# **Functional basis of nutritional diversity in aquatic ecosystems**

## Abstract

While the role of aquatic species in food provisioning is one of the most widely acknowledged ecosystem service provided by aquatic ecosystems, the role of seafood as a source of valuable micronutrients scarce in the human diet is often overlooked. As such, while the ecological mechanisms responsible for fisheries productivity are well studied, the ecological mechanisms responsible for a nutritionally diverse set of seafood species are not well understood. A primary challenge in ecosystem service science in general, and with respect to the issue of human nutrition in particular, is to understand the relationship between functional traits of aquatic species and their content of nutrients essential to the human diet. Here we draw on allometric scaling theory and ecological stoichiometry to generate predictions about how a species’ nutritional profile is related to physiological traits such as growth rate or body size. Predicting a species’ nutritional value in the human diet requires knowledge of not only the ecological drivers of a species’ nutrient content, but also human dietary practices, which may influence which body parts are consumed, and thus the nutritional value of an edible portion. In this study we test whether functional traits can predict species’ nutritional value to people by using dietary food composition data. These data reflect the functional basis of organismal elemental composition and human dietary practices. We find that a trait-based approach is effective at simplifying the complexity of aquatic food webs into a few key axes that strongly control the composition of micronutrients in fish assemblages. We find that for some, but not all, nutrients we analyzed (e.g. Ca, Hg, EPA, DHA), the nutrient content of edible portions varies predictably with latitude and body size, consistent with the functional roles of micronutrients in fish physiology. These results suggest that the availability of micronutrients from local fish assemblages may depend on geography and the body size distribution of the catch.

**Introduction**

While it has long been recognized that ecosystems provide essential benefits to humanity, a mechanistic understanding of the ecological processes underlying the supply of ecosystem services remains elusive. One of the most widely studied and universally important benefits that humans derive from natural ecosystems is food provisioning. Indeed, many coastal human communities rely on wild harvests from local aquatic ecosystems to meet nutritional requirements for macronutrients, such as protein and fats, and micronutrients, such as vitamins and minerals (Kuhnlein and Receveur 2007, Kuhnlein et al. 2009, Kawarazuka and Bene 2011).

Thus, the ecology of food security, an important ecosystem service, is not just about predicting yields, it is about understanding the ecological conditions that lead to a stable supply of nutritionally diverse foods. Although fisheries productivity is studied extensively (refs), there has been surprisingly little consideration of the drivers of the nutritional quality of fisheries yields. Furthermore, which attributes of a food web’s structure influence its ability to provide the essential elements of a human diet remains an open question.

**Nutrition as an ecosystem function and service**

The nutritional quality of prey species plays a fundamental role in ecosystem function. Food webs characterized by nutritionally valuable prey support higher consumer and predator biomass and have higher trophic transfer efficiencies (Hecky 1984, Muller Navarra et al. 2000, Brett et al. 2009). Changes in forage fish communities from lipid-rich to lipid-poor fish in the Pacific Ocean have caused predatory marine birds and mammals to shift their diet to less nutritionally valuable lipid-poor fish and suffer population declines (Rosen and Trites 2006; Romano et al. 2006, Osterblom et al. 2008).

For human consumers, fish are a good source of high quality protein, a range of micronutrients and essential fatty acids (Tacon and Metian 2013). In 2009, aquatic species accounted for 16.6% of the global total supply of animal protein, providing more than three billion people with almost 20% of their average per capita intake of animal protein (FAO ref). However, the role of fish as a source of essential micronutrients may be even more important than as a source of protein (Allison et al. 2007). Fish is important in the diets of many poor populations suffering from vitamin and mineral deficiencies (Roos et al. 2007, Tacon and Metian 2013). In many vulnerable communities around the world, fish consumption plays an important role in combating micronutrient deficiencies (Kawarazuka and Bene 2011). For example, in rural Bangladesh, some of the poorest communities are heavily dependent on small fish from capture fisheries to meet their micronutrient needs. Consumption of small indigenous fishes contributes 40% and 31% of the total recommended intakes of vitamin A and calcium, respectively, at household level in the peak fish production season (Roos et al. 2007). Locally caught seafood contributes significantly to micronutrient intakes in Arctic Canadian Indigenous populations (Kuhnlein and Receveur 2007, Johnson-Down and Egeland 2010).

