MASTER SML

SCIENCES DE LA MER ET DU LITTORAL

MENTION

SCIENCES BIOLOGIQUES MARINES

SPECIALITE

ECOSYSTEMES MARINS

Lutier Mathieu

Effect of different rearing conditions on growth performance and nutritional quality of the macroalgae *Ulva rigida* (C.Agardh, 1823) and *Hydropuntia cornea* (J.Agardh, M.J. Wynne, 1989) cultivated in IMTA

Mémoire de stage de Master 2

Année Universitaire 2016-2017

Structure d'accueil : Grupo de Investigación en Acuicultura
Universidad de Las Palmas de Gran Canaria

Tuteur universitaire : Ar Gall Erwan

Maîtres de stage : Courtois de Viçose Gercende & Viera Maria del Pino

UBO
université de bretagne occidentale

L'Université est une chance

ENGAGEMENT DE NON PLAGIAT

Je soussigné-e Mathieu LUTIER

assure avoir pris connaissance de la charte anti plagiat de l'université de Bretagne occidentale.

Je déclare être pleinement conscient-e que le plagiat total ou partiel de documents publiés sous différentes formes, y compris sur Internet, constitue une violation des droits d'auteur ainsi qu'une fraude caractérisée.

Je m'engage à citer toutes les sources que j'ai utilisées pour rédiger ce travail.

Signature

Acknowledgments

I would like to thank all the GIA team for their welcome, their kindness and for their patience despite the difficulties to communicate in Spanish. I particularly want to thank Marco Hernándes and Jess Theilen for their help and the nice atmosphere during the experiments. I am grateful to Carmen Quintana Luzardo for all the explanations about biochemical technics and for his patience in communicating in Spanish with me. I would also like to thank Dr Juan Luis Gómez Pinchetti and Yolanda García Fernández, from the Banco Español de Algas, for all the technical support. I would like to thank Manon for giving me the PDF title and Justine the bold and Canelle for the help and the support during the difficult times. Finally, I would like to thank Dr Gercende Courtois de Viçose and Dr Maria del Pino Viera Toledo for preparing all the experimental design, for all the help and especially for their kindness and for helping me to do substantial progress in Spanish and English with a lot of patience. Thank you!

View of the Marine Science and Technology Park of Taliarte ©Mathieu Lutier

Table of contents

I. Introduction

I.1. Aquaculture and environment: traditional monoculture versus integrated multi-trophic aquaculture (IMTA)

Aquaculture is probably the fastest-growing food production sector. In 1974, it supplied only 7 percent of the total fish production for human consumption while in 2014 its contribution overtook that of wild caught fish for the first time. This important growth of the sector is expected to continue in the future (FAO, 2016). However, many authors point out the unsustainability of traditional intensive monoculture with a lot of associated environmental concerns. First, the discharge of large amounts of nutrients can cause the toxicity, the eutrophication and the deoxygenation of coastal waters (Pillay, 2004; Gowen & Bradbury, 1987). These organic and inorganic nutrient inputs can also foster the development of pathogens, parasites and harmful microalgae which may, in turn, negatively impact biodiversity (Ferreira et al., 2011) but also the farming species (Jegatheesan et al. 2011; Neori et al., 2004). Moreover, monoculture practices are highly dependent of exogenous source of food such as small pelagic fishes linked with overfishing (Natale et al., 2013) or expensive artificial foods (Neori et al., 2004). They are also highly vulnerable to catastrophic destructions like diseases or extreme weather events (Barrington et al., 2009). There is an increasing public concern about aquaculture practices and fish quality resulting in negative social image of the sector (Barrington et al., 2009). Simultaneously, aquaculture is subject to stricter legislations regarding water treatment (Neori et al., 2004). Therefore, alternative aquaculture practices like integrated multi-trophic aquaculture (IMTA) and bioremediation processes are now considered as the logical next step in the aquaculture future development (Barrington et al., 2009).

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 wastewater chemical components (mainly ammonium) released by cultivated species, like fish, by other organisms, like bacteria or algae, for their biological activities. Unlike bacteria, seaweeds have an assimilative activity and reintroduce energy in the system by the way of photosynthesis and inorganic nutrient assimilation (Barrington et al., 2009; Demetropoulos and Langdon, 2004; Sphigel and Neori, 1996). This enable to use the

maximum amount of energy stored in expensive processed feed instead of flushing energy out of the system (Barrington et al., 2009; Chopin et al., 2001; Neori et al., 1998; Sphigel and Neori, 1996). In addition to cleaning wastewaters, seaweeds can act as oxygen producers (via photosynthesis) for other cultivated animals by means of water recirculation (Schuenhoff et al., 2003). For these reasons, IMTA has a high profitability potential and requires a continued research effort (Chopin, 2013; Barrington et al., 2009; Neori et al., 2004; Chopin et al., 2001). These integrated aquaculture systems can be developed offshore or in land-based systems. In land-based systems, organisms are separated into different ponds and the link between them is assured by circulating water (Neori et al., 1998, Shpigel and Neori, 1996). Such systems allow to avoid the problems inherent to offshore aquaculture like predation, poaching, extreme weather events, pathogens, poisoning and harmful wild organism proliferations (Barrington et al., 2009; Neori et al., 2004; Manzi and Castagna, 1989).

For the reasons quoted above, land-based integrated aquaculture based on seaweeds is considered one of the most suitable IMTA system. 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 a food source for herbivore macro-invertebrate species, like urchins or abalones, with high added value (Tenore, 1976). This constitutes thus a simplified integrated aquaculture system with three compartments: finfishes, seaweeds and macro-algivores, as described in Neori et al. (1998) and Sphigel and Neori (1996).

I.2. IMTA in Canary Islands: fish-algae-abalone integrated system

The Canarian subspecies of the green ormer, *Haliotis tuberculata coccinea* (Reeve, 1846), an herbivorous gastropod belonging to the family *Haliotidae* (L., 1758), is among the organisms of interest for aquaculture diversification and IMTA development in the Canary Islands (Viera et al., 2005a). This is partly due to their high economic value and the important decreasing of *H.tuberculata* wild populations since the second half of the 20th century which supported the emergence of numerous research projects about their production in aquaculture (Neori et al., 1998; Mercer et al., 1993; Tenore, 1976). Since wild algae biomass availability and exploitation are insufficient in the Canary Islands to sustain a commercial production of abalone (Viera et al., 2005a), there is a need to develop production methods to obtain a suitable source of feed. Another factor who often limits abalone production is the expensive cost of commercial food (Sphigel and Neori, 1996). That is why, the implementation of an IMTA system, integrating fish, algae and abalone production, has been considered as a successful local way of production in Canary Islands

Ulva rigida (C.Agardh, 1823) and *Hydropuntia cornea* (J.Agardh) Wynne, 1989 were selected to filter wastewaters of fish ponds in the GIA (*Aquaculture Research Group*, University of Las Palmas de Gran Canaria) integrated system for *H.tuberculata coccinea* production (Viera et al., 2016; 2011; 2005a,b). *U.rigida* is a cosmopolitan green algae of the phylum *Chlorophyta* characteristics of intertidal sheltered rocky zones. Its thallus is thin and shaped as a sheet. *U.rigida* is known as an opportunistic species resistant to stressful conditions and commonly found in nutrient-rich areas (University of Hawai'i Botany Department, 2001). *H.cornea* is a red algae (*Rhodophyta*), of the family *Gracilariaceae*, living in the Atlantic Ocean tropical zone where it is commonly found from the low intertidal zone to subtidal areas. Its thallus is large, branched and fleshy-cartilaginous (Núñez-Resendiz, 2015). These species can reproduce by means of both asexual and sexual reproduction. *Ulva spp.* and *Gracilaria spp.* have long been used in IMTA-systems since they possess numerous characteristics suitable for IMTA: high growth rates, high nutrient absorption capacity, known life-cycle, controlled cultivation process and clonal reproduction (Abreu et al., 2011; Neori et al., 2004, 2000). Moreover, *Ulva*, by its opportunistic nature, is a genus highly resistant to epiphytes (Neori et al., 2004). This is not the case for *Gracillaria spp.* although production methods allow to avoid epiphyte proliferation with a high algae stocking density. *Hydropuntia cornea,* formerly named *Gracilaria cornea*, possesses the same properties than *Gracillaria spp.* (Bird et al., 1992), and has been yet successfully cultivated in IMTA systems (Figueroa et al., 2012; Viera et al., 2016, 2011, 2009, 2005a,b). Viera et al. (2011) have shown that abalone growth rate is better when they are feed with a mix of *U.rigida* and *H.cornea* than with mono-specific diets due to a better input of essential nutrients.

In addition to available seaweed biomass, algal nutritional quality has a primordial importance for the abalone production efficiency (Viera et al., 2005a). For instance, proteins are the major dietary component determining the nutritional value of seaweeds for abalone (Mai et al., 1994). Hence, the optimal necessary amount of protein for abalone growth is 24 to 47% of algae dry weight (DW) and proteins often represent only 11 to 19%DW in wild seaweed (Bansemer et al., 2016). Therefore, Viera et al. (2011) have shown that algae cultivated as biofilter in nutrient enriched aquaculture wastewater have a higher protein content: up to 29 to 34%DW respectively in *U.rigida* and *H.cornea*. Abreu et al. (2011) have found similar results in *Gracilaria vermiculophylla* (Ohmi) Papenfuss, 1967. Viera et al. (2005) shown that *H.tubercula coccinea* fed with these macro-algae has a growth rate comparable to that obtained with artificial food. In that respect, the use of macro-algae produced in aquaculture waste waters to feed abalone is relevant (Viera et al., 2016, 2011, 2009, 2005; Barrington et al., 2009; Neori

et al., 2004) and *U.rigida* and *H.cornea* are particularly adapted to produce *H.tuberculata coccinea* in IMTA. Nevertheless, seaweed production efficiency is highly dependent of cultivation parameters (Abreu et al., 2011; Jiménez del Río et al., 1996, 1994; Sphigel and Neori; 1996, Lapointe and Ryther, 1979, 1978). Furthermore, even if land-based systems enable to control the nutrient allocation to maintain the seaweed nutritional quality (Neori et al., 2004), a better understanding and control of rearing parameters is required to optimise the system functioning.

I.3. Algal culture conditions: control of productivity and nutritional quality

It is well-known that algal production varies according to seasonal variation of environmental parameters such as temperature and light (Abreu et al., 2011; Mata et al., 2006). Interestingly, manageable rearing parameters can be settled to optimise algal production. This is the case of algal stocking density which is a primordial parameter who controls the penetration of light into the pond. If it is too high or too low this can cause respectively light limitation or photoinhibition of growth (Abreu et al., 2011; Neori et al., 2004). It is also linked with the competition for other essential resources such as macronutrients (nitrogen, phosphates), micronutrients and inorganic carbon (Viaroli et al., 1996; Debusk et al., 1986, Duke et al., 1986). Another major parameter is the wastewater flow rate which determines the amount of nutrients provided to seaweeds per unit of time (Jiménez del Rio et al., 1996, Debusk et al., 1986; Lapointe and Ryhther, 1979). Generally, increase the water flow rate allows to enhance the specific growth rate and cans even balance the negative effect of winter unfavourable conditions (Abreu et al., 2011; Jiménez del Rio, 1996, 1994; Lapointe and Tenore, 1981, DeBoer et al., 1978).

The nutrient availability is directly linked with seaweed proximal composition in flow-through land-based cultivation systems (Figueroa et al., 2012, Gómez-Pinchetti et al., 1998; Rosenberg and Ramus, 1982; Ryther et al., 1981; Lapointe and Ryther, 1979). Consequently, it is likely that water flow rate and stocking density control to some extent the nutritional quality of cultivated seaweeds since they control nutrient supply and resource access respectively. Nevertheless, relatively little is known about the effect of these manageable rearing parameters on seaweed proximal composition with a lack of research conducted on this subject. However, some studies showed the high importance of nutritional quality in the abalone production performances (Viera et al., 2005; Mai et al., 1994). In contrast, it is well known that algal proximal composition varies according to environmental conditions (light, temperature, salinity, nitrogen source) but also with seaweed maturity and gender (Schiener et al., 2015;

Khotimchenko, 2005, Rosenberg and Ramus, 1982). The amount of carbohydrates in the thallus is positively correlated with temperature, light and salinity. It is maximal during summer in both macro-algae corresponding with the highest growth rate and photosynthesis (Schiener et al., 2015, Rosenberg and Ramus, 1982). At the opposite, the protein content is inversely correlated with light and temperature and is maximal during winter (Schiener et al., 2015, Rosenberg and Ramus, 1982). Similarly, the lipid content varies according to the season (Khotimchenko, 2005). The link between algal stocking density and light exposition also suggests a possible effect of this rearing parameter on seaweed proximal composition. For these reasons, further studies are required to determine the link between manageable cultivation parameters (e.g. water flow rate and algae stocking density) on seaweed production and nutritional quality. This is particularly the case for fish-algae-abalone integrated systems to insure a suitable seaweed quality and availability for ormers all along the year and thus optimise the system functioning.

I.4. Objectives

In order to maximise the production efficiency of the GIA IMTA system, the main aim of this study was to evaluate the effect of two different cultivation parameters, algal stocking density and water flow rate, on the growth rates and biochemical compositions of *U.rigida* and *H.cornea*. Hence, two different algal stocking densities and wastewater flow rates were tested and environmental conditions were monitored throughout the experimental period (irradiance, temperature, nutrient availability). Growth rate and algae proximal composition (ash, protein, lipid and carbohydrate contents) were evaluated, under the different rearing conditions. In order to attest the interest of IMTA for seaweed production in Canary Islands, these experiments where conducted in both fresh seawater and nutrient-rich GIA wastewaters. The efficiency of the integrated production system was thus analysed by comparison between the two set of experiments. This took place in the context of the installation of new biofiltration ponds in the facilities with the need of setting rearing parameters for *U.lactuca* and *H.cornea* production.

