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

Emmanuelle ROQUE D'ORBCASTEL, Mathieu LUTIER, Emilie LE FLOC'H, François RUELLE, Sébastien TRIPLET, Patrik LE GALL, Clarisse HUBERT, Martine FORTUNE, Thierry LAUGIER, Thibault GEOFFROY, Anaïs CROTTIER, Angélique GOBET, Eric FOUILLAND

Abstract

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

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

Abbreviations:

ARA: arachidonic acid

EPA: eicosapentaenoic acid

HRAP: high-rate algal pond

IMTA: Integrated multi-trophic aquaculture

PUFA: polyunsaturated fatty acids

RAS: recirculating aquaculture systems

RE: removal efficiency

SGR: specific growth rate

1. Introduction

1.1 Food system paradigms

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

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

1.2 From monospecific to multispecies production systems

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

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

1.3 Higher diversity in farmed species could help social acceptability

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

By integrating species with complementary diets, IMTAs reproduce a simplified trophic chain (Fig. 1), in which both primary producers and consumers play a key role. Microalgae or macroalgae nourished by inorganic liquid effluents use photosynthesis to grow, reintroducing energy into the system and acting both to fix CO_2 and to provide O_2 and food for other organisms (Shpigel and Neori 1996; Demetropoulos and Langdon 2004; Barrington et al. 2009). They can serve as a food source for high value-added species, e.g. grazers or filter-feeders such as abalone, urchins, oysters or clams (Tenore 1976; Hugues-Games 1977; Gordin et al. 1981; Borges et al. 2005).

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

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

1.4 Current and future challenges of IMTA

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

Land-based IMTAs present several advantages: easier control of flows, less pressure from predators or pathogens, and negligible exposure to extreme climatic events (Manzi and Castagna 1989; Neori et al. 2004; Blancheton et al. 2009). Of the systems widely used for land-based fish production, recirculating aquaculture systems (RAS) offer many advantages, such as reduced water consumption based on a bacteria treatment loop (Piedrahita 2003; Martins et al. 2010). Wastewater flow in an RAS is consequently reduced compared to traditional flow-through systems, but carries inorganic compounds such as PO_4^{3-}, NO_3^- and CO_2. To reduce these compounds, one of the existing treatments proposed by IMTA is to integrate a compartment of primary producers. Macroalgae have frequently been included in these systems as biofilters, e.g. *Ulva spp.* and *Gracilaria spp.* (Neori et al. 2004; Lopez Figueroa et al. 2012; Neori et al. 2017), but microalgae are less frequently used (Milhazes-Cunha and Otero 2017). Like macroalgae, microalgae add value in terms of feeding macro-invertebrates (i.e. filter-feeders), but they could be even more promising because of their higher photosynthesis rate and greater surface-area-to-volume ratio (i.e. higher nutrient uptake) (Milhazes-Cunha and Otero 2017).

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

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

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

2. From IMTA theory to a new way of production

2.1. Materials and methods

2.1.1. Land-based marine IMTA system

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

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

The IMTA microalgal raceways were inoculated with RAS effluent seawater (4:5 of the mix) and a local microalgal reservoir (1:5) containing a consortium dominated by Chlorellales (mainly *Schizochlamydella* sp and *Picochlorum* sp). Silicate ($Na_2SiO_3,5H_2O$) was added to reach a N:Si:P molar ratio of nearly 10:5:1 in order to favour diatom dominance (Lefebvre et al., 1996). Nanostream electronic pumps (Turbelle®) were added in order to limit biodeposition and ensure light access.

The microalgae cultures (chlorophyll a concentration of 6.3 $mg.l^{-}1$) were then mixed in a tank with airstones and distributed to 3 outdoor tanks containing juvenile Pacific oysters (*Crassostrea gigas*). The microalgae flow rate of 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 feeding ration of 6–8% dry weight (DW). This food ration is frequently used to meet the energy requirements of *C. gigas* broodstock in hatcheries (Utting and Millican 1997; Fabioux et al. 2005; Delaporte et al. 2006); and represents a ration 3 times higher than in a previous study (Li et al. 2019). Food distribution was optimized by the inclusion of a homogenization pump (Turbelle®) and an airlift and by replacing baskets clogged by biofouling with new ones every two weeks.