The value of a fish species in terms of human nutrition benefits can be quantified as the nutrient content in an edible portion relative to Dietary Reference Intakes (DRI) values. The DRI is the daily intake level of a nutrient that is considered to be sufficient to meet the requirements of 97–98% of healthy individuals in every demographic (National Academies of Sciences 2011). Fish species vary widely in their concentration of essential nutrients (USDA 2011). For example, assuming a serving size of 85g of fish, sardines (1.9 g DHA per 100 g tissue) and Pacific herring (0.83 g DHA/100g) provide the recommended level of 1.0g/day EPA and DHA in a single serving, while pink salmon, canary rockfish and surf smelt would require 1.2-1.5 servings, while pacific hake (0.15g/100g DHA) and pollock (0.24 g DHA/100g) would require 4-5 servings to meet the recommended daily requirements (Hyuhn and Kitts 2009). Thus, not all species are equally nutritionally valuable. This variability in essential fatty acid (EFA) content per serving size is related to the total fat content of the fish: the characteristically lean fish like pollock and hake have relatively low contents of EFAs, while the more lipid-rich fish have higher EFA contents.

HERE TALK ABOUT THE THRESHOLDS IN RDI. GIVEN THAT FISH IS AN IMPORTANT AND NON-REDUNDANT SOURCE OF MICRONUTRIENTS, AND PEOPLE COUNT ON FISH AS A MAJOR SOURCE OF VITAMINS, MINERALS AND FATTY ACIDS, WE CONSIDER FISH SPECIES THAT REACH 25% OF DRI IN A SINGLE PORTION TO BE A ‘GOOD’ SOURCE OF A GIVEN NUTRIENT.

**While it has been shown that fish species vary widely in their nutrient profiles (Tacon and Metian 2013), the ecological drivers of this nutritional diversity are unknown.** Correlations between species’ traits and their nutritional value have been observed in terrestrial crop species. For example, the same traits of the chili pepper, *Capsicum*, that make it nutritionally valuable to humans (i.e. its high vitamin A, C, beta carotene and antibacterial properties) are functional traits that evolved as chemical defense compounds (Tewksbury et al. 2008). The high protein content of legume seeds (i.e. beans and peanuts) is likely an adaptive trait for growth in low nitrogen soils (Anderson et al. 2004). In aquatic systems, the nutrients that make fish a nutritionally valuable part of the human diet, such as the calcium, iron, and essential fatty acids contained in fish tissues, play important physiological roles. The main functions of essential elements in fishes include formation of skeletal structure, maintenance of colloidal systems, and regulation of acid-base equilibrium. Calcium and phosphorus are required for the formation of the skeletal structures. Fe, Mn, Cu, Co, Zn, Se, etc are associated with specific proteins in metalloenzymes, which produce unique catalytic functions. Some biologically important compounds, such as hemoglobin and vitamin B12 contain minerals as an inherent part of their structure.

**We hypothesize that species’ nutritional profiles are linked to functional traits because elements that are of nutritional value to humans also serve functional roles in organismal physiology**. Functional traits such as trophic position may be related to the nutritional content of seafood species because they affect the dietary range and quantity of nutrients to which an organism is exposed, as well as the physiological constraints on body composition (Simpson and Raubenheimer 2012). In aquatic food webs, some evidence suggests that nutritional profile may be related to species traits: the distribution of trace metals in fish tissues has been related to feeding guild (Bernhard and Andreae 1984), body size, stage of life cycle and reproductive status (Shearer 1984). Disease state, water quality and other environmental factors may also modify tissue mineral distribution (Lall and Olivier 1993). Although aquatic organisms readily absorb metals from the environment, the ability to regulate abnormal concentrations varies among species. Certain species are able to excrete high proportions of excessive metal intake and regulate the concentration in the body at relatively normal levels (Bryan 1976). This occurs for essential elements such as Cu, Zn, and Fe. However, non-essential minerals such as Hg, Cd, and Pb are more poorly regulated (Karimi et al. 2006). Different metals localize or accumulate in different organs or tissues. For example, Fe concentrations are highest in hematopoietic tissues while Hg is most abundant in the muscle tissues. Since each element is under different levels of homeostatic control, we expect the relationship between elemental content and species’ traits to vary by nutrient. For those nutrients that are more tightly regulated, we expect more consisten patterns across species. Below we discuss the physiological role of several nutrients essential to the human diet and our predictions for how species’ traits may be related to nutrient content.

