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Role of algal diversity in the stability of aquaculture systems: functioning of an integrated multitrophic (IMTA) system in a changing environment

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Glossary

ARA: ARachidonic Acid
B_1: Thiamine
B_7: Biotin
B_{12}: Cobalamin
C: Carbon
C1, C2, C3: Algal consortia selected from Vasco2 (C1, C3) or SW (C2) diversity reservoirs
C1A, C1J: Ingestion experiments with the algal consortium 1 (C1) fed to adult (A) or juvenile (J) oysters
C3A, C3J: Ingestion experiments with the algal consortium 3 (C3) fed to adult (A) or juvenile (J) oysters
Chla: Chlorophyll *a*
DHA: DocosaHexaenoic Acid
DW: Dry Weight
EAA: Essential Amino Acids
EPA: EicosaPentaenoic Acid
HRAP: High-Rate Algal Pond
IMTA: Integrated MultiTrophic Aquaculture
IMTA-EFFECT: Integrated MultiTrophic Aquaculture for EFFiciency and Environmental ConservaTion
N: Nitrogen
NH4: Ammonium
NO2: Nitrite
NO3: Nitrate
OD: Optical Density
P: Phosphorus
PO4: Phosphate
Pp: *Porphyridium purpureum*
PUFA: Polyunsaturated Fatty Acids
RAS: Recirculating Aquaculture System
RE: Removal Efficiency of nutrients
Seawater : SW
Si: Silicon
Ts: *Tetraselmis suecica*
TSS: Total Suspended Solids
TW: Oyster Total fresh Weight (shell + flesh)
Vasco2 : *VAlorisation et Stockage du CO_2*
α: Statistical significance threshold
ρ: Spearman's rank correlation coefficient

Summary

I. Introduction

I.1. Aquaculture & environment: benefits of the Integrated MultiTrophic Aquaculture (IMTA) concept

Aquaculture is the fastest-growing food production sector. Thus, in 1974, it supplied only 7% of the total fish production for human consumption while in 2014 its contribution overtook that of wild caught for the first time. This important growth of the sector is expected to continue in the future (FAO, 2016). However, the sector development model is still today widely based on intensive monospecific farming that is, in some case, pointed out by many authors for his unsustainability. Firstly, this aquaculture process is commonly associated with a lot of environmental concerns. For instance, the discharge of large amounts of nutrients (i.e. organism excretions, unconsumed food) cans cause the toxicity, the eutrophication and the deoxygenation of coastal waters when environmental carrying capacity is exceeded (Gowen & Bradbury, 1987; Pillay, 2004). This cans also foster the development of pathogens, parasites and harmful microalgae which may, in turn, negatively impact biodiversity, human health, but also the farming species (Neori et al., 2004; Burridge et al., 2010; Jegatheesan et al., 2011). Moreover, monoculture practices are still dependent of exogenous expensive source of food such as fishmeal and fish oil of small pelagic fishes potentially related with overfishing (Neori et al., 2004; Natale et al., 2013). Lastly, intensive monocultures are usually more vulnerable to catastrophic destructions like disease outbreaks or climatic extreme events (Barrington et al., 2009). In recent years, this was concomitant with an increasing public concern about aquaculture practices and fish quality resulting in a negative social image. In addition, aquaculture was subject to a global strengthening of legislations regarding water treatment and quality and, more broadly, good aquaculture practices (Neori et al., 2004; Talla Takoukam & Erikstein, 2013; Neori et al., 2017). Therefore, alternative aquaculture practices like integrated multi-trophic aquaculture (IMTA) and bioremediation processes were considered as the logical next-step in the aquaculture future development (Barrington et al., 2009). This was firstly evocated and investigated during early 70's and conducted to a strong increase in the research effort on this specific subject over the last decades (Ryhter et al., 1972; Goldman et al., 1974; Barrington et al., 2009; Neori et al., 2017). This is still relevant in Europe with international research projects currently ongoing (ANR, 2015; Science for Environment Policy, 2015).

According to Chopin (2013), "IMTA is the farming, in proximity, of species from different trophic levels and with complementary ecosystems 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". This is based on the bioremediation concept which is the use of waste waters inorganic and organic (particulate and dissolved matter) compounds released by a cultivated species, like finfish, by other organisms, like bacteria or algae, for their biological activities. Unlike bacteria, besides of simple heterotrophy, algae have an autotrophic activity and reintroduce energy in the system by the way of photosynthesis and inorganic nutrient assimilation (Shpigel et al., 1993; Shpigel & Neori, 1996; Demetropoulos & Langdon, 2004; Barrington et al., 2009). This enable the use of the maximum amount of energy stored in expensive processed feed instead of flushing energy out of the system. In addition to cleaning wastewaters, algae also act as oxygen providers (via photosynthesis) for other cultivated organisms by means of water recirculation. This also allows the diversification of the aquaculture activity, with the development of secondary crops, that could enhance the economic security facing to market instabilities and sudden mortalities (e.g. diseases, storms; Shpigel & Neori; 2007; Barrington et al., 2009). For these reasons, IMTA has a high profitability potential and requires a continued research effort. These IMTA systems can be developed

directly in sea or on land. In land-based systems, organisms are separated into different ponds and the link between them is assured by circulating water (Shpigel & Neori, 1996). Such systems allow avoiding or alleviating problems inherent to sea-based aquaculture like predation, poaching, extreme weather events, pathogens, poisoning and harmful wild organism proliferations (Manzi & Castagna, 1989; Neori et al., 2004; Barrington et al., 2009). Management procedures (e.g. access to the facilities, control of trophic link between organisms) are also considerably facilitated for such production systems (Neori et al., 2004, 2017). Furthermore, this prevents the escape of cultivated organisms which constitutes a major treat for local wild organism populations (O'Ryan & Pereira, 2015). Therefore, due to the numerous advantages, the domination of land-based systems in global aquaculture is expected to continue in the future (Edwards, 2015).

I.2. Finfishes-microalgae-oysters integrated systems: limits and research needs

Land-based integrated aquaculture with algae (i.e. macro or micro) is considered one of the most suitable IMTA system. Nevertheless, to insure the economic viability of such systems it is important to efficiently valorise the produced algal biomass (Neori et al., 2004). One way to achieve this is to use this biomass as source of food for grazers, like urchins and abalone, or filter-feeders, such as bivalves, macroinvertebrate species with a high commercial value (Tenore, 1976; Shpigel & Neori, 1996, 2007; Neori et al., 2004, 2017). This secondary production of macro-invertebrates allows thus to compensate the high maintenance and functioning costs of land-based aquaculture systems principally related to energy expenses (e.g. water pumping, mechanical filters) and could even generate some benefits (Shpigel & Neori; 2007; Barrington et al., 2009). IMTA-system based on macroalgae have been intensively studied during the last decades with less interest for microalgae (Milhazes-Cunha & Otero, 2017). Thus, the predominant algae cultivated as biofilter in land-based IMTA-systems are currently the genus of green and red seaweeds *Ulva* and *Gracilaria* which are frequently associated with abalone or urchin productions (Shpigel & Neori, 1996, 2007; Neori et al., 2004; Neori et al., 2017). Nonetheless, Milhazes-Cunha & Otero (2017) recently reviewed studies on this subject and showed that the use of microalgae could be more promising. Indeed, among other things, they are able to support better nutrient removal efficiency, due to higher relative surface area, than macroalgae and harvests are not obligatory to feed macro-invertebrate (i.e. filter-feeders) which reduced the staff requirement. That is why R&D about microalgae integration in aquaculture systems is still required and relevant (Neori et al., 2017).
Unlike seaweeds, the use of microalgae allows the conversion of inorganic nutrients into particulate organic matter (POM) that remains suspended in the aquaculture effluents. This thereby complicates the recirculation in the aquaculture system or the discharge of the treated wastewater in the environment (Neori et al., 2004; Shpigel & neori, 2007). That is why a filter-feeder compartment, often a bivalve production, is usually added to remove them from wastewaters and convert these particles into valuable biomass (Goldman et al., 1974; Shpigel et al., 1993). This is of great interest since bivalves currently represent a major part of the global aquaculture production (FAO, 2016). This is particularly true in Europe where they dominated the sector production (632 000t produced in 2014) with Mediterranean mussel and Pacific oyster being the main cultivated species, with a high marketability (Eurostat, 2017). Thus, the integration of microalgae in IMTA system could be more economically relevant in Europe than the use of macroalgae. Consequently, our study focused on an IMTA-system constituted of three compartments: finfishes, microalgae and Pacific oysters, on the same principle that evocated in Lefebvre et al. (2004) and Shpigel et al. (1993).

Even if the number of studies about finfish-microalgae-oyster land-based IMTA systems is limited, their potential was already investigated and demonstrated (Lefebvre et al., 1996; Lefebvre et al., 2000; Lefebvre et al., 2004; Neori et al., 2017). Thus, oysters in

integrated aquaculture with microalgae shown significant better growth performances, condition index, biochemical composition and lower mortalities, owing to the probiotic activity of the microalgae, than in the natural environment. They were always free of human pathogens and suitable for human consumption (Goldman et al., 1974; Shpigel & Blaylock, 1991; Shpigel et al., 1993). Moreover, the quality of treated wastewaters was generally high at the system output and permitted recirculation or releasing into the environment (Borges et al., 2005; Milhazes-Cuhna & Otero, 2017).
However, in such system, the productivity of the oyster compartment is mainly related to the quantity and nutritional value of the microphytes provided (Soletchnik et al., 2000; Milhazes-Cuhna & Otero, 2017). Hence, among the microalgae biochemical quality, lipid content as well as essential amino acids (EAA) and polyunsaturated fatty acids (PUFA; e.g. DHA, EPA, ARA) composition play a major role in the diet energy content and oyster assimilation which directly control oyster biomass productivity (Brown et al., 1997 and references therein; Kheder et al., 2010; Anjos et al., 2017). This nutritional value is mainly related to the microalgal diversity present in the IMTA-system with diatoms being the most suitable diet for oyster production (Brown et al., 1997 and references therein). Thus, in 1993, Shpigel et al. noticed that diversity of the microalgal diet provided in an IMTA system was the major parameter controlling oyster growth performances. However, microalgae nutritional quality is also linked with physicochemical conditions like nutrient availability, light, temperature and salinity. For instance, nutrient deprivations can increase lipid content of microalgae, propriety exploited for biofuel valorisation purposes (Chen et al., 2011; Gao et al., 2013). It was also noticed that the quantity of microalgae provided to the oyster should be as constant as possible in order to avoid mass losses with too low or too high particle concentrations (gills clogging) negatively affecting the oyster filtration process (Ward, 2004; Nielsen et al., 2017). Microalgae quantity available in the IMTA-system for biovalorisation purposes was directly related to the specific growth rates of the dominant species (Chen et al., 2011; Gao et al., 2013), determining the possibility to maintain a constant food ration for oysters.

Since outdoor raceways are the predominant commercial system of microalgae production worldwide (low operating costs), it is difficult to control algae physicochemical growth conditions due to the environmental variability (Milhazes-Cuhna & Otero, 2017; Moreno-Garcia et al., 2017). That is why, the vast majority of studies investigating the functioning of finfishes-microalgae-oysters IMTA did not controlled the microalgae development even if this affects the algal community structure, diversity and stability and ultimately the oyster production capacity. Hence, the inability to promote the dominance of desirable microalgal species is currently considered as the major limit for the broader development of IMTA based on microalgae (Milhazes-Cunha & Otero, 2017). One rudimentary process to orientate the algal community development is to adapt the Si:P molar ratio of the fishpond effluent, by manual addition of silicate, to promote the development of diatoms (Lefebvre et al.,1996, 2000, 2004). Other ways to control algal diversity were evocated including 1) the manipulation of C:N:P:Si molar ratios, 2) the choice of the initial inoculum source and 3) the selection of suitable microalgae according to the cultivation system physicochemical conditions (e.g. temperature, light, nutrient composition: N-sources or C-sources; Lefebvre et al., 1996, 2000, 2004; Hussenot, 2003; Borges et al., 2005). Thus, by taking into account the algal species-specific environmental preferences and particular growth requirements, it should be theoretically possible to orientate algal development towards the dominance of suitable species for oyster production. Nevertheless, these suggestions were timidly addressed but not further investigated.
It is however important to also consider the microalgae bioremediation efficiency since it is the initial purpose of IMTA. This remediation efficiency is also dependent of algal diversity in relation with the specific characteristics of fishpond effluents. Indeed, algal growth performances and related nutrient uptake rates are more or less efficient for a species according to the inherent characteristics of a given effluent (e.g. N-source, C-sources, oligo-nutrients; Milhazes-Cunha & Otero, 2017 and references therein). Hence, effluent nutrient composition cans change according to rearing parameters such as temperature, fish species,

fish density or type of feed used (Lin et al., 2002; Yeo et al., 2004; Schneider et al., 2005). That is why cultured algal species must be selected based on the features of each IMTA-system (Borges et al., 2005).
Hence, the ideal microphyte community must be a trade-off between nutritional quality and availability for oysters and bioremediation efficiency in order to optimise the overall functioning of finfish-microalgae-oyster IMTA-systems. The way to achieve it is currently the main research need in this field.

I.3. IMTA pilot-scheme of Palavas-Les-Flots: context and objectives of the study

In France, shellfish production is the dominant mariculture sector with 160 000t produced per year, accounting to 550 million euros (Agreste Bretagne, 2016). Since *Crassostrea gigas* (Thunberg, 1793) is the dominant produced species, it was chosen for the implementation of the France IMTA pilot-scheme at the aquaculture research station of Palavas-Les-Flots (Ifremer), being integrated in a seabass-microalgae-Pacific oyster production system. Indeed, its high marketability gives an economic relevance to the project, which could not be possible in other country, sometimes causing the abandonment of ongoing IMTA projects (Edwards; 2015). The particularity of this pilot, is that IMTA-system is connected to a recirculating aquaculture system (RAS) for seabass production. RAS is another innovative aquaculture system allowing, among other things, to economise water and energy and thus to diminish the running costs, but also to reduce the disease risks (Martins et al., 2010). Indeed, in RAS, fishpond wastewaters are treated by bacteria biofilter, oxygenated and recirculated, reducing the dependence to seawater pumping into the environment. However, the IMTA-system is not fully integrated to the RAS but only connected as a supplementary module who treats a part of the effluents who not recirculate and are then rejected into the sea, as presented in figure 1. In this system, microalgae are cultured in high-rate algal ponds (HRAP) which act as waste stabilisation ponds and are designed to optimise the algal production in continuous renewal conditions with sampling to feed oysters (Milhazes-Cunha et al., 2017).

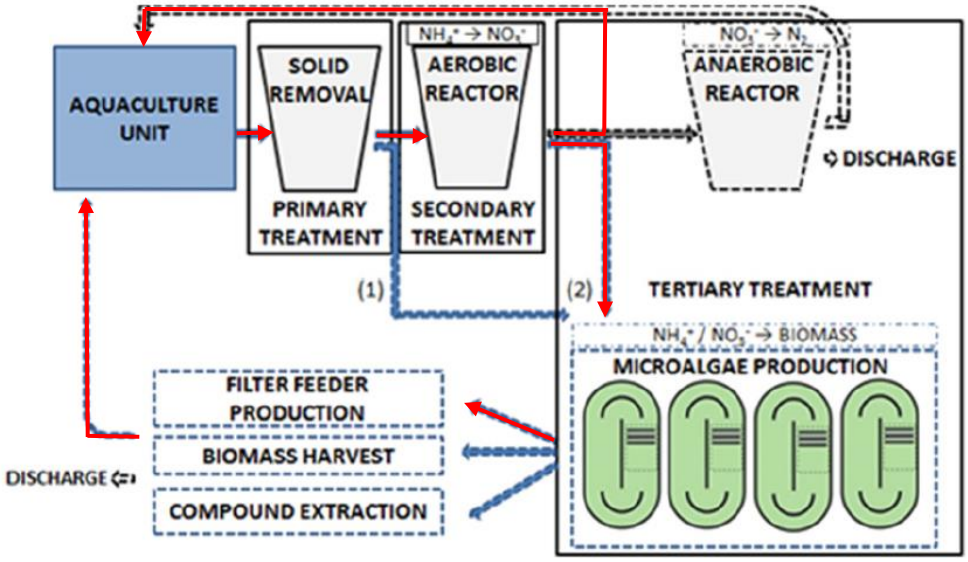

Figure 1. *Representation of different possibilities of microalgae integration in aquaculture systems from Milhazes-Cunha & Otero (2017). In our case, wastewaters are recirculated in the aquaculture unit (fish = RAS) or redirected in the microalgae production unit directly after solid removal and treatment in an aerobic reactor to mineralise ammonium (absence of anaerobic reactor). After passing in the filter feeder unit, water is discharged. Palavas IMTA-RAS system functioning is shown with red arrows*

The IMTA-system functioning was first tested over a 60-days period between spring and summer 2017 (Li et al., 2018, in revision). Microalgae development was orientated towards a diatom-dominance by means of modification of the Si:P molar ratio of the RAS effluent. The microalgal production and its bioremediation efficiency met expectations (more than 96% of effluents N and P removed) for the IMTA first purpose: effluent treatment. However, even if stable carbon isotope analysis shown that diatoms in presence were assimilated by oysters, no growth was observed during the experiments for both juvenile and adult *C.gigas*. Yet, other additional experiments demonstrated that oysters were perfectly able to ingest the dominant algal species. Two explanations were advanced: (1) the biochemical composition of microalgae did not meet oyster growth requirements (2) there was a phytoplankton limitation access to oysters in the IMTA-system with non-optimal hydrodynamic conditions.

We conducted further experiments to optimise the oyster production in the IMTA-system in relation to algal diversity. Hence, the general aim of this study was to find a way to conciliate (1) bioremediation of the RAS-effluents and (2) suitable and stable inputs of food for oysters, in the IMTA-system variable environment, in term of quality, quantity and ability to selection and ingestion by juvenile and adult *C.gigas.* The overall performance of the RAS-IMTA-system was studied to provide data in the context of the European research project IMTA-EFFECT (ANR, 2015).
Thus, the role of algal biodiversity in the stability of integrated aquaculture systems was investigated. In fact, interest was given to the algal unit inoculum source as it was previously recommended by some authors (Borges et al., 2005). Two different reservoirs of algal biodiversity were tested for their ability to promote the growth of suitable algal species in the IMTA system. The first reservoir was natural Mediterranean seawater whereas the second was a microalgal community cultivated in another ongoing projects for bioremediation of industrial CO_2 rejects (Vasco2 project; Port de Marseille Fos, 2018). Indeed, there is an interest to use these two reservoirs of biodiversity for the IMTA-system inoculation since they are directly available in the immediate vicinity, adapted to local environmental conditions (i.e. climatic variations) but must present significant differences in term of community structure and algal diversity (poor v/s enriched nutrient conditions, natural environment v/s intensive culture).
To achieve the objectives, we followed a procedure inspired of that discussed in Borges et al. (2005) who recommended to (1) evaluate the effluent composition and its capacity as culture medium for suitable microalgae (2) optimised the biomass production in realistic condition and (3) evaluate the biofiltration efficiency, and oyster feeding in our case, in field conditions. Thus, the study was separated in two different approaches:
(1) laboratory experiments: selection of algal species in controlled conditions and determination of their performances for remediation efficiency and as feeders for oysters.
(2) IMTA-system functioning: field process selection of the algae according to the effluent and environmental characteristics and determination of the system overall performances.

The first approach (Phase I) was conducted in laboratory under simulated realistic environmental conditions and, after a long selection-period, allowing the emergence of native microalgae consortia adapted to the specific physicochemical characteristics of the IMTA-system. The suitability of these consortia to sustain oyster production was primarily tested by determining specific algal growth rates in different f/2 culture media modified from Guillard (1975) and close to the nutrient conditions in the fishpond effluents (Li et al. 2008, in revision). Growth rate was considered as a relevant index of suitability for cultivation in the IMTA-system because it is directly related with production yield and bioremediation efficiency (Lefebvre et al., 2004). Performances of theses consortia were compared to those of referenced microalgae, commonly used in aquaculture, originating from a culture collection. The ability of these consortia to be consumed by both juvenile and adult oysters was thus determined. A particular focus was done on the role of micronutrients (e.g. trace metals and vitamins) in the specific growth requirements of the suitable microalgae as it was rarely done in IMTA studies. These laboratory selection experiments will allow to select the best inoculum source for the IMTA-system.

The second approach (phase II) consisted to inoculate the IMTA-system HRAP from the biodiversity reservoir showing the most promising results from the Phase I, and thus to follow the evolution of the microalgal community structure, productivity and its ability to sustain oyster growth and to remediate fish/RAS effluent over a full production period, in natural environmental conditions.
This will allow the selection of local species adapted to the local physical conditions (i.e. weather) and the effluent characteristics as it was recommended by some authors to optimise the system performances (Hussenot et al., 2003).

II. Material & Method

II.1. Phase 1: laboratory experiments

II.1.a. Microalgae selection

In order to determine the best inoculum source for the IMTA system, algal selection was performed from two different biodiversity reservoirs. They were chosen for their composition of local species already adapted to local conditions (i.e. weather) and their difference of characteristics likely linked with a difference in algal biodiversity (i.e. variable v/s continuous nutritional and hydrodynamic conditions, natural v/s enriched in nutrients):
(1) a HRAP running for the project Vasco2 ("*Valorisation et stockage du CO_2*"; culture medium highly and continuously enriched in CO_2 and nutrients, see global characteristics in annex I) located at the Ifremer Station of Palavas-Les-Flots (France).
(2) the natural Mediterranean seawater (SW), filtered with a 100µm pore diameter, pumped near the coast in the immediate vicinity of the Palavas-Les-Flots Ifremer station.
Samples (1L) were collected on December 19th 2017 (14h00) in the Vasco2 HRAP and in natural seawater (SW) respectively. Thereafter, samples were brought to the UMR Marbec Sète centre (France) for the algae consortia selection process in controlled conditions.

