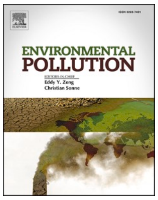
Environmental Pollution 337 (2023) 122598

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol



Fatty-acid based assessment of benthic food-web responses to multiple stressors in a large river system☆

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ARTICLE INFO

Keywords:
Athabasca River
Diatoms
Macroinvertebrates
Nutrient enrichment
Oil sands
Polyunsaturated fatty acids

ABSTRACT

Rivers are often exposed to multiple stressors, such as nutrients and contaminants, whose impacts on the river food webs may not be distinguished by sole assessment of biological community structures. We examined the benthic algal assemblages and the fatty acids (FA) of benthic macroinvertebrates in the lower Athabasca River in Canada, aiming to assess the changes in algal support and nutritional quality of the benthic food web in response to cumulative exposure to natural bitumen, municipal sewage discharge (hereafter, "sewage"), and oil sands mining ("mining"). Data show that the decline in water quality (increases in nutrient concentrations and total suspended solids) was associated with decreases in benthic diatom abundance, and was driven mainly by sewage-induced nutrient enrichment. Responses in nutritional quality of benthic macroinvertebrates, indicated by their polyunsaturated FA (PUFA) concentrations, were taxon- and stressor-specific. Nutritional quality of the larval dragonfly predator, *Ophiogomphus*, decreased nonlinearly with decreasing benthic diatom abundance and was lowest at the sewage-affected sites, although exposure to natural bitumen also resulted in reduced *Ophiogomphus* PUFA concentrations. In contrast, the PUFA concentrations of mayfly grazers/collector-gatherers were not affected by natural bitumen exposure, and were higher at the sewage and sewage+mining sites. The PUFA concentrations of the shredder *Pteronarcys* larvae did not change with cumulative exposure to the stressors. Sediment metal and polycyclic aromatic compound concentrations were not associated with the macroinvertebrate FA changes. Overall, we provide evidence that sewage induced reduction in trophic support by PUFA-rich diatoms, and was the predominant driver of the observed changes in FA composition and nutritional quality of the benthic macroinvertebrates. Fatty-acid metrics are useful to untangle effects of concurrent stressors, but the assessment outcomes depend on the functional feeding guilds used. A food-web perspective using multiple trophic levels and feeding guilds supports a more holistic assessment of the stressor impacts.

1. Introduction

Rivers and their biodiversity are valuable natural resources that are often exposed to multiple stressors, such as nutrients and contaminants, caused by human activities and environmental changes (Dudgeon et al., 2006; Heino et al., 2009; Vörösmarty et al., 2010). The traditional approach for evaluation of such stressors on riverine health focuses on examining community structure and the subsequent comparison of biological pattern to assessment thresholds (e.g., Council of the European Communities, 2000; Glozier et al., 2002). However, structural assessments may not be able to distinguish among the effects of commonly co-occurring stressors on biota, and often lack the capacity to detect stressor-induced functional changes of individual taxa or trophic levels. Such functional differences can occur before or in tandem with shifts in biological assemblages (Hixson and Arts, 2016; Keva et al., 2021). Hence, the constraints of structural assessments indicate the requirement for new complementary approaches for more holistic evaluation of the stressor impacts. The analysis of fatty acids (FA) in biota is proven effective in detecting individual and collective impacts of stressors on biological communities (Filimonova et al., 2016; Bergström et al., 2020; Lau et al., 2021). It also can detect taxon- or trophic level-specific functional responses to stressors (Hixson and Arts, 2016;

☆ This paper has been recommended for acceptance by Sarah Harmon.
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https://doi.org/10.1016/j.envpol.2023.122598
Received 28 February 2023; Received in revised form 21 August 2023; Accepted 19 September 2023
Available online 21 September 2023
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Taipale et al., 2016; Keva et al., 2021), and is a unique measure able to elucidate causal pathways of stressor effects on food webs (Filimonova et al., 2016; Bergström et al., 2020). However, application of this approach has largely been restricted to lake or marine ecosystems, and thus warrants investigation into its application to lotic freshwaters.

Fatty acids in biota can reflect how food web pathways are affected by stressors. Nutrient enrichment, for example, will alter primary production and assemblages of benthic algae, which are the main basal resource in northern clear waters (Vadeboncoeur et al., 2003; Vadeboncoeur and Power, 2017). Changes in basal resources will affect their food quality for consumers, since there are differences in biochemical composition among basal resources (Brett and Müller-Navarra, 1997; Napolitano, 1999; Taipale et al., 2013), especially in their concentrations of long-chain polyunsaturated fatty acids (PUFA) that are synthesized de novo by algae and are necessary for animal physiology (Sargent et al., 1995). Nutrient enrichment of inland waters thus can result in both structural (i.e., assemblages and biomass) and functional (i.e., PUFA production) changes in primary producers (Cashman et al., 2013; Whorley and Wehr, 2018). In particular, municipal sewage effluents are often phosphorus (P)-rich and can alter the relative availability of nitrogen (N) and P for primary producers, i.e., reduce the dissolved inorganic N to total-P (DIN:TP) ratio of river water. Decreases in water DIN:TP ratio will intensify N-limitation in primary producers (Bergström et al., 2020; Fork et al., 2020; Lau et al., 2021), subsequently stimulating the growth of PUFA-deficient cyanobacteria and reducing that of long-chain PUFA-rich taxa, such as diatoms (Diehl et al., 2018). Decreases in water DIN:TP have been shown to reduce PUFA accumulation in pelagic grazers (Bergström et al., 2020; Lau et al., 2021). The shifts from diatoms-dominated to cyanobacteria-dominated assemblages can reduce their long-chain PUFA supply, potentially affecting the growth of invertebrates and subsequently the fish production (Müller-Navarra et al., 2004; Brett et al., 2017; Keva et al., 2021).

Exposure to anthropogenic contaminants can alter aquatic communities by successively eliminating pollution-sensitive and favouring pollution-tolerant taxa (Johnson et al., 1993; Lavoie et al., 2012). However, the presence of both nutrients and contaminants contained within pollution exposures poses difficult challenges for distinguishing the cause of biological effect (Culp et al., 2000). For example, the growth, taxon richness, and diversity of benthic diatoms in rivers have been shown to decrease with increasing metal contamination (Lavoie et al., 2012; Vidal et al., 2021), while shifts to more N-limited conditions in water similarly will reduce the predominance of diatoms and favour cyanobacteria in benthic primary producers (Diehl et al., 2018). Contamination of stream sediment by metal and organic pollutants, such as polycyclic aromatic compounds (PACs), also has been found to reduce abundance and taxon richness of benthic macroinvertebrates (Beasley and Kneale, 2002; Scoggins et al., 2007). These contaminants can bioaccumulate in aquatic food webs and have adverse effects on animal health and physiology (Alegbeleye et al., 2017 and references therein), including lipid metabolism and PUFA accumulation (Filimonova et al., 2016; Hansen et al., 2020). The analysis of FA in consumers thus is potentially useful for assessing the ecological impacts of contaminants apart from those of nutrient enrichment (Filimonova et al., 2016).

The Athabasca oil sands development in north-eastern Alberta, Canada, provides an excellent location to assess whether FA metrics can diagnose the effects of multiple stressors. This is because freshwater environments in the region, such as the lower Athabasca River, receive inputs of heavy metals and PACs from natural erosion of oil containing sands (i.e., bitumen) (Akre et al., 2004; Culp et al., 2021). Fluvial transport of naturally eroded bitumen results in natural metal and PAC exposure that poses potential ecotoxicological effects on the river biota (Cardoso et al., 2020, 2023). Aerial emissions from mining and industrial oil extraction from bitumen may also release metals and PACs, which deposit to snowpack and on the landscape, and subsequently enter the river during snowmelt and precipitation events (Kelly et al., 2009, 2010; Culp et al., 2021), although the metals in snow are found mainly in particulate instead of dissolved form (Shotyk, 2022). Additionally, treated municipal sewage effluent is discharged to the river, causing simultaneous exposure to contaminants and nutrients (Culp et al., 2021). To date, benthic macroinvertebrate assemblages at river sites within the area of intense oil sands mining activity are characterized as having more pollution-tolerant taxa than sites from reference areas, but are more strongly associated with sewage-induced nutrient enrichment than with diffuse contamination from either natural bitumen or oil sands mining (Culp et al., 2018, 2020). Although the overall taxon richness and diversity of benthic macroinvertebrates do not differ between the references sites and the sites exposed to natural bitumen, sewage, and/or oil sands mining (Culp et al., 2018), nutrient enrichment is expected to alter algal biomass and assemblages as well as the resultant bottom-up effects on macroinvertebrates. Also, chronic exposure to contaminants may pose sub-lethal effects and impair physiological functions. The subsidy-stress gradients produced by the combined effects of contaminants and nutrients could therefore mask the chronic effects of low-level contaminant exposure (Wagenhoff et al., 2011; Alexander et al., 2016). In this study we tested if FA metrics could help resolve this confounding effect.

