	10		8.85	
			8	0.063	0.0029-0.238	6.25	20	0.705	8.87	8.86
Streetsia sp.	Hyperiid	California	7	0.055	0.04-0.091	3.14	5	2	4.45	4.45
Paraphronima	Hyperiid	California	13	0.0667	0.022-0.134	1.08	5	2	1.52	1.52
Abyssochromeni plebs	Gammarid	Ross Sea Antarctica	22	0.152	0.0161-0.4248	1.26	-2	2	2.89	
			21	0.177	0.023-0.4547	1.28	0	2	2.49	
			15	0.176	0.0201-0.4311	2.05	2	2	3.72	3.03
Epimeriid sp.	Gammarid	Ross Sea Antarctica	15	0.04	0.0138-0.0658	3.03	-2	2	6.95	
			7	0.042	0.0157-0.0814	4.19	2	2	7.29	7.12

Table 2. Experimental specimens original to this study, including samples sizes, temperature at which experiments were conducted, and corrected metabolic rates. MO_2=metabolic rate. ETNP= Eastern Tropical North Pacific. Q_{10} is the temperature coefficient that quantifies the factorial change in metabolic rate with 10°C change in temperature.

Table 3:

Species	Clade	Transparent	Collection location	MDO (m)	n	Mean wet mass (g)	Mean MO_2 umol g-1 hr-1	Temp °C	a for habitat temp	scaled to .25	MO_2 corrected to 10°C	a for scaling 10°C	scaled to .25g 10°C	ref
Chuneola spinifera	Physosomata	n	Western Subarctic Pacific	150	1	1.52	0.19	1.5	0.21	0.3	0.34	0.38	0.53	Ikeda 2012
Cranocaplalus scleroticus	Physocephalata	y	ETNP	50*	16	0.0335	Table 2	Table 2	Table 2	Table 2	8.86	3.79	5.59	this study
Cyllopus lucasii	Physocephalata	n	Weddell Sea Antarctica	0	16	0.126	9.84	0.5	5.86	8.29	19.01	11.33	16.02	Donnelly 2004
Cyllopus lucasii	Physocephalata	n	Scotia/ Weddell Sea Antarctica	0	5	0.197	6.65	0.5	4.43	6.27	12.85	8.56	12.1	Torres et al 1994
Hyperia galba	Physocephalata	n	Southern California	25	6	0.055	4.86	10	2.35	3.33	4.86	2.35	3.33	Childress, 1975
Hyperiella antarctica	Physocephalata	n	Weddell Sea Antarctica	0	1	0.0672	2.99	0.5	1.52	2.15	5.78	2.94	4.16	Donnelly 2004

Hyperiella dilatica	Physocephalata	n	Ross Sea Antarctica	0	19	0.0102	Table 2	10	3.75	5.3	11.8	3.75	5.53	this study
Hyperoche medusarum	Physocephalata	n	Weddell Sea Antarctica	0	1	0.0621	7.46	0.5	3.72	5.27	14.41	7.19	10.17	Donnelly 2004
Lanceola loveni	Physosomata	n	Western Subarctic Pacific	500	4	0.0628	0.38	2	0.19	0.27	0.65	0.33	0.46	Ikeda 2012
megalanceoloides remipes	Physosomata	n	Weddell Sea Antarctica	150	1	0.4189	1.88	0.5	1.51	2.13	3.62	2.91	4.12	Donnelly 2004
megalanceoloides stephensemi	Physosomata	n	Weddell Sea Antarctica	150	1	4.9971	0.36	0.5	0.53	0.75	0.69	1.03	1.46	Donnelly 2004
Oxychephalus clausi	physocephalata	y	Coral Sea	0-25 *	1	0.0641	18.52	27.5	9.32	13.18	5.51	2.77	3.92	Ikeda and McKinnon 2012
Paraphronima	Physosomata	y	California coast	50 *	13	0.0667	1.08	5	0.55	0.77	1.52	0.77	1.14	this study
Phronima sedentaria	Physocephalata	y	North Atlantic	0-25*	4	0.278	3.46	10	2.51	3.55	3.46		4.98	Elder and Seibel in Prep
Phronima sedentaria	Physocephalata	y	Gulf of California	0-25*	49	0.274	2.99	20	2.16	3.06	1.67		2.7	Elder and Seibel in Prep
Phronima sedentaria	Physocephalata	y	ETNP	0-25*	39	0.288	3.65	20	2.67	3.78	1.99		2.65	Elder and Seibel in Prep
Phronimella elongata	Physocephalata	y	ETNP	0-25*	5	0.021	2.5	10	0.95	1.35	2.5	0.95	1.4	this study

