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Recovery of eutrophized marine ecosystems using the European flat oyster, *Ostrea edulis*

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Abstract

1. The development of tourism and intensification of agriculture has released large amounts of nitrogen and phosphorus into the Mar Menor coastal lagoon in South-east Spain, resulting in a phytoplankton bloom in 2016. This bloom turned the clear and transparent waters turbid and greenish, and killed approximately 85% of benthic macrophytes.
2. Nutrient bioextraction by flat oysters, *Ostrea edulis*, has been proposed for remediation of these eutrophication events and water quality recovery.
3. This research aims to quantify the clearance rate and investigate the genetic origin of Mar Menor oysters under eutrophized conditions for potential applications to bioremediation projects. Oligotrophic and eutrophic conditions were replicated in the laboratory, and oyster feeding behaviour (i.e. clearance rates, ingestion rates, absorption efficiency and absorption rates) were studied using a flow-through system.
4. The genetic characterization of oysters showed no significant difference between individuals from the Mar Menor and individuals collected from a nearby Mediterranean bed (Tabarca Island).
5. Based on the physiological results observed, oysters were grouped into high-feeder (HF) and low-feeder (LF) categories according to their clearance rate, that was 3-fold higher in the HF group. Different responses in feeding behaviour were observed under eutrophic conditions in both oyster groups. Constraints in the absorption capacity of LF oysters seemed to be related to their reduced filtering activity. Lower body condition of LF oysters was evidenced by their negative scope for growth value.
6. From this work, several conclusions can be drawn for future restoration/bioextraction actions: (i) the recovery of half of the oyster population that existed in the past would act as an effective top-down control on the phytoplankton community; (ii) using clearance rate measurements is recommended to select

oysters as broodstock; and (iii) Mediterranean oysters could be used as broodstock in the event that Mar Menor oysters fail to reproduce.

KEYWORDS
clearance rates, eutrophication, flat oysters, genetics, nutrient bioextraction, restoration

1 | INTRODUCTION

The degradation of marine ecosystems has directly resulted in environmental and socio-economic losses world-wide. Due to this, great efforts have been made towards the conservation and restoration of marine habitats and the valuable ecosystem services they provide (Chen et al., 2021). Studies have shown that the protection, conservation, and restoration of marine ecosystems can help in the adaptation and mitigation of climate change effects as well as improving human health and wellbeing (Kabisch et al., 2016; Raymond et al., 2017). Successful restoration of an ecosystem, however, should not only be restricted to natural recovery, it also requires building a self-sustaining system that will provide long-term services in the future (Howie & Bishop, 2021).

The Mar Menor, located in south-east Spain is one of the largest coastal lagoons in the Mediterranean region and is an ecologically important Spanish wetland (Jiménez-Martínez, Aravena & Candela, 2011). The lagoon houses a large variety of marine species, which have been negatively affected by two main drivers: (i) the pressure exerted by tourism, which greatly increased in the 1960s; and (ii) the effects of intensive agriculture, which began in the 1970s. Both events drastically increased the input of nitrogen (N) and phosphorus (P) into the lagoon causing it to eutrophy (Erena et al., 2019; Álvarez-Rogel et al., 2020; Ruiz et al., 2020). The impacts of this nutrient pollution led to the collapse of the lagoon in 2016, when an 'ecosystem disruptive algal bloom' (EDAB) occurred. In general, EDABs are composed of small cyanobacteria and microalgae that disrupt the structure and functioning of eutrophic ecosystems (Mercado et al., 2021). In Mar Menor, however, the EDAB was primarily composed of the cyanobacteria *Synechococcus* spp., as similarly reported in other eutrophic lagoons (Villena & Romo, 2003; Mercado et al., 2021; Phlips et al., 2021). The concentration of microalgae in Mar Menor remained high from 2016 to 2019 (Erena et al., 2019), with particulate organic matter (POM) as high as 6.5 mg L^{-1} in 2016, but decreasing to 4 mg L^{-1} in mid-2018 and late 2019 (Ruiz et al., 2020). Thus, because of these ecological challenges that Mar Menor is facing, it is urgent to find innovative approaches for ecological restoration and conservation.

Globally, there has been significant progress in reducing the input of nutrients into marine systems with the goal of mitigating coastal eutrophication (Boesch, 2019). One proposed strategy is to extract nutrients using bivalves as a nature-based solution (NbS) to improve water quality (Rose et al., 2015). NbSs were defined by the International Union for Conservation of Nature as "actions to protect, sustainably manage, and restore natural or modified ecosystems, which address societal challenges effectively and adaptively, simultaneously providing human well-being and biodiversity benefits" (Cohen-Shacham et al., 2016).

Bivalves can help to mitigate eutrophication as they can filter large amounts of water, pulling organic matter out the water column and taking it up into their tissues. The potential of bivalves to mitigate eutrophication can thus be assessed by measuring physiological feeding parameters such as clearance rates, i.e. the volume of water cleared of particles in a given period of time, among other measures. According to Cranford, Ward & Shumway (2011), bivalves can filter, on average, 2–3 L g^{-1} dry weight h^{-1}. Such values can be used as a first approximation of the total clearance capacity of a population, which can be extrapolated to the potential effect it could have on water clarity of a degraded ecosystem. Due to filtering capabilities, bivalve aquaculture (i.e. oysters, clams, mussels etc.) and wild restoration efforts have proven to be efficient in lowering the impacts of eutrophication in many estuaries globally (Ferreira et al., 2009; zu Ermgassen et al., 2013; Pollack et al., 2013; Humphries et al., 2016; Reitsma et al., 2017; Bricker et al., 2018). For example, in the Great Bay Piscataqua River Estuary (New Hampshire, USA), a seed density of 100 *Crassostrea virginica* (eastern oyster) per m^2 was able to remove 72 kg N $acre^{-1}$ $year^{-1}$ (Bricker et al., 2020). In Huangdun Bay, China, a seed density of 100 *Ostrea plicatula* (Chinese oyster) per m^2 was able to remove 265 kg N $acre^{-1}$ $year^{-1}$ (Ferreira et al., 2009).

The European flat oyster (*Ostrea edulis* L.) is a traditional source of food that once covered vast areas of the open North Sea and other European coastal waters including the Mediterranean Sea (Pogoda et al., 2019). The presence of *O. edulis* in the Mar Menor was described in the 1980s, when the salinity dropped to 42–44 PSU after a channel between the lagoon and the Mediterranean Sea was dredged. During the 1980s and 1990s, the Mar Menor housed a large population of natural flat oysters estimated at over 100 million individuals (García García et al., 1989; Cano et al., 1993; Rosique, 1994; Rosique & García García, 1997). However, the last survery of flat oysters conducted in 2006 revealed a drastic reduction down to 6 million individuals (Rosique, 2006). The recovery of the flat oyster population has been proposed by the authors of the present study (https://noraeurope.eu/spain-the-mar-menor-oyster-initiative/) as an NbS to improve water quality and restore the functionality of the lagoon.

However, any action to recover an oyster population requires the supply of seed from broodstock in hatcheries. Ideally, the broodstock should, if possible, come from the same ecosystem looking to be restored, both for biosecurity reasons (zu Ermgassen, 2020) and to help ensure the best possible chance of survival under local conditions

(Preston et al., 2020). However, if obtaining individuals from the same location is not possible, understanding the origin and genetic similarity of the Mar Menor population with neighbouring (Mediterranean) or more distant populations (Atlantic) is necessary in order to understand the best options for broodstock collection. The goal of this study was therefore to calculate the filtering potential of flat oysters from Mar Menor under different environmental conditions, including those similar to the collapse in 2016. Moreover, the genetic origin of Mar Menor oysters was investigated by comparing local specimens with other populations along the Mediterranean and Atlantic coasts of Spain. This study aims to gain knowledge on the Mar Menor oysters that can be used for future bioremediation or restoration purposes by understanding their genetic origin and providing data on filtration responses under eutrophic conditions. The acquired data can be useful in assessing the potential of oysters as an NbS in Mar Menor and initiate future management actions.

