

CHARACTERIZING FUNCTIONAL BIODIVERSITY ACROSS THE
PHYLUM CTENOPHORA USING PHYSIOLOGICAL
MEASUREMENTS

by

Telissa M. Wilson

A Thesis

Submitted in partial fulfillment
of the requirements for the degree
Master of Environmental Studies
The Evergreen State College

December 2018

© 2018 by Telissa M. Wilson. All rights reserved.

This Thesis for the Master of Environmental Studies Degree

By

Telissa M. Wilson

Has been approved for

The Evergreen State College

by

Erik V. Thuesen, Ph.D.

Member of the Faculty, Zoology

Date

ABSTRACT

Characterizing functional biodiversity across the phylum Ctenophora using physiological measurements.

Telissa M. Wilson

Ctenophores are marine predators that are well known for their bioluminescence and the diffraction of light off their many cilia. Ctenophores are the largest organisms to utilize cilia to power locomotion as opposed to muscle. Specimens of Ctenophora belonging to a range of habitat depths and six distinct orders (Beroida, Cydippida, Thalassacalycida, Platyctenida, Cestida, and Lobata) were collected for this study. Examinations of their enzyme activities in relation to specimen wet mass, feeding morphology, minimum depth of occurrence, number of comb plates and specific ctene row surface area were conducted. Specific topics addressed within this research include 1) relationships between the muscle enzyme creatine kinase and ctenophore locomotion, 2) whether members of different groups within Ctenophora display unique metabolic scaling patterns, relationships between metabolic scaling patterns and feeding strategies, and 3) whether ctenophores support the ‘visual interactions hypothesis’. Results indicate a significant relationship between creatine kinase activity and comb plate density. The number of comb plates does not appear to change in correlation with overall body mass, whereas the specific surface area of ctene rows does show increase, and specific density of comb plates shows significant decrease. When considered as a whole, members of Ctenophora show no significant correlation of body mass to the metabolic enzymes studied. Yet when treated individually, different groups such as Beroida and Lobata displayed species-specific scaling. Over all members studied, the order Lobata showed comparatively lower CK and CS activity. Species-specific scaling is also visible in the creatine kinase scaling patterns of Beroida vs Lobata. Ctenophores were found to support the visual interactions hypothesis. This research shows support for the diversity of biochemical adaptations found throughout the phylum Ctenophora, and subsequent studies may benefit by treating each group within the phyla individually.

Table of Contents

LIST OF FIGURES.....	vi
LIST OF TABLES.....	viii
ACKNOWLEDGEMENTS.....	ix
CHAPTER ONE: BACKGROUND.....	1
Introduction.....	1
Ctenophore Taxonomy.....	3
Ctenophore Morphology and Ecology.....	4
Functional Biodiversity.....	6
<i>Measuring Functional Biodiversity.....</i>	<i>6</i>
<i>Metabolism as an Index of Functional Diversity.....</i>	<i>8</i>
<i>Enzymatic Measurements as Proxies for Metabolism.....</i>	<i>9</i>
Comparative Ecophysiological Studies in other Marine Taxa.....	10
Visual Interactions Hypothesis.....	11
CHAPTER TWO: MANUSCRIPT.....	13
Introduction.....	15
Materials and Methods.....	17
<i>Specimen Collection.....</i>	<i>17</i>
<i>Enzymatic Activity Measurements.....</i>	<i>18</i>
<i>Morphometric Measurements.....</i>	<i>20</i>
<i>Statistical Analysis.....</i>	<i>21</i>

Results.....	21
<i>Enzymatic Activities</i>	22
<i>Metabolic Scaling</i>	22
<i>Morphometric Characteristics</i>	23
<i>Enzymatic Activities in Relation to Depth</i>	24
Discussion.....	24
<i>Enzymatic Activity</i>	24
<i>Scaling</i>	25
<i>Morphometrics</i>	26
<i>Enzyme Activities in Relation to Depth</i>	28
Conclusion.....	28
CHAPTER THREE: CLOSING REMARKS.....	30
Conclusion.....	30
Figures.....	32
Tables.....	41
References.....	45

List of Figures

MANUSCRIPT:	
FIGURES.....	32
Figure 1. a) Creatine kinase activity as a function of body mass in 27 species of Ctenophora. b) Citrate synthase activity as a function of body mass (g).....	32
Figure 2. a) Creatine kinase activity as a function of body mass for beroid, cydippid, and lobate ctenophores. b) Citrate synthase activity as a function of body mass for beroid, cydippid, and lobate ctenophores.....	34
Figure 3. The relationship between creatine kinase and citrate synthase metabolic potential provide a method to examine the relative importance of locomotion (CK) vs overall aerobic metabolism (CS) for individual species.....	36
Figure 4. a) Citrate synthase activity as a function of body mass viewed by feeding morphology b) Creatine kinase activity as a function of body mass viewed by feeding morphology.....	37

Figure 5. Ctenophore comb plate densities, total numbers of combs and
ctene row surface areas as a function of total body wet mass.....39

Figure 6. Citrate synthase and creatine kinase activities of California
ctenophores compared to those of pelagic fishes from the same
region.....40

List of Tables

MANUSCRIPT: TABLES.....	41
Table 1. Enzymatic activities of ctenophores collected off California and Washington.....	41
Table 2. Scaling relationships of enzymatic activities of ctenophores from California & Washington.....	43
Table 3. Morphometric characteristics of oceanic ctenophores from California and Washington.....	44

Acknowledgments

I would like to extend heartfelt thanks to my advisor Dr. Erik V. Thuesen. This work would not have been possible without the constant support, unfailing guidance, gentle prodding, and inspiration that he consistently provided. I am grateful to have him as a mentor.

Thank you also to Sappitah Ahmath, Tiffany Bachtel, and Jojo Froehlich who helped invaluablely by performing numerous enzyme assays with me in the lab. Thank you to Steve Haddock and the Western Flyer crew at Monterey Bay Aquarium Research Institute for allowing us to participate in research cruises to collect specimens. Special thanks to Kaile Adney and the staff at the Science Support Center for their help in acquiring necessary research supplies. I also could not have made it this far without the support of my colleagues from the WSDA Plant Pathology Lab. I am grateful for their support and flexibility.

Thank you to the Evergreen State College Foundation and National Science Foundation awards DEB-1542673 and DEB-1542679 for funding this research.

Lastly, I would like to thank my husband Gabriel Harder. I am forever grateful for his support and help. It was no small feat to hold up our

household as a single parent while I was away on long research cruises and working through weekends. Thank you also to my parents Julie Martin, Caleb Martin, Brad Wilson, and Marty Wilson, who supported me throughout the entire process and allowed me to work long hours and late nights, while providing care for my young daughter and cooking us meals. All of you helped to raise her while I was away, and the comfort of knowing she was in loving hands helped me to accept the time I couldn't spend with her.

CHAPTER ONE: BACKGROUND

Introduction

The planet's five oceans hold more ecological space than all the terrestrial biomes put together. The phylum Ctenophora makes up a small group of macrozooplankton with a large ecological role throughout the world's oceans, both in surface waters, midwater, and bathypelagic realms. All orders are pelagic, with the exception of Platyctenida which is composed of benthic ctenophores that creep across substrate or attach to something on the sea floor. Ctenophores are gelatinous predators that range in size from the tiny *Minictena luteola*, with a 1.5 mm diameter, to the massive *Cestum veneris*, measuring up to 3 meters long. These predators are important players throughout the oceanic ecosystem, and sometimes large increases called 'blooms' can cause extensive damage to typical ecosystem functioning, including economically important fisheries (Mills, 2001). As our planet's climate changes, and these changes are felt in the ocean through increased acidity, temperature, salinity and hypoxia, it is expected that such changes can have profound effects on marine invertebrates and their food webs (Mills, 1995; Mills, 2001; Thuesen et al., 2005). Research has suggested that biodiversity is critical to ecosystem health, and that human

driven climate change and other anthropogenic effects are generating negative impacts on ocean biodiversity (Worm & Lotze, 2016; Hooper et al., 2005). There is a real need to characterize the biodiversity in the world's oceans before these negative impacts result in irrecoverable damage. The objective of this research is to address gaps in our understanding of ctenophore ecophysiology and functional biodiversity.

Until recently, most ctenophore research has focused on surface dwelling specimens due to the difficulties involved with observing and capturing species from the depths and also the fragility of the deeper specimens (Mills, 1995; Haddock, 2004; Robison, 2004). Improvements in SCUBA collection methods (Haddock & Heine, 2005) and remotely operated vehicles (ROVs) have increased our ability to study these organisms. Despite these advances, most current publications are focused on the same few species of ctenophores, mostly because they are readily available, physically robust, and because of the attention they have garnered from impacts to economically important fisheries. Additionally, many species have yet to be described, and almost nothing is known about their physiology and functional biodiversity. This study will utilize physiological measurements to elucidate characteristics of functional biodiversity found

throughout the phylum. This study is the first of its kind to characterize functional biodiversity across multiple orders of Ctenophora.

Ctenophore Taxonomy

The phylum Ctenophora is comprised of eight presently recognized orders; Platyctenida, Cestida, Beroida, Cydippida, Lobata, Cambojiida, Cryptolobiferida, and Thalassocalycida. Currently there are over 150 described species, and it is estimated that this only accounts for half of the extant species, with several still undescribed and many more undiscovered (Appeltans et al., 2012; Mills, 1998). The current ctenophore family tree is constructed mainly from physiological and morphological evidence of homology, with new versions based on gene regions and transcriptome data being published yearly. Many researchers have questioned the traditionally assigned orders in Ctenophora (Harbison, 1985; Mills, 1998; Ryan et al., 2013; Podar et al., 2001; Moroz et al., 2014; Borowiec et al., 2015), and there has been a large debate regarding the phylogenetic placement of the phylum Ctenophora as an early branching metazoan lineage (Nosenko et al., 2013; Dunn et al., 2008; Wallberg et al., 2004; Ryan et al., 2013; Moroz et al., 2014). A few studies have posited that Porifera is the sister lineage to metazoan, and not ctenophores as suggested by others (Simion et al., 2017; Borchiellini et al., 2001; Jekely et al., 2015). Novel methods are being

utilized to clarify the uncertainties in this deep phylogenetic problem, and more and more researchers are finding support for Ctenophora as the sister lineage to all of Metazoa (Shen & Rokas, 2017; Whelen et al., 2017). Piecing together early metazoan evolution will become easier with sequencing of additional Ctenophora and Porifera genomes.

Ctenophore Morphology and Ecology

Ctenophores come in a large array of diverse body structures, from large sac-like beroids to flat belt shaped cestids. All ctenophores are characterized by a body plan involving rotational symmetry and numerous specialized morphological and physiological features unique to the phylum (Dunn et al., 2015). Ctenophores are unique in Metazoa in that they are the only organisms known to have colloblasts (adhesive cells used in prey capture) and they are the largest organism to utilize cilia for locomotion. Most ctenophores have eight rows of ciliated comb plates which extend from the aboral end of the organism up the sides towards the oral end. Some species, such as *Cestum veneris* have severely reduced comb rows (Harbison et al., 1978). A few genera also utilize cilia on appendages called auricles, which are used in feeding behavior (Haddock, 2007). Ctenophores are known to possess the enzyme creatine kinase, yet it is unknown whether or not this enzyme plays a role in ciliary locomotion in these organisms.

