



Mollusc-shell debris derived from aquaculture can promote macrofaunal communities with a high bioturbation capacity

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ABSTRACT

Marine aquaculture releases mollusc shell debris which can accumulate on the seabed. Due to its low degradability, mollusc shell debris becomes integrated into the sediment, modifying both its structure and associated biogeochemical processes. These changes are expected to influence the biological assemblages within the sediment, which play a key role in the biogeochemical processes due to bioturbation. We performed an *in-situ* experiment to test the effects of mollusc shell-hash on the bioturbation capacity of the macrofaunal assemblages within the sediments affected by marine aquaculture. The aquaculture facilities chosen were a fish farm in an oligotrophic area of the Mediterranean over a sandy sediment, and a mussel farm in the Atlantic located in an estuary over silty sediment. Mollusc shell-hash promoted macrofaunal assemblages with a high bioturbation potential, regardless of the influence of aquaculture. Nevertheless, in the sediment from the mussel-farm, the shell-hash did not have a marked effect on the macrofaunal assemblages. The effect of shell-hash on promoting macrofaunal communities with a high bioturbation potential capacity is therefore dependent on the existing environmental conditions in the area, including the natural organic matter input and grain size of the sediment. Shell-hash could be used a strategy to improve the ecosystem function of sediments as a circular economy-oriented management practice.

1. Introduction

Aquaculture is a widespread activity in coastal marine areas (FAO, 2020) with associated environmental drawbacks (Edgar et al., 2010; Mckindsey et al., 2011; Riera et al., 2013). The export of waste is one of the main impacts of marine aquaculture. Generally, studies have focused on the organic waste derived from uneaten pellets, faeces, and pseudofaeces from the species being farmed (Crawford et al., 2003; Hargrave et al., 2008; Sanz-Lazaro and Marín, 2008), but waste is also generated by the biofouling linked to aquaculture facility structures (Fitridge et al., 2012; Sanchez-Jerez et al., 2019a, 2019b). The potential impacts of the aquaculture industry on the benthos depend on the characteristics of the seabed, including grain size (Martinez-Garcia et al., 2015), habitat type (Sanz-Lazaro and Marín, 2008), oceanographic characteristics in the area, and the natural organic matter (OM) load (Kanaya, 2014; Papa-georgiou et al., 2010; Sweetman et al., 2014).

Biofouling grows in the structures of fish farm facilities, and, naturally or due to its mechanical removal as maintenance activities to prevent damage, such as the excessive increase of weight and limitation of water exchange (Bannister et al., 2019) these debris settle in the sediment. While the labile organic part of the biofouling is rapidly mineralised in the sediment, the shell of bivalves can accumulate (Fitridge et al., 2012; Sanchez-Jerez et al., 2019a, 2019b) due to their low degradability (Jenner et al., 1998; Rajagopal et al., 2003). In the case of mussel aquaculture, mussel shell accumulation becomes largely important, due to the input of mussel shell debris of the cultured species (Grenz, 1989; Matisson and Lindén, 1983). Mussel shell debris (referred to as 'shell-hash' Wilding, 2012) can have a long-term effect on sediments, modifying their structure and indirectly ameliorating the effects of organic enrichment (Casado-Coy et al., 2017). Additionally, mussel shell-hash can increase the habitat complexity of sediments, generating new niches for macrofaunal species (Gutierrez et al., 2003), and,

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therefore, directly affect the seabed surrounding the principal coastal aquaculture systems. However, to date, the effects of shell-hash on macrofaunal assemblages have been little studied (Crawford et al., 2003; Hartstein and Rowden, 2004; Weise et al., 2009).

Benthic macrofauna play a key role in the recycling of OM in marine sediments through bioturbation (Braeckman et al., 2010; Kristensen and Kostka, 2013; Meysman et al., 2006). Bioturbation, in the widest sense, is defined as the displacement of particles and solutes by organisms that results in modifications to the sediment stratigraphy and an increased supply of electron-acceptors for bacteria (Kristensen et al., 2012). Bioturbation, therefore, promotes water exchange between the water column and the sediment (Casado-Coy et al., 2020), and enhances the metabolic capacity of the sediment (Aller and Aller, 1998; Sanz-Lazaro and Marin, 2011; Volkenborn et al., 2010). This process boosts the functioning of the marine ecosystem, prevents organic matter accumulation, and favours a good ecological status (Pennafirme et al., 2019).

Although there has been a large research effort into the biological succession resulting from the organic enrichment linked to aquaculture (Hargrave et al., 2008; Pearson and Rosenberg, 1978), there has been little work on the effect mussel shell-hash has on the biological succession. Moreover, aquaculture-associated modifications of the bioturbation capacity of the macrofaunal assemblage within sediments are not sufficiently understood (Adámek and Marsálek, 2013; Nickell et al., 2003; Papageorgiou et al., 2009). Due to the limitations on studying the bioturbation capacity of entire macrofaunal assemblages, indices have been developed to approximate this parameter. These use the life traits of individual species, or other taxonomic levels, along with abundance and biomass data (Renz et al., 2018; Solan et al., 2004). The Bioturbation Potential Capacity (BPC) Index for the macrofaunal community (Queirós et al., 2013; Solan et al., 2004) is among the most widely used index (Foshtomi et al., 2015; Gogina et al., 2020; Gogina et al., 2018; Zhang et al., 2019).

The aim of this work is to study the effect mollusc-shell debris has on the benthic macrofauna community, focusing on the bioturbation capacity in sediment influenced by marine aquaculture. We carried out *in situ* experiments on the sediments of two typical marine aquaculture systems, fish and mussel farming (APROMAR, 2020; FAO, 2020), in areas with contrasting environmental conditions and sediment types (eutrophic-silty and oligotrophic-sandy). We examined the changes in physicochemical variables, macrofaunal assemblages, and their associated bioturbation capacity due to the influence of aquaculture and shell-hash accumulation.