Consortia were isolated and cultivated under controlled temperature, nutritional and irradiance conditions, close to those observed in the IMTA system from April to July. To this end, SW and HRAP samples were inoculated (initial dilution factor of 8.7) in both **poor** f/2 medium (Guillard, 1975) containing nitrate and phosphate, only, and representing N:P molar ratio close to the IMTA-system effluent evaluated during 2017 experiments (Li et al., 2018, in revision). The consortia were also cultivated using **complete** f/2 culture medium (including trace metals and vitamins). In each culture medium, cultivations were performed both with and without silicate enrichment in order to select diatom-dominant consortia v/s other dominant species. Culture media compositions are shown in table 1.
All the inocula were incubated with diurnal temperature variation of 20±5°C (annex II), with a constant photoperiod of 12:12h and moderate diurnal irradiance variations and intensities (maximum of 300µmol photons.m^{-2}.s^{-1}) to avoid photo-inhibition at the beginning of the selection process. Incubations took place in *MIR-154-PE Cooled Incubator (*phcbi®) under a constant culture homogenisation provided by an orbital shaker *Unimax 1010* (heidolph®). Photoperiod and light intensity were regulated with a custom-made LED system (*Carte - Biolight – 4R – REB – V1*) produced by Meodex®, associated with the control software *Sun Insolation V1.5.1.0* provided by the "Observatoire océanologique de Banyuls-sur-Mer" (Banyuls-sur-Mer, France, CNRS©, jean-luc.aucouturier@obs.banyuls.fr).

Following the first cultivation period (2 weeks), flasks with positive algae development were used for subculturing. Subculturing was renewed often as necessary, about every 2-3 weeks, in order to maintain a good physiological status of the selected algae. This consortia selection period took place from 19/12/17 to the beginning of March 2018. Simultaneously,

from mid-February, the same selection process was performed with single algae strain, commonly used in aquaculture, originating from the CCAP (The Scottish Association for Marine Science, Oban, UK) culture collection. Cultivated strains were *Isochrysis galbana* (Parke, 1949) CCAP 927/1, *Diacronema lutheri* (Droop; Bendif & Véron, 2011) CCAP 931/1, *Porphyridium purpureum* (Bory; Drew & Ross, 1965) CCAP 1380/11, *Thalassiosira pseudonana* (Hasle & Heimdal, 1970) CCAP 1085/12, *Chaetoceros calcitrans var. pumilus* (Paulsen; Takano, 1968) CCAP 1010/11, *Tetraselmis suecica* (Kylin; Butcher, 1959) CCAP 66/4 and *Skeletonema marinoi* (Sarno & Zingone, 2005) CCAP 1077/5. Diatoms were cultivated with silicate.

Table 1. *Composition of the different culture media based on the f/2 culture medium described in Guillard (1975). Silicate ($Na_2SiO_3,9H_2O$) was added only for the cultivation of diatom species.*

Culture medium	Macronutrients (M)			Trace Metals (M)			
	$NaNO_3$	$NaH_2PO_4\,H_2O$	$Na_2SiO_3\,9H_2O$	$FeCl_3\,6H_2O$	$Na_2EDTA\,2H_2O$	$CuSO_4\,5H_2O$	$Na_2MoO_4\,2H_2O$
Poor	$8.82.10^{-4}$	$3.62.10^{-5}$	$1.06.10^{-4}$	X	X	X	X
Effluent	$8.82.10^{-4}$	$3.62.10^{-5}$	$1.06.10^{-4}$	X	X	X	X
Complete	$8.82.10^{-4}$	$3.62.10^{-5}$	$1.06.10^{-4}$	$1.17.10^{-5}$	$1.17.10^{-5}$	$3.93.10^{-8}$	$2.60.10^{-8}$
Boosted complete	$4.41.10^{-3}$	$1.81.10^{-4}$	$5.30.10^{-4}$	$1.17.10^{-5}$	$1.17.10^{-5}$	$3.93.10^{-8}$	$2.60.10^{-8}$

Culture medium	Trace Metals (M)			Vitamins (M)			Medium basis
	$ZnSO_4\,7H_2O$	$CoCl_2\,6H_2O$	$MnCl_2\,4H_2O$	B1	H	B12	
Poor	X	X	X	X	X	X	0.2µm filtered SW
Effluent	X	X	X	X	X	X	0.2µm filtered effluent
Complete	$7.65.10^{-8}$	$4.20.10^{-8}$	$9.10.10^{-7}$	$2.96.10^{-7}$	$2.05.10^{-9}$	$3.69.10^{-10}$	0.2µm filtered SW
Boosted complete	$7.65.10^{-8}$	$4.20.10^{-8}$	$9.10.10^{-7}$	$2.96.10^{-7}$	$2.05.10^{-9}$	$3.69.10^{-10}$	0.2µm filtered SW

II.1.b. Growth rates determination

Growth rates of selected strains/consortia were determined when exposed to environmental conditions close to those observed in Palavas-Les-Flots HRAP in early April: diurnal temperature variation of 20±5°C and photoperiod & irradiance variations (43.520° latitude) from April 1st to 5th with 10% light attenuation (maximum of 1400µmol photons.m^{-2}.s^{-1}, annex II). Those parameters were regulated in the incubators by the same technical system as described before.

Growth rates were calculated for each strain/consortium cultivated in different media: **poor** f/2 medium (N/P ± Si), **complete** f/2 medium (N/P/trace metal/vitamin ± Si) and **effluent** f/2 medium (N/P ± Si). **Effluent** f/2 medium is the equivalent of the **poor** f/2 medium but was constituted using filtered IMTA-system effluent (nitrate concentration: $6.41.10^{-5}$ ±$0.25.10^{-5}$M, ammonium concentration 4.99±$0.25.10^{-5}$M) sampled directly in IMTA-system fishponds the 15/03/18, instead of filtered natural seawater, as described in table 1. It was not autoclaved in order to preserve fragile organic compounds such as vitamins. The objective was to determine the importance of micronutrients (e.g. trace metals, vitamins) in fishpond effluents by means of comparison between growth rates in the **poor**, **effluent** and **complete** f/2 culture media.

Growth experiments took place during two different experimental weeks: W1 from 12/03 to 16/03/2018 and W2 from 26/03 to 30/03/18. In both case, the tested strain/consortium was acclimatised to the experimental conditions (i.e. culture medium, temperature, photoperiod & irradiance) one week prior to the beginning of the experiments. Thus, they were inoculated in the tested culture medium, with a 5-dilution factor, in microplates (1mL-48 wells format) with five replicates per strain/consortium x culture medium. Each experimental period was 5-days long and the optical density at 680nm (680nm-OD) was measured each day in the early morning (9h) in each well of the microplate using a *Multilabel Microplate Reader* (Plate Chameleon^{TM}, Hidex®). In each experiment, the culture medium was used as blank. 680nm-OD was used as a proxy of algal biomass (chlorophyll *a*) concentration per well and finally allowed the growth curves construction. Growth rate (number of cellular divisions per day, d^{-1}) was thus determined as the slope of the linear part of the curve ln(680nm-OD) as a function of time.
Concentrations in the algal cultures were too low to allow to count initial cells density on Malassez haemocytometer. Thus, algal concentrations at the beginning of each experiments were only controlled by 680nm-OD. Microscopic observations allowed us to confirm that consortium diversity was not strongly modified during the growth experiments (i.e. comparison between the debut and the end of the growth period).

Due to logistical problems, experiments in the **poor** f/2 culture medium were not performed for all the strains/consortia but only for two of them.

II.1.c. Microalgae selection by the oysters

Both juvenile and adult *C.gigas* were fed alternatively with two of the selected consortia, C1 (diatom-dominated) and C3 (*Chlorophyceae*-dominated), in order to determine the selectivity of food intake with respect to microalgae characteristics. Thus, this constituted 4 different experiments with juvenile (J) or adult (A) oysters fed with C1 or C3 consortia: C1J, C1A, C3J, C3A. The volume of the selected cultures was progressively implemented to reach 5L in glass bottles with air bubbling and homogenisation by magnetic agitator. This cultivation process took place in a **boosted complete** f/2 medium (N/P/trace metal/vitamin ± Si) with a macronutrient concentration (N,P±Si) 5-time higher than in the basic medium (table 1). This culture medium was chosen to reach the algal biomass necessary for the ingestion experiments concentrated in a culture volume as small as possible. Both cultures were incubated in the same temperature and light conditions than for the growth experiments.

• **Experimental design:** 30g total fresh weight of 20months-Adult or 8months-juvenile Pacific oysters were placed, in triplicate, in 5L-beaker (22±1 juveniles/beaker v/s 1 adult/beaker) two days prior the ingestion experiment (i.e. acclimation period). In addition to this triplicate, adult (n=1) and juveniles (n=22), corresponding to 30g of total fresh weight, were sacrificed and shell(s) were placed in an individual beaker to be used as a control during the experiment. Beakers were filled with 5L fresh 100µm-filtered seawater and oysters were immediately fed with the tested consortium/strain in a quantity chosen to reach an optimum (Fabioux et al., 2005; Delaporte et al., 2006) of 6-8% algae dry weight (DW) per oyster dry weight unit (determined with allometric relations previously constructed for both oysters and algae consortia). Homogenisation was continuously maintained using a magnetic stirring system at the bottom of the beaker, below the oysters placed on a grid. Moreover, a continuous air flux was provided to increase homogenisation and oxygenation. Oysters were maintained every day under an artificial continuous light approximately from 8h to 18h00. Temperatures were not regulated. Experimental device is presented in annex III. One day before the experiment, in the late afternoon, beakers were cleaned and seawater was renewed.
Just before the ingestion experiment, beakers were cleaned, filled with 5L fresh 100µm-filtered seawater, and oysters were left undisturbed for one hour. The experimental design was greatly inspired by the protocol used in Nielsen et al. (2017) for the determination of *Ostrea edulis* (L.,

1758) and *C.gigas* ingestion rates. At the beginning of the experiment, the tested consortium was added in the beaker in the same quantity as during the acclimation period (6-8%DW). Each experiment duration was 6h30 and the algal biomass dynamic was followed by measuring the chlorophyll *a* (Chla) concentration as a function of time. 40 to 60mL were sampled in each beaker for Chla determination at 0, 60, 120, 210, 330 and 390 min after the beginning of the experiment. They were filtered on 0.7µm-pore size GF/F filters (Whatman®). Filters were then held at -80°C before further analysis. These experiments occurred during two experimental weeks, from 13/04 to 20/04/2018. Temperature in the beakers was 15.6±0.7°C at 7h00 and 20.8±1.4°C at 17h00. Oxygen concentration kept at 96.1±2.3% saturation. Salinity and pH were respectively 36.5±0.5PSU and 8.3±0.4.

• **Chlorophyll a determination:** After thawing, Chla on filters was extracted in absolute ethanol during 12h after a quick sonication. Then, the supernatant was collected after centrifugation (4000rpm, 10min), filtered on 0.7µm-pore size GF/F filters, and absorbance was read at 750, 665, 649 and 632nm with a *UV-1800 UV-Vis Spectrophotometer* (Shimadzu®). Chla concentration (mg/L) was then calculated according to the formulae given in Ritchie et al. (2006):

(1) for diatoms (C1):

$$Chl\ a\ =\ [11.49 \times (665nm - 750nm) - 1.401 \times (632nm - 750nm)] \times \frac{Ve}{Vf}$$

(2) for *Chlorophyceae* (C3):

$$Chl\ a\ =\ [11.867 \times (665nm - 750nm) - 5.201 \times (649nm - 750nm)] \times \frac{Ve}{Vf}$$

Chl a was the Chla concentration in the beaker, expressed in $mg.L^{-1}$. *750nm*, *665nm*, *649nm* and *632nm* were the absorbances read at the corresponding wavelengths. *Ve* was the ethanol volume, expressed in mL, used during the extraction. *Vf* was the sample filtered volume, expressed in mL.

• **Food ration:** For each ingestion experiment, the applied food ration, in %DW was retrospectively calculated.
The determination of algae dry weight provided to the oysters for each experiment was done as described in Strickland & Parsons (1972) for the determination of total suspended solids (TSS) in natural seawater. Just prior the beginning of the ingestion experiment, a known volume of the pure culture was filtered on calcinated (4h at 450°C) pre-weighed (balance precision = 0.001g) 0.7µm-GF/F filter. Filter is then rinsed with ammoniac formate ($68g.L^{-1}$) in order to eliminate salts. Finally, filter was weighed after 24h-drying at 110°C. For each analysis, a blank was done using culture filtrate. This allowed thus to calculate the algae DW fed to the oyster, in each ingestion experiment, with the following formulae:

(3) $$Algae\ DW = (mf - m0) \times \frac{Vfed}{Vf}$$

Algae DW was the dry mass (g) of algae provided to the oysters. *m0* and *mf* were respectively the initial and final filter dry mass (g). *Vf* was the culture filtered volume (mL). *Vfed* was the culture volume fed to the oysters (mL).
Oysters dry weight was also determined. At the end of the experimental period, individuals were sacrificed, opened, and total flesh was pooled for each beaker. Thus, oysters flesh dry weight was determined for each beaker with a simple weighing after a 48h 110°C-drying. It was then possible to calculate the quantity of algae fed to the oysters in each beaker of each experiment with the following formulae:

$$(4)\ Food\ ration\ =\ \frac{Algae\ DW}{Oysters\ DW} \times 100$$

Food ration was the quantity of algae fed to the oysters, expressed in %DW. *Algae DW* was the dry mass of algae (g) poured in each beaker. *Oysters DW* was the oyster total flesh dry weight per beaker (g).

II.1.d. Statistical analysis

All statistical analyses and graphical representations were performed with the *R software version 3.4.0* (R Core Team, 2017).

• **Growth experiments:** Multiple linear regression model was constructed to represent the evolution of growth rate according to the tested algae (strain/consortium), the culture medium (poor, effluent or complete) and the interaction of these two factors. The best explicative model was chosen according to the Akaike Information Criterion based on the principle of parsimony (Akaike, 1974). Logarithmic transformation of growth rate data was necessary to respect the normality hypothesis. Thus, a model testing the effects of culture medium, strain and their interaction on log(growth rate) was selected. The associated 2-way ANOVA (type III) was then performed. The normality, normality of residuals and homoscedasticity model's assumptions were checked respectively with Levene and Shapiro tests (significance level, $\alpha = 0.05$) and also by means of graphical methods. Tukey HSD a posteriori test was performed to differentiate the statistical groups ($\alpha = 0.05$).

• **Ingestion experiments:** For each ingestion experiment, a linear regression model was constructed to represent the evolution of Chla as a function of time. The model's assumptions were checked by the same way as described before. Simultaneously, a linear curve was constructed, with the method of least squares (Plackett, 1972), to represent the evolution of Chla in the control beaker. The slope of the linear regression model and his confidence interval were determined. They were then compared with the slope of the control linear curve using a Student t-test ($\alpha = 0.05$). Significant difference between the two slopes was considered as a significative consumption of the tested consortium by the oysters.

II.2. Phase 2: Field experiments and IMTA-system functioning

II.2.a. RAS-IMTA system and experimental design

• **Description of the RAS-IMTA system:** The RAS-IMTA system of the Palavas-Les-Flots aquaculture station is presented in annex IV and constituted of three modules built in series:
(1) The seabass RAS production unit as described by Blancheton (2000). In this unit, *Dicentrarchus labrax* (L., 1758) were fed daily with 1.8kg of a commercial diet *Neo Grower Extra Marin-Coul 5* (Le Gouessant®), using self-feeders, and supplied with oxygen. Particulate matter (unconsumed food and faeces) contained in the fishpond effluent was removed by faecal trap and mechanical filter (30µm mesh). Then, after passing through UV-treatment and bacterial biofilter, the vast part of effluents was recirculated in the fish unit whereas a little fraction (about 1.3%) was directed towards the IMTA-system. This unit was designed in triplicate with three independent RAS for seabass production whose effluents were mixed in order to feed the IMTA system. The initial finfish stocking density was 48.7±2.3kg.m^{-3} (460±26 fish per tank, 425±134g.ind^{-1}). They were reared under an artificial 12:12h photoperiod.
(2) The outdoor microalgae production unit in HRAP. Each algal raceway contained 6m^3 working volume (area = 12m^2 and water depth = 0.50m) and was almost continuously filled with the mixed effluents which represented a renewal rate of about 4.2d (input flow rate =

1L.min^{-1}). Circular and bottom-up movements were maintained in each algal raceway by means of two pumps *nanostream electronic* (Turbelle®, 58 watt) located at the two extremities of the HRAP, in order to mix correctly the water column, limit biodeposition and ensure a good light-access for the microphytes. A low airflow was provided in the HRAP with an air diffuser to provide additional source of CO_2 for photosynthesis. The microalgae cultures in triplicate were then mixed in a mixing tank (named "Pool" hereafter) with airstones
(3) The outdoor oyster production unit continuously supplied with the produced microalgae from the Pool tank at a flow rate of 2.7L.h^{-1} with 100 L.h^{-1} of fresh filtered seawater (dilution factor = 37) in order to approach a daily feeding ration of 6-8%DW. *C.gigas* were reared in ponds with a 0.5m^3 working volume and about 5h renewal rate. They were covered with a semi-opaque blanket to limit epiphytic algae development on the pond surfaces and oyster shells but also to avoid predation by seagulls. In this unit, juveniles and adults were produced in triplicate accounting for a total of 6 rearing ponds. The food distribution problem observed last year in the oyster ponds (Li et al., 2018, in revision) was solved by the inclusion of a homogenisation pump *nanostream electronic* (Turbelle®, 5-7 watt) besides of an airlift and by changing the baskets where oysters were located.

• **Experimental design:** The HRAP were inoculated with the Vasco2 culture. Silicate was added in the fishpond effluents, so as to approach a N:Si:P molar ratio of 10:5:1, in order to favour diatom dominance, as described in Lefebvre et al. (1996).
Thus, the 03/04/18, algal raceways previously filled with RAS effluents were inoculated at 20% of their working volume with the Vasco2 culture. Metasilicate (Na_2SiO_3,5H_2O) were dissolved in each algal raceway at a concentration of 8.33 mg.L^{-1} the April 13th, 17th and 19th and then the same quantities were added thrice a week during the rest of the experimental period.
The 17/04/18, 8-months juveniles (252±3 per tank, 1.55±0.33g.ind^{-1}, length: 22±3mm.ind^{-1}) and 20-months adult *C.gigas* (30 per tank, 55.63±16.02g.ind^{-1}, length: 86±13mm.ind^{-1}) were placed in the production ponds. This was considered as the first day of the experimental period. From the 17/04/18 and until the culture crashes, the evolution of the RAS-IMTA system functioning was monitored as described hereafter. Phase II ended the 14/05/18 with the collapse of the three algal cultures.

II.2.b. System functioning indicators

• **Algal growth:** The *in situ* algal growth was followed, three times a week at 10:30 a.m., by Chla determination in each algal raceway and in the pool. This was done using the same method as described before. Calculations were realised with the equation (1) considering that diatoms were dominant in the IMTA-system during the whole experimental period. Growth rate (number of cellular divisions per day, d^{-1}) was thus determined as the slope of the linear part of the curve ln(Chla) as a function of time.

• **Dominant algal species:** The dominant algal species were weekly (Monday) identified in each HRAP by microscopic observations conducted by taxonomists. Due to logistical problems, counting and abundance determinations were not performed.

• **Bioremediation efficiency:** The evolution of the main nutrients was followed three times a week with sampling at 01:45 p.m. at two points of the IMTA system (RAS effluents and algal raceways). The water samples were primarily filtered on GF/F filters (Whatman®) and then stored at -80°C. The analyses were finally carried out with a continuous-flow auto-analyser *Futura* (Alliance®) for determination of nitrate-N (NO3-N), nitrite-N (NO2-N), ammonium-N (NH4-N) and phosphate-P (PO4-P) concentrations. Removal efficiency, RE (%), was then determined for each nutrient and algal raceway with the following formulae:

$$(5)\ RE = \frac{C_{EFF} - C_{AR}}{C_{EFF}} \times 100$$

RE was the removal efficiency for a given nutrient, expressed in %. C_{EFF} and C_{AR} were respectively the nutrient concentrations in the effluents and in the algal raceways, expressed in $mg.L^{-1}$.

• **Oyster growth:** For both adult and juvenile oysters, biometrics were performed at the beginning (17/04/2018) and the end of the experiments (17/05/2018). The total fresh weights (TW, flesh & shell) were determined using a precision scale (precision of 0.001g). Length were also measured with a calliper (precision of 0.01mm). Oyster mass/length net gain (%), during one month of experiments, was calculated using the following formulae:

$$(6)\ Net\ Gain\ = \frac{X_f \times 100}{X_i} - 100$$

Net gain was the net mass or length gain, expressed in %. X_i and X_f were respectively the mean weight (g) or length (mm), per pond, measured at the beginning and at the end of the experimental period.

