

REVIEW

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Assessing diatom-mediated fatty acids in intertidal biofilm: a new conservation concern

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Abstract

Biofilm communities on intertidal mudflats are recognized as major producers of nutrients, especially fatty acids. The rising threats posed by both climatic and anthropogenic stressors increase the necessity of understanding and conserving these communities. Shorebirds provide a proxy for studying the complex ecology of biofilm communities because of their heavy reliance on fatty acids from diatomaceous biofilm for successful long-distance migration. Herein, we review biofilm feeding patterns by migratory shorebirds, experimental design considerations for sampling and studying the fatty acid content of biofilm, and the literature describing established and emerging analytical methodology. Techniques for fatty acid analysis include the commonly employed gas chromatography–flame ionization detection (GC/FID) and gas chromatography–mass spectrometry (GC/MS) with derivatization. Liquid chromatography–mass spectrometry (LC/MS) and liquid chromatography–quadrupole time of flight (LC/QTOF) are newly emerging techniques that enable derivatization to be eliminated. In addition, Fourier transform infrared spectroscopy (FT/IR), a common instrument in chemistry laboratories, has applications in fatty acid research, specifically for screening. Using a combination of sampling and analytical methods is necessary for improved understanding of intertidal biofilm, both as a source of essential fatty acids in aquatic systems and a critical food for shorebirds.

Keywords: Biofilm, Diatoms, Fatty acids, Mudflats, Climate change, Migratory shorebirds, Gas chromatography, Liquid chromatography

Background

Reduced fatty acid production by diatoms in aquatic systems worldwide has emerged as an overarching issue exacerbated by global climate change, with major ramifications to both terrestrial systems and human health (Hixson and Arts 2016; Colombo et al. 2017). Concomitantly, threats to diatomaceous biofilm on intertidal mudflats from climate change and anthropogenic stressors are raising new conservation concerns related

to shorebirds (Beninger 2018). Worldwide declines in the abundance of most migratory shorebird species (Clemens et al. 2016; Studds et al. 2017; Rosenberg et al. 2019; Canham et al. 2021) have occurred in tandem with average losses to their intertidal flat habitats at 16% of their total area between 1984 and 2016 (Murray et al. 2019). Mudflat habitats have long been known to provide wide-ranging ecosystem services that support fish and wildlife (Beninger 2018) but have assumed even more importance in recent years with the discovery that many shorebird species consume intertidal biofilm rich in fatty acids produced by diatoms to support their long-distance migration (Schnurr et al. 2019, 2020). Analysis of fatty acids from intertidal biofilm provides critical information on the value of mudflats as habitats for birds, as well as autecological insights into the microbial communities that compose intertidal biofilm (Schnurr et al. 2020;

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Passarelli et al. 2014). However, techniques for sampling biofilm and assessing the fatty acid content have yet to be fully evaluated or standardized and this will form part of this study's discussion.

The role of biofilm as a food source for shorebirds appears closely linked to both the physical properties of biofilm itself and the chemistry of diatom-mediated fatty acid production. However, as biofilm is highly labile and logistically challenging to collect, experiments based on biofilm sampling from mudflats need to consider a complex array of spatial–temporal factors, as well as issues including fatty acid sample degradation, and dewatering. For analysis of the samples, an array of techniques exists, including gas chromatography–flame ionization detection (GC/FID) with derivatization (Alfaro et al. 2006; Quinn et al. 2017; Schnurr et al. 2019, 2020), gas chromatography–mass spectrometry (GC/MS) with derivatization (Dahl et al. 2000, 2003; Bergamino et al. 2014), liquid chromatography–mass spectrometry (LC/MS) (Schlotterbeck et al. 2018; Pérez-Navarro et al. 2019), liquid chromatography–quadrupole time of flight (LC/QTOF) (Nguyen et al. 2015; Molina-Calle et al. 2017), and Fourier transform infrared spectroscopy (FT/IR) (Karunathilaka et al. 2017; Aryee et al. 2009; Gieroba et al. 2020). Each technique has a different practicality and suitability for analysing complex mixtures of fatty acids. Furthermore, previously established methods in soil science (Buyer and Sasser 2012) and naphthenic acid analysis (Hao et al. 2005; Shang et al. 2013; Brunswick et al. 2015, 2016, 2017; Bowman et al. 2019) may offer new options with crossover to fatty acid analysis.

The purpose of this review is to evaluate experimental design considerations for sampling and analysing the fatty acid content of biofilm. In order to do so, information on the ecology of intertidal biofilm and the emerging literature on biofilm use by migratory shorebirds was collated to identify the challenges to understanding such an extremely complex medium. Based on the context, we provide initial guidance for assessing diatom-mediated fatty acids in intertidal biofilm and underscore the urgency for further research into this new conservation priority.

What is biofilm?

Microphytobenthos assemblages on intertidal mudflats are primarily composed of microalgae, heterotrophic bacteria, and cyanobacteria, and are frequently dominated by photosynthetic diatoms (Passarelli et al. 2014). These diatoms, together with other microorganisms, secrete a matrix of extracellular polymeric substances (EPS) composed of polysaccharides and proteins, which form biofilms or mats at the surface of the mud. The matrix cover binds the microorganism community onto

the sediment, while also adding protection from harsh environmental conditions (Hope et al. 2020). Due to the photosynthetic properties of diatoms and the limited depth of sunlight exposure, biofilm is most abundant in the top 0.5–3 mm of sediment (Macintyre et al. 1996). The biofilm appears either almost colorless or with a green–brown hue, and has a consistency ranging from a low-viscosity slime to a tightly packed gel (Hope et al. 2020; Decho 2000). Microphytobenthic biofilm is a fundamental base to primary production of estuarine ecosystems (Decho 2000). Epibenthic grazers and deposit feeders have a direct dependence on biofilm, which contributes to energy and fatty acid transfer into higher trophic organisms (Herman et al. 2000). Primary production from biofilm is dependent on various environmental conditions, including temperature, salinity, tidal dynamics, light, and nutrients (Underwood and Kromkamp 1999) but, overall, biofilm is estimated to be responsible for approximately 50% of carbon fixation in estuaries (Underwood and Kromkamp 1999). Biofilm also plays a mechanical role in sediment stabilization and protection against scouring by tides and currents (Decho 2000). In the presence of biofilm, the critical erosion threshold of sediments was reported to increase threefold due to the excretion of mucus-like EPS from microorganisms such as diatoms (De Deckere et al. 2001).

Intertidal biofilm is an important food source for migratory shorebirds. Elnor et al. (2005) established that western sandpipers (*Calidris mauri*) and dunlin (*Calidris alpina*) graze biofilm at a major stopover site on the Fraser River estuary, British Columbia, Canada, on their northward journey from Central and South America to Alaska for breeding. Their findings used scanning electron microscopy to show that the tongues of such birds had bristle-like structures capable of scraping biofilm from the sediment surface. The initial discovery fostered new research on the dynamics and ecology of shorebird feeding on biofilm (Beninger and Elnor 2020). Kuwae et al. (2008) investigated western sandpiper grazing using a combination of stable isotope, video recording, and stomach content analysis techniques, and estimated that 45–59% of their diet consisted of biofilm. Subsequently, many other species of shorebirds in tidal flats around the world have been shown to graze biofilm, including the semipalmated sandpiper (*Calidris pusilla*) and red-necked stint (*Calidris ruficollis*) (Beninger and Elnor 2020; Quinn and Hamilton 2012). The underlying rationale for the birds' novel food choice may be partly explained by the fact that diatoms in biofilm produce large quantities of lipids and fatty acids (Scholz and Liebezeit 2013; Stonik and Stonik 2015; Mathot et al. 2018), essential nutrients that are not as readily available in their invertebrate prey. Moreover, the consumption

