PROXIMATE BIOCHEMISTRY OF BENTHIC AND BENTHOPELAGIC CHONDRICHTHYANS: ANALYSIS OF METABOLIC POISE AND RELATIVE TROPHIC POSITION WITH DEPTH

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ABSTRACT

Understanding the energetic requirements and trophic positions of understudied chondrichthyan species (sharks, skates, rays and chimaeras) is important in terms of monitoring their overall ecological importance with depth because of increasing fishing pressure and global climate change. Data on deep-dwelling species is almost entirely lacking in terms of metabolic rates and quantitative trophic level information. In order to address this gap in knowledge, biochemical indices of aerobic and anaerobic metabolic capacity and bulk analyses of stable nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotopic compositions were measured in 14 species of benthic and benthopelagic chondrichthyan fishes over a broad depth range (~90 – 2200m) off the west coast of the U.S.A. The aerobic enzymes citrate synthase (CS) and malate dehydrogenase (MDH) and the anaerobic enzymes lactate dehydrogenase (LDH) and pyruvate kinase (PK) were analyzed in both red (RM) and white muscle (WM) at a common reference temperature of 10˚C in order to determine metabolic poise and locomotory capacity of each species. Significant decreases observed in WM anaerobic enzyme activities with increasing median depth of occurrence (MDO) are most likely explained by the "visual-interactions hypothesis (VIH)." The VIH explains these trends as the result of a relaxed selective pressure for burst locomotion with declining light levels and smaller reactive distances between predator and prey. Phylogeny and locomotory mode are shown to have very little influence on aerobic and anaerobic metabolic poise compared to MDO, and similar metabolic potential is observed in co-occurring demersal sharks and rays. Bulk stable isotope analysis reveals a decrease in $\delta^{13}C$ with depth that may indicate increased dietary reliance on pelagic vertical migrators and/or epipelagic carrion that are depleted in ^{13}C

relative to nearshore and benthic prey sources. Furthermore, the observed enrichment in ^{15}N is likely due, in part, to the ^{15}N enrichment in glutamic acid leaving the liver for the formation of muscle protein as a result of a decrease in urea:TMAO with increasing depth. This suggests a potentially unique increase in trophic discrimination factors (TDF) with depth in chondrichthyans as well as a current overestimation of trophic level calculations for these species at depth when using a standard 3.4‰ TDF. The low metabolic activities and potentially overestimated trophic levels observed in these top-predator, k-selected species makes them more susceptible to changes in the environment than previously thought. This information is key to accurately portraying ecosystem based food web and energetic dynamics in the deep-sea.

TABLE OF CONTENTS

LIST OF TABLES

Chapter 1:

Chapter 2:

LIST OF FIGURES

Chapter I:

Chapter 2:

INTRODUCTION

As fishing pressure and deeper fisheries continue to increase, the need to study the ecological importance of understudied marine taxa, such as chondrichthyan fishes, is becoming ever more important across all depth ranges. Quantitative data, such as trophic position and metabolic rate, allows relative estimations of the transfer of energy through individuals and populations in complex systems, such as the deep sea. This essential information regarding the energetics of chondrichthyan species is needed in order to better understand their ecological importance and provide accurate fisheries management policies.

Data on deep-dwelling chondrichthyan metabolic rates is almost entirely lacking. Understanding the energetic requirements of top-predators, such as elasmobranchs, is important in terms of their overall ecological importance. In most fishes, the largest and most variable portion of these energetic budgets is metabolism (Lowe 2001); however, metabolism is difficult to quantify. Additionally, there is no easy way to measure swimming speeds and energy requirements of active sharks (Dickson et al. 1993), skates and rays, and deep-living species. In their place, biochemical indices of muscle enzyme activity are utilized. Enzymatic assays represent both aerobic and anaerobic metabolic activity, as well as locomotor capabilities. Previous studies on muscle tissue enzymatic activities of chondrichthyan species have focused primarily on shallow-living and active, pelagic species (Alp et al. 1976, Battersby et al. 1996, Bernal et al. 2003, Crabtree and Newsholme 1972, Dickson et al. 1993, Moon and Mommsen 1987, Suarez et al. 1986, Sullivan and Somero 1980, Zammit et al. 1978). Treberg et al. (2003) is the only study, thus far, to make a direct comparison between species of shallow and deep-living

elasmobranchs by comparing two species of squaloid shark. This leaves a major gap in the knowledge of depth related trends in metabolism of chondrichthyan fishes.

In addition, despite the fact that sharks are believed to play an important role in structuring aquatic food webs as apex predators, surprisingly little quantitative information is available on their diets and trophic levels (Cortes 1999). Large and abundant consumers, such as chondrichthyan fishes, are likely to influence the structure and function of the communities in which they live. Myers (2007) suggests that a cascading top-down effect may be a predictable outcome of eliminating functional groups of apex predators, such as sharks, in a given environment. The need for determining trophic positions is, therefore, extremely important. Stable nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotope ratios are routinely used to establish trophic levels and relationships, estimating trophic position and carbon flow to consumers in food webs (Post 2002).

Ecologically, there is a great need to understand the energetics of these K-selected, top-predator species due to increasing fishing pressure in the deep-sea. Deep-sea fisheries appear to already effect the maximum attainable depths known for chondrichthyan fishes (García et al. 2008, Priede et al. 2006). If this is the case, over-exploitation and extinction of these species may occur before they are even understood and may also have unknown long-term ecological implications. Overall, this project will attempt to fill in several gaps in our knowledge and has the potential to provide a basic structure that will assist in the fundamental understanding of benthic/benthopelagic energetics with depth through the examination of the trophic dynamics and metabolic rates of these species. Additionally, examining the roles of these top-predators is essential to the understanding of large-scale population dynamics and potential

implementation of ecosystem-based fisheries management in dynamic systems, such as the deep-sea.

The objectives of this study are to: (1) evaluate metabolic rate using biochemical indices in RM and WM in sharks, skates and chimaeras by combining my data with that of previously published enzyme work; and (2) determine the relative trophic positions (via $\delta^{15}N$ and $\delta^{13}C$ values) of benthic and benthopelagic chondrichthyans off the west coast of the U.S.A.

CHAPTER I

Metabolic enzyme activities in chondrichthyans: Implications for metabolic poise and locomotory capacity.

ABSTRACT

Biochemical indices of aerobic and anaerobic metabolic capacity were measured in 14 species of benthic and benthopelagic chondricthyan fishes over an extensive depth range (~90 – 2200m). These were combined with previously published elasmobranch data making this the first metabolic study to represent a broad range of depths, phylogeny and locomotory modes in these species. The aerobic enzymes citrate synthase (CS) and malate dehydrogenase (MDH) and the anaerobic enzymes lactate dehydrogenase (LDH) and pyruvate kinase (PK) were analyzed in both red (RM) and white muscle (WM) at a common reference temperature of 10˚C. Significant decreases observed in WM anaerobic enzyme activities and a lack of significant trends in RM enzyme activities with increasing median depth of occurrence (MDO) is most likely explained by the visual-interactions hypothesis (VIH). The VIH explains these trends as the result of declining light levels and consequential reduction in the reactive distances between predator and prey, which effectively relaxes the selective pressure for burst locomotory capacity. However, the lack of significant decreases in WM aerobic enzymes is inconsistent with trends seen in previous teleost data. This, as well as a lack of the previously established correlation between CS and LDH, is most likely the result of specific physiological differences between teleost and chondrichthyan fishes. In contrast to Priede et al. (2006), who suggest relatively higher energetic demands in sharks, this study reveals a similarity in metabolic potential between teleosts and chondrichthyans across similar depth ranges that suggests that metabolism may not be a factor in the depth limitation of chondrichthyan fishes. Overall, this study indicates that phylogeny and locomotory mode

have little influence on aerobic and anaerobic metabolic poise as compared to MDO, and suggests similar metabolic potential in co-occurring demersal sharks and rays. Furthermore, these broad interspecific comparisons allow for inferences to be made regarding the locomotory capacity of specific species that are otherwise difficult to study. For instance, the aerobic enzyme capacity of the two deepest dwelling skates studied, *Amblyraja badia* and *Bathyraja mircotrachys*, most likely reflects highly migratory behavior on a large horizontal scale. The low metabolic poise observed in chondrichthyan species at depth, irrespective of their phylogeny, suggests a very slow pace of life for sharks and skates alike. This information should be taken into account by managers of deeper fisheries and deep-sea systems threatened by global climate change.

INTRODUCTION

There has been an ongoing need to study the ecological importance of understudied marine taxa, such as chondrichthyan fishes, across all depth ranges with increasing fishing pressures and deeper fisheries. Important information, such as metabolic rate, energetic requirements, and activity patterns, is needed to better understand these species' ecological importance and to inform accurate fisheries management policies.

Ecologically, there is a great need to understand the metabolism and energy requirements of these K-selected, top-predator species with increasing fishing pressure on the deep-sea. As shallow coastal fish stocks face serial depletion, fishing pressure has increased at depth along the continental slope (Haedrich 2007). Deep-sea fisheries appear to have already reached the maximum attainable depths known for chondrichthyan fishes (Garcia et al. 2008; Priede et al. 2006). Bailey et al. (2009) also suggest a significant decrease in overall fish abundance between 1977 and 2002 in the northeast Atlantic at all depths from 800-2500m. This would suggest that the effects of fishing efforts are seen well below the maximum depth of commercial fishing (approximately 1600m) in that area. If this is the case, current fishing strategies are affecting most, if not all, species of chondrichthyans. Over-exploitation and extinction of these species may occur before basic information such as age, growth, reproduction, distribution, resource utilization, and energy requirements are understood (Garcia et al. 2008; Kyne and Simpfendorfer 2007; Compagno 1990; Ebert and Compagno 2007). The depletion of these upper trophic level fishes may have unknown long-term ecological

implications on prey and predator biomass, population viability, and the potential for species replacement (Stevens et al. 2000; Meyers et al. 2007; Koslow et al. 2001).

Data on deep-dwelling chondrichthyan metabolic rates is almost entirely lacking. Numerous data on the differences between deep and shallow-living species of teleosts are available in regards to metabolic rate and biochemical adaptations. Metabolism at depth is generally thought to be low and constrained by several environmental factors. The "Metabolic Theory of Ecology" claims that all variability in metabolic rate can be explained solely by temperature and animal mass (Gillooly et al. 2001; Brown et al. 2004). However, pelagic teleost species show a marked decline in metabolism that cannot be explained by correcting for either of the aforementioned factors (Drazen and Seibel 2007; Childress and Somero 1979; Sullivan and Somero 1980; Torres et al. 1979).

The leading theory for this decline in metabolic rate with depth is the "visual interactions hypothesis" (VIH). The VIH suggests that as a result of declining light levels with depth, the selective pressure for high locomotory capacity in visual species decreases as the reactive distances between predator and prey are reduced. These distances are highly dependent on light and visual capacity. The demand for energy, or metabolic rate, declines due to reductions in the maintenance and operation costs associated with high performance locomotion (Childress 1995; Drazen and Seibel 2007; Childress et al. 1990a; Somero 1992). This theory is supported by the fact that non-visual animals, such as chaetognaths, medusa, and worms, do not exhibit depth related metabolic declines, but visual taxa do (Drazen and Seibel 2007; Childress and Somero 1979; Sullivan and Somero 1980; Thuesen and Childress 1993a, 1993b, 1994). In addition, the metabolic declines among visual taxa are predominantly reduced in the first

500m, and level off to a fairly constant rate below approximately 1000m where visible light is absent (Lythgoe 1988; Warrant and Locket 2004; Childress 1995).

An important distinction exists in terms of the VIH between pelagic and benthic animals. Differences in the slope of metabolic rate declines between benthic and benthopelagic teleosts have been shown, with benthic fishes generally showing a less pronounced decline with depth (Drazen and Seibel 2007). Benthic declines in metabolism may be less pronounced compared to pelagic realms due to the availability of refuge from predators at all depths (Childress et al. 1990a; Childress 1995; Somero 1992; Drazen and Seibel 2007). Unlike the pelagic zone, the benthic zone substrate provides the opportunity for crypsis and shelter. Therefore, the benthic substrate may further reduce the selective pressure for both burst and sustained swimming behavior. Previous studies have shown that benthic crustaceans, octopods, and echinoderms generally show no decline in metabolic rate with depth (Seibel and Childress 2000; Seibel and Drazen 2007), while more mobile caridean shrimps, which spend a portion of their time in the water column, show a decline (Childress et al. 1990a, Company and Sarda 1998).

Alternative hypotheses strive to explain depth related patterns in metabolism with factors such as pressure and food availability. Some enzymes adapted to high pressure are inefficient, and could therefore limit metabolic rate (Somero and Siebenaller 1979). However, constant levels of enzymatic activity in heart and brain tissue of fishes regardless of depth have shown that these organisms have adapted to maintain a level of enzymatic capacity and performance regardless of pressure (Hochachka and Somero 2002; Childress and Somero 1979; Sullivan and Somero 1980; Siebenaller et al. 1982). This has also been confirmed in the comparison of the enzymatic activity of heart muscle

tissue between a deep and shallow living squaloid shark, *Centroscyllium fabricii* and *Squalus acanthias*, respectively (Treberg et al. 2003). Additionally, metabolic rate studies in fishes and crustaceans show no effect of variable pressure (Childress 1977; Meek and Childress 1973; Belman and Gordon 1979). Thus, alterations in muscle tissue biochemistry with depth may reflect changes associated with reduced metabolic and locomotory demands.

Many earlier studies propose that lower food availability with depth is the driving factor in lower metabolic rates of deep-sea animals (Childress 1971; Smith and Hessler 1974; Dalhoff 2004). This theory is based on the existence of an order of magnitude exponential decline in animal biomass from 0-1000m (Haedrich and Rowe 1977; Angel and Baker 1982; Thurston et al. 1994; Priede et al. 2006). However, food supply does not seem to be a selective factor for metabolic rates. Animals in shallow oligotrophic waters have metabolic rates higher than animals in deep-sea eutrophic regions. If food supply constrained metabolism interspecifically , one would expect these values to be approximately the same (Cowles et al. 1991; Seibel et al. 1997; Seibel and Drazen 2007). Additionally, Seibel and Drazen (2007) show a general lack of correlation between food supply and metabolic rates in the pelagic deep-sea.

Understanding the energetic requirements of top-predators, such as elasmobranchs, with depth is important in terms of their overall ecological importance. In most fishes, the largest and most variable portion of these energetic budgets may be metabolism (Lowe 2001). Data compiled from trawling, baited hooks, baited cameras, and archive data suggest that chondrichthyes reach depths of 3000m, and rarely 4000m; whereas bony fish are found to reach depths greater than 8000m. Priede et al. (2006)

suggest that decreasing metabolism with depth, in conjunction with the energy needed to maintain large-lipid rich livers, is the decisive factor in the known depth ranges of these chondrichthyan fishes. Metabolism is difficult to quantify; and there is also no easy way to measure swimming speeds, activity patterns, and energy requirements of active sharks (Dickson et al. 1993), skates and rays, and deep-living species. In place of direct metabolic measurements, biochemical indices of muscle activity and metabolism can be used.

Metabolic enzyme activities can represent both aerobic and anaerobic metabolic activity, as well as locomotor capabilities, and shed light on the metabolic poise of an individual. The enzymes pyruvate kinase (PK), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), and citrate synthase (CS) are often assayed due to their key regulatory roles in aerobic and anaerobic adenosine triphosphate (ATP) production necessary for muscle contraction (Childress and Somero 1979; Siebenaller et al. 1982). Several studies have found that they correlate well with oxygen consumption rates (Dalhoff 2004; Moyes and Lemoine 2005; Seibel 2007; Drazen and Seibel 2007). These enzymes have also been shown to be stable in frozen tissue (Dickson et al. 1993).

It was previously hypothesized that sharks had lower metabolic rates than teleosts of comparable size and lifestyle based on early metabolic rate work on relatively sluggish, cold-water sharks (Piiper and Schuman 1967; Metcalf and Butler 1984; Brett and Blackburn 1978). However, recent work by Dickson et al. (1993) reveals that elasmobranchs and teleosts with comparable locomotor habits have similar muscle enzyme activities. This refutes the original idea that sharks have lower metabolic rates than teleosts.

Additionally, previous studies on teleosts suggest that interspecific differences in feeding and locomotory habits may partially explain differences seen in WM enzyme activity at a given depth (Sullivan and Somero 1980; Siebenaller et al. 1982; Dickson et al. 1993). WM tissue is powered by anaerobic metabolism and is specialized for short duration accelerations and burst activity for both predator avoidance and the capture of prey (Bone 1966; Sullivan and Somero 1980). This reveals the potential for a wide variation in glycolytic enzyme activities with different locomotory capacities. Protein and water content have been used as additional proxies for locomotory capacity. Pelagic fish and crustaceans show a decline in protein and a rise in water contents, along with a decrease in body robustness and potential locomotory capacity, with depth (Childress and Nygaard 1973, 1974; Bailey and Robison 1986; Stickney and Torres 1989; Childress et al. 1990b).

Interspecific variability in body morphology and activity patterns play a central role in ecology and lends to the diversity of feeding and swimming modes among fish species (Hennemann 2001; Wearmouth and Sims 2009). Differences in behavior and the potential influence of phylogenetic differences may lend to particular metabolic adaptations (Goolish 1991; Gibbs 1997). Pelagic species generally maintain a streamlined morphology independent of phylogeny that allows for high speed and continuous swimming in the illuminated open ocean (Seibel and Drazen 2007). In pelagic sharks, continuous swimming is required for hydrodynamic lift and ram-ventilation, or forced water flow over the gills for respiration (Lowe 1998). Conversely, morphology and locomotory strategy becomes more diverse in mid-water and demersal species where the environment offers protection from predators (Verity et al. 2002;

Seibel and Drazen 2007). Biochemical measurements in cephalopods reveal a tendency towards more efficient, sluggish routine swimming with depth (Seibel et al. 1998, 2000). This exemplifies the reduced need for speed in these environments. Specialization in locomotor and foraging modes has generally been characterized as ranging from sedentary, ambush predation to continuous active foraging (Webb 1984). Skates are universally thought to be less active, ambush predators that spend long periods resting compared to sharks due to their dorsally flattened body morphology adapted for benthic living. Indeed, standard metabolic rates of skates and rays are comparable to cold-water, similar-sized, less active sharks, while active sharks have higher maximum metabolic rates than do more sedentary sharks (Carlson et al. 2004). Dickson et al. (1993) show this same general pattern with decreasing metabolic enzyme rates from active pelagic sharks, bottom associated sharks, to benthic skates and rays.

Previous studies on muscle tissue enzymatic activities of chondricthyan species have focused on shallow living and active, pelagic species (Alp et al. 1976; Bernal et al. 2003; Crabtree and Newsholme 1972; Dickson et al. 1988; Dickson et al. 1993; Moon and Mommsen 1987; Sullivan and Somero 1980; Zammit et al. 1978). Treberg et al. (2003) is the only study, thus far, to make a direct comparison between species of shallow and deep-living elasmobranchs by comparing two species of squaloid sharks. This leaves a major gap in the knowledge of the metabolism of deep living chondrichthyan fishes. Teleost studies have shown a general decrease in LDH, PK, MDH and (to a lesser extent) CS with minimum depth of occurrence (Childress and Somero 1979; Sullivan and Somero 1980; Drazen and Seibel 2007). Thus, it may be reasonable to assume similar depth-related trends in chondrichthyan fishes.

Furthermore, almost all muscle enzyme activity data has been generated using WM. There is an apparent distinction between the roles of WM and RM tissue. During short burst swimming activity powered by WM, energy is supplied by glycolytic enzyme activity (such as LDH and PK) through the degradation of glycogen stores to lactic acid, and aerobic enzymes (such as CS and MDH) are of little importance (Bilinski 1974; Sullivan and Somero 1980). Conversely, RM fibers power sustained slow aerobic swimming that is driven largely by aerobically poised enzymes, such as CS, and lower concentrations of anaerobic enzymes (Bone 1966, 1988; Johnston 1981; Somero and Childress 1980). More data on the metabolic poise of RM could greatly expand our understanding of elasmobranch metabolism and locomotion.

The focus of this study is to evaluate metabolism and locomotory performance of benthic and benthopelagic chondrichthyans off the western coast of the U.S.A using biochemical indicators. This information will help expand our knowledge of these top-predators biology and ecology, as well as assist with potential implementation of ecosystem-based fisheries management in dynamic systems, such as the deep-sea.

MATERIALS AND METHODS

Tissue collection

Elasmobranchs were caught, utilizing beam and otter trawls, along contour during two cruises in Monterey Bay, California in April and October of 2009 at target depths of 100-2000m. Additional specimens were caught in June 2010 during a Fishery Resource Analysis and Monitoring (FRAM) Division (NOAA/NMFS) groundfish slope survey that sampled from Coos Bay, Oregon to San Francisco, California.

Specimens were placed on ice immediately upon being sorted from the trawl. WM tissue was excised from sharks and chimaeras dorsolaterally below the first dorsal fin and dorsally, both from the thickest part of the pectoral fin and next to the vertebra, in skates. Most skates are known to be undulatory appendage propulsors that swim by passing waves down the pectoral fin in the posterior direction (Daniel 1922). As such, both pectoral fin and vertebral muscle were sampled for energetic comparison with a primary focus on the analysis of pectoral fin WM. Sampling *Torpedo californica* presented difficulty because both specimens were small juvenile males (0.49 – 0.70kg). The pectoral fins of both were composed mainly of the electric organ and extra care had to be taken to sample only WM tissue.

RM tissue was also sampled for some species. However, homogenous samples of RM tissue were not large enough in the shark specimens, with the exception of the two largest specimens taken from *Somniosus pacificus*. RM samples were also obtained in sufficient amounts dorsally located above the WM sample in skates, with the exception of *Raja inornata*. Tissue samples were placed in cryovials and frozen in liquid nitrogen

until being transferred to a -80˚C freezer. Samples were stored for up to 22 months prior to analysis. Dickson et al. (1993) showed that storage for up to 44 months had no affect on the activities of enzymes in elasmobranches. Whole specimens were then frozen on board and weighed ashore.

Enzyme assays

The maximal rates of the aerobic enzymes (CS and MDH) and anaerobic enzymes (LDH and PK) were analyzed (Figure 1). Aerobic metabolism involves the oxidation of fuels (lipids, carbohydrates, and amino acids) that supplies resting energy requirements and some low-intensity behaviors, such as sustained swimming (Bennett 1980). The enzyme CS is a good indicator of aerobic metabolism, representative of mitochondrial density, and generally scaling to whole animal metabolic rate. CS catalyzes the initial reaction in the citric acid cycle, which during the oxidation of glucose or fatty acids ultimately creates energy in the form of ATP that is important in sustained muscle activity (Alp et al. 1976; Johnston 1981; Torres and Somero 1988). Similar to aerobic metabolism, CS negatively scales with body size (Somero and Childress 1980). In addition, MDH also plays a key role in the regulation of the citric acid cycle. MDH is indicative of intermediary metabolism with its role in the regulation of redox balance in WM tissue (Sullivan and Somero 1980; Siebenaller et al. 1982).

