Modeling the growth and sporulation dynamics of the macroalga *Ulva* in mixed-age populations in cultivation and the formation of green tides

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Abstract

Ulva is a widespread green algal genus with important ecological roles and promising potential as a seagrass crop. One of the major challenges when cultivating Ulva is sudden biomass disappearance, likely caused by uncontrolled and unpredicted massive sporulation. However, the dynamics of this process are still poorly understood. In this study, we propose a mathematical model describing the biomass accumulation and degradation of Ulva, considering the potential impact of sporulation inhibitors. We developed a differential equation model describing the time evolution of Ulva biomass. Our model simulates biomass in compartments of different Ulva ‘age’ classes, with varying growth and sporulation rates. Coupled with these classes is a differential equation describing the presence of a sporulation inhibitor, produced and secreted by the algae. Our model mimics observed Ulva dynamics. We present Ulva’s biomass accumulation under different initial algae population age distributions and sporulation rates. Furthermore, we simulate water replacement, effectively depleting the sporulation inhibitor, and examine its effects on Ulva’s biomass accumulation. The model developed in this work is the first step towards understanding the dynamics of Ulva growth and degradation. Future work refining and expanding our results should prove beneficial to the ecological research and industrial growth of Ulva.

Keywords: Ulva, sporulation, sporulation inhibitor, modeling, green tides, gametogenesis, aging, aquaculture management.
1. Introduction

The genus *Ulva* (Ulvales, Chlorophyta) comprises a group of green macroalgae, which are cosmopolitan species, both ecologically and economically. Its highly adaptive nature allows it to flourish in various environments, as can be seen from its widespread presence from the Arctic and Antarctic seas to the Equator. In natural populations, *Ulva* spp. are very common in littoral and sublittoral areas. As *Ulva* in nature is a holobiome, its ecological role is vast and includes multiple interactions with other players of the marine ecosystems, such as protista, fungi, bacteria, viruses and various marine fauna. *Ulva* is highly relevant for aquaculture due to its fast growth rates and potential food, feed, materials, chemicals and energy applications. Hence, *Ulva* is considered as a potential crop for controlled biomass production, onshore and offshore (Fernand et al., 2017). Multiple reports in the last decade addressed *Ulva* aquaculture alone or in multitrophic systems. In addition, *Ulva* biorefinery-enabling processes and technologies have made immense progress in producing of starch, protein, cellulose, ulvan, salts, methane, biocrude, biodiesel, bioethanol, and polyhydroxyalkanoates, just to mention a few. Over the years, various systems including plastic sleeves, raceway ponds, tanks, dripping, ropes, nets, rafts and aerated cages have been proposed for *Ulva* biomass cultivation. The variation of cultivation systems ranges from closed, artificial and seawater, onshore systems with fresh seawater to near shore and far offshore production. Yet, one of the significant risks in *Ulva* cultivation is the sudden biomass loss when the algal tissue disintegrates and bleaches, most probably caused by uncontrolled and unpredicted massive sporulation.

Opposite to the controlled cultivation, *Ulva* green tides are massive, rapid natural accumulations of unattached green macroalgae biomass usually associated with eutrophicated marine environments. Various *Ulva* species (e.g., *U. prolifera, U. compressa, U. linza*) are commonly linked to the formation of green tides which eventually reach coastal marine areas. Green tides seriously damage the coastal marine environment on occasions modifying the shoreline structure, or affecting biodiversity and damaging ecosystem services such as navigation, fishery and recreation. In addition, decaying seaweed biomass causes anoxia yielding hydrogen sulfide at toxic levels in coastal waters and on the shores (Nedergaard et al., 2002; Nedergaard et al., 2002; Castel et al., 1996; Viaroli et al., 1995).

Various explanations have been proposed for the rapid accumulation and simultaneous collapse of *Ulva* dominated green tides. Favorable environmental conditions for *Ulva* habitats, such as temperature, salinity, hydrodynamics and nutrient levels affect the rapid biomass growth. In addition, recent studies showed that blooming leads to the selection of rapidly growing strains (Fort et al., 2020) with potentially differentially expressed genetic signatures (He et al., 2021). Furthermore, for *Ulva prolifera*, a strain
dominating the Yellow Sea bloom, 91.6–96.4 % of the released spores developed into young seedlings, suggesting that 1 gFW thallus was able to produce about $2.8 \times 10^8$–$2.7 \times 10^9$ new younger seedlings, of free-floating biomass (Zhang et al., 2013).

