MHTWG - Pelagic DO Study

Particulate Organic Matter - Source, Composition and Degradation (Objective 2)

Ross, D.J.[1]; Revill, A.T.[2]; Bodrossy, L.[2] and Nichols, P.D.[2]

[1]Institute for Marine and Antarctic Studies, UTAS, Hobart

[2]CSIRO Oceans and Atmosphere, Hobart, Tasmania

September 2016

Prepared for the Tasmanian Salmonid Growers Association

Institute for Marine and Antarctic Studies, University of Tasmania, Private Bag 49, Hobart TAS 7001

Enquires should be directed to:
Dr Jeff Ross
Institute for Marine and Antarctic Studies
University of Tasmania
Private Bag 49, Hobart, Tasmania 7001, Australia
Jeff.Ross@utas.edu.au
Ph. (03) 6226 8281
Fax (03) 6226 8035

Contents

1 EXECUTIVE SUMMARY

The combined biomarker approach (C:N, isotopes, fatty acids and genomics) provided valuable insight into the source, composition and transformation of organic matter in the harbour. Elevated concentrations of the bulk organic matter properties in the river samples are consistent with a significant terrestrial/riverine source of particulate organic carbon and nitrogen to the harbour; further supported by the relatively depleted C isotope signature of particulate organic matter in harbour surface waters. However, it is well recognised that a large fraction of catchment derived organic material is likely to be refractory. Comparison of fatty acid profiles of the organic matter in the harbour and key external sources (e.g. river, ocean and aquafeed) confirmed a lower proportion of the fatty acids indicative of more labile and reactive organic material in river samples.

A key finding from the fatty acid and genomic analyses was the significance of organic material produced internally in the harbour water column via microbial production. This has important implications for our current understanding of carbon, nitrogen and oxygen dynamics in the harbour. The prevalence of chemolithoautotrophs (nitrifying bacteria and archaea), that create organic matter using chemical rather than light energy, provides a new source of carbon that will lead to an oxygen demand when mineralised. In the case of these nitrifying organisms, ammonium is the source of energy used to fix carbon (CO_2), and our genomic results demonstrate that the distinct physiochemical gradients of Macquarie Harbour influence the distribution and abundance of these microorganisms. Bacterial nitrifiers were more common in the surface waters and ubiquitous throughout the water column compared to archaeal nitrifiers that were far more common at depth. Comparisons with nitrification rate measurements points to a more active role for the archaeal nitrifiers at depth, however, more work is required to confirm this relationship. Either way, the prevalence of nitrifiers in the water column, their conversion of ammonium to nitrate and consumption of oxygen clearly has direct implications for nitrogen cycling and oxygen dynamics.

A key objective of this study was to try and use biomarkers to identify and track the influence of aquafeed waste in the water column. The concentration of linoleic acid and oleic acid and their contribution to the fatty acid profile provided a clear signature of aquafeed waste in the environment, however, only in the cage itself. At the closest distance sampled to stocked cages, 100m, there was no detectable aquafeed fatty acid signature. This is perhaps not that surprising when considering the highly diffusive nature of the water column, the relatively low stocking densities at the time of sampling and the difficulty in matching sampling with

feeding and fish extraction. The highly labile nature of aquafeed further adds to this challenge. It is also important to consider that indirect pathways i.e. emissions of dissolved inorganic nutrients (e.g. ammonium) and the subsequent incorporation into organic matter would not be detected using fatty acids. Given the likely importance of nitrifying organisms in the harbour, future work should consider measuring the N isotope of ammonium to trace this pathway. In this study we did intend to measure the isotopic signature of particulate organic nitrogen, but unfortunately concentrations were too low and not amenable to analysis.

We recommend that future sampling focus on the peak stocking period in summer and in closer proximity to the cages (e.g. 0-100m). This would provide greater sensitivity in determining the water column footprint of aquafeed waste. Concurrent sampling more broadly across the harbour and from the key external sources of organic matter, would provide a valuable addition to this study given that the current conclusions on the major sources, composition and transformation of organic matter are limited to the one survey in April 2016. This study highlighted the significant role that microbes are likely to be playing in system dynamics and as such, understanding their phenology (e.g. life cycle and environmental influences) and role in the harbour should be an important focus of future research.

2 BACKGROUND

Monthly monitoring of water quality in Macquarie Harbour has highlighted that nitrate (NO_3^-) concentrations are approaching the established compliance level (at the surface) set by DPIPWE. Dissolved oxygen (DO) in waters below the halocline has also been observed to decrease since 2009, which coincides with the expansion of fish cage aquaculture.

Fish cage aquaculture can influence water column DO through the microbial processing interacting with fish feed and fish wastes via aerobic respiration (using DO to break down organic matter) and nitrification (using DO to convert ammonia to nitrate). Together these two processes contribute to the loss of DO in the water column that we refer to as pelagic oxygen demand (POD).

The availability of labile organic matter and the presence of microbial organisms are fundamental to these processes. This report describes the findings of the biomarker (C:N, isotopes, fatty acids and genomics) study carried out to identify the source and composition of particulate organic matter, and the presence and abundance of the key microorganisms likely to be responsible for nitrification in the Macquarie Harbour water column.

This report encompasses Objective 2 of the larger MHTWG MH Pelagic DO Study. Objectives 1 & 3 measured POD and nitrification in the water column of Macquarie Harbour and the results are covered in a separate MHTWG Pelagic DO Report produced by ADS (MHTWG MH Pelagic DO Study, Objective 1 &3, 2016). That report complements this report and should be considered concurrently.

3 PARTICULATE ORGANIC MATTER - SOURCE, COMPOSITION AND DEGRADATION

3.1 INTRODUCTION

The availability of labile organic matter is fundamental to a variety of heterotrophic processes such as oxygen respiration, denitrification, sulphate reduction and methogenesis. As such, identifying the origin, composition and abundance of the organic matter in Macquarie Harbour is critical to understanding the drivers of oxygen demand and nutrient transformation in the water column.

Although the pool of organic matter in systems like Macquarie Harbour is no doubt large, a significant fraction is likely to be refractory and mostly unreactive. Thus, only a proportion of the total organic matter supports the heterotrophic process such as those consuming oxygen. Furthermore, there are also likely to be range of sources of organic matter, including terrestrial organic matter delivered from the surrounding catchments, organic matter produced within the rivers entering the Harbour, marine-derived organic matter entering through Hells Gates. Organic matter produced internally via phytoplankton and microbial production may also be important in the Harbour and of course, organic matter inputs via aquaculture is likely to be significant.

To understand the relative importance of the sources of particulate organic matter in Macquarie Harbour we used multiple indicators; fatty acids, stable isotopes and C:N ratios. This suite of indicators is commonly used to evaluate the origin and nature of organic matter, see review by Volkman & Tanoue (2002) and applications in San Francisco Bay (Canuel et al., 1995) and Chesapeake Bay (Canuel and Zimmerman 1999), and has been successfully applied to Macquarie Harbour sediments. To identify the microorganisms present in the water column and those likely to be responsible (e.g. sulphate reducing bacteria, nitrifying and denitrifying bacteria, methanogenic archaea) for the transformation of the organic matter, consumption of oxygen, and the cycling of key elements such as nitrogen, the genomic DNA of collected samples was also analysed.

Fatty Acids

Fatty acids (lipids) are long-chain hydrocarbons capped by a carboxyl group (COOH) and are a necessary component in the diet of all animals. Whilst they do occur naturally in the

environment, they are also an essential additive in fish feed and as such can be a valuable tool in ecological studies. Such an approach has been used both in Tasmania and overseas to identify the presence of material derived from fish farming (Macleod et al., 2004, Holdsworth et al., 2008, Black et al., 2012). Fatty acids from terrestrial oil and meal sources used in aquafeeds have a unique signature in the marine environment, with a fatty acid profile dominated largely by C_{18} components including linoleic acid (LA; 18:2w6) and oleic acid (OA; 18:1w9) (Nichols et al., 2014). In contrast, natural marine ecosystems are dominated by long-chain ($\geq C_{20}$) omega-3 polyunsaturated fatty acids (w3 LC-PUFA), such as eicosapentaenoic acid (EPA; 20:5w3) and docosahexaenoic acid (DHA; 22:6w3), which are produced in substantial quantities by marine phytoplankton (Dalsgaard et al., 2003). Groups of compounds and fatty acid ratios can also be used to indicate the presence and/or sources of certain types of organisms or biological material. For example, bacteria specifically synthesise a series of odd-carbon number C15-C17 and 18:1w7 fatty acids and their sum can be used to trace their presence in aquatic systems (e.g. Dalsgaard et al., 2003, Parrish et al., 2005). As such, the unique chemical structures of fatty acids, when identified and viewed as a profile, can be used to help delineate the major sources and the transfer of organic material through food webs (Parrish et al., 2015).

Stable Isotopes and C:N ratios

Stable isotope analysis can similarly be used to identify sources of organic material and how they might be transferred through the food chain. All biologically active elements exist in a number of different isotopic forms, with some being more stable than others. For example, most carbon is present as ^{12}C, with approximately 1% being ^{13}C. The ratio of these two isotopes may be altered by biological processes, and when isotopic information in organic matter pools and biota is available is possible to draw direct inferences regarding the sources and pathways of organic matter. For example, the $\delta^{13}C$ value of terrestrial organic matter (25 to -33‰) and freshwater phytoplankton (-25 to -30‰) is typically more depleted compared with marine particulate organic matter (-22 to -18‰) (see Middelburg and Nieuwenhuize, 1998). Similarly, marine organic matter usually has $\delta^{15}N$ values of 5 - 7‰ indicating it is derived from phytoplankton, whereas terrestrial organic matter generally has $\delta^{15}N$ values < 4‰ (see Middelburg and Nieuwenhuize, 1998). Stable isotope analysis of Macquarie Harbour sediments revealed that sites in the southern part of the harbour generally showed relatively depleted $\delta^{13}C$ and $\delta^{15}N$ values, consistent with higher levels of terrestrial or freshwater inputs, whereas sites closer to Hells Gates (and the marine influence) were more enriched in both $\delta^{15}N$ and $\delta^{13}C$ (Ross et al., 2016). The C:N ratio of organic matter can also be used to infer its likely source and C:N ratios have also been used as a proxy for carbon quality with lower ratios indicating increased organic matter lability. Terrestrial organic matter

generally has a high C:N (>20) compared to marine organic matter (phytoplankton ~6.6). In Macquarie Harbour sediments, the C:N ratio of sediment organic matter showed a similar estuarine gradient; higher ratios (>20) were generally found at those sites closer to terrestrial inputs from the Gordon and King river, with ratios decreasing (<20) as you move towards the marine influence at Hells Gates. The influence of farming could also be seen superimposed on the estuarine gradient. Sediment samples collected in leases often had more enriched $\delta^{15}N$ and $\delta^{13}C$ values and the C:N ratio was lower relative to the background harbour signature and typical of fish feed (Crawford et al., 2003; Chen et al., 2003; Wang et al., 2013), however, the strength of the signal appeared to be dependent on farm history and intensity (Ross et al., 2016).

Genomics

Given the importance of nitrogen in estuarine and coastal waters the study focused on the genes that are markers for the microorganisms that can carry out important nitrogen cycling processes. In Macquarie Harbour, the particular interests are in the microorganisms associated with nitrification (the oxidation of ammonia to nitrite and nitrate) due to their potential influence on both dissolved nitrogen and oxygen concentrations. Ammonia oxidisation is controlled by the enzyme ammonia monooxygenase which is partly encoded by the *amoA* gene, and thus, the *amoA* gene is often used as the molecular marker for ammonia-oxidising microorganisms in the environment (e.g. Zhang et al. 2015). Previous studies have shown that ammonia-oxidising bacteria (AOB) and ammonia-oxidising archaea (AOA) are potential contributors to nitrification and that environmental factors play a key role in driving shifts in their abundance and community composition (e.g. Bollmann and Laanbroek 2002, Francis et al., 2007). As such, the distinct physiochemical gradients of Macquarie Harbour have the potential to influence the distribution and abundance of these microorganisms and in turn, their potential contribution to nitrification and oxygen consumption. In subsequent reporting, tag sequencing will be used to look more broadly at microbial genetic diversity through the water column at the study sites.

3.2 METHODS

Samples for particulate organic matter analysis came from the carboys of site water collected for POD during campaign 3, April 19-21 2016. For a more detailed description of the collection method and study design, see MHTWG MH Pelagic DO Study (Objectives 1 & 3) 2016. Suspended particulate matter (SPM) was measured for all POD sites/depths, and all other indicators (fatty acids, stable isotopes, C:N ratios and genomics) were measured from

all sites/depths on the southern transect, and from the surface sample (typically 3m) only at all other sites (see Table 1).

Water samples were mixed well and poured through a filter (47 mm GF/F filters) using a vacuum pump to collect as much material as possible. Three separate filters were required per sample i.e. SPM, fatty acids and isotopes (particulate organic carbon and nitrogen are also measured on this filter). Once the filter was clogged with particulate matter, the volume filtered was recorded (typically 1-5 l), the filters were folded in half (sediment on the inside) and placed in the muffled aluminium foil cups and frozen prior to analysis. For genomics the water was pumped through a 0.22 μM Sterivex filter using a peristaltic pump to collect all microorganisms. The filter was wrapped in foil and placed in a zip lock plastic bag and immediately frozen.

3.2.1 Suspended Particulate Matter

The filters were dried for 24 -48 hours at 500C and the weighed. SPM concentration (l^{-1}) was determined by subtracting the original filter weight from the combined filter-particulate weight, and dividing by the volume of sample filtered.

3.2.2 Isotopes, C and N content

Nitrogen and carbon contents and $\delta^{15}N$ and $\delta^{13}C$ of the filtered particulate matter were analysed using a Carlo Erba NA1500 CNS analyser interfaced via a Conflo V to a Thermo Scientific Delta V Advantage isotope ratio mass spectrometer operating in the continuous flow mode. Combustion and oxidation were achieved at 1090°C and reduction at 650°C. Samples were analysed at least in duplicate and values corrected via a multi-point correction curve based on international standards. %C and %N were calculated based on the response of the mass spectrometer using a standard calibration curve. Results are presented in standard δ notation:

$$\delta\ (‰) = [(Rsample/Rstandard) - 1]\ 1000$$

Where:

Rsample and Rstandard are the relevant isotopic ratios measured in the sample and standard. The standard for carbon is Vienna Pee Dee Belemnite (VPDB) and for nitrogen is Air.

3.2.3 Fatty Acids

The fatty acid composition of the filtered particulate matter was determined using gas chromatography (GC), with component confirmation by GC-mass spectrometry (GC-MS). All samples were analysed following the direct transmethylation technique outlined in Parrish et

al (2015). Sediment was homogenised and approximately 0.25 g of freeze dried sediment was weighed into pre-weighed and tared glass tubes. All tubes were reweighed and samples directly transmethylated in methanol: dichloromethane: concentrated hydrochloric acid (10:1:1 v/v). Samples were kept for 2 hrs at 80°C, before tubes were allowed to cool and 1 ml of Milli-Q® water added, along with 1.8 ml hexane:dichloromethane (4:1 v/v). Tubes were then vortexed and centrifuged at 2000 rpm for 5 minutes to break phase, with the upper, organic layer removed. This step was repeated twice. The organic layer was reduced under a stream of nitrogen gas and chloroform, with a known concentration of internal injection standard (19:0 FAME) was added.

FAME samples were analysed using an Agilent Technologies (Palo Alto, CA, USA) 7890B GC equipped with a non-polar Equity™-1 fused silica capillary column (15 m x 0.1 mm internal diameter and 0.1 μm film thickness). Samples (0.2 μl) were injected at an oven temperature of 120°C with helium as the carrier gas. The oven temperature was raised to 270°C at a rate of 10°C per minute, then to 310°C at 5°C per minute. Peaks were quantified using Agilent Technologies ChemStation software, with initial identification based on comparison of retention times with known and laboratory standards. Confirmation of component identification was performed by GC-MS of selected samples and was carried out on a ThermoScientific 1310 GC coupled with a TSQ triple quadrupole. Samples were injected using a Tripleplus RSH auto sampler with a non-polar HP-5 Ultra 2 bonded-phase column (50 m x 0.32 mm i.d. x 0.17 μm film thickness) used. The HP-5 column was of similar polarity to the column used for GC analyses. The initial oven temperature of 45°C was held for 1 min, followed by temperature programming at 30°C per min to 140°C then at 3°C per min to 310°C where it was held for 12 min. Helium was used as the carrier gas. Mass spectrometer operating conditions were: electron impact energy 70 eV; emission current 250 μA, transfer line 310°C; source temperature 240°C; scan rate 0.8 scan/sec and mass range 40-650 Da. Mass spectra were acquired and processed with Thermo Scientific XcaliburTM software (Waltham, MA, USA).

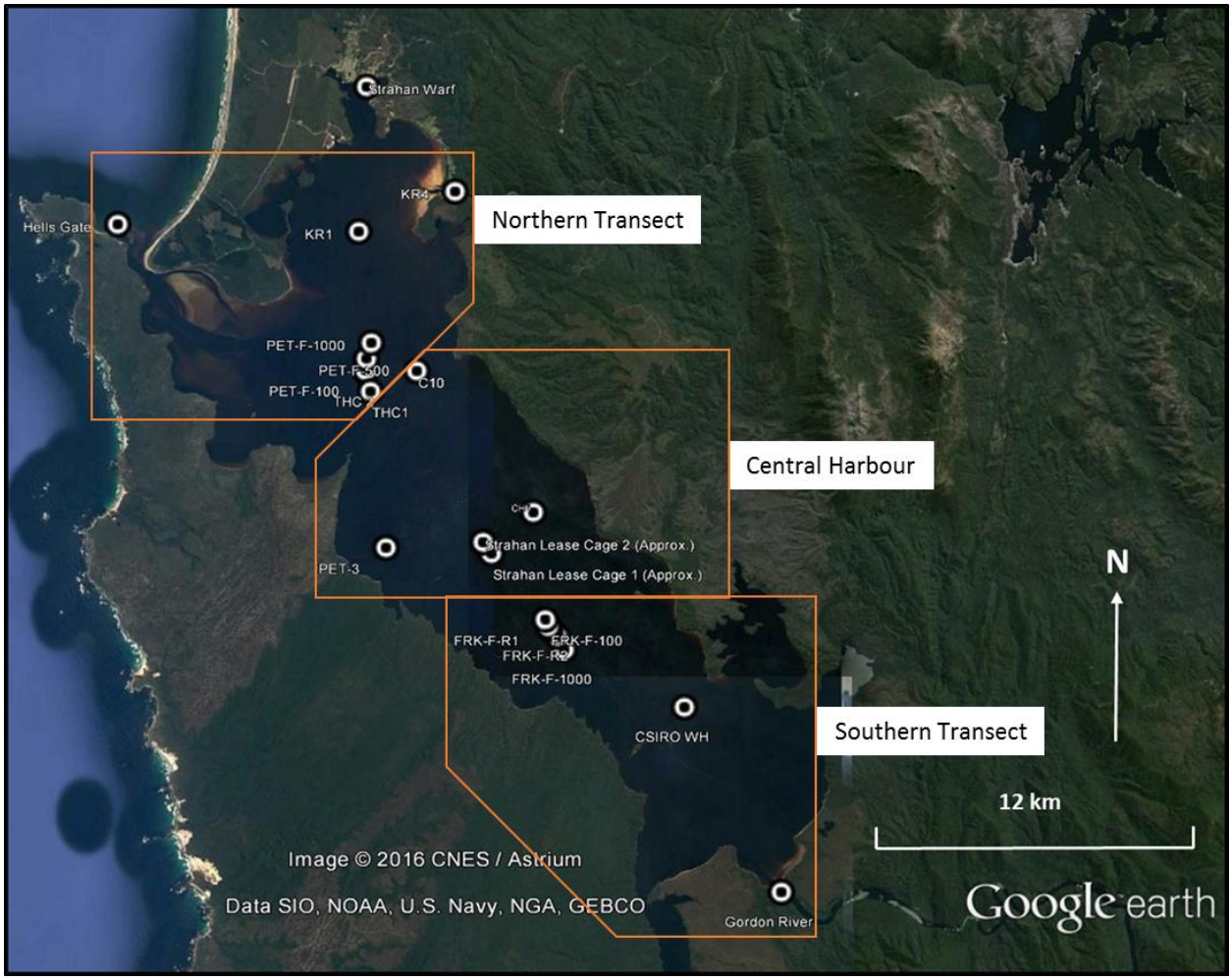

Figure 1 Map of Macquarie Harbour and Sampling Sites

Table 1 Sampling Sites, Depths, Dates, and Locations

Region	Site	Site Abbriv.	Longitude	Latitude	Number of Depths	Specific Depths Sampled	Dates Incubated
Northern Transect	Table Head Central Lease	PET-F-R1	145°19'37.73"E	42°15'47.19"S	3	3m, 8m, 20m	21/04/2016
	Table Head Central Lease	PET-F-R2	145°19'30.05"E	42°15'22.98"S	4	3m, 8m, 20m, 36m	21/04/2016
	Petuna Transect 1 (100m)	PET-F-100	145°19'29.67"E	42°15'20.36"S	4	3m, 8m, 20m, 38m	21/04/2016
	Petuna Transect 2 (500m)	PET-F-500	145°19'30.99"E	42°15'05.87"S	4	3m, 8m, 20m, 28m	21/04/2016
	Petuna Transect 3 (1000m)	PET-F-1000	145°19'39.10"E	42°14'45.60"S	3	3m, 8m, 15m	21/04/2016
	King Basin	KR1	145°19'18.11"E	42°12'24.42"S	4	3m, 8m, 20m, 32m	21/04/2016
	Hells Gates	HG3	145°12'38.89"E	42°12'14.82"S	1	5m	20/04/2016
	King River	KR4	145°21'57.96"E	42°11'33.84"S	1	1m	21/04/2016
Central Harbour Sites	Strahan Lease	STR-F-R1	*145°21'57.13"E	* 42°18'08.68"S	4	3m, 8m, 20m, 40m	20/04/2016
	Strahan Lease	STR-F-R2	*145°22'03.09"E	*42°18'14.42"S	4	3m, 8m, 20m, 36m	20/04/2016
	Central Harbour	C10	145°20'54.37"E	42°15'21.35"S	4	3m, 8m, 20m, 40m	20/04/2016
	Central Harbour	CH5	145°24'06.82"E	42°18'19.93"S	4	3m, 8m, 20m, 36m	20/04/2016
	PET3	PET 3	145°20'03.37"E	42°19'04.38"S	3	3m, 8m, 15m	20/04/2016
Southern Transect (POD)	Franklin Lease in Farm	FRK-F-R1	145°24'26.29"E	42°20'34.87"S	4	3m, 8m, 20m, 30m	19/04/2016
	Franklin Lease in Farm	FRK-F-R2	145°24'30.78"E	42°20'43.17"S	4	3m, 8m, 20m, 28m	19/04/2016
	Franklin Transect 1 (100m)	FRK-F-100	145°24'33.20"E	42°20'46.35"S	4	3m, 8m, 20m, 28m	19/04/2016
	Franklin Transect 2 (500m)	FRK-F-500	145°24'46.69"E	42°20'56.22"S	4	3m, 8m, 20m, 27m	19/04/2016
	Franklin Transect 3 (1000m)	FRK-F-1000	145°24'57.43"E	42°21'12.49"S	4	3m, 8m, 20m, 28m	19/04/2016
	Gordon River	GR 1	145°30'54.27"E	42°26'15.56"S	2	2m, 8m	19/04/2016
	World Heritage Site	CSIRO WH	145°28'15.60"E	42°22'24.72"S	4	3m, 8m, 20m, 30m	19/04/2016

3.2.4 Analysis of Gene Abundance

Quantitative PCR (qPCR) was performed as previously described (Abell et al. 2013), with bacterial and archaeal *amoA*, *nosZ*, and bacterial 16S rRNA genes quantified from DNA extractions using a 7500 real-time PCR system (Applied Biosystems, Carlsbad, CA). The nosZ gene was included because it is a marker gene for denitrification and bacterial 16S represents total bacteria. PCR reactions (15 μL) comprised 10 μg/μL BSA, 10 pmol of each primer, 7.5 μL of Q-PCR master mix (Applied Biosystems) and 1 μL of template DNA (comprising ~10–20 ng of DNA). Fluorescence acquisition was performed at the temperatures indicated for each gene in Table 2, at which temperature all primer dimers had melted, but specific products had not. The cycling conditions were 95 °C for 3 min, followed by 40 cycles of 40 s at 95 °C, 30 s at the specific annealing temperature followed by 1 min at 72 °C and then 20 s at the appropriate acquisition temperature (Table 2). Standards for qPCR were generated by serial dilutions of known copies of PCR fragments of the respective functional gene generated using M13 PCR from clones generated during the present and previous studies. Samples were assayed as previously described (Abell et al. 2010) to ensure that there was no significant effect of PCR inhibition on the qPCR data.

