Integrated Multitrophic Aquaculture: Filter Feeder Bivalves as Efficient Reducers of Wastes Derived from Coastal Aquaculture Assessed with Stable isotopes

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Introduction

The organic enrichment is recognized as the most important problem associated to open-water aquaculture as a direct result of the release of dissolved and particulate nutrient loads. Uneaten pellet and fresh food supplied at fish cages, together with the excretion products from cultured fishes are the source of the nutrient loads released (Cheshuk *et al.*, 2003). Whereas the dissolved compounds are easily dispersed and diluted in the water column, the particulate compounds sink to the sea floor, causing severe modifications of the physical and chemical characteristics of the sediment and the community dynamics of marine seagrass and benthic fauna (Karakassis *et al.*, 2000; Mente *et al.*, 2006). The effects have been widely studied in the Atlantic and the Pacific Ocean, specially in relation to the salmon industry, however knowledge of fish farming impacts in the oligotrophic waters of Mediterranean Sea is scarce (Vizzini & Mazzola, 2004). Integrated multitrophic aquaculture (IMTA) has emerged as a potential tool to mitigate the environmental impact of organic enrichment by integrating fish with low-trophic organisms (molluscs and/or algae) during farming through the recycling of particulate and dissolved compounds.

To decipher whether integrated multitrophic aquaculture is an effective method for minimizing and reducing waste inputs into the Mediterranean coastal ecosystem we have designed a multitrophic integrated system involving filter feeders bivalves *Mytillus galloprovincialis* (Lamarck, 1819*)* and *Mimachlamys varia* (Linnaeus, 1758), and fin fish *Argyrosomus regius* (Asso,1801). Through determination of carbon and nitrogen stable isotopes to the several organisms and trophic strategies, the following aims will be accomplished:

i. Define the isotopic composition of the trophic food web of integrated multitrophic aquaculture in relation to two reference stations.

ii. Study the temporal variability in waste matter fluxes at the different treatments, hence annual and seasonal variability.

iii. Calculate the relative contributions of wastes (fresh food, pellets, plankton) to the fish farmed and to the filter feeders at the IMTA.

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Methods

The study was conducted from years 2008 and 2011 at a research experimental station (LIMIA), in Andratx Bay, on the SW coast of Mallorca. The installations included 6 floating cages with cultured *Argyrosomus regius*, with a total fish stock of 12-15 t year $^{-1}$. Two reference sites were selected to evaluate the effects of aquaculture wastes in the adjacent environment, an external site (control 1) located approximately 350 m away from the fish cages within the Andratx Bay, and a second site (control 2) located 21 nautical miles away. Filter feeder bivalves *Mytilus galloprovincialis* and *Mimachlamys varia* were chosen for this study as they are native to the region, fast growing and commercially viable. They were collected from the harbour area in Andratx Bay between April and July 2007 and transferred to plastic bags which were placed hanging on fish cages, and the same amount was simultaneously attached to the rope buoys in control 1 and control 2.

In the experimental site (hereafter called cage site), approximately 10 adult specimens of *Argyrosomus regius*, and 6 samples of *Mytilus galloprovincialis* and *Mimachlamys varia* were sampled seasonally. Additionally, potential food sources derived from aquaculture wastes as fish faeces and pellet and fresh food were sampled; as well as the other components of the marine food web, phytoplankton, zooplankton, particulate organic matter (POM) and sedimentary organic matter (SOM). Moreover, 5 sedimentivorous echinoderms *Holothuria (panningothuria) forskalii* Delle Chiaje, 1823 were also collected. Similarly, in control sites 6 specimens of filter feeder bivalves *Mytilus galloprovincialis* and *Mimachlamys varia* were sampled. In control 1, *Mimachlamys varia* could not be sampled since the polyethylene bags were lost after severe storm periods. Moreover, phytoplankton, zooplankton, particulate organic matter (POM) and sedimentary organic matter (SOM) were sampled seasonally and 5 samples of sedimentivorous echinoderms *Holothuria (panningothuria) forskalii* were sampled annually following the same procedure as in cage site. All samples were frozen immediately after sampling and kept at -20ºC till further processing.

Samples were dried in an oven at 60ºC for 24h and subsequently grounded to a fine powder using a mortar and pestle. A minimum of two replicates for each sample was analyzed for $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures, except for commercial dry pellet feed which had a stable and controlled composition (Trouw S.A). SOM samples for $\delta^{13}C$ isotopic analysis were acidified by adding 2N HCl, while for $\delta^{15}N$ analysis, non- acidified replicates were used. From each sample 2 ± 0.1 mg of dry weight was placed in tin cups to determine the stable isotope ratios of carbon and nitrogen.