The primary pathway among vertebrates for anaerobic metabolism is glycolysis, the conversion of large carbohydrates to lactic acid. Glycolytic enzymes PK and LDH indicate anaerobic metabolism. LDH maintains redox balance with the reversible reaction pyruvate + NADH <–> lactate + NAD^+ and is indicative of burst locomotor

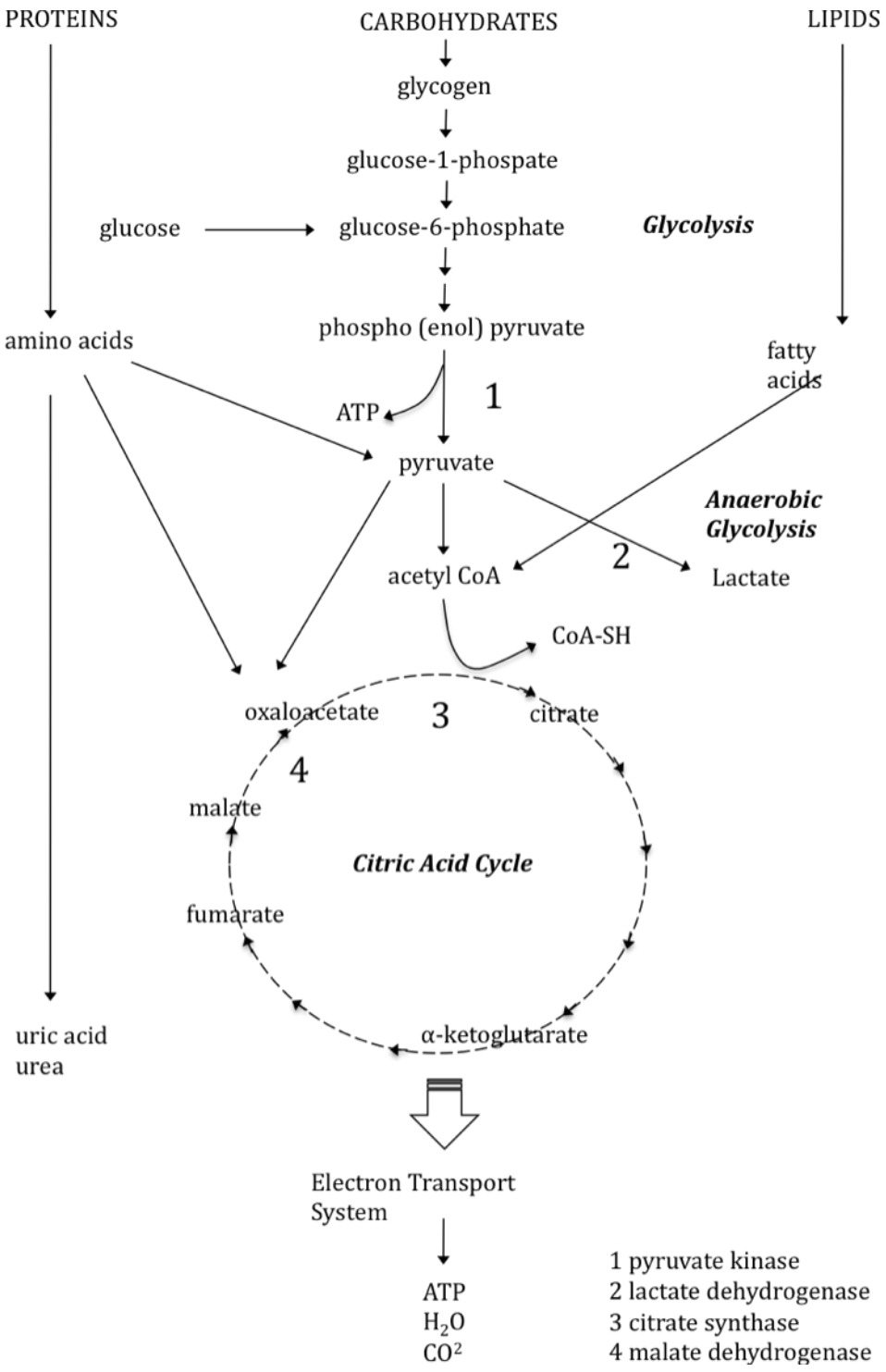

Figure 1. Basic pathways of ATP production in fish myotomal muscle, including the enzymes pyruvate kinase, lactate dehydrogenase, citrate synthase and malate dehydrogenase.

capacity (Childress and Somero 1979). LDH activity correlates well with burst locomotory capacity, in part, due to its facilitation of ATP production when oxygen supplies are low or rapidly depleted (Somero and Childress 1980; Dalhoff 2004). Overall, rate measurements of glycolytic enzymes tend to be much more varied between species and over depth than CS measurements (Crabtree and Newsholme 1972; Moon and Mommsen 1987; Dickson et al. 1993; Treberg et al. 2003).

Frozen muscle tissue was weighed and homogenized in nine volumes of ice-cold 10 mM Tris HCl buffer (pH 7.55 at 10 °C) on ice in a Kontes Duall ground-glass homogenizer. Homogenates were centrifuged (5000 g) for 5 minutes after the completion of the CS assay. Care was taken to avoid any resulting lipid layer and the supernatant was used for LDH, PK and MDH assays. Supernatants were kept on ice, without further purification, until use the same day.

All assays were run in a total volume of 2.0ml at 10˚C using a Shimadzu UV 1601 spectrophotometer with a temperature controlled water bath and water-jacketed 12-cell cuvette holder. A temperature of 10˚C was chosen because it is within the temperature range experienced by most of these species. Enzymatic activity is proportional to the change in absorbance at 340nm (for MDH, PK, and LDH) and at 412nm (for CS) over time and is reported in international units (IU; µmol substrate converted to product per min) per gram tissue wet mass. Enzyme assays were run under saturating substrate conditions as follows: Citrate synthase: 0.1 mM dithiobis-nitrobenzoic acid, 0.1 mM acetyl CoA, 2 mM $MgCl_2$, 50 mM Imidazol HCl (pH 8 at 10˚C). Reaction was initiated by 0.5 mM oxaloacetate. Pyruvate kinase: 0.1 mM fructose 1,6 bisphospate, 5.0 mM ADP, 0.15 mM NADH, 10 U of LDH, 10 mM $MgSO_4$, 100 mM

KCl, 80 mM Tris HCl (pH 7.8 at 10˚C). Reaction was initiated by 1.0 mM phospho(enol)pyruvate. Malate dehydrogenase: 0.15 mM NADH, 0.5 mM oxaloacetate, 20 mM $MgCl_2$ 100 mM Tris HCl (pH 8.1 at 10˚C). Lactate dehydrogenase: 0.15 mM NADH, 2 mM sodium pyruvate, 100 mM KCl, 80 mM Imidazole HCl (pH 7.8 at 10˚C).

Enzyme activities have been shown to scale with body size- with a general increase in anaerobic and a decrease in aerobic activities with body mass (Somero and Childress 1980; Somero and Childress 1985). Regression analyses were performed between body mass and individual enzyme activities for both RM and WM for species with sample sizes large enough for analysis.

Previous studies have run all assays, including CS, after centrifuging homogenates over a range of speeds and durations (see Somero and Childress 1980; Sullivan and Somero 1980; Dickson et al. 1993; Bernal et al.2003; and Treberg et al. 2003 for example). This clear (particle free) supernatant is assayed in order to reduce background noise in the spectrophotometer. CS is found within the mitochondrion (Childress and Somero 1979) and significant activity may be lost in the assay due to the process of spinning out mitochondrial rich particles in the homogenate. To evaluate the effects of centrifugation, CS activity was analyzed across a range of species for both teleosts (*Microstomus pacificus, Anoplopoma fimbria,* and *Coryphaenoides acrolepis*) and elasmobranchs (*R. inornata, A. brunneus,* and *A. badia*) using unspun homogenates, quickspun supernatants (20sec at 5000g), and fullspun supernatants (5min at 5000g). Comparisons of this data to that in the literature for other chondrichthyan species of varying depth ranges and activity levels were made. Previous data on elasmobranches centrifuged their samples anywhere from 5000-12000g for 5-10 min (Dickson et al. 1993;

1998; Bernal et al. 2003; Treberg et al. 2003; Moon and Mommsen 1987). For the purposes of comparison, these previous data are considered fullspun.

Median depth of occurrence

Previous studies on teleosts examine depth related trends in metabolic variables using the minimum depth of occurrence for each species (for example Childress and Somero 1979; Sullivan and Somero 1980; Drazen and Seibel 2007). This depth is defined as the depth below which 90% of the adult population of a given species is captured (Childress 1995; Seibel and Drazen 2007). This approach takes into account the fact that a given fish may not always occupy a single depth due to possible diel and ontogenetic migration (Collins et al. 2005; Jacobson et al. 2001). However, it is common knowledge that many species of elasmobranch have ontogenetic depth shifts and segregate in depth and location by sex, age of maturity, and/or size. In this case, the minimum depth of occurrence may be only representative of a small portion of the species population. This study will use the median depth of occurrence (MDO) in order to be more representative of a given species as a whole. Known minimum and maximum depths of occurrences were obtained for all species, and the median was taken between the two values (Table 1; Ebert 2004, Carrier et al. 2010, Froese and Pauly 2004).

Proximate chemistry

Body composition analyses were done in order to look for potential trends with depth. Protein and lipid content of dorsal WM tissue was determined in duplicate on tissue homogenized in distilled water. The bicinchoninic acid (BCA) protein assay (Smith

et al. 1985) was used with bovine serum albumin as a standard. Lipids were extracted according to Bligh and Dyer (1959) as modified by Reisenbichler and Bailey (1991), and assayed using the sulfuric acid charring method of Marsh and Weinstein (1966) using glyceryl trioleate as a standard. Tissue samples (~0.05 – 0.2g) were also dried in a 60˚C oven in triplicate for 24 hours, or until dry, to determine water content by taking the difference between wet and dry masses of the tissue.

Statistical analyses

Model I and II regression analyses were applied to explore relationships between depth, body mass, proximate chemistry, and enzymatic activity. A model II regression (Ricker 1973; Laws 1997) was used when comparing enzyme rates to median depths of occurrence because both variables are not controlled, and hence, both contain error. A log-log transformation was conducted prior to model II regressions when necessary. Data from previously published literature was included in the analysis of trends in enzyme activity with depth, and in the analysis of the potential effects of both phylogeny and locomotory mode on WM enzyme activity (see table 1 for corrected literature values). Differences in sampling sites for WM in skates were analyzed using one-way analysis of variance (ANOVA) with a tukey-kramer post-hoc procedure to analyze enzyme activities as a percentage of pectoral muscle activity values versus vertebral muscle. A Kruskal-Wallis test with a tukey-kramer post-hoc procedure was used to test for interspecific differences in enzymatic activity in this data, and also for phylogenetic effects on WM enzyme activity in both this data and literature values (excluding groups

with only one value). All tests were run at a significance level of $\alpha = 0.05$ using Statistica 7.1 (Stat Soft, Inc., Tulsa, Oklahoma).

In order to further test interspecific differences in metabolic poise, a hierarchical cluster analysis was performed using CS and LDH values for all available data. The data was transformed into a Bray-Curtis similarity matrix and cluster distances were determined by using group average values. This allows for comparisons that take into account both an aerobic and anaerobic metabolic indicator. Prior to analysis, each enzyme was standardized to the maximum value from 0 to 100. MDH and PK were not included due to the paucity of data in previous literature. Cluster analyses were performed with Primer 6.1 (PRIMER-E Ltd.). A Q_{10} of 2.0 was used to correct literature data to a temperature of 10˚C for comparisons to my data where needed.

RESULTS

Fourteen elasmobranch species were sampled over a broad depth range in this study: 9 skate species from ~90-570m and 830-2200m; 3 shark species from ~450-1200m; 1 torpedo ray at ~90m; and 1 species of chimaera from ~80-540m (Figure 2). Although evolutionarily divergent, Chimaeriformes, such as *Hydrolagus colliei*, have been included in this study, as they have been shown to have a similar metabolic organization to elasmobranchs (Speers-Roesch et al. 2006). All chondrichthyans caught in the thirty-nine trawls conducted from approximately100-2000m in Monterey Bay were sampled. Select specimens from the NOAA/NMFS slope survey were utilized to add additional species and increase samples sizes.

Enzyme analyses

Examining the difference between pectoral and vertebral WM enzyme activities of the skates revealed few significant differences between the two muscle types. The aerobic CS enzyme activity tended to be higher in pectoral muscle, while MDH was highly variable. Anaerobic activities (LDH and PK) tended to be about the same or lower in pectoral muscle (Figure 3). CS and LDH were fairly consistent in terms of their percentage of pectoral activity, with the exception of *T. Californica*. This species had statistically lower LDH and higher CS activity in pectoral muscle relative to vertebral muscle compared to all other species (p-values <0.01). Due to this fact, and the difficulty sampling pectoral muscle in the juvenile torpedo rays, vertebral muscle for *T. californica* was used in all further analyses, while pectoral muscle was used for all skates.

Table 1 summarizes the mean activities of CS, MDH, LDH and PK in WM (vertebral and pectoral) and RM for all species. Few body mass effects were seen in the species from this study (Figures 4 and 5). This is likely the result of small sample sizes and size ranges for many of the species (Table 1). Among those trends seen there was a consistent decrease in CS activity in WM and RM, an increase in PK activity in WM, and a decrease in MDH in RM with increasing body size. Due to paucity of significant trends, comparisons between species were made using means without scaling for body size.

Mean maximal WM activities for data from this study were highly variable with the skate *Amblyraja badia's* CS activity being statistically higher than all other species (p-value= <0.0001). Mean RM activities were less variable interspecifically than their WM counterparts with the skate *R. binoculata* PK activity being statistically higher than all other species (p-values= <0.01).

Examination of differing rates of homogenate centrifugation was performed in order to make accurate comparisons to literature data. There was a highly significant linear relationship between the unspun assay and both the quickspin and fullspin assays ($p<0.001$; Figure 6). The statistically similar slopes and y-intercepts reveal that spinning the homogenate reduces CS activity values, and the effect occurs with even a 20 sec spin. As a result, for comparison to my data, previously published CS values were corrected using the equation: unspun CS = 1.29* (fullspun CS) + 0.41, where published data were considered fullspun.

Among all available data, including CS and temperature corrected literature values (Table 1), differences were seen in enzyme activity rates of CS, MDH, LDH and PK between RM and WM. Aerobic enzymes were consistently much higher in RM than

WM in all species. Anaerobic enzymes were more variable with either approximately equal or much higher activities in WM in most shark species, and conversely approximately equal or slightly elevated activities in RM in most skate species.

Depth trends were evaluated in WM and RM using all available data. Sharks, rays, and chimaeras were categorically marked in these trends due to the decreasing predominance of shark species data with increasing depth. Despite marked morphological differences, all three categories seemed to fall along the same regression line. WM anaerobic enzymes (LDH and PK) show a marked statistically significant decline in activity with increasing MDO (Figure 7). The decline is most predominant from 0 to 500m, and begins to level off at deeper depths past 1000m. Aerobic enzyme (CS and MDH) trends are far less pronounced (Figure 8). CS shows a statistically insignificant increase in activity with increasing MDO. This trend is driven by the two deepest dwelling skates *A. badia* (MDO of 1585m) and *B. microtrachys* (MDO of 2448m). *A. badia*, as mentioned previously, has a CS activity that is statistically greater than all other species, while *B. microtrachys* is most similar to species of intermediate depths between 100-500m. Removing these two values reveals a statistically insignificant decrease in CS activity with increasing MDO among all species. CS activity was additionally examined in this studies data alone in order to remove any potential bias from corrected literature data (Figure 9). This data, with the exclusion of the two deepest skates, shows an insignificant decrease in CS activity with MDO. MDH shows a statistically insignificant increase driven in part by the low values of *T. californica* and *R. erinacea,* as well as the high variation in the deepest skate, *B. microtrachys.* In general, the sharks with MDOs between 500 and 1000 meters have lower MDH activities than all

other values for the rays and the chimaera *H. colliei*. In addition, there is a strong significant correlation between MDH and CS ($r= 0.55$, p-value <0.0001) among all individual fish studied (Figure 10). The RM aerobic and anaerobic enzymes showed no significant trends with increasing MDO with an insignificant increase in MDH, an insignificant decrease in LDH, and relatively no change in CS and PK (Figure 11).

To examine the effects of phylogeny on enzymatic rate, the species from all available data were grouped by family and by order (Figure 12). Not all families are represented by activity values of each enzyme due to the paucity of MDH and PK data in the literature. Families with 3 or more representative species (Arhynchobatidae, Rajidae, Scyliorhinidae, and Carcharhinidae) were examined statistically. Among these families, there existed only two significant differences: carcharhinids had statistically higher LDH than all other families (p-values <0.01); while the scyliorhinids were statistically lower (p-values <0.03) than the two skate families. Examining general trends among all families revealed that the mean values for CS, LDH and PK were consistently highest in the family Lamnidae. The family Somniosidae consistently had among the lowest values for CS, MDH, LDH and PK. Additionally, the two families of skates, Arhynchobatidae and Rajidae, had the highest mean values among those families for which MDH activities were measured. Statistical analyses of species grouped by order (with the exclusion of the single species order Chimaeriformes) revealed no statistically significant difference in CS activity. PK activity was significantly highest in Lamniformes (p-values <0.01). LDH was more variable, with Lamniformes and Carcharhiniformes being significantly higher than most other orders. MDH activity was statistically higher in the order Rajiformes compared to Carcharhiniformes (two individuals of the family

Scyliorhinidae) and Squaliformes (p-values <0.05). However, the relative variability in the range of MDH activities in the Rajiformes was extremely large.

Hierarchical clustering of WM CS and LDH activity data from this and published studies reveals a more complex picture (Figure 13). Clusters seem to primarily follow the high variability of LDH activity, with the variability in CS driving within cluster significant relationships. *A. badia* is the extreme exception to this trend, with the highest recorded CS activity and relatively low LDH activity. This species clusters with the lamnid endothermic sharks *Isurus oxyrinchus*, *Lamna ditropis*. At approximately 80% similarity, three clusters exist for the remaining data. From the top of the figure, the first cluster is comprised of mostly shallow benthic rays and benthopelagic sharks, all of which are statistically indistinguishable from one another. The epipelagic oceanic shark *Alopias vulpinus* is also clustered in this group. This cluster is the most similar to that of the Lamnid sharks and *A. badia*. The second cluster is composed mainly of shallower dwelling benthic skates, with the exception of the deep-sea skate *B. microtrachys*. The cluster also includes the Squalid *S. acanthias*, the benthopelagic deeper-dwelling shark *Scyliorhinus canicula*, and the shallow ray *T. californica*. The final cluster is composed of mainly deeper dwelling benthic skates and benthopelagic Squalid and Scyliorhinid sharks. This includes one shallower species of skate, *R. stellulata*. The one major exception is the highly migratory, slow-swimming epipelagic shark, *Prionace glauca*. This shark is statistically similar to the Squalid *Centroscyllium fabricii* and the skates *B. kincaidii, R. stellulata, and B. abyssicola.* There are no statistically significant clusters for RM.

Proximate chemistry

Table 2 summarizes the mean values for % protein, water and lipid content of each species. The % protein content for the fish assayed decreases insignificantly with increasing MDO (p-value = 0.25; Figure 14a); % water content shows a positive, but insignificant, trend with increasing MDO (p-value = 0.224; Figure 14b); and lipid content shows relatively no trend with MDO (p-value = 0.82; Figure 14c). *S. pacificus* was shown to be statistically far higher in lipid contents then all other species (p-value <0.0001). Additionally, there is a statistically significant decrease in % protein with log scaled body mass (p-values<0.0001; Figure 15).

Table 1. Summary of the mean optimal enzyme activities (units/g wet weight) of WM, both pectoral (WMp) and vertebral (WMv), and RM CS, MDH, PK, and LDH at 10˚C ± 1 standard deviation where applicable. Literature data were corrected for CS (CS= 1.29*(fullspun CS)+0.41) and for temperature using a Q_{10} of 2.0. Median depths of occurrences were obtained from Ebert (2003), Froese and Pauly (2004) and Carrier et al. (2010). If the sample size was different than the reported "n" for a given assay, the number was reported in ( ) below the value. Sources: [1] Alp et al. 1976, [2] Bernal et al. 2003, [3] Crabtree and Newsholme 1972, [4] Dickson et al. 1988, [5] Dickson et al. 1993, [6] Moon and Mommsen 1987, [7] Sullivan and Somero 1980, [8] Treberg et al. 2003, [9] Zammit et al. 1978.