2.1.2. Nutrient remediation and algal biomass production

Throughout the 31-day experiment, the water was sampled 3 times a week to monitor nutrient parameters (inlet and outlet) in the photosynthetic extractive compartment (the 3 outdoor microalgal raceways). Water samples (n total = 49) were filtered (GF/F, WhatmanTM) after each sampling and stored (at -25°C) for NO_3-N, NO_2-N, NH_4-N, PO_4-P analysis (Alliance® auto-analyser). Nutrient removal efficiency (RE, %) was calculated (see Li et al. 2019).

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

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

2.1.3. Oyster growth

On d1, 756 8-month juvenile oysters (n_{total}=756) were split into 3 outdoor tanks (n_{tank}= 252±3). The initial fresh 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} respectively. The final fresh weight and length were measured after 32 days. The specific growth rate (SGR) was calculated with the equation:

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

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

2.2. Results & discussion

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

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

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

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

seasonal period but using a natural seawater inoculum (Li et al., 2019). The microalgal crash around d24 was probably due to rapid algal CO_2 consumption leading to elevated pH (maximum pH of 10) and CO_2 depletion. The results also confirmed the feasibility of bioremediating fish nutrients using an inoculum from a local HRAP. The remediation of phosphates was high, 85 ± 25% (with a maximal value of 97.6%), but was lower for nitrogenous elements, with 49 ± 21% for NH_4-N (maximal value of 93.5%), and 24.9 ± 11.1% for NO_3-N (maximal value of 51.3%).

The algal yields – and as a consequence bioremediation efficiency – could be improved, for example, by reusing CO_2 released by the fish in the RAS loop in the algae culture in order to maintain pH at seawater value (*ca* 8) and to avoid CO_2 depletion. Another research avenue would be to integrate detritivorous organisms (e.g. mullet fish, holothurians, nematodes) with algae cultures, as the former would be able to feed on deposits.

Juvenile oysters more than doubled in weight and length

During the 1-month period, both weight and length gain in the oysters was significant (p <0.001), more than doubling, with a final weight of 3.7±0.9 g ind^{-1} (n=754). No mortality was observed (0.2%). Despite strong variability in algal production within the IMTA-microalgae raceways, the produced biomass allowed significant oyster growth over the experimental period: a very encouraging result. The oyster growth rate of 1.04% was in the same order of magnitude as that of juveniles reared in a nearby natural lagoon (0.97% in Li et al., 2019).

As mentioned by Troell et al. (2009), the integration of bivalves in an IMTA is not straightforward. With the addition of silicates, the phytoplankton that grows with the input of fish nutrients is suitable food for filter-feeders and can have a positive (Lefebvre et al. 2000, 2004) or insignificant (Li et al. 2019) effect on bivalve growth. Those growth results are mainly explained by determining environmental factors, such as ambient concentrations of nutrient availability, particulate organic matter or seston content (Troell and Norberg 1998), but also indirectly by fish-feeding variability (i.e. its duration and quantity).

In our case study, the IMTA microalgal inoculum was mostly composed of Chlorellales – these are not the common microalgae used for juvenile oyster feed, which is usually composed of diatoms or *Prymnesiophyceae* (McCausland et al. 1999; Ponis et al. 2003). Indeed, juvenile oysters are usually not able to retain small particles, e.g. < 4 µm for *C. gigas* when sestonic load is low, <12 µm when sestonic load is higher (Barillé et al. 1993), and even 20 µm for *Ostrea chilensis* (Dunphy et al. 2006). Chlorellales such as *Schizochlamydella* sp and *Picochlorum* sp are rather small (2–10 microns and 1.5–3 microns, respectively) and consequently not assimilable by juvenile oysters (Korshikov 1953; Tsarenko 2011; Henley et al. 2004). The addition of silicates permitted a shift in the

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

IMTA supplementary biomass production

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 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}.

