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Fatty acids as tracers of trophic interactions between seston, mussels and biodeposits in a coastal embayment of mussel rafts in the proximity of fish cages

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ABSTRACT

We traced the food sources of mussel *Mytilus galloprovincialis* cultured in suspension in Ría Ares-Betanzos (N.W. Spain) by means of fatty acid (FA) biomarkers. The FA profile of seston, mussels' mantle, digestive gland and feces was analyzed during five seasons. Due to the proximity of a fish farm to the bivalve aquaculture site, we also tested if mussels and seston situated 170 m distant from the fish cages incorporated fish feed FA markers compared with samples obtained 550 m away. The principal FA in the mussels' organs were 16:0, 16:1ω7, EPA (20:5ω3) and DHA (22:6ω3), while 16:0 predominated in the feces. Seasonal fluctuations in the seston composition were mirrored in the FA signature of mussels' organs and feces, although the digestive gland had the closest resemblance to the seston FA profile. In general, diatom and bacteria derived-biomarkers predominated in mussels' organs and feces during the upwelling period (spring–summer), while dinoflagellates were the dominant dietary source during downwelling (autumn–winter). The higher concentration of EPA and DHA in both organs and the feces compared with the seston suggested a preferential accumulation of these ω3 FA in the mussels' tissues. The results showed a lack of assimilation of fish feed FA biomarkers in the seston and mussel samples. This might be due to the dispersion of uneaten feed particles by high current velocity, substantial distance between the fish and mussel culture, the limited amount of nutrient waste released by the fish farm and dilution of feed particles in the large mussel standing stock.

1. Introduction

Fatty acids (FA) are valuable biochemical markers to trace the flow of organic matter along different trophic levels in marine food webs (Dalsgaard et al., 2003; Kelly and Scheibling, 2012). Fatty acids provide a qualitative measurement of the energy transferred from primary producers up to higher trophic levels (Dalsgaard et al., 2003) and have the advantage that once stored in the body they don't undergo major changes (Graeve et al., 1994; Xu and Yang, 2007; Kelly and Scheibling, 2012). According to the 'you are what you eat' principle, the FA profile of consumers reflects the composition of their diet and their trophic relationships, even if most taxa lack a fat-storage organ and are capable of modifying their FA composition depending on the environmental characteristics, the physiological status and the turnover rate of each tissue (Ventrella et al., 2008; Kelly and Scheibling, 2012; Richoux et al., 2014). Previous studies have successfully used FA markers to investigate the trophic interactions and feeding ecology of mussels and their food sources (Budge et al., 2001; Alkanani et al., 2007; Shin et al., 2008; Ventrella et al., 2008; Ezgeta-Balić et al., 2012; Najdek et al., 2013; Zhao et al., 2013; Richoux et al., 2014). Unlike the analysis of the stomach food content, the fatty acid signature of mussels' tissues can reveal bivalves' food sources in a long-term basis (Ezgeta-Balić et al., 2012). Mussels are generalist filter-feeders that feed on seston and could potentially incorporate the fatty acid signatures of the diatoms, dinoflagellates, bacteria and other suspended particulate organic matter sources into their tissues. The fluctuations in the FA composition of the mussels' diet can influence their growth and lipid composition (Alkanani et al., 2007; Narváez et al., 2008). Freites et al. (2002a) found that the nutritional quality of the natural seston explained the variance of almost all FA of energetic importance to mussel *Mytilus galloprovincialis*, even if selective retention was observed for 20:4ω6 during winter for reproductive processes rather than food ingestion.