Table 2 PCR primers and PCR conditions used in this study. T_{Ann} denotes the annealing temperature used and T_{Acq} denotes the temperature at which fluorescence acquisition was performed during qPCR.

Primer	Target gene	Sequence (5′-3′)	T_{Ann}/T_{Acq}
Arch amoA-1F	*amoA* AOA	STAATGGTCTGGCTTAGACG	56/78
Arch amoA-	*amoA* AOA	GCGGCCATCCATCTGTATGT	56/78
amoA-1F	*amoA* β-AOB	GGGGTTTCTACTGGTGGT	55/78
amoA-2R	*amoA* β-AOB	CCCCTCKGSAAAGCCTTCTTC	55/78
nosZF	*nosZ*	CCTAYTGGCCGCCRCART	55/82
nosZ1622R	*nosZ*	CGSACCTTSTTGCCSTYGCG	55/82
27f	Bacterial 16S rRNA	AGAGTTTGATCCTGGCTCAG	55/76
1492r	Bacterial 16S rRNA	GGTTACCTTGTTACGACTT	55/76

3.3 RESULTS

To assist with interpretation of the data with respect to the key questions, namely how does the composition of particulate organic matter vary with respect to the major external sources (aquaculture, river and ocean) and according to depth in the harbour, the data is pooled and presented according to distance from the aquaculture cage and water depth. The full data sets (i.e. at the individual site level) are presented in Appendix A[1].

[1] Appendix figures are denoted with 'A' proceeding the figure number i.e. Figure A1

3.3.1 Suspended Particulate Matter: Bulk Properties

Suspended particulate matter (SPM) concentrations varied between sites and across depths (Figure 2, Figure A1). In the surface samples (3 m) SPM concentrations were clearly higher in the river samples and lower in the ocean sample (Figure 2). There was no clear evidence that SPM concentrations at the cage sites were higher relative to the other harbour sites at the time of sampling; the exception was at cage 1 in the Franklin lease (Figure A1). Overall, SPM concentrations were higher in the surface and bottom samples (Figure 2).

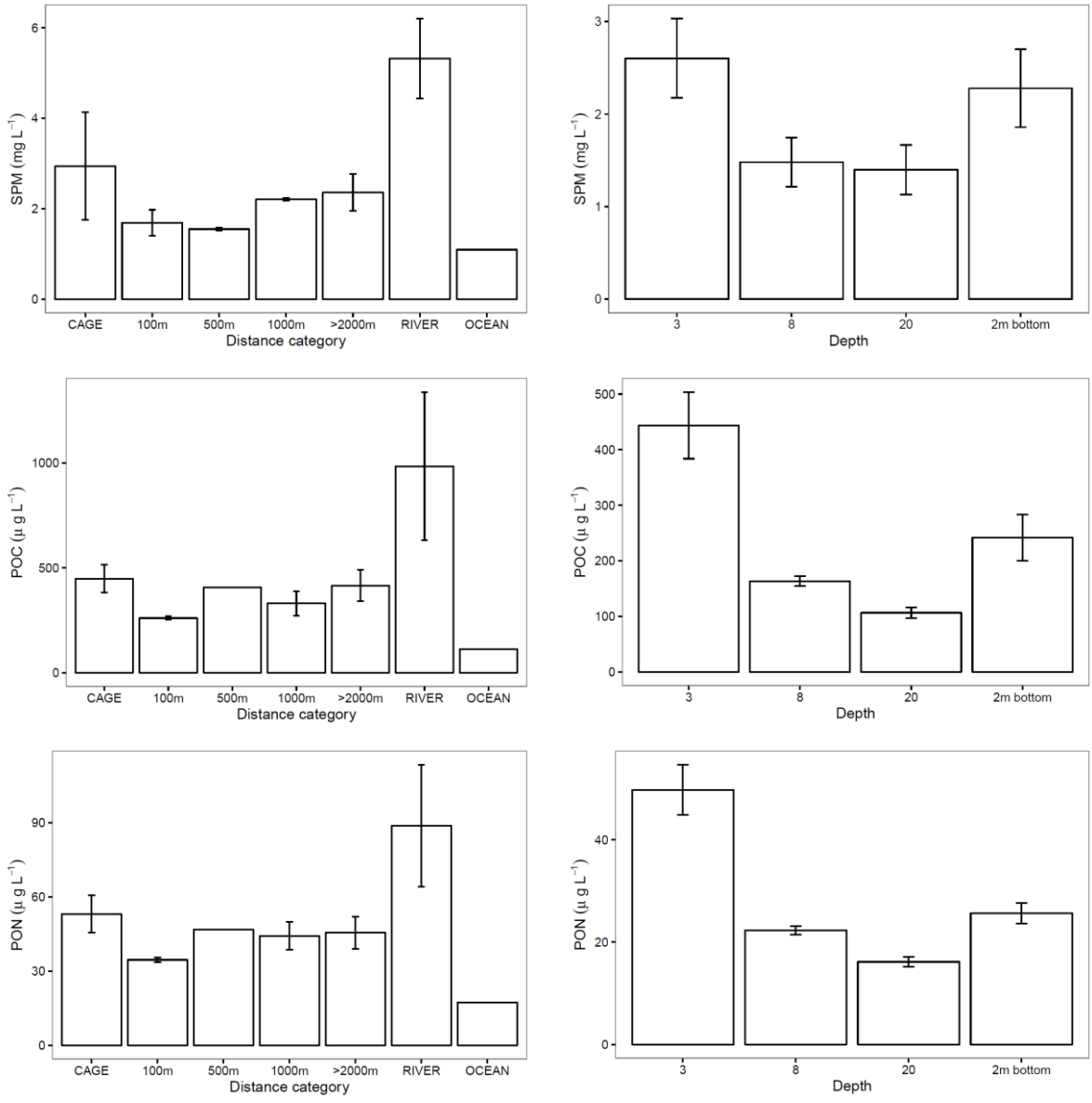

Figure 2 Concentrations of SPM, POC and PON from water samples (µg L-1) collected at 3 m water depth and pooled according to distance from cage with river (GR1 and KR4) and ocean sources (HG) grouped separately (left hand panels). Right hand panels show the same parameters pooled according to depth for the southern transect only.

A similar pattern was observed for particulate organic carbon (POC) and nitrogen (PON) concentrations (Figure 2; Figure A2 & Figure A3). Concentrations were high in the river samples, lower in the ocean sample and there was no clear pattern associated with fish farming (Figure 2). Concentrations were also higher in the surface and bottom water samples, however, surface waters clearly had the highest concentrations of POC and PON.

Total bacterial concentrations and fatty acid concentrations were also clearly higher in surface samples compared to the other depths (Figure 3, Figure A4 Figure A5). Unlike SPM, POC and PON, bacterial concentrations were higher in harbour samples compared to the river and ocean sources, however, there was no clear pattern associated with fish farming. Fatty acid concentrations were also low in the ocean sample, and the highest concentrations were associated with the cage sites, notably the 2 cages at Franklin and 1 of the cages at Table Head (Figure A5).

3.3.2 Isotopes and C:N ratio

The stable carbon isotopic composition (δ^{13}) of the suspended particulate matter (POC) ranged from -22 ‰ at the ocean site (i.e. HG in Figure A6) to ~ -28‰ in the surface samples (i.e. lowest salinity and most indicative of incoming waters) of the rivers (Figure 4; Figure A6).

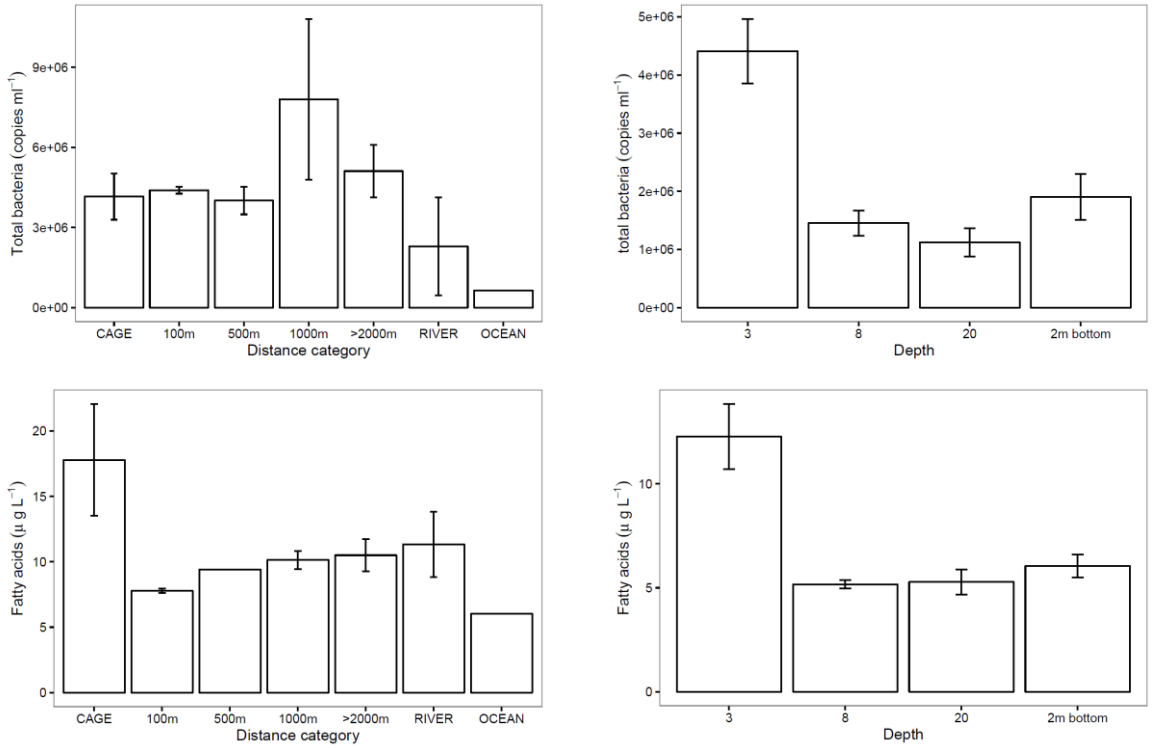

Figure 3 Total bacteria (copies ml-1) and fatty acid concentrations (µg L-1) from water samples (µg L-1) collected at 3m depth and pooled according to distance from cage with river (GR1 and KR4) and ocean sources (HG) grouped separately (left hand panels). Right hand panels show the same parameters pooled according to depth for the southern transect only.

The $\delta^{13}C$ was relatively depleted (<-27 ‰) at all the harbour sites indicative of the lower salinities and freshwater influence in surface waters, and at depth $\delta^{13}C$ became more enriched (Figure 4) There was no clear $\delta^{13}C$ signature associated with farming.

The C:N ratios of the suspended particulate matter increased from closely resembling Redfield ratio (6.6) for marine phytoplankton at the ocean site (6.4) to the highest ratios (~10-12) in the river surface samples (Figure 4), with the exception of the bottom sample collected at the 500m site on the southern transect that had a C:N ratio of 15 (Figure A7). The C:N of surface and bottom water samples were higher than those at the mid depths (Figure 4). There was no clear C:N signature associated with proximity to farming at the time of sampling (Figure 4).

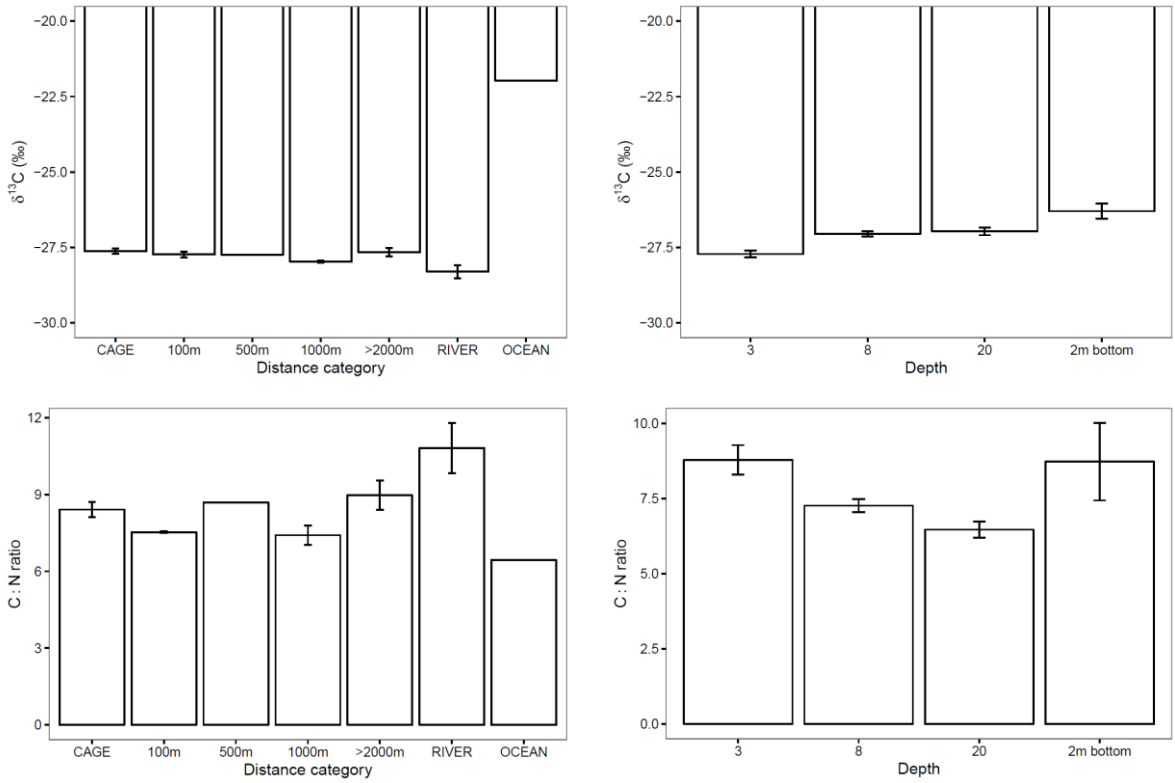

Figure 4 Stable carbon isotopes (δ13C) and C:N ratios of particulate organic matter in water samples collected at 3m depth and pooled according to distance from cage with river (GR1 and KR4) and ocean sources (HG) grouped separately(left hand panels). Right hand panels show the same fatty acids pooled according to depth for the southern transect only.

3.3.3 Fatty Acids

Individual fatty acid compounds, groups of fatty acids and fatty acid ratios were used to distinguish the presence of different sources of organic matter. Concentrations of 15:0, i17:0 md 17:0 and 18:1w7c were summed and used as a bacterial marker (e.g. Dalsgaard et al. 2003, Parish et al., 2005), the sum of long-chain saturated fatty acids ($\geq C_{20}$ SFA; 20:0, 22:0, 24:0) as an indicator of terrestrial plant material (e.g. Carpenter et al. 1991, Canuel et al., 1995) and the concentration of polyunsaturated fatty acids (PUFA) as an indicator of labile

organic material (Shaw & Johns 1985, Zimmerman & Canuel 2001). The long-chain ($\geq C_{20}$) omega-3 polyunsaturated fatty acids - docosahexaenoic acid (DHA; 22:6w3) and eicosapentaenoic acid (EPA; 20:5w3) - were used as indicators of dinoflagellates and diatoms respectively, and their ratio (DHA:EPA) as an indicator of their relative dominance (Nichols et al., 1984, Volkman et al., 1989). To identify the presence of aquafeed waste, linoleic acid (LA; 18:2w6) and oleic acid (OA; 18:1w9c) which are both present in high concentration in fish feed were used as tracers (Nichols et al. 2014).

The most abundant fatty acids in the suspended particulate matter were 16:0, 16:1w7c, 18:0 and 18:1w7c, however, their relative contribution varied between sources (i.e. cage, river and ocean; Table 3) and with depth (Table 4). Notably, both 16:1w7c and 18:1w7c made up a greater % of the total fatty acids in the harbour samples (typically >10%) compared to their contribution (typically <10%) in river and ocean samples. In terms of changes with depth, of the more common fatty acids, 18:1w7c, showed the greatest change, making up a greater % of fatty acids in the 3 m and 8 m samples compared to the two deeper sampling stations.

Shorter chain saturated fatty acids ($\leq C_{18}$ SFA) were the most abundant group of fatty acids across all sites and depths (Table 6). In contrast, long-chain saturated fatty acids ($\geq C_{20}$ SFA) represented only a small % of the total fatty acids in the harbour (typically ≤2%), but were more abundant in the river samples (5-7%). Polyunsaturated fatty acids (PUFA) made up ~10% of total fatty acids in 3 m samples in the harbour compared with 5.5% in the river samples and 22% in the ocean sample. Fatty acids indicative of bacteria made up a greater % of total fatty acids in the harbour 3 m sites compared to the river and ocean sites (Table 5) and their contribution at 3 m and 8 m was greater than at 20 m and 2 m off the bottom (Table 6).

Of the key fatty acids indicative of aquaculture inputs, both linoleic acid (LA; 18:2w6) and oleic acid (OA; 18:1w9) were found in higher concentrations and made up a greater % of total fatty acids at cages and in surface samples (Figure 5). The 2 cages at Franklin and one cage at Table Head were largely responsible for the elevated concentrations detected in cages (Figure A8Figure A9). Beyond the cages there was no relationship with proximity to farming at the time of sampling. Eicosapentaenoic acid (EPA; 20:5w3) and docosahexaenoic acid (DHA; 22:6w3), which are produced in substantial quantities by marine phytoplankton, were in relatively low concentrations in harbour and river samples, contributing on average <2 and <1% of total fatty acids respectively. As expected, both EPA and DHA represented a greater % of total fatty acids in the ocean sample (~3.5%). EPA (more indicative of diatoms), was more abundant and represented a greater % of total fatty acids in the shallower depths, whereas DHA (more indicative of dinoflagellates) was more ubiquitous throughout the water

column in terms of the % of total fatty acids, but concentrations were greater at depth (Figure 5; Table 3Table 4). The ratio of DHA:EPA was lower in the harbour (at 3m) compared with the river and ocean sites (Table 5) and lower at 3 m and 8 m compared to 20 m and 2 m off the bottom (Table 6).

Multivariate analysis using Principal Coordinate Analysis was also used to explore potential changes in fatty acid composition in relation to the major external sources of organic matter and depth in the water column. The PCO of surface samples shows that the main separation in fatty acid composition occurred for the river, ocean and cage samples (Figure 6). The ocean sample appears to separate out due to the contribution of EPA and the polyunsaturated fatty acid 18:3w3, and the cage sites due to the contribution of OA and LA. The grouping of harbour samples appears to be associated with a number of the fatty acids indicative of bacteria. The PCO of samples collected on the southern transect shows the separation according to depth (Figure 7). The surface samples were positively correlated with fatty acids 18:3w6, 18:1w7 and 16:1w13 and the long chain monounsaturated fatty acids (LC-MUFA e.g. 20:1w11, 20:1w9 and 20:1w7), typically derived from copepods, helped distinguish the deeper samples.

