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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 CO₂ and to
54
55 86 provide O₂ 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 PO_4^{3-} , NO_3^- and 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 ± 134 g ind⁻¹), which were
153 reared at a density increasing from 51 kg m⁻³ (d1) to 56 kg m⁻³ (d32); this unit was considered a nutrient provider.
154 Each was connected to an outdoor microalgal raceway (6 m³, n=3), which continuously received RAS wastewater
155 with a fixed flow rate (1 L.min⁻¹), 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 (Na₂SiO₃·5H₂O) 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⁻¹) 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⁻¹ was diluted with 100 L.h⁻¹ 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 NO₃-N, NO₂-N, NH₄-
173 N, PO₄-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 ± 0.33 g.ind⁻¹ and 22 ± 3 mm.ind⁻¹
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⁻¹, 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⁻¹
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 CO₂ consumption leading to elevated pH (maximum pH of 10) and CO₂ 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 NH₄-N (maximal value of 93.5%), and 24.9 ± 11.1% for NO₃-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 CO₂ 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 CO₂ 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⁻¹ (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
63
64
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⁻¹ were distributed, giving a growth ratio of 2.39
29
30 248 kg of fish.d⁻¹. The nutrients excreted by fish and taken in by microalgae fed an oyster biomass of 0.022 kg.d⁻¹.

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

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

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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).
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12

13 507 **Competing Interest**

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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**

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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**

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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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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
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 CO₂ and to
54
55 86 provide O₂ 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
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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
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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
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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).

36
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
40
41 109 al. 2009). Of the systems widely used for land-based fish production, recirculating aquaculture systems (RAS)
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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 PO_4^{3-} , NO_3^- and 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 ± 134 g ind⁻¹), which were
153 reared at a density increasing from 51 kg m⁻³ (d1) to 56 kg m⁻³ (d32); this unit was considered a nutrient provider.
154 Each was connected to an outdoor microalgal raceway (6 m³, n=3), which continuously received RAS wastewater
155 with a fixed flow rate (1 L.min⁻¹), 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 (Na₂SiO₃·5H₂O) 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⁻¹) 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⁻¹ was diluted with 100 L.h⁻¹ 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 NO₃-N, NO₂-N, NH₄-
173 N, PO₄-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 ± 0.33 g.ind⁻¹ and 22 ± 3 mm.ind⁻¹
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⁻¹, 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⁻¹
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 probably due to rapid algal CO₂ consumption leading to elevated pH (maximum pH of 10) and CO₂ depletion.
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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
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8 208 nitrogenous elements, with 49 ± 21% for NH₄-N (maximal value of 93.5%), and 24.9 ± 11.1% for NO₃-N (maximal
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10 209 value of 51.3%).
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12 210 The algal yields – and as a consequence bioremediation efficiency – could be improved, for example, by reusing
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14 211 CO₂ 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 CO₂ depletion. Another research avenue would be to integrate detritivorous organisms (e.g. mullet fish,
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18 213 holothurians, nematodes) with algae cultures, as the former would be able to feed on deposits.
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21 214 *Juvenile oysters more than doubled in weight and length*
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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⁻¹ (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
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31 219 same order of magnitude as that of juveniles reared in a nearby natural lagoon (0.97% in Li et al., 2019).
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34 220 As mentioned by Troell et al. (2009), the integration of bivalves in an IMTA is not straightforward. With the
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36 221 addition of silicates, the phytoplankton that grows with the input of fish nutrients is suitable food for filter-feeders
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38 222 and can have a positive (Lefebvre et al. 2000, 2004) or insignificant (Li et al. 2019) effect on bivalve growth.
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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
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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,
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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

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
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8 237 (> to 25 µm; Reimann and Lewin 1964) with a particular shape that could favour its retention on oyster gills and
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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
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14 240 ARA (Keerthi et al. 2012). Other constituents may also have played a role in the oyster food chain, as ciliates and
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16 241 flagellates from 4 to 72 µm are known to be retained by the oyster (Dupuy et al. 1999). Future experiments on the
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18 242 entire microbial food web are necessary to delve further into the microorganism communities assimilable by
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20 243 oysters in an IMTA oyster-growing context. To reach optimal conditions for bivalve production, these experiments
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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⁻¹ were distributed, giving a growth ratio of 2.39
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30 248 kg of fish.d⁻¹. The nutrients excreted by fish and taken in by microalgae fed an oyster biomass of 0.022 kg.d⁻¹.