2. Material & methods

2.1. Study area

The study was carried out during summer 2016, at a fish farm in the Mediterranean Sea and a mussel farm in the Atlantic Ocean, both typical types of aquaculture, with contrasting sediments and oceanographic characteristics (Fig. S1). At the fish-farm, the seabed was unconsolidated sandy sediment with a naturally low OM content, typical of carbonate sediments. At the mussel-farm, the seabed was silty with a high OM content and the typical terrigenous influence from *rías*, narrow inlets formed by the partial submergence of a river valley (Evans and Prego, 2003). The fish farm (El Campello, Alicante, SE Spain: 38°25'34.7"N 0°22'29.3"W) raised gilthead seabream (*Sparus aurata* Linnaeus, 1758), with a production of around 600 t per year, which were fed daily with pellets. The fish farm comprised 12 round cages and was located offshore (2.8 km from the coast and at 34 m water depth) in oligotrophic conditions (Fig. S1). The mussel farm (Ría de Vigo, NW Spain 42°16'35.04"N 008°43'23.477"W) cultured *Mytilus galloprovincialis* Linnaeus, 1758, and was located 1 km from the coast, at 12 m water depth (Fig. S1). The system employed at the mussel farm involved a floating raft (a *batea*), with floating platform farms configured into a rectangular shape occupying an area of ca. 100 m² (Camacho et al.,

1991).

2.2. Experimental set-up

To test what effect shell-hash has on sediment influenced by aquaculture, a manipulative experiment was performed at each farm. The experimental units used were plastic containers (24 × 15 × 6 cm with an upper lid) filled with the corresponding sediment from each farm (Fig. S2). The sediment used at the fish-farm was sandy (79.56% sand, 16.13% clay, and 4.3% silt) with a low OM content (0.83%). The sediment was collected from Gola beach in Santa Pola, SE Spain (38° 11,018' 81" N 0° 35,034' 55" W). This sediment was comparable to the sediment below the fish-farm where the experimental units were placed (Krüger et al., 2020). It was taken from the first 10 cm of the surface using a shovel. The sediment used at the mussel-farm was silty (11.94% sand, 11.21% clay, and 76.83% silt) with a high OM content (3.64%). It was collected from Ría de Vigo, NW Spain (42°16.544'N 008°43.494'W), using a Van-Veen grab with a surface area of 0.04 m² and a maximum penetration of 10 cm. This sediment was comparable to the sediment below the mussel-farm where the experimental units were placed (Moreira et al., 2006). On each location, 30 l of sediment was sieved through a 0.5 mm mesh to remove macrofauna.

At each farm, 48 experimental units with defaunated sediment were emplaced. To simulate the influence of aquaculture (IA), 24 experimental units were positioned on the sediment below the aquaculture system and 24 experimental units were placed on the sediment in a control area, at least 200 m from the aquaculture facility, far enough away so that there was a negligible input of OM and shell-hash from the aquaculture facility (Sanz-Lazaro et al., 2011a; Wilding, 2011). The experimental units located under each aquaculture system were equally enriched to simulate a comparable organic enrichment of the seabed below the two kinds of aquaculture systems, as OM enrichment is the main input to sediments from aquaculture systems (Armstrong et al., 2020; Holmer and Barry, 2005; Yang et al., 2018). Organic enrichment was performed by adding 92 g of labile OM per kg of sediment in the form of finely ground fish feed [L-4 Alternate CMX 20 2P BB2, SKRETTING (46.5% protein, 20% fats and oils, 6.1% minerals, 2.2% fibre, 1% phosphorus, 0.9% calcium and 0.4% sodium)]. This level of OM input corresponds to 26 mol POC·m², which is similar to the annual organic enrichment of the seabed under the influence of fish and mussel farms (Callier et al., 2006; Morrisey et al., 2000; Sanz-Lazaro et al., 2011b). To test the effect of shell-hash (SH) on the sediment, we added 1910 g of mussel shell-hash per m² in half of the experimental units. This corresponds to a realistic input under both fish farms (Sanchez-Jerez et al., 2019a, 2019b) and mussel farms (Wilding and Nickell, 2013). As the experiment lasted around one month, the biodeposition of shell-hash from the aquaculture facilities and the dissolution of the shell-hash was expected to be minimal (Tomasových et al., 2014; Wilding, 2011). Shell-hash was obtained from empty mussel valves which were broken to generate fragments between 1.5 and 4 cm (Fig. S2). The experimental units were independent and deployed randomly across the seabed by a scuba diver, so that the macrofaunal colonisation would be similar in all the units. Three random sites were selected for each treatment on each farm, with four replicates of the experimental units being deployed at each of these sites (Fig. 1). The experimental units were buried to the level of the seabed surface to prevent the possible border effects from the plastic containers, which could have hampered macrofaunal colonisation of the experimental units.

After 35 days, the experimental units were closed and retrieved by divers. A small portion of the surface sediment (100 cm²) of each experimental unit (160 cm³) was extracted using a syringe to determine the redox potential, the %OM content of the sediment, the acid volatile sulphates (AVS-S) and the NH₄⁺ and PO₄³⁻ concentrations in the pore-water. The sediment subsamples for the different analyses were taken at the moment that the experimental units were opened in the boat, and these were stored in a fridge at 2 °C until they reached the laboratory.