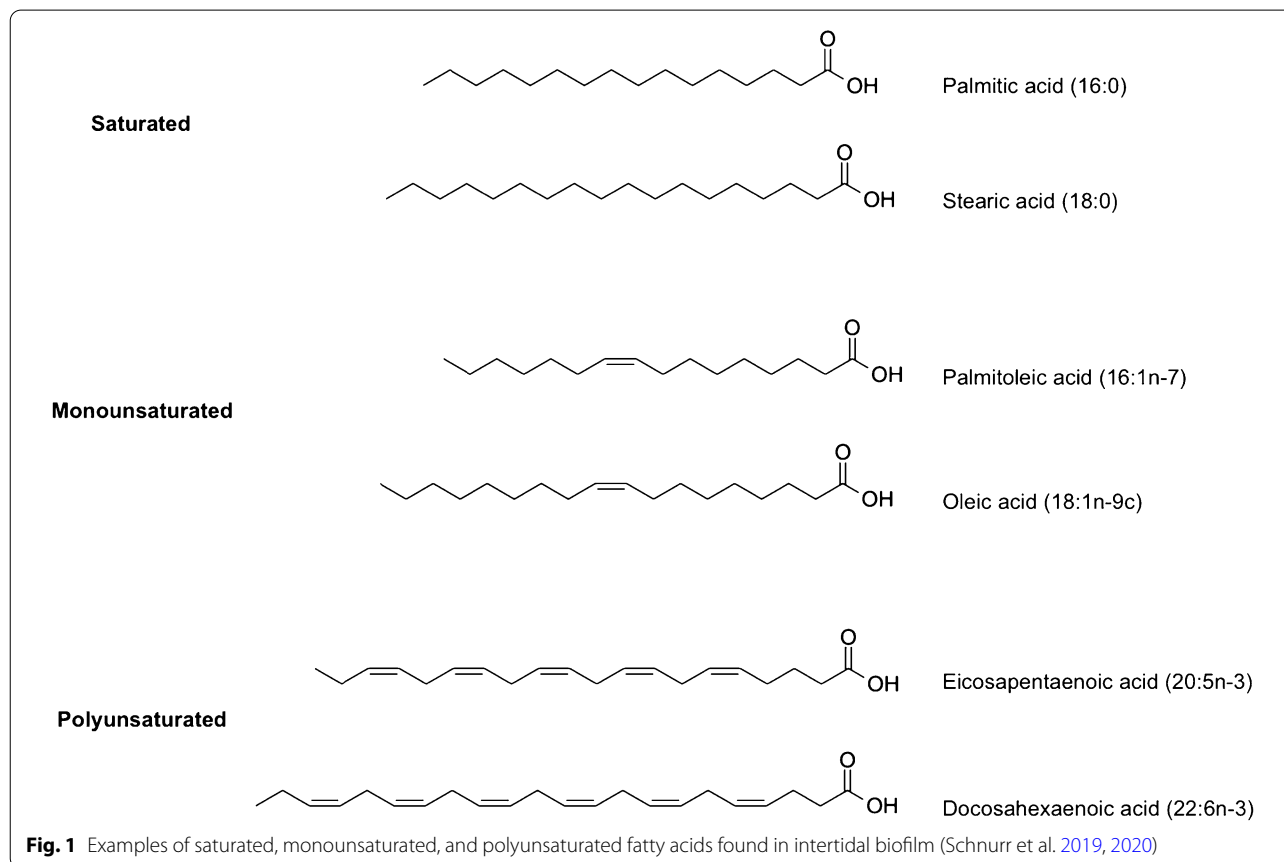
of biofilm has been shown to physiologically enhance muscle performance for long distance flight, as well as provide other benefits for successful migration and reproduction (Jenni and Jenni-Eiermann 1998; Price 2010; Guglielmo 2010).

The abundance of diatoms in biofilm is responsible for its high fatty acid content (Passarelli et al. 2014; Scholz and Liebezeit 2013; Stonik and Stonik 2015; Mathot et al. 2018). Fatty acids are organic molecules with a structural formula of $\text{CH}_3(\text{CH}_2)_n\text{COOH}$, characterized by a carboxylic acid group and an aliphatic chain of varying length. The aliphatic chain may be saturated, termed saturated fatty acids (SFA), or unsaturated with one or more double bonds, designated monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), respectively (Fig. 1). The content profiles of biofilm lipid and fatty acids are not consistent across a mudflat. Changing environmental conditions affect biofilm composition and productivity, consequently altering fatty acid production in diatoms (Schnurr et al. 2019, 2020; Passarelli et al. 2014; Scholz and Liebezeit 2013). Factors such as temperature, light, season, pH, salinity, and tidal cycles all affect diatom growth, and, thus, the concentration of fatty acids in biofilm (Schnurr et al. 2019, 2020; Hu et al.

2008; Li et al. 2014; Hu and Gao 2006; Venkata Mohan and Devi 2014; Sharma et al. 2012). When conditions for growth are favorable, diatoms produce membrane glycerolipids, which primarily consist of PUFAs that play structural roles (Hu et al. 2008). In contrast, under environmental stress when diatom growth is unfavorable, there is a higher production of SFAs and MUFAs which contribute to storage lipids such as triacylglycerol (TAG) (Li et al. 2014).

What can the study of fatty acids reveal?

Temporally changing mudflat conditions result in fluctuating lipid and fatty acid profiles (Schnurr et al. 2019, 2020; Quinn and Hamilton 2012). This affects the organisms that thrive and forage in estuaries, such as migratory shorebirds. As noted previously, different fatty acids accumulate in biofilm depending on environmental conditions (Schnurr et al. 2019, 2020; Hu et al. 2008; Li et al. 2014; Hu and Gao 2006; Venkata Mohan and Devi 2014; Sharma et al. 2012). As the type of fatty acids present in biofilm help determine its nutritional quality, not all intertidal biofilm is equal. The benefits that specific fatty acids have for shorebirds are extensive, with SFAs, MUFAs, and PUFAs all contributing in different ways



(Jenni and Jenni-Eiermann 1998; Price 2010; Guglielmo 2010). Both SFAs and MUFAs comprise a major portion of the energy requirements of migratory birds during long-distance flight. PUFAs, particularly omega-3 (n-3), and omega-6 (n-6) fatty acids, are critical as birds are not able to synthesize these de novo (Stevens 1996) and they have physiological benefits for migration, including increased flight muscle performance and recovery (Price 2010; Guglielmo 2010; Arts et al. 2001; Maillet and Weber 2006). The n-3 fatty acids, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) are all essential nutrients for both birds and other organisms (Maillet and Weber 2007).

Recent studies have linked seasonal changes in fatty acid content of intertidal biofilm to shorebird presence at their migratory stopover site in the Fraser River estuary, such that the period of highest fatty acid abundance aligns with western sandpiper arrival en masse during their northward spring migration (Schnurr et al. 2019, 2020). Similarly, during their southward migration via the upper Bay of Fundy (New Brunswick, Canada), staging semipalmated sandpipers were shown to ingest more biofilm in areas of the mudflats where PUFAs were most abundant (Quinn et al. 2017). While the fundamental importance of fatty acids to the health of aquatic systems is well established (Maillet and Weber 2007), targeted multidisciplinary research is needed to test the various hypotheses on the nutritional properties of biofilm and the effect that its different fatty acid compositions have on avian migratory performance (Schnurr et al. 2019; Price 2010).

The use of fatty acids as biomarkers has broad applications in benthic ecosystems. Although not a perfect trophic marker, they can provide useful qualitative information (Schnurr et al. 2020; Dalsgaard et al. 2003; Kelly and Scheibling 2012). The fatty acid profile of mudflat biofilm can provide insight into its microorganism community composition. However, fluctuations in diatom growth conditions that result in changes in fatty acid content and the fact that multiple estuarine invertebrates may contain the same fatty acids are considered complications to using fatty acids as biomarkers (Dalsgaard et al. 2003; Kelly and Scheibling 2012). Schnurr et al. (2020) studied seasonal changes in the ratios of specific fatty acids compared to the total fatty acid concentrations for particular microorganism groups as chemotaxonomic biomarkers in the Fraser River estuary. They observed community compositional changes in fatty acid biomarkers for bacteria (odd-chain length fatty acids), cyanobacteria (18:2n-6), dinoflagellates (22:6n-3 and 18:1n-9), and diatoms (16:1n-7 and 20:5n-3). Further, from spring to summer, diatom abundance in the intertidal biofilm decreased, while the abundance of cyanobacteria,

bacteria, and dinoflagellates increased. Thus, the availability of diatoms appears correlated with the seasonal abundance of shorebirds and may help explain why their southward summer migration through the Fraser River estuary is protracted and dissipated relative to the concentrated spring migration (Schnurr et al. 2020).