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32
33 249 In the experimental conditions tested, the results showed that an IMTA with reduced human intervention allowed
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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
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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
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39 252 be provided per year. This yield could be even higher if 100% RAS water was used to supplement the microalgal
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41 253 compartment (5% of RAS water was used in this study), as in this case 20 microalgal raceways could be run.
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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
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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.
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4 265 By transforming waste into reusable material and additional biomass, IMTAs can reduce environmental impact,
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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,
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14 270 with specific attention paid to analysing their effects on the biology and welfare of candidate species and the
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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

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54 498 55 499 **Statements & Declaration**

56 57 500 **Ethical**

501 All sampling and animal handling respected good scientific practice and animal welfare rules. The number of fish
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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).
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12

13 507 **Competing Interest**

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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**

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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**

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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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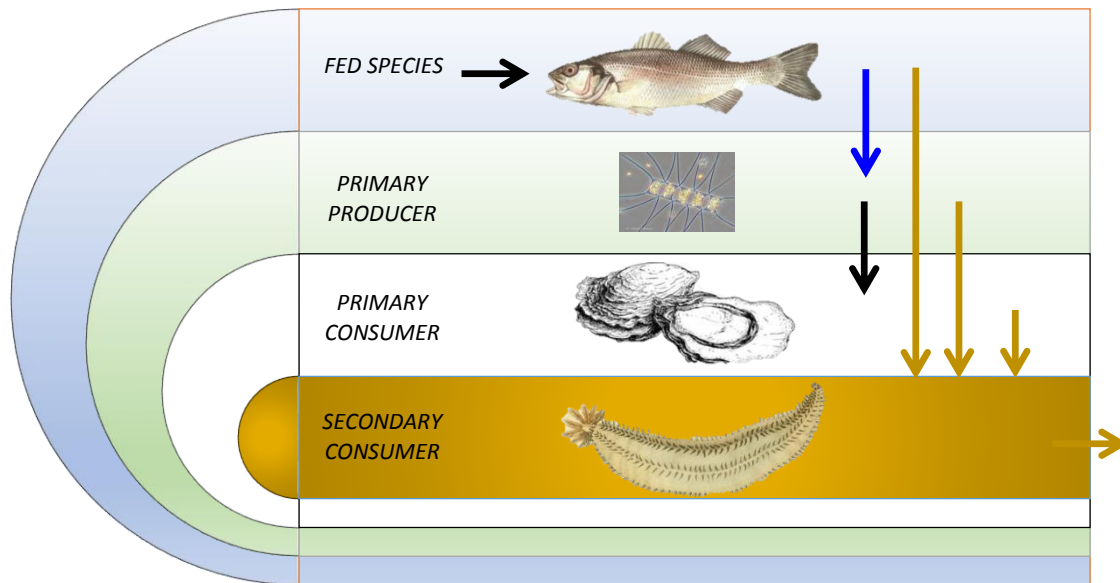


Fig. 1. Theoretical representation of an IMTA assemblage, with triangles representing the different trophic compartments and arrows representing the different flows (blue for inorganic, black for feed, brown for organic) - illustrations from: lapecheenligne.com, ©N.Neaud-Masson, shutterstock.com and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

