#### ABSTRACT

Modeling Macroalgae Growth to Optimize Biomass

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The EFDC-MPI model was amended to include the growth kinetics of macroalgae (seaweed) in the water-quality and hydrodynamic calculations. While EFDC has historically simulated macroalgae grown only on the sediment bed, this restriction was lifted to facilitate simulation of kelp farms where the macroalgae substrate was specified at the depth in the water column yielding maximum growth due to optimum light intensity and temperature. The vegetative drag forces on the water column (along with commensurate changes to turbulence intensity and its length scale) were calculated using a new approach based on aquaculture studies and their effects on flow. The macroalgae metabolized nitrates (NO3) or ammonium (NH4) and phosphates (PO4) into dissolved organic nitrogen and phosphorus as well as both labile and refractory particulate organic nitrogen and phosphorus according to the CE-QUAL water-quality model built into EFDC. The ability to specify both nutrient concentrations and point-source masses was added to simulate "fertilization" (supplementation of nutrient-rich water at various locations in the kelp farm). Finally, data assimilation was included to nudge the open boundary conditions to ensure stability when all boundaries were specified-pressure time

series, which allowed this model to be forced by a regional-scale EFDC or ROMS model. Developments integrated with a domain decomposition-based MPI parallelization for computational efficiency and results output in CF-compliant NetCDF. This model was developed to support uncertainty quantification and sensitivity analyses with the goal of maximizing biomass production by optimizing nutrient loading, depth in the water column (to control sunlight intensity and temperature), and location of fertilization points (to minimize "nutrient shadows" downstream in the kelp farm). Large (100-ha) kelp farms are under consideration by the Department of Energy as a source of biofuel (MARINER Program) and numerical modeling is required to ensure that these systems are developed in the most cost-effective manner. Modeling Macroalgae Growth to Optimize Biomass

by

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## CHAPTER ONE

## Introduction

As fossil fuel usage for transportation, industry, and private enterprises increased since the 1900s, global carbon emissions rose accordingly. The rate of emissions also trended upwards, with emissions increasing by over 16 times between 1900 and 2008, and about 1.5 times during 1990–2008 alone (Climate Change, 2021). Environmental challenges of fossil fuel use, along with other issues, such as dynamic swings in crude oil prices and challenges in energy security, have made the replacement of these environmentally harmful and unsustainable fuels by renewable and sustainable alternatives necessary (Dragone, 2010). Bioethanol, which is considered a renewable energy source, can potentially reduce transportation emissions in addition to replacing a portion of the petroleum-based fuel supply (Alvarado-Morales et al, 2013; Philippsen, 2010), even though its current production is not enough to meet all of current fuel demand.

There is increasing recognition and understanding of the vital life-supporting services provided by the ocean. Ocean processes offer numerous solutions for climate change mitigation and efforts to support ocean economies are motivated by the hope of building sustainable food and energy systems. According to conservative estimates, the ocean economy generated \$1.5 trillion in 2010 and has the potential to outperform the growth rate of the global economy in terms of generated value and employment (OECD, 2016). The Organization for Economic Cooperation and Development conservatively

projects that the ocean economy could reach an output of \$3 trillion in 2030 (OECD, 2016).

Biofuel production has long been seen as a means to achieve a more sustainable existence and secure energy source. A simplified path to carbon neutrality using algal biofuels is this: algae convert sunlight into chemical energy the form of triacylglycerides. The CO2 taken up by algae during photosynthesis is the same CO2 released when the algae-turned-biogas is burned for energy (Murphy et al, 2015).

Macroalgae have been a commercially valuable resource for centuries. Historically, seaweed has been harvested for food, fertilizer, livestock feed, and pharmaceutical products and at the turn of the 21st century, seaweeds became the second largest aquaculture product worldwide (behind fish) in terms of biomass (Food and Agricultural Organization of the United Nations, 1996).

Since algae grow in marine waters (Philippsen, 2010) algal biofuels, which are considered "third-generation biofuels" (Dragone, 2010), may help change the food vs. fuel argument. Yes, it is true that large-scale adoption of this approach can potentially have negative effects, but it can allow highly productive land to be used for food production as opposed to crops for bio- fuels. Macroalgae, or seaweed, has no lignin but high moisture (70–90%) and ash (21.5–33.4%) levels (Jung, 2017). Low amounts of lignin in macroalgae render it well suited for biogas production in anaerobic digesters (Dave et al, 2013). On the other hand, easily fermentable carbohydrates, including laminarin and mannitol, especially in brown macroalgae, are suitable for bioethanol conversion (Philippsen, 2010; Borines et al, 2011).

The breakeven selling price for electricity generated from seaweed-based aquaculture farming has been estimated at around \$154 Mega-Watt-hours (MW-h) (Borines et al, 2011). This price may be acceptable compared with other renew- able energy prices, such as solar thermal (\$251/MW-h), solar photovoltaic (\$157/MW-h), and biomass-generated electricity (\$120.2 /MW-h).

Current feedstocks for bioethanol production (predominantly corn and sugarcane) compete directly with human foods by using arable lands, water, fertilizer, and other resources, and arguably may have negative effects on food prices (Philippsen, 2010; Bringezu et al, 2007). Therefore, much attention is now focused on producing biofuels from lignocellulosic biomass, agricultural wastes, and other biological materials. Although these feedstocks do not compete directly with human food resources, they can compete indirectly if they are cultivated in available arable lands (Eisentraut, 2010). Also because cellulosic biomass has high lignin content, its conversion into biofuels can be difficult and cost-prohibitive (Philippsen, 2010). Interest in macroalgae is driven by the food-fuel debate and impacts of land-use change to cropland. The ethics of using arable land (0.2 ha of arable land per capita worldwide) for energy and not for food is questionable (Brusima, 2017). Because macroalgae grown in open-ocean environments do not compete with terrestrial crops, these debates are obviated.

Seaweed can also be used to remediate excess nutrients in hypoxic regions (dead zones, e.g., nutrient-rich waters associated with salmon farms) (Murphy et al, 2015). Algae have several advantages over other energy crops, including high growth yields and rates and the capacity to capture CO<sub>2</sub>. Also, macroalgae (seaweed) have high

carbohydrate concentrations, making them good substrates for biogas production (Costa, 2013).

In the quest for new biofuels, anaerobic digestion of macroalgae is promising. This involves nutrient recycling, biogas production, and waste reduction (Appels, 2011; Mottet et at, 2010). Natural production of seaweed (kelp) is approximately 1 tonne dry wt./ha/yr, which is much greater than most terrestrial plants. Large amounts of seaweed are washed up on beaches (most notably Caribbean) every winter and can provide the feedstock for biofuel (Murphy et al, 2015; Mar'echal et al, 2017).

