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# Seasonal and interannual production of sea lettuce (*Ulva* sp.) in outdoor cultures based on commercial size ponds

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## Abstract

The many nutritional benefits reported in seaweeds have increased their demand in the western world for human consumption. In order to supply this demand, it is necessary to cultivate seaweeds both offshore and onshore. Offshore cultivation is highly vulnerable to climate variation. Cultures on land can be operated while essential variables can be controlled (nutrient supply) or partially regulated (light and temperature) providing a more uniform quality and continuous production. In this study, we present the results of pond-culture in a commercial pilot-facility on the Pacific, temperate coast of Mexico, which has been continuously running for 2½ years. Ponds of 100 $m^3$ were seeded with 3 kg/$m^2$ of a previously selected strain of *Ulva*. Pulse fertilization and a full water exchange were made twice a week. Ponds were fully harvested every 3 weeks and re-seeded with the initial density. Seaweed production showed a bimodal distribution with a strong peak in spring (258–290 g $m^{-2}$ $day^{-1}$), a minor peak in autumn, and lower production in summer and much lower in winter (40–85 g $m^{-2}$ $day^{-1}$). Highest growth performance occurred when the average temperature remained between 17 and 23°C. This study provides a realistic baseline for annual seaweed production on a commercial pilot-scale aquaculture farm.

**KEYWORDS**
growth temperature, onshore cultivation, pond cultivation, tumble culture, *Ulva*

## 1 | INTRODUCTION

Around five decades ago, the green alga *Ulva* was proposed, among other seaweeds, as a valuable marine resource due to its multiple uses as food for human consumption, animal feed, biofilter, pharmaceuticals, and even as biomass for biofuels (Lapointe & Ryther, 1979, Lapointe & Tenore, 1981). Numerous studies have been carried out since then, aimed to support the commercial cultivation of this genus under conditions sufficient to control its biomass production and chemical composition. Nevertheless, so far, only a few cases of seaweed cultivation onshore have succeeded commercially (Hafting, Critchley, Cornish, Hubley, & Archibald, 2012). Reliable data on biomass production at a commercial scale are too scarce and variable to motivate or justify the necessary investment.

Since the early 70s, several studies have evaluated the physiological performance and chemical composition of *Ulva* spp. under different culture conditions of temperature, light, and salinity (Mohsen, Khaleata, Hashem, & Metwalli, 1974; Mohsen, Nasr, & Metwalli, 1973; Mohsen, Nasr, & Metwally, 1972). These studies were followed by research focused on the optimization of the biomass productivity and changes in the chemical composition, when *Ulva* is grown in outdoor tanks of different sizes (Lapointe & Tenore, 1981).

The interest on Integrated Multi-Trophic Aquaculture (IMTA) (Chopin et al., 2001; Neori, Cohen, & Gordin, 1991; Neori et al., 2004) considered *Ulva* as an ideal seaweed to be used in abalone and fish farms (Al-Hafedh, Alam, Buschmann, 2015; Bolton, Robertson-Andersson, Shuuluka, & Kandjengo, 2009; Ben-Ari et al., 2014; Cohen & Neori, 1991; Mata et al., 2003; Mata, Schuenhoff, & Santos, 2010; Robertson-Andersson et al., 2007; Shpigel et al., 2017). More recently, its high productivity has driven additional studies of its cultivation at a larger scale for the production of biomass as a source of biofuels (Bruhn et al., 2011; Mata, Magnusson, Paul, & de Nys, 2016).

