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# Food web in Mediterranean coastal integrated multi-trophic aquaculture ponds: Learnings from fatty acids and stable isotope tracers

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**Abstract :**

Ecosystem responsible aquaculture practices are today imperative to feed the world increasing population. The culture of extractive species such as oyster with fed species such as fish is a promising solution to recycle waste streams, which would be otherwise lost and discharged into the surrounding environment. In Mediterranean earthen ponds, meagre (Argyrosomus regius), white seabream (Diplodus sargus) and grey mullet (Mugil cephalus) are three fish species of different trophic levels that are good candidates to be raised in integrated multi-trophic aquaculture (IMTA) pond with oysters (Magallana gigas) and macroalgae (Ulva flexuosa). Trophic links between species raised in IMTA ponds were inferred using the analysis of body fatty acid composition as well as in carbon and nitrogen stable isotopes. A combination of 3 treatments was tested: fish, oysters, phytoplankton and macroalgae (T1); fish, oysters and phytoplankton (T2); fish, phytoplankton and macroalgae (T3). Our results highlighted that the three fish species were in competition for food sources as they mainly fed on commercial feed whatever the treatment. However, grey mullet also consumed polychaetes that naturally grown in ponds. Macroalgae were not used by fish. In T1 and T2, oysters actively consumed the excess of phytoplankton. In T2, the absence of macroalgae reduce nutrient competition for phytoplankton growth and oysters have more available food. . In earthen ponds, the production of fish with macroalgae or fish with oyster should be privileged compared to usual semi-intensive fish polyculture as the presence of extractive species reduced fish wastes.

**Highlights**

► Integrated Multi-Trophic Aquaculture is promising to preserve environment. ► Fish are good candidates to be raised with oysters and macroalgae. ► Trophic link between organisms were elucidated using fatty acids and stable isotopes ► The 3 fish species were in competition for food sources (commercial feed). ► Macroalgae removal reduce competition with phytoplankton that beneficiate to oyster.

**Keywords** : IMTA, Biochemical markers, Argyrosomus regius, Diplodus sargus, Mugil cephalus

## 1. Introduction

Human fish consumption has steadily increased of 3% per year since 1961 and in 2013 aquaculture has become the main fish supply (FAO, 2022). Ecosystem responsible aquaculture practices are today imperative to feed the world increasing population and ensure the viability of this sector (Naylor et al., 2009; Thomas et al., 2021). One of such practices is combining the culture of extractive species with fed species in the same mariculture sites and referred as Integrated Multi-Trophic Aquaculture (IMTA, Barrington et al., 2009; Chopin, 2006). Extractive species improve the environment by removing waste materials and lowering the nutrient load when farmed in the same enclosure with fed species. Extractive species perform a truly *in-situ* bio-mitigation of waste streams which would be otherwise lost and discharge into the surrounding environment. For example, the introduction of macroalgae helps to reduce level of inorganic nutrients and can be an additional food source for herbivorous or omnivorous fish (Chopin et al., 2001). Another example are bivalves (oysters, mussels…) that actively consume suspended particulate organic matter (SPOM). In addition to environmental benefits, the presence of extractive species in the fishponds, can increase both the profits of farmers and social acceptance of their production systems (Barrington et al., 2009).

Marine semi-intensive fish culture in ponds is the main production system used in southwestern European countries. Such ponds can be compared to confined lagoon environments with sporadic communication with the sea. Excess amounts of particulate (i.e. feces and unfed nutrients) and dissolved (i.e. nitrogen and phosphorus) nutrients produced by aquaculture activities are considered to be one of the main sources of pollution in coastal environments as waste are directly discharged into shallow coastal waters

(Holmer et al., 2003; Tovar et al., 2000). Dissolved nutrients released in seawater are mainly assimilated by primary producer conducting to a massive development of phytoplankton and macroalgae leading to imbalanced environment where eutrophication events are observed with the death of some resident populations (Carballeira Braña et al., 2021). These are events that prompted the necessity for understanding the ecological processes within the pond ecosystems and to help manage the IMTA farming. For instance, little is known about trophic relationships and nutrient fluxes between organisms living together in IMTA ponds whereas the concept of IMTA is based on trophic species complementarity. To help farmers to develop sustainable IMTA systems, the understanding of complex trophic interactions between fed organisms and extractive species is necessary. Species assemblage must be adapted depending on natural productivity and biodiversity of ponds. The understanding of trophic interaction between species and environment could be facilitated through the development of theoretical ecological models (Gamito et al., 2020; Thomas et al., 2021) but models require previous knowledge of the trophic relationship and nutrient fluxes between organisms living together in IMTA ponds which is scarcely known (Cunha et al., 2019a).

Trophic relationship between organisms in IMTA ponds can be elucidate using two independent and complementary methods: fatty acid (FA) profiling and carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope analyses. While both of these approaches have limitations, the combination of these techniques has proved to be a powerful tool to determine trophic interactions within complex food web (Boecklen et al., 2011; Dalsgaard et al., 2003). These techniques are based on the principle that an animal's diet is reflected in the patterns of FA, $\delta^{13}C$ and $\delta^{15}N$ of their tissues. They provide information on the diet integrated over a period of time and allow quantitative diet analysis that is an important advantage compared to

older methods such as stomach contents analysis. The use of FA is based on the concept that food sources lay down certain FA patterns that may be transferred conservatively to consumers (Dalsgaard et al., 2003). For example, fish commercial feed contain specific FA that are transferred and conserved along the trophic chain allowing their use as trophic tracers (Baltadakis et al., 2020; Redmond et al., 2010). Analyzing the FA profile of consumers enables to trace predator-prey relations by direct comparison of FA profiles. The combined analysis $\delta^{13}C$ and $\delta^{15}N$ values is a common method to elucidate trophic structure and for inferring energy and matter flows in food webs (Middelburg, 2014). The use of natural stable isotopes assumes that isotope values of consumers reflect those of assimilated dietary sources. The $\delta^{13}C$ values of consumer tissues are usually similar to those of their diets, which helps to identify the origin of food sources (DeNiro and Epstein, 1978). In contrast, $\delta^{15}N$ values become enriched from a prey to consumer and thus are typically used to estimate the trophic position of the consumer (Minagawa and Wada, 1984). The use of both methods offers a reliable approach for examining trophic interactions in IMTA ponds but food sources must have different FA profiles as well as $\delta^{13}C$ and $\delta^{15}N$ values.

Meagre (*Argyrosomus regius*), white seabream (*Diplodus sargus*) and grey mullet (*Mugil cephalus*) are three fish species of different trophic levels that are good candidates to be raised in a fed IMTA pond with oysters (*Magallana gigas*) and macroalgae (*Ulva flexuosa*) (Cunha et al., 2019a). This study was conducted in marine Mediterranean ponds submitted to annual physico-chemical variations to be close to the conditions encountered by farmers form these regions. The present work investigated the expected trophic links between organisms cohabiting in IMTA ponds using FA as well as $\delta^{13}C$ and $\delta^{15}N$ values (Fig. 1). The main objective of this study was to establish the diets of the three fish species by varying the presence of macroalgae or oysters, having an impact on the availability of

nutrients in the environment. We expected that meagre and white seabream two carnivorous species only fed on commercial feed whereas grey mullet an omnivorous fish fed on commercial feed, natural occurring polychaetes and macroalgae. Food preferences of oyster were also elucidated to better understand their role to bio-mitigate waste streams. Our hypothesis was that oyster, a filter feeder, beneficiated of suspended particulate organic matter composed by a pool of phytoplankton, zooplankton, fish faeces and unfeed fed.

## 2. Material and methods

### *2.1. Experimental design*

From April to November 2016, the experimental trial was performed at the Aquaculture Research Station of the Portuguese Institute of the Sea and Atmosphere (37º 02´ N; 07º 49´W) in six rectangular earthen ponds with 750 $m^3$ of water volume (500 $m^2$ surface and a mean depth of 1.5 m). All ponds were operated in a semi-intensive system with continuous water renewal using unfiltered water from Ria Formosa (a mesotidal coastal lagoon) collected in a reservoir and pumped to the ponds. The water quality in the reservoir is naturally improved by the presence of seagrass that dominate the bottom and retain particulate matter on their leaves and sediment, and nutrients in their tissues (De los Santos, et al., 2020). Daily water renewal ranged between 60 to 150% according to temperature and feeding rate. Three IMTA treatments with different functional groups were compared in duplicated ponds. The combinations in each treatment were as follow: treatment 1 (T1) fish + oysters + phytoplankton + macroalgae; treatment 2 (T2) fish + oysters + phytoplankton; treatment 3 (T3) fish + phytoplankton + macroalgae. The three treatments were composed by three fish species of different trophic levels: a carnivore (meagre, *Argyrosomus regius*), an omnivore (white seabream, *Diplodus sargus*) and an omnivore/detritivore (grey mullet, *Mugil cephalus*). The

fish, reared at the Aquaculture Research Station, were pre-fattened in similar ponds to be well adapted to the environment. Fish were daily fed with a commercial diet at an average 1.5% body weight during all the trial. In T1 and T2, commercial seeds of an organic extractive species (the filter feeder oyster, *Magallana gigas*) were added in each pond after 24h acclimation to the pond conditions. They were farmed in traditional oyster mesh bags suspended close to the surface were air exposed every week for 24h to avoid biofouling. Zootechnical parameters of fish and oysters are presented Table 1. Inorganic extractive species that naturally developed in the ponds were phytoplankton and macroalgae. In the treatments with macroalgae (T1 and T3), the autochthonous sea lettuce (*Ulva flexuosa*) was cultivated in 6 rafts of 1 $m^2$ each at a density of 30 g and the other two macroalgae naturally present in ponds (*Ulva* spp. and *Rizoclonium riparum)* were let grown. In T2, all natural floating macroalgae were manually removed with the help of a fishing net every week. Macroalgae over the bottom was not removed to avoid disturbances to the system (sediment resuspension and fish stress). For detailed methodology in the rearing system see Cunha et al. (2019b) and in the *Ulva* sp. cultivation see Favot et al. (2019). Environmental parameters (i.e., water temperature, dissolved oxygen, pH, salinity and water turbidity) were monitored twice a day and results are available in Cunha et al. (2019a).

### *2.2. Sampling collection*

To study trophic interaction within the IMTA ponds, all reared organisms (i.e., fish, oyster and *U. flexuosa*), the main food source (i.e., commercial feed) as well as natural food sources available in the ponds (i.e., suspended particulate organic matter including phytoplankton, large zooplankton, polychaetes, *Ulva spp.* and *R. riparium*) were sampled. Sampling was performed at the end of the 9 months of experimentation in all six ponds. From each ponds, three specimens of each fish species were individually euthanized by

immersion in a water bath containing an excess of benzocaine (Nahon et al., 2017). Fish were euthanized according to EU legal frameworks, relating to the protection of animals used for scientific purposes (*i.e.* Directive 2010/63/EU). Animals were considered dead following cessation of opercula movement. After death, white dorsal muscle tissue was dissected from above the lateral line. Oysters were collected in triplicate on culture bags from T1 and T2. Total flesh of oyster was separated from the shells. In each pond, two pools of polychaetes were collected in the first 20-30 cm sediment layer using a shovel. Polychaetes were considered in their entirety and individuals were not identify at species level. Suspended organic particulate matter (SPOM, a bulk of matter including phytoplankton, small zooplankton and fish waste (i.e. feces and feed debris), were collected in triplicate with a bucket (4 L) in each pond and pre-filtered through a 200 µm mesh to remove large particles. SPOM were then recovered by filtration on pre-combusted (5h, 450°C) Whatman GF/F filters. Large zooplankton was sampled in triplicate in each ponds using a 200 µm net pulled horizontally around each pond, removed from the cod-ent, and concentrated into tubs. Cultured sea lettuce (*U. flexuosa*) was collected on the raft structures whereas natural occurring macroalgae (*Ulva* spp. and *R. riparium*) were collected from stones along the pond's margins. The whole macroalgae were conserved. *Ulva* spp. that naturally grown in ponds were not identify at the species level. All samples were carefully rinsed with ultrapure water (milliQ®; MerckMillipore, Molsheim, France), frozen, freeze-dried and stored at -80°C until FA and stable isotope analyses. Before analysis, samples were ground to a fine homogeneous powder using a Precellys® grinder mill (Bertin Technologies, Montigny-le-Bretonneux, France).

*2.3. Fatty acid analysis*

FA were extracted and methylated on a sub-sample of each sample using a direct acid transesterification procedure described by Lewis et al. (2000) and Indarti et al. (2005). Briefly, 50 to 500 mg of powder (depending of FA richness in each type of samples) or half filters for SPOM were placed in Teflon-lined screw cap vials with 4 ml of a transesterification solution [methanol:sulfuric acid:dichloromethane (1.7:0.3:2, v/v/v)]. Dichloromethane was supplemented with an antioxidant (50 µg $ml^{-1}$ of buthylhydroxytoluene, (Christie and Han, 2012). Samples were flushed with $N_2$, vortexed and then, placed in a preheated oven at 90°C during 90 min. The extraction vials were allowed to cool before the addition of 1 ml of MilliQ water. The extracts were centrifuged (1328 g, 5 min, 4°C) and the lower organic phase containing the FA methyl esters (FAMEs) was transferred in another tube. The upper aqueous phase was rinsed with 2 mL of heptane-dichloromethane (4:1) and centrifuged (1328 g, 5 min, 4°C). This procedure was repeated twice, and the pooled organic phases were rinsed with a 2% solution of potassium carbonate (4 ml). After centrifugation (1328 g, 10 min, 4°C), an aliquot of the organic phase (6 ml) was evaporated under a gentle $N_2$ flush, and FAMEs were re-dissolved in 100 to 1000 µl of heptane prior to analysis. Fatty acids as methyl esters were analysed using a Varian Star 3900 CP gas chromatograph coupled with a flame ionization detector. One microliter of sample was injected in a split/splitless injector maintained at 260 °C with a split ratio of 100:1. The carrier gas was helium (constant column flow 1 ml $min^{-1}$). FAME separation was performed on a DB wax column (30 m × 0.25 mm ID, 0.25 µm thickness) from Agilent using the following temperature program: 100°C-180°C at 8°C $min^{-1}$, 180-220°C at 4°C $min^{-1}$ and a constant temperature of 220°C during 20 min. FAMEs in samples were identified by comparison of retention times with commercial standards: Supelco 37, PUFA

n°3, BAME (SUPELCO, France) and C16:3ω4 from Cayman Chemical Compagny. Individual FA were expressed as a percentage of total FAME identified.

*2.4. Carbon and nitrogen stable isotope analysis*

Analysis of $\delta^{13}C$ values of animal tissues may require lipid extraction when C/N ratios are superior to 3.5 as lipids are naturally $^{13}C$-depleted (DeNiro and Epstein, 1977; Post et al., 2007). Prior performing stable isotope analysis, preliminary tests have been carried out to determine C/N ratios of untreated samples as a potentially good predictor of the influence of lipids on $\delta^{13}C$ values. As C/N ratios were not significantly higher than 3.5 for meagre (3.27 ± 0.23, n = 18), white seabream (3.78 ± 0.45, n = 18) and grey mullet (3.34 ± 0.23, n = 18), lipid extraction was not necessary and approximately 1 mg of each fish sample was directly weighed and packed into tin capsules for simultaneous analysis of carbon and nitrogen stable isotopes. C/N ratios of oyster, polychaetes and large zooplankton were 4.66 ± 0.55 (n = 12), 4.36 ± 0.43 (n = 12) and 4.38 ± 0.76 (n = 18), respectively. The need for lipid extraction have been evaluated on a case-by-case basis as some studies have showed that it was not always pertinent when C/N ratios are superior to 3.5 (Chouvelon et al., 2014). Samples of oyster, polychaetes and large zooplankton have been randomly selected to test the influence of lipids on $\delta^{13}C$ values. In order to remove naturally $^{13}C$-depleted lipids, samples were treated with cyclohexane as described by Chouvelon et al. (2014). For each sample, approximately 20 mg of powder were weighted in glass vials. A volume of 4 ml of cyclohexane was added and after 1 hour, samples were centrifuged (4000 g, 10 min, 10°C). The supernatant was discarded, and the procedure was repeated twice. Samples were then dried in a dry bath at 45°C before isotopic analysis. This method has been chosen as it does

not impact $\delta^{15}N$ values (Chouvelon et al., 2014), compared to commonly used chloroform-methanol or dichloromethane-methanol mixtures (Post et al., 2007; Schlechtriem et al., 2003). Approximately 1 mg of sample with or without lipids was weighed and packed into a tiny capsule for simultaneous analysis of $\delta^{13}C$ and $\delta^{15}N$. Results of these preliminary tests showed that lipid extraction was necessary to assess $\delta^{13}C$ of oyster and polychaetes but not for zooplankton. Differences between $\delta^{13}C$ values with and without lipid were -1.17 ± 0.16‰ and -0.70 ± 0.10‰ for oyster and polychaetes respectively, (t-test, $p < 0.01$) and 0.37 ± 0.52‰ for large zooplankton (t-test, $p > 0.05$). After lipid extraction, approximately 1 mg of oyster or polychaete tissue was weighed and packed into tiny capsules for simultaneous analysis of carbon and nitrogen stable isotopes. Other samples (i.e. large zooplankton, macroalgae, SPOM and food) were packed without treatment. $\delta^{13}C$ and $\delta^{15}N$ values of samples were analyzed by an Euro EA3000 (Pavia, Italia) elemental analyzer coupled with a GVI Isoprime (Manchester, England) isotope ratio mass spectrometer used in continuous-flow mode. The $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratios are expressed in conventional delta notation in per mil (‰) relative to the levels of $^{13}C$ in Vienna Pee Dee Belemnite and $^{15}N$ in atmospheric air. Repeated measurements on alanine exhibited a precision of ± 0.11‰ and ± 0.12‰ for $\delta^{13}C$ and $\delta^{15}N$ values, respectively. Commercial standards, alanine, wheat flour and corn flour from IsoAnalytical Lab (Crew, United Kingdom), IAEA-N-1, IAEA-N-2 and IAEA-CH3 cellulose and USGS24 graphite from National Institute of Standard and Technology (Gaithersburg, USA) were used for a multipoint calibration.