Studies conducted on the Central Florida Atlantic coastline in the late 1970s compared growths of red, green, and brown algae in outdoor, flow- through cultures (Lapointe, 1978). In year-round viability, growth rate, and dry weight yields, the red algae Gracilaria tikvahiae ranked highest of the algal species assessed. Notably, G. tikvahiae has an impressive growth rate (maximum growth rate of Pmax = 0.6/d) relative to other macroalgae (Lapointe, 1978).

Moreover, G. tikvahiae is an excellent candidate for a bioenergy crop because it reproduces vegetatively (not sexually) through fragmentation of thalli and is relatively easy to maintain as a monoculture (Hanisak, 1987).

However, cultivating G. tikvahiae is difficult in a laboratory setting and all the more operationally challenging and expensive in open-ocean environments. This is why modeling is a necessary tool for aquaculture farmers; it can guide and inform site design and operations and obviate trial-and-error approaches. Additionally, modeling can inform the profitability of seaweeds as energy crops. To realize the promise of cost-effective macroalgal biofuels, it is necessary to estimate algal growth rates (as well as its

uncertainties) as a function of irradiance, nutrient concentrations, temperature, salinity, and even local flow conditions. Effective modeling informs site design (e.g., farm layout, plant density, spatial arrangement of the inoculum, etc.) as well as site operations (e.g., seeding times, harvest frequency, maintenance schedules, etc.) to maximize biomass production. Questions to be addressed include:

(1) What areal density of *G. tikvahiae* and aquaculture farm layout (dimensions, extent, etc.) and operations (harvest triggers) maximized productivity and (2) What production variability could be expected over the course of the year?

#### CHAPTER TWO

#### Literature Review

As fossil fuel usage for transportation, industry, and private enterprises increased since the 1900s, global carbon emissions rose accordingly. Studies on the production ecology of Chilean-grown G. tikvahiae began in the 1970s when government agencies realized the severe economic consequences of continued over-exploitation of natural kelp (Pizarro, 1986). To maintain and even augment G. tikvahiae populations, researchers sought to understand the kelp's life cycle. A kelp's thallus (Figure 2.1) refers to the combined features of an individual kelp plant from its anchoring holdfast to its branching blades. When exposed to sufficient light, thalli grow into erect axes and branches: the "shrubbery" characteristic of G. tikvahiae. As a plant grows larger and heavier, fragments are more likely to break off by moving water, either cast ashore or deposited onto the ocean floor where they are buried and act again as anchored thalli (Pizarro, 1986).

Across several studies in Florida, G. tikvahiae has been shown to grow fastest in July and slowest in January (Hanisak et al, 1988; Pizarro, 1986; Hanisak, 1987). Hanisak (1990) also measured G. tikvahiae growth, which peaked in late July at 46 g dry wt./(m2 d) and fell to 12 g dry wt./(m2 d) in late January (Hanisak, 1987).

Physiological needs of G. tikvahiae include light, nitrate and phosphate nutrients, appropriate temperatures, and salinities. As part of a year-round study, maximum growth rate (0.6/d) was observed when grown with a culture density of 0.4 kg wet wt./m2 in

summertime conditions (Lapointe and Ryther, 1978). The highest year-round biomass yields were obtained when the kelp were maintained at areal densities of 2–3 kg wet wt./m2. Over the course of this study, 20 < S < 36 %, 12 < T < 31 °C, 72.6 < I < 218.1 W/m2 (Lapointe and Ryther, 1978). Algae were grown in outdoor growth chambers sustained with sea water obtained from a lagoon connecting the Indian River to the Atlantic Ocean. Experiments receive78d nutrient enrichment of nitrogen and phosphorous (as NaNO3 and NaH2PO4) at an atomic N:P ratio of 10:1. From July to December, a close correlation between light intensity and G. tikvahiae biomass yield was observed; a lesser correlation was apparent from January to June.

Lapointe and Ryther (1978) also reported that Gracilaria growth depended upon seawater exchange and that growth increased with flow rate independent of nutrient concentrations past a threshold value. From these experiments, the best growth of G. tikvahiae was obtained at a culture density of about 2 kg wet wt./m2, with a rapid exchange of seawater medium (flow rate of 22 culture volume exchanges per day of seawater enriched with 15  $\mu$ mol/NO3N and 4  $\mu$ mol/NO3N). This demonstrated that nutrient flux is at least as important as nutrient concentration (Lapointe and Ryther, 1978).

Studies of Gracilaria noted that finely branched thallus forms tend to have higher production rates than coarsely-branched species (Kanwisher, 1966; King and Schramm, 1976; Littler and Arnold, 1982). When clones of G. tikvahiae from outdoor tank cultures with varying morphologies were evaluated for photosynthetic and growth rates, the surface area:volume ratio of the plant correlated with productivity. Ranging from 20–52 cm3/cm2 (Hanisak et al, 1988), this ratio was higher when G. tikvahiae clones were

highly branched with numerous smaller, finer branches (Figure 2.1) than when clones were thicker with more coarsely branched thalli (Figure 2.1). Because nutrient uptake is the metabolic process by which algae actively transport compounds across membrane surfaces, the rate of uptake depends on the relative surface area available for such transport; thus, a thallus form with a high surface-area:volume ratio achieves more rapid nutrient uptake (Hanisak et al, 1909).

Leveraging the relationship between morphological form and photosynthetic performance can inform harvest practices (i.e. how the algae are pruned upon harvest) and seeding density (spacing between algae within the aquaculture farm) and maximize biomass retrieved from each harvest period. The significance of drawing this connection between algae morphology and photosynthetic productivity lies in the ability to make predictions about algal photosynthetic processes without being constrained to a specific geographic location or environment (Littler and Arnold, 1982).

This morphology-photosynthetic rate relationship suggests that farmers maintain a high surface-area: volume ratio when pruning once the algae have reached maturity in their life cycle (Littler and Arnold, 1982). Self-shading obstructs sufficient light from reaching the algae's photosynthetic apparatus, and pruning strategies that maintain a high surface area: volume ratio effectively mitigate this phenomena. Additionally, this functional-form model could be used to identify the most productive portions of algal thalli.



Figure 2.1. Herbarium sheets of *G. tikvahiae* showing morphological variations.

#### CHAPTER THREE

Site Description (Kona, Hawai'i)

Currently, a large portion of the U.S. Exclusive Economic Zone (EEZ) is inappropriate for commercial macroalage cultivation. Nutrient-poor surface waters are a major contributing factor to this limitation. A significant swath of the U.S. EEZ that lacks surface nutrients also possesses deep, nutrient-rich seawater, a feature that an aquaculture site might use to supply nutrients to the system. This is the case for the tropical ocean waters that surround Hawai'i (Kampachi Farms, 2019).

