The contribution of salmon farming material to sediment accumulation on reefs in established farming regions.

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Executive Summary

This study was commissioned by the Department of Primary Industries (DPIPWE), Tasmanian Abalone Council (TAC) and representatives of the Tasmanian salmonid farming industry (Tassal Pty. Ltd and Huon Aquaculture Pty. Ltd) to establish whether salmon farming has a major contribution to sedimentation on reef ecosystems in farming regions and to determine what analyses and methods might be most appropriate for detecting fish farm signatures. The findings show that sedimentation rates were not noticeably elevated in areas where salmon farming is undertaken relative to areas without salmon farming and that the "dust" collecting on macroalgae in the Huon and D'Entrecasteaux Channel is not salmon food or faeces. It was not possible to identify a reliable indicator of salmon derived material that could be used to characterise salmon inputs into the broader environment. The isotope and fatty acid signals of sites closest to the salmon farm leases were generally similar to control sites. This reflects the background signal inputs which also showed high variability for several markers. The approaches assessed in the current study were confounded by the diverse nature of the other background inputs into the region.

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1. Introduction

The Institute for Marine and Antarctic Studies (IMAS) was asked by the Department of Primary Industries (DPIPWE), Tasmanian Abalone Council (TAC) and representatives of the Tasmanian salmonid farming industry (Tassal Pty. Ltd And Huon Aquaculture Pty. Ltd) to investigate "dust" material associated with inshore reefs in SE Tasmania, particularly in the lower Huon and D'Entrecasteaux Channel region. It had been proposed that this "dust" may be directly related to salmonid farming activities. Consequently, a study was developed to establish firstly whether sedimentation was in fact greater in areas where fish farming occurs than in other similar regions, and secondly to what extent marine farming of salmonids might contribute to these sediments.

Comparison of sedimentation load and rates of deposition between different areas can be readily achieved using sediment traps. However, the collection and quantitative sampling of the dust material already accumulated on reef algae is a more difficult task and it has been recognised that there is a current need to further develop the analyses and methods appropriate for detecting fish farm signatures on reefs in the Tasmanian context. This project sought to specifically develop a suitable sample collection technique.

Characterisation of the resultant sediment material and identification of specific indicators of fish farm derived materials is also quite complex. 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 and have 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 have a large number of unique chemical structures that, when identified, can be viewed as a profile to provide a distinctive "signature"; these signatures help describe the transfer of dietary material through marine and terrestrial food webs (Parrish et al. 2014).

Stable isotope analysis can similarly be used to identify and inform how extraneous sources of organic material might be transferred through the food chain. All biologically active elements exist in a number of different isotopic forms, with some being more abundant

relative to others and some being more stable than others. For example most carbon is present as 12C, with approximately 1% being 13C. The ratio of these two isotopes may be altered by biological processes, and when isotopic information is available on the various components of the food web it is possible to draw direct inferences regarding sources and diet and trophic level interactions.

Redmond et al. (2010) used a combination of fatty acid profiles and stable isotopes (a parameter similarly used to understand source links) to successfully trace the assimilation of salmon pellets in the blue mussel, *Mytilus edulis*. Stable isotope signatures have also been employed to successfully monitor the footprint of a barramundi farm in Northern Queensland, where isotopic nitrogen (^{15}N) from aquaculture feeds was identified in the leaves of mangrove trees along a creek system (McKinnon et al. 2010).

In the current study it was proposed that a combination of fatty acid (lipid) and stable isotope analysis be used to characterise both the collected sediment material (dust) and potential sources. Port Esperance was specifically identified as a site where sediment deposition appeared to have increased. It is also the location of one of the earliest fish farm leases, and therefore assessment of the sediments associated with reef systems in this area should provide useful information regarding the nature of the depositing material.

This study aims to:

a) Establish whether salmon farming contributes to sedimentation on reef ecosystems in farming regions
b) Identify what analyses and methods are most appropriate for detecting fish farm signatures

The research will help clarify for both the salmon and abalone industries, whether salmon derived materials have the potential to contribute to the broader sedimentation regime on rocky reef habitats in SE Tasmania.

2. Methods

This study established a reliable and objective approach to sample, characterise and compare sediments associated with reef macroalgae. This included development of a practical approach for sampling sediments in the field and the analytical techniques to distinguish the signatures of potential sediment sources (i.e. catchment inputs through rivers, sewage treatment and fish processing plant inputs, oceanic and salmon farm sources). In combination this provides an approach that will enable a better understanding of the relative contributions of the various potential sources to the broader sediment loads. Comprehensive sampling within Port Esperance will clarify the drivers and sources of sedimentation throughout the bay (Fig. 1). Comparing the sediment signatures from reefs within existing marine farming regions (Channel/Huon) with those from reefs with similar hydrodynamic situations but remote from farming will help establish whether salmon farming is contributing to sedimentation (Fig. 2).

2.1 Sample Collection

2.1.1 Sediment Traps

Sediment traps were deployed at seven sites within Port Esperance, three sites within the D'Entrecasteaux Channel and Huon Estuary, and five reference sites (Recherche Bay, Southport, Port Arthur, Triabunna and Chinaman's Bay) (Table 1). At each site, two lots of triplicate sediment traps were deployed approximately 10 m from one another. Traps were constructed of 50 mm PVC tubing and were 315 mm long. Sediment traps had small plastic dividers placed in the top of each tube to help prevent vertebrates entering the trap and to reduce sediment re-suspension. Traps were anchored to concrete blocks placed on the benthos by divers. Sediment traps were collected by diver after 14 days, lids were placed on each trap underwater, and they were then transferred to the boat. Sediment traps were stored overnight to settle in a 4°C fridge prior to processing (see below).

2.1.2 Algal "Dust"

Sargassum was identified as the standard plant from which to collect "dust" samples on the basis that it is ubiquitous throughout the system, plays an important (structuring) role in the reef communities and the configuration of the plant and fronds make it more likely to retain sediment material than other macroalgal species. Sargasum plants were haphazardly identified by divers on each of the chosen reefs from a consistent depth (approximately 5m),

and were collected approximately 10 - 20m apart. Fine sediment or 'dust' settled on the plants was collected by placing a large plastic bag very carefully over an individual branch of Sargassum before shaking to dislodge the sediment, the plant was then gently withdrawn and the bag sealed. The contents of the bag were transferred to a 5 L carboy on the boat, transported to the lab, and stored in a 4°C fridge overnight prior to processing (see below).

2.1.3 Potential Source Material

Four subsurface 5 L carboys were collected at three locations in Port Esperance which were deemed to be potential waterborne sources of sediment i.e. Port Esperance river mouth (catchment input), sewerage treatment plant discharge, and the fish processing plant discharge (Fig.1, Table 1). Samples of salmon feed, salmon farm net wash, salmon faeces, and mussel line net wash were also collected. All potential source samples were analysed for fatty acid composition and isotopic (C and N) signatures by CSIRO.

Samples of other potential aquaculture sedimentation sources were also collected:

i. 2 x salmon feed samples:
   - 4mm pellets (smolt diet) and 9mm pellets (grow out diet)

ii. 2 x composite salmon faeces samples:
   - 3 x smolt individuals and 3 x grow out individuals

iii. Mussel line fouling material (Dover Bay)

iv. Salmon farm net wash material (Stringers Lease):
   Four replicate samples were collected using a small plankton net (150 ml), which was trawled alongside a pen (monofilament net) to a depth of 8 m during netwashing. The sample was transferred into a container and rinsed with a small amount of site water.

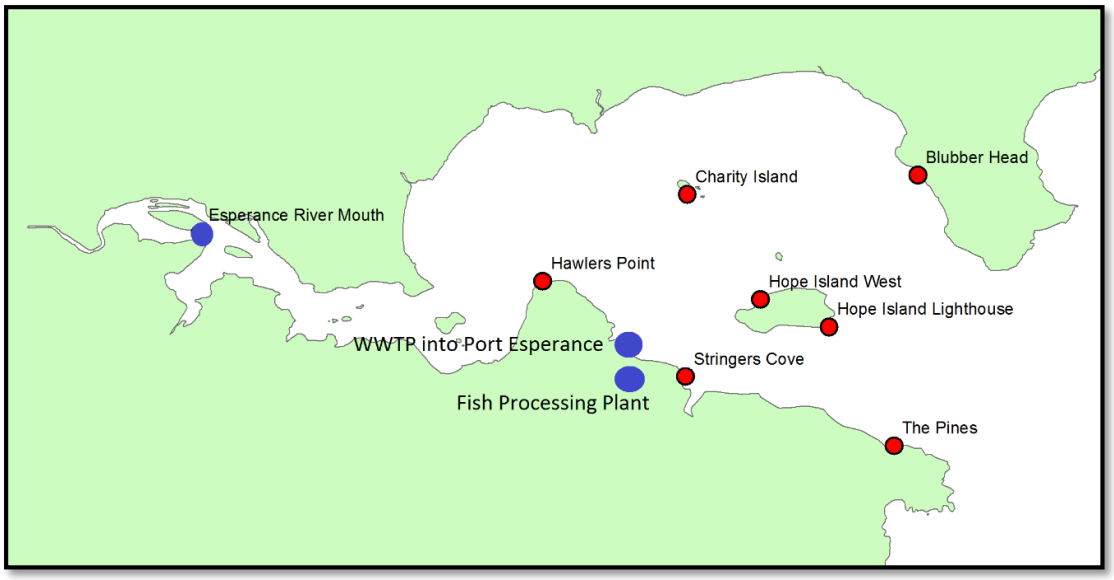

Figure 1. *Map showing sampling sites on reefs in Port Esperance, including the three potential water source sites.*

