



Research article

Mussels do not directly assimilate fish farm wastes: Shifting the rationale of integrated multi-trophic aquaculture to a broader scale



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ABSTRACT

Pollution is one of the most significant issues that is currently impeding the development of fish farming. Integrated multi-trophic aquaculture (IMTA) has the potential to reduce the accumulation of organic wastes in the environment by using taxa of lower trophic levels such as filter feeders. However, the capacity of filter feeders to assimilate significant quantities of fish farm wastes has not yet been fully tested *in situ*. We analyzed the stable isotopes $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in mussels from six fish farms and from six other areas that were not influenced by fish farming, at two water strata (surface and mid-water) across a marked gradient of eutrophication along more than 900 km of coastline in the Western Mediterranean. We found that the mussels did not directly assimilate fish farming wastes. Consequently, fish farming wastes did not constitute a major component of mussel diet, irrespective of local productivity and depth in the water column. These outcomes do not necessarily mean that IMTA is not suitable in other cases, but rather that there should be a shift in the rationale of IMTA by modifying the concept of direct assimilation of wastes to a more general approach of IMTA based on regional budgets of nutrients.

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1. Introduction

The importance of aquaculture for food production is growing at an ever increasing rate, and this trend is expected to continue in the coming decades. Marine environments are particularly important in this regard, as the space is much less restricted compared to other food production activities that take place entirely on land (FAO, 2014). However, the rate of establishment of aquaculture is impeded by the impacts of pollution that may generate. In particular, fish farming generates a large quantity of organic wastes, mainly derived from feeding, in the form of uneaten feed and fish faeces (Sanz-Lazaro and Marin, 2008). Uneaten feed and fish faeces consist of large and small particles. Large faecal particles and uneaten feed sink rapidly and may accumulate in sediments on the seafloor where they may be consumed by detritus-eating animals. Small particles of waste can remain in suspension and then be consumed by filter-feeding zooplankton or by visual feeders, such as fish, in the water column, or by mussels. When accumulate on the seabed, these wastes lead to oxygen depletion and the prevalence of anaerobic metabolic pathways, deteriorating of the

ecological status of the benthic system and consequently on its ecological functions (Holmer et al., 2005; Karakassis et al., 1999; Sanz-Lazaro and Marin, 2011).

Under this scenario, integrated multi-trophic aquaculture (IMTA) has emerged as a potential tool to help fish farming become a more environmentally friendly activity, by culturing combinations of species at different trophic levels (Neori et al., 2004). The purpose of IMTA is two-fold, seeking to reduce environmental impacts while increasing production. It aims to limit the wastes derived from aquaculture by culturing species with a low trophic level that can feed on the wastes generated by cultured species at higher trophic levels. This approach is expected to maximize the production of the low-trophic-level species due to an increase on the availability of food derived from the release of wastes by high-trophic-level species (Soto, 2009).

IMTA has been designed using generally fish as the high-trophic-level species, and, usually, extractive and filter-feeding species as the low-trophic-level species, for which mussels and oysters are the main groups used in marine IMTA (Cranford et al., 2013). The concept of IMTA is appealing and promising. Bivalves have the capacity to accumulate fish farm wastes (Handa et al., 2012b; MacDonald et al., 2011; Redmond et al., 2010): models simulating bivalve production predict significantly greater yields when cultured under IMTA compared to monocultures (Ferreira

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et al., 2012; Sara et al., 2012). Nevertheless, the implementation of IMTA using fish and bivalves has yielded contrasting results. In some cases bivalves have had a higher growth rate close to fish farms (Jones and Iwama, 1991; Wallace, 1980), but in other cases the fish farms did not seem to influence their growth (Cheshuk et al., 2003; Navarrete-Mier et al., 2010; Peharda et al., 2007), or only had an influence at certain times of the year (Handa et al., 2012a). Nevertheless, the observed changes in growth rate do not necessarily prove the assimilation of fish farming wastes by bivalves.

Isotopes have been widely used in ecology to decipher trophic pathways, allowing measurements of time-integrated assimilation of foods (Hobson and Welch, 1992) and differentiation in the origin (terrestrial or marine) of food sources (Darimont et al., 2009). Isotopes have previously been used to trace fish farm wastes (Holmer et al., 2007; Sara et al., 2004), and are suitable to test the assimilation of fish farming wastes (Sanz-Lazaro et al., 2015; Vizzini and Mazzola, 2004; Yokoyama et al., 2015). They can be distinguished from other sources of marine food because a considerable part of the fish food ingredients have a terrestrial origin, which is $\delta^{13}\text{C}$ depleted (Ytrestol et al., 2015). As a result, this isotope has been used to evaluate the assimilation of fish farming wastes in different organisms and communities (Dolenec et al., 2006; Irisarri et al., 2015; Navarrete-Mier et al., 2010; Sanz-Lazaro et al., 2011a,b).