The most relevant justification for the implementation of onshore cultivation is the potential control on the production of functional seaweed products for human usage. Compared with offshore culture methods, cultivation in tanks or ponds offers a better opportunity to control some variables driving the seaweed metabolism (e.g., photosynthesis, nutrient uptake), and its vegetative productivity per unit area (Hafting et al., 2012; Hafting et al., 2015; Lapointe & Tenore, 1981). Frequently, however, the best conditions for optimizing productivity are not optimal for the production of secondary metabolites. Although variables such as fertilization can be fully controlled in terms of the type of nutrient, frequency, and doses, other variables (such as light and temperature) can only be partially regulated. Light, for instance, can be directly conditioned by the seaweed density; temperature is not economically feasible to control at large scales but can be regulated by the rate and timing of the seawater flushes. Other engineering characteristics, such as the shape and depth of the tanks, can strongly influence light availability and biomass production per unit area (Bidwell, McLachlan, & Lloyd, 1985). Even with these and other potential constraints, land-based technology is still considered particularly important for the production of high-value products obtained from seaweed genera that do not conform to current offshore techniques (Hafting et al., 2015).

Among the greatest advantages of land-based cultivation are: the ability to be adapted to a wide range of seaweed genera; nutrients can be added efficiently; vessels are three dimensional, making efficient use of available area; effluents can be treated and continuous or semi-continuous production can be maintained (Hafting et al., 2012, Hafting et al., 2015, Pereira, Yarish, & Critchley, 2013). Studies by Craigie and Shacklock (1995) and Bidwell et al. (1985) on the domestication of *Chondrus crispus* (Irish Moss) set the standard approach for onshore tank cultivation (known as tumble culture); a suitable method for the cultivation of seaweeds in tanks and ponds. This method consists of basically maintaining the seaweeds in a continuous resuspension between the bottom and the surface of the water column by aeration provided from the bottom of the tank. It is ideal for species that can grow vegetatively and their morphology or size is not compromised by the "tumbling" movement in the tanks. The studies on *C. crispus* provided many essential engineering facts, particularly relevant while considering the commercial scaling of the cultures. For instance, these authors demonstrated a better efficiency of providing water movement by air agitation instead of paddlewheels, and the advantage of a rectangular shape against the oval, among other features (Craigie, Cornish, & Deveau, 2019).

The feasibility of the commercial cultivation of *Ulva* will depend strongly on the biomass yield (expressed as biomass/area/time). There are many studies in the literature that provide the biomass production (from g $m^{-2}$ $day^{-1}$ to tons $ha^{-1}$ $year^{-1}$) of different species and strains of *Ulva* under different engineering designs, culture treatments, and climates (Bruhn et al., 2011; Cohen & Neori, 1991; Jiménez del Río, Ramazanov, & Reyna, 1996; Mhatre et al., 2018; Neori, Shpigel, & Ben-Ezra, 2000; Neori et al., 2003). Although data are very variable, these are necessary in order to justify commercial cultivation because they provide critical scenarios of maximum and minimum productivity. However, most of these studies have strong limitations in order to be reliable for commercial cultivation due to three main reasons: (a) the scale of the culture system (size and depth of tanks) are usually too small; (b) the length of the cultivation period is usually very short (few months of the year); and (c) the difference in post-harvesting methods used to report biomass increase (wet: dry ratio and salt content). Most of the studies do not cover a full year of cultivation and consequently, annual values of biomass production (tons $ha^{-1}$ $year^{-1}$) are inferred from a few months of data. Additionally, none of these studies maintained the same strain for more than a year.

The aim of this research was to provide a case study of *Ulva* spp. cultivation at a scale in size (commercial pilot-scale, 100 $m^3$ and length of time of 30 months) sufficient to identify seasonal and interannual variations. The cultivation method was the "tumbling culture", proposed by Bidwell et al. (1985) and Craigie and Shacklock (1995). Three *Ulva* strains were previously tested on the same scale for 6 months. The strain with the best results was maintained in culture for 24 additional months. Interannual and seasonal productions are discussed in relation to changing culture conditions of light and temperature.

## 2 | MATERIAL AND METHODS

### 2.1 | Collection of strains

*Ulva* samples were collected from Bahia San Quintin, Baja California, Mexico, a coastal lagoon on the east Pacific coast (30° 26′N, 115° 59′W). In the laboratory, three different foliar strains were selected and, based on their morphology, were classified as *Ulva lactuca* types A and B, and *Ulva fasciata* type C. Samples from each collection were placed on herbarium sheets, photographed, and maintained in the laboratory for future record.