Table 1*. Sites sampled in existing farming areas (Port Esperance and Huon/Channel), on reefs in similar hydrodynamic situations remote from farming (Reference), and at potential sediment source areas (Source).*

Area	Site	Samples Collected
Port Esperance	*The Pines*	*6 sediment traps* *5 L algal dust samples*
	Stringers Cove	
	Hope Island Lighthouse	
	Hope Island West	
	Hawlers Point	
	Charity Island	
	Blubber Head	
Huon/Channel	*Satellite Island North*	*6 sediment traps* *5 L algal dust samples*
	Huon River	
	Conningham (Shepherds)	
Reference	*Triabunna*	*6 sediment traps* *5 L algal dust samples*
	Chinaman's Bay	
	Recherche Bay	
	Southport	
	Port Arthur	
Source	*Port Esperance River Mouth*	*5 L water samples*
	Sewerage treatment plant discharge (Port Esperance)	
	Fish processing plant discharge (Port Esperance)	

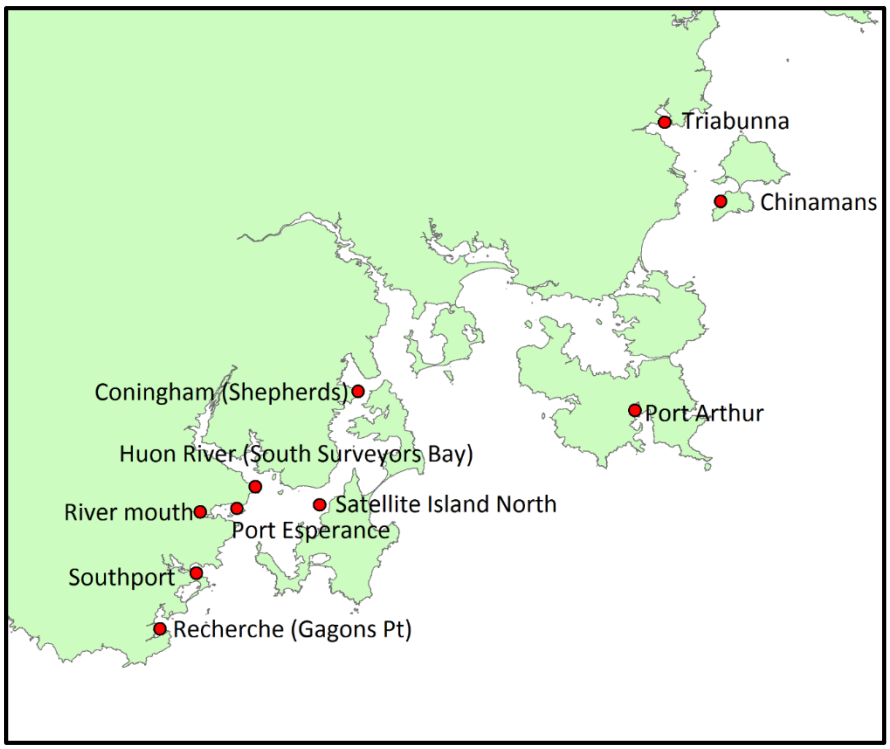

Figure 2. *Map showing sampling sites on reefs in existing farming areas (Channel/Huon) and on reefs in similar hydrodynamic situations but remote from farming.*

2.2 Sample Processing

2.2.1 Sediment Traps

After settling overnight the sediment traps were handled extremely carefully so as to not disturb the contents. The plastic dividers were removed from the top of the sediment traps and the overlying water removed using a siphon. The remaining sample was then mixed well and transferred to a measuring cylinder where the total volume was recorded and any obvious fauna or plant matter removed. This sample was centrifuged at 3500 rpm/ 4°C for 3 minutes in a 50 ml vial. The overlying water was again carefully siphoned off and the remaining sample centrifuged a second time; this step was repeated once more to concentrate the material in a single vial. The samples were then stored in a -30°C freezer until freeze dried using a LABCONCO Freezone 4.5/ -57°C for approximately 4 days and the sample dry weights recorded for sedimentation rate calculations.

2.2.2 Algal "Dust"

The top ~ 4 L of sample was siphoned off being careful not to remove any fine material. The remaining ~1 L of sample was poured through a 1 mm sieve (to remove any large plant material or fauna) and transferred to a volumetric cylinder and left to settle for a further 3 hours. After that time, ~500 ml of water was siphoned off each sample (concentrating the

algal ‘dust’ in to a final ~500 ml sample). Samples were then transferred to 50 ml vials and centrifuged at 3500 rpm at 4°C for 3 mins. Overlying water was carefully removed and any remaining sample was poured in to the same vial and centrifuged again. This step was repeated to concentrate all material in to the one vial. Samples were stored in a -30°C freezer until freeze dried at using a LABCONCO Freezone 4.5 at -57°C for approximately 4 days.

2.2.3 Potential Source Material

Water source samples were mixed well and poured through a filter (47mm GF/F filters) using a vacuum pump to collect as much material as possible. Two filters were required per sample. Once all of the sample was filtered, the filters were folded in half (sediment on the inside) and placed in the muffled aluminium foil cups in a muffle furnace at 450°C for 4 hours. Other source samples were freeze dried using a LABCONCO Freezone 4.5 at -57°C for approximately 4 days.

2.3 Laboratory Analysis

Samples were analysed to determine the proportion of isotopes (C:N) and specific fatty acid markers for salmon farming and/ or other sources.

2.3.1 Fatty Acids (Lipids)

Fatty acid composition of sediments 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 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 analysis 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).

2.3.2 Isotopes of C and N

Sediment samples for nitrogen isotope analysis were weighed into tin cups. For the analysis of carbon, samples were weighed into aluminium cups and acidified using sulphurous acid (6% w/w min; Australian Chemical Reagents, Queensland; Verardo, D. J. *et al.*, (1990)) to remove any mineral carbonate. Samples were then analysed for nitrogen and carbon contents, δ15N and δ13C 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:

$$\delta\ (‰) = [(R_{sample}/R_{standard}) - 1]\ 1000$$

Where:

R_{sample} and $R_{standard}$ are the relevant isotopic ratios measured in the sample and standard. The standard for carbon is VPDB and for nitrogen is Air.

3. Results & Discussion

Sedimentation rates did not differ markedly between the sites in Port Esperance, Port Arthur, the Huon Estuary, the Channel or Triabunna with rates at each of these sites ranging from approximately 10-60 g/m^2/ d (Fig. 3). However, rates were much higher at Chinaman's Bay with individual traps collecting 168 g/d/m^2 and 436 g/d/m^2. The rates in Port Esperance in this study (mean 20 g/m^2/ d) are similar to those observed in previous studies from this area - Macleod et al (2004) reported background sedimentation rates in Stringers Cove, Port Esperance of between 20 – 40 g/m^2/ d and whilst levels directly under/ adjacent to salmon farms were not consistently elevated they could on occasions be up to three times that of background levels (Macleod et al, 2004). Studies overseas have shown similar sedimentation rates around salmon farms; Sutherland et al. (2001) recorded sedimentation rates of approximately 18 g/m^2/ d adjacent to salmonid cages whilst Black (2001) reported rates of between 11-33 g/m^2/ d directly beneath cages in Scotland.

A number of studies have suggested that the deposition of particulate material from salmon farms tends to be highly localized (e.g. Brown et al., 1987, Hevia et al., 1996, Carrol et al., 2003, Cromey et al., 2003, Macleod et al., 2006, Keeley et al., 2013), often with the main impact footprint restricted to within 30-50m of the cages. The relatively consistent sedimentation rates observed at most sites in the current study would tend to support this suggestion, although it should be noted that finer particulate material may be carried more widely (albeit at much lower loads).