*Ulva* sp. has a complex reproduction strategy with alternation of generations in which both isomorphic gametophytes and sporophytes coexist. The gametophytes produce biflagellated haploid gametes through mitosis while the sporophytes produce quadriflagellated haploid zoids through meiosis (Wichard 2015). The initiation of a green tide requires simultaneous sporulation and release of gametes of multiple thalli. How this sporulation is achieved and controlled at the initial population is still a puzzling question. The simultaneous release of zoids and gametes in large numbers over a short period, combined with favorable environmental conditions, would provide the prerequisites for the formation of green tides. Indeed, mechanical or other factors, fragmentation of *Ulva* thalli would produce large amounts of spores giving rise to the rapid proliferation of the seaweed under field conditions. This explanation likely supports the rapid accumulation of the vast biomass of *U. prolifera* in the green tide that occurred in Qingdao, 2008 (Gao et al., 2010).

Ecological studies indicate that sporulation in *Ulva* is seasonal, and when it occurs a significant amount of parental biomass contributes to the massive production of swarmers (Amsler and Searles, 1980; Littler and Littler, 1980; Niesenbaum, 1988). It has been also shown that the formation and release of swarmers is inhibited by “sporulation-inhibiting substances” excreted into the growth medium by the whole thalli, or their fragments (Nilsen and Nordby, 1975). A later study identified two sporulation inhibitors in both *Ulva mutabilis* and *Ulva linza*. The first sporulation inhibitor 1 (SI-1), is a glycoprotein isolated from the thalli media or the cell wall’ and the second sporulation inhibitor 2 (SI-2), is a small molecular weight compound that was isolated from the inner space between the two blade cell layers (Stratmann et al., 1996; Jönsson et al., 1985; Kessler et al., 2018; Vesty et al., 2015). It was also shown that the production rate of the SI-1 decreases with the maturation of the thallus and the production rate of SI-2 is constant (Stratmann et al., 1996).

Furthermore, it was discovered that the formed gametes were only released slowly and asynchronously in the presence of another substance known as a swarming inhibitor, the removal of which resulted in nearly immediate and complete swarming (Stratmann et al., 1996; Wichard and Oertel, 2010). This precise control of swarmer formation and release suggests that *Ulva* developed a tightly regulated mechanism to guarantee simultaneous release of swarmers to the environment, observed initially by Smith at the Pacific coast (Smith, 1947), probably to maximize the likelihood of sexual reproduction. Indeed, most recent studies on the floating *Ulva prolifera* showed that all tested thalli were sporophytes with sexual
reproductive patterns (Zhao et al., 2019). Additional studies showed that if the thalli are ageing (i.e. no growth) they become insensitive to the artificially added sporulation inhibitor and release swarvers even in its controlled presence in the growing media (Alsufyani et al., 2017).

Mathematical models are essential tools to study and predict the behavior of complex biological systems. Several models have been developed to predict seaweed biomass growth and decomposition, the behavior of harmful green tides and the seaweed biomass production in seagriculture. Indeed, long-term ecological models that predict macroalgal productivity and seasonal blooms in prone ecosystems (Martins and Marques, 2002; Solidoro et al., 1997; Ren et al., 2014; Martins et al., 2007; Port et al., 2015; Brush and Nixon, 2010; Aldridge and Trimmer, 2009; Lavaud et al., 2020; Seip, 1980; Aveytua-Alcázar et al., 2008; Duarte and Ferreira, 1997) or culture models that focus mostly on onshore photobioreactors (Friedlander et al., 1990; Oca Baradad et al., 2019) and offshore cultivation (Broch and Slagstad, 2012; Petrell et al., 1993; Hadley et al., 2015) were developed. These models, which pursue a basic understanding of the thermodynamics of individual algae thalli and photobioreactors (Zollmann et al., 2018), provide important tools to predict the productivity and seasonal environmental effects on the seaweed population dynamics. However, such models treat the macroalgae population as a bulk and do not differentiate between ages of individual thalli within the population. As discussed above, thalli age is an important factor affecting the activity of sporulation inhibitors in the alga and the ultimate release of swarvers.

Furthermore, these models do not consider the possible interthalli chemical interactions, some of which can be based on SI-1 secreted to the environment. The production and secretion to the environment of molecules such as SI-1 could provide insights into the molecular mechanisms behind the synchronization of massive spore release at the population level - a phenomenon crucial for both green tide formation and sudden biomass disappearance in Ulva seagriculture.

