



Variation on the standing stock of *Gracilaria* sp. in a temperate estuary under single-stressor and multiple-stressor climate change scenarios

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ABSTRACT

The seaweed *Gracilaria* is not only relevant to the functioning of coastal ecosystems as it also has an important economic value particularly linked to agar extraction. Climate change is expected to affect the production of marine macrophytes and hence their related processes. Baring this in mind, our aim was to develop a numerical model capable of predicting variations on *Gracilaria* sp. growth and standing stock under climate change scenarios, including temperature rise (RCP2.6 and RCP8.5), hypersalinity and enhanced nitrogen (N) runoff. Single- and multiple-stressor simulations were run to check for possible interactions among different stressors and the resulting impacts on *Gracilaria* sp. growth. Results indicate that the effects of isolated and combined stressors on *Gracilaria* sp. standing stock differ. The tested temperature rise scenarios induced decreases of 29% (RCP2.6) and 57% (RCP8.5) on the red algae biomass, with more severe effects under the highest emission scenario (RCP8.5), which were related to enhanced algal respiration, decomposition and grazing pressure. Seasonal hypersalinity caused a reduction of 35% on the net productivity of *Gracilaria* sp., while the simulated N runoff increase (25%) had no effect on the annual biomass of *Gracilaria* sp. Contrarily to the moderate effects of single-stressors, multiple-stressor scenarios had severe impacts on *Gracilaria* sp. annual standing stock with reductions > 90%, pointing out to the occurrence of synergistic effects of temperature and salinity on the production of *Gracilaria* sp.

Overall, these results indicate that within the next decades, the interaction of different environmental stressors will pose significant constraints to the production of *Gracilaria* sp. on natural systems with subsequent effects to therein associated ecological services.

1. Introduction

Macroalgae are important primary producers that dominate coastal areas worldwide [1]. Among their major ecosystem functions and services are the provisioning of habitat for many species of fish and invertebrates, bioremediation of nutrient-enriched waters (e.g. aquaculture effluents; [2–4]) and mitigation of climate change, acting as a carbon dioxide sink and reducing the effects of ocean acidification and de-oxygenation [5]. The role of macroalgae go far beyond their important ecological roles, as they are also used for global food [6] and feed production, including the diets of fish [7–9], dairy goats [10], as well as the production of derivatives and biofuel. This highlights the importance of macroalgae exploration as a remarkable and profitable economic source [11]. Within Rhodophyta, the genus *Gracilaria* has a significant importance worldwide [11,12], due to its high economic

yield associated to commercially valuable extracts, namely, agar [13,14]. The chemical and physical properties of the agar make it suitable for a diversity of uses in medical, pharmaceutical, industrial and food applications [15–17]. In fact, the use of *Gracilaria* for feed-stuffs and extraction of agar materials goes back to the time when seaweeds were harvested from natural populations [6,18]. However, to give response to the increasingly demand for both the food and feed industry in the 20th century [19], successful efforts on the industrial farming of *Gracilaria*, including IMTA (Integrated Multi-Trophic Aquaculture) systems, have been conducted in several countries and using different cultivation methods [6,12,18], enabled by their fast and efficient growth [20].

Several key environmental factors have been identified to affect *Gracilaria* cultivation, namely light, air and water temperature, salinity, nutrients, cultivation depth, water movement and presence of

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herbivorous fish [21–23]. In a global climate change context, the increase of temperature influences biochemical reactions with direct consequences on key physiological rates such as photosynthesis and respiration of macroalgae [24], with effects on *Gracilaria* growth, agar yield and quality of colour strains [25,26]. Moreover, the direct and indirect consequences of increased water temperature on marine communities can be rather complex if multiple stressors interact, leading to expressive changes in range shifts and abundance of red seaweeds of the genus *Gracilaria*, as different species from temperate and tropical environments have different thermal tolerance [27,28].

For some *Gracilaria* species growth has been positively related with temperature within the range 15 and 35 °C [29], whereas for *G. lemaneiformis* death has been reported for water temperature above 26 °C [6]. At the temperate waters of the Portuguese Atlantic coast, the optimum temperature for *G. vermiculophylla* is 20 °C [30]. *Gracilaria* species tolerate salinity values within the range 5–35 psu [6], while the optimum range is reported within 15–35 psu [20,25].

In relation to nutrients, it has been shown that the growth of *Gracilaria* sp. increased when the N/P concentrations rose from 50/3.13 μM to 400/25 μM but decreased significantly when the former values were exceeded [31]. On the other hand, for *G. conferta* an N supply up to 333 μmol N l⁻¹ counteracted the stressful effects of increased irradiance and temperature and had a positive effect on the seaweed photosynthesis [32].

Therefore, the objective of this work is to develop a dynamic model able to simulate the production of *Gracilaria* sp. in natural systems, followed by the run of different scenarios forced by the long-term projections of climate change (2081–2100) [33], to ultimately address the effects of multiple stressors on *Gracilaria* growth, standing stock and production.