We used FA to elucidate the impacts of nutrient enrichment as well as natural and/or anthropogenic contaminants on benthic macroinvertebrates of different functional feeding groups in the lower Athabasca River. Our objectives were to: (i) assess the cumulative effects of exposure to natural bitumen, sewage discharge, and oil sands mining on the FA composition and nutritional quality (i.e., tissue long-chain PUFA concentrations) of the benthic macroinvertebrates, (ii) unravel the main stressor that drives their FA changes, (iii) assess whether the FA and nutritional responses of benthic macroinvertebrates are consistent between trophic levels and feeding guilds, and (iv) determine whether FA metrics are useful for disentangling the effects of multiple stressors. We predicted that changes in water quality due to natural bitumen, sewage discharge, and/or oil sands mining activity would pose bottom-up effects on the diversity of benthic primary producers and the abundance of benthic diatoms (i.e., the predominant PUFA-rich benthic algal taxa at the study sites), as well as on the FA composition and nutritional quality of benthic macroinvertebrates. We expected that the invertebrate FA were sufficiently sensitive to reflect the effects of contaminants from natural bitumen or mining activity, although these effects could be weaker than those resulting from changes in water quality, because sediment PAC and metal concentrations at sites exposed to natural bitumen alone or with mining were within limits of sediment quality guidelines (MacDonald et al., 2000; Culp et al., 2020). We further predicted that the changes in FA composition and nutritional quality caused by the stressors would be more prominent in benthic grazers than in shredders, and that the effects would propagate to predators which normally have a greater demand for long-chain PUFA than do primary consumers (Lau et al., 2012; Strandberg et al., 2015). We expected that FA metrics would be useful to complement taxonomy-based assessments of biological communities and to diagnose the ecological impacts of multiple stressors.

2. Materials and methods

2.1. Study sites

We selected 10 sampling sites with similar habitat characteristics (see Environment Canada, 2011a,b; Culp et al., 2020), depicted M0 to M9, at the lower Athabasca River of Canada (Table 1; Fig. 1), which is an 8th order river near Fort McMurray, Alberta, and has a catchment area of ca. 95,000 km^2. These sites extended ca. 600 km from the Town of Athabasca (M0) to the edge of Wood Buffalo National Park (M9) (Fig. 1). The most upstream site M0 was located outside the bitumen deposits, while sites M1 to M9 were within the landscape with the presence of natural bitumen. Thus, M1 to M9 were exposed to local natural bitumen deposits and/or fluvial transport of naturally eroded bitumen from

Table 1
Study sites and their exposure to natural bitumen, municipal sewage effluent, or oil sands development at the lower Athabasca River. High, Moderate, and Low refer to the level of exposure. Site category was used as the fixed factor in nested ANOVA. Exposure pathways: a, natural erosion of bitumen at local reaches (Droppo et al., 2018; Cardoso et al., 2020, 2023); b, fluvial transport of naturally eroded bitumen from upstream sources (Droppo et al., 2018; Cardoso et al., 2020, 2023); c, nutrient inputs from municipal sewage effluent (Regional Municipality of Wood Buffalo, 2012); d, airborne deposition of industrial-derived contaminants from stack emissions and fugitive dust (Kirk et al., 2014), and meltwater from snow with airborne contaminant deposits (Kelly et al., 2009, 2010; Kirk et al., 2014).

Site	Exposure			Site category	Exposure pathway
	Natural bitumen	Municipal sewage effluent	Oil sands development		
M0	No	No	No	Reference	Not applicable
M1	Yes	No	No	Natural bitumen	a, b
M2	Yes	No	No	Natural bitumen	a, b
M3	Yes	High	No	Sewage	a, b, c
M3B	Yes	Moderate	High	Sewage+mining	a, b, c, d
M4	Yes	Moderate	High	Sewage+mining	a, b, c, d
M6	Yes	Moderate	High	Sewage+mining	a, b, c, d
M7	Yes	Moderate	High	Sewage+mining	a, b, c, d
M7C	Yes	Moderate	Moderate	Sewage+mining	a, b, c, d
M9	Yes	Moderate	Low	Improvement	a, b, c, d

upstream sources. Further, sites M3 to M9 were located below the municipal sewage effluent (hereafter referred to as "sewage") discharge from Fort McMurray (Fig. 1). The sewage discharge at Fort McMurray constituted 88% of the total sewage discharge and was the major source of nutrient inputs in the study region, although there were a few sewage discharge points downstream that altogether constituted 12% of the total sewage discharge (Regional Municipality of Wood Buffalo, 2012). The five sites downstream of M3, i.e., from M3B to M7C, were in areas with active oil sands mining (hereafter referred to as "mining") (Fig. 1). The downstream site M9 was outside the mining area and used either to detect biological responses to the cumulative stressor effects or as an improvement zone. The sites were classified into five categories (Table 1; Culp et al., 2020), such that the study design allowed us to assess the effects of exposure to natural bitumen alone (by comparing M0 with M1 and M2) and the additional effects of the Fort McMurray sewage (by comparing M1 and M2 with M3). Also, sites M3B to M9 represent the gradient of mining from high to low activity. Sites M3B to M7C were expected to receive stronger combined effects of sewage and mining (hereafter referred to as "sewage+mining") than did the improvement site M9 (Table 1). The comparisons of these sites with M3 allowed the assessment of the changes in FA composition and nutritional quality of benthic macroinvertebrates in response to mainly the intensity of mining activity (Table 1; Fig. 1; Culp et al., 2020). All sampling was conducted in late September of 2012.

The study sites generally differ in benthic macroinvertebrate composition (Culp et al., 2020), but the overall taxon richness and diversity of benthic macroinvertebrates are similar among the sites (Culp et al., 2018). There are also no among-site differences in the overall richness of the pollution-sensitive EPT taxa (i.e., total mayflies, stoneflies, and caddisflies; Culp et al., 2018). Culp et al. (2020) found that the assemblages differed between the upstream reference site and the sites exposed solely to natural bitumen. These sites had higher abundances of Hydropsychidae, but fewer taxa of mayflies and dragonflies, as well as fewer pollution-tolerant taxa (e.g., oligochaetes and chironomids) than the sewage and sewage+mining sites. The downstream improvement site had higher abundances of mayflies, stoneflies, and caddisflies, and fewer pollution-tolerant taxa compared to the sewage and sewage+mining sites. Stoneflies, mayflies, and dragonflies were also more abundant at the improvement site than the reference sites. Nutrients and sediment PACs and metals were identified as environmental correlates with the spatial pattern of benthic macroinvertebrate assemblages at the lower Athabasca River, although the nutrient effects were stronger (Culp et al., 2020).

2.2. *Benthic macroinvertebrate sampling*

At each site we collected benthic macroinvertebrates at five equally spaced locations along a 250–500 m reach at the near-shore shallow habitat by kick-sampling with a 400-μm mesh kick net (Culp et al., 2020). Benthic macroinvertebrates for FA analysis were sorted to genus level on-site, focusing on the dragonfly *Ophiogomphus* (Gomphidae, Odonata; a predator), the stonefly *Pteronarcys* (Pteronarcyidae, Plecoptera; a shredder), the mayflies *Ametropus* (Ametropodidae, Ephemeroptera; a collector-filterer), *Heptagenia*, and *Rhithrogena* (Heptageniidae, Ephemeroptera; grazers), since these taxa were commonly present in most study sites (Culp et al., 2020) and represent multiple functional feeding guilds and trophic levels. Shredders generally do not feed on benthic primary producers on hard substrates, but they may ingest and assimilate the producers that have settled and/or grown on surfaces of plant detritus (Guo et al., 2016). Thus, we included the shredder *Pteronarcys* although we expected its FA responses to the stressors would be weaker than those of the mayflies and *Ophiogomphus*. The macroinvertebrates were stored in liquid nitrogen immediately after collection and kept at −80 °C in the laboratory until further processing for FA analysis.