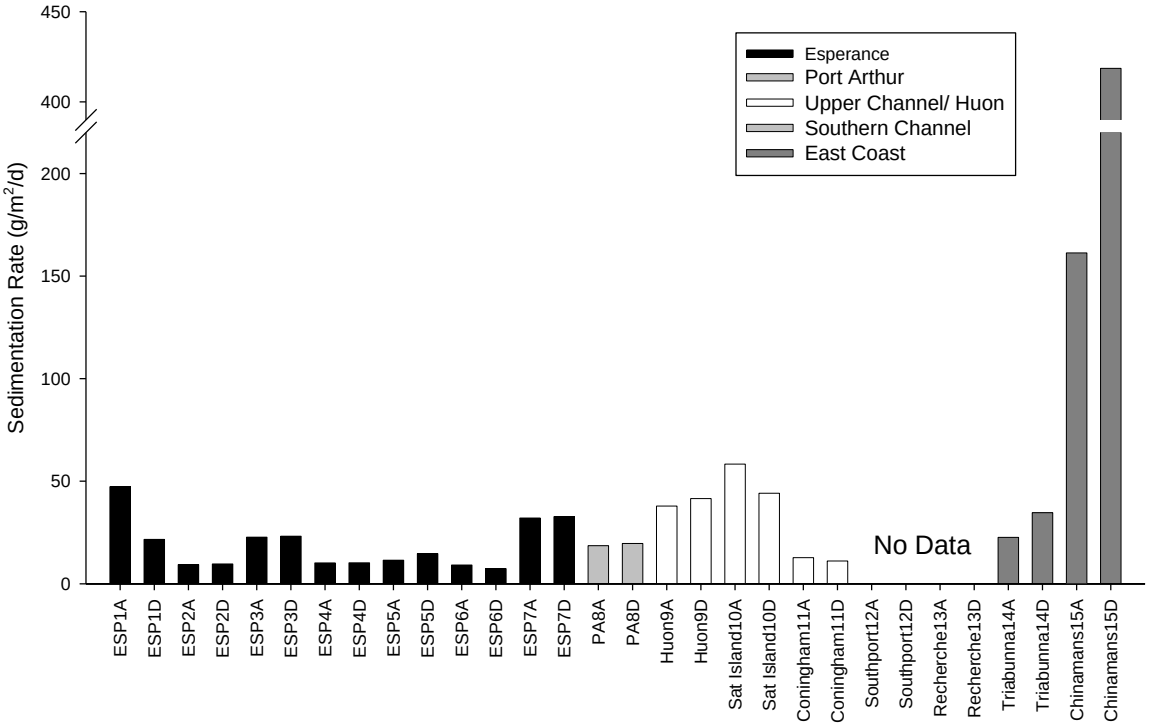

Figure 3. *Sedimentation rate (mg/d/m2) for each of the two sediment traps (A and D) at each site.*

The percentage carbon in the two different types of fish feed was very similar (46-47%), however the percentage nitrogen was greater in the 4mm smolt diet than in the 9mm grow out diet (8.4% cf 5.8%) (Fig. 4). Levels of both carbon and nitrogen were reduced in fish faeces relative to the feed but were still markedly higher than the levels obtained in the dust material collected from the Sargassum plants through the Huon and D'Entrecasteux Channel. The percentage carbon and nitrogen in the algal dust samples were very similar within regions, with all of the Port Esperance, Huon and Channel samples having carbon levels below 10% and nitrogen levels for the most part less than 1% - a marked reduction on the feed/ faeces results which would tend to suggest that the dust material is not comprised of waste salmon feed/ faeces specifically. Levels of both carbon and nitrogen at Chinaman's Bay were slighty higher (1.5% N and 12-13% C) but still not comparable with the salmonid waste materials directly. Interestingly, but perhaps not surprisingly given they both represent fouling communities, the signals for the netwash and mussel fouling samples were similar. Levels of both carbon and nitrogen observed in the environmental and netwash samples collected in this study are broadly consistent with previous observations (i.e. a study looking at epiphytes and sediment from Corner Inlet (Nichols et al., 1985) and more recently from a study of abalone diet preferences in Tasmania (Guest et al, 2013)).

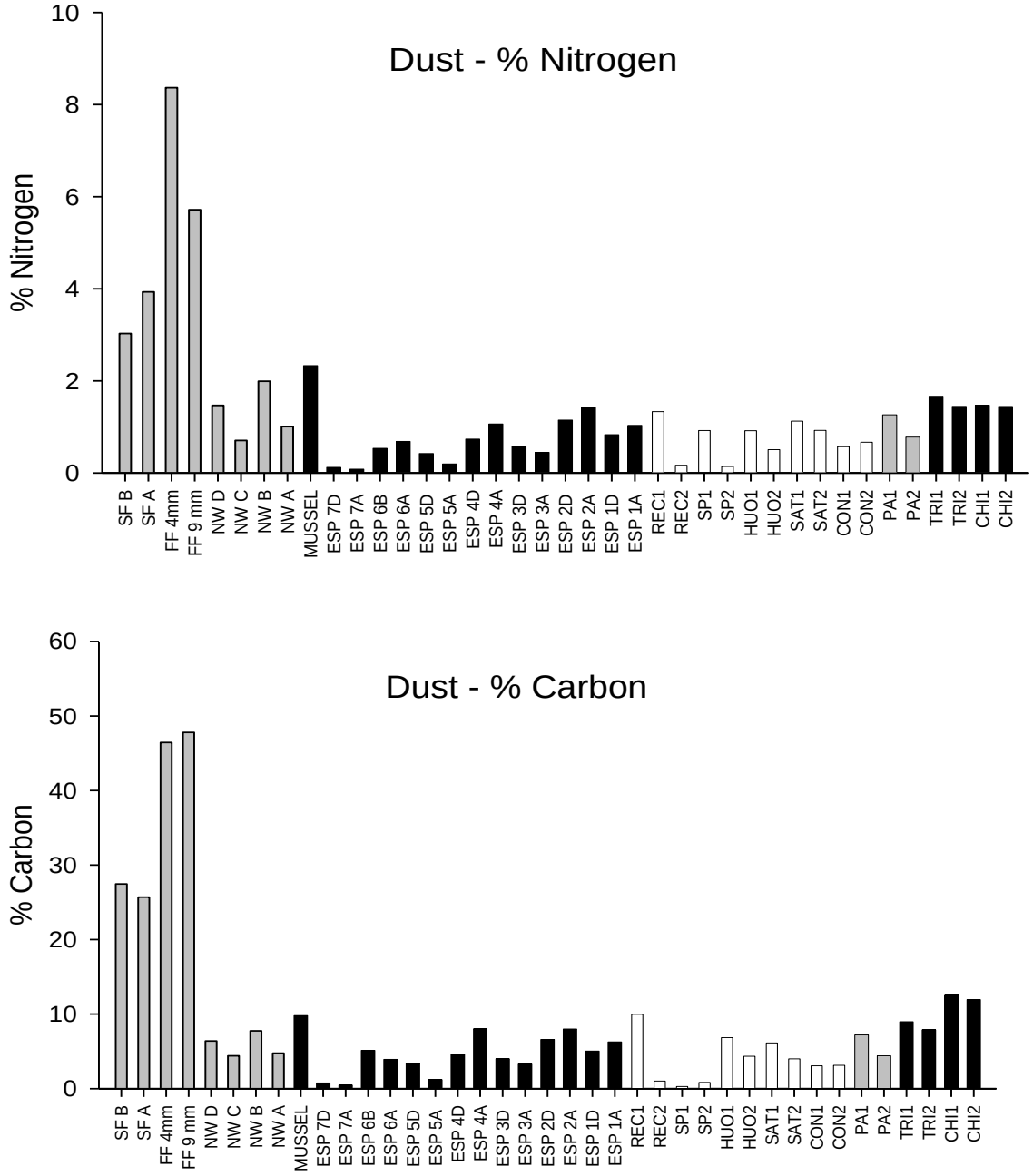

Figure 4. *Percentage nitrogen (% N) and carbon (%C) in dust samples collected from duplicate Sargassum plants (A and D) at each of the study sites (Port Esperance (ESP), Recherche Bay (REC), Southport (SP), Huon (HUO), Satellite Island (SAT), Conningham (CON), Port Arthur (PA), Triabunna (TRI), Chinamans Bay (CHI), the Mussel farm in Port Esperance (MUS)) and the levels associated with potential sources (fish feed (FF), salmon faeces (SF) and netwash (NW)).*

The percentage carbon and nitrogen in the sediment collected in traps at each site showed a very similar pattern to that of the dust collected from the macroalgae (Fig. 5). The spatial distribution was similar as were the absolute levels, again there is little here to suggest that farm derived materials are the main contributors to the overall sediment loads and composition.