II. Material & Methods

II.1. Algal cultivation systems

The green and red macroalgae, *U.rigida* and *H.cornea*, were both cultivated, in a land-based integrated fish-algae-abalone production system and in Atlantic seawater from January 24th until April 04th 2017 in the GIA aquaculture facilities based in the Marine Science and

Technology Park of Taliarte (ULPGC, Telde, Gran Canaria, Canary Islands, Spain). Prior to the beginning of experiments, *U.rigida* and *H.cornea* were both cultivated in the GIA IMTA system for years, so they were already adapted to the flow-through production system.

The IMTA system for algae production included six elliptical fiberglass opaque tanks with a surface of 2.2m^2 and a volume of 2500L. These tanks were supplied with the effluents of finfishes and abalone production tanks which were previously channelled in a 11m^3 sedimentation pond to eliminate the particulate matter. The finfish species were *Argyrosomus regius* (Asso, 1801), *Sparus aurata* (Linnaeus, 1758) and *Dicentrachus labrax* (Linnaeus, 1758), bred at a low stocking density of 3.56 $kg.m^{-3}$ and high renewal rate of 24 $vol.d^{-1}$. Those breeding parameters were considered constant during the 10 experimental weeks. The nutrient contribution of the abalone production tanks to the effluent was insignificant. The ammonium concentration measured in the input of the seaweed tanks was very low during all the experimental period. The maximum amount of ammonium was recorded two hours after the feeding of finfishes and was about 4.0µM. This experimental algal culture in the effluent of the aquaculture facilities was called "EF experiment".

Simultaneously, algae where cultivated in six circular plastic tanks, with a surface of 0.2m^2 and a volume of 90L, supplied with fresh non-filtered seawater pumped from the Atlantic Ocean. The ammonium concentration in the seawater was far below the µmole per litter (undetectable by spectrophotometry). This experimental algal culture was called "SW experiment". The two experimental systems are represented in annex 1 and pictures are showed in annex 2. Algal species were cultured in triplicates in both EF and SW experiments. In each tank, a bottom linear pipeline provided aeration. This air flow allowed to ensure a correct light exposition for the macroalgae via a bottom-up continuous movement.

II.2. Experimental design

The effects of two rearing factors, algal stocking density and water flow rate, on the seaweed growth performance and nutritional quality were evaluated in this study. Each parameter was tested at low and high intensity: low (LF) and high (HF) water flow rate and low (LD) and high (HD) stocking density for *U.rigida* and *H.*cornea respectively. The algae stocking density was lower for *U.rigida* than for *H.cornea* in order to adapt this parameter to their distinct physiology and ecology. The higher stocking density for *H.cornea* allowed to regulate the presence of epiphyte seaweeds (Neori et al., 2004). Those rearing controlled parameters were crossed which constituted 4 different trials (HFLD, HFHD, LFHD, LFLD) for both EF and SW experiments, as summarised in the Table 1. The duration of the trials HFLD

and HFHD was extended to 3 weeks instead of 2 weeks because of technical and logistical problems. Water flow was regulated manually every two days. To regulate the algal stocking density, weekly harvests were conducted during the experimental period. Seaweeds were manually squeezed to remove water and weighted (Fresh Weight). The tanks were emptied and cleaned with bleach. Seaweeds were then re-inoculated at the required stocking density (depending of the experiment in progress, Table 1) in the corresponding tank. At the beginning of a new rearing trial, the tanks of the SW experiment were inoculated with algae from the EF experiment. Weekly weighing allowed thus to calculate algal growth rate.

Table 1. Summary of the different rearing trials and the corresponding water flow (HF: High Flow, LF: Low Flow) and stocking density (HD: High Density, LD: Low Density) for both EF (Effluent) and SW (Sea Water) experiments

Trial (EF & SW)	**Water Flow** (vol.d^{-1})	**Algae Stocking Density** (gFW.L^{-1})		**Dates of the experiments (EF & SW)**
		U.rigida	*H.cornea*	
HFLD	10	2.5	8	24/01 - 14/02 (3 weeks)
HFHD	10	5	12	14/02 - 07/03 (3 weeks)
LFHD	5	5	12	07/03 - 21/03 (2 weeks)
LFLD	5	2.5	8	21/03 - 04/04 (2 weeks)

II.3. Algal growth rate

GR, the algal daily growth rate, was determined using the formulae described by DeBoer et al. (1978):

$$\mathrm{GR} = 100\frac{\ln\left(\frac{N_t}{N_0}\right)}{t} \text{ expressed in \%.d}^{-1}$$

N_t is the algae fresh weight at the time t of the harvest. N_0 is the algae fresh weight inoculated at the beginning of the cultivation trial. t, expressed in days, is the time elapsed between the inoculation and the harvest (7 days).

II.4. Seaweed proximal composition

At the end of the second week of each trial homogeneous samples from each tank were collected to be analysed for nutrient composition in both EF and SW experiment. The collected samples were rinsed with distilled water to remove salt and epibionts, dried with paper towel and frozen at -80°C until their lyophilisation. All the seaweed samples were lyophilised, reduced to powder after mixing and stored in a fridge at 4°C before analysis. Due to logistical problems, samples of the HFLD trial were collected at the end of the third week.

• **Humidity:** The percentage of humidity in the lyophilised samples was determined as described in AOAC (1995): the sample was weighted before and after a 12h desiccation at 110°C. This humidity percentage allowed thus to determine the dry weight of each sample with the following formulae:

$$\mathrm{DW} = \mathrm{WW} - \frac{\mathrm{WW} \times \%\mathrm{humidity}}{100}$$

DW is the lyophilised sample calculated dry weight. *WW* is the lyophilised sample wet weight which is determinable by weighing. *%humidity* is the humidity percentage determined after desiccation of the lyophilised sample.

• **Ash content:** The ash content was determined using the method described in AOAC (1995): the sample was weighted before and after a 12h incineration at 600°C. The ash content was calculated with the following formulae:

$$\mathrm{Ashes} = 100\ \frac{Ash\ Weight}{DW}\ \text{expressed in \%DW}$$

Ash Weight is the weight of the sample after the incineration.

• **Protein content:** The protein content was determined using the Kjeldahl method as described in AOAC (1995). This consists in determining the total nitrogen composition of a sample after his digestion in concentrated sulfuric acid at 400°C. The total nitrogen is ultimately converted into borate, dosed by hydrochloric acid. Finally, the Kjeldahl factor allows to determine the protein content from the total nitrogen content. This factor is based on the fact that proteins contain an average of about 16% of nitrogen. The protein content was calculated using the following formulae:

$$\mathrm{Proteins} = \frac{(\mathrm{V_{HCl\ Sample}} - \mathrm{V_{HCl\ Blank}}) \times \mathrm{N} \times \mathrm{AW} \times \mathrm{K} \times 100}{\mathrm{DW}}\ \text{expressed in \%DW}$$

$V_{HCl\ Sample}$ and $V_{HCl\ Blank}$ are the amounts of hydrochloric acid (0.1N), in mL, poured to reach the equivalence point during the titration of the sample and the blank respectively. *N* is the normality of the hydrochloric acid, here 0.1N. *AW* is the atomic weight of the nitrogen, 14.007 $g.mol^{-1}$. *K* is the Kjeldahl coefficient which is 6.25. *DW* is the lyophilised sample calculated dry weight expressed in mg in this formulae.

• **Lipid content:** The lipid content was determined using the method described in Folch et al. (1957) which is an extraction of the total lipids in a chloroform/methanol mix (2:1) with 0.01%

BHT. The lipid content was calculated using the formulae:

$$\text{Lipids} = 100\ \frac{Lipid\ Weight}{DW} \text{ expressed in \%DW}$$

Lipid Weight is the weight of the lipids after the organic solvent evaporation.

• **Carbohydrates:** The carbohydrate content was calculated using the difference between the ash, protein, lipid relative contents and the total proximate composition (AOAC, 1995):

$$\text{Carbohydrates} = 100 - \text{Ashes} - \text{Proteins} - \text{Lipids} \text{ expressed in \%DW}$$

II.5. Environmental parameters

• **pH and temperature:** Water pH and temperature were measured two times per day (from Monday to Friday), 9:00 and 15:00, in each tank of the two experiments with a Waterproof Double Junction pHTestr 34 (Oakton®) and a U14292 Tube Thermometer Graduated (3B Scientific®; mercury thermometer graduated from -10 to 110 °C) respectively.

• **Irradiance:** The irradiance was monitored at high frequency by the meteorological station of the BEA (*Banco Español de Algas*) located on the Marine Science and Technology Park of Taliarte (BEA, personal communication, 2017). The photons flux density, PFD, expressed in µmol photons.m^{-2}.s^{-1} was measured in the "Photosynthetically Active Radiation" (PAR) spectral range which extends from 400 to 700nm.

II.6. Statistical analysis

• **Algal growth rate:** In both EF and SW experiments, 6 replicates per trial corresponding to two similar weeks of production were considered for statistical analysis (triplicate of one week plus triplicate of the other week). In the "SW" experiment the two chosen weeks were necessarily the two first experimental weeks. A series of 2-way ANOVA with permutations (1000) were used to analyse the effects of algal stocking density and water flow rate on the algal growth rate. When the homoscedasticity prerequisite was not respected, non-parametric statistical tests of Scheirer-Ray-Hare (SRH) were used. T-tests with Welch correction were used to determine the growth differences between the two cultivation experiments (EF and SW) for each algal species. When the normality prerequisite was non-respected, permutations (1000) were used. When the homoscedasticity prerequisite was non-respected, equivalent non-parametric U-tests of Mann-Whitney-Wilcoxon were used.

• **Seaweed proximal composition:** The effects of algal stocking density and water flow rate on the seaweed proximal composition (ash, protein, lipid and carbohydrate contents) were determined in the same way with a series of 2-Way ANOVA with permutations (1000) or Scheirer-Ray-Hare tests (SRH). The difference between cultivation systems (EF and SW) was analysed using t-tests (with or without permutations) or U-tests.

The normality conditions were determined using Shapiro-Wilk tests and homoscedasticity conditions were assessed using Bartlett tests. All statistical analysis were carried out with R (R Core Team).

III. Results

III.1. Algal growth rate

Growth rates of tested seaweeds are summarised in Table 2. The results of the statistical tests testing the effects of water flow rate and algal stocking density on the seaweed growth rates are summarised in Table 3.

Table 2. Growth rate (Mean ± SD; average of two experimental weeks) of *U.rigida* and *H.cornea* in both experimental systems, EF and SW, for each condition of flow and density

Growth Rate (%.d^{-1})	EF experiment				SW experiment			
	HFLD	HFHD	LFHD	LFLD	HFLD	HFHD	LFHD	LFLD
U.rigida	8.6 ± 0.5	4.5 ± 1.0	3.3 ± 1.3	5.4 ± 1.8	10.4 ± 0.8	5.4 ± 1.2	6.0 ± 1.2	8.6 ± 2.0
H.cornea	1.2 ± 0.9	0.8 ± 0.4	1.5 ± 1.3	1.7 ± 0.9	0.7 ± 0.9	2.4 ± 1.4	3.7 ± 0.5	3.0 ± 0.3

The algal growth rate as a function of rearing trial, species and experiment is showed in figure 1. Growth rate was significantly higher in the SW than in the EF experiment for both *U.rigida* ($P<0.01$) and *H.cornea* ($P<0.001$) with an exception noticed for *H.cornea* in the HFLD trial (Table 2, Fig. 1). In the EF experiment, algal stocking density ($P<0.001$) and water flow rate ($P<0.001$) significantly impacted *U.rigida* growth rate (Table 3). It substantially increased with decreasing density, differently according to the water flow rate (two factors interactions significant at the 10% treshold), being 1.9 (high flow) to 1.6 (low flow) fold higher under low density conditions. Under the same density condition, *U.rigida* growth rate significantly

increased with increasing water flow rate (Fig. 1).

Table 3. Determination of the effects of water flow rate (F) and algae stocking density (D) and their interaction (F*D) on the growth rate. Statistical test used is specified: 2-way ANOVA with permutations (AOVP) or non-parametric test of Scheirer-Ray-Hare (SRH). Significant probability values are indicated in bold: P<0.05(*), P<0.01(**), P<0.001(***)

Effluent	*U.rigida*	*H.cornea*	**Sea water**	*U.rigida*	*H.cornea*
Flow (F)	**<0.001*** **	0,181	Flow (F)	0,380	**0.006** **
Density (D)	**<0.001*** **	0,571	Density (D)	**<0.001*** **	**0.010***
F*D	0.073	0,980	F*D	**0.043***	0,817
Statistical Test	*AOVP*	*AOVP*	*Statistical Test*	*AOVP*	*SHR*

In the SW experiment, *U.rigida* growth rate was only significantly affected by algal stocking density (P<0.001). As in the EF experiment, growth rates increased with decreasing density, differently according to the water flow rate (two factors interactions, P<0.05), being 1.9 (high flow) to 1.4 (low flow) fold higher under low density conditions. No significant trend emerged for *H.cornea* growth rate in the EF experiment. Conversely, in the SW experiment, both algal stocking density (P<0.05) and water flow rate (P<0.01) significantly affected *H.cornea* growth rate. Growth rate increased with decreasing water flow rate and increasing algal stocking density with a minimum of 0.7±0.7%.d^{-1} observed in HFLD and a maximum of 3.7±0.5%.d^{-1} reached in LFHD. Weekly growth rates during the experimental period are shown in annex 3.