These waters are thermally stratified into a well-mixed warm upper layer overlying cooler nutrient-rich deep seawater (DSW). This stratification hinders the supply of nutrients upwelled into the photic zone, which results in a nutrient-limited "oligotrophic" phytoplankton community having low biological productivity. Oligotrophic oceans are the oceanic equivalent of terrestrial deserts (Grandelli, 2012).

Pumping DSW into the upper waters should enhance algal growth. A balance must be struck between providing sufficient nutrients and not bringing up too much cold water, as doing so could lower the temperature below that of ideal growing conditions. Ideally, the system will be irrigated with seawater flows deep enough so that the discharged nutrients remain below the photic zone. At the same time, it is crucial that G. tikvahiae be planted shallow enough that light needed for growth is optimum (Grandelli, 2012).

Oceanographically, Kona is an ideal location for offshore macroalgal culture as waters on the leeward side of the Big Island of Hawai'i are protected from trade winds

and prevailing seas. Also, a steep offshore slope allows for easy access to nutrient-dense DSW from greater depths. Ocean Era (formerly Kampachi Farms), a Hawai'i-based mariculture company, has collected site- specific data (temperature, nutrient concentrations, irradiance, and salinity) that were used to populate models (Kampachi Farms, 2019).

As depicted in Figure 3.1 The Ocean Era MARINER (Macroalgae Research Inspiring Novel Energy Resources) Blue Fields Offshore Macroalgae Demonstration Project is being deployed in the offshore waters adjacent to Pawai Bay and the Old Airport County Recreation Park, in Kailua-Kona, Hawai'i. The submersible macroalgae culture array offers distinct operational and economic advantages, as the potential damage from storm surf or hurricanes is greatly reduced and normal wear-and-tear on the array and the moorings is minimized by the dampening of the day-to-day wind and wave action at the surface. This demonstration project is currently active and is projected to continue into 2022.



Figure 3.1. The Ocean Era MARINER (Macroalgae Research Inspiring Novel Energy Resources) Blue Fields Offshore Macroalgae Demonstration Project Site Location and the site of this study. Underlying chart: 19327 West Coast of Hawai'i

The submersible algae platform (see Figure 3.2) is situated approximately 1.5 nautical miles offshore and will normally sit below the water surface. The demonstration array is moored to the ocean bottom in approximately 120m (400 ft) water depth, to minimize significant impacts on water quality, coral reefs, or dolphin resting activity.



Figure 3.2. Schematic of the algae growing platform (not to scale). This submersible macroalgae culture array offers distinct operational and economic advantages, as the potential damage from storm surf or hurricanes is greatly reduced, normal wear-and-tear on the array and the moorings is minimized by the dampening of the day-to-day wind and wave action at the surface.

This Hawai 'i-based demonstration project aims to validate technologies that facilitate aquaculture cultivation using only the energy that exists in the natural environment (wind, wave, current, and solar energies) while focusing solely on native Hawaiian macroalgae species, G. tikvahiae included. Numerous native species were tested for suitability in on-shore trials at the Kampachi Farms' research yard at the Natural Energy Laboratory, in Kona, Hawai'i. Modeling efforts of the following research study were based on the details of this Hawai'i-based demonstration project.

# Irradiance Data

Figure 3.3 shows hourly data of downwelling photosynthetically active radiation obtained from the Natural Energy Laboratory of Hawai'i Authority (measured near Kona, Hawai'i) at https://midcdmz.nrel.gov/apps/ go2url.pl?site=NELHA. The LI-COR LI-190

quantum sensor (coordinates: 19.728 144°N, 156.058 936°W, elevation: 4 m AMSL, Hawai'i Standard Time) measures PAR in the 400 to 700 nm waveband (Olson and Andreas, 2012). Seasonality is evident.

# Light Attenuation

Like all plants, macroalgae require some minimal amount of light to survive. Their actual rates of growth depend upon the intensity of light that is available to them. Light attenuates exponentially with depth in the water column according to the Beer-Lambert Law (Abdelrhman, 2016) as shown in Figure 3.4. An average light-attenuation coefficient of  $K_d = 0.161/m$  was calculated for Kona waters by fitting the Beer-Lambert Law to measured light intensities at various depths in the top 10 m of the water column:

$$I(z) = I_0 \exp(K_d z)$$
, (3.1)

where I0 (W/m2) is the light intensity at the surface and z (m) is depth.



Figure 3.3. Light data 2014--2018. Illumination for plants, also known as "irradiance", can be measured in PAR (photosynthetically active radiation) with the units of watts per square meter. Notice the annual cyclicity of PAR in Kona, Hawai'i between 2014 and 2018. Periods of peak PAR (often July and August of the summer months) overlap with *G. tikvahiae's* growing season. Recall that *G. tikvahiae's* maximum reported growth rate of 0.6/day was observed when grown in summertime conditions.



Figure 3.4. Best-fit curves of PAR with depth are shown. Red data and symbols are for noontime and purple data and symbols are for 9 AM and 3 PM.

#### Monthly Temperature Data

Figure 3.5 shows the monthly temperatures of deep and shallow Kona waters. The shallow warm waters largely ranged from 25-35 °C, while the cold, deep waters ranged from 5.5 to 7.5 °C.

Temperatures in shallow Kona waters peak around November each year. The most extreme outlier within the temperature data, occurring November 2015, was so great that it also corresponded with the maximum deep Kona waters temperature (7.5 °C).

Because nutrient concentrations vary widely, one must be strategic when supplying deep seawater to the algae growing platform. If excessive cold water is supplied from the pumping system to the growing platform, one runs the risk of expending too much energy and decreasing the water temperature to a non-optimal ranges.



Figure 3.5. Temperatures of shallow (orange) and deep (green) Kona waters. Both landbased and offshore macroalgae cultivation studies observe G. growth in waters with temperatures ranging from 12°C to 31°C. Chapter 4 explores the modeling efforts to identify ideal seeding and growth temperature ranges for this specific site.

### Monthly NO3 and NH4 Data

Figure 3.6 shows monthly concentrations of [NO3] and [NH4] in deep and

shallow Kona waters. In shallow, warm waters, [NO3] ranged from 0.1 to 2.3 µM and

[NH4] ranged from 0.1 to 1.3 µM. In colder, deep Kona waters, [NO3] ranged from 35

to 70  $\mu$ M and [NH4] ranged from 0.05 to 0.8  $\mu$ M.



Figure 3.6. [NO<sub>3</sub>] (red) and [NH<sub>4</sub>] (blue) in (a) shallow and (b) deep Kona waters. While nitrate and ammonium values vary in shallow water, both hover near a concentration of roughly 0.4M. In deep seawater, nitrate concentrations on average increase greatly (by two orders of magnitude), while ammonium values remain roughly similar.