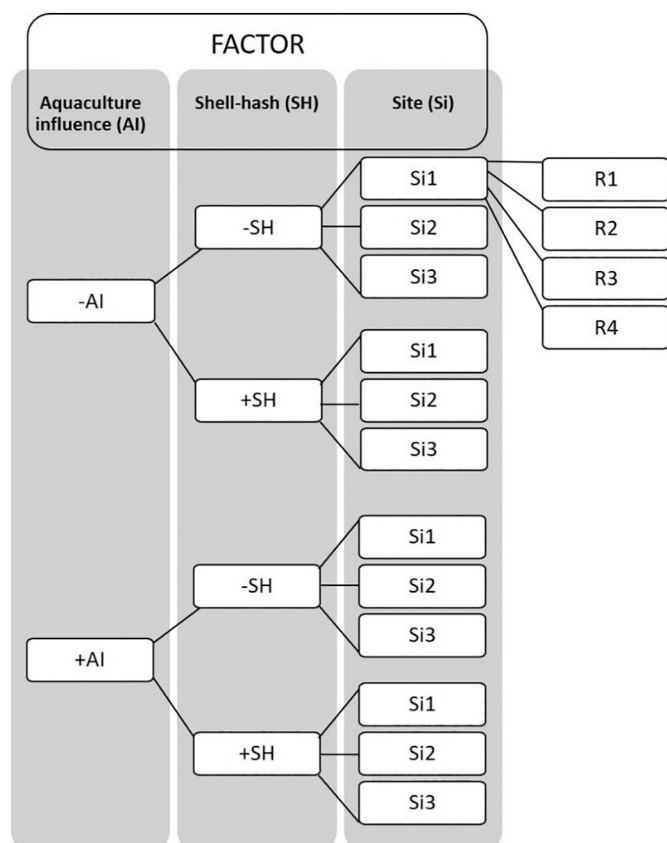


Fig. 1. Experimental design of each farm. Factors were aquaculture influence (-AI and + AI), shell-hash (-SH and + SH) and sites ($n = 4$).

The redox potential was measured in the first 3 cm of the sediment using an electrochemical sensor (Hamilton Liq-Glass ORP) when the experimental units were opened in the boat. In the laboratory, the %OM content was measured by the loss on ignition method at 450 °C over 4 h for 8 g of sediment. AVS-S accumulation was quantified with the distillation method proposed by Allen et al. (1993) using 5 ml of wet sediment. To measure the NH_4^+ and PO_4^{3-} concentrations in the sediment porewater, the porewater was extracted from 20 ml of wet sediment using a vacuum pump. The porewater was filtered (syringe filter – 13 mm Ø 0.22 µm) and transferred to 15 ml plastic vials and frozen (–20 °C) until analysis. The NH_4^+ and the PO_4^{3-} concentrations were measured using an Automated Wet Chemistry Analyser– Continuous Flow Analyser (Skalar Analytical B.V., Breda, the Netherlands). The remainder of the sediment, ca. 2.000 cm³ from each experimental unit, was the basis of the macrofauna study. This sediment was sieved through a 0.5 mm mesh and the retained material was preserved in a 70% ethanol seawater solution. In the laboratory, individual macrofauna were separated into four groups: annelids, arthropods, molluscs, and a fourth group containing other taxa, and stored in a 70% alcohol solution for later identification. The benthic groups were classified to the lowest practical taxonomic level. The macrofaunal dry weight (constant mass at 60 °C) was measured separately for each taxon of each experimental unit to determine the macrofaunal biomass. The macrofaunal abundance was reported as individuals·m⁻² and the macrofaunal biomass data was reported as g·m⁻². The community bioturbation potential (BPC) per m² was calculated by combining the macrofaunal abundance and biomass data of each taxon from each experimental unit with information about the life traits of the individual species or taxonomic groups, specifically their mobility and reworking capacity (Queirós et al., 2013; Solan et al., 2004). If the taxon was not on the list published in Queirós et al., 2013, the mobility and reworking potential

was assigned according to the phylogenetically closest taxon. Moreover, the Shannon-Wiener index (H') was calculated to determine the diversity of the macrofaunal assemblages from the experimental units (Shannon and Wiener, 1963).

2.3. Data analysis

The data set from each farm was analysed separately because the environmental conditions (such as sediment type and oceanographic conditions) were not comparable. The experimental design (Fig. 1) involved a three way-factorial where the factors were: 1) aquaculture influence [fixed and orthogonal; two levels: without and with AI (-AI and + AI, respectively)]; 2) shell-hash [fixed and orthogonal; two levels: without and with shell hash (-SH and +SH, respectively)]; 3) site, random and nested in aquaculture influence and shell-hash interaction with three levels. Four replicates were used for each combination of treatments.

Non-parametric multivariate analyses of variance (PERMANOVA) and principal component ordination (PCO) were used to assess changes in the structure of the macrofaunal assemblages in terms of abundance and biomass. Analyses were made based on the Bray–Curtis dissimilarity index (Bray and Curtis, 1957) selecting variables which contributed at least 3% of the total. A PERMDISP analysis was run to test for heterogeneity of variances. If the data did not meet the abovementioned assumption, the level of significance of PERMANOVA was lowered to 0.01. A univariate analysis of variance (ANOVA) was performed for the response variables: the %OM content, redox potential, AVS-S accumulation, NH_4^+ and PO_4^{3-} porewater concentrations; and its biological descriptors: H' diversity and BPC index. Prior to the ANOVA, the homogeneity of variance and normality of the data was checked using Cochran's C test and p-p plots, and the data was transformed whenever necessary (Underwood, 1997). If, after being transformed, the data did not meet the abovementioned assumptions, the significance level of the ANOVA was lowered to 0.01. When differences were found between any combination of factors, the Student-Newman-Keuls *post-hoc* test for multiple comparisons was applied. The physicochemical variables of the sediments were compared using the macrofaunal assemblage matrices of abundance and biomass, utilising BIOENV to identify the physicochemical variables of the sediments that most contributed to the variation between the macrofaunal assemblages. Similarly, matrices of the physicochemical sediment parameters were calculated using normalised Euclidean distances and the correlations were calculated using the Spearman rank correlation coefficient (Pw) (Clarke and Ainsworth, 1993). When the ANOVA detected a significant difference in the BPC index of the different treatments, SIMPER analyses were used to identify which taxon contributed most to those differences (Clarke, 1993). The multivariate analyses were conducted using PRIMER V.6 + PERMANOVA (Plymouth Marine Laboratory, UK) and the ANOVA analyses were performed in the R software environment (version 3.4.4) with the GAD package (Sandrini-Neto and Camargo, 2014). All the statistical tests were conducted with a significance level of $\alpha = 0.05$. The data was reported as the mean \pm the standard error (SE), while the effect size was reported with a 95% confidence interval (CI) (Nakagawa and Cuthill, 2007). Any differences that were not significant were not included in the results section.

