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## Marine ecological aquaculture: a successful Mediterranean integrated multi-trophic aquaculture case study of a fish, oyster and algae assemblage

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### ► To cite this version:

Emmanuelle Roque d'Orbcastel, Mathieu Lutier, Emilie Le Floc'H, François Ruelle, Sébastien Triplet, et al.. Marine ecological aquaculture: a successful Mediterranean integrated multi-trophic aquaculture case study of a fish, oyster and algae assemblage. *Aquaculture International*, 2022, 10.1007/s10499-022-00953-0 . hal-03753874

**HAL Id: hal-03753874**

**<https://hal.science/hal-03753874v1>**

Submitted on 18 Aug 2022

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1 **Marine ecological aquaculture: a successful Mediterranean integrated multi-trophic aquaculture**  
2 **case study of a fish, oyster and algae assemblage**

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7 **Abstract**

8 Inspired by agroecology, ecological aquaculture proposes an alternative model that uses ecology as a paradigm to  
9 develop innovative, more eco-friendly aquaculture with environmental, economic and social benefits. Integrated  
10 multi-trophic aquaculture (IMTA) is one application of this principle. Inspired by the natural trophic chain, it  
11 associates primary producers with primary or secondary consumers, providing a new source of biomass without  
12 requiring supplementary feed by recycling inorganic and organic wastes. Of these systems, land-based IMTAs  
13 demonstrate several advantages, especially easier control of nutrient flows, contaminants and/or predators. This  
14 study focused on a land-based marine IMTA, combining a recirculating aquaculture system for fish consecutively  
15 with a natural marine polyculture of microalgae and oyster cultivation. The objective was to assess the ability of  
16 the microalgal polyculture both to bioremediate fish nutrients and to sustain oyster growth. For the first time in a  
17 Mediterranean climate, we confirmed the feasibility of developing a microalgae community of interest for oysters  
18 maintained by fish effluent. Despite strong variability in microalgae production, this IMTA system resulted in  
19 significant oyster growth over the experimental period of one month, with growth results of the same order of  
20 magnitude as natural juvenile growth. In the conditions tested, this IMTA with reduced human intervention  
21 allowed a gain in recoverable biomass of 3.7 g of oyster produced per kg of fish feed distributed. By transforming  
22 waste into additional biomass, IMTAs offer a more promising, ecological avenue for aquaculture, based on a  
23 circular economy, which may in turn increase the social acceptability of fish farming. (250 words)

24 **Key words** Integrated Multi-trophic Aquaculture, nutrient recycling, oysters, fish, microalgae.

25 **Abbreviations:**

26 ARA: arachidonic acid

27 EPA: eicosapentaenoic acid

28 HRAP: high-rate algal pond

29 IMTA: Integrated multi-trophic aquaculture

30 PUFA: polyunsaturated fatty acids

31 RAS: recirculating aquaculture systems

32 RE: removal efficiency

33 SGR: specific growth rate

34

## 35 **1. Introduction**

### 36 *1.1 Food system paradigms*

37 Today, the global food system and food security face multiple challenges, with a convergence of population growth  
38 and increasing hunger and malnutrition (FAO 2016). Additional pressure, e.g. anthropic activity and  
39 environmental pollution, threatens natural resources, while the consequences of climate change are leading to the  
40 loss of biodiversity and unbalanced production systems (IPBES 2019). A transition is required towards more  
41 sustainable production and consumption.

42 In recent decades, the general paradigm in animal or vegetal feedstock production, especially in Europe, has been  
43 to grow one selected species under conditions of intensive cultivation. This method of production is highly  
44 dependent on the species' ability for high and stable growth and resilience to sudden environmental changes. In a  
45 context of global change, with extreme climatic events occurring more frequently, in aquaculture systems, high  
46 water temperatures potentially increase the risk of some diseases (Karvonen et al. 2010). Monospecific systems  
47 are based on species selected for their ability to dedicate their energy to growth and have little adaptability to  
48 exogenous pressures; thus, xenobiotics (e.g. antimicrobials) are often needed to maintain productivity in intensive  
49 systems (Smith et al. 1999). Of animal production systems, aquaculture is growing 7.8% per year, exceeding all  
50 others (Troell et al. 2014). Monospecific aquaculture (especially to raise finfish) is today common worldwide and  
51 is often associated with the use of antibiotics (Miranda 2011; Lulijwa et al. 2019), potential chemical contaminants  
52 such as heavy metals or dioxins, or hormones (FAO 2003). Another disadvantage is the discharge of large amounts  
53 of nutrients (i.e. from organism excretions and unconsumed food), which can cause the eutrophication and  
54 deoxygenation of coastal waters when environmental carrying capacity is exceeded (Gowen and Bradbury 1987;  
55 Pillay 2004). This can foster the development of pathogens and parasites, which may in turn negatively impact  
56 biodiversity, human health, as well as the farmed species (Neori et al. 2004; Jegatheesan et al. 2011), leading to  
57 sometimes dramatic environmental concerns due to the presence of potential harmful residues impacting the health  
58 of end consumers (Okocha et al. 2018).

### 59 *1.2 From monospecific to multispecies production systems*

60 A major paradigm shift is essential to improve not only aquaculture food safety, but also its social acceptability.  
1  
2 61 In the agricultural sector, research into alternative agriculture applying the principles of ecology emerged in the  
3  
4 62 1980s (Altieri 1983). The ‘agroecology’ paradigm is based on several principles: e.g. input reduction, recycling,  
5  
6 63 animal health, biodiversity and synergy (Wezel et al. 2020). It aims to take into account interactions between the  
7  
8 64 plants, animals, humans and the environment within agricultural systems and to imitate the natural processes  
9  
10 65 involved in ecosystem productivity, stability and resilience (Malézieux 2012) in order to develop new production  
11  
12 66 methods (Snapp 2017).

13  
14  
15 67 Inspired by this approach, ‘ecological aquaculture’ has been put forward as an alternative model that uses ecology  
16  
17 68 as a paradigm to develop aquaculture with environmental, economic and social benefits. It maintains that  
18  
19 69 aquaculture should mimic the structure and functions of natural ecosystems and practice nutrient recycling through  
20  
21 70 waste reuse (Costa-Pierce 2015; Aubin et al. 2017). Based on the natural trophic chain, integrated multi-trophic  
22  
23 71 aquaculture (IMTA) is in this sense one of the logical next steps in alternative aquaculture development  
24  
25 72 (Barrington et al. 2009), representing a way to improve existing systems.

### 26 27 28 73 ***1.3 Higher diversity in farmed species could help social acceptability***

29  
30 74 Studies looking into the possibility of IMTA began in the early 1970s (Ryther 1975; Goldman et al. 1974), with  
31  
32 75 research efforts increasing over the last two decades, both in marine ecosystems (Chopin et al. 2001; Hussenot  
33  
34 76 2003; Barrington et al. 2009 in FAO 2009; Milhazes-Cunha and Otero, 2017; Buck et al. 2018) and freshwater  
35  
36 77 ecosystems (Wongkiew et al. 2017). As mentioned by Chopin (2013), the concept of IMTA can in fact be traced  
37  
38 78 back to the origins of aquaculture (in 2200–2100 BCE, You Hou Bin detailed the integration of fish with aquatic  
39  
40 79 plants). Chopin defines IMTA as “*The farming, in proximity, of species from different trophic levels and with*  
41  
42 80 *complementary ecosystem functions in a way that allows one species’ uneaten feed and wastes, nutrients and by-*  
43  
44 81 *products to be recaptured and converted into fertilizer, feed and energy for the other crops, and to take advantage*  
45  
46 82 *of synergistic interactions among species while biomitigation takes place.*”

47  
48  
49 83 By integrating species with complementary diets, IMTAs reproduce a simplified trophic chain (Fig. 1), in which  
50  
51 84 both primary producers and consumers play a key role. Microalgae or macroalgae nourished by inorganic liquid  
52  
53 85 effluents use photosynthesis to grow, reintroducing energy into the system and acting both to fix CO<sub>2</sub> and to  
54  
55 86 provide O<sub>2</sub> and food for other organisms (Shpigel and Neori 1996; Demetropoulos and Langdon 2004; Barrington  
56  
57 87 et al. 2009). They can serve as a food source for high value-added species, e.g. grazers or filter-feeders such as  
58  
59 88 abalone, urchins, oysters or clams (Tenore 1976; Hugues-Games 1977; Gordin et al. 1981; Borges et al. 2005).

89 Organic compounds released by farmed species or from unused external feed can also be consumed by primary or  
1 90 secondary consumers such as crustaceans, or echinoderms such as holothurians and urchins (Chopin 2013). The  
2  
3 91 resulting reduced environmental impact of IMTA could increase the social acceptability of aquaculture (Alexander  
4  
5 92 et al. 2016; Knowler et al. 2020), representing a perceived improvement over current monoculture practices for  
6  
7 93 the public (Barrington et al. 2010).