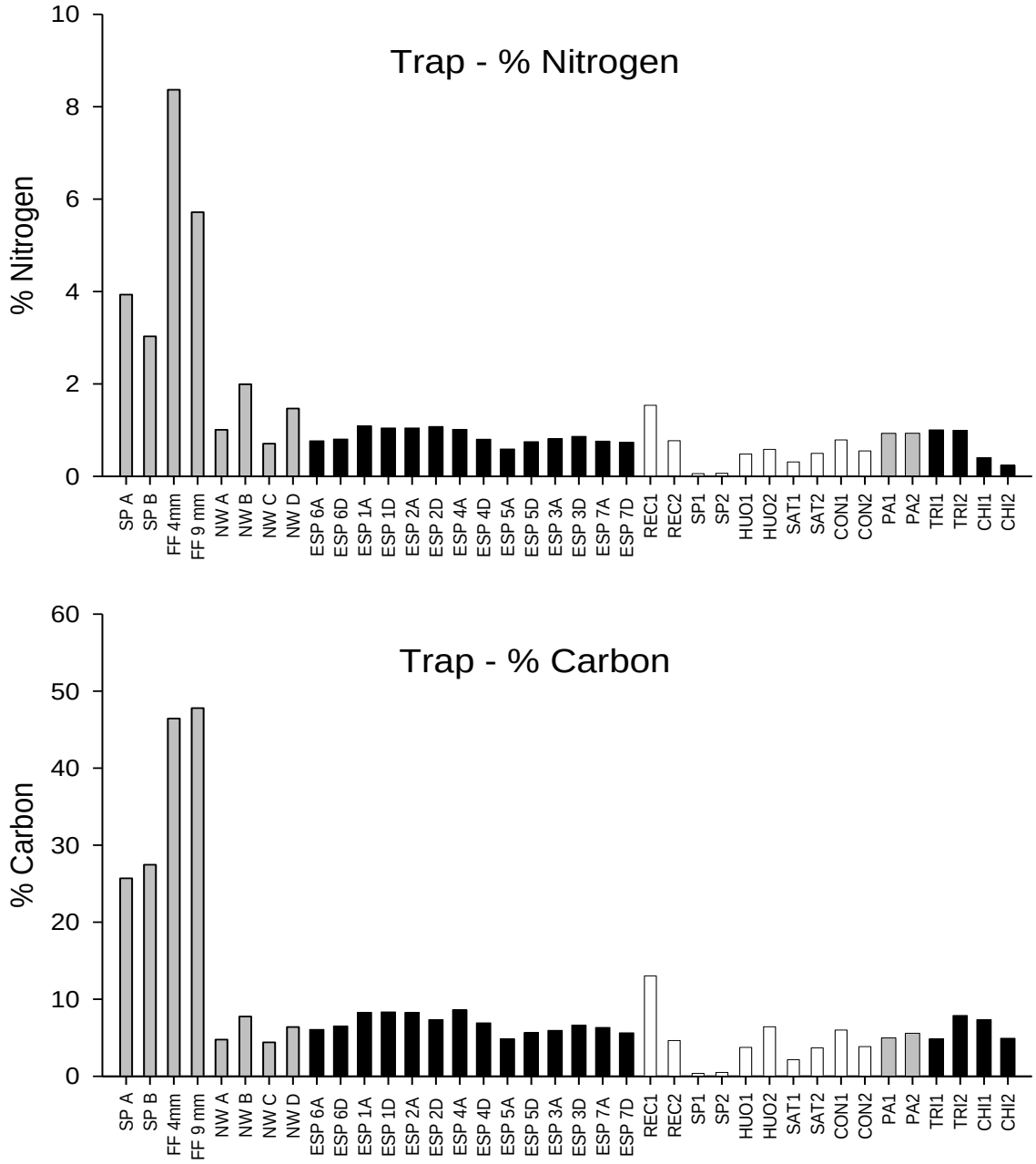

Figure 5. *Percentage nitrogen (% N) and carbon (%C) in duplicate sediment traps (A and D) at each of the study sites (Port Esperance (ESP), Recherche Bay (REC), Southport (SP), Huon (HUO), Satellite Island (SAT), Conningham (CON), Port Arthur (PA), Triabunna (TRI), Chinamans Bay (CHI), the Mussel farm in Port Esperance (MUS)) and the levels associated with potential sources (fish feed (FF), salmon faeces (SF) and netwash (NW)).*

Oleic acid is a monounsaturated omega-9 fatty acid that occurs naturally in vegetable fats and fish oil and linoleic acid is a polyunsaturated omega-6 fatty acid which is derived from dietary oils (Nichols et al., 2014) and have been reported as being useful indicators of the presence of salmon farming wastes (feed and faeces) in the environment. Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are both omega-3 fatty acids that are commonly found in marine algae but which are concentrated through the food chain and

therefore are prolific in higher trophic order fish (salmonids) and may have the potential to be indicators of change in the environment that could be related to salmon production.
The results clearly show that oleic and linoleic acid levels were proportionally high, in terms of the overall contribution to the total fatty acid load, in both the feed and faeces samples, and also were a significant presence in the lipid profile from the fish processing outfall (Fig. 6). Whilst there was clear evidence of elevated levels of oleic and linoleic acid in the salmon sources (fish feed and faeces), levels were also elevated in one sample from the Esperance River, this may be a function of other activities in the catchment (e.g. agriculture or forestry) and has implications for the use of these markers as indicators of salmon derived inputs. The two samples from the Esperance river were very different in their composition, a higher level of replication might help clarify the patterns observed. In contrast docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) comprised a much greater proportion of the fatty acid complement in the net wash and mussel fouling samples than in the salmonid source samples (feed and faeces), perhaps reflecting algal species in amongst these communities. Biofouling communities in general can be quite high in DHA and EPA (Gonzalez-Silvera et al. 2015).

Oleic and linoleic acid represented a lower proportion of the fatty acid complement in the dust samples than in the source samples, and there was little evidence of any marked spatial differences in these particular fatty acids (Fig. 7). The DHA proportions in the dust samples were broadly comparable with the sources assessed, with the exception of the dust samples collected at Triabunna where DHA was noticeably elevated. EPA signals from the dust were quite high, but with no particular spatial distinctions, and therefore may simply reflect the broader environmental conditions and the fact that microalgal and/or microphytobenthic contributions to the broader system are significant.

In contrast there were some clear spatial distinctions in the levels of these markers in the sediment trap samples (Fig. 8). Linoleic acid comprised a markedly higher proportion of the samples collected at Triabunna and was also elevated in one of the replicates from Satellite Island. A possible explanation for the Triabunna difference might be the presence of the fish processing plant . The actual mean regional loads of linoleic acid in this study were as follows: Esperance 10-15 mg/g, Huon 30 mg/g, Chinaman's 35 mg/g, Triabunna 70 mg/g. There was only one sample from the Esperance sampling that showed any marked difference in proportion of oleic acid, and the same sample also contained an increased level of DHA.

Given the potential for both of these markers to be associated with elevated plankton inputs it is possible that this might reflect a localized event. Perhaps the most interesting comparison is that for EPA, as this marker seems to suggest a more generalized elevation throughout the Esperance region more broadly, with a doubling in relative contribution at locations 4, 5 and 6.