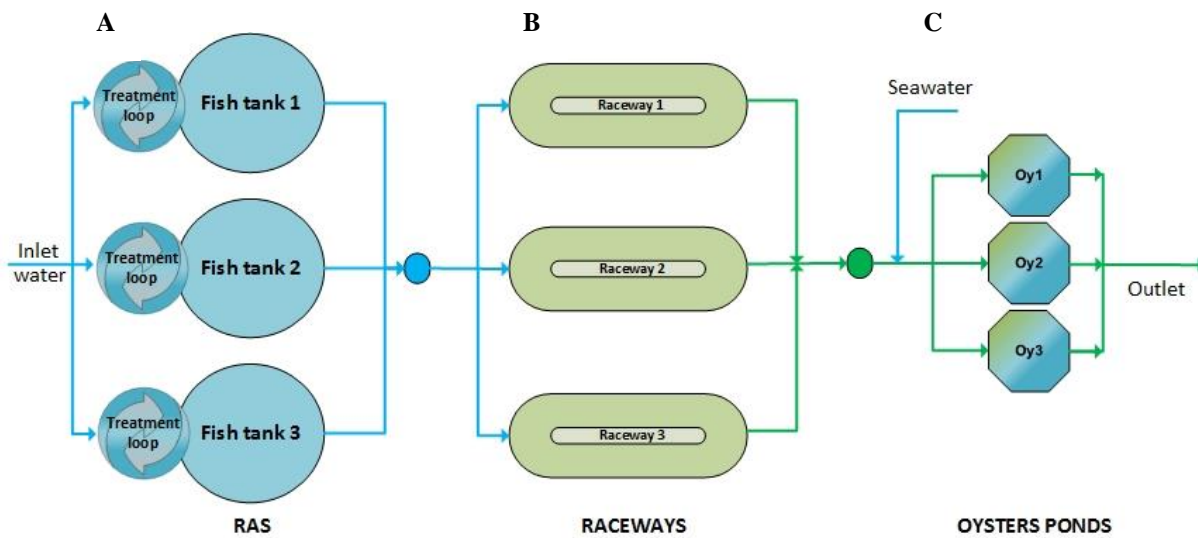


Fig. 2. Experimental land-based IMTA (Ifremer, Palavas) with three compartments: (A) fish tanks operating with water recirculation; (B) outdoor microalgal raceways receiving fish effluent from A; (C) oyster ponds receiving microalgae from lagoons in B. Total surface area = 150 m².

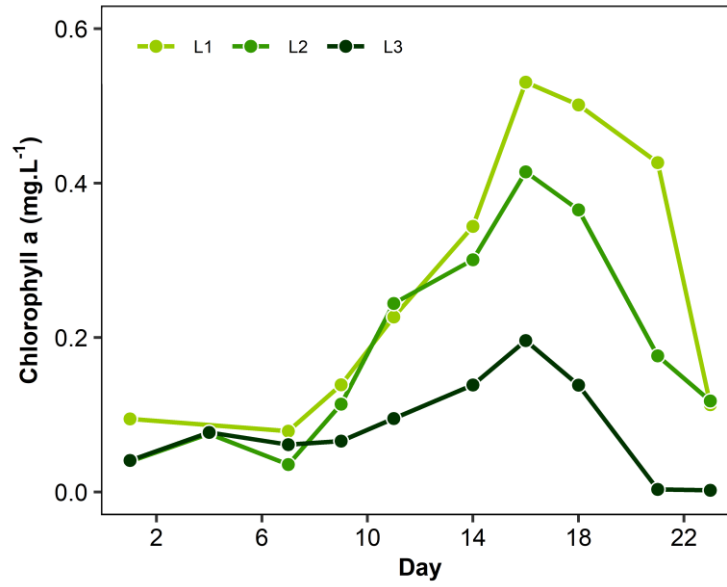


Fig. 3. Temporal Chla concentrations (in mg.L⁻¹) during the experiment in the three IMTA-microalgae replicates: L1, L2 and L3.

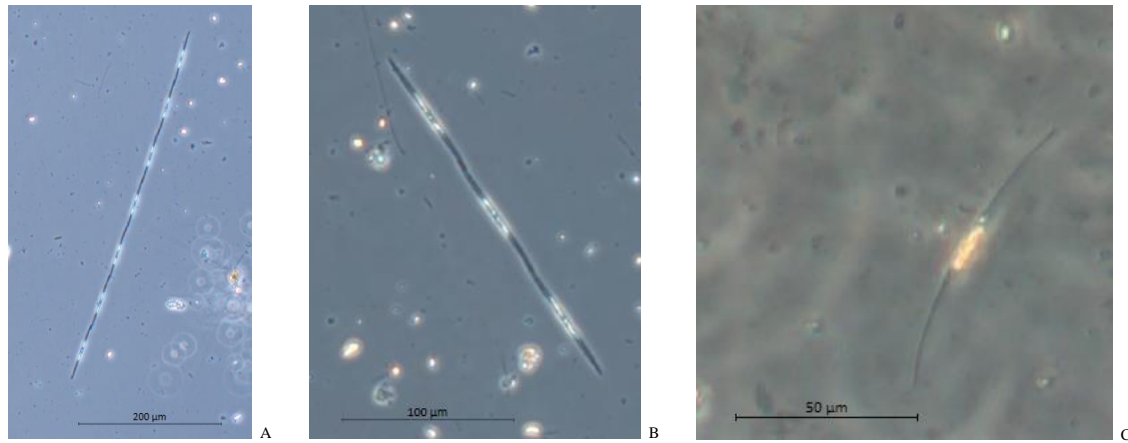


Fig. 4. *Pseudonitzschia* (A and B) and *Cyndrotheca closterium* (C) identified in the three IMTA-microalgae replicates (photos © Elise Caillard).