# Monthly PO4 Data

Figure 3.7 demonstrates that [PO4] was consistently around 0.2 µM in shallow

waters, while [PO4] in deeper waters was most often 3 µM with a few elevated values

between 5 and 6 µM. Note that [PO4] are elevated in November 2016.



Figure 3.7. [PO<sub>4</sub>] in shallow (orange) and deep (green) Kona waters.

# Monthly Salinity Data

Biomass was not limited by salinity, as both shallow (34.8–34.3 ‰, mean: 35.3 ‰) and deep (34.2–34.9 ‰, mean: 34.5 ‰) seawater salinity concentrations in Kona fell within the optimal salinity range (between 24 and 36 ‰) for G. tikvahiae growth, as shown in Figure 3.8.



Figure 3.8. Salinity in shallow (orange) and deep (green) Kona waters. Of the environmental factors discussed in this study, salinity has historically had the least impact on *G. tikvahiae* growth. Also, Kona waters at the surface and at depth tend to maintain an average density that approaches 35ppt.

#### CHAPTER FOUR

#### Basic Growth Model

The governing equation for algal biomass growth is shown in Equation 4.1(Cerco and Cole, 1995)

$$\frac{\partial B}{\partial t} = \left( P - B_M - P_R - \frac{\partial w_s}{\partial z} \right) B, \tag{4.1}$$

where B is the algal biomass expressed as carbon equivalents (g-C/m3), P is the production rate (1/d), BM is the basal metabolic rate (1/d), PR is the predation rate (/d), and  $w_s$  is the settling velocity (m/d) (Cerco and Cole, 1995). The production rate (P) is limited by seaweed density and the availability of light and nutrients, as well as temperature and salinity; the effect of each is multiplicative as shown in Equation 4.2 (Cerco and Cole, 1995).

$$P = P_{max}f(I)g(T)h(N)i(S)j(\rho), \qquad (4.2)$$

where  $P_{max}$  is production (growth rate) under optimal growth conditions (1/d), f (I) is the effect of non-optimal illumination ( $0 \le f(I) \le 1$ ), g(T) is the effect of non-optimal temperature ( $0 \le g(T) \le 1$ ), h (N) is the effect of non-optimal nutrient concentrations ( $0 \le h(N) \le 1$ ), I (S) is the effect of non-optimal salinity ( $0 \le I(S) \le 1$ ), and f ( $\rho$ ) is the effect of self-shading due to increasing biomass areal density ( $0 \le j(\rho) \le 1$ ) (Cerco and Cole, 1995). All of these functions are time and space-dependent and their values vary according to incident solar radiation, temperature, local nutrient concentrations, salinity, and biomass areal density.

In most large-scale algal mariculture systems, it is not economically feasible to control certain physical factors such as temperature, salinity, and light, which can have large diurnal seasonal fluctuations. However, knowledge of how macroalgae respond to these factors that determine growth potential is key to understanding seasonal patterns of yield and in assessing the viability of potential cultivation sites (Hanisak, 1987).

## Maximum Productivity

In studies led by Hanisak, G. tikvahiae was fed pulsed nutrients (phosphorus as NaH2PO4, nitrogen as NaNO3 and NH4Cl) and grown in outdoor containers (Hanisak, 1987). The maximum growth rate reported in the literature is P = 0.6/d and this suggests only minimal limitations due to non-optimal temperatures, nutrients, salinities, and culture densities (Hanisak, 1987).

## Light

No light is available to the algae at night, so no algae growth occurs during this period. Several clones of G. tikvahiae were isolated from outdoor or greenhouse samples and cultured under controlled laboratory conditions (Hanisak, 1987). In the lab, light saturation typically occurred at about 21.9 W/m2 (100  $\mu$ E/(m2 s)), levels that are typical for populations of Gracilaria under natural conditions in turbid water (Hanisak, 1987; Lapointe and Hanisak, 1985). Light inhibition occurred at relatively high photon flux of 109.5 W/m2 (500  $\mu$ E/(m2 s)) (Friedlander and Zelikovitch, 1984).

In Florida, where these experiments took place, surface light intensities ranged from 175.2 to 394.2 W/m2 (306.6 W/m2 in August when growth was maximum) at the water surface, but the culture system was 1 m deep in turbid waters.

Based on the analysis by Hanisak (1987), light limitation in Figure 4.1 is described by Equation 4.3 (Cerco and Cole, 1995) :

$$f(I) = \begin{cases} \frac{I}{I_{opt}} exp\left(1 - \frac{I}{I_{opt}}\right) for \ I < I_{opt} \\ 1 & for \ I \le I_{opt} \le I_{max}, \\ \frac{I_{max}}{I} & for \ I > I_{max} \end{cases}$$
(4.3)

where  $I_{\text{opt}}$  = 21.9 W/m² and Imax = 109.5 W/m². Note that Steele's Equation was

used for the increasing limb of the light-limitation function (I < Iopt). Without specific data on growth limitation due to photoinhibition, an asymptotically decreasing light limitation function was assumed when I > Imax. Hence, the limitation function described by Hanisak (1987) was implemented.



Figure 4.1. This piecewise function describes *G. tikvahiae* light limitation. *G.tikvahiae* growth, as a function of irradiance, is maximized when f(I) = 1.0 (from light saturation at 21.9 W/m<sup>2</sup> to light inhibition at four to five times that energy flux at 109.5 W/m<sup>2</sup>).

#### *Temperature*

Experiments were conducted on *Gracilaria tikvahiae* with temperatures  $12^{\circ}C < T < 36^{\circ}C$  with optimal growth between  $24^{\circ}C < T < 30^{\circ}C$  (Hanisak, 1987). Lapointe (1984) indicated that *Gracilaria* did not grow well below 15 °C (growth was about 25% of the maximum observed at 27 °C). Figure 4.2 depicts the temperature growth-limitation function:

$$g(T) = \begin{cases} 1 \text{ for } 24 \le T \le 30^{\circ}\text{C} \\ exp[-K_{T_2}(T-30)^2] \text{ for } T > 30^{\circ}\text{C} \end{cases}$$
(4.4)

where KT1 = 0.017 °C<sup>-2</sup> and KT2 = 0.064 °C<sup>-2</sup>. These coefficients were

determined by fitting the preceding equation such that g(15 °C) = 0.25 and g(36 °C) = 0.1



Figure 4.2. *G. tikvahiae* temperature limitation function. Temperature dependent growth is optimal between 24 °Cand 30 °C. As temperatures approach the lower temperature bound, macroalgae growth rises steadily, and as temperatures increase past the upper temperature bound, macroalage growth declines more rapidly than it rose. When mixing deep and shallow seawaters, one should aim for a mixing ratio that favors the lower end of the ideal temperature range. This can allow for a temperature buffer and a prolonged period of maximal growth.