Scenarios of temperature rise, hypersalinity and eutrophication were chosen according to future scenarios expected for South-Western Europe regions [33,34] and empirical knowledge based on professional experience gained at the study site [35–37].

Because the model can be adapted to other systems, it may be further used as a management tool, foreseeing the optimisation of seaweed farming under climatic variability and thus supporting cost-effective decision by seaweed farmers.

2. Material and methods

2.1. Study site and field data

The study site is a warm-temperate system, the Mondego estuary, located on the west coast of Portugal (40°08 N, 8°50 W). The estuary is about 7 km long, with an area of approximately 1072 ha of wetland habitats, divided in two channels, north and south, separated by an alluvial island (Fig. 1). The north channel is deeper (5–10 m during high tide; tidal range 1–3 m, residence time: 1–2 days), while the south channel is shallower (2–4 m during high tide, tidal range 0.5–3.5 m, residence time: 3 days) and the location of important biological communities of primary producers and consumers [35]. Due to the upstream rice fields and sluices, the south arm environment is highly influenced by hydrodynamics, which in turn depends on the seasonal amount of rainfall and rice fields' management, where the amount of water available for the rice crop is controlled through a system of sluices. For a more detailed characterisation of the system, see e.g. Martins et al. [38] and Lillebø et al. [36].

In the Mondego estuary, *Gracilaria gracilis* is present all year round showing a biomass peak during summer [39]. During the 90's, when the system was highly eutrophic, macroalgal assemblages were strongly dominated by *Ulva intestinalis* [38,40]. However, in 1998 due to the implementation of a management plan, which led to an effective decrease on the nitrogen load of the system [36], there was a shift on the dominance pattern of macroalgal species, whereas *U. intestinalis* presented a marked decrease of biomass, *G. gracilis* average biomass

increased by 133% (from 17.63 to 41.14 gAFDWm⁻²) [39]. Such shift is attributed to a more efficient mechanism of internal nitrogen storage by *G. gracilis* compared to *U. intestinalis* [41,42], which under situations of lower nitrogen availability seems to provide a competitive advantage of *G. gracilis* over *U. intestinalis* [39]. Moreover, it was shown that compared to *U. intestinalis*, the effects of *Gracilaria* algal mats on the local macroinvertebrate communities was less detrimental and that *Gracilaria* is able to sustain enriched macroinvertebrate communities [40].

An intensive monitoring program was carried out during the 1990's and the beginning of the 2000's [35] aiming to characterise the biological communities established at the intertidal mudflats from the south arm of the estuary (e.g. [35,43]). At each site, six replicate cores were taken along a transect to a depth of 20 cm (13 cm inner diameter) and then pooled together for biological material analyses. The sediment was washed through a 500 mm mesh sieve and the biological material preserved in 4% buffered formalin. Animals and plants were identified to species level and subsequently dried at 70 °C for 72 h to estimate biomass as dry weight (DW), and ash free dry weight (AFDW) after combusting samples for 8 h at 450 °C [35]. *Gracilaria* sp. data (g DWm⁻²) used in the present study were retrieved from that dataset, corresponding to samples collected fortnightly, between January and December 1993, at one sampling station (*Zostera* site- [35]) with a downward location at the south arm of the estuary (Fig. 1). Simultaneously, water temperature (°C), salinity (psu), dissolved oxygen (mg l⁻¹ and %) and pH were measured in situ and water samples (approximately 250 ml) were collected for estimating dissolved inorganic nutrients (PO₄-P, NO₃-N, NO₂-N and NH₄-N) (Fig. 2). Sediment samples were also collected to quantify the organic matter content. For the model validation, an independent data set collected at the same location between January and June 1994 was used.

2.2. Model conceptualization and formulation

The model includes three state variables, *Gracilaria* sp. biomass (Grac, gDW m⁻²), *Gracilaria* sp. nitrogen content (N_{int}, μmolN (gDW)⁻¹) and phosphorus content (P_{int}, μmolP(gDW)⁻¹) (Fig. 3), and their variation through time is described by the following equations, respectively:

$$\frac{dGrac}{dt} = NPP - Rep - Graz - Dec - Adv \quad (1)$$

NPP- net primary productivity (d⁻¹), Rep- reproduction (d⁻¹), Graz- grazing (d⁻¹), Dec- decomposition (d⁻¹), Adv- advection (d⁻¹).

$$\frac{dNint}{dt} = N_{upt} - N_{cons} \quad (2)$$

N_{upt}- nitrogen uptake (μmolN(gDW)⁻¹d⁻¹), N_{cons}- nitrogen consumption (μmolN(gDW)⁻¹d⁻¹).

$$\frac{dPint}{dt} = P_{upt} - P_{cons} \quad (3)$$

P_{upt}- phosphorus uptake (μmolP(gDW)⁻¹d⁻¹), P_{cons}- phosphorus consumption (μmolP(gDW)⁻¹d⁻¹).