Species	n	Fish wt range (kg)	Min depth occurrence (m)	Max depth occurrence (m)	MDO (m)		Oxidative metabolism		Glycolytic/anaerobic metabolism		Source
							CS	MDH	PK	LDH	
Sharks											
Alopias vulpinus (common thresher shark)	6	6.3 - 43.1	0	366	183	WM	1.03 ± 0.32			207.4 ± 41.15	[2]
						RM	17.67 ± 2.01			100.7 ± 10.55	
Apristurus brunneus (brown catshark)	7	0.048 - 1.0	33	1298	665.5	WM	0.60 ± 0.22	7.27 ± 1.54	14.56 ± 7.63 (6)	17.55 ± 5.94	this study
Carcharhinus acronotus (Atlantic blacknose shark)	2	0.5 - 3.1	9	64	36.5	WM				532.45	[2]
Carcharhinus limbatus (blacktip shark)	1		0	64	32	WM	0.78		63.8	385	[4]
						RM	30.63		26.6	46.85	
Carcharhinus plumbeus (sandbar shark)	2		1	280	140.5	WM	0.96		80	193	[4]
						RM	12.19		25.6	58.1	
Centroscyllium fabricii (black dogfish)	5	0.03 - 0.09	180	1600	890	WM	0.84 ± 0.48	5.73 ± 1.46	26.02 ± 7.42	50.77 ± 13.79	[8]
						RM	11.05 ± 1.83	106.21 ± 13.72	37.34 ± 12.78	47.38 ± 11.02	
Isurus oxyrinchus (shortfin mako shark)	37	4.8 - 60.8	0	500	250	WM	1.57 ± 0.33		196.8 ± 37.8	551.35 ± 27.0	[2, 5]
						RM	15.19 ± 1.46		59.9 ± 18.8	112.78 ± 9.6	
Lamna ditropis (Salmon shark)	2	127 - 136	0	375	187.5	WM	1.9			627.25	[2]
						RM	23.02			124.95	
Mustelus californicus (gray smoothhound shark)	2	0.19 - 0.26	0	46	23	WM	0.83		126.65	339.70	[5]
Parmaturus xaniurus (filetail catshark)	4	0.18 - 0.33	91	1251	671	WM	0.73 ± 0.11	9.38 ± 4.15	46.68 ± 3.66	12.98 ± 9.92	this study
Prionace glauca (blue shark)	4	34 - 59	1	350	175.5	WM	0.74 ± 0.27		51.65 ± 2.75	74.55 ± 9.55	[5]
						RM	14.51 ± 4.26		14.35 ± 1.45	35.7 ± 3.95	
Rhizoprionodon terraenovae (Atlantic sharpnose shark)	8	0.1 - 0.7	10	280	145	WM				462.35 ± 26.15	[2]
Scyliorhinus canicula (dogfish)	3		10	780	395	WM	1.18		96.17	116.67	[1, 3, 9]
						RM	90.65		35.71	38.89	
Somniosus pacificus (Pacific sleeper shark)	3	2.84 - 49.18	0	2000	1000	WM	0.49 ± 0.08	7.29 ± 2.50	43.44 ± 6.63	37.28 ± 13.10	this study
						RM	6.80 ± 1.54 (2)	103.02 ± 57.85 (2)	22.23 ± 5.30 (2)	56.02 ± 35.57 (2)	

Table 1 (continued)

Species	n	Fish wt range (kg)	Min depth occurrence (m)	Max depth occurrence (m)	MDO (m)		Oxidative metabolism		Glycolytic/anaerobic metabolism		Source
							CS	MDH	PK	LDH	
Sphyrna lewini (scalloped hammerhead shark)	10	0.5 - 0.8	0	512	256	WM	0.76 ± 0.23			323.3 ± 19.85	[2]
						RM	22.05 ± 0.91			76.45 ± 6.2	
Squalus acanthias (spiny dogfish)	7		0	1460	730	WM	0.98	4.78 ± 0.54	42.01	116.39	[7, 8]
						RM	18.49 ± 3.1	72.12 ± 8.41	41.86 ± 7.44	28.43 ± 7.04	
Triakis semifasciata (leopard shark)	9	0.14 - 0.81	1	91	46	WM	0.72 ± 0.25		117.55 ± 12.30	330.60 ± 10.75	[5]
						RM	13.80 ± 1.43		28.90 ± 2.30	94.70 ± 4.10	
Rays											
Amblyraja badia (broad skate)	6	0.04 - 2.94	846	2324	1585	WMv	0.98 ± 0.24 (3)	12.90 ± 2.15 (3)	35.72 ± 9.30 (3)	54.29 ± 16.55 (3)	this study
						WMp	2.65 ± 0.66	20.46 ± 5.61	34.77 ± 9.41	48.07 ± 12.60	
						RM	15.35 ± 7.11 (5)	99.51 ± 25.68 (5)	42.08 ± 8.78 (5)	50.22 ± 11.78 (5)	
Bathyraja abyssicola (deepsea skate)	4	3.87 - 10.02	362	2906	1634	WMv	0.37 ± 0.05 (3)	14.13 ± 7.57 (3)	28.44 ± 6.90 (3)	64.57 ± 21.35 (3)	this study
						WMp	0.70 ± 0.13	14.42 ± 3.59	32.00 ± 10.25	46.32 ± 15.44	
						RM	22.95 ± 1.45 (3)	137.87 ± 19.64 (3)	35.28 ± 0.70 (3)	55.46 ± 54.47 (3)	
Bathyraja kincaidii (sandpaper skate)	8	0.12 - 1.04	55	1372	713.5	WMp	1.11 ± 0.78 (6)	15.73 ± 4.67 (6)	54.71 ± 16.89 (6)	70.30 ± 15.72 (6)	this study
						RM	14.61 (1)	133.72 (1)	33.62 (1)	87.0 (1)	
Bathyraja microtrachys (fine-spined skate)	5	1.18 - 1.97	1995	2900	2447.5	WMv	0.41 ± 0.05 (3)	10.05 ± 1.51 (3)	26.10 ± 11.57 (3)	45.26 ± 17.60 (3)	this study
						WMp	1.68 ± 1.01	21.89 ± 8.64	41.59 ± 21.84	59.93 ± 27.92	
						RM	23.61 ± 3.65 (4)	150.51 ± 8.14 (4)	40.17 ± 11.53 (4)	82.84 ± 11.26 (4)	
Bathyraja trachura (roughtail skate)	7	1.46 - 3.9	213	2550	1381.5	WMv	0.41 ± 0.06 (3)	14.64 ± 3.58 (3)	57.80 ± 14.82 (3)	66.63 ± 14.55 (3)	this study
						WMp	0.57 ± 0.16	15.64 ± 4.45	64.85 ± 14.79	63.67 ± 14.20	
						RM	17.42 ± 3.07 (5)	140.93 ± 17.76 (5)	55.17 ± 14.75 (5)	112.79 ± 10.51 (5)	
Platyrhinoidis triseriata (thornback ray)	4	0.09 - 0.19	1	137	69	WM	0.66 ± 0.26		106.60 ± 23.70	185.15 ± 14.20	[5]
Raja binoculata (big skate)	6	0.82 - 14.85	3	800	401.5	WMp	1.70 ± 0.19	20.26 ± 6.89	67.98 ± 19.48	85.63 ± 21.38	this study
						RM	29.41 ± 2.97 (3)	186.04 ± 16.13 (3)	105.71 ± 12.97	149.66 ± 14.42 (3)	

Table 1 (continued)

Species	n	Fish wt range (kg)	Min depth occurrence (m)	Max depth occurrence (m)	MDO (m)		Oxidative metabolism		Glycolytic/anaerobic metabolism		Source
							CS	MDH	PK	LDH	
Raja erinacea (little skate)	6	164.5	1	329	165	WM	1.22 ± 0.68	11.96 ± 1.07	49.77 ± 3.1	104.9 ± 7.81	[6]
						RM	20.93 ± 4.07	94.67 ± 11.9	52.03 ± 12.57	59.34 ± 4.16	
Raja inornata (California skate)	5	0.32 - 1.4	17	671	344	WMp	1.35 ± 0.17 (4)	17.85 ± 3.20 (4)	59.87 ± 16.19 (4)	106.79 ± 8.60 (4)	this study
Raja rhina (longnose skate)	17	0.06 - 6.12	9	1069	539	WMv	0.74 ± 0.15	18.11 ± 5.18 (3)	61.69 ± 32.18 (3)	67.32 ± 24.38 (3)	this study
						WMp	1.15 ± 0.35	15.30 ± 4.84	33.05 ± 12.46 (13)	51.23 ± 18.03	
						RM	21.98 ± 7.01 (2)	156.94 ± 36.35 (2)	62.01 ± 12.75 (2)	83.55 ± 22.89 (2)	
Raja stellulata (Pacific starry skate)	1	1.4752	18	732	375	WMp	0.74	18.72	52.94	80.07	this study
						RM	19.55	154.63	65.51	92.35	
Rhinobatos productus (shovelnose guitarfish)	8	0.06 - 1.00	1	91	46	WM	0.78 ± 0.28		145.70 ± 16.0	287.95 ± 55.85	[5]
Torpedo californica (Pacific torpedo ray)	2	0.49 - 0.70	3	200	101.5	WMv	1.29 ± 0.07	9.88 ± 1.38	39.93 ± 0.75	50.10 ± 1.16	this study
						WMp	1.05	6.62	20.25	15.51	
Urobatis halleri (round sting ray)	3	0.09 - 0.31	1	91	46	WMv	0.62 ± 0.21		91.40 ± 7.65	184.40 ± 20.50	[5]
Chimaera											
Hydrolagus colliei (spotted ratfish)	8	0.09 - 1.13	1	971	486	WM	1.54 ± 0.19	12.39 ± 1.91	36.04 ± 6.56 (7)	62.89 ± 18.18	this study

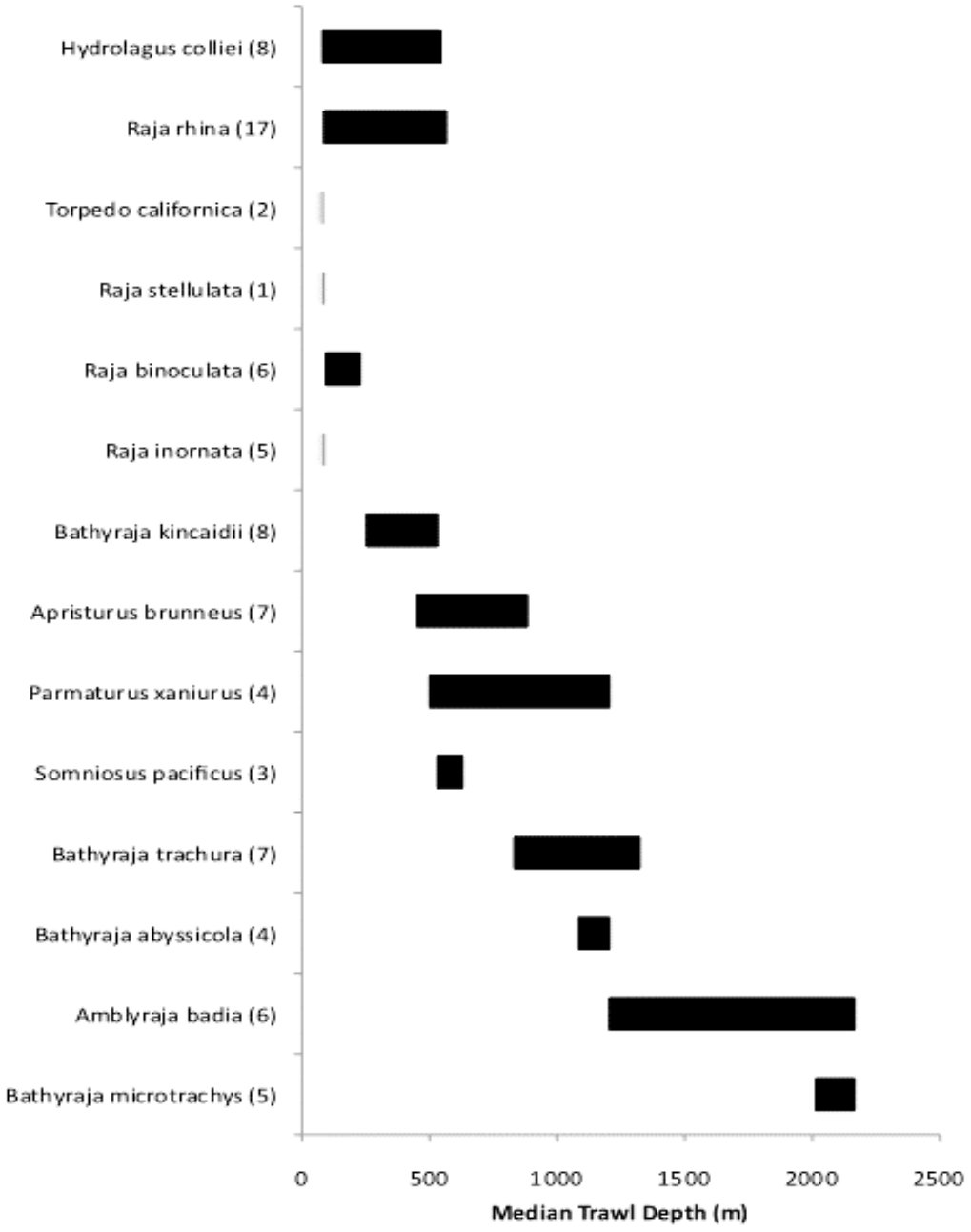

Figure 2. Range of depth of capture for 14 species of chondrichthyans collected during three research cruises off the west coast of the U.S.A. Median depths of along contour trawls were used and the sample size for each species is given in parentheses.

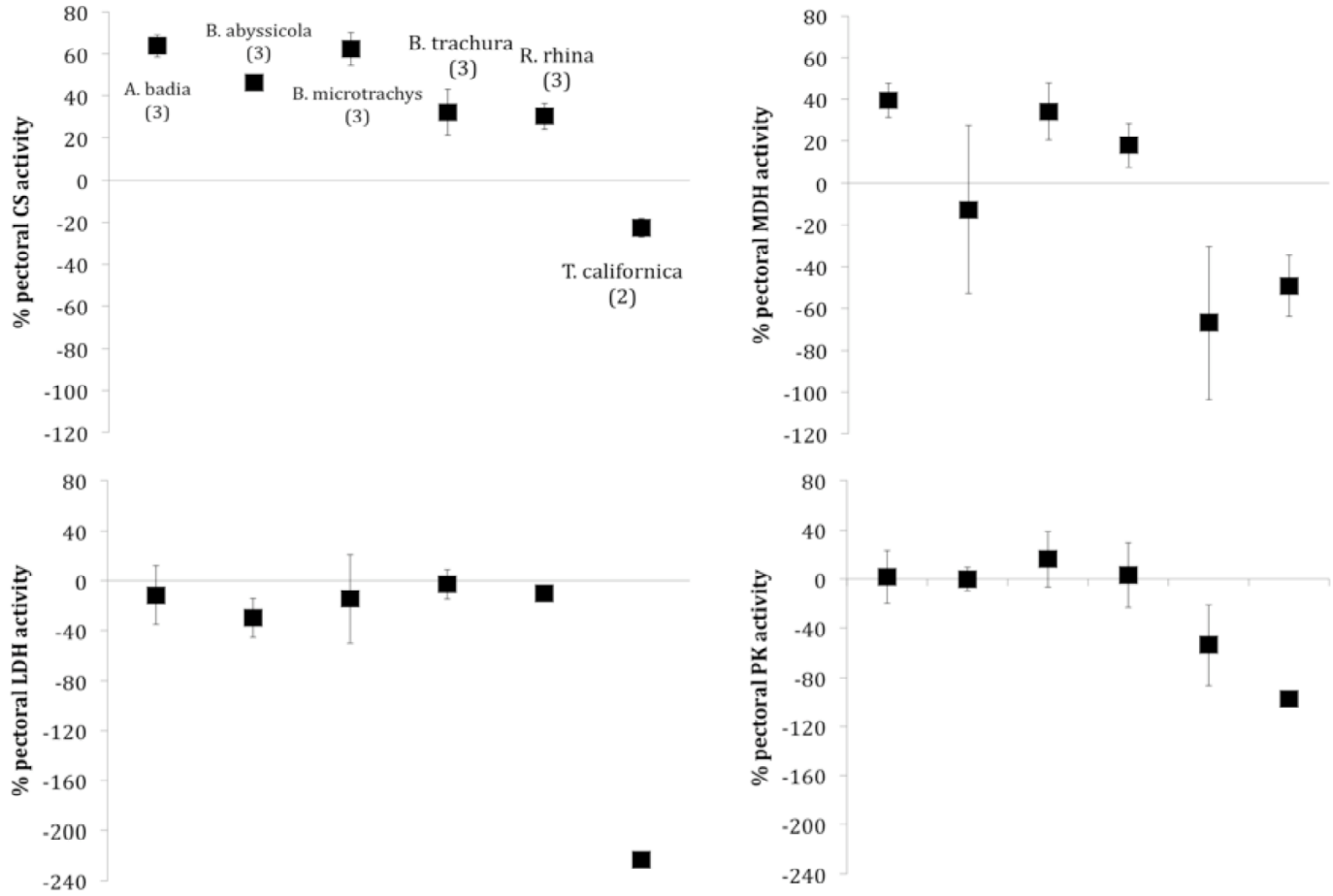

Figure 3. Plot of the % CS, MDH, LDH, and PK activities in pectoral WM versus vertebral WM averaged per species (± 1 standard error) for *A. badia, B. abyssicola, B. microtrachys, B. trachura, R. rhina,* and *T. californica* (shown in order from left to right in each plot). Plots above the 0 value line indicate higher activities in pectoral muscle, while plots below indicate higher activities in vertebral muscle.

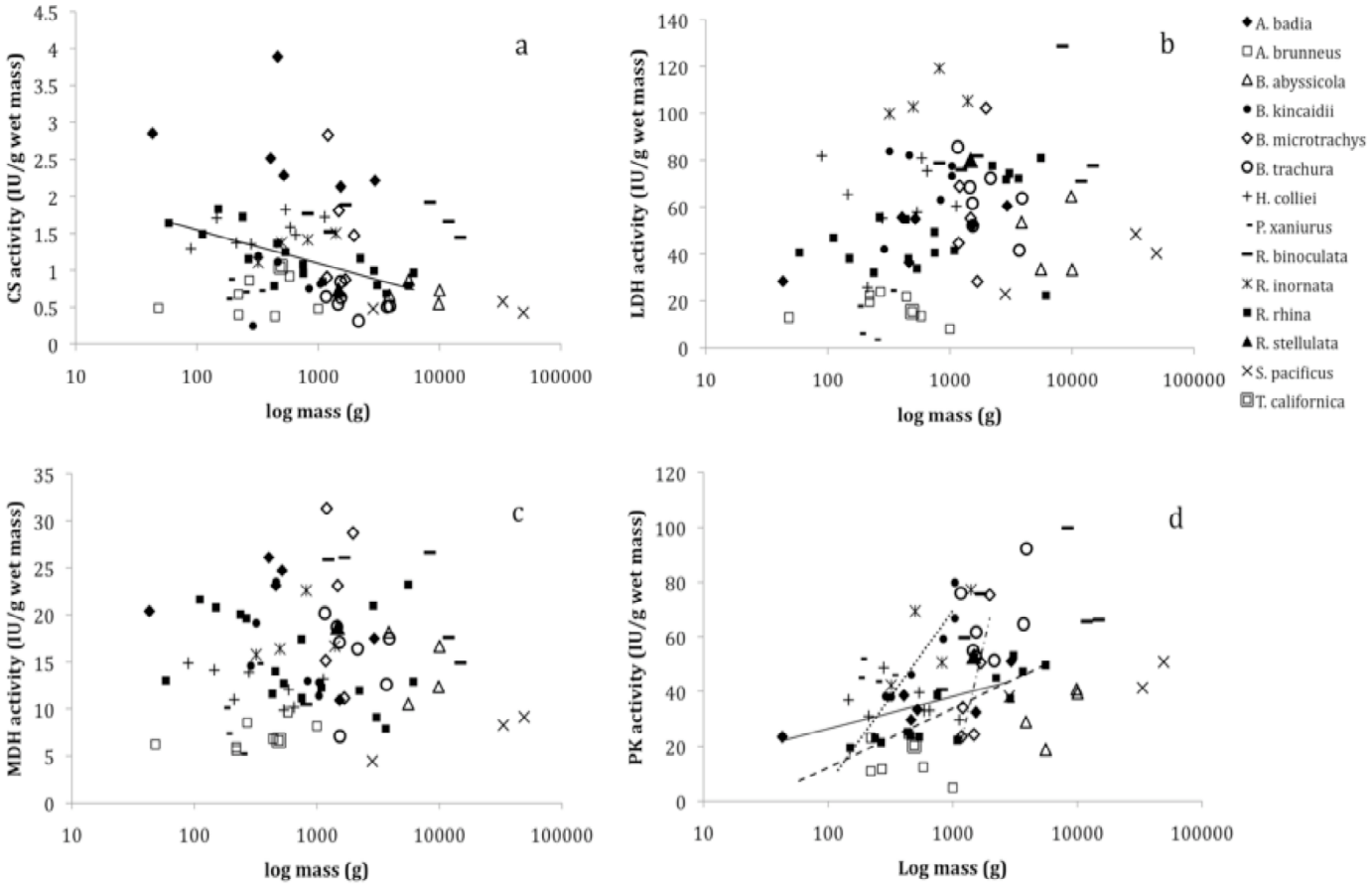

Figure 4. Relationships between WM CS, MDH, LDH, and PK activity (IU/g wet mass) at 10˚C with log mass (g). (a) Shows one significant decreasing linear trend in CS activity with increasing mass (p-value= 0.012) in *R. rhina.* (b,c) MDH and LDH activity respectively, showed no significant trends. (d) Shows four significant increasing linear trends in PK activity with increasing mass in *A. badia* (p-value= 0.039), *B. kincaidii* (p-value= 0.002), *B. microtrachys* (p-value= 0.043) and *R. rhina* (p-value<0.001).

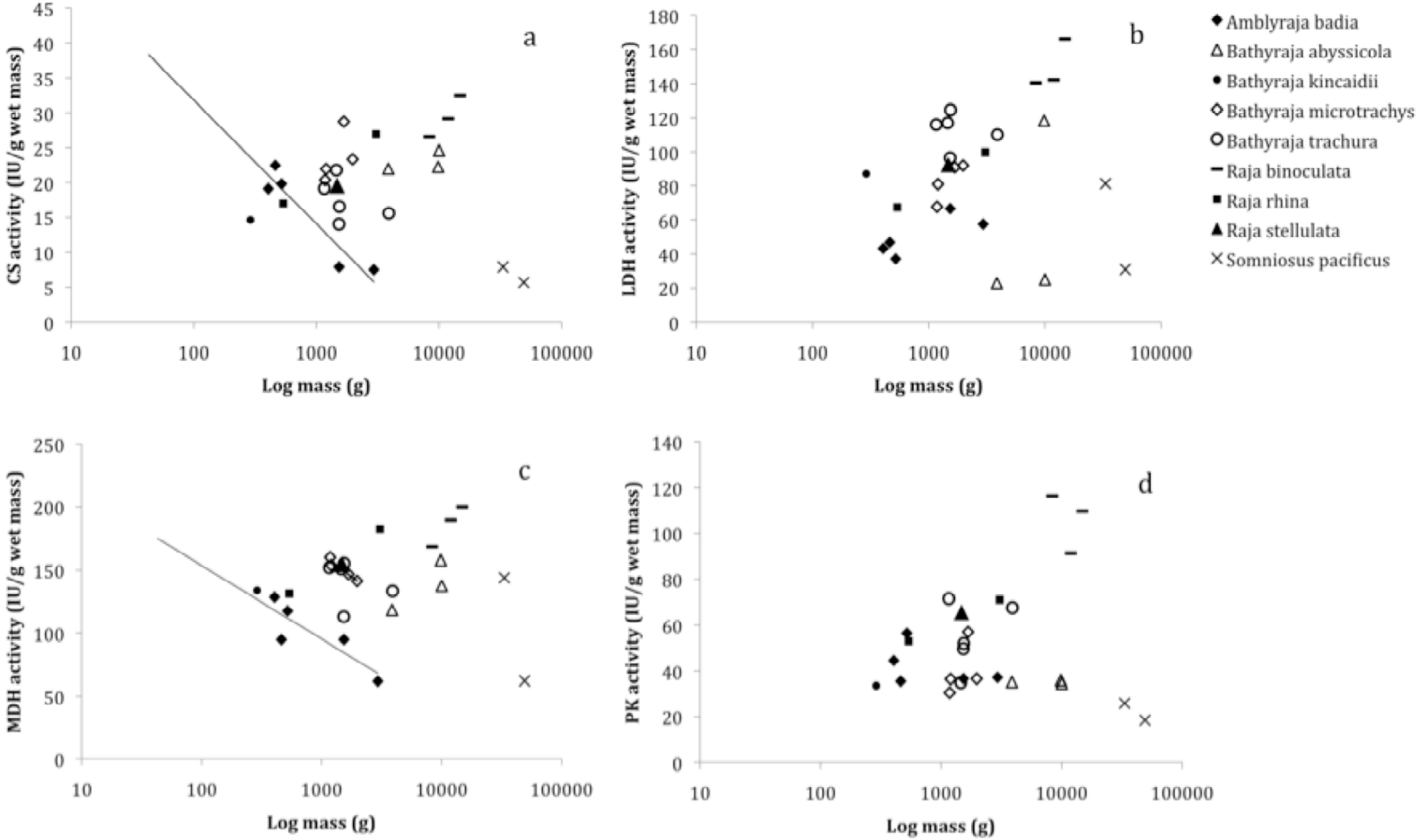

Figure 5. Relationships between RM CS, MDH, LDH, and PK activity (IU/g wet mass) at 10˚C with log mass (g). (a) Shows one significant decreasing linear trend in CS activity with increasing mass (p-value=0.047) in *A. badia.* (b) Shows two significant decreasing trends in MDH activity with *A. badia* (p-value= 0.046) and *B. microtrachys* (p-value= 0.042). (c,d) Show no significant trends in either LDH or PK activities with increasing log mass.

