Canadian Science Publishing
NRC Research Press journals | Publishing Services

Environmental Reviews
Dossiers environnement

Production, Distribution, and Abundance of Long-Chain Omega-3 Polyunsaturated Fatty Acids: A Fundamental Dichotomy between Freshwater and Terrestrial Ecosystems

Journal:	*Environmental Reviews*
Manuscript ID	er-2015-0029.R1
Manuscript Type:	Review
Date Submitted by the Author:	18-Aug-2015
Complete List of Authors:	Hixson, Stefanie; Ryerson University, Chemistry and Biology Sharma, Bhanu; University of Toronto- Mississauga, Kainz, Martin; WasserCluster - Biologische Station Lunz, Wacker, Alexander; University of Potsdam, Institute of Biochemistry and Biology Arts, Michael; Ryerson University, Chemistry and Biology
Keyword:	aquatic ecosystems, conservation, eicosapentaenoic acid, docosahexaenoic acid, food webs

SCHOLARONE™
Manuscripts

Production, Distribution, and Abundance of Long-Chain Omega-3 Polyunsaturated Fatty Acids: A Fundamental Dichotomy between Freshwater and Terrestrial Ecosystems

Stefanie M. Hixson[1], Bhanu Sharma[2*], Martin J. Kainz[3], Alexander Wacker[4], Michael T. Arts[1]

[1]Department of Chemistry and Biology, Ryerson University, 350 Victoria Street, Toronto, Ontario, Canada

[2]Department of Biology, University of Toronto- Mississauga, 3359 Mississauga Rd, Mississauga, Ontario, Canada

[3]WasserCluster - Biologische Station Lunz, 3929 Lunz am See, Austria

[4]Institute of Biochemistry and Biology, University of Potsdam, Am Neuen Palais 10, Potsdam, 14469 Germany

*Current affiliation: Toronto Rehabilitation Institute, University Health Network, 550 University Ave, Toronto, Ontario, Canada M5G 2A2

Corresponding author: Stefanie M. Hixson
Department of Chemistry and Biology
Ryerson University
350 Victoria Street
Toronto Ontario Canada M5B 2K3
+1 (416) 979-5000 ext. 3216
shixson@ryerson.ca

Word count: 6,841

Draft

Abstract

Long-chain polyunsaturated fatty acids (LC-PUFA) are critical for the health of aquatic and terrestrial organisms; therefore, understanding the production, distribution, and abundance of these compounds is very important. Although the dynamics of LC-PUFA production and distribution in aquatic environments has been well documented, a systematic and comprehensive comparison to LC-PUFA in terrestrial environments has not been rigorously investigated. Here we use a data synthesis approach to compare and contrast fatty acid profiles of 369 aquatic and terrestrial organisms. Habitat and trophic level were interacting factors that determined the proportion of individual omega-3 (n-3) or omega-6 (n-6) PUFA in aquatic and terrestrial organisms. Higher total n-3 content compared with n-6 PUFA and a strong prevalence of the n-3 PUFA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) characterized aquatic versus terrestrial organisms. Conversely, terrestrial organisms had higher linoleic acid (LNA) and alpha-linolenic acid (ALA) contents than aquatic organisms; however, the ratio of ALA: LNA was higher in aquatic organisms. The EPA + DHA content was higher in aquatic animals than terrestrial organisms, and increased from algae to invertebrates to vertebrates in the aquatic environment. An analysis of covariance revealed that fatty acid composition was highly dependent on the interaction between habitat and trophic level. We conclude that freshwater ecosystems provide an essential service through the production of n-3 LC-PUFA that are required to maintain the health of terrestrial organisms including humans.

Keywords: Aquatic ecosystems, conservation, eicosapentaenoic acid, docosahexaenoic acid, food webs

Draft

Introduction

Long-chain (i.e. ≥ 20 carbons long) polyunsaturated fatty acids (LC-PUFA) are critically involved with key physiological functions of aquatic and terrestrial vertebrates, including humans, in supporting brain function, cardiovascular health, growth, reproduction, and the immune response (Arts et al. 2001; Brenna et al. 2009; Simopoulos 2011; Parrish 2013). The LC-PUFA with distinct critical functions for vertebrate health include eicosapentaenoic acid (EPA; 20:5n-3), docosahexaenoic acid (DHA; 22:6n-3), and arachidonic acid (ARA; 20:4n-6). The long carbon chain and highly unsaturated nature of these compounds is important for cell membranes, as it allows for both structure and fluidity (Arts and Kohler 2009), as well as quick conformational changes (Sargent et al. 2002). Because of its unique structure, DHA plays a critical role in the development and functioning of neural and ocular tissue (brain and eye) (Parrish 2009; Lands 2009; Raji et al., 2014), but also has been shown to have important roles in cognition, behaviour, and mood (Kidd, 2007). In addition, ARA is crucial for brain functioning, cell signalling and is a precursor for endocannabinoids (Turcotte et al. 2015), and eicosanoids (Calder, 2015a). The omega-3 PUFA EPA and DHA are known to have anti-inflammatory effects, lower risks of cardiovascular disease, influence immune functions and defense against infections, and protect against some cancers (as reviewed by Calder, 2015b).

These LC-PUFA are produced from their omega-3 (n-3) and omega-6 (n-6) precursors: alpha-linolenic acid (ALA; 18:3n-3) and linoleic acid (LNA; 18:2n-6) (Fig. 1). Vertebrates lack the enzymes necessary to form ALA (via delta-15 desaturase) and LNA (via delta-12 desaturase; Cook and Mcmaster 2004) from 18:1n-9, thus these short-chain fatty acids are considered essential in their diet. However, their direct physiological function in organisms is limited, as their main purpose is to act as a precursor to the physiologically-essential LC-PUFA: EPA,

DHA, and ARA (Tocher 2003). ALA and LNA are found in reasonable abundance and are typically not limiting to animals (Cunnane 2000). While consumption of the n-3 and n-6 precursor is an essential requirement for all vertebrates, consuming pre-formed EPA, DHA, and ARA is highly advantageous for many vertebrates, especially if they have a limited ability to synthesize them (Parrish 2009).

LC-PUFA are mostly synthesized by primary producers at the base of freshwater and marine food webs. Some algal taxa (e.g. diatoms, dinoflagellates, cryptophytes) produce relatively large amounts of EPA and DHA (Brett and Müller-Navarra 1997, Galloway and Winder 2015) and these LC-PUFA are progressively consumed and generally selectively retained by other aquatic organisms (e.g. zooplankton, benthic invertebrates, molluscs, and fish) higher up in the food chain, which makes these fatty acids effective dietary biomarkers in food webs (Daalsgard et al. 2003; Kainz et al. 2004; Lands 2009; Taipale et al. 2013). The LC-PUFA composition of algae is an important determinant of food quality for consumers, and is a powerful tool to track different consumer diets in an aquatic food web (Budge et al. 2002; Dalsgaard et al. 2003; Taipale et al. 2013). Conversely, primary producers in terrestrial ecosystems produce ALA and LNA; and the evidence is lacking that they have the ability to synthesize EPA, DHA and ARA (Sayanova and Napier 2004). The inherent difference in LC-PUFA production between aquatic and terrestrial ecosystems is largely rooted at the base of food webs in these habitats, and therefore has important physiological consequences for all consumers.

It is generally assumed that fatty acid composition (including the production, abundance and distribution) of aquatic vs. terrestrial species are distinctly different, mainly on account of LC-PUFA (Olsen 1999; Gladyshev et al. 2009); however, this has yet to be systematically and

quantitatively supported; particularly for freshwater species. The role of LC-PUFA in the aquatic environment has been well documented (Arts and Wainmann 1999; Kainz et al. 2004; Arts et al. 2009; Parrish 2013); however, a direct and quantitative comparison has not been made to the terrestrial environment. It is also poorly documented whether all animals in the terrestrial environment have a universal dependency on LC-PUFA. However, there is evidence to suggest that LC-PUFA are needed for functioning of some tissues (e.g. neural, ocular; Böhm et al. 2014), and/or certain stages in development, and/or during certain seasons (Gladyshev et al. 2009). Yet terrestrial animals (most studies focus on humans or human models) are known to be poor at desaturating and elongating ALA and LNA to their LC-PUFA products (Supplementary Table S1). The amount of LC-PUFA in terrestrial animal tissues may be related to their accessibility to available dietary LC-PUFA, as well as their ability to synthesize LC-PUFA. It is therefore important to distinguish differences in the production, distribution and abundance of LC-PUFA between aquatic and terrestrial organisms.

Freshwater ecosystems are of particular interest in terms of the potential for LC-PUFA transfer from aquatic to terrestrial organisms because there is a high level of connectivity between freshwater and surrounding terrestrial landscapes in these systems (Gladyshev et al. 2009; Gladyshev et al. 2013). Because of this high degree of connectivity, wetlands are likely particularly efficient sources of LC-PUFA and easily spread to terrestrial animals that live in and around these habitats. The transfer of LC-PUFA may occur via direct or indirect dietary trajectories from wetlands to terrestrial consumers (Gladyshev et al. 2013). Some species have both aquatic and terrestrial life cycles, such as insects and amphibians. Alternatively, terrestrial consumers may have direct access to aquatic diets, for example piscivores like herons, eagles, osprey, otters, bears, etc. Thus, we must be cognizant of the production and transfer of LC-PUFA

Draft

from freshwater to surrounding terrestrial environments. However, it must also be established whether there is indeed a systematic difference in LC-PUFA production between organisms in these environments, and whether freshwater ecosystems are providing an essential resource to animals living in adjacent terrestrial ecosystems.

The distinction between the types and amounts of PUFA in aquatic and terrestrial environments must be well documented in order to investigate the degree of terrestrial dependency on LC-PUFA produced in aquatic ecosystems. The n-3 and n-6 PUFA, in particular ALA, LNA, EPA, DHA, and ARA, are of special interest due to their essentiality and physiological functions in organisms (Parrish 2009); therefore we focused on these fatty acids. We focused on freshwater organisms due to the high level of connectivity between freshwater and surrounding terrestrial ecosystems and the potential for LC-PUFA transfer. The primary objective of this data synthesis was to define and quantify the difference in fatty acid profiles (ALA, LNA, EPA, DHA, ARA) in freshwater and terrestrial organisms at varying trophic levels to more rigorously quantify the distinct and natural variation in LC-PUFA production, distribution, and abundance that exists between these ecosystems.