### *2.5. Statistical analysis*

Only FA representing more than 1% of total FA were considered for data analysis. The global FA composition of fish, oyster and their potential food sources was represented through non-metric multidimensional scaling (nMDS) based on Euclidean distances. The effects of species and treatments on FA composition were assessed through permutational variance analyses (PERMANOVA, Kelly and Scheibling, 2012). Similarity-based statistical techniques were used for FA data because they do not require homogeneity of covariances or multivariate normality and can be used with a large number of variables (Clarke, 1993). $\delta^{13}C$ and $\delta^{15}N$ values of fish, oyster and their potential food sources were compared using non-parametric Kruskal-Wallis (KW) tests, followed by Conover-Iman multiple comparison tests with Bonferroni's adjustment method as residuals were not normal nor homoscedastic (tested with Shapiro-Wilk and Bartlett tests, respectively). The contribution of food sources (i.e., feed, SPOM, large zooplankton, *Ulva* spp., *R. riparium*, *U. flexuosa* and polychaetes) to fish and oyster diets were estimated using Bayesian stable isotope mixing models (*simmr* package of R software, Parnell et al., 2013). For all sources, carbon and nitrogen trophic discrimination factors used were respectively 1.74 ± 1.09‰ and 3.5 ± 1.28‰ for fish (Sweeting et al., 2007b, 2007a); respectively 1.9 ± 0.2‰ and 3.8 ± 0.2‰ for oyster (Dubois et al., 2007). The models were run considering the concentration of carbon and nitrogen in each food sources of fish. This was not the case for oyster since such concentrations were not estimated for SPOM. All statistical analyses and graphics were performed with the free software R (Core Team, 2017) and the R Version 3.4.1 (2017 06 30).

## 3. Results

Percentage of total fatty acid as well as $\delta^{13}C$ and $\delta^{15}N$ of fish, oyster and all potential food sources in T1 (fish, oyster, phytoplankton and macroalgae), T2 (fish, oyster and phytoplankton) and T3 (fish, phytoplankton and macroalgae) are presented in supplementary 1 to 11.

### *3.1. Fatty acid composition of fish, oyster and their potential food sources*

#### *3.1.1. Potential food sources of fish and oyster*

FA profiles of potential food sources of fish and oyster were significantly different (PermANOVA, $p < 0.001$, Fig. 2 and Table 2). Excepted for large zooplankton, no differences between treatments were showed (PermANOVA, $p > 0.05$, Supplementary Tables 1 to 7). The commercial feed contained 9 FA with a contribution superior to 1% of total FA (Supplementary Table 1). In commercial feed, the main FA were the saturated fatty acid (SFA) C16:0, the monounsaturated fatty acid (MUFA) C18:1ω9 and the polyunsaturated fatty acid (PUFA) C18:2ω6. The C14:0, C18:0, C16:1ω7, C18:1ω7, C18:3ω3 and C22:6ω3 occurred in lower proportion (between 1.5 and 4%, Table 2). SFA, MUFA and PUFA represented 29%, 26% and 45% of total FA, respectively. Polychaetes sampled in the three IMTA ponds belonged to three different genera (*Diopatra* spp., *Marphysa* spp. and *Nereis* spp). FA composition of polychaetes was relatively similar to that of commercial feed with significant proportion of C18:1ω9 and C18:2ω9 (Fig. 2, Table 2). However, polychaetes contained higher diversity and proportion of PUFA as well as

significant lower proportion of C16:0 compared to commercial feed. Polychaetes were characterized by significant proportion of PUFA C16:4ω3, C18:2ω6, C20:2ω6, C20:4ω6, C20:5ω3, and C22:6ω3. Minor PUFA such as C21:5ω3, C22:2ω6 and C22:4ω6 were only measured in polychaetes. The three macroalgae species exhibited low proportions of C18:0 and C18:1ω9 as well as high proportions of MUFA C18:1ω7 and PUFA C16:4ω3, C18:2ω6, C18:3ω3 and C18:4ω3. However, despite similarity between FA composition of *Ulva* spp., *U. flexuosa* and *Rizoclonium riparium*, proportions of main FAs were significantly different, especially their proportions of PUFA C18:2ω6, C18:3ω3 and C18:4ω3 (PermANOVA, $p < 0.001$, Table 2 and Fig. 2). SPOM contained a total of 19 FA with a contribution superior to 1% of total FA that were similar between T1 and T2 (PermANOVA, $p > 0.05$, Supplementary Table 6). The main FAs were C16:0, C18:0 and C18:1ω9 (Table 2). SPOM was characterized by high proportion of bacterial FAs with C15:0, C15:0 iso and ante, C16:0 iso, C17:0, C17:1ω7 and C18:1ω7 represented 16% of total FAs, C14:0 and C16:1ω7. The ratios C16:1ω7/C16:0 and C20:5ω3/C22:6ω3 were lower than 1. In SPOM, proportion of PUFA was lower and proportion of SFA was higher compared to other potential food sources. FA profile of SPOM was very different that those of feed pellet (Table 2, Fig. 2). Large zooplankton contained a high proportion of SFA C16:0 and C18:0, MUFA C18:1ω7 and C18:1ω9 and PUFA C18:2ω6, C20:2ω6, C20:5ω3 C22:6ω3 (Table 2). Large zooplankton contained higher proportion of PUFA compared to other food sources. In fact, FA profile of large zooplankton were intermediate between those of SPOM and feed pellets (Table 2, Fig. 2). FA profile of zooplankton from T2 was significantly different than that from T1 (Supplementary Table 7). In T2, the proportion of C18:2ω6 was significantly higher than in T1 (PermANOVA, $p < 0.001$).

*3.1.2. Fish and oyster*

Whatever the treatment, FA composition of meagre, white seabream and grey mullet was similar among species with a total of 16 FA identified with a contribution higher to 1% of total FAs (Fig. 3). FA composition of fish was similar to that of feed pellets with SFA C16:0 and C18:0, MUFA C18:1ω9 and PUFA C18:2ω6 and C22:6ω3 largely predominant overall the FA pools (Fig. 3). However, FA proportions significantly varied between fish species leading to a good discrimination (PermANOVA, $p < 0.001$, Fig. 2 and 3). In the 3 treatments, MDS representation showed that FA profiles of meagre and white seabream were closer from commercial feed than that of grey mullet. In fact, FA profile of grey mullet was intermediate between those of commercial feed and polychaetes. Grey mullet had lower proportions of C18:1ω9, C18:2ω6 and C18:3ω3 and higher proportion of C20:4ω6, C20:5ω3, C22:5ω3 and C22:6ω3 than those of meagre and white seabream (Fig. 3). A similar pattern was observed when FA proportions from polychaetes were compared to those from commercial feed. In T1 and T3, with macroalgae, FA profile from the three fish species was very different than that from macroalgae (Fig. 2). Moreover, C16:4ω3, a FA found in high proportion in macroalgae, was not detected in fish. In white seabream, FA proportions were similar between the three treatments (PermANOVA, $p > 0.05$, Supplementary Table 9). For meagre and grey mullet, slight differences of FA proportions appeared between T1 and T2 as well as T1 and T3 (PermANOVA, $p < 0.05$, Supplementary Table 8 and 10). Meagre from T2 and T3 had slightly higher proportion of C22:6ω3 than those from T1 (7% and 5% respectively, PermANOVA, $p < 0.001$). For grey mullet, differences between treatments were due to the proportions of SFA C14:0, C18:0, MUFA C16:1ω7, C18:1ω9 and PUFA C20:4ω6 and C22:6ω3. The mean concentration of PUFA

C22:6ω3 was significantly higher in T1 (15 %) than in T2 and T3 (7%, PermANOVA, $p < 0.001$).

Oyster contained a total of 16 FA with a contribution superior to 1% of total FA (Supplementary Table 11). SFA proportion (47%) with C16:0, C14:0, C17:0 and C18:0 was relatively high. The proportion of MUFA was 17% with a higher contribution of C16:1ω7, C18:1ω7 and C18:1ω9 whereas the proportion of PUFA was 36% with a higher contribution of C18:2ω6, C18:3ω3, C18:4ω3, C20:5ω3 and C22:6ω3. FA composition of oyster were closed to those of SPOM and large zooplankton and farther to those of feed pellets (Fig. 2). As for large zooplankton, FA proportions differed between T1 and T2 (Fig. 4, PermANOVA, $p < 0.001$). In T1, oysters had higher proportion of MUFA and lower proportions of SFA and MUFA than in T2.

### *3.2. Carbon and nitrogen stable isotope values of fish, oyster, and their potential food sources*

#### *3.2.1. Potential food sources of fish and oyster*

$\delta^{13}C$ and $\delta^{15}N$ values significantly differed between food sources (Fig. 5, Supplementary Table 1 to 7, KW test, $p < 0.001$). $\delta^{13}C$ and $\delta^{15}N$ values of food sources ranged from -23.54 ± 0.25‰ for commercial feed to -12.61 ± 0.24‰ for *U. flexuosa* and from 6.41 ± 0.24‰ for commercial feed to 9.81 ± 0.52‰ for polychaetes, respectively (Fig. 5). Commercial feed was $^{13}C$- and $^{15}N$-depleted compared to polychaetes and macroalgae (Post Hoc tests, $p < 0.05$). Polychaetes were significantly $^{13}C$-depleted and $^{15}N$-enriched compared to the three macroalgae species (Post Hoc test, $p < 0.05$). The three macroalgae

species had similar $\delta^{15}N$ values (KW test, $p > 0.05$) but $\delta^{13}C$ values significantly differed between *R. riparium* and *U. flexuosa*. *R. riparium* was significantly $^{13}C$-depleted compared to *U. flexuosa*. $\delta^{13}C$ and $\delta^{15}N$ values did not significantly differed between the three treatments for the three macroalgae (KW test, $p > 0.05$). In T1 and T2, SPOM and large zooplankton had similar $\delta^{13}C$ values (Post Hoc test, $p = 0.05$) but different $\delta^{15}N$ values (Post Hoc test, $p < 0.01$). $\delta^{15}N$ values of SPOM were significantly lower than those of large zooplankton. For SPOM, $\delta^{13}C$ and $\delta^{15}N$ values were significantly different between T1 and T2 (Wilcoxon test, $p < 0.01$). For large zooplankton, only $\delta^{15}N$ values differed between treatments. In T1, $\delta^{13}C$ values of commercial feed were lower than those of SPOM and large zooplankton (Post Hoc test, $p < 0.01$) whereas $\delta^{15}N$ values of commercial feed and SPOM were similar (Post Hoc test, $p > 0.05$) but $^{15}N$-depleted compared to large zooplankton (Post Hoc test, $p < 0.01$). In T2, $\delta^{13}C$ values of commercial feed were similar to those of SPOM and large zooplankton (Post Hoc test, $p > 0.05$). $\delta^{15}N$ values of commercial feed were similar than those of zooplankton (Post Hoc test, $p > 0.05$) but higher than those of SPOM (Post Hoc test, $p < 0.01$). $\delta^{13}C$ and $\delta^{15}N$ values of oyster significantly differed between treatments (Wilcoxon test, $p < 0.01$).

*3.2.2. Fish and oyster*

$\delta^{13}C$ and $\delta^{15}N$ values significantly differed between fish species (KW test, $p < 0.001$, Fig. 5, Supplementary Table 8 to 10). Grey mullets were $^{13}C$-enriched compared to meagre and white seabream (Post Hoc test, $p < 0.05$); white seabream were $^{15}N$-enriched compared to meagre and grey mullet. Within each fish species, no differences of both $\delta^{13}C$ and $\delta^{15}N$ values were observed between treatments (KW test, $p > 0.05$). Mixing model indicated that

commercial feed was the main source used by the three fish species (Fig. 6). Commercial feed contributed to 71 ± 4%, 75 ± 6% and 52 ± 5% to the diet of meagre, white seabream and grey mullet, respectively. Contribution of polychaetes and the three species of macroalgae to the diet of meagre and white seabream were inferior to 15%. For grey mullet, contribution of polychaetes to their diet was estimated to 22 ± 10% whereas contribution of the three macroalgae species were estimated to be less than 10%.

Oysters from T1 were significantly $^{13}C$- and $^{15}N$-enriched compared to oysters from T2 (Fig. 5, Supplementary Table 11). Mixing model indicated that SPOM was the main source of feed used by oyster in both treatments with a contribution to the diet from 48 ± 17% and 71 ± 22% in T1 and T2, respectively (Fig. 7). In T1, commercial feed and large zooplankton contributed to 36 ± 14% and 15 ± 11% to the diet of oyster, respectively. In T2, commercial feed contributed to 11 ± 9% and large zooplankton to 18 ±18% of the diet of oyster.

## 4. Discussion

In the present work, the food preferences of meagre (*Argyrosomus regius*), white seabream (*Diplodus sargus*) and grey mullet (*Mugil cephalus*) raised in three different IMTA ponds were elucidated using FA as well as $\delta^{13}C$ and $\delta^{15}N$ analysis. Fish diets were compared when fish were reared in ponds together with phytoplankton, macroalgae and oysters (treatment 1, T1); just with phytoplankton and oyster (no macroalgae, treatment 2, T2); and with phytoplankton and macroalgae (no oyster, treatment 3, T3). Food preferences of oyster were also discussed to better understand their role to bio-mitigate waste streams.

### *4.1. Fish diet*

FA profiles as well as $\delta^{13}C$ and $\delta^{15}N$ values of meagre and white seabream highlighted that the three fish species were mainly fed on commercial feed whatever the treatment. FA composition of meagre and white seabream was very similar to that of commercial feed with high proportions of C18:1ω9 and C18:2ω6 as well as low proportions of nutritionally important C20:5ω3 (eicosapentaenoic acid, EPA) and C22:6ω3 (docosahexaenoic acid, DHA). Soybean oil, known to contain approximately 50% of C18:2ω6, 20% of C18:1ω9 and neither EPA nor DHA (Rombenso et al., 2016), was the main source of oil present in commercial feed (oil composition indicated by the supplier: 10% of soybean oil and 1% of fish oil). This result confirms that marine carnivorous fish incorporate in their tissues FA from their diets with little or no modification (Sargent et al., 2003). Fish have limited ability to convert C18:2ω6 to ω3 long chain PUFA, such as EPA and DHA, due to the weak activity of fatty acyl desaturases (Castro et al., 2016; Xie et al., 2013). Meagre and white seabream were enriched by 2.84‰ and 2.67‰ in $^{13}C$ and by 3.99‰ and 4.81‰ in $^{15}N$, respectively, compared to commercial feed. Similar high trophic discrimination factor (+ 5.91‰) have been recorded for red seabream (*Pagrus major*) fed with commercial feed in IMTA pond (Park et al., 2021). In our experiment, commercial feed were mainly composed by plant based ingredients that are known to increase trophic discrimination factor compared to animal based ingredients (Nahon et al., 2020b). Results of mixing model indicated that commercial feed contributed to most of meagre and white seabream diets (more than 70%). However, carbon and nitrogen trophic discrimination factors were probably higher than those used in the mixing models (i.e., $1.74 \pm 1.09$‰ and $3.5 \pm 1.28$‰ respectively, Sweeting et al., 2007b, 2007a). A multiplicity of factors (e.g., environment, taxon, fish size, diet…) strongly affects trophic discrimination factor and despite incorporating standard deviation

to trophic discrimination factor, results of mixing models are still sensitive to the values used for trophic discrimination factor (Parnell et al., 2013). The contribution of commercial feed to meagre and white seabream diets should be underestimated whereas the contribution of macroalgae should be overestimated by the mixing model.

In natural environment, adult meagre is considered to be a carnivorous species mainly consuming hyper benthos such as crustaceans, fish, and polychaetes (Pasquaud et al., 2010). However, meagre have high trophic plasticity giving them the ability to adapt their diets to new environments (Valero-Rodriguez et al., 2015). White seabream is also a carnivorous species consuming benthic copepods, amphipods and polychaetes (Ventura et al., 2017). In the three treatments, FAs profiles of meagre and white seabream were farther to those of polychaetes than commercial feed. Carbon and nitrogen stable isotope analysis confirmed that meagre and white seabream consumed low proportion of polychaetes (around 13% for both fish species according to the mixing model). Commercial feed supplied every day was enough to cover nutritional needs of both fish species without benefiting of resources naturally produced by IMTA ponds. The supply of commercial pellets, that represent a high cost for farmers in pond aquaculture, should be reduced to force fish to complete their diets with natural resources present in ponds. In T1 and T3, meagre and white seabream tissues did not contain C16:4ω3 a FA found in high proportion in the three species of macroalgae. $\delta^{13}C$ and $\delta^{15}N$ values of fish tissues were highly depleted in $^{13}C$ compared to macroalgae. Both biochemical markers indicated that macroalgae were not consumed by both fish species.