9  
10 94 In comparison to other systems, IMTA is not only more environmentally friendly, but can also potentially provide  
11  
12 95 more economic stability through product diversification (Granada et al. 2016), although the overall capital gain  
13  
14 96 (via greater degree of productivity) has not yet been demonstrated at an industrial level, apart from in one Asian  
15  
16 97 study (Fang et al. 2019).

#### 18 19 98 **1.4 Current and future challenges of IMTA**

20  
21 99 IMTAs can be developed both in open sea or inland areas (Shpigel and Neori 1996; Neori et al. 1998). Among  
22  
23 100 current IMTAs challenges, there are (i) biological challenges with the choice of candidate species adapted to the  
24  
25 101 environmental and societal contexts, (ii) economical challenges to demonstrate their performance and rentability  
26  
27 102 in comparison with conventional systems (Yu et al. 2017) and (iii) zoo-technological challenges to determinate  
28  
29 103 key variables to cultivate candidate species (Buck et al. 2018) and to ensure nutrient fluxes management (Granada  
30  
31 104 et al. 2016), especially for offshore systems (Buck et al. 2018) where the connectivity between compartments need  
32  
33 105 to be documented. Moreover, new planification of aquaculture area have to be considered, with extensive areas  
34  
35 106 required for species coping with low natural densities, such as holothurian (Tolon et al. 2017; Chary et al. 2019).

36  
37 107 Land-based IMTAs present several advantages: easier control of flows, less pressure from predators or pathogens,  
38  
39 108 and negligible exposure to extreme climatic events (Manzi and Castagna 1989; Neori et al. 2004; Blancheton et  
40  
41 109 al. 2009). Of the systems widely used for land-based fish production, recirculating aquaculture systems (RAS)  
42  
43 110 offer many advantages, such as reduced water consumption based on a bacteria treatment loop (Piedrahita 2003;  
44  
45 111 Martins et al. 2010). Wastewater flow in an RAS is consequently reduced compared to traditional flow-through  
46  
47 112 systems, but carries inorganic compounds such as  $\text{PO}_4^{3-}$ ,  $\text{NO}_3^-$  and  $\text{CO}_2$ . To reduce these compounds, one of the  
48  
49 113 existing treatments proposed by IMTA is to integrate a compartment of primary producers. Macroalgae have  
50  
51 114 frequently been included in these systems as biofilters, e.g. *Ulva spp.* and *Gracilaria spp.* (Neori et al. 2004; Lopez  
52  
53 115 Figueroa et al. 2012; Neori et al. 2017), but microalgae are less frequently used (Milhazes-Cunha and Otero 2017).

54  
55 116 Like macroalgae, microalgae add value in terms of feeding macro-invertebrates (i.e. filter-feeders), but they could  
56  
57 117 be even more promising because of their higher photosynthesis rate and greater surface-area-to-volume ratio (i.e.  
58  
59 118 higher nutrient uptake) (Milhazes-Cunha and Otero 2017).

119 One challenge for land-based IMTA systems is ensuring microalgae culture stability to allow both optimal nutrient  
120 remediation for the primary culture (e.g. fish) and optimal feeding for the associated culture (e.g. filter species). A  
121 monospecific algal culture may be selected in order to meet the needs of the other IMTA species. However, several  
122 studies have reported that an algal polyculture consisting of an assemblage of several species presents higher  
123 resilience to disturbances and ensures greater efficiency in resource use (Newby et al. 2016). In particular,  
124 multispecies algal cultures could better cope with climatic and fish-effluent variability. Some authors have  
125 demonstrated that natural plurispecific algal cultures can both grow on finfish effluent and ensure nutrient  
126 remediation (Lefebvre et al. 1996, 2004; Neori et al. 2017, Galès et al. 2020). Of course, within the IMTA,  
127 microalgae must also fulfil the feeding requirements of filter species. In one study, the addition of silicate in an  
128 algal culture initially filled with natural seawater and continuously supplemented with nitrate-enriched RAS  
129 wastewater led to a diatom-based algal culture with a remediation capacity equivalent to that of macroalgae (Li et  
130 al. 2019). In the microalgae biochemical composition, lipid content as well as essential amino acids and  
131 polyunsaturated fatty acids play a major role in the diet energy content, directly controlling oyster assimilation and  
132 biomass productivity (Brown et al. 1997 and references therein; Ben Kheder et al. 2010; Anjos et al. 2017). This  
133 nutritional value is mainly related to microalgal diversity, with diatoms being the most suitable diet for oyster  
134 growth (Brown et al. 1997 and references therein).

135 The use of a continuous nutrient-enriched multispecies algal culture as an inoculum reservoir, if the culture is well  
136 controlled, stabilized and monitored, may help: (i) to reduce the risk of producing undesirable (i.e. toxic) algal  
137 species (when using natural coastal seawater inoculum), (ii) to accelerate the time required to reach the maximal  
138 microalgal biomass, ensuring optimal inorganic matter remediation and oyster feeding.

139 This study focused on combining a RAS (for sea bass) with algal polycultures and oyster cultures. We used an  
140 inoculum from a year-round algal culture (a high-rate algal pond or HRAP, located close to the IMTA) initially  
141 inoculated with natural local seawater. The aim was to assess the algal polyculture's ability to sustain oyster growth  
142 from an associated compartment when continuously supplemented with wastewater from a fish-based RAS in an  
143 IMTA context.

## 144 **2. From IMTA theory to a new way of production**

### 145 ***2.1. Materials and methods***

#### 146 *2.1.1. Land-based marine IMTA system*

147 The experiment ran over 31 days, from 17 April 2018 (day 1) to 17 May 2018 (day 32), at the French Institute for  
148 Ocean Science (Ifremer) station in Palavas-les-Flots (southern France), which has a Mediterranean climate. The  
149 experimental IMTA system (Fig. 2) was adapted following Li et al. (2019), with improved hydrodynamics by  
150 including additional recirculation pumps in the outdoor microalgal raceways and oyster tanks.

151 Three separate units were created to conduct the experiment. An indoor RAS (in triplicate) was dedicated to sea  
152 bass (*Dicentrarchus labrax*) (n=1380 fish, split into 460 per tank, initial weight of  $425 \pm 134$  g ind<sup>-1</sup>), which were  
153 reared at a density increasing from 51 kg m<sup>-3</sup> (d1) to 56 kg m<sup>-3</sup> (d32); this unit was considered a nutrient provider.  
154 Each was connected to an outdoor microalgal raceway (6 m<sup>3</sup>, n=3), which continuously received RAS wastewater  
155 with a fixed flow rate (1 L.min<sup>-1</sup>), ensuring a hydraulic retention time of 4.2 d.

156 The IMTA microalgal raceways were inoculated with RAS effluent seawater (4:5 of the mix) and a local  
157 microalgal reservoir (1:5) containing a consortium dominated by Chlorellales (mainly *Schizochlamyde* sp  
158 and *Picochlorum* sp). Silicate (Na<sub>2</sub>SiO<sub>3</sub>·5H<sub>2</sub>O) was added to reach a N:Si:P molar ratio of nearly 10:5:1 in order  
159 to favour diatom dominance (Lefebvre et al., 1996). Nanostream electronic pumps (Turbelle®) were added in  
160 order to limit biodeposition and ensure light access.

161 The microalgae cultures (chlorophyll a concentration of 6.3 mg.l<sup>-1</sup>) were then mixed in a tank with airstones and  
162 distributed to 3 outdoor tanks containing juvenile Pacific oysters (*Crassostrea gigas*). The microalgae flow rate of  
163 2.7 L.h<sup>-1</sup> was diluted with 100 L.h<sup>-1</sup> of fresh filtered seawater (dilution factor = 37), in order to approach a daily  
164 feeding ration of 6–8% dry weight (DW). This food ration is frequently used to meet the energy requirements of  
165 *C. gigas* broodstock in hatcheries (Utting and Millican 1997; Fabioux et al. 2005; Delaporte et al. 2006); and  
166 represents a ration 3 times higher than in a previous study (Li et al. 2019). Food distribution was optimized by the  
167 inclusion of a homogenization pump (Turbelle®) and an airlift and by replacing baskets clogged by biofouling  
168 with new ones every two weeks.

### 2.1.2. Nutrient remediation and algal biomass production

170 Throughout the 31-day experiment, the water was sampled 3 times a week to monitor nutrient parameters (inlet  
171 and outlet) in the photosynthetic extractive compartment (the 3 outdoor microalgal raceways). Water samples (n  
172 total = 49) were filtered (GF/F, Whatman™) after each sampling and stored (at -25°C) for NO<sub>3</sub>-N, NO<sub>2</sub>-N, NH<sub>4</sub>-  
173 N, PO<sub>4</sub>-P analysis (Alliance® auto-analyser). Nutrient removal efficiency (RE, %) was calculated (see Li et al.  
174 2019).