Fatty acid biomarkers have been used extensively for studying a range of consumer and food web relationships (Dalsgaard et al. 2003; Kelly and Scheibling 2012; Dahl et al. 2003; Alfaro et al. 2006; Van Der Heijden et al. 2019). Dahl et al. (2003) discerned food chain linkages by studying the fatty acid profiles in muscle tissues of three bird species: common eider (*Somateria mollissima*), black-legged kittiwake (*Rissa tridactyla*), and northern fulmar (*Fulmarus glacialis*). From the fatty acid composition, they found that, of the three species, the common eider had the lowest trophic level and the strongest relationship with the benthic food chain. The northern fulmar was found to have the highest trophic level, followed by the black-legged kittiwake, with both of these species associated with the pelagic food chain. Alfaro et al. (2006) investigated the use of fatty acid biomarkers and stable isotope analysis to study estuarial food webs in northern New Zealand. Information on the preferred food sources of various organisms including fish, filter feeding invertebrates, and shrimp was found using fatty acid biomarkers. Using this method, they also found that higher consumers have a mixed diet consisting of a wide variety of prey from different trophic linkages (Alfaro et al. 2006). When used in tandem with other methods, such as stable isotope analysis and compound specific isotope analysis, fatty acid biomarkers become even more powerful tools for studying consumer-resource systems and community composition (Dalsgaard et al. 2003; Kelly and Scheibling 2012; Alfaro et al. 2006; Van Der Heijden et al. 2019).

What experimental conditions and designs should be considered?

When designing experiments for studying the relationship between fatty acids, biofilm, and ecosystems, researchers must take spatial and temporal variability into account. On intertidal mudflats, the composition of biofilm varies spatially between different areas and temporally throughout the year (Bergamino et al. 2014). Frankenbach et al. (2020) studied the primary productivity of microphytobenthos in the Ria de Aveiro Estuary, Portugal, and placed an emphasis on characterizing the local variability. To study spatial variability, samples were collected in areas with differing sediment grain size, salinity, hydrodynamics, and distances to the mouth of the estuary. To study temporal variability, samples were taken each hour, fortnight, and season. Including these

spatial and temporal variables in the study allowed them to acquire detailed data on photosynthetic productivity, enabling accurate estuary carbon-fixation estimates. Similarly, Bergamino et al. (2014) examined spatial and temporal changes in organic material and sediments in the Kowie Estuary, South Africa. Sampling took place in each of the four seasons and were collected in three areas of the estuary with different marine influences. Using both fatty acid and stable isotope analyses, the organic compositions were found to vary spatially and temporally. While adopting a spatial and temporal approach to estuarial research is time-consuming and complex, it should be explicitly considered as it provides deeper insight into ecosystem dynamics (Bergamino et al. 2014; Frankenburg et al. 2020).

Biofilm contamination and degradation are unavoidable factors that must also be carefully considered. Quinn et al. (2017) noted that sediment contamination is inescapable in biofilm samples collected in the field and that the presence of sediment can alter the measured fatty acid content. In Schnurr et al. (2019), while invertebrates larger than 0.5 mm were removed from biofilm samples before analyses, meiofaunal invertebrates smaller than 0.5 mm remained. As a result, a small portion of the total fatty acids and biomass measured could be attributed to meiofauna. The degradation of fatty acid samples can also corrupt targeted organic compounds unless samples are frozen promptly after collection (Rudy et al. 2016). Degradation can occur through hydrolysis of samples by lipases present in aqueous conditions and through other chemical reactions such as oxidation. Both processes result in changes to the total fatty acid content, potentially compromising the integrity of a study. To avoid this, if immediate analysis is not available, samples should ideally be stored at $-80\text{ }^{\circ}\text{C}$ following collection (Rudy et al. 2016; Metherel et al. 2013; Sasaki and Capuzzo 1984). The low temperature reduces deterioration of the fatty acids present in the samples.

Other sediment effects, such as compaction and dewatering, can change how algal biomass data is processed and interpreted. A study in the Eden Estuary, Scotland (Perkins et al. 2003) investigated the effects of sediment compaction and dewatering on algal biomass data. The results differed based on whether the algal biomass data

(expressed as chlorophyll *a* and EPS) was presented as a concentration ($\mu\text{g Chl } a$ per mg dry weight sediment) or as content ($\mu\text{g Chl } a$ per cm^2 sediment). A theoretical model was used to predict the effects that these sediment transformations had on Chl *a* and EPS values over the course of a 6-h tidal emersion period. The predicted algal biomass values from the theoretical model were then compared to experimentally obtained Chl *a* and EPS values for diel and seasonal emersion periods. The predicted biomass Chl *a* values expressed in units of concentration and content showed opposing trends in the face of dewatering effects (Table 1). These findings highlight the importance of correcting for dewatering effects in sediment data and understanding the implications of expressing algal biomass as either concentration or content.

What analytical techniques are available for fatty acid analysis?

Gas chromatography

Gas chromatography (GC) based methods are cost effective, robust, and commonly employed to identify and quantify fatty acids (Fisk et al. 2014). While these aspects make GC a widely accepted technique, due to the low volatility of targeted fatty acids, extensive sample preparation and derivatization is often required (Fisk et al. 2014; Drozd 1975). Sample preparation for fatty acids frequently involves a lipid extraction method, such as the Folch Method (Folch et al. 1957). Another well-known lipid extraction method is the Bligh and Dyer method (Bligh and Dyer 1959), which, along with the Folch method, have many published modifications involving different solvents and solvent ratios (Kumar et al. 2015; Ren et al. 2017). The optimal choice of extraction method depends on the lipid chemical behavior and the nature of the sample matrix, as well as the researcher's specific recovery goals and time frame (Kumar et al. 2015; Work and Work 1972; Ulmer et al. 2018). Extraction preferentially also includes an internal standard that displays similar behaviour to the analyte, in order to give an indication of fatty acid recovery. Deuterated fatty acids and odd-numbered long chain fatty acids such as tridecanoic, heptadecanoic, and nonadecanoic acids have previously been used as internal standards (Quehenberger et al. 2011; Reich et al. 2012; Politz et al. 2013).

Table 1 Effects of compaction on biomass data expressed as a concentration or content (Perkins et al. 2003)

| | Concentration ($\mu\text{g Chl } a$ per mg dry weight sediment) | Content ($\mu\text{g Chl } a$ per cm^2 sediment) |
|-----------------------|---|---|
| Effect of compaction | Enrichment from deeper sediment levels increases dry bulk density | Increase in dry bulk density is offset by inclusion of deeper, less productive layers |
| Trend seen in results | Increase in Chl <i>a</i> * concentration | Decrease in Chl <i>a</i> * content |

*Chl *a* refers to chlorophyll *a*, a proxy for algal biomass that can be expressed as either a concentration or a content

Extracted fatty acids are frequently analysed by both GC/FID and GC/MS (Tang and Row 2013). GC/FID does not generally require prior derivatization of the fatty acids before column separation and FID detection. FID detection applies a destructive air–hydrogen flame with the electric current from this combustion being measured by a detector. The drawbacks of FID detection are that all peaks are determined by current response rather than accurate mass, as is the case by a mass spectrometer-based method. For fatty acids analysis, GC/MS in positive electron ionization mode (EI+) is more sensitive than GC/FID, has better specificity, and provides more compound structural information. By GC/MS, compounds are separated on a GC capillary column, with eluting analytes ionized by electron bombardment, resulting in fragmentation ions that are detected by their mass to charge ratios. However, to be amenable to GC/MS EI+ detection, fatty acids require derivatization (Fisk et al. 2014). Derivatization involves the replacement of a fatty acid functional group in order to increase the volatility of the analyte (Fisk et al. 2014; Drozd 1975). A commonly used derivatization method for fatty acids involves methylation with reagents such as boron trifluoride (BF₃) and methanol to create fatty acid methyl esters (FAME), which possess volatilities sufficient for GC analysis (Topolewska et al. 2015). Other less common derivatization methods used for analysis of fatty acids include silylation to generate trimethylsilyl (TMSi) esters, as well as the production of *tert*-butyldimethylsilyl (TBDMSi) derivatives (Woo and Kim 1999; Moon et al. 2018). The choice of derivatization method generally depends upon the demonstrated stability of the product analyte and can be dependent upon the matrix. Each