### 2.2 | Cultivation

Seaweed fronds were individually selected and rinsed with filtered seawater in order to remove any sediment and epizooties before being placed in 1.2 $m^3$ outdoor tanks. The tanks were provided with aeration from the bottom in order to keep the seaweeds in continuous resuspension. Tanks were seeded with 1–4 kg/$m^3$ in order to select a density that provided the best yield in a period of 3 weeks. Once sufficient biomass was generated, one 100 $m^2$ pond (4 x 25 m) was seeded with 3 kg fresh weight (fw) of each of the *Ulva* strains (i.e., 300 kg per pond) and maintained for 3 weeks before being harvested to its original density. The three strains were cultured simultaneously for 6 months (February–June 2015) in the commercial ponds. After that, the best performing strain (C) was maintained in culture for 24 additional months in three 100 $m^2$ ponds.

Seawater was replaced twice a week (previously filtered through a 10 μm filter). Cultures were fertilized twice a week with 500 μm $NH_3NO_3$ and 50 μm of $H_3PO_4$ for 12 hr before replacing the seawater. Pulse fertilization and full water exchange were practiced twice a week. Ponds were harvested every 3 weeks and re-seeded with the initial density. Harvesting was carried out by hand using scoop fishnets. The biomass was placed in plastic containers (18 x 12 x 10.5″) and squeezed manually to remove excess water. Fresh weight was recorded using a Torrey scale (model EQB-W) with a 0.02 kg precision. The containers were maintained overnight in a warehouse and dried the next morning in an automated conveyor drier.

Seawater temperature and irradiance data were obtained from the REDMAR oceanographic data center from the Federal Research Center CICESE (www.redmar.cicese.mx, Centro de Investigación Científica y Educación Superior de Ensenada). The temperature within the ponds was recorded daily using maximum/minimum thermometers.

The amount of salt that remained on a dry sample was estimated by rinsing a sub-sample with tap water for 15 min and compared with an unrinsed sample.

### 2.3 | Chemical composition

At the end of every harvest period, scheduled to be every 3 weeks and 1 day after fertilization, seaweed samples were collected to determine their chemical composition. Samples were dried at 60°C until reaching constant weight and stored until analysis. Total nitrogen was analyzed according to the method of micro-Kjeldahl. Lipids were extracted with methylene chloride and determined by Folch et al. (1957). Ash content was evaluated by the gravimetric method as recommended by Sluiter et al. (2008). Fresh samples were kept frozen at −18°C until analysis, Chl *a* and Chl *b* were extracted in 10 ml of 80% acetone, pigment concentrations were determined by spectrophotometry following Lichtenthaler and Wellburn (1983).

### 2.4 | Salt content and fw:dw ratio

The fresh weight: dried weight ratio (fw:dw) and the amount of salt after rinsing with tap or fresh water were estimated in the laboratory. Rinsed samples were placed in distilled or tap water for 5 min, respectively, then spun for 20 s using a handheld vegetable spinner before dried in an oven at 60°C until reaching constant weight.

## 3 | RESULTS

### 3.1 | Light

Solar irradiance ($W/m^2$) varied among the years of this study (Figure 1). Maximum total irradiance occurred in 2016 and minimum in 2018. Total irradiance was 8% less in 2017 than in 2016. The annual difference between 2016 and 2018 was 13.7%.