Although this distinction was considered in the model, Pmax is reported using the convention of the experiments that implicitly consider no growth at night by dividing by the fraction of the day that is light.

# Nutrients

Lapointe (1987) noted that phosphate was the limiting nutrient in the Florida Keys. Maximum yield occurred at nutrient enrichments of 10 to 100  $\mu$ M nitrogen and 1 to 10  $\mu$ M phosphorus (Hanisak et al, 1990). There was a pattern of winter N and P limitation and summer P limitation (Lapointe 1987). The Michaelis- Menten (Monod) equation is often used to estimate nutrient limitation for micro- (Hein, 1995) and macroalgae (Li, 2016; Pederson and Borum, 1997) where:

$$h([NO_3]), ([NH_3]), ([PO_4]) = \min\left[\left(\frac{[PO_4]}{k_{[PO_4]+[[PO_4]]}}\right), max\left(\frac{[NH_4]}{k_{[NH_4]+[[NH_4]]}}, \left(\frac{[NO_3]}{k_{[NO_3]+[NO_3]}}\right)\right)\right],$$

$$(4.5)$$

Until such time as experimental data are available to confirm half-saturation constants, values of  $\kappa_{PO4} = 0.1$ ,  $\kappa_{NH4} = 0.3$ , and  $\kappa_{NO3} = 0.4 \,\mu\text{M}$  were implemented because these yielded growth rates consistent with observations by Lapointe (1987). The preceding equation indicates that growth is either nitrogen or phosphorus limited.



Figure 4.3. G. tikvahiae nutrient limitation. Until such time as experimental data are available to confirm half-saturation constants, values of  $\kappa_{PO4} = 0.1$ ,  $\kappa_{NH4} = 0.3$ , and  $\kappa_{NO3} = 0.4 \,\mu\text{M}$  were implemented because these yielded growth rates consistent with observations by Lapointe (1987). The preceding equation indicates that growth is either nitrogen or phosphorus limited. Greater concentrations of nitrogen seem to be required to observe optimal growth than those of phosphorus.

# Salinity

Growth was observed over a salinity range of 6 to 42‰, with optimal growth

between 24 and 36 ‰ (Capo et al, 1999. The salinity growth limitation function in Figure 4.4 is:

$$i(S) = \begin{cases} exp[-K_{S_1}(24-S)^2] \text{ for } S < 24\%_0 \\ 1 & \text{for } 24 \le S \le 36\%_0 \\ exp[-K_{S_2}(S-36)^2] \text{ for } S < 36\%_0 \end{cases}$$
(4.6)

where  $K_{S1} = 0.007$  and  $K_{S2} = 0.063$  ppt<sup>-2</sup> obtained by setting g (6) =

g(42) = 0.1 because of the observed growth range noted by Capo (1999).



Figure 4.4. G. tikvahiae salinity limitation. Recall that salinity at the study site is fairly sconstant; variations that deviate greatly from 35ppt are unlikely.

#### Culture Density

In a series of outdoor, continuous-flow seawater cultures, Lapointe (1978) investigated the effects of culture density on G. tikvahiae growth rates. They reported maximum growth rates at a low density ( $\rho_{opt} = 0.4 \text{ kg/m2}$ ) with decreasing rates thereafter. These effects are likely due to a combination of self-shading (although decreased nutrient availability could also limit growth, nutrients were in excess in their experiments,). Digitizing their data, the fit of growth limitation as a function of culture density was (Figure 4.5):

$$j(p) = \begin{cases} 1 \text{ for } p < p_{opt} \\ exp[-K_p(p - p_{opt})] \text{ for } p \ge p_{opt} \end{cases}$$
(4.7)

where  $K_{\rho} = 0.513 \text{ m}^2/\text{kg}$ .

#### Basal Metabolism

A photosynthesis:respiration ratio of 20:1 suggests that the basal metabolic rate is  $B_{\rm M} = -0.05 \ (P_{\rm max} = -0.03/d)$  [LTD84]. Because the maximum measured growth rate of 0.6/d is the net growth rate, the basal metabolic rate has already been considered in this

estimate so it need not be explicitly considered in the model. *G. tikvahiae* growthlimitation model parameters are listed in Table 4.1.

Experiments indicate that light, nutrients, and temperature more strongly affect algae growth than salinity and culture density. Light and temperature patterns are seasonal, while the nutrients made available to the aquaculture system can be strategically curated by way of the deep seawater (DSW) pumping system. As such, suggestions offered to farmers of this modeled aquaculture site should prioritize forming a seeding schedule that aligns with optimal light and temperature conditions throughout the year.



Figure 4.5. *G. tikvahiae* growth as function of culture density. As the macroalgae culture grows beyond 0.4 kg/m<sup>2</sup>, its growth rate decreases exponentially. This knowledge will inform harvest frequency and technique (depending on the extent of self-shading).

Table 4.1. Growth model parameters

Parameter	Value
Maximum growth rate, $P_{\text{max}}$	1.04/d
Light saturation, Iopt	21.9 W/m2
Photoinhibition, Imax	109.5 W/m2
Temperature constants, KT and KT2	0.017 and 0.064 °C–2
Half-saturation constants, knh4, kno3, kpo4,	0.1, 0.3, and 0.4 µM
Salinity constants, KS and KS	0.007 and 0.063 ppt-2
Density saturation, popt	0.4 kg/m2
Density constant, Kp	0.513 m2/kg2

#### CHAPTER FIVE

#### **Results and Discussion**

A set of conditions for the basic growth model was initially specified according to expert judgement (Lapointe and Ryther, 1978), hereinafter referred to as the baseline model: a culture depth of d = 5 m, a mixing ratio of r = 0 (no irrigation), hmax = 4.0 kg/m2 and hmin = 0.4 kg/m2 (biomass to trigger harvest and pruned biomass). The baseline model run yielded an average growth rate of P = 0.048/d and cumulative biomass grown over one year (2014) of 61.0 kg wet wt./(m2 yr).

For G. tikvahiae cultivation in Florida, one practice was to harvest at the upper end of the optimal range at hmax = 4 kg wet wt./m2 and prune back to hmin = 0.4 kg wet wt./m2 ((Lapointe and Ryther, 1978). This scheme typically required harvesting approximately every week in the summer and every two weeks in the winter (Hanisak, 1987; Lapointe and Ryther, 1978).

An optimization was performed on the baseline model to maximize the cumulative biomass harvested per square meter for 2014. These optimal values were supplied to model runs over 12 months (January to December of 2014) and the range of productivities was recorded. Adjustable parameters were culture depth, d, to control light intensity (i.e., too shallow will lead to photoinhibition while too deep does not allow sufficient light), mixing ratio, r, which is the fraction of cold, nutrient-rich, deep water with which the system is irrigated (i.e., too little mixing can cause insufficient nutrients while too much deep water will yield sub-optimal temperatures), and harvest triggers,

hmax and hmin, to control density limitation (i.e., the density trigger for harvest and the minimum density to maintain while pruning).