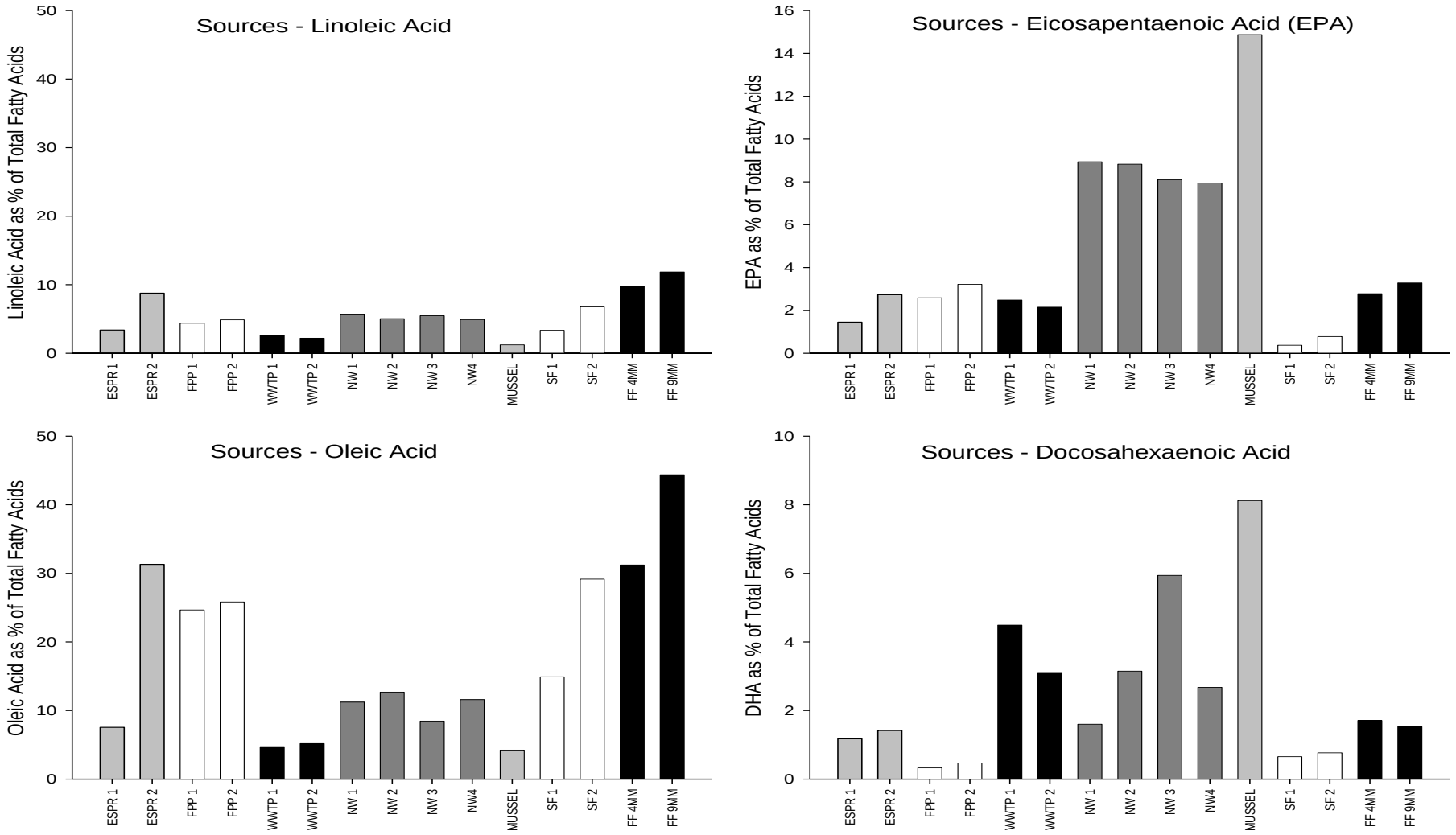

Figure 6. *Percentage fatty acid (Linoleic acid, Eicosapentaenoic acid, Oleic acid, and Decosahexaenoic acid) in samples collected from potential sources within Port Esperance (i.e. Esperance River (ESPR), Fish Processing Plant (FPP),Waste Water Treatment Plant (WWTP), Net Wash (NW), Mussel Lines (MUSSEL), Salmon Faeces (SF) and Fish Feed (FF)).*

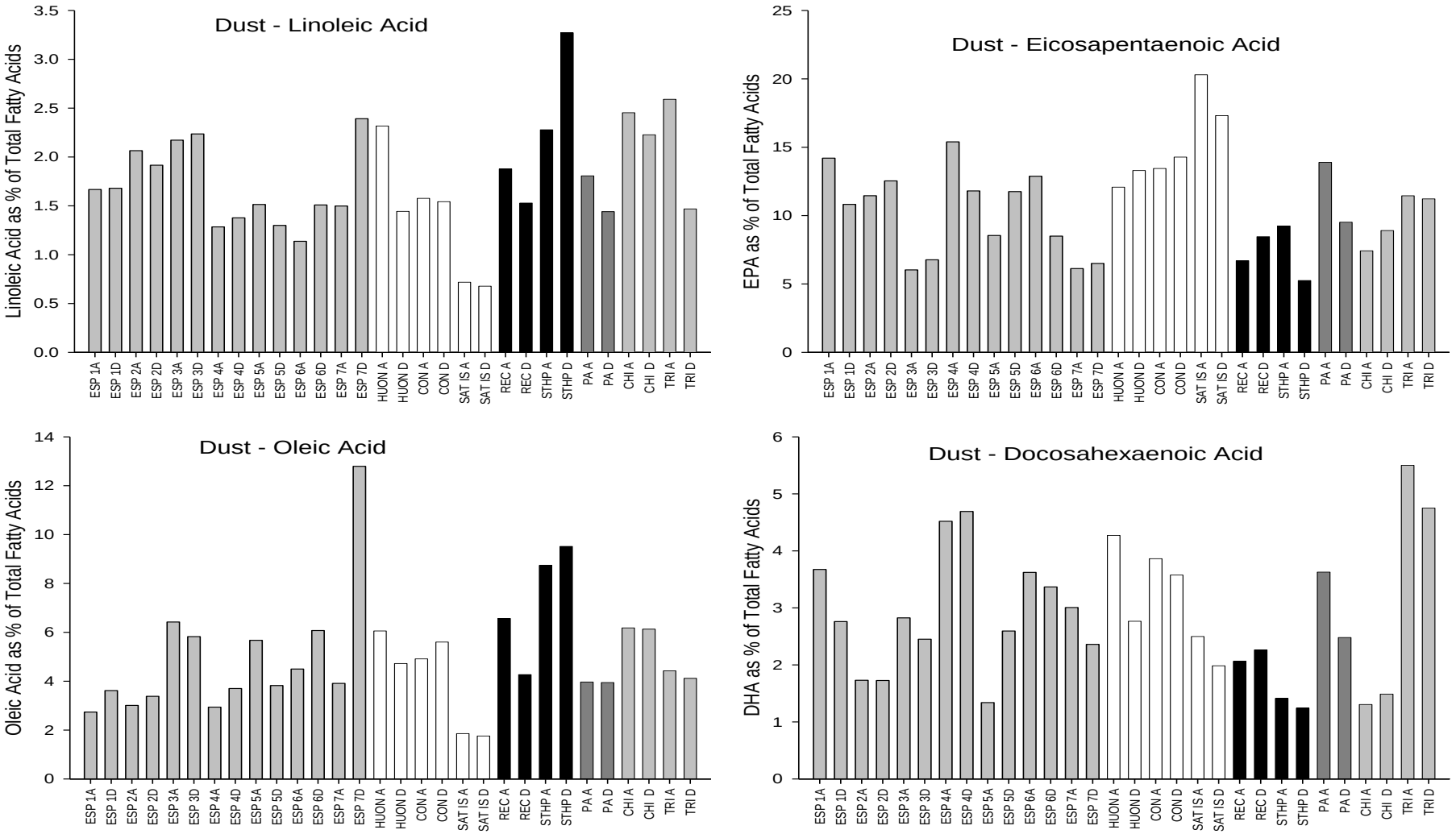

Figure 7. *Percentage fatty acid (Linoleic acid, Eicosapentaenoic acid, Oleic acid, and Decosahexaenoic acid) in samples collected from "dust" associated with Sargassum in Port Esperance (ESP) – light grey bars, Huon and Upper Channel (Huon (HUO), Conningham (CON), Satelite Island (SAT))- white bars, Lower Channel (Recherche Bay (REC), Southport (STHP) – black bars, Tasman Peninsula (Port Arthur (PA)- dark grey bars and East coast (Chinamans Bay (CHI), Triabunna (TRI) – white bars.*

The comparison of the isotopic signatures (15N and 13C) from both the dust and the sediment traps alongside what have been considered the main sources of sedimentation (feed, faeces and water) shows quite clearly the distinction between the signatures for the dust and sediment trap samples (Fig. 9). The traps seeming slightly more aligned with the signatures for the water column sources, but neither being clearly aligned with feed as a source. The distinction between the "dust" and the sediment traps may reflect the relative age of the material being sampled (i.e. the dust may have deteriorated somewhat or there may be differences in the trophic levels of the material deposited over time).

The ordination of the isotopic signatures of the various source samples (fish feed, salmon faeces, net wash, and mussel line biofouling) shows the netwash (NW) samples to sit apart from the other sources along both the carbon and nitrogen axes and this likely reflects the microalgal contribution to that signal (Fig.10). The mussel fouling signature lies much higher on the N axis, which would tend to suggest a trophic increment in this material (i.e. the signals sits higher than that of the netwash and the primary producer signal), and may reflect the presence of filter feeding omnivorous hydroids and/or ascidians in the samples as these have been noted as common components of netwash material (Hodson et al, 1997, Baxter et al., 2012, Yaxley, 2015).

The equivalent ordination of the macroalgal "dust" and sediment trap samples based on the isotopic signatures provides little separation of sites overall. Spatial differences were slightly more pronounced in the dust samples than in the sediment trap material. The greatest differences in the dust results would appear to be that Triabunna has an elevated N signal again suggesting the material may be sourced from a higher trophic level, and Chinaman's Bay where the C isotopic signature was higher (Fig.11). A higher C signature also differentiated Chinaman's Bay in the sediment trap samples but not quite so clearly. Again this may be a function of the fact that the trap material is relatively fresh, whilst the "dust" is comprised of a range of material at different levels of deterioration.

When all of the fatty acid and isotopic information is combined then it can be seen that the samples of feed and faeces can be easily differentiated from all other signals, and quite clearly stand apart from the other samples (Fig. 12). The next level of differentiation distinguishes the mussel fouling communities, suggesting this community is quite different to the other samples. Thereafter the samples from the sediment trap at Chinaman's Bay and the

dust sample from Triabunna were sequentially distinguished, but still at quite a high level, suggesting that there may be biotic process differences in relation to depositing sediment on the East Coast. However, these differences do not seem to be directly related to salmonid feed or faecal deposition.