### 3.2 | Temperature

Seawater temperature, an annual basis, showed little difference among the years. On average, 2017 was slightly cooler than in 2016 and 2018 (–0.23 and – 0.20°C, respectively). The difference between 2016 and 2017 was only 0.03°C. The seawater temperature at the pond, however, was up to 4°C warmer in summer and – 5°C cooler winter

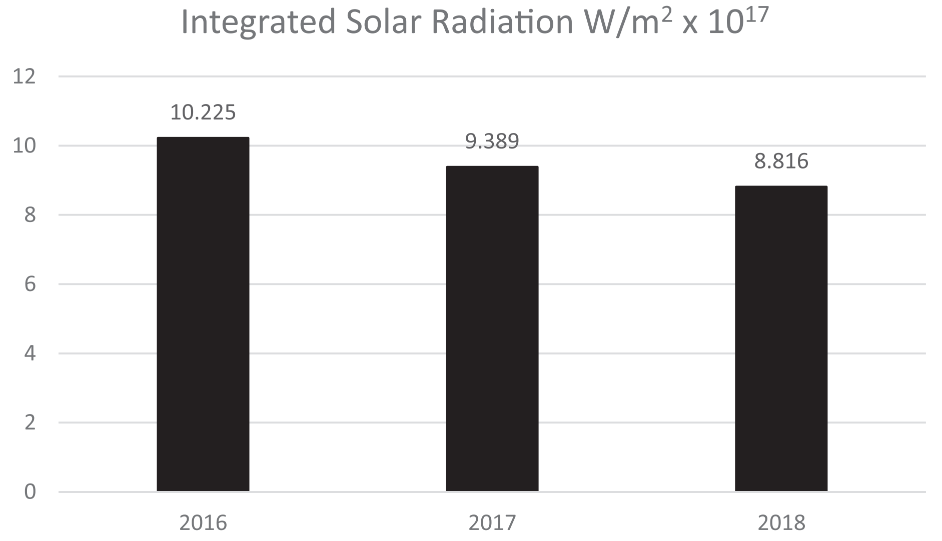


**FIGURE 1** Annual integrated solar radiation at Ensenada, Baja California. Data obtained from redmar.cicese.mx

**TABLE 1** Seasonal temperature average and strain C biomass yield for 2016 and 2017

| | 2016 | | 2017 | |
|---|---|---|---|---|
| Season | T (°C) | Yield (g $m^{-2}$ $year^{-1}$) | T (°C) | Yield (g $m^{-2}$ $year^{-1}$) |
| Spring | 23.7 | 230.0 | 21.1 | 216.1 |
| Summer | 22.3 | 181.9 | 24.0 | 160.5 |
| Autumn | 16.8 | 180.8 | 19.6 | 158.6 |
| Winter | 14.6 | 122.4 | 14.9 | 143.2 |
| Annual average | **19.35** | **178.8** | **19.9** | **169.6** |

(Figure 2). In 2017, the summer and autumn temperatures were 1.7 and 2.8°C warmer than in 2016. The spring temperature of 2016, however, was 2.6°C warmer (Table 1).

## 3.3 | Strain selection

The biomass average yield (g $m^{-2}$ $day^{-1}$) among the strains was different. Higher yields (222 g $m^{-2}$ $day^{-1}$) were observed in strain C and lower yields in strain A (160.2 g $m^{-2}$ $day^{-1}$). The production of strain C was 27.9% and 15.3% higher than strains A and B, respectively (Figure 3).

### 3.3.1 | Strain C

Strain C was cultured continuously for 32 months (until the end of the study). Seaweed production (g $m^{-2}$ $day^{-1}$, fresh weight) showed a bimodal distribution with a strong peak in spring and a minor peak in autumn, and lower production in summer and much lower in winter. The best performance occurred when the average temperature range