This paper aims to introduce a novel framework for the description of population dynamics and collective thalli behavior of Ulva biomass, presumably controlled by shared sporulation inhibitors. We propose that various environmental and internal biological changes on the single thallus level predetermine the ability of the individual thallus to produce, and to donate to and receive from the population environment, factors that regulate the synchronized formation and release of swarvers. A natural tool to describe this process is offered by population dynamics models, often employed to describe bacterial and animal population dynamics (Succurro and Ebenhöh, 2018; Friedman and Gore, 2017).

In the following sections we develop and simulate such a mathematical model in an attempt to characterize the dynamics of Ulva biomass formation and degradation.
2. Methods

The model presented below consists of n+1 ordinary differential equations (ODEs), where n is set as the number of cultivation days (also equal to the number of age group equations). ODEs 0...n-1 describe the rate of change of biomass of n discrete age classes of Ulva thalli, denoted ai, and additional n+1 equations for the rate of change of the inhibitor I. As described previously, there are at least three types of inhibitors involved in the process of Ulva swarmers release: SP-1, SP-2 and a swarming inhibitor (SWI). We aggregate these inhibitors into a single quantity that controls the simultaneous swarmers’ release from thalli, followed by the biomass decrease.

The n ODEs follow a simple discretized version of a partial differential equation of $m(a,t)$ where $m$ is the biomass of Ulva in controlled volume (a bioreactor or a given sea volume, for example), $a$ is the age of algae, and $t$ is time. As previously shown, the growth rate for thalli decreases with age(Alsufyani et al., 2017).

The following equations specify the dynamics of the biomass of each age class $a_i$, coupled with the dynamics of the inhibitor $I$, in the growth environment:

$$\frac{da_i}{dt} = r_i \left( 1 - \frac{\sum_{j} a_j}{K} \right) + \lambda_i a_{i-1} - \lambda_{i+1} a_i - \sigma a_i f(I) \quad i = 1, 2, \ldots, n$$

(1)

$$\frac{dI}{dt} = \sum_{i=1}^{n} a_i (\theta_i - I(\sum_{i=1}^{n} \mu_i a_i) - \xi I + \gamma I$$

(2)

Table 1: model parameters, their interpretation, and values.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Meaning</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>$r_i$</td>
<td>Growth rate of algae age $a_i$</td>
<td>$0.45(0.1 + e^{-0.5\log(2)/30})$</td>
</tr>
<tr>
<td>$f(I)$</td>
<td>Limiting factor due to inhibitor concentration, I</td>
<td>$1 - (1 + e^{-10(0.5 - I)})^{-1}$</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>Degradation constant</td>
<td>0.3</td>
</tr>
<tr>
<td>$\xi$</td>
<td>Inhibitor loss function to the environment (for example, water replacement)</td>
<td>$0.45(0.1 + e^{-0.5\log(2)/120})$</td>
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The model assumes a logistic growth of biomass with a growth rate parameter $r_i$ for each algae age class $a_i$ and a carrying capacity $K$, defined as the maximum Ulva biomass density in the growth environment. Biomass moves between compartment $a_i$ to $a_{i+1}$ at rates $\lambda_i$, defining the “natural aging” of algae. At each age class $a_i$, biomass is degraded at a rate $\sigma_f(I)$, where $\sigma_i$ is the maximal destruction rate, and $f(I)$ is a monotonically decreasing function of the inhibitor $I$, scaled between zero and one. Furthermore, we define $\mu_i$ as the SI1 uptake rate of each age class $a_i$; $\xi$ the leakage or injection of the inhibitor $I$, that can be managed externally to the system (e.g., washing the algae, destroying the algae, injection nutrients); and $\gamma$ is the nutrient supply flux in the units of inhibitor concentration. Model parameters are also summarised in Table 1.

<table>
<thead>
<tr>
<th>$\theta_i$</th>
<th>Age-dependent inhibitor generation function</th>
<th>$0.45(0.1 + e^{-r_\log(2)/120})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu_i$</td>
<td>Age-dependent inhibitor uptake</td>
<td>$0.45(0.05 + e^{-r_\log(2)/120})$</td>
</tr>
<tr>
<td>$\gamma_i$</td>
<td>Constant inhibitor addition or extraction</td>
<td>$0.0 - 0.1$ (varies in the figures)</td>
</tr>
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3. Results and Discussion

Here we study the Ulva biomass population dynamics, controlled by sporulation inhibitor production and absorption, by stimulating the biomass accumulation under various scenarios for mixed-age populations, for 100 days (Stratmann et al., 1996; Alsufyani et al., 2017). In all the following simulations, we assumed the initial density of seaweed in the cultivation media is $a_{ini} = 0.2$ kg m$^{-3}$ and at the maximum carrying capacity, the biomass can reach a density of 10 kg m$^{-3}$ ($a_{max}$). In addition, for the initial population conditions, we denoted young thalli as a population with $a_i = 0$ at $t = 0$ and old thalli as a population with $a_i = 120$ days at $t = 0$. From a physiological point of view, young thalli are those thalli, whose cell differentiation is controlled by SI and old thalli are those thalli, which are insensitive to SI and do not produce it.