From a bottom-up point of view, certain FA and FA ratios can be used as biomarkers to characterize the seasonal contribution of phytoplankton classes and bacteria to the mussels' diet (Budge et al., 2001; Handå et al., 2012). Diatoms are rich sources of 16:1ω7 and 20:5ω3 (eicosapentaenoic acid or EPA) and are characterize by a ratio 16:1ω7/16:0 > 1 and 20:5ω3/22:6ω3 > 1 (Budge et al., 2001; Dalsgaard et al., 2003). On the other hand, FA 16:0, 18:1ω9, 18:4ω3, 22:6ω3 (docosahexaenoic acid or DHA) together with the ratio 22:6ω3/20:5ω3 > 1 are typical dinoflagellate markers (Budge et al., 2001 Dalsgaard et al., 2003). These distinct FA can be

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transferred from primary producers up to the mussel tissues and indicate a preferential ingestion of diatoms or dinoflagellates. In addition, odd-numbered branched FA like 15:0 and 17:0, 18:1ω7 and the ratio 18:1ω7/18:1ω9 > 1 can indicate a bacterial input in the mussels' diet (Budge et al., 2001).

Even if phytoplankton is the primary food source for mussels, several studies have reported that mussels can effectively utilize excess particulate organic matter coming from fish cages when reared in proximity to the net-pens (Handå et al., 2012). The analysis of the fatty acid profile of mussels cultured near fish cages or directly exposed to fish effluents can be used to trace the assimilation of uneaten fish feed in the mussels' tissues (Gao et al., 2006; Redmond et al., 2010; Both et al., 2011, 2012; George and Parrish, 2012; Handå et al., 2012; Both et al., 2013). Mono-unsaturated FA (MUFA) 20:1ω9, 22:1ω11 and EPA are traditional fish feed biomarkers, originated from the sardine, herring and capelin utilized for feed manufacturing (NRC, 1993). Recently, fish feed have started to contain elevated amounts of vegetable fatty acids derived from terrestrial seed oils and meals that are not generally found in the marine food chains and thus, these terrestrial FA can be indicators of feed waste assimilation by mussels. These fish feed markers include high percentages of oleic acid 18:1ω9 and polyunsaturated fatty acids (PUFA) such as linoleic acid 18:2ω6, α-linolenic acid 18:3ω6 along with eicosenoic acid 20:1ω9, arachidonic acid (20:4ω6) and a low ω3/ω6 ratio (Gao et al., 2006; Redmond et al., 2010; Both et al., 2011; Handå et al., 2012).

The principal objective of this study was to analyze the trophic interactions between seston and mussels *M. galloprovincialis* cultured in suspension in a commercial raft polygon in the Ría de Ares-Betanzos (Galicia, N.W. Spain) using fatty acid trophic markers. Due to the fact that some of the mussel rafts are located proximate to a fish aquaculture site, a second aim of this study was to assess if mussels cultured in a raft near fish cages were assimilating any uneaten fish feed (i.e. from feed 'fines' up to particles <50 μm) as part of their diet. We compared the fatty acid profile of mussels cultured in a raft close to fish cages of red sea bream (*Pagellus bogaraveo*) with mussels reared in a raft distant from the net-pens. Insights on the effectiveness of the utilization of fish feed by mussels will be of great importance for the implementation of integrated mussel-fish systems worldwide, but especially for Galicia, a region where mussel farming is the principal aquaculture activity and provides 9000 and 20,000 direct and indirect jobs, respectively (Labarta et al., 2004).

2. Material and methods

2.1. Study site

The Ría de Ares-Betanzos is located between Cape Fisterra and Cape Prior in Galicia, on the N.W. coast of the Iberian Peninsula (43°22′39.20″ N, 8°12′39.77″W; Fig. 1). Seston and mussel samples were collected at two commercial mussel rafts in Lorbé polygon: raft P-14, placed in the proximity (170 m North) of fish net-pens (43°23′19.62″N, 8°17′17.71″ W) and raft P-46, located further away (550 m North) from the cages (43°23′19.62″N, 8°17′17.71″W). Rafts P-14 and P-46 had the same dimensions (550 m^2) and mussels were cultured following the traditional commercial protocols (Fig. 1). Mussel seeds originated from the same location and the average density was 700 mussels m^{-1} rope. Lorbé raft polygon is situated on the southern shore of the Ría Ares-Betanzos and is the main area of shellfish farming with 107 culture units grouped in parallel rows.

