



## Mediterranean fouling communities assimilate the organic matter derived from coastal fish farms as a new trophic resource



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### ABSTRACT

Currently, the lipid content of fish feeds includes high amounts of terrestrial vegetable oils, rich in n-6 fatty acids and poor in n-3 fatty acids. Sinking organic matter in the shape of fragmented pellets and fish faeces could be ingested by the surrounding fauna attracted to the submerged structures of aquaculture facilities or living in natural benthic habitats. Fatty acids contained in feed pellets were used as trophic markers to shed light on the assimilation and incorporation of aquaculture wastes by the invertebrate fauna associated to sea-cages. Eighteen macroinvertebrate species, and zooplankton, seaweeds and sediments were collected from two fish farms, one of which (control) had not been used as such for two years. This study demonstrates that macroinvertebrate fauna present in fouling can take up sinking organic matter from farms. Further research should be directed at assessing the potential implications of aquaculture production for the surrounding ecosystem.

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### 1. Introduction

Anthropogenic perturbations in coastal marine ecosystems include the physical modification of habitats, the introduction of nutrients and pollutants, and alterations to populations of wild species as a result of fishing and other processes (Dempster et al., 2009). Fish farms have an ecological effect on the surrounding environment, which is noticeable not only at a benthic level (e.g. Naylor et al., 2000; Karakassis et al., 2000; Black, 2001; Dempster et al., 2004), due to sinking organic matter in the form of uneaten feed pellets and faeces (Fernandez-Jover et al., 2009; Black et al., 2012). Changes in the invertebrate assemblage structure (Fabi et al., 2009; Madin et al., 2009; Fernandez-Gonzalez et al., 2013) have been extensively studied in relation to the increase of organic matter sedimented from fish farms.

Most farmed marine fish are piscivorous species that require marine ingredients in their feed in order to achieve an optimal growth rate and health status (Naylor et al., 2000; Sargent et al., 2002; Fernandez-Jover et al., 2011). Nevertheless, current feeds also include high levels of vegetable oils in their composition, such as

linseed, soybean, rapeseed or palm oil that are rich in saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) like oleic acid (18:1n-9, OA) and polyunsaturated fatty acids (PUFA) like linoleic acid (18:2n-6, LA) and  $\alpha$ -linolenic acid (18:3n-3, LNA). Additionally, these vegetable oils present poor levels of the long chain PUFAs eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA), which are abundant in fish oils (Turchini et al., 2010; Fernandez-Jover et al., 2011) and become essential for fish, as well as arachidonic acid (20:4n-6, ARA). Large amounts of organic matter in the form of high energy fish feed rich in vegetable fatty acids are introduced into the environment, where uneaten pellets are consumed by wild fish which assemble near farms. In addition, fragmented pellets and faeces might represent a new trophic source for fouling organisms, providing them with a new source of terrestrial fatty acids such as LA and LNA, which are unusual in marine environments, and should be investigated (Sara et al., 2004; Fernandez-Jover et al., 2008; Arechavala-Lopez et al., 2011). If the above is true, it could modify their fatty acid composition; however, our knowledge of the extent to which the commercial feed affects different trophic levels is limited. It remains unclear which ecological compartments might be influenced by commercial feed entering the food chain. Changes in the fatty acid profile of the aggregated fauna have been detected by several authors (Skog et al., 2003; Fernandez-Jover et al., 2007, 2009) but there is no full picture of the influence of fish farming on marine communities.

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Due to the composition of feed, fatty acids could be used as markers of feed consumption by marine communities attached to sea-cage structures, and as markers of lipidic waste accumulation on the sea-floor and in algal organisms. Several studies have proposed the use of fatty acid analysis to check the influence of aquaculture on the fatty acid composition of cage-associated fauna (zooplankton, sea-urchins, mussels, shrimps, fish and also sediments) (Samuelsen et al., 1988; Henderson et al., 1997; Cook et al., 2000; Skog et al., 2003; Gao et al., 2006; Fernandez-Jover et al., 2007; Olsen et al., 2009; Barberá et al., 2011), and many studies based on fatty acid profiles have concluded that food web relationships exist (Graeve et al., 1994; Scott et al., 1999). The effect of lost pellets have been studied by several authors (Sara et al., 2004; Vita et al., 2004; Sanz-Lázaro et al., 2011a, 2011b), mainly focusing on aggregated wild fish, and not taking into account the effect on other organisms such as macroinvertebrate species, zooplankton or seaweeds, that colonize the submerged fish farm structure. The present research focuses on the transfer of vegetable fatty acids from aquaculture wastes to the surrounding ecosystem, and looks for possible to detect changes in trophic behaviour due to the assimilation of organic matter derived from aquaculture activities. For this, we analyzed the modification of the fatty acid profile in different ecological compartments, with special attention to the incorporation of terrestrial fatty acids in marine macroinvertebrate communities, including a wide range of species belonging to different taxa, and zooplankton. We also analyzed the sediments below the sea-cages and various seaweed species because of their importance as natural trophic resources for fouling organisms.