In the following plots, we simulated the behavior of populations with a mixed-age composition. Each population was labeled by the percentage of young and old thalli at initial population. Thus, for example, 100/0 labels an entirely young initial population (0.2 kg m$^{-3}$ of thalli with $a_i = 0$ at $t = 0$); 0/100 labels a completely old initial population (0.2 kg m$^{-3}$ of thalli with $a_i = 120$ days at $t = 0$); and 50/50 represents an initial population comprised of equal parts of old and young algae (0.1 kg m$^{-3}$ of thalli with $a_i = 0$ at $t = 0$ and 0.1 kg m$^{-3}$ of thalli with $a_i = 120$ days at $t = 0$).
The biomass yield (density increase due to growth; i.e. initial density subtracted from final density) over time for various mixed-age populations is shown in Fig. 1a. The growth of mixed aged populations with 100/0, 80/20 and 50/50 population mix of old and young thalli showed a typical sigmoidal growth, reaching 90% of the maximum biomass density (9 kg m$^{-3}$) at 27, 30 and 37 days, respectively. Populations with predominantly old biomass at the beginning (20/80) showed a long lag phase but exhibited positive growth, reaching 90% of the maximum biomass density at day 87 (Fig. 1b). The population with only old algae (0/100) at the beginning of the cultivation showed degradation of the biomass from day 1 and never showed positive growth (Fig. 1b). The population with a 10/90 mix of initial ages showed a small growth (positive yield) during the first 40 days but then showed biomass degradation and never reached 90% of the maximum density (Fig. 1a, b).

As simulated in our model, such dynamics of the biomass growth could be explained by the dynamics of the production of the sporulation inhibitor (Fig. 1c). For populations 100/0, 80/20, 50/50 and 20/80 the rate of sporulation inhibitor generation is positive and increases over time, while for the 10/90 and 0/100 populations, although initial production of the sporulation inhibitor is observed, it is reduced over time (Fig. 1c). These findings show that sporulation inhibitor production by a small (20% in our simulations) young population could potentially provide enough inhibitor to prevent the old algae population from biomass loss, thus leading to overall positive biomass production. Additionally, the rate of biomass accumulation increases with the increased fraction of the young thalli in the initial population. Nevertheless, the maximum inhibitor production decreases with time in all populations as all thalli age (Fig. 1c).
Figure 1. a. Yield (biomass gain in kg/m³ up to the limit of 10 kg/m³ in a bioreactor with a starting density of 0.2 kg/m³) for populations with various initial age mixes. b. Ulva biomass growth kinetics as a function of the initial age distribution of thalli in the population. Values of the 0/100, and 10/90 populations are not presented as they did not reach 90% of the maximal carrying capacity. c. Inhibitor production in the population over time.

As seaweeds rarely grow in closed bodies of water, where the inhibitor could accumulate continuously in the environment, we sought to stimulate the impact of inhibitor removal from the seaweed environment by water replacement. We assume that each event of water replacement completely removes the inhibitor produced by the seaweed during the time interval from the previous water replacement. Figure 2a shows the dynamics of various initial age mixed populations yields when the inhibitor is removed by water replacement. In the scenario where water is replaced every 14 days, only few, relatively “young” populations (100/0, 90/10, 80/20 and 70/30) achieved the 90% of the maximum yield. Moreover, the time to reach this yield level increased from 27-30 days (without replacement for these four age groups) to 37-40 days, in the 100/0, 90/10, 80/20 and 70/30 populations, respectively (Fig. 2b). The growth yield shows fluctuating dynamics, showing in the initial overall growth for populations with predominantly young thalli during the first 60 cultivation days, followed by the overall reduction of the yield in the aging populations. Interestingly, these fluctuating yield dynamics were previously reported by us during a 12-month offshore cultivation work with Ulva harvesting every week (Chemodanov et al., 2018). It could happen that the weekly removal of the whole seaweed biomass from the sea and cages for weighting also removed the sporulation inhibitor accumulated in the boundary layer near the thalli. This suggestion, of course, requires further detailed experiments investigating the ability to monitor the dynamics of sporulation inhibitors production and accumulation/diffusion in the thalli environment. Increasing the frequency of water exchange, and thus inhibitor removal (Fig. 2c), reduced the ability of populations with a large portion of old algae to show positive yield during the whole cultivation period (Fig. 2a).
Figure 2. a. Yield (biomass gain in kg/m³ up to the limit of 10 kg/m³ in a bioreactor with a starting density of 0.2 g/m³) for populations with various initial age mixes with 14 days water replacement frequency. b. Ulva biomass growth kinetics as a function of the initial age distribution of thalli in the population with various frequencies of water replacement. c. Inhibitor production in the population of the time with 14 days of water replacement frequency.

