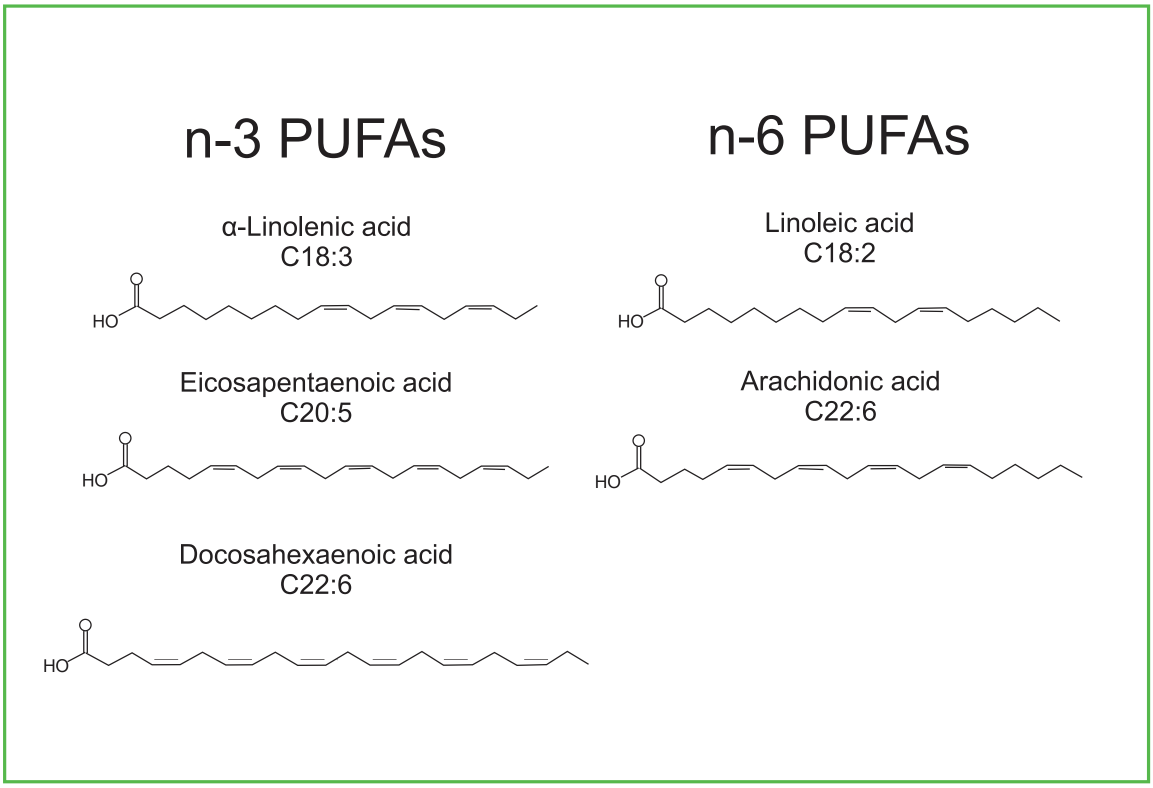
# The use of fatty acid lipids in freshwater ecological research



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**June 2020**

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**This report should be cited as:**
Growns, I, Frost, L. Ryder, D. McInerney, P., Bond, N. and Lester, R. (2020) The use of fatty acod lipids in freshwater ecological research. University of New England, Armidale.

ISBN 978-0-6484756-8-2

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This review forms part of and was funded by the Environmental Water Knowledge and Research Project which was financed by the Australian Government's Department of the Environment and Energy and coordinated by the Murray–Darling Freshwater Research Centre.

Front plate adapted from Hoppenbrouwers Tamara, Cvejić Hogervorst Jelena H., Garssen Johan, Wichers Harry J., Willemsen Linette E. M. (2019). Long Chain Polyunsaturated Fatty Acids (LCPUFAs) in the Prevention of Food Allergy.Frontiers in Immunology, 10, 1118.

## *Abstract*

Fatty acids (FA) are a class of lipids that act in essential physiological functions in organisms and are transferred via trophic interactions in food webs. The literature regarding the roles of FA has a long history in marine systems, but is yet to receive the same attention in freshwater systems. In this review we discuss the scientific literature that uses FA in freshwater ecology but limited to experimental studies. We identified 178 papers that have used FA to either examine food webs, anthropogenic disturbance, subsides between ecosystems, seasonal or spatial variation and as biomarkers in freshwater ecosystems. The majority of research concerned the use of FA for examining food web structures and has been subject to several recent reviews so is not covered here. We examined the remaining research topics and identified six areas where FA could be used to develop new knowledge in freshwater ecology. Our review assists natural resource management by highlighting consistencies among studies but also identifies where research is inconclusive and requires further work to establish ecological generalisations.

*Key words: Fatty acids; freshwater; review; future research; river management*

## *Introduction*

Lipids (or fats and oils) are defined as substances that are soluble in organic solvents (Ratnayake and Galli 2009). Lipids are an important component of living cells and together with carbohydrates and proteins, are the main constituents of plant and animal cells. Fatty acids (FA) are a class of lipids that act in important functions for physiological processes in all organisms, including antecedents to anti-inflammatory eicosanoids (Pernet et al. 2007), preservation of cell membranes and their functions (Guschina and Harwood 2009), and for metabolism including energy storage (Dalsgaard et al. 2003). The number and composition of FA (or FA profile) in any particular material or organism is influenced by many factors, including but not limited to, environmental influences (e.g. temperature), phylogeny, diet and metabolism (Piorreck et al. 1984, Burns et al. 2011, Lang et al. 2011).

Two forms of FA, omega-3 (termed n-3) and omega-6 (n-6) poly-unsaturated FA (PUFA – a list of acronyms used throughout the remainder of this review is provided in Table 1), are produced almost exclusively by plants, especially phytoplankton, benthic algae and macrophytes (Dalsgaard et al. 2003), and are critical nutrients for heterotrophs, including invertebrates, fish, and humans (Arts et al. 2001). Because most heterotrophs cannot make n-3 and n-6 PUFA at rates sufficient to meet their fundamental biochemical requirements (Parrish 2009), these molecules are thought to be EFA. Aquatic (marine and freshwater) ecosystems are the primary source of n-3 FA in the environment, therefore supporting both aquatic and terrestrial heterotrophs via the trophic transfer of these key EFA through food webs (Gladyshev et al. 2013). FA are a promising biochemical metric to use as a proxy for ecosystem-scale food quality due to the large body of research detailing how growth and reproduction of many aquatic consumers may be constrained by the limitation of certain EFA (Galloway and Winder 2015).

FAs have been used within marine ecosystems to investigate predator-prey relationships and to help understand process dynamics within food webs for over four decades (Dalsgaard et al. 2003, Suzumura 2005, Mrozik et al. 2014). In contrast, the use of FA in freshwater ecosystems is relatively novel. Here, we present a review of the scientific literature on the use of FA in experimental studies in freshwater ecology and identify future areas of potential research.

## ***Methods and Results***

We retrieved relevant publications by conducting a search of two scientific databases, Web of Science® and Scopus®, using the search phrase ("fatty acid" AND freshwater). The searches yielded 1120 and 1645 citations respectively. A large number of references were mainly concerned with human nutrition, aquaculture and/or biofuels and manually filtering studies relating to freshwater ecosystems yielded 178 references (Supplementary material). Papers just describing the fatty acid composition of material or biota were ignored e.g. Bell et al. (1994). The earliest paper was published in 1979 (Figure 1). This paper concerned the effects of diets with different dietary FA and temperature on the production of a cladoceran (D'Abramo 1979). However, the majority of papers have been published since 2009. The increase in publications is probably due to technological advances in the ability to analyse FA profiles in biological material (Tang and Row 2013) and subsequent reductions in the cost of analyses, but also the acknowledgement by the scientific community of the value of FAs in ecological research.