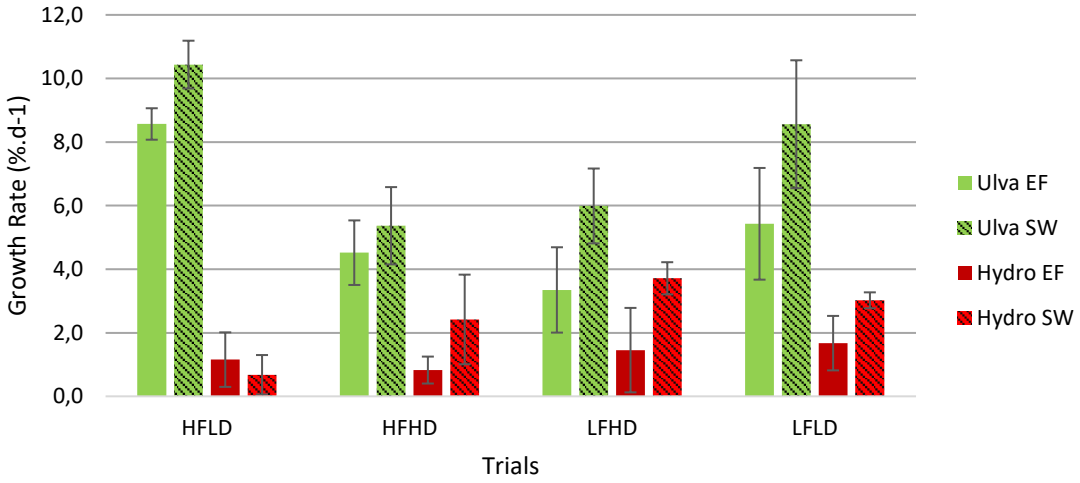


Figure 1. Growth rate (Mean ± SD; average of two experimental weeks) of *U.rigida* and *H.cornea* in both experimental system, EF and SW, for each condition of flow and density

III.2. Seaweed proximal composition

Biochemical proximal compositions of tested seaweeds are summarised in Table 4. The results of the statistical tests testing the effects of water flow rate and algal stocking density on seaweed biochemical composition are gathered in the Table 5.

Table 4. Proximate composition (Mean ± SD) of *U.rigida* and *H.cornea* in both experimental systems, EF and SW, for each condition of flow and density at the end of the second experimental week ([1]third in the case of HFLD)

***U.rigida* (%DW)**	EF experiment				SW experiment			
	HFLD[1]	HFHD	LFHD	LFLD	HFLD[1]	HFHD	LFHD	LFLD
Ashes	30.7±1.9	24.3±0.1	26.2±1.1	28.9±1.8	29.4±0.4	27.8±1.2	25.3±2.4	30.7±1.7
Proteins	15.9±1.0	16.5±0.6	11.1±0.8	8.7±0.4	5.0±0.3	6.8±1.1	6.9±0.6	6.1±0.5
Lipids	3.3±0.1	3.9±0.2	2.9±0.3	2.6±0.1	2.5±0.3	1.6±0.4	2.5±0.3	2.3±0.2
Carbohydrates	50.0±1.2	55.4±0.8	59.7±0.3	59.9±1.8	63.1±0.7	63.7±0.6	65.3±2.6	60.9±1.5
***H.cornea* (%DW)**	EF experiment				SW experiment			
	HFLD[1]	HFHD	LFHD	LFLD	HFLD[1]	HFHD	LFHD	LFLD
Ashes	41.3±0.7	39.0±2.2	37.7±3.3	37.1±2.0	33.5±1.9	32.7±0.5	35.2±0.7	31.2±1.6
Proteins	15.5±1.6	14.3±1.0	12.6±0.7	10.4±0.7	7.6±0.3	8.4±0.2	9.1±0.6	8.2±0.5
Lipids	2.9±0.5	2.2±0.5	3.0±0.6	3.0±0.2	1.5±0.1	1.9±0.2	2.3±0.2	2.8±0.4
Carbohydrates	40.3±1.9	44.5±3.8	46.7±2.1	49.5±1.8	57.5±2.0	57.0±0.5	53.5±0.9	57.8±1.8

Table 5. Determination of the effects of water flow rate (F) and algae stocking density (D) and their interaction (F*D) on the ash, protein, lipid and carbohydrate contents. Statistical tests used were 2-way ANOVA with permutations (AOVP, 1000 permutations). Significant probability values (P) are indicated in bold: P<0.05(*), P<0.01(**), P<0.001(***)

U.rigida	Ashes	Proteins	Lipids	Carbohydrates
Effluent	P	P	P	P
Flow (F)	0.843	**<0.001*** **	**<0.001*** **	**<0.001*** **
Density (D)	**0.001****	**0.007****	**0.008****	**0.008****
F*D	0.068	**0.033***	0.592	**0.010***
Sea Water	P	P	P	P
Flow (F)	0.667	0.133	0.116	0.655
Density (D)	**0.001****	**0.014***	0.114	**0.017***
F*D	0.077	0.160	**0.011***	0.090
H.cornea	Ashes	Proteins	Lipids	Carbohydrates
Effluent	P	P	P	P
Flow (F)	0.063	**<0.001*** **	0.120	**0.010***
Density (D)	0.667	0.308	0.262	0.594
F*D	0.491	**0.017***	0.250	**0.035***
Sea Water	P	P	P	P
Flow (F)	1.000	**0.019***	**<0.001*** **	0.146
Density (D)	0.077	**0.009****	0.863	**0.021***
F*D	**0.010***	1.000	**0.016***	0.066

• **Ash content:** The ash content as a function of rearing trial, species and experiment is represented in figure 2. Ash content was significantly higher in the EF than in the SW experiment for *H.cornea* (P<0.001) whereas no significant difference was demonstrated for *U.rigida*. In the EF experiment, *U.rigida* ash content was significantly affected by algal stocking density (P<0.01; Table 5) being 1.1 to 1.3 fold higher under low density conditions (Table 5, Fig. 2).

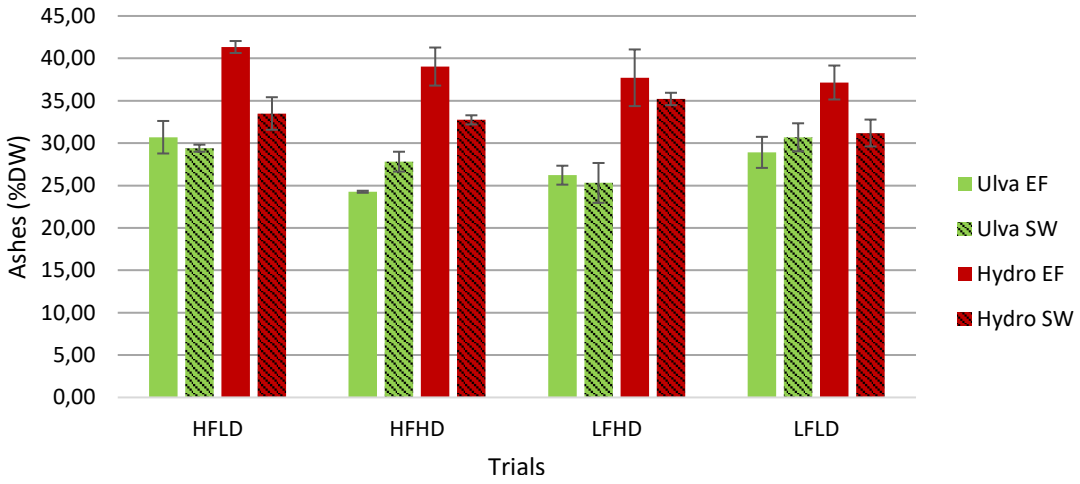


Figure 2. Ash content (Mean ± SD) of *U.rigida* and *H.cornea* in both experimental system, EF and SW, for each condition of flow and density at the end of the second experimental week (third in the case of HFLD).

In the SW experiment, *U.rigida* ash content was also significantly affected by stocking density (P<0.01) being 1.1 to 1.2 fold higher at low densities. In the EF experiment, no significant trend was observed for *H.cornea* ash content which still appeared to increase with water flow rate (significant effect at 10% threshold; Table 5) with a maximum of 41.3±0.7DW reached at HFLD. On the contrary, in the SW experiment, whereas the two factors did not have significant effect separately, their interaction significantly affected (P<0.05) the *H.cornea* ash content. While at high flow ash content was similar between densities, at low flow it was about 4%DW higher at high density.

• **Protein content:** The protein content as a function of rearing trial, species and experiment is illustrated in figure 3. Protein content was significantly higher in the EF than in the SW experiment for both *U.rigida* (P<0.001) and *H.cornea* (P<0.01). In the EF experiment, *U.rigida* protein content was significantly (Table 5) affected by both water flow rate (P<0.001) and algal stocking density (P<0.01). It substantially increased with water flow rate, differently according to the stocking density (significant two factor interaction, P<0.05), being 1.5 (high density) to 1.8 (low density) fold higher under high flow conditions. Under the same density condition,

U.rigida protein content increased with algal stocking density. In the SW experiment, *U.rigida* protein content was significantly affected by algal stocking density ($P<0.05$) being 0.8 to 1.8%DW higher at high densities. In the EF experiment, *H.cornea* protein content was significantly affected by water flow rate ($P<0.001$). It substantially increased with water flow rate, differently according to the stocking density (significant interaction of the two factors, $P<0.05$), being 1.1 (high density) to 1.5 fold higher at high flows. Contrary, in the SW experiment, *H.cornea* protein content was significantly affected by both water flow rate ($P<0.05$) and algal stocking density ($P<0.01$). It increased with density and decreasing water flow rate to reach a maximum of 9.1±0.6%DW at LFHD and a minimum of 7.6±0.3%DW at HFLD (Fig. 3).

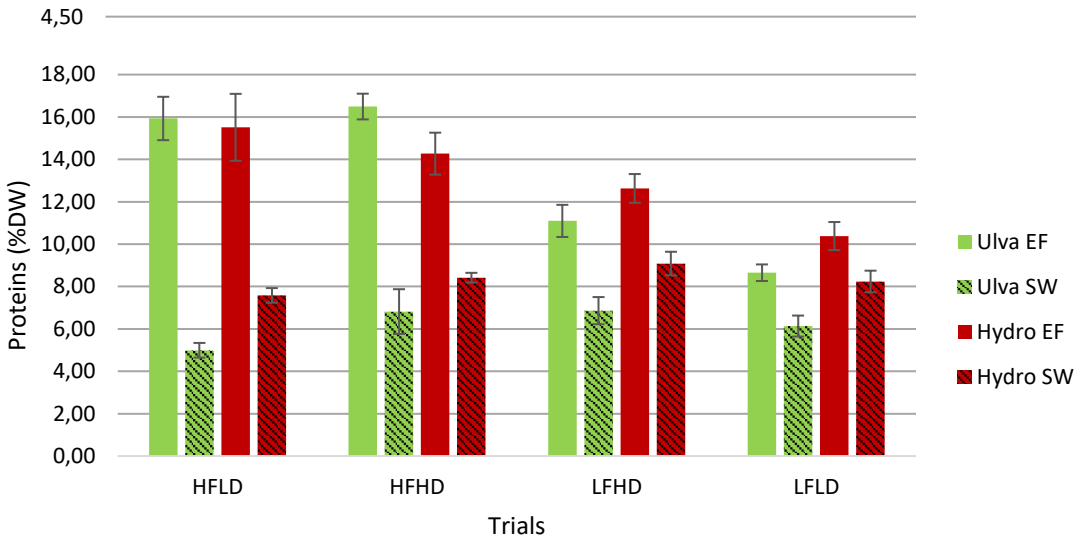


Figure 3. Protein content (Mean ± SD) of *U.rigida* and *H.cornea* in both experimental system, EF and SW, for each condition of flow and density at the end of the second experimental week (third in the case of HFLD).

• **Lipid content:** The lipid content as a function of rearing trial, species and experiment is shown in figure 4. Lipid content was significantly higher in the EF than in the SW experiment for both *U.rigida* ($P<0.001$) and *H.cornea*. ($P<0.001$). In the EF experiment, *U.rigida* lipid content was significantly affected by both water flow rate ($P<0.01$) and stocking density ($P<0.01$; Table 5). It substantially increased with water flow rate and, to a lesser extent, with algal stocking density with a maximum of 3.9±0.2%DW reached at HFHD and a minimum of 2.6±0.1 at LFLD (Fig. 4). In the SW experiment, no significant trend was detected for *U.rigida* lipid content according to water flow rate and stocking density separately. However, in this experiment, it was significantly affected by algal stocking density and water flow rate interaction ($P<0.05$). Indeed, while *U.rigida* lipid content was similar between densities at low flow, at high flow it was 1.6 fold lower at high density (HFHD). In the EF experiment, no

significant trend was demonstrated for *H.cornea* lipid content (Table 5). In contrast, In the SW experiment, it was significantly affected by water flow rate ($P<0.001$). As a matter of fact, *H.cornea* lipid content increased with decreasing water flow rate, differently according to stocking density (significant interaction of the two factors, $P<0.05$), being 1.2 (high density) to 1.9 (low density) fold higher under low water flow conditions (Fig. 4).