Ctenophores lack advanced muscle tissues, but are known to have very large smooth muscle cells, and one genus, *Euplokamis*, has striated muscle tissue (Tamm & Tamm, 1989; Mackie & Mills, 1992). Another aspect of this research is to investigate the potential role of the muscle enzyme creatine kinase in ctenophore locomotion.

There are three main feeding strategies that have been observed in ctenophores, although several variations on the three themes exist (Haddock, 2007). The three most common feeding strategies use either tentacles, lobes, or an engulfing mechanism. Most members assigned to Cydippida feed entirely via the use of two tentacles. Also, the cydippid larval stage present in all ctenophores (except the Beroids) typically use tentacles for feeding. Members that feed using tentacles are typically sit-and-wait predators. They hang out in the water column with their tentacles fully extended and wait for prey. When contact with prey is made, the numerous sticky colloblasts adhere to the prey and the tentacles rapidly retract, pulling the prey close to the organism's body, and then the animal rotates to bring its mouth to the prey. Ctenophores eat copepods, other zooplankton, and even other ctenophores. While tentacles are the main feeding mechanism for some ctenophores, others utilize different strategies but still have reduced tentacles present (Haddock, 2007).

The lobate feeders are harder to generalize. Lobates are characterized by small to large oral lobes, presence of auricles, and reduced tentacles near the mouth. Lobate feeders swim towards prey, passively or actively (depending on the species), and when contact is made, the lobe(s) bring the prey towards the mouth (Harbison et al., 1978; Haddock, 2007). In addition to feeding, some lobate ctenophores also use their lobes to propel themselves through the water (Haddock, 2007).

Species that feed solely by engulfing belong to the order Beroida. Their body is shaped like a large sack, with an oral opening on one end. Engulfers swim through the water with their mouths open, and are thought to use chemical signals to attract and/or sense prey (Haddock, 2007). As mentioned previously, members of Beroida do not have a cydippid larval stage, and so are restricted to this one form of feeding throughout life.

Measuring Functional Biodiversity

Measuring functional biodiversity in the oceans is an important step to predicting how environmental perturbations may affect marine ecosystems. Biodiversity is critical to ecosystem functioning, and there is a large gap in our knowledge regarding the functional biodiversity within the phylum Ctenophora. Biodiversity is considered the “number and composition of the

genotypes, species, functional types, and landscape units in a given system” (Diaz & Cabido, 2001). Functional diversity is the total sum of functional traits that organisms possess that play a role in how they interact with and/or affect their environment and ecosystem. Functional traits can be measurements based on morphology, physiology, ecology, behavior, phenology, energetics, or almost any other relevant categorical or quantitative functional trait. There are no unanimously accepted functional traits that are approved for use in a functional diversity study, and the definition of functional diversity across multiple disciplines and publications is often ambiguously defined.

Analysis of functional diversity has become increasingly prevalent in studies aimed at predicting ecosystem impacts from declines in biodiversity. Functional traits are now considered to be superior measures to use over previous methods which assessed biodiversity using species richness and species abundance (Schleuter et al., 2010; Diaz & Cabido, 2001; Petchey et al., 2006). There are numerous studies that lay down various methods and indices for analyzing functional diversity, yet, as with selecting traits there is still no agreed-upon one proper way to go about it (Petchey et al., 2006; Rosado et al., 2013). Several researchers do agree that using multiple traits is

a better approach than just using a single functional trait (Lefcheck et al., 2015; Vileger et al., 2008).

Metabolism as an index of Functional diversity

Since the introduction of the term ‘functional diversity’, there has been an explosion of different indices used by researchers to better understand species’ ecological roles. In the marine literature, physiological traits such as the rate of metabolism have been used extensively to understand species’ ecological niches, phenotypic adaptations, and biodiversity (Thuesen et al., 1998a & 1998b; Seibel & Childress, 2000; Thuesen & Childress, 1994). In the case of many marine studies on zooplankton, the chosen trait (metabolism) is studied comparatively with information regarding habitat depth, body size, taxonomic groupings, and any available life history knowledge (Barnett et al., 2007; Pomerleau et al., 2015; Thuesen & Childress, 1994; Thuesen et al., 1998a; Seibel et al., 1997). Measurements of metabolism used in this context can provide a window into the functional diversity of difficult to observe marine taxa. Metabolism is traditionally measured using oxygen consumption data, and preferably on specimens in a resting state. As the organism respire it uses up the available oxygen, resulting in consumption rates per unit time. Oxygen consumption rates provide useful information regarding metabolism in robust ctenophore

species. Unfortunately, many ctenophores cannot withstand the experimental procedures and disintegrate completely, sometimes even before successful transfer to a respiration chamber.

Enzymatic measurements as proxies for metabolism

The difficulties working with fragile specimens and the restrictions involved with assaying living specimens prompted researchers to evaluate alternative methods for characterizing metabolism. Preliminary research by King and Packard (1975) showed significant correlation between electron transport chain activity and respiration in several members of zooplankton (King & Packard, 1975). Subsequent research on marine fishes investigated the use of individual aerobic and anaerobic metabolic enzyme activities, and also found correlation with respiration rates (Childress & Somero, 1990; Torres & Somero, 1988). The same key metabolic enzymes were assayed and found to be good indicators of metabolism in pelagic chaetognaths, nemertean, and annelids (Thuesen & Childress, 1993a; Thuesen & Childress, 1993b). Of the enzymes studied, citrate synthase (CS), pyruvate kinase (PK), and lactate dehydrogenase (LDH) were all found to be good indicators of metabolic potential. CS plays a key role in the Krebs's cycle and is critical in aerobic metabolism. The latter enzymes, PK and LDH, are considered anaerobic metabolic proxies, as they are enzymes that mostly

contribute to glycolysis, although PK has minor roles in aerobic metabolism as well. In addition to providing valuable ecophysiological information about hard to study, fragile organisms, these metabolic proxies have successfully been used in models to predict ecological relationships and bloom dynamics (Dahlhoff, 2004; Purcell, 2009). Researchers have also shown that when combined with accurate population estimates, metabolic proxies can be used to estimate oceanic carbon flux inputs (Childress & Thuesen, 1992).

Comparative Ecophysiology studies in other marine invertebrates

Physiological characteristics such as oxygen consumption rates and key metabolic enzyme activities have been successfully used to elucidate ecophysiological traits and aspects of functional biodiversity in other marine organisms. For instance, studies of medusae and chaetognaths have shown that species that live or migrate within the Oxygen Minimum Zone (OMZ) tend to be anaerobically poised and shallower species displayed higher aerobic metabolic potentials (Thuesen & Childress, 1994; Thuesen & Childress, 1993b). Comparison of oxygen consumption rates and metabolic enzyme activities in some organisms has shown support for the use of certain enzymes as indicators of metabolic potential (Thuesen & Childress, 1993b). Enzymatic activities of different species of copepods were able to

reveal distinct groups that were associated with aspects of their morphology and ecology (Thuesen et al., 1998b). All of the ecophysiological comparative studies mentioned above show that Chaetognatha, Medusae, and Copepoda are composed of physiologically distinct groups of species, which suggests that they occupy different functional niches in their environment.

Visual Interactions Hypothesis

Several studies have shown that organisms with image-forming eyes have metabolic rates that decrease with increasing depth, in amounts that are higher than expected once factors such as pressure and temperature are accounted for (Childress & Mickel, 1985; Seibel et al., 1997; Seibel & Drazen, 2007; Torres et al., 1994; Torres & Somero, 1988). Interestingly, organisms such as medusa, copepods, and chaetognaths, which do not contain image-forming eyes have shown little correlation between metabolic activity and increased depth (Childress, 1995; Thuesen & Childress, 1994; Thuesen & Childress, 1993; Thuesen et al., 1998b). The ‘visual interaction hypothesis’ posits that visually orientating animals involved in predator-prey interactions have higher metabolic rates in surface waters than the metabolic rates of animals living in the deep, dark ocean (Childress & Somero, 1979; Childress & Mickel, 1985). Reduction of light with depth reduces the

evolutionary pressure for burst swimming involved in such predator-prey interactions and results in lower metabolic rates. There is currently no data published on ctenophores as a group, with respect to the visual interactions hypothesis. The physiological measurements taken in this study provide a means to test the validity of the visual interactions hypothesis for the first time in this abundant group of non-visual marine predators.

CHAPTER TWO: MANUSCRIPT

Formatted and prepared for: PLOS ONE

This manuscript has been prepared for submission to a peer-reviewed journal. It is presented here as a preliminary draft to fulfill graduation requirements for The Evergreen State College Master of Environmental Studies program.

Ctenophore Ecophysiology

Ecophysiological characteristics of temperate ctenophore species utilizing physical, morphometric, and enzymatic measurements

Wilson, T.M. ^{1*}, Thuesen, E.V. ¹, Haddock, S.D.H. ²

¹ The Evergreen State College, Olympia, Washington 98505;

² Monterey Bay Aquarium Research Institute, Moss Landing, California 95039.

*To whom correspondence should be addressed. Email:

wiltel16@evergreen.edu

-list of key words: Ctenophore, ctene plate, ctene row, morphometric, metabolic scaling, cilia, creatine kinase.

Introduction

Ctenophores are gelatinous macrozooplankton found throughout the world's oceans. While most are planktonic, about 20 percent are actually benthic (Mills, 1998). Originally thought to be a sister clade of Cnidarians, recent research has placed them much closer to Porifera, as the most basal multicellular organisms (Dunn et al., 2008; Ryan et al., 2013; Moroz et al., 2014). Ctenophores inhabit all depth ranges of the ocean from upper epipelagic zones to 6700 meters. Ctenophore locomotion is powered by eight rows of ciliated combs, making them the largest organisms to utilize cilia for movement. Because of their fragility and habitat, ctenophores are very difficult to collect and study, and as a result, there is a large lack of information regarding their physiology, ecology, and evolution (Haddock, 2004). Ctenophores typically feed by one of three mechanisms: tentacles, feeding lobes, or engulfing (Haddock, 2007). Because of their important role as predators of many commercial and noncommercial fish and shellfish larvae, they merit further research into many aspects of their biology and ecology (Shiganova, 1998). Furthermore, through biochemical analysis and reliable population predictions, their role in oceanic carbon cycling can be estimated (Childress & Thuesen, 1992). The taxonomy and evolutionary history of Ctenophora is still largely disorganized, with many species

remaining undescribed, and many more species currently clumped into families to which they may not belong (Appeltans et al., 2012; Haddock, 2004; Mills, 1998). The aim of the current project is to utilize physiological measurements to help profile the functional diversity within Ctenophora.

Previous studies have shown support for the use of metabolic enzymes in estimating overall metabolism. Muscle enzymes such as lactate dehydrogenase, creatine kinase, malate dehydrogenase, citrate synthase, as well as electron transport system components have been used to estimate metabolism in fish and zooplankton (King & Packard, 1975; Childress & Somero, 1979; Somero & Childress, 1990; Thuesen & Childress, 1993a; Thuesen & Childress, 1993b). All of the organisms for which previous researchers have used enzymatic activities as metabolic proxies utilize muscle driven locomotion. Ctenophores are unique in that they rely upon a non-muscle associated locomotion system (Tamm & Tamm, 1989), and we are uncertain if such proxies will work for ctenophores.

