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Polyunsaturated fatty acids in stream food webs – high dissimilarity among producers and consumers

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Keywords: freshwater fish, invertebrates, algae, trophic transfer, biomarker

Abstract

1. Streams and rivers provide crucial sources of long-chain polyunsaturated fatty acids (LC-PUFA, a subset of PUFA with ≥ 20 C in their acyl chains) for freshwater and terrestrial animals. LC-PUFA are primarily produced by particular algal taxa and are subsequently transferred to fish and higher consumers, including humans. Although research on the trophic transfer of LC-PUFA in streams is very limited, studies on the PUFA composition of organisms at various trophic levels within stream food webs are well documented. Here, we systematically analyze the literature to synthesize LC-PUFA distribution and retention in stream ecosystems.
2. Generally, stream food webs are highly enriched in omega-3 LC-PUFA, in particular eicosapentaenoic acid (EPA, 20:5 ω -3) and docosahexaenoic acid (DHA, 22:6 ω -3). Algae from temperate rivers had more EPA compared with algae from subtropical rivers. Stream invertebrates from both temperate and subtropical rivers preferentially retain algal EPA and their PUFA tend to vary with algal PUFA. Invertebrates may be capable of regulating their fatty acid (FA) composition, but this ability is limited, to the extent that they must obtain physiologically important LC-PUFA primarily from algae.
3. DHA is selectively and highly retained in all fish taxa irrespective of their feeding sites (i.e., temperate or subtropical rivers). However, DHA content is variable among stream fish taxa and is likely linked to resource utilization and their endogenous

metabolism. Freshwater salmonids have a very high DHA content, which suggests they preferentially retain this FA or biosynthesize it from EPA. The PUFA profiles of other fish taxa more closely reflect their dietary PUFA supply. However, so far very few studies have considered the differences in the PUFA biosynthesis of fish taxa in rivers or compared the dietary PUFA effect on different fish taxa.

4. The supply of LC-PUFA from streams and rivers is known to be affected by altered light availability, greater nutrient inputs and other anthropogenic activities, and any decrease in algal LC-PUFA production will negatively affect the consumers that are nutritionally dependent on these molecules. Further investigations are needed to understand how environmental changes affect LC-PUFA production and retention in stream ecosystems, and how to protect streams from threats to this essential ecosystem service.

Keywords: freshwater fish, invertebrates, algae, trophic transfer, biomarker

Introduction

Streams and rivers are strongly linked with terrestrial ecosystems compared with other systems (Allan, 2004), and provide crucial sources of long-chain polyunsaturated fatty acids (LC-PUFA) for freshwater and terrestrial animals (Hixson, Sharma, Kainz, Wacker & Arts, 2015; Twining *et al.*, 2016). Long chain-PUFA, such as docosahexaenoic acid (DHA, 22:6 ω -3), eicosapentaenoic acid (EPA, 20:5 ω -3) and arachidonic acid (ARA, 20:4 ω -6), are essential for physiological functions supporting the maintenance, growth, and reproduction of consumers (Tocher, 2003; Arts, Ackman & Holub, 2001; Sargent, Bell, McEvoy, Tocher & Estevez, 1999). These LC-PUFA can be synthesized from shorter-chain PUFA alpha-linolenic acid (ALA, 18:3 ω -3) and linoleic acid (LIN, 18:2 ω -6), which are abundant in some basal food sources, e.g., green algae and terrestrial plants (Guo, Kainz, Sheldon & Bunn, 2016b). However, LC-PUFA are primarily produced by certain taxa of algae and transferred through the food web to fish and humans. Many metazoan consumers will grow best when a sufficient amount of LC-PUFA is directly available in their diet (Brett & Müller-Navarra, 1997). High retention of LC-PUFA in metazoan consumers means high availability for the next trophic level. Most current studies on the trophic transfer of LC-PUFA are from lacustrine systems, and the characterization of LC-PUFA distribution and retention in stream ecosystems is largely unknown.

Algal LC-PUFA content provides an important measure of nutritional quality for consumers in aquatic food webs. Stream diatoms (Bacillariophyta) are considered to be high quality food because of their high EPA content, but they are generally low in DHA (Torres-Ruiz, Wehr & Perrone, 2007) and distinctly different from diatoms in marine and lake ecosystems (Guo *et al.*, 2016b; Brett, Müller-Navarra & Persson, 2009). Green algae are regarded as medium quality food due to their high content of ALA and LIN, while cyanobacteria typically lack LC-PUFA (Temina, Rezankova, Rezanka & Dembitsky, 2007; Lang, Hodac, Friedl & Feussner, 2011) and sterols (Martin-Creuzburg, von Elert & Hoffmann, 2008), and are considered to be a poor quality food for aquatic invertebrates (Napolitano, 1999; Napolitano *et al.*, 1994). High dietary LC-PUFA content, especially EPA, has been shown to support faster growth rates of stream invertebrates (Guo, Kainz, Sheldon & Bunn, 2016a; Guo, Kainz, Valdez, Sheldon & Bunn, 2016c). Since herbivorous stream invertebrates have a limited ability to synthesize LC-PUFA, they must directly obtain LC-PUFA from algae, and can have a PUFA composition that largely reflects the algal PUFA composition (Guo *et al.*, 2016c; Torres-Ruiz, Wehr & Perrone, 2010). In turn, basal consumers feeding on high-quality algae are also of high nutritional quality food for secondary consumers, such as fish (Brett & Müller-Navarra, 1997; Brett, Müller-Navarra, Ballantyne, Ravet & Goldman, 2006; Ravet, Brett & Arhonditsis, 2010). The growth and reproduction of freshwater fish is significantly improved by increased dietary LC-PUFA content (Tocher, 2003), and

a high fish LC-PUFA content is in turn nutritionally beneficial for subsequent consumers (e.g., Gladyshev, Sushchik and Makhutova (2013)), including humans (e.g., Mozaffarian, Prineas, Stein and Siscovick (2006)).