• **Oyster food ration:** Algal dry weight was determined thrice a week, in triplicate, in the pool (mixing tank). This was done using the same method as described before for the determination of TSS in seawater. Oyster food ration was calculated three times a week from these data. For the adults, oyster dry weight calculation was based on the TW data measured in biometrics and an allometric equation previously constructed with field data originating from a scientific campaign conducted in the Thau lagoon in 2013-2014 (*Pronamed-II project*, n=886 19-20months *C.gigas*; Franck Lagarde personal communication). The established linear relation (R^2 = 0.60) was: Oyster DW = 0.0273*TW-0.1654. The food ration, in %DW, was then determined using the equation (4), considering the dilution rate (F=37) of the algal culture in the oyster ponds. Since no relation between TW and DW was available for juveniles *C.gigas*, we did not calculated the food ration for them in %DW. Indeed, relation between TW (flesh and shell) and flesh weight varies differently according to the age (Butler, 1952). Consequently, to allow a comparison between adult and juvenile oysters, food rations were also expressed in $\%DW_{algae}.TW_{oysters}^{-1}.d^{-1}$. Because biometrics were not realised weekly, the oyster weight reference used for the calculations were both initial and final weights for juveniles whereas it was a mean between initial and final weights for the adults.

• **Environmental conditions:** pH, temperature and salinity were daily measured at 9:30 a.m. in the algal raceways using a multi-parameter *Professional Plus* probe (YSI®). Light irradiance data (total solar radiations), in $J.cm^{-2}.d^{-1}$, were recorded continuously and obtained from the meteorological station located at 8.3km of the experimental facility, in the Montpellier airport (Gregory Messiaen personal communication).

II.2.c. Statistical analysis

All statistical analyses and graphical representations were performed with the *R software version 3.4.0* (R Core Team, 2017). The eventual relations between the different variables of the IMTA-system functioning were tested using non-parametric Spearman's rank correlation tests (α = 0.05). The differences between the initial and final oyster weights or lengths were tested by means of non-parametric Wilcoxon-Mann-Whitney tests for both adults and juveniles (α = 0.05).

III. Results

III.1. Phase 1: laboratory experiments

III.1.a. Microalgae selection

At the end of the selection period, 3 consortia were observed: C1 (diatom-dominant) and C2 & C3 (*Chlorophycae*-dominants). C1 diatom-dominant species was identified as *Amphora sp.* by microscopy. Only two single strains from the culture collections showed a substantial growth: the red algae (Pp) *P.pupureum* and the green algae (Ts) *T.suecica*. The selected strains/consortia and their associated selection processes are summarised in table 2.

Table 2. *Presentation of the selected consortia and single strains and their selection characteristics.*

Consortium / Strain	Characteristics	Origin	Selection medium
C1	diatom-dominant (*Amphora sp.)*	Vasco2	**poor** f/2 medium (N/P/Si)
C2	*Chlorophycae*-dominant little round cells	Vasco2	**complete** f/2 medium (N/P/trace metal/vitamins)
C3	*Chlorophycae*-dominant little round cells	SW	**poor** f/2 medium (N/P)
Pp	*Porphyridium purpureum*	Culture collection	**poor** f/2 medium (N/P)
Ts	*Tetraselmis suecica*	Culture collection	**poor** f/2 medium (N/P)

III.1.b. Growth experiments

A multiple linear regression model explaining the log(growth rate) variations according to the factors strain/consortium, culture medium, and their interaction, was constructed. The model was statistically significant ($p<0.001$***). The associated ANOVA was performed and explained 97,93 % of the observed variance. The factors strain/consortium ($p<0.001$***), culture medium ($p<0.001$***) and their interaction ($p<0.001$***) had a statistically significant effect on growth rate. Thus, the factors strain/consortium, culture medium and their interaction explained respectively 27.53 %, 6.46 % and 63.94 % of the observed variance. The results of Tukey HSD, a posteriori, test were represented in figure 2.

In general, growth rate was significantly greater in the complete medium than in the effluent medium for each consortium/strain. For instance, a maximum growth rate of 1.2±0.1 d^{-1} was observed in the complete medium for consortium C1, which was about 1.9-fold higher than in the effluent medium. However, C1 growth in the effluent medium (0.7±0.1 d^{-1}) was significantly greater (about 2.5-fold) than in the poor medium (0.3±0.0 d^{-1}).
An exception was noticed for the C3 consortium. Indeed, its growth was significantly higher in the effluent medium (1.4-fold higher) than in the complete medium (0.6±0.0 d^{-1}) with a growth rate of 0.9±0.0 d^{-1}.
Interestingly, the Pp growth rate in the poor medium was the highest recorded with 2.0±0.1 d^{-1}. This was nearly 2-fold higher than in all the other experiments for others strain/consortium and culture media.

It was interesting to compare the different strain/consortium growth rates in the effluent medium, which present the nutrient characteristics closest to the nutrient conditions encountered in the IMTA-system. When cultivated in effluent medium, C3 consortium showed the highest growth rate, significantly greater to the C1 consortium (about 1.4-fold higher) which was significantly higher than the C2 growth rate (about 1.8-fold).
In this effluent medium, Ts and Pp strains had intermediate growth rates. Ts growth rate was not significantly different from those of C3 and C1. Lastly, Pp growth rate was similar to TS and C1 growth rates.

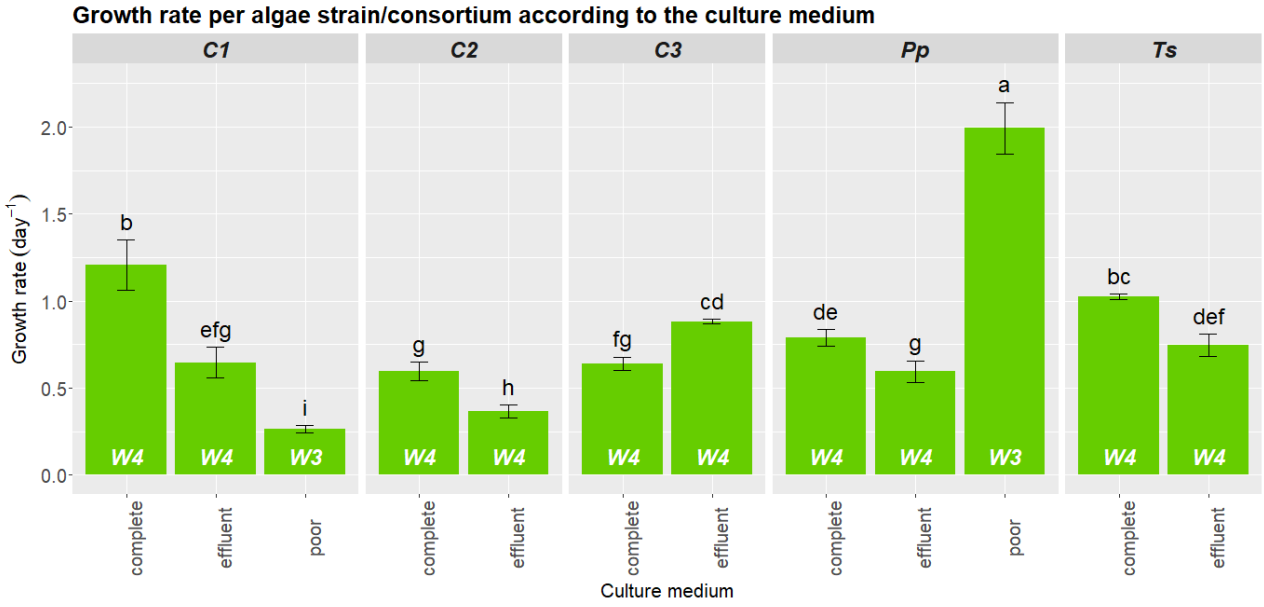

Figure 2. *Presentation of mean algal growth rates (d^{-1}) as a function of strain/consortium and culture medium. Standard deviations are shown for each experiment as error bars. Experimental week is shown, in white, for each experiment. Finally, Tukey HSD test results are presented: groups with the same alphabetic letter are not significantly different at a 0.05 significance level.*

III.1.c. Ingestion experiments

In spite of the initial objective of 6-8%, the food ration was always close to 11-12%DW for the two C1 experiments and close to 5-7%DW for the two C3 experiments. The experimental plan and the different characteristics associated with each experiment were summarised in table 3.
Linear regression models were constructed to explain the Chla dynamic as a function of time in each ingestion experiment. These models were presented in figure 3, 4, 5 and 6 for C1J, C1A, C3J and C3A experiments respectively. In each figure, R^2 indicated the proportion of the observed Chla variance explained by the linear model: 88,3%, 85,8% and 75,0% respectively for C1J, C1A and C3A experiments. The statistical significance (p-value) of each model was indicated. In agreement with this information, C3J linear model was unreliable (p-value >> 0.05 significance level; figure 4). Moreover, the C3J model's assumptions (e.g. normality, homoscedasticity) were not respected and this model was considered as invalid. Results of the student t-tests, testing the difference between the slope of the control and the experimental linear regressions, were summarised in table 3. The Student t-test results showed that the Chla decrease rate was significantly higher in the ingestion experiment than in the control beaker for both C1J, C1A and C3A experiment. Thus, we assumed that C1 consortium was ingested by both juveniles and adults whereas C3 consortium was only consumed by adult oysters. In fact, no significant temporal variation was observed for the Chla concentration in the C3J experimental beakers (according to statistics: no linear relation between Chla and time; figure 5).

Table 3. *Presentation of the experimental plan for the ingestion experiments. The Student t tests results are indicated: significance degree of the difference between the control and the experiment (p<0.05*, p<0.01**, p<0.001***). Due to the invalidity of the linear model, t test was not performed (NP) for C3J*

Consortium	Oysters			Experience	Date	Food Ration (%DW)	Student t test significance level
	Individuals	n	DW (g)				
C1	Adults	1	0.73±0.25	C1A	19/04/2018	12±4	**
C3	Adults	1	0.73±0.25	C3A	13/04/2018	7±3	**
C1	Juveniles	21±1	0.93±0.04	C1J	18/04/2018	11±1	*
C3	Juveniles	21±1	0.93±0.04	C3J	20/04/2018	5±1	NP

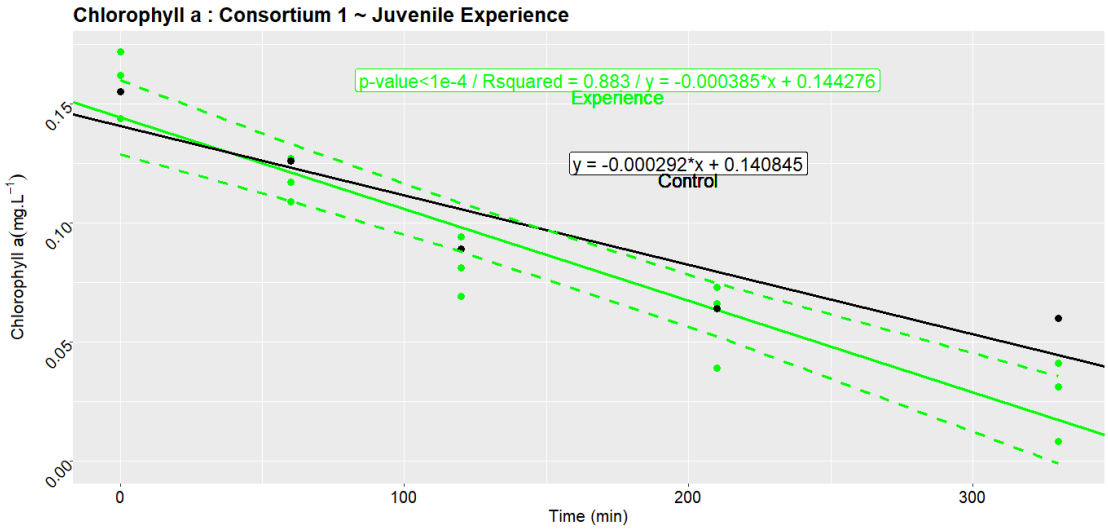

Figure 3. *C1*Juveniles experiment (C1J): chlorophyll a (mg.L^{-1}) evolution in the experimental beakers as a function of time (min) and the regression linear model associated (green). The green dotted line represented the slope confidence interval. Significance degree of the model was presented (p-value) with the associated R^2 (Rsquared) and the linear equation. The linear regression in the control beaker was shown in black (R^2 = 0.86) with the linear equation associated.*

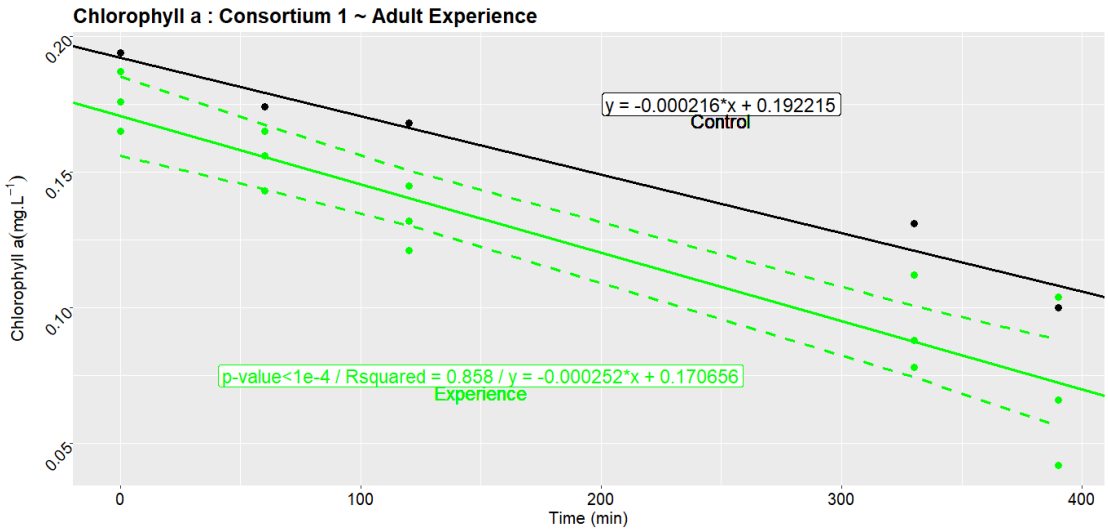

Figure 4. *C1*Adults experiment (C1A): chlorophyll a (mg.L^{-1}) evolution in the experimental beakers as a function of time (min) and the regression linear model associated (green). The green dotted line represented the slope confidence interval. Significance degree of the model was presented (p-value)*

with the associated R^2 (Rsquared) and the linear equation. The linear regression in the control beaker was shown in black (R^2 = 0.96) with the linear equation associated.

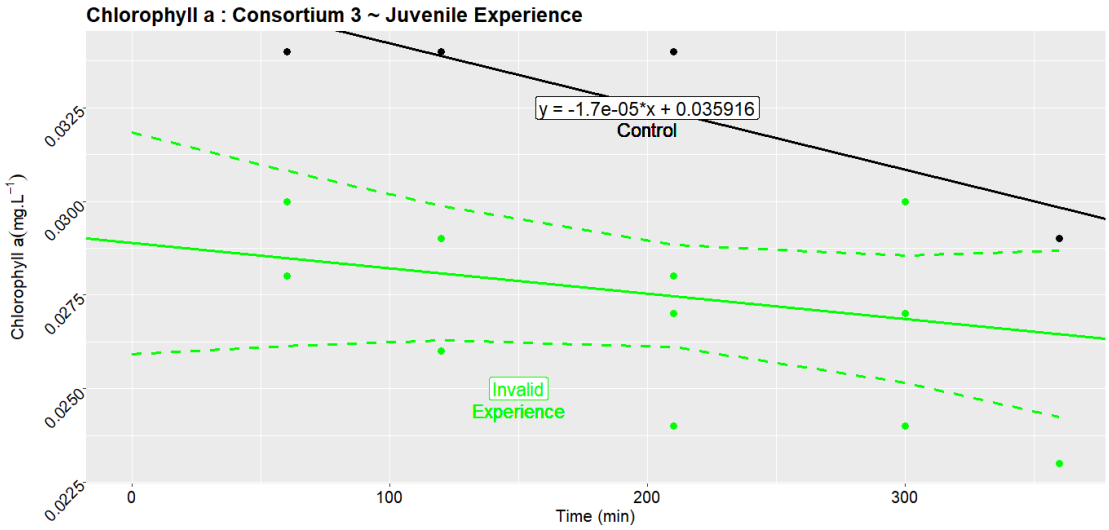

Figure 5. *C3*Juveniles experiment (C3J): chlorophyll a (mg.L^{-1}) evolution in the experimental beakers as a function of time (min) and the regression linear model associated (green). The green dotted line represented the slope confidence interval. The linear regression in the control beaker was shown in black. Both experiment and control linear regression models were unreliable according to their significance level and non-respected model's assumptions.*

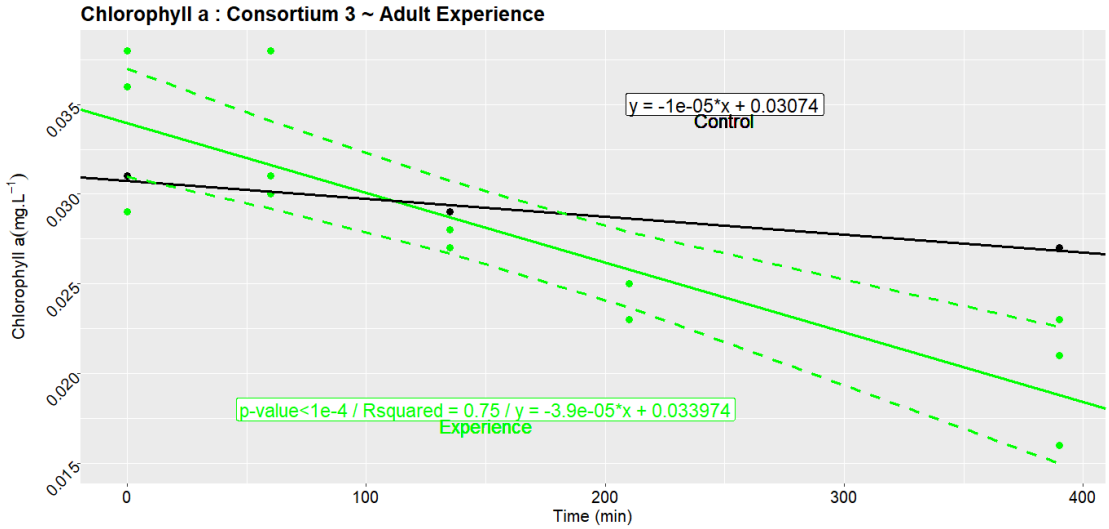

Figure 6. *C3*Adults experiment (C3A): chlorophyll a (mg.L^{-1}) evolution in the experimental beakers as a function of time (min) and the regression linear model associated (green). The dotted line represented the slope confidence interval. Significance degree of the model was presented (p-value) with the associated R^2 (Rsquared) and the linear equation. The linear regression in the control beaker was shown in black (R^2 = 0.97) with the linear equation.*

III.2. Phase 2: Field experiments and IMTA-system functioning

III.2.a. Algae production and environmental parameters

The dynamics of algal biomass, expressed as Chla concentration, in the HRAP are shown in figure 7. Due to logistical problems, silicates were not always added three times a week. Dates where they were provided were indicated in figure 7. Chla globally showed the same three-phased pattern in the different HRAP. (1) From 17/04 to 23/04, Chla concentrations were low, 0.06mg.L^{-1} on average, which was concomitant with a potential cyanobacteria dominance. (2) After the 23/04, a rapid increase in algal biomass was observed in each HRAP, with maximum Chla concentrations reached on the 02/05 ranging from 0.20 to 0.53 mg.L^{-1}. This corresponded to growth rates ranging between 0.1d^{-1} (HRAP3) and 0.4 d^{-1} (HRAP2). This was also concomitant with a shift in the community dominant species from cyanobacteria to the diatom *Cylindotheca closterium* (Ehrenberg; Reimann & Lewin, 1964). (3) Finally, the *C.closterium*-dominated community declined in each HRAP after the 02/05 and collapsed between the 07/05 (HRAP1) and 11/05 (HRAP3). It was also important to note that Chla concentrations were much lower in the HRAP3 than in the other HRAP.