As seaweed in the natural environment usually live in high energy conditions, we also studied the coupled effects of mechanical destruction ($\sigma$) and population age distribution on the ability of the population biomass yield (Fig. 3a). Higher rates of mechanical destruction prevent positive yields in all mixed-age populations. Lower mechanical destruction affects a smaller portion of the population with higher initial portions of young thalli (Fig. 3a) showing again the regenerative ability of the populations with high growth (lower sporulation) capabilities.

Figure 3. a. Time to achieve 90% of the maximum carrying capacity as a heatmap (color represents the time in days) depending on the initial age distribution (x-axis) and the mechanical destruction parameter $\sigma_j$ (y-axis). b. Time to 90% as a heatmap (color represents the time in days) depending on the initial age distribution (x-axis) and the addition of the external inhibitor $\gamma$ (y-axis). White color means that the population never achieves 90% of the maximum carrying capacity.
Finally, after confirming experimentally that direct application of SI-1 prevents gametogenesis in *U*. *mutabilis* (*Supplemental Information and Fig S1*), we investigated the impact of direct addition of the sporulation inhibitor to the seaweed growth media computationally.

Adding an external sporulation inhibitor (up to 0.1) reduced the time to achieve 90% of the maximum yield from 27 days (without inhibitor addition) to 21 days for 100/0 group, 29 days (without inhibitor addition) to 24 for 80/20 group, from 35 days (without inhibitor addition) to 28 for the 50/50 group and from 60 (without inhibitor addition) to 45 days for the 20/80 group. No effects at this maximum concentration have been observed for the 0/100 group.

### 4. Conclusions

In this study, we aimed to further understand the growth and sporulation dynamics of *Ulva* using a mathematical model. We found that successful accumulation of *Ulva* biomass depends on the age distribution of the algae population, where older starter populations produce lower yields. However, this age-dependent effect can be mitigated, leading to prolonged maintenance of *Ulva*'s aquacultures, by external addition of sporulation inhibitors.

Indeed, the sporulation phenomenon creates unique constraints on the age structure of *Ulva* populations. In higher plants, sexual reproduction and vegetative propagation compete for nutrients, but the competition may be mitigated by separating these processes through time (Evans and Black, 1993). However, in *Ulva*, the whole thallus can be transformed into gametangia and sporangia while flowering plants assign only a specific portion of biomass to reproductive structures. *Ulva* thus requires a strict regulation of sporulation, e.g. through the age-dependent production of SI. Only if the SI-1 synthesis ceases during the *Ulva*'s development cycle and its concentration falls below a critical threshold concentration, gametogenesis is induced at positions of the blade where the SI-2 concentration between the cell layers is also sufficiently low (Stratmann et al., 1996). Our findings thus imply that the more SI provided by young algae in mixed cultures, the higher the growth rate and biomass yields. As purified SIs are not yet widely available in large quantities, the use of mixed-aged cultures can be an important tool to maintain them at adequate nutrient levels e.g. in integrated multi trophic aquaculture. The modeling of *Ulva*'s growth indicates the importance of SI-producing algae for sustainable and successful seagricultures, and paves the way for a better understanding of the green tide formation in coastal areas.
5. Code availability
The model creation process is explained in detail in the Methods. Code for creating the figures is available upon request.

6. Author contributions
AG and AL conceived the initial idea for the study; UO, AG and AL designed the study and the mathematical model; AL produced the results; TW produced the experimental results; All authors interpreted the results and wrote the manuscript.

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8. Competing interests
The authors declare that they have no conflict of interest.

9. References
Brush, M. J. and Nixon, S. W.: Modeling the role of macroalgae in a shallow sub-estuary of Narragansett