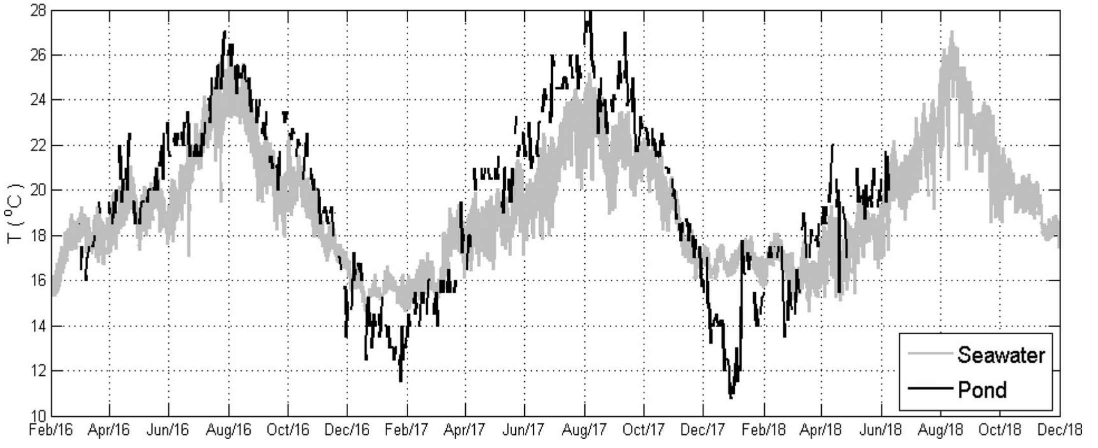


**FIGURE 2** Sea water temperature in ponds and surface ocean temperature from February 2016 to December 2018 (data obtained from www.redmar.cicese.mx). The temperature within the ponds is the daily average obtained from maximum/minimum thermometers

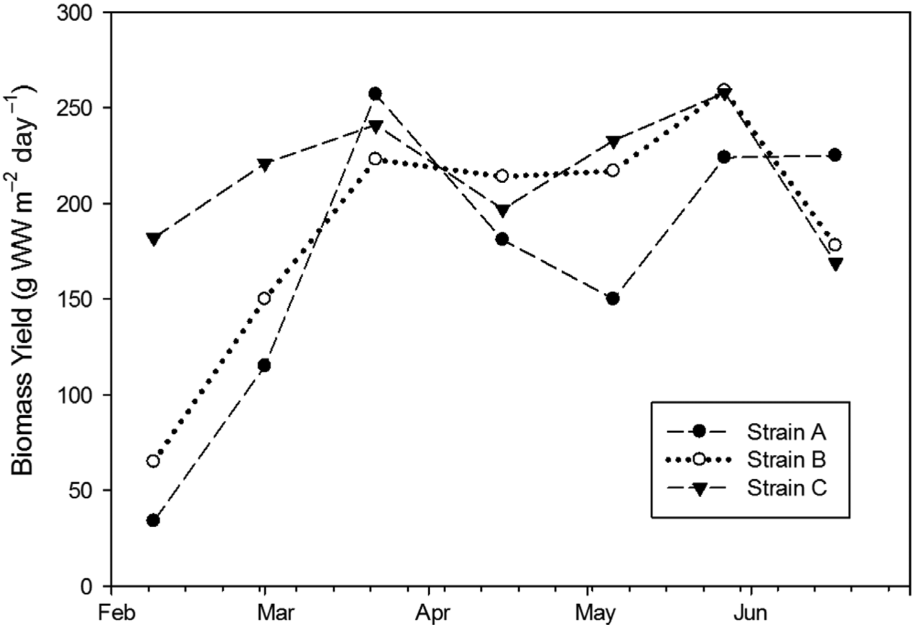


**FIGURE 3** Biomass yield of three different strains of *Ulva* cultured from February to July 2015. Each inflection in the curves indicates the harvesting date (approximately every 3 weeks)

remained between 17 and 23°C. Above or below these temperatures, biomass production decreased. Maximum production occurred in the spring (258–290 g m$^{-2}$ day$^{-1}$) with a minimum in winter (40–85 g m$^{-2}$ day$^{-1}$). A slight net loss of biomass (– 36.6 g m$^{-2}$ day$^{-1}$) was recorded during the first week in January of 2017 and last week of December of 2017, coinciding with the minimum temperature and shorter photoperiods. However, the stock remained and the production recovered in a week (Figure 4).