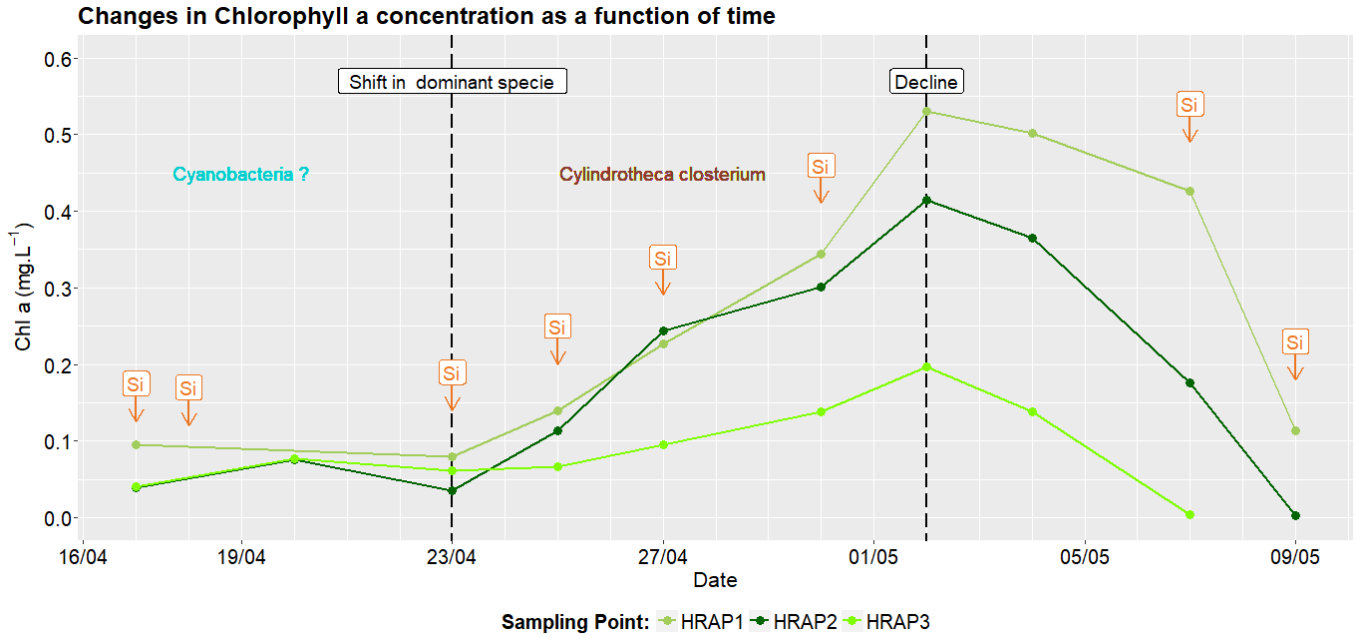

Figure 7. *Changes in the chlorophyll a concentration (mg.L^{-1}), in the different HRAP, as a function of time. "Si" labels represent the dates when silicate was added into the algal raceways. The figure is divided into three sections with dash-lines representing the algal community evolution tendencies observed during the running period. Dominant species in the community are indicated..*

Water temperature and pH variations, measured at 9:30 a.m., in the HRAP during the experimental period, were presented in figure 8. According to Spearman correlation test, morning temperature was negatively correlated with Chla concentration (*, ρ = -0.51). Indeed, the temperature decreased during the second period when the Chla increased and reached a minimum of 12°C the 02/05 which was concomitant with the maximum Chla recorded concentration. Then, during the decline of the algal community, temperature risen. Conversely, pH followed the same pattern that Chla concentration with which it is significantly positively correlated (*, ρ = 0.55). Hence, the highest pH, ranging from 10.0 to 10.3 were recorded the 02/05, during the Chla peak in the HRAP. Due to technical problems, pH values measured in the HRAP1 were not reliable and were not presented here.

Means of salinity and irradiance, were respectively 35.5±0.7 (measured at 9:30 a.m.) and 2149±433J.cm^{-2}.d^{-1} during the experimental period (annex V). Salinity increased almost linearly during this period with 32.7±0.4 the 17/04, 35.3±0.2 the 23/04, 36.7±0.2 the 02/05 and a maximal value of 39.1±0.0 reached the 09/05.

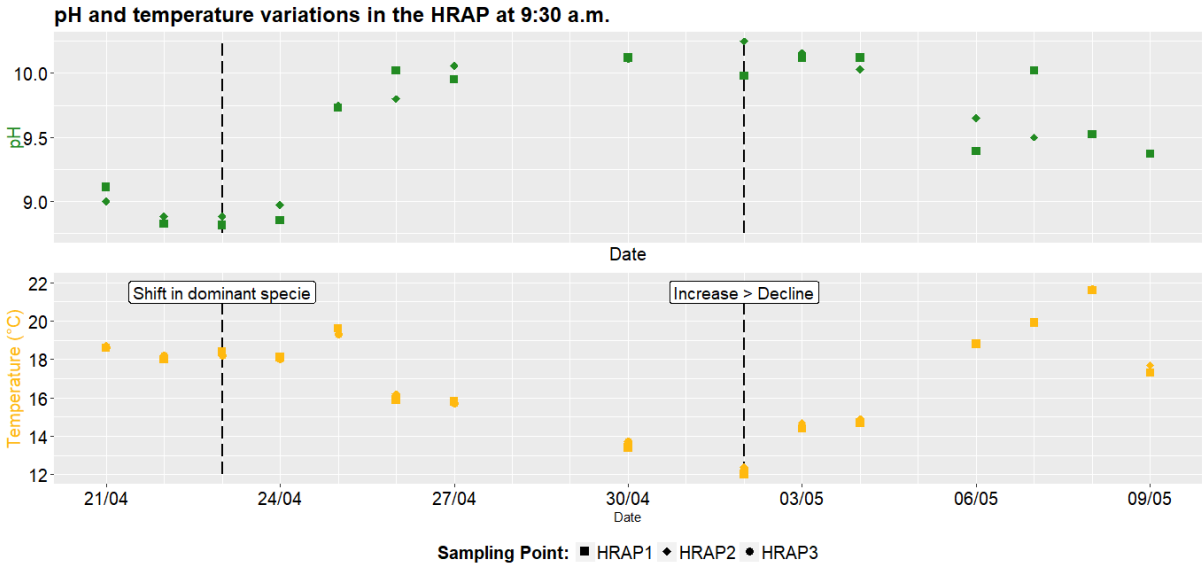

Figure 8. *Evolution of pH and temperature (°C), measured at 9:30 a.m., as a function of time in the different HRAP. The figure was divided into three sections with dash-lines representing the algal community evolution tendencies observed during the running period (see figure 7).*

III.2.b. Bioremediation efficiency

During the experimental period, the effluent Si:N:P molar ratio was on average 1:27:1. The evolution of removal efficiencies (RE) for NO3-N, NH4-N and PO4-P as a function of time in the HRAP are shown in figure 9.

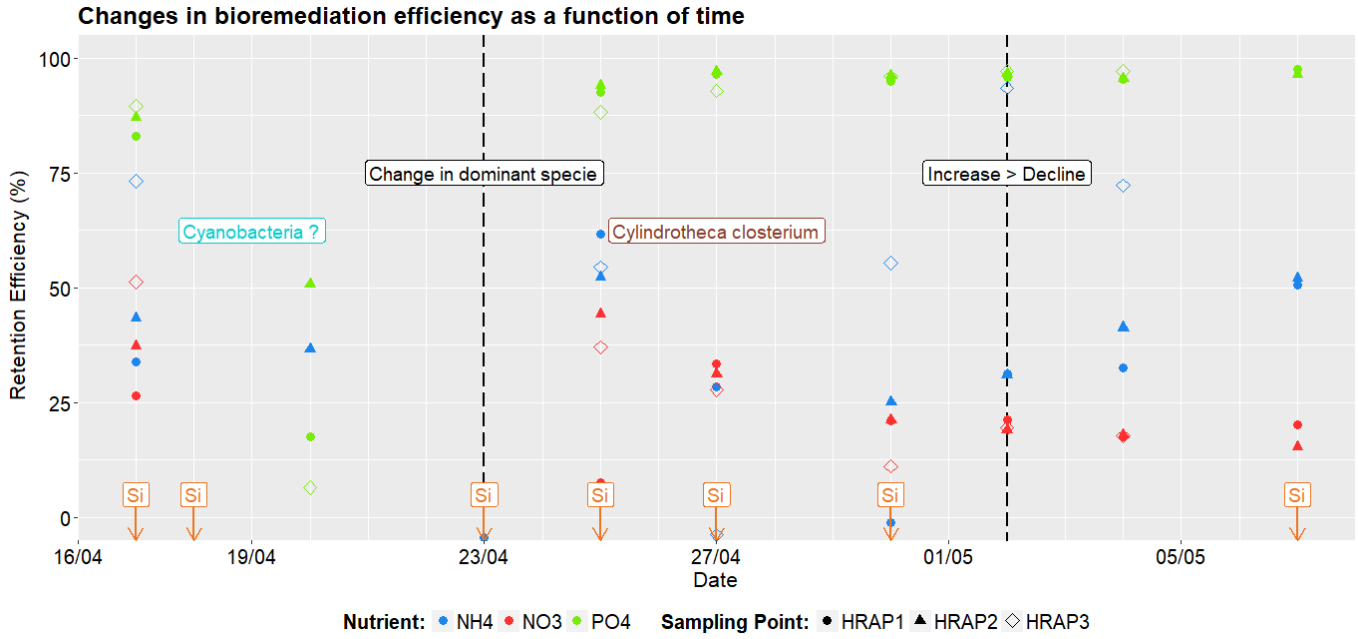

Figure 9. *Evolution of removal efficiencies, RE (%), for three nutrients, in the HRAP, as a function of time: NH4-N (NH4), NO3-N (NO3) and PO4-P (PO4). "Si" labels represent the dates when silicate was added in the algal raceways. The figure is divided into three sections with dash-lines representing the algal community evolution tendencies observed during the running period (see figure 7). Dominant species in the community are shown. In this figure, only values representing nutrient removals are indicated (i.e. no negative values = nutrient production).*

Phosphates were the highest removed nutrient with a RE of 95.3±2.3% of the input concentration being consumed by the algal consortium after the shift in dominant species (23/04). From the 27/04, PO4-P RE reached a plateau in all 3 HRAP and, concomitantly, retention efficiencies started to decrease for nitrate and ammonium.
After the 23/04, RE was globally higher for ammonium than for nitrate. However, it was highly erratic ranging from 25.2 to 93.5%. Conversely, NO3-N RE was less variable with an average of 22.5±9.4% from 23/05 to 07/05. In the HRAP1 and HRAP2 (in full points in figure 8), for the three nutrients, RE decreased until the 23/04, where they were negative or close to zero, and increased quickly after the beginning of *C.closterium* dominance. The sharpest increase was observed for phosphate and then for ammonium. The 27/04, when PO4-P reached its highest value, RE started to decrease for nitrate and ammonium. The decrease in NO3-N RE is then observed until the collapse of the algal community, while NH4-N RE increased after the 02/05 when community began to decline. As for Chla concentration (figure 7), HRAP3 was a particular case where NH4-N RE dropped to 0% as soon as the 27/04 and then immediately increased to reach 93.5% the 02/05. From this date, N-NH4 RE remained high until the collapse of the culture.

During the whole period, nitrite concentrations (figure 10) were always higher in the HRAP (0.041±0.015mg.L^{-1}) than in the fishpond effluents (0.014±0.001mg.L^{-1}). They were low in comparison with nitrate (5.685±1.809mg.L^{-1}), ammonium (0.816±0.370mg.L^{-1}) and phosphates (0.546±0.194mg.L^{-1}) concentrations in the effluent. RE was also calculated for N-NO2 but was always negative corresponding to a production rate of nitrite in the HRAP. Spearman test showed that this NO2 production was significantly positively correlated with N-NH4 RE in the HRAP during the whole period (*, ρ = 0.39). Indeed, in the HRAP 1 and 2, the maximum productions rates of NO2 were observed when NH4-N RE increases and particularly after the consortium collapsing where maximum production value of 0.0605 and 0.0585 mg. L^{-1} NO_2 were noticed. This was also the case for HRAP3 with a NO2 production peak measured the 02/05 when NH4-N RE reached is maximum value.

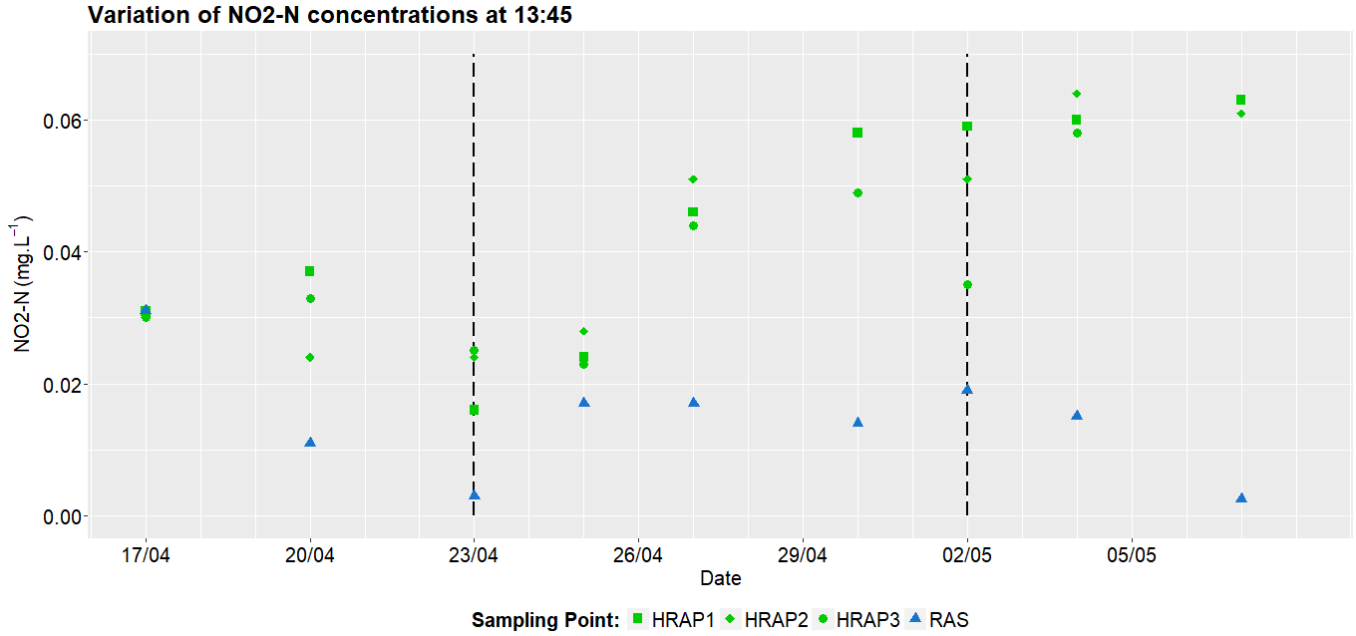

Figure 10. *Evolution of NO2-N concentrations (mgN.L-1) as a function of time in the different HRAP (green) and in the RAS effluent (blue). The figure is divided into three sections with dash-lines representing the algal community evolution tendencies observed during the running period (see figure 7).*

III.2.c. Oyster production

The mean of oyster juvenile total fresh weight increases from 1.55±0.33g.ind^{-1} at the beginning of the experiment to 3.17±0.71g.ind^{-1} at the end of the running period. This accounted for a doubling of the weight with a net mass gain of 104.8±4.3%.ind^{-1}. Thus, 1.26kg total fresh weight of juvenile were produced during the experimental period in the triplicate. In parallel, net length gain was 36.88±1.79%.ind^{-1}. Wilcoxon-Mann-Whitney tests showed that both length (***) and weight (***) juvenile growths were significant. Conversely, for adult *C.gigas* no significative growth was observed for weight nor length during this period, as demonstrated by Wilcoxon-Mann-Whitney tests. This was concomitant with mortalities in the adult production unit with 0%, 3% and 27% in the three ponds respectively. Moribund oysters were systematically collected and analysed by specialised laboratory with both histology and molecular methods (Histalim©, LDV34©). Results showed an infection by the oyster pathogen *Vibrio aesturianus*.

Food rations are presented in figure 11, expressed per oyster total fresh weight unit (%DWalgae.TWoyster^{-1}.d^{-1}). For juveniles, food rations at the end of the experiments (final total fresh weight as reference) were about 2-fold lower than at the beginning (initial total fresh weight as a reference) of the experiments. This is in concordance with weight growth as described before. However, food rations were always superior for juveniles than for adults (average weight as reference because of the absence of growth) during the whole experimental period. The mean food rations provided to the adult oysters during the experimental period were also expressed per oyster total dry weight unit. During this period, average food ration was 6.6±2.25%DWalgae.DWoyster^{-1}.d^{-1}. A maximum was reached from 25/04 to 09/05/2018 with food rations always superior to 6%DW.d^{-1}.

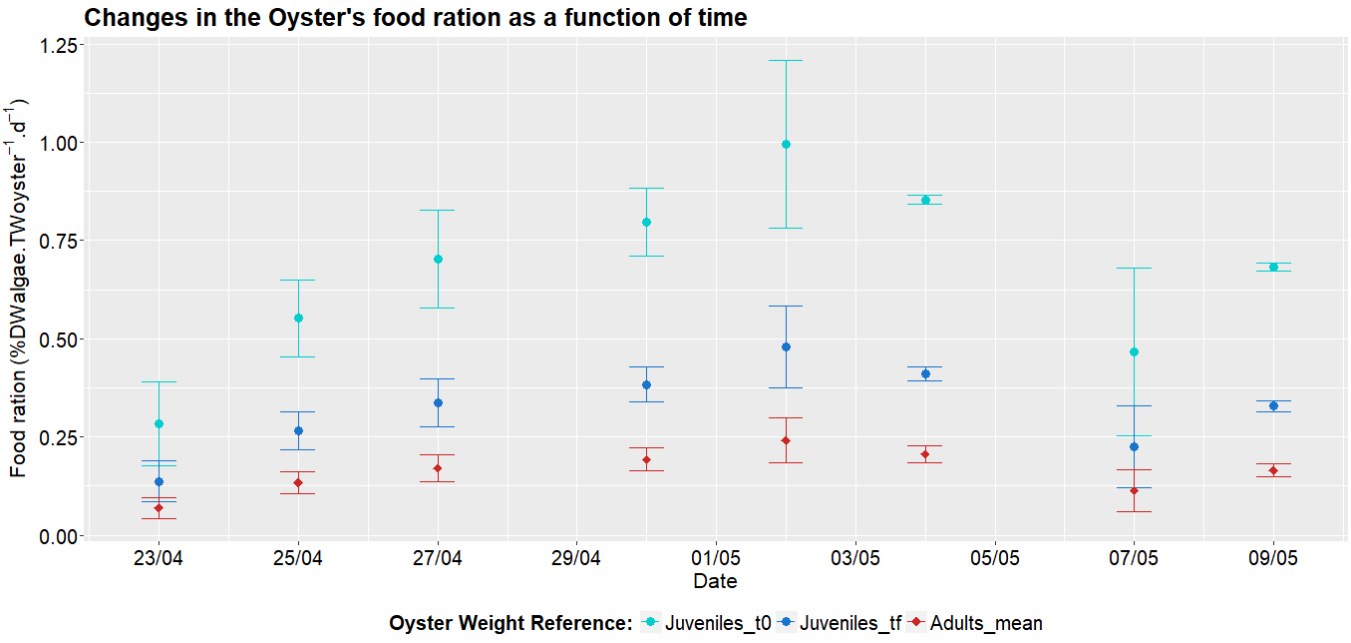

Figure 11. *Changes in the mean oyster's food ration, expressed in %DWalgae.TWoyster^{-1}.d^{-1}, in the oyster production unit, as a function of time. Mean and standard deviations were shown. Juvenile food rations were represented with round-shape points: light blue and deep blue points were respectively calculated using initial (t0) or final (tf) juvenile total fresh weights as a reference. Adults food rations were plotted in red diamond-shape points. They were calculated using the mean of t0 and tf adult total fresh weights as a reference (similar).*

IV. Discussion

IV.1. Phase 1: laboratory experiments

The aim of this experimental phase was to select interesting microalgae species for cultivation in the IMTA system, in term of productivity, bioremediation efficiency and ability for feed both adult and juvenile Pacific oysters. This selection process was performed using two different algal diversity reservoirs: natural Mediterranean SW v/s HRAP with high nutrient and CO_2 enrichment (Vasco2). This in order to select the best inoculum source for the IMTA-system as it was previously recommended by some authors (Borges et al., 2005; Hussenot et al., 2003). Laboratory selection process was performed in physicochemical conditions close to that observed in April in the IMTA-system (20±5°C) with f/2 modified media chosen for their similarity in main nutrient (N and P) composition with the RAS effluents. This similarity was confirmed since we determined a mean N:P ratio of 27±5 in the effluents from 17/04 to 05/07/2018 which was close to 24, that of f/2 medium. Consequently, those media were characterised by a P limitation (Redfield et al., 1963). In these conditions three consortia emerged and two single strains, commonly used in aquaculture, grew:

- Diatom-dominant C1 mainly constituted of *Amphora sp.* (Vasco2)
- *Chlorophyceae*-dominant C2 (SW) and C3 (Vasco2)
-Single strains, Pp: *Porphyridium purpureum* and Ts: *Tetraselmis suecica*

Suitability of these selected algae for productivity and bioremediation efficiency in the IMTA-system are discussed according to the determination of their growth performances in f/2 modified media and under realistic physical conditions (20±5°C, light variations characteristics of April in the HRAP: maximum of 1400µmol photons.m^{-2}.s^{-1}, annex II) simulated in laboratory. In this context, the importance of micronutrients (trace metals, vitamins) is discussed by comparison between **poor**, **effluent** and **complete** f/2 media which were almost identical in macronutrient (N/P ± Si) composition.
Finally, C1 and C3 were tested for their ability to be selected and ingested by juvenile and adult *C.gigas*.