Table 1. Definitions of acronyms related to fatty acids used throughout the text.

| Acronym | Phrase | Definition or formulae |
|---|---|---|
| ADA | Arachidonic acid | 20:4n6 |
| ALA | α-linolenic acid | 18:3n3 |
| BAFA | Bacterial fatty acids | 15:0, 17:0 and their branched homologues |
| DHA | Docosahexaenoic acid | 22:6(n-3). |
| DPA | Docosapentaenoic acid | any straight chain 22:5 fatty acid |
| EFA | Essential fatty acid | Fatty acids that animals must ingest because the body requires them for good health but generally cannot synthesize them |
| EPA | Eicosapentaenoic acid | 20:5n3 |
| HUFA | Highly unsaturated fatty acid | Polyunsaturated fatty acids containing 20 or more carbon atoms in the carbon chain |
| IA | Isoseptadecanoic acid | 17:0iso |
| LCPUFA | Long-chain poly unsaturated fatty acid | Fatty acids with 18–20 carbons or more and the constituent hydrocarbon chain possesses two or more carbon–carbon double bonds |
| LIN | Linoleic acid | 18:2n6 |
| MUFA | Monosaturated fatty acid | are fatty acids that have one double bond in the fatty acid chain with all of the remainder carbon atoms being single-bonded. |
| OA | Oleic acid | C18:1n9c |
| PAL | Palmitoleic acid | 16:1n-7 |
| PA | Palmitic acid | 16:00 |
| PLA | Palmitoleic acid | 16:1n7 |
| PLFA | Phospholipid fatty acid | A molecule made of two fatty acid chains joined to a phosphate group by a glycerol molecule |
| PUFA | Polyunsaturated fatty acid | Fats in which the constituent hydrocarbon chain possesses two or more carbon–carbon double bonds |
| SCFA | Short chain fatty acid | are fatty acids with less than six carbon atoms |
| SFA | Saturated fatty acids | A saturated fat is a type of fat in which the fatty acid chains have all or predominantly single bonds |
| TeFA | Terrestrial Fatty acids | 22:0, 24:0 |
| TFA | Total fatty acid | Sum of all fatty acids |

Table 2. Percentage of publications in different habitats, subject groups, number of groups and research topics.

| Habitat | Publications (%) | Subject Group | Publications (%) | Number of subject groups | Publications (%) | Major themes | Publications (%) |
|---|---|---|---|---|---|---|---|
| Laboratory/mesocosm | 37 | Sediment | 5 | 1 | 12 | Food web | 54 |
| Lentic | 41 | Seston | 13 | 2 | 28 | Disturbance | 28 |
| Lotic | 25 | Leafpacks | 3 | 3 | 8 | Subsidy between ecosystems | 5 |
| Estuary | 2 | Periphyton | 10 | 4 | 4 | Seasonal or spatial variation | 9 |
| | | Cyanobacteria | 4 | 5 | 1 | Biomarkers | 11 |
| | | Bacteria | 9 | 6 | 1 | | |
| | | Phytoplankton | 26 | | | | |
| | | Aquatic plant | 1 | | | | |
| | | Zooplankton | 42 | | | | |
| | | Benthic invertebrates | 16 | | | | |
| | | Mollusc | 12 | | | | |
| | | Amphibians | 1 | | | | |
| | | Fish | 23 | | | | |
| | | Birds | 1 | | | | |
| | | Mammal | 1 | | | | |

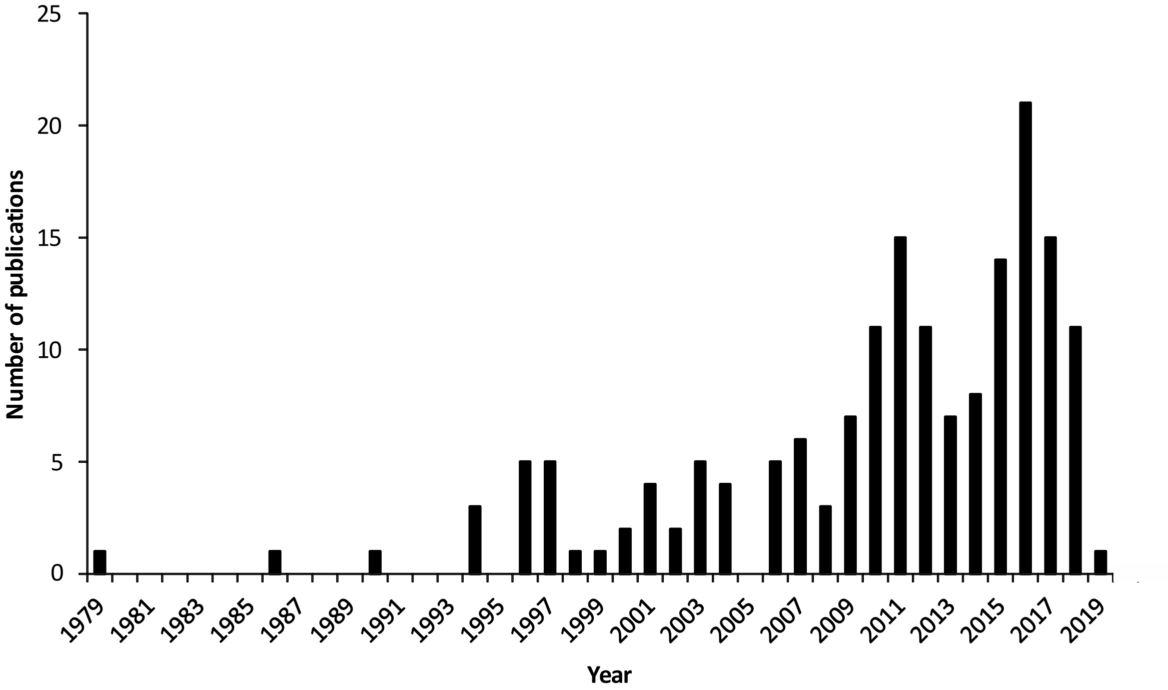

Figure 1. Number of publications by year using fatty acids in freshwater ecological research.

The bulk of freshwater research interrogating FA profiles has occurred in laboratory and mesocosm experiments or taken place in lentic habitats such as ponds and lakes (Table 2). Despite research occurring across broad range of subject groups within freshwater ecology, the majority of studies using FA focus on zooplankton and phytoplankton. In contrast, we only identified one (0.6%) publication for each of amphibians, birds and mammals. Typically research using FA has been quite specific, dominated by studies investigating with only one or two biotic groups, with only eight papers from the 178 concerning four or more biotic groups. Each of these eight papers were mainly concerned food webs and the different groups were examined for trophic linkages between them e.g. (Czesny et al. 2011, Sanseverino et al. 2012, Volk and Kiffney 2012, Carassou et al. 2017). FA have been used for a range of topics, but more than half of the studies have investigated food webs and trophic interactions. Reviews of FA use in exploring food webs in lentic and lotic habitats have recently been published e.g. Guo et al. (2016) and Brett et al. (2017), instead we focus on the use of FA other subject areas in freshwater ecology (e.g. response to disturbance, seasonal/spatial variation, capacity as biomarkers) and highlight areas of potential future research.

## ***Effects of disturbance or environmental factors on fatty acid composition***

### *Climate change and temperature*

Climate modelling predicts that both surface air temperatures and the frequency of extreme heat days will continue to increase worldwide (Team et al. 2014), and it is thought that temperatures in lentic ecosystems will increase concordantly. Effects are likely to be most pronounced in water bodies with large surface-to-volume ratios (i.e., small ponds and large shallow lakes (McKee et al. 2002, Van Doorslaer et al. 2007). Hixson and Arts (2016) conducted an exploratory data synthesis with 952 FA profiles from six major groups of marine and freshwater phytoplankton. They found temperature was strongly correlated with a decrease in the proportion of n-3 LCPUFA and an increase in omega-6 FA and saturated FA. (Hixson and Arts 2016). The authors suggested that reduced production of these EFA would negatively affect species that depend on these compounds for optimum physiological function, with cascading effects throughout the world's ecosystems. However, this work was a correlative study and there may have been other confounding effects contributing to their findings e.g. their literature search may have imposed a bias on the types of phytoplankton that were included, variation within taxa groups and different culture methods.

Empirical experimental evidence exploring the effects of temperature, and therefore potentially climate change, on the FA composition in freshwater ecosystems and biota is limited and contradictory. Temperature is assumed to have strong influence on the quantity and quality of FA in both marine and freshwater phytoplankton, since acclimatisation to increasing temperature requires decreasing PUFA content in cell membranes, while, simultaneously, increasing saturated FA (SFA) to maintain cell membrane structural rigidity (Rousch et al. 2003, Fuschino et al. 2011). Several studies have shown that PUFA concentration increases in green algal phytoplankton from low temperatures (~15°C) to moderate temperatures (~20°C) before reaching a threshold (~25°C) and declining (Renaud et al. 2002, Hodaifa et al. 2010, Fuschino et al. 2011, Sikora et al. 2014, von Elert and Fink 2018). However, we expect temperature effects on FA composition to differ with freshwater phytoplankton species as it does for marine species (Thompson et al. 1992).