175 The microalgal biomass was monitored 3 times a week in the 3 outdoor microalgal raceways; chlorophyll a (Chla)  
176 was determined after water sample filtration using GF/F filters (Association 1995), and the pigments were  
177 extracted with methanol (Ritchie 2006). Chla concentration was measured using a spectrophotometer and  
178 calculated using the Ritchie (2006) equation. The main microalgal species were identified by microscopy with an  
179 Olympus IMT2 inverted light microscope, following the protocol of the French Observation and Monitoring  
180 Programme for Phytoplankton and Hydrology (REPHY) in coastal waters (Neaud-Masson 2020).

181 Some environmental parameters (i.e. temperature, salinity, pH) were monitored daily in all tanks (i.e. fish, algae  
182 and oyster) using a YSI® probe.

### 2.1.3. Oyster growth

184 On d1, 756 8-month juvenile oysters ( $n_{\text{total}}=756$ ) were split into 3 outdoor tanks ( $n_{\text{tank}}= 252\pm 3$ ). The initial fresh  
185 weight and length of the juvenile oysters were measured, with values of  $1.55\pm 0.33$  g.ind<sup>-1</sup> and  $22\pm 3$  mm.ind<sup>-1</sup>  
186 respectively. The final fresh weight and length were measured after 32 days. The specific growth rate (SGR) was  
187 calculated with the equation:

$$SGR \text{ (in \%)} = \frac{\ln(\text{final weight} - \text{initial weight}) * 100}{\text{number of days}}$$

189 The non-parametric Wilcoxon-Mann-Whitney statistical test was used to assess median differences between  
190 experimental conditions.

## 2.2. Results & discussion

### *An IMTA-microalgae community of interest, but highly variable Chla production*

194 The results confirmed the feasibility of cultivating a microalgae community on fish effluent in a Mediterranean  
195 climate.

196 In all raceways, the Chla pigment concentrations, indicator of algal biomass, showed a similar three-phased pattern  
197 (Fig. 3): (1) from d1 to d9, a low initial concentration of around 0.06 mg Chla.L<sup>-1</sup>, with *cyanobacteria* dominance  
198 observed in microscopy, (2) from d9 to d16, an increase in production (reaching a maximum of 0.58 mg Chla.L<sup>-1</sup>  
199 at d15), with the dominance of *Cylindrotheca closterium* (Ehrenberg) Reimann and Lewin 1964, and finally, (3)  
200 after d16, a decline in algal growth, still with *Cylindrotheca closterium* (at d23) as the main algal species, and  
201 *Pseudo-nitzschia* and *cyanobacteria* at the end of the culture (d28).

202 Using an inoculum from a local intensive algal culture to start the microalgal culture, we observed that the maximal  
203 Chla production was reached after 16 days, twice as fast as in a previous experiment performed during the same



204 seasonal period but using a natural seawater inoculum (Li et al., 2019). The microalgal crash around d24 was  
1  
2 205 probably due to rapid algal CO<sub>2</sub> consumption leading to elevated pH (maximum pH of 10) and CO<sub>2</sub> depletion.  
3  
4 206 The results also confirmed the feasibility of bioremediating fish nutrients using an inoculum from a local HRAP.  
5  
6 207 The remediation of phosphates was high, 85 ± 25% (with a maximal value of 97.6%), but was lower for  
7  
8 208 nitrogenous elements, with 49 ± 21% for NH<sub>4</sub>-N (maximal value of 93.5%), and 24.9 ± 11.1% for NO<sub>3</sub>-N (maximal  
9  
10 209 value of 51.3%).  
11  
12 210 The algal yields – and as a consequence bioremediation efficiency – could be improved, for example, by reusing  
13  
14 211 CO<sub>2</sub> released by the fish in the RAS loop in the algae culture in order to maintain pH at seawater value (*ca* 8) and  
15  
16 212 to avoid CO<sub>2</sub> depletion. Another research avenue would be to integrate detritivorous organisms (e.g. mullet fish,  
17  
18 213 holothurians, nematodes) with algae cultures, as the former would be able to feed on deposits.  
19  
20  
21 214 *Juvenile oysters more than doubled in weight and length*  
22  
23 215 During the 1-month period, both weight and length gain in the oysters was significant ( $p < 0.001$ ), more than  
24  
25 216 doubling, with a final weight of 3.7±0.9 g ind<sup>-1</sup> (n=754). No mortality was observed (0.2%). Despite strong  
26  
27 217 variability in algal production within the IMTA-microalgae raceways, the produced biomass allowed significant  
28  
29 218 oyster growth over the experimental period: a very encouraging result. The oyster growth rate of 1.04% was in the  
30  
31 219 same order of magnitude as that of juveniles reared in a nearby natural lagoon (0.97% in Li et al., 2019).  
32  
33  
34 220 As mentioned by Troell et al. (2009), the integration of bivalves in an IMTA is not straightforward. With the  
35  
36 221 addition of silicates, the phytoplankton that grows with the input of fish nutrients is suitable food for filter-feeders  
37  
38 222 and can have a positive (Lefebvre et al. 2000, 2004) or insignificant (Li et al. 2019) effect on bivalve growth.  
39  
40 223 Those growth results are mainly explained by determining environmental factors, such as ambient concentrations  
41  
42 224 of nutrient availability, particulate organic matter or seston content (Troell and Norberg 1998), but also indirectly  
43  
44 225 by fish-feeding variability (i.e. its duration and quantity).  
45  
46  
47 226 In our case study, the IMTA microalgal inoculum was mostly composed of Chlorellales – these are not the common  
48  
49 227 microalgae used for juvenile oyster feed, which is usually composed of diatoms or *Prymnesiophyceae*  
50  
51 228 (McCausland et al. 1999; Ponis et al. 2003). Indeed, juvenile oysters are usually not able to retain small particles,  
52  
53 229 e.g. < 4 µm for *C. gigas* when sestonic load is low, <12 µm when sestonic load is higher (Barillé et al. 1993), and  
54  
55 230 even 20 µm for *Ostrea chilensis* (Dunphy et al. 2006). Chlorellales such as *Schizochlamydeella* sp and *Picochlorum*  
56  
57 231 sp are rather small (2–10 microns and 1.5–3 microns, respectively) and consequently not assimilable by juvenile  
58  
59 232 oysters (Korshikov 1953; Tsarenko 2011; Henley et al. 2004). The addition of silicates permitted a shift in the  
60  
61  
62  
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65

233 initial algal community towards diatoms of the *Bacillariophyceae* family, whether these were present (but not  
1  
2 234 detectable) in the 1:5 inoculum at the beginning of the period, or in the 4:5 water from the RAS effluent. This shift  
3  
4 235 in the microalgal composition allowed the growth of the juvenile oysters, indicating that the feed composition was  
5  
6 236 both assimilable and resulted in growth gain. *C. closterium*, which grew in the microalgal raceways, are long cells  
7  
8 237 (> to 25 µm; Reimann and Lewin 1964) with a particular shape that could favour its retention on oyster gills and  
9  
10 238 be suitable microalgae for juvenile oyster growth. Its high nutritional value for secondary consumers has  
11  
12 239 previously been described: it has high lipid content and is particularly rich in essential PUFAs such as EPA and  
13  
14 240 ARA (Keerthi et al. 2012). Other constituents may also have played a role in the oyster food chain, as ciliates and  
15  
16 241 flagellates from 4 to 72 µm are known to be retained by the oyster (Dupuy et al. 1999). Future experiments on the  
17  
18 242 entire microbial food web are necessary to delve further into the microorganism communities assimilable by  
19  
20 243 oysters in an IMTA oyster-growing context. To reach optimal conditions for bivalve production, these experiments  
21  
22 244 can give rise to improvements in the system – for example, daily uniform fish feeding and therefore, nutrient  
23  
24 245 excretion – to smooth out variations in the microalgae cultures and decrease the risk of crop crashing.

#### 26 246 *IMTA supplementary biomass production*

27  
28 247 At a daily feeding rate of 1% of the fish biomass, 5.89 kg of feed.d<sup>-1</sup> were distributed, giving a growth ratio of 2.39  
29  
30 248 kg of fish.d<sup>-1</sup>. The nutrients excreted by fish and taken in by microalgae fed an oyster biomass of 0.022 kg.d<sup>-1</sup>.