FA profile of grey mullet was intermediate between the FA profile of commercial feed and those of polychaetes. $\delta^{13}C$ and $\delta^{15}N$ values of grey mullet tissues confirmed that grey mullet fed on both commercial feed and polychaetes (52% and 22%, respectively according

to mixing model). Polychaetes were not identify at the level species. We made the hypothesis that all species were indifferently consumed by grey mullets. FA profiles of polychaetes were close to that of commercial feed with high level of C18:1ω9 and C18:2ω6 indicating that polychaetes probably beneficiated of unfed food that settled and accumulated on surface sediments of IMTA ponds. Unfortunately, FA profile of sediment that will confirmed this hypothesis was not available. Salvo et al. (2015) showed similar results from the opportunistic polychaetes (*Ophryotrocha cyclops*) most likely consuming fish pellets when living in sediments of salmonids aquaculture sites. Compared to commercial feed, polychaetes contained lower proportion of C18:2ω6 and higher levels of PUFAs especially C20:2ω6, DHA and EPA. This result confirms previous studies showing that FA profile of polychaetes reflected FA profile of their diet with higher proportions of essential PUFA *de novo* biosynthesized (Bischoff et al., 2009; Jerónimo et al., 2021; Yousefi-Garakouei et al., 2018). We noticed that polychaetes were highly $^{13}C$-enriched (around 4.4‰) when compared to commercial feed indicating they should beneficiate from other food sources present in sediments such as fish feces, bacteria, and microphytobenthos. The recovery of nutrients from unfed commercial feed by deposit feeders such as polychaetes offers two major advantages. The first is to remove excess of particulate organic material that would be lost to the environment and negatively impact adjacent aquatic ecosystems (Edwards, 2015). The second is to recycle wasted nutrients in new food source for fish, moreover enriched in DHA and EPA (Marques et al., 2018; Nederlof et al., 2019). Grey mullet tissues contained higher proportion of DHA and EPA than meagre and white seabream. Polychaetes bioremediation increased nutritional value of grey mullet for human consumption. The lack of essential PUFA when replacing fishmeal and fish oil derived from wild fish by plant sources could be counterbalanced by the polychaetes

bioremediation in IMTA ponds. In T1 and T3, grey mullet did not consume macroalgae since specific FA found in macroalgae were not detected in their tissues. Contrary to meagre and white seabream, this result was surprising since adult grey mullet is known to be omnivorous consuming diverse items such as algae, plant materials, annelids, crustaceans, bivalves, and detritus (De Silva and Wijeyaratne, 1977; Soyinka, 2008). In IMTA ponds, in which nutritional rich commercial feed was available, grey mullet did not consume macroalgae. Similar results were found in IMTA ponds for the omnivorous carps (*Cyprio carpus*) preferring commercial feed, with higher nutritional value, compared to poor natural food sources such as macroalgae (Nahon et al., 2020a; Schultz et al., 2012).

Our results supported trophic interactions modeled by Gamito et al. (2020) using Ecopath with Ecosim (EwE software, Christensen, 1998). This study, based on the same three IMTA treatments, showed that meagre and white seabream mostly fed on commercial feed whereas grey mullet fed both on commercial feed and polychaetes. Meagre and white seabream had higher $\delta^{15}N$ values than grey mullet, confirming their higher trophic level in the three IMTA ponds (Hobson and Welch, 1992; Post, 2002). Grey mullet exploiting commercial feed and natural polychaetes is a good candidate to be co-raised in IMTA ponds with carnivorous fish. On the other hand, meagre and white seabream were in competition for commercial feed. The presence of macroalgae and/or oyster did not affect trophic preference of the three fish species. However, the role of macroalgae is crucial in IMTA ponds to enhanced water quality that lead to improve fish growth and performance in T1 and T3 compared to T2 (Table 1, Cunha et al., 2019a).

### *4.2. Oysters feed*

Oysters are active filter feeders known to consume a fraction of SPOM mainly composed by plankton and bacteria (Coutteau and Sorgeloos, 1992; Dupuy et al., 2000; Naskar et al., 2022). In this study, SPOM was a bulk of matter from 0.47 µm to 200 µm including phytoplankton, small zooplankton and fish waste (i.e. feces and feed debris). FA profile of SPOM was very different from that of commercial feed and larger zooplankton. The presence of macroalgae did not seem to affect SPOM FA profile since no significant differences were detected between T1 and T2. Bacterial FA (i.e. C15:0 iso and ante, C16:0 iso and C18:1ω7/C18:1ω1 < 1) were identified in small proportion in SPOM that act as microcosm for attached bacteria (Allan et al., 2010; Bachok et al., 2003). The presence of dinoflagellates seemed to be higher than diatoms in the phytoplankton pool of SPOM since the ratios 16:1ω7/16:0 and 20:5ω3/22:6ω3 were lower than 1 (Dalsgaard et al., 2003; Kelly and Scheibling, 2012). Cunha et al. (2019a) identified a dominance of diatoms and chlorophytes (*Chlorodendrophyceae* spp.) in T1 and a dominance of diatoms and phytoflagellates in T2. Since seawater samples were not collected at the same time such differences could reflect seasonal variations in microalgae blooms (Ahlgren, 1993). According to FA results, differences in $\delta^{13}C$ and $\delta^{15}N$ values of SPOM between T1 and T2 should be related to differences between environmental conditions rather than differences between phytoplankton populations. Cunha et al. (2019a) have recorded than in T1, phytoplankton was in competition for nutrients with macroalgae. FA profile of large zooplankton as well as their $\delta^{13}C$ and $\delta^{15}N$ values were closed to those of SPOM and commercial feed. Large zooplankton grazed on SPOM but was also able to use commercial feed. Similar results have been previously observed in different aquaculture ponds (Fernandez-Jover et al., 2009; Grey et al., 2004). Compared to SPOM, zooplankton was enriched by 0.6‰ in $^{13}C$ and 4‰ in$^{15}N$. High levels of PUFA, such as EPA and DHA, have

been measured on natural zooplankton feeding only on phytoplankton (Dalsgaard et al., 2003). In T1 and T2, the high proportion of C18:2ω6 measured in zooplankton was derived from the intake of commercial feed. Similar results have been observed by (Fernandez-Jover et al., 2009) who found high levels of C18:2ω6 pellet-derived FA in zooplankton samples associated with fish farms. In T2, the proportion of C18:2ω6 in zooplankton was higher than in T1 probably due the lower concentration of phytoplankton found in this pond (Cunha et al., 2019a).

FA profile of oysters was close to those of SPOM and large zooplankton but farther away to those of commercial feed indicating that their food was preferentially the previous food items. Stable isotope values of oyster tissues confirmed that SPOM and zooplankton were the main food sources used by oyster. According to mixing model, oysters consumed 48% of SPOM and 36% of zooplankton in T1; and 71% of SPOM and 18% of zooplankton in T2. Our results confirmed that oyster actively fed on phytoplankton (Dupuy et al., 1999). The biomass of phytoplankton was higher in T2 than in T1 as the density of macroalgae was controlled in T2 and therefore there was a reduced competition for dissolved nutrients. The higher levels of phytoplankton in this treatment led to a better growth of oyster compared to T1 (Cunha et al., 2019a). FA differences between oysters from T1 and T2 reflected the food sources used. Oyster raised in T1 had higher proportion of C18:2ω6 and C18:3ω3 provided by zooplankton whereas oyster raised in T2 had higher proportion C16:1ω7 and C18:1ω7 provided by phytoplankton. Low proportion of FA characteristics of bacteria were identified in oyster tissues raised in T1 and T2. This result confirmed that oyster are capable to eat bacteria attached to SPOM as shown in previous studies (Langdon and Newell, 1990; Xu and Yang, 2007). Stable isotope and FA profiles of oysters revealed that debris of commercial feed did not contribute to their diet. Oyster had low level of

C18:1ω9 and C18:2ω6, the specific FA found in commercial feed. Our study confirmed results of previous *in situ* studies showing that fish farming wastes do not make up a substantial part of the diet of filter-feeders such as oysters and mussels (Aguado-Giménez et al., 2014; Irisarri et al., 2015; Sanz-Lazaro and Sanchez-Jerez, 2017). Oyster benefited indirectly of fish wastes by using phytoplankton whom the growth is stimulated by fish wastes. Oysters and their potential food sources were sampled at the end of the experimentation and temporal variations were not considered during this study. The contribution of the different sources to oyster growth differ depending of the period of the year (Marin Leal et al., 2008). Such variations could be considered in further study to understand the annual role of oyster to bio-remediate fish wastes.

## 5. Conclusions

This study confirmed that both tools combined fatty acids as well as carbon and nitrogen stable isotopes were efficient to trace trophic transfer across benthic and pelagic compartment of in IMTA ponds. Our results highlighted that fish, oyster and macroalgae produced together in earthen ponds is an improved system compared to the usual semi-intensive fish polyculture. The three fish species meagre, white seabream and grey mullet, were good candidates to be raised in IMTA ponds but they are in competition for food as they mainly fed on commercial feed whatever the treatment. Grey mullet complemented its diet by consuming polychaetes that naturally grown in ponds. The combination of polychaetes and grey mullet in IMTA systems showed to be an epitome of “circular economy” with food resources produced by men being returned to men in the form of protein. Polychaetes were an important trophic link as they recycled fish detritus into

nutritionally essential FA that accumulated into grey mullet. To increase detritivorous performances and decrease food competition on commercial feed, grey mullet should be raised in separated cages bellow the other fish species. T3 including fish, phytoplankton and macroalgae was comparable to the usual Mediterranean earthen pond production. The presence of macroalgae is necessary to absorb and reduce the excess of nutrients, to oxygenate the water during day light hours and to control phytoplankton proliferation that can be detrimental to fish survival during the night due to the increased oxygen consumption. The introduction of oysters in T1 and T2 largely improved IMTA ponds as oysters actively consumed phytoplankton. In such ponds, phytoplankton was in competition with macroalgae for nutrients. Since macroalgae growth naturally in ponds and are not consumed by fish, their growth should be controlled when oysters are present in IMTA ponds to improve phytoplankton biomass and enhance oyster growth rate. Food web of IMTA ponds should be complicated by introducing species consuming macroalgae such as sea urchins or abalones.

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## References


Aguado-Giménez, F., Hernández, M.D., Cerezo-Valverde, J., Piedecausa, M.A., García-García, B., 2014. Does flat oyster (*Ostrea edulis*) rearing improve under open-sea integrated multi-trophic conditions? Aquac. Int. 22, 447–467. https://doi.org/10.1007/s10499-013-9653-6

Ahlgren, G., 1993. Seasonal variation of fatty acid content in natural phytoplankton in two eutrophic lakes. A factor controlling zooplankton species? SIL Proc. 1922-2010 25, 144–149. https://doi.org/10.1080/03680770.1992.11900076

Allan, E.L., Ambrose, S.T., Richoux, N.B., Froneman, P.W., 2010. Determining spatial changes in the diet of nearshore suspension-feeders along the South African coastline: Stable isotope and fatty acid signatures. Estuar. Coast. Shelf Sci. 87, 463–471. https://doi.org/10.1016/j.ecss.2010.02.004

Bachok, Z., Mfilinge, P.L., Tsuchiya, M., 2003. The diet of the mud clam *Geloina coaxans* (Mollusca, Bivalvia) as indicated by fatty acid markers in a subtropical mangrove forest of Okinawa, Japan. J. Exp. Mar. Biol. Ecol. 292, 187–197. https://doi.org/10.1016/S0022-0981(03)00160-6

Baltadakis, A., Casserly, J., Falconer, L., Sprague, M., Telfer, T., 2020. European lobsters utilise Atlantic salmon wastes in coastal integrated multi-trophic aquaculture systems. Aquac. Environ. Interact. 12, 485–494. https://doi.org/10.3354/aei00378

Barrington, K., Chopin, T., Robinson, S., 2009. Integrated multi-trophic aquaculture (IMTA) in marine temperate waters, in: Integrated Mariculture: A Global Review. pp. 7–46.

Bischoff, A.A., Fink, P., Waller, U., 2009. The fatty acid composition of *Nereis diversicolor* cultured in an integrated recirculated system: Possible implications for aquaculture. Aquaculture 296, 271–276. https://doi.org/10.1016/j.aquaculture.2009.09.002

Boecklen, W.J., Yarnes, C.T., Cook, B.A., James, A.C., 2011. On the use of stable isotopes in trophic ecology. Annu. Rev. Ecol. Evol. Syst. 42(1), 411–440. https://doi.org/10.1146/annurev-ecolsys-102209-144726.

Carballeira Braña, C.B., Cerbule, K., Senff, P., Stolz, I.K., 2021. Towards environmental sustainability in marine finfish aquaculture. Front. Mar. Sci. 8. https://doi.org/10.3389/fmars.2021.666662

Castro, L.F.C., Tocher, D.R., Monroig, O., 2016. Long-chain polyunsaturated fatty acid biosynthesis in chordates: Insights into the evolution of Fads and Elovl gene repertoire. Prog. Lipid Res. 62, 25–40. https://doi.org/10.1016/j.plipres.2016.01.001

Chopin, T., 2006. Integrated multi-trophic aquaculture. What it is and why you should care... and don't confuse it with polyculture. North. Aquac. 12, 4.

Chopin, T., Buschmann, A.H., Halling, C., Troell, M., Kautsky, N., Neori, A., Kraemer, G.P., Zertuche-González, J.A., Yarish, C., Neefus, C., 2001. Intergating seaweeds into marine aquaculture systems: a key toward sustainability. J. Phycol. 37, 975–986. https://doi.org/10.1046/j.1529-8817.2001.01137.x

Chouvelon, T., Chapuis, A., Bustamande, P., Lefebvre, S., Mornet, F., Guillou, G., Violamer, L., Dupuy, C., 2014. Trophic ecology of European sardine *Sardina pilchardus* and European anchovy *Engraulis encrasicolus* in the Bay of Biscay (north-east Atlantic) inferred from $\delta^{13}C$ and $\delta^{15}N$ values of fish and identified

mesozooplanktonic organisms. J. Sea Res. 85, 277–291. https://doi.org/10.1016/j.seares.2013.05.011

Christensen, V., 1998. Fishery-induced changes in a marine ecosystem: insight from models of the Gulf of Thailand. J. Fish Biol. 53, 128–142. https://doi.org/10.1111/j.1095-8649.1998.tb01023.x

Christie, W.W., Han, X., 2012. Chapter 7 - Preparation of derivatives of fatty acids, in: Christie, W.W., Han, X. (Eds.), Lipid Analysis (Fourth Edition). Woodhead Publishing, pp. 145–158. https://doi.org/10.1533/9780857097866.145

Clarke, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. Austral Ecol. 18, 117–143. https://doi.org/10.1111/j.1442-9993.1993.tb00438.x

Coutteau, P., Sorgeloos, P., 1992. The use of algal substitutes and the requirement for live algae in the hatchery and nursery rearing of bivalve molluscs: an international survey. J. Shellfish Res.

Cunha, M.E., Quental Ferreira, H., Parejo, A., Gamito, S., Ribeiro, L., Moreira, M., Monteiro, I., Soares, F., Pousão, P., 2019a. Understanding the individual role of fish, oyster, phytoplankton and macroalgae in the ecology of integrated production in earthen ponds. Aquaculture 512, 734297. https://doi.org/10.1016/j.aquaculture.2019.734297

Cunha, M.E., Quental-Ferreira, H., Parejo, A., Gamito, S., Ribeiro, L., Moreira, M., Monteiro, I., Soares, F., Pousão-Ferreira, P., 2019b. Methodology for assessing the individual role of fish, oyster, phytoplankton and macroalgae in the ecology of integrated production in earthen ponds. MethodsX 6, 2570–2576. https://doi.org/10.1016/j.mex.2019.10.016

Dalsgaard, J., St. John, M., Kattner, G., Müller-Navarra, D., Hagen, W., 2003. Fatty acid trophic markers in the pelagic marine environment, in: Advances in Marine Biology. Elsevier, pp. 225–340. https://doi.org/10.1016/S0065-2881(03)46005-7

De Silva, S.S., Wijeyaratne, M.J.S., 1977. Studies on the biology of young grey mullet, *Mugil cephalus L.* II. Food and Feeding. Aquaculture 12, 157–167. https://doi.org/10.1016/0044-8486(77)90183-1

DeNiro, M.J., Epstein, S., 1978. Influence of diet on distribution of carbon isotopes in animals. Geochim. Cosmochim. Acta 42, 495–506. https://doi.org/10.1016/0016-7037(78)90199-0

DeNiro, M.J., Epstein, S., 1977. Mechanism of carbon isotope fractionation associated with lipid synthesis. Science 197, 261–263.

Dubois, S., Jean-Louis, B., Bertrand, B., Lefebvre, S., 2007. Isotope trophic-step fractionation of suspension-feeding species: Implications for food partitioning in coastal ecosystems. J. Exp. Mar. Biol. Ecol. 351, 121–128. https://doi.org/10.1016/j.jembe.2007.06.020

Dupuy, C., Le Gall, S., Hartmann, H., Bréret, M., 1999. Retention of ciliates and flagellates by the oyster *Crassostrea gigas* in French Atlantic coastal ponds: protists as a trophic link between bacterioplankton and benthic suspension-feeders. Mar. Ecol. Prog. Ser. 177, 165–175. https://doi.org/10.3354/meps177165

Dupuy, C., Vaquer, A., Lam-Höai, T., Rougier, C., Mazouni, N., Lautier, J., Collos, Y., Le Gall, S., 2000. Feeding rate of the oyster *Crassostrea gigas* in a natural planktonic community of the Mediterranean Thau Lagoon. Mar. Ecol. Prog. Ser. 205, 171–184. https://doi.org/10.3354/meps205171

Edwards, P., 2015. Aquaculture environment interactions: Past, present and likely future trends. Aquaculture 447, 2–14. https://doi.org/10.1016/j.aquaculture.2015.02.001

FAO, 2022. Résumé de la situation mondiale des pêches et de l'aquaculture 2022. Vers une transformation bleue. FAO, Rome. https://doi.org/10.4060/cc0463fr

Favot, G., Cunha, M.E., Quental-Ferreira, H., Álvares Serrão, M.E., 2019. Production of *Ulva* sp. in Multitrophic Aquaculture in Earthen Ponds. *Aquac Fish Stud.* Volume 1(1), 1-8. https://doi.org/10.31038/afs.2019111

Fernandez-Jover, D., Sanchez-Jerez, P., Bayle-Sempere, J.T., Arechavala-Lopez, P., Martinez-Rubio, L., Jimenez, J.A.L., Lopez, F.J.M., 2009. Coastal fish farms are settlement sites for juvenile fish. Mar. Environ. Res. 68, 89–96. https://doi.org/10.1016/j.marenvres.2009.04.006

Froese, R., Pauly, D., 2022. FishBase. World Wide Web electronic publication. www.fishbase.org, version (02/2022)

Gamito, S., Quental-Ferreira, H., Parejo, A., Aubin, J., Christensen, V., Cunha, M., 2020. Integrated multi-trophic aquaculture systems: energy transfers and food web organization in coastal earthen ponds. Aquac. Environ. Interact. 12, 457–470. https://doi.org/10.3354/aei00375

Grey, J., Waldron, S., Hutchinson, R., 2004. The utility of carbon and nitrogen isotope analyses to trace contributions from fish farms to the receiving communities of freshwater lakes: a pilot study in Esthwaite Water, UK. Hydrobiologia 524, 253–262. https://doi.org/10.1023/B:HYDR.0000036144.49795.dd

Hobson, K., Welch, H.E., 1992. Determination of trophic relationships within a high arctic marine food web using $\delta^{13}C$ and $\delta^{15}$. Mar. Ecol.-Prog. Ser. 84, 9–18.