Table 3 Fatty acid profile of suspended particulate matter expressed as a percentage composition (mean ±SE) of total fatty acids for samples collected at 3m depth and pooled according to distance from cage, together with river (KR4 and GR1), ocean (HG) and aquafeed sources.

	CAGE	100m	500m	1000m	>2000m	OCEAN	RIVER	AQUA-FEED
i14:0	0.27 (0.05)	0.40 (0.05)	0.45	0.33 (0.09)	0.40 (0.06)	0.21	0.24 (0.02)	0.02
14:1	0.24 (0.1)	0.24 (0.1)	2.46	0.31 (0)	0.33 (0.06)	0.48	0.04 (0)	0.12
14:0	3.61 (0.32)	4.61 (1.15)	5.65	4.43 (0.9)	4.63 (0.4)	7.25	1.92 (0.04)	2.89
4,8,12TMTD	0.10 (0.02)	0.16 (0.04)	0.30	0.20 (0.04)	0.07 (0.03)	0.14	0.24 (0.04)	0.00
i15:0	1.45 (0.28)	2.04 (0.23)	2.38	1.93 (0.22)	2.32 (0.25)	0.70	1.87 (0.17)	0.06
a15:0	1.19 (0.24)	1.76 (0.21)	2.14	1.61 (0.12)	1.62 (0.1)	0.54	1.06 (0.26)	0.04
15:0	0.99 (0.14)	1.01 (0.04)	1.12	0.91 (0.15)	1.18 (0.07)	1.08	0.82 (0.07)	0.25
i16:0	0.56 (0.08)	0.79 (0.02)	0.80	0.71 (0.04)	0.69 (0.04)	1.35	0.59 (0.06)	0.00
16:1w9c	1.05 (0.22)	1.69 (0.24)	1.15	1.20 (0)	1.60 (0.23)	0.92	0.99 (0.19)	0.36
16:1w7c	10.20 (1.54)	14.54 (0.48)	14.46	12.58 (0.85)	12.83 (0.63)	9.72	10.19 (4.19)	6.36
16:1w7t	0.10 (0.03)	0.17 (0.01)	0.07	0.13 (0.01)	0.05 (0.03)	0.19	0.25 (0.01)	0.00
16:1w5c	0.35 (0.07)	0.55 (0.03)	0.50	0.50 (0.03)	0.45 (0.03)	0.34	0.87 (0.01)	0.14
16:1w13t	0.37 (0.07)	0.55 (0.14)	0.61	0.62 (0.03)	0.37 (0.02)	1.64	0.38 (0)	0.07
16:0	25.62 (2.27)	22.87 (2.54)	21.71	26.43 (0.05)	24.18 (0.42)	26.10	26.76 (2.25)	20.10
br17:1a	0.07 (0.04)	0.00 (0)	0.00	0.10 (0.03)	0.06 (0.03)	0.00	0.00 (0)	0.00
br17:1b	0.01 (0.01)	0.00 (0)	0.00	0.05 (0.05)	0.01 (0.01)	0.00	0.02 (0.02)	0.00
16:0FALD	0.06 (0.02)	0.05 (0.05)	0.06	0.07 (0.07)	0.02 (0.02)	0.14	0.10 (0.1)	0.00
i17:0	0.50 (0.09)	0.62 (0.08)	0.63	0.55 (0.12)	0.70 (0.04)	0.48	0.64 (0.05)	0.08
17:1w8c+a17:0	0.62 (0.08)	0.83 (0)	0.73	0.77 (0.03)	0.79 (0.04)	0.51	0.66 (0.05)	0.31
17:1	0.03 (0.02)	0.00 (0)	0.00	0.03 (0.03)	0.03 (0.02)	0.07	0.02 (0.02)	0.00
17:0	0.68 (0.05)	0.71 (0.06)	0.68	0.80 (0.07)	0.76 (0.05)	0.82	0.72 (0.1)	0.35
18:3w6	1.45 (0.47)	3.39 (1.05)	3.72	3.49 (0.29)	2.20 (0.26)	2.48	0.63 (0.52)	0.12
18:4w3	1.36 (0.88)	0.00 (0)	0.00	0.00 (0)	0.13 (0.13)	5.06	0.72 (0.11)	0.41
i18:0	0.06 (0.02)	0.07 (0.07)	0.00	0.04 (0.04)	0.02 (0.02)	0.31	0.53 (0.1)	0.06
18:2w6 LA	2.96 (0.8)	1.83 (0.22)	1.78	1.55 (0.22)	1.57 (0.1)	1.84	1.73 (0.23)	9.81
18:3w3	1.84 (0.58)	2.00 (0.38)	1.82	2.29 (0.47)	1.50 (0.33)	4.44	1.11 (0.52)	1.64
18:1w9c OA	11.70 (4.28)	4.88 (0.07)	4.63	4.39 (0.31)	5.13 (0.63)	4.90	7.64 (0.99)	31.23
18:1w7c	11.12 (1.9)	15.66 (0.36)	13.58	13.50 (0.7)	13.01 (0.85)	5.02	9.98 (5.07)	2.72
18:1w7t	0.20 (0.01)	0.23 (0.02)	0.20	0.23 (0.02)	0.26 (0.03)	0.31	0.32 (0.03)	0.16
18:1a	0.31 (0.08)	0.28 (0.06)	0.32	0.38 (0.09)	0.34 (0.05)	0.52	0.45 (0.08)	0.00
18:1b	0.02 (0.01)	0.08 (0.04)	0.17	0.04 (0.04)	0.09 (0.03)	0.20	0.17 (0.03)	0.00
18:1c	0.06 (0.02)	0.00 (0)	0.00	0.06 (0.06)	0.04 (0.03)	0.18	0.07 (0.07)	0.00
18:0	12.16 (2.21)	9.35 (2.64)	8.63	12.47 (2.64)	12.23 (0.87)	8.61	15.85 (6.01)	14.01
18:1FALD	0.13 (0.05)	0.17 (0.17)	0.15	0.07 (0.07)	0.11 (0.04)	0.13	0.00 (0)	0.06
18:0FALD	0.23 (0.03)	0.21 (0.01)	0.34	0.17 (0.03)	0.28 (0.04)	0.33	0.27 (0.03)	0.00
19:1a	0.05 (0.01)	0.03 (0.03)	0.09	0.02 (0.02)	0.06 (0.02)	0.07	0.04 (0.01)	0.04
19:1b	0.06 (0)	0.07 (0)	0.05	0.05 (0.01)	0.09 (0.02)	0.06	0.07 (0.01)	0.03
19:0	0.34 (0.05)	0.32 (0.07)	0.41	0.25 (0.04)	0.44 (0.06)	0.32	0.68 (0.03)	0.00
20:4w6	0.26 (0.04)	0.24 (0.05)	0.22	0.27 (0.03)	0.25 (0.02)	0.36	0.14 (0.04)	0.66
20:5w3 EPA	1.39 (0.28)	1.52 (0.45)	1.65	1.42 (0.27)	1.43 (0.09)	3.56	0.47 (0.11)	2.78
20:3	0.02 (0.02)	0.04 (0.03)	0.00	0.00 (0)	0.01 (0.01)	0.00	0.02 (0.01)	0.00
20:3w6	0.06 (0.02)	0.09 (0.09)	0.16	0.05 (0.05)	0.05 (0.02)	0.08	0.00 (0)	0.12
20:4w3	0.11 (0.04)	0.16 (0.03)	0.08	0.09 (0.08)	0.15 (0.07)	0.29	0.07 (0.01)	0.14
C20PUFA	0.20 (0.05)	0.14 (0.09)	0.40	0.00 (0)	0.16 (0.07)	0.19	0.05 (0.05)	0.00
20:2w6	0.15 (0.04)	0.11 (0.05)	0.23	0.10 (0.06)	0.12 (0.01)	0.22	0.09 (0.01)	0.09
20:1w11c	0.32 (0.05)	0.40 (0.01)	0.35	0.32 (0.07)	0.55 (0.11)	0.34	0.44 (0.16)	0.00
20:1w9c	0.73 (0.11)	0.60 (0.07)	0.57	0.45 (0.13)	1.65 (0.69)	0.47	0.93 (0.44)	0.45
20:1w7c	0.24 (0.02)	0.33 (0.04)	0.28	0.22 (0.03)	0.32 (0.03)	0.23	0.26 (0.02)	0.08
20:1w5c	0.10 (0.02)	0.12 (0.02)	0.08	0.09 (0.06)	0.10 (0.02)	0.09	0.18 (0.03)	0.00
20:0	0.74 (0.06)	0.60 (0.01)	0.58	0.53 (0.01)	0.61 (0.04)	0.50	1.12 (0.18)	0.30
21:0	0.09 (0.01)	0.09 (0.01)	0.07	0.09 (0.01)	0.08 (0.02)	0.07	0.20 (0)	0.02
22:5w6	0.03 (0.02)	0.02 (0.02)	0.04	0.00 (0)	0.04 (0.02)	0.11	0.00 (0)	0.09
22:6w3 DHA	0.87 (0.2)	0.74 (0.29)	0.88	0.79 (0.25)	0.81 (0.06)	3.62	0.52 (0.04)	1.71
22:4w6	0.02 (0.01)	0.03 (0.03)	0.00	0.00 (0)	0.01 (0.01)	0.09	0.02 (0.02)	0.11
22:5w3	0.06 (0.02)	0.05 (0.04)	0.07	0.04 (0.04)	0.06 (0.01)	0.03	0.01 (0.01)	0.33
22:1w11c	0.24 (0.06)	0.16 (0.05)	0.25	0.11 (0.02)	0.63 (0.3)	0.17	0.35 (0.29)	0.00
22:1w9c	0.35 (0.06)	0.59 (0.09)	0.58	0.30 (0.29)	0.67 (0.03)	0.24	1.47 (0.38)	0.03
22:1w7c	0.07 (0.01)	0.07 (0.02)	0.10	0.05 (0.02)	0.09 (0.02)	0.05	0.16 (0.03)	0.00
22:0	0.70 (0.14)	0.62 (0.01)	0.60	0.53 (0)	0.51 (0.06)	0.34	1.70 (0.38)	0.08
24:1w11c	0.08 (0.04)	0.05 (0)	0.07	0.08 (0.03)	0.08 (0.03)	0.28	0.03 (0.03)	0.00
24:1w9c	0.16 (0.04)	0.09 (0.01)	0.08	0.13 (0.01)	0.21 (0.08)	0.34	0.17 (0.01)	0.06
24:1w7c	0.13 (0.1)	0.13 (0.09)	0.14	0.21 (0.18)	0.09 (0.04)	0.11	0.21 (0.04)	0.00
24:0	0.76 (0.15)	0.84 (0.01)	0.74	0.89 (0.08)	0.71 (0.04)	0.73	2.75 (0.21)	0.04

Table 4 Fatty acid profile of suspended particulate matter expressed as a percentage composition (mean ±SE) of total fatty acids for samples collected at each depth on the southern transect.

	3m	8m	20m	2m bottom
i14:0	0.26 (0.05)	0.42 (0.07)	0.38 (0.06)	0.30 (0.05)
14:1	0.14 (0.04)	0.20 (0.04)	0.13 (0.03)	0.21 (0.04)
14:0	3.28 (0.47)	4.31 (0.48)	3.16 (0.36)	3.52 (0.32)
4,8,12TMTD	0.13 (0.04)	0.08 (0.02)	0.13 (0.05)	0.08 (0.04)
i15:0	1.56 (0.24)	1.85 (0.12)	2.31 (0.72)	2.13 (0.61)
a15:0	1.30 (0.18)	1.47 (0.12)	1.06 (0.14)	1.10 (0.11)
15:0	0.89 (0.1)	1.50 (0.08)	1.28 (0.1)	1.14 (0.03)
i16:0	0.68 (0.07)	1.03 (0.12)	1.10 (0.1)	1.23 (0.14)
16:1w9c	1.31 (0.24)	1.96 (0.24)	3.04 (0.54)	2.59 (0.3)
16:1w7c	12.01 (1.32)	13.00 (0.99)	9.15 (1.57)	15.32 (2.15)
16:1w7t	0.14 (0.04)	0.33 (0.11)	0.66 (0.12)	1.11 (0.14)
16:1w5c	0.48 (0.1)	0.62 (0.05)	0.43 (0.06)	0.55 (0.06)
16:1w13t	0.44 (0.06)	0.35 (0.05)	0.21 (0.05)	0.22 (0.04)
16:0	24.41 (0.77)	25.53 (1.09)	26.82 (2.17)	21.69 (1.79)
br17:1a	0.02 (0.01)	0.07 (0.02)	0.07 (0.02)	0.10 (0.02)
br17:1b	0.01 (0.01)	0.00 (0)	0.01 (0.01)	0.00 (0)
16:0FALD	0.04 (0.02)	0.12 (0.02)	0.14 (0.03)	0.14 (0.03)
i17:0	0.52 (0.08)	0.58 (0.02)	0.62 (0.09)	0.61 (0.07)
17:1w8c+a17	0.68 (0.07)	0.90 (0.06)	0.81 (0.04)	1.04 (0.06)
17:1	0.04 (0.01)	0.08 (0.02)	0.05 (0.01)	0.09 (0.03)
17:0	0.72 (0.05)	0.87 (0.04)	0.80 (0.05)	0.76 (0.04)
18:3w6	1.89 (0.47)	1.14 (0.2)	0.48 (0.18)	0.47 (0.12)
18:4w3	1.24 (0.9)	0.28 (0.19)	0.20 (0.15)	0.34 (0.13)
i18:0	0.13 (0.1)	0.08 (0.03)	0.46 (0.15)	0.23 (0.04)
18:2w6 LA	2.60 (0.83)	1.68 (0.22)	0.98 (0.1)	1.00 (0.15)
18:3w3	2.52 (0.45)	0.85 (0.1)	0.44 (0.17)	0.41 (0.14)
18:1w9c OA	9.46 (4.19)	6.74 (0.93)	6.92 (1.65)	7.77 (1.3)
18:1w7c	13.06 (1.49)	11.94 (1.12)	5.37 (0.67)	6.63 (0.59)
18:1w7t	0.24 (0.02)	0.28 (0.04)	0.24 (0.03)	0.48 (0.1)
18:1a	0.36 (0.09)	0.24 (0.05)	0.15 (0.05)	0.24 (0.03)
18:1b	0.06 (0.02)	0.06 (0.03)	0.05 (0.02)	0.10 (0.02)
18:1c	0.06 (0.02)	0.03 (0.02)	0.07 (0.06)	0.08 (0.03)
18:0	10.43 (0.39)	12.49 (1.06)	15.82 (2.61)	13.07 (2.31)
18:1FALD	0.01 (0.01)	0.01 (0.01)	0.05 (0.02)	0.08 (0.03)
18:0FALD	0.23 (0.03)	0.24 (0.02)	0.41 (0.08)	0.35 (0.03)
19:1a	0.03 (0.01)	0.02 (0.01)	0.05 (0.04)	0.06 (0.03)
19:1b	0.07 (0.01)	0.06 (0.01)	0.21 (0.06)	0.37 (0.2)
19:0	0.29 (0.07)	0.31 (0.04)	0.42 (0.06)	0.35 (0.04)
20:4w6	0.22 (0.04)	0.22 (0.03)	0.39 (0.09)	0.24 (0.02)
20:5w3 EPA	1.20 (0.23)	1.30 (0.16)	0.68 (0.15)	0.71 (0.16)
20:3	0.02 (0.01)	0.07 (0.02)	0.39 (0.15)	0.37 (0.13)
20:3w6	0.03 (0.02)	0.07 (0.02)	0.07 (0.03)	0.09 (0.02)
20:4w3	0.21 (0.05)	0.16 (0.02)	0.21 (0.08)	0.16 (0.02)
C20PUFA	0.07 (0.04)	0.07 (0.04)	0.20 (0.09)	0.13 (0.06)
20:2w6	0.14 (0.04)	0.21 (0.08)	0.86 (0.26)	0.21 (0.06)
20:1w11c	0.36 (0.03)	0.46 (0.06)	0.57 (0.31)	0.56 (0.26)
20:1w9c	0.56 (0.09)	0.41 (0.11)	3.24 (2.06)	2.43 (1.67)
20:1w7c	0.28 (0.02)	0.26 (0.02)	1.82 (0.53)	0.91 (0.17)
20:1w5c	0.15 (0.01)	0.15 (0.02)	0.25 (0.08)	0.31 (0.11)
20:0	0.72 (0.09)	0.80 (0.1)	0.81 (0.05)	0.86 (0.15)
21:0	0.12 (0.02)	0.13 (0)	0.16 (0.01)	0.25 (0.12)
22:5w6	0.05 (0.02)	0.11 (0.02)	0.19 (0.07)	0.14 (0.04)
22:6w3 DHA	0.72 (0.18)	0.88 (0.14)	0.47 (0.17)	0.71 (0.18)
22:4w6	0.00 (0)	0.04 (0.01)	0.03 (0.01)	0.05 (0.03)
22:5w3	0.06 (0.02)	0.05 (0.01)	0.57 (0.52)	0.19 (0.09)
22:1w11c	0.10 (0.02)	0.13 (0.02)	1.04 (0.87)	0.95 (0.68)
22:1w9c	0.67 (0.26)	0.52 (0.1)	0.78 (0.22)	1.14 (0.45)
22:1w7c	0.10 (0.02)	0.11 (0.01)	0.57 (0.27)	0.32 (0.11)
22:0	0.86 (0.15)	0.66 (0.11)	0.59 (0.08)	1.13 (0.38)
24:1w11c	0.08 (0.04)	0.10 (0.03)	0.19 (0.06)	0.18 (0.07)
24:1w9c	0.14 (0.03)	0.24 (0.05)	0.31 (0.24)	0.59 (0.3)
24:1w7c	0.25 (0.09)	0.25 (0.1)	0.20 (0.05)	0.19 (0.05)
24:0	1.14 (0.3)	0.85 (0.1)	0.73 (0.12)	1.23 (0.55)

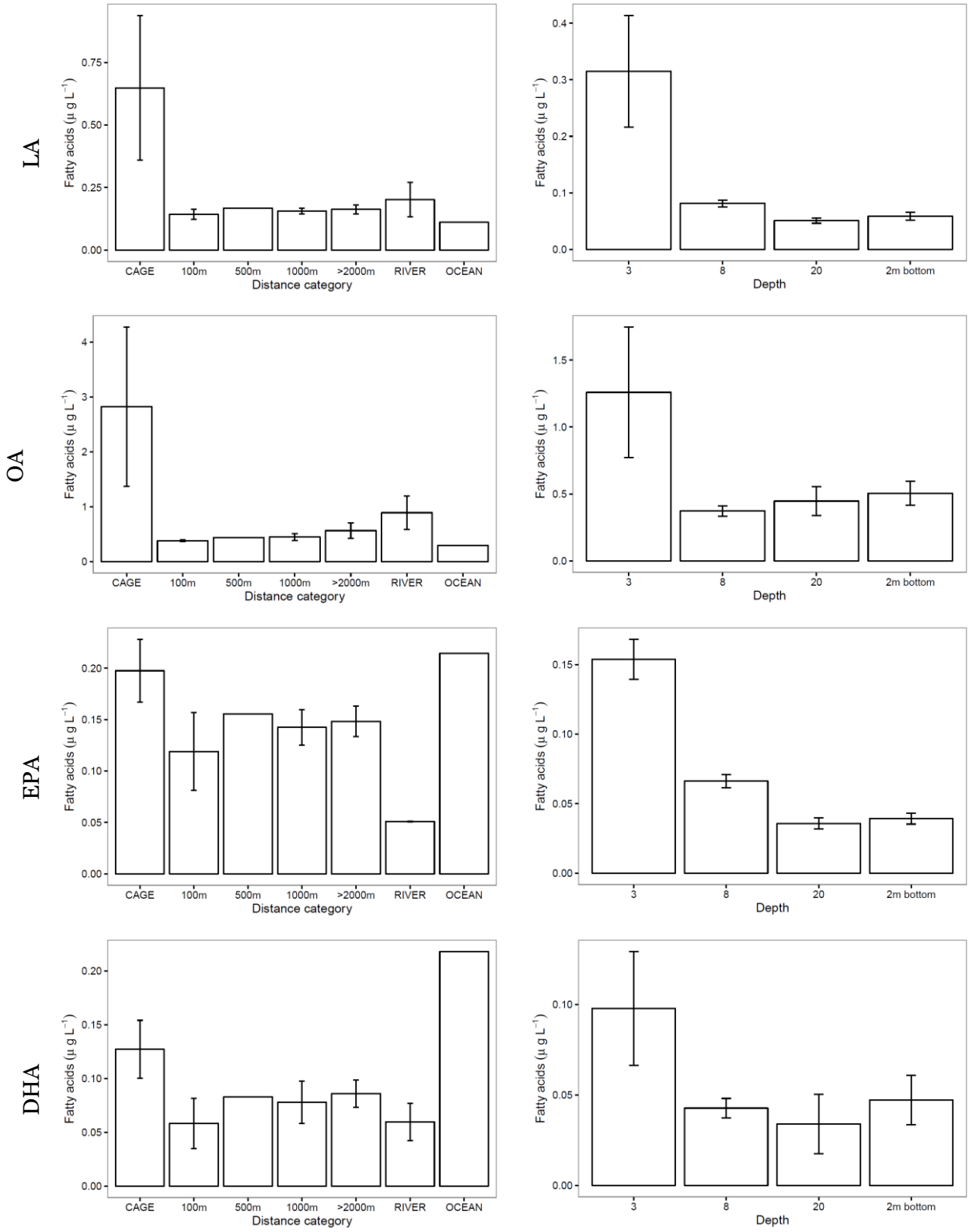

Figure 5 Fatty acid concentration of LA, OA, EPA and DHA from water samples (µg L^{-1}) collected at 3 m depth and pooled according to distance from cage with river (GR1 and KR4) and ocean sources (HG) grouped separately(left hand panels). Right hand panels show the same fatty acids pooled according to depth for the southern transect only.