Here, we characterize the range of variability in fish tissue nutrient content among fish species consumed around the globe, and test whether nutrient profiles of commonly consumed fish species are related to species’ traits, including body size, trophic position and habitat associations, which are broadly related to the function and form of species in aquatic systems (Woodward et al. 2005, other ref?). Given that fish is known to be a high quality source of essential micronutrients, but fish consumption may be limited to a small number of portions per week (reference?), we sought to identify which species are the most nutrient dense and contribute at least 25% of DRI in a single portion. We chose to analyze a selection of ions and compounds that affect the nutritional value to human consumers, while also being implicated in a range of biologically important processes that affect growth and reproduction. We examined fatty acid concentration (which reflects fish diet and physiological condition), a toxic metal for which increased concentrations are negative for humans (Hg), two metals beneficial at low concentrations but toxic at high concentrations (Zn and Fe) and one mineral (Ca).

*Calcium*

Calcium is one of the most abundant cations in the body of a fish and is an important component of bone formation and maintenance of skeletal tissues. Other functions of calcium include muscle contraction, blood clot formation, nerve transmission, maintenance of cell membrane integrity, and the activation of several important enzymes. Generally, the rate of uptake, the deposition pattern and retention by skeletal tissues is similar in freshwater and marine species, and also independent of bone types (ref). We expected that whole-body calcium content in fish would scale allometrically with fish skeleton mass since most calcium in fish is stored in the skeleton (Hendrixson et al. 2002). Fish skeleton mass scales isometrically across a wide range of fish taxa and life stages (Berrios-Lopez et al. 1996). Thus, we expected whole-body calcium content in fish to scale isometrically as well, meaning that whole body calcium content should increase with body size.

*Iron*

Iron is an essential element in the cellular respiratory process through its oxidation-reduction activity and electron transfer. It is found in the body mainly in complex form bound to proteins such as heme compounds. In rainbow trout, iron is absorbed in the peritoneal cavity and stored at higher concentrations in the liver, spleen, and head kidney (Walker and Fromm 1976). In the Atlantic cod, *Gadus morhua*, blood volume scales with negative allometry (i.e. with a slope of 0.82) (Skov and Steffensen 2003). Assuming that iron content in fish is proportional to blood volume, we expected that whole body iron content should scale with negative allometry (i.e. slope <1). HOWEVER, SINCE STORAGE OF IRON IS SPECIES SPECIFIC, THIS SCALING PATTERN MIGHT VARY BY SPECIES.

*Zinc*

Zinc is an essential constituent of several metalloenzymes involved in the metabolism of nucleic acids, proteins, carbohydrates and fatty acids and in replication and transcription of DNA. Whole body zinc content is known to vary with body size and diet type. For example, among killifishes, whiting, and two species flatfish, whole body Zn content decreases with increasing body size ( ref 6). However, in the hake, *Merluccius productus*, Zn in muscle tissue increases with body size (ref 39). Zinc is known to accumulate in excess of the organism’s immediate needs. In aquatic food webs, zinc accumulation patterns are related to diet type. For example, planktivorous fish have been shown to accumulate more Zn than piscivores (Bernhard and Andreae 1984). Thus, we expected Zn content to be related to body size and trophic position.

*Mercury*

Mercury is a heavy metal that has no known biological functions, and is toxic at high levels (ref). Mercury tends to accumulate in the muscle tissue flesh of elasmobranchs and teleosts. Older and larger fish contain more mercury per unit wet weight than do young fish. This is well documented for several species including dogfish, eel, hake, snappers, bluefish, striped bass and striped mullet (ref). Levels of mercury in muscle from adult tunas, billfishes and other carnivores are higher than those in young fishes with lower trophic positions, suggesting that mercury concentrations in fish tissues is related to predatory behavior, longevity and body size (ref). Thus, we expected mercury concentrations to be related to trophic position and body size.