Fish PUFA composition has been extensively studied in marine and freshwater ecosystems, and also in aquaculture. In contrast to marine fish, whose PUFA profile is mainly determined by diet, fish in streams and rivers face environments that are low in dietary DHA. In fish, DHA plays an important role in neural development and visual acuity, and is therefore highly conserved (Sargent *et al.*, 1999; Tocher, 2003). Some freshwater fish, such as Arctic charr (*Salvelinus alpinus*), rainbow trout (*Oncorhynchus mykiss*), and European perch (*Perca fluviatilis*) have been shown to convert, at various efficiencies, ALA into DHA when their diets do not contain EPA or DHA (Henrotte *et al.*, 2011; Murray, Hager, Tocher & Kainz, 2014; Hixson, Parrish & Anderson, 2014). Given this ability, it is likely that the PUFA composition in fish from streams and rivers will be less similar to their food sources, i.e., invertebrates and algae. Previous field investigations of fish PUFA composition in streams and rivers have mainly focused on temporal and spatial changes (e.g., Dayhuff (2004)), and a direct, quantitative comparison of fish PUFA to their food sources has yet to be made.

Long-chain PUFA are selectively retained and accumulated within food webs because of

the high physiological requirements in animals (Kainz, Arts & Mazumder, 2004; Strandberg *et al.*, 2015). In lotic consumers, there are some clear differences in consumer abilities to retain certain LC-PUFA. For example, insect larvae in streams do not retain DHA, but require EPA and ARA for their somatic growth and reproduction (Ahlgren, Vrede & Goedkoop, 2009; Guo *et al.*, 2016c). In contrast, salmonids in streams retain high amounts of DHA (Heissenberger, Watzke & Kainz, 2010), whereas European perch in lakes retain both EPA and DHA (Taipale *et al.*, 2016). In addition, LC-PUFA retention in some consumers is strongly affected by environmental changes. For example, LC-PUFA retention in stream invertebrates was less in river sections with greater water temperature (Gladyshev *et al.*, 2016), and varied in channel catfish, carp, and bluegill with longitudinal locations from upstream to downstream of rivers (Young, Whitley & Trushenski, 2016; Rude, Trushenski & Whitley, 2016; Dayhuff, 2004). It is important to determine which physiological factors cause the differences in LC-PUFA retention among fish and invertebrates in order to improve our understanding of trophic PUFA dynamics and production in stream ecosystems. To date, no studies have documented such differences in LC-PUFA retention amongst stream consumers.

In ways both subtle and obvious, LC-PUFA production and retention in streams may influence the health of both aquatic and terrestrial animals, including humans. Although research on the trophic transfer of LC-PUFA through stream food webs is limited, studies

on PUFA profiles in organisms at various trophic levels within stream food webs are well documented. It is timely to systematically analyze and synthesize this information across various stream ecosystems to better understand PUFA distribution and retention in aquatic food webs. In this review, we focus on LC-PUFA DHA, EPA and ARA, and the shorter-chain PUFA ALA and LIN due to their essential physiological functions in animals (Arts, Brett & Kainz, 2009). The objectives of this review were: (1) to quantify differences in PUFA composition of fish and their food sources, i.e., invertebrates and algae; and (2) to assess the LC-PUFA retention in different fish taxa and invertebrates in streams and rivers.

Methods

Data collection and synthesis

Fatty acid data in organisms at various trophic levels within river and stream food webs were collected from the databases Web of Science and Google Scholar. The following search terms were used: “fatty acid”, and “river” or “stream”, combining with different terms for food web organisms, i.e., “algae” or “periphyton” or “biofilm”, and “invertebrate”, and “fish” or “salmon” or “trout” or “grayling” or “perch” or “bass” or “carp” or “roach” or “rudd” or “catfish” or “pike”. All related studies published before September 2016 were included. This search generated 522 hits, including 194 studies for algae (henceforth referred to as algae, periphyton and biofilm), 68 for invertebrates and

260 for fish (referred to as fish and all fish taxa).

Data were selected according to the following four criteria: (1) Laboratory or field experimental data were not included, as algal fatty acid profiles are sensitive to controlled or manipulated conditions (Cashman, Wehr & Truhn, 2013; Hill, Rinchard & Czesny, 2011; Guo *et al.*, 2016a), some of which are unrepresentative of natural rivers or streams. In addition, natural food sources for fish are often changed or replaced in field cultures (Bell, Ghioni & Sargent, 1994); (2) data for DHA, EPA, ARA, ALA, LIN, and the sum of saturated fatty acids (SAFA) and monounsaturated fatty acids (MUFA) were included; (3) FA data in published studies were presented as relative FA (%), and; (4) only FA data in fish muscle tissues were included. The studies did not further differentiate between various muscle tissues, such as dorsal and ventral.

Following the above criteria, we examined individual studies to confirm the validity of the data. The fish taxa present were Salmoniformes, Perciformes, Cypriniformes and Siluriformes. Since the FA composition of pike was reported in only a single study, it was excluded. Effects of fish movement, fish gender and sampling date were not considered as this review focuses on the general pattern of PUFA distribution and retention in streams. Outliers were detected by reviewing the data compiled for each FA within an organism of the stream food web (algae, invertebrates or each fish taxa). The Grubb's

outlier test was applied to identify significant outliers ($p < 0.05$) (Hixson *et al.*, 2015). If a significant outlier was detected, the original data point was examined. Further, within each site, a grand mean of each species was calculated from the FA data regardless of season.