31  
32  
33 249 In the experimental conditions tested, the results showed that an IMTA with reduced human intervention allowed  
34  
35 250 a gain in recoverable biomass: i.e. 3.7 g of oyster produced per kg of fish feed distributed. For a production unit  
36  
37 251 of 100 tonnes of fish fed at 1% of the biomass twice a day, an additional production of 2.7 tonnes of oyster could  
38  
39 252 be provided per year. This yield could be even higher if 100% RAS water was used to supplement the microalgal  
40  
41 253 compartment (5% of RAS water was used in this study), as in this case 20 microalgal raceways could be run.  
42  
43 254 Moreover, only 2% of the microalgae biomass was used to feed the 3 oyster compartments: if 100% was used to  
44  
45 255 feed the oysters, 37 times more oyster biomass would be produced. Further work is needed to investigate the  
46  
47 256 economic viability of such a system in more detail, in order to establish whether there is an overall economic  
48  
49 257 benefit to implementing such a system.

50  
51 258

#### 53 259 **Conclusion**

54  
55 260 This study demonstrated the feasibility of using an inoculum of a microalgal polyculture (favouring diatoms of  
56  
57 261 interest) from a local intensive basin together with an appropriate oyster–food ratio to feed oyster juveniles in an  
58  
59 262 IMTA community. The experiment resulted in equivalent Chla production in a shorter time frame than a previous  
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61  
62  
63  
64  
65

263 published study (15 days less to reach the same production). This IMTA design could still be improved, in  
1 particular regarding technical and environmental factors, in order to better control algal culture variability.  
2

3  
4 265 By transforming waste into reusable material and additional biomass, IMTAs can reduce environmental impact,  
5  
6 266 giving a more positive, ecological image to aquaculture. It could potentially be promoted with a specific ‘circular  
7  
8 267 aquaculture’ certification. However, despite its promise, in Europe the development of IMTA still faces various  
9  
10 268 issues. Some of the obstacles could be overcome by pursuing research on both biological, economic and social  
11  
12 269 aspects of IMTA and of end consumer perceptions of its products. Innovative zero-waste designs should be studied,  
13  
14 270 with specific attention paid to analysing their effects on the biology and welfare of candidate species and the  
15  
16 271 quality of the end products. Increasing the economic and ecological sustainability of aquaculture should help build  
17  
18 272 the case for its social acceptance.  
19

## 20 21 273 **Acknowledgements**

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23  
24 274 This study was conducted in the framework of the Cooperation in Fisheries, Aquaculture and Seafood Processing  
25  
26 275 (COFASP) European Research Area Network (ERA-Net) project (IMTA-Effect project) with funding from the  
27  
28 276 French National Research Agency (ANR-15-COFA-0001-06) (for installations, experiments, analyses) and the  
29  
30 277 Marine Biodiversity and Conservation (MARBEC) research group (which funded M. Lutier’s Master’s degree).  
31  
32 278 The authors would also like to thank colleagues from MARBEC and the French National Research Institute for  
33  
34 279 Agriculture, Food and the Environment (INRAE) for their contribution in the fish biometry: Bastien Sadoul, Eric  
35  
36 280 Gasset, Frédéric Clota, Benjamin Geffroy and the two students Maxime Petit Jean and Baptiste Bal. We also thank  
37  
38 281 Claire Carre for her expertise on phytoplankton, Audrey Mandonnet for her help in the laboratory, Elise Caillard  
39  
40 282 for her photos, and Cyrille Przybyla, manager of the VASCO2 project that provided us with the algal inoculum.  
41  
42 283 Finally, many thanks to the editor and referees for their constructive comments on the initial version of this  
43  
44 284 manuscript, and to Elise Bradbury for the English proofreading.  
45  
46

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## 55 56 499 **Statements & Declaration**

### 57 58 500 **Ethical**

501 All sampling and animal handling respected good scientific practice and animal welfare rules. The number of fish  
1  
2 502 sampled was limited to that strictly necessary: the reared fish were shared with another ERA-Net project (Animal  
3  
4 503 Health and Welfare – Welfare, Health and Individuality in Farmed Fish, ANIHWA WINFISH, 2015–2018).

5  
6 504 **Funding**

7  
8  
9 505 This work was supported by the ERA-Net COFASP project (IMTA-Effect project) with funding from The French  
10  
11 506 National Research Agency (ANR-15-COFA-0001-06).

12  
13 507 **Competing Interest**

14  
15  
16 508 The authors declare that they have no conflict of interest concerning this manuscript and no competing financial  
17  
18 509 interests that could influence the work reported in this paper.

19  
20 510 **Data availability**

21  
22  
23 511 The datasets generated during and/or analysed during the current study are available from the corresponding author  
24  
25 512 on reasonable request.

26  
27 513 **Author contributions**

28  
29  
30 514 ERO and EF designed the study and wrote the manuscript. ML and FR managed the day-to-day experiment. ML,  
31  
32 515 ERO and EF collected the samples. ML, ERO, FR, ST, TL, AC, PLG performed the biometrics. ML, AG, ELF,  
33  
34 516 CH performed the microalgae analyses. TG performed the nutrient analyses. ML performed the statistics. All  
35  
36 517 authors read and approved the final manuscript.

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45 521 Angélique GOBET (AG). All authors approved the manuscript submission.

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1 **Marine ecological aquaculture: a successful Mediterranean **integrated multi-trophic aquaculture****  
2 **case study of a fish, oyster and algae assemblage**

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6  
7 **Abstract**

8 Inspired by agroecology, ecological aquaculture proposes an alternative model that uses ecology as a paradigm to  
9 develop innovative, more eco-friendly aquaculture with environmental, economic and social benefits. Integrated  
10 multi-trophic aquaculture (IMTA) is one application of this principle. Inspired by the natural trophic chain, it  
11 associates primary producers with primary or secondary consumers, providing a new source of biomass without  
12 requiring supplementary feed by recycling inorganic and organic wastes. Of these systems, land-based IMTAs  
13 demonstrate several advantages, especially easier control of nutrient flows, contaminants and/or predators. This  
14 study focused on a land-based marine IMTA, combining a recirculating aquaculture system for fish consecutively  
15 with a natural marine polyculture of microalgae and oyster cultivation. The objective was to assess the ability of  
16 the microalgal polyculture both to bioremediate fish nutrients and to sustain oyster growth. For the first time in a  
17 Mediterranean climate, we confirmed the feasibility of developing a microalgae community of interest for oysters  
18 maintained by fish effluent. Despite strong variability in microalgae production, this IMTA system resulted in  
19 significant oyster growth over the experimental period of one month, with growth results of the same order of  
20 magnitude as natural juvenile growth. In the conditions tested, this IMTA with reduced human intervention  
21 allowed a gain in recoverable biomass of 3.7 g of oyster produced per kg of fish feed distributed. By transforming  
22 waste into additional biomass, IMTAs offer a more promising, ecological avenue for aquaculture, based on a  
23 circular economy, which may in turn increase the social acceptability of fish farming. (250 words)

24 **Key words** Integrated Multi-trophic Aquaculture, nutrient recycling, oysters, fish, microalgae.

25 **Abbreviations:**

26 ARA: arachidonic acid

27 EPA: eicosapentaenoic acid

28 HRAP: high-rate algal pond

29 IMTA: Integrated multi-trophic aquaculture

30 PUFA: polyunsaturated fatty acids

31 RAS: recirculating aquaculture systems

32 RE: removal efficiency

33 SGR: specific growth rate

34

## 35 **1. Introduction**

### 36 *1.1 Food system paradigms*

37 Today, the global food system and food security face multiple challenges, with a convergence of population growth  
38 and increasing hunger and malnutrition (FAO 2016). Additional pressure, e.g. anthropic activity and  
39 environmental pollution, threatens natural resources, while the consequences of climate change are leading to the  
40 loss of biodiversity and unbalanced production systems (IPBES 2019). A transition is required towards more  
41 sustainable production and consumption.

42 In recent decades, the general paradigm in animal or vegetal feedstock production, especially in Europe, has been  
43 to grow one selected species under conditions of intensive cultivation. This method of production is highly  
44 dependent on the species' ability for high and stable growth and resilience to sudden environmental changes. In a  
45 context of global change, with extreme climatic events occurring more frequently, in aquaculture systems, high  
46 water temperatures potentially increase the risk of some diseases (Karvonen et al. 2010). Monospecific systems  
47 are based on species selected for their ability to dedicate their energy to growth and have little adaptability to  
48 exogenous pressures; thus, xenobiotics (e.g. antimicrobials) are often needed to maintain productivity in intensive  
49 systems (Smith et al. 1999). Of animal production systems, aquaculture is growing 7.8% per year, exceeding all  
50 others (Troell et al. 2014). Monospecific aquaculture (especially to raise finfish) is today common worldwide and  
51 is often associated with the use of antibiotics (Miranda 2011; Lulijwa et al. 2019), potential chemical contaminants  
52 such as heavy metals or dioxins, or hormones (FAO 2003). Another disadvantage is the discharge of large amounts  
53 of nutrients (i.e. from organism excretions and unconsumed food), which can cause the eutrophication and  
54 deoxygenation of coastal waters when environmental carrying capacity is exceeded (Gowen and Bradbury 1987;  
55 Pillay 2004). This can foster the development of pathogens and parasites, which may in turn negatively impact  
56 biodiversity, human health, as well as the farmed species (Neori et al. 2004; Jegatheesan et al. 2011), leading to  
57 sometimes dramatic environmental concerns due to the presence of potential harmful residues impacting the health  
58 of end consumers (Okocha et al. 2018).