Although lacking a true mesodermal layer, ctenophores do possess the largest known smooth muscle cells, and some forms even have striated muscle cells (Mackie et al., 1988; Martindale, 2005). Recent genome sequencing results have shown that the ctenophore *Mnemiopsis leidyi* possesses the gene for the ATP/ADP regulating enzyme creatine kinase

(Blast:<http://research.nhgri.nih.gov/mnemiopsis/ontologies/kegg/hsa00330.shtml>). Typically, in other eukaryotic organisms, isoforms of creatine kinase are expressed in tissues that require large amounts of ATP, such as muscle tissue, cardiac tissue, and mitochondria (Wallimann et al., 1992). In addition to buffering intracellular ATP levels, CK also plays a role in regulating pH and buffering ADP concentrations (Wallimann et al., 1992). In the current study, due to the unique pairing of muscle and ciliary systems present in some members of Ctenophora (Tamm & Tamm, 1989), we hypothesized that if CK provides the necessary energy for powering cilia, then CK activity will scale allometrically with morphological measurements of ctenes and ctene rows.

Material and Methods

Specimen collection

Ctenophore specimens were collected during research cruises of the R.V. *Western Flyer* off central California utilizing SCUBA and ROV collection methods during May 17-23, 2014, September 16-22, 2014, December 13-18, 2016, and July 24-31, 2018. Specimens were collected by ROV *Doc Ricketts* using detritus samplers, or suction samplers for the hardier species (Haddock et al., 2017). When possible, all specimens used in this study were

photographed live on board the ship. Specimens free of parasites and with cleared gut contents were frozen in liquid N₂ at sea, shipped on dry ice to Olympia, Washington, and later transferred to -80 °C freezer for storage. All specimens were analyzed within 6 months of capture. *Pleurobrachia bachei* (A. Agassiz, 1860) specimens were collected utilizing 1-L “jelly-catchers” in southern Puget Sound at Oakland Bay Marina, in Shelton, Washington in July 2014, and in Budd Inlet, Olympia, Washington in July 2016.

Enzymatic activity measurements

Rates of citrate synthase (CS) and creatine kinase (CK) of 26 different species of ctenophores sampled from a range of depths were assayed. CS was chosen as a predictor of overall specimen metabolism, based on its ability to correlate well with overall metabolic rate, resistance to freezing, and applicability to fragile specimens (Thuesen & Childress, 1994). CK activity was chosen due to its potential role in ciliary locomotion. Both enzymes were assayed in duplicate for each specimen.

Frozen specimens were weighed on a Metler Toledo analytical balance, and homogenized in 10 mmol TRIS/HCL buffer using handheld glass homogenizers (15 ml or 40 ml) on ice, or when specimen size required, a Waring commercial blender. Homogenate was centrifuged ten minutes at

4°C, 6600 g. All activity was measured within one hour of homogenization using a Hewlett- Packard diode array spectrophotometer with a temperature controlled cuvette held at 20°C. All biochemical reagents for enzymatic assays were obtained from Sigma Aldrich, with the exception of NADH (AcrosOrganics).

The enzymatic activity of creatine kinase was assayed utilizing a coupled enzymatic reaction involving the secondary enzymes, hexokinase and glucose-6-phosphodehydrogenase (Dawson, 1970; Szasz et al., 1976). In this assay, CK dephosphorylates phosphocreatine, resulting in the production of ATP. The ATP is further reacted by hexokinase to phosphorylate glucose, which then is reacted with G6-PDH to produce NADPH. The production of NADPH is followed spectrophotometrically at 340 nm, and is directly proportional to CK activity. The final cuvette reaction volume of 1 ml contained 100 mM Imidazole Buffer (pH 7.1), 10 mM MgCl₂, 20 mM glucose, 1.8 mM ADP, 3.0 mM phosphocreatine, 1.3 mM NADP, 1600 Units L⁻¹ G-6-PDH, and 2800 Units L⁻¹ hexokinase. The activity was measured for up to six minutes, excluding a lag phase of approximately 100 seconds. Efficacy of the assay conditions were confirmed using tissue from store bought cod filets (results not shown) (Somero & Childress, 1990).

Due to differences of pH presented in CK assay protocols (Dawson & Eppenberger, 1970; Szasz et al., 1976; Somero & Childress, 1990), a range of pH conditions were tested to determine the optimum pH for CK activity measurements in ctenophore tissue homogenates. Specimens of *P. bachei* were assayed in triplicate with solutions at pH of 6.5, 6.7, 6.9, 7.1, 7.3, and 7.5 (results not shown). A pH of 7.1 was chosen as the optimum pH for CK activity measurement in ctenophores.

The enzymatic activity of citrate synthase was measured as an increase of absorbance at 412 nm via its reaction with the substrate 5, 5-Dithiobis (2-nitrobenzoic acid) (DTNB). The final cuvette reaction volume of 1 mL contained 50 mM imidazole/HCL buffer (pH 7.8 at 20°C), 0.5 mM oxaloacetate, 0.1 mM acetyl-CoA, 0.1 mM DTNB, and 1.5 mM MgCl₂ (Childress & Somero, 1979). CS activity was measured for up to six minutes, with addition of oxaloacetate after background was detected, and any background rate was subtracted from the latter rate. All activities are given in units (μmols of substrate converted to product per minute) g⁻¹.

Morphometric measurements

Morphometric measurements from 13 different species were analyzed.

Morphometric analyses were carried out on a subset of the total specimens

(N=24). All specimens were photographed live with reference scale bars onboard ship, with the intent to capture width, height, length, and area of ctene rows. A combination of live footage stills (shot prior to capture by cameras on the ROV *Doc Ricketts*) and photos of live specimens onboard ship were used to calculate total ctene plate numbers. At least four of the eight ctene rows were included in the ctene plate counts per specimen. Due to the size and shape of each specimen, it was impossible to obtain counts from all eight rows from any one photo, and means of visible rows were used. All CS and CK measurements were analyzed for relationships between enzymatic activities and morphometric characteristics.

Statistical analysis

Power regressions were carried out on log-transformed measurements to improve linearity, utilizing KaleidaGraph, version 4.5.2 statistical software. A confidence level of 95% was applied when interpreting correlations.

Results

A total of 125 specimens of 26 species were used in this study. Of the total, 117 specimens were from California and eight were from the Puget Sound in Washington.

Enzymatic activities

Enzymatic activities were successfully measured on 125 different specimens from 26 different species, belonging to six different orders, Lobata, Cydippida, Beroida, Platyctenida, Thalassocalycida, and Cestida (Table 1). Thirteen of the species are new to science and were given operational names. *Haeckelia beehleri* displayed the highest CS activity, whereas *Kiyohimea usagi* displayed the lowest, 0.1920 units g⁻¹ and 0.0030 units g⁻¹, respectively (Table 1). *Beroe gracilis* displayed the highest CK activity, whereas *Lampocteis* sp. B displayed the lowest, 0.6408 units g⁻¹ and 0.0115 units g⁻¹ respectively (Table 1).

Metabolic scaling

Over the 26 species sampled, CS and CK activities did not significantly correlate to body mass when species were viewed individually ($p > 0.05$, Fig. 1 and Fig. 2). CS and CK activities for specimens belonging to the order Lobata were comparatively lower than activities displayed by orders Beroida and Cydippida (Fig. 2 and Fig. 3). CK activities showed a decrease with body mass for the orders Beroida, Cydippida, and Lobata ($y = 0.128x^{-0.289}$; $R = 0.74$, $p < 0.0012$; $y = 0.0623x^{-0.109}$; $R = 0.17$, $p > 0.05$ and $y = 0.0394x^{-0.0855}$; $R = 0.34$, $p > 0.05$ respectively, Fig. 2a, Table 2). CK

activities of engulfer, tentacle, and lobe feeders showed a significant decrease with body mass (g) ($y=0.128x^{-0.289}$; $R=0.749$, $p<0.0012$, $y=0.0867x^{-0.207}$; $R=0.478$, $p<0.059$, and $y=0.0413x^{-0.111}$; $R=0.449$, $p<0.0087$ respectively, see Fig. 4.b and Table 2). CS activity did not correlate significantly with body mass when viewed by feeding strategy ($p>0.05$, Fig. 4.a). CS activity of engulfers displayed an inverse scaling relationship comparative to that of tentacle and lobe feeders (Fig. 2.b).

Morphometric characteristics

An undescribed *Nephaloctena* (sp. A) displayed the highest comb plate density, 1528.3 (comb plates g^{-1}), whereas *Beroe abyssicola* had the lowest, 27.9 (comb plates g^{-1}) (Table 3). Comb plate density showed a significant decrease with body mass ($y = 190.52x^{-0.87}$; $R = 0.96$, $p < 0.0001$; Fig. 5), while comb row area (mm^2) showed a significant increase with body mass ($y = 4.64x^{0.52}$; $R = 0.63$, $p < 0.01$; Fig. 5). Surface area of comb plates (mm^2) and CK activity were not correlated ($p<0.05$, figures not shown). CK and CS activities did not correlate with comb plate density or number of combs ($p<0.05$, figures not shown). The total number of combs did not significantly correlate with body mass (Fig. 5).

Enzymatic activities in relation to depth

The minimum depth of occurrence (MDO) was considered to be the depth below which 95% of the population of each species lives (Childress, 1995). This was calculated for each species using the MBARI Video Annotation and Reference System database (VARs, Schlising & Jacobsen Stout, 2006) for ROV observations over twenty-eight years and by personal communication for undescribed species (Table 1). CS and CK activities showed no significant decline with minimum depth of occurrence in ctenophores. CS activity of pelagic fishes from the same region significantly declined with depth (Childress & Thuesen, 1995) ($y=10.355x-0.56$; $R = 0.77$, $p < 0.001$ Fig. 6).

Discussion

Enzymatic activity

Enzyme activities yielded some interesting characteristics when specimens were grouped by the three orders with the most abundant number of specimens, Beroida, Lobata, and Cydippida. Lobata displayed the lowest CK and CS activities, and may represent a group of ctenophores that are less active feeders. This has been supported by observations that some members of Lobata are indeed very slow foragers (Matsumoto & Harbison, 1993).

Ctenophores that feed using the engulfing strategy displayed positive scaling with CS activity, whereas all other feeding types for CS activities displayed negative scaling trends. Lobe and tentacle feeding ctenophores are typically considered filter feeders and sit and wait predators, respectively, while the Beroids that comprise the ‘engulfers’ feeding type actively swim towards prey (Matsumoto & Harbison, 1993). The positive scaling trend observed for CS activity of engulfers may suggest that Beroids rely mostly upon anaerobic metabolism during feeding interactions. However, current studies suggest that traditionally held monophyletic orders such as Lobata and Cydippida may in fact be polyphyletic. One study, which sampled 26 species, from 5 orders, compared 18s ribosomal RNA sequences and found that traditional assigned orders based on morphological data need to be reassigned (Podar et al., 2001) and that several species are more closely related to members assigned to different orders. It may be worth revisiting the enzymatic measurements as a function of body mass when grouped by order once ctenophore phylogenetics are resolved.