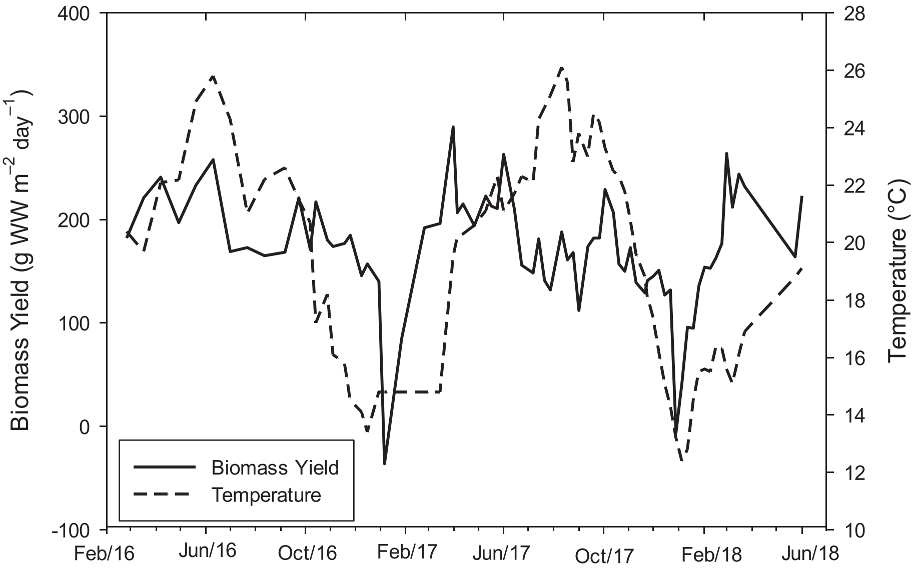


**FIGURE 4** Biomass yield (g WW m$^{-2}$ year$^{-1}$) of *Ulva* strain C from February 2016 to June 2018. Each inflection in the curves indicates the harvesting date (approximately every 3 weeks)

**TABLE 2** Average chemical composition of strain C culture from February 2016 to July 2018

| Unit | Average | Min | Max |
|---|---|---|---|
| % nitrogen | 2.86 | 1.21 | 4.78 |
| % lipids | 1.85 | 0.87 | 5.93 |
| % ash | 36.64 | 22.80 | 60.38 |
| Chl *a* μg/g fw | 875.62 | 687.37 | 1,576.54 |
| Chl *b* μg/g fw | 619.94 | 575.38 | 1,427.22 |

**TABLE 3** *Ulva* fresh and dried weight ratios and amount of salt estimated as the difference in weight after rinsing ($n$ = 3)

| Treatment | Fresh weight (g) | Dried weight (g) | Fw:dw | Difference in weight (%) |
|---|---|---|---|---|
| No rinse | 3.0 | 0.5568 | 5.5 | |
| Tap water rinse | 3.0 | 0.5255 | 5.7 | 5.6 |
| Distilled water rinse | 3.0 | 0.5146 | 5.9 | 7.6 |

## 3.4 | Chemical composition

The average nitrogen content, lipids, and ash were 2.8, 1.8, and 36.6%, respectively. Average Chl *a* and Chl *b* were 875.6 and 619.94 μg g$^{-1}$ fw, respectively (Table 2). Minimum values in nitrogen and pigments occurred on few occasions, due to weather conditions, when harvesting could not be performed the day after fertilization.

## 3.5 | Salt content and fw:dw ratio

The fw:dw ratio varied from 5.5–5.9. Higher values corresponded to samples rinsed in distilled water and lower values to not rinsed. The amount of salt in samples rinsed with tap and distilled water were 5.6 and 7.6%, respectively (Table 3).