2.3. *Periphyton sampling*

Periphyton was sampled at each benthic macroinvertebrate sampling location by using a scalpel to scrape epilithic biofilm material from 5 randomly picked fist-sized rocks. The estimated total rock surface area scraped was ca. 300 cm^2. These samples were combined as a single composite sample for each site and preserved in 95% ethanol. Laboratory analysis for taxonomy of diatoms and non-diatom species initially consisted of allowing aliquots of diluted samples to settle overnight in sedimentation chambers using Utermöhl's procedure described in Lund et al. (1958). For non-diatom analysis, a minimum of 400 units (cells, filaments, or colonies) from randomly selected transects were viewed under a Zeiss Axiovert 40 CFL inverted microscope (500 × magnification). At this stage, the diatoms were not identified to species. Identification of diatoms to species involved further processing of the sample to make diatom slides. For each sample, diatom slides were then prepared by boiling subsamples in concentrated nitric acid and hydrogen peroxide, then washing several times (by centrifugation) with deionized water (Patrick and Reimer, 1966; Carr et al., 1986). A couple of drops of diluted diatom slurry were deposited on a cover slip and placed on a slide warmer, and allowed to evaporate overnight. A drop of Naphrax was placed on a glass slide and the coverslip with diatoms inverted on to it. A minimum of 500 diatom frustules were counted at 1000× magnification under oil immersion on the compound microscope. Diversity metrics including species richness and Simpson's diversity index of the whole benthic primary producer community were calculated for individual sites.

2.4. *Water quality and sediment sampling*

Water samples were collected at the most downstream kick sampling location at each site, and analysed for dissolved and particulate organic carbon (DOC and POC), dissolved inorganic nitrogen (sum of NO_3^--N, NO_2^--N, and NH_4^+-N; DIN), particulate organic N (PON), total dissolved N (TDN), total dissolved phosphorus (TDP), and total P (TP), total hardness (i.e., $CaCO_3$), total suspended solids (TSS), and total dissolved solids (TDS) (Table S1), according to the National Laboratory of Environmental Testing (NLET) protocols (Environment and Climate Change

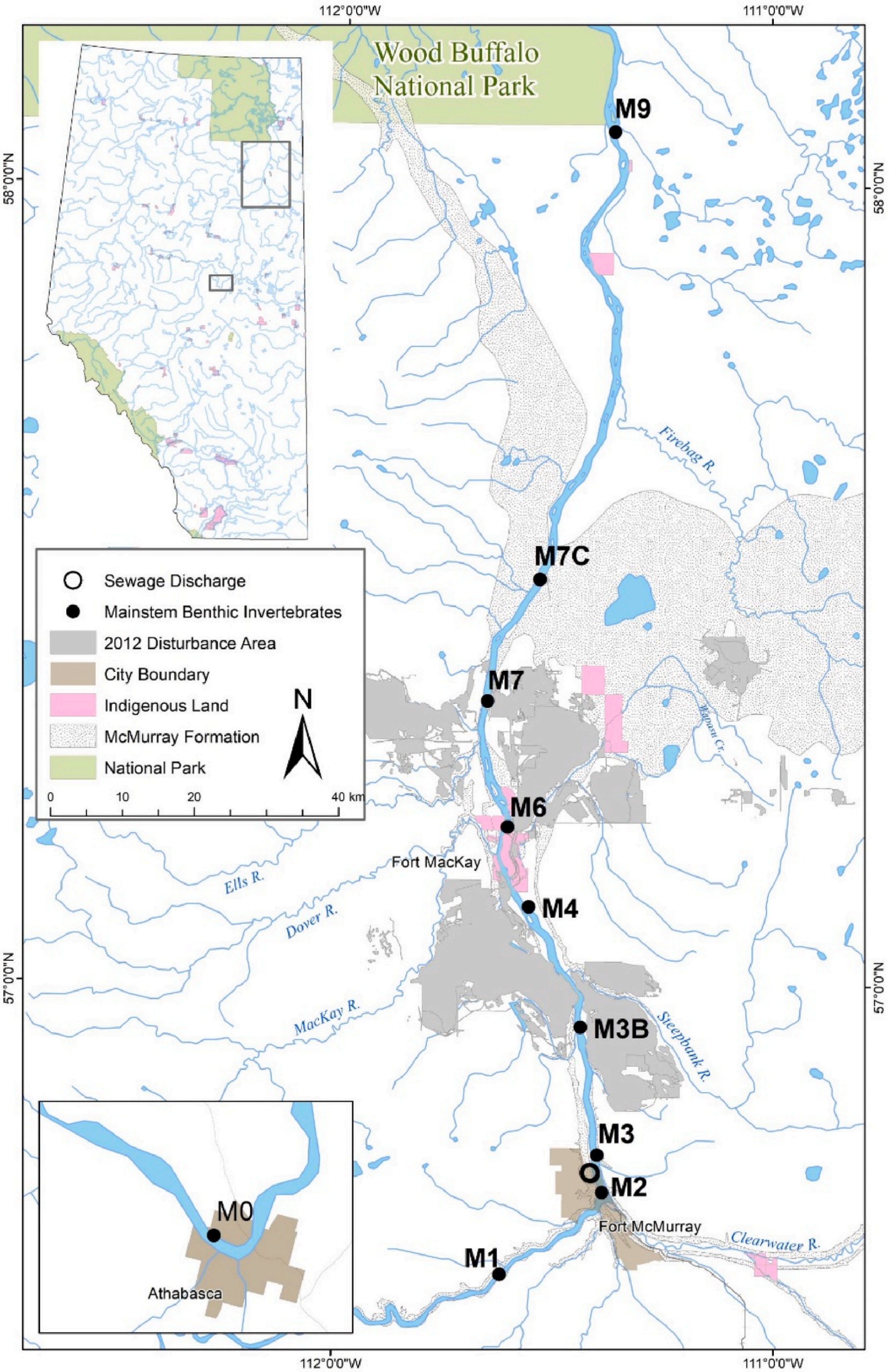

Fig. 1. Locations of the study sites at the lower Athabasca River of Canada. Areas in light grey indicate surface mineable bitumen deposits, where areas with active mining activity are indicated in grey. M0, upstream reference site outside the natural bitumen geology; M1 and M2, sites with exposure to natural bitumen; M3, with natural bitumen and municipal sewage effluent discharge from Fort McMurray; M3B-M7C, with natural bitumen, sewage discharge, and oil sands development; M9, improvement site.

Canada, 2019). Conductivity was measured in situ using a handheld multiparameter meter (ProPlus YSI multimeter). To quantify organic and metal contaminants, a stainless-steel spoon was used to collect depositional sediment that was placed into glass containers and stored at 4 °C until analysis. Sediment samples were analysed for polycyclic aromatic compounds (PACs) and trace metals based on the NLET analytical protocols (Environment and Climate Change Canada, 2019).

2.5. *Fatty-acid analysis*

Fatty-acid compositions of benthic macroinvertebrates were analysed within six months after collection following the methods described in Lau et al. (2012). The macroinvertebrates were freeze-dried and pulverised using a tissue homogeniser (Precellys, Bertin Corp., Maryland, USA). For *Ametropus*, *Heptagenia*, and *Rhithrogena*, samples from individual locations of each site were pooled to ensure sufficient material for FA analysis. We then homogenised the dried samples (5–30 mg) and extracted their lipids in 2 mL 3:2 (v:v) hexane-isopropanol solution (homogeniser: Ultra-Turrax, Janke & Kunkel, IKA Werke, Staufen, Germany), followed by adding 0.7 mL 6.7% Na_2SO_4 to remove non-lipids. The mixture was vortexed and centrifuged at 2000 rpm (281 *g*) and 4 °C for 5 min. The top organic phase was separated, evaporated under N_2, and the resultant lipid extract was dissolved in 0.5 mL hexane. The solution was then spiked with 8 μg internal standard methyl 15-methylheptadecanoate (Larodan, Malmö, Sweden), saponified with 2 mL 0.01 M NaOH in dry methanol, and methylated with 3 mL 14% methanolic BF_3 solution at 60 °C for 10 min. After cooling, 2 mL 20% NaCl and 2 mL hexane were added. The mixture was then vortexed and centrifuged (281 *g*, 4 °C, 5 min). The lipid layer containing the FA methyl esters was extracted, dried under N_2, and dissolved in 0.5 mL hexane.

The FA methyl esters were analysed using a GP3800 gas chromatograph (Varian AB, Stockholm, Sweden) installed with a flame ionisation detector and a BPX70 silica capillary column (50.0 m length, 0.22 mm internal diameter, 0.25 μm film thickness; SGE, Austin, Texas, U.S.A.). Split injection (1:10) was applied and the oven temperature was set at 158 °C for 5 min, then increased by 2 $°C\ min^{-1}$ to 220 °C which was finally maintained for 8 min. Carrier gas was helium with a constant flow rate of 0.8 $mL\ min^{-1}$. The GLC-461 FA methyl ester mixture (Nu-Chek Prep Inc., Elysian, Minnesota, U.S.A.) and the FA methyl esters iso- and anteiso-15:0 and 17:0 (Larodan, Malmö, Sweden) were used to identify individual FA peaks of the sample. These standards contained the FA that are specific to the common basal food resources in aquatic ecosystems, i.e., algae, terrestrial organic matter, and bacteria. Concentrations of individual FA in the samples were quantified based on the internal standard and are reported as mg FA g^{-1} dry mass.