2 | METHODS

2.1 | Sampling and stocking of oysters

Thirty oysters, approximately 10 cm in size, were manually collected in August 2020 by diving from along Baron Island (37°41′46″N 0°45′13″W) in the Mar Menor lagoon (SE Spain). Oysters were brought to the laboratory at the Instituto Español de Oceanografía (San Pedro del Pinatar, Murcia, http://www.mu.ieo.es/) and maintained in sea water pumped directly from the Mar Menor lagoon (filtered to 0.5 μm). Oysters were placed in baskets inside a 500-L tank and the sea water was changed three times a week. Salinity was periodically monitored and ranged between 40 and 42 PSU. The water temperature was set to 18 ± 1°C and oysters were held under these conditions for 2 months to acclimate prior to the start of experiments. Oysters were fed a mixture of cultured microalgae, made up of *Tisochrysis lutea* (formerly known as *Isochrysis galbana*, clone T-Iso) and *Tetraselmis suecica*. The daily food ration was set at 3% microalgae in relation to the oyster meat dry weight. Food was supplied through a peristaltic pump throughout the day in order to maintain a food concentration below the pseudofaeces threshold (≈2 mm^3 L^{-1}, ≈1 mg L^{-1} of particulate organic matter; Bayne, 1993). Oyster stock were maintained under the described laboratory conditions for 6 months before they were exposed to eutrophized conditions.

2.2 | Experimental design and set-up

From the oyster stock, 10 animals (plus five more for replacement if necessary) were selected for the experiments (Table 1). The first experiment (*Exp I*) aimed to estimate the components of the energy balance of the Mar Menor oysters under oligotrophic conditions (0.82 mg POM L^{-1}), in order to characterize their physiological response and to estimate their scope for growth (SFG). Characterization of the oysters was completed through the biometric and genetic studies detailed below. In two additional experiments (*Exp II* and *Exp III*), two levels of eutrophication (2.50 and 4.83 mg POM L^{-1} for *Exp II* and *Exp III*, respectively) observed during the successive EDABs in the Mar Menor (Ruiz et al., 2021) were simulated and the physiological responses of the oysters were measured. Each experiment lasted 3 days, where the first was used to condition organisms, and physiological measurements were carried out during the following 2 days. The same animals were used for the three experiments unless replacement of individuals was necessary due to death (three oysters died during the period of physiological measurements over 1 month). Oysters were individually placed in a flow-through system composed of 12 experimental chambers, where 10 individuals were used and two chambers were left empty as controls. Each chamber was rectangular in shape with a volume of 4,000 ml with the inflow in the bottom and the outflow in the top on opposite sides. The chambers were fed by a 12-channel peristaltic pump (ISMATEC MCP) that pumped in the experimental sea water with the pre-defined particle concentration. Flow rate was adjusted to obtain a difference <40% between inflow and outflow concentrations. Temperature (18 ± 1 °C) and salinity (41 ± 1 PSU) were the same as during acclimation (see previous section). Particles used to simulate the three environmental scenarios were the cultured microalgae (*T. lutea*) as organic matter source and previously ashed (600 °C) and sieved (40 μm) fine marine sediment from reference areas as an inorganic matter source. This mix of microalgae and sediment was prepared every day in 10-L round bottom flasks and supplied to the flow-

TABLE 1 Experimental conditions used to assess the physiological responses of the Mar Menor oyster bed to various levels of particulate matter as observed in the Mar Menor lagoon during a phytoplankton bloom. Particulate matter was composed of the microalgae, *Tisochrysis lutea*, as a source of organic matter and ashed marine sediment as a source of inorganic matter. The mean value for 16 samples per *Exp* (eight samples per day of clearance measurements) and standard deviations are displayed.

Experimental conditions	TPM mg L^{-1}	PIM mg L^{-1}	POM mg L^{-1}	POM %	VOL mm^3 L^{-1}	CELLS *10^6 L^{-1}	POM/VOL mg $(mm^3)^{-1}$
Exp-I	1.27 ± 0.09	0.45 ± 0.05	0.82 ± 0.08	64.7 ± 4.0	2.02 ± 0.24	42.6 ± 9.9	0.41 ± 0.04
Exp-II	3.03 ± 0.18	0.53 ± 0.14	2.50 ± 0.21	82.7 ± 4.7	6.96 ± 0.37	125 ± 19	0.36 ± 0.03
Exp-III	5.55 ± 0.61	0.72 ± 0.16	4.83 ± 0.46	87.4 ± 1.6	11.76 ± 0.62	252 ± 22	0.41 ± 0.02

Abbreviations: CELLS, concentration in cells number; PIM, particulate inorganic matter; POM, particulate organic matter; TPM, total particulate matter; VOL, particulate volume concentration.

through system by a second multichannel peristaltic pump adjusted to a flow rate to simulate the pre-defined particle concentration.

2.3 | Physiological measurements

Clearance rate (CR, expressed in L ind^{-1} h^{-1}) was calculated from the difference between inflow and outflow concentrations in the experimental system according to the Hildreth & Crisp (1976) equation $CR = f^*(C_i - C_o)/C_i$. In this equation, f is the flow of water expressed in L h^{-1}, C_i is the inflow concentration (considered as the control outflow), and C_o is the outflow concentration, both of which are expressed in particulate volume units, mm^3 L^{-1}. The flow rates ranged between 1.1 to 5.5 L h^{-1} depending on the particle concentration tested (Exp I, II and III) and the feeding behaviour of the oysters (HF vs. LF) so that the difference between inlet and outlet never exceeded 40%. The CR equation was selected as the design of the flow-through chambers avoids recirculation of filtered water (Filgueira, Labarta & Fernández-Reiriz, 2006). Particle concentration was measured with a Coulter Counter, model Multisizer III fitted with a 100-μm orifice diameter tube. Clearance rate measurements were carried out four times per day over two consecutive days (days 2 and 3 of each experiment).

Ingestion rate (IR, mg POM h^{-1}) was obtained by multiplying the CR (L h^{-1}) by the concentration of the diet expressed as mg POM L^{-1}. Seston characterization was performed by filtering 2 L of water ($n = 16$ filters per each diet) from the outflows of the control chambers through previously rinsed, ashed and weighed Whatman GF/C filters (1.2 μm pore size). The filters were later rinsed with a 0.5 M ammonium formate solution, dried for 24 h at 100 °C and ashed at 450 °C for 4 h. Total particulate matter expressed as mg L^{-1} was determined as the dry weight of the suspension on the filter after drying and particulate inorganic matter was given as the weight remaining after ashing. The difference between dry weight and ashed weight was considered as the organic weight (POM).

Absorption efficiency (AE), was calculated from the percentage of organic matter in the food and in the faeces according to Conover's ratio (Conover, 1966), $AE = ((F - E)/([1 - E) F])^*100$. Here, F is the percentage of organic matter in the food and E is the percentage of organic matter in the faeces. All biodeposits produced by each individual were siphoned with a pipette, filtered onto Whatman GF/C filters and processed in a similar way to that described for seston samples. Biodeposits were collected twice (overnight and after CR measurements) during the last 2 days of each experiment (days 2 and 3 after placing the oysters in the flow-through system). Absorption rate (AR, mg POM h^{-1}) was obtained by multiplying the ingestion rate by the absorption efficiency ($AR = IR^*AE$).

Respiration rate (RR mg O_2 ind^{-1} h^{-1}) was calculated from the decline in oxygen concentration recorded during 1–1.5 h (oxygen was not allowed to decline past 35% of the initial value). Oxygen concentrations were measured every 15 minutes therefore obtaining four to six measurements per oyster using a YSI 52 DO instrument connected to a YSI 5905 self-stirring BOD probe. Each oyster was transferred into an individual respirometer chamber with a volume of ~800 ml filled with 18 ± 1 °C aerated filtered sea water. RRs were calculated according to the equation $RR = [[(O_2^{bl} - O_2^{exp})/t]]^*V$, where O_2^{bl} and O_2^{exp} are the oxygen concentrations in the control (respirometer without oysters) and experimental respirometers, respectively, expressed in mg O_2 L^{-1}; t is the time, expressed in h, and V is the volume, expressed in L. RRs were measured in the oysters at the beginning of *Exp I*.