Net productivity (NPP) is described by:

$$NPP = GPP - R \quad (4)$$

where GPP is gross productivity (d⁻¹) and R is respiration (d⁻¹).

$$GPP = \mu_{max} \times fI \times fT \times fS \times fNP \quad (5)$$

where μ_{max} is maximum growth rate (d⁻¹) multiplied by the dimensionless growth limiting factors of light (fI), temperature (fT), salinity (fS) and nutrients (fNP), respectively.

$$R = R_{max20} \times \Theta^{(T-20)} \quad (6)$$

where R_{max20} is the maximum respiration rate at 20 °C (d⁻¹), Θ is an empirical coefficient (dimensionless) and T is temperature (°C).

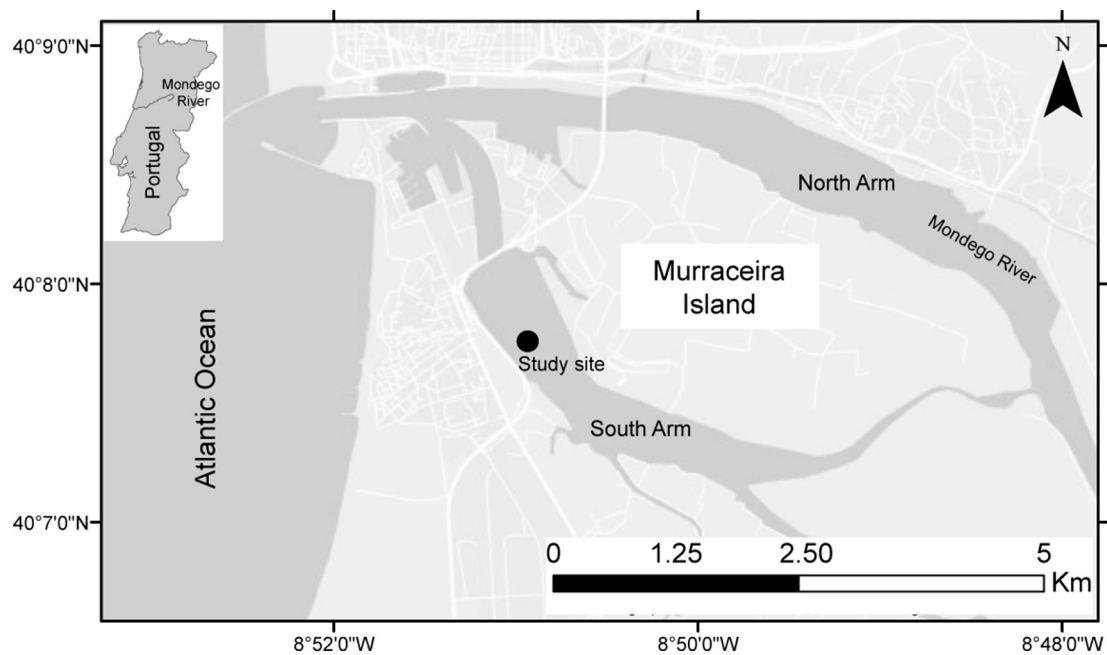


Fig. 1. The Mondego estuary (NW coast of Portugal) with location of the study site.

$$fI = \frac{I}{I_{opt}} \times e^{\left(1 - \frac{I}{I_{opt}}\right)} \quad (7)$$

I is photon flux density ($\mu\text{mol m}^{-2} \text{s}^{-1}$) and I_{opt} ($\mu\text{mol m}^{-2} \text{s}^{-1}$) is the optimum photon flux density.

$$fT = e^{\left[-2.3 \times \left(\frac{T - T_{opt}}{T_x - T_{opt}}\right)^2\right]} \quad (8)$$

T is temperature ($^{\circ}\text{C}$), T_{opt} is the optimum temperature for growth ($^{\circ}\text{C}$), $T_x = T_{min}$ for $T \leq T_{opt}$ and $T_x = T_{max}$ for $T > T_{opt}$. T_{min} , is the lower temperature limit below which growth ceases ($^{\circ}\text{C}$) and T_{max} is the upper temperature limit above which growth ceases ($^{\circ}\text{C}$).

If salinity (S) $\geq S_{crit}$, then

$$fS = 1 - \left(\frac{S - S_{opt}}{S_x - S_{opt}}\right)^m \quad (9)$$

where $S_x = S_{min}$ and $m = 2.5$ for $S < S_{opt}$; $S_x = S_{max}$ and $m = 2$ for $S \geq S_{opt}$.

If salinity $< S_{crit}$, then

$$fS = \frac{S - S_{min}}{S_{opt} - S_{min}} \quad (10)$$

S_{opt} is optimum salinity at which growth rate is maximum (psu); S_{min} , lower salinity limit at which growth rate ceases (psu); S_{max} is upper salinity limit at which growth ceases (psu); S_{crit} is the salinity value at which growth slows down (psu).