2.6. *Data analysis*

All water physicochemical variables and invertebrate FA data were log_{10}-transformed for normal-distribution approximation. We also calculated the DIN:TP molar ratios (log_{10}-transformed) of river water samples to indicate spatial changes in the relative availability of N and P. The water DIN:TP ratio is known to affect the nutrient limitation regime and long-chain PUFA supply from phytoplankton for aquatic food chains (Bergström et al., 2020; Lau et al., 2021).

We used principal component analysis (PCA) to assess the spatial changes in FA composition of the benthic macroinvertebrates with all taxa together and for individual taxa separately. The ephemeropteran genera (i.e., *Ametropus*, *Heptagenia*, and *Rhithrogena*) were run as a group because of their lack of replicates and patchy distribution among sites. Except for the long-chain ω6 PUFA arachidonic acid (20:4ω6; ARA) and ω3 PUFA eicosapentaenoic acid (20:5ω3; EPA) and docosahexaenoic acid (22:6ω3; DHA), which are known to be particularly important for animal growth and physiological functions (Brett and Müller-Navarra, 1997; Brett et al., 2017), the other identified FA were classified into major groups before PCA: short- (with <20 carbon) and long-chain (with ≥20 carbon) saturated FA (ShortSAFA and LongSAFA), monounsaturated FA (MUFA), bacteria-specific FA (BAFA), and other ω3 and other ω6 PUFA (ω3other and ω6other) (Table S2).

We further used nested ANOVA to compare the PUFA groups (i.e., ARA+EPA+DHA, ω3other, and ω6other) and ω3:ω6 FA ratios of *Ophiogomphus* and *Pteronarcys* between site categories. Sample ARA, EPA, and DHA were grouped together as they showed similar spatial changes based on PCA. In nested ANOVA, site categories were used as the fixed factor, and individual sites as the random factor nested within site categories. Post-hoc Tukey HSD tests were conducted when nested ANOVA detected significant effects of site categories (i.e., $p < 0.05$).

We sought to elucidate the stressors that underlay the spatial patterns of the invertebrate FA. Separate PCA were therefore conducted to examine the cross-site differences in (i) physicochemical characteristics of river water, and (ii) polycyclic aromatic compound (PAC) and (iii) metal concentrations in sediment. We then used the first principal components, i.e., $PC1_{Water}$, $PC1_{PAC}$, and $PC1_{Metal}$, which captured 63–74% of the variance in their respective data, to represent the stressor gradients across sites. To elucidate the bottom-up effects caused by changes in water quality on (i) the abundance of benthic diatoms, (ii) the diversity metrics of overall benthic primary producers, and (iii) the nutritional quality of benthic macroinvertebrates, we regressed diatom abundance (cells) and producer diversity metrics against $PC1_{Water}$. We then regressed the invertebrate PUFA groups and ω3:ω6 FA ratios against diatom abundance, as diatoms dominated the benthic primary producer community at most study sites, contributing to 37–100% of total primary producer abundance (Table S1). Green algae were found at low abundance (i.e., <2% of total primary producer abundance) at two sites (M0 and M2) (Table S1). Cyanobacteria were absent at M0, M3B, and M7, and their contribution to total primary producer abundance was high at M1 (63%) and M3 (53%), and was 4–24% at the other sites (Table S1). Regression was also used to test whether the invertebrate FA responded to changes in the diversity metrics of benthic primary producers. Similarly, we regressed the invertebrate PUFA groups and ω3:ω6 FA ratios against $PC1_{PAC}$ and $PC1_{Metal}$ to determine whether sediment PAC and metal concentrations affected PUFA accumulation in the benthic macroinvertebrates. In all regression analysis, both linear and nonlinear (i.e., exponential) models were examined, as the invertebrate FA responses might not be unidirectional and/or of similar magnitude (Filimonova et al., 2016). The model with the lowest corrected Akaike information criterion (AIC_C) was selected as the best model.

Nested ANOVA, Tukey HSD tests, and regressions were conducted using JMP® Pro 15.0.0 (SAS Institute Inc., Cary), and PCA was run using the vegan package (Oksanen et al., 2020) in R version 4.0.3 (R Core Team, 2020). All variables were standardized before PCA. Statistical significance level (α) was set at 0.05.

3. Results

3.1. *Patterns in invertebrate FA among sites*

PCA showed apparent variations in FA composition both between and within the benthic macroinvertebrate taxa, which were indicated by the first (PC1; accounted for 54% of the total variance) and second principal components (PC2; 23%), respectively (Fig. 2a). Concentrations of the long-chain PUFA, i.e., ARA, EPA, and DHA, and the ω3:ω6 FA ratio were negatively correlated with PC2, and were generally higher in the ephemeropteran grazers and collector-filterers (i.e., *Ametropus*, *Heptagenia*, and *Rhithrogena*), intermediate in the predatory dragonfly *Ophiogomphus*, and lower in the shredding stonefly *Pteronarcys*. In particular, EPA was most dominant among the long-chain PUFA, and constituted 58–89% of the ARA+EPA+DHA concentrations in the consumers (Table S2). Ranges of site-average EPA concentrations were 1.70–4.82 mg FA g^{-1} dry mass for ephemeropterans, 1.76–7.80 mg FA g^{-1} dry mass for *Ophiogomphus*, and 0.85–1.82 mg FA g^{-1} dry mass for