Table 5 Percentage composition (mean ±SE) of saturated fatty acids (≤C_{18} & ≥C_{20}), polyunsaturated fatty acids, fatty acids consistent with a bacteria source (15:0, $_i$17:0, 17:0 and 18:1$_{w7}$) and the ratio of DHA:EPA for samples collected at 3m depth and pooled according to distance from cage, together with river (KR4 and GR1), ocean (HG) and aquafeed sources.

	CAGE	100m	500m	1000m	>2000m	OCEAN	RIVER	AQUA-FEED
≤C_{18} SFA	43.05 (3.95)	38.55 (3.93)	37.79	45.03 (2.01)	42.98 (1.3)	43.86	46.07 (8.26)	23.24
≥C_{20} SFA	2.20 (0.25)	2.05 (0.01)	1.92	1.94 (0.1)	1.82 (0.12)	1.56	5.57 (0.78)	0.43
PUFA	10.59 (1.88)	10.08 (2.54)	10.81	9.95 (0.56)	8.30 (0.6)	22.01	5.50 (0.63)	17.65
Bacteria	13.49 (2.14)	18.23 (0.16)	16.21	15.98 (0.34)	15.91 (0.95)	7.72	12.49 (5.01)	3.55
DHA:EPA	0.62 (0.04)	0.47 (0.05)	0.53	0.54 (0.07)	0.57 (0.03)	1.02	1.17 (0.35)	0.61

Table 6 Percentage composition (mean ±SE) of saturated fatty acids (≤C_{18} & ≥C_{20}), polyunsaturated fatty acids, fatty acids consistent with a bacteria source (15:0, $_i$17:0, 17:0 and 18:1$_{w7}$) and the ratio of DHA:EPA for samples collected at each depth on the southern transect.

	3m	8m	20m	2m bottom
≤C_{18} SFA	39.73 (1.24)	44.71 (1.52)	47.87 (4.19)	40.18 (3.74)
≥C_{20} SFA	2.72 (0.45)	2.32 (0.26)	2.13 (0.22)	3.22 (1.06)
PUFU	10.73 (1.83)	6.83 (0.59)	5.50 (0.86)	4.59 (0.5)
Bacteria	15.42 (1.65)	15.18 (1.14)	8.31 (0.62)	9.62 (0.58)
DHA:EPA	0.60 (0.06)	0.70 (0.08)	1.07 (0.45)	1.27 (0.34)

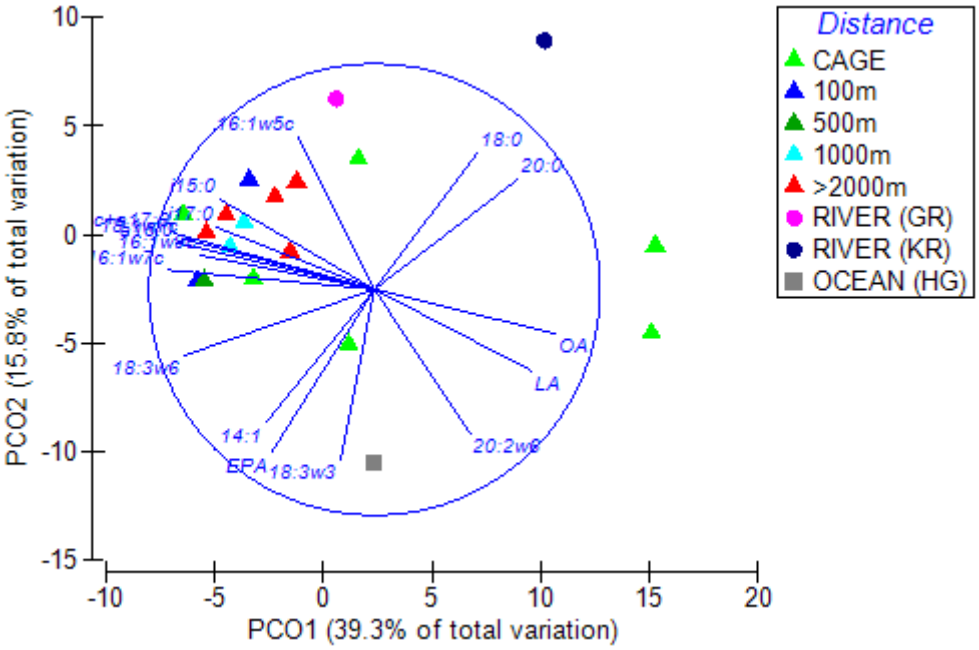

Figure 6 Biplots of the first two principal components calculated by PCO using fatty acid composition of all 3 m samples labelled according to distance from cage and river (GR1 and KR4) and ocean sites. Correlations between the first two principal components are shown for highest Pearson correlations for individual fatty acids (r > 0.75). The lengths of the lines indicate the strength of the correlation to the PCO axes.

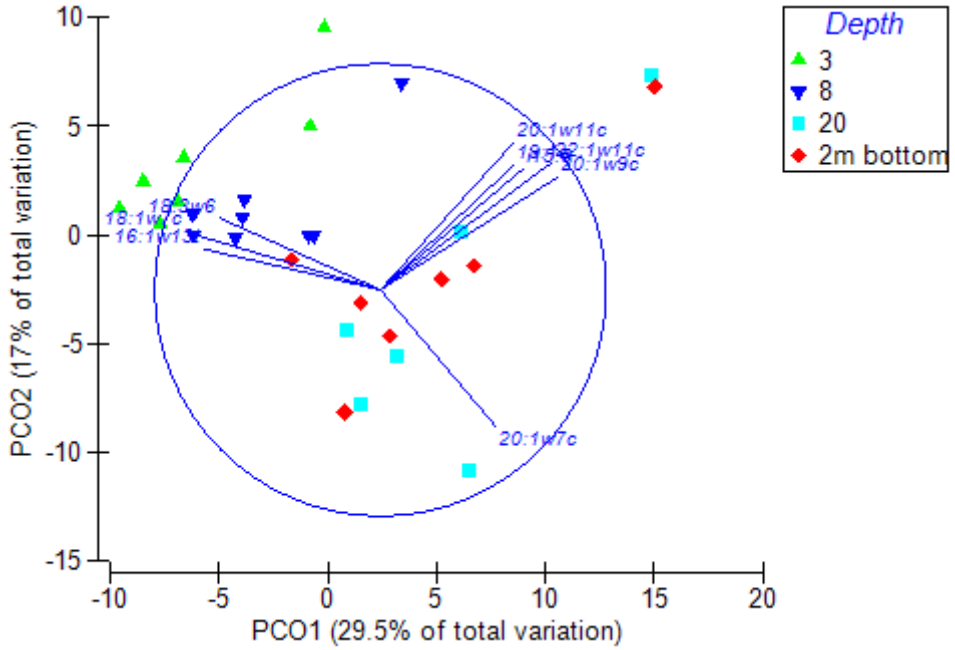

Figure 7 Biplots of the first two principal components calculated by PCO using fatty acid composition for samples collected on the southern transect and labelled according to depth. Correlations between the first two principal components are shown for highest Pearson correlations for individual fatty acids ($r > 0.75$). The lengths of the lines indicate the strength of the correlation to the PCO axes.

3.3.4 Gene Abundance

There were distinct differences in the distribution and abundance of the marker genes for archaeal (AOA) and bacterial (AOB) nitrifiers in relation to the major external sources of organic matter and depth in the water column (Figure 8). The abundance of both AOA and AOB were generally higher in the harbour compared to in the river and ocean samples. Interestingly though, the abundance and AOB (Figure A13) and total bacteria (Figure A4) were relatively high in the Gordon, but in contrast, their abundance was relatively low in the King River.

There was no evidence of a gradient in the abundance of AOA or AOB in relation to proximity to farming (Figure 8). Archaeal nitrifiers were far more abundant than bacterial nitrifiers at depth (>3m), and in contrast, bacterial nitrifiers were more abundant than archaeal nitrifiers in surface (3m) samples (Figure 8).

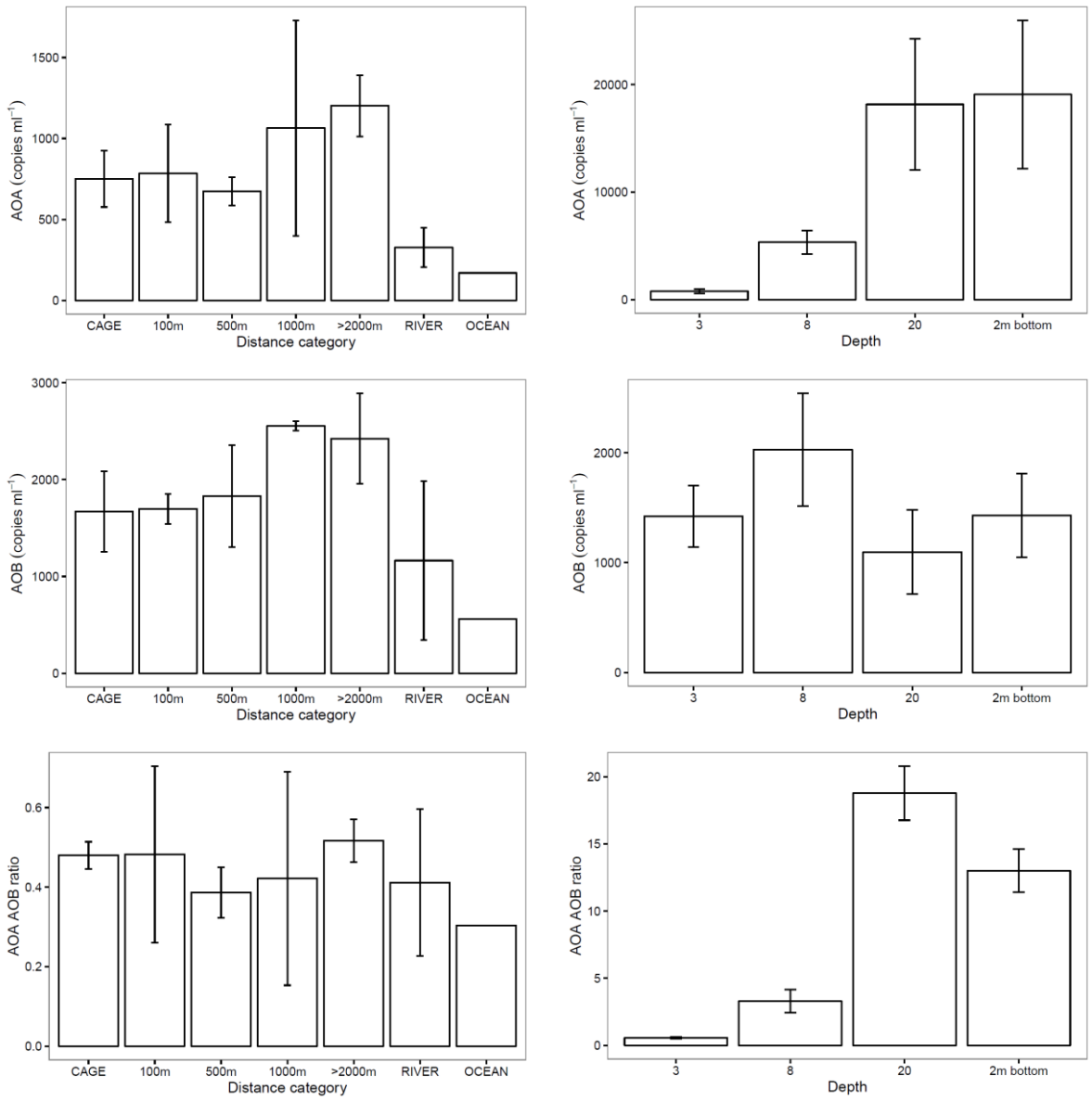

Figure 8 Concentrations (copies ml^{-1}) of AOA, AOB and the AOA/AOB ratio from water samples collected at 3 m depth and pooled according to distance from cage with river (GR1 and KR4) and ocean sources (HG) grouped separately (left hand panels). Right hand panels show the same parameters pooled according to depth for the southern transect only.

3.4 DISCUSSION

Suspended particulate matter concentrations were highest in the Gordon and King river surface samples (i.e. lowest salinity and most indicative of incoming waters) consistent with a significant terrestrial/riverine source of particulate organic carbon and nitrogen to the harbour. The more depleted isotope values of the particulate organic carbon in the harbour surface samples ($\delta^{13}C$ = -27 - -28‰) is also consistent with the influence of riverine/terrestrial sources of carbon which was similarly depleted ($\delta^{13}C$ ~-28‰) compared with the more enriched ocean ($\delta^{13}C$ ~ -22‰) and aquafeed (δ13C ~ -24.8‰). These values are consistent with expectations from the literature with the $\delta^{13}C$ values of terrestrial organic matter (-25 to -33‰) and freshwater phytoplankton (-25 to -30‰) typically more depleted compared with marine particulate organic matter (-22 to -18‰) (see Middelburg and Nieuwenhuize, 1998).

The C:N ratios of the particulate organic matter in the harbour surface waters (8.0±0.2) were intermediate between the river (10.8±1) and the ocean (6.4) and aquafeed (5.6) sources, again consistent with the influence of the terrestrial/riverine organic component, but also demonstrating the presence of other sources of organic matter (e.g. marine, aquafeed, internal production). The relatively low C:N ratio of the river samples suggests that freshwater phytoplankton may be a significant component of the catchment organic matter entering the harbour given that the C:N ratio of freshwater phytoplankton is typically lower than that of terrestrial vegetation and soils (e.g. Cloern et al., 2002; Fazhu et al., 2015).

There was no evidence of a shift in either the C:N ratio or $\delta^{13}C$ in proximity to farming. In contrast, the isotopic (C & N) value and C:N ratio of sediment organic matter has proven effective is identifying the spatial extent of deposited waste feed and faeces in the harbour sediments (Ross et al., 2014; 2016). The difficulties in detection in the water column are perhaps not that surprising given the dynamic nature (e.g. horizontal and vertical advection and dispersion) of the water column and the transient nature of feeding and waste production relative to benthic sediments that integrate deposition over much longer time periods.

Fatty acid profiles in the water column did, however, identify the presence of material derived from fish farming. Total fatty acid concentrations were higher and the concentrations of both linoleic acid (LA; 18:2w6) and oleic acid (OA; 18:1w9) used in aquafeed were present in elevated concentrations in cage samples. Revill et al., (2016) also reported elevated total fatty acid concentrations and elevated concentrations of LA close to cages in Macquarie Harbour during sampling in December 2015. Fatty acid profiles of sediments in the Macquarie Harbour (Revill et al., 2015, White et al., unpub data) and

elsewhere (e.g. Van Biesen & Parrish 2005, Black et al., 2012) have also documented the shift towards the composition of aquafeed in sediments beneath farms. White (unpub data) documented a major shift in fatty acid composition between 0 and 50 m from cages, and was able to detect the presence of elevated, albeit much lower, total fatty acids and LA to between 250 and 500 m and attributed this to acute and diffuse deposition zones respectively.

In this study, there was no evidence of elevated concentrations of total fatty acids or the key aquafeed markers LA and OA beyond the cages, with the next closest sample station at 100 m. Again, this likely highlights the difficulty of detecting farms inputs in the water column due to rapid diffusion and the transient nature of waste products following feeding and fish digestion. The large variability between cages, with 3 of the 6 sampled cages largely responsible for the elevated concentrations, most probably reflects the timing of sampling in relation to feeding and fish digestion. It also points to the highly transient nature of farm inputs. Lipids, of which fatty acids are the major components, provide a very dense form of energy, and thus, which are highly labile (Parrish et al., 2015) and are likely to be rapidly consumed and assimilated. Sampling during peak stocking times in summer, at a finer spatial resolution (between 0-100m) and more closely associated with feeding is expected to offer greater sensitivity in detecting the influence of farm wastes in the water column. The use of compound specific isotopes may also further help distinguish LA and other fatty acids sourced from farm inputs with those derived naturally from the surrounding catchment.

The fatty acid profiles of samples collected throughout the harbour also provided greater insight into the composition and source of suspended particulate matter. The essential fatty acids, EPA (mainly diatoms) and DHA (mainly dinoflagellates) made up a relatively small proportion of total fatty acids in the harbour surface samples, typically less than 2% and 1% respectively, compared with ~3.5% for both DHA and EPA in the ocean sample. This is consistent with low microalgal production in the water column due to the tannin rich water waters in the harbour (Koehnken 1996). However, it is also important to note that sampling at the surface in this study was at 3 m and much of the light attenuation occurs above this depth. For example, in February only 10% of the surface irradiance reached 2m below the surface (MHTWG MH Pelagic DO Study, Objective 1 &3, 2016). As such, the biomass and productivity of microalgae is likely to be higher in the top 1-2 m of the water column in the harbour.

Long-chain saturated fatty acids ($\geq C_{20}$ SFA) which are indicators for terrestrially derived plant material were, as expected, in higher concentrations and made up a greater proportion of total fatty acids in the river samples (5-7%) compared to the harbour (1-3%), aquafeed (0.4%) and ocean (1.6%) samples. These fatty acids are likely to represent a relatively

refractory load to the harbour. Polyunsaturated fatty acids (PUFA) on the other hand are more susceptible to degradation than other fatty acids and are often used as an indicator of labile 'fresh' organic matter (Shaw and Johns 1985, Haddad et al., 1991, Zimmerman and Canuel 2001). In the river samples, PUFA represented 5.5% of the fatty acids compared with 8-12% in the harbour, indicating that some of the labile organic matter in the harbour is either internally produced or sourced from aquafeed or the ocean. There was some indication that the relative contribution of PUFA was higher in closer proximity to farming, however, the substantial variability between sites precludes any definitive conclusion.

A major finding of this study is the importance of bacterial production. Bacterial specific fatty acids (15:0, 17:0, i17:0 and 18:1w7[2]) represented between 10 and 20% of total fatty pool in harbour surface samples, indicating that bacterial production is a major source of organic matter in the harbour. These findings are supported by the genomics, which found the highest abundance of bacteria (using the 16S gene) in the harbour surface waters. It is also notable that the concentrations of bacterial fatty acids correspond to where the total fatty acid pool and PUFA were most concentrated. This suggests that bacteria may be preferentially utilizing the more labile organic matter in surface waters. In contrast, the more refractory organic matter, such as the long-chain saturated fatty acids are more ubiquitous throughout the water column, and in the case of 22:0 and 24:0 are relatively more abundant in the bottom water samples. Also of interest, bacterial abundance estimated by both fatty acid biomarkers and genomics was very low in the King River compared to the Gordon River. This may well reflect the effects of heavy metal contamination on suppressing microbial production in the King River waters compared to the far more pristine catchment of the Gordon.

The characterization of the ammonium oxidizers using genomics provided further insight into the importance of microbially mediated production for the cycling of nitrogen in the harbour water column. Both ammonium oxidizing bacteria (AOB) and archaea (AOA) were in elevated concentrations in the harbour relative to the river and ocean samples consistent with internal production of both AOB and AOA in the harbour. The fatty acid data provided further evidence of the prevalence of nitrifying organisms. High levels of the fatty acid 18:1w7 (vaccenic acid) has previously been reported in *Nitrobacteria* (Lipski et al., 2001) which play a critical role in the nitrification process, converting nitrites into nitrates; this was the dominant bacterial fatty acid, representing between 10 and 15% of total fatty acids in harbour surface samples. Ammonium oxidizing bacteria (AOB) were present throughout the water column, whereas the fatty acid 18:1w7 was clearly more abundant at 3 and 8 m relative

[2] Although not included in the calculation, a portion of 16:1 fatty acid are likely to be bacterial in origin

to 20 m and 2 m off the bottom. Ammonium oxidizing archaea (AOA) on the other hand were far more abundant at depth and an order of magnitude more abundant than the AOB at depth. It is well documented that environmental factors such as salinity, oxygen, temperature and light are likely to be important drivers shaping ammonia-oxidising communities. For example, Zhang et al. (2015) found that archaeal and bacterial nitrifiers in the estuary of the Yong River in China were strongly impacted by salinity. From freshwater to marine sites archaeal nitrifiers increased by as much as 13-22 times, whereas bacterial nitrifiers increased by 1-2 times; these patterns are remarkably similar to those found in this study when comparing brackish surface samples to the more marine deeper samples.

The relationship between the abundance of ammonium oxidisers and activity (i.e. actual rates of nitrification) is ambiguous in estuarine and coastal waters. A number of studies have reported that ammonia oxidation activity is positively correlated with archaeal nitrifier abundance and not bacterial nitrifier abundance (Caffrey et al. 2007; Lam et al. 2007; Zhang et al. 2015). However, other studies have found that bacterial nitrifiers were the major contributors to ammonia oxidation, especially in high ammonia habitats where bacterial nitrifiers were quantitatively dominant. The actual rates of nitrification (NTR) measured in this study, albeit from surveys carried out earlier in the year, suggest that nitrification rates increase markedly with depth (MHTWG MH Pelagic DO Study, Objective 1 &3, 2016). Given that the archaeal nitrifiers were far more abundant than bacterial nitrifiers, and notably more abundant at depth, these results suggest that the archaeal nitrifiers may be responsible for nitrification in Macquarie Harbour. However, more nitrification rate measurements and concomitant assessments of the microbial assemblage are required to confirm this relationship. In particular, concomitant quantification of the expression levels of the enzyme responsible for ammonia oxidation by nitrifying archaea and bacteria will be important to support this finding.

As discussed elsewhere, the nitrification process, whether by AOA or AOB, has important implications for both nitrogen and oxygen dynamics due to the transformation of ammonium to nitrate and the concomitant consumption of oxygen. A key finding from this study is the significance of organic material produced internally in the harbour water column via microbial production. This has important implications for our current understanding of carbon cycling. For example, nitrifiers are chemolithoautotrophs that fix carbon dioxide and get their energy from external chemical compounds rather than light, in this case ammonium. As such, unlike heterotrophic bacteria that utilise and convert organic matter into bacterial biomass, chemoautotrophs create new organic matter as they fix CO_2. This has the implication of increased oxygen demand when this organic matter is mineralised.