We identified two studies that examined the effects of temperature related changes in FA composition of phytoplankton on higher order consumers and these had opposing findings. The first study hypothesized that increased temperatures and nutrient would lead to a shift to cyanobacterial communities which would then reduce the efficiency of feeding and growth of zooplankton (Przytulska et al. 2015). They tested the growth and FA composition of *Daphnia* sourced from two regions with different temperature regimes and fed them either cyanobacteria or green algae. They found that there was a significant negative effect of increased temperature and cyanobacterial food on zooplankton fatty acid content and composition. The combined effect of temperature and food quality on the performance of the low temperature *Daphnia* was greater than their effects added separately, further indicating the potentially strong negative indirect effects of climate warming on aquatic food web processes.

The second study examined the relative influences of abiotic selection and food web effects associated with climate change on intraspecific differences and interrelationships between HUFA content, body size, and fecundity of freshwater copepods (Charette and Derry 2016). In artificial ponds climate-driven temperature changes favoured the coexistence of a diversity of phytoplankton species with different temperature optima and nutritive quality.

The change resulted in positive relationships between temperature, copepod DHA content and body size. Temperature correlated positively with algal biovolume, and arbitrated relationships between copepod HUFA content, body size and fecundity. The presence of brook trout further increased these positive effects in warm ponds, likely through nutrient input and stimulation of phytoplankton resources. These results suggest that climate change may have previously unrecognised positive effects on higher order consumers. The small number of studies and contrasting results of the effects of temperature increases derived from climate change on primary producers and consumers suggests this topic should be an important area for future research.

### *Altered land use and agriculture*

Altered land use and agricultural activities have the potential to significantly modify the physical, chemical, and biological properties that comprise stable and healthy stream ecosystems. Their effects include riparian vegetation removal or alteration, elevated sediment loads, increased dissolved nutrients, and inputs of environmental pollutants (Ehrman and Lamberti 1992, Osborne and Kovacic 1993, Strayer et al. 2003). One of the major impacts of nutrient addition and altered light regimes from riparian clearing is changes in the composition of primary producers, which has obvious implications in terms of basal food resources and therefore the nutritional quality and FA profiles of and higher order consumers (Larson et al. 2013). We identified six papers that examined the potential of land use change and agriculture to affect the FA composition in stream organic matter and biota.

Two studies specifically examined agricultural land use effects on primary producers and reported contrasting results. Boëchat et al. (2011) examined how the FA in benthic microbial mats differed between tropical streams located in protected pristine and agricultural savannah areas. Streams in agricultural areas had lower total biomass of benthic microbial mats than pristine streams. However, the higher concentrations of essential PUFA, such as LIN, ALA and EPA, that were observed in agricultural streams suggest enhanced lipid complexity and a higher nutritional quality of the autotrophically dominated microbial community relative to pristine streams (reflecting a dominance of heterotrophic microbial communities). Whorley and Wehr (2018) investigated the nutritional contribution of benthic algae in the form of FA in streams with different ages of improved environmental management practices (EMP) versus pristine streams. Their results indicated that EMPs were able to reduce agricultural effects, as evidenced by a significantly lower benthic algal total FA content in reference compared to agricultural streams. However, FA content did not differ between EMP-age categories, a result suggesting that EMPs were effective within one to two years.

The four remaining studies examined the effects of land-use on FA composition of suspended organic matter in streams and rivers. Boëchat et al. (2014) analysed land-use effects on the FA composition and concentrations in suspended particulate organic matter (SPOM) along a fourth-order tropical river. Urbanisation was the only the land-use category correlating with both FA composition and concentrations. Higher concentrations of saturated FAs, especially C16:0 and C18:0, which are the main components of domestic sewage, were observed at sampling stations downstream of urban centres. They suggest that FA can be used as biomarkers to identify anthropogenic disturbances. Larson et al. (2013) investigated variation in the fatty acid (FA) composition of seston and primary consumers within and among 11 tributary systems of Lake Michigan, USA. They hypothesised that among-system and among-habitat variation in FAs at the base of food webs would be related to algal production, which in turn is influenced by three different land uses. Of the three land cover characteristics, only intense land use appeared to be strongly related to seston and consumer FA and this effect was only strong in river mouth and nearshore lake sites. River seston and consumer FA composition was highly variable, but that variation did not appear to be driven by watershed land cover characteristics. Lu et al. (2014) compared the FA composition of POM from eight headwater streams influenced by different types of watershed land use. The contribution of bacterial FA differed significantly among sampling times, but not among land use types. However, the concentrations of total long-chain polyunsaturated FA, EPA and ARA showed that the nutritional value of POM in forest streams was lower than in urban streams. Ngugi et al. (2017) used lipid biomarkers in suspended particulate OM (SPOM) at seven river discharge sites in Lake Victoria, in an effort to evaluate the potential impacts of upstream catchment land use. At sites receiving discharge from industrial effluent the FAs were dominated by SCFA, PUFA, MUFA and branched FAs. Sites receiving discharge from substantial agricultural activity in the catchment were dominated by LCPUFA reflecting vascular plant terrestrial input. They suggested that human land use in upstream watersheds affect the source, composition and distribution of SPOM discharged by rivers but may also change the quality of SPOM. Of the six studies examining land-use impacts only Larson et al. (2013) investigated whether changes in the basal food source was transferred to consumers. We would thus argue that further research is required to evaluate if the effects of altered land use and agriculture on basal food sources are broadly reflected in the production of species higher in the food web.

### *Herbicides*

All three publications that investigated the influence of herbicides on FA composition focussed on algae. Chalifour et al. (2014) investigated the effect of low temperature on the toxicity of Norflurazon (Nf) and Fluridone (Fd) herbicides on a *Chlamydomonas reinhardtii*. These two herbicides are widely used for the control of grasses and invasive aquatic weeds. The fatty acid composition was modified by temperature and the level of unsaturation noticeably increased at 15 compared with 25°C. MUFA and PUFA decreased in the presence of Nf and Fd at 25°C but not at either 8 or 15°C. El-Sheekh et al. (1994) showed the herbicide atrazine concentrations induced a general inhibition on growth, photosynthesis, and dark respiration in the green alga *Chlorella kessleri*. The herbicide had a preferential effect on the associated fatty acid composition of total and phospholipids. After 24 and 48 hr the herbicide stimulated FA synthesis at concentrations where photosynthesis was inhibited. However, stearic and miristic acids disappeared at 15 µM after 24 hr and the total polyunsaturated FA were not affected after 48 hr. Fatty acid synthesis was sensitive to treatment at 72 hr by 5 and 10 µM atrazine, whereas the total saturated FA were completely inhibited. Both studies therefore demonstrated a change in the FA composition of algae with herbicide exposure. The third study examined whether consumers of algae could be affected by herbicide application. da Silva et al. (2016) compared the reproductive potential of *Daphnia magna* fed a diet of *Raphidocelis subcapitata* exposed to the herbicide pendimethalin with control mesocosms. The chemical composition of *R. subcapitata* exposed to the herbicide revealed a slight decrease in total fatty acid levels, with a particular decrease of essential omega 9 monounsaturated fatty acids. Female *D. magna* exposed to the herbicide diet experienced a 16% reduction in reproduction, measured as the total number of offspring produced per female. Although they suggest it is difficult to discriminate the contribution of the pesticide (as a toxic agent transferred through the food web) from that of the food with a poor quality-compromised by the same pesticide, there are no doubts that, under environmentally relevant concentrations of pesticides, both pathways may compromise the populations of freshwater grazers in the long term, with consequences in the control of the primary productivity of these systems.

### *Nutrients and cyanobacterial blooms*

The effects of both increased and decreased nutrient loading on FA in biota in lentic and lotic systems have been examined in the literature. Changes in nutrient inputs can have overwhelming effects upon the quality of receiving waters (Carpenter et al. 1998, Correll 1998). The most common effect of increased important nutrients, nitrogen and phosphorus, to aquatic ecosystems is an increase in the biomass of algae and aquatic plants and a shift in phytoplankton composition to bloom-forming species, many of which may be toxic, or which may not be consumed effectively by aquatic grazers (Smith 2003).