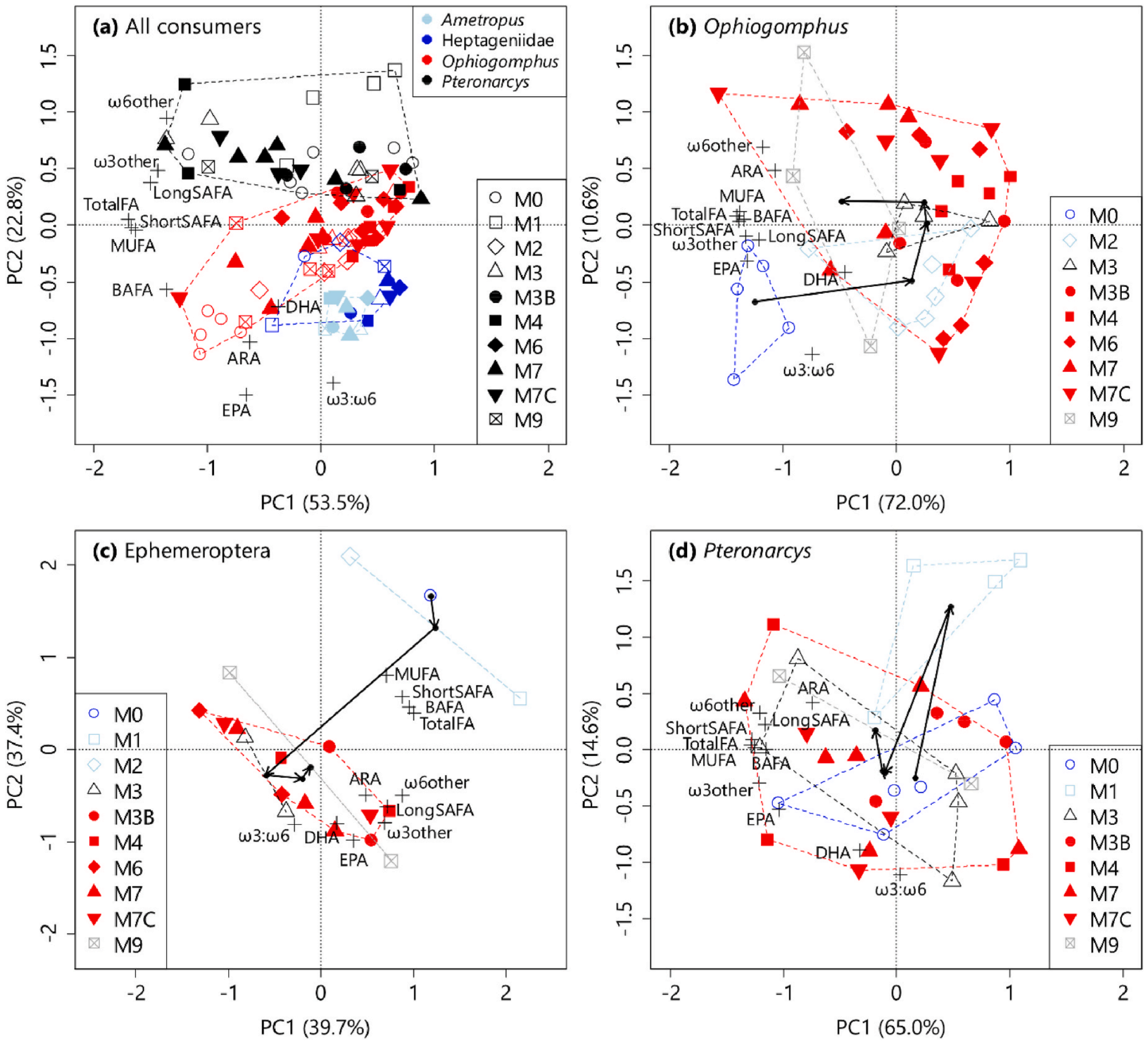

Fig. 2. Principal component analysis (PCA) of the fatty-acid (FA) compositions of (a) all benthic macroinvertebrate taxa, (b) *Ophiogomphus*, (c) Ephemeroptera, and (d) *Pteronarcys*. Variance percentages explained by the PCA axes are indicated in parentheses. Dotted lines indicate convex hulls of individual taxa (a) or sites (b–d). In (b–d), black dots are centroids of individual site categories and black arrows indicate FA changes of the invertebrates from the upstream reference site to the downstream improvement site. ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ShortSAFA and LongSAFA, total short- (<20 carbon) and long-chain (≥20 carbon) saturated FA respectively; MUFA, total monounsaturated FA; BAFA, total bacterial FA; ω3other, total omega-3 FA excluding DHA and EPA; ω6other, total omega-6 FA excluding ARA; ω3:ω6, omega-3 to omega-6 FA ratio; TotalFA, overall total FA.

Pteronarcys (Table S2). Concentrations of the other FA groups (e.g., MUFA, BAFA, ω3other, and ω6other) varied within individual taxa, and were negatively correlated with PC1. Fatty-acid compositions of *Ophiogomphus* were more similar to those of the ephemeropterans (i.e., these taxa were closer on the PCA biplot) at the upstream sites without (M0) or with natural bitumen (M1 and M2) and at the improvement site (M9), but were more similar to those of *Pteronarcys* at the sites with sewage discharge and mining (e.g., M3B, M4) (Fig. 2a).

The PCA of individual taxa showed strong spatial patterns in FA compositions of *Ophiogomphus* and the ephemeropterans (Fig. 2b,c). The concentrations of all FA including ARA, EPA, and DHA of *Ophiogomphus* were negatively correlated with PC1, which explained 72% of the FA variance (Fig. 2b). All these FA concentrations in *Ophiogomphus* were 33.7% (for LongSAFA) to 73.1% (for BAFA) lower in the upstream site exposed to natural bitumen (M2) than in the reference site without natural bitumen (M0) (Table S2; Fig. 2b). *Ophiogomphus* had further lower concentrations of these FA at sites with sewage (M3) or sewage+mining (M3B, M4, and M6) (Table S2; Fig. 2b). At the downstream sites M7-M9, all FA in *Ophiogomphus* generally increased again, although *Ophiogomphus* in M9 had larger variabilities in FA composition (i.e., a larger convex hull on the PCA biplot) than *Ophiogomphus* in M0 and M2 (Table S2; Fig. 2b).

The ephemeropterans showed a different spatial pattern in FA than that of *Ophiogomphus*. In the PCA of ephemeropterans, similar proportions of the FA variance were explained by PC1 (40%) and PC2 (37%) (Fig. 2c). Despite lacking replicates within individual sites, the ephemeropterans showed generally lower PUFA concentrations (ARA, EPA, DHA, ω3other, and ω6other) and ω3:ω6 FA ratios at the upstream reference sites without (M0) or with natural bitumen (M1 and M2) than at the other sites (M3-M9) (Table S2; Fig. 2c), contrary to the FA patterns (i.e., concentrations of most FA were higher at M0) observed in *Ophiogomphus*. Ephemeropterans at M0-M2, however, had higher BAFA, MUFA, ShortSAFA, and total FA concentrations than those at M3-M9 (Table S2; Fig. 2c).

In the PCA of the stonefly *Pteronarcys*, PC1 and PC2 captured 65% and 15% of the FA variance respectively (Fig. 2d). Except for DHA and the ω3:ω6 FA ratio, which were negatively correlated with PC2, all other FA were negatively associated with PC1. *Pteronarcys* had lower DHA concentrations and ω3:ω6 FA ratios at M1 than at the other sites, where the FA compositions of *Pteronarcys* did not show apparent cross-site patterns because of their large within-site variabilities (Table S2; Fig. 2d).

Nested ANOVA largely confirmed the PCA results, and indicated that *Ophiogomphus* at the most upstream reference site (M0, without exposure to natural bitumen) had higher ARA+EPA+DHA concentrations (mean ± SE = 9.10 ± 0.63 mg FA g^{-1} dry mass) than at the other site categories (i.e., sites with exposure to natural bitumen (M1 and M2), sewage (M3), sewage+mining (M3B-M7C), and the improvement site (M9) (from 2.61 ± 0.34 to 4.76 ± 0.73 mg FA g^{-1} dry mass)) (Tables 2 and S2). *Ophiogomphus* ARA+EPA+DHA concentrations did not differ between sites with natural bitumen, sewage, sewage+mining, and the downstream improvement site (Tables 2 and S2).

The ω3other PUFA concentrations of *Ophiogomphus* did not differ between reference and improvement sites (M0 and M9), and between sites exposed to natural bitumen, sewage, and sewage+mining (Tables 2 and S2). *Ophiogomphus*, however, had higher ω3other PUFA concentrations at M0 and M9 than at sites with natural bitumen and sewage+mining (Tables 2 and S2). The ω3other PUFA concentrations of *Ophiogomphus* at the sewage site (M3) were lower than those at the reference site (M0), but were not different from those at the other site groups (Tables 2 and S2).

Differences in ω6other PUFA concentrations of *Ophiogomphus* between site categories were near-significant ($p = 0.056$; Tables 2 and S2). *Ophiogomphus* tended to have higher ω6other PUFA concentrations at the reference and improvement sites (M0 and M9) (Fig. 2b). The ω3:ω6 FA ratios of *Ophiogomphus* were higher at the reference site (M0) than at the sewage (M3) and sewage+mining sites (M3B-M7C), while those at sites with natural bitumen alone (M1 and M2) and the improvement site (M9) were not different from all other site groups (Tables 2 and S2).

Concentrations of ARA+EPA+DHA in *Pteronarcys* were near-significant ($p = 0.062$; Tables 2 and S2), and tended to be lower at the site with natural bitumen alone (M1) than at other site categories (Fig. 2d). *Pteronarcys* ω3other and ω6other PUFA concentrations were similar between site categories (Tables 2 and S2). *Pteronarcys* had higher ω3:ω6 FA ratios at the reference site (M0) and sites with sewage (M3) or sewage+mining (M3B-M7C) than at the site with sole natural bitumen (M1) (Tables 2 and S2). But the ω3:ω6 FA ratios of *Pteronarcys* at the improvement site (M9) did not differ from those at the other site groups (Table 2 and S2).

3.2. Patterns in water chemistry among sites

PCA of water physicochemical attributes showed that $PC1_{Water}$ explained 73% of the total variance, which was much greater than that (14%) explained by $PC2_{Water}$ (Fig. 3a). Increasing $PC1_{Water}$ scores indicated a decreasing water quality, as concentrations of TSS, DOC, POC, and nutrients including TDN, TDP, and TP were positively associated with $PC1_{Water}$ and higher at the site with sewage (M3) than at the other sites (Table S1; Fig. 3a). Specifically, TSS, TDN, and TP were 20.0–67.6 mg L^{-1}, 0.020–0.429 mg L^{-1}, and 0.048–0.119 mg L^{-1} higher, respectively, at M3 than at the other sites (Table S1). In contrast, the water DIN:TP ratio, conductivity, total hardness, and TDS concentrations were negatively correlated with $PC1_{Water}$. The water DIN:TP ratio, conductivity, total hardness, and TDS concentrations were 0.72–2.91, 340–361 μS cm^{-1}, 133–147 mg L^{-1}, and 167–178 mg L^{-1}, respectively, at reference sites M0 and M1 (i.e., the upstream sites without and with exposure to natural bitumen, respectively), and were higher than those at the other sites (ranges of DIN:TP = 0.38–0.66, conductivity = 195–291 μS cm^{-1}, total hardness = 61–107 mg L^{-1}, TDS = 97–141 mg L^{-1}) (Table S1; Fig. 3a). The improvement site (M9) and the sewage+mining sites (M3B-M7C) had similar physicochemical characteristics of river water, as indicated by their proximity on the PCA biplot (Table S1; Fig. 3a).