## 2. Material and methods

### 2.1. Study site and field sampling

The study area was located off the south-east coast of Spain, in the western Mediterranean Sea (Fig. 1). Two fish farm facilities were selected to carry out the study: impact (an active fish farm facility; producing 900 t of fish per year) and control (a fish farm not used for production for the previous two years). From June to September 2010, samples of sediments below the sea cages, seaweeds, feed pellets, fish faeces and surrounding organisms were collected. Fish faeces suspended in the water column were collected using sediment traps deployed for 24 h. Horizontal plankton hauls were performed to collect zooplankton communities of each fish farm according to the main current. Fouling communities were collected by scraping mooring ropes covering the main habitats of algae, hydroids and mussels. After collection, samples were trans-

ferred to the laboratory in a cooling box with sea water, ice and air supply prior to identification. Sessile fouling organisms and the associated fauna were sorted, identified to species level whenever possible and frozen at  $-80^{\circ}\text{C}$ . As well as the feed used at the sampling time, we tested the feed used in winter, because the presence of winter pellets in the area should not be discarded. At least three replicates were taken for each item, except for faeces (only one pool of faeces could be analyzed because of the difficulty involved in obtaining a big enough sample for fatty acid analysis).

### 2.2. Fatty acid analysis

Total lipids were extracted from 0.5 to 3.0 g of sample by homogenizing in 20 ml of chloroform/methanol (2:1 v/v) in an Ultra Turrax tissue disrupter (IKA ULTRA-TURRAX T 25 digital, IKA-WERKE). Total lipids were prepared according to the method described by Folch et al. (1957) and non-lipid impurities were removed by washing with 0.88% (w/v) KCl. The weight of lipids was determined gravimetrically after evaporation of the solvent and overnight desiccation in vacuum. Fatty acid methyl esters (FAME) were prepared by acid-catalyzed transesterification of total lipids according to the method of Christie (1982), and the total lipid samples were trans-methylated overnight in 2 ml of 1% sulphuric acid in methanol (plus 1 ml of toluene to dissolve neutral lipids) at  $50^{\circ}\text{C}$ . The methyl esters were extracted twice in 5 ml hexane-diethyl ether (1:1, v/v) after neutralization with 2 ml of 2%  $\text{KHCO}_3$ , dried under nitrogen and redissolved in 0.5 ml of iso-hexane. FAME were separated and quantified by gas-liquid chromatography using an SP<sup>TM</sup> 2560 flexible fused silica capillary column (100 m long, internal diameter of 0.25 mm and film thickness of 0.20 mm; SUPELCO) in a Hewlett-Packard 5890 gas chromatograph. The oven temperature of the gas chromatograph was programmed for 5 min at an initial temperature of  $140^{\circ}\text{C}$ , and increased at a rate of  $4^{\circ}\text{C}/\text{min}$  to  $230^{\circ}\text{C}$ , further increased at a rate of  $1^{\circ}\text{C}/\text{min}$  to  $240^{\circ}\text{C}$  and then held at that temperature for 6 min. The injector and flame ionization detector were set at  $260^{\circ}\text{C}$ . Helium was used as carrier gas at a pressure of 300 kPa, and peaks were identified by comparing their retention times with appropriate FAME standards purchased from the Sigma Chemical Company (St. Louis, MO, USA). Individual fatty acid concentrations were expressed as percentages of the total content.

### 2.3. Fatty acid markers

The n-3/n-6 index was used to detect alterations of the fatty acid profile due to feed consumption. Lower values indicate a reduction of the total n-3 fatty acid content, usually present in high

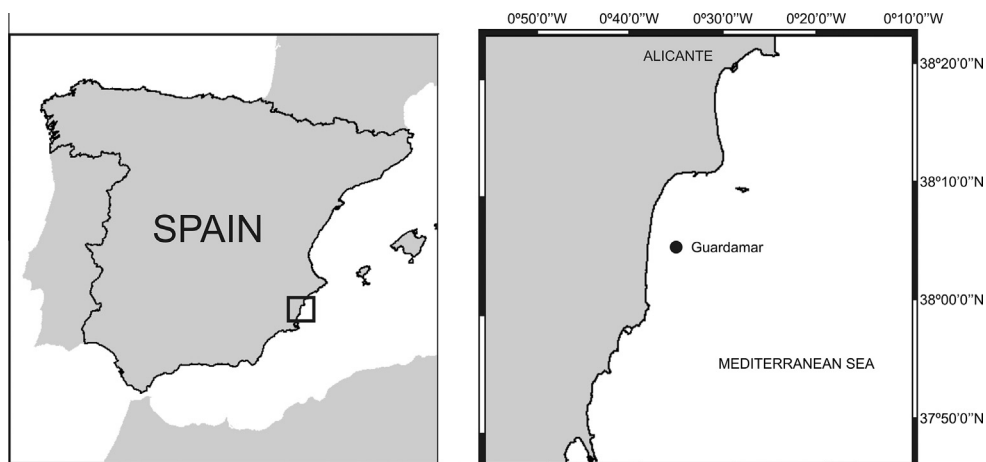


Fig. 1. Location of the studied farms in Guardamar Bay, south-east coast of Spain, in the Western Mediterranean Sea.

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