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Does temperature shift justify microalgae production under greenhouse?

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Abstract

We analyze the influence of greenhouses in the cultivation of phytoplankton. For this we propose a model for the marine green algae *Tetraselmis suecica*, and adapt it to four other species (*Spirulina platensis*, *Dunaliella salina*, *Phaeodactylum tri-cornutum* and *Chlorella vulgaris*). Experiments under a greenhouse were carried out for the marine green algae *Tetraselmis suecica*, shifting the temperature of two raceways compared to a reference raceway with free evolving temperature. The productivity model was then parametrized and validated accounting for the recorded evolution of temperature and light. The yearly raceway pond production and the benefit of greenhouse usage was assessed under different scenarios for the five considered species. At year scale, greenhouse efficiency is notable only for few species, e.g. *Spirulina platensis*, where productivity can be increased by 20 %. Based on these results, cultivation under greenhouse is beneficial mainly to protect the culture against contamination and to increase productivity in cold regions for species susceptible to photoinhibition with optimal growth in high temperatures. Rotation of the cultivated species is also a good strategy to improve annual productivity.

Keywords: Microalgae, Temperature, Raceway, Modelling, Greenhouse

1. Introduction

Microalgae are a promising and environmental-friendly source of high valuable chemicals for green chemistry and, on a longer run, for biofuel (Mobin and Alam, 2017). Despite its potential, large scale production of algal biofuel is challenging, and

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to date not economically and energetically viable (Baudry et al., 2017, Morales et al., 2019a). Maximizing productivity through a better understanding and optimization of the growth conditions is still necessary so that industrial production of microalgae becomes a reality (Barsanti and Gualtieri, 2018, Bernard, 2011).

The two most widespread processes for producing microalgae are closed photobioreactors and open raceways (Schade and Meier, 2019). Photobioreactors can lead to a higher production output with a better resistance to biological contaminants, but the energy input necessary for mixing and cooling strongly penalize the economical and environmental balances (Tan et al., 2018, Schade and Meier, 2019). The more rustic raceways are a simpler and cheaper way for producing microalgae outdoors. They need less energy input and the functional design is simpler. The drawback is the higher contamination in the culture by grazers, bacteria, viruses or even other competitive microalgae species (Williams and Laurens, 2010, Mata et al., 2010). Using a greenhouse is an interesting trade-off to reduce contamination while modulating climate. Indeed, outdoor production is susceptible to external weather changes, especially temperature and solar radiation fluctuations, leading to growth conditions significantly different from the constant and optimal ones often maintained in the laboratory.

Microalgae are autotrophic organisms that fix CO_2 from the atmosphere through the Calvin cycle, using the photon energy from photosynthetically active radiations (PAR). Photosynthesis rate responds differently according to light intensity, which can be separated in 3 regions: photolimited, saturated, and photo inhibited (Béchet et al., 2013, Williams and Laurens, 2010). In the first region growth is limited by the quantity of absorbed photons. In the light saturated region, the photosynthetic system functions at its full capacity so growth does not increase with more light intensity. Surpassing a given intensity, photo inhibition takes place, and growth starts to diminish because of deactivation of key proteins in the photosynthetic apparatus (García-Camacho et al., 2012).

Temperature has also a strong influence on the growth rate of microalgae, since it affects the rate of enzymatic reactions. Moreover, it also modulates the solubility of several key molecules, such as CO_2 and O_2 that impact the growth rate (Ketheesan and Nirmalakhandan, 2013). The direct effect on growth rate for moderated temperatures is well represented by the Arrhenius Equation, representing how the reaction rates are enhanced by temperature (Grimaud et al., 2017). But above the optimal temperature – the temperature for which the growth is maximized- the structure of some proteins changes, especially in the electron transport chain, leading to a rapid drop in the net growth rate concomitant with a mortality increase (Serra-Maia et al., 2016). Above a maximum temperature, the algae can no longer grow (Grimaud et al., 2017).

Climate control in greenhouses is an option to adapt the thermal conditions to

the physiological optimum of the species. Shadowing for reducing photoinhibition can also be implemented in greenhouses, using nets or even photovoltaic panels (Martínez et al., 2017, Morales et al., 2019b). Artificial light is also a possibility for mitigating the natural daily and seasonal fluctuations for outdoor production. The low winter temperatures diminish productivity, while in summer extreme light intensities might inhibit growth (Serra-Maia et al., 2016) and elevated temperatures may even lead to culture collapse. Greenhouses thus offer an interesting way to alleviate growth reduction due to weather, either passively or with additional energy to cool down or warm up depending on the season. The benefit of a greenhouse must then be assessed and compared with the additional costs in terms of energy and infrastructure.

Growth models for microalgae have been successfully used to predict the productivity in response to different conditions and factors, such as nutrient concentration, temperature, and light (Béchet et al., 2013, Bernard, 2011). Most models are only validated in indoor conditions within a steady environment, which simplifies experimental setting, but extrapolating the use of these models to an outdoor dynamic environment is uncertain (Darvehei et al., 2018). Examples of validated models for raceway cultivation are scarce, even more for long-term production. An artificial neural network model was validated for cultivation in open raceway ponds, while confirming that temperature and light intensity are very important factors influencing productivity (Supriyanto et al., 2019). Some models taking into account hydrodynamics were validated using mass balance of various components, but they do not take into account temperature changes (Fernández et al., 2016, Ranganathan et al., 2017). Furthermore, long-term prediction models that have been developed to account for seasonal and diurnal changes in temperature and light have rarely been validated (Banerjee and Ramaswamy, 2017, Casagli et al., 2021).

In this work, we explore the effects of seasonal and daily temperature changes in microalgae production using raceways in a greenhouse. The model is used to predict annual production and the influence of greenhouses in the cultivation of microalgae accounting for temperature and light in a dynamic outdoor environment. The model is calibrated and validated using original experiments with *Tetraselmis suecica*, a microalga widely used in aquaculture as a food source, and which can tolerate a variety of temperatures and salinity (Molina et al., 1991, Fabregas et al., 1984). The experiments were carried out in 3 raceways under a greenhouse with three different temperature regimes.