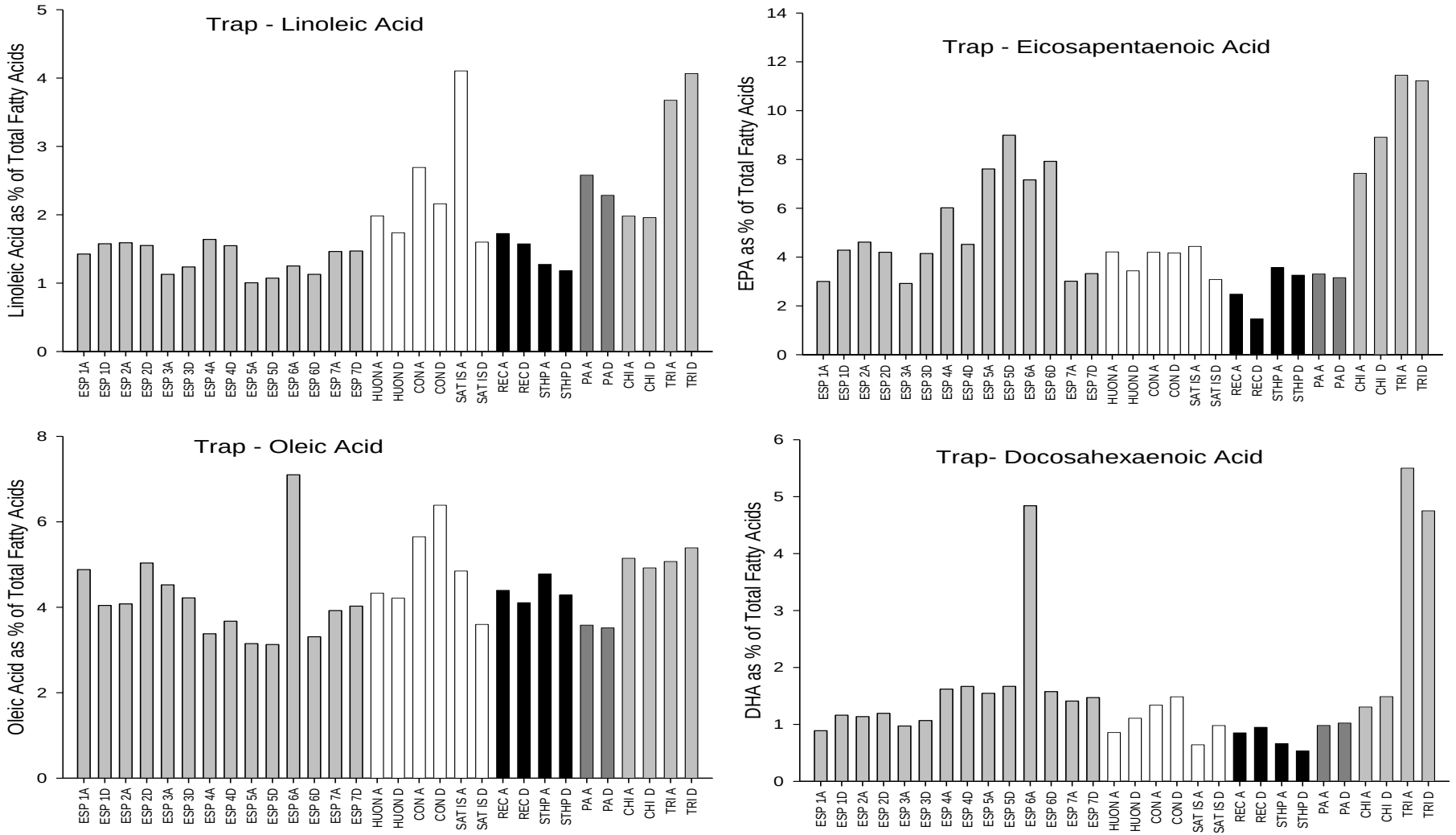

Figure 8. *Percentage fatty acid (Linoleic acid, Eicosapentaenoic acid, Oleic acid, and Decosahexaenoic acid) in samples collected from sediment trap contents in Port Esperance (ESP) – light grey bars, Huon and Upper Channel (Huon (HUO), Conningham (CON), Satelite Island (SAT))- white bars, Lower*

Channel (Recherche Bay (REC), Southport (STHP) – black bars, Tasman Peninsula (Port Arthur (PA)- dark grey bars and East coast (Chinamans Bay (CHI), Triabunna (TRI) – white bars.

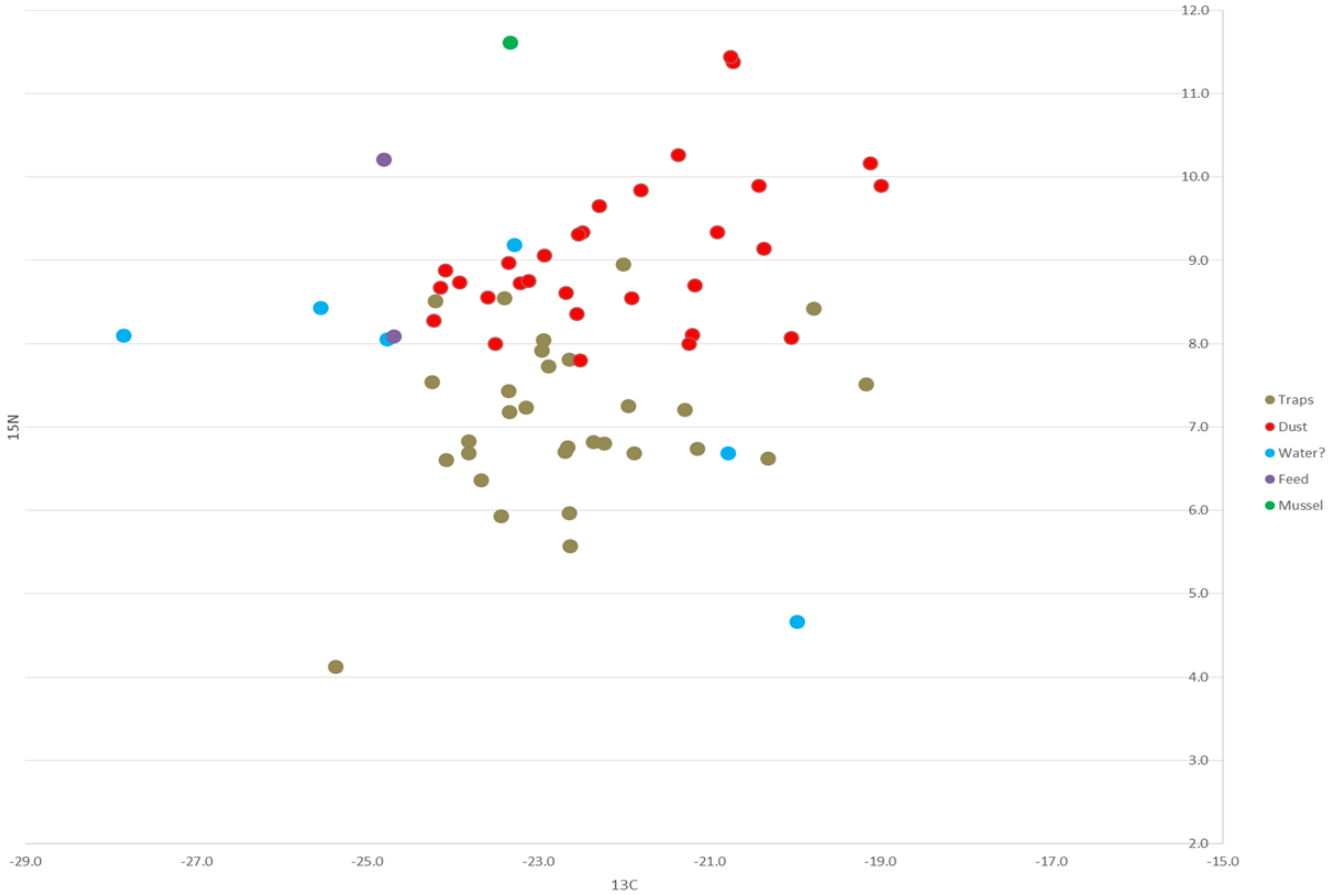

Figure 9. *Scatter plot of 15N and 13C isotopes from all samples; includes sediment traps, algal 'dust', water, feed and mussel line biofouling.*