derivatization method has benefits as well as drawbacks; for example, the synthesis of FAME derivatives using BF₃ and methanol have been found to have a lower reaction yield compared to the production of TBDMSi derivatives by silylation using *N-tert*-butyldimethylsilyl-*N*-methyltri-fluoroacetamide (MTBSTFA) as a reagent (Topolewska et al. 2015). Additionally, TBDMSi derivatives have higher hydrolytic stability compared to TMSi derivatives, which can easily hydrolyse when exposed to water (Woo and Kim 1999). Several previously mentioned papers employed GC, as well as the FAME method of derivatization, including Schnurr et al. (2019, 2020), Quinn et al. (2017), Dahl et al. (2003), Alfaro et al. (2006), and Bergamino et al. (2014). A summary of the advantages and disadvantages of GC/FID and GC/MS, as well as other analytical techniques discussed in following sections, is provided in Table 2. In addition to the previously mentioned techniques, the use of comprehensive two-dimensional gas chromatography (GC × GC) has been reported for the analysis of algal fatty acids (Akoto et al. 2008; Gu et al. 2011). This technique has been found to have lower detection limits and improved separation, as compared to traditional GC analysis (Akoto et al. 2008; De Geus et al. 2001).

GC methods are well established in soil science, in which the use of phospholipid fatty acids (PLFAs) as biomarkers has been gaining popularity since the 1990s (Frostegård et al. 2011). Mudflats and terrestrial soils have many similarities and involve the study of microorganisms living in complex substrates. Likely, PLFAs could be adapted for studying intertidal biofilm. Buyer and Sasser (2012) used a high throughput GC sample preparation method for soil analysis that could potentially be

Table 2 Advantages and disadvantages of various analytical methods for fatty acid analysis

| Method | Advantages | Disadvantages |
|---|--|--|
| Gas chromatography flame–ionization detection (GC/FID) (Tang and Row 2013; Fisk et al. 2014; Schnurr et al. 2019, 2020) | Established for mudflat FA analysis Cost-effective | Extensive sample prep and derivatization Destructive Insensitive Low specificity Does not provide structural information |
| Gas chromatography mass spectrometry (GC/MS) (Tang and Row 2013; Fisk et al. 2014; Bergamino et al. 2014) | Established for mudflat FA analysis Provides structural information | Extensive sample prep and derivatization required |
| Liquid chromatography mass spectrometry (LC/MS) (Brondz 2002; Schlotterbeck et al. 2018) | Limited sample preparation required Provides structural information Sensitive | Less established in mudflat FA analysis Expensive |
| Liquid chromatography quadrupole time-of-flight (LC/QTOF) (Molina-Calle et al. 2017; Schlotterbeck et al. 2018) | Limited sample preparation Provides structural information High resolution High mass accuracy | Less established in mudflat FA analysis Expensive |
| Fourier transform infrared (FT/IR) spectroscopy (Karunathilaka et al. 2017; Aryee et al. 2009; Gieroba et al. 2020) | Rapid Non-destructive | Spectra can be distorted by water Provides limited data compared to other methods |

FA fatty acid

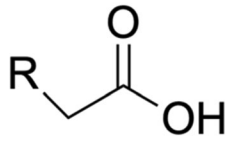

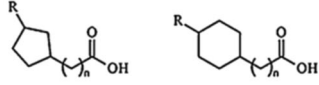
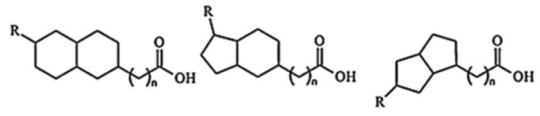
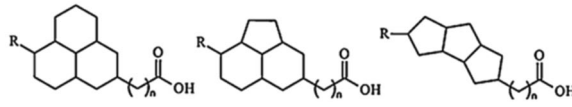
applied to biofilm. Their method described the preparation of ninety-six samples, as well as blanks, in only a day and a half. The previously mentioned Bligh–Dyer lipid extraction (Bligh and Dyer 1959) was used, as well as a solid phase extraction. Such an efficient method could reduce the extensive preparation time when preparing biofilm samples for GC analysis. Due to similarities between fatty acids and naphthenic acids, it is likely that the two will behave in comparable ways when analyzed in the laboratory. Therefore, additional potential procedures based on this association need to be reviewed. Fatty acids and naphthenic acids are very similar chemical species, both containing a carboxylic acid with an aliphatic chain (Table 3) (Shang et al. 2013). The main difference between the two is the presence of saturated ring structures, such as cyclohexyl and cyclopentyl, on naphthenic acids. These descriptions encompass the classic definition of naphthenic acids, which can be described with the formula $C_nH_{2n+z}O_2$ (Shang et al. 2013). The “z” in this formula is either zero, or an even, negative integer, which represents the hydrogen deficiency. It is expected that established methods from naphthenic acid studies could be applied to fatty acids in the future, reducing the time required for planning and designing an experiment. Comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry ($GC \times GC/TOFMS$) has been used for naphthenic acid mixture characterization along with the use of mass deconvolution software and spectral database searches (Hao et al. 2005). Recently, this $GC \times GC/TOFMS$ technique has

been applied to the naphthenic acid monitoring of a wetland reclamation site in the Athabasca oils sands region in northern Alberta, Canada (Bowman et al. 2019). Both of these studies involved the derivatization of the samples into methyl esters. The internal standard used in the second method was methyl 2-hexyl-decanoate dissolved in dichloromethane (Bowman et al. 2019). Techniques for liquid chromatography analysis of naphthenic acids are discussed in the following section.

Liquid chromatography

Liquid chromatography is a separation technique that can be applied to fatty acid analysis (Pérez-Navarro et al. 2019; Molina-Calle et al. 2017; Nguyen et al. 2015; Bronz 2002). While sample clean-up and derivatization prior to LC analysis can improve the selectivity and sensitivity of the instrument, it is not absolutely required (Bronz 2002; Snyder and Kirkland 1979; Kataoka 2017). When performing trace analysis in ppb and ppt, contamination is a major problem, especially when studying fatty acids that are commonly found in the laboratory environment (Mizuike and Pinta 1978). Application of direct LC analysis without derivatization can avoid the time-consuming preparative steps that risk contamination from ubiquitous fatty acids in the lab. Palmitic acid and stearic acid are fatty acids that have been found to be common laboratory contaminants in disposable plasticware used to avoid cross contamination between samples (Cheng and Yu 2020). Using glass, rather than plastic, helped to reduce the contamination and decrease the limit of

Table 3 Comparison between fatty acids and naphthenic acids (Shang et al. 2013)

| Fatty acids | Naphthenic acids |
|--|--|
| Structural formula $CH_3(CH_2)_nCOOH$ | $C_nH_{2n+z}O_2$ |
| Representative structures | |
|  <p>R=Hydrocarbon Chain</p> | <p>Z = 0</p>  <p>Z = -2</p>  <p>Z = -4</p>  <p>Z = -6</p>  |

detection when studying these fatty acids. Ubiquitous fatty acid contaminants have also been found in commercially available solid phase extraction columns, which can be particularly troubling when using these products during sample preparation (Cavonius and Carlsson 2015). It is important to be aware of these pervasive fatty acids and plan experiments to minimize the effect of contamination. As with other chromatographic techniques, compound separation occurs by selection of a column phase that shows affinity for the analytes of interest, in combination with complementary mobile phases. There are numerous publications listing LC parameter choices including the LC column, mobile phases, and eluent gradients that have achieved separation of fatty acids. Chu et al. (2009), Samburova et al. (2013), and Schlotterbeck et al. (2018) are examples of literature that describe the use of ultra and ultra-high performance liquid chromatography to study fatty acids, with Schlotterbeck et al. (2018) describing methods for both targeted and untargeted analysis. The LC parameters used in these papers are summarized in Table 4 for the benefit of future researchers.