The model is then further consolidated for four other species (*Dunaliella salina*, *Spirulina platensis*, *Phaeodactylum tricornutum* and *Chlorella vulgaris*) based on parameters available in the literature. Productivity simulations for different locations in France are carried out. These simulations exemplify which characteristics of a species are important when cultivated at different seasons and locations.

2. Materials and methods

2.1. Experiments in raceway ponds

The green microalga *Tetraselmis suecica* AC 254 (Algobank Caen, Université de Caen Normandie, France), was inoculated in three 2.61 m² raceway ponds operated in a transparent greenhouse, located in *Villefranche-sur-Mer*, France. The water depth was 16 cm on average, for a total culture volume of 417 L. Fresh water was added daily to compensate evaporation. The temperature of the first raceway was not controlled, while that of the others was controlled to be respectively 5 °C above and 5 °C below the first one. Two experiments are shown in this work, the first one took place between the 12th and 29th of January, which was used to validate the model, and the second one between 06th and 26th of February, which was used to calibrate the model.

2.2. Culture medium

The medium consisted in 0.2µm filtered sea water, enriched with an industrial solution (Kanieltra 15 Fe DTPA) used for agricultural purposes providing trace elements and metals. No vitamins were added. pH was kept at 7.5 by CO₂ injection when pH value was above this setpoint. The concentrations for every element in the medium are listed at Table .1.

Temperature was regulated with a Lauda cryostat connected to a thermal exchanger made of inox tubes place in the raceway. A ramp program with a set of 12 temperatures was used for each raceway where the temperature was changed. Temperature and light intensity of the three raceways were recorded every 30 seconds. At the beginning of each batch, culture medium was renewed and raceways were inoculated with the same inoculum of *Tetraselmis suecica* at the same concentration.

[Table 1 about here.]

2.3. Dry weight and cell counting

Biomass dry weight was measured on samples of 50 mL collected in duplicates, centrifuged for 5 minutes at 3000 g and rinsed 3 times with freshwater to reduce the salt concentration. It was verified that *Tetraselmis suecica* cells do not break in fresh water or due to centrifugation. Once the centrifugation supernatant was removed, the cell pellet was resuspended in about 1 mL and filtered through a preweighted 1.2 m GF/C glass fiber filter. Filters were stored inside an oven at 75°C for 24 hours and then weighted with a precision balance.

The concentration of cells was measured with an optical cell counter (HIAC, model 9703) after dilution of the culture with 0.22 µm filtered seawater. Concentration was calculated counting all particles whose diameters range from 2.65 m to 20 m.

2.4. Laboratory calibration experiments

Additional experiments were conducted in the laboratory to measure the response of the growth rate to different light intensities and temperatures. For this purpose, we used the MC 1000-OD multicultivator which is equipped with 8 culture flasks placed in a thermostatically controlled bath and individually illuminated by a controlled light source. This device also allows us to measure automatically every 5 minutes the optical density of the cultures at 680 and 720nm. Simultaneous measurements of cell density have shown that, over a given concentration range, the relationship between cell concentration and OD is linear. For each of the 2 experiments, the samples taken from the raceway ponds were diluted so that the initial cell concentrations in 8 culture flasks were identical. For the light and temperature conditions tested (see paragraph 2.6), the maximum growth rate was measured during the exponential growth phase, i.e., when the slope of the data on a logarithmic scale is linear.

2.5. Model hypotheses

To represent algal cultivation under a greenhouse submitted to actual environmental fluctuations, the model needs to consider variations in light intensity, light attenuation along depth due to algae, and temperature. The nutrients are introduced in excess so that they are not limiting growth. We consider a modified version of the simple model validated by Bernard and Rémond (2012). The growth rate is represented by the product of two functions of light (I) and temperature (T), considering a multiplicative effect:

$$\mu(T, I) = \phi(T)\mu_{opt}(I) \quad (1)$$

$$\phi(T) = \frac{(T-T_{max})(T-T_{min})^2}{(T_{opt}-T_{min})((T_{opt}-T_{min})(T-T_{opt})-(T_{opt}-T_{max})(T_{opt}+T_{min}-2T))} \quad (2)$$

$$\mu_{opt}(I) = \mu_{max} \frac{I}{I + \frac{\mu_{max}}{\alpha} \left(\frac{I}{I_{opt}} - 1 \right)^2} \quad (3)$$

This equation represents the growth rate at the depth where the light intensity equals I . The average growth rate over the water column must account for the light attenuation with depth, due to turbidity. We assume that I decreases exponentially with depth, according to the Beer-Lambert law (Benson and Rusch, 2006):

$$I(L) = I_0 \exp(-\sigma XL) \quad (4)$$

With I_0 being the light intensity at the surface, σ the extinction coefficient, X the algae concentration and L the depth of water. The analytical expression of the integrated form of Equation (3) along depth gives the average growth, $\overline{\mu_{opt}}$. Its mathematical expression was given by Martínez et al. (2018). The extinction coefficient σ is dependent on the biomass. As suggested by Morel (1988) it can be written in the form of a power function, i.e.

$$\sigma = AX^B \quad (5)$$

The other modification consists in the addition of a mortality rate function as in Béchet et al. (2017):

$$\lambda(T) = \lambda_0(t) \exp(\beta T) \quad (6)$$

$\lambda_0(t)$ is considered to take two possible values. One for daytime when photosynthesis is active, and one for nighttime when only respiration takes place. The net growth rate is then given by:

$$\mu_{net}(T, I) = \phi(T) \overline{\mu_{opt}}(I) - \lambda(T) \quad (7)$$

The ordinary differential equation describing biomass dynamics is thus:

$$\frac{dX}{dt} = \mu_{net} X \quad (8)$$

2.6. Model calibration

In order to calibrate the response curves to temperature and light for *Tetraselmis suecica* growth rates obtained at different light intensities and temperatures from the Multi-Cultivator were divided into four data sets. The $\mu_{opt}(I)$ function is calibrated using data from two different temperatures, 26 °C and 30 °C, at 8 different light intensities: 50, 100, 150, 250, 400, 600, 900, 1023 $\mu\text{mol.m}^{-2}\text{s}^{-1}$. The $\phi(T)$ function is calibrated using data from two different light intensities, 250 and 400 $\mu\text{mol.m}^{-2}\text{s}^{-1}$, at 5 different temperatures: 15, 21, 26, 30, 33 °C. The biomass concentration in the multicultivator ranged from 0.05 to 0.6 g/L.