We identified six studies that examined increased nutrient effects in lentic environments, two of these only examined the direct effects on algal species. Hu and Gao (2006) tested the effects of different levels of nitrogen (N), phosphorous (P), salinity and temperature on the green algae *Nannochloropsis* sp. Increased levels of N and P raised protein and PUFA contents but decreased carbohydrate, total lipid and TFA contents. Piepho et al. (2012) tested the influence of phosphorus (P) supply, light intensity and temperature on FA concentrations of four freshwater algae. The effect of increased P on FA was complex and was affected by both light intensity and temperature and differed between species. For example, changes in several saturated and unsaturated FA concentrations with higher light intensity were more distinct in the low-P treatments than in the high-P treatments. Interactive effects of temperature and P supply on various FA concentrations were observed in all four species, but there was no consistent pattern. However, the TFA, SFA and MUFA content of three of the algal species decreased with P addition.

Three studies examined the effects of eutrophication changes in FA in basal food sources on higher order consumers. Larson et al. (2016) examined the effects of eutrophication in Lake Erie on the border of the USA and Canada on secondary production and FA content in freshwater mussels. They found secondary production was highest in the more eutrophic sites and lowest in the less eutrophic sites. Mussel tissues in the eutrophic sites also included more EPA and DPA, but fewer bacterial FAs, suggesting more algae at the base of the food web in the more eutrophic sites. Cyanobacterial abundance among sites was not correlated to secondary production, but was positively related to EPA and DPA content in the mussels, suggesting more of the EFA in locations with more cyanobacteria. These results suggest that growth of secondary consumers is correlated with the availability of EFA in the more eutrophic waters. In contrast, Müller-Navarra et al. (2000) showed that low transfer efficiencies between primary producers and consumers exist during cyanobacteria bloom conditions caused by eutrophic conditions are related to low relative EPA content of the primary producer community. Zooplankton growth and egg production were strongly related to the primary producer EPA to carbon ratio. This indicates that limitation of zooplankton production by this essential fatty acid is of central importance at the pelagic producer–consumer interface. Similarly, Bartsch et al. (2017) demonstrated that increased nutrient and sediment loading in rivers have caused observable changes in algal community composition, and thereby, altered the quality and quantity of food resources available to native freshwater mussels. The authors suggested that areas dominated by cyanobacteria caused by eutrophication may not provide enough food quality to promote or sustain mussel growth.

One study examined sedimentary organic matter composition in lakes with different trophic states (as indicated by N and P concentrations) and attempted to identify the sources using fatty acid biomarkers (Xu et al. 2014). The FA profile of organic matter indicated that there were several sources including microalgae, aquatic plants, terrestrial plants and bacteria. They found that terrestrial plant derived organic carbon was a useful predictor for sediment TOC. However, with increasing trophic state the aquatic source contribution to sedimentary organic matter pool increased.

We found two studies that examined the effects of decreased nutrients on FA in lentic systems. In laboratory experiments Ahlgren et al. (1998) demonstrated that phosphorus limitation affects both overall FA quality and PUFA in diatoms and green algae. Phytoplankton grown at low P concentrations had reduced PUFA but increased SFA compared with higher concentrations. Hartwich et al. (2012) compared the elemental and biochemical composition of seston in oligotrophic and mesotrophic phases of a lake. They also conducted *Daphnia* growth experiments using the oligotrophic seston supplemented with n-3 PUFA. In the oligotrophic phase, particulate carbon and phosphorus concentrations were lower, FA concentrations were higher, and the taxonomic composition of phytoplankton was less diverse. The limitation by food quantity (in terms of carbon) and quality (i.e. ALA) during the mesotrophic phase changed to a complex multiple nutrient limitation facilitated by food quantity, phosphorus, and n-3 FA in the following oligotrophic phase. Growth of *Daphnia* was limited by either phosphorus or n-3 fatty acids. The authors suggested that seasonal and annual changes in nutrient availabilities can create complex co-limitation scenarios for consumers, which may also affect the efficiency of energy transfer in food webs.

We identified two studies that examined eutrophication in lotic ecosystems. Ziegler and Lyon (2010) used in situ 13C-labeling experiments conducted in six streams representing a gradient in nutrient enrichment to explore how FA, stoichiometry, and the composition of active biofilm phototrophs may regulate C cycling in epilithic biofilms. Carbon cycling was tracked through epilithic biofilm communities by assessing net primary production (NPP) and 13C-labeling of biofilm PUFA. The absolute quantities of PUFA were highest in the two most nutrient rich sites. In addition, the quantity of new C incorporated into PUFA was generally higher in the two more nutrient-rich streams. Dalu et al. (2016) compared EFA in the phytobenthos (benthic algae) growing on different substrate types (bricks, clay tiles, rocks, macrophytes, and sediments) at 2 river sites subject to differing nutrient concentration. They hypothesized that the FA content of phytobenthos would change in response to shifts in local nutrient availability but not substrate type. EFA content, including ADA, ALA, DHA, EPA and LIN, in the phytobenthos differed overall among substrates, sites, and seasons and was generally greater in summer than in autumn and winter. EFA content was significantly greater on artificial than natural substrates and was greater at the nutrient-enriched downstream site than at the upstream site. These studies, along with those discussed previously in relation to altered land-use, suggest that eutrophication in streams and rivers increases the nutritional value of basal food sources. However, future research is required to examine how the changes would relate to the production of higher order consumers.

We found two investigations that examined the effect of cyanobacterial blooms on FA profiles that did not necessarily consider eutrophication per se. Wacker and Martin-Creuzburg (2007) suggested that cyanobacteria are deficient in some essential lipids that can lead to a decline in the growth and fecundity of *Daphnia*. Thus, they investigated *Daphnia magna*'s allocation of lipids such as PUFA and cholesterol during an experimental non-toxic cyanobacterial bloom. They found considerable maternal investment of the particularly important omega-3 PUFA, especially EPA, into the eggs. The concentration of EPA was 2.4-fold higher in eggs than in somatic tissue, a cumulative effect, which was not significantly changed under cyanobacterial food conditions. Under deprived food conditions, *D. magna* not only decreased the number of eggs produced but reduced the previously high concentrations of EPA in both eggs and somatic tissues. In contrast to EPA, the concentrations of ALA and cholesterol, although lower than EPA, were more homeostatic in eggs than in somatic tissues, in which concentrations decreased. When food quality was enhanced, *D. magna* were able to recover completely the FA concentrations in their somatic tissues and eggs. This suggests that some PUFA are primarily needed for reproduction.

Groendahl and Fink (2017) conducted a laboratory experiment in which they fed the herbivorous freshwater gastropod *Lymnaea stagnalis* single non-toxic cyanobacterial and unialgal diets or a mixed diet to test whether diet-mixing may enable these herbivores to control non-toxic cyanobacterial abundances. The treatments in which *L. stagnalis* were fed non-toxic cyanobacteria and a mixed diet provided a significantly higher shell and soft-body growth rates than the average of all single algal. However, the increase in growth provided by the non-toxic cyanobacteria diets could not be related to typical determinants of dietary quality such as toxicity, nutrient stoichiometry or essential FA content. The authors suggest that their results strongly contradict previous research which describes non-toxic cyanobacteria as a low quality food resource for freshwater herbivores in general and that freshwater gastropods may be able to control benthic non-toxic cyanobacteria blooms.