LC/MS and LC/QTOF are widely used LC instruments that are highly selective and sensitive, with their ability to provide analyte structural information showing promise for applications to fatty acid analysis. Advantages and disadvantages for LC/MS and LC/QTOF are provided in Table 2 to contrast the previously mentioned GC methods. Pérez-Navarro et al. (2019) used liquid chromatography tandem mass spectrometry (LC/MS/MS) to determine the free fatty acid profile in *Vitis vinifera* grape cultivars. Molina-Calle et al. (2017) tentatively identified fatty acids using LC/QTOF to research Stevia leaves. Nguyen et al. (2015) used a combination of GC/QTOF

and LC/QTOF to analyze both fatty acids and triacylglycerols from fruits in the *Apiaceae* family. Additionally, several established LC techniques for naphthenic acid analysis have potential applications in fatty acid method development. Shang et al. (2013) described a specific and sensitive LC/MS method for quantification of naphthenic acids that included the use of large volume injection. Sample preparation was simple, with samples only being centrifuged after the addition of the internal standard. Brunswick et al. (2015, 2016, 2017) reported a high resolution, high throughput, LC/QTOF method that also involved limited sample preparation by pH adjustment to an alkaline pH > 10. Applicability of the naphthenic acid methodology to fatty acids is supported by their use of deuterated fatty acids as internal standards and reference standards.

Fourier transform infrared (FT/IR) spectroscopy

Fourier transform infrared (FT/IR) spectroscopy is a technique used to analyse the characteristic molecular absorption of infrared light to determine structure, typically in the region of 4000–400 cm⁻¹ (Griffiths 1983; Faix 1992). It is a rapid and non-destructive analytical technique with a broad range of applications and is readily available in many laboratories for routine work (Gieroba et al. 2020). FT/IR was used by Gieroba et al. (2020) to analyze bacterial biofilm on human teeth. They were able to identify lipids and proteins in biological samples but noted caution because the presence of water can distort results. The use of FT/IR to study free fatty acids in fish oils was described by Aryee et al. (2009). FT/IR was demonstrated to be an adequate method for determination of free fatty acids due to its simplicity and reproducibility. FT/IR could have potential applications for studying

Table 4 Examples of published liquid chromatography parameters for fatty acid analysis

| | LC column | Mobile phases | Eluent gradients |
|---|---|--|---|
| Chu et al. (2009) | ACQUITY UPLC BEH-C18, 130 Å, 1.7 µm, 2.1 mm × 50 mm, waters | Solvent A: water Solvent B: acetonitrile | 70% A, 30% B decreasing to 25% A, 75% B over 10 min Increasing to 100% B over 10 min |
| Samburova et al. (2013) | ACQUITY UPLC BEH-C18, 130 Å, 1.7 µm, 2.1 mm × 50 mm, waters | Solvent A: acetonitrile Solvent B: 10 mM ammonium formate in methanol | 20% A, 80% B for 5 min Increasing to 100% B over 10 min Held at 100% B for 45 min |
| Schlotterbeck et al. (2018) (untargeted analysis) | Kinetex, 100 Å, 2.6 µm, 150 × 2.1 mm; C8, TMS end capped | Solvent A: 10 mM aqueous ammonium acetate buffer Solvent B: 55% ACN, 40% IPA and 5% 10 mM aqueous ammonium acetate buffer | 60% A, 40% B, increasing to 100% B in 15 min Held at 100% B for 5 min |
| Schlotterbeck et al. (2018) (targeted analysis of FA(16:4) _{n-3}) | Kinetex, 100 Å, 2.6 µm, 150 × 2.1 mm; C8, TMS end capped | Solvent A: 10 mM aqueous ammonium acetate buffer Solvent B: 55% ACN, 40% IPA and 5% 10 mM aqueous ammonium acetate buffer | 60% A, 40% B, increasing to 100% B in 5 min Held at 100% for 2 min |

FA(16:4)_{n-3}: hexadeca-4Z,7Z,10Z,13Z-tetraenoic acid

biofilm but is most likely reserved as a rapid and less sensitive screening tool to initially assess the composition of the sample.

Summary

The present review synthesizes literature on the roles and properties of intertidal biofilm, with special reference to migratory shorebirds, and underscores the value of diatoms as a major source of fatty acids. In support of future studies, experimental design factors such as sample storage, spatial–temporal sampling, and accounting for dewatering effects on sediment are emphasized. The established methodologies of biofilm fatty acid analysis including GC/FID and GC/MS remain effective, but more forthcoming techniques, including LC/MS and LC/QTOF should be considered, alongside the possible incorporation of FT/IR as a screening tool. In addition, potential is noted for applying soil science and naphthenic acid techniques to fatty acid research.

Worldwide, a growing appreciation for the benefits of intertidal mudflat habitats has given alarm to the potential negative effects of climate change and coastal development to both mudflat quality and quantity. In particular, as the quality of intertidal biofilm can be gauged by the production of diatom-mediated fatty acids (Schnurr et al. 2020; Canham et al. 2021), the predicted reduced production of polyunsaturated fatty acids in algae due to global warming (Hixson and Arts 2016; Colombo et al. 2020) compounded by losses in mudflat quantity (Murray et al. 2019) are likely to result in irreversible and fundamentally adverse changes to these coastal systems. Establishing robust and routine analytical techniques to temporally monitor the quality and condition of mudflats, in tandem with research programs to achieve a deeper understanding of mudflat ecology, are core to effective conservation. In particular, determining the quality of biofilm as a food source for migratory shorebirds is of prime importance. In this regard, the determination of specific SFAs, MUFAs, and PUFAs ratios are a key goal. Perhaps the overarching challenge is to discover the specific triggers to the lipid accumulation in diatoms and related responses in intertidal biofilm, given the fundamental role of mudflats in supporting not only migratory shorebirds but also the whole ecosystem of surrounding wildlife and fisheries (Mathot et al. 2018).

Abbreviations

GC/FID: Gas chromatography–flame ionization detection; GC/MS: Gas chromatography–mass spectrometry; LC/MS: Liquid chromatography–mass spectrometry; LC/QTOF: Liquid chromatography–quadrupole time-of-flight; FT/IR: Fourier transform infrared spectroscopy; EPS: Extracellular polymeric substances; SFA: Saturated fatty acid; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; TAG: Triacylglycerol; n-3: Omega-3; n-6: Omega-6; EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid; Chl α : Chlorophyll α ;

El+: Positive electron ionization mode; BF₃: Boron trifluoride; FAME: Fatty acid methyl esters; TMSi: Trimethylsilyl; TBDMSi: *tert*-Butyldimethylsilyl; MTBSTFA: *N-tert*-Butyldimethylsilyl-*N*-methyltrifluoroacetamide; GC \times GC: Two-dimensional gas chromatography; PLFAs: Phospholipid fatty acids; GC \times GC/TOFMS: Two-dimensional gas chromatography time-of-flight mass spectrometry; LC/MS/MS: Liquid chromatography tandem mass spectrometry.

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Authors' contributions

JEO was the primary writer of the manuscript and played a major role in literature curation and paper visualization. CCC contributed to writing, literature curation, and paper visualization. PB contributed substantively to revising the manuscript. RWE contributed substantively to revising the manuscript and conceptualization of the work. OB contributed to the project design and investigation. MK provided analytical methodology expertise in revising the manuscript. GV played a role in project administration and supervision. MCD played a central role in the conceptualization of the project aims, revising the manuscript, and project administration. DS played a critical role in the development of the research, project administration, and manuscript revision. All authors read and approved the final manuscript.