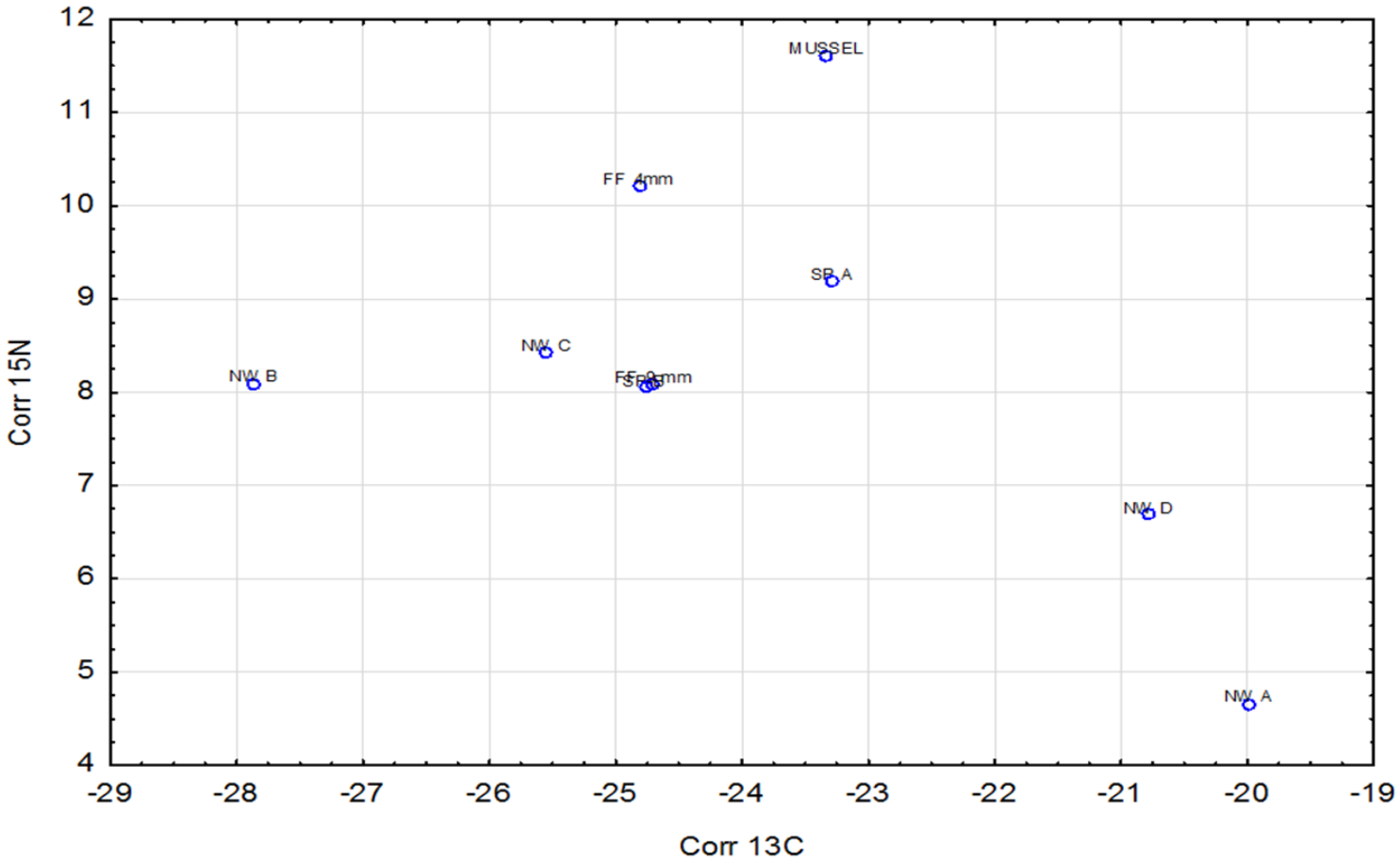

Figure 10. *Ordination (2D MDS plot) of source samples (fish feed, salmon faeces, net wash, and mussel line biofouling) based on the full breakdown of 15N and 13C isotopes in all replicates from those regions.*

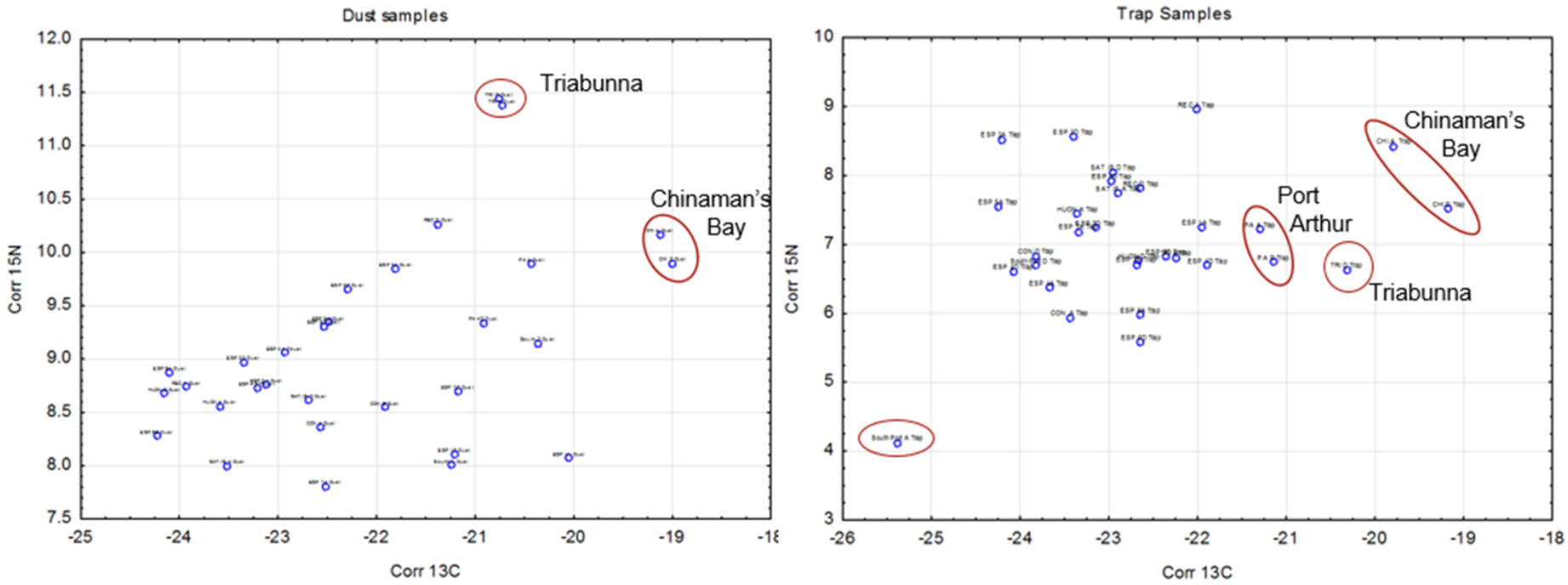

Figure 11. *Ordination (2D MDS plot) of 15N and 13C isotope signatures for both the Sargassum "dust" samples and the sediment trap samples from all locations.*