3. Results

3.1. Physicochemical parameters

The OM content of the experimental sediment units from the fish farm increased by $0.60 \pm 0.22\%$ under the influence of aquaculture and by $0.16 \pm 0.34\%$ with shell-hash. The redox potential of the experimental sediment units decreased by 234.1 ± 23.7 mV under the influence of aquaculture and by 28.8 ± 79.2 mV with shell-hash. The AVS-S accumulation in the experimental units from the fish farm only changed

under the influence of aquaculture, where it increased by 151.7 ± 108.1 $\text{mg}\cdot\text{kg}^{-1}$ compared to the samples that were not influenced by aquaculture. The NH_4^+ porewater concentration in the experimental units increased by $522.8 \mu\text{M}$ under the influence of aquaculture, while shell-hash increased it by $47.0 \pm 31.5 \mu\text{M}$ where there was no aquaculture influence and by $229.6 \pm 124.0 \mu\text{M}$ where there was, compared to the samples with no shell-hash. The PO_4^{3-} porewater concentration of the experimental unit sediments only changed under the influence of aquaculture, where it increased by $67.9 \pm 15.7 \mu\text{M}$ (Fig. S3; Table 1 & S1).

The OM content of the sediment with shell-hash from the mussel farm increased by $1.00 \pm 0.22\%$ where there was no aquaculture influence. Where there was an influence, the OM content of the sediment with shell-hash was $0.16 \pm 0.23\%$ lower compared to sediment with no shell-hash. The redox potential of the sediment under the influence of mussel farming decreased by 21.5 ± 10.3 mV compared to the sediment not affected by the aquaculture. With regard to the AVS-S accumulation in the sediment from the mussel-farm, the influence of aquaculture and shell-hash had significant and opposite effects. In the experimental units that were not influenced by the aquaculture and which had shell-hash, the AVS-S accumulation in the sediment increased by 171.0 ± 95.5 $\text{mg}\cdot\text{kg}^{-1}$ compared to the sediment with no shell-hash; while in the experimental units that were influenced by aquaculture, the shell-hash did not exert a significant effect on the AVS-S accumulation. Shell-hash decreased the NH_4^+ porewater concentration of the experimental units at the mussel farm by $3.06 \pm 1.63 \mu\text{M}$ where there was no influence from the aquaculture, and by $0.60 \pm 0.48 \mu\text{M}$ under the influence of this. The PO_4^{3-} porewater concentration of the experimental sediment units from the mussel farm that were influenced by aquaculture decreased by $2.18 \pm 1.62 \mu\text{M}$ compared to the sediment not affected by aquaculture; while the shell-hash did not have a significant effect on the PO_4^{3-} porewater concentration of the experimental units (Fig. S3; Table 1 & S1).

3.2. Macrofauna community

3.2.1. Fish farm sediment

A total of 5239 individuals belonging to 91 different taxa were identified; these were mainly polychaetes (35 taxa), crustaceans (33 taxa) and molluscs (23 taxa). In the sediment of the experimental units in the fish-farm area, 1825 individuals of macrofauna were identified, with the most abundant taxon being the family Nassariidae (Table S2). The macrofaunal biomass of the experimental sediment unit in the fish-farm area ranged between 0.07 and $2.40 \text{ g}\cdot\text{m}^{-2}$, with the family Nassariidae also being the taxon with the greatest biomass (Table S2). The PERMANOVA analysis indicated significant differences in the structure of the macrofaunal assemblages under the influence of aquaculture, for abundance and biomass (Table S3), which was reflected, to some extent, in the ordering of the samples in the PCO analysis of macrofaunal abundance and biomass (Fig. S4). Shell-hash did not show any effect in the PERMANOVA analysis of macrofaunal abundance and biomass in

the sediment from the fish farm (Table S3). The physicochemical variables of the experimental sediment units that primarily correlated to macrofaunal abundance and biomass was the redox potential (0.566 for the abundance data and 0.548 for biomass data) (Table S4).

The H' of the macrofaunal assemblages from the experimental units from the fish farm ranged between 2.32 and 4.10. Shell-hash increased the H' by 0.51 ± 0.31 where there was no aquaculture influence; while it showed no effect on the H' under the influence of aquaculture (Fig. 2; Table 2). The BPC of the macrofaunal assemblages from the experimental units from the fish farm ranged between 25.7 and 421.0 per m^2 . Under the influence of aquaculture, the BPC of the macrofaunal assemblages increased by $128 \pm 51 \text{ per m}^2$ compared to those where there was no aquaculture influence (Fig. 2; Table 2). The taxa which contributed predominantly to this increase in BPC were the families Nassariidae (30%) and Nereididae (20%) (Table 3 and S2). Shell-hash increased the BPC of the macrofaunal assemblages by $77 \pm 61 \text{ per m}^2$ without any influence from aquaculture (Fig. 2; Table 2); the main taxa that contributed to this increase was the family Nassariidae (23%) (Table 3). Where there was no aquaculture influence, shell-hash increased the BPC of the macrofaunal assemblages in the sediment by $95 \pm 65 \text{ per m}^2$ (Fig. 2; Table 2); the main taxa that contributed to this increase were the families Gammaridae (12%) and Galatheididae (12%) (Table 3). However, under the influence of aquaculture, shell-hash had no significant effect size on the BPC (Fig. 2; Table 2).

3.2.2. Mussel farm sediment

3414 individuals of macrofauna were identified in the experimental units from the mussel farm, the most abundant taxon being the family Capitellidae ($5916 \text{ individual}\cdot\text{m}^{-2}$), and the taxon with the greatest biomass was the family Buccinidae (Table S2). The PERMANOVA analysis of the macrofaunal abundance and biomass at the mussel farm showed significant differences in the structure of the macrofaunal assemblages linked to the influence of aquaculture (Table S3), despite there not being a clear ordering of the samples in the PCO according to this influence (Fig. S4). Shell-hash did not show any effect in the PERMANOVA analysis of the macrofaunal abundance and biomass at the mussel farm (Table S3). The physicochemical sediment variables that mainly correlated to macrofaunal abundance were AVS-S and OM content (0.119), and redox potential and OM content (0.340) correlated to the macrofaunal biomass (Table S4).

