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Dispersal and assimilation of an aquaculture waste subsidy in a low productivity coastal environment

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ABSTRACT

To understand dispersal and assimilation of aquaculture waste subsidies in a naturally low-productivity environment, we applied a novel, rapid transmethylation technique to analyse sediment and biota fatty acid composition. This technique was initially validated at Atlantic salmon farms in Macquarie Harbour, Australia, where sediments were collected at farm and control locations. Subsequently, sediment, benthic polychaete and zooplankton were sampled at sites 0, 50, 250, 500 and 1000 m distant from multiple cages. Results demonstrated an acute deposition zone up to 50 m from cages and a diffuse zone extending 500 m from cages. Changes in sediment concentration of linoleic acid, oleic acid and total fatty acids were effective tracers of farm deposition. Bacterial biomarkers indicated that aquaculture waste stimulates bacterial productivity in sediments, with elevated biomarker concentrations also detected in benthic polychaetes. Overall, fatty acid analysis was a sensitive technique to characterize the benthic footprint of aquaculture influence.

1. Introduction

Trophic subsidies arising from anthropogenic activities are increasingly common in coastal marine environments (Iwama, 1991; Smith et al., 1999; Bouwman et al., 2005). Globally, the aquaculture of carnivorous fishes is rapidly expanding in coastal waters and represents a substantial trophic resource where farming intensities are high. A significant portion of feed used in aquaculture flows to the environment as metabolic waste products, faeces and waste feed (Carroll et al., 2003; Holmer et al., 2005; Wang et al., 2012). Faeces and waste feed are available to primary consumers directly, either in sediments or as debris in the water column, whereas metabolic waste products, such as nitrogen, may be available indirectly through assimilation in the water column by phytoplankton. Consequently, there is increasing concern over the impact aquaculture has on adjacent marine ecosystems. Organic enrichment of sediments directly beneath fish farms is common (e.g. Strain and Hargrave, 2005; Bannister et al., 2014), which can lead to major changes in benthic communities (Edgar et al., 2005; Kutti et al., 2007a; Macleod et al., 2007) and local oxygen depletion and anoxia (Holmer et al., 2005; Hargrave, 2010). Whilst sediments directly below farms are subject to the bulk of organic enrichment, the zone beyond receives particulate and dissolved nutrients, generally in quantities that can be assimilated via natural processes and pathways (Kutti et al., 2007b).

The spatial range, magnitude and temporality of the trophic subsidy from aquaculture depends largely upon the local environment (Urbina, 2016). Sedimentation and resuspension of materials link benthic and pelagic environments and influence the scale of impact. The extent of sedimentation from aquaculture, and its intensity, varies in distance and concentration, from localised to diffuse (Findlay et al., 1995; Kutti et al., 2007b). Subsequent resuspension of waste material can also lead to the dispersal of farm waste to large areas (Keeley et al., 2013), with both sedimentation and resuspension dependent on the hydrodynamics of the receiving environment. In the pelagic environment, excessive outputs of nitrogen and phosphorus can stimulate plankton productivity and drive community change (Tsagaraki et al., 2013; Sestanovic et al., 2016; Fernandez-Jover et al., 2016). However, this pathway is difficult to detect empirically in the water column, as background nutrient levels and biological processes make detection and attribution to aquaculture waste difficult (Skejic et al., 2011; Price et al., 2015). Impacts of point-source outputs from aquaculture in the acute deposition zone are relatively well understood. Indeed, biota across multiple trophic levels assimilate and re-distribute aquaculture derived inputs (e.g. Dempster et al., 2009; Fernandez-Jover et al., 2011; Gonzalez-Silvera et al., 2015), as well as enable bulk recovery of sediments (Macleod et al., 2007; Kutti et al., 2008). Less well understood are

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Table 1
Fatty acid biomarkers commonly used for tracing dietary sources in the marine environment.

Fatty acid biomarker	Dominant source	Reference
LA (18:2n-6), OA (18:1n-9), ALA (18:3n-3), ΣLA,OA,ALA	Terrestrial-oil based aquafeeds	Turchini et al., 2009 Fernandez-Jover et al., 2011 Olsen et al., 2012 Gonzalez-Silvera et al., 2015
DHA (22:6n-3), EPA (20:5n-3), Σn-3 LC-PUFA	Marine phytoplankton	Dalsgaard et al., 2003
EPA, 16:1n-7	Diatoms	Graeve et al., 1994a Kharlamenko et al., 2001
DHA	Dinoflagellates	Viso and Marty, 1993 Bachok et al., 2003
20:1n-9, 20:1n-11, 22:1n-9, 22:1n-11, DHA	Copepods	Graeve et al., 1994b Kharlamenko et al., 2001 Dalsgaard et al., 2003
Σ15:0 & 17:0, *iso*- and *anteiso*-branched acids, 18:1n-7	Bacteria	Kharlamenko et al., 1995 Budge and Parrish, 1998 Kharlamenko et al., 2001
26:0, 28:0, 30:0	Vascular plants	Meziane and Tsuchiya, 2000 Meziane et al., 2006

NB: LA: linoleic acid, OA: oleic acid, ALA: α-linolenic acid, DHA: docosahexaenoic acid, EPA: eicosapentaenoic acid, LC-PUFA: long-chain polyunsaturated fatty acid.

interactions between aquaculture outputs and the environment in the diffuse deposition zone, where bulk carbon and nitrogen values from aquaculture are often below detection levels and impacts are masked by biological processes.