For comparative purposes, physiological rates were standardized to the dry weight of oysters. CR was standardized to 1 g of dry specimen and was calculated using the allometric exponent $b = 0.791$. This exponent relates the variation in this physiological rate to the soft tissue weight of the animal at 20 °C in the flat oyster according to Haure et al. (1998). For RR, the allometric exponent used was 0.825 as established by the same authors at a similar temperature (20 °C) to the present study. Standardized physiological rates (Y_{st}) were calculated as follows: $Y_{st} = (W_{st}/W_{exp})^{b*}Y_{exp}$, where W_{st} and W_{exp} are the standardized (1 g of dry weight) and the experimental weights, respectively. Y_{exp} is the measured physiological rate and b is the allometric exponent used. When physiological measurements were completed, the oysters were sacrificed and their biometric parameters measured (see below).

To estimate SFG, the physiological rates were transformed into energy equivalents (J g^{-1} h^{-1}). The following energy equivalents were used as recommended by Widdows & Johnson (1988): 1 mg organic matter is equivalent to 23 J; 1 ml oxygen is equivalent to 20.33 J; and 1 mg oxygen is equivalent to 0.6998 ml oxygen (Ansell, 1973). SFG was then calculated from the energy balance equation according to the expression $SFG = (I - F) - R = (I^*AE) - R$, where I is the consumption of the energy available in the diet, F is the energy lost in the faeces, AE is the absorption efficiency and R is the energy consumed by respiration. SFG was calculated for the oysters at the beginning of *Exp I*.

2.4 | Physiological data statistical analysis

Physiological data were tested for normality and homogeneity of variances (assessed by the Levene's test) prior to conducting statistical analyses. If data did not meet the required assumptions, they were log transformed; however, AE data had to be subjected to angular transformation (Arcsine√percentage). From the clearance rates observed in *Exp I*, oysters were classified in two groups according to their feeding behaviour: high (HF) and low feeders (LF). A two-way ANOVA was performed to investigate the effect of particle concentration (POM) and feeding behaviour to establish the physiological response of oysters to variation in particle concentration according to their feeding behaviour. A Student–Newman–Keuls test was used to differentiate between groups (*post-hoc* test) and a Student t-test was used to compare the two sets of data (HF and LF). The

relationship between CR and particle concentration was established by a linear regression for each oyster group. Comparison between these regressions was performed by means of an analysis of covariance (ANCOVA). The comparison between intercepts of the regression equations provides insight to the differences in CR between oyster groups, while comparison of the slopes provides insights to the differences in the effect of particle concentration on CR. Statistical significance was set to 0.05 for all analyses. The statistical analyses were performed using Statgraphics Centurion 16.1.3.

2.5 | Biometric measurements

Upon conclusion of the experiments, all oysters were measured and dissected for biometric measurements and a piece of gill tissue was preserved for genetic analysis. Shell measurements were taken according to Bayne (2017) where height (H) was measured from the umbo to the ventral margin of the valves, length (L) was the maximal distance between anterior and posterior margins and width (W) was the maximal distance between the two valves. Several indices were calculated from these dimensions including elongation (H/L), compactness (W/L) and convexity (W/H). Dry weights (DW) of valves and meat (gills, gonads, muscle, digestive gland and remaining tissues) were recorded after drying tissues in an oven at 100°C for 24 h. The following indices were calculated from soft tissues data: (i) gill index (gill DW / meat DW)*100); (ii) hepato-somatic index ((digestive gland DW / meat DW)*100); (iii) gonado-somatic index ((gonad DW / meat DW)*100); (iv) muscle index ((muscle DW / meat DW) *100); and (v) remaining tissues index ((remaining DW /meat DW)*100). For comparison purposes the shell condition index, CI_s ((meat DW /valves DW)*100) (Lucas & Beninger, 1985; Davenport & Chen, 1987) and the total condition index, CI_t ((meat WW/total weight)*100, where *meat WW* is the fresh weight of soft tissues, and *total weight* is the weight of the whole oyster before dissection (Ansell, Loosmore & Lander, 1964)) were calculated.

2.6 | Genetic study

In total, 32 specimens of *O. edulis* from the Mar Menor lagoon (hereafter OMM, Mar Menor oysters) were analysed for genetic characterization, including the oysters used in the physiological analyses. Genetic characterization was done using a gill fragment. To study the levels of genetic diversity and population structure of the OMM bed, a Mediterranean population from Santa Pola in Alicante (ALI, 30 individuals) and six Galician beds (Ría Eo, Ortigueira, Ferrol, Pontedeume, Ría de Noia, Ría de Pontevedra) previously analysed (569 specimens, see Vera et al. (2016), for bed descriptions) were included in the genetic analysis of this study (Table S1, locations described in the table).

DNA extraction was performed with gill material using the eZN. An E-96 mollusc DNA kit (Omega Bio-Tek) following the protocol provided by the manufacturer. Sixteen microsatellite loci (OeduT5, OeduO9, OeduU2, OeduJ12, Oed177a, Oed181, Oed202a, Oed202b, Oed212b, Oed240, Oed243, Oed325, Oed327, OE03, OE27, OE11) were analysed (Launey et al., 2002; Lallias et al., 2009; Vera et al., 2015) being amplified in three multiplex polymerase chain reactions following the methodology described in Vera et al. (2016). The genotyping of all microsatellites for each specimen was carried out in an ABI 3730 XL automatic sequencer (Applied Biosystems, Forest City, CA, USA) at the Fragment Analysis and Sequencing Unit of the University of Santiago de Compostela (Campus Lugo), using the GeneMapper 4.0 program (Applied BioSystems) and the size standard GeneScan™ 500 LIZ® (Applied Biosystems) for allele scoring.

Levels of genetic diversity (measured as observed heterozygosity (Ho), expected heterozygosity (He), number of alleles (A) and allelic richness (Ar)), the conformance to the Hardy–Weinberg expectations (HWE), and coefficients of intrapopulational differentiation/inbreeding coefficients (F_{IS} values) were analysed with the programs FSTAT 2.9.3 (Goudet, 2001) and GENEPOP 4.0 (Rousset, 2008). The global and pairwise coefficients of population differentiation (F_{ST}) for all included beds were carried out with FSTAT and their significance was tested with 10,000 permutations. The relationship between the different animals and beds was also analysed with a discriminant analysis of principal components with the ADEGENET 2.0.0 program (Jombart & Ahmed, 2011) using R (R Development Core Team, 2014). Data were transformed using principal component analysis and an appropriate number of principal components and discriminant functions were retained to explain > 90% of variance. Finally, a hierarchical analysis of molecular variance (AMOVA) using the program ARLEQUIN (Excoffier et al., 2005) was carried out to study the distribution of genetic variation within and among groups of beds created by their slope (i.e. Mediterranean and Atlantic groups). Their significance was tested with 10,000 permutations.

3 | RESULTS

3.1 | Experimental conditions

Three sets of experimental conditions were used to replicate the range of particle concentrations observed in the Mar Menor since the lagoon collapsed in 2016 (Table 1; Ruiz et al., 2021). Oligotrophic conditions examined in *Exp-I* showed a particle concentration (POM) of 0.82 mg L^{-1}. Two levels of eutrophication were subsequently created in *Exp-II* (2.50 mg L^{-1} POM) and *Exp-III* (4.83 mg L^{-1} POM).