*Polyunsaturated fatty acids*

Fish require three polyunsaturated fatty acids (PUFA) for their normal growth and reproduction, including reproduction: docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (AA). These PUFA play an important role in maintaining the structural and functional integrity of cell membranes and they are precursors to a group of highly active hormones, the eicosanoids. We predicted that omega-3 fatty acid content in fish would increase for species occupying colder waters due to the functional role of polyunsaturated fatty acids in maintaining cell membrane fluidity at cold temperatures (Farkas et al. 1984). We test this prediction using fish collected over a range of latitudes from tropical to polar waters. We assume that latitude is a reasonable proxy for cold-water tolerance over such a broad spatial scale (Sunday et al. 2011).

## Methods

We aimed to document the range of variation in nutrient content across finfish species. To understand how nutrient profile varies among species, we tested whether ecological traits known to be both biologically important and exhibit predictable scaling relationships could explain this variation. WE COLLECTED SPATIALLY AND SIZE EXPLICIT DATA, TO IDENTIFY THRESHOLDS IN GEOGRAPHY AND OR BODY SIZE THAT INDICATE THAT A SPECIES ACHEIVES 25% RDI IN A SINGLE PORTION.

### Literature search and data collection

To test how nutrient profile varies with ecological traits, we assembled a dataset of nutrient content in the edible portions of 354 aquatic species. We defined the nutritional profile of a species as the quantity of a given nutrient in 100 g of edible tissue - a metric that is commonly used in the human food composition literature (Nowak et al. 2014). We aimed to include as many finfish species as possible, from marine and freshwater systems, covering a wide geographic range. We searched the literature for analytical compositional values for each of these species. We searched the peer-reviewed literature as well as food composition databases or tables, such as the Food and Agriculture Organization’s INFOODS database (FAO/INFOODS 2014) and the United States Department of Agriculture’s Nutrient Files (USDA 2012). We restricted our analysis to include only the edible portions of wild, raw fish (thus excluding prepared or farmed seafood items). Our dataset includes quantities for the following microelements: calcium; iron; zinc; mercury and two fatty acids: eicosapentaenoic acid (EPA); and docosahexaenoic acid (DHA). To address inconsistencies in fatty acid data reporting, we standardized fatty acid measurements using the fatty acid conversion factors proposed by Nowak et al. (2014).

Ecological trait information was collected for each species from FishBase (Froese and Pauly 2014) and SeaLifeBase (Palomares and Pauly 2014). We included body size (maximum length), fractional trophic position, temperature preference (using latitude as a proxy) and habitat preference (Marine, Freshwater, brackish…). We converted body length data into body mass, using established length-mass relationship data (*mass*= *a\*length^b*). We used species-specific or taxon-specific *a* and *b* parameter values published in Froese et al. (2013).

### Statistical Analysis

We modeled the relationship between nutrient content and species’ traits with linear regression models using a log-transformed power function. The full model included the entire set of predictors:

ln(nutrient content) = *Β0.i* + *Β1.i*\*ln(body size)\*latitude + *Β2.i*\*ln(body size)\*(trophic position) + *Β3.i*\*ln(body size)\*(habitat) + εi

We created models from subsets of the full model that represented hypotheses based on the known physiological roles of micronutrients **and their relationships to our set of predictors**. We identified the best subset of models using the Akaike Information Criterion, adjusted for small sample sizes (AICc). We used AICc, δaic and Akaike weights (w) to compare models. We ranked models based on w, and selected the set of models that produced a cumulative w > 0.95, meaning that we are 95% confident that the chosen set includes the best model (Burnham and Anderson 2002).

We report all models with AICc differences ( δaic = AICi − AICmin) less than or equal to two ( δaic ≤ 2). In cases where we could not obtain measurements of all traits for all species, we performed model selection on reduced datasets without missing values (Appendix A). To account for model uncertainty, we performed model averaging of coefficients in all models with δaic < 2, and included zeros as coefficients when variables did not enter a particular model (Burnham and Anderson 2002). We conducted all our analyses in R version 3.1.2 (R Core Development 2014) using the MuMIn package (<http://r-forge.r-project.org/projects/mumin/>).

-somewhere here, need to address multi-collinearity of predictor variables etc.