## 4 | DISCUSSION

This study provided a continuous record of the variability of a yearly *Ulva* biomass yield cultured under natural conditions at a pilot-scale (100 $m^2$ ponds) and highlighted relevant sources of variability that may mask productivity estimates. The biomass yield is subject to the environmental conditions that vary daily, seasonally, and from year to year. Therefore, rather than provide a precise number, the goal is to provide a better understanding of the possible range of variability in the biomass yield. In agriculture, for instance, modeling of biomass production and yield of horticultural crops is a major field. Many descriptive and explanatory models are used for crop yield forecasting. Functional models based on photosynthesis performance for dry biomass production (Basso & Liu 2019) to statistical models based in large records of crop production (see Marcelis et al. 1998) set an example of what eventually could be possible for on-land seaweed cultivation.

Under natural conditions, most species within the genus *Ulva* are considered opportunistic with strong seasonal occurrence. Thus, a relevant concern in the commercial cultivation of *Ulva* spp. is to assure the possibility to maintain the same strain growing vegetative from year to year. In our case, we demonstrated the feasibility to maintain the same *Ulva* strain in culture for more than 2 years. The strain remained healthy until the end of the experiment. Sporadic sporulation events were observed but only on few plants. This success was based on assuring that the cultures did not suffer nutrient depletion and carrying out regular harvesting, which restricts the occurrence of light limitation within the ponds associated to fronds self-shading.

It is important to consider that there will always be annual variability in macroalgae biomass yield due to climate differences in solar irradiance and temperature from year to year in nature or in culture. These differences can be very significant in the harvesting of natural populations, such as the reduction in 90% of the regular harvesting of *Macrocystis pyrifera* during El Niño years due to a reduction in the wild kelp beds (Casas-Valdez, Serviere-Zaragoza, Lluch-Belda, Marcos, & Aguila-Ramírez, 2003; Ladah & Zertuche-González, 2004). Similar effects can be expected in commercial cultures in the open ocean such as in the case of eucheumatoid cultures in Asia (Largo, Chung, Gerung, & Sondak, 2017). One of the advantages of considering on-shore cultures is to reduce effects of variability in solar irradiance, photoperiod, and seasonal temperature, which cause differences in the potential annual biomass yield.

In our study, biomass production of strain C exhibited a seasonal bimodal distribution that could be related to seasonal light and temperature variations (Figure 4). The highest productivity occurs in the late winter/early spring. During the summer, when the water temperature was at a maximum, a reduction in the biomass yield was observed. When the temperature decreased below 23°C in autumn, the cultures tended to recover. In December, when the temperature fell below 16°C, there was a drastic reduction in productivity. The strain, however, persisted and was able to perform a vigorous recovery in February. This collapse in the yield in December coincides with the shortest and coolest days of the year.

Seaweed production in-land culture is directly influenced by yearly solar radiation, which can vary in the order of 10 ± 2%. The integrated annual irradiance between 2016 and 2017 varied by 8% and in the period 2016–2018 varied 13.7%. The annual biomass production of 2017 was around 5% lower than in 2016 (Table 4). Although our dataset is too short to correlate light and biomass production, it can be assumed that the decrease in annual irradiance of 8% between 2 years was responsible for a proportional reduction in the biomass yield of 2017.

**TABLE 4** Biomass annual production for 2016 and 2017

| Year | Total production in 300 $m^2$ (ton fw) | Annual production per $m^2$ (kg fw) | Projected production per ha (ton fw) | Projected production per ha (ton fw:dw) ratio, 7.5:1 | Production ($g\ m^{-2}\ day^{-1}$ fw) | Production ($g\ m^{-2}\ day^{-1}$ dw) |
|---|---|---|---|---|---|---|
| 2016 | 19.6 | 65 | 600 | 80.0 | 179 | 23.9 |
| 2017 | 18.6 | 62 | 530 | 70.7 | 170 | 22.6 |

The seasonal variation in temperature among years can also be responsible for the variation in biomass yield. In addition, it would be relevant to consider that temperature, the second most important factor in pond cultivation (assuming the nutrient supply is controlled), would have a higher variation within the ponds. In our study, water temperature in the ponds was up to 4°C warmer in the summer and−5°C in the winter than ocean temperature (Figure 2). The summer temperature was equivalent to an anomaly of a prevailing El Niño event. Thus, the optimal temperature range at which any cultivar of seaweed could perform according to laboratory experiments (Craigie & Wen, 1984; Duke, Litaker, & Ramus, 1989; Jie et al., 2016; Kalita & Tytlianov, 2003; Kim, Kang, Park, Lee, & Kim, 2011; Mohsen et al., 1973) would be shortened by the temperature effect of the ponds.