3.2 | Physiological characterization under oligotrophic conditions (*Exp-I*)

During *Exp I*, at the lowest POM concentration, some oysters filtered at much higher rates than others, even after standardizing the data to 1 g of DW. Thus, the standardized CR ranged from 0.2 to 2.5 L g DW^{-1} h^{-1}, with two distinct groups observable; the HF filtering

more than 1 L g DW^{-1} h^{-1}, and LF filtering less than 1 L g DW^{-1} h^{-1} on average. HF oysters filtered at an average rate of 1.73 L g DW^{-1} h^{-1}, which was significantly different (2.7-fold higher; Student *t*, $P < 0.05$) than LF oysters (0.63 L g DW^{-1} h^{-1}; Table 2). Similarly, ingestion rate was significantly higher ($P < 0.05$) in HF than LF (HF/LF = 2.7; Table 2). Absorption efficiency in both groups showed high values when compared to the literature (Bayne, 2017), although AE was significantly higher ($P < 0.05$) in HF (81.2%) than in LF (70.5%) oysters. As a consequence, differences in AR between both groups (1.09 and 0.35 mg g DW^{-1} h^{-1} for HF and LF, respectively) was slightly higher than differences in IR. Significant differences were also observed in metabolic rates, although results were the inverse of what has been described for energy acquisition rates. Thus, RR was significantly higher ($P < 0.05$) in LF oysters (1.35 and 0.85 mg O_2 g DW^{-1} h^{-1} for LF and HF, respectively). LF oysters showed a negative SFG (−11.2 J g DW^{-1} h^{-1}) due to their low energy input, as a consequence of low CR and AE and high energy demands. In contrast, HF oysters showed a positive SFG of 13.0 J g DW^{-1} h^{-1}.

3.3 | Feeding responses to eutrophized conditions (*Exp-II* and *Exp-III*)

The physiological responses to increasing particle concentrations in the water column were different for each group of oysters. The results of the two-way analysis of variance (ANOVA, Table 3) indicate that both particle concentration and feeding behaviour significantly influence all measured physiological rates, except for AE. The interaction of the two factors is also significant for all the physiological rates measured ($F = 3.8$, $P < 0.05$ for CR; $F = 9.9$, $P < 0.001$ for IR; $F = 7.9$, $P < 0.01$ for AR; $F = 6.2$, $P < 0.01$ for SFG) except for AE ($F = 0.06$, $P > 0.05$), showing that the response of physiological rates to the increase in particle concentration is different in each group of oysters.

The increase in particle concentration led to a 2-fold decrease in CR in the HF oysters, that ranged from 1.73 L h^{-1} at the lowest POM (0.82 mg L^{-1}, *Exp-I*) to 1.15 L h^{-1} at the highest POM (4.83 mg L^{-1}, *Exp-III*; Figure 1). CR at the intermediate POM (2.50 mg L^{-1}, *Exp-II*) was similar (1.79 L h^{-1} $P > 0.05$) to the lowest POM, and statistically different from the highest POM (*Exp III*). The only significant differences were observed between *Exp III* and the other two concentrations examined. In contrast, the LF oysters showed a similar CR (Figure 1) in the three particle concentrations tested, with values between 0.53 and 0.63 L h^{-1}, although these differences were not significant ($P > 0.05$). The comparison between the CR of oyster groups (Student *t*) for each experiment showed that CR was significantly higher in HF than in LF oysters, in all three cases.

The regression fitted between particle concentration and CR for each oyster group (Figure 2) showed no significant relationship between CRs and POM in low feeding oysters ($P = 0.41$). However,

TABLE 2 Physiological rates measured under oligotrophic conditions (total particulate matter = 1.27 ± 0.09 mg L^{-1}, particulate organic matter = 0.82 ± 0.08 mg L^{-1}) in high (HF) and low (LF) feeding oysters from the Mar Menor. All rates were standardized to 1 g of oyster dry weight. Significant differences between the oyster groups are denoted with an asterisk (Student *t*, $P < 0.05$). The ratio between HF and LF for each physiological rate is also shown. Scope for growth has been estimated by the integration of all physiological rates and is expressed in joules in the energy balance. The means and standard deviations obtained are displayed for the physiological measurements done on 2 consecutive days on 4 (HF) and 6 (LF) oysters from each group.

Physiological rates	HF	LF	HF/LF
Clearance rate (L g DW^{-1} h^{-1})	1.73 ± 0.67	0.63 ± 0.32*	2.7
Ingestion rate (mg OM g DW^{-1} h^{-1})	1.34 ± 0.38	0.49 ± 0.24*	2.7
Absorption efficiency (%)	81.2 ± 2.7	70.5 ± 5.6*	1.2
Absorption rate (mg OM g DW^{-1} h^{-1})	1.09 ± 0.31	0.35 ± 0.17*	3.1
Respiration rate (mg O_2 g DW^{-1} h^{-1})	0.85 ± 0.15	1.35 ± 0.21*	0.6
Scope for growth (J g DW^{-1} h^{-1})	13.0 ± 8.0	−11.2 ± 3.6*	--

TABLE 3 Results of the two-way-ANOVA on the physiological responses to the particle concentration for each oyster group (Mar Menor oysters). F values from the two-way-ANOVA are shown. The two factors are the particle concentration (particulate organic matter (POM)) and the oyster feeding behaviour (FB). F-values for the interaction between the factors (POM*FB) are also shown.

Physiological rate	Particle concentration (POM)	Feeding behaviour (FB)	Interaction POM*FB
Clearance rate (L g DW^{-1} h^{-1})	5.6**	100.4***	3.8*
Ingestion rate (mg g DW^{-1} h^{-1})	67.5***	97.7***	9.9***
Absorption efficiency (%)	1.8	0.13	0.06
Absorption rate (mg g DW^{-1} h^{-1})	62.3***	87.1***	7.9**
Scope for growth (J g DW^{-1} h^{-1})	56.8***	97.2***	6.2**

Note: Significant F values at a level of * ($P < 0.05$), ** ($P < 0.01$) or *** ($P < 0.001$).

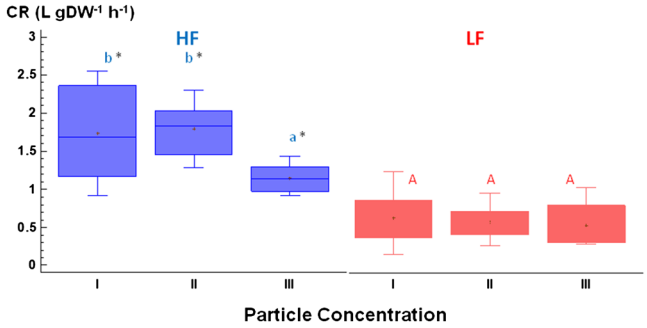

FIGURE 1 Clearance rates (CR, L g DW^{-1} h^{-1}) in high (HF, in blue) and low (LF, in red) feeding oysters from the Mar Menor measured under three particle concentrations, I (total particulate matter (TPM) = 1.27 ± 0.09 mg L^{-1}, particulate organic matter (POM) = 0.82 ± 0.08 mg L^{-1}), II (TPM = 3.03 ± 0.18 mg L^{-1}, POM = 2.50 ± 0.21 mg L^{-1}) and III (TPM = 5.55 ± 0.61 mg L^{-1}, POM = 4.83 ± 0.46 mg L^{-1}). All rates were standardized to 1 g of oyster dry weight. Results of the Student–Newman–Keuls *post-hoc* test (significance level, $P < 0.05$) used to check differences between particle concentrations in each oyster group are also shown: capital letters for LF oysters and lowercase letters for HF oysters. Significant differences between oyster groups for each particle concentration are denoted with an asterisk (Student *t*, $P < 0.05$).

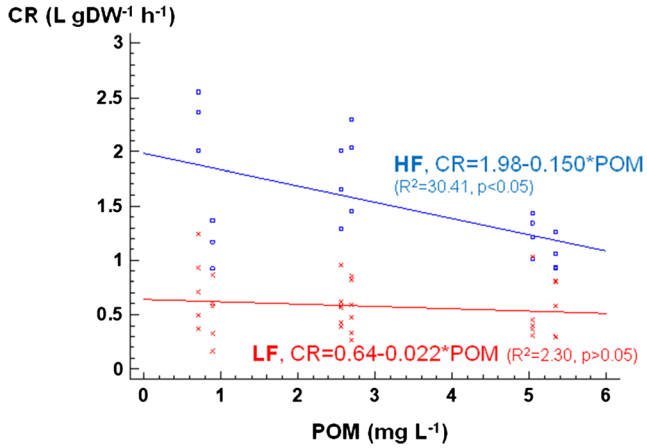

FIGURE 2 Regression lines fitted between standardized (to 1 g of oyster dry weight) clearance rates (CR), expressed as L g DW^{-1} h^{-1}, and particulate organic matter (particulate organic matter, mg L^{-1}) in high (HF, in blue) and low (LF, in red) feeding oysters from the Mar Menor.

the slope of the regression line in HF oysters was significant (b = −0.150, $P < 0.001$) and negative, indicating a decrease in CR as the concentration of particles increases in the water column.