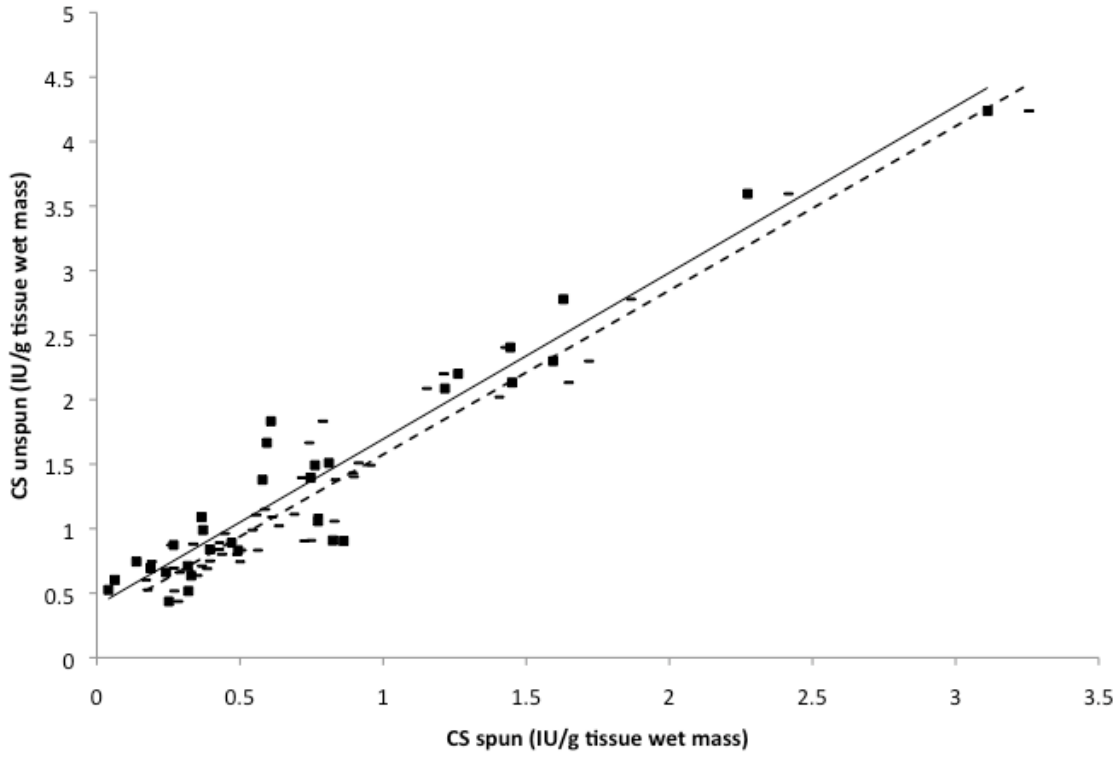

Figure 6. Relationship between unspun CS homogenate assays and CS quickspun (20sec at 5000g, dotted line and dash symbols; y=1.27x+0.30, r^2=0.94) and CS fullspun (5min at 5000g, solid line and squares; y= 1.29x+0.41, r^2=0.92) supernatant assays at 10˚C. Points are individual assays per fish for the species *M. pacificus, A. fimbria, C. acrolepis, R. inornata, A. brunneus, and A. badia.*

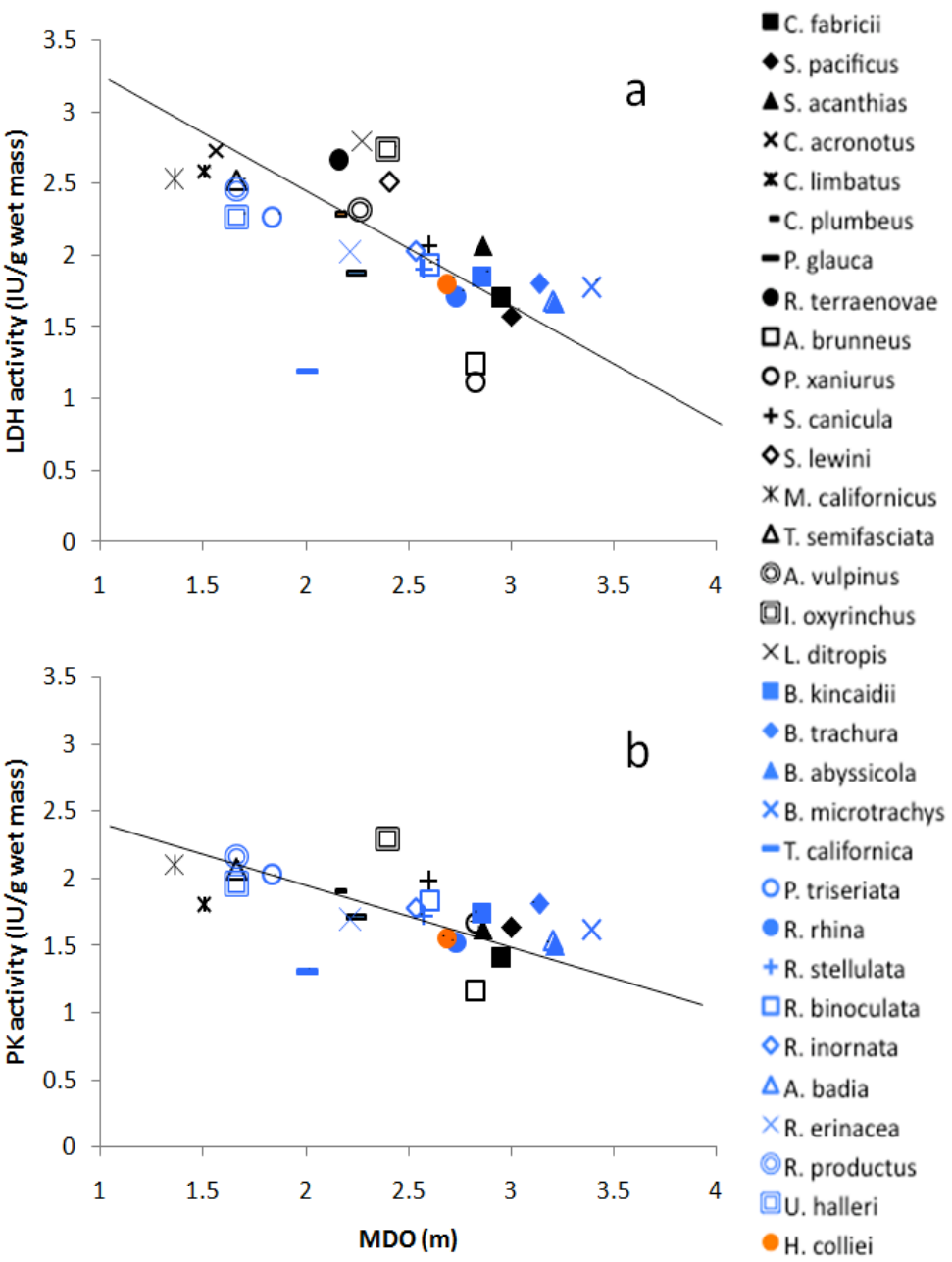

Figure 7. (a) Log-log plot of mean WM LDH activity (IU/g wet mass) per species at 10˚C plotted versus MDO (m). log(y)= -0.830*log(x) + 4.058 (r^2= 0.29, p-value= <0.001). (b) Log-log plot of mean WM PK activity (IU/g wet mass) per species at 10˚C plotted versus MDO (m). log(y)= -0.460*log(x) + 2.859 (r^2= 0.16, p-value= 0.002). Symbols: black= sharks; blue=skates and rays; orange= chimaera.

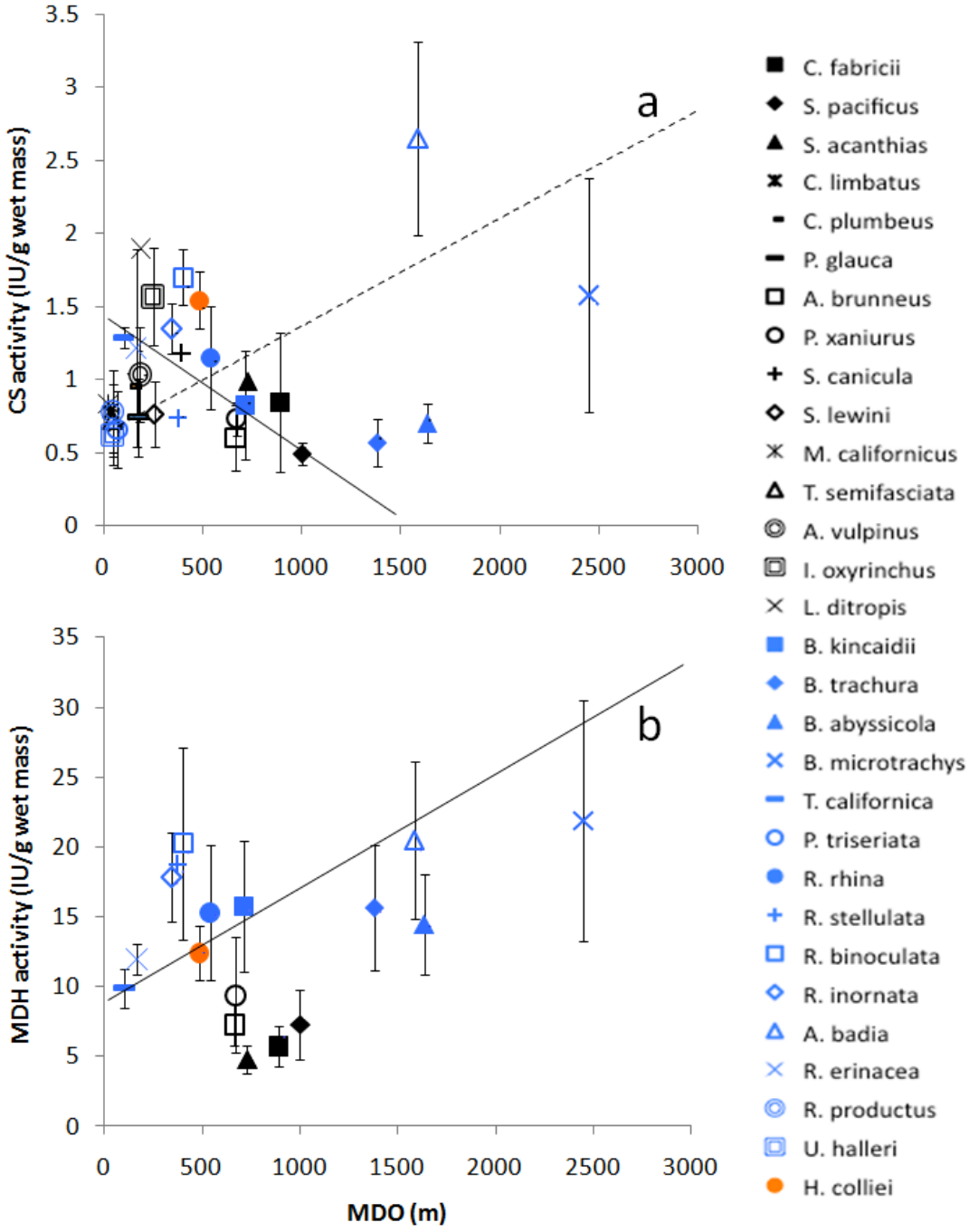
a
b
CS activity (IU/g wet mass)
MDH activity (IU/g wet mass)
MDO (m)
0
0.5
1
1.5
2
2.5
3
3.5
5
10
15
20
25
30
35
500
1000
1500
2000
2500
3000
C. fabricii
S. pacificus
S. acanthias
C. limbatus
C. plumbeus
P. glauca
A. brunneus
P. xaniurus
S. canicula
S. lewini
M. californicus
T. semifasciata
A. vulpinus
I. oxyrinchus
L. ditropis
B. kincaidii
B. trachura
B. abyssicola
B. microtrachys
T. californica
P. triseriata
R. rhina
R. stellulata
R. binoculata
R. inornata
A. badia
R. erinacea
R. productus
U. halleri
H. colliei

Figure 8. (a) Mean WM CS activity (IU/g wet mass) per species (±1 standard deviation) at 10˚C plotted versus MDO (m). The dashed linear regression includes all data points: y=0.62 + 0.0008x (r^2= 0.20, p-value= 0.20). The solid line excludes the two deepest skates (*B. microtrachys* and *A. badia*): y = 1.46 – 0.001x (r^2= 0.48, p-value= 0.20). (b) Mean WM MDH activity (IU/g wet mass) per species (±1 standard deviation) at 10˚C plotted versus MDO (m). y= 8.68 + 0.009x (r^2= 0.04, p-value= 0.15). Symbols: black= sharks; blue=skates and rays; orange= chimaera.

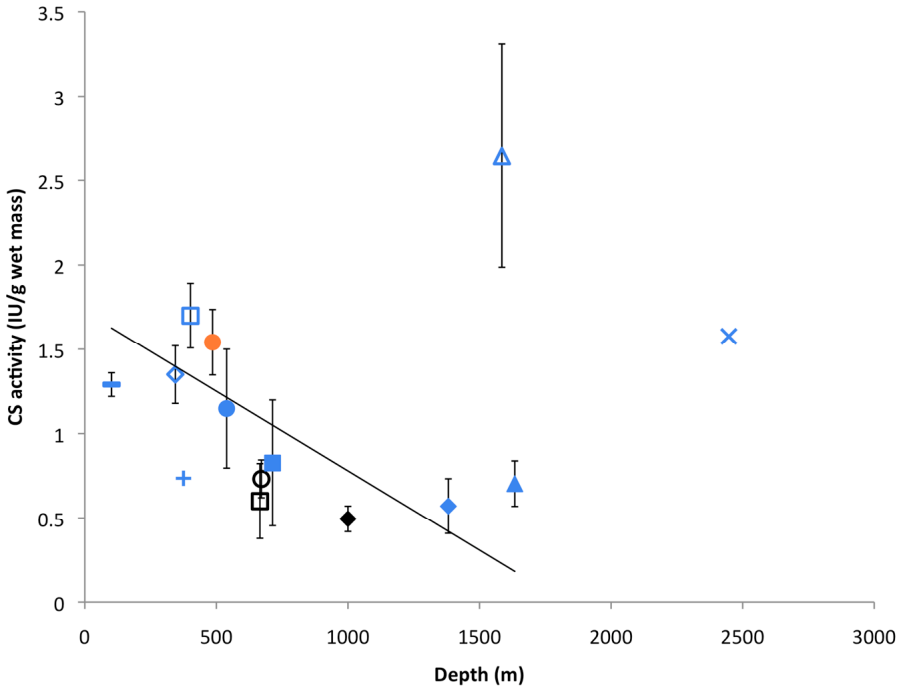

Figure 9. Mean WM CS activity (IU/g wet mass) per species (±1 standard deviation) at 10˚C plotted versus MDO (m) for data from this study only. The solid line excludes the two deepest skates (*B. microtrachys* and *A. badia*) and shows an insignificant decrease in CS activity with depth: y = 1.72 - 0.0015x (r^2= 0.18, p-value= 0.15). Symbols: black= sharks; blue=skates and rays; orange= chimaera.

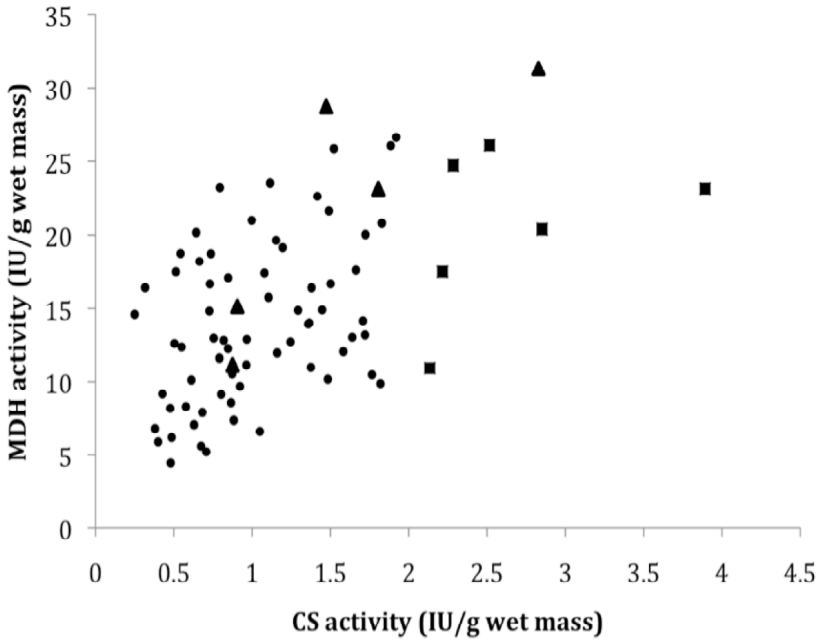

Figure 10. Correlation of WM CS and MDH activity (units/g wet weight) at 10˚C per individual fish. y = 5.1629x + 8.8758, r-value = 0.55, p-value = <0.0001. The two deepest dwelling skates are highlighted: squares= *A. badia* and triangles= *B. microtrachys*.

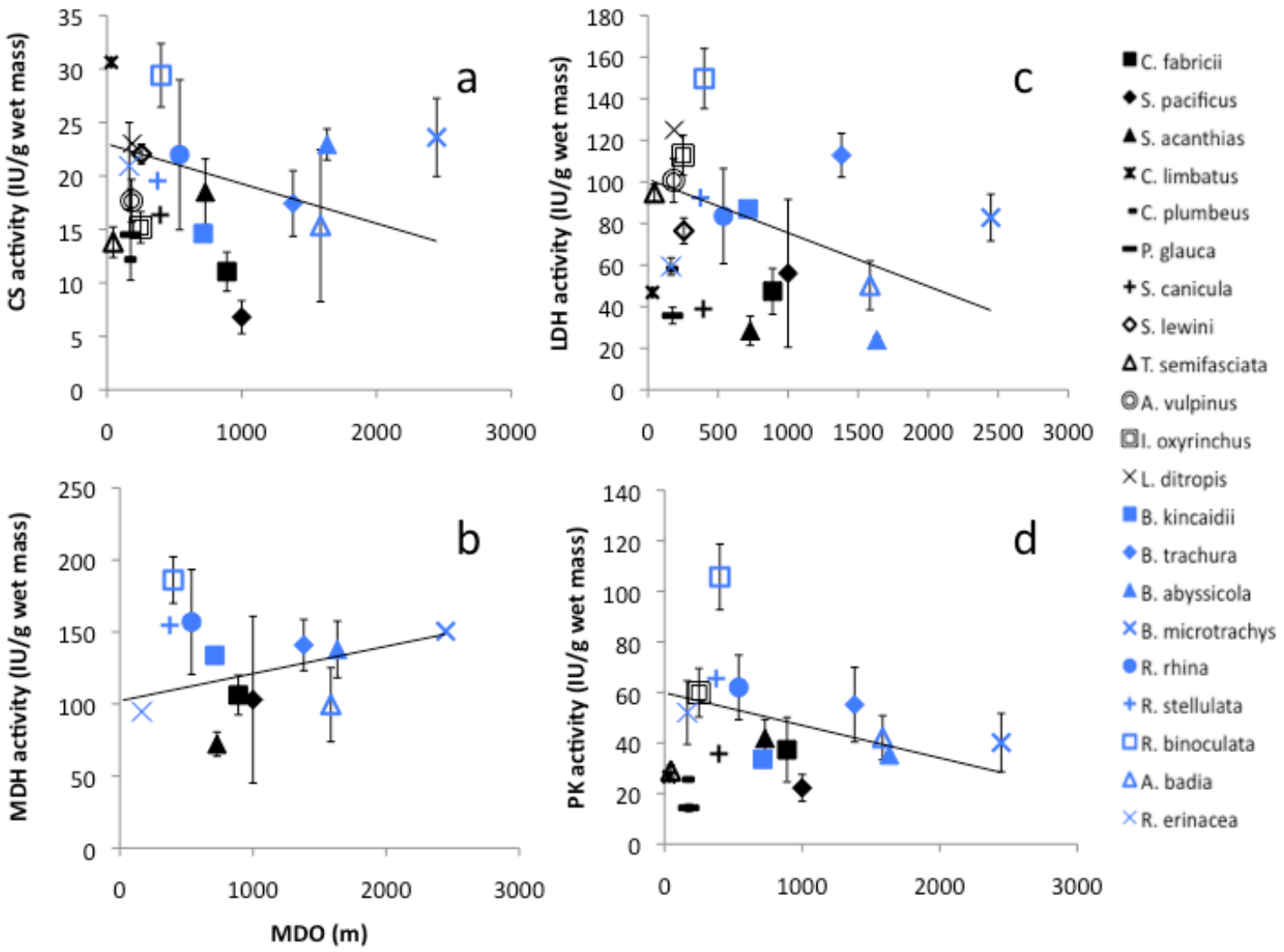
a
b
c
d
CS activity (IU/g wet mass)
MDH activity (IU/g wet mass)
LDH activity (IU/g wet mass)
PK activity (IU/g wet mass)
MDO (m)
C. fabricii
S. pacificus
S. acanthias
C. limbatus
C. plumbeus
P. glauca
S. canicula
S. lewini
T. semifasciata
A. vulpinus
I. oxyrinchus
L. ditropis
B. kincaidii
B. trachura
B. abyssicola
B. microtrachys
R. rhina
R. stellulata
R. binoculata
A. badia
R. erinacea

Figure 11. Mean RM CS, MDH, LDH and PK activity (IU/g wet mass) per species (± 1 standard deviation) at 10˚C plotted versus MDO (m). (a) CS: statistically insignificant decreasing linear trend y= 22.98 - 0.009x (r^2= 0.29, p-value= 0.92). (b) MDH: statistically insignificant increasing linear trend y= 102.17 + 0.05x (r^2= 0.18, p-value= 0.94). (c) LDH: statistically insignificant decreasing linear trend y= 101.24 - 0.05x (r^2= 0.17, p-value= 0.66). (d) PK: statistically insignificant decreasing linear trend y= 59.85 – 0.03x (r^2= 0.26, p-value= 0.91). Symbols: black= sharks; blue=skates and rays; orange= chimaera.