The H' of the macrofaunal assemblages from the experimental units from the mussel farm ranged between 1.30 and 3.69. However, aquaculture influence and shell-hash had no significant effect on the H' (Fig. 2). The BPC of the macrofaunal assemblages ranged between 9.3 and 782.2 per m^2 , where under the influence of aquaculture the BPC decreased by $95 \pm 76 \text{ per m}^2$. The main taxa that contributed to this decrease in BPC were the families Capitellidae (21%) and Cirratulidae (12%) (Table 3 and S2). Shell-hash, however, had no effect on the BPC of the macrofaunal assemblages (Fig. 2; Table 2).

Table 1

Summary of physicochemical parameters of sediment from the fish-farm and sediment from the mussel-farm, analysed at the end of the experiment. Factors were aquaculture influence (-AI and + AI) and shell-hash (-SH and + SH), the levels of the factor site were pooled ($n = 12$, mean \pm SE).

	OM content (%)	Redox (mV)	AVS-S ($\text{mg}\cdot\text{kg}^{-1}$)	NH_4^+ porewater (μM)	PO_4^{3-} porewater (μM)
Fish farm					
-AI -SH	0.83 ± 0.03	-14.67 ± 8.55	14.43 ± 2.08	81.94 ± 15.03	9.71 ± 0.77
-AI +SH	1.03 ± 0.05	-61.37 ± 12.45	16.40 ± 5.43	128.90 ± 4.37	12.10 ± 0.99
+AI -SH	1.46 ± 0.08	-262.67 ± 12.41	156.14 ± 11.27	513.38 ± 27.20	78.00 ± 12.01
+AI +SH	1.61 ± 0.10	-278.00 ± 8.62	178.14 ± 89.79	743.02 ± 49.35	79.60 ± 8.12
Mussel farm					
-AI -SH	3.64 ± 0.06	-404.41 ± 5.95	165.21 ± 20.60	90.48 ± 0.38	37.46 ± 0.66
-AI +SH	4.64 ± 0.08	-404.92 ± 5.02	336.20 ± 38.17	87.42 ± 0.63	38.15 ± 0.25
+AI -SH	4.08 ± 0.06	-426.33 ± 2.99	515.49 ± 50.62	90.36 ± 0.18	36.54 ± 0.60
+AI +SH	3.91 ± 0.09	-426.08 ± 4.78	401.54 ± 61.10	89.76 ± 0.12	34.69 ± 1.11

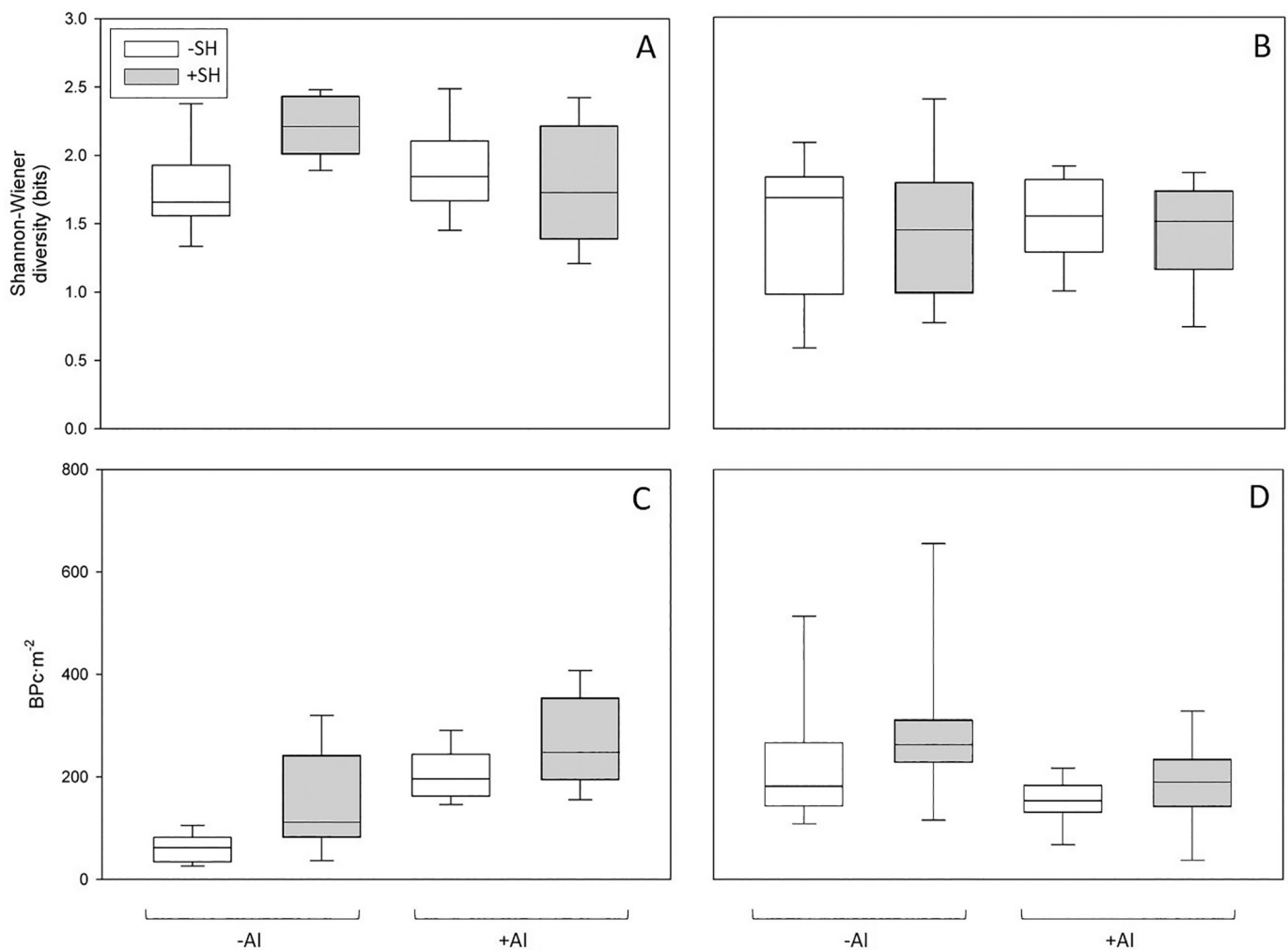


Fig. 2. Shannon-Wiener diversity (H') of macrofauna community of sediment from the fish farm (A) and of sediment from the mussel farm (B); Bioturbation potential capacity (BPC) of macrofauna community of sediment from the fish farm (C) and of sediment from the mussel farm (D) per m², where the biomass was calculated with the dry weigh. Factors were aquaculture influence (-AI and +AI), and shell-hash (-SH and +SH). Line is the median, and the box have 10th, 25th, 75th and 90th percentiles as vertical boxes with error bars. Factor site was pooled (n = 12).