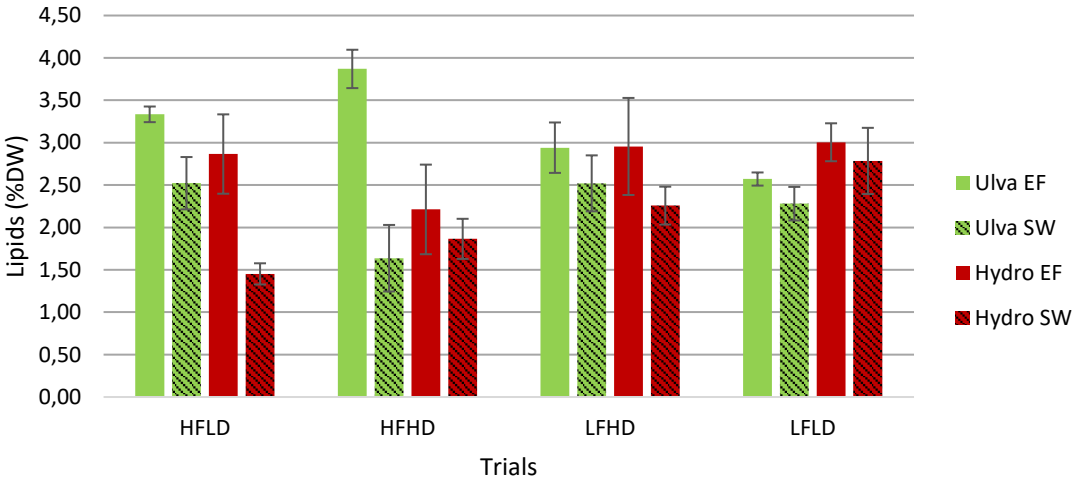


Figure 4. Lipid content (Mean ± SD) of *U.rigida* and *H.cornea* in both experimental system, EF and SW, for each condition of flow and density at the end of the second experimental week (third in the case of HFLD).

• **Carbohydrates:** The carbohydrate content as a function of rearing trial, species and experiment is showed in figure 5. Carbohydrate content is significantly higher in the SW than in the EF experiment for both *U.rigida* ($P<0.001$) and *H.cornea* ($P<0.001$). In the EF experiment, *U.rigida* carbohydrate content was significantly affected by both water flow rate

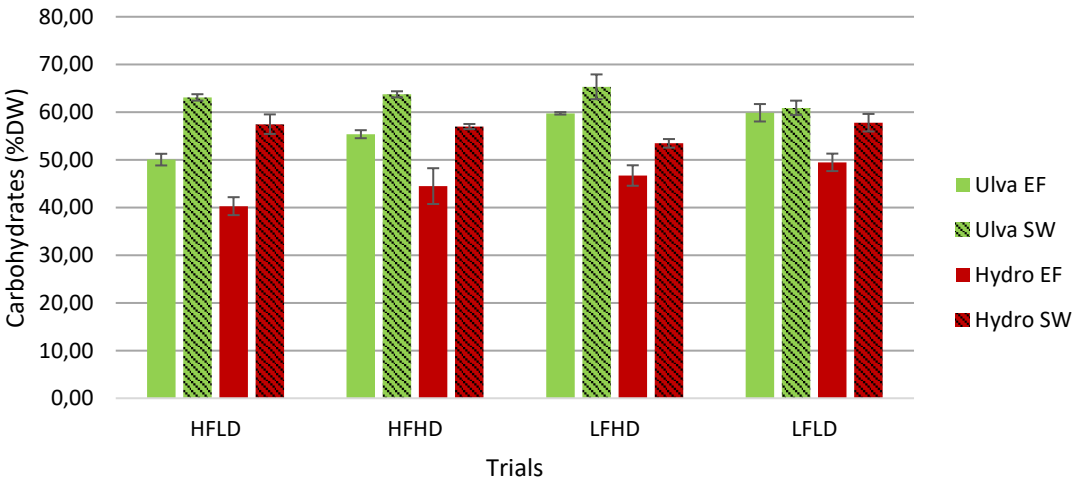


Figure 5. Carbohydrate content (Mean ± SD) of *U.rigida* and *H.cornea* in both experimental system, EF and SW, for each condition of flow and density at the end of the second experimental week (third in the case of HFLD).

($P<0.001$) and stocking density ($P<0.01$, Table 5). It substantially increased with decreasing water flow, differently according to the stocking density (significant interaction of the two

factors, P<0.05), being 4.3%DW (high density) to 9.9%DW higher (low density) at low water flows. At high Flow, the carbohydrate content increased with stocking density whereas no clear effect was observed at low water flows. In the SW experiment, only the stocking density had a significant effect (P<0.05) on *U.rigida* carbohydrate content. In fact, it slightly increased with increasing stocking density (Fig. 5). In the EF experiment, *H.cornea* carbohydrate content was significantly affected by the water flow rate (P<0.05). Indeed, it substantially increased with decreasing water flow rate, differently according to the algal stocking density (significant interaction of the two factors, P<0.05), being 2.2%DW (high density) to 9.2%DW higher (low density) under low water flow conditions. In contrast, in the SW experiment, *H.cornea* carbohydrate content was significantly affected by algal stocking density (P<0.05). In fact, it slightly increased with decreasing stocking density, differently according to the water flow rate (two factor interaction significant at 10% threshold; Table 5), being 0.5 (quite similar at high flow) to 4.3%DW (high flow) higher at low densities (Fig. 5).

III.3. Environmental parameters

• **pH and temperature:** Average temperature and pH recorded during the morning and the afternoon in each trial are shown in figure 6. Morning average pH was practically equal between trials and experiments ranging between 8.7 and 8.9. In contrast, important variations were recorded during the afternoon with average pH ranging between 9.1 and 10.0. It seems increase with increasing stocking density and decreasing water flow for both species in both EF and SW experiment. During the afternoon, pH was globally similar for *U.rigida* between the two experiments whereas it was globally higher for *H.cornea* in the SW experiment. Daily temperature was much variable in the SW than in the EF experiment with the minimal values recorded during the morning and the highest during the afternoon. Temperature was similar between the *U.rigida* and *H.cornea* replicates of a same experiment except in the EF experiment where afternoon temperature were much higher in the *H.cornea* tanks.

• **Irradiance:** The maximum PFD recorded during the experimental period was 442µmol photons.m^{-2}.s^{-1} and the average at noon was 214±78µmol photons.m^{-2}.s^{-1}.

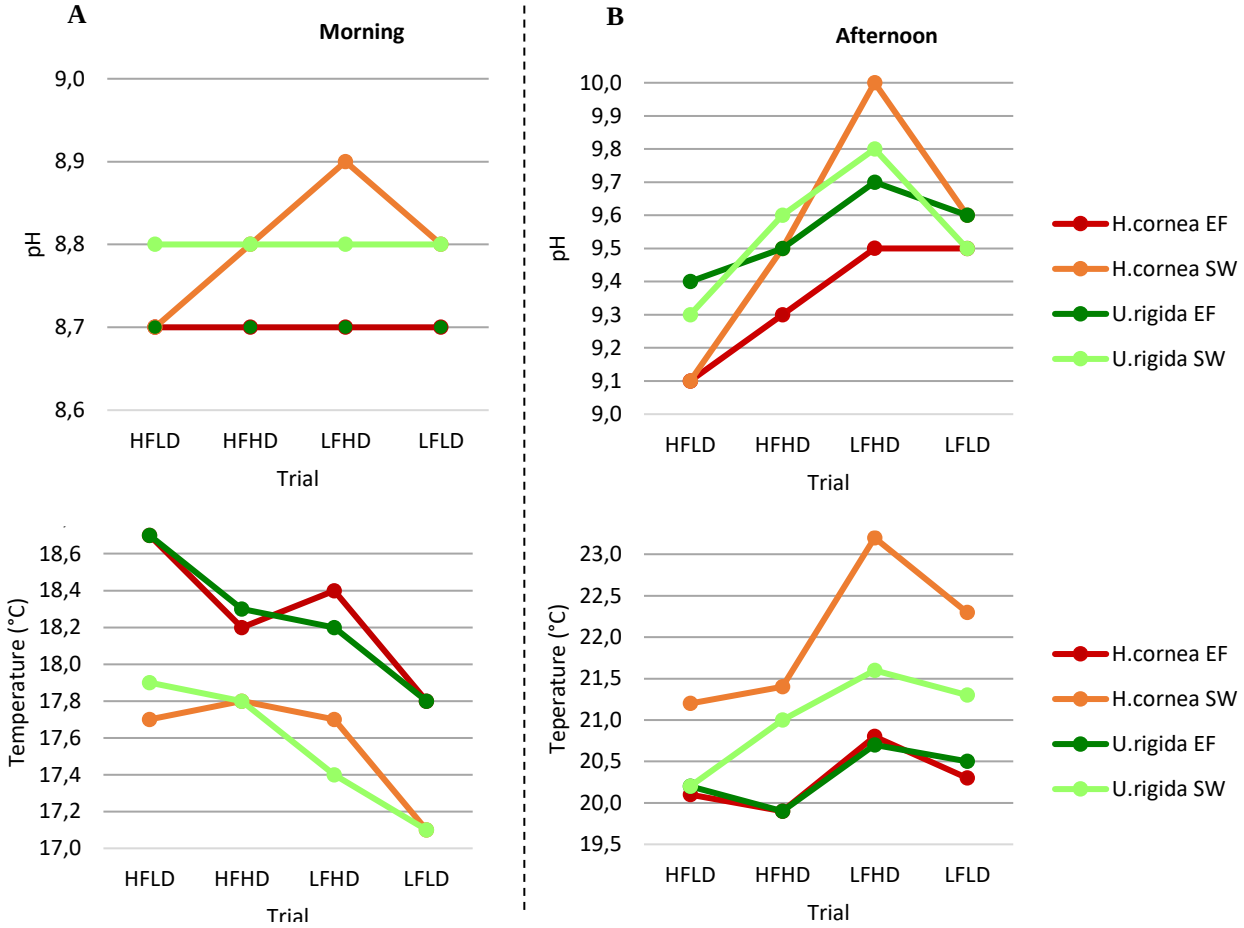


Figure 6. Average pH and Temperature per Trial recorded at 9:00 (A) and 15:00 (B) for each species in each experiment (EF and SW).

IV. Discussion

It is well known that nitrogen (N) is the main chemical element that limits primary productivity in marine ecosystems by its availability (Howarth, 1988). This is especially the case for macroalgae growing in flow-through cultivation systems: that is well documented for both *Gracilaria* (genus genetically close to *Hydropuntia*) and *Ulva* genus (DeBusk et al., 1986; Rosenberg and Ramus, 1982, Ryther et al., 1981; Lapointe and Ryther, 1978, 1979; DeBoer et al., 1978). For those genus, ammonium (NH_4^+) is the inorganic nitrogen form most widely used and supports the highest algal growth rates. In the EF experiment, the maximum diurnal concentration of ammonium was limited to 4µM which is very low compared to high concentrations often recorded in nutrient-rich aquaculture wastewaters (Table 6). This can be related to the low fish stocking density with a relatively high dilution ratio in contrast with previous studies (Figueroa et al., 2012; Viera et al., 2009; Neori et al., 2000; Gómez-Pinchetti et al., 1998; Jiménez del Río, 1996, 1994). Generally other macronutrients essentials for algal

growth such as inorganic carbon (C) and phosphates are found in excess in aquaculture wastewaters. However, they were not measured in our experiments. In the SW experiment, NH_4^+ concentration was undetectable by the colorimetric Solórzano method (Solórzano, 1969) and consequently far below the µmole per litter which is in accordance with the oligotrophic nature of the Canarian seawater (Arístegui et al., 2001). Light and temperature are the other main environmental factors determining the macroalgae productivity in natural environments and flow-through cultivation systems (Abreu et al., 2011; Neori et al., 2004; Robertson-Andersson, 2003; Rosenberg and Ramus, 1982) by their control of energy input (photosynthesis) and metabolic rates respectively. In our experiments, photons flux densities were relatively low compared to those recorded in other studies conducted in the Technology Park of Taliarte (Gran Canaria). However, wavelength range of measurement and seasons were often not specified which complicates comparisons. Despite this, it is possible to consider that irradiances were globally equal or lower in our study (from January to April 2017) than those in these previous studies conducted outdoor or in greenhouse in the same geographical area, potentially during seasons where irradiances were higher (Viera et al., 2016, 2011, 2009, 2005b; Gómez Pinchetti et al., 1998; Jiménez del Río, 1996, 1994). From late January to early April, water temperature ranged between 17.8 (9:00) and 20.7°C (15:00) in the EF experiment. This is much lower than water temperatures recorded by Viera et al. (2009), which ranged between 20.3 (9:00, December) and 24.4°C (18:00, August), and Viera et al. (2005b) which ranged from 19 to 23°C. This could be explained by the different water volumes which influence heat

Table 6. Summary of various studies investigating the effects of different rearing conditions on *H.cornea* and *U.rigida* growth parameters.

H.cornea	NH_4^+ (µM)	Stocking density		Water Flow ($vol.d^{-1}$)	GR ($\%.d^{-1}$)
		$gFW.L^{-1}$	$Kg.m^{-2}$		
Viera et al. (2009)	5.6-10.16	4	2.9	4-24	2.69-5.08
Viera et al. (2005b)	70	6		8	5.08
U.rigida	NH_4^+ (µM)	Stocking density		Water Flow ($vol.d^{-1}$)	GR ($\%.d^{-1}$)
		$gFW.L^{-1}$	$Kg.m^{-2}$		
Viera et al. (2009)	5.6-10.16	1	0.7	4-24	3.14-8.63
Viera et al. (2005b)	70	2		8	10.24
Gómez Pinchetti et al. (1998)	27.8-94.5	2.5		8	12-16
Jiménez del Rio et al. (1996)	50-66.7	2.5	1.1	2-12	6.8-9.6

exchanges. Thus, in the SW experiment, with very low water volume (90L), water temperatures

variations were higher (Fig. 8) with the lowest and highest recorded temperatures. Temperatures were higher in the *H.cornea* SW tanks likely due to a better light exposition during the afternoon (Annex 1).