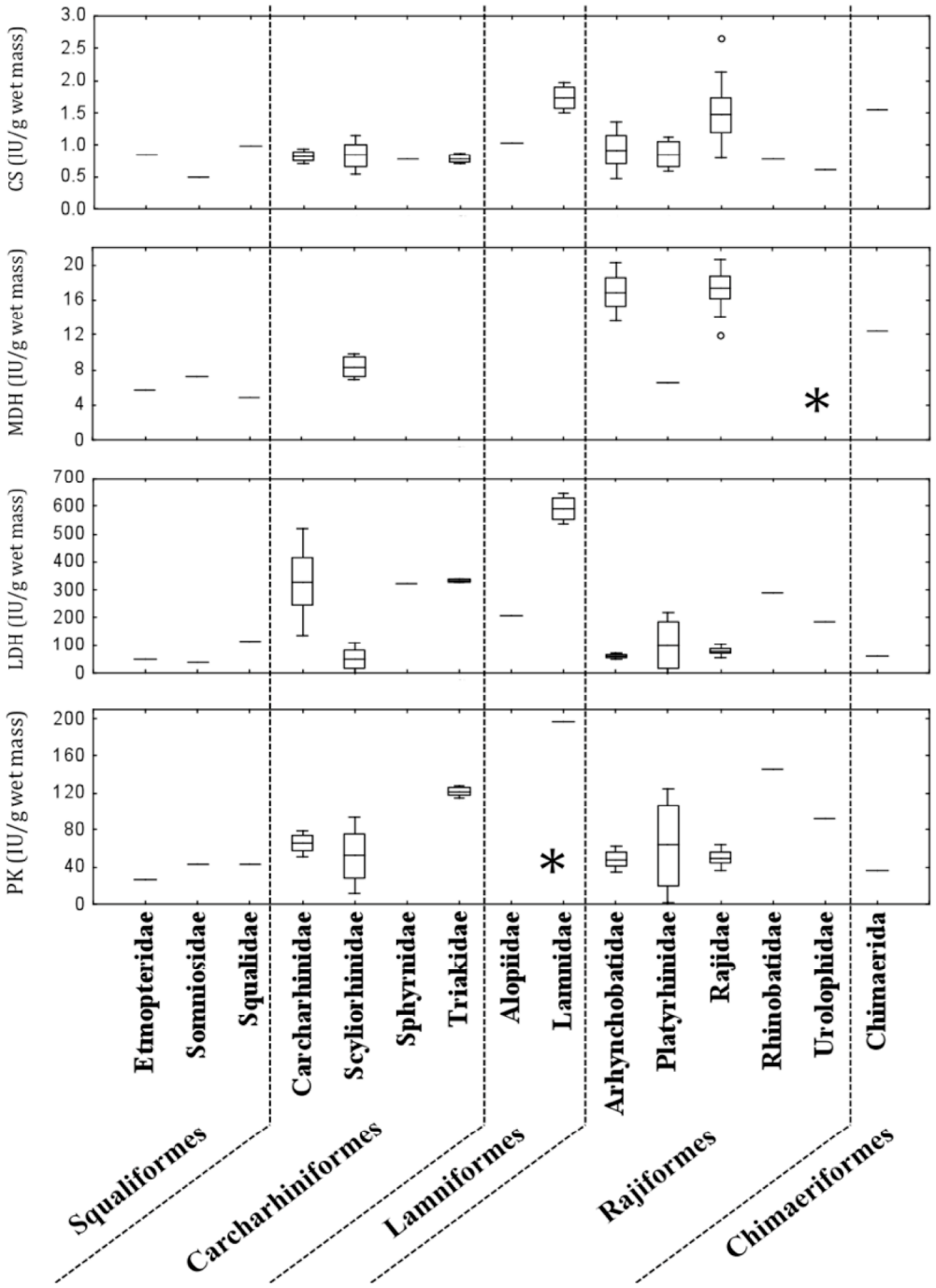

Figure 12. Boxplots of families in the orders Squaliformes, Carcharhiniformes, Lamniformes, Rajiformes, and Chimaeriformes (in order left to right) for CS, MDH, LDH, and PK activity (IU/g wet mass) at 10˚C. Boxplots: line is the mean, box is the standard error, error bars are standard deviation. Orders that are significantly higher than all others (PK of Lamniformes and MDH of Rajiformes) are indicated with an *.

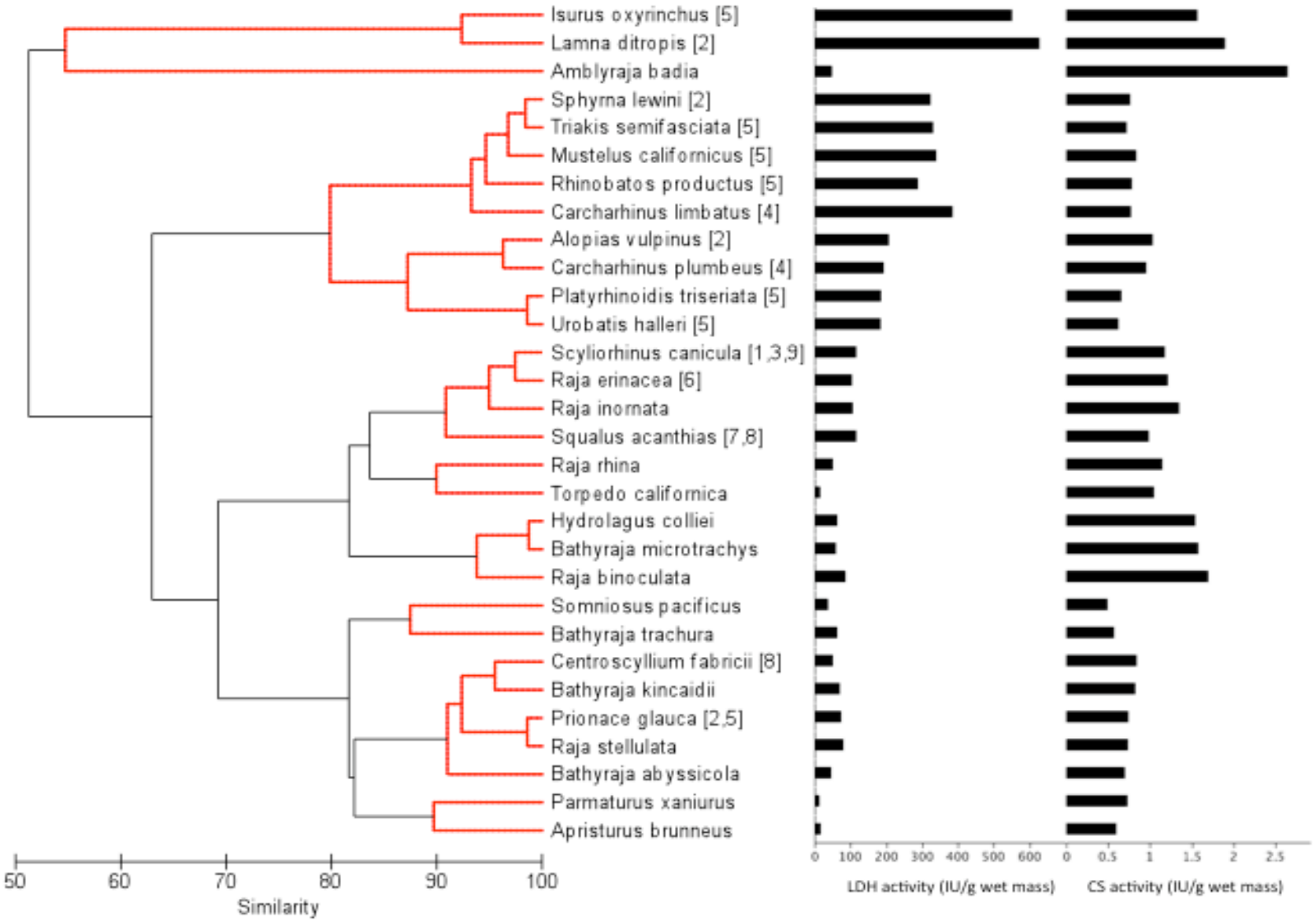
Isurus oxyrinchus [5]
Lamna ditropis [2]
Amblyraja badia
Sphyrna lewini [2]
Triakis semifasciata [5]
Mustelus californicus [5]
Rhinobatos productus [5]
Carcharhinus limbatus [4]
Alopias vulpinus [2]
Carcharhinus plumbeus [4]
Platyrhinoidis triseriata [5]
Urobatis halleri [5]
Scyliorhinus canicula [1,3,9]
Raja erinacea [6]
Raja inornata
Squalus acanthias [7,8]
Raja rhina
Torpedo californica
Hydrolagus colliei
Bathyraja microtrachys
Raja binoculata
Somniosus pacificus
Bathyraja trachura
Centroscyllium fabricii [8]
Bathyraja kincaidii
Prionace glauca [2,5]
Raja stellulata
Bathyraja abyssicola
Parmaturus xaniurus
Apristurus brunneus
50
60
70
80
90
100
Similarity
0
100
200
300
400
500
600
LDH activity (IU/g wet mass)
0
0.5
1
1.5
2
2.5
CS activity (IU/g wet mass)

Figure 13. WM hierarchical cluster analysis based on standardised CS and LDH activity values at 10˚C from this and previously published studies. CS values were adjusted according to the equation: unspun CS = 1.29* (fullspun CS) + 0.41, where published data were considered fullspun. Values were adjusted with a Q_{10} of 2.0 when necessary. Black lines represent statistically different clusters, while red lines indicate statistcally indistinguishable species within clusters. [1] Alp et al. 1976, [2] Bernal et al. 2003, [3] Crabtree and Newsholme 1972, [4] Dickson et al. 1988, [5] Dickson et al. 1993, [6] Moon and Mommsen 1987, [7] Sullivan and Somero 1980, [8] Treberg et al. 2003, [9] Zammit et al. 1978. On the right are the mean LDH and CS activity (IU/g wet mass) values for each individual species.

Table 2. Mean values for % water, protein and lipid content per species ± 1 standard deviation. If sample size was different than the reported "n" for a given assay, the number was reported in ( ) next to the value.

Species	n	% H_2O	% Protein	% Lipid
Apristurus brunneus (brown catshark)	7	84.78 ± 3.32		
Parmaturus xaniurus (filetail catshark)	4	79.26 ± 0.62	8.82 (1)	0.56 (1)
Somniosus pacificus (Pacific sleeper shark)	3	84.12 ± 1.49	4.99 ± 0.48 (2)	2.94 ± 0.18 (2)
Amblyraja badia (broad skate)	6	82.15 ± 0.83	6.78 (1)	0.36 (1)
Bathyraja abyssicola (deepsea skate)	4	80.02 ± 0.38		
Bathyraja kincaidii (sandpaper skate)	8	78.43 ± 0.89 (7)	9.99 ± 0.10 (3)	0.66 ± 0.05 (3)
Bathyraja microtrachys (fine-spined skate)	5	80.61 ± 3.70	7.99 (1)	0.72 (1)
Bathyraja trachura (roughtail skate)	7	80.43 ± 0.44		
Raja binoculata (big skate)	6	77.29 ± 0.44		
Raja inornata (California skate)	5	77.56 ± 0.72		
Raja rhina (longnose skate)	17	78.27 ± 0.89 (15)	10.74 ± 1.32 (9)	0.66 ± 0.08 (9)
Raja stellulata (Pacific starry skate)	1	77.80		
Torpedo californica (Pacific torpedo ray)	2	82.03 ± 0.99	9.41 ± 0.34	0.65 ± 0.05
Hydrolagus colliei (spotted ratfish)	8	79.26 ± 0.86	9.20 ± 1.07 (5)	0.93 ± 0.08 (5)

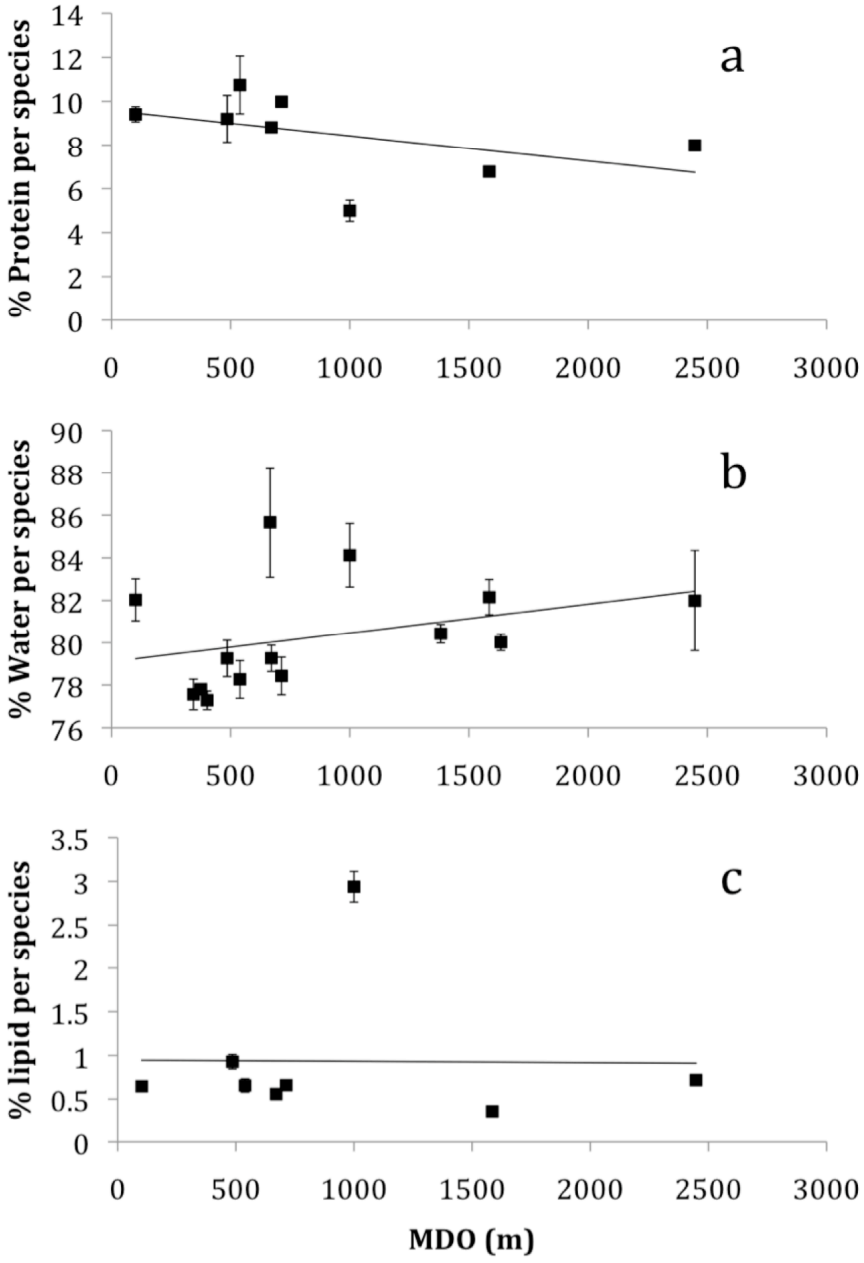

Figure 14. (a) % Protein content per species plotted versus MDO (m). $y = 9.58 - 0.0012x$ ($r^2 = 0.22$, p-value = 0.25). (b) % Water content per species plotted MDO (m). $y = 79.1 + 0.0014x$ ($r^2 = 0.12$, p-value = 0.22). (c) % Lipid content per species MDO depth (m). $y = 0.74 + 9.89E^{-5}x$ ($r^2 = 0.007$, p-value = 0.82).

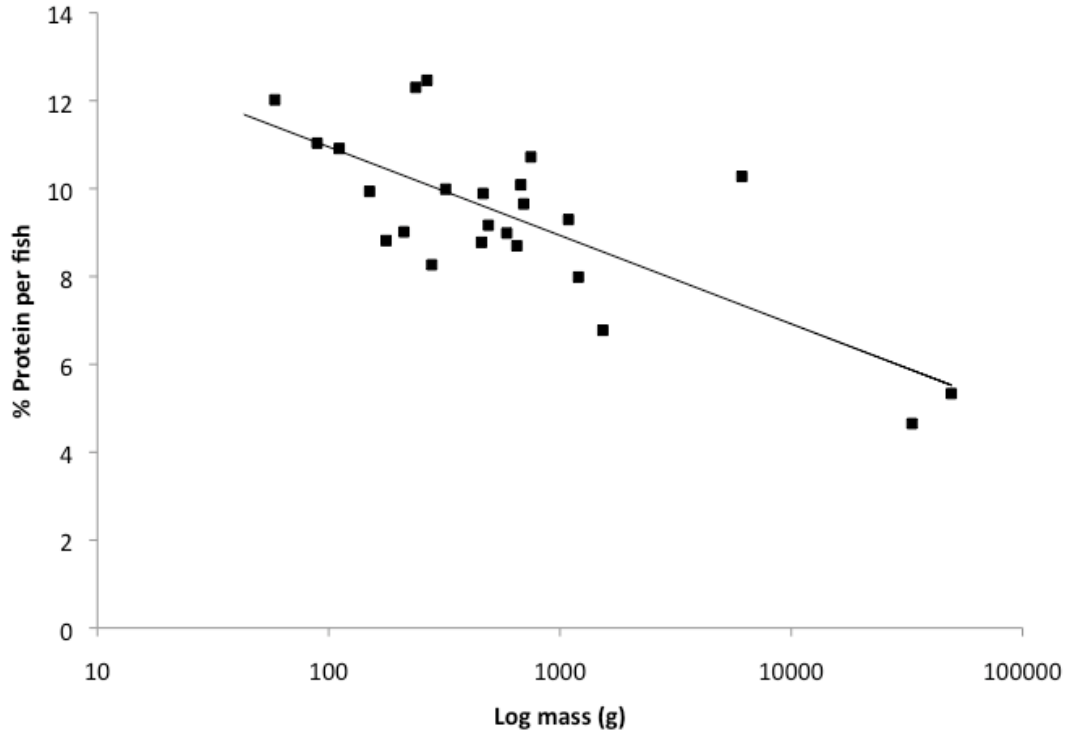

Figure 15. Significantly decreasing trend between % protein and log mass (g) for all individuals. y= -0.875*log(x) + 14.98 (r^2 = 0.56, p-value = <0.0001).

DISCUSSION

This study is the first to examine the metabolism of benthic and benthopelagic chondrichthyans over a broad depth range using multiple enzyme activities. This study has added a great deal of muscle metabolic biochemical data to existing literature. In doing so, it reveals important information about metabolic depth trends, locomotory capabilities and energy requirements of chondrichthyans.

Enzymatic trends with depth

Several hypotheses and environmental factors attempt to explain previously observed declines in enzyme activities and metabolism with increasing depth of occurrence in fishes. In this study, across a range of species (Table 1) both LDH and PK show a significant decline with MDOs ranging from 0 to 1000m, with declines most pronounced in the first few hundred meters. After this depth, both trends begin to level off to a depth of ~2500m (Figure 7). Enzyme dilution, the presence of oxygen minimum zones, food limitation, and the visual interactions hypothesis are examined here in order to provide insight into the pronounced declines in anaerobic enzyme activities, the lack of significant aerobic trends seen in this data; and to provide important information on the potential locomotory capabilities of chondrichthyan fishes with depth.

Enzyme dilution

Although depth declines were clearly seen in anaerobic enzyme activities, these trends are not likely the result of enzyme dilution. A decline in protein and an increase in water contents with depth have been observed for pelagic fishes (Childress and Nygaard

1973; Bailey and Robison 1986; Stickney and Torres 1989; Childress et al. 1990b), and crustaceans (Childress and Nygaard 1974) reflecting a reduced swimming capacity. Sullivan and Somero (1980) found a significantly higher protein content in shallow-versus deep-living fishes. Thus, depth-related trends in muscle enzymatic activity may be partially due to enzyme dilution in deeper, less robust fishes. However, this difference was extremely small when compared to the difference between shallow- and deep-living species LDH, MDH and PK activity. This study shows insignificant changes in water and protein content with depth for chondrichthyans (Figure 14), which indicates that the large depth related differences seen in LDH and PK enzymatic activity are caused by factors other than enzyme dilution.

Oxygen minimum zones

Most of the specimens obtained in this study were caught off the coast of California where waters are characterized by an OMZ between approximately 600 and 1000m (Levin 2002); however, this data suggests that the presence of this low oxygen layer does not explain the metabolic enzyme activities of deeper-dwelling chondrichthyans. OMZs have been shown to have a large regional influence on the abundance, distribution and physiology of marine organisms (Childress and Seibel 1998; Helly and Levin 2004). Species dwelling within the OMZ have been shown to utilize adaptive strategies such as the suppression of metabolic rates, reduction of high locomotory function, and morphological adaptations (i.e. increased gill surface area) for energy conservation and maintenance in these high-stress environments (Seibel 2011; Childress and Seibel 1998). If OMZs alone play a significant part in the suppression of metabolic poise with depth, one would expect to see a correlated increase in oxygen

concentration and metabolism below the OMZ. However, organisms that live in higher oxygen concentrations below the OMZ have been shown to have lower metabolic rates (Childress 1995; Childress and Seibel 1998). Furthermore, previous teleost studies have shown a continued decrease in metabolic enzyme rates at depth despite these oxygen layers (ex. Somero and Childress 1980; Drazen and Seibel 2007).

