



Supplement of

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

Uri Obolski et al.

Correspondence to: Uri Obolski (uriobols@tauex.tau.ac.il) and Alexander Liberzon (alexlib@tauex.tau.ac.il)

The copyright of individual parts of the supplement might differ from the article licence.

Content:

- Materials and methods for adding sporulation inhibitor 1 (SI-1) to *U. mutabilis* culture to suppress gametogenesis
- Figure S1 Sensitivity analysis of initial algae distribution

Materials and methods for adding sporulation inhibitor 1 (SI-1) to *U. mutabilis* culture to suppress gametogenesis

Purification of the sporulation inhibitor (SI-1) and bioassay

Extraction. Stratmann et al. (1996) established extraction protocols for the SI-1, which were slightly modified and applied *U. mutabilis* (Kessler et al., 2018; Vesty et al., 2015). 500 mL of the *Ulva* culture medium (UCM) from 2–3 week old axenic *U. mutabilis* cultures was stirred with 50 mL phenol (saturated with 100 mmol L⁻¹ Tris-HCl, 1 mmol L⁻¹ EDTA, pH 7.5) in a 1 L two-neck round-bottom flask for 20 minutes at 20 °C to extract the SI-1 from the UCM. The phenol phase was transferred into plastic tubes after centrifugation (3800 g, 10 minutes). The extraction process was repeated once more, and the phenol phases were combined. After re-extracting with 100 mL of 10 mmol L⁻¹ Tris-HCl (pH 8.0), the phenol phase was mixed with three volumes of acetone and incubated at -20 °C for 30 minutes. Centrifugation (3800 g, 20 min, 0 °C) was used to collect the precipitate, washed three times with pre-cooled ethanol (-20 °C). After lyophilisation, the precipitate was suspended in 100 mmol L⁻¹ Tris-HCl (pH 8.0) and stored at -20 °C. A dilution series tested the activity of the partly purified sporulation inhibitor.

Estimation of SI activity. *Ulva* fragments were washed twice in a fine sieve after being chopped. The fragments ($N_{\text{Fragments (total)}} = 100 \pm 30$) were transferred into 96 multiwell dishes (Nunc, Roskilde, Denmark) containing 100 µl UCM for gametogenesis analysis. The concentration of the SI-1 was determined through a dilution series of the partially purified compounds with UCM. (Due to the nature of the discrete dilution series, measurement variance is also affected by the interval between dilution steps).

$$\text{Inhibitory rate (\%)} = \frac{N_{\text{Fragments (total)}} - N_{\text{Fragments with gametangia}}}{N_{\text{Fragments (total)}}} \times 100$$

One unit of the SI is defined as the minimal amount of the factor that inhibits differentiation of a blade cell into gametangia with an *Inhibitory rate* of 50% in 1 mL UCM within three days under standard conditions (Stratmann et al., 1996). 10 Units were applied in the bioassay.

Bioassay. The gametogenesis of a mature thallus can be induced by changing the UCM and opening the tube (Alsufyani et al., 2017). Those thalli were used to test the activity of the sporulation inhibitor, ten units of the extracted SI-1 from the *Ulva* culture medium of a young *Ulva* population (100/0) were added to one Petri dish, while the other part of the thallus was simply incubated in UCM.

Sensitivity analysis of initial algae distribution

Figure S1: Analogous to Figure 2 in the main text ((a) yield, (b) time to 90% of the maximum carrying capacity and (c) inhibitor amount), with a different initial age distribution. We model the initial age distributions as an exponential distribution truncated to $[0,120]$, and vary its scale parameter between 1 and 50, where increased scale parameters means an older initial population.. That is, the same initial density of 0.2 kg/m was distributed among the different a_i classes, but instead of a bimodal distribution of $i=0$ or $i=120$, we used the distribution described above. Qualitatively similar results are obtained.