Scaling

Overall, ctenophores displayed higher CK activities than CS activities. Creatine kinase in muscle tissue provides adenosine triphosphate (ATP) for muscle contraction. Similarly, our results suggest that CK is responsible for

providing ATP to drive cilia, similar to its role in muscle tissue. The high number of giant mitochondria located in the polster cells associated with the comb rows (Horridge, 1964) suggests that ctenophores are very aerobic despite their low CS activity. In contrast to studies of CS activity in other gelatinous zooplankton, members of this study did not display the common allometric scaling relationships of decreasing activity with increasing body mass. Overall, ctenophores displayed variability among the same species and between species for the enzymatic activities studied. Although the ctenophores studied here possessed variable scaling relationships, when viewed collectively they did tend to have an overall negative allometric scaling relationship of CK activity. Our results suggest that creatine kinase plays an important role in ctenophore metabolism.

Morphometrics

This study is the first of its kind investigating the possible role of CK between ctenophore locomotion and ctenophore cilia. Morphometric results were obtained for a total of 13 different species, representative of five different orders. Our results indicate high metabolic diversity among species within the same given order, including members of Beroida despite high morphological similarities within the order. Our results do not suggest a relationship between ctenophore characteristics and CK activity. Arginine kinase

is an ancient phosphagen kinase, present in many invertebrates, which may be worth investigating for its potential role in supplying ATP for ciliary locomotion. Previous research has suggested that arginine kinase may supply the needed energy in the form of ATP to drive ciliary motion in the single celled organism *Paramecium caudatum* (Noguchi et al., 2001). Adenylate kinase is another potential candidate worth investigating for supplying ATP in ctenophore cilia. In addition to investigating other phosphagen kinases, the ctenophore *Velamen parallelum* warrants further study. This species had the lowest number of ctenes and among the highest CK activity. Due to the ribbon-like nature of this species, photographing and counting of ctenes was difficult and unfortunately, this species was not included in our morphometric analysis. *Velamen parallelum* is known to escape predation using a rapid undulating muscle driven motion (Stretch, 1982), which may account for its high CK activity despite low ctene counts. Future morphometric studies of CK activity in this organism could be interesting.

Both Beroida and Cydippida have relatively developed muscle tissue for ctenophores, but this tissue is known to be associated with the mouth and the tentacular sheath, respectively (Tamm & Tamm, 1989; Mackie et al., 1988). This observation may support our finding that ctene morphometrics did not

positively scale with CK activity in these groups. The significant scaling found between CK activity and mass when viewed by feeding strategy also support this observation.

Enzymatic activities in relation to depth

Ctenophores, which lack image-forming eyes, support the visual interactions hypothesis. Despite enzymatic activities that range over two orders of magnitude for both pelagic fishes and ctenophores, there were no significant effects of habitat depth on enzymatic activities of ctenophores. Although the visual interactions hypothesis has been challenged (Childress et al., 2008), overwhelming evidence supports this evolutionary hypothesis that animals involved in visual predator-prey relationships in surface waters have much higher metabolic rates than animals living in the perpetually dark, deep sea.

Conclusion

Members of Ctenophora covered in this study clearly display unique patterns of biochemical diversity. The significant functional groups that emerged in this study suggest that ctenophores have evolved different physiological strategies to adapt to their environment. Because of such physiological diversity, future studies should take care not to lump different species together when analyzing metabolic characteristics, but should take morphological, functional, and taxonomical differences into consideration

during analyses. While the role between CK and cilia is still unclear in ctenophores, our study suggests CK plays a vital role in ctenophore metabolism. It would be interesting to see if CK activity has potential for use as a metabolic indicator for the phylum. Lastly, a more complete knowledge of ctenophore physiology is crucial to establishing a baseline for their role in oceanic ecosystems, predicting and understanding bloom dynamics, and perceiving how they may be impacted by both anthropogenic-driven climate change, and natural environmental fluxes (Childress & Thuesen, 1992; Mills, 2001).

CHAPTER THREE: CLOSING REMARKS

Conclusion

Enzymatic activities of ctenophores sampled for this study suggest that the group is highly diverse physiologically, as well as morphologically. When all specimens sampled were evaluated for enzymatic scaling with body mass, there were no significant trends visible. While there were no significant scaling patterns between either CK or CS when viewed by individual specimen wet mass, several significant patterns emerged when comparing enzymatic measurements within different functional groups. Of the functional groups investigated, the three most common feeding strategies were particularly good indicators of functional diversity when viewed by CK activity. The feeding strategies used in this study are not all inclusive, and there are many variations that exist within the phylum. Future studies would benefit by characterizing and including the other feeding strategies which exist within the phylum. The feeding strategies described by engulfing, tentacles, and lobate did not cover all of the species used within this current study.

Morphometric investigation of the role of CK in powering ciliary movement in ctenophores was inconclusive. This study focused on a subset of the total

specimens collected, and future studies may benefit by including more specimens per species sampled. A single small and a large representative was chosen for each species used in our morphometric study. In future studies, it may be worthwhile to investigate specimens that constitute a larger size range, and also explore activities of different phosphagen kinases. Additionally, further studies using ctene preps and the biochemicals required for CK activity could help to elucidate the relationship between ciliary motion and CK.

Depth related decline in CS and CK activities were not detected for the species included in this study. The species sampled represent six of the eight orders currently assigned in the phylum, and suggest that ctenophores show support for the visual interactions hypothesis. Additionally, it was observed that the deepest specimen collected and analyzed, an undescribed *Tjalfiella* sp., had among the highest CK activity measured. It is worth noting that CK and CS activities have not been thoroughly tested for their potential to be suitable metabolic proxies in ctenophores. This is partly due to the difficulties in obtaining respiration measurements on fragile and rare species. However, a comparison of oxygen consumption rates and CK activity in the more robust species of ctenophores may provide useful insights.

Figures

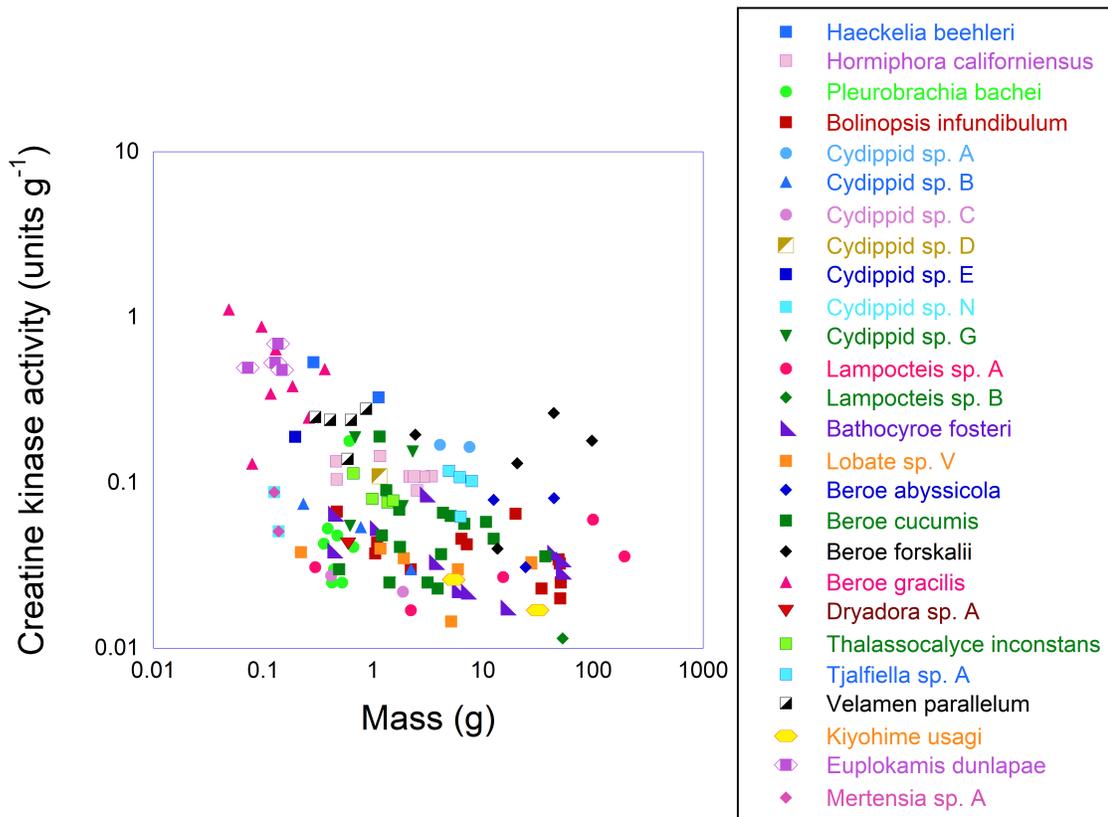


Figure 1. a) Creatine kinase activity as a function of body mass in 27 species of Ctenophora. Ctenophores do not show significant scaling in creatine kinase activity (units g⁻¹) over all species tested.

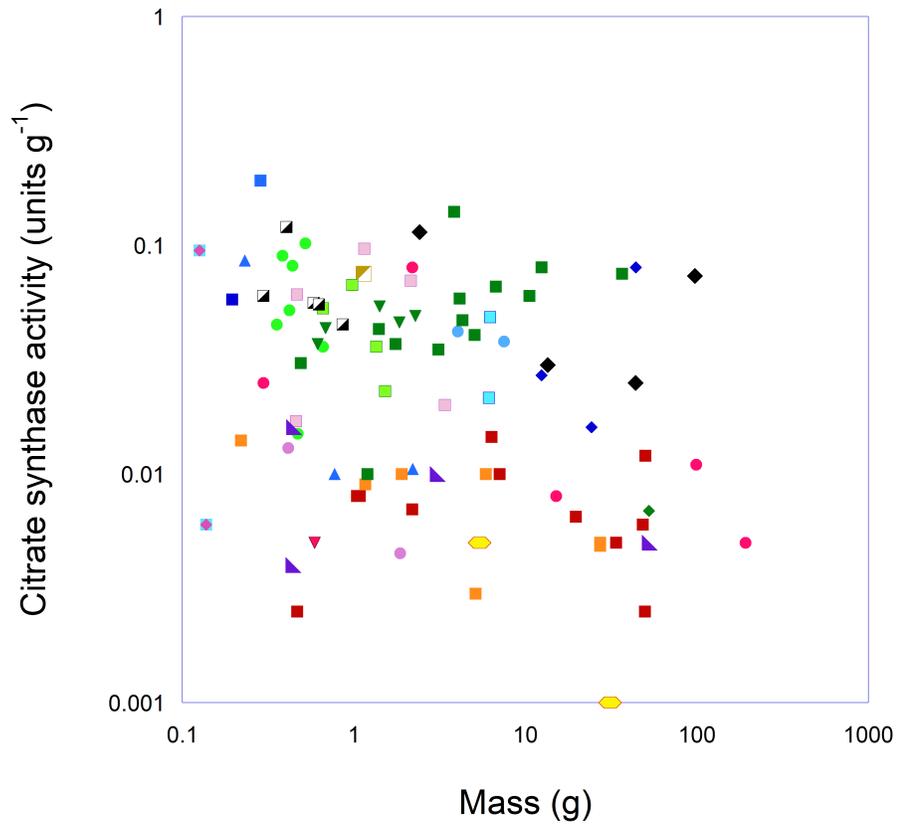


Figure 1. b) Citrate synthase activity as a function of body mass (g).