In the experimental conditions tested, the results showed that an IMTA with reduced human intervention allowed a gain in recoverable biomass: i.e. 3.7 g of oyster produced per kg of fish feed distributed. For a production unit of 100 tonnes of fish fed at 1% of the biomass twice a day, an additional production of 2.7 tonnes of oyster could be provided per year. This yield could be even higher if 100% RAS water was used to supplement the microalgal compartment (5% of RAS water was used in this study), as in this case 20 microalgal raceways could be run. Moreover, only 2% of the microalgae biomass was used to feed the 3 oyster compartments: if 100% was used to feed the oysters, 37 times more oyster biomass would be produced. Further work is needed to investigate the economic viability of such a system in more detail, in order to establish whether there is an overall economic benefit to implementing such a system.

Conclusion

This study demonstrated the feasibility of using an inoculum of a microalgal polyculture (favouring diatoms of interest) from a local intensive basin together with an appropriate oyster–food ratio to feed oyster juveniles in an IMTA community. The experiment resulted in equivalent Chl*a* production in a shorter time frame than a previous

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

By transforming waste into reusable material and additional biomass, IMTAs can reduce environmental impact, giving a more positive, ecological image to aquaculture. It could potentially be promoted with a specific 'circular aquaculture' certification. However, despite its promise, in Europe the development of IMTA still faces various issues. Some of the obstacles could be overcome by pursuing research on both biological, economic and social aspects of IMTA and of end consumer perceptions of its products. Innovative zero-waste designs should be studied, with specific attention paid to analysing their effects on the biology and welfare of candidate species and the quality of the end products. Increasing the economic and ecological sustainability of aquaculture should help build the case for its social acceptance.

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Statements & Declaration

Ethical

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

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Competing Interest

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

Data availability

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

Author contributions

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

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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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Abstract

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

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

Abbreviations:

ARA: arachidonic acid

EPA: eicosapentaenoic acid

HRAP: high-rate algal pond

IMTA: Integrated multi-trophic aquaculture

PUFA: polyunsaturated fatty acids

RAS: recirculating aquaculture systems

RE: removal efficiency

SGR: specific growth rate

1. Introduction

1.1 Food system paradigms

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

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

1.2 From monospecific to multispecies production systems

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

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

1.3 Higher diversity in farmed species could help social acceptability

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

By integrating species with complementary diets, IMTAs reproduce a simplified trophic chain (Fig. 1), in which both primary producers and consumers play a key role. Microalgae or macroalgae nourished by inorganic liquid effluents use photosynthesis to grow, reintroducing energy into the system and acting both to fix CO_2 and to provide O_2 and food for other organisms (Shpigel and Neori 1996; Demetropoulos and Langdon 2004; Barrington et al. 2009). They can serve as a food source for high value-added species, e.g. grazers or filter-feeders such as abalone, urchins, oysters or clams (Tenore 1976; Hugues-Games 1977; Gordin et al. 1981; Borges et al. 2005).

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

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

1.4 Current and future challenges of IMTA

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

Land-based IMTAs present several advantages: easier control of flows, less pressure from predators or pathogens, and negligible exposure to extreme climatic events (Manzi and Castagna 1989; Neori et al. 2004; Blancheton et al. 2009). Of the systems widely used for land-based fish production, recirculating aquaculture systems (RAS) offer many advantages, such as reduced water consumption based on a bacteria treatment loop (Piedrahita 2003; Martins et al. 2010). Wastewater flow in an RAS is consequently reduced compared to traditional flow-through systems, but carries inorganic compounds such as PO_4^{3-}, NO_3^- and CO_2. To reduce these compounds, one of the existing treatments proposed by IMTA is to integrate a compartment of primary producers. Macroalgae have frequently been included in these systems as biofilters, e.g. *Ulva spp.* and *Gracilaria spp.* (Neori et al. 2004; Lopez Figueroa et al. 2012; Neori et al. 2017), but microalgae are less frequently used (Milhazes-Cunha and Otero 2017). Like macroalgae, microalgae add value in terms of feeding macro-invertebrates (i.e. filter-feeders), but they could be even more promising because of their higher photosynthesis rate and greater surface-area-to-volume ratio (i.e. higher nutrient uptake) (Milhazes-Cunha and Otero 2017).