For nutrients, it is assumed that

$$fNP = \begin{cases} 1, & \text{if } 12 \leq N_{int}:P_{int} \leq 16 \\ fN, & \text{if } N_{int}:P_{int} < 12 \text{ (indicates nitrogen limitation)} \\ fP, & \text{if } N_{int}:P_{int} > 16 \text{ (indicates phosphorus limitation)} \end{cases} \quad (11)$$

if $N_{int}:P_{int} \geq 12$ and $N_{int}:P_{int} \leq 16$, then $fNP = 1$

if $N_{int}:P_{int} < 12$, then $fNP = fN$, which indicates nitrogen limitation; if $N_{int}:P_{int} > 16$, then $fNP = fP$, which indicates phosphorus limitation. Thus, fX , where X corresponds to the limiting nutrient (either N or P), is described by:

$$fX = \frac{X_{int} - X_{min}}{kq_x + X_{int} - X_{min}} \quad (12)$$

The uptake of X (the limiting nutrient) is described by:

$$X_{up} = \left(\frac{X_{max} - X_{int}}{X_{max} - X_{min}}\right) \times \left(\frac{V_{max} \times X_{ext}}{k_x + X_{ext}}\right) \quad (13)$$

where X_{int} is internal nutrient concentration ($\mu\text{molX(gDW)}^{-1}$); X_{max} , maximum internal concentration of nutrient ($\mu\text{molX(gDW)}^{-1}$); X_{min} , minimum internal concentration of nutrient ($\mu\text{molX(gDW)}^{-1}$); V_{max} , maximum uptake rate of nutrient ($\mu\text{molX(gDW)}^{-1} \text{d}^{-1}$); K_x , half-saturation constant for the uptake of nutrient (μmolL^{-1}); X_{ext} , external concentration of nutrient (μmolL^{-1}).

The assimilation of nutrients, X_a , is defined by:

$$X_a = X_{int} \times X_{req} \quad (14)$$

where X_{int} is the internal nutrient concentration ($\mu\text{molX(gDW)}^{-1}$) and X_{req} is the internal requirement of nutrient for growth ($\mu\text{molX(gDW)}^{-1}$).

Macroalgae reproduction, grazing and decomposition are described by Eqs. (16), (17) and (18), respectively:

$$Rep = Rep_{max} \times \theta^{(T-20)} \quad (15)$$

Rep_{max} - maximum reproduction rate (d^{-1}), T- temperature ($^{\circ}\text{C}$), θ - empirical coefficient (dimensionless).

$$Graz = Graz_{max} \times f(T) \quad (16)$$

$Graz_{max}$ - maximum grazing rate (d^{-1}), $f(T)$ - temperature-dependent function (dimensionless).

$$Dec = Dec_{max} \times \theta^{(T-20)} \quad (17)$$

Dec_{max} - maximum decomposition rate (d^{-1}), T- temperature ($^{\circ}\text{C}$), θ - empirical coefficient (dimensionless).

Advection (Adv) accounts for the portion of macroalgal tissue dragged out of the system through hydrodynamics. Based on experimental evidence for the study site [37,44], advection is highly dependent on the upstream sluice state (open or close), which is described by a binary effect, which varies between 0.4 and 10% of macroalgal tissue drifting out of the system into the adjacent coastal area.