Holmer, M., Pérez, M., Duarte, C.M., 2003. Benthic primary producers—a neglected environmental problem in Mediterranean maricultures? Mar. Pollut. Bull. 46, 1372–1376. https://doi.org/10.1016/S0025-326X(03)00396-5

Indarti, E., Majid, M., Hashim, R., Shu-Chien, A., 2005. Direct FAME synthesis for rapid total lipid analysis from fish oil and cod liver oil. J. Food Compos. Anal. 18, 161–170. https://doi.org/10.1016/j.jfca.2003.12.007

Irisarri, J., Fernández-Reiriz, M.J., Labarta, U., Cranford, P.J., Robinson, S.M.C., 2015. Availability and utilization of waste fish feed by mussels *Mytilus edulis* in a commercial integrated multi-trophic aquaculture (IMTA) system: A multi-indicator assessment approach. Ecol. Indic. 48, 673–686. https://doi.org/10.1016/j.ecolind.2014.09.030

Jerónimo, D., Lillebø, A.I., Maciel, E., Domingues, M.R.M., Cremades, J., Calado, R., 2021. Unravelling the fatty acid profiles of different polychaete species cultured under integrated multi-trophic aquaculture (IMTA). Sci. Rep. 11. https://doi.org/10.1038/s41598-021-90185-8

Kelly, J., Scheibling, R., 2012. Fatty acids as dietary traces in benthic food webs. Mar. Ecol. Prog. Ser. 446, 1–22. https://doi.org/10.3354/meps09559

Langdon, C.J., Newell, R.I.E., 1990. Utilization of detritus and bacteria as food sources by two bivalve suspension-feeders, the oyster *Crassostrea virginica* and the mussel *Geukensia demissa*. Mar. Ecol. Prog. Ser. 58, 299–310.

Lewis, T., Nichols, P.D., McMeekin, T.A., 2000. Evaluation of extraction methods for recovery of fatty acids from lipid-producing microheterotrophs. J. Microbiol. Methods 43, 107–116. https://doi.org/10.1016/S0167-7012(00)00217-7

Marín Leal, J.C., Dubois, S., Orvain, F., Galois, R., Blin, J.-L., Ropert, M., Bataillé, M.-P., Ourry, A., Lefebvre, S. 2008. Stable isotopes ($\delta^{13}C$, $\delta^{15}N$) and modelling as tools to estimate the trophic ecology of cultivated oysters in two contrasting environments. Mar.Biol. 153, 673-688. https://doi.org/10.1007/s00227-007-0841-7.

Marques, B., Lillebø, A., Ricardo, F., Nunes, C., Coimbra, M., Calado, R., 2018. Adding value to ragworms (*Hediste diversicolor*) through the bioremediation of a super-intensive marine fish farm. Aquac. Environ. Interact. 10, 79–88. https://doi.org/10.3354/aei00255

Middelburg, J.J., 2014. Stable isotopes dissect aquatic food webs from the top to the bottom. Biogeosciences 11, 2357–2371. https://doi.org/10.5194/bg-11-2357-2014

Minagawa, M., Wada, E., 1984. Stepwise enrichment of $^{15}N$ along food-chains. Further evidence and the relation between $\delta^{15}N$ and animal age. Geochim. Cosmochim. Acta 48, 1135–1140. https://doi.org/10.1016/0016-7037(84)90204-7

Nahon, S., Roussel, J.-M., Jaeger, C., Menniti, C., Kerhervé, P., Mortillaro, J.-M., Aubin, J., 2020a. Characterization of trophic niche partitioning between carp (*Cyprinus carpio*) and roach (*Rutilus rutilus*) in experimental polyculture ponds using carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes. Aquaculture 522, 735162. https://doi.org/10.1016/j.aquaculture.2020.735162

Nahon, S., Séité, S., Kolasinski, J., Aguirre, P., Geurden, I., 2017. Effects of euthanasia methods on stable carbon ($\delta^{13}C$ value) and nitrogen ($\delta^{15}N$ value) isotopic compositions of fry and juvenile rainbow trout *Oncorhynchus mykiss*. Rapid Commun. Mass Spectrom. 31, 1742–1748. https://doi.org/10.1002/rcm.7958

Nahon, S., Séité, S., Lefebvre, S., Kolasinski, J., Aguirre, P., Geurden, I., 2020b. How protein quality drives incorporation rates and trophic discrimination of carbon and

nitrogen stable isotope ratios in a freshwater first-feeding fish. Freshw. Biol. 65, 1870–1882. https://doi.org/10.1111/fwb.13578

Naskar, S., Biswas, G., Kumar, P., De, D., Sawan,t P.B., Das, S., Roy, U., 2022. Effects of estuarine oyster, *Crassostrea cuttackensis* as the extractive species at varied densities on productivity and culture environment in brackishwater integrated multi-trophic aquaculture (BIMTA) system. Aquaculture 554, 738128. https://doi.org/10.1016/j.aquaculture.2022.738128.

Naylor, R.L., Hardy, R.W., Bureau, D.P., Chiu, A., Elliott, M., Farrell, A.P., Forster, I., Gatlin, D.M., Goldburg, R.J., Hua, K., Nichols, P.D., 2009. Feeding aquaculture in an era of finite resources. Proc. Natl. Acad. Sci. 106, 15103–15110. https://doi.org/10.1073/pnas.0905235106

Nederlof, M., Jansen, H., Dahlgren, T., Fang, J., Meier, S., Strand, O., Sveier, H., Verdegem, M., Smaal, A., 2019. Application of polychaetes in (de)coupled integrated aquaculture: production of a high-quality marine resource. Aquac. Environ. Interact. 11, 221–237. https://doi.org/10.3354/aei00309

Park, M.S., Kim, J.K., Shin, S., Min, B.H., Samanta, P., 2021. Trophic fractionation in an integrated multi-trophic aquaculture off Tongyoung Coast: A stable isotope approach. Aquaculture 536, 736454. https://doi.org/10.1016/j.aquaculture.2021.736454

Parnell, A.C., Phillips, D.L., Bearhop, S., Semmens, B.X., Ward, E.J., Moore, J.W., Jackson, A.L., Grey, J., Kelly, D.J., Inger, R., 2013. Bayesian stable isotope mixing models. Environmetrics n/a-n/a. https://doi.org/10.1002/env.2221

Pasquaud, S., David, V., Lobry, J., Girardin, M., Sautour, B., Elie, P., 2010. Exploitation of trophic resources by fish under stressful estuarine conditions. Mar. Ecol. Prog. Ser. 400. https://doi.org/10.3354/meps08387

Post, D.M., 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83, 703–718. https://doi.org/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2

Post, D.M., Layman, C.A., Arrington, D.A., Takimoto, G., Quattrochi, J., Montana, C.G., 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152, 179–189. https://doi.org/10.1007/s00442-006-0630-x

Redmond, K.J., Magnesen, T., Hansen, P.K., Strand, Ø., Meier, S., 2010. Stable isotopes and fatty acids as tracers of the assimilation of salmon fish feed in blue mussels (*Mytilus edulis*). Aquaculture 298, 202–210. https://doi.org/10.1016/j.aquaculture.2009.10.002

Rombenso, A.N., Trushenski, J.T., Schwarz, M.H., 2016. Fish oil replacement in feeds for juvenile Florida pompano: Composition of alternative lipid influences degree of tissue fatty acid profile distortion. Aquaculture 458, 177–186. https://doi.org/10.1016/j.aquaculture.2016.03.009

Salvo, F., Hamoutene, D., Dufour, S.C., 2015. Trophic analyses of opportunistic polychaetes (*Ophryotrocha cyclops* ) at salmonid aquaculture sites. J. Mar. Biol. Assoc. U. K. 95, 713–722. https://doi.org/10.1017/S0025315414002070

De los Santos, C.C., Olivé, I., Moreira, M., Silva, A., Freitas, C., Luna, R.L., Quental-Ferreira, H., Martins, M., Costa, M.M., Silva, J., Cunha, M.E., Soares, F., Pousão-Ferreira, P., Santos, R., 2020. Seagrass meadows improve inflowing water quality

in aquaculture ponds, Aquaculture, Volume 528, 735502, https://doi.org/10.1016/j.aquaculture.2020.735502

Sanz-Lazaro, C., Sanchez-Jerez, P., 2017. Mussels do not directly assimilate fish farm wastes: Shifting the rationale of integrated multi-trophic aquaculture to a broader scale. J. Environ. Manage. 201, 82–88. https://doi.org/10.1016/j.jenvman.2017.06.029

Sargent, J.R., Tocher, D.R., Bell, J.G., 2003. The Lipids, in: Fish Nutrition. Elsevier, pp. 181–257. https://doi.org/10.1016/B978-012319652-1/50005-7

Schlechtriem, C., Focken, U., Becker, K., 2003. Effect of different lipid extraction methods on $\delta^{13}C$ of lipid and lipid-free fractions of fish and different fish feeds. Isotopes Environ. Health Stud. 39, 135–140. https://doi.org/10.1080/1025601031000113565

Schultz, S., Vallant, B., Kainz, M.J., 2012. Preferential feeding on high quality diets decreases methyl mercury of farm-raised common carp (*Cyprinus carpio* L.). Aquaculture 338–341, 105–110. https://doi.org/10.1016/j.aquaculture.2012.01.006

Soyinka, O.O., 2008. The feeding ecology of *Mugil cephalus* (Linnaeus) from a high brackish tropical lagoon in South-west, Nigeria. Afr. J. Biotechnol. 7, 4192–4198.

Sweeting, C.J., Barry, J., Barnes, C., Polunin, N.V.C., Jennings, S., 2007a. Effects of body size and environment on diet-tissue $\delta^{15}N$ fractionation in fishes. J. Exp. Mar. Biol. Ecol. 340, 1–10. https://doi.org/10.1016/j.jembe.2006.07.023

Sweeting, C.J., Barry, J.T., Polunin, N.V.C., Jennings, S., 2007b. Effects of body size and environment on diet-tissue $\delta^{13}C$ fractionation in fishes. J. Exp. Mar. Biol. Ecol. 352, 165–176. https://doi.org/10.1016/j.jembe.2007.07.007

Thomas, M., Pasquet, A., Aubin, J., Nahon, S., Lecocq, T., 2021. When more is more: taking advantage of species diversity to move towards sustainable aquaculture. Biol. Rev. 96, 767–784. https://doi.org/10.1111/brv.12677

Tovar, A., Moreno, C., Mánuel-Vez, M.P., García-Vargas, M., 2000. Environmental implications of intensive marine aquaculture in earthen ponds. Mar. Pollut. Bull. 40, 981–988. https://doi.org/10.1016/S0025-326X(00)00040-0

Valero-Rodriguez, J.M., Toledo-Guedes, K., Arechavala-Lopez, P., Izquierdo-Gomez, D., Sanchez-Jerez, P., 2015. The use of trophic resources by *Argyrosomus regius* (Asso, 1801) escaped from Mediterranean offshore fish farms. J. Appl. Ichthyol. 31, 10–15. https://doi.org/10.1111/jai.12649

Ventura, D., Bonhomme, V., Colangelo, P., Bonifazi, A., Jona Lasinio, G., Ardizzone, G., 2017. Does morphology predict trophic niche differentiation? Relationship between feeding habits and body shape in four co-occurring juvenile species (Pisces: Perciformes, Sparidae). Estuar. Coast. Shelf Sci. 191, 84–95. https://doi.org/10.1016/j.ecss.2017.04.014

Xie, B., Qin, J., Yang, H., Wang, X., Wang, Y.-H., Li, T.-Y., 2013. Organic aquaculture in China: A review from a global perspective. Aquaculture 414–415, 243–253. https://doi.org/https://doi.org/10.1016/j.aquaculture.2013.08.019

Xu, Q., Yang, H., 2007. Food sources of three bivalves living in two habitats of Jiazhou Bay (Qingdao, China): indicated by lipid biomarkers and stable isotope analysis. J. Shellfish Res. 26, 561–567. https://doi.org/10.2983/0730-8000(2007)26[561:FSOTBL]2.0.CO;2

Yousefi-Garakouei, M., Kamali, A., Soltani, M., 2018. Effects of rearing density on growth, fatty acid profile and bioremediation ability of polychaete *Nereis diversicolor* in an

integrated aquaculture system with rainbow trout (*Oncorhynchus mykiss*). Aquac. Res. https://doi.org/10.1111/are.13918

**Table**

**Table 1.** Initial and final zootechnical parameters (mean ± sd) of fish and oysters reared in IMTA ponds. Trophic levels are given according to FishBase (Froese and Pauly, 2022)

| Species | number pond$^{-1}$ | Biomass (kg m$^{-3}$) | Mean weight (g) | Mean total length (cm) | Final biomass (kg m$^{-3}$) | | | Trophic level |
|---|---|---|---|---|---|---|---|---|
| | | | | | T1 | T2 | T3 | |
| *Argyrosomus regius* | 1450 | 0.39 | 204.5 ± 63.30 | 26.6 ± 2.92 | 1.11 ± 0.01 | 1.15 ± 0.01 | 0.80 ± 0.08 | 4.3 |
| *Diplodus sargus* | 850 | 0.08 | 51.5 ± 18.61 | 14.2 ± 1.47 | 0.21 ± 0.01 | 0.18 ± 0.01 | 0.19 ± 0.02 | 3.4 |
| *Mugil cephalus* | 565 | 0.08 | 117.6 ± 95.75 | 19.4 ± 5.63 | 0.16 ± 0.01 | 0.12 ± 0.01 | 0.14 ± 0.01 | 2.5 |
| *Magallana gigas* | 18000 | 0.07 | 0.5 ± 0.09 | | 0.24 ± 0.01 | 0.23 ± 0.01 | | |

**Table 2.** Percentage of total fatty acids (mean ± sd, n = 18) of potential food sources. Only fatty acids superior to 1% of total fatty acids in at least one sample are presented.

| | Feed | Polychaetes | *U. flexuosa* | *Ulva* spp. | *R. riparium* | SPOM | Large zooplankton |
|---|---|---|---|---|---|---|---|
| C14:0 | 2.16 ± 0.43 | 2.39 ± 0.89 | 1.56 ± 1.01[a] | 1.82 ± 0.89[a] | *tr* | 5.64 ± 0.88 | 2.81 ± 0.04 |
| C15:0 | *tr* | *tr* | *tr* | *tr* | *tr* | 1.80 ± 0.28 | tr |
| C15:0 iso | *tr* | *tr* | *tr* | *tr* | *tr* | 2.17 ± 0.34 | tr |
| C15:0 ante | *tr* | *tr* | *tr* | *tr* | *tr* | 1.38 ± 0.45 | tr |
| C16:0 | 21.61 ± 0.29 | 16.77 ± 6.12 | 30.94 ± 4.45 | 32.34 ± 2.99 | 28.82 ± 2.13 | 32.47 ± 4.32 | 26.24 2.29 |
| C17:0 | *tr* | 1.32 ± 0.49 | *tr* | *tr* | *tr* | 1.22 ± 0.24 | 1.05 ± 0.10 |
| C18:0 | 4.01 ± 0.19 | 6.31 ± 1.25 | *tr* | *tr* | *tr* | 10.24 ± 1.94 | 8.05 ± 1.18 |
| C20:0 | *tr* | *tr* | *tr* | *tr* | *tr* | 1.25 ± 0.28 | tr |
| C22:0 | *tr* | 1.01 ± 0.63 | *tr* | *tr* | *tr* | 1.15 ± 0.29 | tr |
| **SFA** | **29.09 ± 1.12** | **29.85 ± 8.11** | **35.47 ± 5.26[a]** | **36.95 ± 3.41[a]** | **31.95 ± 2.51[b]** | **58.58 ± 3.64** | **40.45 ± 2.43** |
| C16:1ω5 | *tr* | *tr* | 2.97 ± 0.66[a] | 4.62 ± 1.16[b] | 3.45 ± 0.54[a] | 1.85 ± 0.69 | tr |
| C16:1ω7 | 2.13 ± 0.21 | 2.10 ± 0.74 | 2.55 ± 0.51[a] | *tr* | 1.12 ± 0.48[b] | 7.40 ± 1.13 | 2.76 ± 0.68 |
| C18:1ω7 | 1.62 ± 0.12 | 2.65 ± 0.43 | 8.61 ± 1.34[a] | 5.29 ± 0.63[b] | 6.32 ± 0.49[c] | 6.82 ± 0.99 | 4.19 ± 1.02 |
| C18:1ω9 | 21.83 ± 0.53 | 11.08 ± 1.54 | *tr* | *tr* | 1.13 ± 0.68 | 9.62 ± 1.12 | 13.34 ± 3.43 |
| C20:1ω9 | *tr* | 1.89 ± 0.74 | n.d. | *tr* | *tr* | 1.18 ± 0.62 | tr |
| **MUFA** | **26.00 ± 0.46** | **18.70 ± 1.98** | **15.08 ± 1.60[a]** | **11.89 ± 0.75[b]** | **12.30 ± 1.60[b]** | **27.96 ± 1.67** | **21.65 ± 3.11** |
| C16:4ω3 | *tr* | 4.60 ± 1.11 | 17.03 ± 2.61 | 16.52 ± 2.54 | 15.26 ± 2.34 | tr | tr |
| C18:2ω6 | 37.07 ± 1.82 | 15.22 ± 2.47 | 3.17 ± 0.36[a] | 4.72 ± 0.67[b] | 6.68 ± 0.84[c] | 3.18 ± 0.59 | 15.31 ± 6.10 |
| C18:3ω3 | 3.92 ± 0.16 | 1.48 ± 0.27 | 9.75 ± 1.18[a] | 15.21 ± 1.95[b] | 18.45 ± 1.66[c] | 1.78 ± 0.48 | 2.36 ± 0.41 |
| C18:4ω3 | *tr* | *tr* | 17.12 ± 2.61[a] | 10.23 ± 1.58[b] | 10.96 ± 1.49[b] | tr | 1.12 ± 0.44 |
| C20:2ω6 | *tr* | 6.85 ± 2.52 | n.d. | *tr* | *tr* | tr | tr |
| C20:4ω6 | *tr* | 2.94 ± 0.80 | *tr* | *tr* | *tr* | tr | 1.65 ± 0.56 |
| C20:5ω3 | *tr* | 6.91 ± 1.74 | *tr* | *tr* | 1.29 ± 0.76[c] | 1.45 ± 0.77 | 7.30 ± 2.69 |
| C21:5ω3 | n.d. | 1.17 ± 2.45 | *tr* | n.d. | n.d. | tr | n.d. |
| C22:2ω6 | n.d. | 1.19 ± 2.76 | n.d. | *tr* | n.d. | tr | n.d. |
| C22:4ω6 | n.d. | 1.64 ± 0.98 | n.d. | *tr* | n.d. | *tr* | n.d. |
| C22:5ω3 | n.d. | 1.54 ± 1.07 | 1.31 ± 1.19 | 1.22 ± 1.25 | tr | 1.25 ± 0.32 | tr |
| C22:6ω3 | 2.02 ± 0.15 | 5.86 ± 2.89 | n.d. | *tr* | *tr* | 1.77 ± 0.98 | 7.25 ± 3.84 |
| **PUFA** | **44.91 ± 1.01** | **51.45 ± 8.64** | **49.45 ± 5.80[a]** | **51.17 ± 3.75[a]** | **55.75 ± 3.54[b]** | **13.46 ± 3.21** | **37.90 ± 2.76** |