Ctenophores do not show significant scaling in either of these enzymes over all species tested. Refer to Figure 1a for symbol legend.

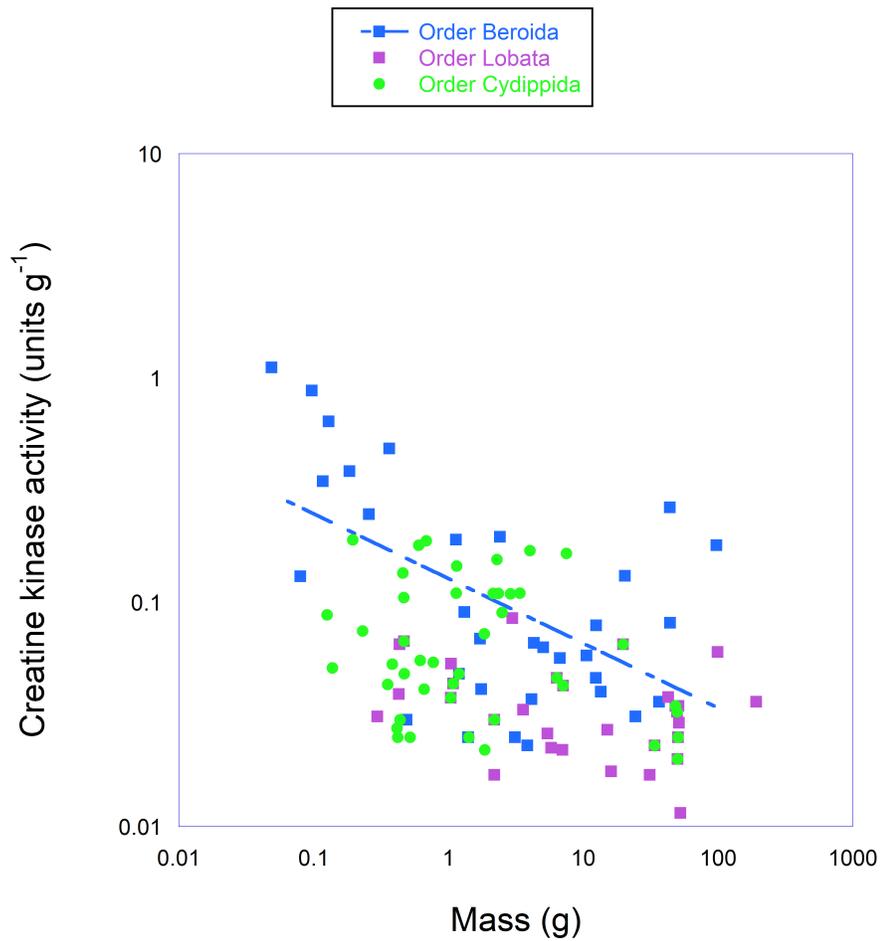


Figure 2. a) Creatine kinase activity as a function of body mass for beroid, cydippid, and lobate ctenophores. When groups are viewed separately, significant correlations were found between CK activity and body mass for members of Beroida ($y=0.128x^{(-0.289)}$; $R=0.74$, $p<0.0012$). Cydippid and lobate species did not display significant results ($y=0.0623x^{(-0.109)}$; $R=0.17$, $p>0.05$ and $y=0.0394x^{(-0.0855)}$; $R=0.34$, $p>0.05$ respectively).

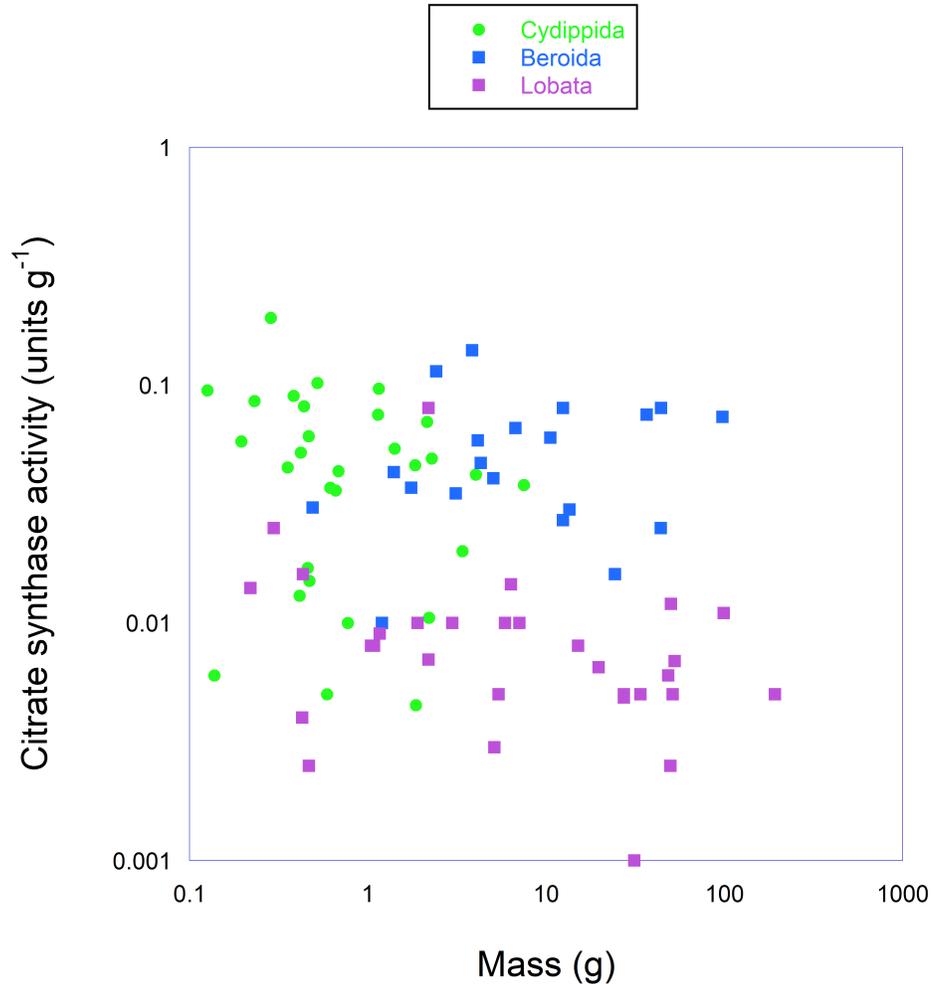


Figure 2. b) Citrate synthase activity as a function of body mass for beroid, cydippid, and lobate ctenophores when viewed separately did not yield significant correlations for CS activity and body wet mass ($y=0.0384x^{(0.0856)}$; $R=0.11$, $p>0.05$; $y=0.0339x^{(-0.109)}$; $R=0.24$, $p>0.05$; and $y=0.00953x^{(-0.126)}$; $R=0.24$, $p>0.05$ respectively). When viewed as a whole, different orders displayed unique scaling patterns, with the order Beroida exhibiting an inverse scaling relationship.

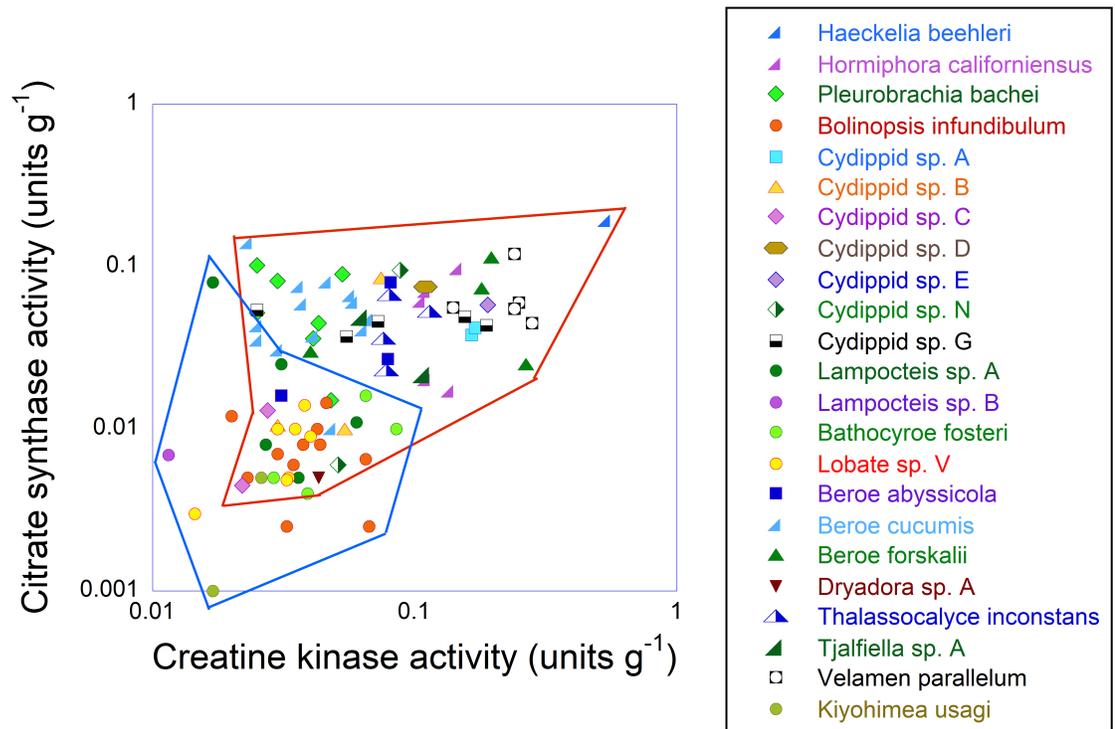


Figure 3. Creatine kinase vs. citrate synthase. Members of Lobata are represented with circle symbols and circled in blue, all other species are circled in red.