Jiménez del Rio et al., 1996 describe the existence of a proportional relation between algal growth rate and increasing water flow rate until an optimum were nutrients are in kinetic saturation for seaweeds. In fact, the water flow controls the essential nutrients supply to the seaweeds per time unit, for instance NH_4^+ or inorganic carbon (Jiménez del Rio et al., 1996, Debusk et al., 1986; Deboer et al., 1986; Lapointe and Ryhther, 1979). It appears that flow rate should be adapted to the wastewater chemical composition to provide enough nutrient income. For example, Viera et al. (2009) tested various water flow rates (4, 12, 24vol.d^{-1}) and observed the best growth rates at 24vol.d^{-1} for both *U.rigida* and *H.cornea*. In contrast, with higher ammonium supplies and comparable stocking density (Table 6), Jiménez del Rio et al. (1996) recorded the kinetic saturation at only 4vol.d^{-1}. This factor also interacts with stocking density which increases the competition for resource access such as inorganic carbon, macronutrients (e.g. NH_4^+), micronutrients and light (Viaroli et al., 1996; Jiménez del Río, 1996, 1994; Debusk et al., 1986; Duke et al., 1986). In the present study, under much lower NH_4^+ conditions and stocking densities equal or greater than previous studies (Table 6), the highest water flow rate tested was only 10vol.d^{-1} probably limiting the nutrient availability. Nevertheless it only affected *U.rigida* growth rates which was 1.4 to 1.6 fold higher with doubling water flow. This supports the hypothesis of N-limitations in our experiments. Parker (1981) showed that increase water flow rate can compensate the N-limitation for *Ulva lactuca* (L., 1753) when light is non-limiting. In fact, Ryther et al. (1981) showed that light availability impacted the N-uptake. In the context of our study, this implies that light was not strongly limiting for *U.rigida.* while it could be always limiting for *H.cornea* whatever the stocking density. It is also possible that water flow rate was always insufficient to provide enough nutrient for the important *H.cornea* biomasses. In the case of *U.rigida,* N-limitation hypothesis was supported by the fact that bleaching of the thalli occurred at low water flows. This indicating that *U.rigida* used and strongly depleted its N-reserves (Gómez Pinchetti et al., 1998) under low flow conditions. The high pH values recorded during the experiments were also consistent with the hypothesis of strong nutrient-limiting conditions in our experiments. Indeed, in the EF experiment, average pH was about 8.7 at 9:00 and ranged from 9.15 to 9.70 at 15:00 (Fig. 6). In comparison, Jiménez del Rio et al. (1994) recorded pH values about 8.1 at 9:00 and 9.1 at 15:00 in their *U.rigida* cultivation system. Authors showed that this high pH characterizes carbon limiting conditions for seaweeds since the main forms of dissolved inorganic carbon usable by seaweeds (e.g. CO_2)

are much less available. This pH rise is linked to photosynthesis using the inorganic carbon supplies that are insufficient to replenish the resource stock. Jiménez del Rio et al. (1994) demonstrated that suitable carbon supply can be maintained, even at high densities, by increasing the water flow rate. In the effluent experiment, seaweeds appeared to be strongly carbon limited with higher pH values than those reported by Jiménez del Rio et al. (1994) but also by Neori et al. (2000; pH:8.5-8.9) for C-limited *U.lactuca* and *Gracilaria conferta* (Montagne, 1846). This limitation appeared to be stronger at high densities and low water flows indicating a stronger competition for resources access in these experiments. *U.rigida* growth rates were higher than those reported for *H.cornea* as it is normally recorded due to its opportunistic nature (Jiménez del Rio et al., 1996, Rosenberg and Ramus, 1982). This higher metabolic rate explain the higher pH observed during the afternoon in the *U.rigida* EF tanks. In the SW tanks, pH were the highest for *H.cornea* which is likely due to higher photosynthetic rates since the temperatures and light exposition where more elevated in these tanks.

In the EF experiment, growth rates decreased with increasing density for *U.rigida*. This relation was already reported for *U.rigida, H.cornea, Ulva spp.* and *Gracilaria spp.* cultivated in flow through production systems (Robledo et al., 2012; Abreu et al., 2010; Viera et al., 2009; Robertson-andersson, 2003; Jiménez del Rio et al., 1996, 1994). This is principally due to the self-shading phenomenon which reduces the amount of light received by the thallus for its photosynthesis with increasing density in addition to increasing competition for nutrient access (Lapointe and Tenore, 1981). Conversely, at low stocking density, photoinhibition of the growth could occur. That is why an optimal stocking density exists for each algal species where the growth is optimal. In two different studies, Jiménez del Rio et al. (1996, 1994) found *U.rigida* optimal stocking density of $1g.L^{-1}$ at $8vol.d^{-1}$, with a GR of $12.6\%.d^{-1}$, and $2.5g.L^{-1}$ at $12vol.d^{-1}$, with a GR of $9.6\%.d^{-1}$. Alternatively, Viera et al. (2009) found $1g.L^{-1}$ as optimal density at $24vol.d^{-1}$ with growth rates reaching $8.63\%.d^{-1}$. This seems perfectly in accordance with *U.rigida* growth rate registered in our study which reached a maximum of $8.6\%.d^{-1}$ at $2.5g.L^{-1}$ for a water flow of $10vol.d^{-1}$. Viera et al. (2009) found that *H.cornea* growth rate reached a maximum of $5.08\%.d^{-1}$ at $4g.L^{-1}$ and decreased until $3.46\%.d^{-1}$ at $6g.L^{-1}$ under $24vol.d^{-1}$. In contrasts, in our study we found no significant different for *H.cornea* growth rate between 8 and $12g.L^{-1}$ with growth rates staying about $1\%.d^{-1}$ and highly variable probably due to the higher stocking density and lower nutrient inflow. The stocking densities applied are unsuitable in the conditions of our study with *H.cornea* being N-limited and light-limited even at $8g.L^{-1}$. Robertson-andersson (2003) stated that there is a linear relation between optimal stocking density and size of the tanks used for cultivation. He also showed that consequently,

growth rate is more reliable with stocking densities expressed in fresh weight per surface unit than per volume unit. In the present experiment, the tested *H.cornea* stocking densities were 9.1kg.m^{-2} (8g.L^{-1}) and 13.6kg.m^{-2} (12g.L^{-1}) which is clearly higher than the stocking densities used in previous studies under more favourable conditions (Table 6). The same effect is observed for *U.rigida* with applied stocking densities of 2.8kg.m^{-2} (2.5g.L^{-1}) and 5.7kg.m^{-2} (5g.L^{-1}) which is above the optimal densities of 0.7 (Viera et al., 2009) and 1.1kg.m^{-2} (Jiménez del Rio et al.,1996) noticed in previous studies. According to that principles, under so unfavourable rearing conditions, it seems unlikely that *U.rigida* sustained growth rates comparable to those observed in the literature (Table 6). For example, in this present study, *U.rigida* growth rate reached a maximum in February equal to that observed by Viera et al. (2009) in summer (higher irradiance) at lower density with higher water flow rate and ammonium supplies. Similarly, they are in the range of the growth rates observed by Jiménez del Río (1996) in summer under more favourable rearing conditions. In those more suitable conditions, authors showed that *U.rigida* growth rate started to decrease for densities higher that 0.7 and 1.1kg.m^{-2} whereas we found comparable growth rates at 2.8kg.m^{-2}. This likely reflects weighing mistakes done in the present study. Indeed, the manual spin-drying, used in our experiments, did not eliminate all the water surplus in the seaweed biomass. In previous studies spin-drying was often done using a centrifugate (Figueroa et al., 2012; Viera et al., 2009, 2005b). Hence, I our study the produced biomass was likely overestimated each week. This is especially true for *U.rigida* that have a morphology retaining large amount of water. Indeed, for *H.cornea*, with probably lower weighing variability, growth rates stayed very low and close to zero which was in accordance with light, nitrogen and carbon limitations. Under not N-limiting conditions, Neori et al. (2000) observed crash stocks of *G.conferta* for stocking densities of 5-13kg.m^{-2}. Abreu et al. (2011) showed that for cultivated *G.vermiculophylla*, which has a morphology close to *H.cornea*, only 15% of incident solar irradiance reaching 15cm depth in the water column at 7kg.m^{-2} causing important light limitation. The fact that water flow do not had any effect on *H.cornea* growth rate and no bleaching occurred suggest that light was probably the most limiting factor in the present study which shows that tested densities, expressed in kg.m^{-2}, were clearly not suitable.

Some previous studies testing the effect of algal transfer from N-enriched wastewater to N-depleted sea water showed that in such conditions growth rate was directly linked with the algal reserves (Figueroa et al., 2012; Gómez Pinchetti et al., 1998; Jiménez del Río et al., 1994). Thus, Lapointe and Ryther (1979) showed that results of production in N-depleted sweater reflecting more the amount of nutrient reserves in the inoculated algae than the influence of the

rearing treatment. Consequently, it seems difficult to show a link with rearing conditions (water flow, stocking density) and algal growth rates in the SW experiment. Gómez Pinchetti et al. (1998) did similar experiments with *U.rigida* cultivated at 2.5g.L^{-1}, 8vol.d^{-1} and 27.78-94.44µM-NH_4^+ during one month prior to be transferred in N-depleted sea water (<3µM-NH_4^+). Authors found that when transferred in seawater, *U.rigida* growth rate falls from 12-16%.d^{-1} to less than 2%.d^{-1} in few days with total stop of the growth after 5 days. Strong bleaching appeared after 14 days. Lapointe et al. (1979) did a similar study with *Gracilaria foliifera* **(**Forsskål) Børgesen, 1932 and showed that growth stayed as high as in the N-enriched medium during the first 6 days in the seawater treatment before to decrease. Ryther et al. (1981) showed that *Gracilaria tikvahiae* (McLachlan, 1987) could growth at a maximum yield in N-depleted seawater during two weeks after being soaked in very high nutrients conditions for 24 hours. No one studies shown higher growth rates in the seawater treatment than in the N enriched treatment as we found in the present study for *U.rigida* and *H.cornea*. Nevertheless, in these studies, both sea water and enriched cultivation experiments were done in similar rearing conditions. In the present study, *H.cornea* stocking densities were 3.6kg.m^{-2} (8g.L^{-1}) and 5.4kg.m^{-2} (12g.L^{-1}) instead of 9.1 and 13.6kg.m^{-2} in the EF experiment. *U.rigida* stocking densities were 1.1kg.m^{-2} (2.5g.L^{-1}) and 2.2kg.m^{-2} (5g.L^{-1}) instead of 2.8 and 5.7kg.m^{-2} in the EF experiment. Consequently, they were much favourable in the SW experiment. Furthermore, temperatures were higher during the afternoon as well as light exposition due to the sun position (annex 1) and the relatively higher transparency of the SW tanks. Yet, Growth rate is correlated with this parameter for many algae species in flow-through cultivation systems (Abreu et al., 2011). It is well known that nitrogen reserves are quickly used to sustain high growth rates when the temperature and light conditions become more suitable. This is commonly observed for macroalgae in natural environments but also in land-based production systems for both *Gracilaria spp.* and *Ulva spp.* (Duke et al., 1986; Rosenberg and Ramus, 1982; Chapman and Craigie, 1977). It is possible that this occurred for both species when transferred in the SW cultivation medium explaining the higher growth rates recorded. However, in all the experimental conditions, bleaching occurred only 1 week after the beginning of the SW experiment indicating that N-reserves were totally depleted. Gómez-Pinchetti et al. (1998) found that *U.rigida* growth rate stopped more than 1 week before the bleaching event whereas in our experiments growth rates stayed high one or two weeks after even if they seemed follow a slight decreasing pattern over time (annex 3). However, growth rates were really high and it is unlikely that both *U.rigida* and *H.cornea* possessed full N-reserves in the EF experiment (poor in nutrient) prior to be inoculated in the SW experiment. It was likely not the case as

shown by the bleaching event occurring in the effluents for *U.rigida*. Consequently, growth rate was certainly overestimated in this experiment. In the SW experiment, inoculated algal biomass were very low (225-1080g), Therefore, weighing was probably more affected by water staying in the algal biomass. Moreover, the scale used had a very low precision (about 0.1kg) which probably accentuated the weighing variability. It was demonstrated that *U.rigida* growth rate increased with decreasing density in the SW experiment. This seems not likely because one of the highest growth rate was observed in the last experiment (LFLD) which was inoculated with algae in poor physiological condition originated from the EF bleaching event. This could be related to higher light availability at low density but, more likely, growth rate was more overestimated at low than at high inoculated densities (stronger weighing variability). In contrasts, in the SW experiment, *H.cornea* growth rate increased with increasing stocking density. In the *H.cornea* tanks, high epiphytic development of algae from the genus *Cladophora* were noticed. The increasing stocking density could beneficiate to *H.cornea* by reducing the epiphyte biomass by the means of friction or self-shading (Hanisak and Ryther, 1984). *H.cornea* growth rate was also enhanced with decreasing water flow. Maybe this was due to an increase in the nutrient residence time in the tank favouring their absorption. Nevertheless, these relations could only be apparent. In the case of *H.cornea*, in addition to weighing variability, it is possible that the estimated growth rates only reflected the epiphyte growths. The highest growth rates were registered for last trials (March-April) where epiphytic development seemed to be stronger potentially following the increasing irradiance or natural cycle in the environment since seawater was not filtered. Moreover, recorded pH values in the SW experiments were globally higher than in the EF experiments which demonstrated a more important carbon limitation (limitation higher at high densities but especially at low flow rates). To study the difference of growth efficiency between aquaculture effluent and N-depleted seawater, it seems more appropriate to use wild collected algae already adapted to the natural local conditions as it was done in some studies. In this case, Authors showed that flow rate was the main factor controlling the algae production (Rosenberg and Ramus, 1982, Lapointe and Ryther, 1979, 1978) whereas it was not the case here.