### *Invasive species*

We identified eight papers that used FA profiles to define the roles of invasive species in lentic systems and none in lotic waters. Four of these investigated invasive bivalve gastropods, two studies explored FA content in fish and two studied crustaceans. The first bivalve study assessed the capability of the invasive freshwater bivalve *Corbicula fluminea* to process and modify the quality of pelagic food sources for subsequent use by benthic invertebrates (Basen et al. 2013). They ran a growth experiment in which juvenile *Gammarus roeselii* were raised either directly on pelagic algae or on the same algae biodeposited by the bivalve either as faeces or pseudofaeces. They showed that growth and survival of *G. roeselii* significantly improved when algae was offered as biodeposited material and suggested that this clam-mediated upgrading of food quality is due to both an increased bioavailability of pelagic food particles, which are packed in mucus during clam processing, and an increased dietary provisioning with essential lipids (sterols and PUFA) originating from the clams. The second study investigated the bivalve *C. fluminea* nutritional requirements for essential lipids in a standardized growth experiment (Basen et al. 2011). Juvenile clams were fed one of three cyanobacterial species or one of two green algae. Somatic growth rates were then correlated with elemental (C:N and C:P) and biochemical (sterol and FA content) components of the food sources and clam tissue. Somatic growth rates were significantly higher when juveniles were fed green algae than when fed cyanobacteria. Linear regression analyses revealed significant positive relationships between somatic growth rates and dietary sterol and polyunsaturated FA content. The result suggests that the growth of *C. fluminea* is partially dependent on the availability of these essential lipids in the diet. The third study compared the feeding spectra of the invasive bivalve (*Dreissena polymorpha*) with a coexisting native bivalve (*Unio tumidus*) using a FA marker analysis (Makhutova et al. 2013). Analyses of FA trophic markers in bivalve tissues showed that *U. tumidus* and *D. polymorpha* mainly consumed algae (greens, diatoms, and dinoflagellates), cyanobacteria, and detritus particles enriched with bacteria. According to the multivariate statistical analysis, the two species had different feeding ranges: *D. polymorpha* fed on planktonic sources, while *U. tumidus* mostly consumed food sources of benthic origin, mainly detritus. In addition, *U. tumidus* and *Dreissena* species differed in percentages of long-chain polyunsaturated FAs of n-3 and n-6 families and specific FA which they could synthesize (20:1n-13 and 22:3 Δ7,13,16). They concluded that the different feeding spectra of the two species and the ability of *U. tumidus* to synthesise of specific FA, might be the basis for the successful coexistence of native species and invaders. The fourth study assessed the effects of the invasive bivalve *Dreissena polymorpha* on the physiology of the native bivalve *Lampsilis siliquoidea* using FA (McGoldrick et al. 2009). Concentrations of total lipid and of individual FA in *L. siliquoidea* and therefore physiological condition were not significantly correlated with *D. polymorpha* infestation, but FA do provide a possible explanation for differences in glycogen concentrations at different sites in the Great Lakes. Unionids from sites in US waters had higher levels of PA and EPA in their tissues, suggesting that they consume a diet rich in diatoms. Unionids collected from on the Canadian side of the delta had higher concentrations of α-linolenic acid ALA and LIN which is indicative of a diet rich in green algae and cyanobacteria, respectively. These results suggest that diatoms are more abundant in less productive US waters, which receive most of the water flowing out of Lake Huron, whereas green algae and cyanobacteria make up larger portions of the planktonic community in the more eutrophic Canadian waters.

The first study to use FA in an invasive fish species examined the effect of dissolved organic carbon (DOC) concentrations of lake water (Scharnweber et al. 2016). They conducted a field study to investigate the connection between morphological divergence and PUFA composition of Redfin perch (*Perca fluviatilis*) from six lakes across a gradient of DOC concentration. They expected a decrease in the concentration of fish PUFA due to the restructuring effects of DOC on aquatic food webs. Proportions of specific PUFA, including ADA, ALA, DHA and EPA did not differ in perch caught in clear and brown-water lakes, indicating no severe limitation of essential FA for perch inhabiting brown water lakes. The second fish study examined the potential effects of the alien bigheaded carp (*Hypophthalmichthys nobilis*) on FA profiles of consumers occupying multiple trophic levels in large rivers (Fritts et al. 2018). FA concentrations in seston were positively associated with omega-3 highly unsaturated fatty acids, indicating that these locations had abundant, high-quality basal food resources despite hosting the greatest bigheaded carp densities. FA profiles of freshwater mussels were similar to the fatty acid values in the seston and were not influenced by bigheaded carp abundances. Hydropsychid caddisflies and bluegill fish did not differ significantly in total FA or percent lipid among spatial locations, indicating that omnivorous species may be relatively unaffected by bigheaded carps. Gizzard shad (a zooplankton predator), however, exhibited the lowest FA concentrations in the locations with the highest relative bigheaded carp densities. Zooplankton densities are negatively affected bigheaded carp's establishment, which may explain the disconnect between the gizzard shad FA and the plentiful, high-quality phytoplankton in those rivers.

One paper described the effects of an invasive crustacean using FA examined the effects of the non-endemic Cladoceran *Bythotrephes longimanus* on a resident crustacean species (*Mysis relicta*) (Nordin et al. 2008). Significant differences in FA quantity and composition of *M. relicta* were found between invaded and non-invaded lakes, and among lakes within these groups. Generally, *M. relicta* in non-invaded lakes had higher concentrations of ADA, ALA, LIN, OA and PA while *M. relicta* in invaded lakes had higher concentrations of DHA. However, differences in FA profiles and gut contents of *M. relicta* between invaded and non-invaded lakes are consistent with competition for Cladocera in the presence of the invader rather than pre-existing differences among lakes. They conclude that the diet of *M. relicta* is affected by the invasion of *B. longimanus.* The second paper examined the FA profile of the invasive Ponto-Caspian mysid *Limnomysis benede*ni (Fink 2013). The author fed this species green algae and young *Daphnia* in mesocosms and found the mysid can biosynthesise eicosanoid FA because they were not present in the experimental foods. Fink (2013) suggested that invasion of mysids capable of trophic upgrading of dietary FA might have positive effects on the fitness of planktivorous fish.

### *Summary*

Environmental disturbance can have positive or negative influences on the FA nutritional value of food sources. Most studies examined the effects on primary producers only. More research is required on how either the positive or negative effects follow on to influence primary and secondary consumers. There is a distinct lack of research on two common human induced environmental disturbance, river regulation and colmation. Using FA analyses in these areas would enhance the identification of the mechanisms these disturbances use for better management of rivers.

### ***Material and energy subsidy between ecosystems***

Fatty acids have been used to elucidate the connection and energy subsidies at freshwater and terrestrial and freshwater and marine interfaces. Our literature search identified eight studies using FA for examining the subsidies of material and energy between ecosystems. We do not include the literature that has been already been reviewed examining the importance of terrestrial litter inputs to freshwater food webs (Guo et al. 2016, Brett et al. 2017).

Heintz et al. (2004) suggested that adult salmon returning to their natal streams represent a significant source of energy, nutrients, and biochemicals. They tested this hypothesis by varying the quantity of dead pink salmon (*Oncorhynchus gorbuscha*) added to stream mesocosms and testing the FA composition of resident reared Coho salmon (*O. kisutch*). The lipid content and triacylglycerols of the Coho salmon increased with increasing carcass density whereas phospholipids decreased. Increased amounts of triacylglycerols accounted for most of the lipid increase. In addition to increasing in concentration, the FA composition of the triacylglycerols also changed with carcass density. Triacylglycerols of juvenile Coho salmon from the control streams had significantly higher omega-3: omega-6 ratios as a result of fivefold and sixfold increases in the concentrations of eicosapentanoic and docosahexanoic fatty acids, respectively. These data demonstrate an immediate nutritional advantage resulting from the introduction of salmon carcasses in juvenile Coho salmon rearing habitat and indicate the usefulness of FA and lipid class analysis for investigating the effects of marine-derived nutrients on juvenile salmonids.

Smits et al. (2016) investigated how sockeye salmon (*Oncorhynchus nerka*) spawning density and stream temperature affect the growth, body condition, and FA composition of juvenile Coho salmon, a known egg predator. They compared mean body size of juvenile coho salmon and found that the largest individuals occurred in warm streams in which sockeye salmon spawned, although overall subsidy magnitude (spawner density) had no effect on consumer body size. Individuals that consumed more salmon eggs (estimated from δ15N) were larger and had altered FA composition but did not have higher relative body condition. These results indicate that effects of marine subsidies on freshwater consumers depend both on local habitat conditions and on individual variation in energy allocation.

Samways et al. (2017) examined whether FA delivered to rivers and streams by spawning by returning Atlantic salmon (*Salmo salar*) are incorporated by freshwater biota. They used stream mesocosms and added marine derived nutrients from Atlantic salmon to observe FA profiles of biofilm, macroinvertebrates, and Atlantic salmon juveniles. Spawning Atlantic salmon had a positive effect on total lipid content in all trophic levels relative to controls. FA profiles of the invertebrates and salmon juveniles from the treatment stream were similar to the FA profile of the salmon eggs. They suggest that the observed responses demonstrate a change in the food web structure resulting from the establishment of this new, marine-based, basal resource. The positive effects of the marine subsidy increasing freshwater productivity are complimented by marine-based lipids that represent surplus energy and an increase in the quality of resources, thereby contributing to the diversity and health of freshwater ecosystems.