## Results

### add SENTENCE SUMMARIZING RESULTS AND SIGNPOSTING.

*We assembled fish from all over the world (map of where your samples came from, color or symbol coded by nutrient?). Most data was available for X nutrient, but we found at least X measurements for each nutrient we tested.*

### Minerals

**Calcium**

Calcium in the edible portion varied by over 6 orders of magnitude in the 83 fish for which we found data, from 1 mg/100 mg in the XX fish to over 7 in XX species (Figure 1). Variation in calcium content was explained by body size, habitat and latitude in the best model (Table 1, Figure 1, R2 = 0.63). Ca content in edible portions declined with increasing latitude of the species, and with increasing body size. Trophic position and an interaction between body size and latitude were retained in the best model set, though their coefficients did not differ significantly from zero, suggesting they are not driving the main effects of body size and latitude. X species had calcium contents that achieved RDI, and 20 species had calcium contents that equaled or exceeded 25% RDI. These species averaged 495.83 g (min=3.71g, max=38.82 kg) and were distributed over X latitude. The remaining 63 species contained less than 25% of calcium RDI in one portion, and had mean body size 6.27 kg (min=31g, max=2532.63kg).

For every 10% increase in body size, calcium content decreases by 3.39% ([Figure 2](https://docs.google.com/drawings/d/1tqpBX1J7E_iW4klju1Uqjd1tqHLm7ZYRjNvE5TbOS-A/edit)a). With each degree of latitude away from the equator, calcium content decreases by 7.25%.

**Iron**

Habitat, latitude and body size all explained variation in iron content of edible portions (Table 2, best model R2=0.32). However, only latitude showed a significant effect. With each degree of latitude away from the equator, iron content decreases by 5% ([Figure 2b](https://docs.google.com/drawings/d/1tqpBX1J7E_iW4klju1Uqjd1tqHLm7ZYRjNvE5TbOS-A/edit)).

**Zinc**

Body size, latitude and body size\* latitude all explained variation in the zinc content of edible portions (Table 3, best model R2=0.27-0.28). Across these models, only latitude had a significant effect. For each degree of latitude away from the equator, zinc content decreases by 3% ([Figure 2c](https://docs.google.com/drawings/d/1tqpBX1J7E_iW4klju1Uqjd1tqHLm7ZYRjNvE5TbOS-A/edit)). Nine out of 84 species have more than 25% of RDI in a single edible portion. These species were found between 11-41 degrees of latitude and had mean body size 1.14kg (min=3.71g, max= 34.0kg).

**Mercury**

Latitude, habitat, trophic level and body size all explained variation in the mercury content of edible portions (Table 4, best model R2=0.27). Latitude, habitat and body size all had significant effects. Each 10% increase in body size is associated with a 2.62% increase in mercury content ([Figure 2d](https://docs.google.com/drawings/d/1tqpBX1J7E_iW4klju1Uqjd1tqHLm7ZYRjNvE5TbOS-A/edit)). With each degree of latitude away from the equator, mercury content decreases by 4%. Marine species contain 50% more mercury than freshwater species. Thirty-three species contained more than the EPA's reference dose limit for mercury (0.1 mcg/kg body weight/day, assuming a 60kg person) in a 100g edible portion. The mean body mass of species above the reference dose limit was 9.35 kg (min=0.4kg, max=2532.63kg) vs. 3.92 kg (min=1.03kg, max=66.92kg) for the species below the reference limit.

### Fatty acids

**EPA**

The top models had moderate explanatory power (R2=0.2) ([Table 5](https://docs.google.com/spreadsheets/d/1Mu6hsNGHwhrEGR5kQp18W3OnPTQYQ5zL-3mYIBYcoWI/edit#gid=1579902761)). Habitat, latitude, body size and trophic level, as well as the interactions between these predictors and body size all entered the top model set. Of these terms, latitude and body size had significant effects. For each 10% increase in body size, EPA content decreases by 1.20% ([Figure 3a](https://docs.google.com/drawings/d/1G9cwEpWRumV-qIRddWOIfSh-QLAija9J4ric4l9Gv98/edit?usp=sharing)). Each degree of latitude away from the equator leads to a 2.49% increase in EPA content. Twenty-nine of 204 species contained 100% of the RDI for EPA in one portion, while 93 species contained at least 25% of RDI in one portion.