Model was calibrated in two steps: A pre-calibration using the Multi-Cultivator data and a final calibration using data from a raceway batch. All numerical calculations were made using *Python* programming language. In the first step, the Trust Region Reflective algorithm - present in the *curve_fit* function from the *Scipy* library - was used to fit the growth function to the data from the Multi-Cultivator. The algorithm to find the parameters functions in a loop, an initial guess for the 6 parameters is used then μ_{max} , I_{opt} and α are calculated applying the *curve_fit* function to $\mu(T, I)$ considering the $\phi(T)$ value from the initial guess, then T_{min} , T_{opt} and

T_{max} are calculated applying the *curve_fit* function to $\mu(T, I)$ considering the value of $\mu_{opt}(I)$ from the previously calculated parameters. These steps are repeated until convergence of the parameters. The respiration rate was taken from Béchet et al. (2017).

In the second step T_{min} , α and μ_{max} are reparametrized using data from one of the raceway experiments (calibration experiment). T_{min} is recalculated because temperatures reached in the cold raceway were much lower than what was possible to reach in the Multi-Cultivator, μ_{max} must be recalculated because it is dependent on light availability and the raceways are submitted to day and night cycles. α is also recalculated, after a posteriori verification of its high sensibility - the parameter α was recalculated to fit the experimental data. It was verified that the new recalibrated value was still correctly predicting the data from the Multi-Cultivator. For this second calibration, we use the L-BFGS-B algorithm present in the *minimize* function from the *Scipy* library, starting from many initial guesses. The final set of parameters with the least squared error is chosen.

The function of the extinction parameter σ was calibrated using a similar method from Béchet et al. (2017). A total of 12 values of σ were obtained measuring the light intensity at the surface and at the bottom of the raceway at different biomass concentrations.

2.7. Model validation criteria

We validate the model by comparing simulations to measurements acquired in the 3 models. We calculate Theil's inequality coefficient (TIC) (Decostere et al., 2016, Theil, 1961):

$$TIC = \frac{\sqrt{\sum_i (y_i - y_{i,m})^2}}{\sqrt{\sum_i y_i^2} + \sqrt{\sum_i y_{i,m}^2}} \quad (9)$$

where y_i is the simulated result and $y_{i,m}$ represents measured data. Values of TIC lower than 0.3 usually indicate a good model performance (Xianmin, 1993).

2.8. Sensitivity analysis

We studied the global sensitivity of the model parameters in relation to the simulated results using the input data from the raceways. The sensitivity coefficient was defined as in Bernard et al. (2001):

$$\sigma_y^{\Delta p} = \frac{1}{t_f} \int_0^{t_f} \frac{y(p + \Delta p, x_0, u, \tau) - y(p, x_0, u, \tau)}{y(p, x_0, u, \tau)} d\tau \quad (10)$$

where y is the simulated output at time τ with parameter p , initial condition x_0 and input variables u (e.g. light intensity and temperature). Global sensitivity is calculated using Morris's screening method (Morris, 1991), but we replace the standard elementary effect by the sensitivity coefficient defined above. The parameters

domain analysed is the region comprised between the base value and $\pm 100\%$ of the standard deviation of the calibrated parameter. The domain of each parameter is divided in 20 points, at each iteration parameters are changed by addition or subtraction of 5/19 of the total length of the domain. Morris algorithm is repeated 100 times, then the mean value of the sensitivity coefficient and its standard deviation are calculated for each parameter.

2.9. Water temperature model

To evaluate the performance of raceway production under greenhouse we parametrized an autoregressive model for raceway water temperature inside and outside of the greenhouse. The autoregressive model is based on external air temperature (given by Meteo-France (Gwennaëlle Larvor et al., 2020)) and light intensity. The model for water temperature inside the greenhouse has one additional constant, T_0 . Note also that the time delays differ between the two models:

$$T_{w_{in}}(t) = aT_{ext}(t - 3) + bT_{ext}(t) + cI(t - 1) + dT_{w_{in}}(t - 1) + T_0 \quad (11)$$

$$T_{w_{out}}(t) = aT_{ext}(t - 4) + bT_{ext}(t) + cI(t) + dT_{w_{out}}(t - 1) \quad (12)$$

where $T_{w_{in}}$ and $T_{w_{out}}$ are, respectively, the water temperature in the raceway inside the greenhouse and the temperature in the raceway outside, T_{ext} is the air temperature outside in degrees Celsius, I is the light intensity reaching the raceway surface in $\mu mol/(m^2s)$ and t is the time in hours.

Time delays were identified in the parameters calibration phase. First, delays were fixed, and the parameters were determined through minimization of squared errors using the calibration dataset. This was repeated for a variety of time delays combinations. The model with the least error for the validation data was kept. Validation and calibration datasets for greenhouse water temperature are each composed of six weeks, for different months of the year. Only one week of data for calibration and one week for validation were available for the external raceway. Calibrated values can be found in Table .2.

[Table 2 about here.]