The Brock model [45] was used to estimate the light intensity at

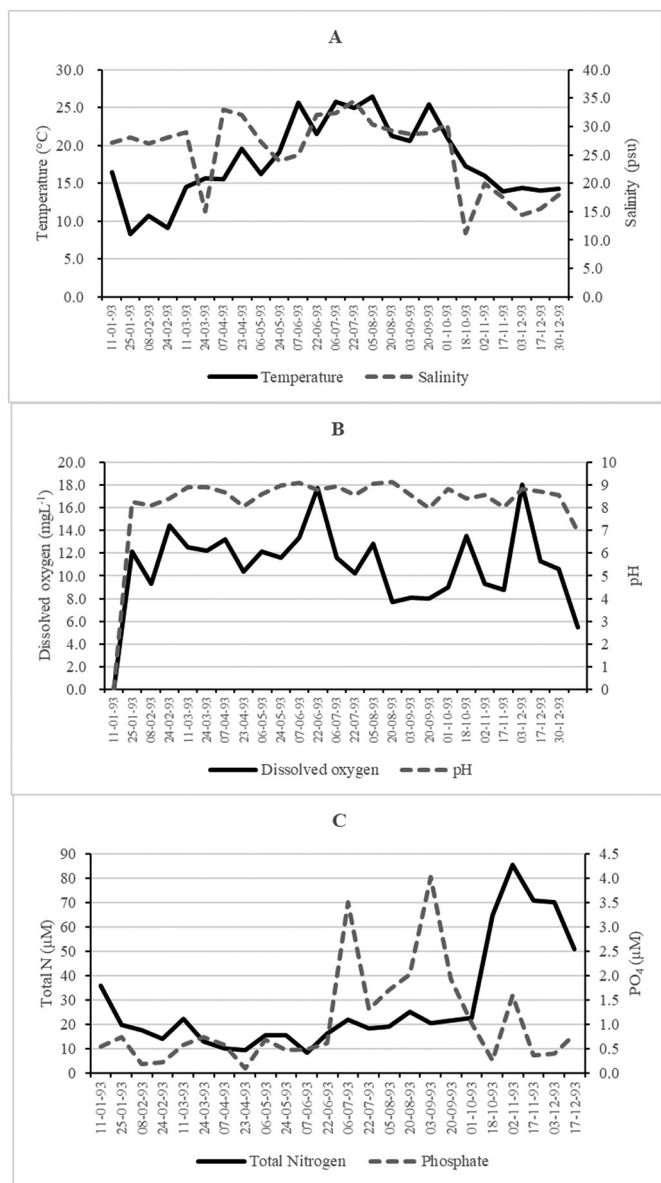


Fig. 2. Base line environmental conditions at the Mondego estuary used in the base run (see text for details). A- Water temperature (°C) and salinity (psu); B- dissolved oxygen (mgL⁻¹) and pH; C- total nitrogen (TN) and phosphate (PO₄) (µM).

surface (I_0), which in turn allowed estimating the photon flux density at surface (PFD_0) by assuming that 42% of the overall energy is available for photosynthesis [46]. Photon flux density at depth z (PFD_z) was estimated through the Lambert-Beer formulation:

$$PFD_z = PFD_0 \times e^{-kz} \quad (18)$$

k - light extinction coefficient (m^{-1}) and z - depth (m).

The light extinction coefficient depends on the amount of suspended and dissolved material in the water, which in turn depends on the upstream sluice state (close = 0 or open = 1) that is managed according to the amount of rainfall and the water requirements of the upstream rice fields [36,38]. When large amounts of macroalgae accumulate, k is also considered biomass-dependent due to macroalgae self-shading. These effects were described by:

$$k = \begin{cases} 2, & \text{if } sluice = 0 \text{ AND } algal \text{ biomass} < 50 \\ 5.59, & \text{if } sluice = 1 \text{ AND } algal \text{ biomass} < 50 \\ 2 + (0.01 \times algal \text{ biomass}), & \text{if } sluice = 0 \text{ AND } algal \text{ biomass} \geq 50 \\ 5.59 + (0.01 \times algal \text{ biomass}), & \text{if } sluice = 1 \text{ AND } algal \text{ biomass} \geq 50 \end{cases} \quad (19)$$

Assuming that the value 0 and 1 corresponds, respectively, to the situation of sluice closed and opened, an algal biomass of 50 g DWm⁻² is assumed to considerably decrease the light reaching algae underneath according to in situ observation (personal observation). The numerical values of k were experimentally obtained at the study site [38].

Tidal height (m) was simulated according to equation:

$$Tidal \text{ height} = \frac{HBM + HPM}{2} \times \cos\left(\frac{2 \times \pi \times Time}{Tide \text{ period}}\right) \quad (20)$$

where HBM (low tide tidal height) and HPM (high tide tidal height) depend on tidal range (local range 0.2–3.7 m), tide period corresponds to semi-diurnal tide (12h25m per cycle) expressed in days.

The calibration simulation run for 360 days, from January to December 1993, with a time step of 15 days. Table I contains definition, values, units and sources of parameters used in the model.

2.3. Sensitivity analysis

Sensitivity analysis was performed after imposing variations within the range $\pm 10\%$ to each parameter while all the others were kept unchanged. The sensitivity of each parameter (S) was estimated according to Jørgensen [53]:

$$S = \frac{\left(\frac{\partial X}{X}\right)}{\left(\frac{\partial P}{P}\right)} \quad (21)$$

X - state variable, P - parameter, ∂ - variation between the final and the initial values.

2.4. Tested scenarios

The model was used to check for the effects of some stressors that are likely to impact temperate estuaries and coastal areas in the next decades. On the other hand, both single- and multiple-stressor scenarios were simulated to check for any stressor's interaction. The single-stressor scenarios accounted for the effect of temperature rise according to two projections from the IPCC [33] for 2081–2100: RCP2.6 (corresponding to the lowest CO₂ emission scenario with average increase of 1 °C imposed to water temperature) and RCP8.5 (corresponding to the highest CO₂ emission scenario, with average increase of 3.7 °C imposed to water temperature), the effect of salinity increase where hypersaline conditions were reached during the warmer seasons (salinity ≥ 40 psu) and an increase of total N caused by coastal eutrophication as expected in the next decades as a consequence of both climate change and increased anthropogenic pressure in coastal areas [54,55].

The two multiple-stressor scenarios accounted for the respective effects of the two-temperature rise scenarios combined with the variation imposed to the other two stressors (Table II).