**DHA**

The top model had moderate explanatory power (R2=0.18) ([Table 6](https://docs.google.com/spreadsheets/d/1Mu6hsNGHwhrEGR5kQp18W3OnPTQYQ5zL-3mYIBYcoWI/edit#gid=531186192)). Habitat, latitude, body size and trophic level, as well as the interactions between these predictors and body size all entered the top model set. Of these terms, all had significant effects except habitat\*body size interaction. With each degree of latitude away from the equator, DHA content increases by 1.54% ([Figure 3b](https://docs.google.com/drawings/d/1G9cwEpWRumV-qIRddWOIfSh-QLAija9J4ric4l9Gv98/edit?usp=sharing)). Each 10% increase in body size leads to a 0.87% decrease in DHA content.

## Discussion

We found that the substantial variation in the nutritional content of an edible portion among seafood species can be explained partly major ecological functional traits of latitude, body size and habitat associations. **The two axes that most strongly control nutritional content of the edible portion are body size and latitude. In order to meet at least 25% of RDI for a range of nutrients, species must be XX size and come from XX latitude. For calcium, all species that contain 25% of RDI in one portion are 6.43kg or smaller.** In contrast to much of the existing work on stoichiometry in fishes, which address patterns of macroelement and macronutrient variability, here we studied variability in microelemental composition. We found even greater levels of variability in micronutrient stoichiometry than have been documented in the literature for macroelements. Calcium content per edible portion varied by more than 500-fold (min=2.99, max=1503.00, median=45.15 mg/100 g). Fatty acids varied by X amount. Essential microelements varied by X fold, while non-essential elements such as mercury varied by X amount. It is possible that we found higher levels of variability than have been previously documented because we focused on micronutrients, which may be more variable across taxa than macronutrients due to their higher degree of functional specificity or lower level of homeostasis (Karimi et al. 2006). In addition, we sampled fishes over a much greater range of taxa, body sizes, geographic origins etc. Further, our data are for the edible portion of fish, which includes different body tissues depending on the species and its size. Sometimes the edible portion contains organs such as liver and skeleton, sometimes it is just muscle tissue. Nonetheless, in spite of the diversity of fishes included in our analysis, we found that our models had relatively high levels of explanatory power, suggesting that we have identified some traits that have a strong influence on whole body stoichiometry as well as the stoichiometry of the edible portion.

The degree of variability we documented in the edible portion is substantially higher than variability documented among whole body measurements. This could be due to the fact we are sampling across much broader habitat/latitudinal/phylogenetic ranges, and/or because nutrient deposition is not uniform across body tissues, so including samples that are are not uniform in their inclusion of body parts could increase the range of variability. Nonetheless, the relevant metric of nutritional value from the human perspective is the nutrient content of the edible portion, not the whole body. Thus, documented variability in whole body elemental composition may underestimate the variability in nutritional value of the edible portion.

**Micronutrient variability**

The elemental stoichiometry of fishes is a trait arising from a variety of evolutionary pressures on form and function (Kay et al. 2005). Diverse stoichiometries among fish taxa are a consequence of the variation in fish body composition and the biochemicals that constitute all living organisms such as proteins, carbohydrates and lipids. The variability in stoichiometry among fishes has been related to allometry (Hendrixson et al. 2007, Vrede et al. 2010), habitat preferences (Childress et al. 1990, Sterner and George 2000, Hendrixson et al. 2007, Vrede et al. 2010), diet type (Pilati and Vanni 2007, Hendrixson et al. 2007, Vrede et al. 2010), ontogeny (Pilati and Vanni 2007), and degree of ossification (Hendrixson et al. 2007, Czamanski et al. 2011). In this study, we documented the degree of variability in micronutrients with respect to these sources of variation, including body size, habitat, trophic position and geographic origin. Of these sources of variation we found consistently strong support for the importance of latitude and body size, mixed evidence for the role of habitat, and weak evidence for the role of trophic position in determining a fish’s nutritional composition.