There was an increase in IR with increasing food concentration, with significant differences between the three conditions tested for each group of oysters ($P < 0.001$; Figure 3). Similar to CR, IR for HF oysters was significantly higher (3-fold for *Exp I* and *II*, and 2-fold for *III*) than for LF oysters for each of the particle concentrations tested.

In contrast to clearance and ingestion rates, the food AE hardly varied with particle concentration (Figure 4). In the case of HF

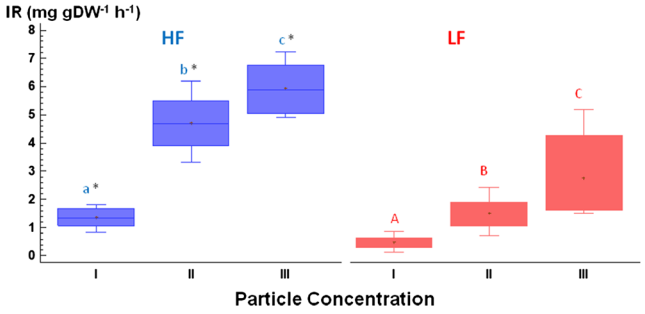

FIGURE 3 Food absorption efficiencies (AE, %) in high (HF, in blue) and low (LF, in red) feeding oysters from the Mar Menor measured under three particle concentrations, I (total particulate matter (TPM) = 1.27 ± 0.09 mg L^{-1}, particulate organic matter (POM) = 0.82 ± 0.08 mg L^{-1}), II (TPM = 3.03 ± 0.18 mg L^{-1}, POM = 2.50 ± 0.21 mg L^{-1}) and III (TPM = 5.55 ± 0.61 mg L^{-1}, POM = 4.83 ± 0.46 mg L^{-1}). Results of the Student–Newman–Keuls *post-hoc* test (significance level, $P < 0.05$) used to check differences between particle concentrations in each oyster group are also shown: capital letters for LF oysters and lowercase letters for HF oysters. Significant differences between oyster groups for each particle concentration are denoted with an asterisk (Student *t*, $P < 0.05$).

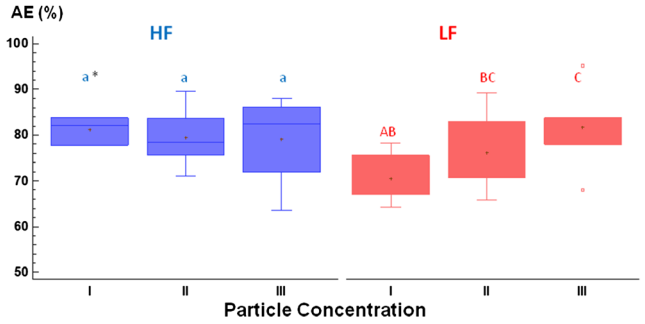

FIGURE 4 Ingestion rates (IR, mg DW^{-1} h^{-1}) in high (HF, in blue) and low (LF, in red) feeding oysters from the Mar Menor measured under three particle concentrations: I (total particulate matter (TPM) = 1.27 ± 0.09 mg L^{-1}, particulate organic matter (POM) = 0.82 ± 0.08 mg L^{-1}), II (TPM = 3.03 ± 0.18 mg L^{-1}, POM = 2.50 ± 0.21 mg L^{-1}) and III (TPM = 5.55 ± 0.61 mg L^{-1}, POM = 4.83 ± 0.46 mg L^{-1}). All rates were standardized to 1 g of oyster dry weight. Results of the Student–Newman–Keuls *post-hoc* test (significance level, $P < 0.05$) used to check differences between particle concentrations in each oyster group are also shown: capital letters for LF oysters and lowercase letters for HF oysters. Significant differences between oyster groups for each particle concentration are denoted as an asterisk (Student *t*, $P < 0.05$).

oysters, the AE was independent of particle concentration ($P = 0.91$) with a value close to 80%. Although similar results were observed in the LF oysters, where AE was similar in *Exp II* and *III* (76.1 and 81.7%, respectively), AE was found to be a significantly lower in oysters at low particle concentration in *Exp I* (70.5%), although differences were only significant between *Exp I* and *III*. Comparison between HF and LF oysters at each experimental condition revealed that only at the low particle concentration (*Exp I*) there was a significant difference in the

AE between both oyster groups, with a lower value in LF oysters (HF = 81.2% and LF = 70.5%).

The response of absorption rate to increases in particle concentration (Figure 5) reflects that described for the IR, as AE is almost independent of particle concentration. Thus, for both oyster groups, AR increases with particle concentration, with significant differences ($P < 0.05$) observed between the experimental conditions. AR ranged from 0.35 to 2.28 mg h^{-1} in LF oysters and from 1.08 to 4.68 mg h^{-1} in HF oysters depending on the particle concentration. Thus, AR in HF oysters was more than 3-fold higher than in LF oysters at *Exp I* and *II*, and differences were reduced to 2-fold in *Exp III*.

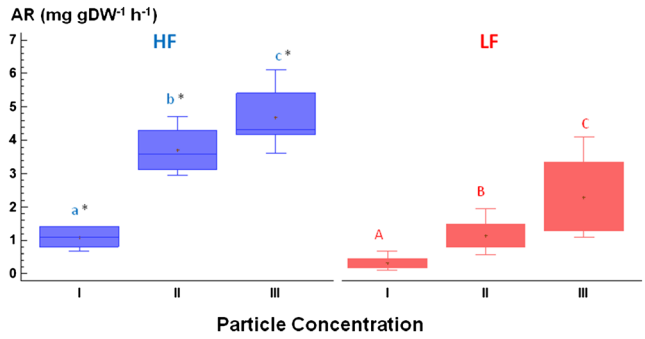

FIGURE 5 Absorption rates (AR, mg DW^{-1} h^{-1}) in high (HF, in blue) and low (LF, in red) feeding oysters from the Mar Menor measured under three particle concentrations: I (total particulate matter (TPM) = 1.27 ± 0.09 mg L^{-1}, particulate organic matter (POM) = 0.82 ± 0.08 mg L^{-1}), II (TPM = 3.03 ± 0.18 mg L^{-1}, POM = 2.50 ± 0.21 mg L^{-1}) and III (TPM = 5.55 ± 0.61 mg L^{-1}, POM = 4.83 ± 0.46 mg L^{-1}). All rates were standardized to 1 g of oyster dry weight. Results of the Student–Newman–Keuls *post-hoc* test (significance level, $P < 0.05$) used to check differences between particle concentrations in each oyster group are also shown: capital letters for LF oysters and lowercase letters for HF oysters. Significant differences between oysters groups for each particle concentration are denoted as an asterisk (Student *t*, $P < 0.05$).

3.4 | Biometric characterization of OMM

No significant differences between feeding behaviour groups were observed in any of the biometric parameters measured, for both valves and soft tissues, or in the indices calculated from them (Table 4). The oysters used in this study had a total live weight of ~190 g of which most was the weight of the shell (~150 g). Total dry weight of soft tissues was 1.22 and 1.40 g for HF and LF oysters, respectively. The highest fraction of soft tissue corresponded to muscle, which accounted for almost 30% of the total meat dry weight, followed by gonads (20–23%) and remaining tissues (around 24%). Hepatosomatic indices comprised between 12–15% and gill indices were around 10% in both oyster groups. Condition index relating the shell and meat weights were 0.85 and 0.97 for HF and LF oysters, respectively.