2.5. Statistical analysis

To assess the fitting level between the observed biomass values and the simulated values in both the model calibration and validation, the root mean square error (RMSE) was calculated between the two sets of data. The RMSE is the standard deviation of the residuals and it measures how far from the regression line data points are.

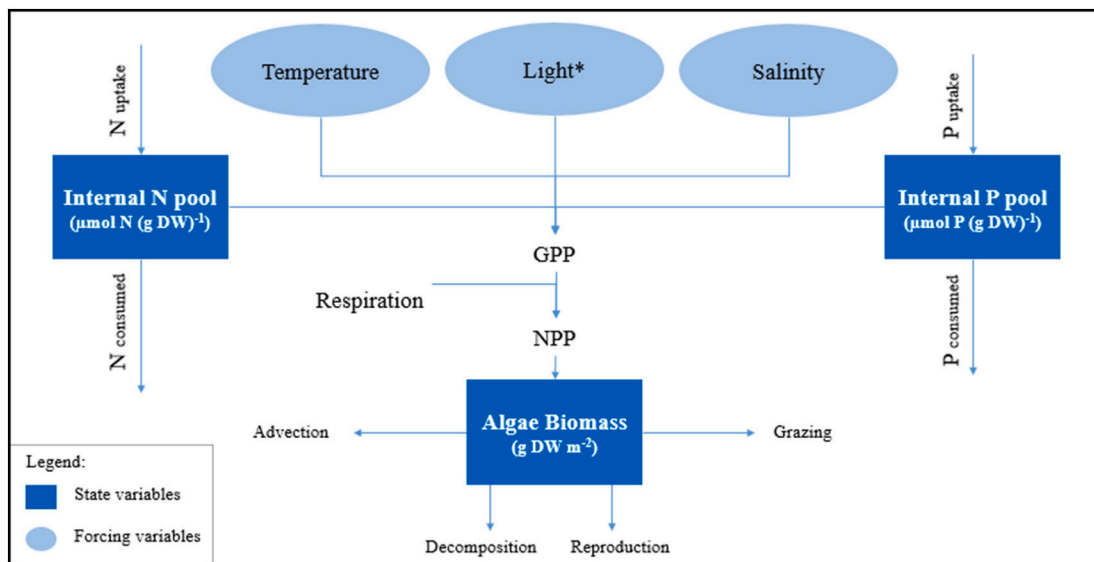


Fig. 3. Simplified conceptual diagram of the model. * Light is measured in PFD – photon flux density ($\mu\text{mol m}^{-2} \text{s}^{-1}$); GPP – Gross Primary Production (d^{-1}); NPP – Net Primary Production (d^{-1}); N – Nitrogen; P – Phosphorus.

$$RMSE = \sqrt{\frac{\sum_{i=1}^n (P_i - O_i)^2}{n}} \quad (22)$$

Pi- predicted values, Oi- observed values, n- number of data points.

3. Results

Input external variables vary according to daily and seasonal fluctuations (Fig. 4).

The simulated biomass of *Gracilaria* sp. followed the same annual trend as the observed biomass (RMSE = 13.183), with the highest value registered in March, followed by a gradual decrease towards the end of the year in the simulated set, while the observed set registered a moderate peak of biomass in July (Fig. 5).

The most sensitive environmental parameters were the maximum temperature for *Gracilaria* growth and the minimum and optimum temperature for grazers, while the most sensitive physiological rates were maximum grazing rate, maximum *Gracilaria* productivity and maximum *Gracilaria* respiration rate (Appendix I). These variations imply a range of variation of results between -23% and $+90\%$ (Fig. 7), highlighting the strong dependency of *Gracilaria* standing stock on maximum production rate and on the optimum temperature for grazers, respectively (Fig. 6).

In the validation run, the root mean square error (RMSE) between the observed and the simulated values was 1.838 (Fig. 7).

Single-stressor scenarios caused different effects on *Gracilaria* sp. depending on the evaluated stressor. Clearly, temperature was the stressor with the strongest effect on the algae standing stock, with

Table I

Parameters used in the model: brief description, units, used value, literature range or value and source.