Table 2

Results of the ANOVAS of Shannon-Wiener Diversity (H') and bioturbation potential capacity (BPC) of macrofaunal assemblages of sediment from the fish-farm and from the mussel-farm, where the biomass was calculated with the dry weigh. Factors were aquaculture influence (-AI and +AI), shell-hash (-SH and +SH) and site (SITE) (n = 4). Df: degrees of freedom; MS: mean square; F: F-distribution.

	Df	H' diversity (bits)						BPC					
		Fish farm			Mussel farm			Fish farm			Mussel farm		
		MS	F	P	MS	F	P	MS	F	P	MS	F	P
AI	1	0.238	1.920	> 0.1	0.000	0.000	> 0.9	196,669	34.175	<0.001	108,441	6.869	< 0.01
SH	1	0.274	2.205	> 0.1	0.023	0.212	> 0.6	72,576	12.611	< 0.01	30,645	1.941	>0.1
AIxSH	1	1.029	8.291	< 0.01	0.029	0.262	> 0.6	3562	0.619	> 0.4	3490	0.221	> 0.6
Site (AIxSH)	8	0.062	0.496	> 0.8	0.590	5.364	<0.001	2393	0.416	> 0.9	6398	0.405	> 0.9
Residuals	36	0.124			0.110			5755			15,787		
Cochran's C test		C = 2215			C = 0.1918			C = 0.2518			C = 0.4769		
		p > 0.05			p > 0.05			p > 0.05			p < 0.05		
Transformation		none			none			none			none		

Significant effects (p < 0.05) are indicated in bold.

4. Discussion

This study shows that shell-hash affects sediments differently, depending on the oceanographic conditions, OM content, and grain size. In the sediment from the fish farm, disregarding any influence from the aquaculture, the shell-hash changed the macrofaunal assemblages,

which appeared in the sediment of the experimental units, promoting macrofaunal assemblages with a high bioturbation capacity, such as the families Nassariidae and Nereididae. However, the shell-hash had no significant effect on any of the macrofaunal parameters of the sediments from the mussel farm.

Table 3

Results of calculation of similarity percentages (SIMPER) on BPC (bioturbation potential capacity) of macrofaunal assemblages of sediment from each farm, where the biomass was calculated with the dry weigh. According to the differences obtained in the ANOVA analyses (Table 2), SIMPER analyses are made to each factor: aquaculture influence (-AI and +AI), shell-hash (-SH and +SH). Av.Abund (average of abundance), Av. Diss (average of dissimilarity), Diss/SD (average of dissimilarity per their standard deviation), Contrib% (percentage of contribution on the dissimilarity), Cum.% (percentage of contribution on the dissimilarity accumulated).

BPC of Fish farm sediment						
Taxa	Av. Abund	Av. Abund	Av. Diss	Diss/SD	Contrib %	Cum. %
	-AI	+AI				
Nassariidae	4.70	95.57	25.16	1.78	29.88	29.88
Nereididae	4.72	56.96	16.64	1.49	19.76	49.64
Gammaridae	18.84	15.86	4.86	1.24	5.78	55.42
Calappidae	1.42	9.01	3.76	0.66	4.46	59.88
Euphasiidae	15.28	4.43	3.64	0.68	4.32	64.20
Galatheidae	13.58	0.00	3.01	0.64	3.58	67.77
Average dissimilarity = 84.19						
	-SH	+SH				
Nassariidae	35.97	61.45	17.51	1.12	23.45	23.45
Nereididae	32.52	26.62	10.53	1.12	14.11	37.56
Gammaridae	14.69	20.39	5.51	0.98	7.38	44.94
Euphasiidae	4.93	15.71	5.11	0.72	6.84	51.78
Galatheidae	2.05	12.58	4.42	0.62	5.93	57.70
Calappidae	7.28	2.61	2.61	0.59	3.50	61.20
Average dissimilarity = 74.65						

BPC of Mussel farm sediment						
Taxa	Av. Abund	Av. Abund	Av. Diss	Diss/SD	Contrib %	Cum. %
	-AI	+AI				
Capitellidae	86.25	45.36	13.69	0.98	21.12	21.12
Cirratulidae	2.66	36.25	8.05	1.19	12.41	33.53
Portunidae	35.91	1.32	7.94	0.84	12.25	45.78
Gammaridae	34.93	27.23	6.64	1.15	10.25	56.03
Buccinidae	29.4	0	3.89	0.33	6	62.02
Nereididae	11.02	7.48	3.14	1.01	4.84	66.87
Average dissimilarity = 64.82						

4.1. Physicochemical sediment parameters

Physicochemical sediment parameters are used as indicators of biological response to organic enrichment from aquaculture (Armstrong et al., 2020; Cranford et al., 2020; Hamoutene, 2014; Hargrave et al., 2008). The different changes produced in the physicochemical parameters of the experimental sediment units from each farm, resulting from the influence of the aquaculture, could be explained by the remarkably different characteristics of the sediments, including their grain size and OM content. The oligotrophic conditions of the Mediterranean Sea, where the fish farm was located, explain why the sediment had a low OM content. The moderate values of the redox potential and the low levels of AVS in the sediment not affected by aquaculture indicated that the conditions in this sandy sediment were mainly oxic. Under the influence of aquaculture, the OM content and AVS accumulation in the sediment increased, and the redox potential values lowered, indicating that the environment was becoming more reduced (Hargrave et al., 2008). In reduced environments, OM input is mineralised through anaerobic metabolic pathways, which are less efficient than aerobic ones (Holmer and Barry, 2005), and the metabolic capacity of the sediment diminishes. As a consequence, the OM content in the sediment could increase. Nevertheless, these values indicate less-reduced conditions compared to the experimental sediment units from the mussel farm that had a lower redox potential and greater accumulation of sulphides, without considering the influence of the aquaculture system (Hargrave et al., 2008).