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

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

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

2. From IMTA theory to a new way of production

2.1. Materials and methods

2.1.1. Land-based marine IMTA system

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

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

The IMTA microalgal raceways were inoculated with RAS effluent seawater (4:5 of the mix) and a local microalgal reservoir (1:5) containing a consortium dominated by Chlorellales (mainly *Schizochlamydella* sp and *Picochlorum* sp). Silicate ($Na_2SiO_3,5H_2O$) was added to reach a N:Si:P molar ratio of nearly 10:5:1 in order to favour diatom dominance (Lefebvre et al., 1996). Nanostream electronic pumps (Turbelle®) were added in order to limit biodeposition and ensure light access.

The microalgae cultures (chlorophyll a concentration of 6.3 $mg.l^{-}1$) were then mixed in a tank with airstones and distributed to 3 outdoor tanks containing juvenile Pacific oysters (*Crassostrea gigas*). The microalgae flow rate of 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 feeding ration of 6–8% dry weight (DW). This food ration is frequently used to meet the energy requirements of *C. gigas* broodstock in hatcheries (Utting and Millican 1997; Fabioux et al. 2005; Delaporte et al. 2006); and represents a ration 3 times higher than in a previous study (Li et al. 2019). Food distribution was optimized by the inclusion of a homogenization pump (Turbelle®) and an airlift and by replacing baskets clogged by biofouling with new ones every two weeks.

2.1.2. Nutrient remediation and algal biomass production

Throughout the 31-day experiment, the water was sampled 3 times a week to monitor nutrient parameters (inlet and outlet) in the photosynthetic extractive compartment (the 3 outdoor microalgal raceways). Water samples (n total = 49) were filtered (GF/F, WhatmanTM) after each sampling and stored (at -25°C) for NO_3-N, NO_2-N, NH_4-N, PO_4-P analysis (Alliance® auto-analyser). Nutrient removal efficiency (RE, %) was calculated (see Li et al. 2019).

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

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

2.1.3. Oyster growth

On d1, 756 8-month juvenile oysters (n_{total}=756) were split into 3 outdoor tanks (n_{tank}= 252±3). The initial fresh 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} respectively. The final fresh weight and length were measured after 32 days. The specific growth rate (SGR) was calculated with the equation:

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

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

2.2. Results & discussion

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

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

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

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

seasonal period but using a natural seawater inoculum (Li et al., 2019). The microalgal crash around d24 was probably due to rapid algal CO_2 consumption leading to elevated pH (maximum pH of 10) and CO_2 depletion. The results also confirmed the feasibility of bioremediating fish nutrients using an inoculum from a local HRAP. The remediation of phosphates was high, 85 ± 25% (with a maximal value of 97.6%), but was lower for nitrogenous elements, with 49 ± 21% for NH_4-N (maximal value of 93.5%), and 24.9 ± 11.1% for NO_3-N (maximal value of 51.3%).

The algal yields – and as a consequence bioremediation efficiency – could be improved, for example, by reusing CO_2 released by the fish in the RAS loop in the algae culture in order to maintain pH at seawater value (*ca* 8) and to avoid CO_2 depletion. Another research avenue would be to integrate detritivorous organisms (e.g. mullet fish, holothurians, nematodes) with algae cultures, as the former would be able to feed on deposits.