In our study, the annual water temperature average in 2016 and 2017 was relatively similar (0.5°C difference) (Table 1). Larger differences, however, were observed in the average seasonal temperature among the years. The average temperature in 2017 for the summer and autumn seasons was higher than the same seasons for 2016 and more frequently the temperature reached peaks above 23°C (Figure 4, Table 1) causing lower biomass production during that period. These effects cannot be observed in short-term experiments, which commonly extrapolate annual biomass yields per hectare based on a few months of data. For instance, the biomass projections presented in this study based on the biomass yield of three strains cultured from February to July are relatively high compared with strain C yields cultured for 30 months. The main reason for this is that the winter collapse observed during long term culture was not included in the first experiment (Figure 3, Table 5).

Similar to the changes in biomass yield, changes in the chemical composition are expected (Table 4). A consistent chemical composition through a year is not possible; however, culture practices can be implemented in order to optimize biomass yield or secondary metabolite production. In our study, it was a priority to assure a minimum value of protein content (> 15%). This was achieved not only by assuring the proper fertilization but also by synchronizing fertilization with the time of harvesting. Minimum values of nitrogen and chlorophyll occurred when the harvesting could not be perform the day to weather conditions (Santana winds or rain).

In most previous studies, besides the inability to see the variability in biomass yield annually or seasonally, there are at least two other factors that can introduce bias when measuring biomass yield; the fresh to dry ratio (fw:dw), and the amount of salt. In controlled laboratory experiments, common fw:dw ratios for species as *Ulva* may vary around 5:1. In these cases, the surface water on the algae is blotted and biomass of grams or less is dry at 60°C until a constant weight is reached. At a pilot-scale or farm operation, in our experience, this ratio can go from 7.5 to 11:1 just by the simple fact of water accumulation when it is collected by nets (in the order of kg). Biomass yield in seaweed culture studies rarely specifies the inclusion or not of salt content in their results. In most cases, one could assume that it is included. This means that biomass production would be overestimated. The amount of salt in the cultured seaweed depends on two kinds of factors: those related to the form of the seaweed and those related to the harvest and post-harvest procedures. Seaweeds with a high area: volume ratio, like *Ulva* or *Pyropia*, would have higher amounts of salt. As the scale of harvesting increases, the amount of water that remains trapped in the harvested volume tends to be higher, such that the amount of water during that the seaweed retains during the drying period would be higher as well as the salt remaining on its surface. Then, once dried, the handling of the biomass may cause some degree of salt loss.

**TABLE 5** Biomass yield and yield projections for strains A, B, and C cultured from February to June 2015

| | Average yield | | Projections | |
|---|---|---|---|---|
| Strain | g $m^{-2}$ $day^{-1}$ | kg $year^{-1}$ $m^{-2}$ | ton/ha wet | ton/ha dry |
| A | 160.2 | 58.5 | 584.7 | 78.0 |
| B | 188 | 68.6 | 686.2 | 91.5 |
| C | 222 | 81.0 | 810.3 | 108.0 |
| All | 190 | 69.4 | 693.5 | 92.5 |

## 5 | CONCLUSION

This study demonstrated the feasibility to maintain an *Ulva* strain culture on-shore, under natural conditions of light and temperature, for more than a year. Yearly biomass production was less variable than seasonal production. Although biomass yield was significantly affected by seasonal variation, this pattern remained the same from 1 year to the next allowing for some predictability of seasonal production. Understanding the seasonal and yearly variation of seaweed production cultivated onshore will allow to define more realistic scenarios for commercial success.

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