IV.1.a. Microalgae selection process

The different abilities of the single strains, from culture collection, to grow in conditions close to the physicochemical characteristics of the IMTA-system pointed out the importance to select species adapted to the local climatic conditions and, above all, to the effluent features. Thus, in our selection experiments, only Ts and Pp were able to survive and flourish in the **poor** f/2 medium used during the selection process of the strains originating from the culture collection (N/P ± Si, table 1, **complete** medium was only used for the consortia selection and not for the single strains). In our culture conditions, strains that have already been cultivated successfully in fishpond effluent, in IMTA, such as *I.galbana*, *C.calcitrans* or *S.marinoi*, were not able to growth (Goldman et al., 1974; Lefebvre et al., 1996, 2000, 2004). This is likely due to the different strategies in nutrient uptake, carbon and energy allocation and thus the distinct nutrient requirements that coexists in the algal diversity (Halsey & Jones, 2015 and references therein). For instance, it was previously demonstrated that *I.galbana* cans not be cultivated in effluents were *Tetraselmis spp.* were very productive (Borges et al., 2005). N:P ratios could have played a major role. Thus, similarly to fishpond effluents, the f/2 medium was characterised by a high N:P ratio where only Pp and Tp could have been able to cope with such P-restricted condition. For example, it was demonstrated that maximum performances of *Skeletonema costatum* (Greville; Cleve, 1873) and *C.calcitrans* where observed at much lower

N:P ratio, 9.5:1 and 12:1 (in a f/2 culture medium) respectively (Lefebvre et al., 1996; Krichnavaruk et al., 2005). Similarly, in 1979, in outdoor culture, Harrison & Davis showed that *S.costatum* and *Chaetoceros spp.* flourished in algal communities only for low N:P ratios, under N-depleted conditions (<16), which was clearly not the case here. The importance of effluent characteristics as yet been demonstrated in IMTA-system by favouriting differentially species dominance with distinct N:P ratio applied (Lefebvre et al., 1996). Hence, algal consortia that emerged at the end of the selection period were adapted to this type of nutrient substrate, with high N:P ratio, corresponding to the IMTA-system fishpond effluents.

Our study also highlighted the importance of micronutrients (i.e. trace metals and vitamins) in the presence and initiation of microalgal growth and dominance as it has already been described in natural environments (Croft et al., 2005, 2006; Bertrand et al., 2007). Indeed, using the SW diversity reservoir, an algal consortium, C2, only emerged in the **complete** f/2 medium whereas no microalgae were able to grow in the **poor** medium. We used **poor** f/2 medium for the algal selection because we hypothesised that vitamins and trace metal presence in the fishpond effluents must be anecdotic. In this case, it would have been relevant to perform selection in the most drastic conditions (i.e. without micronutrients). Yet, this is probably not the case and the **poor** f/2 medium could have been not representative of the fishpond effluents in term of micronutrient composition, as discussed below. Consequently, this represents a limit of the study since single strains could have possibly grown if the selection was performed using less-limited oligonutrients f/2 medium (such as the **effluent** f/2 medium, as discussed below). This was previously observed for some algal strains with no growth in **poor** f/2 culture medium with absence of trace metals and/or vitamins (Grossart, 1999). For the Vasco2 experiments, similar consortia in term of diversity (C1 and C3) were observed in the **poor** and **complete** f/2 media suggesting that they were more resilient to micronutrient deprivations. For the growth and ingestion experiments, C1 and C3 consortia selected in the most drastic conditions, the **poor** f/2 medium, were retained instead of those selected in the **complete** (Table 1).

As it is well-known and well-documented, the inclusion of silicate in the culture medium caused a major change in the consortium species composition for the benefit of diatoms (Hussenot & Gautier, 1994; Lefebvre et al., 1996, 2000, 2004). Thus, Vasco2 sample inoculated in a **poor** f/2 medium conducted to the emergence of *Chlorophyceae*-dominant consortium, C3, whereas with the supplementation of silicate in the medium, a diatom-dominant consortium, C1, appeared with *Amphora sp*. as the main species.

Selection process was also conditioned by the environmental tolerance range since optimum for growth of the different selected species are related with environmental parameters, mainly with temperature and irradiance (Qi-Hua et al., 1997, 1998; De La Peña et al., 2007). In the laboratory conditions, Ts and Pp were in their range of environmental tolerance, in particular for temperature with their growth optima demonstrated to be at 19-21°C and 21-26°C respectively (Golueke & Oswald, 1961; Fabregas et al., 1984; Cohen et al., 1988; Abu-Rezq et al., 1999). The consortia were selected from SW and Vasco2 samples collected in winter and not in April. Consequently, this could explain the little number of algae selected, particularly in SW with only one emerging consortium (*Chlorophyceae*-dominant C2), since they were suddenly transferred from winter to April physical conditions. Thus, the selected species must present a high environmental tolerance which could represent an advantage in outdoor highly variable conditions such as in the IMTA-system.

Sampling in December represent a limit of the experimental procedure since abundance and phytoplankton diversity are generally the lowest at this time of the year in Mediterranean north-western lagoon (Bec et al., 2005). Consequently, SW was probably a poor reservoir of diversity in December, which would have been different in April. This could explain why no diatoms emerged for SW with the inclusion of silicate in the culture media since diatoms are generally not well represented in the algal diversity in this period (Del Amo et al.,

1999; Bec et al., 2005). This not significates that SW cans not be a suitable inoculum source for IMTA-systems since it is successfully used in many IMTA-systems to sustain production of interesting diatoms (Shpigel et al., 1993; Lefebvre et al., 1996, 2000, 2004). Indeed, Li et al. (2018) were previously able to select the diatom *Phaeodactylum tricornutum* (Bohlin, 1898) in the IMTA-system from an inoculum of spring Mediterranean SW. This means that SW diversity reservoir his highly variable and follow the variation of natural communities throughout the year. Consequently, whereas in unfavourable months (i.e. autumn and winter) microalgal diversity falls in natural SW (Del Amo et al., 1999; Bec et al., 2005), diversity is much more stable in highly nutrient enriched HRAP, Vasco2, that stays suitable for IMTA-system inoculation. Thus, if we collected our samples in April, results of the selection process in laboratory experiments would have been different. Species composition is probably also different in the Vasco2 HRAP between April and December. Anyhow, IMTA-system requires a suitable inoculum source, able to favour the dominance of interesting species, throughout the year. Only Vasco2 HRAP algal diversity reservoir was suitable to achieve this.

IV.1.b. Growth performances and implications for IMTA

• Global growth performances:

We determined and compared the growth rates of the different selected algal strains and consortia in several f/2 culture media, representatives of the RAS effluents N:P molar ratio but with different micronutrient compositions (i.e. trace metals and vitamins). It was noticed that the different tested factors, kind of strain/consortia and culture medium (i.e. quantity of micronutrients) but also their interaction, significantly affected algal growth rates.

The observed differences in growth rates between strains/consortia are attributable to the distinct algal growth and ecological strategies that coexist in the environment with different stratagems of carbon and energy allocation (see Halsey & Jones, 2015 and references therein). Thus, growth rates are distinct according to the taxa and even sometimes inside the genus (Griffiths & Harrison, 2009). Moreover, for the consortia, even if the algal species were in their range of tolerance for the applied physiochemical conditions, they were not necessarily all in their optimal range of environmental conditions where maximal growth rates occur, as discussed before. These environmental preferences are widely species-specific likely accounting for the differences between strains/consortia (Qi-Hua et al., 1997, 1998; Abu-Rezq et al., 1999; De La Peña, 2007). Thus, it was interesting for us to determine the selected species that sustained the best performances in the physicochemical conditions of the IMTA-system. As a comparison, growth rates recorded in the literature in laboratory experiments in f/2 and associated culture media are presented in table 4 for axenic (i.e. no bacteria nor predators) Ts, axenic Pp and some axenic species of *Amphora spp.*, with the culture conditions associated. Comparisons have to be considered carefully since physicochemical parameters, especially temperature and light conditions (photoperiod and irradiance), and cultivation systems were various between studies which affected growth rates differently. Thus, differences in growth rates between our study and that of other authors are probably mainly attributable to the differences in temperature and irradiance since they were variable in our experiments whereas they were kept constants in the vast majority of other studies (table 4). Indeed, temperature and light are the primary parameters controlling microalgal growth rates with a major influence in outdoor cultivations (Andersen, 2005; Milhazes-Cuhna & Otero, 2017; Moreno-Garcia et al., 2017). Even if the effects of photoperiod on algal growth are globally demonstrated, effects of light and daily temperature variations are not well-studied (Csavina et al., 2011 and references therein).
Anyway, these variable conditions are more realistic in the context of outdoor cultivation in our IMTA-system and consortia/strains were globally able to sustain good growth performances. Indeed, globally, whatever the cultivation medium, the strains/consortia showed good growth performances with 83% growth rates being higher to 0.5 divisions per day and even some

exceeding 1.d^{-1}. This was close to the average growth rate of marine species which is around 0.88d^{-1} (Griffiths & Harrison, 2009).

Table 4. *Compilation of some growth rates data for T.suecica, Amphora sp. and P.pururem, and the associated physicochemical growth conditions, found in the literature. f/2 is the basic medium described in Guillard (1975) whereas f is the same with doubled nutrient concentrations as described in Guillard & Ryther (1962). Boosted f/2 is a strongly modified version described in Carballo-Cárdenas et al. (2003): among other things, N is 18-fold more concentrated, P concentration is 28-fold higher and this is also the case for micronutrients (e.g. 2-fold more Fe). Moreover, 5mM of NaH_2CO_3 is added as C-source. Compositions of optimised f/2 media were all optimised by means of statistical methods (e.g. response surface methodology): (1) optimised concentrations of Na_2SiO_3, $NaNO_3$ and vitamin B_1 (2) optimised N-source: 2337µM ammonium instead of nitrate (3) optimised N:P ratio: 50 instead of 22 in the basic f/2 medium. For each parameter, standard errors are indicated when they were provided in the publications.*

Species	Culture medium	Irradiance (µmol photons.m^{-2}.s^{-1})	Photoperiod (day:night h)	Temperature (°C)	Bubbling	Growth rate (d^{-1})	Reference
T.suecica	f/2	150	12:12	15	no	0.96±0.07	Ribalet et al., 2007
T.suecica	boosted f/2	50-70	16:08	22	no	1.2	Carballo-Cárdenas et al., 2003
Amphora subtropica	f/2	60	21:03	34	yes	0.5	BenMoussa-Dahmen et al., 2016
Amphora sp.	optimised f/2 (1)	80	?	30	no	0.81	Chtourou et al., 2015
Amphora sp.	f	50 (PPFD)	24:01	20±1	no	0.61±0.02	Romero-Romero & Sánchez-Saavedra, 2016
Amphora sp.	optimised f/2 (2)	1400 lux	?	25	no	1.5	Hutagalung et al., 2014
P.purpureum	optimised f/2 (3)	98	18:06	23±1	no	0.19	Razaghi et al., 2013
P.purpureum	f/2	150	18:06	19-21	yes	0.26-0.27	Fuentes-Grünewald et al., 2015
P.purpureum	f	3500 lux	12:12	20±2	no	0.52	Aizdaicher et al., 2014

In the complete f/2 medium, Ts growth rate, 1.0±0.0 d^{-1}, agreed with those observed by some authors in the same medium at 15 and 22°C (table 4; Carballo-Cárdenas et al., 2003; Ribalet et al., 2007). It was also similar to those observed by Borges et al. (2005), 1.01-1.14 d^{-1} for the same species in seabass fishpond effluents with N:P of 3:1 at 19±1°C. In our **effluent** f/2 medium (f/2 medium without supplementation of nutrients, table 1), growth rate was 0.7±0.1 d-1. This was in the same range than growth rates observed by Borges et al. (2005) for Ts in a turbot effluent: 0.65 and 0.75 d^{-1} with N:P ratios of 16:1 and 14:1 respectively. It is important to note that at such growth rates in the turbot effluents, they observed a high removal efficiency close to 90% for NH4-N, from 41 to 91% for NO3-N and from 52 to 63% for PO4-P. These results were considered as adapted for incorporation in an IMTA-system since ammonium was almost totally removed and it is the main toxic product released in fishpond effluents (Neori et al., 2004 and references therein; Borges et al., 2005). Thus, it is possible to suggest that, in our effluent conditions, with Ts, remediation efficiency could be high and suitable for the IMTA-system. By comparison with Borges et al. (2005), this also showed that Ts is adapted to perform high productivity and remediation efficiency in a wide range of N:P ratios from 3 to as high as 24 which is clearly suitable for incorporation in our IMTA-system with variable and high

N:P ratios: 27±5. Moreover, in a reference medium, with a close composition to f/2 and N:P ratio of 20, Borges et al. (2005) even recorded growth rates as high as 1.16-1.21 d^{-1} and remediation efficiencies greater than 90% for both NO3 and NH4.

In a complete revue, Griffiths & Harrison (2009) showed that *P.purpureum* generally exhibited little growth rates in cultivation experiments with an average of about 0.4 d^{-1}. Unusually, Pp realised strong growth performances in our experiments. Thus, Pp growth rates were superior in our **complete**, 0.8±0.1 d^{-1}, and **effluent**, 0.6±0.1 d^{-1}, f/2 media than in previous studies conducted in **complete** f/2 and in N:P 50 optimised f/2 medium (table 1). More surprisingly, a considerable growth rate, 2.0±0.1 d^{-1} was measured in the **poor** f/2 medium, without micronutrients, which represented the highest growth recorded in our experiments for all the strains/consortia. It was even superior to the maximum growth rate of 0.52 d^{-1} found in the literature with a f medium at 20°C. On the basis of literature, there are no explanations for this phenomenon. For instance, this was totally in disagreement with Croft et al. (2005) who demonstrated that *P.purpureum* was unable to growth in minimal culture medium without the supplementation of at least 10ng.L^{-1} vitamin B_{12}. The only logical explanation to this phenomenon was that our Pp strain was not dependant of vitamin B_{12} to growth (i.e. possessed the B_{12}-independent form of methionine synthase) has never been mentioned in the literature. As discussed before, our study was characterised by strong selection conditions. This drastic selection could have induced the expression of genes favouriting growth under micronutrients deprivation. Thus, the importance of acclimatisation process to new growth conditions has been yet demonstrated (Borges et al., 2005). Further investigations must be conducted in our Pp strain in order to clarify this issue since it seems unique according to its performances after strong selections under micronutrient deprivation.

In our experiments, growth performances of the *Amphora sp.*-dominant consortium were inferior to the average of 0.8 generally measured in laboratory experiments for *Amphora spp.*, according to Griffiths & Harrison (2009), in the **effluent** medium, 0.6±0.1 d^{-1}, but not in the **complete** f/2 medium, 1.2±0.1 d^{-1}. However, C1 growth rate in the **effluent** medium was in the same range than growth rates measured for single *Amphora spp.* reared in f or f/2 medium for biovalorisation or invertebrate feed purposes (Chtourou et al., 2015; BenMoussa-Dahmen et al., 2016; Romero-Romero & Sánchez-Saavedra, 2016; Table 4). Thus, this consortium could have been able to sustain high productivity and remediation efficiencies in the IMTA-system.
Globally, C3 also sustained high growth performances, particularly in the effluent f/2 medium, 0.9±0.0 d^{-1}, with a growth rate above the average for marine microalgae cultivated in good growth conditions (Griffiths & Harrison, 2009). This underline the excellent ability of this consortium to be cultivated in fishpond effluents. Since this consortium possessed the highest growth rate in this medium (significantly higher than Ts), it could be the most suitable strain/consortium to inoculate in the IMTA-system for bioremediation purpose.
Lastly, the C2 consortium growth rates were very low when compared with the others strain/consortia (0.6±0.1 d^{-1} in the **complete** and 0.4±0.0 d^{-1} in the **effluent** f/2 medium). As suggested before, it was probably not in his optimum of temperature and light conditions. In addition, C2 was the only strain/consortium selected on a complete f/2 medium. Consequently, it could have higher micronutrient requirements than the other tested microalgae which could explain his poor performances in each culture medium. Consequently, this consortium was the less adapted for cultivation in the IMTA-system.

• Role of micronutrients:

Growth experiments also showed that micronutrients (i.e. trace metals and vitamins) controls growth rates in culture media with close or similar N:P (±Si) ratios and composition. Thus, for the vast majority of consortia, growth rates were statistically greater in the **complete** than in the **effluent** medium. Since the main difference between these two media was the inclusion or not of oligonutrients, changes in growth rates can be mainly attributable to vitamin

and trace metal availabilities. This important effect of micronutrients was already evocated, for instance in a f/2 medium (Grossart, 1999), but not in the context of IMTA despite the control that they could have on remediation efficiency and productivity through growth rates. Even if the effects of trace metals and vitamins are not distinguishable in our study, the major role of these two kinds of compounds in biological activities and control of productivity in natural ecosystems is well-known.

For instance, iron, cobalt, zinc and copper are trace metals obligatory required by microalgae for growth, notably because of their role in metalloenzymes functioning. For example, phytoplankton development is limited for free Zn concentrations inferior to $10^{-11.5}$M (Price & Morel, 1990). Iron is a major oligo-nutrient due to his role in photosynthetic pigment biosynthesis (Pushnik et al., 1984). His availability notably controls the primary productivity in the major part of the vast Southern Ocean, playing an important role in biogeochemical cycles (Bertrand et al., 2007). For its part, Co is important in microalgae metabolism for its use, among other things, in the constitution of cobalamin, the B_{12} vitamin (Price & Morel, 1990; Croft et al., 2005, 2006). Essential trace metals were almost all provided in the **complete** f/2 medium whereas they were not added in the **poor** and **effluent** f/2 media (table 1). Thus, these trace metals availability has likely a direct positive effect on algal growth rates in our experiments with **complete** medium being much less-limited than the **effluent**. This assumption is in accordance with previous studies (Erickson et al., 1970; French & Evans, 1988; Chen et al., 2011).

Other essential nutrients for microalgae growth are the thiamine (B_1), biotin (B_7) and cobalamin (B_{12}) vitamins. In fact, they are often indispensable in algal metabolism as enzyme co-factors (Carlucci & Silbernagel, 1969; Croft et al., 2006 and references therein; Bertrand et al., 2007; Kazamia et al., 2012). Thus, B_7 cans be essential in the fatty acid synthesis and B_1 acts frequently as cofactor in carbohydrates and amino acids synthesis. Finally, B_{12} often plays a major role as cofactor for methylmelonyl-coA mutase, involved in the synthesis of methionine. Even if they are typically autotrophs, microalgae are often unable to synthesise these vitamins and need external input from the surrounding environment. Thus, according to Croft et al. (2005) more than half of micro-algal species obligatory require B_{12} to growth. In another study conducted on 306 species of micro and macroalgae, they demonstrated that 22% obligatory required B_1 to growth whereas 5% needed B_7. Those requirements were so important that Bertrand et al. (2007) demonstrated that phytoplankton primary production was partly limited by B_{12} in the Ross Sea. Thus, it is very likely that growth rates in the **complete** medium where stimulated by the inclusion of essential vitamins. However, this vitamin requirement is widely species-specific and is not related to taxonomy with differences often occurring in the same genus (Croft et al., 2005; 2006). Thus, Borowitzka (1999) demonstrated that absence of vitamins did not affect growth rate of Ts whereas it interrupted the growth of other species such as the diatom *C.calcitrans*. On contrary, B_{12} obligatory requirement was demonstrated for Pp with no growth in its absence (Croft et al., 2005). Thus, better growth in the **complete** medium could be partly explained by the supplementation of vitamins for Pp whereas it was probably only related to trace metals for Ts. Anyway, the role of vitamin in growth rate control must be considered, notably for consortia, since the majority of microalgae obligatory require vitamins to grow.

In our experiments we observed that growth rate was significantly lower in the **poor** than in the **effluent** medium for the C1-consortium. Growths experiments were also performed in the **poor** medium for the other strains/consortia but they were not presented and not included in the statistical analysis since they were not directly comparable with those performed in **complete** and **effluent** experiments. Indeed, acclimatisation to the physicochemical conditions was not performed or dilution rate was lower (dilution rate = 4) when they were inoculated in **poor** medium in the microplate. These two factors were able to negatively affects growth rate by photoinhibition in the case of non-acclimatisation or by self-shading and increasing in competition for nutrient access in case of lower dilution (Erickson et

al., 2015). Nonetheless, cell densities were really low at the beginning of the experiments and self-shading and competition had probably not a strong impact. Whatever the case, recorded growth rates were lower than in the effluent medium for non-acclimatised Ts, with 0.5±0.1 d^{-1}, or even negative and undeterminable for C3 diluted 4 times instead of 5. Irrespective of the surprising case of Pp, these pointed out the fact that **effluent** medium was probably richer in micronutrients than **poor** medium. Indeed, trace metals (i.e. Zn, Mn, Fe, Cu) were present as additives in the fish food *Neo Grower Extra Marin* (Le Gouessant®) used in the IMTA-system. They could be concentrated in the fishpond seawater, that constituted **effluent** f/2 medium, by the dissolution of both fish faeces and unconsumed food (Hossain & Jauncey, 1989; Reid et al., 2009; Burridge et al., 2010). Vitamins were not present but essential amino acids, as methionine, were directly incorporated in the fish food. The limitation of growth by B_{12} is mainly related to perturbation of methionine synthesis (Croft et al., 2005, 2006; Bertrand et al., 2007). Thus, microalgae that obligatory required B_{12} to growth (e.g. potentially C3 with no growth in **poor** medium) could have avoided this limitation by the direct uptake of methionine present in the surrounding environment as it was already observed before (Croft et al., 2005). Contrary, deprivation of trace metals is frequent in north-western Mediterranean SW that constituted the **poor** f/2 medium, even with the influence of the Rhône river (Sayed et al., 1992), and vitamin are in anecdotic concentrations in natural SW (Provasoli & Carlucci, 1974).
This importance of micronutrients could explain why extremely low cell densities where observed in our cultures for strains/consortia selected with poor medium (i.e. too low to be counted on haemocytometer) whereas high densities are generally observed in classic f/2 medium (Carballo-Cárdenas et al., 2003; Ribalet et al., 2007; Razaghi et al., 2013; Chtourou et al., 2015; BenMoussa-Dahmen et al., 2016). Thus, in absence of micronutrients, selected strains/consortia were potentially only able to maintain itself and not to grow efficiently. Their maintenance in micronutrient deprivation (**poor** f/2 medium) could have been possible by recycling of dead cells by bacteria which replete the nutrient pool (e.g. micronutrients) since our cultures were not axenic (Jürgens & Güde, 1990; Goldman & Denett, 1991; Croft et al., 2005, 2006).
These experiments showed that micronutrient availability, originating from the dissolution of unconsumed food and fish faeces, likely played an important role in remediation efficiency, productivity and possibly also in species selection in the microalgal unit of IMTA-systems. This not significates that oligonutrients must be manually added in the HRAP like it is the case for silicate. Indeed, these compounds are very expensive, and this would be not economically relevant. However, it seems important to determine the micronutrient cycling in the IMTA-system, from the fishpond effluent, to the treatment in the bacteria biofilter until the consumption in the HRAP. We could not measure the concentrations of vitamins and trace metals which constituted a limit of our study and require further experiments. Yet, bacteria of the biofilter were probably able to chelate trace metals and consume organic compounds, such as methionine, which probably reduced the availability in the RAS effluents (Hutchins et al., 1999, Michaud et al., 2006). Thus, in our case, **effluent** f/2 medium was constituted with water sampled directly in the fishponds and not in the RAS effluents after the treatment of the fishpond wastewaters in the bacteria biofilter. Hence, the benefits of this concentration of micronutrients in the fishponds could be diminished after passing in bacteria biofilter in the IMTA-system. Thus, it would have been interesting to compare growth rate in different media prepared with samples collected before and after passing in the bacteria biofilter. This type of experiments was conducted by Borges et al. (2005) and demonstrated that productivity of *P.tricornutum* was better in effluents without previous anaerobic treatment.