The strong existing relationship between the algal protein content and the nitrogen availability appears clearly in our experiments. First of all, protein contents were 1.4-3.2 and 1.3-2.0 fold higher in the EF experiment than in the SW experiment for both *U.rigida* and *H.cornea*. In addition, the water flow rate positively increased the protein content in the EF experiment for both species by increasing the nitrogen supply. This relation with water flow rate was reported for all the N-organic compounds (proteins, pigments, free amino acids) in

H.cornea, U.rigida, Gracilaria spp. and *Ulva spp.* cultivated in flow through production systems (Figueroa et al., 2012; Gómez Pinchetti et al., 1998; Rosenberg and Ramus, 1982; Lapointe and Ryther, 1979). Here the Kjeldhall method not allowed to differentiate these different N-coumpounds and considered them as proteins (AOAC, 1995). In the present study, this relation shows the major role played by water flow rate in the maintenance of algal nutritional quality in poor nutrient conditions. In the EF experiment (<4µM-NH_4^+), protein content was 8.7-15.9%DW and 10.4-15.5%DW for *U.rigida* and *H.cornea* respectively. In comparison, at 10-30M-NH_4^+, Viera et al. (2016) found 18,8%DW and 25%DW for both species whereas in previous studies the same authors found 29.35%DW and 33.76%DW at 10-400µM-NH_4^+ (Viera et al., 2011) and 33-41%DW at 70µM-NH_4^+ (Viera et al., 2005b) for *U.rigida* and *H.cornea* respectively. This illustrates the importance of ammonium availability link with water flows which were 12, 4 and 8vol.d^{-1} respectively in these studies. The majority of macroalgae are able to store nitrogen when it is present in excess in the ambient environment and other factors limit the growth. Nitrogen cans be stored in the form of inorganic compounds, pigments, free amino acids or proteins (Figueroa et al., 2012; Pinchetti et al., 1998; Rosenberg and Ramus, 1982; Lapointe and Ryther, 1979). This explains the high proteins content regularly observed in the algae cultivated in fish pond nitrogen-rich wastewaters. In the EF experiment, nitrogen supply seemed sufficient to allow the growth of seaweed without a strong depletion of their internal reserves at 10vol.d^{-1}. However, protein contents strongly decreased at 5vol.d^{-1} which proves that N became strongly limiting in this condition. At low flow, strong bleaching occurred for *U.lactuca* and, to a lesser extent, *H.cornea* also presented a modified aspect. This indicates that their amino-acids and inorganic N-reserves were already depleted and they used their pigment N-reserves. In the last trial (LFLD) both *U.rigida* and *H.cornea* presented the lowest protein content which indicate that N-reserves were not reconstituted and continue to be depleted. This was unlikely a negative effect of decreasing density at low water flow as statistics suggested for *U.rigida*. In Canarian oligotrophic seawater (4vol.d^{-1}), Viera et al. (2011) found protein contents of 16.6%DW and 11.27%DW for *U.rigida* and *H.cornea* respectively. These were similar to the protein contents observed in our EF experiment. This could be explained by the 6 to 12fold higher stocking densities for each algal species in the present study (in kg.m^{-2}) since the competition for ammonium access was stronger and seaweed received lower light levels which negatively affects N-uptake (Rosenberg and Rabus, 1982). In the SW experiment, *U.rigida* protein content was 5.0-6.9%DW and 7.6-9.1%DW for *H.cornea*. This is far below of the protein content observed by Viera et al. (2011). Such results could be explained by stocking densities 2.6 to 5.3fold higher in our experiments (in kg.m^{-2}) which should considerably

complicate the ammonium uptake in this oligotrophic condition. Moreover, bleaching was systematically observed for each seaweeds species. Statistics showed that protein content increased with increasing density for the two species and increased with decreasing water flow for *H.cornea* (Tab. 5). It is more likely that proximal compositions observed in the SW experiment only reflects the nutritional history of the inoculated seaweeds. This was previously observed in similar experiments (Lapointe and Ryther, 1978, 1979; Ryther et al., 1981). Thus, protein contents in the SW experiment were directly linked to N-reserves of the inoculated algae originated from the previous trial in the EF experiment. This relation is more or less observable here (Fig. 4). It is important to consider that water temperature and irradiance vary between experiments since these parameters affect the depletion rate of N-reserves (Duke et al., 1986; Rosenberg and Ramus, 1982; Chapman and Craigie, 1977). This is in agreement with the observation done by Figueroa et al. (2012) and Gómez Pinchetti et al. (1998) that indicated subsequent depletion of N-compounds in *H.cornea* and *U.rigida* two weeks after their inoculation in Canarian seawater. In the SW experiment the lower protein contents were always observed at the end of the HFLD trial. However, biochemical samples were sampled after three weeks which was a week later than for the other trials. Consequently, reserves should be more depleted and it was not possible to compare HFLD proximal compositions with the other trials. Hence, statistics were probably biased. In unfavourable conditions, such as low flow in the EF experiment or in the whole SW experiment, *U.rigida* protein content decreased more than the one of *H.cornea*. This corroborate the fact that depletion rate of N-reserves is directly correlated with the growth rate (Rosenberg and Ramus, 1982). By its opportunistic nature and high growth rates, *U.rigida* use up its N-reserves faster than *H.cornea*. Consequently, at low ammonium concentrations, *U.rigida* protein content is more dependent on water flow rate. This illustrate the fact that *Ulva spp.* are less resistant to poor nutrient conditions than red seaweeds (Rosenberg and Ramus, 1982) which could be related to the bleaching event occurring in the EF experiment for *U.rigida*.

It is often noticed that macroalgae carbohydrate content is inversely correlated to protein content. This is due to an ecological strategy that consists to focus on carbohydrate synthesis when nitrogen is limiting (Figueroa et al., 2012; Gómez Pinchetti et al., 1998; Lapointe and Ryther, 1979; Neish and Shacklock, 1971). In agreement, both *U.rigida* and *H.cornea* carbohydrate contents substantially increased with decreasing water flow rate while the opposite was found for the protein content. In the EF experiment, carbohydrate contents were 50.0-59.9 and 40.3-49.5%DW for *U.rigida* and *H.cornea*. In their experiment in seawater, where protein contents were close to those registered in our EF experiment, Viera et al. (2011)

found carbohydrate contents of 56.4 and 58%DW for *U.rigida* and *H.cornea* respectively. Thus, in our experiment *H.cornea* carbohydrate content was not as high as expected according to the protein content. The main difference between Viera et al. (2011) and the present study is that in our case stocking densities ($kg.m^{-2}$) were 5.5-8.2 fold higher and *H.cornea* was likely subject to high light limitations as discussed before. Consequently, carbohydrate synthesis by the mean of photosynthesis was probably inhibited. In addition, it was showed that inorganic carbon was probably limiting for both *U.rigida* and *H.cornea*. Considering this, it is important to note that, in our case, the positive link between decreasing water flow rate and carbohydrate content was likely indirect. In fact, increase water flow rate enhances the nutrient renewal rate (Debusk et al., 1986). Hence, in poor nutrient conditions, high water flow rate is primordial for both carbon and nitrogen supplies. The apparent negative relationship between water flow and carbohydrate content reflects more likely the fact that, under N and C-limiting conditions, protein synthesis is prioritised prior to carbohydrate synthesis. At low flow, protein synthesis becomes anecdotic and carbohydrate relative content rises accordingly. Statistics showed that at high flow, *U.rigida* carbohydrate content increased with increasing density. Anyhow, this relation was not well pronounced and this is probably an artefact due to the variability in the carbohydrate calculation method (by difference). In the SW experiment, carbohydrate contents were high: 60.9-65.3 and 53.5-57.8%DW for *U.rigida* and *H.cornea* respectively. This is perfectly in accordance with the fact that carbohydrate content is negatively correlated with protein content even in highly C-limited conditions. *H.cornea* carbohydrate content increased with the stocking density which is exactly the opposite trend that noticed for protein content. For *U.rigida* this relation between carbohydrate and protein content was not clearly marked, particularly in the HFLD condition where protein content was very low. Nevertheless, after three weeks in this condition, poor physiological condition might have impaired to observe the relation.

Inversely to carbohydrates, macroalgae lipid content could be directly linked to the inorganic carbon availability in the ambient environment since lipids can act as a carbon reserves (Pinchetti et al., 1998; Pohl and Zurheide, 1979). Indeed, in the EF experiment, *U.rigida* lipid content substantially increased with water flow rate which represent conditions where carbon was less limiting. Another positive relation, less pronounced, was establish between *U.rigida* lipid content and algae stocking density. This variation is not related with pH variation. It is possibly that this reflects mechanisms more complex which control differentially the synthesis of the different kind of lipid compounds. For example, Gómez Pinchetti et al. (1998) demonstrated that the synthesis of poly-unsaturated and mono-unsaturated fatty acids are stimulated by opposite environmental factors. Considering the limited existing literature on

the control of lipid synthesis by rearing parameters, in flow-through cultivation system, further studies are required to better understand these mechanisms. In conditions not likely C-limited, Viera et al. (2016, 2011) recorded lipid contents ranging from 3.7 to 6.3%DW for *U.rigida* and from 4.3 to 5.4%DW for *H.cornea*. In the EF experiment, lipid contents were globally lower for both species: 2.6-3.9%DW for *U.rigida* and 2.2-3.3%DW for *H.cornea*. In seawater culture, Viera et al. (2011) registered lipid contents of 3.7 and 5.4%DW for *U.rigida* and *H.cornea* respectively. In comparison in the SW experiment, lipid contents were much lower: 1.6-2.5%DW for *U.rigida* and 1.5-2.8%DW for *H.cornea*. These results are in accordance with C-limiting conditions in EF and SW experiments with depletion of lipid carbon reserves to sustain photosynthesis. In the SW experiment, at high flow rate, *U.rigida* lipid content fall at high density. In contrast, *H.cornea* lipid content substantially increased with decreasing water flow rate. Finer analysis will be required to understand the explanation mechanisms.

Previous studies showed that macroalgae ash content is positively correlated with growth rate in flow-through cultivation systems (Pinchetti et al., 1998; Lapointe and Ryther, 1979). This correspond to the present observations. Indeed, *U.rigida* ash contents substantially increased with decreasing densities in both EF and SW experiments following exactly the same variation pattern as growth rates. These results showed that, in spite of a possible overestimation due to weighing mistakes, growth rates were really higher at low density in the SW experiment likely due to lower light limitation. Similarly, *H.cornea* ash content did not follow particular variation pattern as it was the case for the growth rate in both EF and SW experiment.

V. Conclusion and prospects

The present studies reveal the existence of strong carbon, light and nitrogen limitation in the GIA flow-through integrated system for macroalgae production. It is very likely that non-determined essential nutrient, like phosphorus, were also limiting in this study. The tested stocking densities and water flow rate were unsuitable under such poor nutrient conditions and the global efficiency of the IMTA system was low during all the experimental period. Indeed, on the one hand, nutritional qualities of *U.rigida* and *H.cornea* were unsuitable for efficient abalone production since they were far below the optimal lipid and protein requirements previously reported for *H.tuberculata coccinea* (Viera et al., 2011, 2005). On the other hand, algae production was low with growth rates closed to zero for *H.cornea* and probably fewer than those previously reported in other IMTA systems for *U.rigida* with a clear overestimation of algal growth rates.

Nevertheless, our study demonstrates that it was possible to ameliorate algal production and nutritional qualities by adapting rearing parameters to low nutrient conditions. In our case, this consisted to regulate water flow rate at more than 10vol.d^{-1} and substantially decrease algal stocking density for both species. Several other studies showed that optimal rearing parameters are species, system but also season dependents (Steyn, 2000; Hanisak and Ryther, 1984). It will be relevant to monitor wastewater nutrient composition all along the year and adapt water flow and stocking density accordingly. This would enable to maintain a constant efficiency of the IMTA system even in period of low finfish stocking density.

Even in such unfavourable rearing conditions, this study demonstrates the interest of IMTA for seaweed production in oligotrophic seawater regions such as Canary Island. Indeed, even with very low nutrient supplies, nutritional quality was substantially better for algae cultivated in the aquaculture effluent than for those cultivated in seawater. It is however important to specify that, in the present study, the SW experiment was not really adapted to allow comparisons. Because of the EF origin of inoculated seaweeds, the evolution of both proximal compositions and growth rates in the SW experiment mainly reflected the amount of N-reserves previously accumulated. Furthermore, temperature, irradiance and stocking densities, expressed in kg.m^{-2}, were different between the experiments. In further studies, seaweeds cultivated as control in seawater will have to be collected in wild populations.

Finally, to allow comparison between different cultivation systems, stocking densities should be expressed per surface unit instead of volume unit. In our case, that would have avoided to obtained such poor results for *H.cornea* which was cultivated under clearly too high stocking densities on the basis of literature data.