Conclusions

The combined biomarker study has provided a unique insight into the source, transformation and fate of organic matter in the harbour, most notably, the significant role that microbes are likely to be playing in the dynamics of carbon, nitrogen and oxygen in the harbour ecosystem. The fatty acids LA and OA also proved to be effective at identifying the presence of aquafeed waste in the water column. However, the study also demonstrated the challenges faced with capturing and documenting the influence of aquafeed waste in the water column given the highly diffusive nature of the water column, the transient nature of feeding and the potentially high reactivity of the waste. The relatively low stocking densities at the time of sampling most likely exacerbated the challenge. Future sampling should focus on the peak stocking period in summer and in closer proximity to the cages (e.g. 0-100 m). This would provide greater sensitivity in determining the water column footprint of aquafeed waste. It is also important to consider that indirect pathways i.e. emissions of dissolved inorganic nutrients (e.g. ammonium) and the subsequent incorporation into organic matter would not be detected using fatty acids. These dissolved nutrients may be helping fuel microbial production; measuring the N isotope of ammonium may help trace this pathway.

Finally, it is important to remember that the biomarker study was conducted once, in April 2016, and as such, the conclusions need to be treated with a degree of caution until the survey is repeated. We recommend that the survey be repeated in spring/summer to provide contrasting stocking and background environmental conditions (e.g. higher water temperatures, water column productivity).

3.5 REFERENCES

Abell, G. C. J., A. T. Revill, C. Smith, A. P. Bissett, J. K. Volkman, and S. S. Robert. 2010. Archaeal ammonia oxidizers and nirS-type denitrifiers dominate sediment nitrifying and denitrifying populations in a subtropical macrotidal estuary. Isme Journal 4:286-300.

Abell, G. C. J., D. J. Ross, J. P. Keane, J. M. Oakes, B. D. Eyre, S. S. Robert, and J. K. Volkman. 2013. Nitrifying and denitrifying microbial communities and their relationship to nutrient fluxes and sediment geochemistry in the Derwent Estuary, Tasmania. Aquatic Microbial Ecology 70:63-75.

Black, K. D., L. A. Calder, T. D. Nickell, M. D. J. Sayer, H. Orr, T. Brand, E. J. Cook, S. H. Magill, T. Katz, N. Eden, K. J. Jones, M. Tsapakis, and D. Angel. 2012. Chlorophyll, lipid profiles and bioturbation in sediments around a fish cage farm in the Gulf of Eilat, Israel. Aquaculture 356:317-327.

Bollmann, A., and H. J. Laanbroek. 2002. Influence of oxygen partial pressure and salinity on the community composition of ammonia-oxidizing bacteria in the Schelde estuary. Aquatic Microbial Ecology 28:239-247.

Caffrey, J. M., N. Bano, K. Kalanetra, and J. T. Hollibaugh. 2007. Ammonia oxidation and ammonia-oxidizing bacteria and archaea from estuaries with differing histories of hypoxia. Isme Journal 1:660-662.

Canuel, E. A., J. E. Cloern, D. B. Ringelberg, J. B. Guckert, and G. H. Rau. 1995. Molecular and isotopic tracers used to examine sources of organic matter and its incorporation into the food webs of San Francisco Bay. Limnology and Oceanography 40:67-81.

Canuel, E. A., and A. R. Zimmerman. 1999. Composition of particulate organic matter in the southern Chesapeake Bay: Sources and reactivity. Estuaries 22:980-994.

Carpenter, P. D., Butler, E. C. V., Higgins, H. W., Mackey, D. J., & Nichols, P. D. (1991). Chemistry of trace elements, humic substances and sedimentary organic matter in Macquarie Harbour, Tasmania. Marine and Freshwater Research, 42(6), 625-654.

Chen, Y. S., M. C. M. Beveridge, T. C. Telfer, and W. J. Roy. 2003. Nutrient leaching and settling rate characteristics of the faeces of Atlantic salmon (Salmo salar L.) and the implications for modelling of solid waste dispersion. Journal of Applied Ichthyology 19:114-117.

Cloern, J. E., E. A. Canuel, and D. Harris. 2002. Stable carbon and nitrogen isotope composition of aquatic and terrestrial plants of the San Francisco Bay estuarine system. Limnology and Oceanography 47:713-729.

Crawford, C., 2003. Environmental management of marine aquaculture in Tasmania, Australia. Aquaculture 226:129-138.

Dalsgaard, J., M. St John, G. Kattner, D. Muller-Navarra, and W. Hagen (2003). Fatty acid trophic markers in the pelagic marine environment. Adv. Mar. Biol. 46: 225-340

Fazhu, Z., S. Jiao, R. Chengjie, K. Di, D. Jian, H. Xinhui, Y. Gaihe, F. Yongzhong, and R. Guangxin. 2015. Land use change influences soil C, N, and P stoichiometry under 'Grain-to-Green Program' in China. Scientific Reports 5:10195.

Francis, C. A., J. M. Beman, and M. M. M. Kuypers. 2007. New processes and players in the nitrogen cycle: the microbial ecology of anaerobic and archaeal ammonia oxidation. Isme Journal 1:19-27.

Haddad, R. I., C. S. Martens, and J. W. Farrington. 1992. QUANTIFYING EARLY DIAGENESIS OF FATTY-ACIDS IN A RAPIDLY ACCUMULATING COASTAL MARINE SEDIMENT. Organic Geochemistry 19:205-216.

Holdsworth, D.G., Revill, A.T., Volkman, J.K. and Swadling, K. (2008). Lipid biomarkers in sediment traps and sediments from North West Bay, Tasmania. Aquafin CRC Project 4.2(2) (FRDC Project No. 2004/074). Aquafin Cooperative Research Centre, Fisheries Research and Development Corporation, Commonwealth Scientific and Industrial Research Organisation. Published by CSIRO Marine and Atmospheric Research.

Koehnken, L., 1996. Macquarie Harbour–King River study. Draft final report. Department of Environment and Land Management, Tasmania.

Lam, P., M. M. Jensen, G. Lavik, D. F. McGinnis, B. Müller, C. J. Schubert, R. Amann, B. Thamdrup, and M. M. M. Kuypers. 2007. Linking crenarchaeal and bacterial nitrification to anammox in the Black Sea. Proceedings of the National Academy of Sciences 104:7104-7109.

Lipski, A., E. Spieck, A. Makolla, and K. Altendorf. 2001. Fatty acid profiles of nitrite-oxidizing bacteria reflect their phylogenetic heterogeneity. Systematic and Applied Microbiology 24:377-384.

Macleod, C. K., C. M. Crawford, and N. A. Moltschaniwskyj. 2004. Assessment of long term change in sediment condition after organic enrichment: defining recovery. Marine Pollution Bulletin 49:79-88.

Middelburg, J. J., and J. Nieuwenhuize. 1998. Carbon and nitrogen stable isotopes in suspended matter and sediments from the Schelde Estuary. Marine Chemistry 60:217-225.

Nichols, P. D., G. J. Jones, J. W. Deleeuw, and R. B. Johns. 1984. THE FATTY-ACID AND STEROL COMPOSITION OF 2 MARINE DINOFLAGELLATES. Phytochemistry 23:1043-1047.

Nichols, P.D., Glencross, B., Petrie, J., Singh, S.P. (2014) Readily Available Sources of Long-chain Omega-3 Oils: Is Farmed Australian Seafood a Better Source of the Good Oil Than Wild-Caught Seafood? Nutrients 6: 1063-1079.

Parrish, C. C., R. J. Thompson, and D. Deibel. 2005. Lipid classes and fatty acids in plankton and settling matter during the spring bloom in a cold ocean coastal environment. Marine Ecology Progress Series 286:57-68.

Parrish, C. C., P. D. Nichols, H. Pethybridge, and J. W. Young. 2015. Direct determination of fatty acids in fish tissues: quantifying top predator trophic connections. Oecologia 177:85-95.

Revill, A.T.; Brock, M. and Nichols, P.D. 2015. Characterising Sediment Organic Matter in Macquarie Harbour Sediments. CSIRO report for TSGA.

Revill, A. T., Ross, J., & Thompson, P. A. (2016). Investigating Dissolved Oxygen Drawdown in Macquarie Harbour. CSIRO, Australia.

Ross, D. Jeff, Neil Hartstein, Catriona M. Macleod, M. Auluck, V. Lucieer, P. Cook, and J. Valentine. 2015. Characterising Benthic Pelagic Interactions in Macquarie Harbour - Organic Matter Processing in Sediments and the Importance for Nutrient Dynamics. FRDC Final.

Ross, D.J, McCarthy, A., Davey, A., Pender, A., Macleod, C.M (2016) Understanding the Ecology of Dorvilleid Polychaetes in Macquarie Harbour: Response of the benthos to organic enrichment from finish aquaculture. FRDC Final Report Project No 2014/038.

Shaw, P. M., and R. B. Johns. 1985. ORGANIC GEOCHEMICAL STUDIES OF A RECENT INNER GREAT BARRIER-REEF SEDIMENT .1. ASSESSMENT OF INPUT SOURCES. Organic Geochemistry 8:147-156.

Van Biesen, G., and C. C. Parrish. 2005. Long-chain monounsaturated fatty acids as biomarkers for the dispersal of organic waste from a fish enclosure. Mar. Environ. Res. 60: 375-388.

Volkman, J. K., S. W. Jeffrey, P. D. Nichols, G. I. Rogers, and C. D. Garland. 1989. FATTY-ACID AND LIPID-COMPOSITION OF 10 SPECIES OF MICROALGAE USED IN MARICULTURE. Journal of Experimental Marine Biology and Ecology 128:219-240.

Volkman, J. K., and E. Tanoue. 2002. Chemical and biological studies of particulate organic matter in the ocean. Journal of Oceanography 58:265-279.

Wang, X. X., K. Andresen, A. Handa, B. Jensen, K. I. Reitan, and Y. Olsen. 2013. Chemical composition and release rate of waste discharge from an Atlantic salmon farm with an evaluation of IMTA feasibility. Aquaculture Environment Interactions 4:147-162.

Ward, B. B., (2008) Nitrification. In: Nitrogen in the Marine Environment. Eds. D. G. Capone, D. A. Bronk, M. R. Mulholland, and E. J. Carpenter. Elsevier, Amsterdam. (199-262)

Ward, B. B., (2011) Measurement and distribution of nitrification rates in the oceans. In Microbial Nitrification and Related Processes. M. G. Klotz, editor, Methods in Enzymology 486 (307-323)

Zhang, Q., F. Tang, Y. Zhou, J. Xu, M. Wang, and H. J. Laanbroek. 2015. Shifts in the pelagic ammonia-oxidizing microbial communities along the eutrophic estuary of Yong River in Ningbo City, China. Frontiers in Microbiology 6.

Zimmerman, A. R., and E. A. Canuel. 2001. Bulk organic matter and lipid biomarker composition of Chesapeake Bay surficial sediments as indicators of environmental processes. Estuarine Coastal and Shelf Science 53:319-341.

3.6 APPENDIX A

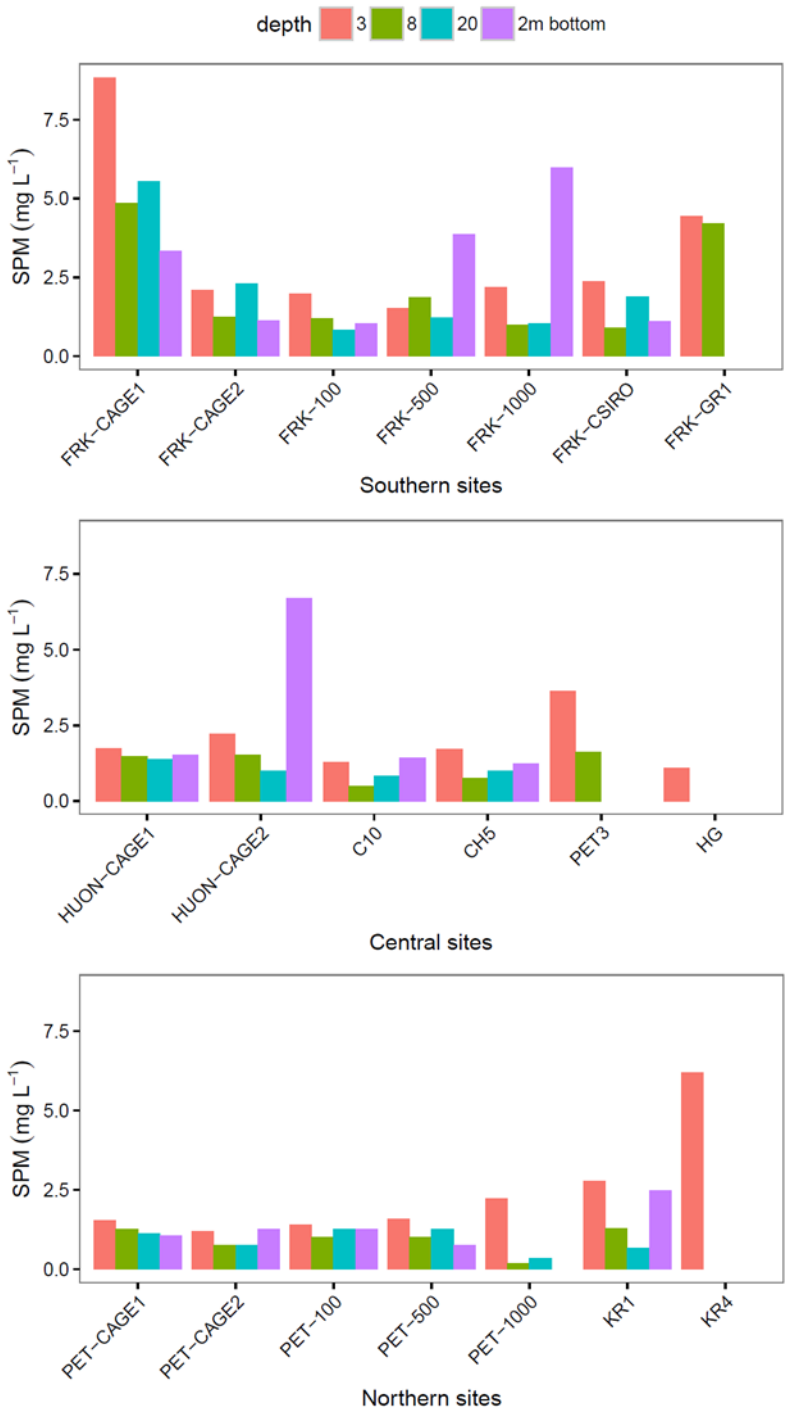

Figure A1 – Suspended particulate matter (SPM) concentrations (mg/l) from water samples collected at each depth (3, 8, 20 and 2m from the bottom) at sites on the southern transect (upper panel), central sites (middle panel) and the northern transect (lower panel).

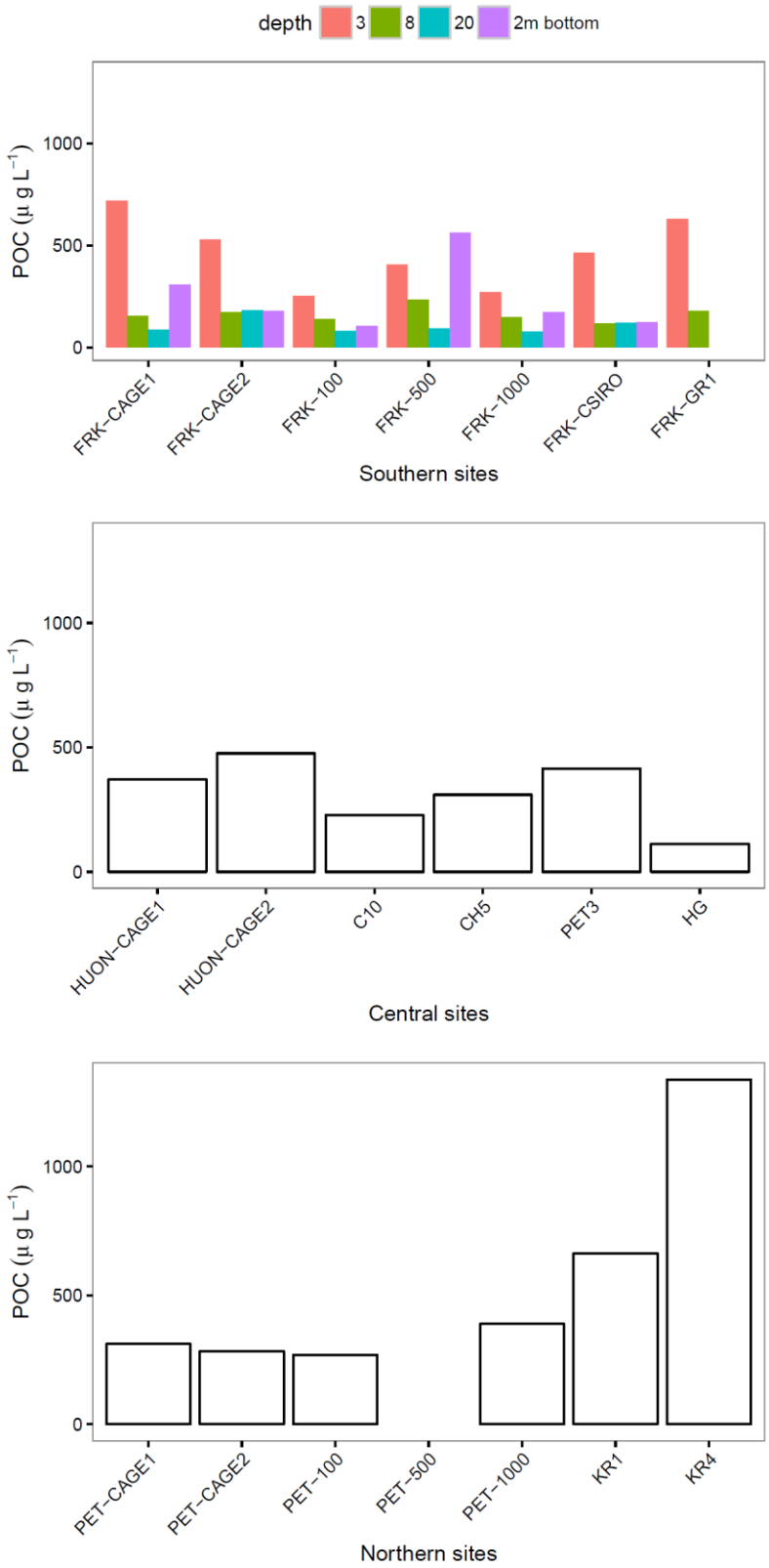

Figure A2 – Particulate organic carbon (POC) from water samples (µg L-1) collected at each depth (3, 8, 20 and 2m from the bottom) at sites on the southern transect (upper panel), and from surface samples (3m) at the central sites (middle panel) and on the northern transect (lower panel).

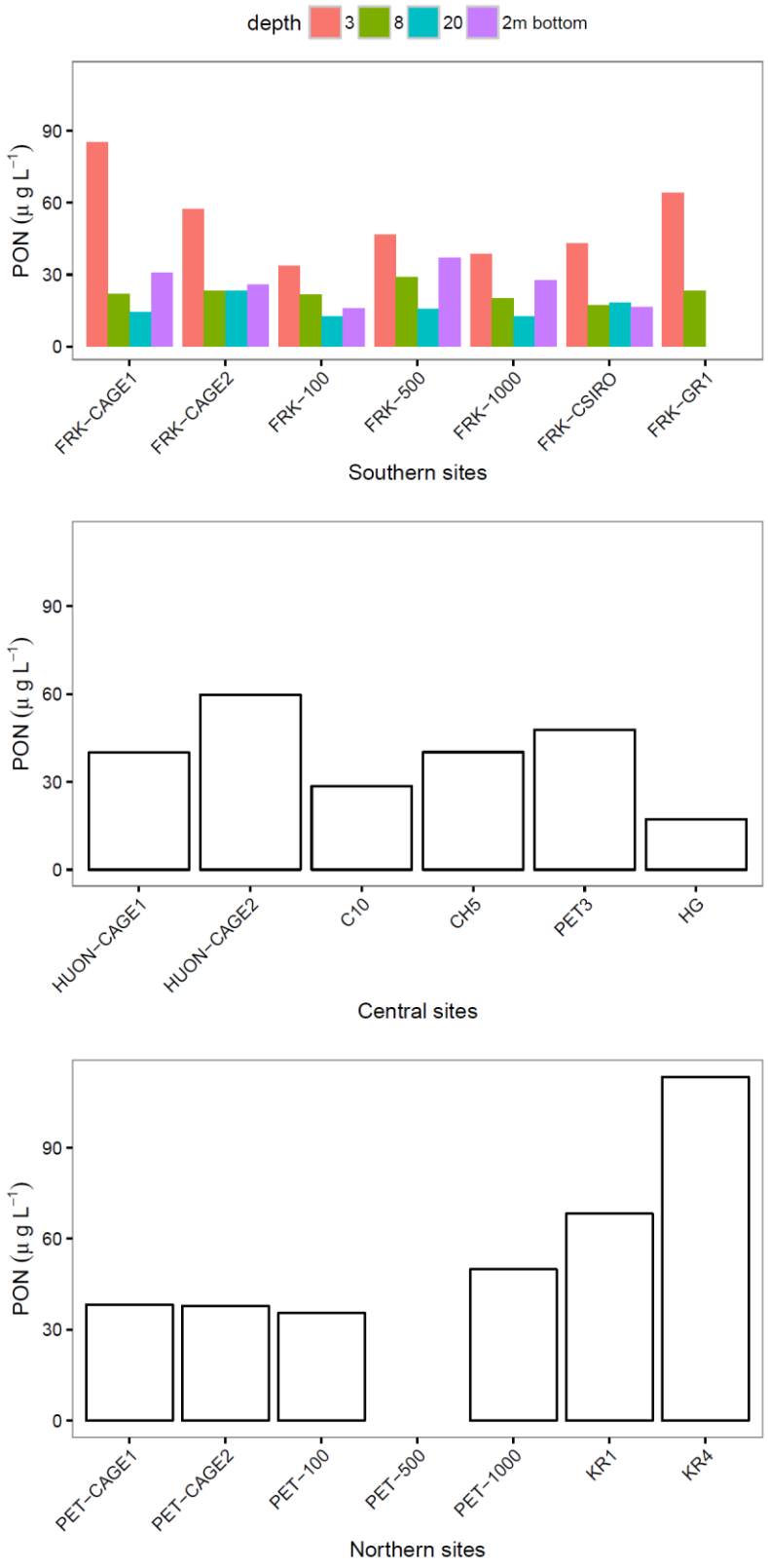

Figure A3 – Particulate organic nitrogen (PON) from water samples (µg L-1) collected at each depth (3, 8, 20 and 2m from the bottom) at sites on the southern transect (upper panel), and from surface samples (3m) at the central sites (middle panel) and on the northern transect (lower panel).