The remaining papers examined various subsidies from non-litter terrestrial sources. The first, Masclaux et al. (2011) examined importance of allochthonous pollen deposition for zooplankton production using growth experiments on two cladocerans (*Daphnia longispina* and *Simocephalus vetulus*). The animals were fed pollen from three species (*Alnus* sp., *Populus* sp., and *Cedrus* sp.) and FA profiles analyses. They found that although lipid analysis revealed high PUFA contents in pollen, both cladoceran species exhibited suboptimal development when directly fed on pollen. The low food value of pollen was attributed to the presence of a refractory wall reducing its digestibility. In a second set of experiments, cladocerans were fed on a mixture of heterotrophic microorganisms (bacteria, chytrids, and protozoa) that had grown on pollen grains (*Cedrus* sp.). The introduction of microorganisms as an intermediate trophic level resulted in cladoceran growth rates that were about double those obtained on pollen alone. Hence, the authors suggest that the carbon in pollen could sustain zooplankton growth indirectly, and highlight the key role of microorganisms, and especially of chytrids, in transferring and upgrading pollen PUFA to higher trophic levels. The second paper built on the first when Masclaux et al. (2013) examined the transfer of pollen organic carbon to aquatic consumers during a pine pollen rain event on an oxbow lake. They found microorganism concentrations were by far higher in the neuston, where pollen grains accumulated, than in the seston. Zooplankton species were also unevenly distributed in the two compartments. Bulk isotope and isotopes of FA analyses showed trophic partitioning among these zooplankton species, with some of the taxa foraging specifically on neuston, where they benefit from pollen-derived carbon. Microorganisms were identified as a key element in the trophic upgrading of pollen food quality and in the transfer of pollen carbon to metazoan consumers. Pollen grains may thus contribute, as an allochthonous food pulse, to aquatic production at specific seasons, but they may also act as a structuring factor in lake habitats. The third paper, Vargas et al. (2011) evaluated the potential sources and composition of organic matter along a lake-river-fjord corridor using stable isotope (δ13C) and FA biomarker analyses. A saturated FA biomarker generally ascribed to higher plants comprised one of the highest fractions of POC FA along the river plumes, up to 68-86%. The authors used this result to suggest that rivers may subsidise fjord ecosystems with allochthonous contributions. The last paper, Martin-Creuzburg et al. (2017) examined the export and therefore a terrestrial subsidy of essential PUFA via emerging insects from a lake. Insect emergence from varying depths and seasonal mean FA concentrations in different insect groups were used to estimate PUFA fluxes. While Chironomidae contributed most to insect biomass and total PUFA export, *Chaoborus* *flavicans* contributed most to the export of EPA, ARA, and especially DHA. The use of a conceptual model developed to examine insect deposition rates on land showed an average total PUFA deposition rate of 150 mg $m^{-2}$ $yr^{-1}$ within 100 m inland from the shore. They suggest that their data indicate that insect-mediated PUFA fluxes from lakes are substantial, implying that freshwater-derived PUFA can crucially influence food web processes in adjacent terrestrial habitats.

In summary, these studies demonstrate that energy and nutrient subsidies can exist between ecosystems. All this research has taken place in permanent water bodies. The interplay in subsidies between intermittent and permanent water sources is an area for future research and would support river management. For example, the benefits of flooding wetlands with environmental water.

### ***Seasonal or spatial variation in fatty acids***

Understanding the size and extent of variation in both the temporal and spatial distribution of FA is important to identify food web functioning and changes in the nutritional value of basal food sources. Trophic organisation of complex food webs may vary at numerous spatial and temporal scales, both in terms of direct trophic connections and the underlying energy pathways that support production. The literature search found 14 papers describing spatial and/or temporal variation in the FA composition of organic matter or biota in both lentic and lotic waters.

#### *Spatial variation in the FA profiles in lentic ecosystems*

Li et al. (2018) used FA biomarkers to examine spatial heterogeneity of sedimentary organic matter (SOM) to identify its sources. Higher relative percentages of odd n-alkanes (C26 to C35) and LCPUFA (C24:0 to C32:0) explained the influence of inflow rivers carrying terrestrial inputs on SOM. The higher relative percentages of n-alkanes from C14 to

C20, ALA, LIN and PA in one lake section demonstrated that local topology was important for the accumulation of algae-derived OM in sediments. Short-chain and middle-chain biomarker compounds revealed a large contribution from macrophytes in another lake section and an obvious algae-derived organic matter accumulation in an algae-type region, respectively.

### *Temporal or seasonal variation in FA in lentic waters*

Pollero et al. (1981) studied the lipid composition and seasonal changes in the freshwater bivalve *Diplodom patagonicus*. The FA composition was especially rich in the ALA and LIN (ca. 25%), and poor in DHA and EPA. The n6/n3 acid ratio was approximately two, which is very high compared to marine bivalves. The FA composition and the n6/n3 acid ratio were constant during the whole year, suggesting a very stable diet, rich in vegetal detritus and poor in diatoms. The influence of environmental temperature fluctuation with season on FA composition also was negligible. Goedkoop et al. (2000) quantified FA concentrations and their seasonal variations for profundal benthic invertebrates, surficial sediment, and sedimenting matter from Lake Erken, Sweden. Food quality for profundal zoobenthos, as indicated by the concentrations of LCPUFA, n3 FA, or EPA in sediment and sedimenting matter, was highest in spring and autumn and markedly lower in summer.

Taipale et al. (2009) investigated the seasonal variation in the diet of *Daphnia* by analysing the phospholipid FA (PLFA) profiles of seston and of the animals themselves, through the open water period in a small, dystrophic lake. Algal PUFA contributed appreciably to total PLFAs in adult *Daphnia* during spring and summer, but less so in autumn. Biomass of *Daphnia* in the lake reached their highest biomass in autumn, when methanotrophic activity was also highest, and the highest magnitude of MOB-specific PLFAs was recorded in both adult and juvenile *Daphnia*. Autumnal mixing evidently stimulates bacterial oxidation of methane from the hypolimnion, and exploitation of the methanotrophic bacteria sustains a high *Daphnia* population late in the season.

Roy Chowdhury et al. (2014) determined rates of potential methane oxidation (PMO) and shifts in methanotrophs over hydrological and seasonal gradients. Surface and subsurface soil samples (0-8 or 8-16cm depths) were analysed for PMO and profiled for methanotroph community structure using PLFA analysis over four seasons (winter, spring, summer and fall) and three landscape positions (upland, intermittently flooded, and permanently flooded sites). PMO rates were highest in the winter. PLFA profiling of methanotrophs showed that both Type I and Type II methanotrophs were dominant in winter. PMO and methanotroph biomass were highest in the winter and in the permanently flooded sites which suggested substrate methane concentration was more important in regulating methanotrophy than redox potential or seasonal shifts in temperature under flooded conditions.

McMeans et al. (2015) investigated how seston FA and water temperature explained seasonal variation in cladoceran and copepod FA over three years in pre-alpine, oligotrophic Lake Lunz, Austria. Using the mostly algal-derived PUFA (ARA, EPA and DHA), TeFA and as source-specific biomarkers, they showed that cladocerans consistently contained more ARA and EPA and copepods more DHA than seston. None of the physiologically important PUFA were connected between zooplankton and seston across the entire study period but copepod DHA increased with seston DHA during the coldest months. EPA, conversely, increased with decreasing water temperature in both zooplankton groups. For the nonessential FA, TFA were lower in zooplankton than in seston and not related to dietary supply or water temperature. However, cladoceran and copepod BAFA increased significantly with increasing seston BAFA and decreasing water temperature. The results suggested that physiological regulation in response to changing water temperature had a significant impact on cladoceran and copepod EPA and the extent of dietary tracking for copepod DHA. TFA available in the seston may not have been consumed or was not readily incorporated by zooplankton, but BAFA were reliable indicators of available resources throughout multiple seasonal cycles. The authors suggested that both FA type and water temperature impact the extent that dietary vs. nondietary processes govern cladoceran and copepod FA in oligotrophic lakes.