### 59 *1.2 From monospecific to multispecies production systems*

60 A major paradigm shift is essential to improve not only aquaculture food safety, but also its social acceptability.  
1  
2 61 In the agricultural sector, research into alternative agriculture applying the principles of ecology emerged in the  
3  
4 62 1980s (Altieri 1983). The ‘agroecology’ paradigm is based on several principles: e.g. input reduction, recycling,  
5  
6 63 animal health, biodiversity and synergy (Wezel et al. 2020). It aims to take into account interactions between the  
7  
8 64 plants, animals, humans and the environment within agricultural systems and to imitate the natural processes  
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10 65 involved in ecosystem productivity, stability and resilience (Malézieux 2012) in order to develop new production  
11  
12 66 methods (Snapp 2017).

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15 67 Inspired by this approach, ‘ecological aquaculture’ has been put forward as an alternative model that uses ecology  
16  
17 68 as a paradigm to develop aquaculture with environmental, economic and social benefits. It maintains that  
18  
19 69 aquaculture should mimic the structure and functions of natural ecosystems and practice nutrient recycling through  
20  
21 70 waste reuse (Costa-Pierce 2015; Aubin et al. 2017). Based on the natural trophic chain, integrated multi-trophic  
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23 71 aquaculture (IMTA) is in this sense one of the logical next steps in alternative aquaculture development  
24  
25 72 (Barrington et al. 2009), representing a way to improve existing systems.

### 26 27 28 73 ***1.3 Higher diversity in farmed species could help social acceptability***

29  
30 74 Studies looking into the possibility of IMTA began in the early 1970s (Ryther 1975; Goldman et al. 1974), with  
31  
32 75 research efforts increasing over the last two decades, both in marine ecosystems (Chopin et al. 2001; Hussenot  
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34 76 2003; Barrington et al. 2009 in FAO 2009; Milhazes-Cunha and Otero, 2017; Buck et al. 2018) and freshwater  
35  
36 77 ecosystems (Wongkiew et al. 2017). As mentioned by Chopin (2013), the concept of IMTA can in fact be traced  
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38 78 back to the origins of aquaculture (in 2200–2100 BCE, You Hou Bin detailed the integration of fish with aquatic  
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40 79 plants). Chopin defines IMTA as “*The farming, in proximity, of species from different trophic levels and with*  
41  
42 80 *complementary ecosystem functions in a way that allows one species’ uneaten feed and wastes, nutrients and by-*  
43  
44 81 *products to be recaptured and converted into fertilizer, feed and energy for the other crops, and to take advantage*  
45  
46 82 *of synergistic interactions among species while biomitigation takes place.*”

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48  
49 83 By integrating species with complementary diets, IMTAs reproduce a simplified trophic chain (Fig. 1), in which  
50  
51 84 both primary producers and consumers play a key role. Microalgae or macroalgae nourished by inorganic liquid  
52  
53 85 effluents use photosynthesis to grow, reintroducing energy into the system and acting both to fix CO<sub>2</sub> and to  
54  
55 86 provide O<sub>2</sub> and food for other organisms (Shpigel and Neori 1996; Demetropoulos and Langdon 2004; Barrington  
56  
57 87 et al. 2009). They can serve as a food source for high value-added species, e.g. grazers or filter-feeders such as  
58  
59 88 abalone, urchins, oysters or clams (Tenore 1976; Hugues-Games 1977; Gordin et al. 1981; Borges et al. 2005).

89 Organic compounds released by farmed species or from unused external feed can also be consumed by primary or  
1 90 secondary consumers such as crustaceans, or echinoderms such as holothurians and urchins (Chopin 2013). The  
2  
3 91 resulting reduced environmental impact of IMTA could increase the social acceptability of aquaculture (Alexander  
4  
5 92 et al. 2016; Knowler et al. 2020), representing a perceived improvement over current monoculture practices for  
6  
7 93 the public (Barrington et al. 2010).

9  
10 94 In comparison to other systems, IMTA is not only more environmentally friendly, but can also potentially provide  
11  
12 95 more economic stability through product diversification (Granada et al. 2016), although the overall capital gain  
13  
14 96 (via greater degree of productivity) has not yet been demonstrated at an industrial level, apart from in one Asian  
15  
16 97 study (Fang et al. 2019).

#### 18 19 98 ***1.4 Current and future challenges of IMTA***

20  
21 99 IMTAs can be developed both in open sea or inland areas (Shpigel and Neori 1996; Neori et al. 1998). Among  
22  
23 100 current IMTAs challenges, there are (i) biological challenges with the choice of candidate species adapted to the  
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25 101 environmental and societal contexts, (ii) economical challenges to demonstrate their performance and rentability  
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27 102 in comparison with conventional systems (Yu et al. 2017) and (iii) zoo-technological challenges to determinate  
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29 103 key variables to cultivate candidate species (Buck et al. 2018) and to ensure nutrient fluxes management (Granada  
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31 104 et al. 2016), especially for offshore systems (Buck et al. 2018) where the connectivity between compartments need  
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33 105 to be documented. Moreover, new planification of aquaculture area have to be considered, with extensive areas  
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35 106 required for species coping with low natural densities, such as holothurian (Tolon et al. 2017; Chary et al. 2019).

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37 107 Land-based IMTAs present several advantages: easier control of flows, less pressure from predators or pathogens,  
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39 108 and negligible exposure to extreme climatic events (Manzi and Castagna 1989; Neori et al. 2004; Blancheton et  
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41 109 al. 2009). Of the systems widely used for land-based fish production, recirculating aquaculture systems (RAS)  
42  
43 110 offer many advantages, such as reduced water consumption based on a bacteria treatment loop (Piedrahita 2003;  
44  
45 111 Martins et al. 2010). Wastewater flow in an RAS is consequently reduced compared to traditional flow-through  
46  
47 112 systems, but carries inorganic compounds such as  $\text{PO}_4^{3-}$ ,  $\text{NO}_3^-$  and  $\text{CO}_2$ . To reduce these compounds, one of the  
48  
49 113 existing treatments proposed by IMTA is to integrate a compartment of primary producers. Macroalgae have  
50  
51 114 frequently been included in these systems as biofilters, e.g. *Ulva spp.* and *Gracilaria spp.* (Neori et al. 2004; Lopez  
52  
53 115 Figueroa et al. 2012; Neori et al. 2017), but microalgae are less frequently used (Milhazes-Cunha and Otero 2017).  
54  
55 116 Like macroalgae, microalgae add value in terms of feeding macro-invertebrates (i.e. filter-feeders), but they could  
56  
57 117 be even more promising because of their higher photosynthesis rate and greater surface-area-to-volume ratio (i.e.  
58  
59 118 higher nutrient uptake) (Milhazes-Cunha and Otero 2017).

119 One challenge for land-based IMTA systems is ensuring microalgae culture stability to allow both optimal nutrient  
120 remediation for the primary culture (e.g. fish) and optimal feeding for the associated culture (e.g. filter species). A  
121 monospecific algal culture may be selected in order to meet the needs of the other IMTA species. However, several  
122 studies have reported that an algal polyculture consisting of an assemblage of several species presents higher  
123 resilience to disturbances and ensures greater efficiency in resource use (Newby et al. 2016). In particular,  
124 multispecies algal cultures could better cope with climatic and fish-effluent variability. Some authors have  
125 demonstrated that natural plurispecific algal cultures can both grow on finfish effluent and ensure nutrient  
126 remediation (Lefebvre et al. 1996, 2004; Neori et al. 2017, Galès et al. 2020). Of course, within the IMTA,  
127 microalgae must also fulfil the feeding requirements of filter species. In one study, the addition of silicate in an  
128 algal culture initially filled with natural seawater and continuously supplemented with nitrate-enriched RAS  
129 wastewater led to a diatom-based algal culture with a remediation capacity equivalent to that of macroalgae (Li et  
130 al. 2019). In the microalgae biochemical composition, lipid content as well as essential amino acids and  
131 polyunsaturated fatty acids play a major role in the diet energy content, directly controlling oyster assimilation and  
132 biomass productivity (Brown et al. 1997 and references therein; Ben Kheder et al. 2010; Anjos et al. 2017). This  
133 nutritional value is mainly related to microalgal diversity, with diatoms being the most suitable diet for oyster  
134 growth (Brown et al. 1997 and references therein).