Chlorophyceae-dominant C3 was a particular case for which the growth rate was 1.4-fold higher in the effluent than in the complete f/2 medium (figure 1). It was shown that growth in f/2 medium is sometimes C limited since, in absence of bubbling, C provisioning is only ensured by CO_2 dissolution by means of gaseous exchanges with the atmosphere (Guillard, 1975; Rocha et al., 2003). In this case, some studies demonstrated that heterotrophy cans represent an alternative source of C carbon in case of inorganic C-source limitation. Organic substances that can be directly consumed by microalgae, such as carbohydrates and amino

acids, are frequently released by unconsumed food and fish excretions in aquaculture effluents (Reid et al., 2009). Thus, this could have benefited tor C3 to sustain a growth rate that could be lower based only on atmospheric CO_2 dissolution. For example, Cerón-García noticed that growth rate of the benthic diatom *P.tricornutum* was enhanced by 148%, compared to photoautotrophy, with the addition of glucose in the culture medium of García Sánchez. In a lesser extent, the presence of organic compounds in the fishpond effluents could have also benefited to other strains consortia since it was demonstrated that Pp, Ts and *Amphora spp.* are able to cope with mixotrophy (i.e. concomitant use of autotrophy and heterotrophy; Antia et al., 1975; Turner, 1979; Admiraal et al., 1987; Bohlin, 1998). Moreover, amino acids can also act as N source which could be also profitable (Turner, 1979). In our study, concentrations of organic compounds in the effluent were not measured. However, if their effect on growth rates was real, this aspect must be studied since, as well as vitamins and trace metals, these substances are probably consumed in the RAS biofilter by heterotrophic bacteria. Furthermore, it could be interesting to further investigate on mixotrophy of microalgae in IMTA-system because this could partially allow to compensate C-limitation that frequently happens in IMTA-system causing the decline of algal cultures (Goldman et al., 1974).

IV.1.b. Ingestion experiments

The final step of the laboratory experiments was the evaluation of the ability of these algae to be selected and ingested by both 8-months juvenile (20±2mm.ind^{-1}) and 20-months adult *C.gigas* (71±6mm.ind^{-1}). It was technically impossible to test all the selected strains/consortia so only 2 consortia were retained for the ingestion experiments: C1 & C3. *Chlorophyceae*-dominated C3 was chosen according to its high growth performances in the **effluent** f/2 medium and, consequently, its probable capacities to ensure high remediation efficiencies and stable food supplies for oysters in the IMTA-system. Even if its growth performances were respectable, we selected *Amphora sp.*-dominated C1 because it is the only selected strain/consortia constituted of diatoms. Indeed, literature widely considers that diatoms are the preferential diets of oysters, including *C.gigas*, in natural and aquaculture environments and possess generally the highest nutritional value for their alimentation (Shpigel et al., 1993; Brown et al., 1997; Soletchnik et al., 2000; Arapov et al., 2010; Kheder et al., 2010; Anjos et al., 2017). Consequently, they are the most likely suitable to sustain the highest *C.gigas* growth performances in the IMTA-system and it is important to test C1 ability to be ingested.

Consumption of C1 was noticed for both juvenile and adult oysters (figures 3 and 4). This was consistent with literature since *Amphora sp.* size, body shape and nutritional value must be well-adapted for a selection by both juvenile and adult oysters. Indeed, the particle size is a fundamental aspect conditioning the ingestion of microalgae by oysters. For instance, Adult *C.gigas* prefers algae with an equivalent spherical diameter of 7-15 µm with a retention efficiency on their gill of 100% for particle superior to 6µm whereas it falls to 49% for 2 µm particles (Nielsen et al., 2017). Yet, even if *Amphora sp.* are globally small-sized diatoms, cell length was often around 11-13 µm and they frequently form aggregates (De La Peña, 2007). It was also theorised that microalgae body shape influences the gill retention of microalgae in *C.gigas*. This is especially the case for diatoms characterized by complex forms of the frustule which favour the retention (Bougrier et al., 1997). It was also previously evocated that oysters are able to select differentially particles according to their nutritional value (Arapov et al., 2010 and references therein). Yet, members of *Amphora* genus generally contain large amount of lipid, are particularly rich in PUFA and, among them, essential n-3 and n-6 unsaturated fatty acids (De La Peña et al., 2007; Courtois de Viçose et al., 2012). Consequently, they are particularly adapted to respond to nutritional needs of Pacific oysters. Thus, in an IMTA-system, Shpigel et al. (1993) reported that diatoms, with a large part of *Amphora spp.*, were dominant in the stomach of 4-5-months *C.gigas* accounting to 80% of the ingested biomass. Kasim & Mukai (2009) demonstrated with an electivity index that, in the Akkeshi-ko estuary,

Amphora sp. was one of the preferentially selected microalgal specie by *C.gigas* during a two-years growth period. Already in 1923, Martin noticed that the biofouling benthic diatom *Amphora spp.* cans be an important part of the *Crassostrea virginica* (Gmelin, 1791) diet in brackish ponds.
Beyond the ingestion aspect, assimilation efficiency is a major parameter that was not determined in our study. Hence, it is the parameter controlling the amount of energy disponible in the algal diet for oyster's metabolism and growth (Pouvreau et al., 2006). According to the high nutritional value of *Amphora spp.*, we can assume that this assimilation efficiency could be probably high for the C1. Hence, Brick (1970) studied the alimentary function of a close species, *C.virginica*, cultivated on raft in Hawaii and found that local *Amphora sp.* was efficiently retained on the gut and was no present in faeces. This suggesting that *Amphora sp.* was well-digested by the oysters and that the absorption of essential organic compound was probably efficient. Accordingly, *Amphora sp.*-dominated C1 constitutes, very likely, an efficient diet for mass production of juvenile and adult Pacific oysters in our IMTA system.

In accordance with literature data, suggesting that *Chlorophyceae* are of poor interest for *C.gigas* nutrition (Brown et al., 1997 and references therein), C3 consortium was not consumed by juveniles but only by adult oysters (figures 5 and 6). Even if we do not have granulometry data and consequently no class size for the C3, we microscopically observed that it was constituted of small round cells much more smaller than C1-*Amphora sp*. Consequently, cell size was inferior to 10µm and consequently probably less efficiently retained on *C.gigas* gills of juveniles. It was previously showed that *C.gigas* feeding behaviour was ontogeny dependant (Gerdes, 1983). Thus, Cannuel & Beninger (2006) determined that completely functional adult gill system for particle acquisition was only attained in 2.4cm juveniles. Hence, 8-months aged juveniles used in our experiments were, in average, smaller than 2.4cm length which indicated that they were not yet able to exploited a wide range of particle size. Consequently, while juveniles were not able to retain C3 microalgae with their not fully developed gills, adults were able to ingest them. Nevertheless, even if this consortium was ingested by adult oysters, it could probably not sustain efficient growth performances because of low assimilation efficiency. In fact, *Chlorophyceae* do not have a good nutritional value for oysters (Brown et al., 1997; Arapov et al., 2010). Hence, we can conclude that C3 was likely not adapted to respond to nutritional requirements of *C.gigas*, especially for juveniles, in our IMTA-system.

IV.2. Phase 2: Field experiments and IMTA-system functioning

The aim of this experimental phase was to assess the IMTA-system functioning in term of bioremediation efficiency and oyster production using an algal inoculum from an intensive algal culture production system (Vasco2). Indeed, laboratory experiments showed the ability of Vasco2 diversity reservoir to provide interesting algal species in term of productivity, bioremediation and nutrition of juvenile and adult Pacific oysters. The main objective was to solve the problem of oyster production, in the system, identified in a 2017 experiment (Li et al., 2018, in revision). To achieve this, our study ambition was to focus research effort on algae diversity with the hypothesis that algal species were potentially not suitable in this previous study, in term of nutritional quality, to sustain the energy requirements of *C.gigas* in the production system. Thus, we tested the ability of a new inoculum source to promote the growth of suitable algae in field experiments, in the IMTA-system, during a month operating period from April to May 2018.

IV.2.a. Performances of the algal production unit

Under the dominance of *C.closterium* (from 17/04), maximum TSS concentration of 58±1 $mg.L^{-1}$ was reached in the POOL. This is less than half of the productivity observed last year at the same period with maximum of 135.3±34.7 $mg.L^{-1}$ TSS (Li et al., 2018, in revision). Growth rates (determined from Chla concentrations) ranged between 0.2 and 0.4d^{-1} which is relatively low compared with growth rates already observed in fishpond effluents for diatom consortia (as high as 1.5 d^{-1} when measured with 680nm-OD, Lefebvre et al., 2004) and, above all, for *C.closterium* in laboratory experiments. Hence, in laboratory experiments, this strain can frequently reach 1.6 to 2d^{-1} in f/2 or close media (Staats et al., 1999; Alcoverro et al., 2000; Keerthi et al., 2012). Even if temperature and light are major factors controlling the productivity in outdoor cultivations systems (Eppley, 1972), this could not explain the poor productivities results observed during our experiments. Indeed, light conditions were relatively constant during the experimental period and *C.closterium* is an eurytherm species able to cope with the low temperatures registered during our experimental period. Thus, it cans growth under a wide range of temperature, from 2 to 30°C and sustained highest growth rates and productivities between 10 and 25°C (Ohgai et al., 1986). In our case, this is illustrated by the negative correlation between temperature and Chla concentration, with the maximum biomass observed at the lowest temperature, as discussed below. Moreover, in our study, we recorded minimal 9:30 a.m. temperature of 12°C and, last year, Li et al. (2018, in revision) recorded temperatures as low as 9.5°C. Furthermore, nutrient concentrations were globally equal or greater in our effluents than in those of the 2017 experiment (Li et al., 2018, in revision) which could not explain the discrepancy in productivity.

In our case, moderate productivities and growth rates, compared with literature and last year experiments, could mainly be explained by some concomitant phenomena:
(1) Lefebvre et al. (1996) demonstrated that, in fishpond effluents, biomass of algal diatom-dominant consortia augmented with increasing Si:P ratio with the highest productivities at 5-6. In our study, silicate additions in the HRAP failed to adjust Si:P ratio to 5:1. Thus, silicate quantities were to low and Si:P ratio was in average 0.8 and ranged between 0.5 and 1.6. This is very low in comparison with that classically maintained in IMTA-studies and applied last year: 5 (Lefebvre et al., 1996, 2004; Borges et al., 2005; Li et al., 2018, in revision). This low Si:P ratio must have also affected growth rates since diatoms, and notably *Cylindrotheca sp.* needed Si not only to constitute frustules, but also for synthesis and replication of DNA (Okita & Volcani, 1978).
(2) Another explanation to low growth rates and productivity was the unsuitable hydrodynamic conditions and homogenisation in the algal ponds. Hence, a constant sedimentation and important fouling was observed during the experimental period. Moreover, electrical short-circuit were induced by important rainfalls and pump stopped sometimes aggravating the phenomenon. This produced biomass was not considered and consequently productivity, estimated from sampling in the water column, was underestimated. This point constitutes the principal weakness of the IMTA-system functioning. In fact, even if the mass loss due to sedimentation was not assessed, it must have been important and represent a vast amount of energy flushed out of the system and unusable to produce oysters. Moreover, these sedimented algae died quickly and formed mud with the incapacity to remediate the effluents. The particular case of HRAP3, with apparent growth rate of 0.2d^{-2}, allowed to illustrate this point. Indeed, the 24/04, homogenisation pumps stopped working for a long-time in this algal pond. Consequently, the vast part of the algae sedimented and algal biomass stayed low during the whole experimental period in comparison with the other HRAP (figure 7).
(3) Observed productivity in the water column (mainly in term of Chla) was likely underestimated because of strong grazing in the HRAP. Thus, we only observed the net growth rate of algae for which the part of production consumed by zooplankton have been subtracted as it frequently occurs in natural and aquaculture ecosystems (Roberts et al., 2003; Lefebvre et al., 2006). This could have been emphasised by the formation of an important layer of mud in the bottom of algal raceways. In fact, it was previously suggested that grazers could be able to flourished and take refuge in this sedimented matter and sustain active vertical migrations to feed on suspended algae (Lefebvre et al., 2006).

(4) Growth rates were also probably reduced by competition with bacteria for nutrient access (Grossart, 1999). This is supported by the existing relation between NH4-N RE and nitrite production in the HRAP indicating competition with nitrifying bacteria for nutrient uptake. It is possible to assume that bacteria flourished in the mud formed by intense sedimentation. Thus, in the HRAP3 where strong sedimentation event occurred, nitrite production was high which was concomitant with a NH4-N RE as high as 93.5% reached the 02/05. That was almost 2-fold greater than those observed in the other HRAP (figure 9 and 10). This competition is also suggested by the fact that ammonium removal increased after the algal community decline (02/05) with maximum production rates of nitrite, as high as 2420%, occurring when culture collapse (figure 10). This likely showed that bacteria out-compete algae at the end of the experimental period.
(5) Growth rates could also have been limited in micronutrients in our experiments since Keerthi et al. (2012) demonstrated their important role, notably trace metals, in controlling the growth of *C.closterium* in laboratory experiments. If this is the case, RAS bacteria biofilter could have played a role as discussed before.

During the *C.closterium* dominance, remediation was efficient for P with an average removal of 95±2% PO4-P whereas it was low for NO3-N, 23±10%, and highly erratic for NH4-N, 49±21%. These results indicate that P was a limiting nutrient for algal growth in the RAS-effluents which could explained the incomplete consumption of N-nutrients in the IMTA-system. This was supported by comparison of N:P molar ratio of the effluents, 27.2±4.5, with the Redfield average N:P ratio of diatom composition: 16 (Redfield et al., 1963; Brezinski et al., 1985). These are poor results since remediation efficiencies for ammonium, nitrate and nitrite can be much higher for diatom-communities cultivated in fishpond effluents with often more than 90% of these compounds removed (Goldman et al., 1974; Lefebvre et al., 2004; Borges et al., 2005). Ammonium is a major targeted-compound in IMTA bioremediation since it is toxic for marine life, his not efficient removal from fishpond effluents complicate the reuse of wastewaters or their release in natural environment (Neori et al., 2004 and references therein). Nitrite is also a toxic compound (Lewis & Morris, 1986) and it is not removed but produced in our algal raceways. Hence, during our experimental period, for N-compounds, algal production unit not well respond to the primary aim of IMTA which is detoxification and clearance of wastewaters. Furthermore, last year, molar N:P ratio of the effluent was greater, 38, and removal efficiencies for ammonium, nitrate, nitrate and phosphate were all greater than 95% (Li et al., 2018, in revision). The main difference is the extremely low Si:P ratio noticed in our effluents, as discussed before. Thus, we can hypothesise that, rather than P, Si was the main limiting nutrient for algal growth in this experiment explaining the low retention efficiencies of N-compounds. Si:P ratio was variable in our effluents during the experimental period. The highest Si:P molar ratio, 1.6, was noticed the 23/04 and was followed, two days later, by high NH4-N and NO3-N RE in the 3 HRAP. Then, even with silicate addition, Si:P ratio decreased to stayed, on average, around 0.6 for the rest of the experiments. This was concomitant with a decreasing of NO3-N until the end of the experiments and with a diminution of NH4-N RE until the community decline, after which it increased, probably because of bacteria activity. Thus, N-compound remediation seemed related to Si availability during the *C.closterium*-community growth period. When bacteria activity augmented (i.e. increasing production of nitrite), ammonium RE was no longer related with Si:P molar ratio. These observations are in agreement with a strong Si-limitation of *C.closterium*-dominant consortium growth and suggested that, if Si:P ratio have been greater, with sufficient amount of silicate added in the effluent, removal rate of N-compounds could have been greater. A non-intuitive phenomenon was that this Si limitation did not appear to affect P-removal efficiency. However, this could be explained by the presence of a strong bacterial activity during the whole experimental period which maintained an high uptake of phosphate even when *C.closterium* was Si-limited. In this case, bacteria such as cyanobacteria, for which the presence was assessed in the IMTA-system during the experiments probably consumed phosphates but not N-compounds since they can used atmospheric dinitrogen for growth (Karl et al., 2002). This assumption was supported by 2 facts: (1) high PO4-P RE was already noticed prior to the

dominance of *C.closterium* and was still observed after algal-community decline (2) nitrite was produced all along the experimental period demonstrated the continual presence of nitrifying bacteria.
Hence, it is possible to assume that growth of the whole community, constituted of bacteria and algae, was P-limited whereas the algal part constituted of diatoms was, before all, Si-limited affecting the N-compound removal efficiency. This will be further investigated by the analysis of samples collected during the experimental period for silicate concentration determination in the HRAP (i.e. see the evolution of silicate consumption in the algal raceways). This pointed out the importance of the adjustment of Si:P molar ratio in the control of remediation efficiency for diatom-dominant consortia in IMTA-system. This also suggests that modification of this molar ratio could affect the competition relationship between algae and bacteria in aquaculture systems. This importance of Si:N:P molar ratio have yet been proved with algae, for instance *C.closterium*, often being disadvantaged in their competition with bacteria for nutrient (e.g. micronutrients, P) depleted conditions with impacts on growth rates (Guerrini et al., 1998; Grossart, 1999). Since bacteria appeared to have a major role in our IMTA-system, it would be relevant to study their role in nutrient cycling.

It is important to note that the important diminution in RE observed for all the nutrients, with the major part becoming negative, after the 17/04 was related to a dilution of the effluent caused by maintenance of the fishpond production unit. Because of the 4.2d renewal rate, this dilution affected more tardily the HRAP and consequently nutrient concentrations were greater in the HRAP than in the effluent causing a bias in RE calculations.

IV.2.b. Selection process and algal community dynamic

The 23/04, collapsing of the previous community (mainly constituted of cyanobacteria) was observed with the formation of an important mud layer at the bottom of the HRAP. This was immediately followed by a rapid increase in Chla concentrations and the emergence of a new kind of algal consortium dominated by the epipelic (grow on mud) benthic diatom *C.closterium*. This was mainly caused by silicate enrichments that started the 13/04. Some previous studies in IMTA-systems and algal cultivation experiments showed that diatom dominance is usually induced after adjustment of the Si:P ratio to values greater than 3 (Hussenot & Gautier, 1994; Lefebvre et al., 1996, 2004). However, we observed a diatom dominance for much lower Si:P ratio with 0.8±0.4. This was probably due to the high competitivity of the dominant-diatom species *C.closterium* even in such unfavourable conditions. Last year, at the same period, algal consortium was dominated by *P.tricornutum* (Li et al., 2018). This species could not been able to growth in effluents with such low Si:P ratio while *C.closterium* flourish in this condition. Indeed, *C.closterium* presents unique characteristics that can be related to his selection and success in the IMTA-system under these particular nutrient conditions.
(1) Unlike the vast majority of other diatoms, this specie became competitive in Si depleted conditions. This is permitted by its unique characteristics such as its weakly silicified frustule (large unsilicified area) and ability to form aggregate around dying cells with the advantage to beneficiate of regenerating source of N, Si, P and dissolved organic nutrients (Harisson & Davis, 1979). In our case, this was likely strongly amplified by the bacterial lysis of algal cells that dyed during the precedent culture crash which released C-rich, N-rich and P-rich compounds as well as important oligo-nutrients such as vitamins (Jürgens & Güde, 1990; Goldman & Dennett, 1991; Croft et al., 2005, 2006). Furthermore, it was previously evocated that during degradation of senescent algal cells, growth factors are released which stimulate the growth of healthy algae (Grossart, 1999).
(2) *Cylindrotheca sp.* possessed features of fast reproductioned r-selective species with the ability to proliferate in perturbated ecosystems like the IMTA-system (Sun et al., 2004).
(3) Environmental conditions were globally favourable to *C.closterium* growth during the experimental period. They were highly variable with strong winds and low atmospheric

temperature causing an important decrease in water temperatures. A significant correlation was found between this drop in water temperature and the increase in *C.closterium*-consortium biomass represented by Chla concentrations. This was likely an indirect relation. It was possible to assume that *C.closterium* became more competitive that its competitors, like bacteria or other algae species, at low temperatures that usually reduced metabolic rates. Thus, as discussed before, *C.closterium* is able to sustain high growth performances for temperature as low as 12°C. Thus, diminution of temperatures turned to the advantage of *C.closterium* and this was probably exacerbated by its ability to excrete allelopathic chemicals that inhibit the growth of competitors (Hiromi et al., 1995). Thus, abundance of *C.closterium* in the community increased until the 02/05 where minimal temperature was reached.