### *Temporal and spatial variation in FA in lentic systems*

Boon et al. (1996) used PLFA indicative of specific bacterial metabolic groups to identify spatial variation between a permanent and an ephemeral wetland. There were no significant differences in the relative proportions of the sum of branched PLFAs in the sediments between the two sites, across seasons, or between the vegetated and bare zones. Concentrations of total saturated and monounsaturated PLFAs in the sediments followed the same trend as those of the total PLFAs. There was no significant difference in overall composition between the early and late flood periods in terms of total saturated or monounsaturated PLFAs. In contrast, saturated PLFAs were proportionally more abundant in the sediments of the permanent wetlands than in sediments from the ephemeral wetland, whereas monounsaturated FA forms were relatively more common. Gross measures such as total PLFA content, the broad distribution of saturated, monounsaturated and branched FA, and varied little between early and late flooding.

Hrycik et al. (2018) examined spatial and temporal variation in stomach contents FA, and stable isotopes of yellow perch (*Perca flavescens*) across seasons and across sites spanning approximately 200 km in Lake Erie's Central Basin (LECB). They found limited spatial variation of biochemical indicators and more pronounced variation across seasons for all three trophic indicators, especially FA profiles. FA profiles were predominantly influenced by seasonal fluctuations in DHA. The results suggest that adult yellow perch in

LECB showed little differentiation in resource use across space, but their diets and biochemical compositions varied seasonally.

Lau et al. (2012) analysed the FAs of 22 taxa of benthic macroinvertebrates, zooplankton and fish collected from the littoral, pelagic and profundal habitats of nine boreal oligotrophic lakes over spring, summer and autumn. They quantified and compared the FA variance partitions contributed by species, site and season using partial redundancy analysis both on all consumers and on benthic arthropods alone. Species identity by itself explained between 84.4 and 72.8% of explained FA variation of all consumers and benthic arthropods, respectively. In contrast the factors of site, season and all joint effects accounted for 0–11.3% only.  Pelagic and profundal consumers showed stronger reliance on autochthonous resources than did their littoral counterparts as reflected by their higher n3 to n6 FA ratios.

### *Spatial variation in the FA profiles in lotic ecosystems*

Guo et al. (2015) investigated nine streams in South-East Queensland, Australia, to identify environmental factors affecting the FA composition of periphyton. Riparian tree canopy extent and N concentrations explained most of the observed variation in periphyton FA profiles. Nitrogen concentrations showed negative relationships with most SFA, while MUFA. PA and PLA were negatively correlated with canopy cover. In contrast, the percentage of HUFA in periphyton was greater with increasing canopy cover regardless of NOx-N concentrations. Variation in riparian canopy cover and nutrients gave rise to opposite outcomes in terms of periphyton food quality and quantity. The highest periphyton food quality, measured by HUFA content, occurred in streams with high canopy cover (and subsequently lower light exposure) and low nutrients, while the highest periphyton food quantity occurred in streams with low canopy cover.

Rude et al. (2016) examined whether differences in FA profiles of the fish Bluegill (*Lepomis macrochirus*) among main channel, and connected and disconnected floodplain lakes. Bluegill FA profiles differed among habitats and river reaches, including differences in levels of individual FAs such as LIN (an indicator of allochthonous inputs), which was higher among main channel fish. The $n-3$:$n-6$ FA ratio, an indicator of aquatic primary productivity, was higher among floodplain lake fish. The differences enabled >87.5% reclassification accuracy of fish to their source environment and the authors suggested that FA profiles can be used to infer recent habitat use and habitat-specific foraging of fishes in large river-floodplain ecosystems.

### *Temporal or seasonal variation in FA in lotic waters*

Sushchik et al. (2007) studied the seasonal composition and concentrations of FA in benthos from pebbly littoral region of the Yenisei River in a sampling site near Krasnoyarsk city (Siberia, Russia). They concentrated on major LCPUFA of the n3 family: EPA and DHA. In the diatom dominated phytobenthos the annual maxima of EPA and DHA pool occurred in spring and early summer. In the zoobenthos, EPA and DHA pool peaked in autumn, due mainly to an increase of the biomass of gammarids and to a moderate increase of the PUFA content per body weight. Seasonal peaks of EPA in overwintering insect larvae (chironomids and caddisflies) generally coincided with those of biomass of these larvae, while there was no such trend for amphipods and oligochaetes. In spring and early summer, the main part of n3 PUFA, 40–97% of total amount, in the littoral region was contained in biomass of producers, i.e., benthic microalgae, and in autumn it was transferred to primary consumers—benthic invertebrates, which contained between 76 to 93% of total n3 PUFA.

Twining et al. (2017) documented foodweb structure and examined FA composition as a metric of food quality in an Adirondack stream throughout the temperate growing season. In spite of major seasonal shifts in environmental factors, such as light availability and temperature, they found limited seasonal variation in the FA composition of basal resources and macroinvertebrates. Instead, we found consistent differences in FA composition between aquatic and terrestrial basal resources and between macroinvertebrate functional feeding groups.

### *Temporal and spatial variation in FA in lotic systems*

Honeyfield and Maloney (2015) examined seasonal patterns in periphyton FA in six minimally disturbed headwater streams in Pennsylvania's Appalachian Mountains, USA. Environmental data and periphyton were collected across four seasons for FA and algal taxa content. The authors observed significant seasonal differences in fatty acids. Summer and fall FA profiles were driven by temperature, overstory cover, and conductivity and winter profiles by measures of stream size. The physiologically important ALA was highest in summer and fall. Winter samples had the highest EPA. Six saturated FA differed among the seasons. In contrast, the authors found very little differences in FA among streams.

Carassou et al. (2017) investigated ontogenetic and seasonal variations in the diet of the freshwater mullet (*Myxus capensis*) across a river–estuary interface using FA dietary tracers. They tested two hypotheses. Firstly, the freshwater mullet diet shifts as individuals grow and migrate from the estuary to the river. Secondly, the dominant food resources utilized by freshwater mullet vary through time, mainly as a function of the seasonal changes in the availability of preferred food items in each habitat. Both hypotheses were sustained, because the results indicated broad dietary flexibility by *M. capensis*, with food items varying from benthic microalgae to insects but this depended on habitat and seasonal patterns in the accessibility of resources.

In summary, significant temporal and spatial variation can exist in FA in both lentic and lotic systems. The benefits of further understanding the quality of food sources through time and space and how this is passed onto primary and secondary consumers will assist with river management into the future.

### *Fatty acids as biomarkers and qualitative and quantitative studies*

Our review identified that 11% of FA papers in freshwater ecosystems were primarily concerned in with using FA as biomarkers to identify basal food sources, e.g. bacterial or green algae etc. (Table 1.). Some lipid species, including FA, are limited to or are synthesised by certain taxa, so if the FA in question is metabolically stable (or retains its basic structure after consumption) it may be used to trace energy transfers through a food web, thus helping to define predatory-prey relationships (Napolitano 1999). Examples of FA specific to particular biotic groups include the bacteria-specific IA (Goedkoop et al. 2000), FA with more than 24 carbons for terrestrial plants (Falk-Petersen et al. 2002) and OA for brown algae (Johns et al. 1979). However, there are some specific FA that have been attributed to different biotic groups by different authors. For example, the FA 16:0 has been used as a biomarker for green algae, cyanobacteria and fungi (Vestal and White 1989, Kelly and Scheibling 2012). Biomarkers for specific species of algae are easily overlapped, such as the 18:3n6 has been simultaneously used as a marker for green algae (Meziane and Tsuchiya 2000, Xu et al. 2014) and cyanobacteria (Hayakawa et al. 2002, de Kluijver et al. 2012). In addition, the one biomarker for a particular group may not be present for environmental reasons. The PLFA 20:5n3 although considered to be a consistent biomarker for diatoms is often not found in large quantities relative to other PLFA (Napolitano 1999) and may be found in lower light or nutrient concentrations (Ahlgren et al. 1992). Given biomarkers for individual species of plankton, aquatic and terrestrial materials are not always specific enough to identify a single source and the fact that the internal biosynthetic capabilities of most organisms have not been elucidated, some authors caution their use in food web studies (Wakeham 1995, Dalsgaard et al. 2003).