2.10. Solar data

Solar data to estimate annual production was obtained from NASA Langley Research Center (LaRC) POWER Project. Daily ground radiation, H , was calculated with the method of Duffie and Beckman (1991), using daily insolation clearness index and the following equations:

$$H = H_0 \cdot K_T \quad (13)$$

where K_T is the insolation clearness index obtained from the NASA database, H_0 is the daily extraterrestrial radiation on a horizontal surface in $J.m^{-2}$ given by:

$$H_0 = c(1 + 0.033 \frac{360n}{365})(\cos \phi \cos \delta \sin \omega_s + \frac{\pi \omega_s}{180} \sin \phi \sin \delta) \quad (14)$$

where c is a constant equals to $3.76 \cdot 10^7$, n is the n^{th} day of the year, ϕ is the latitude, δ is the sun's declination angle and ω_s is the sunset hour angle. Average hourly irradiance, I , in $W.m^{-2}$ can be calculated by:

$$I = H \times \frac{\pi(a + b \cos \omega)}{24 \times 3600} \frac{\cos \omega - \cos \omega_s}{\sin \omega_s - \frac{\pi \omega_s}{180} \cos \omega_s} \quad (15)$$

where a and b are coefficients dependent on sunset solar angle (Duffie and Beckman, 1991) and ω is the hour angle. We assume a conversion factor of 2.02 from $W.m^{-2}$ to $\mu mol.m^{-2}.s^{-1}$ (Hassika et al., 1997). Also, based on our data we consider that the film covering the greenhouse attenuates 20 % of the solar radiation.

2.11. Yearly cultivation prediction and greenhouse efficiency

The yearly production was simulated by the model for *Tetraselmis suecica*, *Dunaliella salina* (Béchet et al., 2017), *Spirulina platensis* (Venkataraman, 1997, Qiang et al., 1998), *Phaeodactylum tricornutum* (Bernard and Rémond, 2012, Fernández et al., 1997, Bitaubé Pérez et al., 2008, Wu et al., 2010) and *Chlorella vulgaris* (Béchet et al., 2015). The model parameters for *Chlorella vulgaris* and *Dunaliella salina* were both validated in Béchet et al. (2015) and Béchet et al. (2017), respectively. The parameters for the three other species were extrapolated from data available from several works, as detailed in Appendix Table 2. We simulated yearly production for locations near Nice, Rennes and Paris which represent three different climates in France. The yearly production was simulated considering a continuous system with a dilution rate D . In this case the equation describing the system is:

$$\frac{dX}{dt} = \mu_{net}X - DX \quad (16)$$

The resulting average daily productivity P is computed as follows:

$$P = \frac{1}{T} \int_0^T D(t)X(t)dt \quad (17)$$

The biomass concentration that maximizes production P for each season, X_{opt} , was computed based on a day representing the average conditions of the season (see Appendix). X_{opt} is used as the initial condition for each season and D is either equal to μ_{net} or zero if $X(t) < X_{opt}$ in order to maximize productivity P .

The yearly productivity was computed, for each location, considering three scenarios: cultivation outside, inside the greenhouse with free temperature and optimal temperature control inside the greenhouse. For this last case it is assumed that a climate control system could reach any temperature between the two extreme former cases. This means that when the temperature inside the greenhouse is above the optimal temperature, it will be regulated nearby the optimal temperature by increasing the air flux entering (and leaving) the greenhouse. X_{opt} is calculated for each season as well as for each cultivation condition.

We define the greenhouse efficiency as the gain in productivity when growing algae in the greenhouse:

$$e_g = \frac{P_{greenhouse}}{P_{outside}} \quad (18)$$

where $P_{greenhouse}$ and $P_{outside}$ are the optimal productivities outside or under greenhouse given the conditions of each season. Greenhouse efficiency is used to analyse the impact of each parameter on the choice of cultivating under greenhouse.

To better understand the factors affecting the greenhouse efficiency we compute its sensitivity with respect to the model parameters:

$$\sigma_{e_g}^{\Delta p} = \frac{e_g(p + \Delta p) - e_g(p)}{e_g(p)} \quad (19)$$

and Δp varies between $\pm 10\%$ of the base values for *Tetraselmis suecica*.

3. Results

3.1. Model calibration

Table .3 shows the model parameters for *Tetraselmis suecica*. The optimal temperature for gross growth rate (T_{opt}) was found to be 30.9 °C (when corrected with respiration, the optimal temperature for net growth is 30.4 °C) which is in the literature range (Tredici et al., 2015, Molina et al., 1991, Weiss et al., 1985). The parameter T_{max} was 34.5 °C, in the upper range compared to literature (25-32 °C) (Molina et al., 1991, Weiss et al., 1985). This can be explained by an acclimation of the cells, since the raceways were inoculated at the end of summer when water temperature frequently exceeded 30 °C. Finally, parameter T_{min} was estimated to be -14 °C, but this parameter has been shown to be rather artificial, and this low value means that growth rate is less sensitive to temperatures changes below the optimal rate (*i.e.* that $\frac{d\mu}{dT}$ for temperature below T_{topt} , is closer to zero when T_{min} is very low).

[Table 3 about here.]

3.2. Sensitivity analysis

Results of the global sensitivity analysis are shown in Table .4. The global sensitivity mean indicates the level of influence of the parameter on the output, negative values indicate that an increase in the parameter will decrease the output of the model (i.e. final biomass concentration). The parameter with the most influence on the output was μ_{max} , followed by the extinction coefficient parameters, T_{opt} and α . The variance indicates if there are coupling effects between parameters or nonlinear responses. The results show that the variance correlates with the absolute value of the sensibility, indicating little coupling in the analyzed range. The weak coupling between parameters is in accordance with our model since the growth rate function is written as a multiplication of two independent functions depending either on light or temperature. However, nonlinear responses could be expected, for example, when the water temperature is close to one of the cardinal temperatures of the model.

[Table 4 about here.]

[Figure 1 about here.]

[Figure 2 about here.]

3.3. Experiments and simulation

Figures .1 and .3 show simulated and measured biomass evolution in the 3 ponds during the 2 experiments. The raceway with +5 °C shift was always the most productive, followed by the raceway at ambient temperature. The heated raceway showed a twofold productivity compared to the cold one for the second experiment in February, demonstrating the strong limitation due to temperature in the algal productivity.