The final dataset was composed of 87 FA profiles from 23 studies (See Appendix), and was stratified into 8 groups according to trophic level, sampling area, and fish taxa. The studies on the FA composition of algae and invertebrates were largely conducted in northern temperate rivers and southern subtropical Queensland, Australia. Therefore, both algae and invertebrate data were grouped as algae-north (10 FA profiles), algae-QLD (13 FA profiles), inverts-north (15 FA profiles), and inverts-QLD (6 FA profiles). Invertebrate data included the FA profiles of Ephemeroptera, Trichoptera, Plecoptera, Coleoptera, Chironomidae, Corixidae, Notonectidae, Gammaridae and Oligochaeta. Since all fish FA data were from northern temperate rivers, except one study in Queensland, Australia (Jardine *et al.*, 2015), fish data were classified into 4 groups according to taxon, i.e., Salmoniformes, Perciformes, Cypriniformes and Siluriformes. There were 8 FA profiles for Salmoniformes, 18 for Perciformes, 7 for Cypriniformes, and 10 for Siluriformes. Fish feeding guilds included omnivores and carnivores.

Data analyses

Relative FA data (%), i.e., DHA, EPA, ARA, ALA, LIN, MUFA and SAFA, were arcsine-squareroot-transformed for normal distribution approximation. Analysis of similarity (ANOSIM) was employed to test for the difference in FA composition between any two groups. ANOSIM calculates an R statistic that assesses the differences between groups, where R values close to 1 indicate complete dissimilarity among sample sets and values near 0 suggest no difference among sample sets (Clarke & Warwick, 2001). Non-metric multidimensional scaling (NMDS) was used to visualize the FA profiles among different groups. NMDS was based on Bray-Curtis dissimilarity matrix, and the stress value <0.2 was accepted (Clarke, 1993). Further, a regression tree was created and structured in a hierarchical fashion to explore patterns between individual FA and organisms at different trophic levels. As homogeneous covariance matrices are not required in regression tree analysis and variables do not need to be normally distributed (Budge, Iverson, Bowen & Ackman, 2002), untransformed percentage data were used. One-way ANOVA with Tukey's HSD post-hoc test for multiple comparisons was also performed to detect the effect of trophic groups on FA compositions. Additionally, we calculated PUFA retention ratios between consumers (fish and invertebrates) and algae by dividing consumer PUFA% by algal PUFA%, i.e., $[\text{PUFA}]_{\text{consumer}}/[\text{PUFA}]_{\text{algae}}$, to assess trophic relationships between algal PUFA supply and PUFA retention in the consumers. The retention ratio was used as trophic comparison and not as a mechanistic path of PUFA transfer. All statistical analyses were conducted in the statistical software R

version 3.3.1 (R Core Team, 2016), with the package Vegan (Oksanen *et al.*, 2013) used for ANOSIM and NMDS and the package Tree (Ripley & Ripley, 2016) for regression tree analysis. Statistical significance was set at $p = 0.05$.

Results

Fatty acid dissimilarity among stream food web organisms

Algae, invertebrates, and fish differed in their FA compositions (ANOSIM global $R = 0.730$, $p = 0.001$) (Table 1). A total of 28 pairwise comparisons were performed (Table 1), 26 of which were significantly different. No significant differences were detected between temperate and subtropical invertebrates ($R = 0.064$, $p = 0.276$) or between Cypriniformes and Siluriformes ($R = 0.118$, $p = 0.096$). The highest dissimilarity was found between Salmoniformes and inverts-QLD ($R = 1.000$, $p = 0.001$), whereas the lowest dissimilarity was between temperate algae and temperate invertebrates ($R = 0.218$, $p = 0.005$). Further, the FA profiles of Salmoniformes were more dissimilar to those of Cypriniformes ($R = 0.708$, $p < 0.01$) and Siluriformes ($R = 0.827$, $p < 0.01$) than to those of Perciformes ($R = 0.336$, $p < 0.05$).

The NMDS plot illustrates the difference in the FA profiles among 8 groups that represented the stream food web organisms of this study (Fig 1). The FA with the greatest effect on sample ordination were DHA, EPA, and ARA. DHA and ARA were primarily

responsible for the distribution of the samples along NMDS1, which separated basal food sources and invertebrates from higher consumers such as Salmoniformes and Perciformes (correlation coefficients, r : DHA = -0.99, ARA = -0.96). NMDS2 expressed differences between periphyton and invertebrates as well as between the major fish groups, and was most strongly correlated with EPA ($r = -0.99$).

Stream food web organisms separated by individual fatty acids

As FA composition was different among the 8 groups, these groups were further separated using a regression tree, combined with ANOVA (Fig 2 and 3). DHA clearly separated fish from algae and invertebrates. As potential fish food sources, algae and invertebrates contained only traces of DHA, thus much less than all of the fish taxa. Similarly, ARA in algae and invertebrates was lower than in all of the fish taxa. In contrast, EPA and ALA in invertebrates from temperate rivers were significantly higher compared with those in fish.

Differences in algal FA were evident in the FA profiles of invertebrates. Algae from temperate rivers had greater percentages of EPA and ALA, but less SAFA compared with algae from subtropical rivers. A similar FA pattern was observed in invertebrates from temperate and subtropical rivers.

Further, invertebrates and algae had several significant differences in FA composition. Invertebrates from temperate rivers had higher EPA, but lower SAFA compared with algae from temperate rivers. Similarly, invertebrates from subtropical rivers also had higher EPA, but lower SAFA compared with algae from subtropical rivers. The proportions of LIN and ARA did not show any major difference between algae and invertebrate groups except that invertebrates from subtropical rivers had significantly lower LIN than algae.