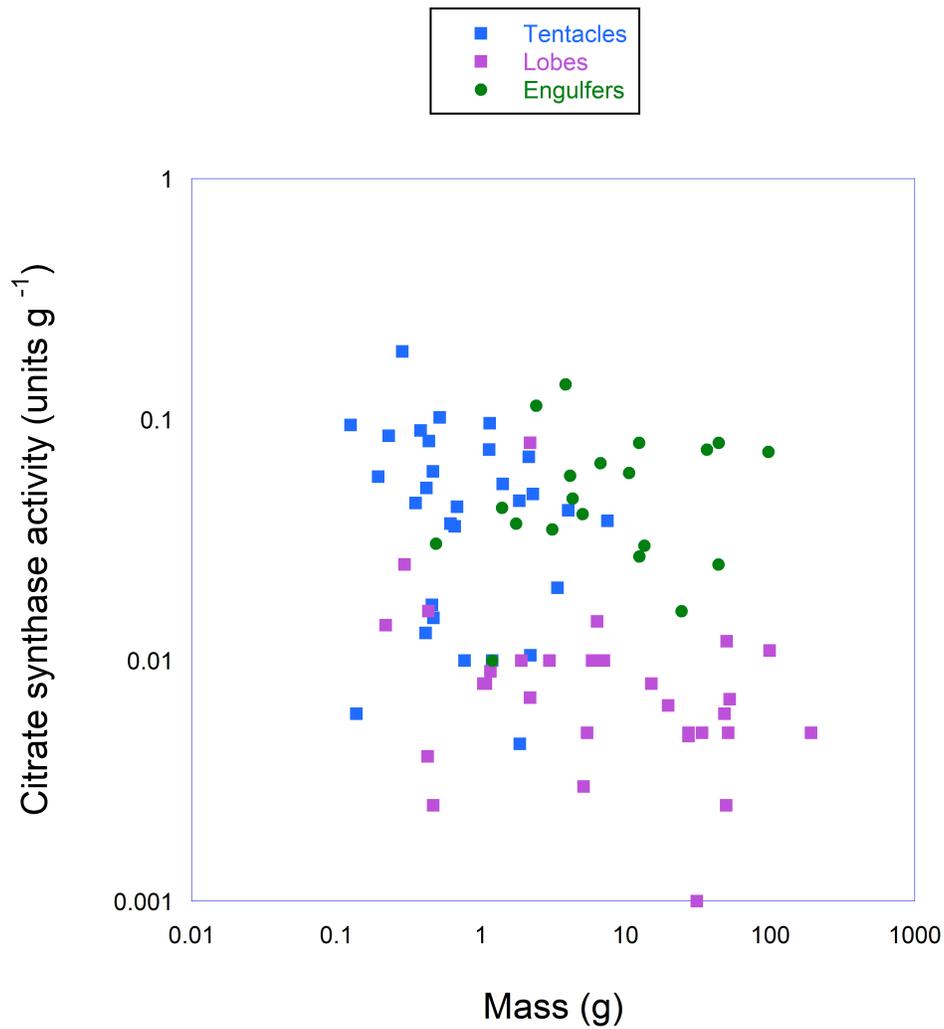


Figure 4. a) Citrate synthase activity as a function of body mass viewed by feeding morphology. Different feeding groups did not display significant correlation with body mass ($P > 0.05$), but appear to display unique clustering by functional group.

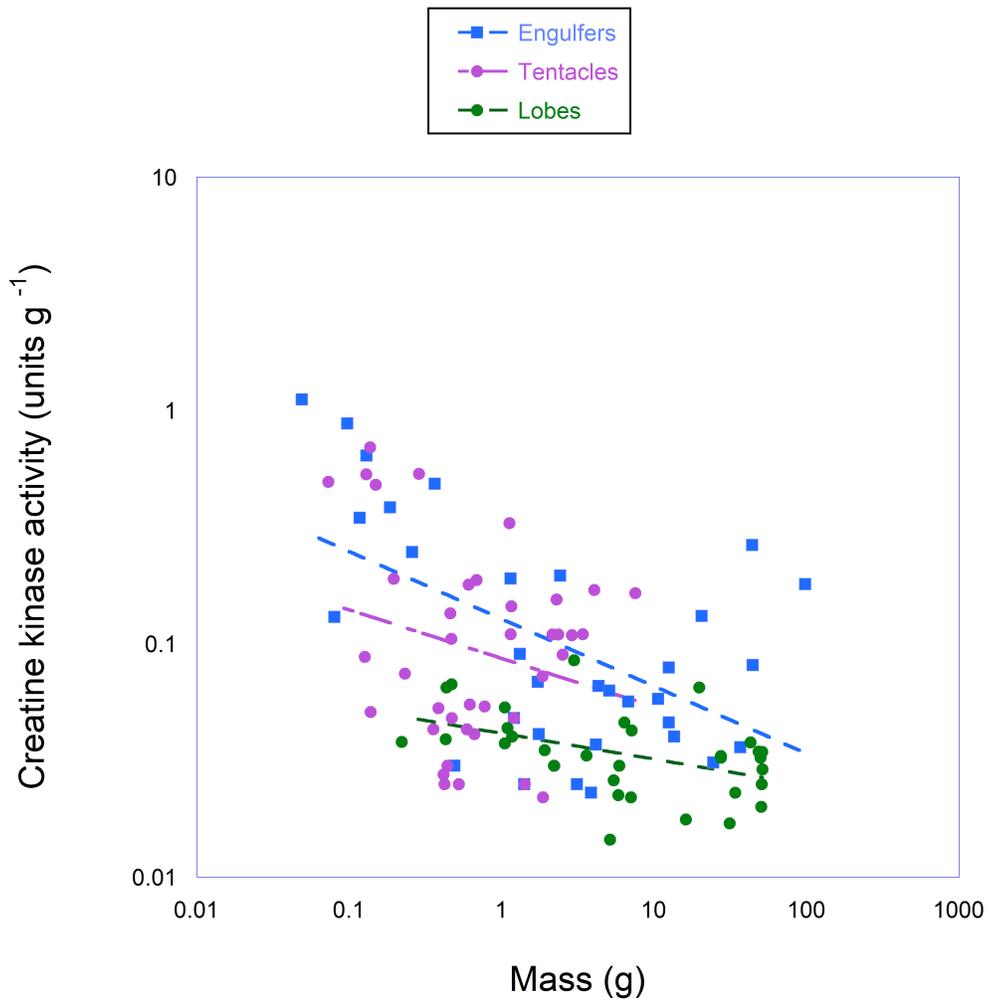


Figure 4. b) Creatine kinase activity as a function of body mass viewed by feeding morphology. CK activities of engulfer, tentacle, and lobate feeding groups significantly scaled with body mass ($y=0.128x^{-0.289}$; $R=0.749$, $p<0.0012$, $y=0.0867x^{-0.207}$; $R=0.478$, $p<0.059$, and $y=0.0413x^{-0.111}$; $R=0.449$, $p<0.0087$ respectively).

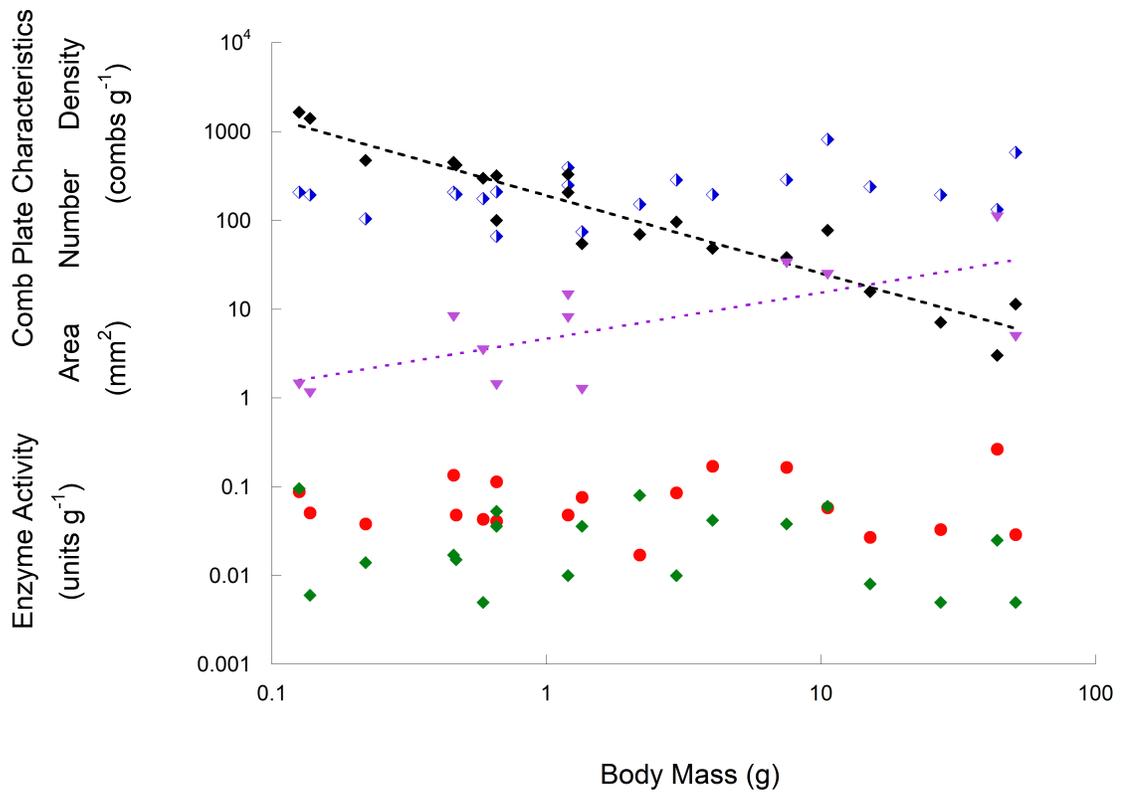


Figure 5. Ctenophore comb plate densities (\blacklozenge), total numbers of combs (\blacklozenge) and ctene row surface areas (\blacktriangledown) as a function of total body wet mass for 13 species, two specimens each (when possible). Comb plate densities are correlated significantly with body mass ($y = 190.52x^{(-0.87)}$; $R = 0.96$, $p < 0.0001$). Comb row surface areas are also correlated significantly with body mass ($y = 4.64x^{(0.52)}$; $R = 0.63$, $p < 0.01$). The metabolic enzymes creatine kinase (CK) (\bullet) and citrate synthase (CS) (\blacklozenge) show no significant difference with body size

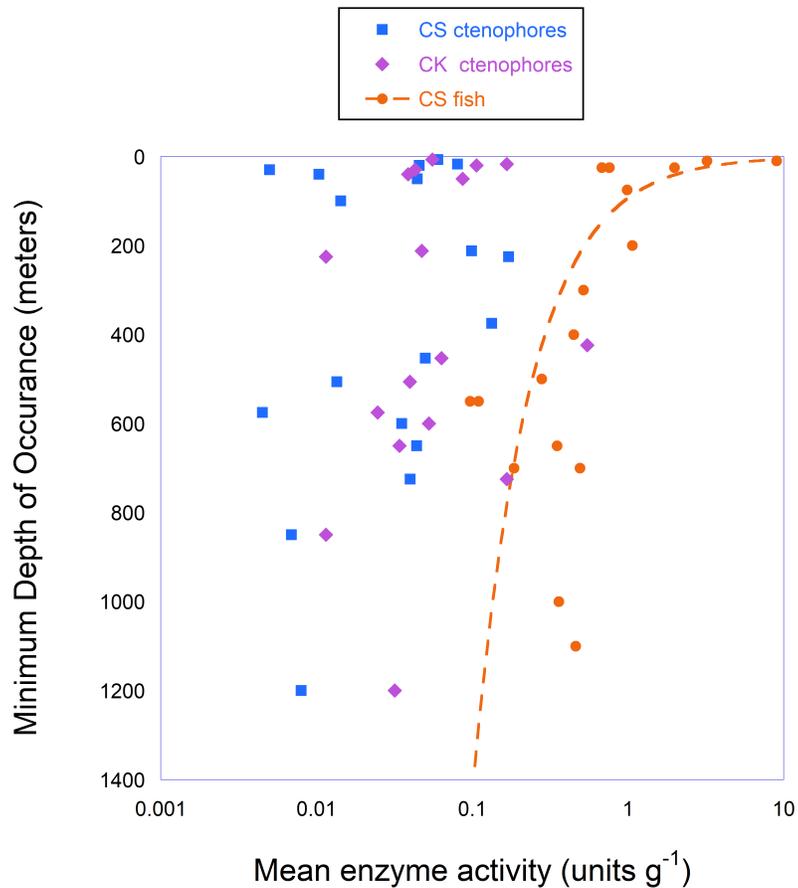


Figure 6. Citrate synthase and creatine kinase activities of Washington and California ctenophores as a function of minimum depth of occurrence compared to pelagic fishes from the same region. The slopes of the regression lines for ctenophores are not significantly different from zero. The slope of the regression line for CS activities of pelagic fishes is $y=10.355x-0.56$; $R=0.77$, $p < 0.001$ (Childress & Somero, 1979; Childress & Thuesen, 1995).