The analyses were run at the SCTI (Scientific-Technical Services) from the Balearic Islands' University using a continuous flow mass spectrometer (Thermo Finnegan Delta x-plus). The analytical precision of the stable isotope analyses was based on the standard deviation of the BSL samples, which were 0.08 ‰ for $\delta^{13}C$ and 0.09 ‰ for $\delta^{15}N$. Isotope ratios were expressed in $\delta^{13}C$ and $\delta^{15}N$, with units of ‰, according to the following equation:

$\delta^{13}C$ or $\delta^{15}N = [(R_{sample}/R_{reference}) - 1] \times 1000$

where R is the corresponding $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio.

Distance based permutation analysis of variance (PERMANOVA) was applied to test the hypothesis that there were no differences in the isotopic signature ($\delta^{13}C$ and $\delta^{15}N$) of filter feeders bivalves between treatments (cage, control 1 and control 2) and sampling periods (years: 2008, 2009, 2010 and 2010; and season: spring, summer and autumn). All statistical computations were performed using the statistical package PRIMER® 6.0 PERMANOVA software. The analysis was not performed on $\delta^{13}C$ and $\delta^{15}N$ sediment data because, as a consequence of the low carbon and nitrogen content in the samples, some replicates did not reach the mass requirements and could not be analyzed in the mass spectrometer.

SISUS Bayesian Mixing model (Stable Isotope Sourcing using Sampling) was applied to quantify the feasible contributions of the potential organic matter sources (phytoplankton, zooplankton, POM, faecal material, pellet and fresh food) to the filter feeders bivalves' diet, based on the analyzed stable isotope ratios. A discrimination of 0.3 ‰ for carbon was assumed for POM and filter feeders bivalves while for muscle samples of *A. regius* a 1.3 ‰ was applied. For nitrogen, a correction of 2.3 ‰ per trophic level was applied (McCutchan *et al.*, 2003).

Results

$\delta^{13}C$ and $\delta^{15}N$ values were determined for the food web components in the four years of study in cage and control sites. Filter feeders bivalves from cage site exhibited isotopic values, ranging from -18.52 ‰ to -21.50 ‰ for ^{13}C and 5.85 ‰ to 8.47 ‰ for ^{15}N in *M. galloprovincialis*, and between -22.34 ‰ to -17.69 ‰ for ^{13}C and 5.70 ‰ to 9.38 ‰ for ^{15}N in *M. varia*. Statistical analyses showed significant differences between cage and control treatments and seasons in the four years of study for ^{13}C and ^{15}N in *M. varia* while *M. galloprovincialis* showed significant differences between treatments, season and year for ^{13}C and between treatments and seasons for ^{15}N. (PERMANOVA, p<0.05). *M. galloprovincialis* from cage site showed an enrichment of 0.11 ‰ in ^{13}C and 1.33 ‰ in ^{15}N compared to bivalves from control 1; greatest differences were found when comparing cage site with control 2, with an enrichment of 1.44 ‰ in ^{13}C and 3.07‰ in ^{15}N (p<0.05). Regarding *M. varia* from cage site, mean values for ^{13}C were -19.80 ± 0.44 ‰ and 7.47 ± 0.70 ‰ for ^{15}N, showing an enrichment of 0.32 ‰ and 1.74 ‰ in ^{13}C and ^{15}N, respectively, compared to control 2 (p<0.05).

According to the bayesian mixing model, the main food source for the fish *A. regius* was pellet food, with a mean global contribution of 72.35 %, while the remaining 27.65 %