*tr* indicates that fatty acids were found in trace amounts (< 1% of total fatty acids). n.d indicates that fatty acids were not detected. SFA, MUFA and PUFA are the sum of saturated, monounsaturated and polyunsaturated fatty acid, respectively. Letters indicate significant differences among fatty acid proportions of the three macroalgae species *U. flexuosa, Ulva* spp. and *R. riparium* (permANOVA, $p \leq 0.05$).

**Figure caption**

**Fig.1.** Expected food web in Mediterranean coastal integrated multi-trophic aquaculture ponds

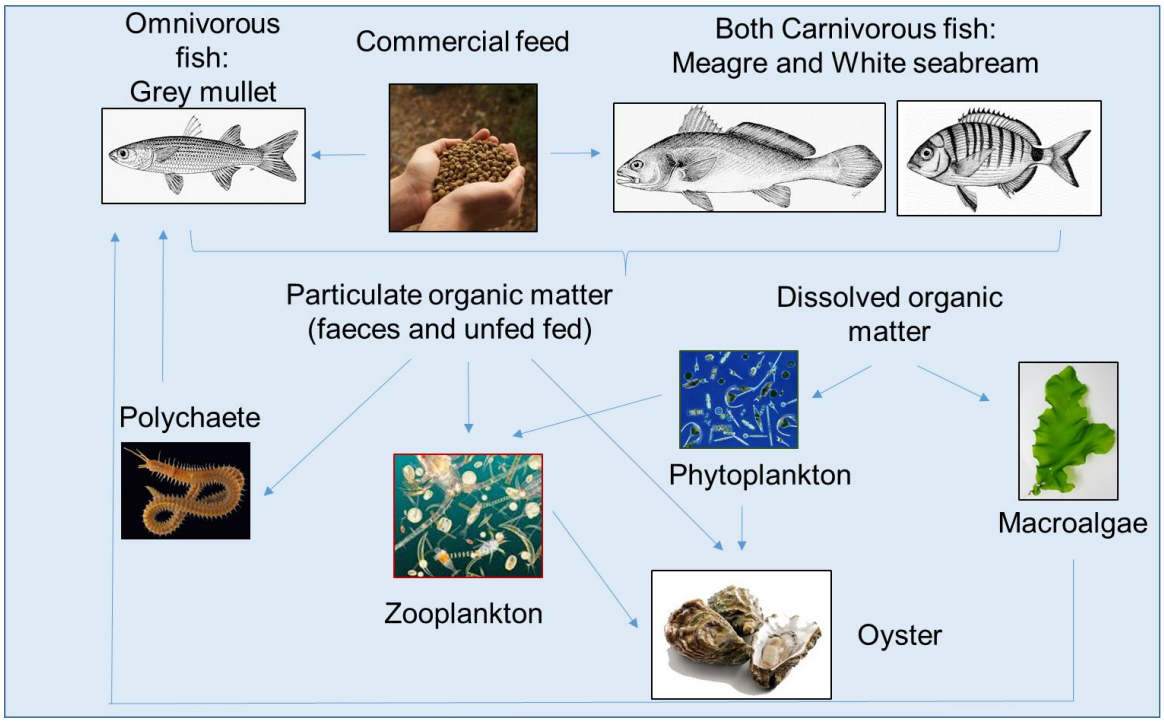


**Fig. 2.** Multidimensional scaling (MDS) plot based on fatty acid profile of potential food sources as well as fish and oyster in T1 (A), T2 (B) and T3 (C) ponds. Meagre are represented by ■, white seabream by ♦, grey mullet by ▼, oyster by ▲, commercial feed pellets by ●, polychaete by ○, SPOM by ◊, large zooplankton by □, *Ulva flexuosa* by ×, *Ulva* spp. by + and *Rhizoclonium riparium* by ⁎**.** Only fatty acids superior to 1% in at least one sample have been considered.

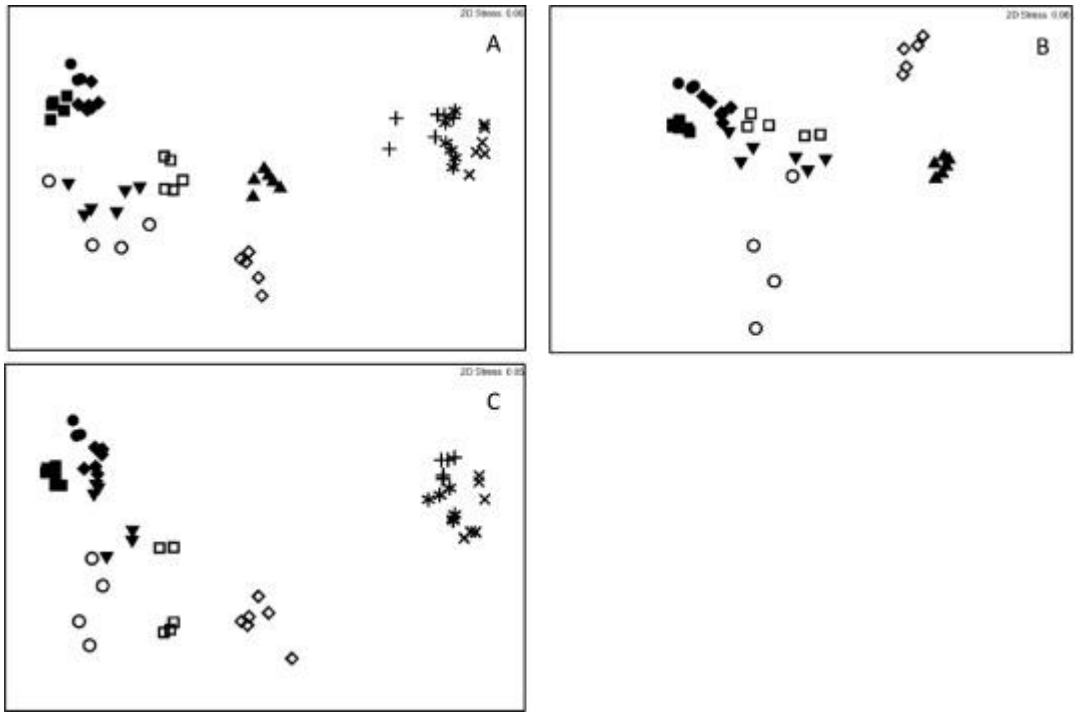


**Fig. 3.** Percentage of total fatty acids (mean ± sd, n = 6) of commercial feed pellet in black, meagre in white, white seabream in light grey and grey mullet in dark grey. Only FA superior to 1% of total fatty acids in at least one sample are presented. Letters indicate significant differences among fish species (permANOVA, $p \leq 0.05$).

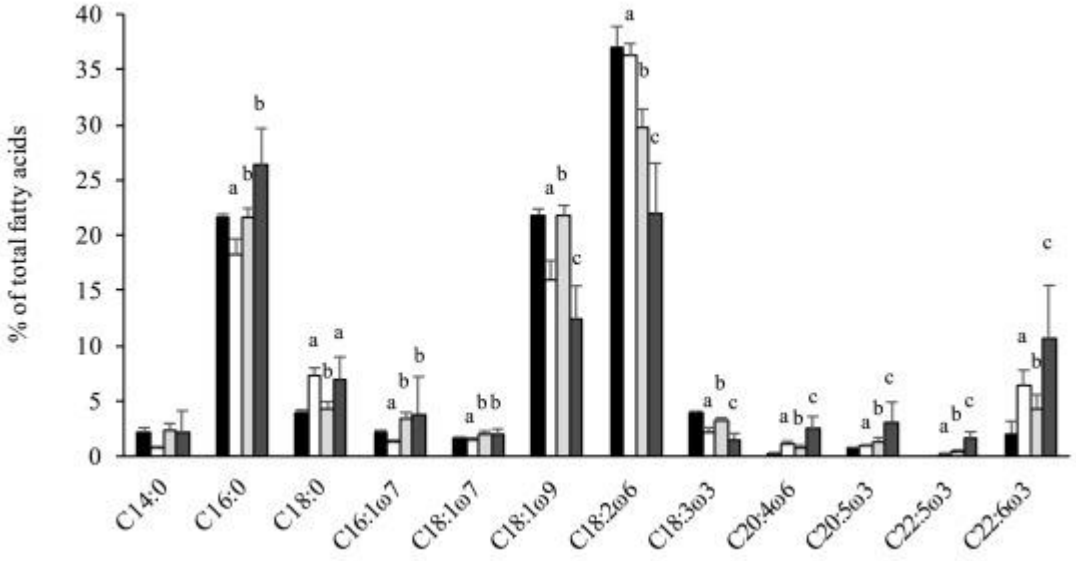


**Fig. 4.** Percentage of total fatty acids (mean ± sd, n = 6) of oyster from T1 and T2 ponds in white and black respectively. Only FA superior to 1% of total fatty in at least one sample are presented. Letters indicate significant differences among fatty acid in the two treatments (permANOVA, $p \leq 0.05$).

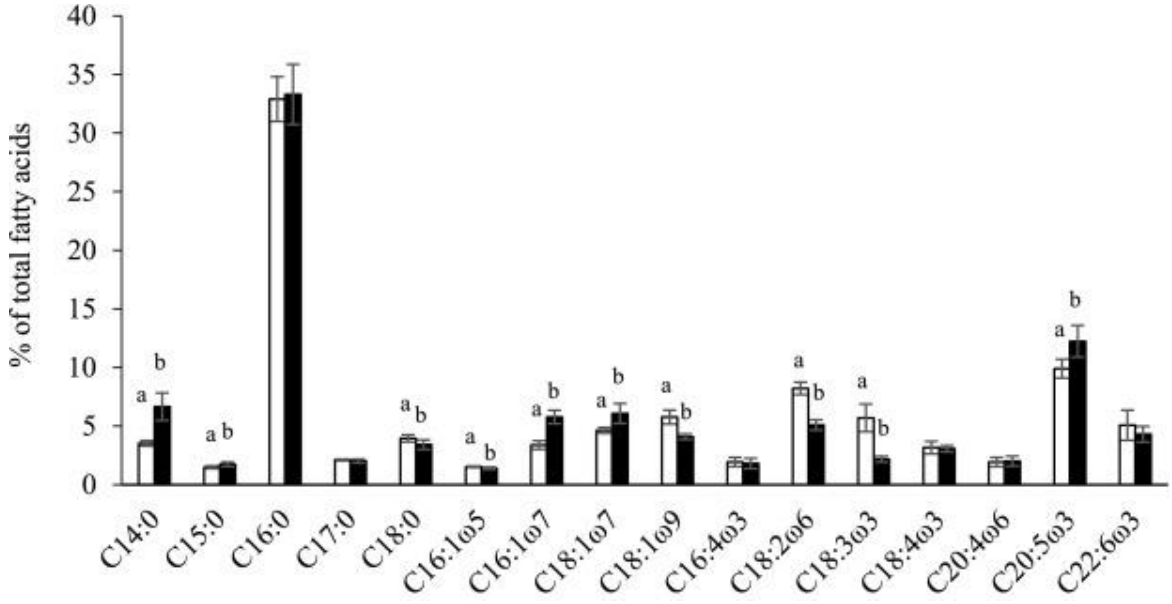


**Fig. 5.** $\delta^{13}C$ and $\delta^{15}N$ values (mean ± sd, n = 6) of potential food sources and fish (A) and potential food sources and oyster (B) in T1, T2 and T3 ponds in black, white and grey, respectively. In (A), meagre are represented by □, seabream by ◊, grey mullet by Δ, commercial feed pellets by **+,** polychaete by ○, *Ulva flexuosa* by ×, *Ulva* spp. by + and *Rhizoclonium riparium* by ⁎. In (B) oyster are represented by Δ, commercial feed pellets by **+,** SPOM by □ and large zooplankton by ◊.

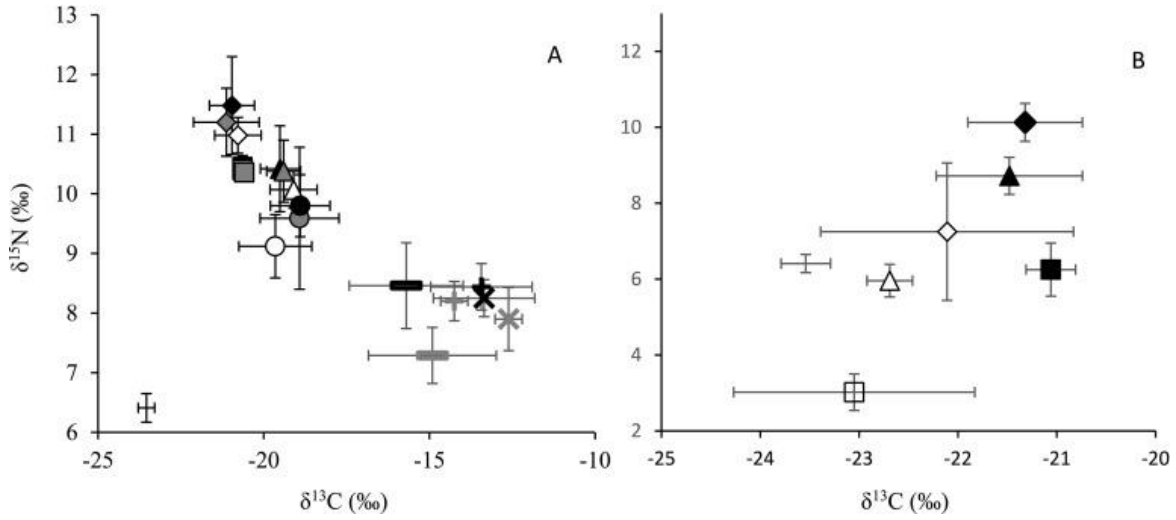


**Fig. 6.** Estimation of the contributions of food sources to diet of meagre (A), white seabream (B), grey mullet (C) and oyster (D). Boxplots are the result of Bayesian mixing model with mean, standard deviation and credible interval (n = 18).