Salmoniformes and Perciformes were more enriched in DHA compared with Cypriniformes and Siluriformes. Salmoniformes also contained significantly less ARA than Perciformes, and EPA was distinctly lower in Siluriformes than in other fish taxa. Finally, Cypriniformes and Siluriformes were more enriched in LIN than other fish taxa.

Polyunsaturated fatty acid retention in fish and invertebrates

These data indicate fish and invertebrates selectively retain algal PUFA (Table 2). The retention ratio between all fish taxa and algae was highest for DHA (8 – 49), much less for ARA (3 – 13), and generally < 1 for EPA and ALA. Conversely, for invertebrates, EPA (1.41 – 2.42) was preferentially retained over DHA (0.05 – 0.66). The retention ratios of EPA, ALA and ARA were all > 1 , while those of DHA and LIN were < 1 .

In northern temperate rivers, the average DHA retention ratios in Salmoniformes (37.7) and Perciformes (35.9) were about 4 times greater than in Cypriniformes (8.3) and Siluriformes (8.1), and about 30 times greater than in invertebrates (0.7). In contrast, the retention ratios of EPA and ALA in invertebrates (1.41 and 1.13, respectively) were around 1.5-6 times greater compared with all fish taxa.

In subtropical rivers, the DHA retention ratio in Perciformes (49.0) was ~ 1.5 times greater than in Cypriniformes (31.4) and Siluriformes (27.8). Conversely, the retention ratios of EPA and ALA in invertebrates (2.4 and 1.1, respectively) were around 3-5 times greater compared to all fish taxa.

Discussion

The results of this study on FA distribution and retention among multiple taxa illustrated that stream food webs were highly enriched in omega-3 LC-PUFA, in particular DHA and EPA, despite the fact that riverine systems typically receive large subsidies of terrestrial matter (Allan, 2004), which are low in LC-PUFA. We interpret the generally high LC-PUFA retention in consumers as 'selective funneling' of dietary PUFA from the base to the top of stream food webs (effect of trophic transfer), and also stress the importance of PUFA bioconversion abilities from precursors to LC-PUFA in consumers at various trophic levels in stream food webs (effect of trophic upgrading).

General patterns of PUFA distribution and retention in stream ecosystems

DHA was highly retained in all of the fish studied, while EPA was more enriched in invertebrates. The highest proportions of DHA were observed in Salmoniformes and Perciformes (~24% of total FA) compared with the average DHA level in freshwater fish (17%) (Hixson *et al.*, 2015), suggesting higher nutritional quality of these fishes for subsequent consumers, irrespective of their feeding sites (i.e., temperate or subtropical rivers). However, levels of DHA varied among stream fish taxa and likely linked with resource utilization or their innate ability to synthesize DHA from dietary precursors. In contrast, invertebrates from both temperate and subtropical rivers were rich in EPA and ALA, suggesting that their biochemical composition largely reflects their algal diet (Lau, Leung & Dudgeon, 2008; Guo *et al.*, 2016a; Torres-Ruiz, Wehr & Perrone, 2010). In addition, recent lake studies suggest that ARA may not have a clear relationship with trophic level (Strandberg *et al.*, 2015), whereas in our study, ARA in all non-salmonid fish was significantly greater than that in invertebrates and algae, indicating the physiological importance of ARA for these stream fishes. However, the function of ARA for stream invertebrates is still poorly understood (Guo *et al.*, 2016b) and requires further clarification.

Polyunsaturated fatty acids in algae

It is clear that the LC-PUFA composition of consumers in stream food webs is strongly influenced by basal algal PUFA composition. In our analysis, algae from temperate rivers had more EPA compared with algae from subtropical rivers. Such a difference at the base of stream food webs may be because EPA is predominantly synthesized by certain algal taxa to endure colder temperatures. This argument for greater levels of EPA, and perhaps other LC-PUFA, in algae of temperate streams is based on the finding that LC-PUFA provide cell membranes greater fluidity when exposed to low temperatures (Guschina & Harwood, 2006). Thus, algae in cold waters may contain more LC-PUFA than algae in warm waters, which could consequently affect the PUFA composition and retention of their consumers.

Polyunsaturated fatty acid distribution and retention in invertebrates

Among PUFA, EPA is preferentially retained in invertebrates. EPA is required for development, reproduction, and hormone regulation in many invertebrates (Stanley-Samuelson, 1994). In our study, most invertebrates were insects with well-developed nervous systems and complex behavior, and they were rich in EPA but very low in DHA. Previous findings suggest that the function of DHA for proper neural and retinal tissue development is probably fulfilled in insects by EPA (Ogg, Howard & Stanley-Samuelson, 1991). Therefore, insects may require more EPA than DHA for somatic growth and reproduction (Ahlgren, Vrede & Goedkoop, 2009).

The trophic enrichment of EPA in invertebrates indicates these organisms may be capable of regulating their PUFA composition, such as converting (elongating) the shorter-chain C₁₈ PUFA ALA to longer chained analogs. In our study, no differences were detected in invertebrate FA profiles between temperate and subtropical rivers, although algal PUFA composition between temperate and subtropical rivers was dissimilar, suggesting invertebrates may mediate their own PUFA composition (Goedkoop, Sonesten, Ahlgren & Boberg, 2000). Conversely, we also observed that invertebrate EPA and SAFA varied with algae in both temperate and subtropical samples, indicating invertebrate biochemical composition largely reflected algal FA, consistent with previous findings (Torres-Ruiz, Wehr & Perrone, 2010). Recent studies showed that stream invertebrates may regulate their PUFA patterns and somatic growth to deal with different dietary LC-PUFA (Guo *et al.*, 2016a). Therefore, combined with previous findings, we suggest that invertebrates may be capable of regulating their FA composition, but this ability is nonetheless limited and they obtain physiologically important LC-PUFA primarily from algae.