Concentrations of most PACs and metals found in the sediment were positively correlated with the first principal components, i.e., $PC1_{PAC}$ and $PC1_{Metal}$, which accounted for 75% and 62% of the total variance in the PAC and metal data, respectively (Fig. S1a,b). M7, one of the sewage+mining sites, had generally higher sediment PAC concentrations, and was therefore separated from the other sites on the biplot (Fig. S1a). The total PAC concentrations were 5.20 μg g^{-1} at M7 and 1.07–2.66 μg g^{-1} at the other sites (Table S3). Yet, the study sites had variable sediment metal concentrations, with the downstream sites (M7-M9; total metal concentrations = 56.64–180.84 mg g^{-1}) generally having higher concentrations than the more upstream sites (M0-M6; total metal concentrations = 17.46–54.20 mg g^{-1}) (Fig. S1b).

Benthic diatom abundance and biomass were strongly and positively correlated (biomass in μg = −2.334 + 0.851 (abundance), $F_{1,8} = 28.81$, $p < 0.001$, $R^2 = 0.78$). Benthic diatom abundance decreased with increasing $PC1_{Water}$ scores (Fig. 3b), and was highest at the reference site (M0; 2.8×10^8 cells) and lowest at the sewage site (M3; 9.6×10^6 cells) (Table S1; Fig. 3b). It was intermediate at sites with exposure to sole natural bitumen (M1 and M2) and the improvement site (M9), and was

Table 2
Nested ANOVA of fatty-acid (FA) concentrations (mg FA g^{-1} dry mass) of *Ophiogomphus* and *Pteronarcys* between site categories (Category). Sites were nested within site categories. FA abbreviations: ARA+EPA+DHA, summed concentrations of arachidonic acid (ARA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA); ω3other, total omega-3 FA excluding EPA and DHA; ω6other, total omega-6 FA excluding ARA; ω3:ω6, omega-3 to omega-6 FA ratio. *P* values < 0.05 are boldfaced. Posthoc Tukey's honest significant difference test was applied for pairwise comparisons when nested ANOVA detected significant effects of site categories. Site category abbreviations: Ref, reference; NatBit, natural bitumen; MSE, sewage; MSE+OS, sewage+mining; Improv, improvement. N.A., not applicable.

Fatty acid	Factor	DF_{Effect}	DF_{Error}	*SS*	*F*	*p*	Tukey HSD	Model R^2
Ophiogomphus								
Log_{10}(ARA+EPA+DHA)	Category	4	36	0.691	13.354	**<0.001**	Ref > Improv = NatBit = MSE = MSE + OS	0.618
	Site[Category]	4	36	0.070	1.355	0.269		
Log_{10}(ω3other)	Category	4	36	0.762	12.378	**<0.001**	Ref ≥ Improv ≥ MSE ≥ MSE + OS = NatBit	0.625
	Site[Category]	4	36	0.176	2.864	**0.037**		
Log_{10}(ω6other)	Category	4	36	0.180	2.548	0.056	N.A.	0.301
	Site[Category]	4	36	0.096	1.358	0.268		
Log_{10}(ω3:ω6)	Category	4	36	0.444	8.369	**<0.001**	Ref ≥ NatBit = Improv ≥ MSE = MSE + OS	0.497
	Site[Category]	4	36	0.035	0.663	0.622		
Pteronarcys								
Log_{10}(ARA+EPA+DHA)	Category	4	25	0.080	2.577	0.062	N.A.	0.320
	Site[Category]	3	25	0.011	0.488	0.694		
Log_{10}(ω3other)	Category	4	25	0.465	1.558	0.216	N.A.	0.238
	Site[Category]	3	25	0.132	0.590	0.628		
Log_{10}(ω6other)	Category	4	25	0.131	0.416	0.795	N.A.	0.176
	Site[Category]	3	25	0.308	1.303	0.295		
Log_{10}(ω3:ω6)	Category	4	25	0.594	6.067	**0.002**	Ref = MSE = MSE + OS ≥ Improv ≥ NatBit	0.507
	Site[Category]	3	25	0.034	0.467	0.708		

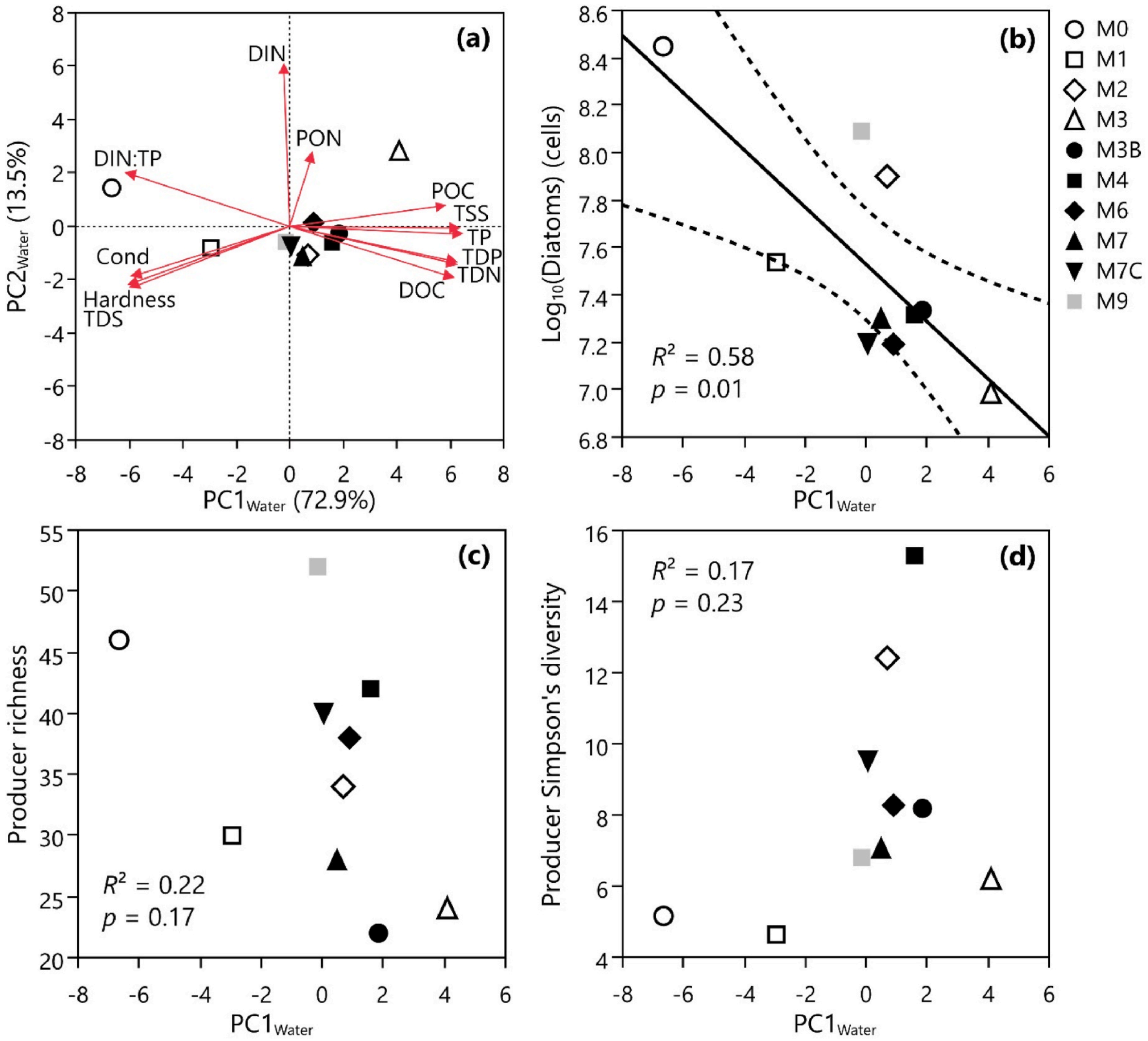

Fig. 3. (a) Principal component analysis (PCA) of water physicochemical characteristics of the study sites. All variables were $\log_{10}$-transformed before PCA. Cond, conductivity; Hardness, total hardness; DOC and POC, dissolved and particulate organic carbon respectively; DIN, dissolved inorganic nitrogen; PON, particulate organic N; TDN, total dissolved N; TDP, total dissolved phosphorus; TP, total P; DIN:TP, DIN to TP molar ratios; TDS, total dissolved solids; TSS, total suspended solids. Variance percentages explained by the PCA axes are indicated in parentheses. (b) Linear regression ± 95% confidence limits of benthic diatom abundance (cells) against the first principal component ($\text{PC1}_{\text{Water}}$) extracted from the PCA of water physicochemical characteristics of the study sites. No significant linear relationship was found for (c) overall species richness and (d) Simpson's diversity index of benthic primary producers with $\text{PC1}_{\text{Water}}$.

lower at sites with sewage+mining (Table S1; Fig. 3b).