The mussel farm is located in an Atlantic estuary, a *ría* that has important OM input due to human activities (Nixon, 1995; Prego, 2002). For this reason, the natural input of OM to the seabed is expected to be notably high compared to oligotrophic areas. This fact, together with the small grain size of this sediment (silty), favour the consumption of oxygen due to the slow diffusion between sediment porewater and the water column. Moreover, it has been demonstrated that a high OM content of sediments produces bacteria bioclogging between the sediment particles, which reduces the acceptor-electron supply in sediments (Hand et al., 2008). As a consequence, the reduced conditions predominate, slowing down the degradation of the OM and promoting their prevalence in sediments (Holmer and Barry, 2005). The response of sediments to aquaculture is therefore dependent on the environmental characteristics of sediments (Martinez-Garcia et al., 2015; Papageorgiou et al., 2010).

Under laboratory conditions, shell-hash can reduce ammonium and sulphide production in sandy sediments with organic enrichment (Casado-Coy et al., 2017). This effect could not be observed in the present experiment as only one sample was taken at one point in time. However, the decreasing trends in OM content, AVS-S, and ammonium concentration when shell-hash was present, especially in the experimental units below the mussel farm, suggest similar positive effects as those observed under laboratory conditions. For this reason, shell-hash may have the capacity to promote physicochemical changes in sediments (Commito and Rusignuolo, 2000; Huettel and Gust, 1992), affecting sediment metabolism (Welsh and Castadelli, 2004).

4.2. Macrofaunal assemblages

The different physicochemical changes observed due to the influence of aquaculture and the addition of shell-hash addition at both farms studied, also produced different effects in terms of the macrofaunal assemblages. The physicochemical variables that principally correlated to the macrofaunal assemblages were different at each farm. The redox potential correlated to the macrofaunal abundance and biomass of the experimental sediment units from the fish farm, while at the mussel farm, these correlated to the AVS-S accumulation, OM content, and redox potential. These results are in line with models that relate physicochemical sediment characteristics with macrofaunal assemblage indices (Hargrave et al., 2008; Nilsson and Rosenberg, 2000). The influence of aquaculture on the sediment from the fish farm produced an increase in macrofaunal abundance, biomass, and BPC, which could indicate a transitory low-pollution condition on the gradient of the organic enrichment (*sensu* Pearson and Rosenberg, 1978), as previously reported (Apostolaki et al., 2007; Papageorgiou et al., 2010). This response could be explained by the fact that this sediment may favour advective oxygen supply due to the grain size and larger porewater space than that of muddy sediments (Janssen et al., 2005; Seibold and Berger, 2017). Thus, in the sediment from the fish farm, to some extent, the OM input resulting from the aquaculture is mineralised through aerobic metabolism. Therefore, oxic conditions prevail and there are no negative effects of the anaerobic metabolism on the macrofaunal assemblages present.

Contrastingly, the influence of aquaculture resulted in a reduction in macrofaunal abundance, biomass, and BPC in the sediments from the mussel farm. This could be due to the consequences of aquaculture on the physicochemical parameters of this sediment, including oxygen depletion. Although anoxic conditions prevailed where aquaculture had no influence, it seems that anaerobic metabolism was enhanced in the presence of aquaculture, which favours the accumulation of toxic by-products in sediments, such as sulphides (Holmer and Barry, 2005; Valdemarsen et al., 2009). These conditions induce an impoverishment of macrofauna assemblages (Pearson and Rosenberg, 1978). Although the macrofaunal diversity is generally lower under the influence of aquaculture, in the present study no effect was observed, there being relatively low values in all the treatments. This finding could be

explained by the incipient nature of the macrofaunal assemblages due to the short duration of the experiment (35 days), meaning the levels of diversity were typical of communities colonising in an early successional stage (Sanz-Lazaro and Marin, 2006). At this point in the succession, diversity levels are not expected to be notably affected by the influence of aquaculture (Martinez-Garcia et al., 2019).

Our results suggests that it should be mandatory to establish a threshold for the organic enrichment resulting from aquaculture, in order to maintain the bioturbation capacity of the macrofaunal assemblages (Sanz-Lazaro et al., 2011a) considering their primary influence on sediment structure, processes, and biogeochemistry (Braeckman et al., 2014; Casado-Coy et al., 2020; Kristensen and Kostka, 2013; Meysman et al., 2006). Moreover, this threshold must be flexible and adapt to specific sediment characteristics and oceanographic conditions due to the fact that aquaculture has a differential effect depending of the grain size and OM content of the sediment (Martinez-Garcia et al., 2015; Sanz-Lazaro et al., 2011a).

Mollusc shell-hash is an important input to sediments influenced by marine aquaculture (Ticina et al., 2020), as this tends to accumulate on the seabed (Sanchez-Jerez et al., 2019a, 2019b; Wilding, 2011). In the present study, shell-hash increased the macrofaunal abundance, diversity, and estimated bioturbation capacity of the sediments from the fish farm under both natural conditions and under the influence of aquaculture. Nassariidae, with moderate mobility and reworking potential, are the main contributors to this increase in BPC in sediments from the fish farm, where they almost doubled their abundance compared to sediment with no shell-hash. Species of this taxon are relatively small (usually <5 cm) and primarily carnivorous scavengers that inhabit predominantly estuarine or shallow marine soft substrates (Beesley, 1998; Iannotta et al., 2009). The increase of this family could be due to the general increase in macrofaunal abundance, which provides food for this taxon. Nereididae, with a high level of mobility and reworking potential is the second most important taxon contributing to the increase in BPC. However, in this case the abundance decreased with respect to sediment with no shell-hash. Species of this taxon play a key role in the structure and functioning of sediments and are considered to be ecosystem regenerators (Kristensen et al., 2012). Regenerators dig and continuously maintain burrows in the sediment; by doing this they transfer sediment from depth to the surface, their burrowing activities moving organic particles around (François et al., 2002). Thus, the abundance of this taxon has a direct effect on the mineralisation of OM (Papasprou et al., 2010). Although the BPC of Nereididae decreased in sediments with shell-hash, the BPC of the entire macrofaunal assemblages increased under these conditions. This result highlights the importance of understanding the ecosystem function of all the macrofaunal assemblages, as well as their abundance and biomass, to predict their effects on the ecosystem services of the sediment, including bioturbation (Biles et al., 2002; Duarte, 2000).