corresponded to fresh food. Although contributions varied annually pellet food always represented the main food source. Pellet food represented an important food source for *M. varia*, with a mean contribution of up to 62.68 % of their isotopic composition in the first three years of study, followed by fresh food, POM, fish faeces and phytoplankton (18.28 %, 11.26%, 5.18 % and 2.58 %, respectively). In 2011, the pattern changed and POM represented the main food source with a mean contribution of 50.74 %. As in *A. regius*, contributions varied both seasonally and annually (Figure 1), however, aquaculture derived products (pellet and fresh food and fish faeces) remained the main food sources along the three first years of study varying from 70.03 % in spring 2009 to the maximum of 93.81 % in autumn 2010. Similarly, pellet food represented the main food source for *M. galloprovincialis,* with a mean contribution of 57.95 %, followed by fresh food (21.46 %), fish faeces (8.90 %), POM (7.84 %) and phytoplankton (3.83 %) during the first three years (Figure 2). Again, in 2011, the trend changed as pellet food decreased to a global mean contribution of 27.78 %, and POM contribution increased to a global mean of 23.44 %. *M. galloprovincialis* also showed a seasonal and annual variation, but again aquaculture derived products remained the main food sources in the four years of study, with mean values varying from 97.31 % in spring 2008 to 53.98 % in spring 2011.
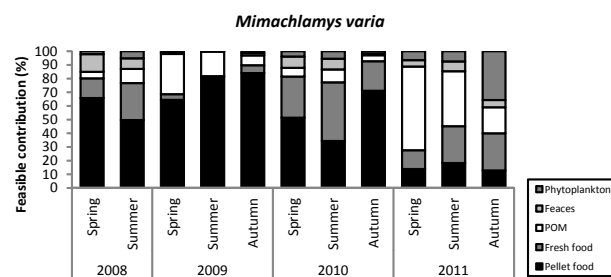
Figure 1. Seasonal and annual variability of the feasible contribution of the main organic matter sources to the diet of *Mimachlamys varia* cultured in fish cages based on Bayesian mixing models.
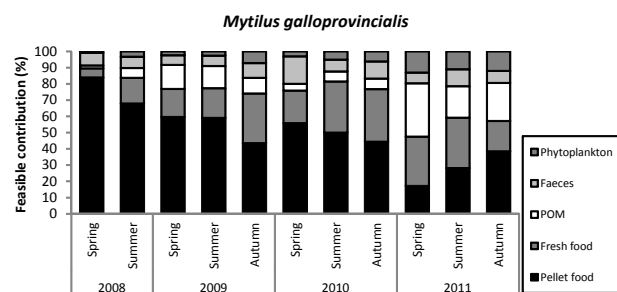
Figure 2. Seasonal and annual variability of the feasible contribution of the main organic matter sources to the diet of *Mytilus galloprovincialis* cultured in fish cages calculated by Bayesian mixing models.

Discussion

The analysis of the $\delta^{13}C$ and $\delta^{15}N$ values of the food web components at each treatment (cage and control sites) depicted different matter fluxes, especially in relation to the nitrogen isotope. ^{15}N is usually used as a viable indicator of the trophic position since it increases amongs trophic level; therefore, organisms placed higher in the trophic web are expected to present higher ^{15}N values. Accordingly, *A. regius* showed higher mean $\delta^{15}N$ values than the other food web components. Analysing bivalves from cage site we observed that their ^{15}N signature was relatively similar to those of phytoplankton, zooplankton, fresh food, fish faeces and benthic sedimentivorous, demonstrating that filter feeder worked efficiently by getting their nitrogen isotopic signature from the filtered substances. A similar pattern was observed in both control sites. Morever, the enrichment in ^{15}N exhibited by *H. forskalii* in cage site is derived by the incorporation of part of aquaculture wastes that sink into the seaflor as it has been observed by other authors, as Dolenec *et al*. (2007). Even though it has been demonstrated that filter feeder assimilate part of aquaculture wastes, there is still a fraction which accumulates in the sediment potentially affecting the sediment and benthic organisms. Higher $\delta^{13}C$ values for *M. galloprovincialis* and *M. varia* exhibited in the cage site compared to control sites are probably linked to the higher water residence time in the inner bay where fish farm is deployed, while control sites are at open areas.

The existing annual variability of the feasible contribution of pellet and fresh food to *A. regius'* diet is atttributed to the nutritional regime instead of a possible change in the intake nutrient strategy. Particularly important are the mixing model analysis in both bivalves, as it strongly demonstrates a contribution of aquaculture wastes in both bivalves' diet. The observed seasonal variations may be attributed to phytoplankton dynamics. Despite pellet food had a very important contribution in both bivalves' diet, we observed some different patterns. For both species, the contribution ssemed to be complementary, in that sense, when one specie increased the feasible contribution of pellet food, the other specie exhibited a decrease in that contribution and vice versa. This could be interpreted as a compotetive strategy between both species, possibly due to the limitation of water income and nutriets for being placed in the same bags.

The study is based on an approach of integrated multitrophic aquaculture to minimize the impact of aquaculture activities to the marine food web and benthic communities through stable isotope signatures of cultured fin fish, filter feeder bivalves, aquaculture derived products, particulate and sedimentary organic matter at the proximity of cages. The results clearly demonstrates that both bivalves are assimilating aquaculture derived wastes efficiently, especially *M. galloprovincialis*, supporting the idea that the co culture of species with different trophic strategies (fish and molluscs) guarantee a good environmental status by improving water quality in fish farms. Additionally, the incorporation of filter feeders in aquaculture installations work in line with the European water framework directive 2000/60/CE which objectives are to guarantee and maintain a good quantitative and qualitative state of water bodies, including marine waters up to one nautical mile from shore.

Acknowledgements

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