The degree of variability in macroelements (C, N and P) and macronutrients (eg lipids and proteins) among fishes is well documented and context-dependent (Sterner and George, 2000, Tanner et al. 2000). In a study of 26 species of neotropical fishes, Vanni et al. (2002) found about 3 fold variation in body P contents, with the highest P species belonging to the *Loricariidae*, a family containing the tropical armoured catﬁsh. Dantas and Attayde (2007) found that N and P contents varied two-fold among eight tropical and temperate species, and for some but not all of these species, N and P contents varied with body size. Vrede et al. (2010) found significant effects of body size, diet and morphology in whole body P content even within a single population of a perch species. However, the effect was not significant when the dorsal muscle tissue was examined separately. Similarly, Hendrixson et al. (2007) found that fish P varied about two fold across 20 species, a pattern associated with body size and feeding type. However, when accounting for the phylogeny, interspecific allometric trends no longer were statistically significant. Patterns in lipid content have also been documented across fish taxa. Spitz et al. (2010) found that lipid contents in 78 marine forage species were highly variable, ranging from 0.3-12% of body mass. Childress et al. (1990) found that lipid content varies with depth preference, perhaps due to differing metabolic requirements associated with living at depth.

**Effects of latitude**

We found that latitude was consistently an important predictor of nutrient content across all the nutrients we tested. Latitude may be important because geographic origin is related to a suite of traits that could affect species' function and form. For example, latitude reflects evolutionary and historical distinctiveness of different ocean basins (Floeter et al. 2005), differences in thermal and metabolic constraints on growth and reproduction (Trip et al. 2014), all of which could influence species' stoichiometry.

We found that increasing latitude is correlated with decreased tissue concentrations of the microelements iron, calcium and zinc, consistent with the Temperature-Constraint Hypothesis (Gaines and Lubchenco 1982, Clements et al. 2009), which suggests that processing and assimilating nutrients in herbivorous fish may be inhibited by cold temperatures at higher latitudes.

In contrast to the patterns for microelements, we found that the concentration of the essential polyunsaturated fatty acids EPA and DHA both increase with increasing latitude. This pattern is consistent with experimental evidence from several fish species showing that polyunsaturated fatty acid concentration is inversely related to temperature (Farkas 1984, Wallaert and Babin 1994), a physiological adaptation to increase cold tolerance by increasing membrane fluidity. Further, variability in fish PUFA content could be related to the concentration of PUFA in zooplankton (Ahlgren et al. 1996), which is known to vary geographically (St John and Lund 1996), and with temperature (ref). Thus, the pattern of increased PUFA concentrations we found in higher latitude species is consistent with cold water adaptations and increased availability of these essential fatty acids in prey items.

**Effects of body size**

The stoichiometry of fishes may vary with body size since habitat preferences, diet and patterns of allocation to different tissues and biochemical compounds all may vary with size. We found significant body size effects for the majority of nutrients we analyzed, including calcium, mercury, EPA and DHA.

In fishes, the majority of calcium content is stored in the skeleton (Hendrixson et al. 2007). In a broad range of fish taxa, skeleton mass scales isometrically (Berrios-Lopez et al. 1996). Thus, whole body calcium content should scale isometrically, and mass specific calcium content should remain constant with increasing body size. In our dataset of the edible portions of fish, which accounted for the calcium contained in bones discarded as plate waste, we found that calcium content decreased significantly with fish body size. That the edible portions of larger fish contain less calcium than the edible portions of smaller fish may be attributed to human dietary practices. While it is customary to eat the whole body, including the bones and head of small fish, such as sardines and XXX (eg Roos et al. 2007), the edible portions of large fish are usually restricted to muscle tissue (fillets), and thus don't contain skeleton-associated calcium stores. Thus, the calcium stored in the skeleton of larger fish is not accessible to the human diet, contributing to the decreased calcium content of large fish. When we account for dietary practices of bone consumption in our analysis, we do not find a significant negative relationship between calcium content and size (supplementary table?).

Consistent with biomagnification of toxic elements in aquatic ecosystems (Chen et al. 2010), we found that mercury content in the edible portion of fish increased with fish body size. More than three quarters of the species in our dataset exceeded the recommended dietary intake levels for mercury in a single portion. The species that did not exceed safe mercury exposure limits, as recommended by the Environmental Protection Agency (EPA ref) were all relatively small species, mean body size 3.92kg. Surprisingly, we did not find an effect of trophic level on mercury content.

**Future directions: Applied ecological stoichiometry**

Most existing stoichiometry theory and experiments address macronutrients, such as carbon, nitrogen and phosphorus. The stoichiometry of micronutrients, such as iron or zinc, is much less well documented. Further, the degree of regulation of micronutrients across a range of fish taxa is unknown. Thus, is it difficult to make predictions about how the content of micronutrients should vary across species. In the context of human nutrition, one of most important benefits that people derive from seafood consumption is not just the calories or protein, but the micronutrients (add refs). As a result, it is important to focus attention on the stoichiometry of micronutrients, particularly iron, zinc, calcium, selenium and essential fatty acids, since those are the most valuable in the human diet.