Juvenile oysters more than doubled in weight and length

During the 1-month period, both weight and length gain in the oysters was significant (p <0.001), more than doubling, with a final weight of 3.7±0.9 g ind^{-1} (n=754). No mortality was observed (0.2%). Despite strong variability in algal production within the IMTA-microalgae raceways, the produced biomass allowed significant oyster growth over the experimental period: a very encouraging result. The oyster growth rate of 1.04% was in the same order of magnitude as that of juveniles reared in a nearby natural lagoon (0.97% in Li et al., 2019).

As mentioned by Troell et al. (2009), the integration of bivalves in an IMTA is not straightforward. With the addition of silicates, the phytoplankton that grows with the input of fish nutrients is suitable food for filter-feeders and can have a positive (Lefebvre et al. 2000, 2004) or insignificant (Li et al. 2019) effect on bivalve growth. Those growth results are mainly explained by determining environmental factors, such as ambient concentrations of nutrient availability, particulate organic matter or seston content (Troell and Norberg 1998), but also indirectly by fish-feeding variability (i.e. its duration and quantity).

In our case study, the IMTA microalgal inoculum was mostly composed of Chlorellales – these are not the common microalgae used for juvenile oyster feed, which is usually composed of diatoms or *Prymnesiophyceae* (McCausland et al. 1999; Ponis et al. 2003). Indeed, juvenile oysters are usually not able to retain small particles, e.g. < 4 µm for *C. gigas* when sestonic load is low, <12 µm when sestonic load is higher (Barillé et al. 1993), and even 20 µm for *Ostrea chilensis* (Dunphy et al. 2006). Chlorellales such as *Schizochlamydella* sp and *Picochlorum* sp are rather small (2–10 microns and 1.5–3 microns, respectively) and consequently not assimilable by juvenile oysters (Korshikov 1953; Tsarenko 2011; Henley et al. 2004). The addition of silicates permitted a shift in the

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

IMTA supplementary biomass production

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 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}.

In the experimental conditions tested, the results showed that an IMTA with reduced human intervention allowed a gain in recoverable biomass: i.e. 3.7 g of oyster produced per kg of fish feed distributed. For a production unit of 100 tonnes of fish fed at 1% of the biomass twice a day, an additional production of 2.7 tonnes of oyster could be provided per year. This yield could be even higher if 100% RAS water was used to supplement the microalgal compartment (5% of RAS water was used in this study), as in this case 20 microalgal raceways could be run. Moreover, only 2% of the microalgae biomass was used to feed the 3 oyster compartments: if 100% was used to feed the oysters, 37 times more oyster biomass would be produced. Further work is needed to investigate the economic viability of such a system in more detail, in order to establish whether there is an overall economic benefit to implementing such a system.

Conclusion

This study demonstrated the feasibility of using an inoculum of a microalgal polyculture (favouring diatoms of interest) from a local intensive basin together with an appropriate oyster–food ratio to feed oyster juveniles in an IMTA community. The experiment resulted in equivalent Chl*a* production in a shorter time frame than a previous

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

By transforming waste into reusable material and additional biomass, IMTAs can reduce environmental impact, giving a more positive, ecological image to aquaculture. It could potentially be promoted with a specific 'circular aquaculture' certification. However, despite its promise, in Europe the development of IMTA still faces various issues. Some of the obstacles could be overcome by pursuing research on both biological, economic and social aspects of IMTA and of end consumer perceptions of its products. Innovative zero-waste designs should be studied, with specific attention paid to analysing their effects on the biology and welfare of candidate species and the quality of the end products. Increasing the economic and ecological sustainability of aquaculture should help build the case for its social acceptance.