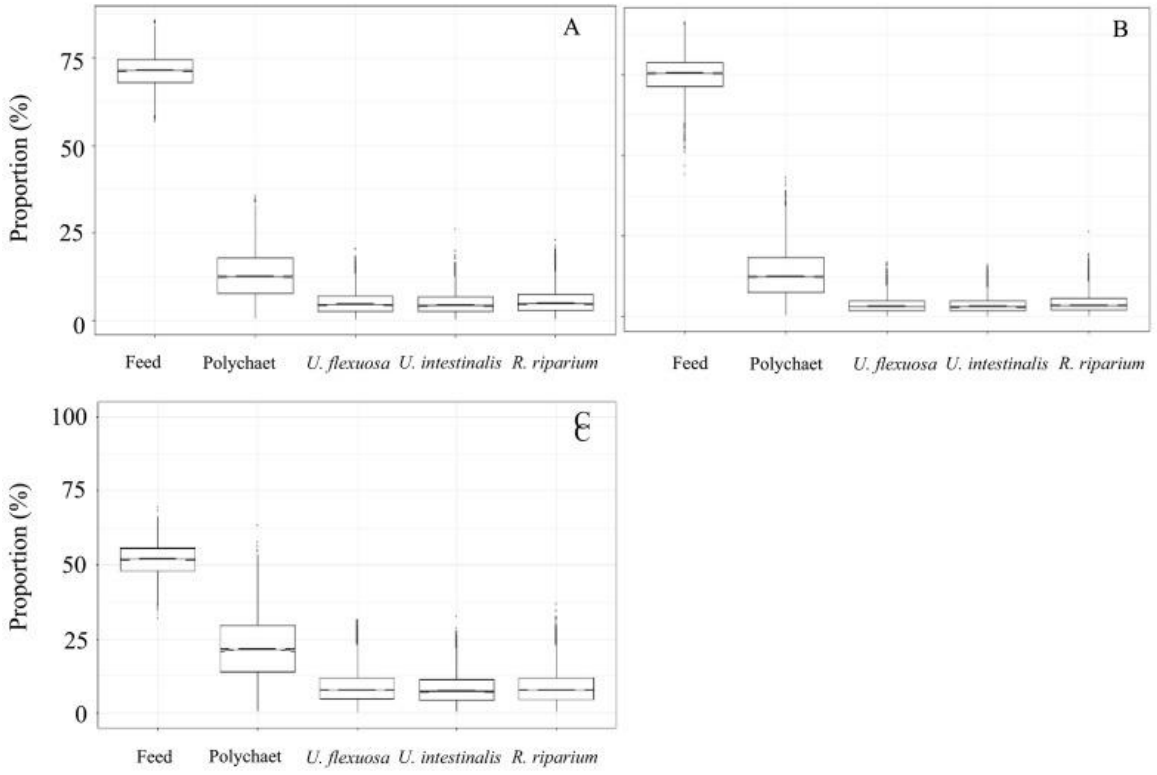
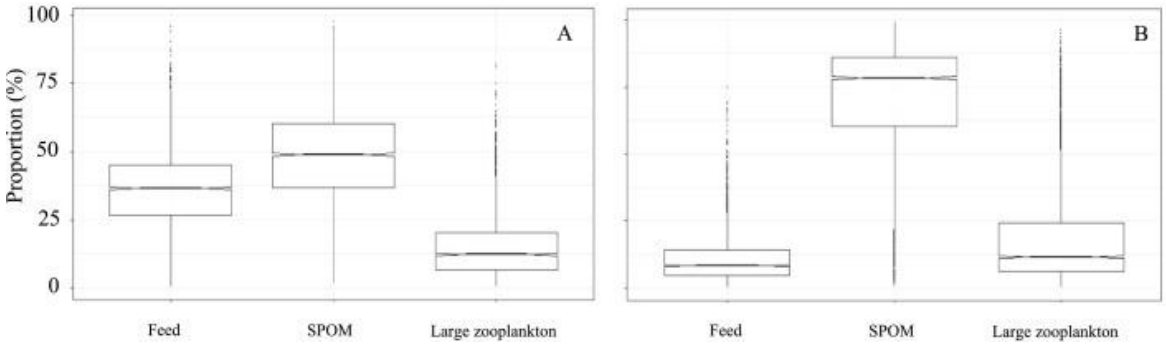



**Fig. 7.** Estimation of the contributions of food sources to diet of oyster in T1 and T2 ponds (A and B, respectively). Boxplots are the result of Bayesian mixing model with mean, standard deviation and credible interval (n = 12).



**Supplementary Table 1.** Percentage of total fatty acid, $\delta^{13}C$ and $\delta^{15}N$ values (‰) of commercial feed pellets (mean ± SD, n = 3). Only fatty acids superior to 0.10% of total FA are presented.

| | Commercial feed pellets |
|---|---|
| C12:0 | *tr* |
| C14:0 | 2.16 ± 0.43 |
| C15:0 | 0.43 ± 0.06 |
| C15:0 iso | *tr* |
| C15:0 ante | *tr* |
| C16:0 | 21.61 ± 0.29 |
| C16:0 iso | *tr* |
| C17:0 | 0.41 ± 0.04 |
| C18:0 | 4.01 ± 0.19 |
| C20:0 | 0.15 ± 0.13 |
| C22:0 | 0.18 ± 0.20 |
| SFA | 29.09 ± 1.12 |
| C14:1ω5 | *tr* |
| C16:1ω5 | *tr* |
| C16:1ω7 | 2.13 ± 0.21 |
| C18:1ω7 | 1.62 ± 0.12 |
| C18:1ω9 | 21.83 ± 0.53 |
| C20:1ω9 | 0.37 ± 0.10 |
| MUFA | 26.00 ± 0.46 |
| C16:2ω4 | 0.27 ± 0.03 |
| C16:3ω4 | 0.27 ± 0.02 |
| C16:4ω1 | *tr* |
| C16:4ω3 | *tr* |
| C18:2ω6 | 37.06 ± 1.82 |
| C18:3ω3 | 3.92 ± 0.16 |
| C18:3ω6 | 0.12 ± 0.01 |
| C18:4ω3 | 0.15 ± 0.01 |
| C20:2ω6 | *tr* |
| C20:4ω6 | 0.27 ± 0.06 |
| C20:5ω3 | 0.70 ± 0.11 |
| C22:6ω3 | 2.02 ± 0.15 |
| PUFA | 44.91 ± 1.01 |
| $\delta^{13}C$ | -23.54 ± 0.25 |
| $\delta^{15}N$ | 6.41 ± 0.24 |

*tr* indicates that fatty acids were found in trace amounts (< 0.10% of total fatty acids). SFA, MUFA and PUFA are the sum of saturated, monounsaturated and polyunsaturated fatty acid, respectively.

**Supplementary Table 2.** Percentage of total fatty acid, $\delta^{13}C$ and $\delta^{15}N$ values (‰) of polychaetes (mean ± SD, n = 3) in T1 (fish, oyster, phytoplankton and macroalgae), T2 (fish, oyster and phytoplankton) and T3 (fish, phytoplankton and macroalgae). Only fatty acids superior to 0.10% of total FA are presented.

| | T1 ponds | T2 ponds | T3 ponds |
|---|---|---|---|
| C12:0 | *tr* | *tr* | *tr* |
| C14:0 | 2.57 ± 0.10 | 1.94 ± 1.37 | 2.70 ± 0.58 |
| C15:0 | 0.96 ± 0.14 | 0.63 ± 0.40 | 1.02 ± 0.11 |
| C15:0 iso | *tr* | 0.20 ± 0.13 | *tr* |
| C15:0 ante | 0.17 ± 0.03 | 0.11 ± 0.07 | 0.20 ± 0.07 |
| C16:0 | 20.84 ± 4.96 | 12.41 ± 7.70 | 18.09 ± 0.18 |
| C16:0 iso | *tr* | *tr* | *tr* |
| C17:0 | 1.72 ± 0.60 | 0.99 ± 0.66 | 1.36 ± 0.22 |
| C18:0 | 7.06 ± 1.80 | 5.76 ± 1.26 | 6.31 ± 0.74 |
| C20:0 | 0.51 ± 0.08 | 0.63 ± 0.34 | 0.41 ± 0.17 |
| C22:0 | 0.98 ± 0.68 | 1.03 ± 0.56 | 1.00 ± 0.85 |
| SFA | 35.34 ± 6.62 | 23.86 ± 9.89 | 31.72 ± 3.13 |
| C16:1ω5 | 0.82 ± 0.07 | 0.46 ± 0.35 | 0.78 ± 0.30 |
| C16:1ω7 | 2.32 ± 0.24 | 1.69 ± 1.01 | 2.35 ± 0.66 |
| C17:1ω7 | *tr* | *tr* | *tr* |
| C18:1ω7 | 2.43 ± 0.20 | 2.60 ± 0.02 | 2.88 ± 0.68 |
| C18:1ω9 | 10.49 ± 0.78 | 11.66 ± 1.17 | 10.95 ± 2.30 |
| C20:1ω9 | 1.43 ± 0.40 | 2.47 ± 0.88 | 1.67 ± 0.46 |
| C22:1ω9 | 0.19 ± 0.17 | 0.20 ± 0.21 | 0.32 ± 0.50 |
| MUFA | 17.71 ± 0.22 | 19.13 ± 1.48 | 19.00 ± 3.08 |
| C16:2ω4 | 0.31 ± 0.16 | 0.21 ± 0.08 | 0.24 ± 0.06 |
| C16:3ω4 | 0.37 ± 0.14 | 0.48 ± 0.26 | 0.50 ± 0.15 |
| C16:4ω1 | 0.35 ± 0.47 | 0.16 ± 0.07 | 0.18 ± 0.04 |
| C16:4ω3 | 4.79 ± 1.57 | 4.12 ± 1.16 | 4.94 ± 0.78 |
| C18:2ω6 | 14.33 ± 1.42 | 16.19 ± 2.15 | 14.92 ± 3.48 |
| C18:3ω3 | 1.34 ± 0.07 | 1.68 ± 0.26 | 1.39 ± 0.29 |
| C18:3ω6 | 0.20 ± 0.07 | 0.15 ± 0.06 | 0.13 ± 0.02 |
| C18:4ω3 | 0.29 ± 0.13 | 0.22 ± 0.04 | 0.33 ± 0.14 |
| C20:2ω6 | 5.67 ± 1.37 | 8.64 ± 3.31 | 5.95 ± 1.47 |
| C20:3ω3 | *tr* | 0.25 ± 0.11 | 0.12 ± 0.12 |
| C20:3ω6 | 0.41 ± 0.13 | 0.61 ± 0.34 | 0.44 ± 0.22 |
| C20:4ω3 | *tr* | 0.11 ± 0.09 | *tr* |
| C20:4ω6 | 2.44 ± 0.53 | 3.30 ± 1.08 | 2.96 ± 0.59 |
| C20:5ω3 | 6.15 ± 1.07 | 7.74 ± 2.76 | 6.64 ± 0.37 |
| C21:5ω3 | 3.05 ± 4.72 | 0.48 ± 0.36 | 0.46 ± 0.53 |
| C22:2ω6 | *tr* | 2.27 ± 4.35 | 0.94 ± 1.88 |

| | | | |
|---|---|---|---|
| C22:4ω6 | 1.50 ± 1.49 | 1.82 ± 0.85 | 1.57 ± 0.96 |
| C22:5ω3 | 1.25 ± 0.68 | 1.81 ± 1.34 | 1.48 ± 1.24 |
| C22:6ω3 | 4.33 ± 2.14 | 6.77 ± 3.27 | 6.09 ± 3.27 |
| PUFA | 46.94 ± 6.76 | 57.02 ± 11.14 | 49.27 ± 5.14 |
| $\delta^{13}C$ | -18.92 ± 1.19 | -19.65 ± 1.1 | -18.90 ± 0.90 |
| $\delta^{15}N$ | 9.59 ± 1.19 | 9.12 ± 0.53 | 9.81 ± 0.52 |

*tr* indicates that fatty acids were found in trace amounts (< 0.10% of total fatty acids). SFA, MUFA and PUFA are the sum of saturated, monounsaturated and polyunsaturated fatty acid, respectively. Letters indicate significant differences between the treatments for fatty acid (permANOVA, $p \leq 0.05$) and stable isotope values (KW, $p \leq 0.05$).

**Supplementary Table 3.** Percentage of total fatty acid, $\delta^{13}C$ and $\delta^{15}N$ values (‰) of macroalgae (*Ulva flexuosa*, mean ± SD, n = 6) in T1 (fish, oyster, phytoplankton and macroalgae) and T3 (fish, phytoplankton and macroalgae). Only fatty acids superior to 0.10% of total FA are presented.

| | T1 ponds | T3 ponds |
|---|---|---|
| C12:0 | *tr* | *tr* |
| C14:0 | 1.47 ± 0.96 | 1.66 ± 1.14 |
| C15:0 | 0.14 ± 0.03[a] | 0.20 ± 0.04[b] |
| C15:0 iso | 0.11 ± 0.06[a] | 0.36 ± 0.27[b] |
| C15:0 ante | *tr*[a] | 0.20 ± 0.06[b] |
| C16:0 | 28.72 ± 1.95 | 33.16 ± 5.28 |
| C16:0 iso | 0.54 ± 0.49 | 0.66 ± 0.48 |
| C17:0 | 0.62 ± 0.13 | 0.67 ± 0.09 |
| C18:0 | 0.22 ± 0.04[a] | 0.34 ± 0.05[b] |
| C20:0 | *tr* | *tr* |
| C22:0 | 0.64 ± 0.36 | 1.10 ± 0.75 |
| SFA | 32.55 ± 2.53 | 38.40 ± 5.81 |
| C14:1ω5 | 0.22 ± 0.08 | 0.22 ± 0.07 |
| C15:1ω5 | *tr* | *tr* |
| C16:1ω5 | 3.00 ± 0.30 | 2.95 ± 0.93 |
| C16:1ω7 | 2.37 ± 0.38 | 2.72 ± 0.59 |
| C17:1ω7 | 0.12 ± 0.07 | 0.10 ± 0.03 |
| C18:1ω7 | 8.09 ± 1.25 | 9.12 ± 1.32 |
| C18:1ω9 | 0.50 ± 0.11 | 0.66 ± 0.28 |
| C20:1ω9 | *tr* | *tr* |
| MUFA | 14.33 ± 1.43 | 15.82 ± 1.51 |
| C16:2ω4 | 0.20 ± 0.05 | 0.21 ± 0.05 |
| C16:3ω4 | *tr* | *tr* |
| C16:4ω1 | *tr* | *tr* |
| C16:4ω3 | 18.56 ± 1.80[a] | 15.49 ± 2.4[b] |
| C18:2ω6 | 3.26 ± 0.34 | 3.08 ± 0.39 |
| C18:3ω3 | 10.66 ± 0.84[a] | 8.83 ± 0.59[b] |
| C18:3ω6 | 0.32 ± 0.04 | 0.33 ± 0.09 |
| C18:4ω3 | 18.42 ± 1.77 | 15.82 ± 2.78 |
| C20:4ω3 | 0.27 ± 0.08 | 0.26 ± 0.17 |
| C20:5ω3 | 0.15 ± 0.12 | *tr* |
| C22:5ω3 | 1.07 ± 0.95 | 1.55 ± 1.45 |
| PUFA | 53.10 ± 1.63[a] | 45.69 ± 6.14[b] |
| $\delta^{13}C$ | -13.35 ± 1.53 | 12.61 ± 0.41 |
| $\delta^{15}N$ | 8.25 ± 0.31 | 7.90 ± 0.53 |

*tr* indicates that fatty acids were found in trace amounts (< 0.10% of total fatty acids). SFA, MUFA and PUFA are the sum of saturated, monounsaturated and polyunsaturated fatty acid, respectively. Letters indicate significant differences between the treatments for fatty acid (permANOVA, $p \leq 0.05$) and stable isotope values (KW, $p \leq 0.05$).

**Supplementary Table 4.** Percentage of total fatty acid, $\delta^{13}C$ and $\delta^{15}N$ values (‰) of macroalgae (*Ulva* spp., mean ± SD, n = 6) in T1 (fish, oyster, phytoplankton and macroalgae) and T3 (fish, phytoplankton and macroalgae). Only fatty acids superior to 0.10% of total FA are presented.

| | T1 ponds | T2 ponds |
|---|---|---|
| C12:0 | *tr* | *tr* |
| C14:0 | 1.56 ± 0.77 | 2.08 ±1.00 |
| C15:0 | 0.19 ± 0.07 | 0.35 ± 0.27 |
| C15:0 iso | 0.24 ± 0.11 | 0.32 ± 0.14 |
| C15:0ante | 0.27 ± 0.10 | 0.26 ± 0.04 |
| C16:0 | 31.18 ± 3.09 | 33.50 ± 2.61 |
| C16:0 iso | 0.62 ± 0.35 | 0.38 ± 0.38 |
| C17:0 | 0.28 ± 0.04 | 0.31 ± 0.07 |
| C18:0 | 0.34 ± 0.10 | 0.30 ± 0.18 |
| C20:0 | *tr* | 0.24 ± 0.24 |
| C22:0 | 0.44 ± 0.19 | 0.86 ± 0.88 |
| SFA | 35.24 ± 3.19 | 38.65 ± 2.31 |
| C14:1ω5 | 0.24 ± 0.06 | 0.23 ± 0.08 |
| C15:1ω5 | *tr* | *tr* |
| C16:1ω5 | 5.22 ± 0.45 | 4.01 ± 1.36 |
| C16:1ω7 | 0.60 ± 0.16 | 0.58 ± 0.07 |
| C17:1ω7 | *tr* | *tr* |
| C18:1ω7 | 5.01 ± 0.43 | 5.57 ± 0.71 |
| C18:1ω9 | 0.80 ± 0.16 | 0.96 ± 0.11 |
| C20:1ω9 | 0.12 ± 0.04 | 0.13 ± 0.06 |
| MUFA | 12.13 ± 0.52 | 11.64 ± 0.80 |
| C16:2ω4 | *tr* | *tr* |
| C16:3ω4 | *tr* | *tr* |
| C16:4ω1 | *tr* | *tr* |
| C16:4ω3 | 18.05 ± 1.18[a] | 14.99 ± 2.67[b] |
| C18:2ω6 | 4.63 ± 0.85 | 4.81 ± 0.50 |
| C18:3ω3 | 15.67 ± 2.53 | 14.74 ± 1.19 |
| C18:3ω6 | 0.90 ± 0.09 | 0.80 ± 0.17 |
| C18:4ω3 | 10.51 ± 0.84 | 9.95 ± 2.14 |
| C20:2ω6 | *tr* | *tr* |
| C20:3ω6 | 0.16 ± 0.04[a] | 0.26 ± 0.09[b] |
| C20:4ω3 | 0.24 ± 0.05 | 0.37 ± 0.22 |
| C20:4ω6 | 0.47 ± 0.10 | 0.46 ± 0.12 |
| C20:5ω3 | 0.88 ± 0.18 | 0.68 ± 0.15 |
| C22:4ω6 | *tr* | 0.24 ± 0.34 |
| C22:5ω3 | 0.82 ± 0.38 | 1.61 ± 1.71 |
| C22:6ω3 | *tr* | 0.55 ± 0.81 |

| | | |
|---|---|---|
| PUFA | 34.58 ± 4.27 | 34.72 ± 5.27 |
| $\delta^{13}C$ | -13.43 ± 0.64 | -14.25 ± 1.09 |
| $\delta^{15}N$ | 8.44 ± 0.39 | 8.20 ± 0.33 |

*tr* indicates that fatty acids were found in trace amounts (< 0.10% of total fatty acids). SFA, MUFA and PUFA are the sum of saturated, monounsaturated and polyunsaturated fatty acid, respectively. Letters indicate significant differences between the treatments for fatty acid (permANOVA, $p \leq 0.05$) and stable isotope values (KW, $p \leq 0.05$).