Establishing the functional role of microelements should enable predictions about how the concentrations of these elements should vary with factors such as body size, temperature and evolutionary history. For example, if some nutrients are under tighter homeostatic control than others, then we might expect those nutrients to be less variable across fish taxa (eg Karimi et al. 2006). For example, in a test of variability of macronutrients, vs. essential micronutrients vs. non-essential elements in aquatic invertebrates, Karimi et al. 2006 found that variation in somatic element concentration increased from macronutrients to non-essential elements.

The allometry of micronutrients is relatively poorly understood relative to macronutrients.

{more to come here…}

Finally, correctly predicting the human nutritional value of fish species depends not just on the elemental stoichiometry of the species, but also on the human dietary practices associated with the species. In general, including a larger proportion of the fish tissues in the edible portion should increase the nutritional value of the fish species. For example, fish eyes and heads can be valuable sources of vitamin A (eg Roos et al. 2007).

**Conclusions**

something that ties together results and future directions in the context of ecology and human nutrition

We have identified two traits, body size and thermal niche, both of which are related to temperature and are correlated with the micronutrient content of fish tissue. With these established scaling relationships, we can estimate how the distribution of traits in an ecosystem, such as the distribution of body sizes, influences the nutritional profile of the fish assemblage as whole. This is an important step towards understanding the mechanisms underlying the nutritional benefits that humans derive from aquatic ecosystems.

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**Figures**

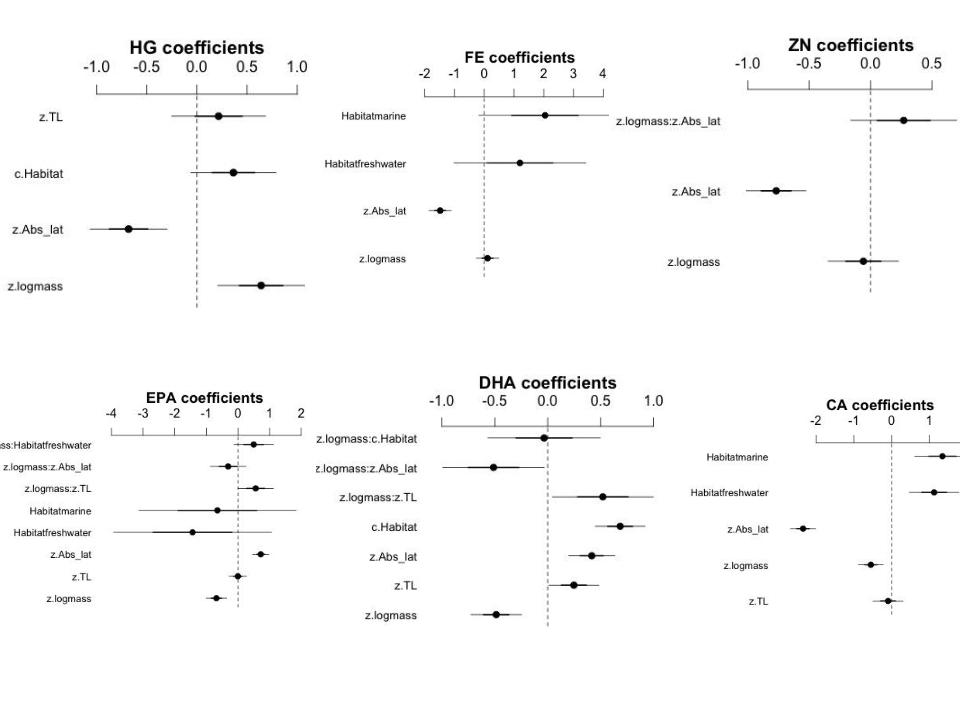


Figure 1. Standardized regression coefficients.

Figure 2.

Table 1. Results of model selection and model averaging for models relating calcium content of 100 g edible portion to species traits.

<https://docs.google.com/spreadsheets/d/1Mu6hsNGHwhrEGR5kQp18W3OnPTQYQ5zL-3mYIBYcoWI/edit?usp=sharing>