Thus, diatom-dominance in our experiment could only be related to the presence of *C.closterium* in the inoculum diversity. This also highlights the suitability of the Vasco2 biodiversity reservoir to inoculate the IMTA-system. The role of Si availability in species selection is also showed. This was also demonstrated in outdoor cultivations by Harrisson & Davis (1979) who observed, with the same inoculum, dominance of *C.closterium* for low Si availability and, conversely, dominance of centric diatoms with more Si in the nutrient pool.

From the 02/05, community started to decline until the culture crash which happened the 07/05 in the HRAP3 and after the 09/05 in the two other ponds. This was likely due to several concomitant phenomena. Firstly, due to logistical problems, silicates were not added during 7-days after the 30/04. It was possible to assume that silicates became critically limiting for *C.closterium* during this long starvation period. Thus, after the 30/04, silicate concentration dropped probably quickly far under the µM. This Si-depletion probably affected the Si-uptake and stopped the growth of *C.closterium* as it is also observed in natural phytoplankton communities dominated by diatoms where it is followed by a decline period (Del Amo et al., 1999). Moreover, we noticed that pH evolution followed the same pattern that algae production, risen to reach maximal values of 10-10.3 the 02/05. This was linked to dissolved inorganic carbon uptake for photosynthesis which breaks the seawater buffering capacity. At as high pH, carbon became likely limiting as it was demonstrated for other cultures and reduced growth and production (Lefebvre et al., 2000; Neori et al., 2017). In fish-microalgae land-based IMTA system, it was previously suggested that algal culture crash occurs when the grazers growth rates (i.e. zooplankton) overtakes that of microalgae (Neori et al., 2017). This was probably the case after the 02/05. This was illustrated by the appearance of large aggregates of chironomid larvae, floating in the HRAP, which are known for they grazing on benthic microalgae and to follow the natural cycle of algal biomass production in natural ecosystems (Goldfinch & Carman, 2000). Moreover, in stress conditions, microalgae reduced their antibiotic production and anti-growth factors which favours competitors such as bacteria (Kellam & Walker, 1989). Thus, this could explain the increase in nitrite production observed during the community decline.
Hence, community decline and collapse were probably mainly due to the overtook of the algal growth rate by predators growth rate due to the 7-days Si and CO_2 depletion that probably stopped the growth of the algal consortium.

IV.2.c. Performances of the oyster production unit

During the one-month operating-period, *C.gigas* juveniles sustained high growth performances., with a biomass doubling. This was higher than the growth rates of 4g-juvenile *c.gigas* observed in a similar land-based IMTA system located in Israel (Eilat). Yet, authors indicated that oyster growth rates were particularly high in this subtropical zone, being 1.5fold greater than in temperate zone (Neori et al., 2017 and references therein). This was even higher than Shpigel et al. (1993) who observed about 80% total fresh weight gain in one month in Eilat-IMTA-system for 5-months juveniles. Juvenile growth rates were also globally higher to those observed in natural ecosystems in France (Mediterranean and Atlantic) and in other

countries (e.g. Portugal, Japan, Canada; Gangnery et al., 2003 and references therein). This indicates the suitability of our IMTA system for Pacific oyster juvenile production and globally the relevance of IMTA for this purpose.

This could be emphasised by the fact that juvenile growth was likely not performed during a complete month but only during the *C.clostridium* dominance period. Indeed, as discussed in the "ingestion experiment" section, *C.gigas* juveniles were likely not able to efficiently retained small cells such as cyanobacteria or small *Chlorophyceae*. Moreover, if they ingested them, they were likely of poor nutritional value. Oppositely, *C.closterium* are long cells (superior to 25µm; UBC, 2018) and have particular shape which could favour its retention on oyster gills as discussed before for *Amphora sp.*. The high nutritional value of *C.closterium* for secondary consumers is already described, with high lipid content and particularly in essential PUFA and fatty acids such as EPA and ARA (Keerthi et al., 2012).

In contrast to these good results for juveniles, adults did not show any growth in the IMTA-system during the whole experiment. During the experimental period, subsequent mortalities occurred in the adult oyster ponds, reaching 27% in the oyster pond 2. The presence of *Vibrio aesturianus* was demonstrated. It is known to cause important mortalities in coastal *C.gigas* production farms. Since infection affects energy allocation this probably explained the absence of adult growth in our experiments (De Decker et al., 2011). Moreover, *Vibrio* infection are mainly related with sexual maturity stage which could explain why juveniles were not affected during the same period (De Decker et al., 2011). It is highly likely that adult oysters were infected prior to enter in the IMTA-system; indeed, some important mortalities were reported in the natural lagoon where our oysters were collected before the experiments (CAT, personal communication). Even if no mortality was observed in the oyster pond 3, infection was present since mortalities were observed after the end of the experiment.

No growth was observed in the 2017 study conducted in the Palavas IMTA system (Li et al., 2018, in revision) although the dominant specie *P.tricornutum* possessed high nutritional value (Céron García et al., 2005) and can usually sustain good growth performances of *C.gigas* (Wilson, 1978). Therefore, nutritional value was not likely limiting for oyster growth rate in this previous study. In 2018, we chose to use an optimal food ration of 6-8%DWalgae.DW oyster^{-1}.d^{-1} in each oyster pond. This food ration is frequently used to respond to important energy requirement of *C.gigas* broodstock conditioning in hatcheries (Utting & Millican, 1997; Fabioux et al., 2005; Delaporte et al., 2006). We decided to applied the same food ration for juveniles. Results showed that the food ration distributed into adult oyster ponds were almost always superior to 6%DW. Since we did not have any allometric relation to convert total juvenile fresh weight into dry weight, we compared food ration between adult and juvenile in %DWalgae.TWalgae.d^{-1}. This demonstrated that optimal food ration was also distributed in juvenile ponds since rations where always greater than those observed in the adult ponds. While we diluted algal culture 37fold in the oyster pond, Li et al. (2018, in revision) diluted 100-fold in the previous experiment. Thus, we could assume that lack of oyster growth observed in 2017 was probably related to food ration limitation. This was exacerbated by the poor hydrodynamic condition in the oyster ponds in this previous study. In IMTA-system food rations are often not taken into account but provided in an uncontrolled manner (Goldman et al., 1974; Shpigel et al., 1993; Lefebvre et al., 2000; Neori et al., 2017). Hence, we demonstrated the importance to control oyster's food ration in IMTA system and we confirmed the suitability of use a food ration of 6-8%DWalgae.DWoyster^{-1}.d^{-1} for juvenile and adult oyster production.

A total of 1.26kg total (shell + flesh) fresh weight of juvenile were produced during the experimental period in the triplicate. This was performed by using 4.5% (8.1L.h^{-1}) of the algal-rich mixed effluents of the HRAP (180L.h^{-1}). Hence, if the totality of these effluents was allocated to *C.gigas* juvenile production during the experimental period, it should be theoretically possible to produce 28.11kg total fresh weight in one month.

V. Conclusion

Our study highlighted the importance of biodiversity in the stability and productivity of aquaculture integrated systems.
Firstly, we confirmed the importance of the inoculum source with highly nutrient enriched HRAP, called “Vasco2”, being able to provide interesting diatom species for cultivation in the IMTA-system, such as *Amphora sp.* and *C.closterium*, in the two experimental phases. Oppositely, Mediterranean SW sampled in December was not able to provide any algal consortium susceptible to sustain high remediation efficiency and oyster production in an IMTA-system. Thus, Vasco2 is a stable biodiversity reservoir for inoculation of the IMTA - system all around the year whereas SW algal diversity was fluctuant and unsuitable in unfavourable months (i.e. winter, autumn). This provides tools for management of IMTA-systems since the inoculation could be performed from intensive production HRAP to another, alternately, throughout the year.
Secondly, the presence of high-competitive diatom *C.closterium* in the initial inoculum diversity allowed the diatom-dominance in nutrient conditions unfavourable for such phenomenon. In its absence, community would have been dominated by other microalgae such as *Chlorophyceae* as it is frequently observed under such Si:N:P molar ratios. In this case, *C.gigas* productivity would probably have been much lower as suggested by the ingestion experiments. Consequently, the success of our IMTA-system functioning could have been partly conditioned by the choice of the inoculum source. Another experiment has been carried out in June 2018 with inoculation of the IMTA-system with natural Mediterranean SW. The future results will allow to discuss about this assumption.

IMTA-system was successful for the production of juvenile Pacific oysters with strong growth performances recorded (weight doubling in one month). This was related to the control of the food ration performed in our oyster’s pond as it is rarely done in fish-microalgae-oyster IMTA-systems. Thus we determined that a food ration of 6-8%DWalgae.DWoyster^{-1}.d^{-1} is appropriated for sustain important juvenile and adult oyster production. Regulation of oyster’s food rations could be an efficient tool for IMTA-systems managing since determination of food availability cans be performed weekly by simple and fast measurement of total suspended matter in the algal raceways. Furthermore, even if remediation was not efficient enough for N-compounds in the algal unit, we assumed that this could be easily ameliorate by addition of suitable amounts of silicate to augment the Si:P ratio which could potentially increases the competitivity of diatoms facing to bacteria. Thus, we pointed out the role of Si:P ratio not only for diatom selection but also for stabilise remediation efficiency. In order to enhance the overall performances of the IMTA-system it would be relevant to ameliorate hydrodynamic conditions in the algal raceways (i.e. reduce the sedimentation rate) and to limit the growth of predators. Control of grazer growth rate cans be easily done by means of 0.1µm-mesh filtration of the fishpond effluents poured in the HRAP, as it was previously suggested (Milhazes-Cunha & Otero, 2017). In our case, better control of silicate additions in the HRAP would also permitted to prolongate the dominance of *C.closterium*. CO_2 was also probably a limiting factor. In the future, a waterwheel will be included in the algal raceways which will resolve the hydrodynamic problems and potentially also the CO_2 limitation by increasing the gaseous exchanges with the atmosphere.

Laboratory experiments demonstrated the role of micronutrients (i.e. trace metals and vitamins) in controlling the growth rates of algal strains in effluent-like culture media. Consequently, oligonutrient availability could likely have an influence on remediation efficiency and algal productivity in IMTA-systems. That is why the role of bacteria biofilter treatment of the effluent prior to circulation in HRAP can be questioning since it probably diminishes the amount of this oligonutrients available for algae. Accordingly, it could be interesting to study the micronutrient cycling in the IMTA-system.

Finally, this study allowed to shown the suitability of two marine diatoms, *Amphora sp.* and *C.closterium*, to growth in the IMTA-system and sustain the production of Pacific oysters. It will be interesting to pursue the study with *Amphora sp.* in field condition (inoculation in the IMTA-system), to study bioremediation efficiency, as it could not be realised in this study for technical reasons. *C.closterium* represents a species of major interest for cultivation in IMTA-system. Indeed, this specie possessed unique ecological characteristics which allowed diatom-dominance in highly variable aquaculture systems (i.e. extreme climatic conditions and nutrient depletions) as in our IMTA-system. Moreover, this species can sustain strong growth performances for juvenile oysters and probably also for adult oysters in healthy status.

VI. Bibliography

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VII. Webography

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VIII. Annexes

Annex I. *Characteristics of the Vasco2 HRAP*
(A) Composition of the agriculture fertilizers (Dynaflor®) added each month (11kg) in the Vasco2 HRAP
(B) Picture of the Vasco2 HRAP (empty)

A

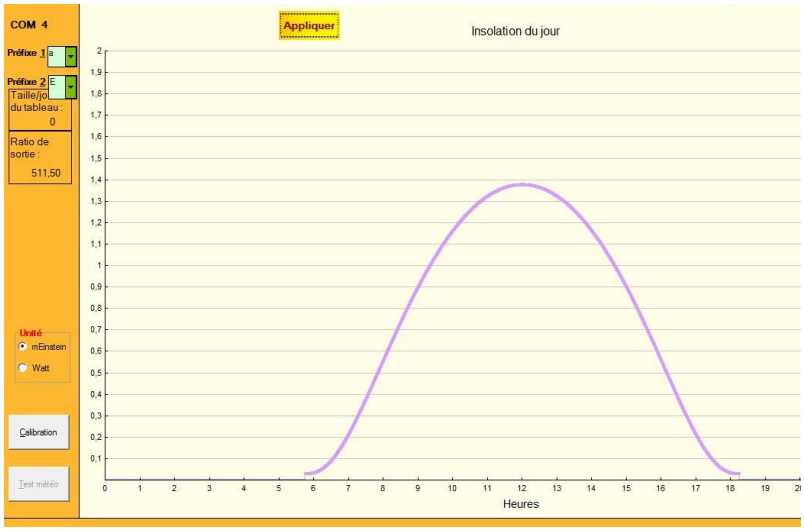
In 125Kg of fertilizer:
-NH_4NO_3 (80kg) -Urea (20kg) -$NH_4H_2PO_4$ (25kg) -Metasilicates (500g)
-Trace metals solution (2L): Bore (240p.p.m), Copper (70p.p.m), Iron (1100p.p.m), Manganese (750p.p.m), Molybdene (60p.p.m), Zinc (340p.p.m)

+ Constant CO_2 enrichment

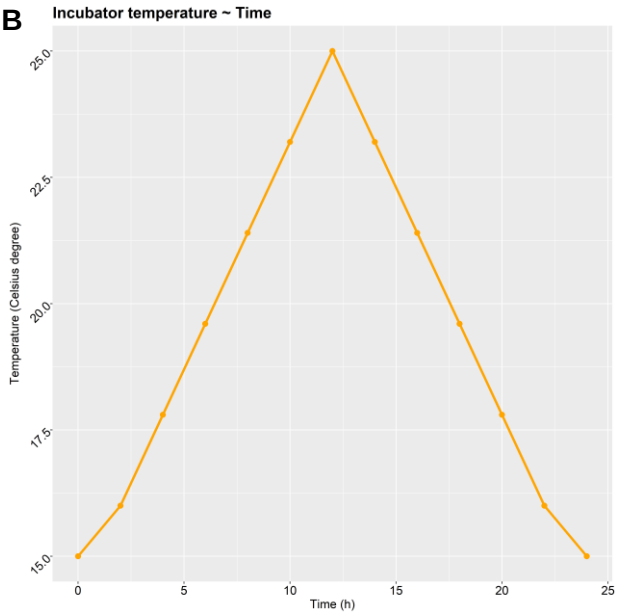
B

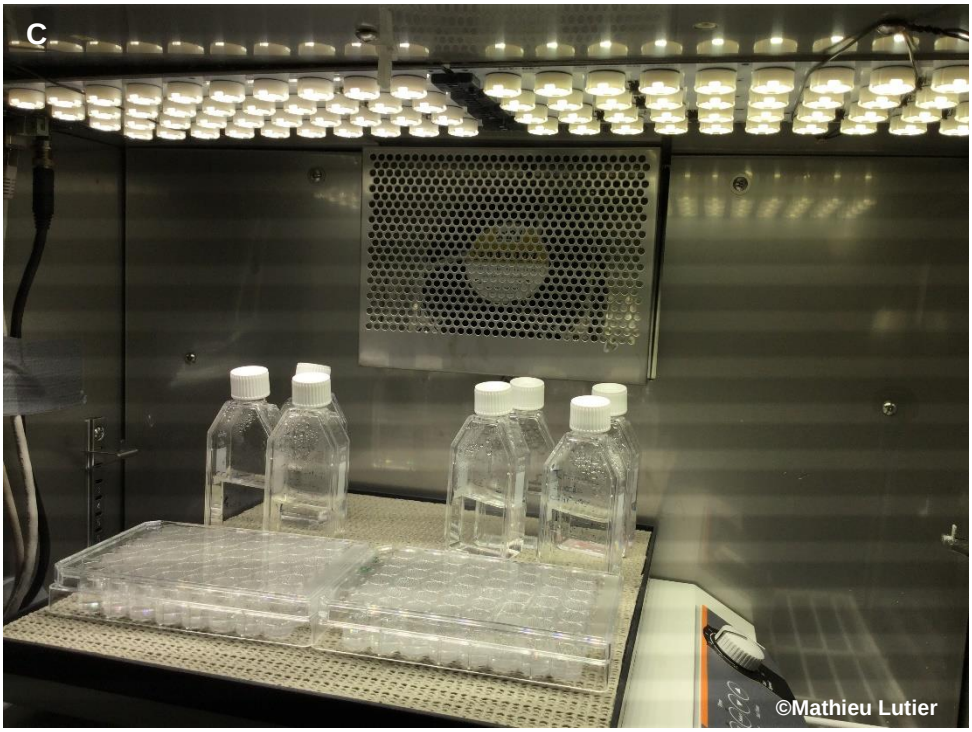
Annex II. *Presentation of some characteristics of the growth experiments.*
Light (A; expressed in mEinstein) and temperature (B, expressed in °C) daily variations in the incubators. (C) View of an incubator during a growth experiments: 2 microplates are incubated