Draft

Methodological Approach

Data collection

Fatty acid data from freshwater and terrestrial species were collected from the primary, peer-reviewed, scientific literature and from the author's unpublished sources. Articles were retrieved from the following databases: JSTOR; Scholar's Portal; Web of Science; and the University of Toronto Article Reserve. The following search algorithm was used when conducting literature reviews: 'Fatty Acid' or 'ALA' or 'LNA' or 'ARA' or 'EPA' or 'DHA' (in

all fields) and 'Freshwater' or 'Terrestrial' or 'Lipid' (in all fields). To qualify for inclusion in the data set, the data were required to present all fatty acids of interest: ALA, LNA, EPA, DHA, ARA; and sums of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and PUFA (or total fatty acids along with a complete list of fatty acids to calculate these sums). The fatty acid data must have been presented as relative fatty acid %. Although it would have been preferable to perform a data synthesis on fatty acid contents expressed as mass-fractions (mg g^{-1}), the majority of studies present fatty acid data on a proportional basis (i.e., %); therefore, using proportional data increased the number of fatty acid profiles available to include in the data synthesis. Although reported values of proportional fatty acid data depend on the total number of identified fatty acids, our main objective was to investigate differences in fatty acid patterns between aquatic and terrestrial organisms, and as such, analytical differences among the investigated studies were considered of minor importance. Outliers were managed by reviewing the data compiled for each fatty acid within a functional group (described below) and a Grubb's outlier test was conducted to determine significant outliers ($p < 0.05$). If a significant outlier was detected, the original source of the data was reviewed and if an error was perceived, the data were removed.

In several cases, fatty acid data (M. T. Arts, Environment Canada, unpublished) for a single species were available for different seasons or from different locations. Within a single location, a grand mean was calculated from the fatty acid data from that location, regardless of season; this value represented the average fatty acid profile of that species in that location. Different locations were considered as separate data and were not amalgamated to provide a grand mean for that particular species.

Data organization

https://mc06.manuscriptcentral.com/er-pubs

The central database (369 fatty acid profiles) was stratified into 9 sub-databases which included the following simplified functional groups: (1) terrestrial plants, (2) terrestrial insects, (3) terrestrial mammals, (4) algae, (5) freshwater insects, (6) zooplankton, (7) benthic invertebrates, (8) freshwater molluscs, and (9) fish.

Multivariate analyses

All multivariate analyses were conducted in PRIMER (Plymouth Routines in Multivariate Ecological Research; PRIMER-E Ltd, Version 6.1.15, Ivybridge, UK). Analysis of similarities (ANOSIM), cluster analysis and multidimensional scaling (MDS) were used to define differences in fatty acid profiles among the different groups (algae, aquatic insects, zooplankton, etc). Fatty acid data were square root transformed prior to analysis in PRIMER to achieve homogeneity of variance in fatty acid data in studies collected from different sources. ANOSIM is multivariate analysis that uses a resemblance matrix, the latter carries out an approximate analogue of ANOVA. ANOSIM generates a value of R that ranges between 0 and 1; a value of zero representing the null hypothesis (no difference among a set of samples) and 1 (complete dissimilarity among set of samples) (Clarke and Warwick 2001). The non-metric Bray-Curtis dissimilarity statistic was used to quantify the compositional dissimilarity between samples (Bray and Curtis 1957). This measure delivers robust and reliable dissimilarity results, and is one of the most commonly applied measurements to express relationships in ecology, environmental sciences and related fields (Clarke and Warwick 2001). The purpose of MDS is to construct the data points in a multi-dimensional space, which configures the data in a similarity/dissimilarity matrix. The MDS method places samples on a two dimensional “map” in such a way that the distance between samples on the map agrees with the rank order of the matching similarity/dissimilarity taken from a similarity matrix (Clarke and Warwick 2001).

https://mc06.manuscriptcentral.com/er-pubs

Therefore, MDS provided a visual representation of the similarities among fatty acid profiles of the different habitats and species groups.

Analysis of covariance

A model was tested to determine if trophic level, habitat or their interaction, affects fatty acid composition. An analysis of covariance (ANCOVA) was performed (Minitab 16 Statistical Software), using fatty acid data (square root transformed) as the response variable (the same data from individuals used in the multivariate statistic analysis, n=369). The model was run for each fatty acid (ALA, LNA, ARA, EPA, DHA) or fatty acid group (SFA, MUFA, PUFA). Factors included in the model were: habitat (fixed categorical variable = aquatic or terrestrial), functional group (loosely based on trophic level or position in a food web, as fixed covariate with 9 levels = terrestrial plant [1], algae [2], aquatic insects [3], terrestrial insects [4], zooplankton [5], benthic [6], mussels [7], fish [8], terrestrial mammals [9]), and the interaction between habitat and trophic level (habitat*trophic level). The functional groups ranged from plants (primary producers) to invertebrates (primary and secondary consumers) to vertebrates (higher consumers). The purpose of arranging these functional groups was to establish a basic hierarchy of positions in a food web within each habitat. However, the groupings we used were approximate because, for example, some invertebrates can differ in their trophic levels, as some can be primary or secondary consumers (e.g. zooplankton), while some vertebrates can be primary or secondary consumers too (e.g. filter feeding planktivorous fish). Therefore, our use of the term "trophic functional groups" only approximates the true trophic position of the various taxa in the respective food webs. Residuals were examined for homogeneity of variance, independence, and normality.

Draft

Data Synthesis Results

Fatty acid profiles

A total of 369 fatty acid profiles (ALA, LNA, EPA, DHA, and ARA as well as total SFA, MUFA, PUFA) from different species of either aquatic or terrestrial habitats were included in the statistical analyses. The fatty acid profiles were further grouped according to taxonomic similarity: terrestrial plants (n = 84), terrestrial insects (n = 50), terrestrial mammals (n = 43), algae (n = 17), aquatic insects (n = 19), zooplankton (n = 21), benthic invertebrates (n = 17), molluscs (n = 31), and fish (n = 87) (Table 1).

Multidimensional scaling

The MDS plots illustrated the difference in fatty acid profiles among trophic groups and habitats that configured the data in a similarity/dissimilarity matrix. Each data point in the plot represents a fatty acid profile (ALA, LNA, EPA DHA, and ARA, total SFA, total MUFA, and total PUFA) for one individual. When individual fatty acid profiles (ALA, LNA, EPA, DHA, ARA, and total SFA, MUFA, PUFA) were grouped according to habitat only (aquatic or terrestrial), there was a divide in the plot, where terrestrial species were located on the top left side of the plane and aquatic species were plotted on the bottom right side of the plane (Fig. 2a). Fatty acid vectors were directionally-oriented in this plot, indicating an association between the vector and the fatty acid profile of individuals in the vicinity of the vector. The LC-PUFA vectors EPA, DHA, and ARA were located on the bottom right side of the plot, indicating an association with aquatic fatty acid profiles. In the opposite direction, the vectors pointing toward the top left side of the plot indicating an association with terrestrial fatty acid profiles were the n-3 and n-6 metabolic precursors (ALA and LNA).

Draft

The separation between fatty acid profiles from organisms in aquatic and terrestrial habitats was still evident when they were organized according to functional group (within taxonomic classification). However, organizing the data by functional group provides greater detail in terms of which category was most responsible for the divide between aquatic and terrestrial fatty acid profiles (Fig. 2b). Terrestrial plants and fish had the least similar fatty acid profiles, as they were more spread spatially from each other. Terrestrial species clustered on the left side of the plot, while aquatic species clustered on the right side of the plot, with FA vectors LNA (terrestrial) and EPA, DHA, and ARA (aquatic) driving this spatial difference. However, data points belonging to a particular functional group did not necessarily tightly cluster together, with the exception of fish. Terrestrial insects tended to group within the "terrestrial" half of the plot, but did not form a tight cluster. Similarly, terrestrial mammals occupied a space between terrestrial plants and fish, but again did not form a distinct cluster. Algae occupied the space between aquatic and terrestrial habitats.

ANOSIM

ANOSIM quantified differences in fatty acid profiles (ALA, LNA, EPA, DHA and ARA, and, total SFA, MUFA, and PUFA) of individuals categorized by habitat (aquatic or terrestrial) and functional group (those of similar taxonomic classification: plants, invertebrates, vertebrates) (Table 2). A total of 34 pairwise comparisons were made. An R-statistic close to 1 indicates that the pair is very different; an R-statistic close to 0 indicates that the pair is not very different. Nearly all pairwise comparisons of fatty acid profiles were significantly different (global R-statistic = 0.421; $p = 0.001$). Only terrestrial plants and terrestrial insects were not significantly different from one another (R-statistic = 0.049; $p = 0.062$). Most of the comparisons were different because the groups were different by both habitat and functional grouping.

Fatty acids and fatty acid group ratios

Because fatty acid content was different in aquatic and terrestrial habitats (based on Fig. 2), these groups were separated along a "trophic gradient" within each habitat (organized by functional groups including plants, invertebrates and vertebrates). The 9 functional groups represent organisms in two habitats (aquatic and terrestrial) and are loosely based on position in a food web. While the hierarchal levels are approximate, there are three major groups in this system, which generally represent producers (plants), primary consumers (invertebrates) and secondary or tertiary consumers (vertebrates). The sum of LNA + ALA was higher in terrestrial than aquatic organisms, while the sum of EPA + DHA was higher in aquatic animals than terrestrial organisms (Fig. 3). The terrestrial plant fatty acid profiles that we had investigated did not contain EPA and DHA. LNA decreased with increasing "trophic level" (from plants to invertebrates to vertebrates), which was sorted by habitat (terrestrial to aquatic), and was higher in terrestrial compared to aquatic organisms (Fig. 4; see ANCOVA results below). Conversely, DHA increased with trophic level (see ANCOVA results below) and was higher in aquatic compared to terrestrial organisms (Fig. 4). The ALA: LNA ratio was higher in primary producers than secondary and tertiary consumers in both aquatic and terrestrial ecosystems (Fig. 5); however did not decrease with increasing trophic level. The mean ALA: LNA ratio was higher in aquatic (1.4: 1) than terrestrial organisms (0.4: 1) according to a two-tailed t-test ($p = 0.033$). The total n-3 PUFA content (sum of ALA, EPA and DHA) in aquatic organisms was higher than terrestrial organisms (Fig. 4). The mean n-3 PUFA content was 19.4% of total fatty acids in aquatic organisms, compared to 7.3% in terrestrial organisms. The total n-6 PUFA content (sum of LNA and ARA) in aquatic organisms was 9.7% of total fatty acids, compared to 24.4% in terrestrial organisms. The mean n-3: n-6 PUFA ratio (based on % total FA) in aquatic organisms

Draft

(3.4: 1) was higher than terrestrial organisms (0.6: 1) according to a two tailed t-test ($p = 0.004$). Aquatic organisms in this study contained 6 times more n-3 PUFA than terrestrial organisms. A summary of the data in this section can be found in Supplementary Table S3.