Primno abyssalis	Physocephalata	n	South Japan Sea	100^	7	0.0746	2.9	0.5	1.52	2.14	5.6	2.93	4.14	Ikeda Hirakawa 1998
Primno abyssalis	Physocephalata	n	Western Subarctic Pacific	100^	17	0.0287	8.35	5	3.44	4.86	11.81	4.86	6.87	yamada and Ikeda 2005
Primno macropa	Physocephalata	n	Weddell Sea Antarctica	0	1	0.0653	11.88	0.5	6	8.49	22.94	11.6	16.4	Donnelly 2004
Primno macropa	Physocephalata	n	Scotia/ Weddell Sea Antarctica	0	6	0.0129	6.61	0.5	2.23	3.15	12.77	4.3	6.09	Torres et al 1994
Scina borealis	Physosomata	n	Western Subarctic Pacific	200*	1	0.0809	0.87	2	0.46	0.65	1.51	0.81	1.14	ikeda 2012
Streetsia	physocephalata	y	California coast	25*	7	0.055	3.14	5	1.52	2.15	4.45	2.16	3.18	this study
Themisto abyssorum	Physocephalata	n	North Atlantic	25*	8	0.0715	6.58	10	3.4	4.81	6.58	3.4	5.02	this study
Themisto gaudichaudii	Physocephalata	n	Scotia/ Weddell Sea Antarctica	0	2	0.314	2.41	0.5	1.8	2.55	4.66	3.49	4.93	Torres et al 1994
Themisto Japonica	Physocephalata	n	South Japan Sea	25	8	0.0179	5.08	0.5	1.86	2.63	9.81	3.59	5.08	Ikeda Hirakawa 1998
Themisto japonica	Physocephalata	n	Western Subarctic Pacific	25	47	0.0135	8.3	5	2.83	4	11.74	4	5.66	yamada and Ikeda 2003

Themisto libellula	Physocephalata	n	Barents sea	30~	11	0.0169	6.67	-0.1	2.41	3.4	12.89	4.65	6.57	Ikeda and Skjoldal 1989
Themisto pacifica	Physocephalata	n	Western Subarctic Pacific	25	22	0.0051	10.35	5	2.77	3.92	14.64	3.92	5.54	yamada and Ikeda 2003
Vibilia sp. 1	Physocephalata	n	ETNP	0*	13	0.016	Table 2	Table 2	Table 2	Table 2	7.62	2.71	4	this study
Vibilia sp. 2	Physocephalata	n	ETNP	0*	30	0.0064	Table 2	Table 2	Table 2	Table 2	8.47	2.4	3.53	this study
Vibilia stebbingi	Physocephalata	n	Weddell Sea Antarctica	0	17	0.0485	15.05	0.5	7.06	9.99	29.07	13.64	19.3	Donnelly 2004
Vibilia stebbingi	Physocephalata	n	Scotia/ Weddell Sea Antarctica	0	2	0.117	6.61	0.5	3.87	5.47	12.77	7.47	10.56	Torres et al 1994

Table 3: Hyperiid amphipod data collected from the literature and this study normalized to a common temperature (10°C) and body mass (0.25g). 11 of the 33 species are original to this study (see table 2). Clade is based on molecular phyolgenetic analysis from Hurt et al. 2013. MDO is minimum depth of occurrence, which is provided in the reference study, or other sources as noted. * distribution from Shulenberger 1977, + distribution from Vinogradov et al. 1996, ~ distribution from Coyle and Pinchuk 2005. When specific distribution values were not available for that species, the MDO is based on known distribution of other species in the same genus based on the cited reference. Q_{10} is the temperature coefficient that quantifies the factorial change in metabolic rate with 10°C change in temperature. Q_{10} from table 2 was used, or literature values when available, otherwise a Q_{10} of 2 was assumed. A scaling coefficient of -.25 was assumed, except for the scaling of the genus *Phronima*, for those rates the regression equations from were used: $MO_2=3.92M^{(-0.263)}$ in the North Atlantic, $MO2=1.907M^{(-0.25)}$ in the Gulf of California and $MO2=2.45M^{(-0.208)}$ in the Eastern Tropical North Pacific (Elder and Seibel in Prep).