Symbol	Description	Units	Used value	Lit. range/value	Source
μ_{\max}	Maximum growth rate at T_{opt}	d^{-1}	0.9	0.001–0.8	[22,71]
$R_{\max 20}$	Maximum respiration rate at 20 °C	d^{-1}	0.1	0.02–0.1	[47]
θ	Empirical coefficient		1.047	1.01–1.2	[47]
I_{opt}	Optimum photon flux density for growth	$\mu\text{mol m}^{-2} \text{s}^{-1}$	200	200–240	[23]
T_{opt}	Optimum temperature for growth	°C	28	20–30	[6]
T_{max}	Upper temperature tolerance limit at which growth ceases	°C	40	30–34	[20,28]
T_{min}	Lower temperature tolerance limit at which growth ceases	°C	10	10–15	[6]
S_{opt}	Optimum salinity for growth	psu	22	20.5–30.5	[18]
S_{max}	Upper salinity tolerance limit at which growth ceases	psu	37	30–37	[25,71]
S_{min}	Lower salinity tolerance limit at which growth decreases	psu	11	5–15	[6,25]
N_{imin}	Minimum internal quota (subsistence quota) for nitrogen	$\mu\text{mol N (gDW)}^{-1}$	500	357	[71]
N_{imax}	Maximum internal quota for nitrogen	$\mu\text{mol N (gDW)}^{-1}$	3000	1928–4285	[48,49]
K_{Ni}	Nitrogen half-saturation constant for growth	$\mu\text{mol N (gDW)}^{-1}$	1786	max. 1786	[47]
V_{maxNO_3}	Maximum nitrate uptake rate	$\mu\text{mol NO}_3 \text{ (gDW)}^{-1} \text{ d}^{-1}$	40	1.06–9.65 $\mu\text{M(gDW)}^{-1} \text{ h}^{-1}$	[72]
V_{maxNH_4}	Maximum ammonium uptake rate	$\mu\text{mol NH}_4 \text{ (gDW)}^{-1} \text{ d}^{-1}$	35	0.32–5.75 $\mu\text{M(gDW)}^{-1} \text{ h}^{-1}$	[72]
K_{NO_3}	Half-saturation constant for nitrate	$\mu\text{mol NO}_3 \text{ l}^{-1}$	3	1–37	[73]
K_{NH_4}	Half-saturation constant for ammonium	$\mu\text{mol NH}_4 \text{ l}^{-1}$	3	1–37	[73]
P_{imax}	Maximum internal quota for phosphorus	$\mu\text{mol P (gDW)}^{-1}$	126	max. 126	[49]
P_{imin}	Minimum internal quota (subsistence quota) for phosphorus	$\mu\text{mol P (gDW)}^{-1}$	16	32.4	[71]
V_{maxPO_4}	Maximum phosphorus uptake rate	$\mu\text{mol PO}_4 \text{ (gDW)}^{-1} \text{ d}^{-1}$	40	105 μM	[71]
K_{PO_4}	Half-saturation constant for phosphorus	$\mu\text{mol PO}_4 \text{ l}^{-1}$	1	1.05 μM	[71]
dec_{Max}	Maximum decomposition rate	d^{-1}	0.0019	0.00015	[71]
$\text{Reprod}_{\text{rate}}$	Reproduction rate-amount of biomass lost by reproduction	d^{-1}	0.02	Max. 0.6	[74]
T_{opGrazSp}	Optimum temperature for grazers	°C	22	15–30	[50]
$T_{\text{maxGrazSp}}$	Upper temperature for grazers	°C	45	–	[51]
$T_{\text{minGrazSp}}$	Lower temperature for grazers	°C	10	–	[51]
Graz_{max}	Maximum grazing rate	d^{-1}	0.043	2–19 $\text{mg (mg animal)}^{-1} \text{ d}^{-1}$	[52]

Table II

Simulated scenarios include 4 single-stressor and 2 multiple-stressor scenarios, 3 stressors: water temperature (T), salinity (S) and nitrogen (N), 2 levels of temperature increase expected for 2081–2100: RCP2.6 and RCP8.5, 1 level of salinity increase: hypersalinity and 1 level of N increase: 25%. Observations are included for further explanation.

Type of scenario	Stressor	Level	Observations
Single-stressor	Temperature	RCP2.6	A water temperature increase of 1 °C was imposed according to the average variation expected for the RCP2.6 scenario [33]
Single-stressor	Temperature	RCP8.5	A water temperature increase of 3.7 °C was imposed according to the average variation expected for the RCP8.5 scenario [33]
Single-stressor	Salinity	Hypersaline	A salinity increase was imposed allowing for the occurrence of hypersaline conditions (salinity ≥ 40) in the summer
Single-stressor	Nitrogen	Eutrophic	A 25% increase on NH_4 and NO_3 concentrations was imposed allowing for the occurrence of high eutrophic conditions
Multiple-stressor	Temperature, salinity and nitrogen	RCP2.6, hypersaline and eutrophic	A combination of the RCP2.6 scenario plus the hypersaline and highly eutrophic scenario was simulated
Multiple-stressor	Temperature, salinity and nitrogen	RCP8.5, hypersaline and eutrophic	A combination of the RCP8.5 scenario plus the hypersaline and highly eutrophic scenario was simulated

RCP2.6 and RCP8.5 scenarios causing a decrease of 29% and 57%, respectively, on the annual biomass of *Gracilaria* (Fig. 8), which was promoted by enhanced algal respiration and decomposition, as well as by the action of grazers on the red algae, which was particularly enhanced on the RCP8.5 scenario (Fig. 9).