This data are consistent with previous studies in that both aerobic and anaerobic rates in deeper species remain approximately the same, or continue to decrease, below the depths of the OMZ. The deeper-dwelling benthopelagic shark *P. xaniurus* has been shown to frequent low oxygen waters to feed, where its enlarged gill slits give it an adaptive advantage (Cross 1988; Ebert 2003). Despite these observations, *P. xaniurus* has enzyme activities in line with shark species that occupy similar depth ranges (which contain known OMZs), including another scyliorhinid, *A. brunneus.* This suggests that OMZs are not the dominant factor explaining the pronounced decreases in anaerobic enzyme activity with depth in chondrichthyans.

Food limitation hypothesis

Many earlier studies proposed that the predominant driving factor in lower metabolic rates of deep-sea animals was a decrease in food availability with depth (Childress 1971; Smith and Hessler 1974; Dalfhoff 2004). This theory is based on the existence of an order of magnitude exponential decline in animal biomass from 0-1000m (Haedrich and Rowe 1977; Angel and Baker 1982; Thurston et al. 1994; Priede et al. 2006) that continues to decrease at deeper depths. It is likely that this hypothesis does not explain observed trends in this data as one would expect to see a continued decrease with increasing depth, and not a leveling off after ~1000m as indicated in this study.

Visual interactions hypothesis

The observed depth trends in metabolic enzyme rates of chondrichthyans tend to follow closely with the predictions of the VIH. The VIH suggests strong declines in metabolism with depth for visual species. The pronounced trends in WM anaerobic enzyme rates corroborate this theory. Across a range of species (Table 1) both LDH and PK show a significant decline with MDOs ranging from 0 to 1000m, with declines most pronounced in the first few hundred meters. After this depth, both trends begin to level off to a depth of ~2500m (Figure 7). The VIH predicts that these strong declines are the result of a relaxed selective pressure for anaerobic, burst-swimming capabilities with decreasing light levels in the first 1000m (Childress 1995; Drazen and Seibel 2007). As WM tissue is powered by anaerobic metabolism and specialized for burst swimming (Bone 1966; Sullivan and Somero 1980), there is great potential for wide variations in glycolytic enzyme activity to be indicative of differences in burst locomotory capacity. This parallels findings in teleost LDH activity with minimum depth of occurrence (Drazen and Seibel 2007; Childress and Somero 1979; Sullivan and Somero 1980), and suggests a corresponding decrease in burst-swimming capacity in chondrichthyans with increasing MDO.

The VIH is further supported by the lack of significant declines in the potential for routine swimming in RM with depth. The VIH predicts changes in WM enzyme activity and subsequent capacity for burst locomotion with decreasing light levels and reactive distances; however, RM fibers, that power sustained slow aerobic swimming (Bone 1966, 1988; Johnston 1981; Somero and Childress 1980), should remain relatively constant with increasing depth irrespective of changes in ambient visibility and burst swimming capacity. Such things as routine migration, foraging, and/or obligate swimming that are

powered by RM metabolism are intrinsic daily energetic requirements and should be maintained with depth. Although interspecific differences in RM metabolic potential may exist due to variation in energy expenditure caused by differences in feeding strategy, migratory distance, and respiration, very little interspecific differences were seen in this data.

It has been suggested that deep living cephalopods have fairly active aerobic metabolism with a highly reduced burst swimming capacity (Seibel et al. 1997, 2000) that closely mimics trends seen in these deeper-dwelling elasmobranchs. Cephalopods show marked depth-related declines in enzymatic activities in mantle muscle that supplies burst-swimming capacity, but not in fin and arm muscles that are utilized for undulatory, slow sustained swimming. The preponderance of deep living species using fin swimming and medusoid arm propulsion indicates that it is a more efficient means of locomotion where high-speed is not required (i.e. deep-sea environments; Seibel et al. 1997, 2000). This shift in locomotory preference with depth may also be seen in elasmobranches, with a preferential adaptive selection for more efficient routine swimming in deeper-dwelling elasmobranchs in conjunction with a decrease in burst locomotory capacity. With the strong exception of the two deepest dwelling skates, *A. badia* and *B. microtrachys*, this is indicative of relatively consistent potential for aerobic routine swimming among these species with depth.

The depth trends seen in WM aerobic enzymes in this data are inconsistent with trends seen in previous studies that support the VIH. A clear relationship between routine and maximum metabolism and burst locomotory capacity has been shown (Seibel and Drazen 2007). Teleost studies show decreases in both anaerobic and aerobic (MDH and to a lesser extent CS) enzyme activities with depth in some species (Childress and Somero

1979; Sullivan and Somero 1980; Drazen and Seibel 2007). In contrast, despite large decreases in anaerobic capacity with depth in WM, chondrichthyans show no significant trends in aerobic capacity (Figure 8). Removing potential error induced by corrected literature CS values, an insignificant decrease is additionally seen in my data alone (Figure 9). This is unexpected, as a strong correlation between WM LDH and CS activity has been suggested for several species of teleosts, as well as between six relatively active shark species, with high CS activities maintained in the mitochondria in the presence of high LDH activities in order to facilitate quick post-burst swimming recovery through the processing of accumulated lactate (Dickson 1995, 1996; Gleeson 1996; Mollet and Cailliet 1996; Bernal et al. 2003). In fishes, lactate generated from burst swimming is converted to glycogen or oxidized to CO_2 and H_2O either in the WM, or is shuttled to other highly aerobically poised tissues such as the liver, heart and gills via the circulatory system in order to facilitate recovery (Bilinkski 1974; Driedzic and Hochachka 1978).

The lack of correlation between aerobic and anaerobic activities likely comes from differences in lactate metabolism between chondrichthyans and teleosts. A lack of a significant relationship between LDH and CS activity in WM in the chondrichthyans examined in this study suggests that WM may not play a significant role in recovery from lactate accumulated after repeated bursts in swimming. Backey (2007) showed that, based on current knowledge of glycogenic lactate processing, not only is WM not the site of lactate conversion to glycogen in three species of shark (*P. glauca, Triakis semifasciata,* and *I. oxyrinchus*), but that the capacity for lactate processing does not correlate with the capacity for lactate production. This contradicts earlier research that indicated retention of lactate in *Squalus acanthias* WM, with the fate of lactate during recovery being gluconeogenesis and glycogen synthesis in place of oxidation (Richards et al. 2003).

Although much further study is needed, this study indicates that chondrichthyans analyzed either: (1) simply oxidize all lactate production with very little conversion to glycogen, using new food sources to synthesize glycogen; or (2) shuttle the majority of lactate into the blood for oxidation or glycogen synthesis in other tissues (such as the heart or liver) with very little reliance on WM CS activity. In either case, the specific physiology of chondrichthyans compared to teleosts explains why no decline in aerobic WM enzyme activity was seen despite strong declines in anaerobic activities.

Metabolic depth limitations

The theory proposed by Priede et al. (2006), which states that the energetic demand of chondrichthyans and maintenance of large-lipid rich livers restricts these species to shallower depths than bony fish, is partially refuted in this data. This study suggests that metabolic trends in WM and RM with depth may not be restricting factors in the depth distribution of chondrichthyan species. Trends in enzymatic activities of LDH, PK, CS and MDH reveal potential decreases in the burst locomotory capacity of chondrichthyans with depth coupled with consistent potential for WM aerobic capacity and sustained swimming behavior. Similar depth trends are observed in teleosts (Sullivan and Somero 1980; Drazen and Seibel 2007). Dickson et al. (1993) additionally came to the conclusion that teleosts and elasmobranches of similar activity level have comparable enzyme activities. This reveals that metabolism is similar between chondrichthyans and teleosts at a given depth, additionally suggesting similar energy demands. This contradicts the supposition proposed by Priede et al. (2006) that energy demands are higher in chondrichthyans compared to bony fish. Most likely, some other biological limitation is causing the shallower depth distribution of chondrichthyan species.

Furthermore, the composition of multiple classes of lipid and fatty acids within the liver, some which are more metabolically active than others, has been shown to vary greatly by species, dietary and habitat preference, and ontogenetic differences (Pethybridge et al. 2010). Further study on the energetic demand and turnover rate of liver tissue, feeding rates, whole body metabolism, and physiological differences of teleosts and chondrichthyans with depth are needed in order to understand important information on the complexities of the energetic demands of these species and to fully analyze Priede's depth limitation hypothesis.

Interspecific and phylogenetic comparisons

Although variability was observed between families within orders of chondrichthyans examined in this study, overall trends suggest that phylogeny has little influence on observed trends with surprisingly few differences seen in enzymatic activities between orders (Figure 12). In general, the active endothermic Lamniformes were the only order to reveal any consistent significant difference in multiple enzyme activities. This suggests that many sharks and skates have similar activity levels when analyzed at a constant temperature. Measurements of enzyme activities of skates and benthic rays in previous literature that indicated lower enzyme activities than species of shallow, active sharks may have been biased by the relatively few species analyzed (Dickson et al. 1993). Among the four families statistically analyzed, the only indication that shallow, active sharks had higher metabolic rates than skates was the statistically higher LDH activity in carcharhinids compared to the two skate families.

Hierarchical cluster analysis performed using CS and LDH (representing potential aerobic and anaerobic capacity) revealed a complex intermixing of phylogenetic groups

that tended to be driven primarily by decreasing LDH activity that corresponded to increasing MDO (Figure 13). Many of the skates and rays cluster significantly with one or more species of shark that are generally found at similar depth ranges. This suggests similar metabolic potential in co-occurring demersal sharks and rays.

In addition, this general analysis reveals relatively little effect of locomotory mode on enzymatic activity patterns in this data. There is a large diversity of locomotory modes among chondrichthyans (characterized as anguilliform to thunniform), with body morphology and swimming mode generally grouping among species in a given family, and generally among orders (Webb and Keyes 1982; Donley and Shadwick 2003). Three main forms are seen in the species in this study: (1) anguilliform swimming where the entire length of the body participates in lateral undulations that is utilized by elongate sharks like scyliorhinids; (2) carangiform swimming seen in more pelagic sharks, such as squalids, most carcharhinids, and some lamniformes, where undulations are confined to the posterior half of the body; and (3) thunniform swimming, representative of most lamnid sharks, where only the tail and caudal peduncle undulate (Webb 1983; Donley and Shadwick 2003). Most batoids in this study are known to utilize undulatory appendage propulsion (Webb 1983). However, many torpedo rays and guitarfish utilize their enlarged tails and caudal fins, laterally undulating much like axial undulating sharks (Rosenberger 2001). As these forms generally group by the orders analyzed in this data, this gives us some idea of the general effect of locomotory mode on enzymatic activity. A lack of clearly established differences between orders, as well as many families, may indicate a potential adaptation to maximize energetic efficiency in marine environments regardless of body type, locomotory mode and activity level. This is additional evidence

that enzyme activities seem to be influenced primarily by MDO. Further study is needed in this area to make more accurate comparisons.

Several interesting comparisons between species of sharks and skates resulted from the cluster analysis. The blue shark, *Prionace glauca*, shows significant similarity to the deep squaloid *C. fabricii* and several skate species. This epipelagic oceanic shark is highly migratory, and has been shown to swim more slowly and be less active than many other pelagic species of carcharhinid sharks (Carey and Sharold 1990). This is indicated by the large difference statistically shown between *P. glauca* and other shallow living carcharhinids in this study. The deeper-dwelling demersal shark *S. pacificus* was shown to be most similar to a deep-water skate, *B. trachura*. *S. pacificus* had among the lowest values measured for CS, PK, MDH and LDH activity out of the species studied. This reveals these individuals to be rather sluggish, slow-swimmers. Two of the three *S. pacificus* were among the largest specimens analyzed. Due to the small sample size, scaling effects were not factored into these analyses. With larger size, higher anaerobic and lower aerobic capacity is expected, as was seen in a few of the species analyzed in this study (Figure 4). If adjusted for size, the aerobic capacity may be more comparable to smaller sharks and skates, however anaerobic capacity would remain significantly lower. This further indicates a smaller potential for burst locomotion in these species. *S. pacificus* is generally considered a bottom feeder that may employ lie-in-wait ambush predation to catch larger more active prey (Ebert et al. 1987; Ebert 2003). However, the enzyme data does not support this contention. As all three individuals were juveniles, one explanation for the observed difference could be a reliance on carrion among smaller individuals of this species, reducing the need for high enzymatic activity and lie-in-wait predation.

Most interesting, however, is the significant similarity between two endothermic lamnid sharks and the deep-water skate *A. badia*. This similarity is driven solely by the relatively high CS activity found in this species of skate. These species of shark regionally conserve metabolic heat derived from RM activity through counter-current heat exchange, elevating the temperature of red and other muscle tissue above that of ambient water (Bernal et al. 2001a,b, 2005; Carey et al. 1971; Anderson and Goldman 2001). Thus, at in-situ muscle temperatures the lamnid sharks' enzymatic activity rates would be far higher than that of *A. badia*. Both *A.* badia and *B. microtrachys*, the two deepest dwelling skates in this study, had unexpectedly high aerobic enzyme activity levels (Figure 8). The fact that these values fell in line with the statistically significant correlation between MDH and CS activity values in my data strongly suggests that these activities are not an artifact of experimental error (Figure 10).

The high WM aerobic values seen in these skates may be, in part, explained by the theory that WM contributes not only to anaerobic burst swimming, but also to intermediate sustained swimming. Gruber and Dickson (1997) show this in leopard sharks. After 6 weeks of endurance training swimming at 60% of maximal sustainable speeds, there was a 3.6% increase in WM fiber diameter and a 34% increase in both CS and LDH activity. This indicates that higher CS in WM is linked to a sustained swimming performance. This leads to the conclusion that these two deep-sea skates may exhibit some form of sustained swimming behavior compared to the other skates examined. The relatively high values of CS and MDH activity in the white pectoral fin muscle compared to the vertebral muscle further suggests some form of sustained, undulatory pectoral fin movement (Figure 3). Tagging studies (mark/recapture and telemetry) for Rajidae species have indicated traveling distances on the order of hundreds of kilometers (Templeman

1984; Walker et al. 1997; Hunter et al. 2005; Sutcliffe et al. 2002; Wearmouth and Sims 2009; King and McFarlane 2010). Comparative studies on sharks and teleosts reveal traveling distances on the order of thousands of kilometers (for ex. Metcalfe and Arnold 1997; Lawson and Rose 2000; Kohler and Turner 2001; Queiroz et al. 2005). In contrast, Chevolot et al. (2007) proposed that the near absence of genetic differentiation in the skate *Amblyraja radiata* over the entire North Atlantic, compared to regional differences seen in other skates, is indicative of a migratory range much greater than previously thought. Therefore, the high aerobic enzymatic capacity seen in the WM of the congener *A. badia* and *B. microtrachys* probably reflect a highly migratory behavior on a large horizontal scale.

Conclusions

Continued exploitation of the deep-sea for resources and fisheries, coupled with the potential impacts of global climate change, makes it ever more important to understand the distribution, activity and energetic demands of understudied chondrichthyan species with depth. Elasmobranchs are generally considered to be long-lived, late maturing, low fecundity species. The very little evidence available suggests that these characteristics are accentuated in the deep-sea (Fowler et al. 2005). The present results further suggest that deep-sea species have very low metabolic rates. This combination of traits makes these species highly susceptible to reduced population viability and extinction with increasing fishing pressure in the deep-sea. Ocean acidification is another anthropogenic affect that threatens marine life. Reductions in habitat pH have been shown to reduce buffering capacity, ion transport, blood oxygen binding, and metabolism (Fabry et al. 2008). It has been suggested that deep-sea species

are highly susceptible to even the smallest changes in ambient pH due to adaptations to the characteristic stability of the deep-sea environment (Childress et al. 1998; Seibel and Walsh 2001; Barry et al. 2002, 2004). In deep species of chondrichthyans that already show reduced metabolism, shown in this study to be due to reductions in locomotory capacity with decreasing light levels, additional metabolic suppression in response to increasing pH will impact important processes such as protein synthesis (Hand 1991), further reducing potential for growth and reproduction. Intense changes, through such processes as CO_2 injection and sequestration in deep-sea areas, may result in cases of widespread mortality of both chondrichthyan species and their prey. This study highlights the importance of using biochemical indicators to analyze deep-dwelling species that could otherwise not be easily studied. Future studies on the metabolic poise of other tissues (such as heart and liver) are needed and could reveal additional information on the complexities of the bioenergetics of these species.

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CHAPTER II

Stable isotope analysis of benthic and benthopelagic chondrichthyans with depth off the west coast U.S.A.

ABSTRACT

Bulk analyses of stable nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotopic compositions of 14 species of benthic and benthopelagic chondrichthyans collected off the west coast of the U.S.A. were conducted in order to evaluate both inter- and intraspecific depth trends, as well as to determine the effect of decreasing urea:TMAO contents with depth on $\delta^{15}N$. A surprisingly significant linear decrease in $\delta^{13}C$ was observed with depth; with benthopelagic sharks having significantly lower $\delta^{13}C$ values compared to benthic skates. In both cases, the decreased ^{13}C enrichment is not likely a result of differing phylogeny, but rather an indication of increased dietary reliance on pelagic vertical migrators and/or epipelagic carrion that are depleted in ^{13}C relative to nearshore and benthic prey sources. Conversely, a statistically significant enrichment in ^{15}N was shown with increasing depth. This is likely due, in part, to the decrease in urea:TMAO ratio with increasing depth. This results in a reduction in the glutamate-glutamine-urea pathway that in turn leads to higher ^{15}N enrichment in the glutamic acid leaving the liver for the formation of muscle protein. This suggests underestimated trophic discrimination factors in chondrichthyans at depth, complicating trophic level calculations for these species. Future studies need to apply additional techniques, such as compound specific amino acid analysis, in order to reveal potential shifts in the isotopic baseline of primary food sources with depth that are not resolved through bulk analysis. This may further explain the observed ^{15}N enrichment with depth. These adjustments must be taken into account in order to accurately portray ecosystem based food-web dynamics in the deep-sea.

INTRODUCTION

Despite the fact that elasmobranchs are believed to play an important role in structuring aquatic food webs as apex predators, surprisingly little quantitative information is available on their diets and trophic levels (Cortes 1999). Large and abundant consumers, such as chondrichthyan fishes, are likely to influence the structure and function of the communities in which they live. Myers (2007) suggests that a cascading top-down effect may be a predictable outcome of eliminating functional groups of apex predators, such as sharks, in a given environment. The development and application of ecosystem-based management relies on the importance of quantifying food-web dynamics and trophic relationships.

The diets and trophic dynamics of chondrichthyans are difficult to identify using traditional methods (i.e. tagging, baited camera studies, stomach content analysis etc). The trophic ecology of elasmobranchs has been conventionally studied through the use of stomach content analysis (Hyslop 1980). Collecting appropriate sample sizes for stomach content analysis in these rare, long-live late maturing species is not only difficult, but may have unknown population and ecological impacts. These problems are exacerbated in the deep sea where abundances are lower and sampling is more difficult. In addition, many elasmobranchs are commonly known to evacuate their gut contents upon capture.

As an alternative approach, stable nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotopic compositions are routinely used to estimate trophic position and carbon flow to consumers in food webs (Post 2002). Stable isotope analysis (SIA) allows quantitative

analysis of food-web dynamics on a range of spatial and temporal scales (Koch 2007). Light stable isotopes undergo mass-dependent sorting (i.e. fractionation) during biochemical and physical processes at a mean average rate per trophic level (Post 2002). As a result of these processes, such as metabolism and respiration, ^{13}C and ^{15}N are strongly retained by the consumer, whereas ^{12}C and ^{14}N are preferentially excreted. Commonly $\delta^{15}N$ values of each animal become an indicator of trophic position relative to the $\delta^{15}N$ value of primary consumers (Minagawa and Wada 1984; Peterson and Fry 1987). The $\delta^{13}C$ values of primary consumers are a useful indicator of dietary carbon source as it is generally conserved, or weakly fractionated, at each trophic step (Peterson and Fry 1987). Comparisons of the isotopic variations of $\delta^{15}N$ and $\delta^{13}C$ values in organic tissues coupled with trophic level enrichment factors can provide key information on food-web position and habitat utilization (Peterson and Fry 1987; Michener and Schell 1994; Post 2002). Over the past decade, this method has been applied to a diversity of taxa (Hobson et al. 1997; Edwards et al. 2002; Sarakinos et al. 2002; Sotiropoulos et al. 2004; Kelly et al. 2006; Post et al. 2007). However, very few studies have investigated elasmobranch ecology using SIA (Fisk et al. 2002; Estrada et al. 2003; Domi et al. 2005; MacNeil et al. 2005; Estrada et al. 2006; Kerr et al. 2006; BOYLE 2010).

Differences in elasmobranch physiology may make comparisons with previously studied taxa difficult and far more complex than previously thought. Unique traits such as urea retention for osmoregulation, lack of adipose tissue, skeletons composed of cartilage as opposed to bone, and a reliance on ketone bodies and amino acids as oxidative fuels in tissue require investigation into how these properties potentially impact $\delta^{15}N$ and $\delta^{13}C$ values. These potential variations may change the interpretation of trophic

enrichment factors and trophic levels in chondrichthyan fishes (Ballantyne 1997; Speers-Roesch and Treberg 2010; Dale et al. 2011; Kim and Koch 2011).

Chondrichthyans are osmoconformers, relying on a combination of organic osmolytes to maintain an extra- and intercellular osmolarity close to that of the environment. They retain high quantities of the waste product urea, $(NH_2)_2CO$, for this purpose. The levels of urea utilized for osmoregulation, in high enough concentrations, pose potential threats to protein folding and binding and the functioning of other cellular components. To counteract the destabilization of proteins, these fishes must also synthesize or ingest other organic osmolytes, such as trimethylamine-N-oxide (TMAO) (Yancey et al. 1982; Yancey 2005); although accumulation is thought to be dietary, this process is poorly understood. TMAO has additionally been hypothesized to counteract protein destabilization due to increasing hydrostatic pressure with depth (Gillet et al. 1997; Yancey and Siebenaller 1999; Yancey et al. 2001, 2004). In shallow-dwelling chondrichthyans a consistent thermodynamically favorable ~2:1 urea to methylamine ratio has been reported to maintain normal protein structure and function (Yancey and Somero 1979; Yancey et al. 1982; Treberg et al. 2006; Wang and Bolen 1997; Street et al. 2006). Recent studies have shown an overall exponential decline in urea:TMAO from 2.96 in the shallowest group to 0.67 in the deepest group, with urea decreasing and TMAO increasing by almost 50% in both cases (Kelly and Yancey 1999; Treberg and Driedzic 2002; Laxson et al. xxxx). TMAO increases with depth are consistent with previous work done on teleosts, squid, and crustaceans (Gillet et al. 1997; Kelly and Yancey 1999; Yancey et al. 2004; Samorette et al. 2007; Kelly and Yancey 1999). In chondrichthyes the decline in urea content with depth is thought to maintain

osmoconformation, while at the same time reducing its perturbing effects that may be compounded by increases in pressure (Kelly and Yancey 1999).