Table 4:

Species	Collection location	MDO (m)	n	Mean wet Mass (g)	Mean MO_2 umol g-1 hr-1	Temp °C	MO2 corrected to 10°C	a for scaling	scaled to .25g	ref
Abyssochromeni plebs	Ross Sea Antarctica	0	36	0.1680	Table 2	Table 2	3.03	1.94	2.75	This study
Cyphocaris challengeri	western subarctic pacific	30~	38	0.0288	3.79	5	5.36	2.21	3.12	Yamada and ieka 2003
Cyphocaris faueri	Scotia/Weddell Sea antarctica	40	8	1.2430	2.46	0.5	4.75	5.02	7.10	Torres et al 1994
Cyphocaris richardi	Scotia/Weddell Sea antarctica	100	5	0.5050	4.15	0.5	8.02	6.76	9.56	Torres et al 1994
Cyphocaris sp.	western subarctic pacific	nd	1	0.0583	2.83	3	4.60	2.26	3.19	Ikeda 2012
Epimeriid	Ross Sea Antarctica	0	22	0.0410	Table 2	Table 2	7.12	3.20	4.53	This study
Euandania gigantea	Scotia/Weddell Sea antarctica	1000	1	5.8130	0.67	0.5	1.29	2.01	2.84	Torres et al 1994
Eusirus antarticus	Scotia/Weddell Sea antarctica	0	26	0.0470	6.12	0.5	11.82	5.50	7.79	Torres et al 1994
Eusirus microps	Scotia/Weddell Sea antarctica	0	1	0.6990	3.71	0.5	7.17	6.55	9.27	Torres et al 1994
Eusirus propeperdentatus	Scotia/Weddell Sea antarctica	0	2	0.0730	3.67	0.5	7.09	3.69	5.21	Torres et al 1994
Paracallisoma coecus	Southern California	500	1	0.2030	1.97	5.5	2.69	1.81	2.55	Childress, 1975
Parandania boeki	Scotia/Weddell Sea antarctica	500	6	0.4650	1.83	0.5	3.54	2.92	4.13	Torres et al 1994

Table 4. Gammarid amphipod data collected from the literature and orignial to this study. 2 of the 12 species are original to this study (see Table 2). Abbreviations are as in Table 3. ~ distribution from Coyle and Pinchuk 2005 .