Species richness and Simpson's diversity index of benthic primary producers were not associated with $\text{PC1}_{\text{Water}}$ scores (Fig. 3c,d). Producer richness was higher at the improvement and the reference sites, intermediate at sites exposed to sole natural bitumen and the sewage+mining sites, and lower at M3 and M3B that were closely downstream of the sewage discharge. Simpson's index was lower at M1 and M0, higher at M4 and M2, and intermediate at the other sites.

Concentrations of ARA+EPA+DHA and ω3other PUFA as well as the ω3:ω6 FA ratios of *Ophiogomphus* increased exponentially with increasing benthic diatom abundance across the sites (Table S4; Fig. 4a–c), illustrating the high nutritional quality of diatoms and their long-chain PUFA supply for *Ophiogomphus* across the food chains. A similar exponential relationship was found between benthic diatom abundance and *Ophiogomphus* ω6other PUFA concentrations (Table S4). These nonlinear models had a lower AIC_C than the linear models (Table S4), thus we interpreted that the nonlinear models could better capture the relationships between *Ophiogomphus* PUFA concentrations and benthic diatom abundance. Based on the exponential regression model, the ARA+EPA+DHA concentrations of *Ophiogomphus* increased from 3.76 mg g^{-1} at the MSE site (M3) to 8.95 mg g^{-1} (i.e., +138%) at the reference site (M0; outside of natural bitumen) across the diatom abundance gradient from 9.6×10^6 to 2.8×10^8 cells cm^{-2} (Fig. 4a). No significant relationship with benthic diatom abundance, neither linear nor nonlinear, was detected for ARA+EPA+DHA, ω3other, and ω6other PUFA concentrations, and for ω3:ω6 FA ratios of the ephemeropterans and *Pteronarcys* (all $F \leq 3.06$, all $p > 0.05$; Table S4).

Concentrations of ARA+EPA+DHA and ω3other PUFA as well as the ω3:ω6 FA ratios of *Ophiogomphus* also increased with increasing richness and decreasing Simpson's diversity index of benthic primary producers (Fig. S2a-f). Yet, these relationships were much weaker than the nonlinear relationships between *Ophiogomphus* PUFA and benthic diatom abundance (Fig. 4a–c). Site orders of *Ophiogomphus* PUFA across the gradients in producer richness and Simpson's index did not follow the spatial pattern in water quality (i.e., across $\text{PC1}_{\text{Water}}$; Fig. 3a). *Pteronarcys* and ephemeropterans PUFA were not associated with producer richness and Simpson's index (all $F \leq 2.73$, all $p > 0.05$).

Except for a positive linear relationship between *Pteronarcys* ARA+EPA+DHA concentrations and $\text{PC1}_{\text{Metal}}$ ($F_{1,31} = 5.90$, $p = 0.02$, $R^2 = 0.16$), no significant linear or nonlinear relationships were observed for

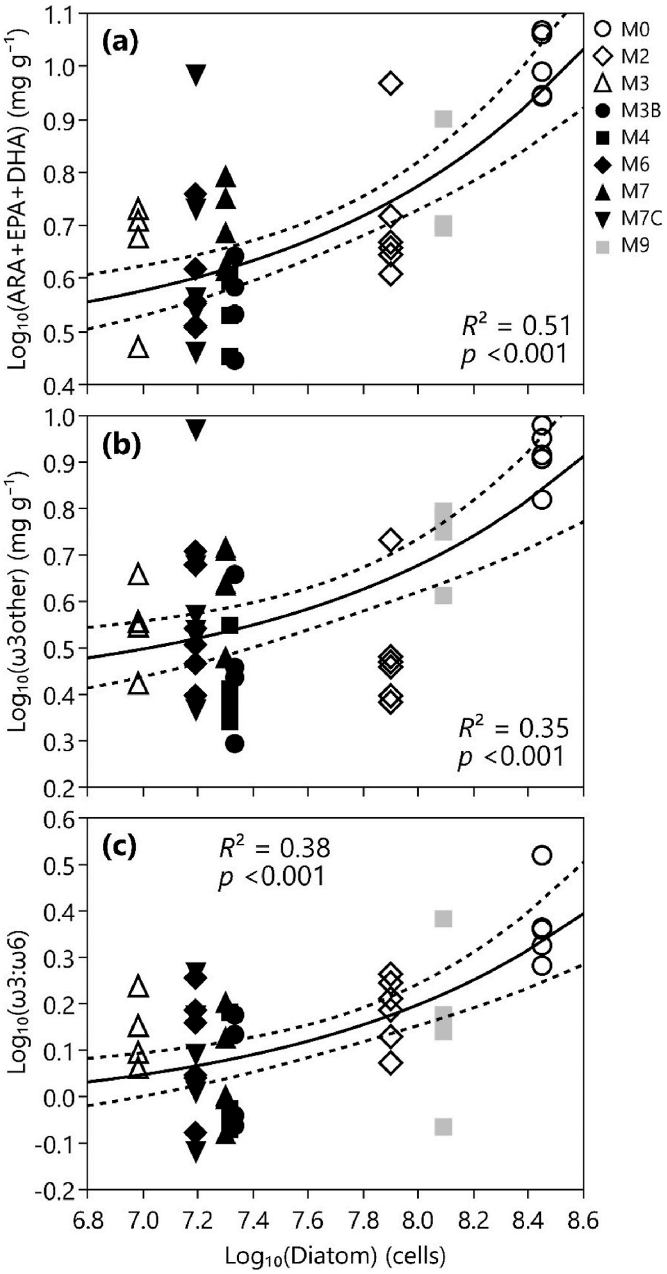

Fig. 4. Exponential regressions of *Ophiogomphus* fatty-acid (FA) concentrations (mg FA g^{-1} dry mass) against benthic diatom abundance (cells) at the study sites. (a) Sum of arachidonic acid (ARA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA); (b) total omega-3 FA excluding DHA and EPA; (c) omega-3 to omega-6 FA ratio. Dotted lines indicate 95% confidence limits of the regression models.

the PUFA concentrations and ω3:ω6 FA ratios of *Ophiogomphus*, *Pteronarcys*, and the ephemeropterans with $PC1_{PAC}$ or $PC1_{Metal}$ (all $F \leq 2.71$, all $p > 0.05$).

4. Discussion

Benthic macroinvertebrate FA compositions showed taxon- or feeding guild-specific changes at sites exposed to natural bitumen and anthropogenic activities, namely sewage discharge and oil sands mining. Changes in nutritional quality indicated by the long-chain PUFA concentration in tissues were more apparent in the dragonfly predator, *Ophiogomphus,* and ephemeropteran grazers/collector-filterers than in the shredder *Pteronarcys.* Nevertheless, the responses of *Ophiogomphus* and ephemeropterans were not unidirectional, as exposure to natural bitumen alone or together with sewage discharge and mining activity was associated with lower nutritional quality of *Ophiogomphus*, with the lowest PUFA concentrations of this dragonfly predator observed at sites closely downstream of the sewage discharge. In contrast, exposure to natural bitumen alone did not result in substantial changes in FA composition in ephemeropterans. However, in the additional presence of sewage discharge, the ephemeropterans generally appeared more PUFA-enriched. These results suggest that the FA composition of ephemeropterans is a useful indicator for tracking impacts of sewage contamination, while that of *Ophiogomphus* can reflect the gradual changes in nutritional quality of the benthic food chains associated with a cumulative response to mainly natural bitumen exposure and sewage, and to a lower degree to mining. Overall, sewage contamination had larger impacts on the FA compositions of these benthic macroinvertebrate taxa than that attributed to natural bitumen or oil sands activity. These findings suggest that the assessment of FA metrics across multiple functional feeding guilds and trophic levels can support a more holistic ecological impact assessment of multiple stressors (Lau et al., 2017, 2021, 2022; Keva et al., 2021).