135 The use of a continuous nutrient-enriched multispecies algal culture as an inoculum reservoir, if the culture is well  
136 controlled, stabilized and monitored, may help: (i) to reduce the risk of producing undesirable (i.e. toxic) algal  
137 species (when using natural coastal seawater inoculum), (ii) to accelerate the time required to reach the maximal  
138 microalgal biomass, ensuring optimal inorganic matter remediation and oyster feeding.

139 This study focused on combining a RAS (for sea bass) with algal polycultures and oyster cultures. We used an  
140 inoculum from a year-round algal culture (a high-rate algal pond or HRAP, located close to the IMTA) initially  
141 inoculated with natural local seawater. The aim was to assess the algal polyculture's ability to sustain oyster growth  
142 from an associated compartment when continuously supplemented with wastewater from a fish-based RAS in an  
143 IMTA context.

## 144 **2. From IMTA theory to a new way of production**

### 145 ***2.1. Materials and methods***

#### 146 *2.1.1. Land-based marine IMTA system*

147 The experiment ran over 31 days, from 17 April 2018 (day 1) to 17 May 2018 (day 32), at the French Institute for  
148 Ocean Science (Ifremer) station in Palavas-les-Flots (southern France), which has a Mediterranean climate. The  
149 experimental IMTA system (Fig. 2) was adapted following Li et al. (2019), with improved hydrodynamics by  
150 including additional recirculation pumps in the outdoor microalgal raceways and oyster tanks.

151 Three separate units were created to conduct the experiment. An indoor RAS (in triplicate) was dedicated to sea  
152 bass (*Dicentrarchus labrax*) (n=1380 fish, split into 460 per tank, initial weight of  $425 \pm 134$  g ind<sup>-1</sup>), which were  
153 reared at a density increasing from 51 kg m<sup>-3</sup> (d1) to 56 kg m<sup>-3</sup> (d32); this unit was considered a nutrient provider.  
154 Each was connected to an outdoor microalgal raceway (6 m<sup>3</sup>, n=3), which continuously received RAS wastewater  
155 with a fixed flow rate (1 L.min<sup>-1</sup>), ensuring a hydraulic retention time of 4.2 d.

156 The IMTA microalgal raceways were inoculated with RAS effluent seawater (4:5 of the mix) and a local  
157 microalgal reservoir (1:5) containing a consortium dominated by Chlorellales (mainly *Schizochlamyde* sp  
158 and *Picochlorum* sp). Silicate (Na<sub>2</sub>SiO<sub>3</sub>·5H<sub>2</sub>O) was added to reach a N:Si:P molar ratio of nearly 10:5:1 in order  
159 to favour diatom dominance (Lefebvre et al., 1996). Nanostream electronic pumps (Turbelle®) were added in  
160 order to limit biodeposition and ensure light access.

161 The microalgae cultures (chlorophyll a concentration of 6.3 mg.l<sup>-1</sup>) were then mixed in a tank with airstones and  
162 distributed to 3 outdoor tanks containing juvenile Pacific oysters (*Crassostrea gigas*). The microalgae flow rate of  
163 2.7 L.h<sup>-1</sup> was diluted with 100 L.h<sup>-1</sup> of fresh filtered seawater (dilution factor = 37), in order to approach a daily  
164 feeding ration of 6–8% dry weight (DW). This food ration is frequently used to meet the energy requirements of  
165 *C. gigas* broodstock in hatcheries (Utting and Millican 1997; Fabioux et al. 2005; Delaporte et al. 2006); and  
166 represents a ration 3 times higher than in a previous study (Li et al. 2019). Food distribution was optimized by the  
167 inclusion of a homogenization pump (Turbelle®) and an airlift and by replacing baskets clogged by biofouling  
168 with new ones every two weeks.

### 2.1.2. Nutrient remediation and algal biomass production

170 Throughout the 31-day experiment, the water was sampled 3 times a week to monitor nutrient parameters (inlet  
171 and outlet) in the photosynthetic extractive compartment (the 3 outdoor microalgal raceways). Water samples (n  
172 total = 49) were filtered (GF/F, Whatman™) after each sampling and stored (at -25°C) for NO<sub>3</sub>-N, NO<sub>2</sub>-N, NH<sub>4</sub>-  
173 N, PO<sub>4</sub>-P analysis (Alliance® auto-analyser). Nutrient removal efficiency (RE, %) was calculated (see Li et al.  
174 2019).



175 The microalgal biomass was monitored 3 times a week in the 3 outdoor microalgal raceways; chlorophyll a (Chla)  
176 was determined after water sample filtration using GF/F filters (Association 1995), and the pigments were  
177 extracted with methanol (Ritchie 2006). Chla concentration was measured using a spectrophotometer and  
178 calculated using the Ritchie (2006) equation. The main microalgal species were identified by microscopy with an  
179 Olympus IMT2 inverted light microscope, following the protocol of the French Observation and Monitoring  
180 Programme for Phytoplankton and Hydrology (REPHY) in coastal waters (Neaud-Masson 2020).

181 Some environmental parameters (i.e. temperature, salinity, pH) were monitored daily in all tanks (i.e. fish, algae  
182 and oyster) using a YSI® probe.

### 2.1.3. Oyster growth

184 On d1, 756 8-month juvenile oysters ( $n_{\text{total}}=756$ ) were split into 3 outdoor tanks ( $n_{\text{tank}}= 252\pm 3$ ). The initial fresh  
185 weight and length of the juvenile oysters were measured, with values of  $1.55\pm 0.33$  g.ind<sup>-1</sup> and  $22\pm 3$  mm.ind<sup>-1</sup>  
186 respectively. The final fresh weight and length were measured after 32 days. The specific growth rate (SGR) was  
187 calculated with the equation:

$$SGR \text{ (in \%)} = \frac{\ln(\text{final weight} - \text{initial weight}) * 100}{\text{number of days}}$$

189 The non-parametric Wilcoxon-Mann-Whitney statistical test was used to assess median differences between  
190 experimental conditions.

## 2.2. Results & discussion

### *An IMTA-microalgae community of interest, but highly variable Chla production*

194 The results confirmed the feasibility of cultivating a microalgae community on fish effluent in a Mediterranean  
195 climate.

196 In all raceways, the Chla pigment concentrations, indicator of algal biomass, showed a similar three-phased pattern  
197 (Fig. 3): (1) from d1 to d9, a low initial concentration of around 0.06 mg Chla.L<sup>-1</sup>, with *cyanobacteria* dominance  
198 observed in microscopy, (2) from d9 to d16, an increase in production (reaching a maximum of 0.58 mg Chla.L<sup>-1</sup>  
199 at d15), with the dominance of *Cylindrotheca closterium* (Ehrenberg) Reimann and Lewin 1964, and finally, (3)  
200 after d16, a decline in algal growth, still with *Cylindrotheca closterium* (at d23) as the main algal species, and  
201 *Pseudo-nitzschia* and *cyanobacteria* at the end of the culture (d28).

202 Using an inoculum from a local intensive algal culture to start the microalgal culture, we observed that the maximal  
203 Chla production was reached after 16 days, twice as fast as in a previous experiment performed during the same