ANCOVA

To determine if habitat (aquatic or terrestrial) and functional group ("trophic level") were significant factors in influencing the fatty acid composition of organisms, a model was designed to test the effect of either habitat or functional group, or the interaction. All individual fatty acids as well as total MUFA and PUFA in all organisms (n=369) depended on the interaction between habitat and functional group (Table 3), while total SFA did not depend on the interaction, functional group or habitat. ARA was the only individual fatty acid that did not depend on habitat ($p = 0.181$). ALA and LNA were negatively related with functional group (trophic covariate coefficients for ALA = -1.11; LNA = -1.18); while EPA (0.57), DHA (1.08) and ARA (0.24) were positively related with functional group.

Draft

Summary and Perspective

Data synthesis

The data synthesis identified quantifiable differences in fatty acid content between freshwater and terrestrial organisms. Both habitat and functional group ("trophic level") were important factors in determining the fatty acid composition of the organisms investigated in this study. Aquatic organisms contained higher n-3 LC-PUFA (EPA and DHA), while terrestrial organisms contained higher LNA content. This fundamental difference caused a significant divide in the fatty acid composition of aquatic vs. terrestrial organisms. While this difference

between aquatic vs. terrestrial organisms has been observed in numerous individual studies, this is the first time this comparison has been made in a systematic and comprehensive study. The collection of fatty acid profiles in this study was thorough, given the selection criteria imposed on data collection. Within each of the 9 functional groups in the study, between 9 and 43 families were represented. Fatty acid profiles of certain functional groups were better represented in the literature than others; for example, terrestrial plants and fish profiles were more easily obtained than benthic invertebrate profiles. Grouping species together also assumed some degree of variability within a functional group, as the fatty acid composition of different species is not identical even when in the same habitat and position in a food web. This natural variation also demonstrated the range of species used within a functional group in the analysis. Phylogeny is often a driver of fatty acid composition (Budge et al. 2002, Dalsgaard et al. 2003, Galloway et al. 2013, Galloway and Winder 2015); therefore, different taxa within a functional group may have different fatty acid compositions. However, despite species differences, the variation within functional groups was minimal compared to the differences between functional groups; and the data analysed were generally representative of organisms in freshwater and terrestrial environments.

The MDS analysis revealed that the fatty acid profiles representing aquatic versus terrestrial organisms broadly formed two clusters (Fig. 2a). While total SFA and total PUFA were characteristic in organisms in both habitats, specific fatty acids were responsible for the distinction between aquatic and terrestrial organisms: EPA, DHA, and ARA were closely associated with aquatic organisms, while ALA and LNA were more closely associated with terrestrial organisms. The position of these vectors coincides with the general assumption that aquatic fatty acid profiles are distinct due to the presence of LC-PUFA (i.e. EPA, DHA, and

ARA). Since the SFA and PUFA vectors were pointing in opposite directions, it suggests a fundamental difference in these fatty acid groups, rather than a difference due to habitat. These vectors drive the overall difference in data points within the MDS plots, demonstrating that freshwater organisms (high in EPA, DHA and ARA) are fundamentally different from terrestrial organisms (high in ALA and LNA).

When the data set was further distinguished by functional group, it became more apparent which groups of organisms overlapped, indicating similar fatty acid compositions (Fig. 2b). The aquatic primary producers (algae) occupied the overlapping space between the terrestrial and aquatic habitat sides of the plot. It is important to note that algae differ in their ability to produce PUFA (Brett and Müller-Navarra 1997). For example, diatoms, cryptophytes, and dinoflagellates synthesize significant amounts EPA and DHA (Ahlgren et al., 1992; Gladyshev et al. 2013). Other algal taxa, such as green algae and cyanobacteria, do not produce EPA and DHA and contain fairly large amounts of ALA and LNA (Gugger et al. 2002, Taipale et al. 2013); therefore, the fatty acid profiles of these taxa resemble that of terrestrial plants (containing ALA and LNA). The data points further away from the overlapping region in aquatic organisms contain increasingly higher LC-PUFA, with increasing trophic level. For example, terrestrial plants and fish were the furthest apart on the plot.

Analysis of individual fatty acids and groups of fatty acids further supported that habitat and functional group ("trophic level") were important determinants of fatty acid composition in organisms in this study. The sum of EPA + DHA was higher in aquatic compared to terrestrial organisms; while the opposite was true for ALA + LNA (Fig. 3). Because of this natural difference in habitats, a trophic gradient was irrelevant without consideration of habitat; therefore the trophic gradient was separated by habitat. Thus, the sum of ALA + LNA (as well as

individual) decreased with increasing trophic level, irrespective of habitat. This relationship indicates that not only does ALA + LNA decrease from terrestrial to aquatic organisms, but primary producers contained higher contents of these fatty acids than secondary and tertiary consumers in each habitat. Conversely, EPA + DHA (as well as each fatty acid individually) increased with trophic level (Fig. 3), indicating that consumers had higher contents of these fatty acids than primary producers. However, the relationship with ARA and trophic level was weaker than that of EPA and DHA (according to ANCOVA) and fits with the observation that accumulation of ARA depends on season, species, and developmental stage (Hartwich et al. 2013) and that ARA may not have a clear relationship with trophic level (Strandberg et al. 2015b). This demonstrates that EPA and DHA in particular are progressively retained by aquatic organisms (e.g. zooplankton, benthic invertebrates, molluscs, and fish) higher up the food chain, reinforcing the findings of Kainz et al. (2004). The assimilation and retention of EPA and DHA in consumers is fundamental to the optimal physiological performance of animals in aquatic food webs (Kainz et al. 2004).

Both LNA and DHA showed particularly strong associations with habitat; where LNA decreased and DHA increased from terrestrial to aquatic habitats (Fig. 4). The predominance of DHA in aquatic organisms as well as the tendency for LNA to decrease going from terrestrial to aquatic food webs has been previously observed (Koussoroplis et al. 2008; Strandberg et al. 2015b). When LNA and ALA were grouped together, there was an association with terrestrial organisms. However, the ALA: LNA ratio clearly demonstrates that aquatic organisms contain relatively higher ALA than LNA content compared to terrestrial organisms (Fig. 5). Both terrestrial and aquatic primary producers synthesize ALA and LNA; however, the n-3 precursor (ALA) is more predominant than LNA in freshwater aquatic ecosystems. This is also apparent

Draft

when considering the n-3: n-6 ratio, which was an average of 6 times higher in aquatic than terrestrial organisms. This estimate of the n-3: n-6 ratio agrees with previous studies that have estimated this difference as 5 to 20 times (Henderson and Tocher 1987; Ahlgren et al. 1994). This reflects the abundance of n-3 PUFA in aquatic ecosystems, and the abundance of n-6 PUFA (LNA) in terrestrial ecosystems (ARA was not strongly associated with either trophic level or habitat). Therefore, gradual transitions in fatty acid proportions are observed from one food web to another (Koussoroplis et al. 2008); and as we report here (Figs. 3 and 4). It is important to note however that terrestrial mammals, although grouped on the terrestrial side of the x-axis (Fig. 3) contain higher EPA and DHA contents in their muscle tissue than terrestrial plants and insects. This suggests that terrestrial mammals obtain EPA and DHA from aquatic resources, or synthesize these LC-PUFA from ALA derived from their diets. Whether EPA and DHA are deemed essential for an organism likely depends on the fatty acid composition of their common prey items, and the extent to which a given species can convert one n-3 or n-6 fatty acid to another (Parrish 2009).

The ANOSIM and ANCOVA models were employed to elaborate on the underlying causes of these differences. In ANOSIM, the main difference observed between groups was due to habitat. Within the same habitat, groups were often different because they occupied different trophic levels. Organisms were likely to have different fatty acid profiles when one was a predator and one was the prey, even within the same habitat. Considering that certain fatty acids with high physiological priority are not only conserved, but are often selectively retained and accumulated within a food web, organisms at higher trophic levels often have higher content of these fatty acids (Kainz et al. 2004; Koussoroplis et al. 2008; Strandberg et al. 2015b). For example, fish and algae were one of the most different pairs in the fatty acid data set according to

ANOSIM. Because both habitat and trophic level are factors that can distinguish fatty acid composition among groups of organisms, most pairwise comparisons were found to be significantly different. Terrestrial plants and terrestrial insects were the only groups compared that were not significantly different. Clearly these groups have very similar fatty acid compositions, despite that many terrestrial insects are herbivores and consume terrestrial plants. It is possible that within the terrestrial habitat, the difference between trophic level is not as great as observed in aquatic habitats, because ALA and LNA are not selectively retained and conserved as EPA and DHA. Therefore, retention of fatty acids in a terrestrial food web is not at a scale that is causing a large difference between predator and prey, as observed in aquatic food webs. Pairwise comparisons among terrestrial organisms (terrestrial mammals vs. plants and insects) were among one of the lowest R-statistics, although still significantly different.

Fatty acid composition was dependent upon functional group (loosely trophic level; grouped by plants, invertebrates and vertebrates), with consideration of habitat (Figs. 3 and 4). We used an ANCOVA to determine which factor (trophic level or habitat) is more influential in determining the fatty acid composition of organisms. Fatty acid composition depended on habitat type (fixed categorical variable), “trophic level” (covariate at 9 levels) and the interaction between the two factors. All individual fatty acids, as well as total MUFA and PUFA, were different among groups due to the interaction between habitat and “trophic level”. This summarizes the observations, analyses and conclusions made from the MDS and ANOSIM analyses that fatty acid composition depends on both habitat and “trophic level”. Total SFA were the exception to this, as they did not differ among organisms in this study as function of habitat type, “trophic level” or the interaction between the two. This was also observed in the MDS, where the SFA vector was positioned at the interface between aquatic and terrestrial groups. This

may indicate that SFA remains constant or in balance regardless of environment and habitat. For example, isopods fed a range of different algal diets in experimental feeding trials had very similar SFA, regardless of the SFA content in diet (Galloway et al. 2014). Although the interaction between habitat and trophic level influences ARA composition, habitat alone did not determine the ARA composition of organisms in this study. Based on this and its weak relationship with "trophic level", it appears that ARA composition in organisms cannot be predicted based on habitat and trophic level alone and that there is little evidence to support the claim that this LC-PUFA is either predominantly an aquatic or terrestrial resource. Therefore, considering the fatty acid profiles of organisms in freshwater and terrestrial habitats and different trophic levels, most fatty acids (except for total SFA) were different in organisms as a function of the interaction between their habitat and trophic level. Freshwater organisms inherently contain high n-3 LC-PUFA, which originate in some taxa of algae and are selectively retained and accumulated successively throughout the food chain in freshwater food webs.