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References

- Akoto L, Stellaard F, Irth H, Vreuls RJJ, Pel R (2008) Improved fatty acid detection in micro-algae and aquatic meiofauna species using a direct thermal desorption interface combined with comprehensive gas chromatography–time-of-flight mass spectrometry. *J Chromatogr A* 1186:254–261. <https://doi.org/10.1016/j.chroma.2008.02.005>
- Alfaro AC, Thomas F, Sergent L, Duxbury M (2006) Identification of trophic interactions within an estuarine food web (northern New Zealand) using fatty acid biomarkers and stable isotopes. *Estuar Coast Shelf Sci* 70:271–286. <https://doi.org/10.1016/j.ecss.2006.06.017>

- Arts MT, Ackman RG, Holub BJ (2001) "Essential fatty acids" in aquatic ecosystems: a crucial link between diet and human health and evolution. *Can J Fish Aquat Sci* 58:122–137. <https://doi.org/10.1139/cjfas-58-1-122>
- Aryee ANA, van de Voort FR, Simpson BK (2009) FTIR determination of free fatty acids in fish oils intended for biodiesel production. *Process Biochem* 44:401–405. <https://doi.org/10.1016/j.procbio.2008.12.004>
- Beninger PG (2018) *Mudflat ecology*. Springer International Publishing, Cham
- Beninger PG, Elner RW (2020) On the tip of the tongue: natural history observations that transformed shorebird ecology. *Ecosphere*. <https://doi.org/10.1002/ecs2.3133>
- Bergamino L, Dalu T, Richoux NB (2014) Evidence of spatial and temporal changes in sources of organic matter in estuarine sediments: stable isotope and fatty acid analyses. *Hydrobiologia* 732:133–145. <https://doi.org/10.1007/s10750-014-1853-1>
- Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 37:911–917. <https://doi.org/10.1139/o59-099>
- Bowman DT, Warren LA, McCarry BE, Slater GF (2019) Profiling of individual naphthenic acids at a composite tailings reclamation fen by comprehensive two-dimensional gas chromatography–mass spectrometry. *Sci Total Environ* 649:1522–1531. <https://doi.org/10.1016/j.scitotenv.2018.08.317>
- Bronz I (2002) Development of fatty acid analysis by high-performance liquid chromatography, gas chromatography, and related techniques. *Anal Chim Acta* 465:1–37
- Brunswick P, Shang D, van Aggelen G, Hindle R, Hewitt LM, Frank RA, Haberl M, Kim M (2015) Trace analysis of total naphthenic acids in aqueous environmental matrices by liquid chromatography/mass spectrometry–quadrupole time of flight mass spectrometry direct injection. *J Chromatogr A* 1405:49–71. <https://doi.org/10.1016/j.chroma.2015.05.048>
- Brunswick P, Hewitt LM, Frank RA, Van Aggelen G, Kim M, Shang D (2016) Specificity of high resolution analysis of naphthenic acids in aqueous environmental matrices. *Anal Methods* 8:6764–6773. <https://doi.org/10.1039/c6ay01912a>
- Brunswick P, Hewitt LM, Frank RA, Kim M, van Aggelen G, Shang D (2017) A traceable reference for direct comparative assessment of total naphthenic acid concentrations in commercial and acid extractable organic mixtures derived from oil sands process water. *J Environ Sci Health A* 52:274–280. <https://doi.org/10.1080/10934529.2016.1253399>
- Buyer JS, Sasser M (2012) High throughput phospholipid fatty acid analysis of soils. *Appl Soil Ecol* 61:127–130. <https://doi.org/10.1016/j.apsoil.2012.06.005>
- Canham R, Flemming SA, Hope DD, Drever MC (2021) Sandpipers go with the flow: correlations between estuarine conditions and shorebird abundance at an important stopover on the Pacific Flyway. *Ecol Evol* 11:2828–2841. <https://doi.org/10.1002/ece3.7240>
- Cavonius LR, Carlsson N-G (2015) Fatty acid contaminations originating from commercially available solid phase-extraction columns. *Chem Sci Rev Lett* 16:1206–1209
- Cheng YY, Yu JZ (2020) Minimizing contamination from plastic labware in the quantification of C16 and C18 fatty acids in filter samples of atmospheric particulate matter and their utility in apportioning cooking source contribution to urban PM2.5. *Atmosphere* 11:1120. <https://doi.org/10.3390/atmos11101120>
- Chu X, Zhao T, Zhang Y, Zhao A, Zhou M, Zheng X, Dan M, Jia W (2009) Determination of 13 free fatty acids in pheretima using ultra-performance LC–ESI–MS. *Chromatographia* 69:645–652. <https://doi.org/10.1365/s10337-009-0962-x>
- Clemens RS, Rogers DI, Hansen BD, Gosbell K, Minton CDT, Straw P, Bamford M, Woehler EJ, Milton DA, Weston MA, Venables B, Weller D, Hassell C, Rutherford B, Onton K, Herrod A, Studts CE, Choi CY, Dhanjal-Adams KL, Murray NJ, Skilleter GA, Fuller RA (2016) Continental-scale decreases in shorebird populations in Australia. *Emu* 116:119–135. <https://doi.org/10.1071/MU15056>
- Colombo SM, Wacker A, Parrish CC, Kainz MJ, Arts MT (2017) A fundamental dichotomy in long-chain polyunsaturated fatty acid abundance between and within marine and terrestrial ecosystems. *Environ Rev* 25:163–174
- Colombo SM, Rodgers TFM, Diamond ML, Bazinet RP, Arts MT (2020) Projected declines in global DHA availability for human consumption as a result of global warming. *Ambio* 49:865–880. <https://doi.org/10.1007/s13280-019-01234-6>
- Dahl TM, Lydersen C, Kovacs KM, Falk-Petersen S, Sargent J, Gjert I, Guliksen B (2000) Fatty acid composition of the blubber in white whales (*Delphinapterus leucas*). *Polar Biol* 23:401–409. <https://doi.org/10.1007/s003000050461>
- Dahl T, Falk-Petersen S, Gabrielsen G, Sargent J, Hop H, Millar R (2003) Lipids and stable isotopes in common eider, black-legged kittiwake and northern fulmar: a trophic study from an Arctic fjord. *Mar Ecol Prog Ser* 256:257–269. <https://doi.org/10.3354/meps256257>
- Dalsgaard J, St. John M, Kattner G, Müller-Navarra D, Hagen W (2003) Fatty acid trophic markers in the pelagic marine environment. *Adv Mar Biol* 46:225–340
- De Deckere EMGT, Tolhurst TJ, De Brouwer JFC (2001) Destabilization of cohesive intertidal sediments by infauna. *Estuar Coast Shelf Sci* 53:665–669. <https://doi.org/10.1006/ecss.2001.0811>
- De Geus HJ, Aidos I, De Boer J, Luten JB, Brinkman UAT (2001) Characterisation of fatty acids in biological oil samples using comprehensive multidimensional gas chromatography. *J Chromatogr A* 910:95–103. [https://doi.org/10.1016/S0021-9673\(00\)01183-3](https://doi.org/10.1016/S0021-9673(00)01183-3)
- Decho AW (2000) Microbial biofilms in intertidal systems: an overview. *Cont Shelf Res* 20:1257–1273. [https://doi.org/10.1016/S0278-4343\(00\)00022-4](https://doi.org/10.1016/S0278-4343(00)00022-4)
- Drozdz J (1975) Chemical derivatization in gas chromatography. *J Chromatogr A* 113:303–356. [https://doi.org/10.1016/S0021-9673\(00\)95303-2](https://doi.org/10.1016/S0021-9673(00)95303-2)
- Elner RW, Beninger PG, Jackson DL, Potter TM (2005) Evidence of a new feeding mode in western sandpiper (*Calidris mauri*) and dunlin (*Calidris alpina*) based on bill and tongue morphology and ultrastructure. *Mar Biol* 146:1223–1234. <https://doi.org/10.1007/s00227-004-1521-5>
- Faix O (1992) *Fourier transform infrared spectroscopy*. Springer, Berlin, pp 83–109
- Fisk HL, West AL, Childs CE, Burdge GC, Calder PC (2014) The use of gas chromatography to analyze compositional changes of fatty acids in rat liver tissue during pregnancy. *J vis Exp* 85:51445. <https://doi.org/10.3791/51445>
- Folch J, Lees M, Sloane GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 226:497–509
- Frankenbach S, Ezequiel J, Plecha S, Goessling JW, Vaz L, Kühl M, Dias JM, Vaz N, Seródio J (2020) Synoptic spatio-temporal variability of the photosynthetic productivity of microphytobenthos and phytoplankton in a tidal Estuary. *Front Mar Sci* 7:170. <https://doi.org/10.3389/fmars.2020.00170>
- Frostegård Å, Tunlid A, Bååth E (2011) Use and misuse of PLFA measurements in soils. *Soil Biol Biochem* 43:1621–1625. <https://doi.org/10.1016/j.soilbio.2010.11.021>
- Gieroba B, Krysa M, Wojtowicz K, Wiater A, Pleszczyńska M, Tomczyk M, Sroka-Bartnicka A (2020) The FT-IR and raman spectroscopies as tools for biofilm characterization created by cariogenic streptococci. *Int J Mol Sci*. <https://doi.org/10.3390/ijms21113811>
- Griffiths PR (1983) Fourier transform infrared spectrometry. *Science* 222:297–302. <https://doi.org/10.1126/science.6623077>
- Gu Q, David F, Lynen F, Vanormelingen P, Vyverman W, Rumpel K, Xu G, Sandra P (2011) Evaluation of ionic liquid stationary phases for one dimensional gas chromatography–mass spectrometry and comprehensive two dimensional gas chromatographic analyses of fatty acids in marine biota. *J Chromatogr A* 1218:3056–3063. <https://doi.org/10.1016/j.chroma.2011.03.011>
- Guglielmo CG (2010) Move that fatty acid: fuel selection and transport in migratory birds and bats. *Integrative and comparative biology*. Oxford Academic, Oxford, pp 336–345
- Hao C, Headley JV, Peru KM, Frank R, Yang P, Solomon KR (2005) Characterization and pattern recognition of oil-sand naphthenic acids using comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry. *J Chromatogr A* 1067:277–284. <https://doi.org/10.1016/j.chroma.2005.01.041>
- Herman P, Middelburg J, Widdows J, Lucas C, Heip C (2000) Stable isotopes as trophic tracers: combining field sampling and manipulative labelling of food resources for macrobenthos. *Mar Ecol Prog Ser* 204:79–92. <https://doi.org/10.3354/meps204079>
- Hixson SM, Arts MT (2016) Climate warming is predicted to reduce omega-3, long-chain, polyunsaturated fatty acid production in phytoplankton. *Glob Change Biol* 22:2744–2755. <https://doi.org/10.1111/gcb.13295>
- Hope JA, Paterson DM, Thrush SF (2020) The role of microphytobenthos in soft-sediment ecological networks and their contribution to the delivery of multiple ecosystem services. *J Ecol* 108:815–830. <https://doi.org/10.1111/1365-2745.13322>