3.5 | Genetic characterization of OMM

Genetic diversity values for the OMM bed (estimated as A, Ar, Ho and He, see Table S2) were similar to the values from Santa Pola (Alicante; all the Mann–Whitney tests for A, Ar, Ho and He with $P > 0.490$) and Galician beds (Mann–Whitney test for Ar, Ho and He with $P > 0.100$) except for A ($P < 0.05$). This difference is probably due to the large difference in the sampling size, which does not affect Ar because this estimator corrects for the number of alleles with the sample size. The OMM bed conformed to HWE for all loci except for Oed325 and Oedu-U2, suggesting random mating. The HWE deviations were

TABLE 4 Biometric parameters (mean and standard deviation obtained from four high feeder (HF) and six low feeder (LF) individuals) of the Mar Menor oysters used in the physiological study. DW denotes the dry weight, while LW denotes live weight. Shell dimensions are height (H), length (L), and weight (W).

Biometrics	Indices	HF	LF
Shells	Live weight (g)	185.44 ± 74.91	193.05 ± 37.16
	Height (H) (cm)	10.40 ± 1.04	10.01 ± 0.86
	Length (L) (cm)	7.81 ± 1.72	8.83 ± 0.57
	Width (W) (cm)	4.19 ± 0.74	4.41 ± 0.42
	Total Shell Weight (g)	147.84 ± 59.50	148.62 ± 33.47
	Elongation H/L	1.38 ± 0.30	1.14 ± 0.15
	Compactness W/L	0.55 ± 0.10	0.50 ± 0.08
	Convexity W/H	0.40 ± 0.05	0.44 ± 0.03
Soft tissues	Total dry tissues (g DW)	1.22 ± 0.50	1.40 ± 0.31
	Gill index	10.85 ± 3.04	10.31 ± 3.29
	Hepato-somatic index	12.55 ± 1.57	15.14 ± 2.65
	Gonado-somatic index	22.91 ± 7.45	20.04 ± 8.19
	Muscle index	29.41 ± 4.62	30.59 ± 5.20
	Rest index	24.28 ± 4.79	23.92 ± 6.18
	Shell condition index	0.85 ± 0.23	0.97 ± 0.26
	Total condition index	2.93 ± 0.79	3.09 ± 1.20

explained by a deficit of heterozygotes (i.e. positive values of F_{IS}), probably due to the presence of null alleles in those loci. This is a common circumstance for microsatellite loci in molluscs due to their high rates of polymorphism (Diz & Presa, 2008; Vera et al., 2016). The global F_{ST} value taking into account all beds was 0.003 ($P < 0.001$). The pairwise F_{ST} values showed non-significant differences between beds within the same slope (i.e. Atlantic or Mediterranean), while the F_{ST} values for the comparisons of beds between different slopes were significant (Table S3), with values practically an order of magnitude higher (F_{ST} values ~ 0.010) than those detected within slopes. Thus, the AMOVA assigned 10 times more genetic variation to differences between slopes (0.00953, $P = 0.036$; 0.95% of the total assigned variation) than between beds within slope (0.00012, $P = 0.471$; 0.01% of the total assigned variation), with the highest percentage of variation assigned to differences between individuals within bed (99.04%), because each specimen has its own multilocus genotype. The discriminant analysis of principal components (Figure 6) confirmed the previous results, clustering the OMM and ALI beds in one group (red) and all the Galician beds in another (in blue).

4 | DISCUSSION

4.1 | Physiological characterization of OMM (under oligotrophic conditions)

The results shown in this study are the first to be published on the clearance potential of flat oysters from the Mar Menor lagoon. Unexpectedly, some oysters filtered faster than others, which led to their classification into two groups, HF and LF oysters (Table 2). CR differences between the two groups of oysters were significant where HF oysters (1.73 L g DW^{-1} h^{-1}, Table 2) fed at almost three times the rate of LF oysters (0.63 L g DW^{-1} h^{-1}). CR in suspension-feeding organisms such as oysters is one of the main physiological parameters used to analyse their feeding behaviour, and is known to be strongly influenced by environmental variables (Bayne, 2017). Cranford, Ward & Shumway (2011) reviewed 25 studies on the CR measurements of various species of oyster and found an average rate of 2.54 L g DW^{-1} h^{-1} in both field and laboratory experiments; this value is not far from the CR obtained with the HF oysters in the present study (1.73 ± 0.67 L g DW^{-1} h^{-1}, Table 2). The literature for flat oysters, however, shows a large span of CR values ranging from 0.8 (Shumway et al., 1985) to 12.6 (Nielsen, Hansen & Vismann, 2017) L g DW^{-1} h^{-1}. These values could be altered by environmental variables such as temperature, particle concentration, food quality, body size, gametogenic stage or nutritive status as these are known to influence the physiological process of feeding (Bayne & Newell, 1983; Griffiths & Griffiths, 1987; Hawkins et al., 1998). Other methodological variables such as the method of measurement (flow-through chamber, Coughlan method or biodeposit method) or the diet supplied (natural vs. monoalgal cultures) can also affect CR estimations, contributing to the range in rates found (Filgueira, Labarta & Fernández-Reiriz, 2006; Bayne, 2017). Upon removing extreme values from the literature and selecting for relatively standard food and temperature conditions, *O. edulis* was found to filter 2–4 L g DW^{-1} h^{-1} (Allen, 1962; Wilson, 1983; Haure et al., 1998; Sawusdee et al., 2015). Therefore, the values found for HF oysters in this study are comparable to published data. The CR obtained for LF oysters, however, cannot be explained with the data obtained in the present study. As the environmental conditions and methodologies applied were the same for all oysters, the difference is probably due to some endogenous variable specific to the organisms studied. Genetic, pollutant content or pathogenic characteristics of LF oysters could potentially explain their low feeding rates.

The genetic analysis in this study was carried using a set of neutral molecular markers (i.e. microsatellite loci). These markers were sufficient to determine the genetic differentiation between the Mar Menor population and others along the Spanish coast, but insufficient for the genomic screening necessary for detecting regions associated with complex traits such as feeding and growth. Therefore, they unfortunately do not help to genetically discriminate between HF and LF individuals. Although there are no recent data on the health status of oysters in the Mar Menor, studies carried out in the 1990s indicated the absence of two of the most common parasites in oysters, *Bonamia* and *Martelia* (Rosique, 2006). Although some organic pollutants have been found in higher concentrations in OMM compared to concentrations found in other bivalves from the lagoon (León et al., 2013), it is not likely that the low filtering activity of LF oysters increases their exposure to contaminants. In any case, bivalve CR is considered one of the most sensitive physiological parameters reflecting the well-being of the organisms and can be used as a biomarker for pollution (Toro, Navarro & Palma-Fleming, 2003; Martinez-Haro et al., 2016).

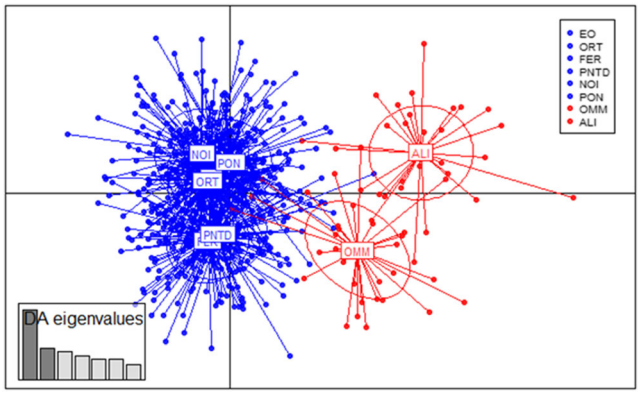

FIGURE 6 Plot of results obtained by discriminant analysis of principal components (DAPCs) with all natural flat oyster beds used. Weight of retained discriminant analysis (DA) eigenvalues to represent > 90% of variance are shown on right bottom box. Bed codes are shown in Table S1. Atlantic beds are shown in blue and Mediterranean beds in red. OMM is the code for the Mar Menor oysters. EO, Ría Eo; ORT, Ortigueira; FER, Ferrol; PNTD, Pontedeume; NOI; Ría de Noia; PON, Ría de Pontevedra; ALI, Alicante

Although to a lesser extent, the AE of LF oysters was significantly lower (70.5%, Table 2) than the AE of HF oysters (81.2%), which increases the differences (3.1-fold, Table 2) between both groups of oysters in terms of absorption rates (1.09 vs. 0.35 mg OM g DW^{-1} h^{-1}) regarding CR differences (2.7-fold). In addition to the lower energy input of LF oysters (lower CR and AE), the group experiences a significantly higher metabolic cost (1.35 mg O_2 g DW^{-1} h^{-1}), at almost 60% higher than of the HF group (0.85 mg O_2 g DW^{-1} h^{-1}). Considering that metabolic costs include the basal metabolism of the organisms together with the cost of their activity, it should be noted that in LF oysters, with much lower filtering activity, there must be some activity with a high energetic cost that is responsible for the observed differences. For example, immune and defence responses (including antioxidant activity) are energy-demanding activities that might explain differences in oxygen consumption between the two oyster groups (Berthe et al., 2004; Carella et al., 2015; Travers et al., 2015; Lassudrie et al., 2020).