The ability to produce and retain high concentrations of urea is thought to have shaped the unique metabolism of elasmobranchs (Ballantyne 1997; Speers-Roesch and Treberg 2010). Unlike teleosts and mammals that rely on fatty acid oxidation as an aerobic fuel source, chondrichthyan muscle tissue has been shown to rely on ketone bodies (Zammit and Newsholme 1979; Moon and Mommsen 1987; Moyes et al. 1989; Chamberlin et al. 1991; Driedzic and DeAlmeida-Val 1996; Ballantyne 1997; Watson and Dickson 2001; Treberg et al. 2003; Speers-Roesch and Treberg 2010). Ketone bodies are high-energy fuels that are primarily produced from acetyl CoA by liver mitochondria and subsequently oxidized in peripheral tissues (Ballantyne 1997; Laffel 1999). Besides this unusual metabolic organization, another distinguishing characteristic is chondrichthyans reliance on amino acids, especially glutamine, as oxidative substrates to supply the energetic requirements of tissues (Chamberlin et al. 1991; Ballantyne 1997). Not only is glutamine an important oxidative substrate in multiple tissues, but it is also the nitrogen donor for urea synthesis instead of ammonia (Chamberlin et al. 1991; Julsrud et al. 1998). Deamination of glutamate by glutamate dehydrogenase (GDH) converts it to α-ketoglutarate for use in the citric acid cycle (Ballantyne 1997; Anderson 1991). An alternative pathway converts glutamate to glutamine with glutamine synthetase, which then acts as the nitrogen donor for urea synthesis (Anderson 1991). A smaller nitrogen isotope fractionation should be associated with the cleavage of the amide functional group of glutamine compared to glutamate. Glutamine has two nitrogen atoms, compared to the single amine group in glutamate, and only one bond is broken (Dale et al. 2011). Hepatic GDH, which has been used to provide a measure of the relative

importance of amino acids as oxidative substrates in tissues, has been shown to have a strong inverse relationship with urea levels in elasmobranchs (Speers-Roesch et al. 2006). Therefore, the increased importance of the glutamate-glutamine-urea pathway in chondrichthyans with higher concentrations of urea could result in lower glutamate catabolism (Speers-Roesch et al. 2006), and therefore lower ^{15}N enrichment in glutamic acid exiting the liver for the formation of muscle proteins (Dale et al. 2011). We therefore hypothesize that the lower concentrations in urea shown with depth in chondrichthyans may result in higher ^{15}N enrichment due to the increased importance of glutamate catabolism.

The aim of this study was to use stable isotope analyses to learn about the relative trophic ecology of an assemblage of demersal elasmobranches over a broad depth range off the west coast of the U.S. The specific goals were to: (1) evaluate both inter- and intraspecific depth trends in $\delta^{15}N$ and $\delta^{13}C$ values; and (2) determine the effect of decreasing urea:TMAO contents with depth on ^{15}N enrichment. This study seeks to provide data that will aid future studies in accurate trophic dynamic studies with the analysis of the feeding ecology and potentially unique enrichment factors in chondrichthyan fishes with depth.

MATERIALS AND METHODS

Bulk stable isotope analysis

White epaxial muscle tissue was sampled in skates, sharks, the chimaera *H. colliei*, and the electric ray *T. californica* over a broad depth range according to the methods in Chapter 1. Cartilaginous tissue was carefully avoided so that the same tissue among all species was analyzed. An average of ~0.1g of frozen tissue was excised placed in clean scintillation vials and dried in a 60˚C oven for 24 hrs, or until dry. Care was taken to remove any frozen water from the sample. Due to the small volume of WM collected from each individual, this process was conducted before pre-treatments in order to conduct water content analyses in conjunction with stable isotope preparation. Samples were ground to a fine powder with a mortar and pestle to allow for thorough chemical treatment before stable isotope analysis was conducted. Between samples, all instrumentation and surfaces were cleaned with ethanol to avoid cross-contamination. Due to the high lipid content seen in many of these species (see Chapter 1 table 2), lipids were extracted in order to keep molar C:N ratios within appropriate measurable limits. A 2:1 methanol to chloroform rinse was performed twice for 24hrs (Hobsen and Welch 1992) with constant agitation. Scintillation vials were lightly capped with Teflon-lined lids to avoid evaporation and contamination. Additionally, a deionized water (DIW) rinse was performed twice for 10 minutes to remove urea from the chondrichthyan tissue.

Recent controversy over preparation methods regarding urea extraction in elasmobranch tissues for stable isotope analysis motivated closer examination of the species analyzed (Logan and Lutcavage 2010; Hussey et al. 2010a, b; Kim and Koch 2011). Urea has a lower $\delta^{15}N$ value than associated tissues, and if retained in the analysis

it could produce artificially low bulk $\delta^{15}N$ values (Fisk et al. 2002). Logan and Lutcavage (2010) found no affect of urea content on the nitrogen stable isotope values in skate and dogfish WM tissue. However, several publications indicate that lipid and urea content may affect diet-tissue discrimination factors and must be addressed in order to obtain accurate interspecific trophic level comparisons and food web modeling due to large differences seen in trophic enrichment factors between primary producers, teleosts, and elasmobranchs (Fisk et al. 2002; Hussey et al. 2010a, b). This was indicated by Kim and Koch (xxxx) who found a preferential loss of ^{14}N and greater C:N ratios with little effect to $\delta^{13}C$ values and the alteration of protein composition and after the removal of urea in elasmobranch tissue. Preliminary analysis on the effects of urea removal on two individuals of *R. rhina* were conducted prior to this study and revealed a consistent increase in both $\delta^{15}N$ values and C:N ratios, with little effect on $\delta^{13}C$ values, for the two individuals (Table 1). This led to the subsequent removal of urea from all tissues analyzed. The organic osmolyte trimethylamine N-oxide (TMAO), principally used to counteract the perturbing effects of urea and pressure on proteins (Yancey et al. 1982; Yancey 2005), is additionally thought to potentially skew C:N ratios. Due to a lack of studies, we cannot predict the isotopic composition of this osmolyte. Due to its solubility in water, however, it has been most likely removed during the DIW rinses along with urea, removing any potential influence.

Table 1. $\delta^{15}N$ (‰), $\delta^{13}C$ (‰) and C:N molar ratios for two individuals of *R. rhina* before and after the removal of urea with deionized water washes.

			Pretreatment			Urea removed		
Species	Mass (g)	Median Depth of Capture (m)	15N (o/oo)	13C (o/oo)	C:N (mol)	15N (o/oo)	13C (o/oo)	C:N (mol)
R. rhina	536.9	533.5	17.1	-15.5	3.21	17.7	-15.2	3.32
R. rhina	751.9	533.5	15.2	-16.2	2.53	15.7	-16.0	3.25

Samples were then dried a second time at 60˚C. Samples (0.3-0.7mg) in tin capsules were analyzed using a Costech ECS 4010 elemental combustion system coupled in continuous flow with a mass spectrometer (Thermo Finnigan Delta XP). Molar C:N ratios ranged from 3.5 to 4.1, indicating proper preparation. Isotope values are reported as δ- values in ‰ relative to Pee Dee Belemnite and atmospheric N_2 standards for carbon and nitrogen respectively (precision ±0.2‰).

Statistical analyses

Species were categorized by broad taxonomic group (sharks, skates, chimaeras or rays) in order to investigate potential phylogenetic differences. Skates, which made up the majority of the species sampled and were captured over the greatest depth range, were additionally separated into depth bins according to their median depths of occurrence (see Chapter 1 Table 1): shallow (<500m), deep (>500m) and abyssal (those species who reach depths below 2000m) to investigate the potential influence of depth in these groups. A depth of 500m was chosen due to the fact that the greatest separation in fish assemblages of the 26 most abundant species in the eastern Pacific has been shown to occur above and below the 500-600m zone (Tolimieri and Levin 2006). One-way

analysis of variance (ANOVA) with a tukey-kramer post-hoc procedure was used to test for both inter-specific and categorical differences in our data. Linear regressions were used to examine the effects of body mass effects on $\delta^{15}N$ and $\delta^{13}C$ values. Investigation into seasonal and sex effects was explored but provided inconclusive results due to small sample sizes.

Laxson et al. (xxxx) concurrently analyzed a subsample of this study's WM samples for TMAO and urea content. TMAO concentrations were determined using a picric acid-ferrous sulfate method based on Wekell and Barnett (1991) and urea concentrations were determined by HPLC, based on the procedure of Wolff et al. (1989). Each was reported in mmol/kg. Linear regression analyses were then used to explore relationships between $\delta^{15}N$, $\delta^{13}C$, molar C:N ratios, depth, and urea:TMAO ratios (see Chapter 1 table 2).

RESULTS

Linear regression analysis revealed very few significant body mass effects intraspecifically. *A. brunneus, H. colliei,* and *R. rhina* $\delta^{15}N$ values (Figure 1a) and *A. badia* and *R. rhina* $\delta^{13}C$ values (Figure 1b) were shown to scale positively with increasing mass. Figure 2 shows a dual isotope plot of $\delta^{15}N$ and $\delta^{13}C$ for each species. Interspecific one-way ANOVA showed $\delta^{15}N$ values for the abyssal skate *B. abyssicola* to be significantly higher than all other species (p-values <0.0001). The shallow skate *R. binoculata* $\delta^{13}C$ values were shown to be significantly higher than most species (p-values <0.001), but statistically insignificant from the shallower dwelling *T. californica, H. colliei,* and the deeper-dwelling *B. abyssicola*. Analysis of general phylogenetic categories revealed that $\delta^{13}C$ values for the benthopelagic sharks were statistically lower than all other groups (p-values <0.0001). The values of $\delta^{15}N$ between these general categories revealed no significant differences.

When separating the skates into three depth categories, $\delta^{15}N$ values for abyssal skates were shown to be statistically similar to *H. colliei* and the benthopelagic sharks, and statistically higher than the deep-skates, *T. californica*, and the both shallow and deep skates (p-values <0.01). Benthopelagic shark $\delta^{13}C$ values remained statistically lower than all other categories (p-values <0.001).

Depth trends revealed an interspecific statistically significant linear increase in $\delta^{15}N$ values and decrease in $\delta^{13}C$ values with increasing depth of capture (Figure 3). *R. rhina* was the only species to show an intraspecific significant increase in $\delta^{15}N$ values with increasing depth. This is probably a reflection of the fact that *R. rhina* had the largest sample size (n=15) collected over a broad depth range (87-566m). Overall, there

is a significant increase in the molar C:N ratio with increasing depth of capture; but it is very slight, increasing from an average of 3.62 to 3.7 from ~80 to 2200m (Figure 4).

With increasing depth, urea:TMAO shows a highly significant linear decrease reflecting decreasing urea content with depth (Figure 5). Subsequently, there is a significant linear decrease in $\delta^{15}N$ values with increasing urea:TMAO ratios (Figure 6).

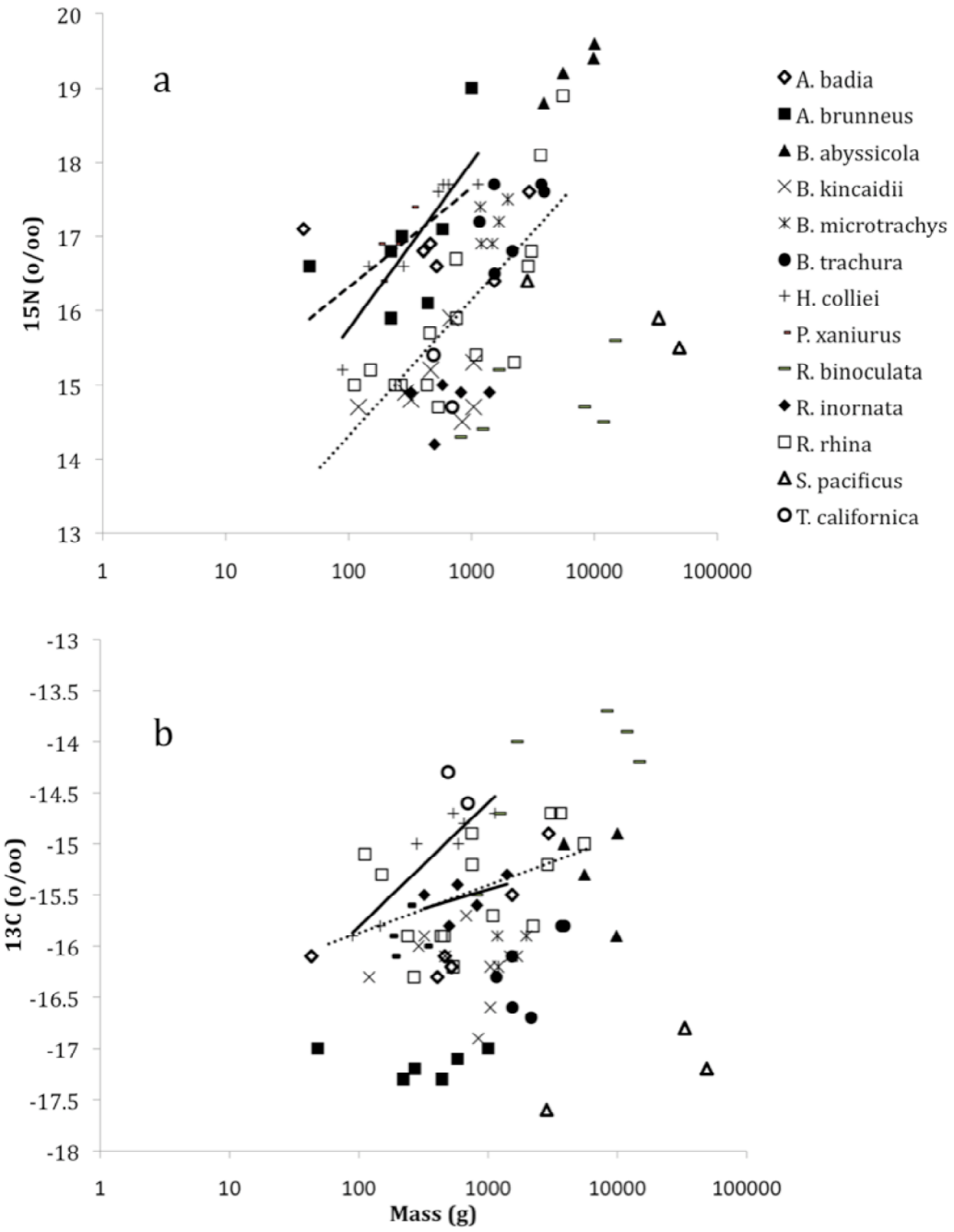


Figure 1. (a) $\delta^{15}N$ (‰) values plotted versus log mass (g). *A. brunneus* (r^2= 0.651, p-value= 0.03), *H. colliei* (r^2= 0.62, p-value= 0.04), and *R. rhina* (r^2= 0.75, p-value= <0.0001) show a linear positive increase in $\delta^{15}N$ with an increase in mass. (b) $\delta^{13}C$ (‰) values plotted versus log-scaled mass (g). *A. badia* (r^2= 0.9, p-value= 0.002) and *R. rhina* (r^2= 0.34, p-value= 0.02) show a linear positive increase $\delta^{13}C$ with an increase in mass.

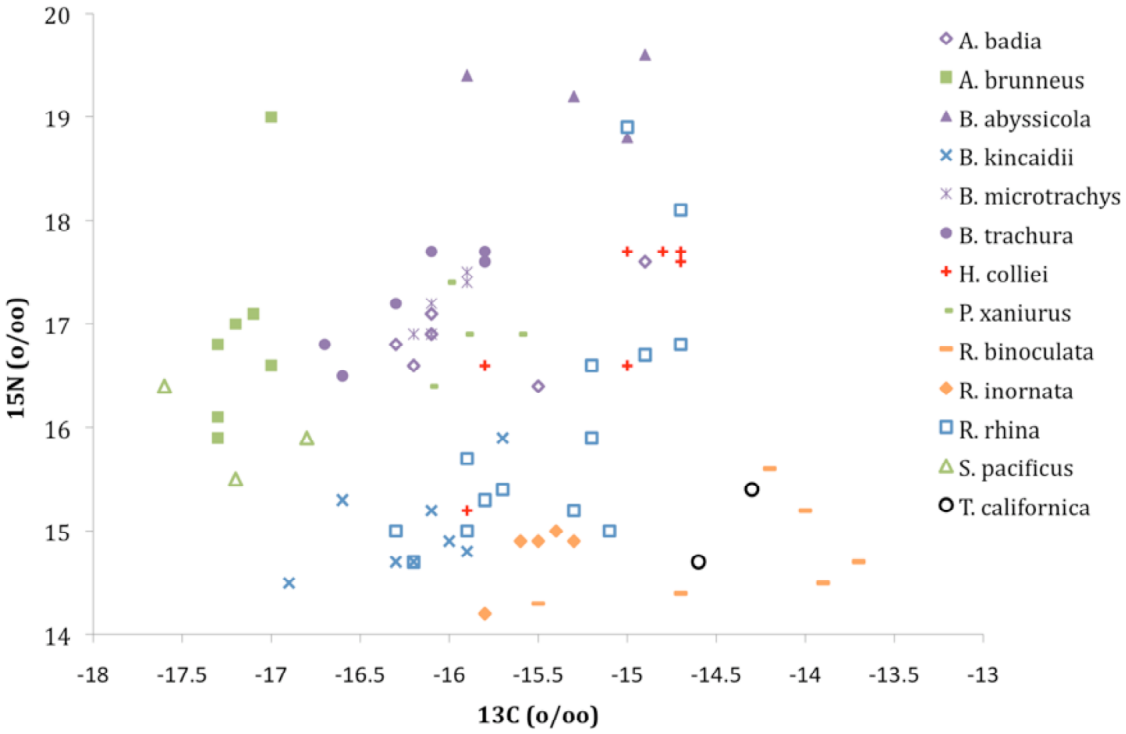
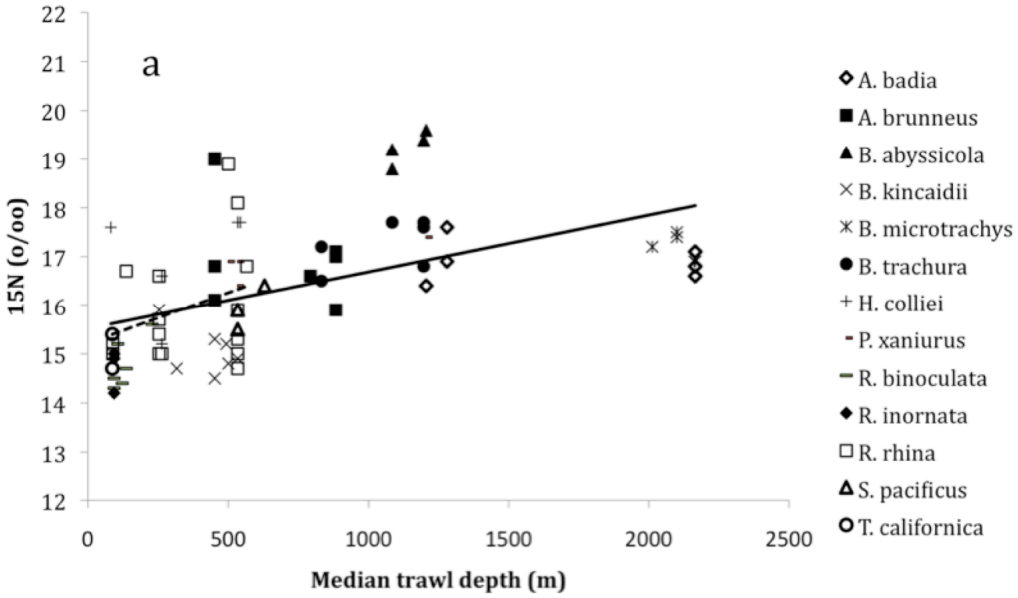


Figure 2. Dual plot of $\delta^{15}N$ (‰) and $\delta^{13}C$ (‰) values by species. Categories used for analysis are separated by color: black= ray; red= chimaera; green= benthopelagic shark; orange= shallow skate (MDO <500m); blue= deep skate (MDH >500m); purple: abyssal skates (those skates whose depth range reaches depths below 2000m).


a
15N (o/oo)
Median trawl depth (m)
A. badia
A. brunneus
B. abyssicola
B. kincaidii
B. microtrachys
B. trachura
H. colliei
P. xaniurus
R. binoculata
R. inornata
R. rhina
S. pacificus
T. californica

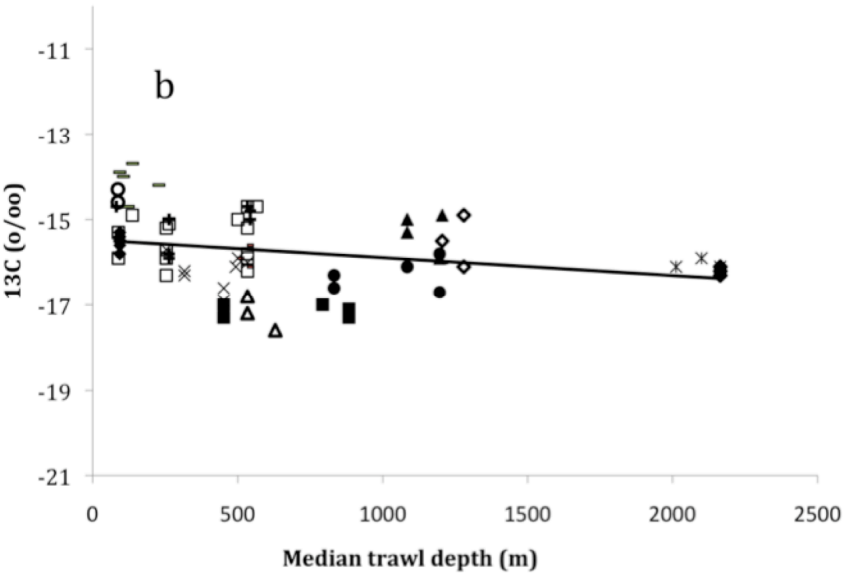

b
13C (o/oo)
Median trawl depth (m)

Figure 3. (a) $\delta^{15}N$ (‰) plotted versus the median depths of along contour trawls (m) at which each individual was captured. *R. rhina* is the only species to show an intraspecific significant linear increase in $\delta^{15}N$ with increasing depth represented by the dashed line ($y=14.7 + 0.004x$, $r^2=0.28$, p-value= 0.035). There is an overall significant linear increase in $\delta^{15}N$ with increasing depth; $y= 15.52 + 0.001x$ ($r^2= 0.28$, p-value= <0.0001). (b) $\delta^{13}C$ (‰) plotted versus median trawl depth (m). A significant linear decrease in $\delta^{13}C$ was seen with increasing depth; $y= -15.47+ 0.0004x$ ($r^2= 0.09$, p-value= 0.009).