The use of FA as biomarkers to identify basal food sources is a qualitative analysis, the FA indicate potential food sources but they do not quantify the amount or percentages of different food sources supporting consumers. Quantitative FA signature analysis (QFASA Iverson et al. (2004)) has become a popular method of estimating diet composition, especially for marine species (Bromaghin et al. 2017). A primary assumption of QFASA is that constants named calibration coefficients, which account for the differential metabolism of individual fatty acids, are known. In practice, however, calibration coefficients are not known, but rather have been estimated in feeding trials with captive animals of a limited number of model species. The impossibility of verifying the accuracy of feeding trial derived calibration coefficients to estimate the diets of wild animals is a foundational problem with QFASA that has generated considerable criticism (Bromaghin 2017). The literature search only identified one paper that had attempted QFASA in a freshwater environment. Taipale et al. (2016) used FA based modelling of field collected cladocerans to generate estimates of dietary resource assimilation by zooplankton of different basal resources. The calibration coefficients used in the modelling consisting of known FA profiles of *Daphnia* fed a diversity of known basal monocultures in controlled laboratory feeding trials. The authors used the Bayesian mixing model FASTAR (Galloway et al. 2015) to estimate the basal food sources of field caught animals in lake ecosystems. A more novel modelling technique has been developed which uses simultaneous estimation of diet composition and calibration coefficients based only on FA signature samples from wild predators and potential prey (Bromaghin 2017, Bromaghin et al. 2017). This modelling technique has been developed for marine species but represents a potentially valuable research tool for both lentic and lotic ecosystems in future.

In summary, the use of FA as biomarkers to identify food sources in food web studies can be problematic. However, the use of QFASA offers a potential to provide better insights into food web studies into the future, particularly in freshwater ecosystems.

### ***Conclusion and recommendations.***

We have demonstrated that FA are now used extensively in freshwater ecosystem research. However, there is still a need to improve the FA interpretation and analyses. This can be achieved by using the capabilities inherent with FA alongside with other biomarkers, such as stable isotopes, using quantitative approaches (e.g. Bayesian approaches) to ultimately improve our understanding of ecosystems.

We identified seven areas which would benefit with the use of FA through this review. These include

- Climate change using multiple consumer levels
- Effects of altered land use on FA composition of secondary consumers
- Invasive species in running waters
- Subsidies between ephemeral or intermittent streams or wetlands and terrestrial ecosystems
- Other anthropogenic impacts, particularly river regulation and colmation.
- Spatial and temporal of primary producers and consumers in lentic and lotic environments.
- Freshwater quantitative FA signature analysis.

Ongoing efforts to provide new knowledge in these areas can only benefit aquatic science and river management.

### ***Acknowledgements***

This review forms part of and was funded by the Environmental Water Knowledge and Research Project which was financed by the Australian Government’s Department of the Environment and Energy and coordinated by the Murray–Darling Freshwater Research Centre.

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Table 1. Definitions of acronyms related to fatty acids used throughout the text.

| Acronym | Phrase | Definition or formulae |
|---|---|---|
| ADA | Arachidonic acid | 20:4n6 |
| ALA | α-linolenic acid | 18:3n3 |
| BAFA | Bacterial fatty acids | 15:0, 17:0 and their branched homologues |
| DHA | Docosahexaenoic acid | 22:6(n-3). |
| DPA | Docosapentaenoic acid | any straight chain 22:5 fatty acid |
| EFA | Essential fatty acid | Fatty acids that animals must ingest because the body requires them for good health but generally cannot synthesize them |
| EPA | Eicosapentaenoic acid | 20:5n3 |
| HUFA | Highly unsaturated fatty acid | Polyunsaturated fatty acids containing 20 or more carbon atoms in the carbon chain |
| IA | Isoseptadecanoic acid | 17:0iso |
| LCPUFA | Long-chain poly unsaturated fatty acid | Fatty acids with 18–20 carbons or more and the constituent hydrocarbon chain possesses two or more carbon–carbon double bonds |
| LIN | Linoleic acid | 18:2n6 |
| MUFA | Monosaturated fatty acid | are fatty acids that have one double bond in the fatty acid chain with all of the remainder carbon atoms being single-bonded. |
| OA | Oleic acid | C18:1n9c |
| PAL | Palmitoleic acid | 16:1n-7 |
| PA | Palmitic acid | 16:00 |
| PLA | Palmitoleic acid | 16:1n7 |
| PLFA | Phospholipid fatty acid | A molecule made of two fatty acid chains joined to a phosphate group by a glycerol molecule |
| PUFA | Polyunsaturated fatty acid | Fats in which the constituent hydrocarbon chain possesses two or more carbon–carbon double bonds |
| SCFA | Short chain fatty acid | are fatty acids with less than six carbon atoms |
| SFA | Saturated fatty acids | A saturated fat is a type of fat in which the fatty acid chains have all or predominantly single bonds |
| TeFA | Terrestrial Fatty acids | 22:0, 24:0 |
| TFA | Total fatty acid | Sum of all fatty acids |

Table 2. Percentage of publications in different habitats, subject groups, number of groups and research topics.

| Habitat | Publications (%) | Subject Group | Publications (%) | Number of subject groups | Publications (%) | Major themes | Publications (%) |
|---|---|---|---|---|---|---|---|
| Laboratory/mesocosm | 37 | Sediment | 5 | 1 | 12 | Food web | 54 |
| Lentic | 41 | Seston | 13 | 2 | 28 | Disturbance | 28 |
| Lotic | 25 | Leafpacks | 3 | 3 | 8 | Subsidy between ecosystems | 5 |
| Estuary | 2 | Periphyton | 10 | 4 | 4 | Seasonal or spatial variation | 9 |
| | | Cyanobacteria | 4 | 5 | 1 | Biomarkers | 11 |
| | | Bacteria | 9 | 6 | 1 | | |
| | | Phytoplankton | 26 | | | | |
| | | Aquatic plant | 1 | | | | |
| | | Zooplankton | 42 | | | | |
| | | Benthic invertebrates | 16 | | | | |
| | | Mollusc | 12 | | | | |
| | | Amphibians | 1 | | | | |
| | | Fish | 23 | | | | |
| | | Birds | 1 | | | | |
| | | Mammal | 1 | | | | |

Figure captions

Figure 1. Number of publications by year using fatty acids in freshwater ecological research.

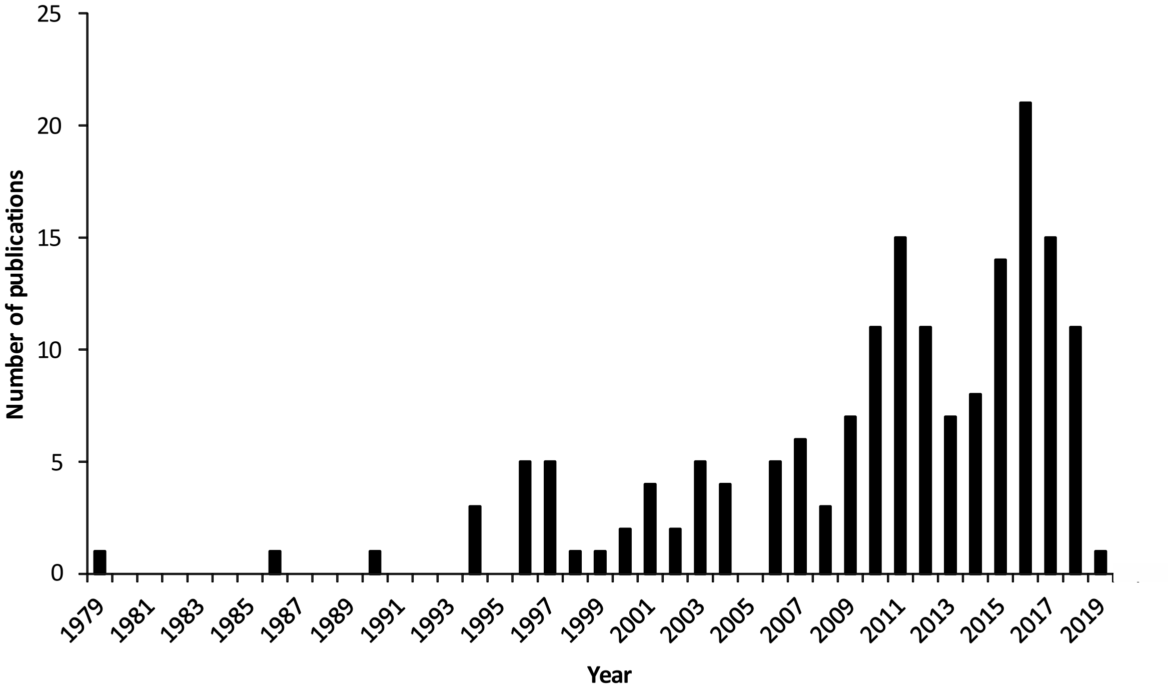
Number of publications
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Figure 1.