**Supplementary Table 5.** Percentage of total fatty acid, $\delta^{13}C$ and $\delta^{15}N$ values (‰) of macroalgae (*Rhizoclonium riparium*, mean ± SD, n = 6) in T1 (fish, oyster, phytoplankton and macroalgae) and T3 (fish, phytoplankton and macroalgae). Only fatty acids superior to 0.10% of total FA are presented.

| | T1 ponds | T2 ponds |
|---|---|---|
| C12:0 | *tr* | *tr* |
| C14:0 | 0.94 ± 0.33 | 0.75 ± 0.34 |
| C15:0 | 0.23 ± 0.07 | 0.19 ± 0.02 |
| C15:0 iso | 0.13 ± 0.07 | *tr* |
| C15:0 ante | 0.19 ± 0.06 | 0.15 ± 0.13 |
| C16:0 | 29.19 ± 2.22 | 28.41 ± 2.20 |
| C16:0 iso | 0.60 ± 0.05[a] | 0.53 ± 0.04[b] |
| C17:0 | 0.35 ± 0.08 | 0.36 ± 0.11 |
| C18:0 | 0.50 ± 0.29 | 0.38 ± 0.06 |
| C20:0 | 0.13 ± 0.08 | 0.15 ± 0.02 |
| C22:0 | 0.24 ± 0.14 | 0.25 ± 0.05 |
| SFA | 32.51 ± 2.67 | 31.27 ± 2.41 |
| C15:1ω5 | *tr* | 0.10 ± 0.06 |
| C16:1ω5 | 3.81 ± 0.48[a] | 3.03 ± 0.16[b] |
| C16:1ω7 | 1.29 ± 0.59 | 0.91 ± 0.20 |
| C17:1ω7 | 0.12 ± 0.05 | 0.12 ± 0.01 |
| C18:1ω7 | 6.38 ± 0.56 | 6.25 ± 0.48 |
| C18:1ω9 | 1.41 ± 0.83 | 0.78 ± 0.20 |
| C20:1ω9 | 0.14 ± 0.12 | *tr* |
| MUFA | 13.21 ± 1.53[a] | 11.22 ± 0.87[b] |
| C16:2ω4 | 0.23 ± 0.12 | 0.15 ± 0.06 |
| C16:3ω4 | 0.14 ± 0.12 | *tr* |
| C16:4ω1 | *tr* | *tr* |
| C16:4ω3 | 14.68 ± 3.07 | 15.96 ± 0.86 |
| C18:2ω6 | 6.67 ± 0.99 | 6.67 ± 0.74 |
| C18:3ω3 | 17.94 ± 1.70 | 19.06 ± 1.55 |
| C18:3ω6 | 0.93 ± 0.09[a] | 1.03 ± 0.04[b] |
| C18:4ω3 | 10.26 ± 1.35 | 11.79 ± 1.31 |
| C20:2ω6 | *tr* | *tr* |
| C20:3ω6 | 0.20 ± 0.05 | 0.19 ± 0.11 |
| C20:4ω3 | 0.23 ± 0.05 | 0.28 ± 0.02 |
| C20:4ω6 | 0.67 ± 0.33 | 0.62 ± 0.09 |
| C20:5ω3 | 1.61 ± 0.94 | 0.92 ± 0.18 |
| C22:5ω3 | 0.56 ± 0.20 | 0.74 ± 0.13 |
| C22:6ω3 | *tr* | *tr* |
| PUFA | 54.28 ± 3.90 | 57.51 ± 2.27 |
| $\delta^{13}C$ | -15.70 ± 1.72 | -14.91 ± 1.93 |

| | | |
|---|---|---|
| $\delta^{15}N$ | 8.46 ± 0.72 | 7.29 ± 0.47 |

*tr* indicates that fatty acids were found in trace amounts (< 0.10% of total fatty acids). SFA, MUFA and PUFA are the sum of saturated, monounsaturated and polyunsaturated fatty acid, respectively. Letters indicate significant differences between the treatments for fatty acid (permANOVA, $p \leq 0.05$) and stable isotope values (KW, $p \leq 0.05$).

**Supplementary Table 6.** Percentage of total fatty acid, $\delta^{13}C$ and $\delta^{15}N$ values (‰) of suspended particulate organic matter (SPOM, mean ± SD, n = 6) in T1 (fish, oyster, phytoplankton and macroalgae), T2 (fish, oyster and phytoplankton) and T3 (fish, phytoplankton and macroalgae). Only fatty acids superior to 0.10% of total FA are presented.

| | T1 ponds | T2 ponds | T3 ponds |
|---|---|---|---|
| C12:0 | 0.35 ± 0.12 | 0.61 ± 0.24 | 0.76 ± 0.47 |
| C14:0 | 5.01 ± 0.51 | 5.89 ± 0.59 | 5.95 ± 1.13 |
| C15:0 | 1.69 ± 0.30 | 1.75 ± 0.22 | 1.93 ± 0.29 |
| C15:0 iso | 2.49 ± 0.34[a] | 2.21 ± 0.05[a] | 1.86 ± 0.18[b] |
| C15:0 ante | 1.49 ±0.26 | 1.64 ± 0.31 | 1.06 ± 0.52 |
| C16:0 | 33.18 ± 2.46 | 31.35 ±5.34 | 32.82 ± 5.14 |
| C16:0 iso | 0.74 ± 0.28 | 0.55 ± 0.19 | 0.77 ± 0.19 |
| C17:0 | 1.32 ± 0.38 | 1.12 ±0.16 | 1.21 ± 0.15 |
| C18:0 | 10.57 ± 1.14 | 10.33 ±1.88 | 9.90 ± 2.67 |
| C20:0 | 1.28 ± 0.43 | 1.21 ± 0.14 | 1.26 ± 0.27 |
| C22:0 | 1.14 ± 0.42 | 1.05 ± 0.16 | 1.25 ± 0.27 |
| SFA | 59.26 ± 2.76 | 57.70 ± 2.95 | 58.76 ± 5.04 |
| C14:1ω5 | *tr* | *tr* | *tr* |
| C16:1ω5 | 2.12 ± 0.80 | 2.03 ± 0.71 | 1.48 ± 0.52 |
| C16:1ω7 | 7.19 ± 0.77 | 7.36 ± 1.90 | 7.61 ± 0.62 |
| C17:1ω7 | 0.75 ± 0.51 | 0.88 ± 0.20 | 0.63 ± 0.33 |
| C18:1ω7 | 6.77 ± 0.60[a,b] | 7.65 ± 1.24[a] | 6.16 ± 0.46[b] |
| C18:1ω9 | 10.00 ±1.16 | 9.14 ± 1.32 | 9.70 ± 0.96 |
| C20:1ω9 | 1.12 ± 0.86 | 1.23 ± 0.43 | 1.19 ±0.63 |
| C22:1ω9 | 0.38 ±0.47 | 0.37 ± 0.40 | 0.20 ± 0.26 |
| MUFA | 28.34 ± 1.76 | 28.66 ± 1.24 | 27.05 ± 1.74 |
| C16:2ω4 | 0.32 ± 0.19 | 0.26 ± 0.28 | 0.52 ± 0.27 |
| C16:3ω4 | 0.14 ± 0.21[a] | 0.18 ± 0.25[a] | 0.66 ± 0.34[b] |
| C16:4ω1 | 0.38 ± 0.40 | 0.43 ± 0.37 | 0.65 ± 0.47 |
| C16:4ω3 | 0.25 ± 0.17 | 0.23 ±0.26 | 0.31 ± 0.38 |
| C18:2ω6 | 3.01 ± 0.38 | 3.60 ± 0.42 | 2.96 ± 0.73 |
| C18:3ω3 | 1.68 ± 0.27[a,b] | 2.25 ± 0.51[a] | 1.48 ± 0.32[b] |
| C18:3ω6 | 0.28 ± 0.19 | 0.24 ±0.17 | 0.38 ± 0.22 |
| C18:4ω3 | 0.74 ± 0.60 | 0.62 ± 0.24 | 0.82 ± 0.21 |
| C20:2ω6 | 0.16 ± 0.15 | 0.32 ± 0.19 | 0.17 ± 0.12 |
| C20:3ω3 | 0.22 ± 0.31 | 0.47 ± 0.19 | 0.24 ± 0.28 |
| C20:3ω6 | 0.13 ± 0.20 | 0.25 ± 0.08 | 0.14 ± 0.22 |
| C20:4ω3 | *tr* | 0.12 ± 0.12 | *tr* |
| C20:4ω6 | 0.22 ± 0.21 | *tr* | 0.14 ± 0.14 |
| C20:5ω3 | 1.26 ± 0.31 | 1.12 ± 1.14 | 1.88 ± 0.53 |
| C21:5ω3 | *tr* | 0.26 ± 0.09 | 0.16 ± 0.26 |
| C22:2ω6 | 0.63 ± 0.67 | 0.36 ± 0.17 | 0.24 ± 0.14 |

| | | | |
|---|---|---|---|
| C22:4ω6 | *tr* | *tr* | *tr* |
| C22:5ω3 | 1.29 ± 0.42 | 1.19 ± 0.24 | 1.27 ± 0.34 |
| C22:6ω3 | 1.59 ± 1.11 | 1.64 ± 0.96 | 2.03 ± 1.00 |
| PUFA | 12.40 ± 2.80 | 13.63 ± 3.21 | 14.19 ± 3.81 |
| $\delta^{13}C$ | -21.06 ± 0.25[a] | -23.05 ± 1.22[b] | -21.09 ± 0.43[a] |
| $\delta^{15}N$ | 6.25 ± 0.70[a] | 3.02 ± 0.48[b] | 5.07 ± 0.56[a] |

*tr* indicates that fatty acids were found in trace amounts (< 0.10% of total fatty acids). SFA, MUFA and PUFA are the sum of saturated, monounsaturated and polyunsaturated fatty acid, respectively. Letters indicate significant differences between the treatments for fatty acid (permANOVA, $p \leq 0.05$) and stable isotope values (KW, $p \leq 0.05$).

**Supplementary Table 7.** Percentage of total fatty acid, $\delta^{13}C$ and $\delta^{15}N$ values (‰) of large zooplankton (mean ± SD, n = 6) in T1 (fish, oyster, phytoplankton and macroalgae), T2 (fish, oyster and phytoplankton) and T3 (fish, phytoplankton and macroalgae). Only fatty acids superior to 0.10% of total FA are presented.

| | T1 ponds | T2 ponds | T3 ponds |
|---|---|---|---|
| C12:0 | *tr* | *tr* | *tr* |
| C14:0 | 2.05 ± 1.23[a] | 1.99 ± 0.23[a] | 4.41 ± 0.99[b] |
| C15:0 | 0.74 ± 0.07[a] | 0.67 ± 0.06[a] | 0.89 ± 0.06[b] |
| C15:0 iso | 0.46 ± 0.08[a] | 0.27 ± 0.16[a] | 0.85 ± 0.35[b] |
| C15:0 ante | 0.18 ± 0.05[a] | 0.15 ± 0.14[a] | 0.31 ± 0.06[b] |
| C16:0 | 26.19 ± 1.80 | 26.62 ± 2.90 | 25.91 ± 2.53 |
| C16:0 iso | 0.23 ± 0.06 | 0.20 ± 0.13 | 0.18 ± 0.12 |
| C17:0 | 1.13 ± 0.02[a] | 0.93 ± 0.05[b] | 1.10 ± 0.05[a] |
| C18:0 | 8.47 ± 1.72 | 7.86 ± 0.49 | 7.81 ± 1.14 |
| C20:0 | 0.35 ± 0.05 | 0.32 ± 0.12 | 0.29 ± 0.16 |
| C22:0 | 0.12 ± 0.06 | 0.16 ± 0.10 | 0.31 ± 0.29 |
| SFA | 40.00 ± 1.21 | 39.26 ± 3.45 | 42.10 ± 1.39 |
| C14:1ω5 | *tr* | 0.12 ± 0.13 | *tr* |
| C16:1ω5 | 0.79 ± 0.07 | 0.53 ± 0.22 | 0.71 ± 0.12 |
| C16:1ω7 | 2.76 ± 0.62 | 2.39 ± 0.62 | 3.13 ± 0.71 |
| C17:1ω7 | 0.24 ± 0.07[a] | 0.12 ± 0.06[b] | 0.20 ± 0.05[a,b] |
| C18:1ω7 | 5.09 ± 0.95[a] | 3.71 ± 1.06[a,b] | 3.77 ± 0.63[b] |
| C18:1ω9 | 13.60 ± 2.14[a] | 16.64 ± 1.67[a] | 9.77 ± 2.04[b] |
| C20:1ω9 | 0.53 ± 0.23 | 0.38 ± 0.08 | 0.42 ± 0.04 |
| MUFA | 23.02 ± 2.66[a] | 23.89 ± 0.45[a] | 18.00 ± 1.08[b] |
| C16:2ω4 | 0.28 ± 0.09[a,b] | 0.38 ± 0.08[a] | 0.20 ± 0.12[b] |
| C16:3ω4 | 0.51 ± 0.30 | 0.48 ± 0.06 | 0.49 ± 0.02 |
| C16:4ω1 | 0.22 ± 0.12 | 0.20 ± 0.04 | 0.20 ± 0.07 |
| C16:4ω3 | 0.85 ± 0.59 | 0.23 ± 0.12 | 0.31 ± 0.03 |
| C18:2ω6 | 13.88 ± 2.35[a] | 21.26 ± 5.21[b] | 10.79 ± 5.02[a] |
| C18:3ω3 | 2.51 ± 0.31 | 2.39 ± 0.63 | 2.19 ± 0.16 |
| C18:3ω6 | 0.28 ± 0.04[a] | 0.24 ± 0.06[a,b] | 0.15 ± 0.07[b] |
| C18:4ω3 | 1.18 ± 0.23[a] | 0.65 ± 0.36[b] | 1.53 ± 0.02[c] |
| C20:2ω6 | 0.79 ± 0.23 | 0.68 ± 0.13 | 0.70 ± 0.16 |
| C20:3ω3 | 0.16 ± 0.11 | *tr* | *tr* |
| C20:3ω6 | *tr* | *tr* | *tr* |
| C20:4ω3 | *tr* | *tr* | *tr* |
| C20:4ω6 | 2.20 ± 0.23[a] | 1.12 ± 0.20[b] | 1.63 ± 0.54[a,b] |
| C20:5ω3 | 7.48 ± 0.81[a] | 4.69 ± 1.95[b] | 9.73 ± 2.24[a] |
| C22:5ω3 | 0.47 ± 0.40 | 0.25 ± 0.27 | 0.45 ± 0.42 |
| C22:6ω3 | 6.12 ± 1.25[a] | 4.10 ± 0.86[b] | 11.51 ± 3.54[c] |
| PUFA | 36.98 ± 1.80 | 36.82 ± 3.43 | 39.90 ± 2.03 |

| | | | |
|---|---|---|---|
| $\delta^{13}C$ | -21.32 ± 0.58 | -22.11 ± 1.28 | -22.39 ± 0.78 |
| $\delta^{15}N$ | 10.13 ± 0.52[a] | 7.25 ± 1.80[b] | 7.57 ± 0.32[b] |

*tr* indicates that fatty acids were found in trace amounts (< 0.10% of total fatty acids). SFA, MUFA and PUFA are the sum of saturated, monounsaturated and polyunsaturated fatty acid, respectively. Letters indicate significant differences between the treatments for fatty acid (permANOVA, $p \leq 0.05$) and stable isotope values (KW, $p \leq 0.05$).