Shell-hash induces sediment structure changes (Commuto and Rusignuolo, 2000) that can enhance diffusion rates between the water column and sediment porewater (Bengt-Owe, 1967). Due to its roughness and structure, shell-hash can modify the advective porewater flux, favouring the formation of microzones (Huettel and Gust, 1992) that are suitable not only for species tolerant to organic enrichment, but also those that are more sensitive (Pearson and Rosenberg, 1978). Moreover, changes to the structure of sediments generated by shell-hash can enhance the sediment habitat complexity (D'Amours et al., 2008; Gutierrez et al., 2003), increasing the number of niches for macrofaunal assemblages (Gutiérrez et al., 2011); this could promote diverse macrofaunal assemblages with different bioturbation traits (Papageorgiou et al., 2009; Sanz-Lazaro and Marin, 2011). However, this shell-hash enhancement of macrofaunal assemblages with higher BPC was not seen in the sediment from the mussel farm. This result could be explained by the fact that the sediment from the mussel farm had a naturally high OM content, and the extra OM load from the aquaculture could have collapsed the system by inducing anoxic conditions

unsuitable for any macrofaunal species, corresponding to the grossly polluted stage (Hargrave et al., 2008; Pearson and Rosenberg, 1978). Perhaps, in other sediments with a low OM content shell-hash could impact macrofaunal assemblages and therefore their BPC. For this reason, it is essential to concentrate more research effort on defining what effect shell-hash has on the BPC of the macrofaunal assemblages in similar sediments.

4.3. Study limitations

Our study focuses on two different farms, one raising fish, the other culturing mussels. Although their sediment and oceanographic characteristics are comparable with the principal areas where these activities are located, more areas should be analysed before the present results can be extrapolated to other farms. Moreover, the limited duration of the experiment was not long enough for the succession to reach a mature stage. Additionally, the structure of the experimental units could block the post-settlement lateral recolonisation of some species with a low movement capacity. These issues could have influenced our results. However, similar experiments using equivalent time spans and structures have been shown to be good proxies for understanding the direct effects of impacts on marine sediments (Fernandez-Gonzalez et al., 2016; Martinez-Garcia et al., 2019). Future experiments involving a higher number of farms and longer time spans, would be desirable to increase our knowledge in this field.

4.4. Management implications

Programmes monitoring the benthic impact of farming activities have traditionally focused on the macrofaunal assemblages in terms of species richness, abundance, biomass, and diversity (Borja et al., 2009; Hargrave et al., 2008; Martinez-Garcia et al., 2013). However, our results show the importance of analysing the sediment macrofaunal assemblages in terms of an ecosystem function approach (Borja et al., 2016; Gammal et al., 2016; Ieno et al., 2006). For example, although the abundance values in the mussel farm sediments were higher under the influence of aquaculture than those of the fish-farm, the bioturbation capacity of the macrofaunal assemblages was higher at the fish farm. Thus, it is expected that the environmental status of the fish farm sediment is greater than that of the mussel farm due to the implications of bioturbation on the biogeochemical fluxes of marine sediments (Braeckman et al., 2010; Casado-Coy et al., 2020; Meysman et al., 2006). The ecosystem functional approach must therefore be considered when assessing the consequences on ecological functions, which in turn affect the ecosystem services that sediments provide to society (Wohlgemuth et al., 2016).

Important research effort has been made to understand the implications of mussel shell-hash accumulation from aquaculture industry on sediment (Bergström et al., 2020; Casado-Coy et al., 2017; Cranford et al., 2009; Hatcher et al., 1994; Tomašových et al., 2014; Wilding, 2012). However, mussel shell-hash derived from biofouling has been scarcely included as an impact in monitoring programmes of farming activities. The results of previous studies indicate that shell-hash can modify marine sediments physically (Commuto and Rusignuolo, 2000; Huettel and Gust, 1992), chemically (Casado-Coy et al., 2017) and ecologically (Commuto et al., 2008; Gutierrez et al., 2003). Moreover, present study suggest that shell-hash can promote macrofaunal assemblages with high bioturbation capacity. Thus, more effort is needed to establish the thresholds of this residue on monitoring programs of farming activities.

5. Conclusions

Our results show that shell-hash from aquaculture can promote macrofaunal assemblages with a high bioturbation capacity in sandy sediments in oligotrophic areas, such as Mediterranean cage farms.

However, the effect of shell-hash can be notably modulated by the environmental characteristics of sediments, including the grain size and natural OM content (Kanaya, 2014; Martinez-Garcia et al., 2015; Papageorgiou et al., 2010). Accordingly, in the case of eutrophic areas, such as estuaries, where mussel production is abundant (APROMAR, 2020), shell-hash does not notably affect the macrofaunal assemblages in sediments. Consequently, it seems that it may not be necessary to retrieve the shell debris derived from the cleaning activities related to aquaculture, but this could be left *in situ* as it may positively affect the ecological status of the seabed in oligotrophic areas (Casado-Coy et al., 2017) and might promote the bioturbation capacity of the macrofaunal assemblages found in these sediments. Moreover, limiting the removal of shell debris in these sediments may avoid perturbations deriving from these processes, such as sediment dredging (Graca et al., 2004; Wilber and Clarke, 2001). Nevertheless, future experiments involving additional levels of shell-hash and other areas with different oceanographic and sediment characteristics should be performed, to increase our understanding of the effects shell-hash has on the bioturbation capacity of macrofaunal assemblages in sediments. This management alternative is cost-effective and could limit, to some extent, the amount of shell debris that ends up as a waste product, as the management of this involves an economic cost (Adams et al., 2011; Morris et al., 2019), hence favouring the circular economy.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aquaculture.2021.737642>.

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