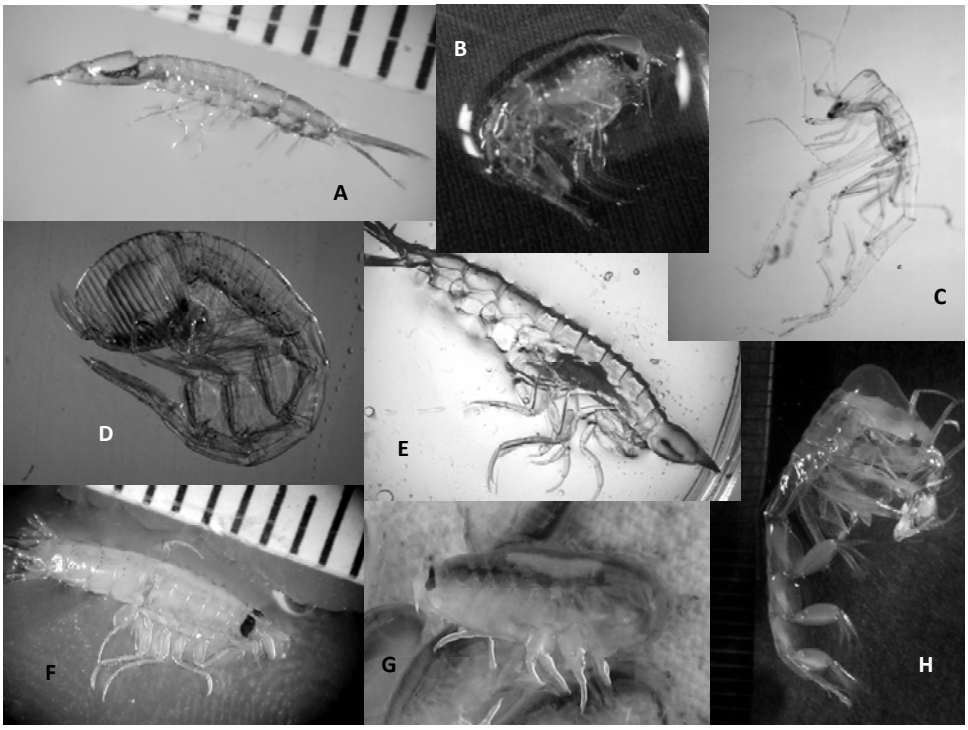

Figure 1. Amphipods from this study. A: *Streetsie* sp., B: *Themisto abyssorum*, C: *Phronimella elongata*, D. *Paraphronima*, E. *Cranocaplalu scleroticuss*, F. *Vibilia* sp.1, G. *Absysochromeni plebs*, H. *Phronima sedentaria* .Table 2 has further details on collection location etc. All photos by L. Elder.

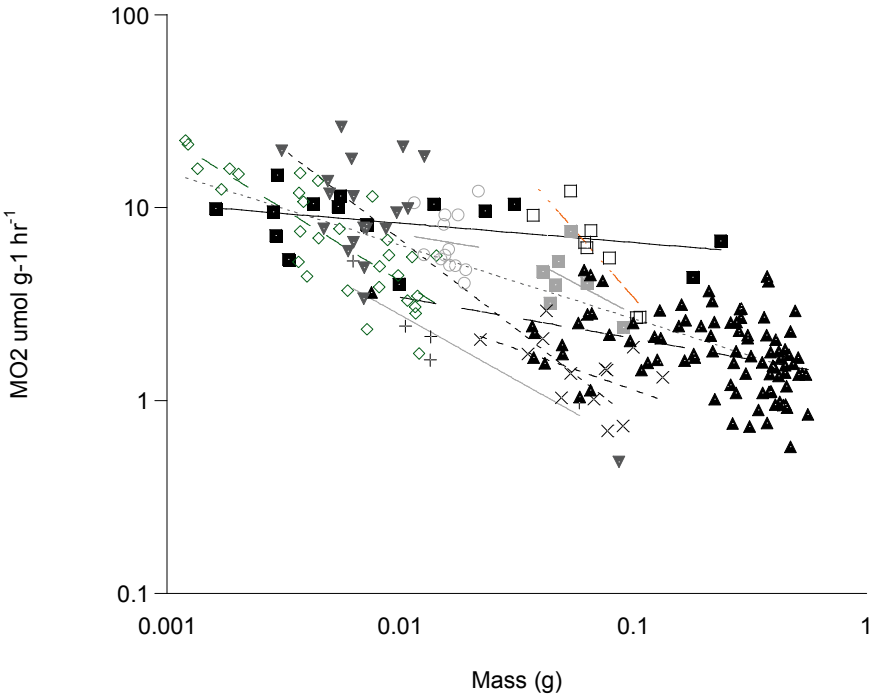

Figure 2. MO_2 for hyperiid amphipods from this study by weight on a log scale. All rates were adjusted to 10C using calculated Q_{10}s or assuming a Q_{10} of 2 where necessary (see table 2). Regression equations for individual species are as follows: *Hyperiella* black upside down triangles, $MO_2=0.0936M^{-0.93}$, $r^2=0.12$, *Themisto*, open square, $MO_2=0.144M^{-1.38}$, $r^2=0.54$, *Vibilia sp. 2* gray open circle, $MO_2=2.911M^{-0.199}$, $r^2=0.001$, *Phronima sedentaria*, black triangle, $MO_2=1.28M^{-0.21}$, $r^2=0.14$, *Phronimella* plus sign, $MO2=0.122M^{-0.67}$, $r^2=0.83$, *Paraphronima*, x, $MO_2=0.429M^{-0.42}$, $r^2=0.27$, *Vibilia sp. 1*, open diamond, $MO_2=0.13M^{-0.75}$, $r^2=0.76$, *Streetsia sp*., gray square, $MO_2=0.639M^{-0.64}$,$r^2=0.1$, *Cranocaplalus scleroticus*, black square, $MO_2=5.24M^{-0.1}$,$r^2=0.15$. The regression equation for all hyperiid amphipods from this study was significant. Iit is: $MO2=1.1074M^{-0.38}$, $r^2=0.54$.