Polyunsaturated fatty acid distribution and retention in fish

DHA is selectively and highly retained over other PUFA in fish, consistent with all reviewed datasets. It is still not clear if the high retention of DHA in fish is mainly driven by dietary DHA (Dayhuff, 2004; Young, Whitledge & Trushenski, 2016; Rude, Trushenski & Whitledge, 2016) or by endogenous PUFA conversion (Tocher & Dick, 1999; Henrotte *et al.*, 2011; Murray *et al.*, 2014; Hixson, Parrish & Anderson, 2014;

Oboh, Betancor, Tocher & Monroig, 2016). Our results showed that DHA retention in fishes from subtropical rivers is greater than that from temperate rivers, suggesting fishes may spend extra energy to synthesize LC-PUFA under high temperature leading to more energy loss (Farkas, Csengeri, Majoros & Oláh, 1980). To date, it is uncertain if the metabolic pathway is sufficient to account for the high DHA content observed in fish from stream ecosystems. High DHA synthesis in fish would lead to small intraspecific variation due to differences in diet composition. However, some recent studies have shown that fish FA profiles differed spatially within large river ecosystems (Dayhuff, 2004; Young, Whitledge & Trushenski, 2016; Rude, Trushenski & Whitledge, 2016). For instance, the FA profiles of bluegill (*Lepomis macrochirus*, Centrarchidae) across Illinois River reaches (Rude, Trushenski & Whitledge, 2016), channel catfish (*Ictalurus punctatus*, Ictaluridae) in the Kaskaskia River (Young, Whitledge & Trushenski, 2016), and sauger (*Sander Canadensis*, Percidae) and white bass (*Morone chrysops*, Moronidae) in the Ohio River (Dayhuff, 2004), were significantly different amongst sites from upstream to downstream of each study river. The spatial variation in fish FA composition was attributed to concurrent differences in the FA composition of primary producers and prey items among different reaches (Dayhuff, 2004; Young, Whitledge & Trushenski, 2016; Rude, Trushenski & Whitledge, 2016). It is most likely that dietary PUFA are the most important PUFA source for the above studied fish, and DHA is effectively retained in their body tissues.

However, the endogenous conversion from dietary precursors, such as ALA and EPA, to DHA seems a key pathway for DHA for stream fish. For example, salmonids from pre-alpine streams may not adjust their PUFA to dietary PUFA, although the DHA content in their diets was significantly different among study streams (Heissenberger, Watzke & Kainz, 2010). When salmonids are grown on feeds containing vegetable oils with only traces of dietary DHA addition, they still retain high amounts of DHA in their muscle tissues (Murray et al. 2014). Accordingly, for river salmonids it is likely that endogenous biosynthesis is sufficient to satisfy their normal physiological requirements (Castro *et al.*, 2012; Leaver *et al.*, 2008). Furthermore, in our study, the FA composition of all fish taxa was distinctly different from invertebrates and algae, suggesting all fish taxa may be able to regulate their PUFA content. To date, only a few studies have considered the differences in the PUFA biosynthesis of fish taxa in rivers or compared the dietary PUFA effect on different fish taxa. Both controlled feeding trials and field studies are needed to differentiate the interaction of diets with endogenous metabolism on the PUFA composition of different fish taxa.

Interestingly, the DHA content in Salmoniformes and Perciformes was significantly greater than for Cypriniformes and Siluriformes. This may be due to differences in feeding guilds as salmonids are more visual predators and may thus require more DHA in

their visual/neuronal tissues. Further, higher DHA in Salmoniformes and Perciformes may also be attributed to differences in thermal requirements of each fish taxa. Salmonids are primarily found in habitats with peak water temperatures below 20 °C (Wehrly, Wang & Mitro, 2007) and these fish are often found in streams where winter water temperatures average 0°C (Heggenes, Krog, Lindås & Dokk, 1993), similar to Perciformes, whereas carp and catfish are typically found in high temperatures (Eaton *et al.*, 1995). DHA is known to act as an ‘anti-freeze-like’ compound in membrane phospholipids by increasing membrane flexibility (Stillwell & Wassall, 2003). Due to its high number of double bonds, DHA has a greater effect on membrane fluidity than other LC-PUFA (i.e., EPA and ARA) (Niebylski & Salem Jr, 1994), even at very low temperatures (Stillwell & Wassall, 2003). These characteristics of DHA may help Salmoniformes and Perciformes to maintain cell membrane fluidity in cold-water temperatures, and may partly explain the low DHA content in Cypriniformes and Siluriformes.

In addition, Salmoniformes and Perciformes occupy habitats where algal EPA is abundant (see above), and the conversion from EPA to DHA may be more efficient than for other fish taxa. In contrast, Cypriniformes and Siluriformes, which typically inhabit in warm waters, and where algal EPA is low, may require more energy to convert dietary ALA to EPA and then to DHA, which would be less efficient. However, it still remains to be determined how different fish taxa utilize dietary PUFA under different nutritional

conditions.