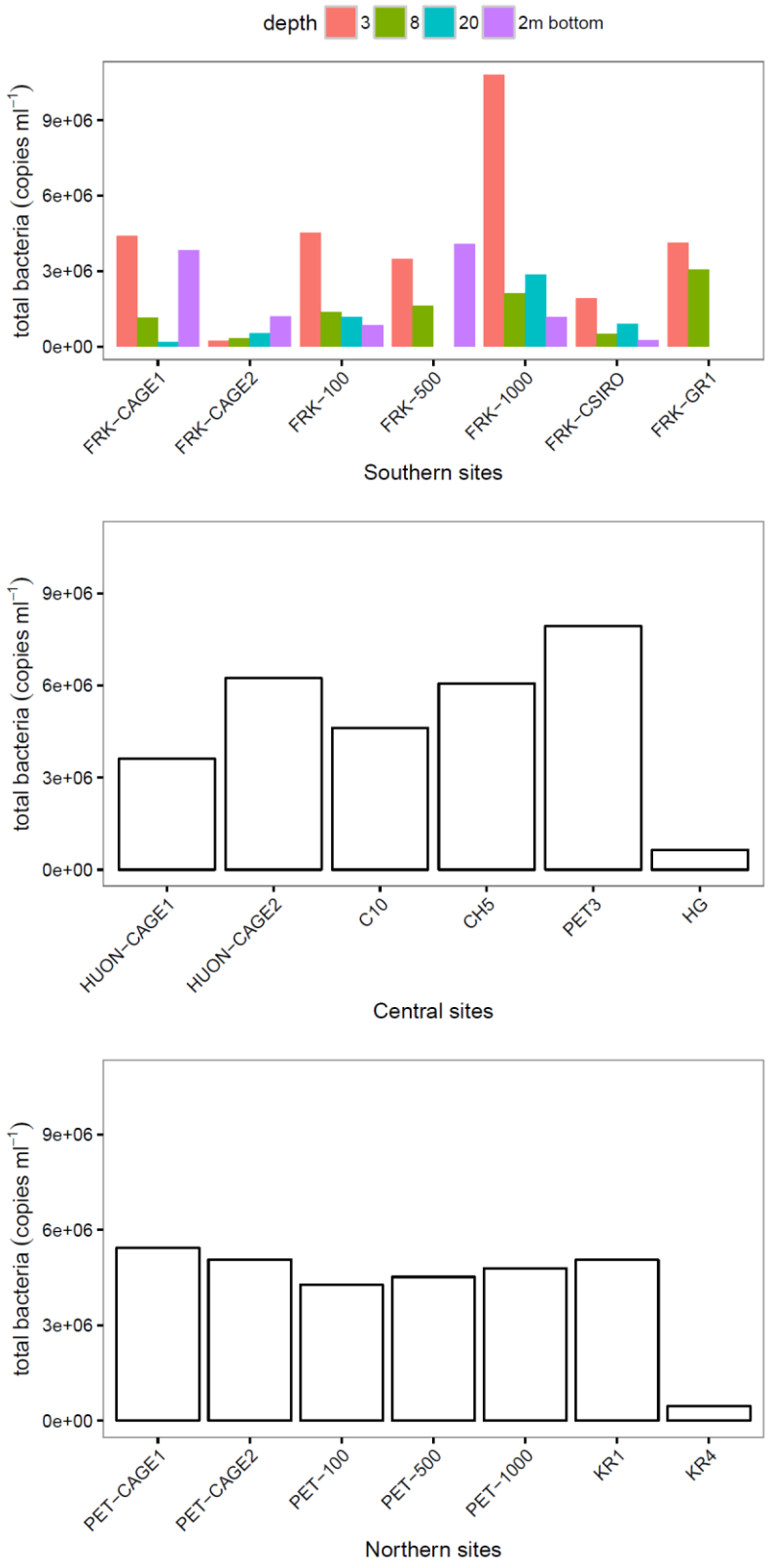

Figure A4 – Total bacteria concentrations (16S) from water samples (copies ml-1) collected at each depth (3, 8, 20 and 2m from the bottom) at sites on the southern transect (upper panel), and from surface samples (3m) at the central sites (middle panel) and on the northern transect (lower panel).

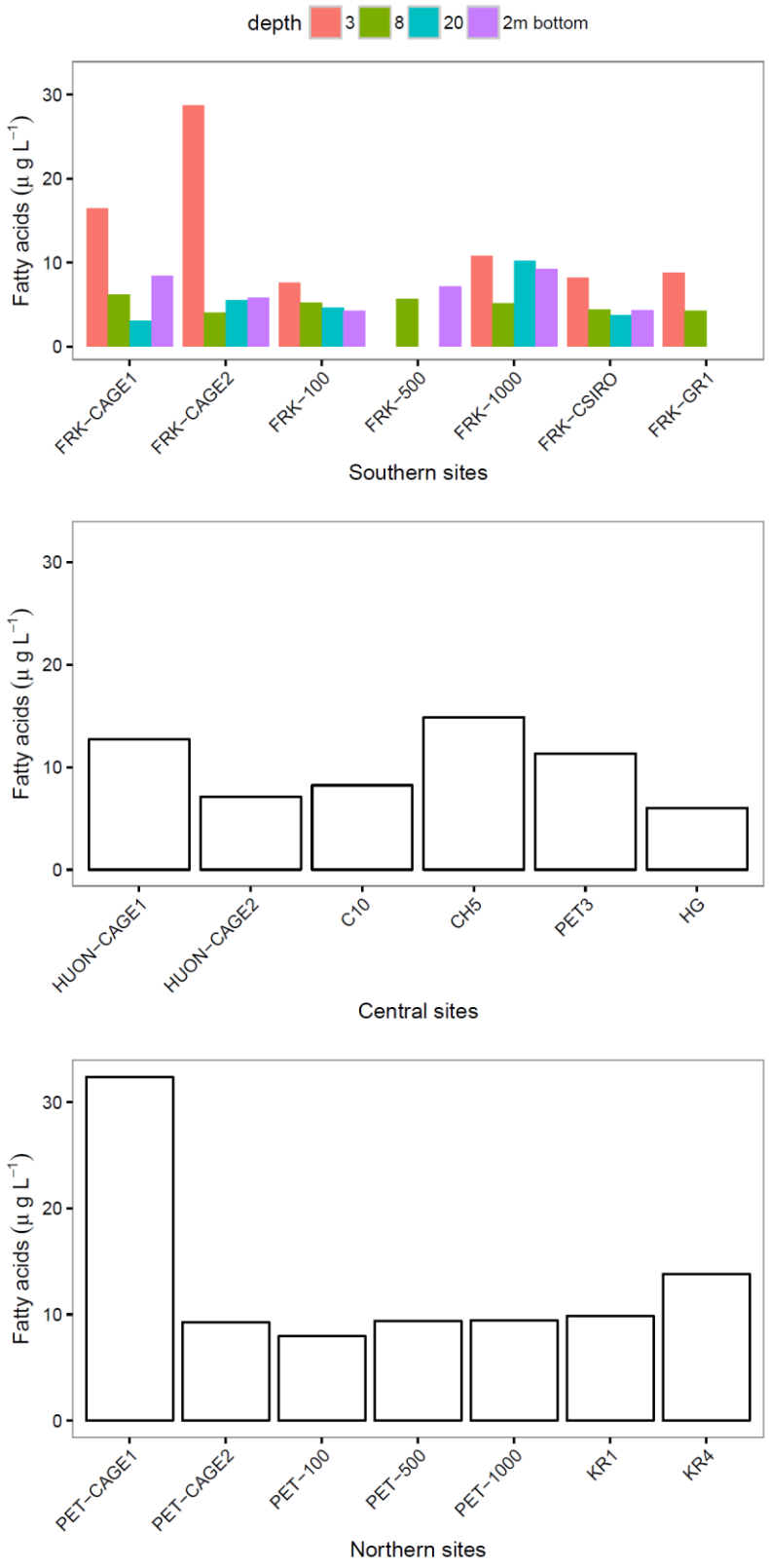

Figure A5 – Total fatty acid concentrations from water samples (µg L-1) collected at each depth (3, 8, 20 and 2m from the bottom) at sites on the southern transect (upper panel), and from surface samples (3m) at the central sites (middle panel) and on the northern transect (lower panel).

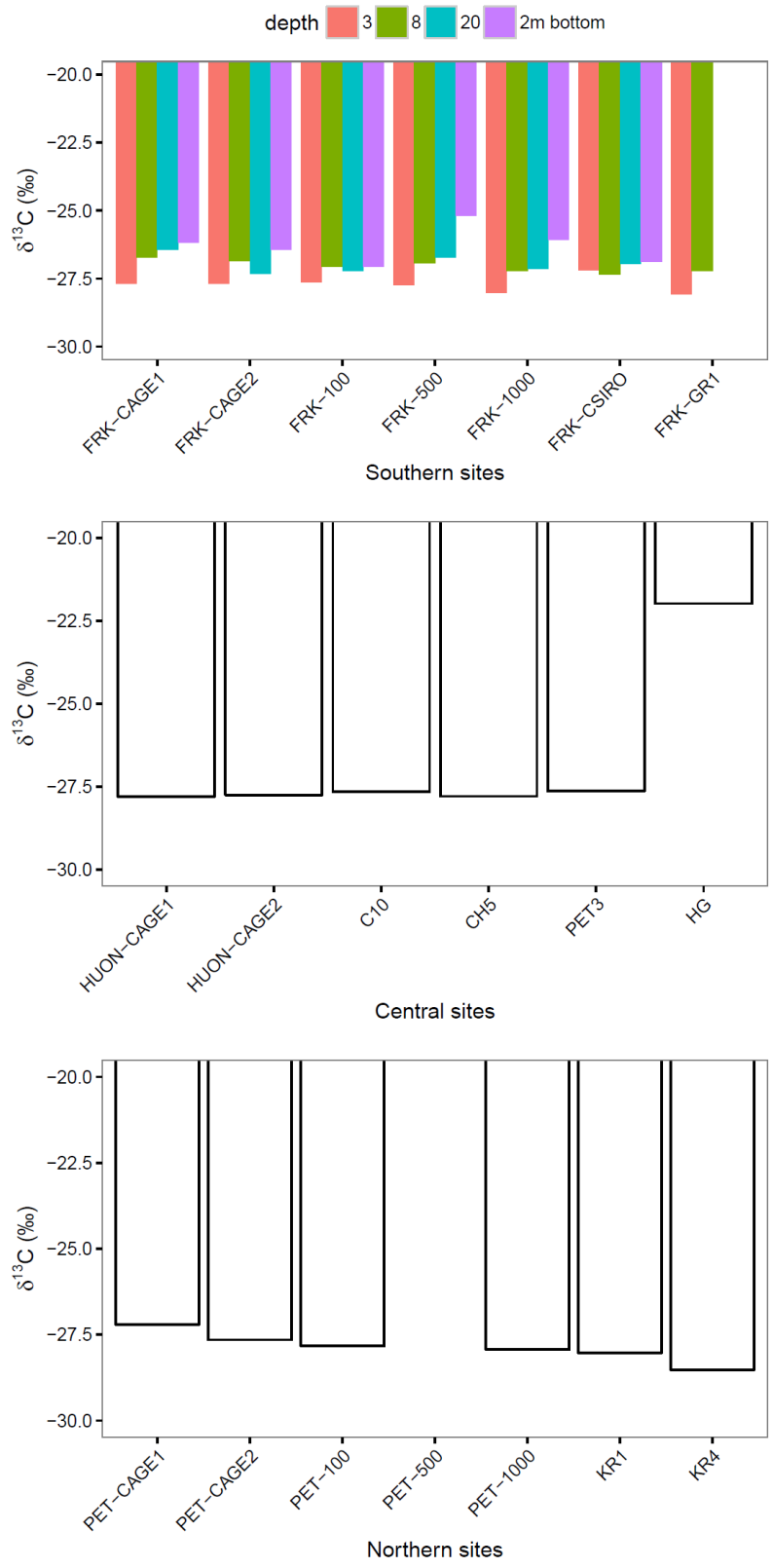

Figure A6 – Stable carbon isotopes (δ13C) of particulate organic matter in water samples collected at each depth (3, 8, 20 and 2m from the bottom) at sites on the southern transect (upper panel), and from surface samples (3m) at the central sites (middle panel) and on the northern transect (lower panel).

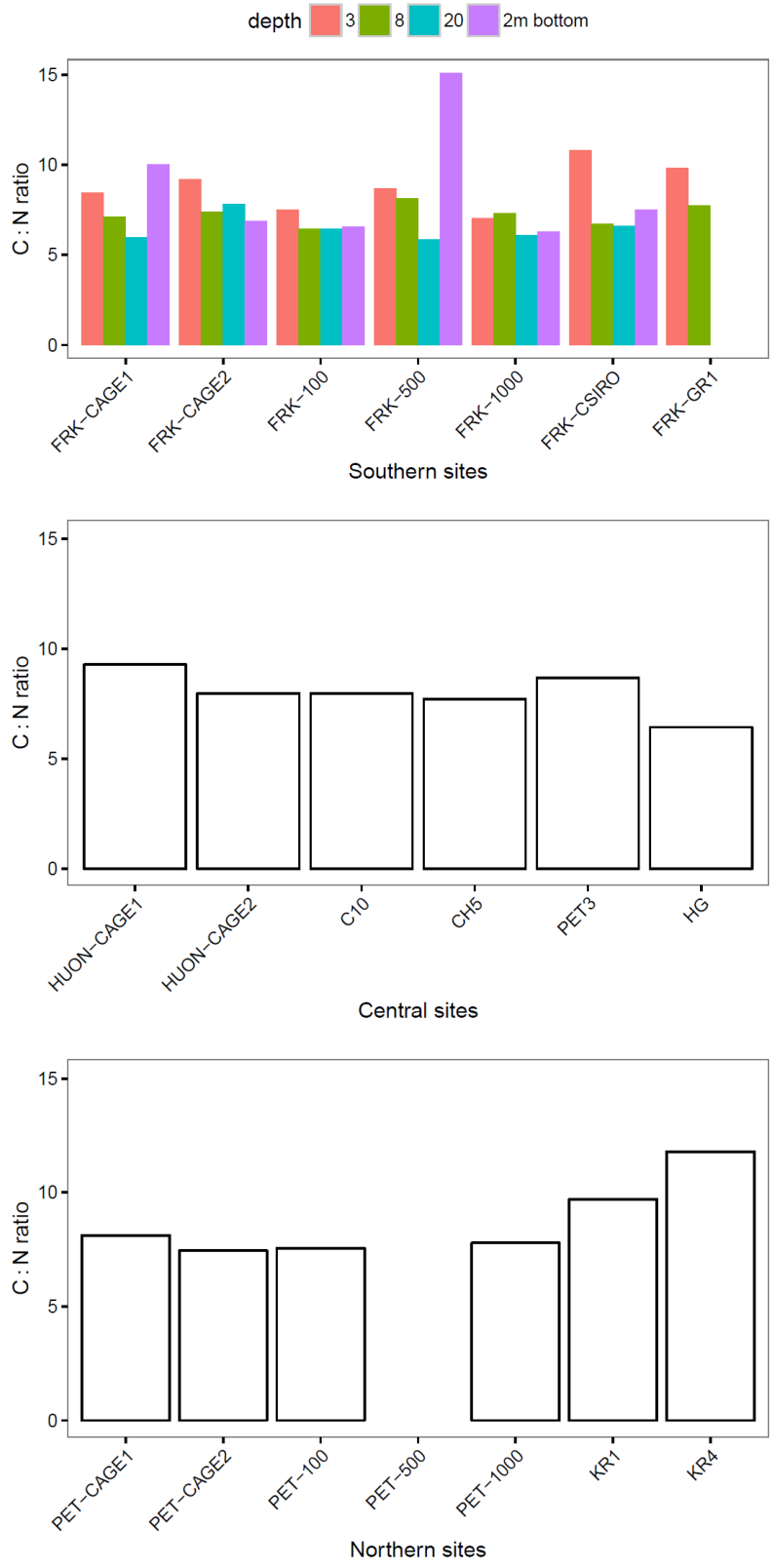

Figure A7 –C:N ratios of particulate organic matter in water samples collected at each depth (3, 8, 20 and 2m from the bottom) at sites on the southern transect (upper panel), and from surface samples (3m) at the central sites (middle panel) and on the northern transect (lower panel).

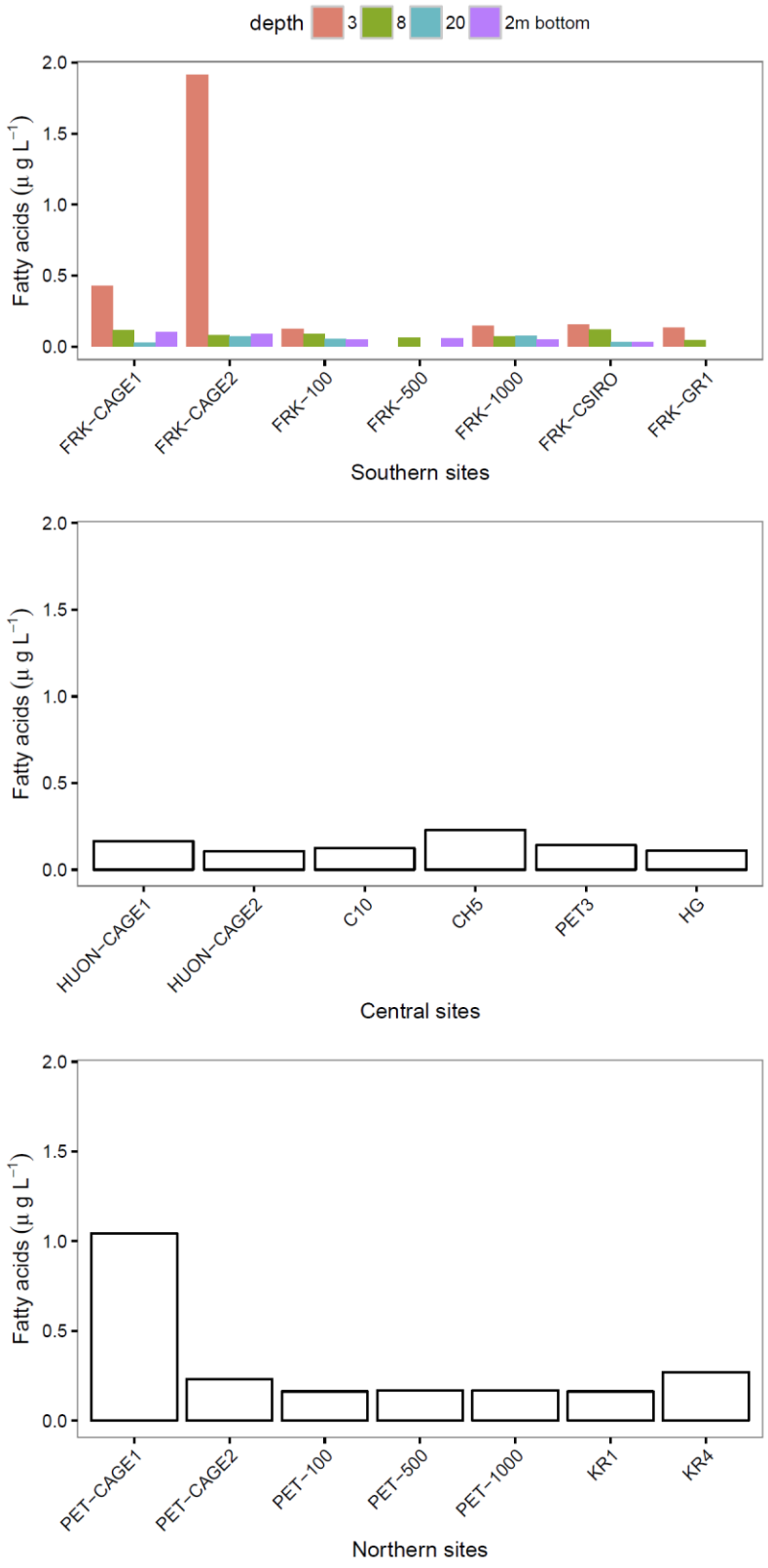

Figure A8 – Fatty acid concentration of LA from water samples (µg L-1) collected at each depth (3, 8, 20 and 2m from the bottom) at sites on the southern transect (upper panel), and from surface samples (3m) at the central sites (middle panel) and on the northern transect (lower panel).