Figure .2 shows the light intensity received by the raceways during the two set of experiments. For the second experiment, raceway ponds were exposed to a more intense radiation, most of the days light intensity reached at least $750 \mu\text{mol.m}^{-2}\text{s}^{-1}$ and the maximum intensity was $1500 \mu\text{mol.m}^{-2}\text{s}^{-1}$, while during the first experiment it never exceeded $700 \mu\text{mol.m}^{-2}\text{s}^{-1}$. Since there is no marked difference in the temperature, this explains the difference in productivity between the two batches. Productivity was never reduced due to photoinhibition at light intensities higher than I_{opt} , since the average growth rate $\overline{\mu_{opt}}$, even at the lowest biomass concentration, would decrease only for light intensities higher than $1400 \mu\text{mol.m}^{-2}\text{s}^{-1}$.

A TIC value of 0.05 was calculated using data from the validation raceway experiment data and a value of 0.08 using data from all raceway batches, which demonstrates the good model predictive capability.

Some situations were less accurately predicted by the model, especially when a lag phase was observed after culture inoculation. Predicting growth in the stress

phase immediately after inoculation is often very challenging. The underestimation of biomass concentration in the validation experiment (Figure .3) can be due to grazing by ciliates in the heated raceway, as it has been regularly observed at higher temperature.

[Figure 3 about here.]

These results highlight the importance of managing the culture temperature to keep an optimal productivity. Since the experiments were carried out during winter, temperature in all raceways was always below the optimal. Our study also shows that the use of a greenhouse in cold climates is a simple and energy efficient (*i.e.* without requiring heating energy) way of increasing productivity by simply keeping the culture at some degrees above ambient temperature.

4. Discussion

4.1. Contamination at higher temperature

Bio contamination is one of the most common problems in raceway culture, either with virus, bacteria or other microalgae species. A greenhouse is a barrier around the culturing environment that contributes to reduce the contaminations. This function of a greenhouse, provided that some basic precautions are respected, is the cheapest way to protect non-aseptic raceways from a direct contact with the biodiversity contained in the aerosols or in the rain (Sialve et al., 2015). A raceway under a greenhouse is thus an economical interesting trade-off between outdoor raceway and photobioreactors which can ensure axenic growth conditions, but for a much higher cost. However, the greenhouse results in a temperature shift, which is a double edged sword because it also enhances the growth rate of the biological contaminants present in the culture. We observed most notably contamination by bacteria and ciliates. Ciliates are predators of microalgae, and their presence can rapidly lead to a crash of the culture, wherein all the biomass can be consumed in a few days. One way to mitigate the growth rate of ciliates despite temperature increase in the greenhouse is to modify salinity (von Alvensleben et al., 2013). *Tetraselmis* is very resistant to changes in salinity and it can grow even in fresh water (Fabregas et al., 1984). We observed under the microscope that dilution with tap water was an effective method to eliminate ciliates while keeping *Tetraselmis* cells mostly intact.

4.2. For which species is the greenhouse beneficial?

The model was used to simulate the yearly productivity of five species along the year. The model accounts for the shift in temperature but also for the light attenuation by the greenhouse film. Results for the estimated productivities over the whole year are shown in Table .6 (full data can be found at the Appendix

Tables 3, 4 and 5). These predictions are in accordance with the average values reported in the literature for outdoor cultivation (Banerjee and Ramaswamy, 2017). The low productivity for *Phaeodactylum Tricornutum* is probably due to the fact that this species does not grow at high temperatures. Silva Benavides et al. (2013) recorded an average productivity of $11.7 \text{ g.m}^{-2}.\text{d}^{-1}$ in summer, regulating water temperature close to the optimal value, while the highest productivity we simulated for non optimal temperature conditions was of $8.9 \text{ g.m}^{-2}.\text{d}^{-1}$. De-Luca et al. (2019) also estimated for *Chlorella Vulgaris* productivities ranging from 37 in summer to $5 \text{ g.m}^{-2}.\text{d}^{-1}$ in winter using optimal control.

Apart from *Dunaliella*, all microalgae had better productivities during winter when cultivated under greenhouse. On spring and fall, greenhouse cultivation starts to be less effective and, in general, a strong greenhouse aeration by implementing air fluxes with exterior becomes regularly necessary to decrease temperature and thus maintain productivity in the same range as outdoor cultivation. In summer, cooling down the raceway by a high air exchange with the external environment becomes imperative, since water temperature can easily surpass the optimal and even maximum temperature. This explains the close to zero productivity for *Phaeodactylum tricornutum* during summer.

Without temperature control in the greenhouse, productivity for *Tetraselmis suecica* during summer, at a location near Nice, is similar to the average during cold seasons, with the risk of culture collapse on the hottest days (see Table 2 of Appendix and Table .6). On the other hand, during winter the greenhouse can increase the productivity by more than 25%. Because during hot seasons the increase in temperature becomes harmful, the average production over the whole year in and out of the greenhouse are similar. Indeed, the months associated with the highest solar flux are more favourable in terms of light intensity, but the expected higher productivities are not met due to overwarming.

Dunaliella salina is a microalgae tolerant to higher temperatures. Naturally thriving in shallow ponds, it can grow at temperatures higher than 40°C while having an optimal temperature for growth around 34°C . Despite this, it performs badly under greenhouse. It can be explained by the fact that its optimal growth rate is reached at very high light intensities, being very resistant to photo inhibition. It results that the light loss due to the greenhouse film deeply impacts productivity. The same explanation holds for *Chlorella*, but since it has an even higher optimal temperature, 37°C , productivity stays similar under both cultivation systems.

The cyanobacteria *Spirulina platensis* was the only species to be more productive under greenhouse during all seasons. Its characteristics are perfect for greenhouse production, i.e. high temperature tolerance and optimal growth at low light intensities. Cultivation under greenhouse could increase winter productivity by more than 80 %, and by more than 20 % over the whole year.

The predictions for these microalgae point out the necessity to adapt the species to be cultivated to the local climate and cultivation system. As in agriculture, it is pointless to cultivate a single species along the year, and the species must be alternated along the seasons. When considering Table .6 (see also Appendix Tables 3, 4 and 5) it turns out that cultivating *Chlorella* in winter, *Tetraselmis* in spring and *Spirulina* in summer and fall is the optimal solution for dry mass production for the region of Nice. This crop rotation strategy would improve annual biomass production by at least 7 % and up to 20 % by comparing it to a monoculture of *Spirulina* and *Chlorella*, respectively. This strategy would slightly change for the regions of Paris and Rennes, where it would be more productive to cultivate *Chlorella* during fall. This highlights how the optimal cultivation strategy depends on the local climate. The qualitative value of each different species also has a primary influence on the cultivation strategy. For example the dry biomass of *D. salina* is ten times more expensive than for *S. platensis*.