Fatty acid biomarkers are an important tool to trace aquaculture-derived subsidies in marine food webs. Aquafeeds used to culture carnivorous fish are a combination of oils and meals from terrestrial and marine origins, with a shift towards greater inclusion of raw products from terrestrial sources (Torstensen and Tocher, 2011; Tacon and Metian, 2015). These include plant materials such as canola, soybean and linseed oils, with animal products (e.g. chicken fat and beef tallow) allowed in some countries (Turchini et al., 2009). Fatty acids from terrestrial oil and meal sources have a unique signature in the marine environment, with a fatty acid profile dominated largely by C_{18} components including linoleic acid (LA; 18:2n-6), oleic acid (OA; 18:1n-9) and alpha-linolenic acid (ALA; 18:3n-3) (Nichols et al., 2014, Table 1). In contrast, natural marine ecosystems are dominated by long-chain ($\geq C_{20}$) omega-3 polyunsaturated fatty acids (n-3 LC-PUFA), which are produced in substantial quantities by marine phytoplankton (Dalsgaard et al., 2003, Table 1). These include eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), which are critical in many biological processes, including cell membrane function and immune system response (Bell and Koppe, 2011). Various other potential food sources in the marine environment may have their own unique fatty acid signature. For example, odd-chain and iso-branched fatty acids can be indicative of bacterial activity (Graeve et al., 2001), longer chain mono-unsaturated fatty acids (LC-MUFA) are produced by marine copepods (Dalsgaard et al., 2003) and long-chain saturated fatty acids (LC-SFA) are indicative of higher plant material materials (Carpenter et al., 1991, Table 1). Fatty acids can be conserved between trophic levels, as it is more energetically efficient to incorporate dietary fatty acids without modification (Parrish, 2013). As a result, information provided by groups of fatty acids, or fatty acid signatures, can delineate carbon cycling and the transfer of material through food webs (Dutto et al., 2014, Parrish et al., 2015, Table 1). In this scenario, fatty acid profiling provides complex information on ecosystem processes and functions.

In the past, fatty acid profiling has been under-utilised in environmental and ecological impact studies, with cost and time associated with sample processing a factor. Furthermore, the large-scale adoption of terrestrial oil sources in the aquaculture industry has been relatively recent (Nichols et al., 2014). Prior to this, the fatty acid signature of aquaculture inputs was analogous to the marine environment, containing large quantities of DHA and EPA derived from fish meal and oil (Turchini et al., 2009) and therefore of limited use as an environmental tracer beyond the acute deposition zone. A novel, rapid fatty acid profiling technique developed by Parrish et al. (2015) and applied to a range of marine fish species, significantly reduces processing time and allows for broad-scale application to environmental and ecological studies (Pethybridge et al., 2015). Here, we expanded the application of the rapid fatty acid profiling technique to sediment, zooplankton and polychaete samples to trace how waste material from the aquaculture of carnivorous fish was dispersed and assimilated in a naturally low productivity environment.

2. Materials and methods

2.1. Study site

Macquarie Harbour is an approximately 250 km^2 embayment on the west coast of Tasmania, Australia (42°18.17′S, 145°23.86′E, Fig. S1). The Harbour has been the site of Atlantic salmon (*Salmo salar*) and Rainbow trout (*Oncorhynchus mykiss*) production for approximately 30 years. The aquaculture industry has expanded recently, from 5000 t total annual production in 2008 to just under 12,000 t in 2013 (DPIPWE, 2016). The Gordon/Franklin river system provides the largest freshwater input into Macquarie Harbour, with waters high in humic substances and higher plant debris. This leads to tannin rich waters with low light penetration in the water column, and low dissolved oxygen below the halocline (Cresswell et al., 1989; Carpenter et al., 1991). Residence time of water in Macquarie Harbour is estimated to be approximately 70 days for freshwater and potentially double this for marine water below 15 m (Koehnken, 1996). Sediments have low faunal biomass and diversity (Edgar et al., 1999; Edgar and Barrett, 2002), and as a whole it is considered a low productivity environment. This low productivity is potentially due to a combination of several factors, including high tannin and humic leachates in the water column, low anthropogenic activities in the catchment, underlying geology, and low incursion of marine-derived nutrients (Koehnken, 1996). Limited data on the fatty acid composition of sediments and primary consumers prior to the introduction of large-scale salmonid aquaculture indicates that sediments were high in shorter chain ($\leq C_{18}$) saturated fatty acids (SC-SFA) indicative of anaerobic microbial communities, and LC-SFA (20:0, 22:0; 24:0, 26:0) derived from higher plant material (Carpenter et al., 1991). As the majority of the catchment and half the embayment is within World Heritage Area, aquaculture represents the only major source of anthropogenic organic material into the Harbour. This, combined with natural low productivity, makes Macquarie Harbour an ideal site for assessing the power and sensitivity of fatty acid biomarkers to detect and map the footprint of anthropogenic influence through aquaculture.

2.2. Sampling design and collection

Sampling was conducted in Macquarie Harbour on three occasions. In November 2012, an initial survey was undertaken, with three farm sites and five control locations sampled across the harbour, to validate the use of fatty acid biomarkers in detecting aquaculture inputs. Three replicate sediment samples were collected at each site collected for fatty acid analysis. All farm samples were taken from directly beneath stocked cages and all control locations were > 2 km from the nearest stocked farm. A sample of aquafeed used on the farms was also obtained