#### Basic Growth Model Optimization

# Depth Optimization

Optimization efforts first focused on determining the culture depth that maximized yield (r, hmax, and hmin held at baseline values). Figure 5.1 shows that algal biomass was maximized at 72.2 kg wet wt./(m2 yr) when algae were planted at depths of 6 to 10 m below the sea surface, which exceeded the baseline results (61.0 kg wet wt./(m2 yr)) by 18.5%. Discontinuities exist in the cumulative harvested biomass data (depicted in red) throughout each optimization step because the aquaculture site can only be harvested as an integer number (1, 2, 3, etc.) of times per year. For example, a site cannot be harvested 6.2 times a year; the site could be harvested either 6 or 7 times per year.

This figure also indicates that seeding 7.81 m below the sea surface minimized light limitation (maximized G. tikvahiae growth as a function of light) achieving f(I) = 0.47 (with 0.5 being the theoretical maximum). For d = 5 m, f(I) = 0.44, so 7.81 m was set as optimal.



Figure 5.1. f(I) (solid blue curve), P (dashed blue curve), and annualized cumulative mass harvested per unit area (symbols) as functions of structure depth, d. At depth d = 7.81m, light-dependent G. *tikvahiae* growth was maximized. The red piecewise function represents cumulative biomass har- vested. These data suggest that roughly 72 kg of algal biomass is available to be harvested when the glowing platform is positioned at depths ranging from 5.5-10.5 m. Since light-dependent G. tikvahiae growth is maximal at 7.81 m, this is an appropriate depth for the platform.

#### Irrigation Mixing Ratio Optimization

Once culture depth was optimized, the relative volumes of deep and shallow waters were varied to determine the ratio that maximized growth (at a fixed depth of d =7.81 m). The goal was to increase nutrient availability without cooling the water too much (thereby inhibiting growth). Biomass was maximized between mixing ratios of 0.08 and 0.22 at 108 kg/(m<sup>2</sup> yr), as shown in Figure 5.2. Ultimately, temperature and nutrient limitation could be co-optimized by setting r = 0.15, achieving g(T) = 0.98 and h([PO<sub>4</sub>], [NH<sub>4</sub>], [NO<sub>3</sub>]) = 0.86 (due primarily to [PO<sub>4</sub>] limitation). This resulted in 108.4 kg/(m<sup>2</sup> yr), a 50.1% increase in cumulative harvested biomass over the depth optimization step and a 77.8% increase over the baseline model (61.0kg wet wt./(m<sup>2</sup> yr)).

As *r* increased from 0 to 0.25, the nutrient-limitation function, h(v), increased while the temperature-limitation function, g(T), decreased. This indicated that as more nutrient-

rich (cool) waters were used to irrigate the algae farm (when *r* was small and increasing), more algae was harvested. The rate at which g(T) decreased was less than the rate of increase of h(v), indicating that optimal nutrient availability was a more sensitive variable than optimal ocean temperature for small values of *r*.



Figure 5.2. Mixing ratio optimization. Since biomass (*P*) was maximized between mixing ratios of 0.08 and 0.22 and the maximum biomass that could be harvested was 108 kg/( $m^2$  yr) between mixing ratios of 0.9 and 0.21, the ideal mixing ratio was established as 0.15.

#### Harvest Parameters Optimization

Next, the trigger for harvest, hmax, and the density to prune back to, hmin, were optimized. Depth and the mixing ratio were fixed at d = 7.81 m and r = 0.15. Figure 5.3 implemented hmax = 4.0 kg wet wt./m2 with hmin varied from 0.01 to 3.99 kg wet wt./m2. As a result, hmin = 1.23 kg wet wt./m2 was the optimal density to prune to that maximized biomass at 119.5 kg/(m2 yr), a 65% increase in cumulative harvested biomass over the mixing-ratio optimization and a 96% increase over the baseline model.

Finally, the trigger for harvest, hmax, was optimized between 1.24 and 8.0 kg wet wt./m<sup>2</sup> with fixed values of depth, mixing ratio, and minimum density to maintain at d = 7.81 m, r = 0.15 and hmin = 1.23 kg wet wt./m2.

As shown in Figure 5.4, P declined nearly linearly as hmax increased. The optimal harvest trigger was hmax = 2.27 kg wet wt./m2, resulting in 129.7 kg/(m2 yr). With these harvest parameters, the predicted harvest frequency was every 3 days. A more efficient strategy would be to harvest algae at hmax = 3.6 kg wet wt./m2, resulting in a this harvest frequency of 7 days and a yield of 123.3 kg/(m2 yr), a 108.5% increase in cumulative harvested mass above the mixing-ratio optimization and an 212.7% increase over the baseline model.

For a more efficient harvesting schedule, a harvest frequency of every 7 days (hmax = 3.6 kg/m2) was selected. This yielded a cumulative harvest biomass of 123.3 kg/(m2 yr).



Figure 5.3. Optimization of hmin when hmax = 4.0 kg/m2. This figure indicates that it is advantageous to initially seed the growing platform with densities that range from 0.5 kg/(m2 yr) to 2.5 kg/(m2 yr), as the minimum amount that can be harvested when seeding densities fall within this range is 110.0 kg/(m2 yr), merely 11% less than the maximum harvest-able amount at this depth and mixing ratio. Knowledge of this suitable range is impactful for farming practices.

#### **Overview of Biological Parameters**

Figure 5.5 compares the cumulative biomass yield for each optimization procedure. Cumulative harvested biomass increased with each optimization of the baseline model. The optimization step between depth and mixing ratio yielded the greatest increase in harvested biomass (65% more than the depth optimization and a 96% increase over the baseline results).



Figure 5.4. Optimization of hmax when hmin= 1.23 kg/m2. The range of suitable densities for hmax I s1.8- $3.3 \text{ kg/m}^2$ . The minimum biomass that can be harvested in this range is  $125 \text{ kg/m}^2$ , with a maximum biomass of  $129.7 \text{ kg/m}^2$ . Beyond this range, harvestable biomass declines rapidly.



Figure 5.5. Comparisons of cumulative biomass harvested at eachoptimization stage: Basic Growth Model, Depth optimization, mixing ratio optimization, seeding density optimization, and harvest trigger density optimization.