- Hu H, Gao K (2006) Response of growth and fatty acid compositions of *Nannochloropsis* sp. to environmental factors under elevated CO₂ concentration. *Biotechnol Lett* 28:987–992. <https://doi.org/10.1007/s10529-006-9026-6>
- Hu Q, Sommerfeld M, Jarvis E, Ghirardi M, Posewitz M, Seibert M, Darzins A (2008) Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. *Plant J* 54:621–639
- Jenni L, Jenni-Eiermann S (1998) Fuel supply and metabolic constraints in migrating birds. *J Avian Biol* 29:521–528. <https://doi.org/10.2307/3677171>
- Karunathilaka SR, Mossoba MM, Chung JK, Haile EA, Strigley CT (2017) Rapid prediction of fatty acid content in marine oil omega-3 dietary supplements using a portable Fourier transform infrared (FTIR) device & partial least-squares regression (PLSR) analysis. *J Agric Food Chem* 65:224–233. <https://doi.org/10.1021/acs.jafc.6b04463>
- Kataoka H (2017) Sample preparation for liquid chromatography. *Liquid chromatography*. Elsevier, Amsterdam, pp 1–37
- Kelly J, Scheibling R (2012) Fatty acids as dietary tracers in benthic food webs. *Mar Ecol Prog Ser* 446:1–22. <https://doi.org/10.3354/meps09559>
- Kumar RR, Rao PH, Arumugam M (2015) Lipid extraction methods from microalgae: a comprehensive review. *Front Energy Res*. <https://doi.org/10.3389/feng.2014.00061>
- Kuwae T, Beninger PG, Decottignies P, Mathot KJ, Lund DR, Elnor RW (2008) Biofilm grazing in a higher vertebrate: the western sandpiper, *Calidris mauri*. *Ecology* 89:599–606. <https://doi.org/10.1890/07-1442.1>
- Li HY, Lu Y, Zheng JW, Yang WD, Liu JS (2014) Biochemical and genetic engineering of diatoms for polyunsaturated fatty acid biosynthesis. *Mar Drugs* 12:153–166. <https://doi.org/10.3390/md12010153>
- Macintyre HL, Geider RJ, Miller DC (1996) Microphytobenthos: the ecological role of the “secret garden” of unvegetated, shallow-water marine habitats. I. Distribution, abundance and primary production. *Estuaries Coast* 19:186–201. <https://doi.org/10.2307/1352224>
- Maillet D, Weber JM (2006) Performance-enhancing role of dietary fatty acids in a long-distance migrant shorebird: the semipalmated sandpiper. *J Exp Biol* 209:2686–2695. <https://doi.org/10.1242/jeb.02299>
- Maillet D, Weber JM (2007) Relationship between n-3 PUFA content and energy metabolism in the flight muscles of a migrating shorebird: evidence for natural doping. *J Exp Biol* 210:413–420. <https://doi.org/10.1242/jeb.02660>
- Mathot KJ, Piersma T, Elnor RW (2018) Shorebirds as integrators and indicators of mudflat ecology. *Mudflat ecology*. Springer International Publishing, Cham, pp 309–338
- Metherell AH, Aristizabal Henao JJ, Stark KD (2013) EPA and DHA levels in whole blood decrease more rapidly when stored at –20 °C as compared with room temperature, 4 and –75 °C. *Lipids* 48:1079–1091. <https://doi.org/10.1007/s11745-013-3827-x>
- Mizuike A, Pinta M (1978) Contamination in trace analysis. *Pure Appl Chem* 40:1519–1529
- Molina-Calle M, Priego-Capote F, Luque de Castro MD (2017) Characterization of Stevia leaves by LC–QTOF MS/MS analysis of polar and non-polar extracts. *Food Chem* 219:329–338. <https://doi.org/10.1016/j.foodchem.2016.09.148>
- Moon JY, Kong TY, Jang HJ, Kang HC, Cho YY, Lee JY, Lee HS (2018) Simultaneous quantification of 18 saturated and unsaturated fatty acids and 7 sterols as their *tert*-butyldimethylsilyl derivatives in human saliva using gas chromatography–tandem mass spectrometry. *J Chromatogr B Anal Technol Biomed Life Sci* 1092:114–121. <https://doi.org/10.1016/j.jchromb.2018.06.003>
- Murray NJ, Phinn SR, DeWitt M, Ferrari R, Johnston R, Lyons MB, Clinton N, Thau D, Fuller RA (2019) The global distribution and trajectory of tidal flats. *Nature* 565:222–225. <https://doi.org/10.1038/s41586-018-0805-8>
- Nguyen T, Aparicio M, Saleh MA, McPhee DJ (2015) Accurate mass GC/ LC–quadrupole time of flight mass spectrometry analysis of fatty acids and triacylglycerols of spicy fruits from the apiaceae family. *Molecules* 20:21421–21432. <https://doi.org/10.3390/molecules201219779>
- Passarelli C, Meziane T, Thiney N, Boeuf D, Jesus B, Ruivo M, Jeanthou C, Hubas E (2014) Seasonal variations of the composition of microbial biofilms in sandy tidal flats: focus of fatty acids, pigments and exopolymers. *Estuar Coast Shelf Sci* 153:29–37. <https://doi.org/10.1016/j.ecss.2014.11.013>
- Pérez-Navarro J, Da Ros A, Masuero D, Izquierdo-Cañas PM, Hermosín-Gutiérrez I, Gómez-Alonso S, Mattivi F, Vrhovsek U (2019) LC–MS/MS analysis of free fatty acid composition and other lipids in skins and seeds of *Vitis vinifera* grape cultivars. *Food Res Int* 125:108556. <https://doi.org/10.1016/j.foodres.2019.108556>
- Perkins RG, Honeywill C, Consalvey M, Austin HA, Tolhurst TJ, Paterson DM (2003) Changes in microphytobenthic chlorophyll a and EPS resulting from sediment compaction due to de-watering: opposing patterns in concentration and content. *Cont Shelf Res* 23:575–586. [https://doi.org/10.1016/S0278-4343\(03\)00006-2](https://doi.org/10.1016/S0278-4343(03)00006-2)
- Politz M, Lennen R, Pflieger B (2013) Quantification of bacterial fatty acids by extraction and methylation. *Bio-Protoc*. <https://doi.org/10.21769/bioprotoc.950>
- Price ER (2010) Dietary lipid composition and avian migratory flight performance: development of a theoretical framework for avian fat storage. *Comp Biochem Physiol A Mol Integr Physiol* 157:297–309. <https://doi.org/10.1016/j.cbpa.2010.05.019>
- Quehenberger O, Armando AM, Dennis EA (2011) High sensitivity quantitative lipidomics analysis of fatty acids in biological samples by gas chromatography–mass spectrometry. *Biochim Biophys Acta Mol Cell Biol Lipids* 1811:648–656
- Quinn JT, Hamilton DJ (2012) Variation in diet of semipalmated sandpipers (*Calidris pusilla*) during stopover in the upper Bay of Fundy, Canada. *Can J Zool* 90:1181–1190. <https://doi.org/10.1139/Z2012-086>
- Quinn JT, Hamilton DJ, Hebert CE (2017) Fatty acid composition and concentration of alternative food of semipalmated sandpipers (*Calidris pusilla*) in the upper bay of Fundy, Canada. *Can J Zool* 95:565–573. <https://doi.org/10.1139/cjz-2016-0246>
- Reich M, Hannig C, Al-Ahmad A, Bolek R, Kümmerer K (2012) A comprehensive method for determination of fatty acids in the initial oral biofilm (pellicle). *J Lipid Res* 53:2226–2230. <https://doi.org/10.1194/jlr.D026260>
- Ren X, Zhao X, Turcotte F, Deschênes JS, Tremblay R, Jolicoeur M (2017) Current lipid extraction methods are significantly enhanced adding a water treatment step in *Chlorella protothecoides*. *Microb Cell Fact* 16:26. <https://doi.org/10.1186/s12934-017-0633-9>
- Rosenberg KV, Dokter AM, Blancher PJ, Sauer JR, Smith AC, Smith PA, Stanton JC, Panjabi A, Helft L, Parr M, Marra PP (2019) Decline of the North American avifauna. *Science* (80-) 366:120–124. <https://doi.org/10.1126/science.aaw1313>
- Rudy MD, Kainz MJ, Graeve M, Colombo SM, Arts MT (2016) Handling and storage procedures have variable effects on fatty acid content in fishes with different lipid quantities. *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0160497>
- Samburova V, Lemos MS, Hiibel S, Kent Hoekman S, Cushman JC, Zielinska B (2013) Analysis of triacylglycerols and free fatty acids in algae using ultra-performance liquid chromatography mass spectrometry. *J Am Oil Chem Soc* 90:53–64. <https://doi.org/10.1007/s11746-012-2138-3>
- Sasaki GC, Capuzzo JM (1984) Degradation of *Artemia* lipids under storage. *Comp Biochem Physiol B* 78:523–531
- Schlötterbeck J, Kolb A, Lämmerhofer M (2018) Free fatty acid profiling in marine algae extract by LC–MS/MS and isolation as well as quantification of the ω-3 fatty acid hexadeca-4,7,10,13-tetraenoic acid. *J Sep Sci* 41:4286–4295. <https://doi.org/10.1002/jssc.201800780>
- Schnurr PJ, Drever MC, Kling HJ, Elnor RW, Arts MT (2019) Seasonal changes in fatty acid composition of estuarine intertidal biofilm: implications for western sandpiper migration. *Estuar Coast Shelf Sci* 224:94–107. <https://doi.org/10.1016/j.ecss.2019.04.047>
- Schnurr PJ, Drever MC, Elnor RW, Harper J, Arts MT (2020) Peak abundance of fatty acids from intertidal biofilm in relation to the breeding migration of shorebirds. *Front Mar Sci*. <https://doi.org/10.3389/fmars.2020.00063>
- Scholz B, Liebezeit G (2013) Biochemical characterisation and fatty acid profiles of 25 benthic marine diatoms isolated from the Solthörn tidal flat (southern North Sea). *J Appl Phycol* 25:453–465. <https://doi.org/10.1007/s10811-012-9879-0>
- Shang D, Kim M, Haberl M, Legzdins A (2013) Development of a rapid liquid chromatography tandem mass spectrometry method for screening of trace naphthenic acids in aqueous environments. *J Chromatogr A* 1278:98–107. <https://doi.org/10.1016/j.chroma.2012.12.078>
- Sharma KK, Schuhmann H, Schenk PM (2012) High lipid induction in microalgae for biodiesel production. *Energies* 5:1532–1553. <https://doi.org/10.3390/en5051532>
- Snyder LR, Kirkland JJ (1979) Introduction to modern liquid chromatography, 2nd edn. Wiley, Hoboken