EPA and DHA requirements and synthesis

Based on these results, it is clear that freshwater ecosystems are a primary originating source of EPA and DHA. It is well known that vertebrates (and mammals in particular) depend on EPA and DHA for different physiologically important functions (Calder, 2015a; Calder, 2015b; Calder, 2014; Stonehouse et al., 2013; Mozaffarian and Wu, 2012; Swanson et al., 2012; Arts et al. 2001). However, if the terrestrial environment does not produce and drive the transfer of EPA and DHA, terrestrial vertebrates without access to marine shorelines must exploit freshwater ecosystems to obtain these fatty acids for health and survival, or synthesize these FA themselves from the precursor ALA. Most terrestrial vertebrates have a limited ability to convert ALA to EPA and DHA. This has been investigated in terrestrial mammals; however, the subjects

of a majority of studies are humans (or rodents as human models). The rate of conversion from ALA to DHA is broadly defined for terrestrial vertebrates, but appears to vary between 0 to 9 percent (Supplementary Table S1). Species, age, diet, and gender are factors that can contribute to the conversion rate (Supplementary Table S1). The question remains that if all vertebrates require EPA and DHA for survival, why have they not evolved the universal ability to synthesize these compounds, or conversely, why have we lost this ability? This is likely related to access to aquatic resources. For example, marine mammals require higher contents of EPA and DHA than cattle to survive. Thus, the ability to synthesize EPA and DHA is likely related to the inverse of access to these LC-PUFA. Organisms without access to aquatic resources rich in EPA and DHA likely have a better ability to synthesize EPA and DHA, while those with access to aquatic resources have a very limited ability because it is available and consumed through the diet. Humans, for example, may have evolved in tandem with dietary access to LC-PUFA from aquatic resources (Joordens et al. 2014), as our ability to synthesize EPA and DHA is very low (Supplementary Table S1). Furthermore, expansion of hominin brain in an environment providing a pre-formed source of DHA is consistent with the developmental requirements in modern humans (Brenna and Carlson, 2014). This is the basis of the shore-based hypothesis of human brain evolution, which proposes that sustained access by certain groups of early *Homo* to aquatic food sources were key to human brain development (Cunnane and Crawford, 2014). In the absence of dietary EPA and DHA, controlled feeding studies have shown that carnivorous marine fish fed a formulated diet without EPA and DHA show up-regulation of the genes responsible for EPA and DHA synthesis; while those fish fed a diet containing sufficient content of EPA and DHA do not upregulate these genes (Xue et al. 2014; Xue et al. 2015). Similarly, results from feeding trials of freshwater fish showed that Arctic charr (*Salvelinus alpinus)* and

Draft

rainbow trout (*Oncorhynchus mykiss*) also converted ALA to EPA and DHA when fed on EPA- and DHA-deprived feeds (Murray et al. 2014; Hixson et al., 2014). Conversely, grazing terrestrial mammals that typically do not consume aquatic resources or live near aquatic ecosystems still contain appreciable contents of DHA in the brain, such as deer (8.9% total fatty acids), elk (9.6%), zebra (18%) and elephant (25%) (Crawford et al. 1976; Cordain et al. 2002); which suggests that these organisms synthesize DHA *de novo* from ALA. To summarize, the necessity to synthesize EPA and DHA depends on of the level of access to pre-formed EPA and DHA in the diet. This also indicates that perhaps independent of habitat and trophic levels, there can also be very strong intrinsic factors in certain taxa to control their fatty acid patterns, through endogenous synthesis of certain fatty acids, namely EPA and DHA.

Aquatic resources provide consistently greater amounts of pre-formed EPA and DHA than those of the terrestrial ecosystem. Dietary DHA is 2.5-100 times higher for equivalent weights of fish or molluscs compared to terrestrial animal muscle tissue (Broadhurst et al. 2002). Terrestrial animals that do not inhabit marine shoreline areas meet their EPA and DHA requirements from their own synthesis or from freshwater ecosystems, such as lakes, rivers, and wetlands. Lakes, rivers, streams, and wetlands are highly connected spatially with terrestrial ecosystems, and this connectivity allows terrestrial organisms to more easily access aquatic food resources (Gladyshev et al. 2009). Thus, these aquatic resources provide the best opportunity for terrestrial organisms to access pre-formed EPA and DHA in their diet, emphasizing the need to conserve these resources globally.

Implications

There are several factors that threaten the production of ALA, EPA and DHA in freshwater ecosystems; in particular increased water temperature due to climate change and

Draft

eutrophication. Temperature has a profound influence on lipid composition and hence the function and stability of cell membranes. Cells adapt to changing temperatures by remodelling the structural integrity and dynamic functioning of their cell membranes. This is accomplished primarily by changes in lipid class and fatty acid composition by varying the length of fatty acids (numbers of carbon atoms) and changing the number of double bonds (Guschina and Harwood 2006; Arts and Kohler 2009; Parrish 2013). As temperature increases, cells adapt by decreasing the number of double bonds in the fatty acids contained in their cell membranes to achieve greater structural rigidity (i.e. less fluidity). As a result, less PUFA is expected as ambient temperature increases (Arts and Kohler 2009; Fuschino et al. 2011). Temperature can also affect ecosystem FA content through re-structuring phytoplankton communities; therefore taxonomic composition as a response to increasing temperature may affect PUFA production (Galloway and Winder 2015). Increasing water temperature as a result of global warming is therefore predicted to reduce the amount of EPA and DHA produced by algae, which will impact aquatic food webs and the terrestrial animals that rely on them.

Increasing nutrient loads results in eutrophication of freshwater ecosystems. Eutrophication (and increased water temperature) favors the growth and population of harmful algal blooms (e.g. cyanobacteria) in planktonic algae (Kosten et al. 2012; Paerl and Paul 2012). Cyanobacteria reduce the DHA and EPA content in the ecosystem in two ways. First, cyanobacteria are rich in 16- and 18- carbon fatty acids, and the majority of species do not produce EPA and DHA (Caramujo et al. 2008). Second, cyanobacteria compete with and often dominate other species of planktonic algae. The mass development of cyanobacteria increases turbidity in the water, which restricts light penetration, and leads to further suppression of algae (e.g. diatoms) that are known to produce EPA and DHA (Paerl and Paul 2012).

Compounding these threats, a recent prediction suggests that the current global supply of EPA is barely sufficient to meet the current nutritional demand of the world population (Budge et al. 2014). As the world's population increases, this resource may become inadequate to meet demands and if EPA and DHA production is further limited due to rising water temperatures and/or eutrophication. The impact may be highly deleterious for both ecosystem and human health. Thus, it is important to conduct research designed to quantify how, where, and when global warming and other factors that limit the supply of LC-PUFA, such as continuous overfishing and eutrophication of freshwater ecosystems, will threaten the production of EPA and DHA in aquatic ecosystems with consequent effects on terrestrial consumers. We must put forth efforts to conserve highly connected freshwater resources, as these valuable areas connect terrestrial organisms with unique aquatic resources, namely EPA and DHA.

Draft

Conclusions

The fatty acid composition of freshwater organisms is distinctly different from organisms in terrestrial ecosystems. Both habitat and trophic level were important factors in determining the proportion of individual n-3 or n-6 LC-PUFA or groups of fatty acids (total MUFA and PUFA) in both aquatic and terrestrial organisms. EPA and DHA show a different relationship with trophic level and habitat compared to ALA and LNA; supporting the hypothesis that EPA and DHA are selectively retained throughout a food web and are also proportionally higher in aquatic than terrestrial food webs. These PUFA are found in different proportions in aquatic and terrestrial organisms due to the interaction between their habitat and trophic level in the food web. Because terrestrial vertebrates require EPA and DHA for health and survival and are generally poor at converting ALA to EPA and DHA, they depend on access to aquatic resources

to obtain these fatty acids, since the production of EPA and DHA in terrestrial ecosystems is orders of magnitude less than in freshwater ecosystems. While ARA is also critically important for vertebrate health, the data suggests that ARA production is not limited to aquatic ecosystems. For terrestrial vertebrates that live away from marine coastlines, access to EPA and DHA must predominantly stem from freshwater ecosystems. These areas are under threat due to climate change and eutrophication. Therefore, in order to retain this essential ecosystem service provided by freshwater we need to focus efforts on wetland and freshwater ecosystem conservation to ensure health of both terrestrial wildlife and humans that derive nutritional benefits from consuming freshwater resources.

Acknowledgements

The authors would like to acknowledge the University of Toronto internship program; Martina Rudy, Jerry Chao, and Susan Wolfaardt for analysis of M. T. Arts' unpublished fatty acid data; and Christian Tegge for assistance in data collection. Aaron Galloway and an anonymous reviewer provided valuable comments which helped us improve the manuscript. MJK was supported by the Austrian Science Fund (P22006-B17), AW by the German Research Foundation (DFG WA2445/8-1) and MTA by the Natural Sciences and Engineering Research Council (NSERC RGPIN-2014-04537).

Draft

References

Ahlgren, G., Gustafsson, I.B., Boberg, M. 1992. Fatty acid content and chemical composition of freshwater microalgae. J. Phycol. **28**: 37-50.

Ahlgren, G., Blomqvist, P., Boberg, M., Gustavsson, I. 1994. Fatty acid content of the dorsal muscle- an indicator of fat quality in freshwater fish. J. Fish Biol. **45**: 131-157.

Arts, M.T., Kohler, C.C. 2009. Health and condition in fish: The influence of lipids on membrane competency and immune response. *In* Lipids in Aquatic Ecosystems. *Edited by* M.T. Arts, M.T. Brett and M.J. Kainz. Springer, New York, pp. 237-256.