Figure 5.6 is a heat map of harvested cumulative biomass overlain by con- tours of harvest frequency in days. Each labeled contour indicates the average number of days required for hmin to grow to a density of hmax, the harvest trigger. While frequent harvests of a day or two yielded 125-130 kg/(m2 yr), this is not feasible from a cost and labor perspective. If instead a harvest frequency of eight days is desired, this heat map indicates that the trigger for harvesting is hmax = 3.6 kg/m2 and the culture should be pruned back to hmin = 1.2 kg/m2. The pixelated appearance of the cumulative mass heat map is due to the integer number of harvest changing within the one-year model simulation. As harvest frequency increased, more harvests were fit into the year with each harvest adding an additional tranche of Gracilaria.

This analysis could prove valuable to *G. tikvahiae* farmers as it provides practical operational guidelines. If for any reason (natural disaster, scheduling error, etc.) the scheduled harvest frequency was no longer tenable, this figure suggests how to maximize biomass (for both more and less frequent harvests).



Cumulative mass  $(kg/m^2)$ 

Figure 5.6. *G.tikvahiae* cumulative biomass yield (2014) overlain by contours (black curves) of average harvest frequency (days).

## CHAPTER SIX

### Conclusions

#### Summary

Recall that certain physical factors (temperature, salinity and light) can have large diurnal seasonal fluctuations that render attempts to manipulate them to enhance macroalgal growth both physically challenging and economically daunting. By incorporating experimental *G. tikvahiae* growth data and our modified equation for macroalgal growth, we were able to establish a range of environmental conditions under which macroalgal production of this species can be maximized. In short, after testing the sensitivities of temperature, salinity, and light, their optimal ranges for growing *G. tikvahiae* were determined 25-35 ppt salinity, 24-30 degrees C temperature, 21.9-109.5 W/m2 light. Nutrient availability (as a means of irrigating the aquaculture system) to the aquaculture system also varies, though employing the DSW pump system offer nutrient regulation.

Next, optimization efforts were focused on factors more readily adjustable: depth of submersible aquaculture system, concentrations of nutrients used to irrigate this system, minimum seeding density and maximum biomass growth before triggering harvest.

When optimized, these values were as follows: a culture depth of d = 7.81 m, a mixing ratio of r = 0.15, hmax = 3.6 kg/m2 (harvest trigger density) and hmin = 1.2 kg/m2 (biomass to trigger harvest and pruned biomass).

#### Concluding Thoughts

These results demonstrate the range of data that can be extracted and analyzed from a model of a macroalgae aquaculture system. We have shown that extant environmental codes such as EFDC coupled with water-quality codes such as CE-QUAL can be used to model algae growth in open-ocean cultivation systems. This strategy allows researchers to avoid time-consuming, costly experiments that represent the majority of current scholarship in this rapidly-emerging field of algal culture for biofuels production. Numerical simulation of the flow system offers the ability to design the macroalgal growth platform and set operational ranges of parameters before construction, thus avoiding the all-too-often used trial-and-error method. Modeling should be used to optimize system parameters to improve efficiency.

When adding to this research, these parameters could include new aquaculture system designs, ideal pumping rates of deep seawater for pulsed irrigation schedules, and optimal harvest times.

Correlation of variable flow rate (from the deep seawater pump) and macroalgal nutrient uptake to solar energy conversion efficiency could yield interesting results. The impact of climate change on growth rates and biomass productivity could also be used to determine long-term feasibility of a project. Moreover, experiment operators can evaluate the impacts of various system conditions (e.g., depth, harvest frequency, flow speeds) without risk of manipulating or damaging the algal biomass under study while saving cost and time.

Most experimental data available are sourced from small-scale tests. This model could be used to determine the feasibility and potential benefits of scaling up. Further,

simulations may be run to quantify the benefits of integrating algae culture ponds with waste treatment plants and fossil-fuel- based power plants, among other possibilities.

## BIBLIOGRAPHY

- Abdelrhman, Mohamed. "Modeling Water Clarity and Light Qual-ity in Oceans". In: Journal of Marine Science and Engineering 4.4 (2016), p. 80.
- Alvarado-Morales, Merlin et al. "Life cycle assessment of biofuel production from brown seaweed in Nordic conditions". In: Biore- source Technology 129 (2013), pp. 92–99. ISSN: 0960-8524. DOI: https://doi.org/10.1016/j.biortech.2012.11.029. URL: https:// www. sciencedirect. com/ science/ article/ pii/ S0960852412017038.
- Appels, Lise et al. "Anaerobic digestion in global bio-energy production: potential and research challenges". In: Renewable and Sustainable Energy Reviews 15.9 (2011), pp. 4295–4301.
- Borines, Myra; de Leon, Rizalinda; and McHenry, Mark. "Bioethanol production from farming non-food macroalgae in Pacific island nations: Chemical constituents, bioethanol yields, and prospec- tive species in the Philippines". In: Renewable and Sustainable Energy Reviews 15 (Dec. 2011), pp. 4432–4435. DOI: 10.1016/ j.rser.2011.07.109.
- Bringezu, Stefan et al. "Towards a Sustainable Biomass Strategy. What we know and what we should know". In: (Jan. 2007).
- Bruinsma, Jelle. World agriculture: towards 2015/2030: an FAO study. Routledge, 2017.
- Buschmann, Alejandro et al. "Mariculture of red algae in Chile". In: World Aquaculture 30 (Jan. 1999), pp. 41–45.
- Buschmann, Alejandro H; Westermeier, Renato; and Retamales, Claudia A. "Cultivation of Gracilaria on the sea-bottom in southern Chile: A review". In: Journal of Applied Phycology 7.3 (1995), pp. 291-301.
- Capo, Tom et al. "Sustained high yields of Gracilaria (Rhodophyta) grown in intensive large-scale culture". In: Journal of Applied Phycology 11 (Apr. 1999). DOI: 10.1023/A:1008077722769.
- Cerco, Carl F and Cole, Thomas. User's guide to the CE-QUAL- ICM three-dimensional eutrophication model. Tech. rep. release version 1.0. Technical Report EL-95-15, US Army Corps of Engineers .1995.
- Climate Change. May 2021. URL: http://www.epa.gov/climatechange/ ghgemissions/global.html.