Filter-feeding bivalves have the capacity to assimilate fish farming wastes under laboratory conditions (Handa et al., 2012b; Reid et al., 2010), although *in situ* pilot studies have shown that fish farming wastes do not make up a substantial part of their diet (Handa et al., 2012a; Irisarri et al., 2015; Navarrete-Mier et al., 2010). This may be because IMTA biomitigation capacity may depend on the trophic state of the water column and/or benthos (Cranford et al., 2013). Thus, the suitability of IMTA is expected to be greater in areas with naturally-low nutrient concentration, as these areas have low densities of plankton with low food quality in comparison to more-eutrophic areas (Both et al., 2012; Lander et al., 2013; Troell et al., 2003).

Previous *in situ* studies have been constrained by the specific environmental characteristics of the single locations at which each experiment was carried out, preventing the extrapolation of the results to other areas. In addition, water depth is expected to be a key parameter in the growth of filter-feeding bivalves (Fuentes et al., 2000), particularly in IMTA (Mazzola et al., 1999), for which the availability of particulate fish farming wastes may be markedly stratified across the water column. However, the importance of depth has not generally been considered in the implementation of IMTA (but see Filgueira et al., 2017). Hence, there is an urgent need for a comprehensive assessment of the feasibility of IMTA systems. This would help to optimize the use of this promising tool to diminish the environmental impact of fish farming, and thus to remove this impediment to the expansion of aquaculture.

The aim of this study is to test whether filter feeders are able to use fish farming wastes as trophic resources in coastal areas close to fish farms, based on the IMTA rationale. We hypothesize that the direct assimilation of organic wastes from fish farming may be related to the natural productivity of the water body, with increased assimilation when primary production is low. To investigate this, we tested whether mussels (*Mytilus galloprovincialis*) could ingest a significant proportion of organic wastes derived from fish farming under different levels of eutrophication and depths in the water column. We analyzed the content of two stable isotopes - $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ - in mussels from six fish farms and from six other areas that were not influenced by fish farming. Samples were taken at two water strata - surface (from 3 to 5 m depth) and mid-water (from 12 to 16 m depth) - covering a marked gradient of eutrophication along the Western Mediterranean coast.

2. Material and methods

2.1. Study area

Mussels were taken from the Spanish coast along the Western end of the Mediterranean, covering more than 900 km of coastline from the southern limits of the Balearic Sea to the upwelling of the Alborán Sea (Fig. 1; Table S1). Although the Mediterranean is generally oligotrophic, the study area has a marked eutrophication gradient ranging from 0.32 to 3.5 mg chl $a\ m^{-3}\ year^{-1}$ (see Fig. S1; D'Ortenzio & D'Alcala, 2009).

2.2. Sampling

To ensure that mussels had enough time to accumulate the isotopic signal we only analyzed mussels that were longer than 45 mm, as the growth rates of *M. galloprovincialis* in the Mediterranean are below 50 mm per year (Abada-Boudjema and Dauvin, 1995; Ceccherelli and Rossi, 1984). Mussels were taken from 12 locations during late summer and autumn of 2015. Current from two oceanographic bouys, Cabo de Gata and Cabo de Palos, showed that for this period of time the current speed was 22.8 and 25.9 $\text{cm}\ s^{-1}$, respectively and the dominant current was in both cases SW (Puertos del Estado, 2017). Six were at fish farms (labelled with "F" and the corresponding number from 1 to 6) culturing gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*), which were fed with pellets of fish feed (mainly composed of plant protein and oil, as well as, marine protein and oil; Fernandez-Jover et al., 2011; Ytrestol et al., 2015) at an annual rate of 300 to 1000 tonnes per year depending on the size of the fish farm. The other six locations were reference sites that were considered unlikely to be influenced by fish farming (labelled with "R" and the corresponding number from 1 to 6); they were generally more than 10 km from the closest fish farm or any other possible source of anthropogenic activity that produced organic wastes. Due to the difficulty of locating structures that supported mussels, reference location R5 was located less than 3 km from fish farm F4. However, R5 was located "upstream" of the main current of the area that passed site F4 (Sanz-Lazaro et al., 2011a), and fish farming wastes at the time of highest production do not reach more than 350 m "downstream" of the main current (Sanz-Lazaro et al., 2011b). In addition, because of the aforementioned difficulty in finding reference sites, two of the reference sites were located 7 km away from each other (Fig. 1; Table S1).

In the fish farm facilities, mussels were taken from ropes and cage structures. In the reference locations we searched buoys of various types, primarily those that delimited marine protected areas. At each location mussels were taken by scuba divers at two water strata, surface (from 3 to 5 m depth) and mid-water (from 12 to 16 m depth). Mussels were collected during the second half of 2014. The distance from each location to the coast was always greater than 200 m in order to avoid possible interferences in the $\delta^{13}\text{C}$ signature between fish feed and other terrestrial sources. In addition to the mussels, two types of fish food used in the fish farms were also analyzed.

2.3. Sample processing

Mussels were chilled in a portable cooler immediately after collection, and after arriving on land they were immediately frozen and transported frozen to the laboratory where they were stored at $-20\ ^\circ\text{C}$. Mussels were then thawed, opened and dissected to remove (and discard) the digestive system. The tissues used for analysis were the mantle, pedal sinus, adductor muscle and gills. They were rinsed with distilled water, lyophilized, ground to a

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