As a consequence of all physiological differences described above, SFG for LF oysters resulted in negative values (−11.2 J g DW^{-1} h^{-1}, Table 2) whereas positive data were obtained in HF oysters (13.0 J g DW^{-1} h^{-1}). Apart from the reasons for the different physiological behaviour of the two groups of oysters, the negative SFG value obtained for the LF oysters indicates the existence of a stress on these organisms that compromises their survival, their reproduction and their potential as bioextractors of nutrients. It is worth noting that SFG has been proposed as a biomarker of stress in marine bivalves and is used in marine pollution monitoring programmes (Widdows & Staff, 2006; Albentosa et al., 2012a; Albentosa et al., 2012b). The differences found in the physiological characterization of the OMM carried out in this study require further studies to investigate potential reasons for their altered physiology and to determine the abundance of LF oysters in the lagoon.

4.2 | Feeding responses to eutrophic conditions

In addition to differences between the two groups of oysters, differences were also found in the response of oysters to the two levels of eutrophication simulated in the laboratory. With respect to CRs, the pattern observed in HF, i.e. a maintenance of CRs between conditions I and II and a decrease at the highest concentration (III; see Figure 1), is similar to that described in the literature (Cranford, Ward & Shumway, 2011). It is widely accepted that suspension-feeding bivalves are able to regulate ingestion by reducing CR as particle concentration increases in order to optimize digestion and absorption of food (see reviews by Lucas (2008) and Bayne (2017)). Furthermore, increases in particle concentration can invoke valve closure under extreme conditions, in which valves can remain closed until the stressor disappears (Vismann, 1991; Le Pennec, Beninger & Herry, 1995).

Ingestion rate increased linearly with particle concentration (Figures 3) within the range examined. In the present study, it seems that the maximum digestive capacity of the OMM has not been reached as the IR is higher at the maximum concentration tested, despite the decrease in CR at this concentration. On the contrary, Tamayo et al. (2014) reported stabilized IR values in *Crassostrea gigas* by regulating CR at medium and high food rations.

In bivalves, rising IR resulting from the increase in phytoplankton concentration has been generally found to promote the reduction of food AE (Bayne, 1993; Ibarrola et al., 2008). Such a negative trade-off between AE and IR is a factor constraining the process of energy absorption and typically represents the consequence of the decline in food retention time with rising IR (Bayne, 2017). In the present study, the trend followed by AE with rising food concentration differs significantly between HF and LF; contrary to expectations, AE increases with increasing IR in LF oysters at high food rations. Such a trend suggests that the constraints in the absorption capacity of LF oysters does not reside in a reduced functional capacity of their digestive organs, but rather in a lowered filtering activity.

By contrast, HF oysters were able to maintain a constant AE (Figure 4) during both eutrophic scenarios. As a consequence, the amount of food absorbed (Figure 5) was increasing with eutrophication. It should be noted that the two eutrophic conditions tested in the present study have high organic matter content, at over 80% (Table 1). It has been also described in the literature (Bayne, 2017) that when the seston shows a low organic content, CR does not decrease with particle concentration but rather is maintained by the production of pseudofaeces. Under these conditions, pre-ingestive selection occurs at the labial palps and the inorganic seston particles are discarded through pseudofaeces, while the ingestion of organic particles increases (Iglesias et al., 1998). One of the characteristics of the phytoplankton blooms observed in the Mar Menor since 2016 is their high organic matter content, with values similar (∼80%) to those simulated in this study (Ruiz et al., 2020). It should be noted, however, that in the bloom of August 2021, the concentration and percentage of organic particulate matter in the seston was lower than the previous events (Ruiz et al., 2020), so further studies would be necessary to elucidate the CR response of oysters to an increase in the seston concentration with a lower organic content.

As mentioned before, the response of LF oysters to increases in particle concentration was different to the response in HF oysters. CR was not related to concentration (Figure 2; $R^2 = 2.30$, $P > 0.05$) and oysters filtered at a slow and constant rate regardless of the increase in food concentration. It seems that the functioning of the gills (the organs responsible for filtration) is limited by some unknown factor that prevents CR from increasing as the particle concentration decreases. In fact, differences in the CR between both oyster groups were higher at the lowest concentration (almost 3-fold) than at the highest concentration (2-fold). The existence of some pathogenic or toxic agent acting at the level of the gill cannot be ruled out.

4.3 | Biometric characterization of OMM

The health of bivalves is often assessed using the condition index (CI), a relationship between body mass (total tissues) and body size (shell

size). There is an extensive list of methods for determining CI in bivalves in the literature, with up to 19 methods having been retrieved by Zeng & Yang (2021) in their recent review. CI_s, is the most widely used CI as it is thought to provide meaningful information about the physiological state of the animal (Lucas & Beninger, 1985). CI_t, is less often used, but there are data in the literature on this index for the OMM for the years when the oyster population was abundant, in the early 1990s (Cano, Rosique & Rocamora, 1997).

Shell CI of OMM was very low in comparison with other *O. edulis* studies, at less than 0.9 for both groups. For instance, Pogoda, Buck & Hagen (2011) described a CI_s range from 1.8 to 7, values much higher than those obtained for the OMM. These large differences might be associated with the different life histories of the oysters, as the Pogoda study used hatchery-raised juveniles that were grown in suspension systems for several years. In contrast, the oysters used in this study were grown on the bottom of the lagoon and wild caught, so their age is unknown. Sawusdee et al. (2015) described higher CI_s in restored oysters maintained on an elevated experimental reef (up to 5) in comparison to those held on the sea bed, mainly due to the higher feeding rates of reef oysters. Lower CI_s (from 1.8 to 3.5 depending on the annual cycle) have been described in natural populations along the Spanish Atlantic coasts (Ruiz et al., 1992). Taking into account that CI_s is the ratio between two weights (meat and shell), it depends not only on the variation of the numerator (meat weight), but also on the denominator (shell weight). It might be possible that the low CI_s recorded in the OMM used in present experiment was related to their high shell weight due to unfavourable habitat or old age. Furthermore, previous literature about the OMM bed during the 1980s and 1990s described them as unappetizing (Abellán Martínez & García-Alcázar, 1991) due to the excessive thickening of their shells and the strong smell of silt due to the inappropriate characteristics of the sea bed of the Mar Menor (Rosique, 2006), as even then the bottom was already muddy and hypoxic.

When comparing the CI_t of oysters in the present study (~3) with values from Cano, Rosique & Rocamora (1997) during the 1990s, when the Mar Menor bed was composed of an active and dynamic oyster population, values reported here are similar to the lowest CI_t (~ 4) recorded after the spawning season (summer and early autumn). It is worth noting that, although our study was done in spring when oysters are at full sexual maturity and therefore have a higher condition, the long period of time in the laboratory may have uncoupled their gametogenic cycle from natural conditions.