- Costa, JC et al. "Biomethanation potential of biological and other wastes". In: Biofuel Technologies. Springer, 2013, pp. 369–396.
- Dave, Ashok et al. "Techno-economic assessment of biofuel development by anaerobic digestion of European marine cold-water seaweeds". In: Bioresource Technology 135 (2013). Biorefineries, pp. 120–127. ISSN: 0960-8524. DOI: https://doi.org/10.1016/ j.biortech.2013.01.005. URL: https://www.sciencedirect. com/science/article/pii/S096085241300028X.
- Dragone, G. et al. "Third generation biofuels from microalgae". In: vol. 2. Jan. 2010, pp. 1355–1366.
- Eisentraut, Anselm. "Sustainable Production of Second-Generation Biofuels". In: (2010). DOI: https:// doi. org/ https:// doi.org/ 10 . 1787 / 5kmh3njpt6r0 - en. URL: https:// www. oecd- ilibrary.org/content/paper/5kmh3njpt6r0-en.
- Kampachi Farms. Final environmental assessment for an off- shore native Hawaiian macroalgae demonstration project. Tech. rep. Kampachi Farms LLC, 2019.
- Food and Agricultural Organization of the United Nations. Cultured Aquatic Species Information Programme, Gracilaria spp. 1996. URL: http://www.fao.org/fishery/culturedspecies/ Gracilaria\_spp/en.
- Friedlander, M and Zelikovitch, N. "Growth rates, phycocolloid yield and quality of the red seaweeds, Gracilaria sp., Pterocla- dia capillacea, Hypnea musciformis, and Hypnea cornuta in field studies in Israel". In: Aquaculture 40.1 (1984), pp. 57–66.
- Grandelli, Pat. Modeling the Physical and Biochemical Influence of Ocean Thermal Energy Conversion Plant Discharges into their Adjacent Waters. Tech. rep. Makai Ocean Engineering, Inc., Kailua, HI (USA), 2012.
- Hanisak, M. D. "Cultivation of Gracilaria and other macroalgae in Florida for energy production". In: Seaweed Cultivation for Renewable Resources. Amsterdam, The Netherlands: Elsevier Science Publishers, 1987, pp. 191–218. URL: http://www. sciencedirect.com/science/journal/00225320.
- Harlin, Marilyn M. "Nitrate uptake by Enteromorpha spp. (Chloro- phyceae): Applications to aquaculture systems". In: Aquaculture 15 (Apr. 1978), p.373– 376. DOI: 10.1016/0044 - 8486(78) 90082-0.
- Hanisak, M Dennis; Littler, Mark M; and Littler, Diane S. "Significance of macroalgal polymorphism: Intraspecific tests of the functional-form model". In: Marine biology 99.2 (1988), pp. 157–165.

- Hanisak, M Dennis; Littler, Mark M; and Littler, Diane S. "Application of the functionalform model to the culture of seaweeds". In: Thirteenth International Seaweed Symposium. Springer. 1990, pp. 73–77.
- Hein, Mette; Pedersen, M Folager; and Sand-Jensen, Kaj. "Size- dependent nitrogen uptake in micro-and macroalgae". In: Marine ecology progress series. Oldendorf 118.1 (1995), pp. 247–253.
- Jara, F. "Epidemic outbreaks of herbivorous annelid worms (Poly- chaeta: Nereididae) in the south of Chile". In: Medio Ambiente 11.1 (1990), pp. 65–75.
- Jung, K et al. "Potentials of macroalgae as feedstocks for biore- finery". In: (2017).
- Kanwisher, J. "Photosynthesis and respiration in some seaweeds." In: Some contemporary studies in marine science. (1966), pp. 407–420.
- King, RJ and Schramm, W. "Photosynthetic rates of benthic marine algae in relation to light intensity and seasonal variations". In: Marine Biology 37.3 (1976), pp. 215– 222.
- Littler, Mark M and Arnold, Keith E. "Primary productivity of marine macroalgal functional-form groups from southwestern North America". In: Journal of Phycology 18.3 (1982), pp. 307–311.
- Lapointe, Brian E. "Phosphorus-and nitrogen-limited photosyn- thesis and growth of Gracilaria tikvahiae (Rhodophyceae) in the Florida Keys: An experimental field study". In: Marine Biology 93.4 (1987), pp. 561–568.
- Lapointe, Brian E and Hanisak, M Dennis. "Productivity and nu- trition of marine biomass systems in Florida". In: Energy from Biomass and Waste IX. Institute of Gas Technology, 1985, pp. 111–126.
- Li, Hongmei et al. "Growth responses of Ulva prolifera to inor- ganic and organic nutrients: Implications for macroalgal blooms in the southern Yellow Sea, China". In: Scientific reports 6 (2016), p. 26498.
- Lapointe, Brian E and Ryther, John H. "Some aspects of the growth and yield of Gracilaria tikvahiae in culture". In: Aqua- culture 15.3 (1978), pp. 185–193.
- Lapointe, BE; Tenore, KR; and Dawes, CJ. "Interactions between light and temperature on the physiological ecology of Gracilaria tikvahiae (Gigartinales: Rhodophyta)". In: Marine Biology 80.2 (1984), pp. 161–170.
- Mar'echal, Jean-Philippe; Hellio, Claire; and Hu, Chuanmin. "A simple, fast, and reliable method to predict Sargassum washing ashore in the Lesser Antilles". In: Remote Sensing Applications: Society and Environment 5 (2017), pp. 54–63.

Mottet, Alexis et al. "Estimating anaerobic biodegradability in- dicators for waste activated sludge". In: Chemical Engineering Journal 160.2 (2010), pp. 488–496.

Murphy, Jerry D et al. A perspective on algal biogas. IEA Bioenergy, 2015.

- Olson, K and Andreas, A. Natural Energy Laboratory of Hawaii Authority (NELHA): Hawaii Ocean Science & Technology Park; Kailua-Kona, Hawaii. Tech. rep. NREL-DATA (National Renew- able Energy Laboratory-Data (NREL-DATA), Golden, CO, 2012.
- OECD. The Ocean Economy in 2030. 2016, p. 252. DOI: https: //doi.org/https://doi.org/10.1787/9789264251724- en. URL: https://www.oecdilibrary.org/content/publication/ 9789264251724-en.
- Pedersen, Morten Foldager and Borum, Jens. "Nutrient control of estuarine macroalgae: growth strategy and the balance between nitrogen requirements and uptake". In: Marine Ecology Progress Series 161 (1997), pp. 155–163.
- Philippsen, Aaron. "Energy Input, Carbon Intensity, and Cost for Ethanol Produced from Brown Seaweed". PhD thesis. University of Victoria, 2010.
- Pizarro, A. "Conocimiento actual y avances recientes sobre el manejo y cultivo de Gracilaria en Chile". In: Monographs in Bi- ology 4 (1986), pp. 63–96.
- Retamales, Claudia A and Buschmann, Alejandro H. "Gracilaria- Mytilus interaction on a commercial algal farm in Chile". In: Hydrobiologia 326.1 (1996), pp. 355–359.
- Ryther, John H.; DeBoer, James A.; and Lapointe, Brian E. "Cultivation of seaweeds for hydrocolloids, waste treatment and biomass for energy conversion". In: Proceedings of the International Sea- weed Symposium. Vol. 9. 1979, pp. 1–16. American Institute of Physics Publication Board. *AIP Style Manual*. 4th ed. New York: American Institute of Physics, 1990.