A

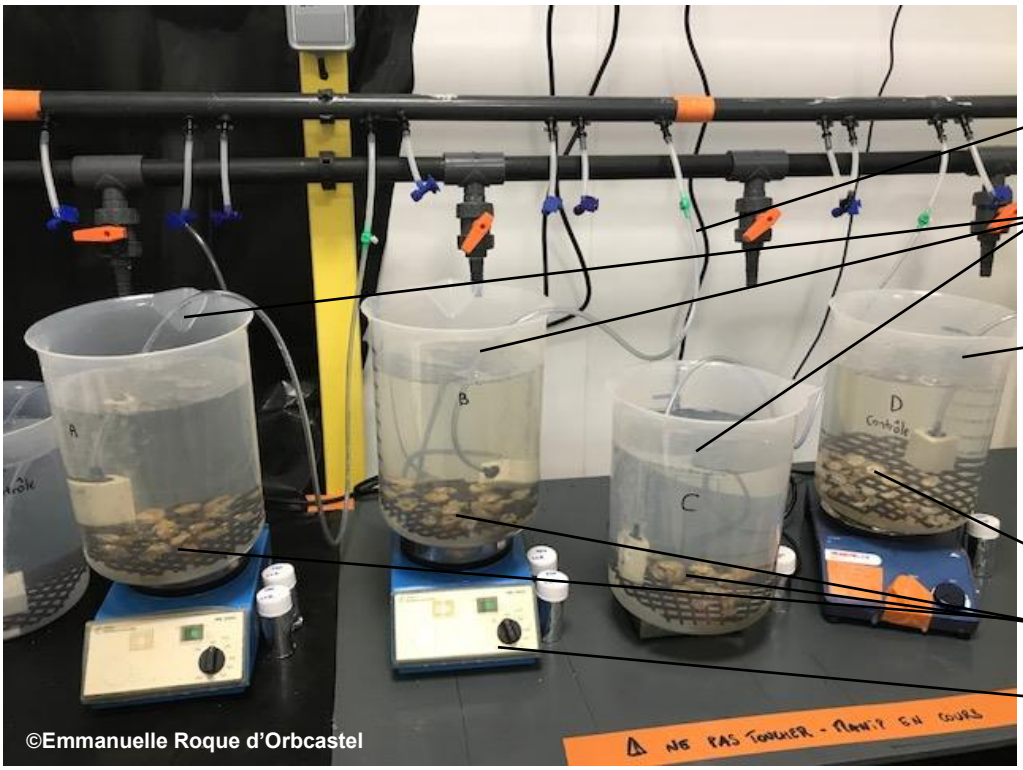


B

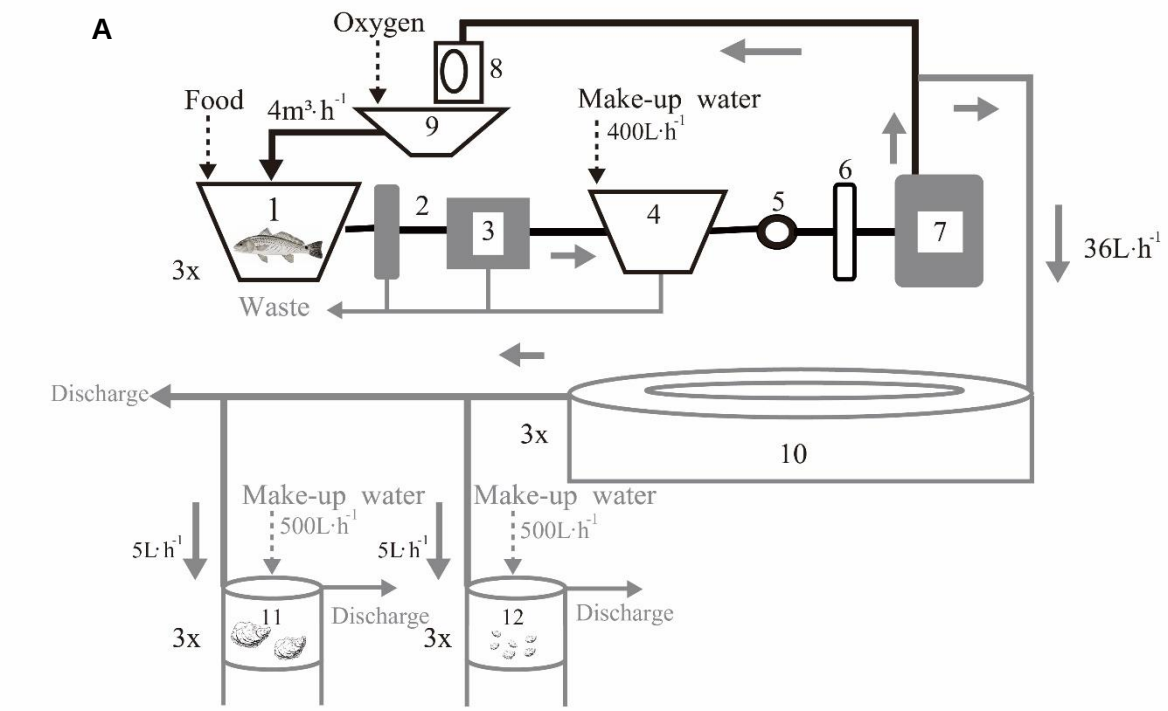




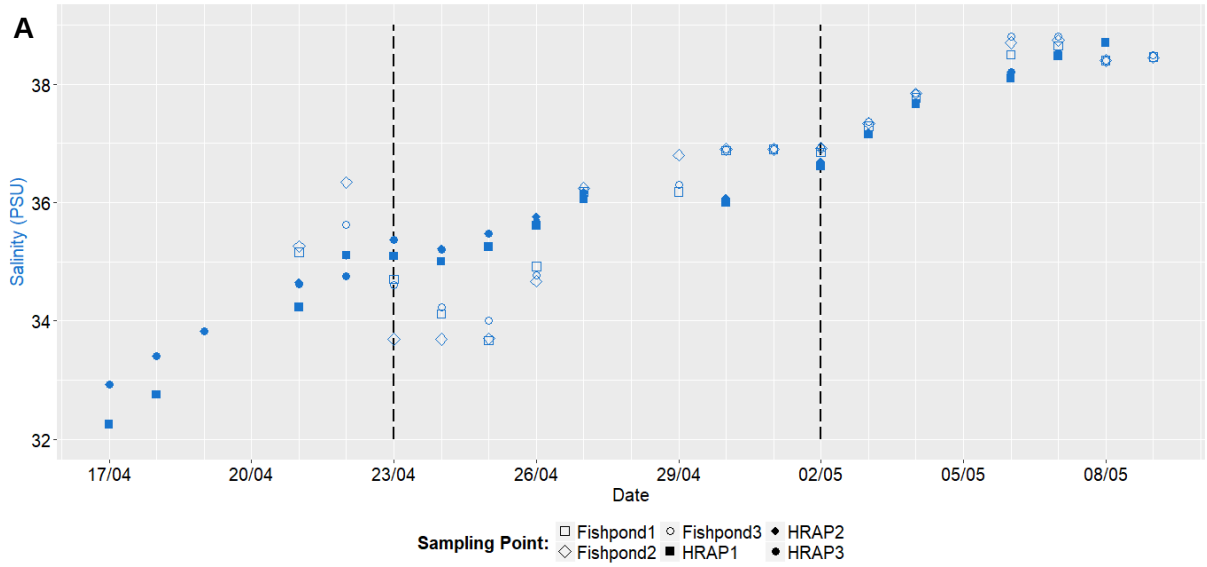
Annex III. *Experimental device used during an ingestion experiment*

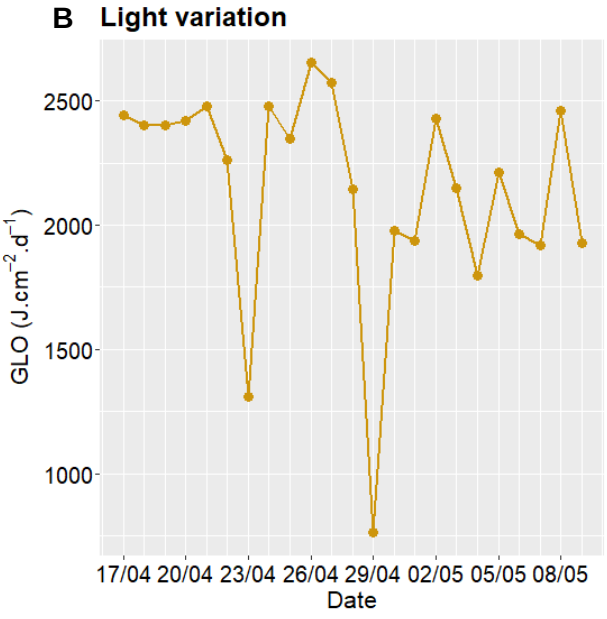


Annex IV. *(A) Schematic diagram of the IMTA-RAS aquaculture system from Li et al. (2018, in revision). Numbers in the diagram mean, 1: Fish tank, 4 m^3; 2: Particle trap; 3: Mechanical filter, 30-µm mesh filter; 4: Pumping tank; 5: Pump; 6: UV lamp; 7: Biological filter; 8: Packed column; 9: Storage tank; 10: microalgal raceway, 6 m^3; 11: Adult oyster tank, 0.5 m^3; 12: Juvenile oyster tank, 0.5 m^3. (B) Picture of a algal raceway (HRAP) during C.closterium dominance (C) Picture of an oyster pond*



Annex V. *Variation of salinity (PSU) at 9:30 a.m. (A) and irradiance (GLO: total solar radiations, J.cm^{-2}.d^{-1}; B) during the experimental period*





IX. Résumé

L'aquaculture est le secteur de production alimentaire à la plus forte croissance. Toutefois, le modèle de développement du secteur est aujourd'hui encore principalement basé sur l'aquaculture intensive monospécifique avec un impact environnemental souvent négatif. En effet, les rejets d'effluents aquacoles, chargés en nutriments, dans le milieu naturel peuvent, entre autres, provoquer des phénomènes d'eutrophisation. Ils peuvent également favoriser le développement de pathogènes, de parasites et d'algues toxiques avec un possible impact négatif, en retour, sur les espèces aquacoles, la biodiversité et la santé humaine. Dans ce contexte, le concept d'Aquaculture MultiTrophique Intégrée (IMTA) à été développé. Il s'agit de l'élevage, à proximité directe, d'espèces de niveaux trophiques différents de manière à ce que les déchets produits par une espèce (e.g. rejets inorganiques et organiques des poissons) soient utilisés par d'autres organismes pour leur croissance (e.g. algues, bactéries). De cette façon, l'excès de nutriments présent dans les effluents est absorbé pour produire une biomasse d'espèces aquacoles secondaires directement valorisables, par exemple des microalgues. Afin d'assurer la durabilité économique de tels systèmes, la biomasse algale produite doit être efficacement valorisée. Pour cela, une production tertiaire de macro-invertébrés herbivores à forte valeur commerciale, comme des bivalves filtreurs est souvent ajoutée. Ainsi, l'intérêt des systèmes d'Aquaculture MultiTrophique Intégré (IMTA) poissons-microalgues-huîtres en bassins (à terre) a déjà été démontré. Dans ce contexte, un système pilote d'IMTA a été mis en place à la station de recherche aquacole de Palavas-Les-Flots (UMR Marbec) avec la culture associée de bars, *Dicentrarchus labrax*, de microalgues dans des lagunes à haut rendement de production algale (HRAP) et d'huîtres creuses. En effet, l'élevage de l'huître creuse, *Crassostrea gigas*, en IMTA est particulièrement pertinent en France où elle est l'espèce aquacole marine dominante en volume produit, avec une forte valeur commerciale.

Une précédente étude conduite en 2017 a toutefois montré l'incapacité de *Crassostrea gigas* adultes et naissains à croître dans le système d'IMTA. La mauvaise qualité nutritionnelle des microalgues cultivées en aurait peut-être été la cause. En effet, la croissance des huîtres est principalement conditionnée par la qualité nutritionnelle des algues cultivées, principalement en termes de teneur en acides gras poly-insaturés (n-3 et n-6), en lipides totaux et en acides aminés essentiels. De ce point de vue, il est largement admis que les diatomées sont la classe de microalgue la plus adaptée en terme de valeur nutritionnelle pour *C.gigas* dont elles constituent généralement la majeure partie du régime alimentaire en milieux naturels et aquacoles. Toutefois, l'incapacité à initier et contrôler la dominance d'espèces de microalgues intéressantes pour la production de macro-invertébrés est aujourd'hui considéré comme la principale limite au développement des systèmes d'IMTA basés sur la production de microalgues. Pour cette raison, notre étude visait à étudier le rôle de la diversité microalgale dans la stabilité et la productivité des systèmes d'IMTA.

Ainsi, deux différents réservoirs de diversité algale, eau de mer Méditerranéenne (SW) v/s HRAP hautement enrichi en nutriments et fonctionnant actuellement à la station de Palavas dans le cadre d'un autre projet de recherche (Vasco2), ont été testés pour leur potentiel à inoculer le système d'IMTA et à y permettre le développement d'espèces algales d'intérêt en terme de productivité, d'efficacité de remédiation (absorption des nutriments présents dans l'effluent) et de capacité à être sélectionné et ingéré par *C.gigas*. Deux approches ont été effectuées en parallèle :

(1) Des expériences de sélection ont été conduites en laboratoire dans des conditions physico-chimiques réalistes simulant celles observées en Avril dans le système d'IMTA. Les consortiums microalgaux sélectionnés ont ensuite été testés pour leur taux de croissance sur différent milieux f/2 (composition proche des effluents) avec l'ajout de différentes

concentrations d'oligonutriments (i.e. métaux traces et vitamines). Enfin, ces consortiums ont été présenté à des huîtres adultes et naissains et testés pour leur capacité à être ingérés. Seul l'inoculum Vasco2 a permis l'émergence d'espèces intéressantes, comme la diatomée *Amphora sp.*, adaptées pour soutenir de bonnes performances de croissance, d'efficacité de remédiation et pour nourrir des huîtres adultes et juvéniles. Le rôle des oligonutriments dans le contrôle des performances de croissance et, en conséquence, d'efficacité de remédiation a été montré dans des effluents aquacoles indiquant leur importance dans les performances globales des systèmes d'IMTA. Cette phase a montré que Vasco2 était un réservoir de diversité algale stable et adapté pour l'inoculation du système d'IMTA alors que l'eau de mer Méditerranéenne est caractérisée par une diversité instable qui suit l'évolution saisonnière des communautés algales dans le milieu naturel. Ainsi, alors que Vasco2 peut-être utilisé toute l'année comme inoculum, SW ne peut pas être utilisée pendant les mois défavorables (i.e. hiver, été) où la diversité reste faible dans le milieu.

(2) Des expériences de sélection et l'étude du fonctionnement du système d'IMTA ont été réalisé en conditions réelles. Ainsi, Vasco2 à été choisi pour inoculer le système d'IMTA et les performances globales du système ont été suivies pendant un mois, du 17/04 au 14/05/2018. L'espèce dominante sélectionnée était la diatomée *Cylindrotheca closterium*. Le taux de croissance de la communauté était de 0.1 à 0.4d^{-1}. L'efficacité de remédiation était de 95±2% pour les phosphates alors qu'elle était erratique pour l'ammonium avec 49±21% et faible pour le nitrate avec 27±5%. La productivité modérée du système et la faible efficacité de remédiation pour les nutriments azotés était vraisemblablement causés par une forte limitation en Si et àune forte compétition avec les bactéries dans le système d'IMTA car l'ajout de silicate n'a pas permis d'atteindre un ratio Si:P recommandé de 5 dans l'effluent. La capacité unique de *C.closterium* à être compétitive dans des conditions limitées en Si et de forte variabilité climatique a été montrée. Cette espèce était aussi capable de soutenir de fortes performances de croissance chez les juvéniles de *C.gigas* avec un doublement du poids frais total en un mois. Ainsi, cette étude a montré qu'il est possible de soutenir une forte production d'huîtres, même avec une production algale modérée, en régulant la ration alimentaire des huîtres à 6-8%MSalgues.MShuîtres^{-1}.jour^{-1} (i.e. contrôle du taux de dilution). Ces résultats montrent que la ration alimentaire était probablement limitante dans les expériences conduites en 2017, et non la qualité nutritionnelle des algues cultivées, expliquant l'absence de croissance des huîtres. Dans notre étude, les huîtres adultes n'ont pas montré de croissance à cause d'une vibriose.

Pour conclure, cette étude montre qu'avec Vasco2 comme inoculum et l'ajout d'une quantité adaptée de silicates dans les effluents (Si:P = 5), notre système d'IMTA pourrait probablement être performant en termes de productivité algale, bioremédiation et production d'huîtres creuses. Des outils doivent également être développés pour réduire la limitation en CO_2 et la croissance des brouteurs afin de prolonger la production d'algues dans le système d'IMTA.

Abstract / Résumé

The suitability of land-based finfish-microalgae-oyster Integrated MultiTrophic Aquaculture (IMTA) has yet been demonstrated. In this context, an IMTA pilot-scheme is currently developed with seabass, microalgae in high rate algal ponds (HRAP) and Pacific oysters in the Palavas-Les-Flots aquaculture research station. A previous experiment showed the incapacity of both juvenile and adult *Crassostrea gigas* to growth in this IMTA-system (Li et al., 2018). It was assumed that this could be due to the unsuitable nutritional value of the microalgae cultivated in the IMTA-system. More broadly, the inability to promote the dominance of desirable microalgal species is currently considered as the major limit for the broader development of IMTA based on microalgae. For this reason, we conducted a study focused on the role of microalgal diversity in the stability and performances of IMTA-systems. Two different reservoirs of algal diversity, natural Mediterranean seawater (SW) and highly nutrient enriched HRAP running in the Palavas station for an ongoing research project (Vasco2) were tested for their ability to inoculate the IMTA-system and promote the development of suitable species in term of productivity, effluent remediation efficiency (removal of nutrients) and ability to be selected and ingested by Pacific oysters. (1) Selection experiments were conducted in laboratory under realistic physicochemical conditions, miming those observed in April in the IMTA-system. Then, the microalgal consortia selected were tested for their growth rates on different effluent-like f/2 culture media with the supplementation of different concentrations of micronutrients. Finally, they were presented to both juvenile and adult *C.gigas* and tested for their ability to be ingested and selected. Only the Vasco2 diversity reservoir allowed the emergence of interesting species, such as the diatom *Amphora sp.*, adapted to sustain good growth performances and remediation efficiency and to feed both adult and juvenile oysters. The role of oligonutrients (i.e. trace metals and vitamins) in controlling the algal growth rates and, consequently, remediation efficiencies was shown in effluent-like culture media indicating their importance in performance of IMTA systems. (2) Selection experiments and study of the IMTA-sytem functioning were performed in field experiments. Thus, Vasco2 diversity reservoir was chosen to inoculate the IMTA-system and the overall performances of the system were followed during a month operating period, from 17/04 to 14/05/2018. The selected dominant species was the diatom *Cylindrotheca closterium*. Algal productivity was moderate, as compared to literature, with a maximum recorded of 58±1 $mg.L^{-1}$ total suspended solid and low community growth rates ranging from 0.1 to $0.4d^{-1}$. Nutrient removal efficiency was 95±2% for phosphate whereas it was erratic for ammonium with 49±21% and low for nitrate with 27±5%. We determined that moderate growth and low N-compound removal efficiencies were likely caused by strong Si-limitations and competition with bacteria in the IMTA-system since addition of silicate failed to reach a suitable Si:P ratio of 5 in the effluent. The unique ability of the diatom *C.closterium* to be competitive under Si-limitation and high climatic variability in aquaculture systems was demonstrated. *C.closterium* was able to sustain high juvenile *C.gigas* growth performances with a doubling in total fresh weight in one month. Hence, we demonstrated that, even with a moderate algal productivity, it is possible to efficiently produce oysters by regulating the input of foods in the oyster ponds to 6-8%DWalgae. $DWoyster.d^{-1}$ (i.e. control of dilution rate). We showed that food ration was probably limiting in the 2017 experiments explaining the absence of oyster growth. In our study, adult oysters did not grow because of a vibriosis. To conclude, we showed that, with Vasco2 HRAP as inoculum source and a correct supplementation of Si in the effluents, our IMTA-system could sustain high overall performances in term of algal productivity, remediation efficiencies and Pacific oyster production. Management tools must also be developed to reduce CO2 limitation and grazers growth to prolongate the algal growth in the IMTA-system.

L'intérêt des systèmes d'Aquaculture MultiTrophique Intégré (IMTA) poissons-microalgues-huîtres en bassins, à terre, à déjà été démontré. Dans ce contexte, un système pilote d'IMTA a été mis en place à la station de recherche aquacole de Palavas-Les-Flots (UMR Marbec) avec la culture associée de bars, de microalgues dans des lagunes à haut rendement de production algale (HRAP) et d'huîtres creuses. Une précédente étude a montré l'incapacité de *Crassostrea gigas* adultes et naissains à croître dans le système d'IMTA. Il a été avancé que cela pouvait être dû à la mauvaise qualité nutritionnelle des microalgues cultivées. Plus généralement, l'incapacité à initier et contrôler la dominance d'espèces de microalgues intéressantes est aujourd'hui considéré comme la principale limite au développement des systèmes d'IMTA basés sur la production de microalgues. Pour cette raison, notre étude visait à étudier le rôle de la diversité microalgale dans la stabilité et la productivité des systèmes d'IMTA. Deux différents réservoirs de diversité algale, eau de mer Méditerranéenne (SW) v/s HRAP hautement enrichi en nutriments et fonctionnant actuellement à la station de Palavas dans le cadre d'un autre projet de recherche (Vasco2), ont été testés pour leur potentiel à inoculer le système d'IMTA et à y permettre le développement d'espèces algales d'intérêt en terme de productivité, d'efficacité de remédiation (absorption des nutriments présents dans l'effluent) et de capacité à être sélectionné et ingéré par *C.gigas*. (1) Des expériences de sélection ont été conduites en laboratoire dans des conditions physico-chimiques réalistes simulant celles observées en Avril dans le système d'IMTA. Les consortiums microalgaux sélectionnés ont ensuite été testés pour leur taux de croissance sur différent milieux f/2 (composition proche des effluents) avec l'ajout de différentes concentrations d'oligonutriments (i.e. métaux traces et vitamines). Enfin, ces consortiums ont été présenté à des huîtres adultes et naissains et testés pour leur capacité à être ingérés. Seul l'inoculum Vasco2 a permis l'émergence d'espèces intéressantes, comme la diatomée *Amphora sp.*, adaptées pour soutenir de bonnes performances de croissance, d'efficacité de remédiation et pour nourrir des huîtres adultes et juvéniles. Le rôle des oligonutriments dans le contrôle des performances de croissance et, en conséquence, d'efficacité de remédiation a été montré dans des effluents aquacoles indiquant leur importance dans les performances globales des systèmes d'IMTA. (2) Des expériences de sélection et l'étude du fonctionnement du système d'IMTA ont été réalisé en conditions réelles. Ainsi, Vasco2 à été choisi pour inoculer le système d'IMTA et les performances globales du système ont été suivies pendant un mois, du 17/04 au 14/05/2018. L'espèce dominante sélectionnée était la diatomée *Cylindrotheca closterium*. Le taux de croissance de la communité était de 0.1 à $0.4d^{-1}$. L'efficacité de remédiation était de 95±2% pour les phosphates alors qu'elle était erratique pour l'ammonium avec 49±21% et faible pour le nitrate avec 27±5%. La productivité modérée du système et la faible efficacité de remédiations pour les nutriments azotés était vraisemblablement causé par une forte limitation en Si et à une forte compétition avec les bactéries dans le système d'IMTA car l'ajout de silicate n'a pas permis d'atteindre un ratio Si:P recommandé de 5 dans l'effluent. La capacité unique de *C.closterium* à être compétitive dans des conditions limitées en Si et de forte variabilité climatique a été montrée. Cette espèce était aussi capable de soutenir de fortes performances de croissance chez les juvéniles de *C.gigas* avec un doublement du poids frais total en un mois. Ainsi, cette étude a montré qu'il est possible de soutenir une forte production d'huîtres, même avec une production algale modérée, en régulant la ration alimentaire des huîtres à 6-8%MSalgues.$MShuîtres^{-1}.jour^{-1}$ (i.e. contrôle du taux de dilution). Ces résultats montrent que la ration alimentaire était probablement limitante dans les expériences conduites en 2017 expliquant l'absence de croissance des huîtres. Dans notre étude, les huîtres adultes n'ont pas montré de croissance à cause d'une vibriose. Pour conclure, cette étude montre qu'avec Vasco2 comme inoculum et l'ajout d'une quantité adaptée de silicates dans les effluents (Si:P = 5), notre système d'IMTA pourrait probablement être performant en termes de productivité algale, bioremédiation et production d'huîtres creuses. Des outils doivent également être développés pour réduire la limitation en CO_2 et la croissance des brouteurs afin de prolonger la production d'algues dans le système d'IMTA.