VI. Bibliography

Abreu M.H., Pereira R., Yarish C., Buschmann A.H., Sousa-Pinto I., 2011. IMTA with *Gracilaria vermiculophylla*: Productivity and nutrient removal performance of the seaweed in a land-based pilot scale system. *Aquaculture*, 312: 77-87

AOAC, 1995. Official methods of analysis (16th ed.). Arlington, VA: Association of Analytical Chemists

Arístegui J., Hernández-León S., Montero M.F., Gómez M., 2001. The seasonal planktonic cycle in coastal waters of the Canary Islands. *Scientia Marina*, 65(1): 51-58

Bansemer M.S., Qin J.G., Harris J.O., Duong D.N., Hoang T.H., Howarth G.S., Stone D.A.J., 2016. Growth and feed utilisation of greenlip abalone (*Haliotis laevigate*) fed nutrient enriched macroalgae. *Aquqculture*, 452: 62-68

Barrington K., Chopin T., and Robinson S., 2009. Integrated multi-trophic aquaculture (IMTA) in marine temperate waters. In D. Soto (ed.). Integrated mariculture: a global review. FAO Fisheries and Aquaculture Technical Paper. No. 529., *FAO*: 7–46

Bird C.J., Rice E.L., Murphy C.A., Ragan M.A., 1992. Phylogenetic relationships in the Gracilariales (Rhodophyta) as determined by 18S rDNA sequences. *Phycologia*, 31(6): 510-522

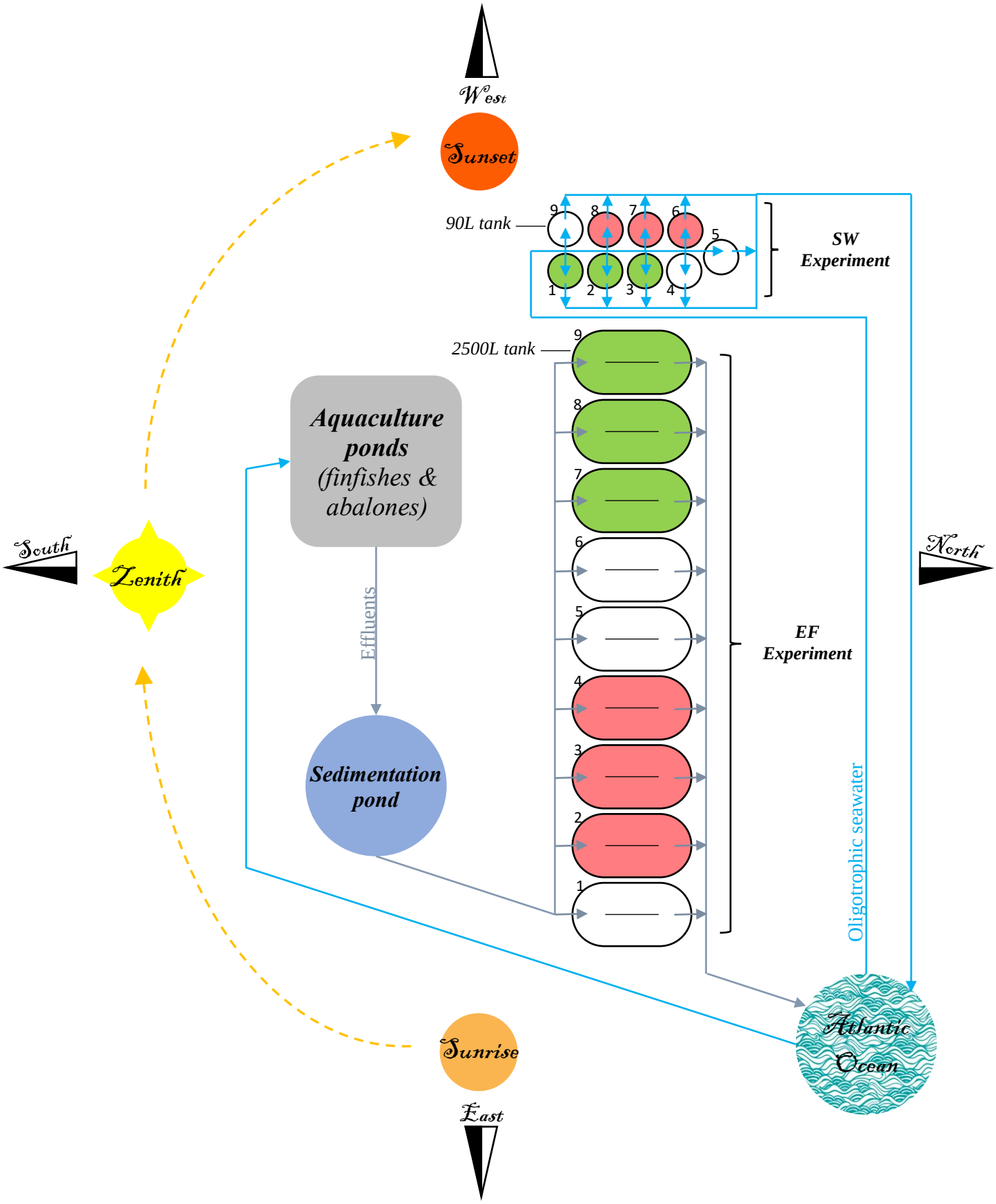
Chapman A.R.O., Craigie J.S., 1977. Seasonal growth in *Laminaria longicruris*: relations with dissolved

inorganic nutrients and internal reserves of nitrogen. *Marine Biology*, 40(3): 197-205
Chopin T., 2013. Integrated Multi-Trophic Aquaculture. http://www2.unb.ca/chopinlab/articles /files/Chopin%202013%20GAA%20IMTA.pdf. 3pp.
Chopin T., Buschmann A.H., Halling C., Troell M., Kautsky N., Neori A., Kraemer G.P., Zertuche-González J.A., Yarish C., Neefus C., 2001. Integrating seaweeds into marine aquaculture systems: a key toward sustainability. *Journal of Phycology*, 37(6): 975-986
DeBoer J.A., Guigli H.J., Israel T.L., D'Elia C.F., 1978. Nutritional studies of two red algae I. Growth rate as a function of nitrogen source and concentration. *Journal of Phycology*, 14: 261-266
DeBusk T.A., Blakeslee M., Ryther J.H., 1986. Studies on the outdoor cultivation of *Ulva lactuca* L.. *Botanica Marina*, 29: 381-386
Demetropoulos C.L., Langdon C.J., 2004. Enhanced production of pacific dulse (*Palmaria mollis*) for co-culture with abalone in a land-based system: nitrogen, phosphorus and trace metal nutrition. *Aquaculture*, 235(1-4): 433-455
Duke C.S., Lapointe B.E., Ramus J., 1986. Effects of light on growth, RuBPCase activity and chemical composition of ulva species (chlorophyta). *Journal of Phycology*, 22(3): 362-370
FAO, 2016. The state of world fisheries and aquaculture 2016. http://www.fao.org/3/a-i5555e.pdf. 220pp.
Ferreira N.C., Bonetti C., Seiffert W.Q., 2011. Hydrological and water quality indices as management tools in marine shrimp culture. *Aquaculture*, 318(3-4): 425-433
Figueroa F.L., Korbee N., Abdala R., Jerez C.G., López-de la Torre M., Güenaga L., Larrubia M.A., Gómez Pinchetti J.L., 2012. Biofiltration of fishpond effluents and accumulation of N-compounds (phycobiliproteins and mycosporine-like amino acids) versus C-compounds (polysaccharides) in *Hydropuntia cornea* (Rhodophyta). *Marine Pollution Bulletin*, 64: 310-318
Folch J., Lees M., Sloane-Stanley G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, 226: 497–509
Gómez Pinchetti J.L., Del Campo Fernández E.C., Moreno Diez P., García Reina G., 1998. Nitrogen availability influences the biochemical composition and photosynthesis of tank-cultivated *Ulva rigida* (Chlorophyta). *Journal of Applied Phycology*, 10: 383-389
Gowen R.J., Bradbury N.B., 1987. The ecological impact of salmonid farming in coastal waters: a review. *Oceanography and Marine Biology*, 25: 563-575
Howarth R.W., 1988. Nutrient limitation of net primary production in marine ecosystems. *Annual Review of Ecology*, 19: 89-110
Jegatheesan, V., Shu, L. and Visvanathan, C. 2011. Aquaculture effluent: impacts and remedies for protecting the environment and human health. In Nriagu, Jerome (ed), Encyclopedia of environmental health. *Elsevier Science*: 123-135
Jiménez del Río M., Ramazanov Z., García-Reina G., 1996. *Ulva rigida* (Ulvales, Chlorophyta) tank culture as biofilters for dissolved inorganic nitrogen from fishpond effluents. *Hydrobiologia*, 326: 61-66
Jiménez del Río M., Ramazanov Z., García-Reina G., 1994. Optimization of yield and biofiltering efficiencies of *Ulva rigida* C. Ag. Cultivated with *Sparus aurata* L. waste waters. *Scientia Marina*, 58(4): 329-335
Khotimchenko S.V., Yakovleva I.M., 2005. Lipid composition of the red alga *Tichocarpus crinitus* exposed to different levels of photon irradiance. *Phytochemistry*, 66(1): 73-79
Lapointe B.E., Tenore K.H., 1981. Experimental outdoor studies with *Ulva fasciata* Delile. I. Interaction of light and nitrogen on nutrient uptake, growth, and biochemical composition. *Journal of Experimental Biology and Ecology*, 53: 135-152
Lapointe B.E., Ryther J.H., 1979. The effects of nitrogen and seawater flow rate on the growth and biochemical composition of *Gracilaria foliifera* var. *angustissima* in mass outdoor cultures. *Botanica Marina*, 22(8): 529-537
Lapointe B.E., Ryhter J.H., 1978. Some aspects of the growth and yield of *Gracilaria tikvahiae* in culture. *Aquaculture*, 15(3): 185:193
Mai K., Mercer J.P., Donlon J., 1994. Comparative studies on the nutrition of abalone, *Haliotis tuberculate* L. and *Haliotis discus hannai* Inno: II. Amino acid composition of abalone and six species of macroalgae with an assessment of their nutritional value. *Aquaculture* 128(1-2): 115-130
Manzi J.J., Castagna M., 1989. Clam Mariculture in North America. *Elsevier*. 461 pp.
Mata L., Silva J., Schuenhoff A., Santos R., 2006. The effects of light and temperature on the photosynthesis of the *Asparagopsis armata* tetrasprorophyte (*Falkenbergia rufolanosa*), cultivated in tanks. *Aquaculture*, 252(1): 12-19
Mercer J.P., Mai K., Donlon J., 1993. Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculate* Linnaeus and *Haliotis discus hannai* Ino I. Effects of algal diets on growth and biochemical composition. *Invertebrate Reproduction & Development*, 23(1-3): 75-88
Natale F., Hofherr J., Fiore G., Virtanen J., 2013. Interactions between aquaculture and fisheries. *Marine Policy*, 38: 205-213
Neish A.C., Shacklock P.F., 1971. Greehouse experiments (1971) on the propagation of strain T4 of Irish Moss.