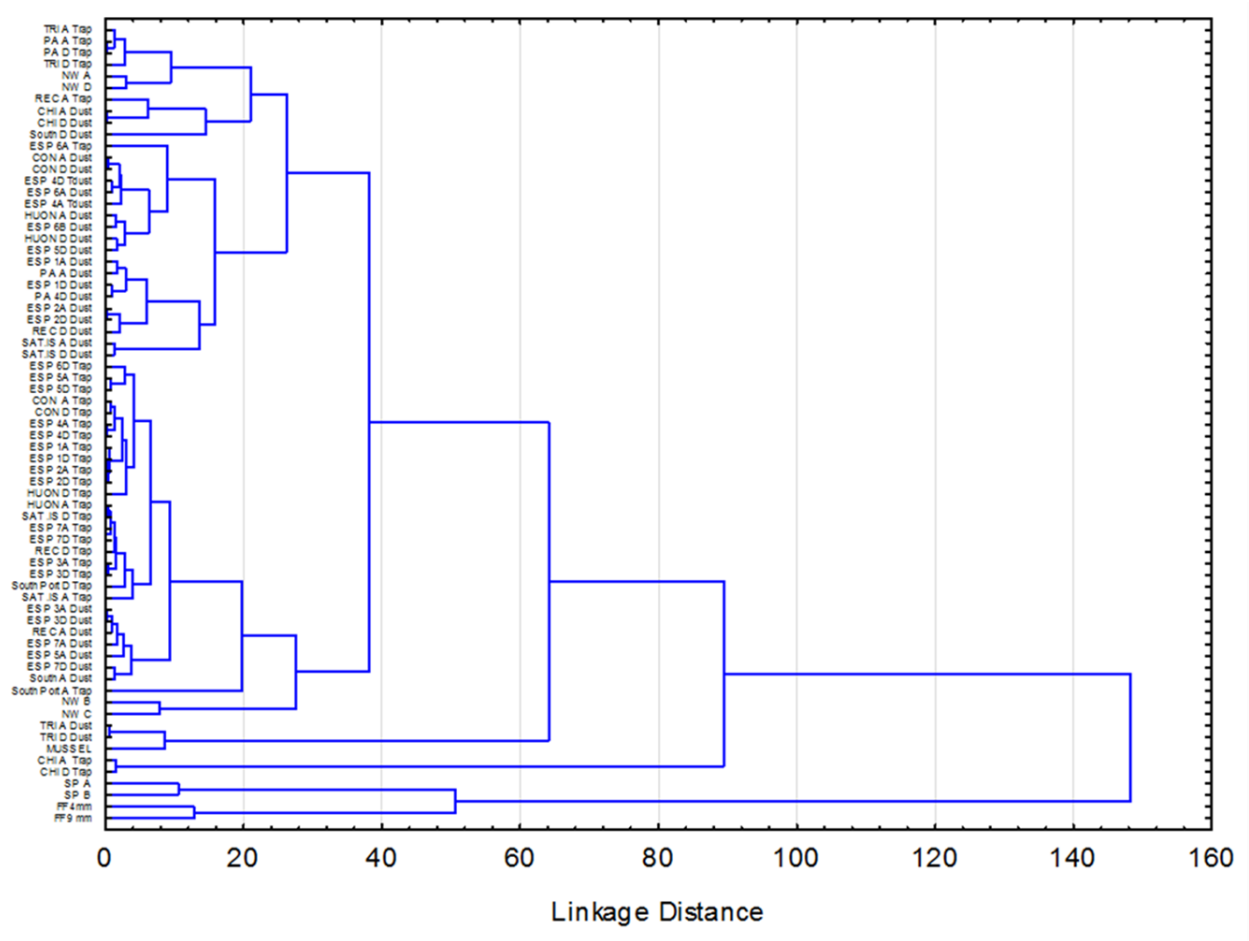

Figure 12. *Cluster analysis – dendogram showing the differentiation of sites based on the combined 15N and 13C isotope and fatty acid results for all samples (sediment trap, dust, and other source samples).*

4. Conclusions

- Whilst sedimentation rates do vary spatially around SE Tasmania, they are not markedly elevated in areas where salmon farming is undertaken relative to areas without salmon farming.
- Current sedimentation rates are comparable with previous studies in Tasmania.
- The dust collecting on macroalgae in the Huon, D'Entrecasteaux Channel or elsewhere around Tasmania is not salmon food or faeces directly.
- The composition of the dust is different to current sediment loads and likely comprises a mix of both current and historic sedimenting material.
- Identification of a reliable indicator of salmon derived material was not possible in the current study. Unfortunately the techniques proposed at the start of the study were confounded by the complexity of signals in the background environmental conditions.
  - Stable isotopes do not appear to be as useful for monitoring/ distinguishing fish farm impacts in SE Tasmania as they were in Macquarie Harbour. This may be a result of the fact that the signatures are harder to distinguish when they are "marine on marine".
  - Fatty acids are also likely to be an ineffective tool for monitoring/ assessments due to the high levels of terrestrial inputs with similar profiles. In this case the problem is that we are trying to distinguish low level terrestrial tracers in an environment where there are substantial terrestrial inputs from other sources.

5. Recommendations & Implications for Future Research

Whilst the dust assessed in this pilot study was not salmon food or faeces directly this does not mean that there is no interaction between salmon farming and the broader environment. Nutrients derived from salmon farming may still play a role in enhancing primary and secondary production. Salmon farming is effectively part of the ecosystem in which it exists and will therefore play a role in the various ecosystem processes and trophic dynamics.

It is important to note that this survey was undertaken at a single point in time and that there may be quite strong temporal differences in both the background and the farming signals. In summer the influence from the farms may be proportionally greater, and therefore sampling at different times may provide a broader understanding of the sediment contributions. That

said the information collected from the dust material off the plants would represent a more temporally integrated sample, and this did not give any strong indication that there was any particular spatial variability that could be directly attributed to salmon farm inputs.

Identifying a particular indicator or signal that can be used to characterize the salmonid contribution to ecosystem interactions/ processes is not an easy task, and it is important to remember that just because you can detect the presence of salmon derived material does not necessarily mean that it is having an adverse effect. Consequently future investigations might be best directed to areas where there is some confidence that i) there is a clear potential for adverse interactions and ii) where the relevant process interactions and mechanism for effect are known. Consequently this may require some preliminary research to more clearly identify those interactions and the components that would make the particular species/ ecosystem vulnerable. The risk based approach in the current FRDC project 2015-024 may provide some understanding of those key interactions with respect to coastal reef systems.

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7. References

Baxter, E. J., Sturt, M. M., Ruane, N. M., Doyle, T. K., McAllen, R., & Rodger, H. D. (2012). Biofouling of the hydroid Ectopleura larynx on aquaculture nets in Ireland: implications for finfish health. Fish Vet J, 13, 17-29.

Black, K. D. (2001). Environmental impacts of aquaculture (Vol. 5). Taylor & Francis US.

Black, K. D., Calder, L. A., Nickell, T. D., Sayer, M. D., Orr, H., Brand, T., ... & Angel, D. (2012). Chlorophyll, lipid profiles and bioturbation in sediments around a fish cage farm in the Gulf of Eilat, Israel. Aquaculture, 356, 317-327.

Brown, J. R., Gowen, R. J., & McLusky, D. S. (1987). The effect of salmon farming on the benthos of a Scottish sea loch. Journal of Experimental Marine Biology and Ecology, 109(1), 39-51.

Carroll, M. L., Cochrane, S., Fieler, R., Velvin, R., & White, P. (2003). Organic enrichment of sediments from salmon farming in Norway: environmental factors, management practices, and monitoring techniques. Aquaculture, 226(1), 165-180.

Cromey, C. J., Nickell, T. D., & Black, K. D. (2002). DEPOMOD—modelling the deposition and biological effects of waste solids from marine cage farms. Aquaculture, 214(1), 211-239.

George, E. M., & Parrish, C. C. (2015). Invertebrate uptake of lipids in the vicinity of Atlantic salmon (Salmo salar) aquaculture sites in British Columbia. Aquaculture Research, 46(5), 1044-1065.

Gonzalez-Silvera, D., Izquierdo-Gomez, D., Fernandez-Gonzalez, V., Martínez-López, F. J., López-Jiménez, J. A., & Sanchez-Jerez, P. (2015). Mediterranean fouling communities assimilate the organic matter derived from coastal fish farms as a new trophic resource. Marine pollution bulletin, 91(1), 45-53.

Guest, M. A., Nichols, P. D., Frusher, S. D., & Hirst, A. J. (2008). Evidence of abalone (Haliotis rubra) diet from combined fatty acid and stable isotope analyses. Marine Biology, 153(4), 579-588.

Herzfeld, M., Parslow, J., Sakov, P. and Andrewartha, J.R. (2005). Numerical hydrodynamic modelling of the D'Entrecasteaux Channel and Huon Estuary.Aquafin CRC Technical report, CSIRO, Hobart, Australia.

Hevia, M., Rosenthal, H., & Gowen, R. J. (1996). Modelling benthic deposition under fish cages. Journal of Applied Ichthyology, 12(2), 71-74.

Hodson, S. L., Lewis, T. E., & Burkea, C. M. (1997). Biofouling of fish-cage netting: efficacy and problems of in situ cleaning. Aquaculture, 152(1), 77-90.

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.

Keeley, N. B., Forrest, B. M., & Macleod, C. K. (2013). Novel observations of benthic enrichment in contrasting flow regimes with implications for marine farm monitoring and management. Marine Pollution Bulletin, 66(1), 105-116.

MacLeod, C, Bissett, AP, Burke, C, Forbes, SE, Holdsworth, D, Nichols, P, Revill, A, Volkman, J (2004). Development of novel methods for the assessment of sediment condition and determination of management protocols for sustainable finfish cage aquaculture operations, Aquafin CRC Final Report, Tasmanian Aquaculture & Fisheries Institute, Hobart, Tasmania, Australia.

Macleod, C. K., Moltschaniwskyj, N. A., & Crawford, C. M. (2006). Evaluation of short-term fallowing as a strategy for the management of recurring organic enrichment under salmon cages. Marine Pollution Bulletin, 52(11), 1458-1466.

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., Nichols, P. D., Pethybridge, H. and Young, J. W. 2015. Direct determination of fatty acids in fish tissues: quantifying top predator trophic connections. Oecologia 177: 85-95. DOI: 10.1007/s00442-014-3131-3 .

Sutherland, T. F., Martin, A. J., & Levings, C. D. (2001). Characterization of suspended particulate matter surrounding a salmonid net-pen in the Broughton Archipelago, British Columbia. ICES Journal of Marine Science: Journal du Conseil, 58(2), 404-410.

Yaxley, B. (2015). Addressing water quality issues with in situ net cleaning in salmon farms in Southeast Tasmania. Report to the Tasmanian Salmonid Growers Association. 100pp.