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Statements & Declaration

Ethical

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

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Competing Interest

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

Data availability

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

Author contributions

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

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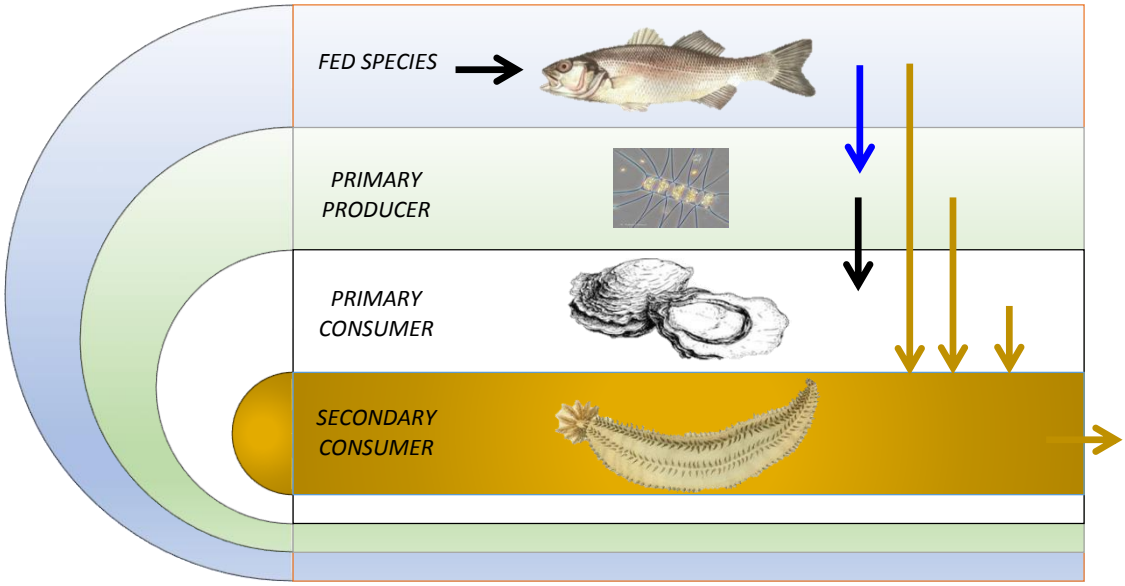

Fig. 1. Theoretical representation of an IMTA assemblage, with triangles representing the different trophic compartments and arrows representing the different flows (blue for inorganic, black for feed, brown for organic) - illustrations from: lapecheenligne.com, ©N.Neaud-Masson, shutterstock.com and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

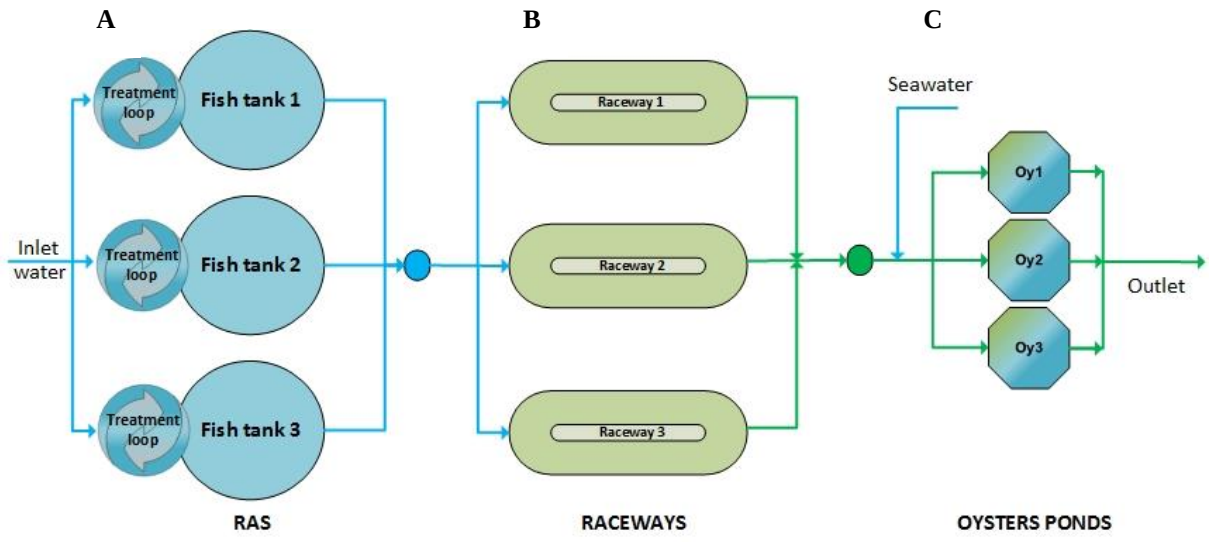

Fig. 2. Experimental land-based IMTA (Ifremer, Palavas) with three compartments: (A) fish tanks operating with water recirculation; (B) outdoor microalgal raceways receiving fish effluent from A; (C) oyster ponds receiving microalgae from lagoons in B. Total surface area = 150 m^2.