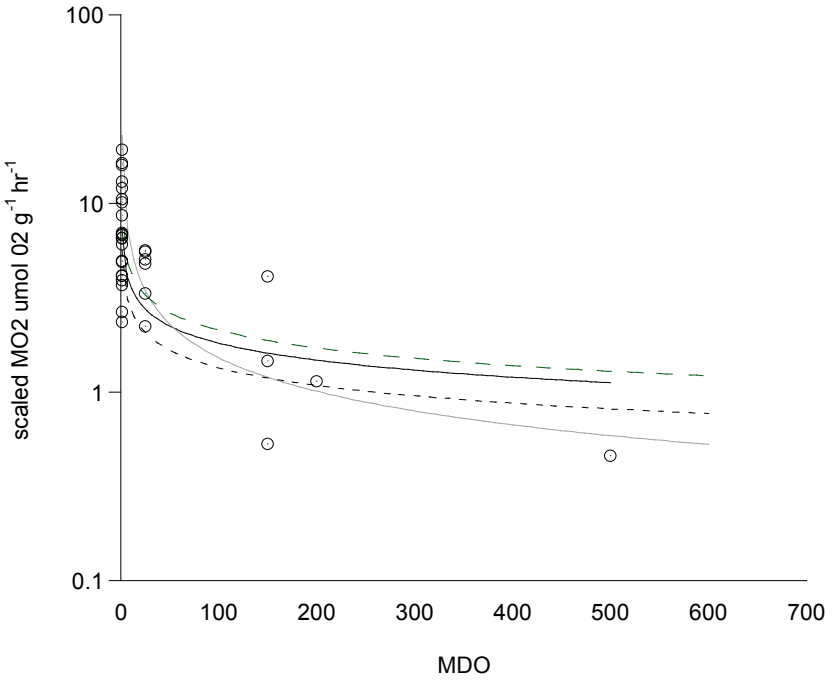

Figure 3: MO_2 for pelagic hyperiid amphipods compared to minimum depth of occurrence (MDO) plotted on a semi log scale. MO_2 was scaled to a common weight of 0.25g and a common temperature of 10°C. Each point is the mean for one species of amphipod (see table 3 for raw data). There is a significant decline in MO_2 with increasing depth of MDO ($F(1,31)=7.84;p<0.0087$). The gray line is for pelagic crustaceans from Childress (1975), the regression equation for that line is $MO_2=23.02MDO^{-0.59.}$ The black regression line is for hyperiid amphipods in this study, regression equation: $MO_2=7.22MDO^{-0.29}$, $r^2$0.28. Top and bottom dotted lines are the 95% confidence limits(cl) for the hyperiid amphipod regression. The regression equation for the lower cl is $MO_2=5.649MDO^{-0.31}$. The regression equation for the upper cl is $MO_2=8.901MDO^{-0.31}$.