The greenhouse, when passively used, can extend the culture duration in the cold seasons, but species that are not prone to photoinhibition must be grown in spring and summer without a greenhouse. Furthermore, simulation shows that a system of temperature control inside the greenhouse, simply by air exchange with the external environment, is able to greatly improve productivity compared to a simple greenhouse. Specially during summer, when temperature can surpass the optimal temperature for the microalgae species. The contamination problem can then become an issue and an air filtering system must probably be implemented.

4.3. Light attenuation by the greenhouse film: a heavy burden

To better understand the consequence of loosing 20% of incoming light due to the greenhouse film, we computed the temperature shift in the greenhouse necessary (ΔT necessary) to compensate this light reduction by providing the same growth rate (see Figure .4). The figure presents this necessary shift in temperature for different light intensity scenarios and various water temperature in a raceway without greenhouse. At high light intensities and low temperatures (a situation which is not common), the greenhouse is more effective. A large shift in temperature can result in significant gains of growth rate. Nonetheless, when temperature in outdoor raceways is already high, the greenhouse can no longer compensate the light attenuation.

[Figure 4 about here.]

To sketch which characteristics of a species would maximize greenhouse efficiency, we consider the parameters having the highest impact (see Table .5), as deduced from the sensitivity analysis (Equation 19). Furthermore, since variances indicate weak coupling between parameters, we can expect the results to be valid for other

species as well. As expected, higher T_{opt} and T_{min} (species adapted to warmer conditions) promote greenhouse usage. Surprisingly, a species with higher T_{max} (but same T_{opt}) would not benefit from the greenhouse. A closer look at equation $\phi(T)$ shows that a higher value of T_{max} reduces $\frac{d\phi(T)}{dT}$ between T_{min} and T_{opt} so that the increase in temperature in the greenhouse results in a less marked gain in growth rate. But as shown in Figure .5, assuming a shift in the temperature growth curve (simultaneous increase in T_{min} , T_{opt} and T_{max}), temperature parameters have a positive impact on greenhouse efficiency. The species with higher I_{opt} (subjected to less photoinhibition), are less interesting for greenhouse cultivation. Growth rate decreases when light intensity is higher than I_{opt} due to stronger photoinhibition. For species where I_{opt} is below the average values of light intensity, the greenhouse is more efficient and protects against photoinhibition.. Figure .5 also demonstrates clearly how greenhouse efficiency increases from summer to winter. These results qualitatively apply to other species, as long as average temperatures stay in the range of T_{min} and T_{max} .

[Table 5 about here.]

[Figure 5 about here.]

[Table 6 about here.]

5. Conclusions

The first motivation to use a greenhouse is the protection against contamination and potential negative effect of rain. By increasing temperature, greenhouses generally improve productivity during winter and autumn, but this effect is less marked in spring and summer as a consequence of light attenuation in conjunction with lethal temperatures. Cultivation under greenhouse should be considered when outdoor water temperature is several degrees lower than the optimal growth temperature for species susceptible to photoinhibition. Alternation of the cultivated species is also a good strategy to improve annual productivity.

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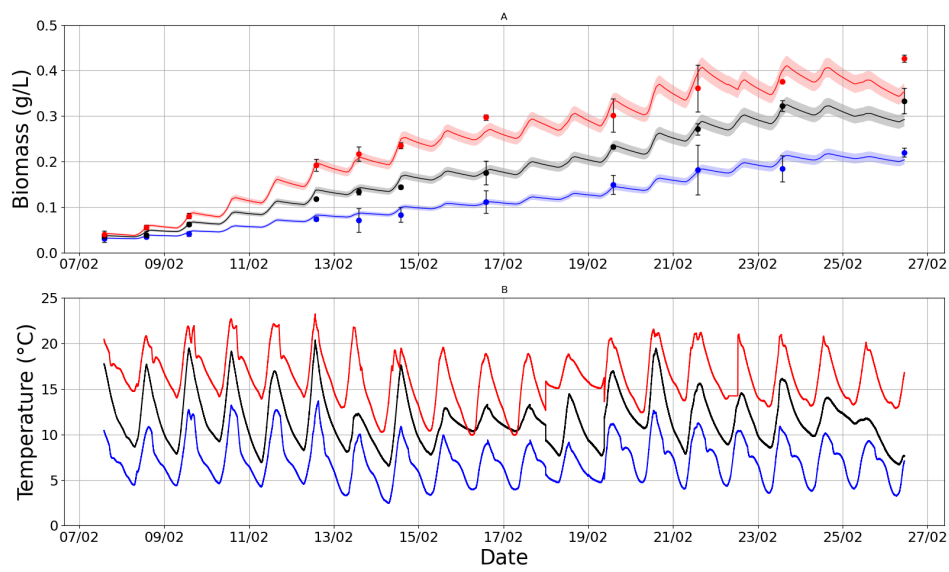


Figure 1: Calibration experiment. Points represent the measured dry weight and its confidence interval. A - curves represent the model simulation, the colored areas represent the region inside \pm the standard deviation. B - measured temperature for the raceways. Red, blue and black colors are the data for the $+5$ °C, -5 °C and at ambient temperature raceways, respectively.