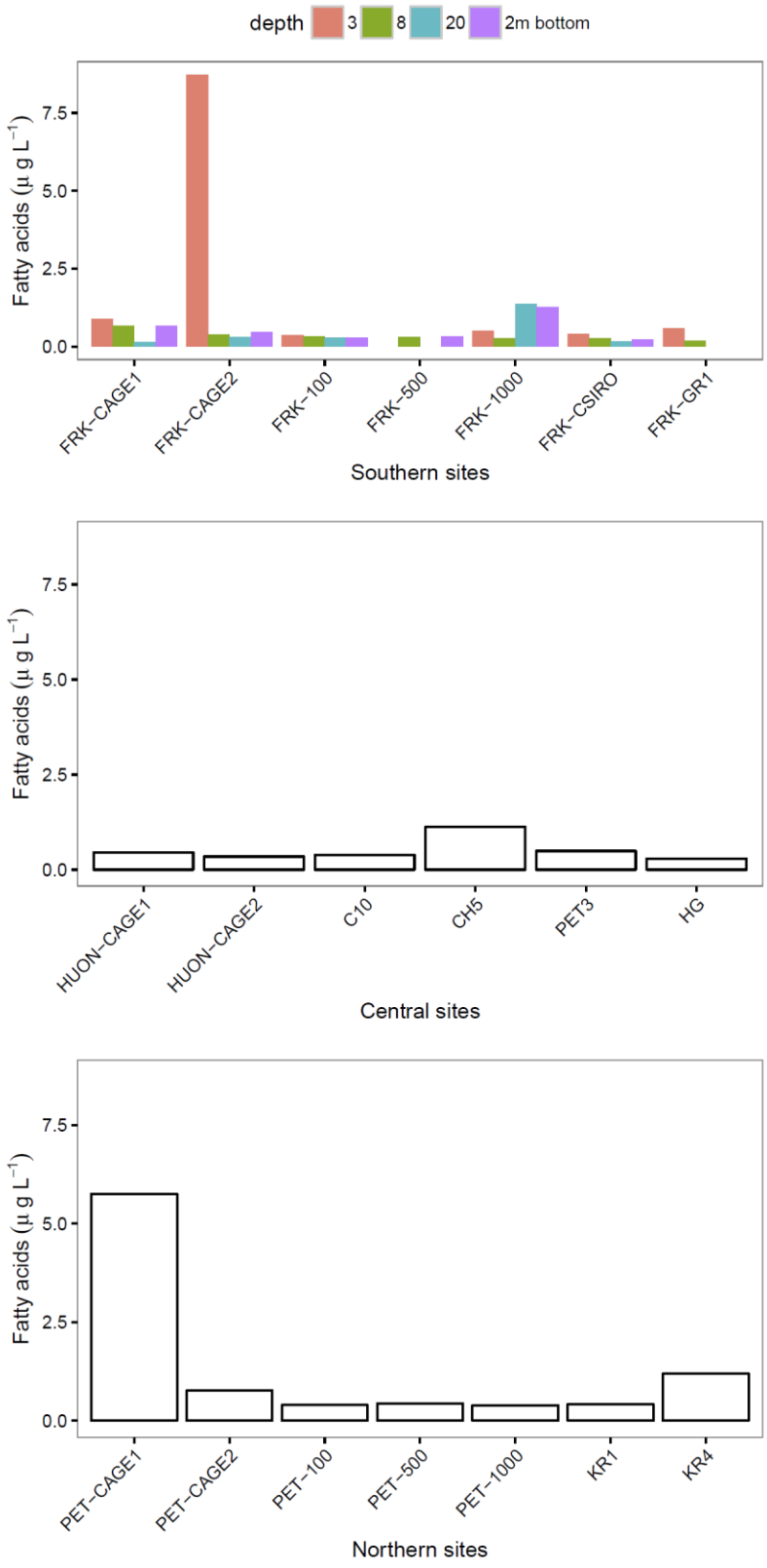

Figure A9 – Fatty acid concentration of OA from water samples (µg L-1) collected at each depth (3, 8, 20 and 2m from the bottom) at sites on the southern transect (upper panel), and from surface samples (3m) at the central sites (middle panel) and on the northern transect (lower panel).

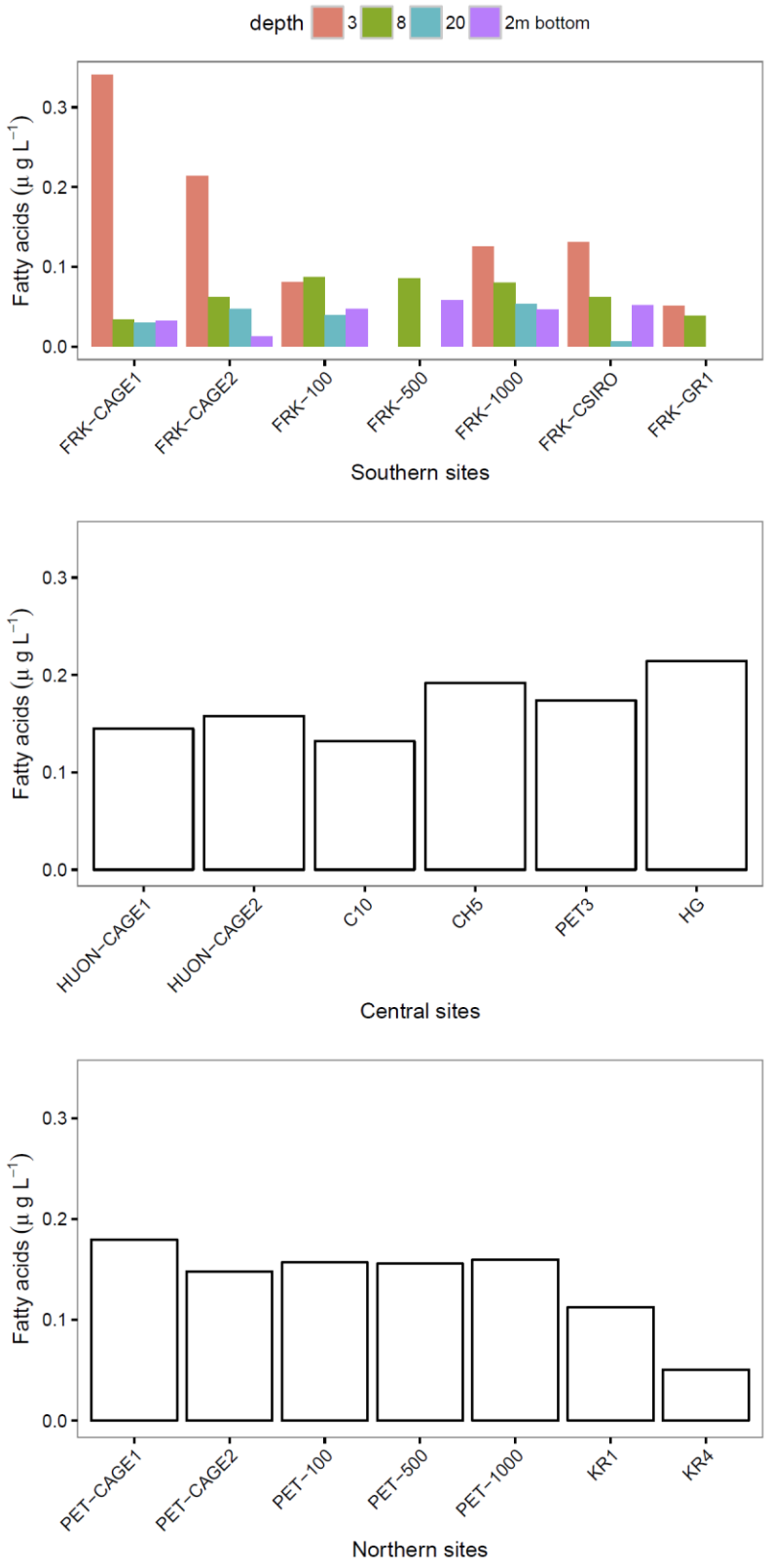

Figure A10 – Fatty acid concentration of EPA from water samples (µg L-1) collected at each depth (3, 8, 20 and 2m from the bottom) at sites on the southern transect (upper panel), and from surface samples (3m) at the central sites (middle panel) and on the northern transect (lower panel).

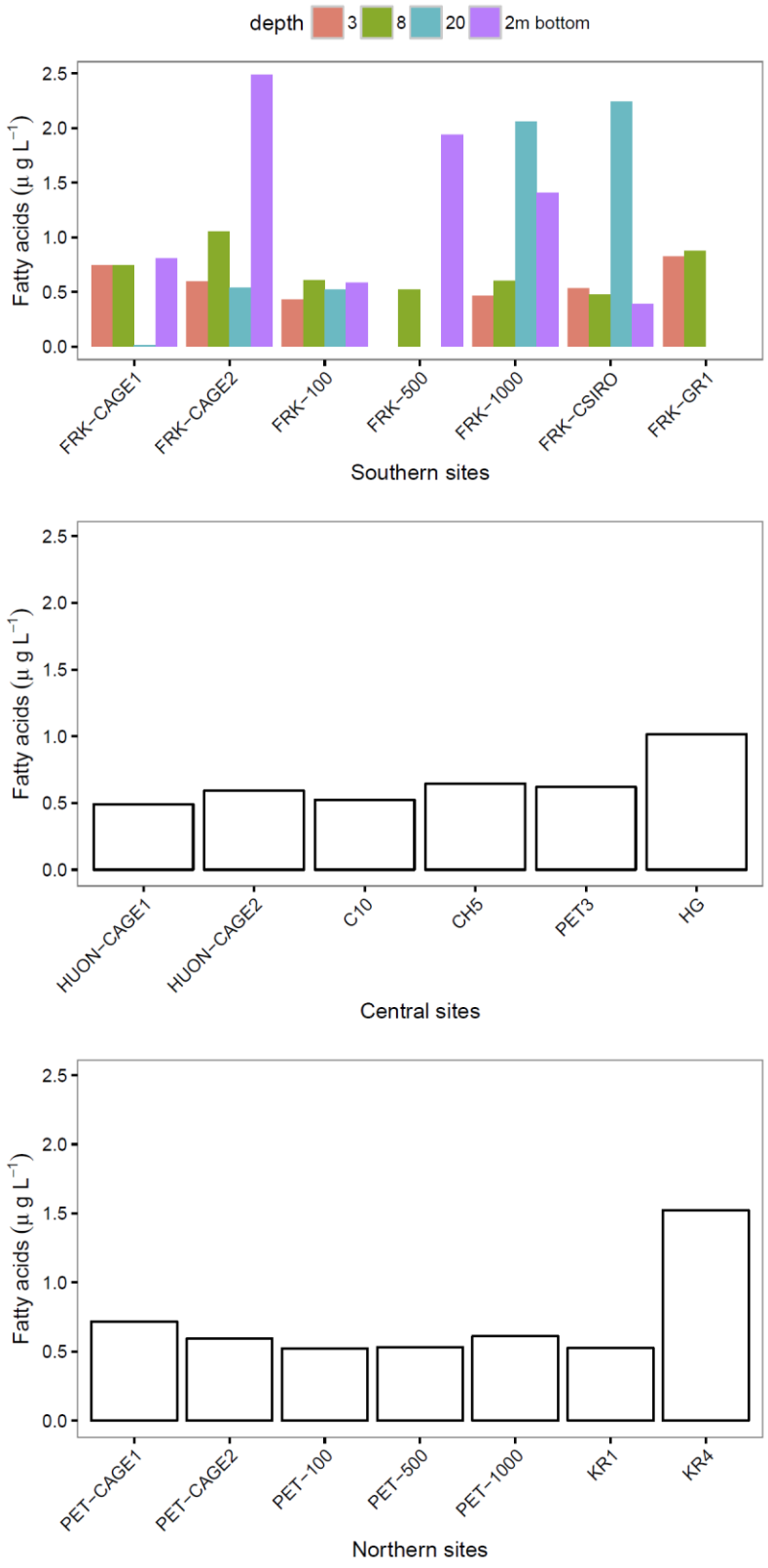

Figure A11 – Fatty acid concentration of DHA from water samples (µg L-1) collected at each depth (3, 8, 20 and 2m from the bottom) at sites on the southern transect (upper panel), and from surface samples (3m) at the central sites (middle panel) and on the northern transect (lower panel).

	CAGE	100m	500m	1000m	>2000m	OCEAN	RIVER
i14:0	0.039 (0.004)	0.031 (0.005)	0.042	0.034 (0.012)	0.043 (0.01)	0.013	0.028 (0.009)
14:1	0.028 (0.005)	0.019 (0.008)	0.232	0.031 (0.002)	0.034 (0.007)	0.029	0.005 (0.001)
14:0	0.583 (0.105)	0.360 (0.097)	0.531	0.455 (0.121)	0.499 (0.092)	0.436	0.218 (0.052)
4,8,12TMTD	0.015 (0.004)	0.012 (0.004)	0.028	0.020 (0.005)	0.007 (0.003)	0.008	0.028 (0.01)
i15:0	0.203 (0.015)	0.159 (0.021)	0.224	0.197 (0.036)	0.254 (0.057)	0.042	0.208 (0.027)
a15:0	0.163 (0.011)	0.137 (0.02)	0.201	0.164 (0.023)	0.167 (0.012)	0.033	0.113 (0.003)
15:0	0.147 (0.018)	0.079 (0.005)	0.105	0.091 (0.009)	0.122 (0.009)	0.065	0.095 (0.028)
i16:0	0.084 (0.011)	0.061 (0)	0.075	0.072 (0.009)	0.070 (0.004)	0.081	0.066 (0.008)
16:1w9c	0.144 (0.007)	0.131 (0.016)	0.108	0.122 (0.008)	0.159 (0.012)	0.055	0.108 (0.003)
16:1w7c	1.491 (0.125)	1.131 (0.062)	1.360	1.280 (0.172)	1.319 (0.089)	0.585	1.049 (0.221)
16:1w7t	0.017 (0.008)	0.013 (0)	0.006	0.013 (0.002)	0.005 (0.003)	0.011	0.028 (0.006)
16:1w5c	0.048 (0.002)	0.043 (0.003)	0.047	0.051 (0.006)	0.047 (0.003)	0.021	0.099 (0.02)
16:1w13t	0.054 (0.01)	0.043 (0.012)	0.057	0.063 (0.007)	0.039 (0.004)	0.099	0.043 (0.01)
16:0	4.813 (1.501)	1.773 (0.159)	2.042	2.678 (0.175)	2.530 (0.273)	1.572	3.086 (0.922)
br17:1a	0.009 (0.004)	0.000 (0)	0.000	0.010 (0.003)	0.007 (0.003)	0.000	0.000 (0)
br17:1b	0.002 (0.002)	0.000 (0)	0.000	0.004 (0.004)	0.002 (0.001)	0.000	0.002 (0.002)
16:0FALD	0.014 (0.007)	0.004 (0.004)	0.006	0.008 (0.008)	0.003 (0.002)	0.009	0.014 (0.014)
i17:0	0.072 (0.01)	0.048 (0.007)	0.059	0.055 (0.008)	0.073 (0.009)	0.029	0.074 (0.022)
17:1w8c+a17:0	0.095 (0.011)	0.064 (0.002)	0.069	0.078 (0.003)	0.082 (0.008)	0.031	0.073 (0.011)
17:1	0.005 (0.002)	0.000 (0)	0.000	0.003 (0.003)	0.003 (0.002)	0.005	0.002 (0.002)
17:0	0.119 (0.03)	0.055 (0.006)	0.064	0.080 (0.002)	0.078 (0.007)	0.049	0.079 (0.007)
18:3w6	0.163 (0.035)	0.265 (0.087)	0.350	0.352 (0.006)	0.228 (0.033)	0.149	0.059 (0.043)
18:4w3	0.266 (0.145)	0.000 (0)	0.000	0.000 (0)	0.013 (0.013)	0.305	0.084 (0.03)
i18:0	0.010 (0.002)	0.006 (0.006)	0.000	0.004 (0.004)	0.002 (0.002)	0.019	0.057 (0.002)
18:2w6 LA	0.648 (0.289)	0.143 (0.02)	0.167	0.156 (0.011)	0.163 (0.018)	0.111	0.202 (0.069)
18:3w3	0.316 (0.114)	0.156 (0.033)	0.172	0.235 (0.064)	0.159 (0.041)	0.267	0.113 (0.03)
18:1w9c OA	2.823 (1.451)	0.379 (0.014)	0.436	0.447 (0.061)	0.564 (0.143)	0.295	0.890 (0.303)
18:1w7c	1.574 (0.107)	1.216 (0.002)	1.277	1.373 (0.163)	1.326 (0.065)	0.302	1.004 (0.325)
18:1w7t	0.037 (0.009)	0.018 (0.002)	0.019	0.023 (0.001)	0.027 (0.003)	0.019	0.038 (0.012)
18:1a	0.044 (0.013)	0.022 (0.005)	0.030	0.038 (0.006)	0.035 (0.004)	0.031	0.049 (0.002)
18:1b	0.004 (0.003)	0.006 (0.003)	0.016	0.004 (0.004)	0.009 (0.003)	0.012	0.020 (0.008)
18:1c	0.011 (0.004)	0.000 (0)	0.000	0.006 (0.006)	0.005 (0.003)	0.011	0.006 (0.006)
18:0	2.295 (0.718)	0.722 (0.189)	0.812	1.246 (0.182)	1.287 (0.169)	0.518	1.945 (1.076)
18:1FALD	0.023 (0.008)	0.014 (0.014)	0.014	0.006 (0.006)	0.011 (0.003)	0.008	0.000 (0)
18:0FALD	0.040 (0.01)	0.017 (0.001)	0.032	0.017 (0.004)	0.029 (0.004)	0.020	0.031 (0.01)
19:1a	0.009 (0.003)	0.002 (0.002)	0.008	0.002 (0.002)	0.007 (0.003)	0.004	0.004 (0)
19:1b	0.011 (0.003)	0.006 (0)	0.004	0.005 (0.001)	0.010 (0.004)	0.004	0.008 (0.003)
19:0	0.053 (0.011)	0.025 (0.006)	0.039	0.025 (0.002)	0.047 (0.01)	0.019	0.078 (0.021)
20:4w6	0.041 (0.007)	0.019 (0.005)	0.020	0.028 (0.001)	0.027 (0.006)	0.022	0.017 (0.008)
20:5w3 EPA	0.197 (0.031)	0.119 (0.038)	0.156	0.142 (0.017)	0.148 (0.015)	0.214	0.051 (0)
20:3	0.003 (0.002)	0.003 (0.003)	0.000	0.000 (0)	0.002 (0.002)	0.000	0.002 (0.001)
20:3w6	0.014 (0.006)	0.007 (0.007)	0.015	0.005 (0.005)	0.006 (0.003)	0.005	0.000 (0)
20:4w3	0.022 (0.009)	0.012 (0.002)	0.007	0.010 (0.009)	0.016 (0.006)	0.018	0.008 (0.003)
C20PUFA	0.031 (0.006)	0.011 (0.007)	0.038	0.000 (0)	0.016 (0.007)	0.012	0.007 (0.007)
20:2w6	0.032 (0.013)	0.009 (0.004)	0.022	0.010 (0.006)	0.013 (0.003)	0.013	0.011 (0.004)
20:1w11c	0.049 (0.008)	0.031 (0.002)	0.033	0.033 (0.009)	0.063 (0.022)	0.021	0.054 (0.03)
20:1w9c	0.135 (0.045)	0.047 (0.007)	0.053	0.047 (0.016)	0.205 (0.112)	0.028	0.116 (0.072)
20:1w7c	0.040 (0.008)	0.026 (0.004)	0.026	0.023 (0.005)	0.034 (0.006)	0.014	0.028 (0.005)
20:1w5c	0.017 (0.005)	0.009 (0.001)	0.007	0.009 (0.007)	0.011 (0.002)	0.006	0.021 (0.008)
20:0	0.139 (0.042)	0.046 (0)	0.055	0.053 (0.005)	0.064 (0.008)	0.030	0.131 (0.049)
21:0	0.015 (0.003)	0.007 (0)	0.007	0.009 (0.002)	0.008 (0.002)	0.004	0.023 (0.005)
22:5w6	0.006 (0.003)	0.002 (0.002)	0.003	0.000 (0)	0.004 (0.002)	0.007	0.000 (0)
22:6w3 DHA	0.127 (0.027)	0.058 (0.023)	0.083	0.078 (0.02)	0.086 (0.013)	0.218	0.060 (0.017)
22:4w6	0.003 (0.001)	0.002 (0.002)	0.000	0.000 (0)	0.002 (0.001)	0.006	0.003 (0.003)
22:5w3	0.014 (0.006)	0.004 (0.003)	0.007	0.003 (0.003)	0.006 (0.002)	0.002	0.001 (0.001)
22:1w11c	0.042 (0.015)	0.012 (0.004)	0.024	0.011 (0.002)	0.079 (0.048)	0.010	0.046 (0.041)
22:1w9c	0.053 (0.009)	0.045 (0.006)	0.054	0.029 (0.027)	0.071 (0.012)	0.014	0.156 (0.007)
22:1w7c	0.014 (0.004)	0.006 (0.001)	0.009	0.005 (0.001)	0.010 (0.003)	0.003	0.018 (0.007)
22:0	0.131 (0.051)	0.048 (0.001)	0.056	0.053 (0.003)	0.051 (0.004)	0.020	0.202 (0.086)
24:1w11c	0.014 (0.006)	0.004 (0)	0.007	0.009 (0.004)	0.009 (0.003)	0.017	0.003 (0.003)
24:1w9c	0.036 (0.016)	0.007 (0.001)	0.008	0.013 (0.002)	0.025 (0.013)	0.021	0.019 (0.003)
24:1w7c	0.024 (0.016)	0.010 (0.007)	0.013	0.022 (0.02)	0.012 (0.006)	0.007	0.025 (0.009)
24:0	0.114 (0.023)	0.065 (0.002)	0.069	0.091 (0.014)	0.073 (0.005)	0.044	0.317 (0.093)

Table A7 Fatty acid profile of suspended particulate matter expressed as a concentration (μg L^{-1}, mean ±SE) of total fatty acids for samples collected at 3m depth and pooled according to distance from cage, together with river (KR4 and GR1), ocean (HG) and aquafeed sources.