The Ría Ares-Betanzos covers an area of 52 km^2, a total volume of 0.65 km^3 and depths between 2 and 43 m (Sánchez-Mata et al., 1999; Álvarez-Salgado et al., 2011). The Ría is a double estuarine system (Asensio-Amor and Grajal-Blanco, 1981) that receives an average flow of 16.5 and 14.1 m^3 s^{-1} from rivers Eume and Mandeo, respectively (Prego et al., 1999). The Ría Ares-Betanzos has great bio-economical importance owing in part to the extensive cultivation of mussel *M. galloprovincialis* on suspended rafts that produce 10,000 Tyr^{-1} (Labarta et al., 2004). Mussel production per raft in the Galician Rías is estimated to range from 60 to 84 Tyr^{-1} and the production cycle lasts 16–18 months, with production and seed ropes coexisting in the same raft to supply commercial demands (Labarta et al., 2004). Floating cages for the culture of red sea bream (*P. bogaraveo*) are installed within

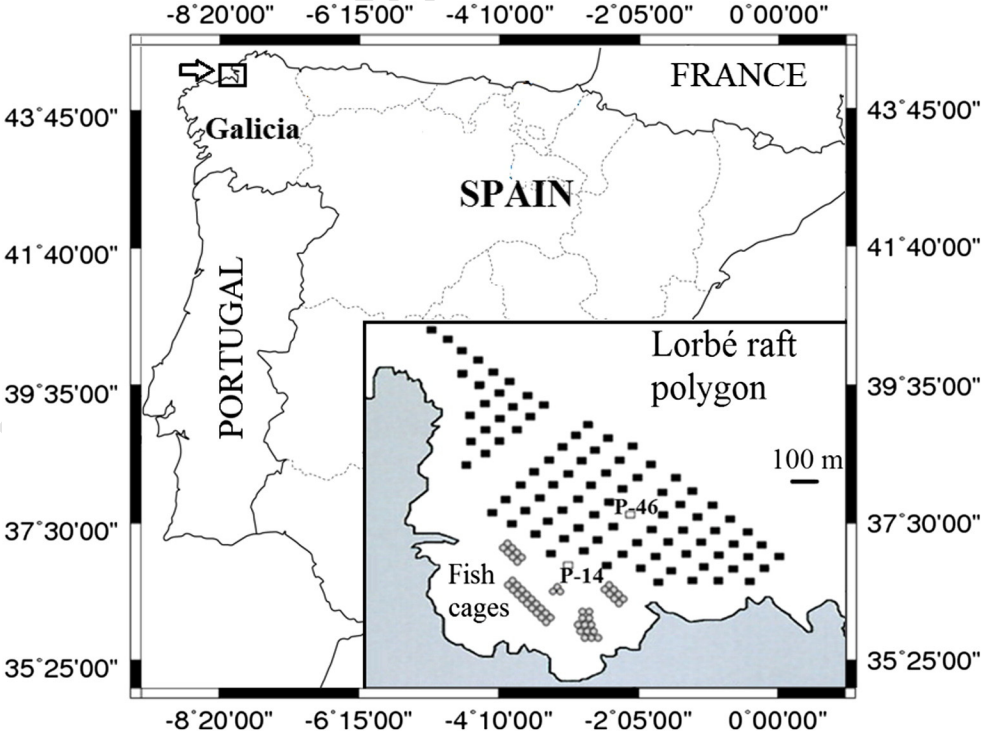

Fig. 1. Geographical position of Lorbé mussel raft polygon in the Ría Ares-Betanzos (Galicia, N.W. Spain). The white squares represent the mussel raft adjacent to the fish farm (P-14; 170 m from the cages) and the raft distant from the cages (P-46; 550 m from the cages). The black squares indicate the position of the remaining mussel rafts moored in the shellfish polygon.

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