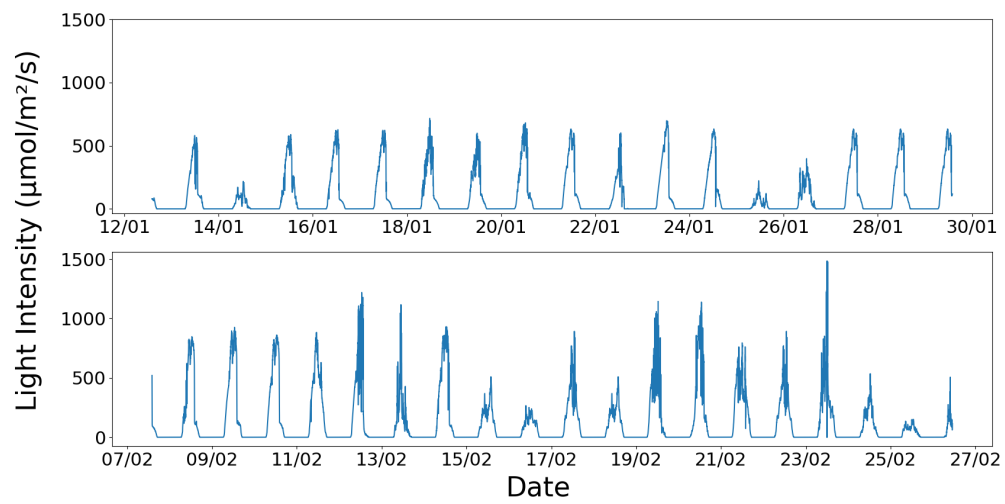


Figure .2: Light intensity for the two experimental campaigns

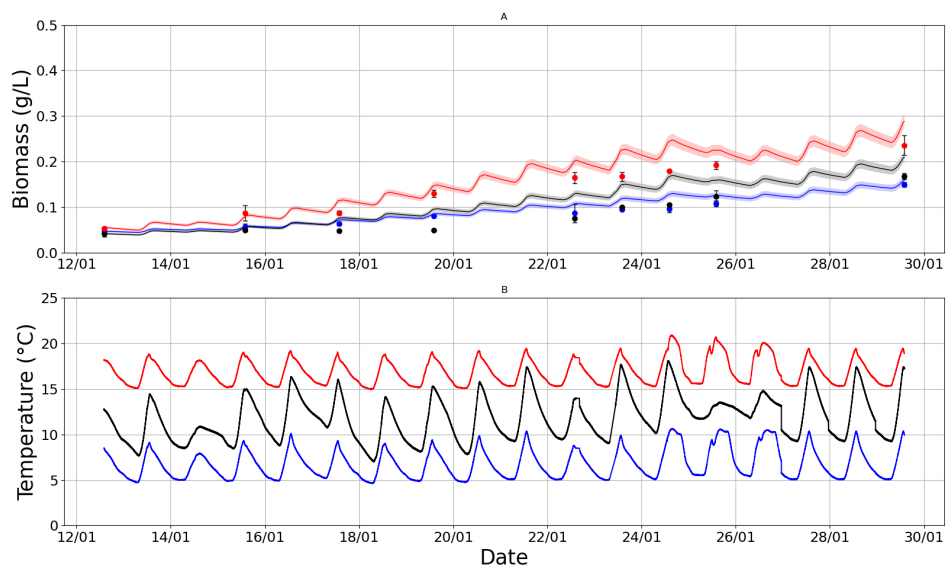


Figure .3: Validation experiment. Points represent the measured dry weight and its confidence interval. A - curves represent the simulation of the model, the colored areas represent the region inside \pm the standard deviation. B - measured temperature for the raceways. Red, blue and black colors are the data for the $+5^{\circ}\text{C}$, -5°C and at ambient temperature raceways, respectively.

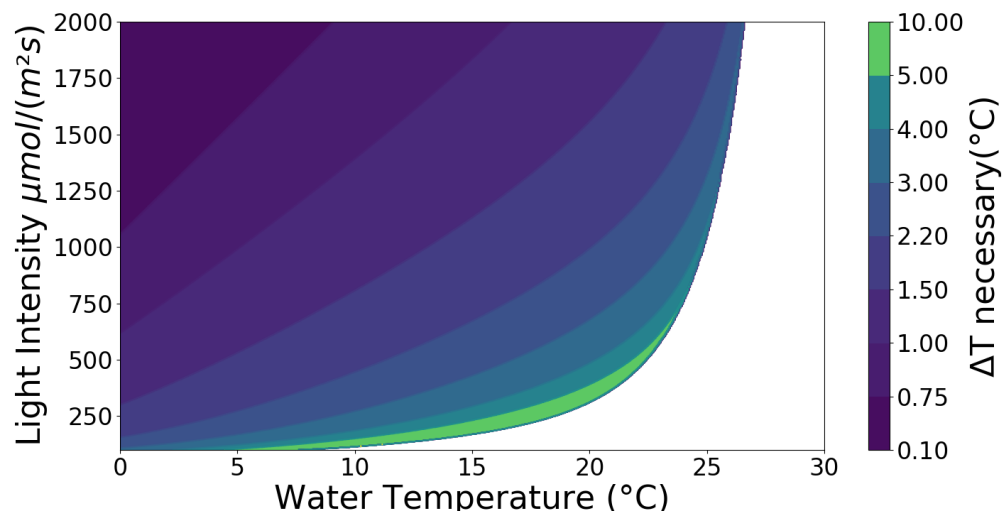


Figure .4: Temperature shift (ΔT necessary) inside the greenhouse to compensate the growth rate loss due to the 20% attenuation of the solar radiation for *Tetraselmis suecica*. In the white region it is not possible to compensate the loss with an increase in temperature.