	3m	8m	20m	2m bottom
i14:0	0.030 (0.005)	0.021 (0.003)	0.021 (0.006)	0.021 (0.006)
14:1	0.016 (0.004)	0.010 (0.003)	0.008 (0.003)	0.013 (0.003)
14:0	0.424 (0.092)	0.218 (0.029)	0.176 (0.052)	0.234 (0.041)
4,8,12TMTD	0.015 (0.005)	0.004 (0.001)	0.008 (0.003)	0.006 (0.004)
i15:0	0.175 (0.016)	0.092 (0.007)	0.157 (0.092)	0.154 (0.065)
a15:0	0.147 (0.014)	0.074 (0.007)	0.054 (0.008)	0.069 (0.008)
15:0	0.110 (0.021)	0.076 (0.006)	0.067 (0.013)	0.074 (0.01)
i16:0	0.081 (0.01)	0.051 (0.006)	0.057 (0.011)	0.078 (0.01)
16:1w9c	0.143 (0.009)	0.099 (0.014)	0.151 (0.025)	0.156 (0.006)
16:1w7c	1.399 (0.122)	0.646 (0.052)	0.462 (0.078)	0.945 (0.118)
16:1w7t	0.020 (0.007)	0.016 (0.004)	0.032 (0.005)	0.069 (0.01)
16:1w5c	0.052 (0.006)	0.031 (0.002)	0.022 (0.003)	0.034 (0.004)
16:1w13t	0.055 (0.011)	0.017 (0.002)	0.011 (0.002)	0.013 (0.002)
16:0	3.177 (0.66)	1.291 (0.128)	1.355 (0.19)	1.393 (0.203)
br17:1a	0.003 (0.002)	0.003 (0.001)	0.004 (0.001)	0.007 (0.001)
br17:1b	0.001 (0.001)	0.000 (0)	0.001 (0.001)	0.000 (0)
16:0FALD	0.006 (0.003)	0.006 (0.001)	0.009 (0.004)	0.010 (0.002)
i17:0	0.059 (0.01)	0.029 (0.001)	0.038 (0.016)	0.041 (0.01)
17:1w8c+a17	0.081 (0.009)	0.045 (0.003)	0.045 (0.011)	0.066 (0.007)
17:1	0.005 (0.001)	0.004 (0.001)	0.003 (0.001)	0.006 (0.003)
17:0	0.089 (0.015)	0.044 (0.004)	0.044 (0.012)	0.049 (0.007)
18:3w6	0.197 (0.041)	0.057 (0.01)	0.024 (0.01)	0.028 (0.006)
18:4w3	0.222 (0.152)	0.014 (0.011)	0.012 (0.008)	0.020 (0.006)
i18:0	0.013 (0.009)	0.004 (0.001)	0.019 (0.004)	0.014 (0.002)
18:2w6 LA	0.483 (0.29)	0.083 (0.01)	0.051 (0.009)	0.064 (0.011)
18:3w3	0.352 (0.105)	0.042 (0.004)	0.021 (0.009)	0.025 (0.007)
18:1w9c OA	1.913 (1.365)	0.342 (0.059)	0.456 (0.231)	0.543 (0.157)
18:1w7c	1.507 (0.119)	0.593 (0.053)	0.280 (0.05)	0.412 (0.033)
18:1w7t	0.031 (0.007)	0.014 (0.003)	0.012 (0.002)	0.032 (0.009)
18:1a	0.039 (0.013)	0.012 (0.002)	0.010 (0.005)	0.016 (0.003)
18:1b	0.006 (0.003)	0.003 (0.001)	0.003 (0.001)	0.006 (0.001)
18:1c	0.008 (0.003)	0.002 (0.001)	0.002 (0.002)	0.005 (0.002)
18:0	1.381 (0.337)	0.622 (0.061)	0.787 (0.156)	0.820 (0.158)
18:1FALD	0.004 (0.004)	0.001 (0.001)	0.003 (0.002)	0.006 (0.002)
18:0FALD	0.028 (0.005)	0.012 (0.002)	0.023 (0.008)	0.024 (0.005)
19:1a	0.005 (0.003)	0.001 (0)	0.004 (0.004)	0.004 (0.002)
19:1b	0.008 (0.002)	0.003 (0)	0.012 (0.005)	0.026 (0.015)
19:0	0.035 (0.007)	0.016 (0.002)	0.025 (0.009)	0.024 (0.005)
20:4w6	0.031 (0.009)	0.011 (0.001)	0.021 (0.007)	0.015 (0.003)
20:5w3 EPA	0.157 (0.043)	0.064 (0.008)	0.035 (0.008)	0.041 (0.007)
20:3	0.002 (0.002)	0.004 (0.001)	0.020 (0.007)	0.021 (0.005)
20:3w6	0.007 (0.005)	0.003 (0.001)	0.004 (0.002)	0.006 (0.002)
20:4w3	0.028 (0.008)	0.008 (0.001)	0.010 (0.003)	0.010 (0.002)
C20PUFA	0.013 (0.008)	0.003 (0.002)	0.011 (0.005)	0.007 (0.003)
20:2w6	0.025 (0.012)	0.011 (0.004)	0.036 (0.006)	0.014 (0.004)
20:1w11c	0.046 (0.008)	0.022 (0.002)	0.046 (0.034)	0.043 (0.026)
20:1w9c	0.086 (0.038)	0.020 (0.006)	0.269 (0.223)	0.205 (0.158)
20:1w7c	0.036 (0.007)	0.013 (0.001)	0.080 (0.01)	0.057 (0.014)
20:1w5c	0.020 (0.004)	0.008 (0.001)	0.011 (0.002)	0.022 (0.01)
20:0	0.107 (0.04)	0.040 (0.005)	0.042 (0.008)	0.058 (0.014)
21:0	0.014 (0.002)	0.007 (0)	0.008 (0.002)	0.017 (0.009)
22:5w6	0.006 (0.003)	0.006 (0.001)	0.008 (0.003)	0.008 (0.002)
22:6w3 DHA	0.098 (0.034)	0.043 (0.005)	0.034 (0.019)	0.047 (0.015)
22:4w6	0.000 (0)	0.002 (0.001)	0.002 (0.001)	0.004 (0.002)
22:5w3	0.010 (0.005)	0.002 (0.001)	0.020 (0.015)	0.015 (0.008)
22:1w11c	0.016 (0.007)	0.006 (0.001)	0.098 (0.091)	0.082 (0.064)
22:1w9c	0.072 (0.022)	0.027 (0.006)	0.052 (0.025)	0.082 (0.036)
22:1w7c	0.013 (0.003)	0.006 (0.001)	0.025 (0.008)	0.023 (0.009)
22:0	0.130 (0.052)	0.032 (0.005)	0.030 (0.004)	0.078 (0.03)
24:1w11c	0.011 (0.006)	0.005 (0.001)	0.013 (0.007)	0.014 (0.006)
24:1w9c	0.023 (0.011)	0.012 (0.003)	0.028 (0.025)	0.046 (0.023)
24:1w7c	0.033 (0.014)	0.012 (0.004)	0.010 (0.002)	0.012 (0.005)
24:0	0.133 (0.03)	0.042 (0.005)	0.037 (0.007)	0.083 (0.041)

Table A8 Fatty acid profile of suspended particulate matter expressed as concentrations (μg L-1, mean ±SE) of fatty acids for samples collected at each depth on the southern transect.

	CAGE	100m	500m	1000m	>2000m	OCEAN	RIVER
Total FA	17.784 (4.271)	7.774 (0.169)	9.406	10.133 (0.683)	10.501 (1.239)	6.021	11.321 (2.496)
≤C_{18} SFA	7.957 (2.342)	2.990 (0.24)	3.554	4.549 (0.103)	4.515 (0.531)	2.641	5.422 (2.086)
≥C_{20} SFA	0.383 (0.1)	0.160 (0.003)	0.180	0.198 (0.023)	0.187 (0.016)	0.094	0.650 (0.227)
PUFU	1.844 (0.499)	0.788 (0.215)	1.017	1.004 (0.012)	0.865 (0.11)	1.325	0.607 (0.066)
Bacteria	1.949 (0.153)	1.417 (0.018)	1.524	1.621 (0.144)	1.625 (0.088)	0.465	1.289 (0.255)
DHA:EPA	0.622 (0.038)	0.475 (0.046)	0.532	0.538 (0.072)	0.569 (0.026)	1.016	1.173 (0.35)

Table A9 Concentrations (µg L^{-1}, mean ±SE) of saturated fatty acids (≤C_{18} & ≥C_{20}), polyunsaturated fatty acids, fatty acids consistent with a bacteria source (15:0, $_{i}$17:0, 17:0 and 18:1$_{w}$7) and the ratio of DHA:EPA for samples collected at 3m depth and pooled according to distance from cage, together with river (KR4 and GR1), ocean (HG) and aquafeed sources

	3m	8m	20m	2m bottom
Total FA	13.440 (3.335)	5.005 (0.296)	5.420 (1.267)	6.518 (0.858)
≤C_{18} SFA	5.183 (1.101)	2.251 (0.191)	2.429 (0.364)	2.570 (0.37)
≥C_{20} SFA	0.370 (0.103)	0.115 (0.012)	0.108 (0.017)	0.218 (0.082)
PUFU	1.593 (0.544)	0.338 (0.028)	0.275 (0.045)	0.288 (0.03)
Bacteria	1.798 (0.163)	0.755 (0.059)	0.441 (0.087)	0.609 (0.057)
DHA:EPA	0.599 (0.064)	0.697 (0.078)	1.075 (0.45)	1.269 (0.337)

Table A10 Concentrations (µg L^{-1}, mean ±SE) of saturated fatty acids (≤C_{18} & ≥C_{20}), polyunsaturated fatty acids, fatty acids consistent with a bacteria source (15:0, $_{i}$17:0, 17:0 and 18:1$_{w}$7) and the ratio of DHA:EPA for samples collected at each depth on the southern transect.

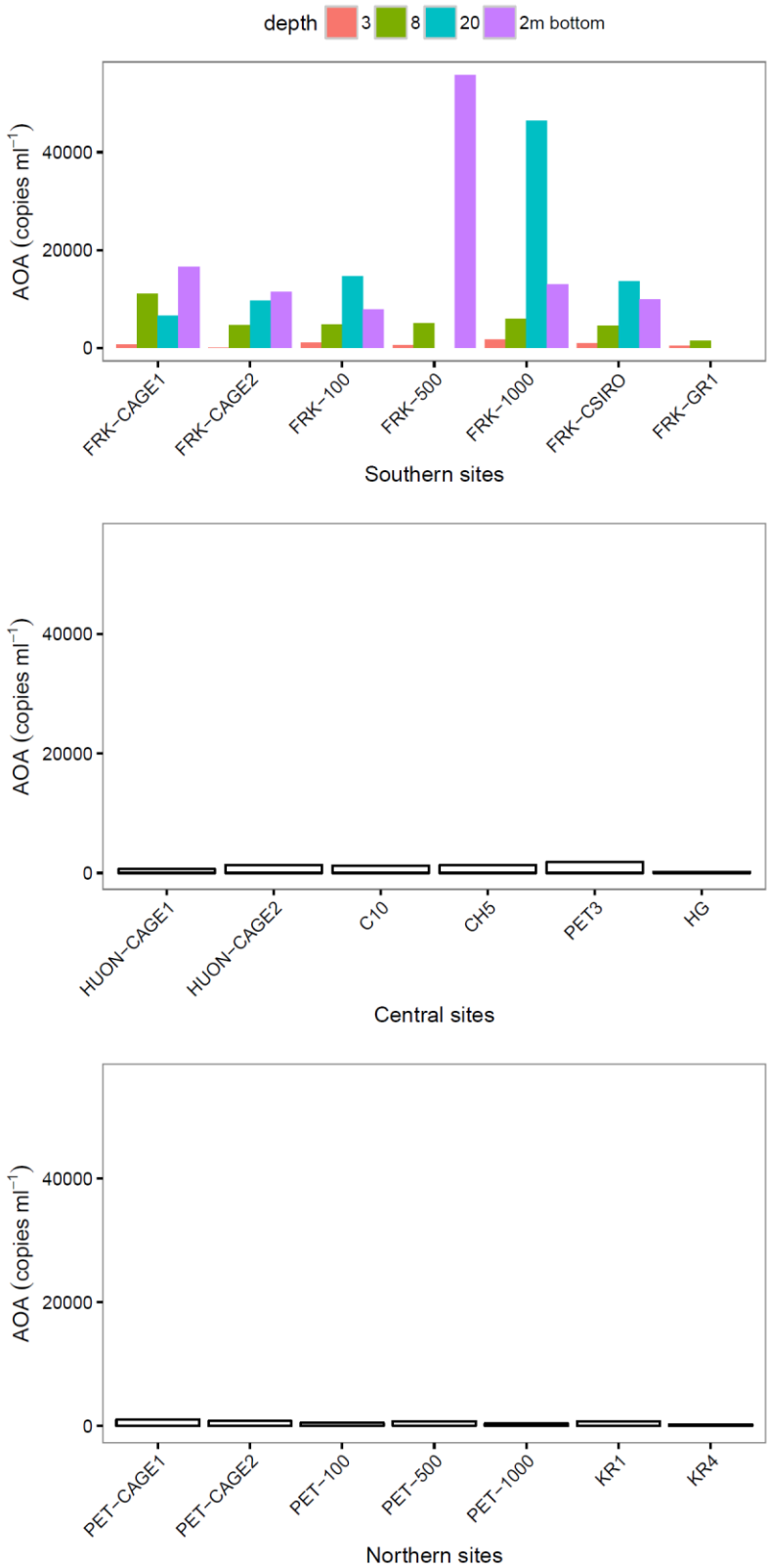

Figure A12 – Abundance of marker genes for archaeal nitrifiers (AOA) from water samples collected at each depth (3, 8, 20 and 2m from the bottom) at sites on the southern transect (upper panel), and from surface samples (3m) at the central sites (middle panel) and on the northern transect (lower panel). Abundances are expressed as copies per ml of water.

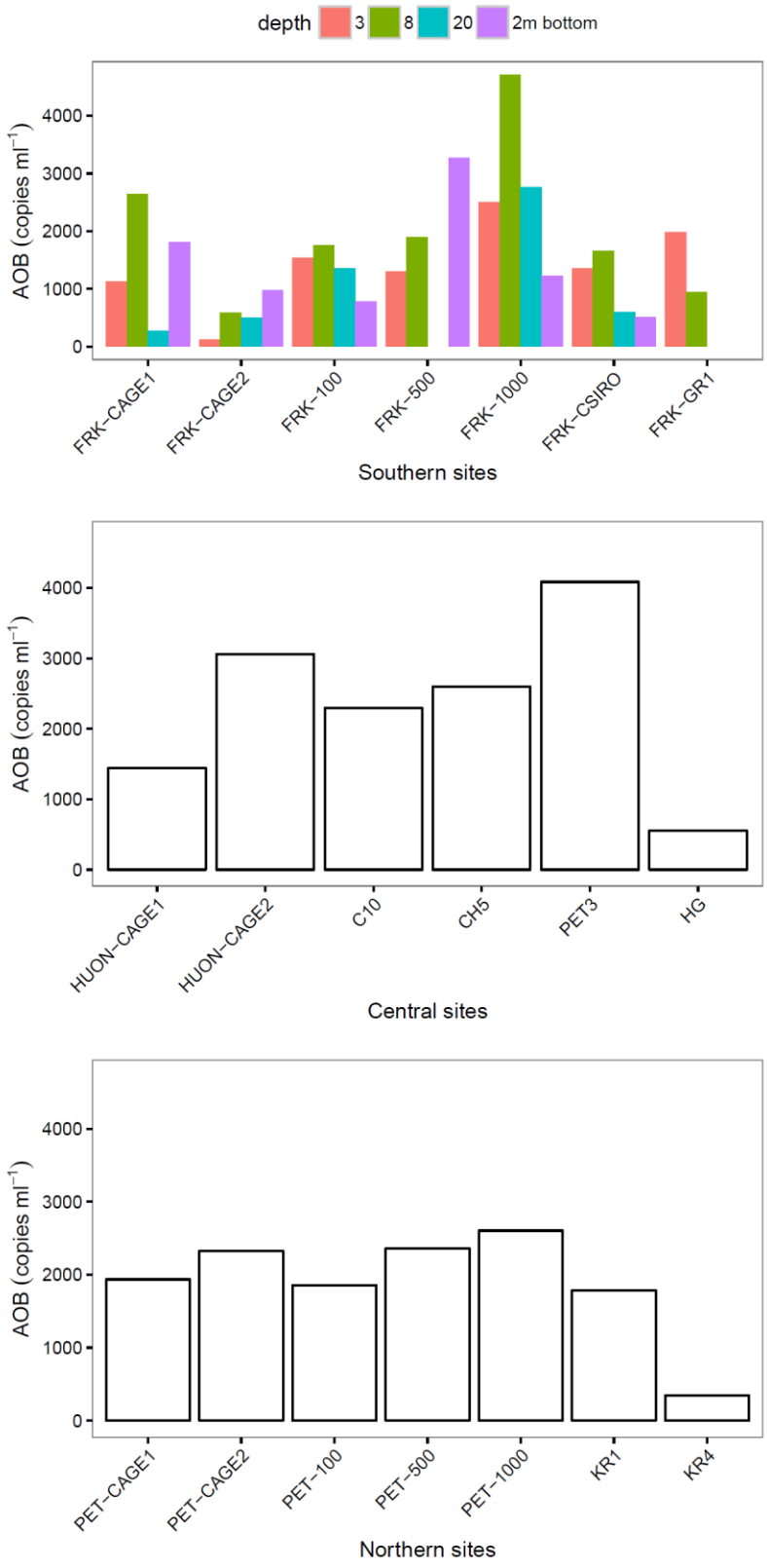

Figure A13 – Abundance of marker genes for bacterial nitrifiers (AOB) from water samples collected at each depth (3, 8, 20 and 2m from the bottom) at sites on the southern transect (upper panel), and from surface samples (3m) at the central sites (middle panel) and on the northern transect (lower panel). Abundances are expressed as copies per ml of water.

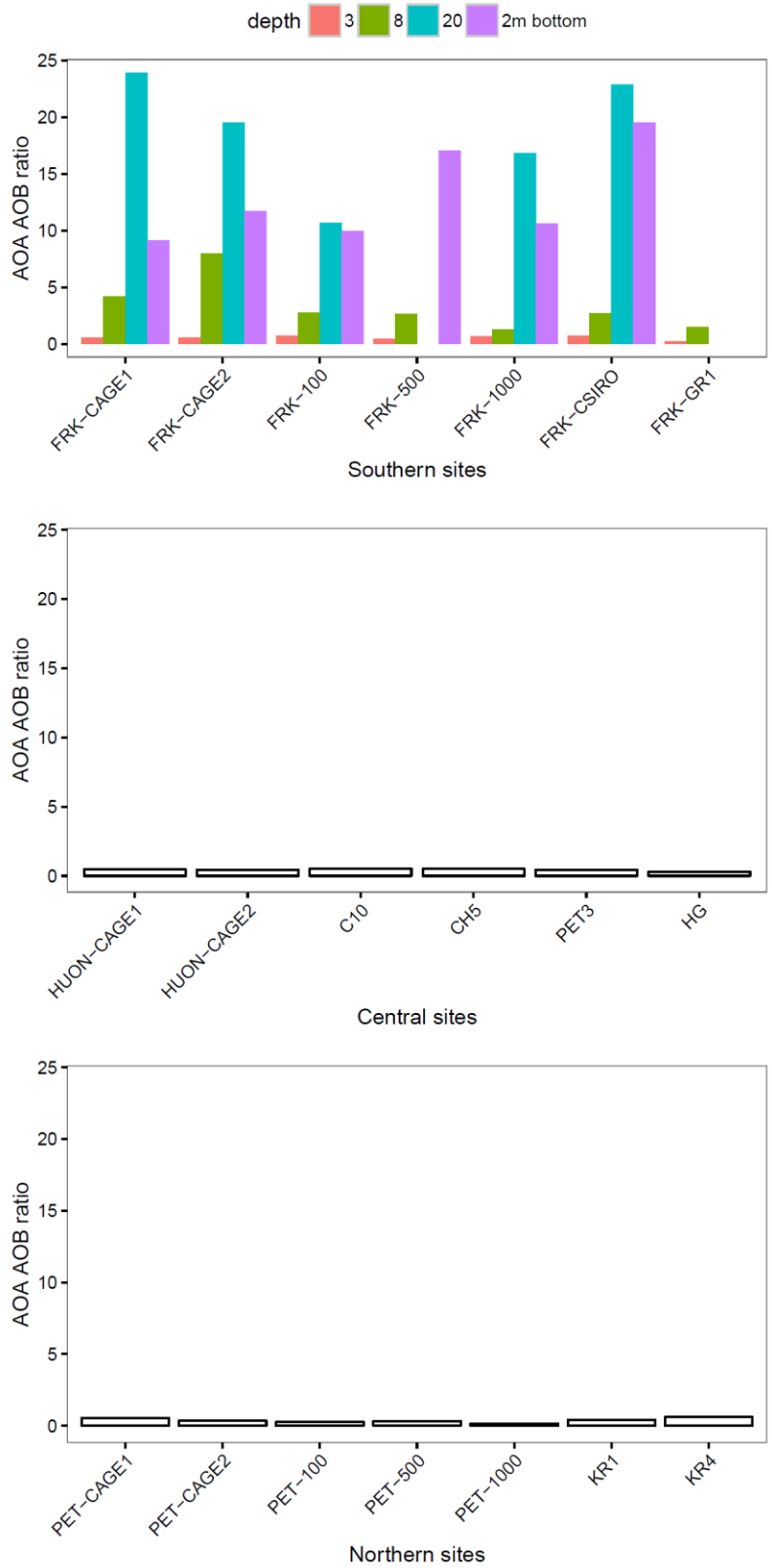

Figure A14 – Ratio of the abundance of marker genes for archaeal (AOA) and bacterial (AOB) nitrifiers from water samples collected at each depth (3, 8, 20 and 2m from the bottom) at sites on the southern transect (upper panel), and from surface samples (3m) at the central sites (middle panel) and on the northern transect (lower panel).