Arts, M.T., Wainmann, B.C. 1999. Lipids in freshwater ecosystems. Springer, New York.

Arts, M.T., Ackman, R.G., Holub, B.J. 2001. Essential fatty acids in aquatic ecosystems: a crucial link between diet and human health and evolution. Can. J. Fish Aquat. Sci. **58**: 122-137.

Arts, M.T., Brett, M.T., Kainz, M.J. 2009. Lipids in aquatic ecosystems. Springer, New York.

Böhm, M., Schultz, S., Koussoroplis, A.M., Kainz. 2014, M.J. Tissue-specific fatty acids response to different diets in common carp (*Cyprinus carpio* L.). Plos One **9**: e0094759

Bray, R., Curtis, J. 1957. An ordination of the upland forest communities of southern Wisconsin. Ecol. Monograph. **27**: 325–349.

Brenna, J.T., Salem, N., Sinclair, A., Cunanne, S.C. 2009. α-Linolenic acid supplementation and conversion to n-3 long-chain polyunsaturated fatty acids in humans. Prostag. Leukotr. Ess. **80**: 85-91.

Brenna, J.T., Carlson, S.E. 2014. Docosahexaenoic acid and human brain development: Evidence that a dietary supply is needed for optimal development. J. Human Evol. **77**: 99-105

Draft

Brett, M.T., Müller-Navarra, D.C. 1997. The role of highly unsaturated fatty acids in aquatic foodweb processes. Freshwater Biol. **38**: 483–499.

Broadhurst, L., Wang, Y., Crawford, M., Cunnane, S., Parkington, J., Schmidt, W. 2002. Brain-specific lipids from marine, lacustrine, or terrestrial food resources: potential impact on early African *Homo sapiens*. Comp. Biochem. Physiol. B. **131**: 653-673.

Budge, S.M., Iverson, S.J., Bowen, D., Ackman, R.G. 2002. Among- and within-species variability in fatty acid signatures of marine fish and invertebrates on the Scotian Shelf, Georges Bank, and southern Gulf of St. Lawrence. Can. J. Fish. Aquat. Sci. **59**: 886–898.

Budge, S., Devred, E., Forget, M., Stuart, V., Trzcinski, M., Sathyendranath, S., Platt, T. 2014. Estimating concentrations of essential omega-3 fatty acids in the ocean: supply and demand. ICES J. Mar. Sci. **71**: 1885-1893.

Calder, P.C. 2014. Very long chain omega-3 (n-3) fatty acids and human health. Eur. J. Lipid Sci. Technol. **116**: 1280-1300.

Calder, P.C. 2015a. Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. Biochim. Biophys. Acta. **1851**:469-484.Calder, P.C. 2015b. Functional roles of fatty acids and their effects on human health. J. Parenter. Enteral. Nutr. DOI: 10.1177/0148607115595980

Caramujo, M., Boschker, H., Admiraal, W. 2008. Fatty acid profiles of algae mark the development and composition of harpacticoid copepods. Freshwater Biol. **53**: 77-90.

Clarke, K., Warwick, R. 2001. Change in marine communities: An approach to statistical analyses and interpretation. Plymouth, UK: Primer-E, Ltd.

Cook, H.W., Mcmaster, C.R. 2004. Fatty acid desaturation and chain elongation in eukaryotes. *In* Biochemistry of lipids, lipoproteins and membranes. New Comprehensive

Biochemistry. *Edited by* D. E. Vance and J. E. Vance. Elsevier. Amsterdam, The Netherlands. pp. 181-204.

Cordain, L., Watkins, B., Florant, G., Kelher, M., Rogers, L., Li, Y. 2002. Fatty acid analysis of wild ruminant tissues: evolutionary implications for reducing diet-related chronic disease. Eur. J. Clin. Nutr. **56**: 181–191.

Crawford, M.A., Casperd, N.M., Sinclair, A.J. 1976. The long chain metabolites of linoleic and linolenic acids in liver and brain in herbivores and carnivores. Comp. Biochem. Physiol.B. **54**: 395–401.

Cunnane, S.C. 2000. The conditional nature of the dietary need for polyunsaturates: A proposal to reclassify 'essential fatty acids' as 'conditionally-indispensable' or 'conditionally-dispensable' fatty acids. British J. Nutr. **84**: 803-812.

Cunnane, S.C., Crawford, M.A. 2014. Energetic and nutritional constraints on infant brain development: Implications for brain expansion during human evolution. J. Human Evol. **77**: 88-98.

Dalsgaard, J., St. John, M., Kattner, G., Müller-Navarra, D., Hagen, W. 2003. Fatty acid trophic markers in the pelagic marine food environment. Adv. Mar. Biol. **46**: 225-340.Fuschino, J., Guschina, I., Dobson, G., Yan, N., Harwood, J., Arts, M.T. 2011. Rising water temperatures alter lipid dynamics and reduce n-3 essential fatty acid concentrations in *Scenedesmus obliquus* (Chlorophyta). J. Phycol. **47**: 763-774.

Galloway, A.W.E., Sosik, E.A., Yeung, J.S., Duggins, D. 2013. Fatty acid and stable isotope biomarkers suggest microbe-induced differences in benthic food webs between depths. Limnol. Oceanogr. **58**: 1452–1462.

Draft

Galloway, A.W.E., Eisenlord, M.E., Dethier, M.N., Holtgrieve, G.W., Brett, M. T. 2014. Quantitative estimates of isopod resource utilization using a Bayesian fatty acid mixing model. Mar. Ecol. Prog. Ser. **507**: 219–232.

Galloway, A.W.E., Winder, M. 2015. Partitioning the relative importance of phylogeny and environmental conditions on phytoplankton fatty acids. Plos One. **10**: e0130053.

Gladyshev, M., Arts, M.T., Sushchik, N. 2009. Preliminary estimates of the export of omega-3 highly unsaturated fatty acids (EPA + DHA) from aquatic to terrestrial ecosystems. *In* Lipids in Aquatic Ecosystems. *Edited by* M.T. Arts, M.T. Brett and M.J. Kainz. Springer, New York, pp. 179-210.

Gladyshev, M., Sushchika, O., Makhutova, N. 2013. Production of EPA and DHA in aquatic ecosystems and their transfer to the land. Prostaglandins Other Lipid. Mediat. **107**: 117-126.

Gugger, M., Lyra, C., Suominen, I., Tsitko, I., Humbert, J.F., Salkinoja-Salonen, M.S., Sivonen, K. 2002. Cellular fatty acids as chemotaxonomic markers of the genera *Anabaena*, *Aphanizomenon*, *Microcystis*, *Nostoc* and *Planktothrix* (cyanobacteria). Int. J. Syst. Evol. Microbiol. b: 1007–1015.Guschina, I., Harwood, J. 2006. Mechanisms of temperature adaptation in poikilotherms. FEBS Letters. **580**: 5477-5483.

Hartwich, M., Martin-Creuzburg, D., Wacker, A. 2013. Seasonal changes in the accumulation of polyunsaturated fatty acids in zooplankton. J. Plankton Res. **35**: 121-134

Henderson, R., Tocher, D. 1987. The lipid composition and biochemistry of freshwater fish. Prog. Lipid. Res. **26**: 281-347.

Draft

Joordens, J., Kuipers, R., Wanink, J., Muskiet, F. 2014. A fish is not a fish: Patterns in fatty acid composition of aquatic food may have had implications for hominin evolution. J. Human Evol. **77**: 107-116.

Kainz, M.J., Arts, M.T., Mazumber, A. 2004. Essential fatty acids in the planktonic food web and their ecological role for higher trophic levels. Limnol Oceanograph. **49**: 1784-1793.

Kidd, P.M. 2007. Omega-3 DHA and EPA for cognition, behavior, and mood: Clinical findings and structural functional synergies with cell membrane phospholipids. Alt. Med. Rev. **12**: 207-227.

Kosten, S., Huszar, V.L.M., Becares, E., Costa, L.S., van Donk, E., et al. 2012. Warmer climates boost cyanobacterial dominance in shallow lakes. Glob. Change Biol. **18**: 118–126

Koussoroplis, A., Lemarchand, C., Bec, A., Desvilettes, C., Amblard, C., Fournier, C., Berny, P., Bourdier, G. 2008. From aquatic to terrestrial food webs: decrease of the docosahexaenoic acid/linoleic acid ratio. Lipids. **43**: 461-466.

Lands, W. 2009. Human life: caught in the food web. *In* Lipids in Aquatic Ecosystems. *Edited by* M.T. Arts, M.T. Brett and M.J. Kainz. Springer, New York, pp. 327-354.

Mozaffarian, D., Wu, JH. 2012. (n-3) fatty acids and cardiovascular health: Are effects of EPA and DHA shared or complementary? J. Nutr. **142**: 614S-625S

Murray, D.S., Hager, H.H., Tocher, D.R., Kainz, M.J. 2014. Effect of partial replacement of dietary fish meal and oil by pumpkin kernel cake and rapeseed oil on fatty acid composition and metabolism in Arctic charr (*Salvelinus alpinus*). Aquaculture. **431**: 85-91.

Draft

Olsen, Y. 1999 . Lipids and essential fatty acids in aquatic food webs: what can freshwater ecologists learn from mariculture? In: Arts M.T., Wainman B.C. (Eds.), Lipids in Freshwater Ecosystems (pp. 161-202). Springer. New York.

Paerl, H., Paul, V. 2012. Climate change: Links to global expansion of harmful cyanobacteria. Water Research. **46**: 1349-1363.

Parrish, C. 2009. Essential fatty acids in aquatic food webs. *In* Lipids in Aquatic Ecosystems. *Edited by* M.T. Arts, M.T. Brett and M.J. Kainz. Springer. New York, pp. 309-326.

Parrish, C. 2013. Lipids in marine ecosystems. [Online]. ISRN Oceanography.

Raji, C.A., Erickson, K.K., Lopez, O.L., Kuller, L.H., Gach, H.M., Thompson, P.M., Riverol, M., Becker, J.T. 2014. Regular fish consumption and age-related brain gray matter loss. Am. J. Prev. Med. **47**: 444-451.

Sayanova, O., Napier, J. 2004. Eicosapentaenoic acid: biosynthetic routes and the potential for synthesis in transgenic plants. Phytochemistry. **65**: 147-58.