- Stevens L (1996) Avian biochemistry and molecular biology. Cambridge University Press
- Stonik VS, Stonik I (2015) Low-molecular-weight metabolites from diatoms: structures, biological roles and biosynthesis. *Mar Drugs* 13:3672–3709. <https://doi.org/10.3390/md13063672>
- Studds CE, Kendall BE, Murray NJ, Wilson HB, Rogers DI, Clemens RS, Gosbell K, Hassell CJ, Jessop R, Melville DS, Milton DA, Minton CDT, Possingham HP, Riegen AC, Straw P, Woehler EJ, Fuller RA (2017) Rapid population decline in migratory shorebirds relying on Yellow Sea tidal mudflats as stopover sites. *Nat Commun* 8:1–7. <https://doi.org/10.1038/ncomms14895>
- Tang B, Row KH (2013) Development of gas chromatography analysis of fatty acids in marine organisms. *J Chromatogr Sci* 51:599–607. <https://doi.org/10.1093/chromsci/bmt005>
- Topolewska A, Czarnowska K, Haliński ŁP, Stepnowski P (2015) Evaluation of four derivatization methods for the analysis of fatty acids from green leafy vegetables by gas chromatography. *J Chromatogr B Anal Technol Biomed Life Sci* 990:150–157. <https://doi.org/10.1016/j.jchromb.2015.03.020>
- Ulmer CZ, Jones CM, Yost RA, Garrett TJ, Bowden JA (2018) Optimization of Folch, Bligh–Dyer, and Matyash sample-to-extraction solvent ratios for human plasma-based lipidomics studies. *Anal Chim Acta* 1037:351–357. <https://doi.org/10.1016/j.aca.2018.08.004>
- Underwood GJC, Kromkamp J (1999) Primary production by phytoplankton and microphytobenthos in Estuaries. *Adv Ecol Res* 29:93–153. [https://doi.org/10.1016/S0065-2504\(08\)60192-0](https://doi.org/10.1016/S0065-2504(08)60192-0)
- Van Der Heijden LH, Graeve M, Asmus R, Rzeznik-Orignac J, Niquil N, Bernier Q, Guillou G, Asmus H, Lebreton B (2019) Trophic importance of microphytobenthos and bacteria to meiofauna in soft-bottom intertidal habitats: a combined trophic marker approach. *Mar Environ Res* 149:50–66. <https://doi.org/10.1016/j.marenvres.2019.05.014>
- Venkata Mohan S, Devi MP (2014) Salinity stress induced lipid synthesis to harness biodiesel during dual mode cultivation of mixotrophic microalgae. *Bioresour Technol* 165:288–294. <https://doi.org/10.1016/j.biortech.2014.02.103>
- Woo KL, Kim JI (1999) New hydrolysis method for extremely small amount of lipids and capillary gas chromatographic analysis as *N*(*O*)-*tert*-butyldimethylsilyl fatty acid derivatives compared with methyl ester derivatives. *J Chromatogr A* 862:199–208. [https://doi.org/10.1016/S0021-9673\(99\)00934-6](https://doi.org/10.1016/S0021-9673(99)00934-6)
- Work TS, Work E (1972) Lipid extraction procedures. Laboratory techniques in biochemistry and molecular biology, vol 3. Elsevier, Amsterdam, pp 347–353. [https://doi.org/10.1016/S0075-7535\(08\)70549-7](https://doi.org/10.1016/S0075-7535(08)70549-7)

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