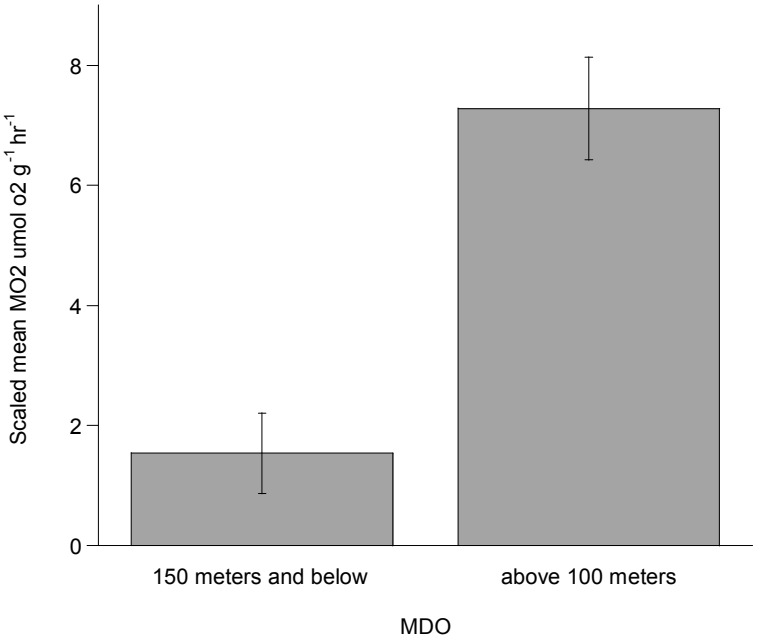

Figure 4: Pelagic hyperiid amphipod mean scaled metabolic rate (MO_2) in µmol 02 g^{-1} hr^{-1} by minimum depth of occurrence (MDO) for species living at 150m and below versus species living above 100m. Specimens with a MDO of 150m or deeper had a significantly lower MO_2 than species with a shallow MDO: ANOVA:$F(1,31)=7.46, p<.0087$. The mean rate for shallow specimens was 7.27±0.85 µmol 02 g^{-1} hr^{-1}, n=28. The mean MO_2 for deep specimens was 1.54±0.67 µmol 02 g^{-1} hr^{-1}, n=5.

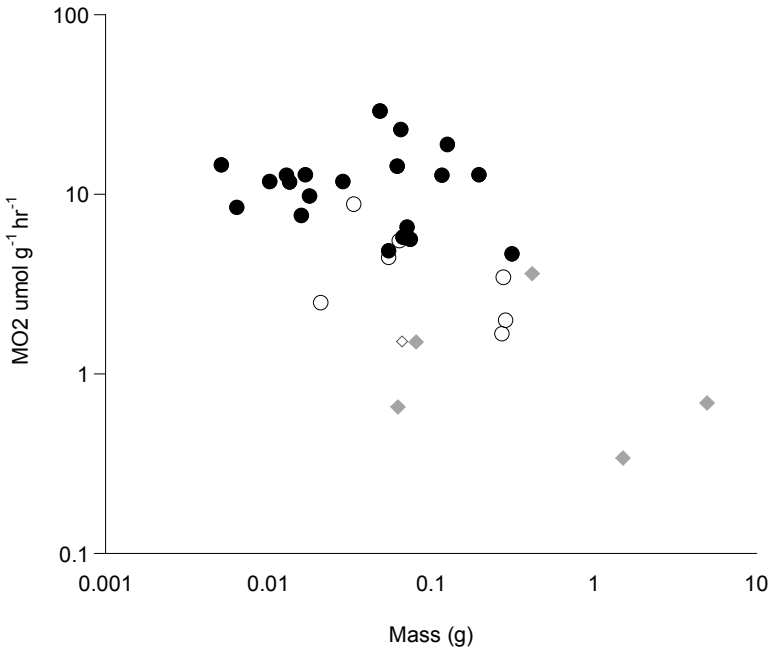

Figure 5: Mean MO_2 values of hyperiid amphipods in µmol O2 g^{-1} hr^{-1} comparing Physocephalata that are non transparent (closed black circles) or transparent (open black circles) and Physosomata that are non transparent (closed gray diamonds) or transparent (open gray diamond, one species). All MO_2s are corrected to 10°C, and reported on a log scale. Specimens are from both this study and literature (Table 3). Transparent amphipods had a significantly lower MO_2 than non-transparent species in the clade Physocephalata (ANOVA: $F(1,26)=13.16$; $p<0.0012$). Non-transparent Physocephalata had a sample size of 20, and the mean MO_2 was 12.00±1.38 µmol O_2 g^{-1} hr^{-1}. The transparent amphipods had a sample size of 8 and a mean MO_2 of 3.74±0.88 µmol O_2 g^{-1} hr^{-1}. Physosomata had a significantly lower metabolic rate (mean 1.38±0.48 µmol O_2 g^{-1} hr^{-1}, n=6) than all Physocephalata (mean 7.29±0.83, n=27) (ANOVA: $f(1,31)=10.14$, $p<0.0033$).