Simopoulos, S. 2011. Evolutionary aspects of diet: The omega-6/omega-3 ratio and the brain. Mol. Neurobiol. **44**: 203-215.

Stonehouse, W., Conlon, C., Podd, J., Hill, S.R., Minihane, A.M., Haskell, C., Kennedy, D. 2013. DHA supplementation improved both memory and reaction time in healthy young adults: a randomized controlled trial. Am. J. Clin. Nutr. **97**: 1134-1143.

Strandberg, U., Taipale, S.J., Hiltunen, M., Galloway, A.W.E., Brett, M.T., Kankaala, P. 2015a. Inferring phytoplankton community composition with a fatty acid mixing model. Ecosphere. **6**: http://dx.doi.org/10.1890/ES14-00382.1

Draft

Strandberg, U., Hiltunen, M., Jelkanen, E., Taipale, S.T., Kainz, M.J, Brett, M.T., Kankaala, P. 2015b. Selective transfer of polyunsaturated fatty acids from phytoplankton to planktivorous fish in large boreal lakes. Sci. Total Environ. **536**: 858–865.

Swanson, D., Block, R., Mousa, S. 2012. Omega-3 fatty acids EPA and DHA: Health benefits throughout life. Adv. Nutr. **3**: 1-7.Taipale, S., Strandberg, U., Peltomaa, E., Galloway, A.W.E., Ojala, A., Brett, M. 2013. Fatty acid composition as biomarkers of freshwater microalgae: analysis of 37 strains of microalgae in 22 genera and in seven classes. Aquat. Microb. Ecol. **71**:165–178.

Tocher, D. 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Rev. Fish. Sci. **11**: 107-184

Turcotte, C., Chouinard, F., Lefebvre, J., Flamand, N. 2015. Regulation of inflammation by cannabinoids, the endocannabinoids 2-arachindonyl-glycerol and arachindonyl-athanolamide, and their metabolites. J. Leukoc. Biol. **97**: 1049-1070.

Xue, X., Feng, C.Y., Hixson, S.M., Johnstone, K., Anderson, D.M., Parrish, C.C., Rise, M. L. 2014. Characterization of the fatty acyl elongase (elovl) gene family, and hepatic elovl and delta-6 fatty acyl desaturase transcript expression and fatty acid responses to diets containing camelina oil in Atlantic cod (*Gadus morhua*). Comp. Biochem. Physiol. Part B. **175**: 9-22

Xue, X. Hixson, S.M., Hori, T.S., Booman, M. Parrish, C.C., Johnstone, K. Anderson, D.M., Rise, M.L. 2015. Atlantic salmon (*Salmo salar*) liver transcriptome response to diets containing camelina products. Comp. Biochem. Physiol. D. **14**: 1-15

Draft

Tables

Table 1. Summary of taxa included in data analysis from each functional group.

Functional Group	Total number	Families	Genera	Species[1]
Terrestrial plants	84	43	69	78
Terrestrial insects	50	21	25	25
Terrestrial mammals	43	12	19	23
Algae	17	14	15	17
Aquatic insects	19	17	14	6+
Zooplankton	21	9	9	9+
Benthic invertebrates	17	9	11	15
Molluscs	31	15	21	24+
Fish	87	21	50	60+

[1] "+" sign indicates that one or more species were represented in a single fatty acid profile from the literature

Draft

Table 2. Pairwise comparison between fatty acid profiles of terrestrial and aquatic organisms; similarities and differences based on ANOSIM results*

Group	Pairwise comparison	R-statistic	P-value
Terrestrial insect	Zooplankton	0.756	0.001
Algae	Fish	0.686	0.001
Terrestrial insect	Fish	0.657	0.001
Terrestrial insect	Mussels	0.655	0.001
Terrestrial plant	Fish	0.634	0.001
Algae	Mussels	0.565	0.001
Terrestrial plant	Zooplankton	0.518	0.001
Terrestrial mammal	Zooplankton	0.506	0.001
Terrestrial mammal	Algae	0.483	0.001
Terrestrial insect	Algae	0.455	0.001
Terrestrial insect	Benthic invertebrate	0.439	0.001
Algae	Zooplankton	0.411	0.001
Aquatic insects	Zooplankton	0.386	0.001
Algae	Aquatic insects	0.377	0.001
Terrestrial mammal	Mussels	0.370	0.001
Terrestrial mammal	Fish	0.365	0.001
Terrestrial insect	Aquatic insect	0.342	0.001
Aquatic insect	Mussels	0.355	0.001
Terrestrial plant	Benthic invertebrate	0.303	0.001
Aquatic insect	Fish	0.300	0.001
Terrestrial plant	Algae	0.266	0.001
Terrestrial plant	Aquatic insect	0.261	0.001
Terrestrial plant	Terrestrial mammal	0.243	0.001

Draft

Terrestrial insect	Terrestrial mammal	0.226	0.001
Benthic invertebrate	Zooplankton	0.213	0.001
Benthic invertebrate	Terrestrial mammal	0.211	0.001
Benthic invertebrate	Algae	0.207	0.001
Zooplankton	Fish	0.198	0.005
Zooplankton	Mussels	0.194	0.002
Benthic invertebrate	Aquatic insect	0.161	0.002
Terrestrial mammal	Aquatic insect	0.149	0.009
Mussels	Fish	0.138	0.005
Benthic invertebrate	Mussels	0.111	0.039
Terrestrial plants	Terrestrial insects	0.049	0.062

*Fatty acid profiles of species (n = 369) were categorized into 9 groups (terrestrial plants, aquatic insects, mammals; aquatic algae, zooplankton, terrestrial insects, aquatic benthic invertebrates, mussels, fish). Pairwise comparisons are listed according to significance and to the R-statistic (higher R-statistics indicate greater difference between two groups, while lower and negative R-statistics indicate a smaller difference between two groups).

Draft

Table 3. Results of ANCOVA model (p-value presented) used to detect differences in fatty acid profiles according to habitat (fixed categorical variable= aquatic or terrestrial) and trophic level* (covariate= 9 trophic levels)

Fatty Acid	Habitat term	Trophic level term	Interaction term
SFA	0.307	0.731	0.392
MUFA	0.002	0.001	0.002
PUFA	0.257	0.099	0.017
ALA	0.010	0.001	0.010
LNA	0.000	0.000	0.003
EPA	0.000	0.021	0.001
DHA	0.021	0.000	0.000
ARA	0.181	0.000	0.003

*Trophic levels (grouped by plant to invertebrate to vertebrate): terrestrial plant (1), algae (2), aquatic insects (3), terrestrial insects (4), zooplankton (5), benthic (6), mussels (7), fish (8), terrestrial mammals (9).

For Peer Review

Figure captions

Figure 1. Biosynthesis pathways of n-3 and n-6 polyunsaturated fatty acids from the saturated 18-carbon fatty acid in vertebrates.

Figure 2. Multidimensional scaling (MDS) of fatty acid profiles of organisms in aquatic and terrestrial ecosystems, organized by, a) habitat type and, b) trophic group.

Figure 3. Comparison of ALA (18:3n-3) and LNA (18:2n-6) with EPA (20:5n-3) and DHA (22:6n-3) content (mean standard ± deviation) in different terrestrial and aquatic plant and animal groups along a trophic gradient (plants to invertebrates to vertebrates), separated by habitat.

Figure 4. The LNA and DHA content (% total fatty acids) in groups of organisms sorted according to habitat (terrestrial to aquatic) and trophic level (from plant to invertebrate to vertebrate).

Figure 5. The ratios of ALA: LNA and total n-3: total n-6 PUFA in groups of organisms sorted according to trophic level (from plant to invertebrate to vertebrate) and habitat (terrestrial to aquatic).