Tables

Table 1. Enzymatic activities of ctenophores collected off California and Washington

Order Family <i>Genus and species</i>	Wet weight range (g)	Enzymatic activity (mean \pm SE, number of specimens)		
		CK (units g ⁻¹)	CS (units g ⁻¹)	MDO (meters)
Cydippida				
Haeckeliidae				
<i>Haeckelia beehleri</i>	0.2860-1.1206	0.4320 \pm 0.103, 2	0.1920, 1	10
Pleurobrachiidae				
<i>Hormiphora californensis</i>	0.4600-3.3950	0.1067 \pm 0.009, 9	0.0458 \pm 0.014, 6	20
<i>Pleurobrachia bachei</i>	0.3548-0.6600	0.0555 \pm 0.018, 8	0.0602 \pm 0.011, 7	7.9
Euplokamidae				
<i>Euplokamis dunlapae</i>	0.1291-0.1487	0.5505 \pm 0.049, 4	<i>na</i>	423.6
Undescribed Cydippida				
Cydippida sp. A	4.0300-7.5000	0.1675 \pm 0.002, 2	0.0400 \pm 0.002, 2	<i>na</i>
Cydippida sp. B	0.2315-2.2040	0.0528 \pm 0.012, 3	0.0353 \pm 0.025, 3	450
Cydippida sp. C	0.4150-1.8610	0.0247 \pm 0.002, 2	0.0086 \pm 0.004, 2	1000
Cydippida sp. D	1.14	0.1100, 1	0.0750, 1	450
Cydippida sp. E	0.195	0.1900, 1	0.0580, 1	200
Cydippida sp. N	0.1260-0.1380	0.0695 \pm 0.018, 2	0.0505 \pm 0.044, 2	33
Cydippida sp. G	0.6160-2.2880	0.0991 \pm 0.031, 5	0.0459 \pm 0.002, 5	750
Dryodoridae				
Dryodora sp. A	0.59	0.0430, 1	0.0500, 1	30
Mertensiidae				

Mertensia sp. A	0.126-0.138	0.0695 ± 0.013, 2	0.0505 ± 0.0315, 2	na
Platyctenida				
Tjalfiellidae				
Tjalfiella sp. A	4.8840-7.8710	0.0977 ± 0.012, 4	0.0350 ± 0.013, 2	2940
Thalassocalycida				
Thalassocalycidae				
<i>Thalassocalyce inconstans</i>	0.6600-1.5200	0.087 ± 0.009, 4	0.0448 ± 0.009, 4	50
Lobata				
Bathocyroidae				
<i>Bathocyroe fosteri</i>	0.4280-51.2500	0.0398 ± 0.006, 11	0.0086 ± 0.002, 4	506.5
Bolinopsidae				
<i>Bolinopsis infundibulum</i>	0.4684-50.6212	0.0388 ± 0.004, 12	0.0075 ± 0.001, 11	40
Eurhamphaeidae				
<i>Kiyohimea usagi</i>	5.4200-31.2200	0.0215 ± 0.004, 2	0.0030 ± 0.002, 2	51.9
Lampoctenidae				
Lampocteis sp. A	0.2970-192.0400	0.0342 ± 0.007, 5	0.0258 ± 0.014, 5	650
Lampocteis sp. B	52.66	0.0115, 1	0.0069, 1	850
Undescribed Lobata				
Lobata sp. V	0.2200-27.3400	0.0319 ± 0.003, 7	0.0079 ± 0.001, 7	1200
Cestida				
Cestidae				
<i>Velamen parallelum</i>	0.2970-0.8600	0.2300 ± 0.023, 5	0.0672 ± 0.013, 5	10
Beroida				
Beroidae				
<i>Beroe abyssicola</i>	12.4430-44.1500	0.0637 ± 0.016, 3	0.0410 ± 0.019, 3	453
<i>Beroe cucumis</i>	0.4898-36.6600	0.0476 ± 0.005, 15	0.0555 ± 0.008, 13	212.1
<i>Beroe forskalii</i>	1.1390-97.5600	0.1672 ± 0.030, 6	0.0606 ± 0.020, 4	17.6
<i>Beroe gracilis</i>	0.0488-0.3636	0.5285 ± 0.117, 8	na	200.1

Table 2. Scaling relationships of enzymatic activities of ctenophores from California and Washington.

Significant relationships presented in bold.

Group	Enzyme	Enzymatic activity g^{-1} wet weight (y) as a function of total body wet weight (M), $y=aM^b$		
		A	b ($\pm 95\%$ C.I., <i>n</i>)	p
Beroida	CK	0.13	-0.28, 32	0.0012
	CS	0.038	0.085, 20	0.4383
Cydippida	CK	0.06	-0.11, 45	0.0891
	CS	0.033	-0.15, 31	0.3588
Lobata	CK	0.04	-0.09, 31	0.0621
	CS	0.0095	-0.14, 30	0.0686
Engulfers	CK	0.12	-0.29, 32	0.0012
	CS	0.038	-0.085, 20	0.4380
Lobes	CK	0.041	-0.11, 32	0.0088
	CS	0.0094	-0.13, 30	0.0685
Tentacles	CK	0.086	-0.21, 40	0.0599
	CS	0.035	-0.16, 30	0.3530

Table 3. Morphometric characteristics of oceanic ctenophores from California and Washington.

Species	Morphometric measurements (mean \pm SE, number of specimens)		
	Comb row area (mm ²)	Comb plate density (comb plates g ⁻¹)	Number of combs
<i>Cydippida</i> sp. A	32.98, 1	43.4 (\pm 5), 2	228 (\pm 46.5), 2
<i>Cydippida</i> sp. N	1.27 (\pm 0.15), 2	1528.3 (\pm 122.5), 2	201 (\pm 7), 2
<i>Cydippida</i> sp. G	2.85 (\pm 4.45), 2	173.5 (\pm 126.25), 2	176 (\pm 332), 2
<i>Dryodora</i> sp. A	3.44, 1	297.8, 1	176, 1
<i>Lobata</i> sp. V	NA	483.7, 1	149 (\pm 45), 2
<i>Haeckelia beehleri</i>	2.24, 1	772.73, 1	221, 1
<i>Hormiphora californensis</i>	11.23 (\pm 3.1), 2	331.5 (\pm 124.5), 2	228 (\pm 20), 2
<i>Pleurobrachia bachei</i>	NA	369.8 (\pm 51.1), 2	203 (\pm 6.5), 2
<i>Thalassocalyce inconstans</i>	1.32 (\pm 0.075), 2	77.4 (\pm 22.6), 2	70 (\pm 4), 2
<i>Bathocyroe fosteri</i>	4.85, 1	45.25 (\pm 39.4), 2	276 (\pm 24), 2
<i>Velamen parallelum</i>	NA	NA	Lowest Observed
<i>Beroe abyssicola</i>	2.45 (\pm 0.55), 2	27.9 (\pm 10.26), 2	575 (\pm 355), 2
<i>Beroe cucumis</i>	16.2 (\pm 8.3), 2	203.4 (\pm 126.3), 2	605 (\pm 211), 2
<i>Beroe forskalii</i>	59.32 (\pm 49.05), 2	177.02 (\pm 356.49), 2	489 (\pm 356.5), 2

References

- Appeltans W, Ahyong ST, Anderson G, Angel MV, Artois T, Bailly N, et al. The magnitude of global marine species diversity. *Current Biology*. 2012; 22(23):2189-202.
- Barnett AJ, Finlay K, Beisner BE. Functional diversity of crustacean zooplankton communities: towards a trait-based classification. *Freshwater Biology*. 2007; 52(5): 796-813.
- Borchiellini C, Manuel M, Alivon E, Boury-Esnault N, Vacelet J, Le Parco Y. Sponge paraphyly and the origin of Metazoa. *Journal of Evolutionary Biology*. 2001; 14(1):171-9.
- Borowiec ML, Lee EK, Chiu JC, Plachetzki DC. Extracting phylogenetic signal and accounting for bias in whole-genome data sets supports the Ctenophora as sister to remaining Metazoa. *BMC Genomics*. 2015; 16(1):987.
- Childress JJ. Are there physiological and biochemical adaptations of metabolism in deep-sea animals? *Trends in Ecology & Evolution*. 1995; 10(1):30-6.

Childress JJ, Mickel TJ. Metabolic rates of animals from the hydrothermal vents and other deep-sea habitats. *Bulletin of the Biological Society of Washington* 1985; 6:249-60.

Childress JJ, Seibel BA, Thuesen EV. 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). *Marine Ecology Progress Series*. 2008; 373:187-91.

Childress JJ, Somero GN. Depth related enzymic activities in muscle, brain and heart of deep-living pelagic marine teleosts. *Marine Biology*. 1979; 52:273-83.

Childress JJ, Somero GN. Metabolic scaling: A new perspective based on scaling of glycolytic enzyme activities. *American Zoologist*. 1990; 30(1):161-73.

Childress JJ, Thuesen EV. Metabolic potential of deep-sea animals: Regional and global scales. In: Rowe GT, Pariente V, editors. *Deep-Sea Food Chains and the Global Carbon Cycle*. Dordrecht: Kluwer Academic Publishers; 1992. p. 217-36.

- Childress JJ, Thuesen EV. Metabolic potentials of deep-sea fishes: A comparative approach. In: Hochachka PW, Mommsen TP, editors. *Biochemistry and Molecular Biology of Fishes*. 5. Amsterdam: Elsevier; 1995. p. 175-96.
- Dahlhoff EP. Biochemical indicators of stress and metabolism: applications for marine ecological studies. *Annual Review of Physiology* 2004; 66:183-207.
- Dawson DM. Creatine kinase from brain: kinetic aspects. *Journal of Neurochemistry* 1970; 17(1):65-74.
- Diaz S, Cabido M. Vive la difference: plant functional diversity matters to ecosystem processes. *Trends in Ecology & Evolution*. 2001;16(11):646-55.
- Dunn CW, Hejnol A, Matus DQ, Pang K, Browne WE, Smith SA, et al. Broad phylogenomic sampling improves resolution of the animal tree of life. *Nature*. 2008; 452(7188):745-9.
- Dunn CW, Leys SP, Haddock SHD. The hidden biology of sponges and ctenophores. *Trends in Ecology & Evolution*. 2015;30(5):282-91.