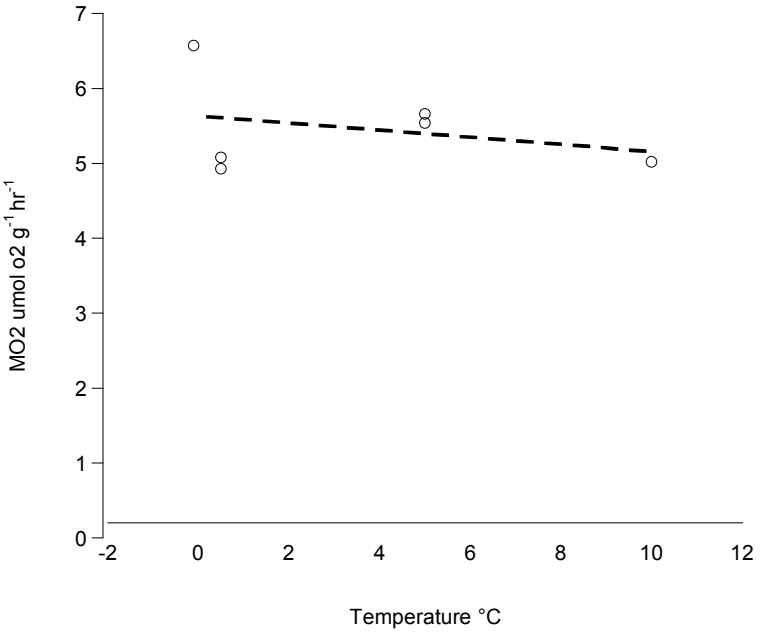

Figure 6. Metabolic rate of different species of the genus *Themisto* normalized to 10°C and scaled to 0.25g, on a linear scale. Species are listed in Table 3. The linear regression is not significant Mo2=5.63-0.048x, R^2=.09. MO_2 is not significantly different between specimens from cold water regions (less than 1C) and specimens from regions with temperature of 5C or higher (t-test: t(4)=-1.84;P<0.1617). Therefore, there is no metabolic cold adaptation in the genus *Themisto*. Table 3 has environmental temperatures and location for each species.

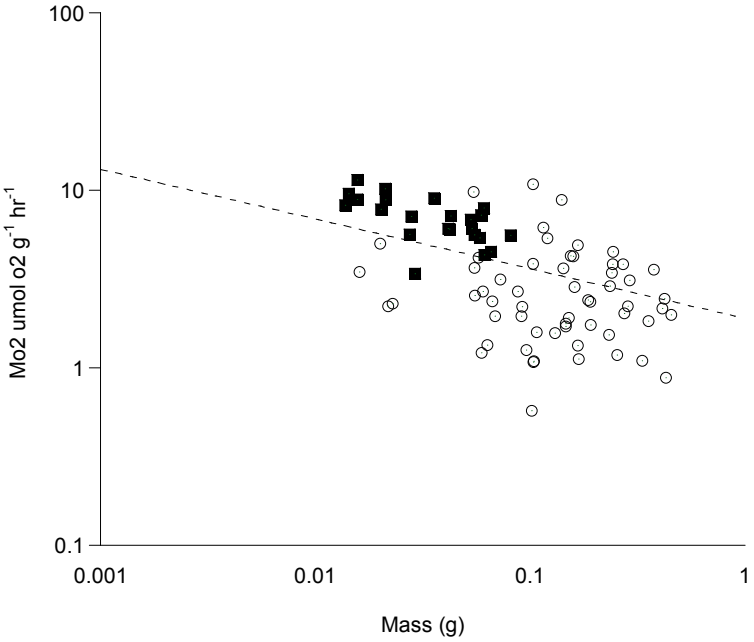

Figure 7. Comparison of metabolic rates for Antarctic gammarid amphipods with published regression equation from Seibel and Drazen (2007, $MO_2=1.9031m^{-0.27}$). All rates are adjusted to 10°C, as is the regression equation, which is derived from a thorough review of benthic amphipods. Antarctic gammarids fall across this regression when adjusted to 10°C. Filled squares are *Epimeriid sp*, open circles are *Abyssochromeni plebs*.