Draft

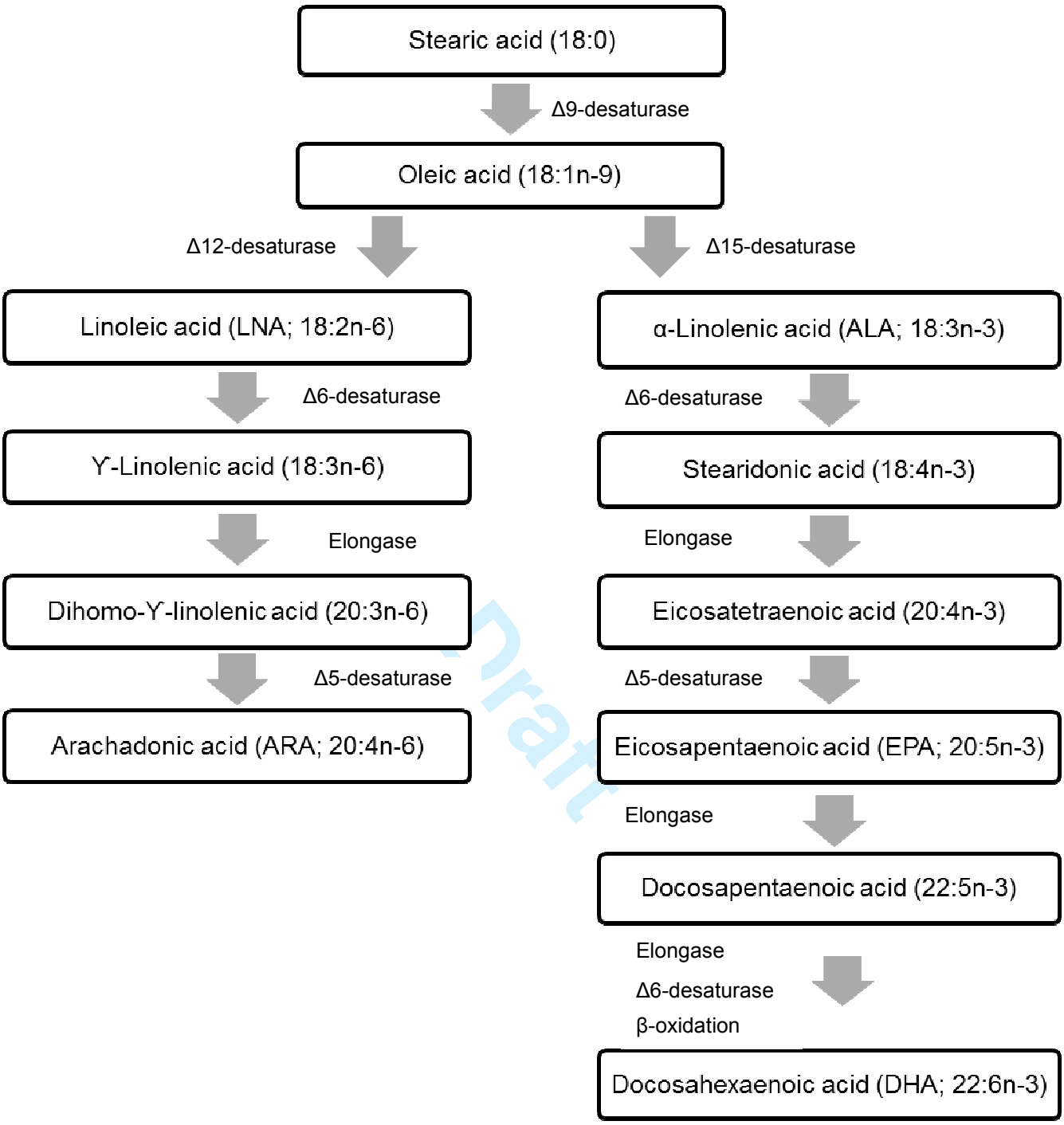
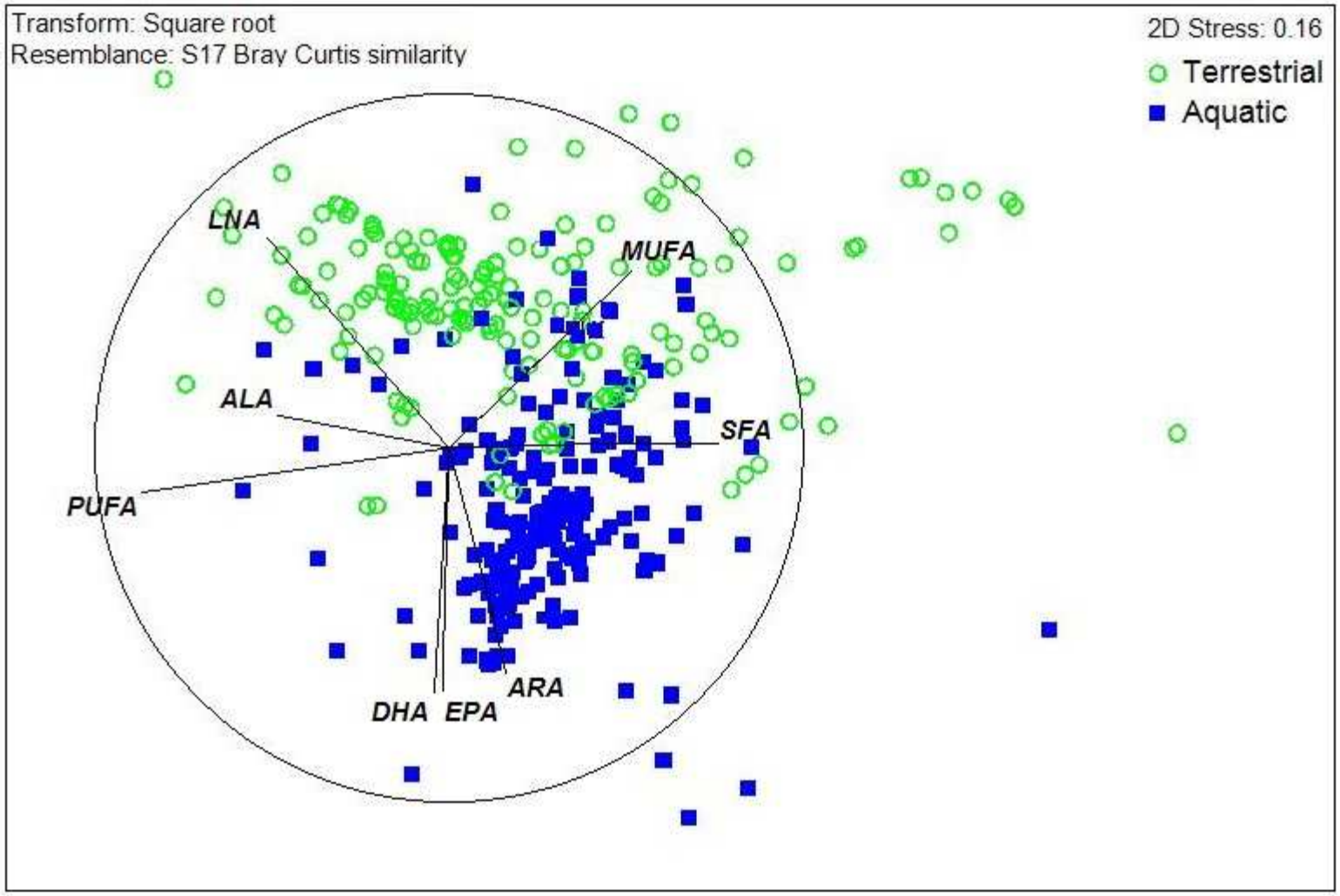
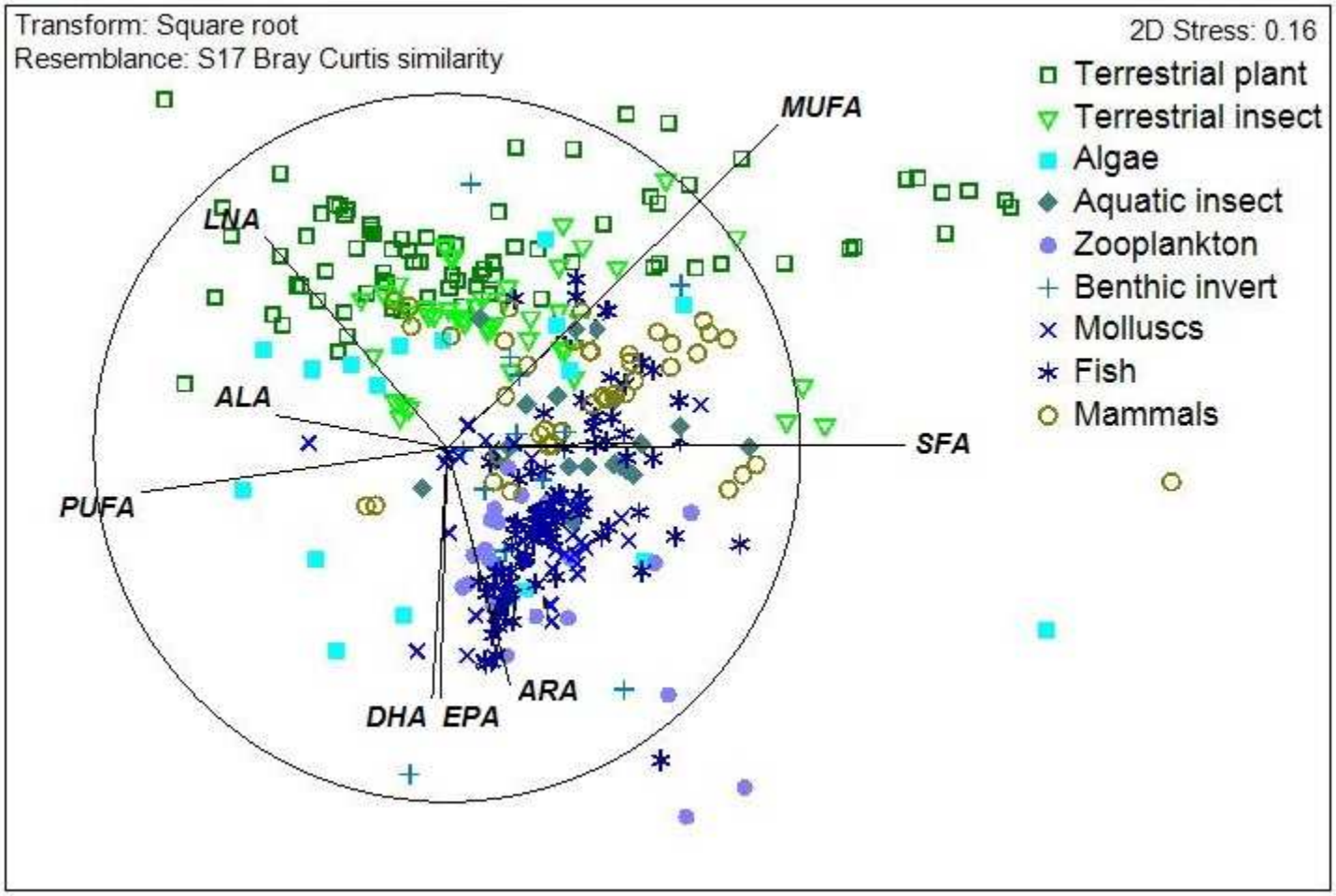

Stearic acid (18:0)
Δ9-desaturase
Oleic acid (18:1n-9)
Δ12-desaturase
Δ15-desaturase
Linoleic acid (LNA; 18:2n-6)
α-Linolenic acid (ALA; 18:3n-3)
Δ6-desaturase
Δ6-desaturase
Y-Linolenic acid (18:3n-6)
Stearidonic acid (18:4n-3)
Elongase
Elongase
Dihomo-Y-linolenic acid (20:3n-6)
Eicosatetraenoic acid (20:4n-3)
Δ5-desaturase
Δ5-desaturase
Arachadonic acid (ARA; 20:4n-6)
Eicosapentaenoic acid (EPA; 20:5n-3)
Elongase
Docosapentaenoic acid (22:5n-3)
Elongase
Δ6-desaturase
β-oxidation
Docosahexaenoic acid (DHA; 22:6n-3)

Fig. 1.


Transform: Square root
Resemblance: S17 Bray Curtis similarity
2D Stress: 0.16
Terrestrial
Aquatic
LNA
MUFA
ALA
SFA
PUFA
ARA
DHA
EPA

a)


Transform: Square root
Resemblance: S17 Bray Curtis similarity
2D Stress: 0.16
Terrestrial plant
Terrestrial insect
Algae
Aquatic insect
Zooplankton
Benthic invert
Molluscs
Fish
Mammals
MUFA
LNA
ALA
SFA
PUFA
ARA
DHA
EPA

b)

Figure 2.