Regardless of the CI used, it is surprising that the conditions of both groups of oysters were the same and no significant differences were observed in either of the two calculated indices (Table 4). Considering that the SFG estimate obtained under the oligotrophic conditions in *Exp I* (similar to those under which the oysters were maintained in the laboratory) was negative for LF, a lower condition index in these oysters would be expected. This surprising result, together with the previously discussed excessive shell development of the OMM, may indicate that neither of the two indices, which include shell weight, is sensitive enough to measure the different physiological states of these two groups of oysters. In fact, Lucas & Beninger (1985) recommended the use of a dynamic condition index that better expresses the physiological status of the animal, instead of a static one such as CI_s or CI_t that relate body mass to shell weight. One of the dynamic indices proposed by Lucas & Beninger (1985) is the SFG as measured in this study. SFG in bivalves is also used in marine pollution monitoring studies as an indicator of their good physiological state, which would reflect the environmental quality of the waters from which these bivalves have been extracted (Widdows & Staff, 2006; Albentosa et al., 2012b). According to the SFG, LF oysters can be considered to be in a poor condition due to the unexplained factors that should be discerned in future research.

4.4 | Genetic characterization of the OMM

Finally, genetic diversity levels found in the OMM were similar to those described for other European oyster beds (Sobolewska & Beaumont, 2005; Lallias et al., 2010; Vera et al., 2016). This suggests no depletion of genetic diversity giving it similar conservation status to other European beds. Moreover, the bed did not show significant deviations from HWE, and thus can be considered as a panmictic unit (i.e. random mating). Pairwise F_{ST} values between beds from different slopes were higher than the value described between European Atlantic beds using the same loci (F_{ST} = 0.0079; Vera et al., 2016), suggesting important differentiation between slopes. This observation strongly supports a Mediterranean origin of the OMM and not a Galician origin as previously speculated (García García et al., 1989). These differences among Iberian populations from both slopes have been described for different marine species, including fish (do Prado et al., 2018; Maroso et al., 2021), molluscs (Diz & Presa, 2008; Sromek et al., 2016) and *O. edulis* (Launey et al., 2002; Diaz-Almela et al., 2004). Moreover, the OMM bed was highly related to the ALI bed (also located on the Mediterranean slope), with no significant differences between these beds. This result is interesting for the management and conservation of the species in the Mar Menor region, allowing for the use of close populations in restoration efforts.

4.5 | Implications for future restoration actions

Studies analysing and modelling the carrying capacity for bivalve aquaculture have provided a useful theoretical framework to understand the impact of bivalves in the ecosystem and, more specifically, the capacity of bivalves to reduce the phytoplankton community and mitigate eutrophication (Small & van Duren, 2019). The extent to which bivalves can exert a top-down regulation on the phytoplankton biomass depends on the balance between the clearance time (CT), defined as the time it takes for the bivalves to filter the water body of a given area, and the water residence time

(RT), which represents the time it takes to renew the water body by the exchange of water from a defined area within the adjacent ecosystem (Smaal & Prins, 1993; Dame, 1996; Dame & Prins, 1998; Prins, Smaal & Dame, 1998). If the clearance ratio – defined as CT/RT by Dame & Prins (1998) is lower than 1 – bivalves filter the water column faster than water is renewed, therefore, bivalves potentially control pelagic processes through their grazing activity. The present study aimed to analyse the clearance potential of local *O. edulis* and the possibilities of using this organism as a tool for bioremediation of the Mar Menor lagoon. Considering the clearance rates recorded in the present experiments (1.73 and 1.15 L h^{-1} at low and high food concentrations, respectively), a putative re-establishment of an oyster population of 135 million specimens, equivalent to that which existed at the beginning of the 1990s (Rosique et al., 1993; Rosique, 1994), would result in a clearance time that would range from 107–161 days to filter the 600 million m^3 of water present in the lagoon. Such a CT value is substantially shorter than the water RT of the lagoon, which has been estimated to be 1 year (Albentosa & Galimany, 2018; Ruiz et al., 2020) and yields a clearance ratio that fluctuates between 0.29 and 0.44. Therefore, according to these indicators of ecosystem processes, the recovery of at least half of the oysters that once populated the Mar Menor would probably result in an effective top-down control of the phytoplankton community.

The quantity of gametes produced in bivalves depends on the quantity and quality of the food available, and consequently is influenced by the energy balance of the broodstocks (Utting & Millican, 1997; Maneiro et al., 2017). The HF oysters showed a positive SFG that may result in more energy available not only for growth but also allow the HF oysters to invest more energy in reproduction and therefore the reproductive output could potentially be better. We may expect that the positive energy balance observed in HF compared to LF oysters may result in a better quality of the gametes produced, resulting in a higher settlement success which would have a positive effect on the restoration and bioremediation. Therefore, HF oysters should be used as broodstock to achieve a higher success in the restoration and the ecosystem services provided by the oysters. The CR could therefore be used as a tool for selecting broodstock for seed production for both restoration and bioextraction actions.

Guidelines for oyster restoration and nutrient bioextraction actions recommend the use of local oysters as broodstock for seed production (Preston et al., 2020) to avoid the risks (introduction of pathogens, invasive non-native species, etc.) involved in the translocation of oysters from one location to another (zu Ermgassen, 2020). Moreover, local populations are better adapted to the particular environmental conditions of the site being restored. In the case of the Mar Menor, where different and successive environmental catastrophes have occurred, the oysters that have survived should have the biological characteristics that make them more resilient to future events. However, it remains to be seen whether it is possible to use oysters from the Mar Menor as broodstock to obtain seed. If it is not possible to use local oysters, the use of organisms from locations as close as possible to the site to be restored is recommended (Preston et al., 2020). The present study has shown the closer genetic proximity of oysters from the Mar Menor to the ALI population, located in the Mediterranean which actually supports a commercial oyster explotation operation (https://www.foodbevg.com/ES/Santa-Pola/469346293133245/Ostres-de-la-Badia). In the case where OMM cannot be reproduced, ALI oysters (more abundant than OMM) could be used as broodstock following the biosecurity procedures recommended in translocation actions (zu Ermgassen, 2020), especially those related to the introduction of pathogens such as *Bonamia*.

5 | CONCLUSIONS

Our study has revealed the existence of two categories of oysters (*O. edulis*) in the Mar Menor lagoon: (i) HF, which have a positive SFG; and (ii) LF, which have a negative SFG. The constraints in the absorption capacity of LF oysters does not reside in a reduced functional capacity of their digestive organs, but rather in a lowered filtering activity.

With the measurements performed in this study, it is not possible to explain the differences in the feeding behaviour of the two groups, so it is necessary to conduct pathological studies and further genetic studies in the future.

The OMM population developed in the 1990s (more than 135 million individuals) would result in a clearance time that would range from 107–161 days to filter the 600 million m^3 of water present in the lagoon. Thus, the recovery of even half of that population could result in an effective top-down control of the phytoplankton community and might act as a mitigation tool of eutrophication in the lagoon.

In the case of future bioremediation and/or restoration actions for the Mar Menor lagoon, a source of oysters is needed. If using OMM as broodstock, the use of the simple and quick measurement of clearance rate to distinguish HF from LF is recommended. HF oysters are recommended for breeding due to their higher and positive SFG.

The genetic results confirm the Mediterranean origin of the OMM. Thus, the Mediterranean population could be used as broodstock to produce the necessary seed in the event that the OMM could not be reproduced.

AUTHOR CONTRIBUTIONS

M. Albentosa: Conceptualization (lead); formal analysis (equal); funding acquisition (lead); project administration (lead); writing—review and editing (lead). **M. I. Akinyemi:** Data curation (supporting); investigation (supporting); writing—original draft (supporting). **M. Vera:** Conceptualization (supporting); formal analysis (equal); investigation (equal); writing—original draft (equal). **I. Ibarrola:** Conceptualization (equal); writing—review and editing (equal). **R. Filgueira:** Conceptualization (equal); writing—review and editing (equal). **E. Galimany:** Conceptualization (equal); writing—review and editing (equal). **F. da Costa:** Conceptualization (equal); writing—review and editing (equal). **B. G. Pardo:** Data curation (equal); investigation (equal).

M. Vázquez-Luis: Writing—review and editing (equal). **A. Hernández:** Writing—review and editing (equal). **S. Hernandis:** Writing—review and editing (equal). **P. Martínez:** Conceptualization (equal); funding acquisition (equal); writing—review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

All authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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