The FA compositions of ephemeropterans showed increased bottom-up support from algae at sites with sewage discharge and oil sands mining. This support likely originated from benthic diatoms that predominated the primary producer community, but it might also be related to the increased amounts of suspended algae at these sites (Culp et al., 2020). In comparison to the upstream sites without or with natural bitumen exposure (i.e., M0 and M1, respectively), the low benthic diatom abundance at sites closely downstream of the sewage discharge was associated with high nutrient levels, high suspended chlorophyll *a* (i.e., an indicator of potamoplankton biomass) and increased TSS concentrations (see Culp et al., 2020). Higher suspended algal biomass and TSS can promote light attenuation (Rügner et al., 2013), with such reductions in light availability enhancing the predominance of diatoms and the long-chain PUFA availability in benthic algae (Guo et al., 2015), although severe light attenuation can limit benthic algal development in shallow habitats (Diehl et al., 2018; Burrows et al., 2021). In addition, benthic algal growth in northern high-latitude freshwater ecosystems is more frequently limited by N than by P (Diehl et al., 2018; Myrstener et al., 2018; Fork et al., 2020), and the sewage-exposed sites also had lower water DIN:TP ratios, implying their stronger N-limitation for algal development. Thus, the observed lower abundance of benthic diatoms at these sites appears to be a consequence of increased light- and N-limitation. Ephemeropterans generally contain high concentrations of long-chain PUFA, particularly EPA (Lau et al., 2012, Vesterinen et al., 2021). However, there was no evident positive correlation between the ARA+EPA+DHA concentrations of ephemeropterans and the benthic diatom abundance in this study. We conjecture that in addition to benthic diatoms, ephemeropterans assimilated potamoplankton and sloughed algal material that had settled on the river bottom, leading to the elevated concentrations of long-chain PUFA in ephemeropterans at sites below the sewage discharge. Although further investigation is needed to elucidate the relative contributions of long-chain PUFA from suspended and benthic algae to the ephemeropterans, it is clear that the long-chain PUFA in ephemeropterans responded positively to mainly sewage-induced nutrient enrichment.

In contrast to the FA patterns in larval mayflies, the dragonfly *Ophiogomphus* larvae had lower long-chain PUFA (i.e., ARA+EPA+DHA) concentrations at sites with sewage (M3) and sewage+mining (M3B-M7C) where benthic diatom abundance was reduced. Although mayflies were available to supply *Ophiogomphus* larvae with long-chain PUFA from benthic algae and associated materials in this river reach, the net spinning filter feeders, *Cheumatopsyche* and *Hydropsyche,* showed greatly reduced abundances at these impaired sites relative to the upstream reference site (Culp et al., 2020). Previous studies have shown that larval caddisflies and mayflies have similar FA compositions (Torres-Ruiz et al., 2007; Lau et al., 2012, Vesterinen et al., 2021). It appears that the trophic transfer of long-chain PUFA to the dragonfly predator was effected at these impaired sites by other benthic primary consumers. Our results suggest that while net-spinning caddisfly larvae likely were a major prey of *Ophiogomphus* larvae at the upstream sites, their PUFA supply to *Ophiogomphus* declined at sites affected by sewage discharge due to their lower abundances. Moreover, there were greater abundances of pollution-tolerant taxa at the sewage and sewage+mining sites, such as oligochaetes and chironomid larvae that generally have lower long-chain PUFA concentrations than larval caddisflies (Torres-Ruiz et al., 2007; Culp et al., 2020; Vesterinen et al., 2021). Increased compensatory feeding on oligochaetes and chironomid larvae thus is also a plausible explanation for the observed lower long-chain PUFA concentrations of *Ophiogomphus* at the sites with sewage discharge. Future FA based assessments can benefit from incorporating additional functional feeding groups (e.g., filter feeders) and the use of mixing models (e.g., fastinR; Neubauer and Jensen, 2015) to quantify the shifts in trophic transfer pathways caused by the stressors.

Ophiogomphus PUFA were associated more strongly with benthic diatom abundance than with the diversity metrics of overall primary producers. Species of diatoms consistently have higher concentrations of long-chain PUFA, such as EPA, than do those of chlorophytes and cyanobacteria (Napolitano, 1999; Taipale et al., 2013). It is thus reasonable that diatom abundance was more important than overall primary producer diversity for the long-chain PUFA transfer in the benthic food chain. The benthic diatom abundance and the ARA+EPA+DHA concentrations of larval *Ophiogomphus* decreased by >70% and >50%, respectively, at the reference site with exposure to natural bitumen (M2) compared to that without (M0). These decreases were probably linked to the effects of natural bitumen on water quality instead of on contaminants, as there were no apparent differences in sediment PAC and metal concentrations between M0 and M2. The sites with most intense mining activity (M3B–M6) also had similar sediment PAC and metal concentrations as did M0 and M2, reflecting that the further decreases in diatom abundance and long-chain PUFA concentrations of *Ophiogomphus* at M3B–M6 were similarly explained by the reduced water quality, but not by contaminants from oil sands mining. Nonetheless, the additive effects of mining activity to natural bitumen exposure were subtle and likely masked by the sewage-induced nutrient enrichment. The sediment PAC and metal concentrations at sites with mining activity were also below thresholds from sediment quality guidelines (MacDonald et al., 2000; Culp et al., 2020). Our results thus provide evidence to support that sewage discharge was the predominant driver of the observed changes in FA composition and nutritional quality of benthic macroinvertebrates in the lower Athabasca River.

Long-chain PUFA are required for fish to maintain good health (Sargent et al., 1995), and riverine fish predators are often dependent on benthic macroinvertebrates for long-chain PUFA (Guo et al., 2022). Although there is potential for lower PUFA per unit benthic macroinvertebrate biomass to negatively affect fish health, previous studies found that fish downstream of the sewage discharge were larger relative to upstream fish (Arciszewski and McMaster, 2021; Culp et al., 2021). We hypothesize that fish downstream of the sewage effluent were able to offset the lower PUFA per unit biomass due to increased food availability, as the macroinvertebrate biomass below the sewage discharge was greater than twice of that at upstream locations (Culp et al., 2020). Hence, findings from this study and literature together suggest that sewage discharge could affect fish health through benthic diatom development and the trophic transfer of long-chain PUFA, though this requires verification through further study.

We did not measure the FA responses of benthic primary producers, due to insufficient producer biomass sampled. The FA composition and long-chain PUFA of benthic primary producers can have strong effects on those of the benthic macroinvertebrates and fish in temperate rivers (Guo et al., 2022), and are subject to change by nutrient stressors via their effects on the producer assemblages (Cashman et al., 2013; Whorley and Wehr, 2018). The invertebrate FA responses observed in this study thus could alternatively be attributed to the stressor-induced changes in assemblage and FA composition of benthic primary producers. Also, the interspecific differences in FA composition of biota are generally greater than the intraspecific differences (e.g., across space and time) (Lau et al., 2012; Taipale et al., 2013; Vesterinen et al., 2021), meaning that the benthic macroinvertebrates can have taxon-specific requirements and selection of dietary FA. A possible limitation of the FA based assessment approach thus is that the FA of benthic macroinvertebrates may not be tightly linked to the assemblages or overall FA of available food sources, because of the selective feeding and/or assimilation by the invertebrates (Grieve and Lau, 2018). In addition, FA in consumers reflect assimilation but not ingestion. The FA metrics therefore may be insufficient to indicate the ecological functions (e.g., leaf-litter breakdown) of certain taxa, such as the shredders, that ingest, but not necessarily assimilate the terrestrial organic matter (Guo et al., 2016; Grieve and Lau, 2018).

5. Conclusions

Overall, our results showed that the declines in water quality (increases in nutrient concentrations and total suspended solids) and benthic diatom abundance in the lower Athabasca River were driven mainly by the sewage discharge, and to a lesser extent by the exposure to natural bitumen or oil sands mining activity. The responses of benthic macroinvertebrates in terms of nutritional quality (tissue PUFA concentrations) were taxon- and stressor-specific. Yet, responses of the predatory *Ophiogomphus* larvae were tightly coupled with the abundance of benthic diatoms, and these larvae were lower in PUFA at sites with sewage discharge. Our study provided evidence that the negative effects of decreased water quality had propagated across the benthic food chains, and that FA metrics are useful to untangle the effects of concurrent stressors on individual trophic levels. Structural diversity metrics of benthic primary producers (this study) and macroinvertebrates (Culp et al., 2018) were insufficient to indicate stressor-induced changes in water quality. Fatty-acid metrics can indicate alterations in trophic pathways and consumer nutritional quality, and complement structural assessments to evaluate the ecological impacts of multiple stressors.

Author contributions

Danny C. P. Lau: Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. Robert B. Brua: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing – review & editing. Willem Goedkoop: Conceptualization, Investigation, Methodology, Resources, Writing – review & editing. Joseph M. Culp: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing – review & editing.

Data statement

Raw data of water chemistry, sediment contaminants, and benthic diatom abundance, as well as a summary of the fatty-acid data that support the findings of this study are provided in the supplementary information. Raw fatty-acid data are available from the corresponding author upon reasonable request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

Technical field and laboratory support was provided by Nancy Glozier, Daryl Halliwell, Eric Luiker, and Allison Ritcey. We thank Jana Pickova for laboratory support in FA analyses. Research funding was provided by and contributes to the Joint Oil Sands Monitoring Program co-led by the Governments of Canada and Alberta but does not necessarily reflect the position of the Program. We also thank the anonymous reviewers for their comments that helped improve an earlier version of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2023.122598.

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