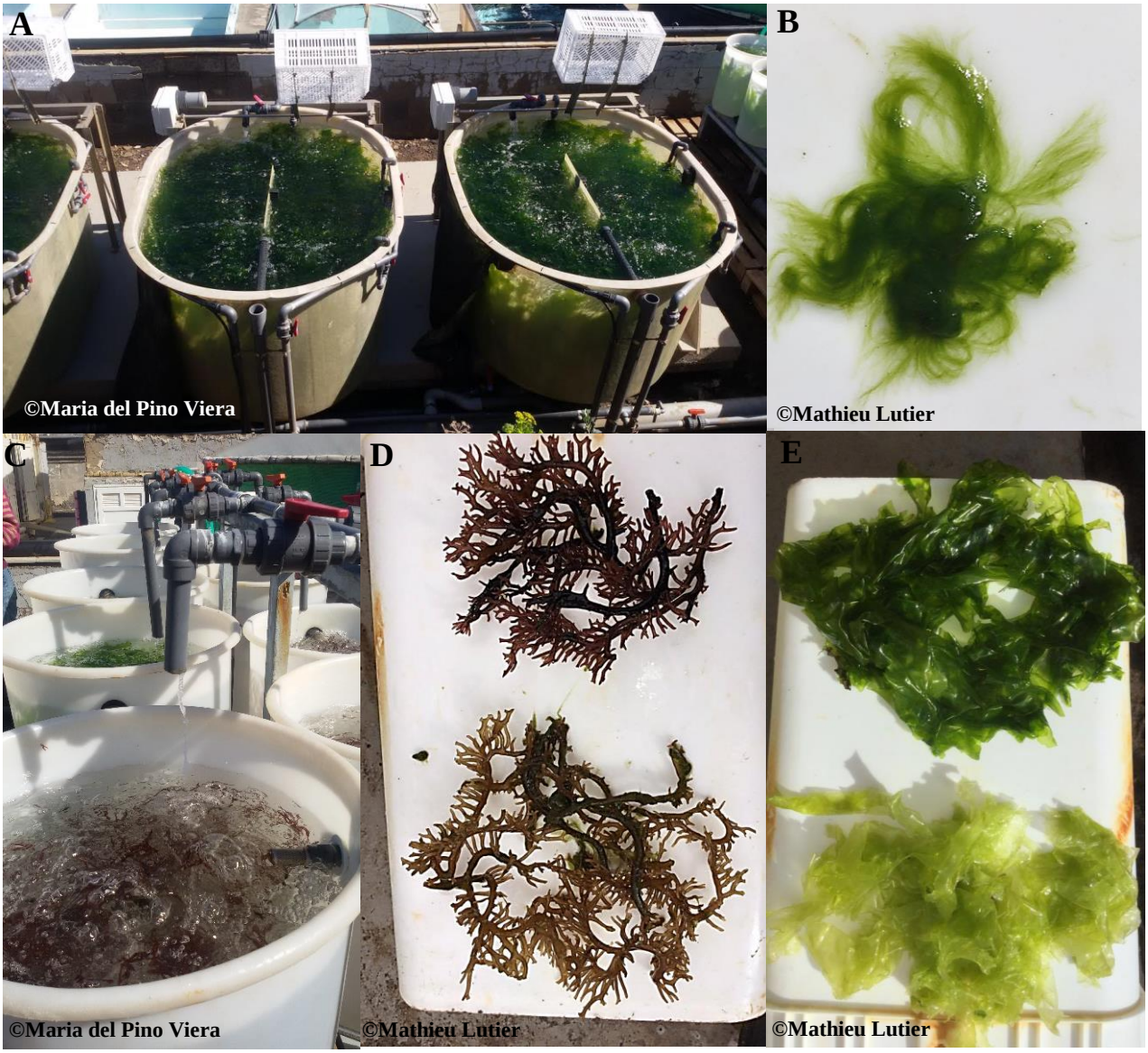
Atlantic Regional Laboratory, National Research Council of Canada
Neori A., Chopin T., Troell M., Buschmann A.H., Kraemer G.P., Halling C., Shpigel M., Yarish C., 2004. Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. *Aquaculture*, 231(1-4): 361-391
Neori A., Shpigel M., Ben-Ezra D., 2000. A sustainable integrated system for culture of fish, seaweed and abalone. *Aquqculture*, 186: 279-291
Neori A., Ragg N.L.C., Shpigel M., 1998. The integrated culture of seaweed, abalone, fish and clams in modular intensive land-based systems: II. Performance and nitrogen partitioning within an abalone (*Haliotis tuberculate*) and macroalgae culture system. *Aquaculture Engineering*, 17(4): 215-239
Núñez-Resendiz M.L., Dreckmann K.M., Sentíes A., Díaz-Larrea J., Zuccarello G.C., 2015. Genetically recognizable but not morphologically: the cryptic nature of *Hydropuntia cornea* and *H. usneoides* (Gracilariales, Rhodophyta) in the Yucatan Peninsula. *Phycologia*, 54(4): 407-416
Parker H.S., 1981. Influence of relative water motion on the growth, ammonium uptake and carbon and nitrogen composition of *Ulva lactuca* (Chlorophyta). *Marine Biology*, 63(3): 309-318
Pillay T.V.R., 2004. Aquaculture and the environment, Second edition. *Blackwell Publishing Ltd. Editorial Offices*. 212pp.
Pohl P., Zurheide F.,1 979. Fatty acids and lipids of marine algae and the control of their biosynthesis by environmental factors. In: Hoppe HA, Levring T, Tanaka Y (eds), Marine Algae in Pharmaceutical Science, Walter de Gruyter, Berlin: 473-523.
Robertson-Andersson D., 2003. The cultivation of *Ulva lactuca* (chlorophyta) in an integrated aquaculture system, for the production of abalone feed and the bioremediation of aquaculture effluent. University of Cape Town, doctoral thesis. https://open.uct.ac.za/handle/11427/6175
Rosenberg G., Ramus J., 1982. Ecological growth strategies in the seaweeds *Gracilaria foliifera* (Rhodophycae) and *Ulva sp.* (Chlorophycae): soluble nitrogen and reserve carbohydrates. *Marine Biology*, 66: 251-259
Ryther J.H., Corwin N., DeBusk T.A., Williams L.D., 1981. Nitrogen uptake and storage by the red alga *Gracilaria Tikvahiae* (McLachlan, 1979). *Aquaculture*, 26: 107-115
Schiener P., Black K.D., Stanley M.S., Green D.H., 2015. The seasonal variation in the chemical composition of the kelp species *Laminaria digitata*, *Laminaria hyperborea*, *Saccharina latissima* and *Alaria escuelenta*. *Journal of Applied Phycology*, 27(1): 363-373
Schuenhoff A., Shpigel M., Lupatsch I., Ashkenazi A., Msuya F.E., Neori A., 2003. A semi-recirculating, integrated system for the culture of fish and seaweed. *Aquaculture*, 221(1-4): 167-181
Shpigel M., Neori A., 1996. The integrated culture of seaweed, abalone, fish and clams in modular intensive land-based systems: I. Proportions of size and projected revenues. *Aquaculture Engineering*, 15(5): 313-326
Solórzano L., 1969. Determination of ammonia in natural waters by the phenol hypochlorite method. *Limnology and oceanography*, 14(5): 799-801
Tenore K.R., 1976. Food chain dynamics of abalone in a polyculture system. *Aquaculture*, 8(1): 23-27
University of Hawai`i Botany Department, 2001. *Ulva rigida* (C. Agardh, 1823). http://www.hawaii.edu/reefalgae/invasive_algae/pdf%20files/ulva_rigida.pdf
Viaroli P., Naldi M., Bondavalli C., Bencivelli S., 1996. Growth of the seaweed *Ulva rigida* C.Agardh in relation to biomass densities, internal nutrient pools and external nutrient supply in the Sacca di Goro lagoon (Northern Italy). *Hydrobiologia*, 329: 93-103
Viera M.P., Courtois de Viçose G., Fernández-Palacios, Izquierdo M.S., 2016. Grow-out culture of abalone *Haliotis tuberculata coccinea* Reeve, fed land-based IMTA produced macroalgae, in a combined fish/abalone offshore mariculture system: effect of stocking density. *Aquaculture Research*, 47: 71-81
Viera M.P., Courtois de Viçose G., Gómez Pinchetti J.L., Bilbao A., Fernández-Palacios H., Izquierdo M.S., 2011. Comparative performance of abalone (*Haliotis tuberculata coccinea* Reeve) fed enriched vs non-enriched macroalgae: effect on growth and body composition. *Aquqculture*, 319: 423-429
Viera M.P., Fidalgo P., Haroun R.J, Courtois de Viçose G., Bilbao, A., Gómez Pinchetti J.L., Izquierdo M.S., 2009. Effect of different rearing conditions on the growth performance of the macroalgae *Ulva rigida*, *Hypnea spinella* and *Gracilaria cornea*. Proceedings of the 7th International Abalone Symposium, Pattaya-Thailand, 107pp.
Viera M.P., Gómez Pinchetti J.L., Courtois de Viçose G., Bilbao A., Suárez S., Haroun R.J., Izquierdo M.S., 2005a. Suitability of three red macroalgae as a feed for the abalone *Haliotis tuberculate coccinea* Reeve. *Aquaculture*, 248: 75-82
Viera M.P., Bilbao A., Suárez Alvarez S., Courtois de Viçose G., Gómez Pinchetti J.L., Hernández-Cruz C.M., Haroun R.J., 2005b. Producción, efficienca de biofiltración y composición nutricional de las macroalgas *Ulva rigida* y *Gracilaria cornea* cultivadas en efluentes de cultivos marinos. https://acceda.ulpgc.es:8443/xmlui/handle/10553/758 Congreso Nacional de Acuicultura, Sesión de diversificación de Especies-Poster: 91-92

~Annexes~

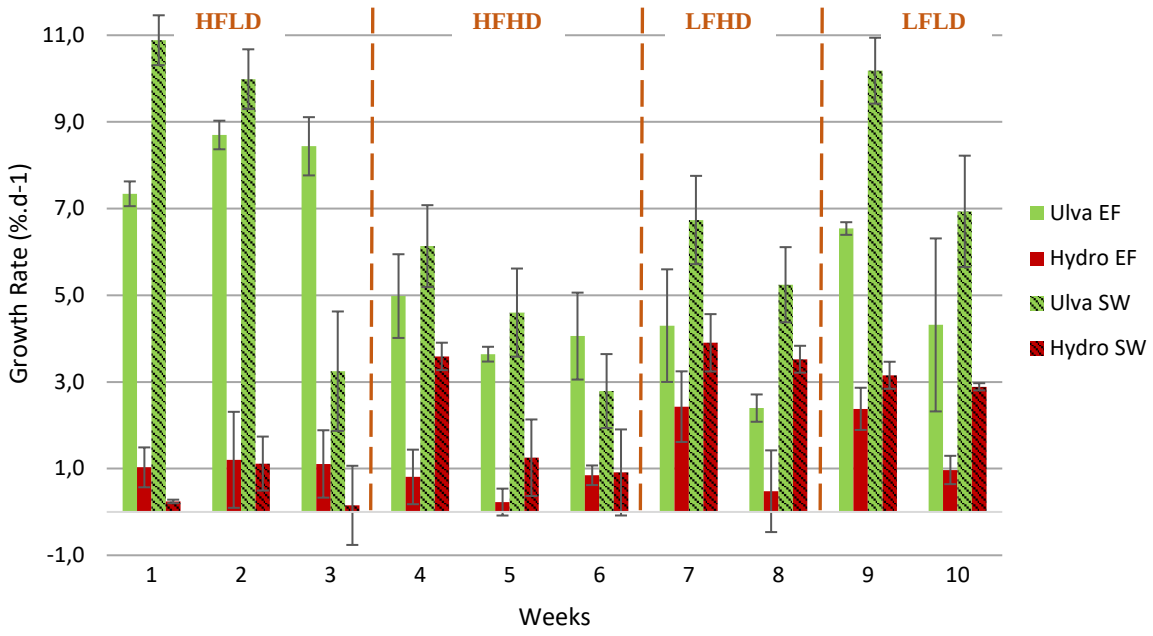
Annex 1. Representation of the GIA facilities: tanks used for the cultivation trials are shown in green and in red for *U.rigida* and *H.cornea* respectively



Annex 2. Pictures of the experimental device: tanks used for the EF cultivation experiment (A), epiphytes found in the *H.cornea* SW tanks during the whole experimental period (B), tanks used for the SW cultivation experiment (C), comparison of the *H.cornea* from the SW (below) and the EF (up) experiment at the end of the HFHD trial (D), comparison of the *U.rigida* from the SW (below) and the EF (up) experiment at the end of the HFHD trial (E)



Annex 3. Weekly growth rates (Mean±SD) for each species, trial and experiment



Abstract

The suitability of the macroalgae *Ulva rigida* and *Hydropuntia cornea*, cultivated in aquaculture effluents (IMTA), as a feed for the ormer *Haliotis tuberculata coccinea* has already been demonstrated in the Canary Islands. Therefore, the efficiency of the whole production system depends of the growth performance and nutritional quality of the cultivated seaweeds which are themselves dependant of rearing parameters such as algal stocking density and water flow rate. The purpose of the present study was to optimise these parameters in the GIA IMTA system. Both *U.rigida* and *H.cornea* were cultivated in the wastewaters of the GIA facilities (EF experiment) and in fresh Atlantic seawater (SW experiment). This allowed thus to determine the efficiency of the IMTA system by comparison. Low and high intensities of both algal stocking density (2.5 and 5g.L^{-1} and 8 and 12g.L^{-1} for *U.rigida* and *H.cornea* respectively) and water flow rate (5 and 10vol.d^{-1}) were crossed to constitute 4 different rearing trials. After 2-3weeks of cultivation per trial, growth rate and algal proximate composition were determined. Ammonium concentrations were lower than 4µM in the EF experiment and under the µmole per litter in the SW experiment. Carbon, Nitrogen and Light limitations of the algal growth were highlighted in both experimental system. This was particularly the case for *H.cornea* with growth rates closed to zero. For *U.rigida* growth was enhanced by decreasing stocking density and increasing water flow rates. Both protein and lipid contents were far lower than those previously reported for these species cultivated in IMTA systems. Consequently, macroalgae were unsuitable to satisfy nutritional needs of the ormers. Nevertheless, it was possible to ameliorate the nutritional quality of *U.rigida* and *H.cornea* by increasing water flow rate and decreasing stocking density. This demonstrates that these parameters should be adapted to maintain the efficiency of the system under poor nutrient conditions. Nutritional quality was clearly better in the EF than in the SW experiment demonstrating the interest of IMTA in the cultivation of algae in oligotrophic seawater regions such as Canary Islands.

Résumé

L'intérêt de l'utilisation des macroalgues *Ulva rigida* et *Hydropuntia cornea*, cultivées dans des effluents aquacoles (IMTA), comme source de nourriture pour l'ormeau *Haliotis tuberculata coccinea* a déjà été démontré aux îles Canaries. Toutefois, l'efficacité de l'intégralité du système de production dépend de la croissance et de la qualité nutritionnelle des algues cultivées qui sont eux-mêmes dépendants de paramètres de culture comme la densité d'élevage et le flux d'eau. Le but de cette étude était d'optimiser ces paramètres dans le système d'IMTA du GIA. *U.rigida* et *H.cornea* ont été cultivés dans les effluents des bassins aquacoles du GIA (expérience EF) et dans de l'eau de mer Atlantique (expérience SW) pour démontrer l'efficacité du système d'IMTA par comparaison. Des faibles et hautes intensités de densité d'élevage (2.5 et 5g.L^{-1} et 8 et 12g.L^{-1} pour *U.rigida* et *H.cornea* respectivement) et de flux d'eau (5 et 10vol.j^{-1}) ont été croisées pour constituer 4 conditions expérimentales de culture. Après 2-3 semaines d'élevage par condition, le taux de croissance et la composition biochimique des algues furent déterminés. Les concentrations en ammonium étaient inférieures à 4µM dans l'expérience EF et inférieure au µmole par litre dans l'expérience SW. Des limitations de la croissance par la disponibilité en carbone, en azote et en lumière furent relevés dans les deux systèmes expérimentaux. C'était particulièrement le cas pour *H.cornea* avec une croissance presque nulle. Le taux de croissance d'*U.rigida* augmentait avec la diminution de la densité d'élevage et l'augmentation du flux d'eau. Les taux de lipides et de protéines étaient très inférieurs à ceux habituellement observés chez ces espèces cultivées en IMTA. En conséquence, les macroalgues n'étaient pas aptes à répondre aux besoins nutritionnels des ormeaux. Néanmoins, il était possible d'améliorer la qualité nutritionnelle d'*U.rigida* et *H.cornea* en augmentant le flux d'eau et diminuant la densité d'élevage. Cette étude démontre qu'il est possible de maintenir l'efficacité du système en adaptant ces paramètres d'élevage aux conditions pauvres en nutriments. La qualité nutritionnelle était nettement meilleure dans l'expérience EF que dans la SW démontrant l'intérêt de l'IMTA pour cultiver des algues dans des régions d'eau de mer oligotrophe comme les îles Canaries.