204 seasonal period but using a natural seawater inoculum (Li et al., 2019). The microalgal crash around d24 was  
1 205 probably due to rapid algal CO<sub>2</sub> consumption leading to elevated pH (maximum pH of 10) and CO<sub>2</sub> depletion.  
3  
4 206 The results also confirmed the feasibility of bioremediating fish nutrients using an inoculum from a local HRAP.  
5  
6 207 The remediation of phosphates was high, 85 ± 25% (with a maximal value of 97.6%), but was lower for  
7  
8 208 nitrogenous elements, with 49 ± 21% for NH<sub>4</sub>-N (maximal value of 93.5%), and 24.9 ± 11.1% for NO<sub>3</sub>-N (maximal  
9  
10 209 value of 51.3%).  
11  
12 210 The algal yields – and as a consequence bioremediation efficiency – could be improved, for example, by reusing  
13  
14 211 CO<sub>2</sub> released by the fish in the RAS loop in the algae culture in order to maintain pH at seawater value (*ca* 8) and  
15  
16 212 to avoid CO<sub>2</sub> depletion. Another research avenue would be to integrate detritivorous organisms (e.g. mullet fish,  
17  
18 213 holothurians, nematodes) with algae cultures, as the former would be able to feed on deposits.  
19  
20  
21 214 *Juvenile oysters more than doubled in weight and length*  
22  
23 215 During the 1-month period, both weight and length gain in the oysters was significant (*p* <0.001), more than  
24  
25 216 doubling, with a final weight of 3.7±0.9 g ind<sup>-1</sup> (n=754). No mortality was observed (0.2%). Despite strong  
26  
27 217 variability in algal production within the IMTA-microalgae raceways, the produced biomass allowed significant  
28  
29 218 oyster growth over the experimental period: a very encouraging result. The oyster growth rate of 1.04% was in the  
30  
31 219 same order of magnitude as that of juveniles reared in a nearby natural lagoon (0.97% in Li et al., 2019).  
32  
33  
34 220 As mentioned by Troell et al. (2009), the integration of bivalves in an IMTA is not straightforward. With the  
35  
36 221 addition of silicates, the phytoplankton that grows with the input of fish nutrients is suitable food for filter-feeders  
37  
38 222 and can have a positive (Lefebvre et al. 2000, 2004) or insignificant (Li et al. 2019) effect on bivalve growth.  
39  
40 223 Those growth results are mainly explained by determining environmental factors, such as ambient concentrations  
41  
42 224 of nutrient availability, particulate organic matter or seston content (Troell and Norberg 1998), but also indirectly  
43  
44 225 by fish-feeding variability (i.e. its duration and quantity).  
45  
46  
47 226 In our case study, the IMTA microalgal inoculum was mostly composed of Chlorellales – these are not the common  
48  
49 227 microalgae used for juvenile oyster feed, which is usually composed of diatoms or *Prymnesiophyceae*  
50  
51 228 (McCausland et al. 1999; Ponis et al. 2003). Indeed, juvenile oysters are usually not able to retain small particles,  
52  
53 229 e.g. < 4 µm for *C. gigas* when sestonic load is low, <12 µm when sestonic load is higher (Barillé et al. 1993), and  
54  
55 230 even 20 µm for *Ostrea chilensis* (Dunphy et al. 2006). Chlorellales such as *Schizochlamydeella* sp and *Picochlorum*  
56  
57 231 sp are rather small (2–10 microns and 1.5–3 microns, respectively) and consequently not assimilable by juvenile  
58  
59 232 oysters (Korshikov 1953; Tsarenko 2011; Henley et al. 2004). The addition of silicates permitted a shift in the  
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233 initial algal community towards diatoms of the *Bacillariophyceae* family, whether these were present (but not  
1  
2 234 detectable) in the 1:5 inoculum at the beginning of the period, or in the 4:5 water from the RAS effluent. This shift  
3  
4 235 in the microalgal composition allowed the growth of the juvenile oysters, indicating that the feed composition was  
5  
6 236 both assimilable and resulted in growth gain. *C. closterium*, which grew in the microalgal raceways, are long cells  
7  
8 237 (> to 25 µm; Reimann and Lewin 1964) with a particular shape that could favour its retention on oyster gills and  
9  
10 238 be suitable microalgae for juvenile oyster growth. Its high nutritional value for secondary consumers has  
11  
12 239 previously been described: it has high lipid content and is particularly rich in essential PUFAs such as EPA and  
13  
14 240 ARA (Keerthi et al. 2012). Other constituents may also have played a role in the oyster food chain, as ciliates and  
15  
16 241 flagellates from 4 to 72 µm are known to be retained by the oyster (Dupuy et al. 1999). Future experiments on the  
17  
18 242 entire microbial food web are necessary to delve further into the microorganism communities assimilable by  
19  
20 243 oysters in an IMTA oyster-growing context. To reach optimal conditions for bivalve production, these experiments  
21  
22 244 can give rise to improvements in the system – for example, daily uniform fish feeding and therefore, nutrient  
23  
24 245 excretion – to smooth out variations in the microalgae cultures and decrease the risk of crop crashing.

#### 26 246 *IMTA supplementary biomass production*

27  
28 247 At a daily feeding rate of 1% of the fish biomass, 5.89 kg of feed.d<sup>-1</sup> were distributed, giving a growth ratio of 2.39  
29  
30 248 kg of fish.d<sup>-1</sup>. The nutrients excreted by fish and taken in by microalgae fed an oyster biomass of 0.022 kg.d<sup>-1</sup>.

31  
32  
33 249 In the experimental conditions tested, the results showed that an IMTA with reduced human intervention allowed  
34  
35 250 a gain in recoverable biomass: i.e. 3.7 g of oyster produced per kg of fish feed distributed. For a production unit  
36  
37 251 of 100 tonnes of fish fed at 1% of the biomass twice a day, an additional production of 2.7 tonnes of oyster could  
38  
39 252 be provided per year. This yield could be even higher if 100% RAS water was used to supplement the microalgal  
40  
41 253 compartment (5% of RAS water was used in this study), as in this case 20 microalgal raceways could be run.  
42  
43 254 Moreover, only 2% of the microalgae biomass was used to feed the 3 oyster compartments: if 100% was used to  
44  
45 255 feed the oysters, 37 times more oyster biomass would be produced. Further work is needed to investigate the  
46  
47 256 economic viability of such a system in more detail, in order to establish whether there is an overall economic  
48  
49 257 benefit to implementing such a system.

#### 51 258 52 53 259 **Conclusion**

54  
55 260 This study demonstrated the feasibility of using an inoculum of a microalgal polyculture (favouring diatoms of  
56  
57 261 interest) from a local intensive basin together with an appropriate oyster–food ratio to feed oyster juveniles in an  
58  
59 262 IMTA community. The experiment resulted in equivalent Chla production in a shorter time frame than a previous  
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263 published study (15 days less to reach the same production). This IMTA design could still be improved, in  
1 particular regarding technical and environmental factors, in order to better control algal culture variability.  
2

3  
4 265 By transforming waste into reusable material and additional biomass, IMTAs can reduce environmental impact,  
5  
6 266 giving a more positive, ecological image to aquaculture. It could potentially be promoted with a specific ‘circular  
7  
8 267 aquaculture’ certification. However, despite its promise, in Europe the development of IMTA still faces various  
9  
10 268 issues. Some of the obstacles could be overcome by pursuing research on both biological, economic and social  
11  
12 269 aspects of IMTA and of end consumer perceptions of its products. Innovative zero-waste designs should be studied,  
13  
14 270 with specific attention paid to analysing their effects on the biology and welfare of candidate species and the  
15  
16 271 quality of the end products. Increasing the economic and ecological sustainability of aquaculture should help build  
17  
18 272 the case for its social acceptance.  
19

## 20 21 273 **Acknowledgements**

22  
23  
24 274 This study was conducted in the framework of the Cooperation in Fisheries, Aquaculture and Seafood Processing  
25  
26 275 (COFASP) European Research Area Network (ERA-Net) project (IMTA-Effect project) with funding from the  
27  
28 276 French National Research Agency (ANR-15-COFA-0001-06) (for installations, experiments, analyses) and the  
29  
30 277 Marine Biodiversity and Conservation (MARBEC) research group (which funded M. Lutier’s Master’s degree).  
31  
32 278 The authors would also like to thank colleagues from MARBEC and the French National Research Institute for  
33  
34 279 Agriculture, Food and the Environment (INRAE) for their contribution in the fish biometry: Bastien Sadoul, Eric  
35  
36 280 Gasset, Frédéric Clota, Benjamin Geffroy and the two students Maxime Petit Jean and Baptiste Bal. We also thank  
37  
38 281 Claire Carre for her expertise on phytoplankton, Audrey Mandonnet for her help in the laboratory, Elise Caillard  
39  
40 282 for her photos, and Cyrille Przybyla, manager of the VASCO2 project that provided us with the algal inoculum.  
41  
42 283 Finally, many thanks to the editor and referees for their constructive comments on the initial version of this  
43  
44 284 manuscript, and to Elise Bradbury for the English proofreading.  
45  
46

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## 55 56 499 **Statements & Declaration**

### 57 58 500 **Ethical**

501 All sampling and animal handling respected good scientific practice and animal welfare rules. The number of fish  
1  
2 502 sampled was limited to that strictly necessary: the reared fish were shared with another ERA-Net project (Animal  
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4 503 Health and Welfare – Welfare, Health and Individuality in Farmed Fish, ANIHWA WINFISH, 2015–2018).

5  
6 504 **Funding**

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9 505 This work was supported by the ERA-Net COFASP project (IMTA-Effect project) with funding from The French  
10  
11 506 National Research Agency (ANR-15-COFA-0001-06).

12  
13 507 **Competing Interest**

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16 508 The authors declare that they have no conflict of interest concerning this manuscript and no competing financial  
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18 509 interests that could influence the work reported in this paper.