The trophic transfer of PUFA in headwater streams

Algal PUFA variations induced by river longitudinal gradients significantly affected fish PUFA composition (Dayhuff, 2004; Young, Whitledge & Trushenski, 2016; Rude, Trushenski & Whitledge, 2016). Interacted with light availability, nutrient inputs and temperature, river longitudinal gradients also determine fish distribution (Buisson, Blanc & Grenouillet, 2008). The River Continuum Concept (RCC) thus implies that fish living in headwater streams are supported by a food web based on terrestrial matter inputs which predominate the upstream reaches of rivers (Vannote, Minshall, Cummins, Sedell & Cushing, 1980). Those terrestrial sources have the short-chain PUFA ALA and LIN, which are usually depleted when autumn leaves are shed and later decomposed (Torres-Ruiz & Wehr, 2010). Modern biochemical tracers, stable isotopes, FA, amino acids, and compound-specific FA have demonstrated that algae are more nutritionally important for stream consumers compared with terrestrial matter (Bunn, Davies & Winning, 2003; Delong & Thorp, 2006; Lau, Leung & Dudgeon, 2009; Brett *et al.*, 2017). However, our results suggest that DHA is highly retained in all fish taxa, even when the supply of DHA is low in algae and invertebrates. It is thus possible that headwater fish may selectively feed on nutritionally high quality food that is rich in EPA, and subsequently use EPA to synthesize DHA. However, to date, it is still not clear how

headwater fish utilize FA from basal food sources, i.e., terrestrial matter and algae.

Disentangling this conundrum will fundamentally improve our understanding of nutritional dynamics and trophic transfer across stream food webs.

Implications

Based on the results we have obtained thus far, algae from subtropical rivers may contain lower percentages of EPA than algae from temperate rivers. Increasing water temperature as a result of global warming is predicted to decrease the global EPA and DHA production in algae; EPA may be reduced by 8.2% and DHA by 27.8% with an increase in water temperature of 2.5°C (Hixson & Arts, 2016). In addition to temperature, algal LC-PUFA production in streams and rivers is also threatened by eutrophication and environmental pollutant inputs (Guo *et al.*, 2016b; Guo, Kainz, Sheldon & Bunn, 2015). Such threats will negatively affect freshwater and terrestrial consumers that rely on algal LC-PUFA. Meanwhile, those factors also directly influence fish endogenous metabolism. Fishes may experience extra stress under high temperature leading to more energy loss given that the DHA retention in fishes from subtropical rivers is greater than that from temperate rivers. All these effects could act in concert, and may significantly affect LC-PUFA supply in stream ecosystems. Therefore, it is important to understand how environmental changes affect the LC-PUFA distribution and retention in stream ecosystems.

Conclusion

This review represents the first step toward documenting differences in the LC-PUFA distribution and retention in stream food webs. The presented results suggest selective retention of PUFA in stream food webs yield high LC-PUFA contents in consumers, particularly DHA in fishes, and EPA in invertebrates. The reasons for the trophic retention of DHA and EPA are still poorly understood, but the analysis suggests that consumers selectively incorporate algal PUFA, and possibly also bioconvert dietary ALA and EPA to DHA in a very wide range of fish taxa. This review integrated individual studies on the FA composition of different food web components, and provided strong evidence of FA differences in temperate and subtropical food webs. Based on this analysis it is apparent that further research, particularly on the role of dietary FA conversion and retention in consumers in various stream ecosystems around the world, is required to better comprehend, predict, and protect the status and trophic retention of nutritionally essential FA.

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Tables and figures

Table 1 Similarity values (ANOSIM) between fatty acid compositions of food web components of rivers and streams. Pairwise comparisons were listed below according to the R statistic (higher R values indicate greater difference between two groups, whereas lower values indicate a small difference between two groups).

	Salmoniformes	Perciformes	Siluriformes	Cypriniformes	Inverts-north	Inverts-QLD	Algae-north
Perciformes	0.3366*						
Siluriformes	0.8274***	0.7615***					
Cypriniformes	0.7081**	0.6709***	0.1175				
Inverts-north	0.9513***	0.9687***	0.8533***	0.7277***			
Inverts-QLD	1***	0.9513***	0.8686***	0.6892***	0.0644		
Algae-north	0.989***	0.9441***	0.927***	0.761***	0.2175**	0.4661**	
Algae-QLD	0.9973***	0.9432***	0.8329***	0.7053***	0.7335***	0.6691***	0.5932***

*** p=0.001, ** p<0.01, * p<0.05

Table 2 Fatty acid retention ratios in northern temperate and southern subtropical rivers, respectively. PUFA retention ratios between consumers (fish and invertebrates) and algae were calculated by dividing consumer PUFA% by algal PUFA% (i.e., $[\text{PUFA}]_{\text{consumer}}/[\text{PUFA}]_{\text{algae}}$). Data used for subtropical rivers were from Jardine *et al.* (2015).

(1) Northern temperate rivers

	LIN	ALA	ARA	EPA	DHA
Salmoniformes	0.67	0.44	3.20	0.79	37.73
Perciformes	0.37	0.18	8.92	0.88	35.93
Cypriniformes	1.14	0.41	5.74	0.72	8.27
Siluriformes	0.89	0.30	5.22	0.22	8.10
Invertebrates	0.96	1.13	2.28	1.41	0.66

(2) Southern subtropical rivers

	LIN	ALA	ARA	EPA	DHA
Perciformes	1.36	0.36	9.25	0.49	48.98
Cypriniformes	3.09	0.46	12.73	0.84	31.36
Siluriformes	0.93	0.35	4.87	0.48	27.78
Invertebrates	0.75	1.06	1.17	2.42	0.05

Fig 1 Non-metric multidimensional scaling (NMDS) of fatty acid compositions of food web components in stream ecosystems (two-dimensional, stress = 0.12)