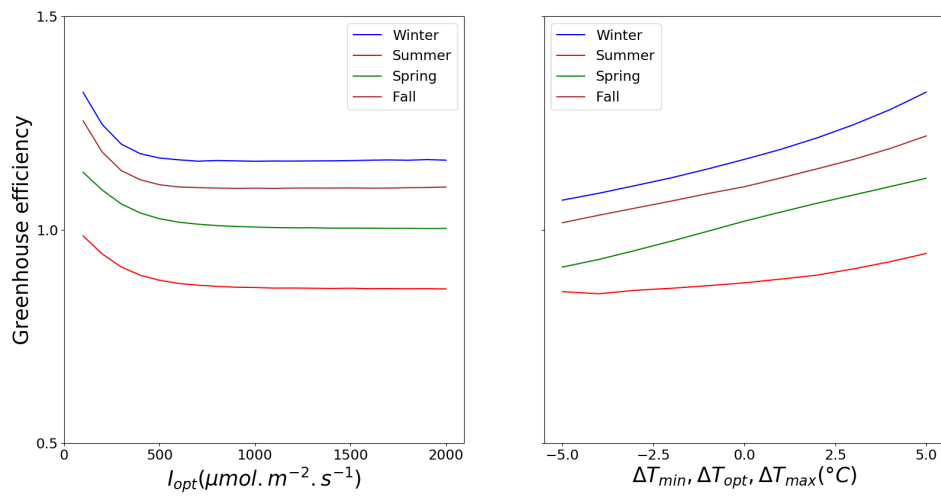


Figure 5: Greenhouse efficiency as a function of the parameter I_{opt} (left) and as a function of a shift ΔT in all the cardinal temperatures parameters (right), for *Tetraselmis suecica* along different seasons of the year at a location near Nice.

Table .1: Composition for the culture medium.

Compound	Conc.(M)	Compound	Conc.(M)
$NaNO_3$	$8.82 \cdot 10^{-4}$	$FeDTPA$	$6.66 \cdot 10^{-6}$
$NaH_2PO_4 \cdot H_2O$	$3.62 \cdot 10^{-5}$	Mn	$2.33 \cdot 10^{-5}$
B	$4.12 \cdot 10^{-5}$	Mo	$5.62 \cdot 10^{-7}$
Cu	$9.44 \cdot 10^{-7}$	Zn	$6.48 \cdot 10^{-6}$

Table .2: Model parameters to calculate water temperature inside the greenhouse or outside.

Parameter	a	b	c	d	T_0
Greenhouse	0.094	0.209	0.0033	0.660	1.207
Outside	0.058	0.151	0.0018	0.760	-

Table .3: Growth model parameters for *Tetraselmis suecica*. In parenthesis the associated standard deviation computed from the covariance matrix.

Parameter	Unit	Value (SD)
μ_{max}	(d^{-1})	3.5 (0.1)
I_{opt}	$(\mu mol.m^{-2}s^{-1})$	571 (73)
α	$(m^2.s.\mu mol^{-1}d^{-1})$	0.024 (0.003)
B	-	-0.635 (0.055)
A	$(L^{B+1}.g^{-B-1}.m^{-1})$	35.30 (7.43)
T_{min}	$(^{\circ}C)$	-14.7 (1.5)
T_{opt}	$(^{\circ}C)$	30.9 (0.9)
T_{max}	$(^{\circ}C)$	35.2 (0.4)
β	$(^{\circ}C^{-1})$	0.0715 (0.0002)
$\lambda_0(day)$	(d^{-1})	0.042 (0.003)
$\lambda_0(night)$	(d^{-1})	0.052 (0.003)

Table .4: Global sensitivity of the model parameters for *Tetraselmis suecica*

Parameter	Global Sensibility mean	Variance
μ_{max}	0.179	0.015
α	0.141	0.012
B	0.137	0.011
T_{max}	0.030	0.001
β	-0.003	0.000
I_{opt}	-0.049	0.003
λ	-0.058	0.002
T_{min}	-0.081	0.006
T_{opt}	-0.153	0.007
A	-0.153	0.014

Table .5: Sensibility of the greenhouse efficiency to model parameters (parameter for *Tetranelmis suecica*)

Parameter	$\sigma_{e_g}^{\Delta p}(10^{-2})$	Variance (10^{-2})
T_{opt}	2.176	0.848
T_{min}	0.897	0.086
B	0.324	0.073
α	0.289	0.048
A	-0.003	0.030
λ	-0.021	0.020
I_{opt}	-0.196	0.055
μ_{max}	-0.229	0.050
β	-0.782	0.104
T_{max}	-1.399	0.530

Table .6: Simulated productivity ($g \cdot m^{-2} \cdot d^{-1}$) during hot (summer and spring) and cold (winter and fall) seasons for raceway cultivation outside, inside greenhouse without and with temperature control

Species		Cold seasons						Hot seasons						Year
		Nice	Paris	Rennes	Nice	Paris	Rennes	Nice	Paris	Rennes	Nice	Paris	Rennes	
<i>Tetraselmis suecica</i>	Greenhouse	5.4	2.7	2.9	13.3	12.2	14.5	9.3	7.5	8.7				
	Outside	5.2	2.5	2.6	25.9	17.5	17.9	15.6	10	10.3				
	Greenhouse T c.	6.3	3.1	3.3	25.2	17.9	18.6	15.8	10.5	10.9				
<i>Spirulina platensis</i>	Greenhouse	7.1	3.5	3.7	24.2	18.6	18.3	15.6	11.1	11				
	Outside	4.5	1.8	1.9	21.5	15.2	14.4	13	8.5	8.1				
	Greenhouse T c.	7.3	3.7	3.9	24.8	19.1	18.6	16	11.4	11.3				
<i>Chlorella vulgaris</i>	Greenhouse	7.5	5.3	5.6	19.5	15.3	15.7	13.5	10.3	10.6				
	Outside	7.7	5.4	5.6	21.1	16.6	16.5	14.4	11	11.1				
	Greenhouse T c.	7.8	5.6	5.8	20	16	16.1	13.9	10.8	10.9				
<i>Dunaliella salina</i>	Greenhouse	3.3	1.9	2	10.9	7.7	8.1	7.1	4.8	5.1				
	Outside	3.8	2	2.2	13.9	9.6	9.8	8.9	5.8	6				
	Greenhouse T c.	3.6	2.1	2.2	11.7	8.3	8.5	7.7	5.2	5.4				
<i>Phaeodactylum tri.</i>	Greenhouse	3.3	2.5	2.7	1	2.5	3.2	2.2	2.5	3				
	Outside	4.1	2.8	3.1	4.1	5.9	7.3	4.1	4.4	5.2				
	Greenhouse T c.	4.5	3.2	3.5	4	6.1	7.4	4.3	4.7	5.5				