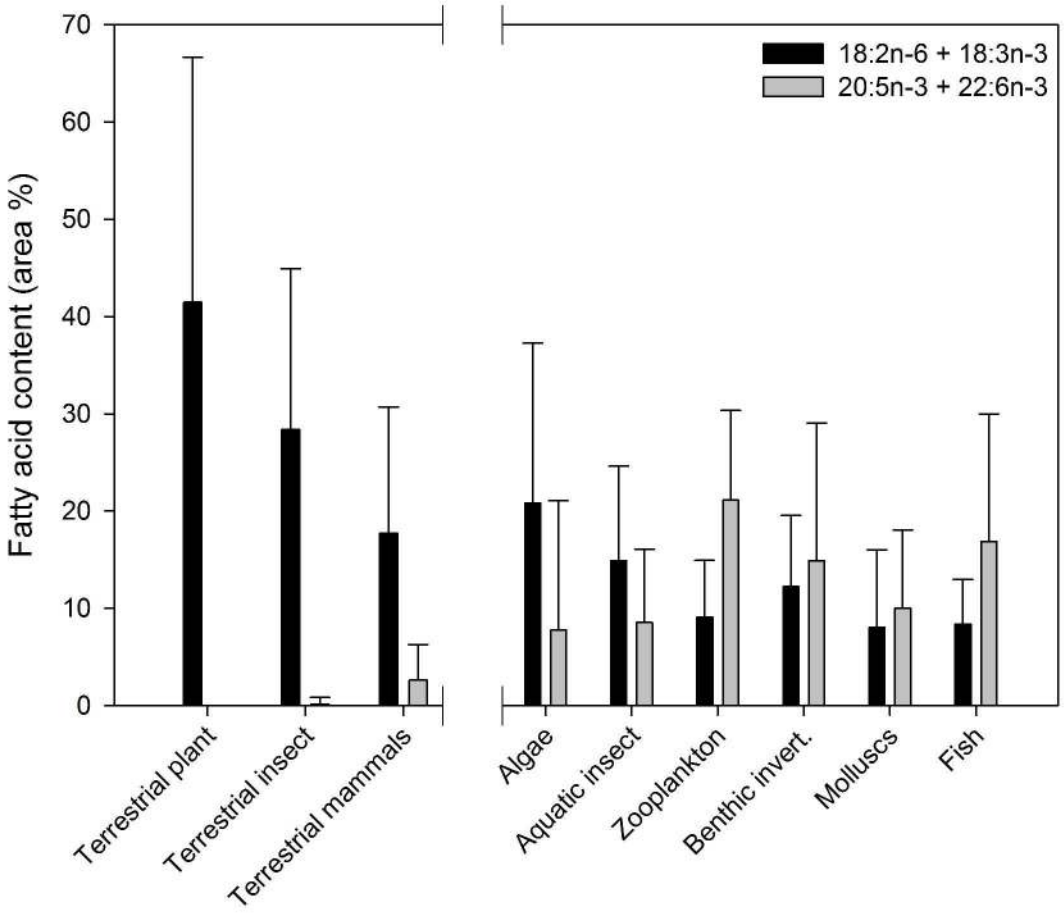

Fatty acid content (area %)
70
60
50
40
30
20
10
0
18:2n-6 + 18:3n-3
20:5n-3 + 22:6n-3
Terrestrial plant
Terrestrial insect
Terrestrial mammals
Algae
Aquatic insect
Zooplankton
Benthic invert.
Molluscs
Fish

Figure 3.

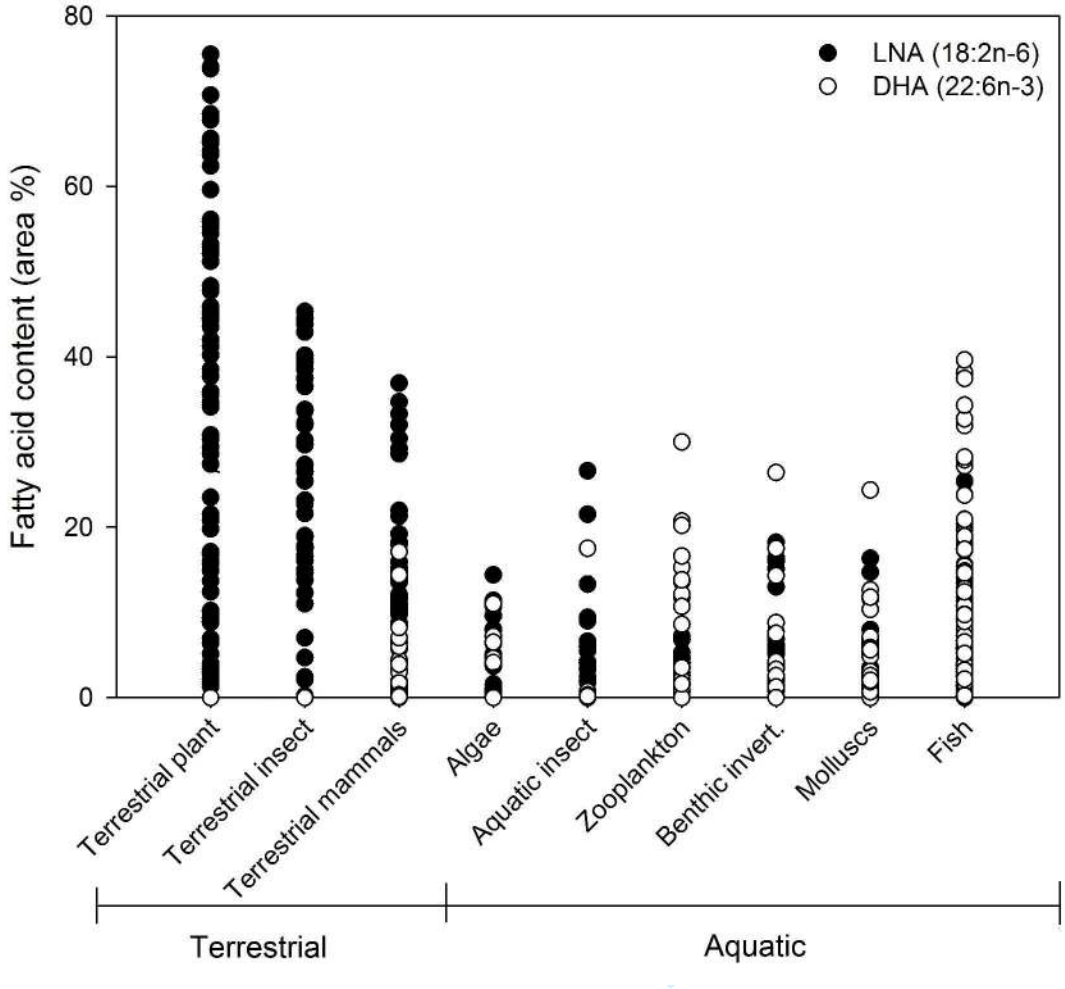

LNA (18:2n-6)
DHA (22:6n-3)
Fatty acid content (area %)
80
60
40
20
0
Terrestrial plant
Terrestrial insect
Terrestrial mammals
Algae
Aquatic insect
Zooplankton
Benthic invert.
Molluscs
Fish
Terrestrial
Aquatic

Figure 4.

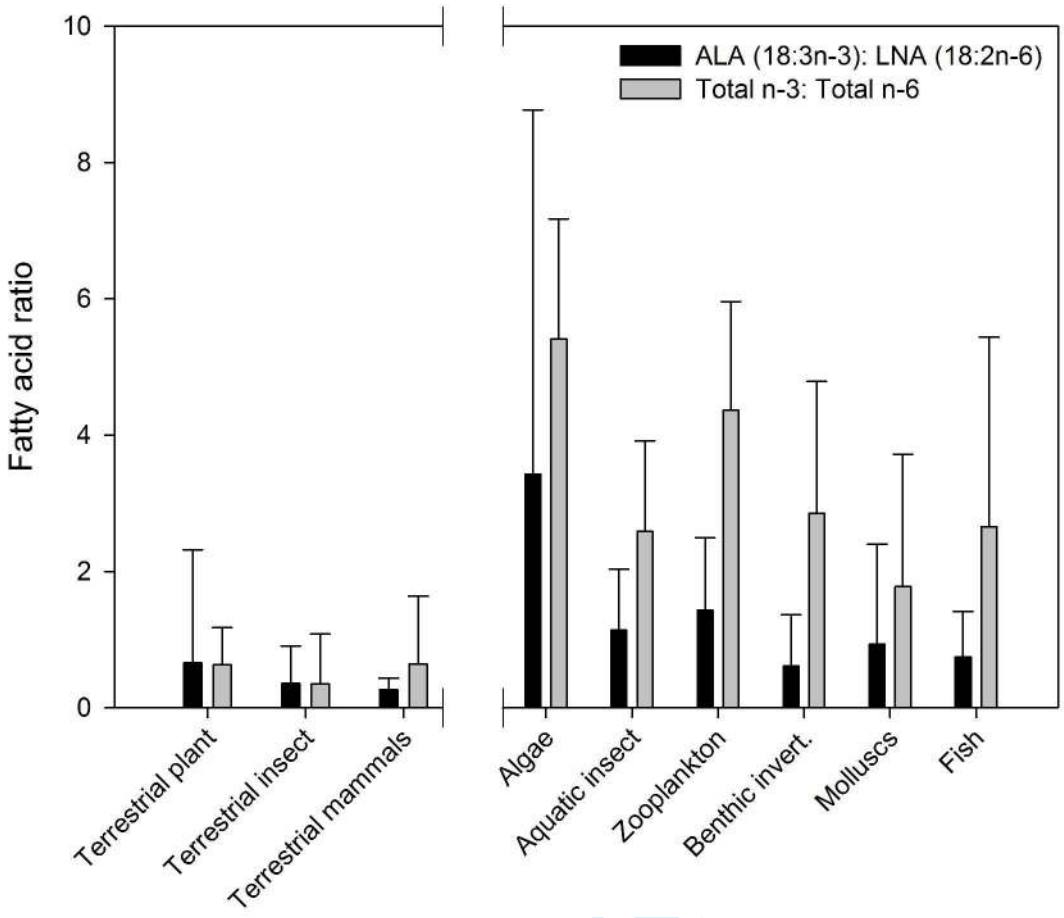

ALA (18:3n-3): LNA (18:2n-6)
Total n-3: Total n-6
Fatty acid ratio
10
8
6
4
2
0
Terrestrial plant
Terrestrial insect
Terrestrial mammals
Algae
Aquatic insect
Zooplankton
Benthic invert.
Molluscs
Fish

Figure 5.