**Supplementary Table 8.** Percentage of total fatty acid, $\delta^{13}C$ and $\delta^{15}N$ values (‰) of meagre (*Argyrosomus regius*, mean ± SD, n = 6) in T1 (fish, oyster, phytoplankton and macroalgae), T2 (fish, oyster and phytoplankton) and T3 (fish, phytoplankton and macroalgae). Only fatty acids superior to 0.10% of total FA are presented.

| | T1 ponds | T2 ponds | T3 ponds |
|---|---|---|---|
| C14:0 | 0.88 ± 0.11 | 0.66 ± 0.07 | 0.69 ± 0.25 |
| C15:0 | 0.24 ± 0.02 | 0.20 ± 0.01 | 0.22 ± 0.04 |
| C16:0 | 20.26 ± 0.97 | 18.32 ± 1.43 | 19.43 ± 1.39 |
| C17:0 | 0.29 ± 0.01 | 0.28 ± 0.03 | 0.29 ± 0.04 |
| C18:0 | 7.24 ± 0.79 | 7.83 ± 0.42 | 8.08 ± 0.63 |
| C20:0 | 0.11 ± 0.05 | 0.14 ± 0.04 | 0.12 ± 0.07 |
| SFA | 29.03 ± 1.29 | 27.42 ± 1.56 | 28.82 ± 1.78 |
| C14:1ω5 | *tr*[a] | 0.15 ± 0.06[b] | 0.18 ± 0.07[b] |
| C16:1ω5 | *tr* | 0.19 ± 0.02 | 0.12 ± 0.09 |
| C16:1ω7 | 1.55 ± 0.09[a] | 1.22 ± 0.15[b] | 1.25 ± 0.25[b] |
| C18:1ω7 | 1.64 ± 0.23 | 1.56 ± 0.17 | 1.56 ± 0.16 |
| C18:1ω9 | 17.97 ± 1.25 | 17.09 ± 1.78 | 15.93 ± 1.61 |
| C20:1ω9 | 0.42 ± 0.06 | 0.45 ± 0.06 | 0.42 ± 0.08 |
| MUFA | 21.71 ± 1.67 | 20.66 ± 2.06 | 19.47 ± 2.06 |
| C16:2ω4 | 0.34 ± 0.04 | 0.27 ± 0.06 | 0.29 ± 0.05 |
| C16:3ω4 | 0.35 ± 0.20 | 0.20 ± 0.04 | 0.41 ± 0.39 |
| C16:4ω1 | 0.23 ± 0.17 | 0.11 ± 0.17 | *tr* |
| C16:4ω3 | 0.31 ± 0.26 | 0.15 ± 0.21 | *tr* |
| C18:2ω6 | 37.97 ± 0.66 | 38.60 ± 0.79 | 38.37 ± 1.73 |
| C18:3ω3 | 2.52 ± 0.53 | 2.32 ± 0.29 | 2.27 ± 0.30 |
| C18:3ω6 | 0.10 ± 0.08 | *tr* | *tr* |
| C18:4ω3 | 0.16 ± 0.20 | *tr* | *tr* |
| C20:2ω6 | 0.20 ± 0.03[a] | 0.27 ± 0.04[b] | 0.24 ± 0.03[b] |
| C20:3ω6 | *tr* | *tr* | *tr* |
| C20:4ω3 | *tr* | *tr* | *tr* |
| C20:4ω6 | 0.99 ± 0.21[a] | 1.23 ± 0.14[b] | 1.37 ± 0.18[b] |
| C20:5ω3 | 0.76 ± 0.07[a] | 0.94 ± 0.08[b] | 1.06 ± 0.17[b] |
| C22:5ω3 | tr | 0.17 ± 0.20 | 0.11 ± 0.17 |
| C22:6ω3 | 5.23 ± 1.16[a] | 7.54 ± 0.84[b] | 7.32 ± 0.92[b] |
| PUFA | 49.26 ± 0.75[a] | 51.92 ± 0.70[b] | 51.71 ± 2.02[b] |
| $\delta^{13}C$ | -20.64 ± 0.15 | -20.64 ± 0.08 | -20.59 ± 0.12 |
| $\delta^{15}N$ | 10.45 ± 0.19 | 10.40 ± 0.15 | 10.36 ± 0.15 |

*tr* indicates that fatty acids were found in trace amounts (< 0.10% of total fatty acids). SFA, MUFA and PUFA are the sum of saturated, monounsaturated and polyunsaturated fatty acid, respectively. Letters indicate

significant differences between the treatments for fatty acid (permANOVA, $p \leq 0.05$) and stable isotope values (KW, $p \leq 0.05$).

**Supplementary Table 9.** Percentage of total fatty acid, $\delta^{13}C$ and $\delta^{15}N$ values (‰) of white seabream (*Diplodus sargus*, mean ± SD, n = 6) in T1 (fish, oyster, phytoplankton and macroalgae), T2 (fish, oyster and phytoplankton) and T3 (fish, phytoplankton and macroalgae). Only fatty acids superior to 0.10% of total FA are presented.

| | T1 ponds | T2 ponds | T3 ponds |
|---|---|---|---|
| C12:0 | *tr* | *tr* | *tr* |
| C14:0 | 2.22 ± 0.49 | 2.15 ± 0.40 | 2.74 ± 0.80 |
| C15:0 | 0.39 ± 0.04 | 0.38 ± 0.06 | 0.45 ± 0.07 |
| C15:0 iso | *tr* | *tr* | 0.11 ± 0.02 |
| C15:0 ante | *tr* | *tr* | *tr* |
| C16:0 | 22.05 ± 0.71 | 21.30 ± 0.34 | 21.69 ± 1.11 |
| C16:0 iso | *tr* | *tr* | *tr* |
| C17:0 | 0.35 ± 0.01 | 0.38 ± 0.06 | 0.37 ± 0.06 |
| C18:0 | 4.23 ± 0.60 | 4.45 ± 0.71 | 4.09 ± 0.54 |
| C20:0 | 0.12 ± 0.02[a,b] | *tr*[a] | 0.12 ± 0.02[b] |
| C22:0 | *tr* | *tr* | *tr* |
| SFA | 29.50 ± 0.72 | 28.98 ± 0.43 | 29.67 ± 1.55 |
| C14:1ω5 | *tr* | *tr* | *tr* |
| C16:1ω5 | *tr* | 0.10 ± 0.05 | *tr* |
| C16:1ω7 | 3.17 ± 0.59 | 3.18 ± 0.50 | 3.18 ± 0.54 |
| C17:1ω7 | 0.19 ± 0.35 | *tr* | *tr* |
| C18:1ω7 | 1.88 ± 0.05[a] | 2.11 ± 0.31[b] | 2.15 ± 0.18[b] |
| C18:1ω9 | 21.72 ± 1.28 | 21.57 ± 0.91 | 21.97 ± 0.93 |
| C20:1ω9 | 0.69 ± 0.06 | 0.71 ± 0.11 | 0.82 ± 0.13 |
| C22:1ω9 | *tr* | *tr* | *tr* |
| MUFA | 27.74 ± 1.57 | 27.76 ± 0.85 | 28.93 ± 1.33 |
| C16:2ω4 | 0.27 ± 0.03 | 0.28 ± 0.06 | 0.28 ± 0.06 |
| C16:3ω4 | 0.41 ± 0.14 | 0.39 ± 0.04 | 0.43 ± 0.02 |
| C16:4ω1 | 0.15 ± 0.04 | 0.16 ± 0.07 | 0.17 ± 0.04 |
| C16:4ω3 | 0.17 ± 0.08 | 0.16 ± 0.16 | 0.26 ± 0.15 |
| C18:2ω6 | 30.71 ± 1.17 | 30.18 ± 2.10 | 28.67 ± 0.99 |
| C18:3ω3 | 3.19 ± 0.32 | 3.22 ± 0.17 | 3.23 ± 0.39 |
| C18:3ω6 | 0.25 ± 0.09 | 0.34 ± 0.08 | 0.28 ± 0.05 |
| C18:4ω3 | 0.32 ± 0.07 | 0.36 ± 0.08 | 0.47 ± 0.18 |
| C20:2ω6 | 0.54 ± 0.09[a,b] | 0.61 ± 0.04[a] | 0.72 ± 0.51[b] |
| C20:3ω3 | 0.14 ± 0.21 | *tr* | *tr* |
| C20:3ω6 | 0.23 ± 0.08 | 0.27 ± 0.05 | 0.22 ± 0.03 |
| C20:4ω3 | 0.16 ± 0.05 | 0.17 ± 0.03 | 0.18 ± 0.10 |
| C20:4ω6 | 0.65 ± 0.19 | 0.77 ± 0.39 | 0.71 ± 0.28 |
| C20:5ω3 | 1.09 ± 0.17 | 1.37 ± 0.48 | 1.43 ± 0.37 |
| C22:5ω3 | 0.33 ± 0.19 | 0.38 ± 0.29 | 0.39 ± 0.21 |
| C22:6ω3 | 4.14 ± 1.40 | 4.71 ± 1.18 | 3.90 ± 1.13 |

| | | | |
|---|---|---|---|
| PUFA | 42.75 ± 1.92 | 43.39 ± 0.79 | 41.40 ± 1.97 |
| $\delta^{13}C$ | -20.96 ± 0.68 | -20.78 ± 0.70 | -21.13 ± 0.99 |
| $\delta^{15}N$ | 11.48 ± 0.82 | 10.98 ± 0.30 | 11.02 ± 0.57 |

*tr* indicates that fatty acids were found in trace amounts (< 0.10% of total fatty acids). SFA, MUFA and PUFA are the sum of saturated, monounsaturated and polyunsaturated fatty acid, respectively. Letters indicate significant differences between the treatments for fatty acid (permANOVA, $p \leq 0.05$) and stable isotope values (KW, $p \leq 0.05$).

**Supplementary Table 10.** Percentage of total fatty acid, $\delta^{13}C$ and $\delta^{15}N$ values (‰) of grey mullet (*Mugil cephalus*, mean ± SD, n = 6) in T1 (fish, oyster, phytoplankton and macroalgae), T2 (fish, oyster and phytoplankton) and T3 (fish, phytoplankton and macroalgae). Only fatty acids superior to 0.10% of total FA are presented.

| | T1 ponds | T2 ponds | T3 ponds |
|---|---|---|---|
| C14:0 | 0.74 ± 0.35[a] | 4.16 ± 2.18[b] | 1.58 ± 0.73[c] |
| C15:0 | 0.34 ± 0.12 | 0.50 ± 0.09 | 0.42 ± 0.12 |
| C15:0 iso | *tr*[a] | 0.13 ± 0.04[b] | *tr*[a,b] |
| C15:0 ante | *tr* | *tr* | *tr* |
| C16:0 | 27.66 ± 3.99 | 24.63 ± 2.19 | 26.66 ± 3.43 |
| C16:0 iso | *tr* | *tr* | *tr* |
| C17:0 | 0.43 ± 0.04[a] | 0.31 ± 0.03[b] | 0.34 ± 0.04[b] |
| C18:0 | 8.57 ± 0.59[a] | 5.22 ± 0.87[b] | 6.94 ± 2.63[a,b] |
| C20:0 | 0.10 ± 0.05 | *tr* | *tr* |
| C22:0 | tr | *tr* | *tr* |
| SFA | 38.25 ± 4.71 | 35.25 ± 3.66 | 36.38 ± 4.98 |
| C14:1ω5 | 0.20 ± 0.16[a] | *tr*[a,b] | *tr*[b] |
| C15:1ω5 | *tr* | *tr* | *tr* |
| C16:1ω5 | 0.10 ± 0.05 | 0.15 ± 0.06 | *tr* |
| C16:1ω7 | 1.25 ± 0.56[a] | 7.03 ± 3.97[b] | 3.16 ± 1.47[b] |
| C17:1ω7 | 0.20 ± 0.28 | *tr* | 0.45 ± 0.48 |
| C18:1ω7 | 1.43 ± 0.31[a] | 2.40 ± 0.18[b] | 2.04 ± 0.40[b] |
| C18:1ω9 | 9.85 ± 1.62[a] | 13.67 ± 2.03[b] | 13.68 ± 3.51[b] |
| C20:1ω9 | 0.33 ± 0.08 | 0.48 ± 0.10 | 0.40 ± 0.16 |
| MUFA | 13.37 ± 2.29[a] | 23.95 ± 3.20[b] | 19.84 ± 5.02[b] |
| C16:2ω4 | 0.27 ± 0.08[a] | 1.06 ± 0.64[b] | 0.23 ± 0.15[a] |
| C16:3ω4 | 0.24 ± 0.19[a] | 1.04 ± 0.62[b] | 0.28 ± 0.22[a] |
| C16:4ω3 | 0.30 ± 0.09 | 0.14 ± 0.11 | 0.24 ± 0.10 |
| C16:4ω1 | 0.34 ± 0.09 | 0.49 ± 0.22 | 0.27 ± 0.11 |
| C18:2ω6 | 20.2 ± 2.7 | 20.5 ± 5.71 | 25.1 ± 3.7 |
| C18:3ω3 | 1.12 ± 0.24[a] | 1.75 ± 0.39[b] | 1.75 ± 0.67[a,b] |
| C18:3ω6 | 0.33 ± 0.01 | 0.40 ± 0.09 | 0.34 ± 0.07 |
| C18:4ω3 | 0.10 ± 0.06[a] | 0.49 ± 0.21[b] | 0.19 ± 0.10[a] |
| C20:2ω6 | 0.61 ± 0.11 | 0.50 ± 0.14 | 0.61 ± 0.08 |
| C20:3ω3 | 0.58 ± 1.21 | *tr* | 0.11 ± 0.15 |
| C20:3ω6 | 0.12 ± 0.03 | 0.10 ± 0.06 | *tr* |
| C20:4ω3 | *tr*[a] | 0.21 ± 0.06[b] | *tr*[a] |
| C20:4ω6 | 3.60 ± 0.81[a] | 1.62 ± 0.41[b] | 2.22 ± 0.95[b] |
| C20:5ω3 | 3.06 ± 2.86 | 3.51 ± 1.13 | 2.65 ± 1.17 |
| C22:4ω6 | 0.14 ± 0.13 | *tr* | *tr* |
| C22:5ω3 | 1.86 ± 0.94 | 1.63 ± 0.41[b] | 1.26 ± 0.26[b] |
| C22:6ω3 | 15.73 ± 3.74[a] | 7.42 ± 3.05 | 8.65 ± 3.03 |

| | | | |
|---|---|---|---|
| PUFA | 48.38 ± 5.56[a] | 40.80 ± 6.10[b] | 43.78 ± 1.87[a,b] |
| $\delta^{13}C$ | -19.51 ± 0.59 | -19.10 ± 0.71 | -19.40 ± 0.51 |
| $\delta^{15}N$ | 10.42 ± 0.72 | 10.07 ± 0.31 | 10.38 ± 0.52 |

*tr* indicates that fatty acids were found in trace amounts (< 0.10% of total fatty acids). SFA, MUFA and PUFA are the sum of saturated, monounsaturated and polyunsaturated fatty acid, respectively. Letters indicate significant differences between the treatments for fatty acid (permANOVA, $p \leq 0.05$) and stable isotope values (KW, $p \leq 0.05$).

**Supplementary Table 11.** Percentage of total fatty acid, $\delta^{13}C$ and $\delta^{15}N$ values (‰) of pacific oyster (mean ± SD, n = 6, *Magallana gigas*) in T1 (fish, oyster, phytoplankton and macroalgae) and T2 (fish, oyster and phytoplankton). Only fatty acids superior to 0.10% of total FA are presented.

| | T1 ponds | T2 ponds |
|---|---|---|
| C12:0 | *tr* | *tr* |
| C14:0 | 3.53 ± 0.23[a] | 6.66 ± 1.18[b] |
| C15:0 | 1.48 ± 0.12[a] | 1.74 ± 0.19[b] |
| C15:0 iso | 0.73 ± 0.05 | 0.68 ± 0.09 |
| C15:0 ante | 0.16 ± 0.03 | 0.14 ± 0.02 |
| C16:0 | 32.90 ± 1.91 | 33.30 ± 2.57 |
| C16:0 iso | 0.46 ± 0.03 | 0.48 ± 0.06 |
| C17:0 | 2.11 ± 0.07 | 2.02 ± 0.17 |
| C18:0 | 3.95 ± 0.30[a] | 3.42 ± 0.41[b] |
| SFA | 45.34 ± 2.18 | 48.44 ± 3.01 |
| C14:1ω5 | *tr* | *tr* |
| C16:1ω5 | 1.52 ± 0.07[a] | 1.41 ± 0.10[b] |
| C16:1ω7 | 3.38 ± 0.37[a] | 5.77 ± 0.58[b] |
| C17:1ω7 | 0.16 ± 0.03[a] | *tr*[b] |
| C18:1ω7 | 4.63 ± 0.25[a] | 6.07 ± 0.86[b] |
| C18:1ω9 | 5.77 ± 0.60[a] | 4.09 ± 0.24[b] |
| C20:1ω9 | 0.36 ± 0.03[a] | 0.26 ± 0.05[b] |
| MUFA | 15.89 ± 0.46[a] | 17.50 ± 1.41[b] |
| C16:2ω4 | 0.30 ± 0.04[a] | 0.62 ± 0.13[b] |
| C16:3ω4 | 0.54 ± 0.11 | 0.59 ± 0.08 |
| C16:4ω1 | *tr* | 0.10 ± 0.07 |
| C16:4ω3 | 1.94 ± 0.40 | 1.81 ± 0.44 |
| C18:2ω6 | 8.22 ± 0.54[a] | 5.10 ± 0.44[b] |
| C18:3ω3 | 5.70 ± 1.18[a] | 2.18 ± 0.24[b] |
| C18:3ω6 | 0.31 ± 0.03[a] | 0.45 ± 0.12[b] |
| C18:4ω3 | 3.16 ± 0.54 | 3.07 ± 0.28 |
| C20:2ω6 | 0.27 ± 0.04[a] | 0.14 ± 0.08[b] |
| C20:3ω3 | *tr* | *tr* |
| C20:3ω6 | *tr* | *tr* |
| C20:4ω3 | 0.36 ± 0.06 | 0.29 ± 0.05 |
| C20:4ω6 | 1.94 ± 0.40 | 2.01 ± 0.43 |
| C20:5ω3 | 9.90 ± 0.80[a] | 12.23 ± 1.36[b] |
| C21:5ω3 | 0.29± 0.18 | 0.47 ± 0.06 |
| C22:4ω6 | *tr* | *tr* |
| C22:5ω3 | 0.51 ± 0.19 | 0.37 ± 0.20 |
| C22:6ω3 | 5.07 ± 1.29 | 4.31 ± 0.67 |
| PUFA | 38.77 ± 2.18[a] | 33.81 ± 2.60[b] |

| | | |
|---|---|---|
| $\delta^{13}C$ | -21.48 ± 0.74[a] | -22.69 ± 0.23[b] |
| $\delta^{15}N$ | 8.72 ± 0.49[a] | 5.96 ± 0.43[b] |

*tr* indicates that fatty acids were found in trace amounts (< 0.10% of total fatty acids). SFA, MUFA and PUFA are the sum of saturated, monounsaturated and polyunsaturated fatty acid, respectively. Letters indicate significant differences between the treatments for fatty acid (permANOVA, $p \leq 0.05$) and stable isotope values (KW, $p \leq 0.05$).