- Haddock SH. Comparative feeding behavior of planktonic ctenophores. *Integrative and Comparative Biology*. 2007;47(6):847-53.
- Haddock SH, Heine JN. Scientific blue-water diving: California Sea Grant College Program, La Jolla; 2005.
- Haddock SHD. A golden age of gelata: past and future research on planktonic ctenophores and cnidarians. *Hydrobiologia*. 2004; 530(1-3):549-56.
- Haddock SHD, Christianson LM, Francis WR, Martini S, Dunn CW, Pugh PR, et al. Insights into the biodiversity, behavior, and bioluminescence of deep-sea organisms using molecular and maritime technology. *Oceanography*. 2017; 30(4):38-47.
- Harbison GR. On the classification and evolution of the Ctenophora. In: *The Origins and Relationships of Lower Invertebrates* (S. Conway Morris, J. D. George, R. Gibson & H. M. Platt, eds.), 1985; 78–100. Clarendon Press, Oxford.
- Harbison GR, Madin LP, Swanberg NR. On the natural history and distribution of oceanic ctenophores. *Deep-Sea Research*. 1978; 25(3):233-56.

Hooper DU, Chapin F, Ewel J, Hector A, Inchausti P, Lavorel S, et al.

Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs*. 2005; 75(1):3-35.

Horridge GA. The giant mitochondria of ctenophore comb-plates. *Journal of Cell Science*. 1964; 3(71):301-10.

Jékely G, Paps J, Nielsen C. The phylogenetic position of ctenophores and the origin(s) of nervous systems. *EvoDevo*. 2015; 6(1):1.

King FD, Packard TT. Respiration and the activity of the respiratory electron transport system in marine zooplankton. *Limnology and Oceanography*. 1975; 20:849–54.

Lefcheck JS, Bastazini VAG, Griffin JN. Choosing and using multiple traits in functional diversity research. *Environmental Conservation*. 2015; 42(2):104-7.

Mackie GO, Mills CE, Singla CL. Structure and function of the prehensile tentilla of *Euplokamis* (Ctenophora, Cydippida). *Zoomorphology*. 1988; 107(6):319-37.

Mackie GO, Mills CE, Singla CL. Giant axons and escape swimming in *Euplokamis dunlapae* (Ctenophora: Cydippida). *Biological Bulletin*. 1992; 182(2):248-56.

Martindale MQ. The evolution of metazoan axial properties. *Nature Reviews Genetics*. 2005; 6(12):917-27.

Matsumoto GI, Harbison GR. In situ observations of foraging, feeding, and escape behavior in three orders of oceanic ctenophores: Lobata, Cestida, and Beroida. *Marine Biology*. 1993; 117(2):279-87.

Mills CE. Medusae, siphonophores, and ctenophores as planktivorous predators in changing global ecosystems. *ICES Journal of Marine Science*. 1995; 52(3-4):575-81.

Mills CE. Jellyfish blooms: are populations increasing globally in response to changing ocean conditions? *Hydrobiologia*. 2001; 451(1-3):55-68.

Mills, C.E. Internet 1998-present. Phylum Ctenophora: list of all valid species names. Electronic internet document available at <http://faculty.washington.edu/cemills/Ctenolist.html>. Published by the author, web page established March 1998, last updated 12 June 2017.

- Moroz LL, Kocot KM, Citarella MR, Dosung S, Norekian TP, Povolotskaya IS, et al. The ctenophore genome and the evolutionary origins of neural systems. *Nature*. 2014; 510(7503):109-14.
- Noguchi M, Sawada T, Akazawa T. ATP-regenerating system in the cilia of *Paramecium caudatum*. *Journal of Experimental Biology*. 2001; 204(6):1063-71.
- Nosenko T, Schreiber F, Adamska M, Adamski M, Eitel M, Hammel J, et al. Deep metazoan phylogeny: when different genes tell different stories. *Molecular Phylogenetics and Evolution*. 2013; 67(1):223-33.
- Petchey OL, Gaston KJ. Functional diversity: back to basics and looking forward. *Ecology Letters*. 2006; 9(6):741-58.
- Podar M, Haddock SHD, Sogin ML, Harbison GR. A molecular phylogenetic framework for the phylum Ctenophora using 18S rRNA genes. *Molecular Phylogenetics and Evolution*. 2001; 21(2):218-30.
- Pomerleau C, Sastri AR, Beisner BE. Evaluation of functional trait diversity for marine zooplankton communities in the Northeast subarctic Pacific Ocean. *Journal of Plankton Research*. 2015; 37(4):712-26.

- Purcell JE. Extension of methods for jellyfish and ctenophore trophic ecology to large-scale research. *Hydrobiologia*. 2009; 616(1):23-50.
- Robison BH. Deep pelagic biology. *Journal of Experimental Marine Biology and Ecology*. 2004; 300(1-2):253-72.
- Rosado BHP, Dias ATC, de Mattos EA. Going back to basics: importance of ecophysiology when choosing functional traits for studying communities and ecosystems. *Natureza & Conservação*. 2013; 11(1):15-22.
- Ryan JF, Pang K, Schnitzler CE, Nguyen AD, Moreland RT, Simmons DK, et al. The genome of the ctenophore *Mnemiopsis leidyi* and its implications for cell type evolution. *Science*. 2013; 342(6164):1242592.
- Schleuter D, Daufresne M, Massol F, Argillier C. A user's guide to functional diversity indices. *Ecological Monographs*. 2010; 80(3):469-84.
- Schlining B, Jacobsen Stout N, editors. MBARI's video annotation and reference system. *Oceans 2006, Proceedings of the Marine Technology Society/Institute of Electrical and Electronics Engineers Oceans Conference*. 2006; Boston, MA.

Seibel BA, Carlini DB. Metabolism of pelagic cephalopods as a function of habitat depth: a reanalysis using phylogenetically independent contrasts. *The Biological Bulletin*. 2001; 201(1):1-5.

Seibel BA, Childress JJ. Metabolism of benthic octopods (Cephalopoda) as a function of habitat depth and oxygen concentration. *Deep-Sea Research Part I: Oceanographic Research Papers*. 2000; 47(7):1247-60.

Seibel BA, Drazen JC. The rate of metabolism in marine animals: environmental constraints, ecological demands and energetic opportunities. *Philosophical Transactions of the Royal Society of London B Biological Sciences*. 2007; 362(1487):2061-78.

Seibel BA, Thuesen EV, Childress JJ, Gorodezky LA. Decline in pelagic cephalopod metabolism with habitat depth reflects differences in locomotory efficiency. *Biological Bulletin*. 1997; 192(2):262-78.

Shen X-X, Hittinger CT, Rokas A. Contentious relationships in phylogenomic studies can be driven by a handful of genes. *Nature Ecology & Evolution*. 2017; 1(5):0126.

Shiganova TA. Invasion of the Black Sea by the ctenophore *Mnemiopsis leidyi* and recent changes in pelagic community structure. *Fisheries Oceanography*. 1998; 7(3-4):305-10.

- Simion P, Philippe H, Baurain D, Jager M, Richter DJ, Di Franco A, et al. A large and consistent phylogenomic dataset supports sponges as the sister group to all other animals. *Current Biology*. 2017; 27(7):958-67.
- Somero GN, Childress JJ. Scaling of ATP-supplying enzymes, myofibrillar proteins and buffering capacity in fish muscle: relationship to locomotory habit. *Journal of Experimental Biology*. 1990; 149(1):319-33.
- Stretch JJ. Observations on the abundance and feeding behavior of the cestid ctenophore, *Velamen parallelum*. *Bulletin of Marine Science*. 1982; 32(3):796-9.
- Szasz G, Gruber W, Bernt E. Creatine kinase in serum: 1. Determination of optimum reaction conditions. *Clinical Chemistry*. 1976; 22(5):650-6.
- Tamm S, Tamm SL. Extracellular ciliary axonemes associated with the surface of smooth muscle cells of ctenophores. *Journal of Cell Science* 1989; 94 (Pt 4):713-24.
- Thuesen EV, Childress JJ. Enzymatic activities and metabolic rates of pelagic chaetognaths: Lack of depth-related declines. *Limnology and Oceanography*. 1993; 38(5):935-48.

Thuesen EV, Childress JJ. Metabolic rates, enzyme activities and chemical compositions of some deep-sea pelagic worms, particularly *Nectonemertes mirabilis* (Nemertea; Hoplonemertinea) and *Poebobius meseres* (Annelida; Polychaeta). *Deep-Sea Research*. 1993; 40(3):937-51.

Thuesen EV, Childress JJ. Oxygen consumption rates and metabolic enzyme activities of oceanic California medusae in relation to body size and habitat depth. *Biological Bulletin*. 1994; 187:84-98.

Thuesen EV, Miller CB, Childress JJ. Ecophysiological interpretation of oxygen consumption rates and enzymatic activities of deep-sea copepods. *Marine Ecology Progress Series*. 1998; 168:95-107.

Thuesen EV, Rutherford LD, Brommer PL. The role of aerobic metabolism and intragel oxygen in hypoxia tolerance of three ctenophores: *Pleurobrachia bachei*, *Bolinopsis infundibulum* and *Mnemiopsis leidyi*. *Journal of the Marine Biological Association of the United Kingdom*. 2005; 85(3):627-33.

- Torres JJ, Aarset AV, Donnelly J, Hopkins TL, Lancraft TM, Ainley DG.
Metabolism of Antarctic micronektonic Crustacea as a function of depth
of occurrence and season. *Marine Ecology Progress Series*. 1994;
113:207-19.
- Torres JJ, Somero GN. Metabolism, enzymic activities and cold adaptation
in Antarctic mesopelagic fishes. *Marine Biology*. 1988; 98(2):169-80.
- Villéger S, Mason NWH, Mouillot D. New multidimensional functional
diversity indices for a multifaceted framework in functional ecology.
Ecology. 2008; 89(8):2290-301.
- Wallberg A, Thollesson M, Farris JS, Jondelius U. The phylogenetic
position of the comb jellies (Ctenophora) and the importance of
taxonomic sampling. *Cladistics*. 2004; 20(6):558-78.
- Wallimann T, Wyss M, Brdiczka D, Nicolay K, Eppenberger HM.
Intracellular compartmentation, structure and function of creatine kinase
isoenzymes in tissues with high and fluctuating energy demands: the
'phosphocreatine circuit' for cellular energy homeostasis. *Biochemical
Journal*. 1992; 281(Pt 1):21-40.

Whelan NV, Kocot KM, Moroz TP, Mukherjee K, Williams P, Paulay G, et al. Ctenophore relationships and their placement as the sister group to all other animals. *Nature Ecology & Evolution*. 2017; 1(11):1737.

Worm B, Lotze HK. Marine biodiversity and climate change. In: Letcher TM, editors. *Climate Change*. 2nd ed. Elsevier; 2016. p. 195-212.