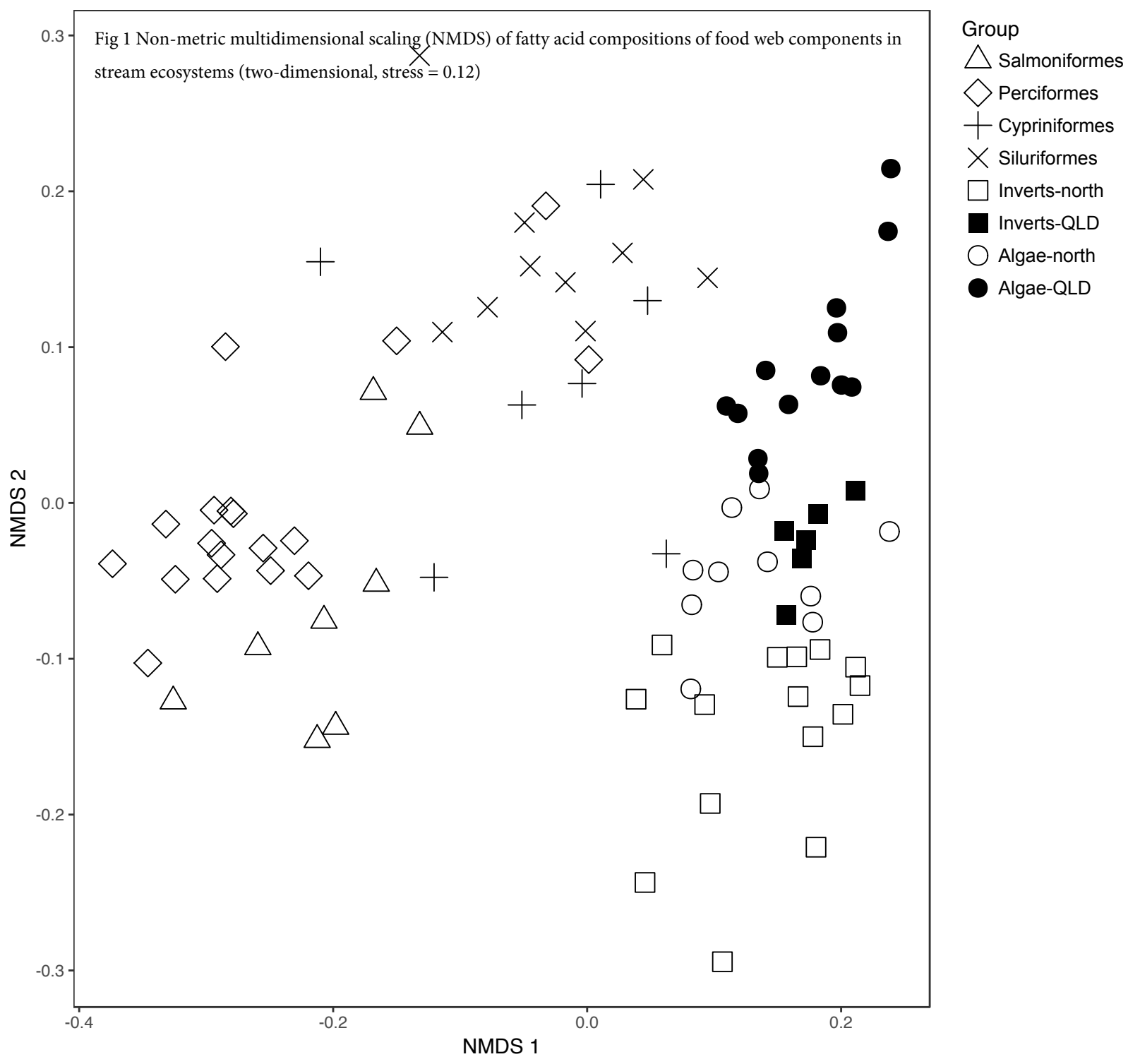
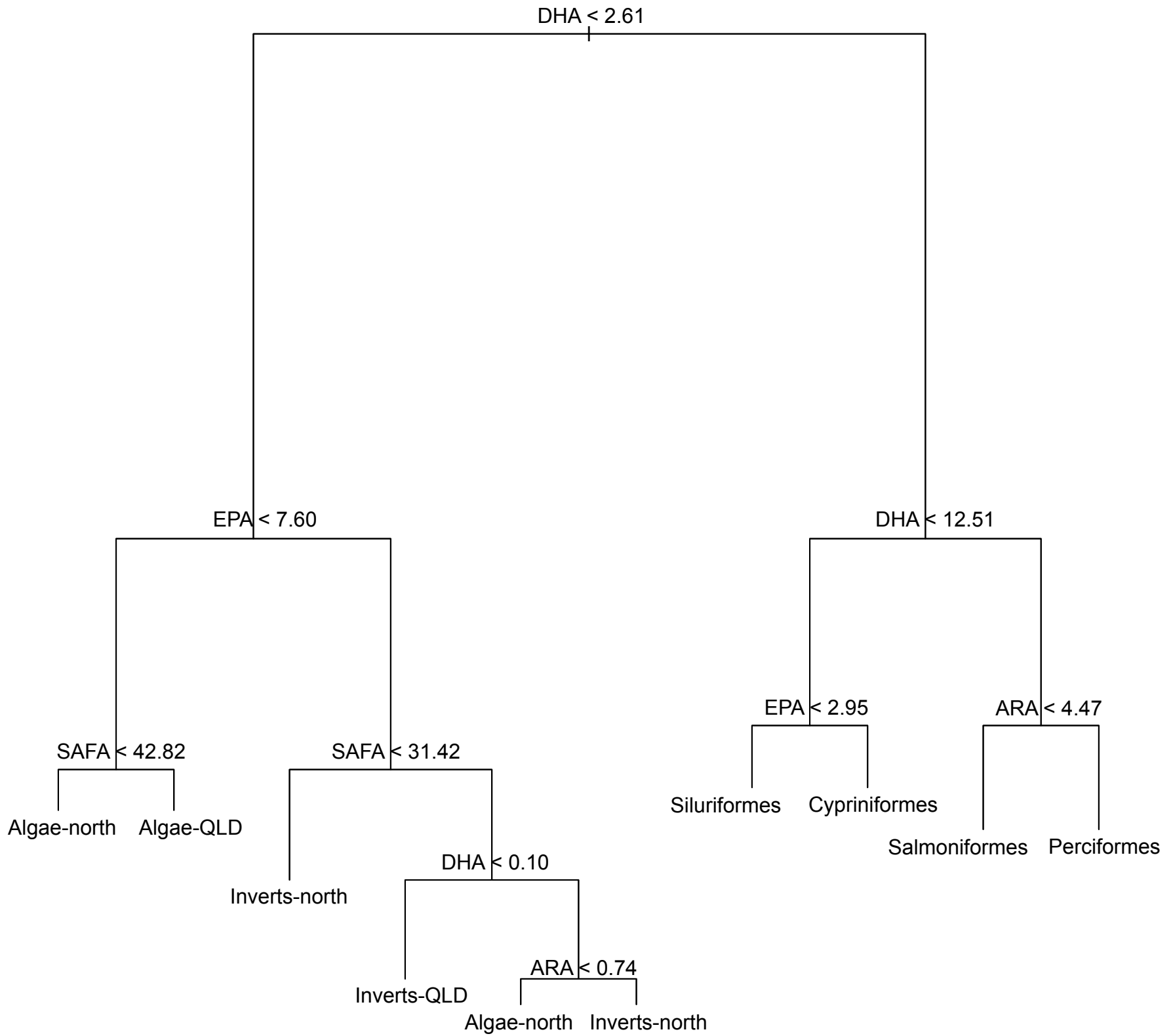
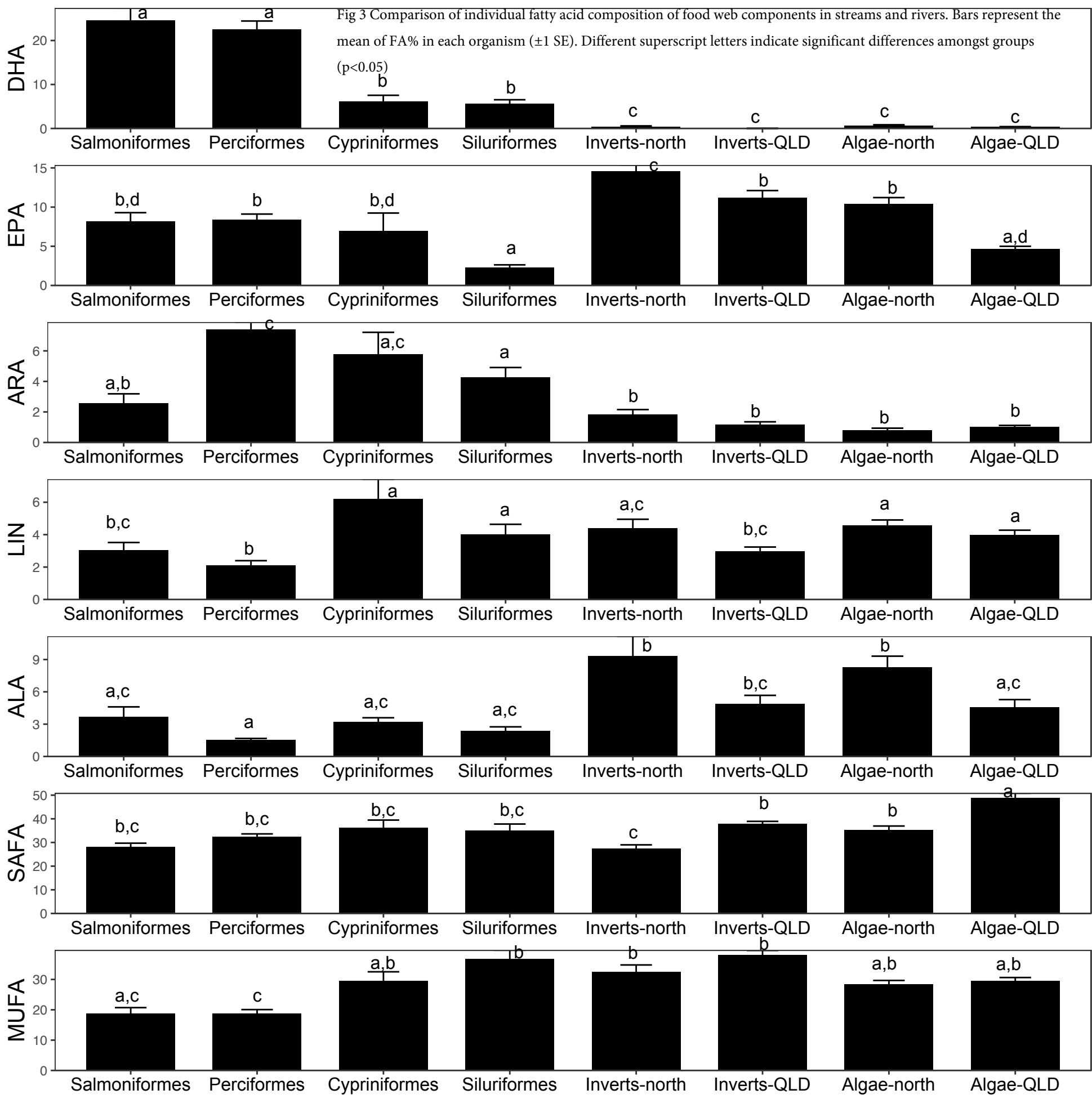


Fig 2 A regression tree for exploring patterns between individual fatty acids and each food web component





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