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20 510 **Data availability**

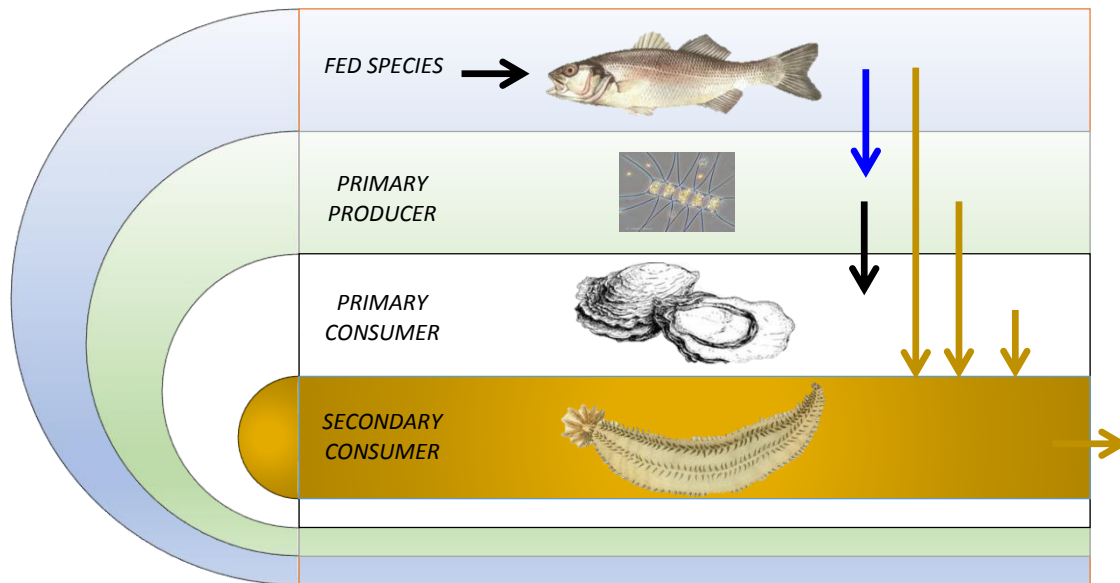
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23 511 The datasets generated during and/or analysed during the current study are available from the corresponding author  
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25 512 on reasonable request.

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27 513 **Author contributions**

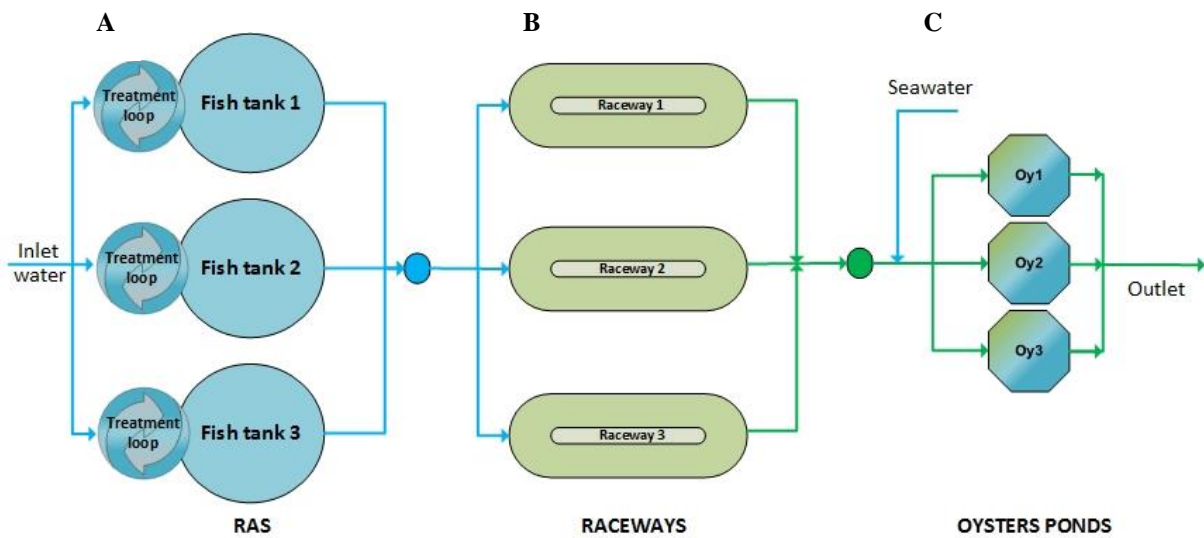
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30 514 ERO and EF designed the study and wrote the manuscript. ML and FR managed the day-to-day experiment. ML,  
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32 515 ERO and EF collected the samples. ML, ERO, FR, ST, TL, AC, PLG performed the biometrics. ML, AG, ELF,  
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34 516 CH performed the microalgae analyses. TG performed the nutrient analyses. ML performed the statistics. All  
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36 517 authors read and approved the final manuscript.

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45 521 Angélique GOBET (AG). All authors approved the manuscript submission.

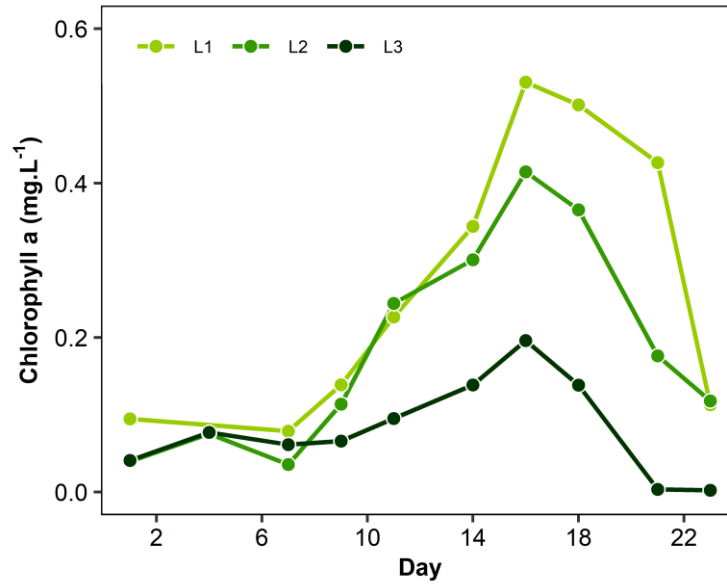
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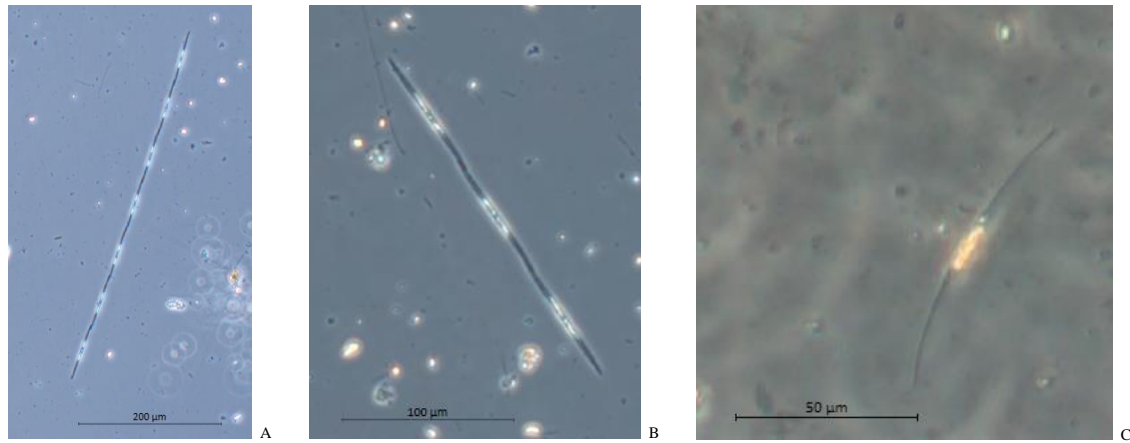
**Fig. 1.** Theoretical representation of an IMTA assemblage, with triangles representing the different trophic compartments and arrows representing the different flows (blue for inorganic, black for feed, brown for organic) - illustrations from: lapecheenligne.com, ©N.Neaud-Masson, shutterstock.com and Manuel d'actinologie ou de zoophytologie Paris ;F.G. Levraut, 1834 – 1836



**Fig. 2.** Experimental land-based IMTA (Ifremer, Palavas) with three compartments: (A) fish tanks operating with water recirculation; (B) outdoor microalgal raceways receiving fish effluent from A; (C) oyster ponds receiving microalgae from lagoons in B. Total surface area = 150 m<sup>2</sup>.



**Fig. 3.** Temporal Chla concentrations (in mg.L<sup>-1</sup>) during the experiment in the three IMTA-microalgae replicates: L1, L2 and L3.



**Fig. 4.** *Pseudonitzschia* (A and B) and *Cylinthrotheca closterium* (C) identified in the three IMTA-microalgae replicates (photos © Elise Caillard).