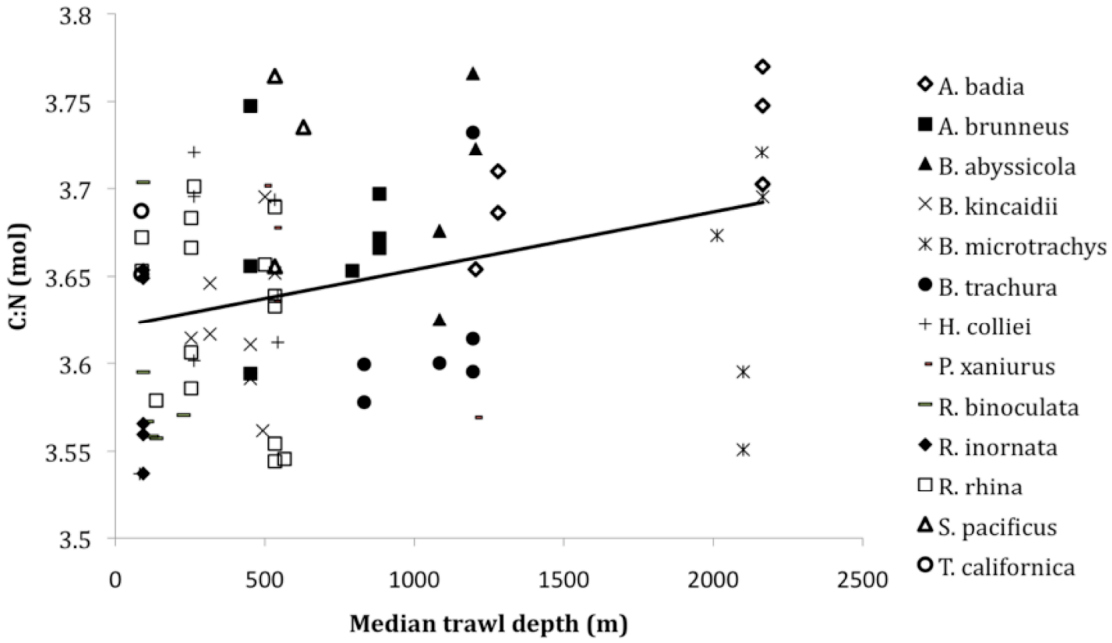


Figure 4. Molar C:N ratio plotted versus median trawl depth (m) for each individual. Relationship shows a significant linear increase in C:N with increasing depth; y= 3.62 + $3.3E^{-5}x$ (r^2= 0.10, p-value= 0.004).

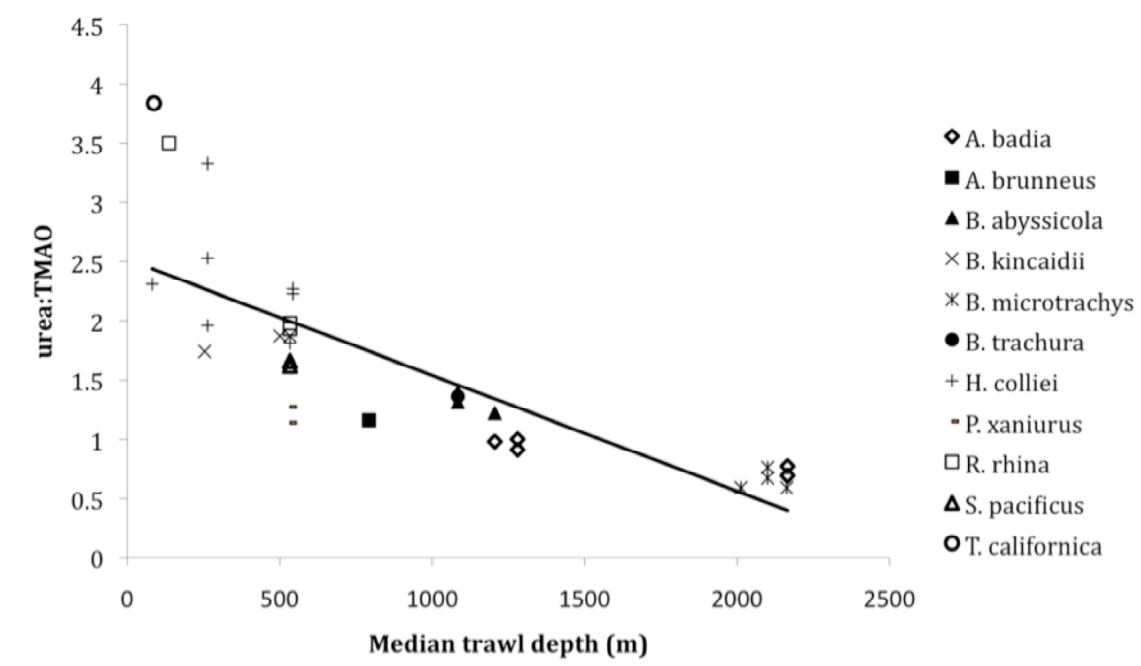


Figure 5. Ratio of urea:TMAO plotted versus median trawl depth (m). Urea:TMAO ratio data was obtained from Laxson et al. (xxxx) and analysed with the median depth of capture for each corresponding individual in this study. A highly signficant linear decrease in urea:TMAO is seen with increasing depth; $y= 2.52 – 0.001x$ ($r^2= 0.64$, p-value= <0.0001).

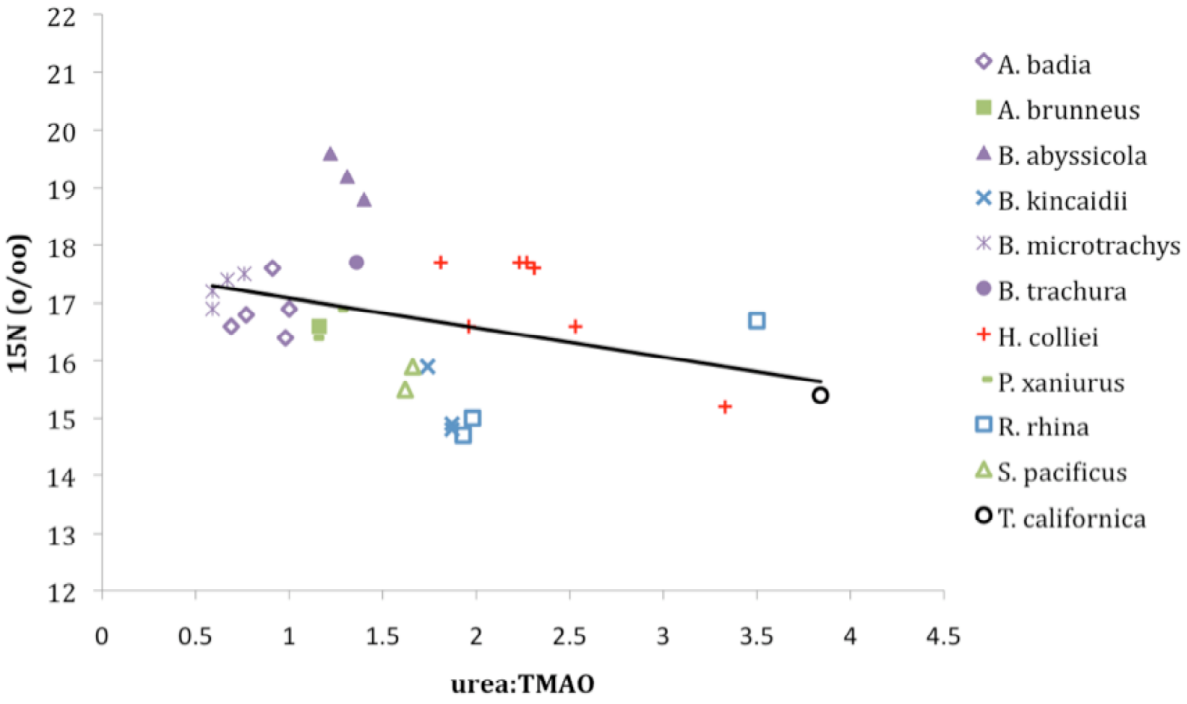


Figure 6. $\delta^{15}N$ (‰) values plotted versus the ratio of urea:TMAO. Urea:TMAO ratio data was obtained from Laxson et al. (xxxx) and analysed with the $\delta^{15}N$ for each corresponding individual in this study. A weak but significant linear decrease was seen in $\delta^{15}N$ with increasing urea:TMAO; y= 17.6 – 0.51x (r^2= 0.12, p-value= 0.05). Categories are again shown by color: black= ray; red= chimaera; green= benthopelagic shark; blue= deep skate (MDH >500m); purple: abyssal skates (those skates whose depth range reaches depths below 2000m).

DISCUSSION

To date, only one previously published study has analyzed the stable isotope values of an elasmobranch species off the California. Boyle (2010) examined the stable isotopes and stomach contents of *B. trachura* off the coast of northern Oregon. The $\delta^{15}N$ fall in line with those of *B. trachura* in this study, ranging from 17.08 – 18.22‰. However, $\delta^{13}C$ are more depleted in ^{13}C (-18.25 - 17.61‰) compared to this study (-16.7 - 15.8‰). As C:N ratios were fairly consistent and within range for both studies, this does not seem to indicate the influence of procedural differences. As potential prey of this study, as indicated by Boyle (2010), are not regionally conserved, future study is needed to look at differences in potential terrigenous carbon input, species coastal population structure, selective or preferential feeding behavior, and other potential regional differences that may explain the variability between these data.

The significantly lower $\delta^{13}C$ values in the benthopelagic sharks compared to the other chondrichthyans in this study may be a function of their tendency to forage in more pelagic zones than the benthic feeding skates (Figure 2). Many aquatic studies have shown ^{13}C enrichment in both nearshore and benthic environments compared to the pelagic (Grebmeier et al. 1988; France 1995; Post et al. 2002; Boyle 2010). *A. brunneus* tends to forage in the midwater column, feeding on mostly pelagic squids, small teleosts and crustaceans. *P. xaniurus* have additionally been shown to feed on mainly pelagic species of crustaceans and small teleosts. In contrast, *S. pacificus* are generally considered bottom feeders. However, they have been shown to eat fast swimming epipelagic species (ex. harbor seals, sea lions, albacore, and salmon) presumably through lie-in wait

predation (Ebert 2003). The torpedo ray, *T. californica* has been shown to utilize two feeding modes: benthic ambush predation during the day and active foraging in the water column at night, stunning their prey with electric organ discharges (Lowe et al. 1994). However, the two individuals examined in this study were small juvenile males that may have a tendency to eat smaller, benthic prey species.

Unexpectedly, ^{15}N values showed little to no significant difference both interspecifically and between general phylogenetic categories. A lack of significant differences in $\delta^{15}N$ values between benthopelagic sharks and the other chondrichthyans in this study may suggest that trophic levels do not differ. Ebert and Bizzarro (2007) found that skates had trophic levels similar to those of several co-occurring demersal shark families, including Scyliorhinidae. This family includes the species *P. xaniurus* and *A. brunneus*. This study suggests that the relatively large *S. pacificus* also has a similar trophic level to these smaller demersal sharks and skates. Biochemical indicators of metabolic poise (Chapter I) indicate this species to be relatively sluggish and slow swimming, with little capacity for burst swimming in comparison to other sharks and rays. This may indicate that these individuals are more feeble swimmers than previously thought, relying more heavily on less active prey species. The two largest specimens caught in this study were immature females, so larger adults may exhibit a higher trophic level. Alternatively, reliance on epipelagic nekton carrion, which has been shown to have significantly lower in $\delta^{15}N$ values than trophically similar species in the benthic food web (Drazen et al. 2008), may influence the lower than expected $\delta^{15}N$ values seen in these species.

Ontogenetic shifts in diet and depth may influence some of the observed trends in this data. Ontogenetic shifts have been shown in the diets of several elasmobranch species

(ex. Orlov 1998; Ebert 2002; Brickle et al. 2003; Dale et al. 2011). *R. rhina* show a significant increase in $\delta^{15}N$ and $\delta^{13}C$ values with increasing mass, suggesting a dietary shift with increasing size (Figure 1). Previous studies validate this finding, showing that *R. rhina* tends to shift from feeding primarily on small crustaceans to larger fish and cephalopods with increasing total length (Robinson et al. 2007; Bizzarro et al. 2007). Bizzarro et al. (2007) additionally show this same general shift in the skates *R. binoculata*, *R. inornata*, and *B. kincaidii*. Previous studies along with the few mass specific trends in $\delta^{15}N$ and $\delta^{13}C$ values reported in this study suggest that most of these species experience size dependent ontogenetic increases in trophic level. On average, larger specimens of *R. rhina* were captured with increasing depth; however, the variability was high. Robinson et al. (2007) showed a diet shift in deeper dwelling *R. rhina*. The subsequent enrichment in ^{15}N with depth of capture in *R. rhina* may therefore reflect an ontogenetic shift in diet and size with depth in this species.

There was a significant interspecific increase in ^{15}N contents with increasing depth (Figure 3a) with the abyssal skates having the highest overall $\delta^{15}N$ values. Three general hypotheses may explain this enrichment with depth: (1) an increase in trophic position with depth; (2) a change in the baseline $\delta^{15}N$ values; and (3) a change in the trophic discrimination factor (TDF).

Increasing trophic position most likely does not explain this trend. An increase of ~3.4‰ in $\delta^{15}N$ values is used to indicate a shift in one trophic level (Post 2002). It has been heavily debated as to whether this value applies to species of elasmobranch, with discrimination factors as low as 2.3‰ reported for some species (e.g. Vanderklift and Ponsard 2003; Hussey et al. 2010a). However, for relative comparison using the general value of 3.4‰ indicates that there is an approximate two trophic level range in this data.

The shallower skates *R. binoculata, R. inornata,* and *R. rhina* and the deeper-dwelling skates *B. kincaidii* and *B. trachura* have been shown to have high dietary overlap at similar sizes (Bizzarro et al. 2007; Boyle 2010) and hence similar trophic levels. Ebert and Bizzarro (2007) calculated similar TLs between the deeper dwelling *B. trachura* (TL 3.78) and two shallower dwelling skates *R. rhina* (TL 3.86) and *R. inornata* (TL 3.76). Very little is known about the diets of the two deepest dwelling skates: *A badia* is known to feed on cephalopods, crustaceans and small bony fishes, and *B. microtrachys* is known to feed on deep-water shrimp (Ebert 2003). Compared to known feeding habits of shallower species (dominated by decapods and fishes in most species), this diet does not indicate that they feed on larger, potentially higher TL, prey species. Additionally, while the deeper dwelling skate *B. abyssicola* (depth of capture ~1080-1200m) has statistically higher $\delta^{15}N$ values, indicating the highest trophic level relative to the other species studied, the shallower-dwelling skate *R. rhina* (depth of capture ~87-566m) has an intraspecific shift of approximately 1.5 trophic levels alone (Figure 2). This indicates that the increase in ^{15}N from ~80-2200m is not influenced by an increase in TL alone.

Enrichment in the ^{15}N in sinking particulate matter with depth may explain the trend seen in these species of chondrichthyans. The abyssal food web may have an isotopic baseline that is enriched in ^{15}N compared to the epipelagic ocean due to $\delta^{15}N$ values being 3-4‰ higher in sinking (i.e. zooplankton fecal pellets, phytoplankton assemblages, etc) versus suspended particulate matter (Altabet et al. 1999). The benthic food web is, therefore, typically more enriched in ^{15}N relative to the pelagic (Peterson and Fry 1987; Drazen et al. 2008; Nilsen et al. 2008). However, Wu et al. (1999) found little to no change in $\delta^{15}N$ values of sinking particulate matter at depths below the photic zone. If baseline $\delta^{15}N$ values were driving the increase in elasmobranch ^{15}N contents then such

changes would be most apparent between the species inhabiting the shallowest waters and those living below the euphotic zone with few apparent changes at greater depths (actual changes in TL aside). In contrast, this study shows a steady increase in $\delta^{15}N$ values past 1000m (Figure 3a). As bulk stable isotope techniques were utilized in this study, changes at the base of the foodweb may not have been seen. Further study is needed (such as amino acid compound specific analysis) in order to determine if there are changes in the baseline $\delta^{15}N$ values that may influence observed relative trophic levels and ^{15}N enrichment with depth.

This study suggests that ^{15}N enrichment with depth is likely due, in part, to the decrease in urea:TMAO ratios with increasing depth (Figure 5). As urea decreases with depth and is subsequently replaced by osmolytes such as TMAO, the glutamate-glutamine-urea pathway is likely to be reduced. This should result in higher nitrogen isotope fractionation with increased reliance on glutamate catabolism, and therefore higher ^{15}N enrichment in glutamic acid leaving the liver for the formation of muscle protein with increasing depth. The weak but statistically significant decrease in $\delta^{15}N$ values with increasing urea:TMAO ratios supports this hypothesis (Figure 6). This suggests a potentially unique increase in TDFs with depth in chondrichthyans. If urea synthesis in chondrichthyans does play a role in TDFs, then increased quantitative knowledge of urea:TMAO ratios in these species with depth will be needed to adjust calculated trophic levels in order to accurately portray ecosystem food-web dynamics in the deep-sea.

The decrease in enrichment of $\delta^{13}C$ values is more difficult to explain (Figure 3b). The lack of statistical differences in mean $\delta^{13}C$ values between taxonomic categories suggests phylogeny is not a likely explanation. Interspecific comparisons reveal that the shallower dwelling *R. binoculata*, *R. inornata*, *T. californica*, and *H. colliei* tend to have

higher values than most of the deeper-dwelling species, with the exception of the deep-water skate *B. abyssicola*. Lipids are ^{13}C-depleted relative to proteins and carbohydrates, so variations in their concentration can affect $\delta^{13}C$ values (Pinnegar and Polunin 1999; Post et al. 2007). However, lipid content in these species shows no change with depth (Chapter I figure 13c), and furthermore was removed as part of this analysis. The decrease is therefore most likely ecologically based. Many baited camera studies have regularly shown many deeper-dwelling benthic/benthopelagic elasmobranch species to be opportunistic scavengers (Jones et al. 2003). A concurrently deployed baited camera study observed *S. pacificus* attracted to the bait at depths of ~500-1000m and *A. badia* at depths of ~2000-2800m (Yeh and Drazen 2011). If deeper species have an increased reliance on carrion, as possibly indicated by ^{15}N enrichment with depth, one would expect to see a concurrent enrichment in ^{13}C as carrion has been shown to enriched relative to benthic prey sources (Drazen et al. 2008). However, Mauchline and Gordon (1991) show the importance of pelagic vertical migrators on the diet of demersal species through the impingement of benthic boundary layers as deep as the abyssal sediment. Depletion with depth may, therefore, reflect the reliance on more pelagic food sources and /or epipelagic carrion as benthic prey biomass declines exponentially with depth (Haedrich and Rowe 1977; Angel and Baker 1982; Thurston et al. 1994; Priede et al. 2006).

Conclusions

Very little is known about the general biology, foraging ecology and habitat use of most deep-sea elasmobranchs. Understanding food web dynamics of the deep-sea is becoming ever more important as it faces increased exploitation with increases in resource limitation in shallow fisheries (Koslow et al. 2000; Haedrich et al. 2001; Roberts et al.

2002; Bailey et al. 2009) and potential impacts of climate change and ocean acidification (Seibel and Walsh 2001; Orr et al. 2005; Bailey et al. 2006; Company et al. 2008). Obtaining information that will allow us to make accurate quantitative comparisons between trophic positions is more important than ever in order to understand the trophic dynamics of this understudied system and monitor ecosystem changes. These results emphasize the need for further study of the $\delta^{15}N$ and $\delta^{13}C$ values in chondrichthyan fishes and in potential size and depth based ontogenetic shifts in feeding and foraging behavior. Future studies need to apply additional techniques, such as amino acid stable isotope analysis (e.g. Dale et al. 2011), in order to establish possible shifts in isotopic baselines and primary food sources with depth. The results of this study also suggest that urea retention in elasmobranchs and resulting shifts in metabolic pathways may have a profound impact on trophic level estimates in chondrichthyans with depth. Further analysis needs to be done examining the relationship between urea content, specific amino acids (such as glutamic acid), GDH activity, and possible enrichments of ^{15}N in glutamic acid in hepatic tissue. This will provide quantitative information on the effects of WM protein formation and potential implications for shifting TDFs with animal activity and depth of occurrence.

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CONCLUSION

This study highlights the relative lack of information regarding the general biology, energetic demands, locomotory capabilities, foraging ecology and habitat use of most deep-sea chondrichthyan fishes. Understanding and monitoring ecosystem-based processes such as food-web dynamics and the transfer of energy are becoming ever more important in the face of increasing fishing pressures and global climate change. This data reveals that the well-established k-selected nature of chondrichthyans is likely compounded by low metabolism at deeper depths. In addition, our understanding of trophic level calculations with depth may be misinterpreted due to unique trophic discrimination factors among chondrichthyans, such as ^{15}N enrichment of glutamic acid with decreasing urea synthesis. Accurate assessments of ecosystem systems and stability must be conducted in order to maintain population viability among these highly susceptible species.

Overall, the lack of profound phylogenetic differences in both metabolic poise and relative trophic position of chondrichthyan fishes reveals similarities between co-occurring demersal sharks and skates. This finding reinforces the importance of using biochemical indicators to analyze species that could otherwise not be studied. Previous assumptions that indicated lower metabolic rates and trophic positions in skates may have been biased by relatively small sample sizes and underrepresentation of deeper living species. This finding may drastically change some representations of both shallow and deep-sea ecosystem-based food webs.

Important potential distinctions between teleosts and elasmobranches due to physiological differences are also revealed in this study. Whether due to differences in

lactate shuttling and processing, the retention of urea for osmoregulation, or the unique use of ketone bodies for metabolism in chondrichthyans, these differences become extremely important when attempting to make biochemical and physiological comparisons across multiple taxa. Future studies on the metabolic poise of tissues such as the heart and liver, and the relationship between osmoregulation, amino acid metabolism and nitrogen isotope dynamics will provide quantitative data needed to refine our understanding of the bioenergetics and trophic positions of these species. Only then can accurate comparisons be made between chondrichthyan and teleost species in order to better inform fisheries management policies.