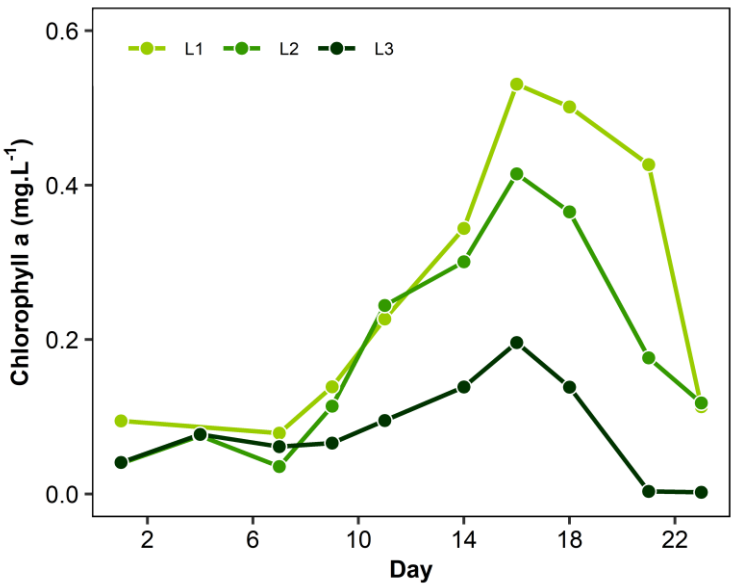

Fig. 3. Temporal Chl*a* concentrations (in mg.L^{-1}) during the experiment in the three IMTA-microalgae replicates: L1, L2 and L3.

Figure Click here to access/download;Figure;fig4.docx

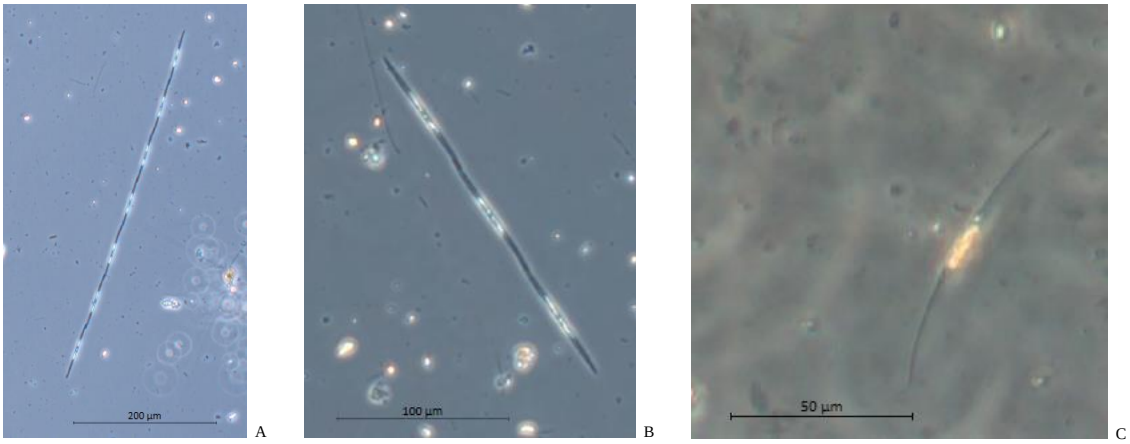

Fig. 4. *Pseudonitzschia* (A and B) and *Cylindrotheca closterium* (C) identified in the three IMTA-microalgae replicates (photos © Elise Caillard).