In the hypersaline scenario, *Gracilaria* biomass was also reduced compared to the base run, specially, in the growing season (Fig. 10). In this case, the reduction of the algal standing stock was caused by an average annual decrease on net productivity of 35%.

Both *Gracilaria* biomass (Fig. 11) and productivity (not shown) were not sensitive to the scenario of 25% increase of NO_3 and NH_4 .

In the two tested multiple-stressor scenarios, encompassing temperature increases (RCP2.6 and RCP8.5, respectively), hypersaline conditions and increased N concentrations, *Gracilaria* growth was

equally and severely affected, without the presence of a spring growing peak and with biomass values close to 0 during most of the year. (Fig. 12).

4. Discussion

The present model was used to check the effects of stressors, which are likely to enhance their impacts on temperate estuaries under expected climate change scenarios, namely, temperature rise, hypersalinity and excessive N runoff [54,55] on the growth and biomass of red algae *Gracilaria* sp.

Results indicate that the standing stock of *Gracilaria* sp. in temperate estuaries may be severely affected by climate change, especially in scenarios where temperature rise and hypersalinity interact and impose

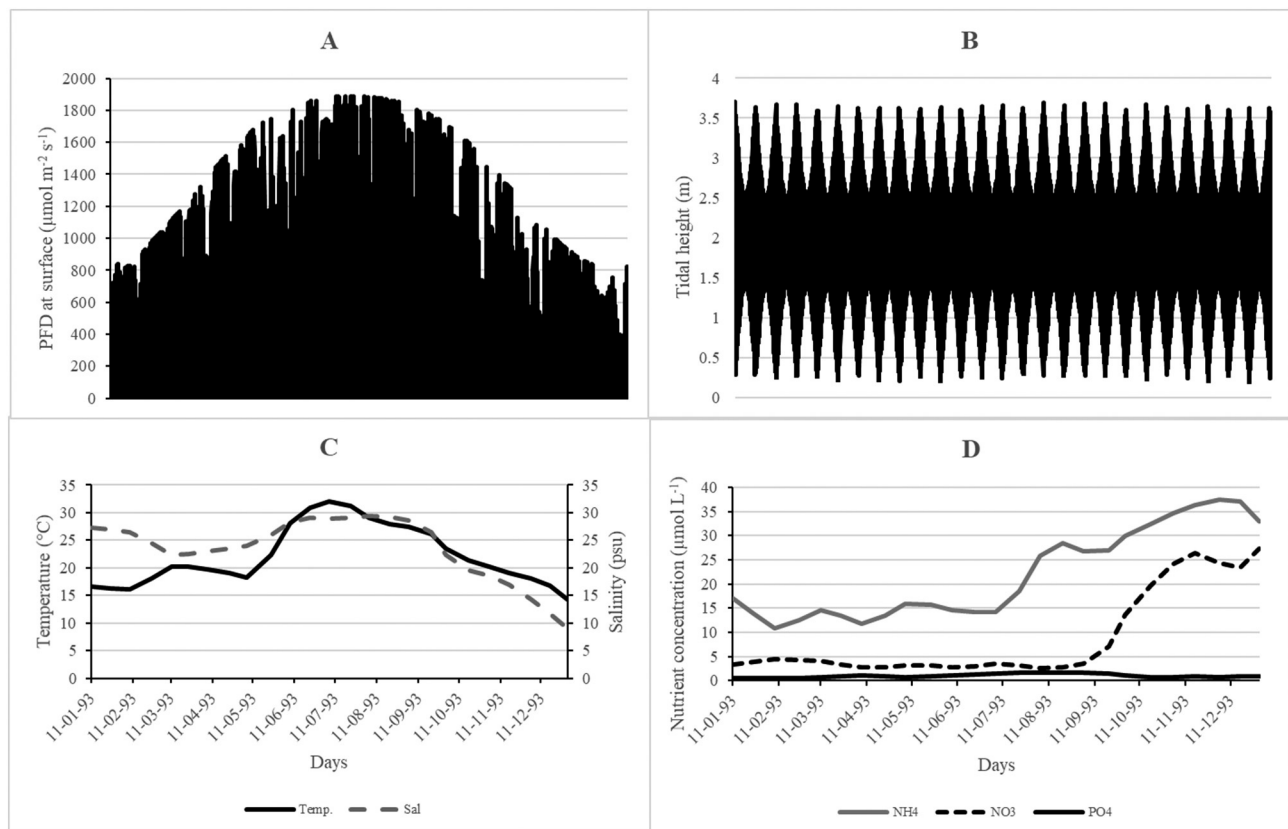


Fig. 4. Variation of photon flux density ($\mu\text{mol m}^{-2} \text{s}^{-1}$) (A), tidal height (m) (B), temperature ($^{\circ}\text{C}$) and salinity (psu) (C) and nutrient concentration (NH_4 , NO_3 and PO_4) ($\mu\text{mol L}^{-1}$) (D) in the base run. X-axis: days of simulation.

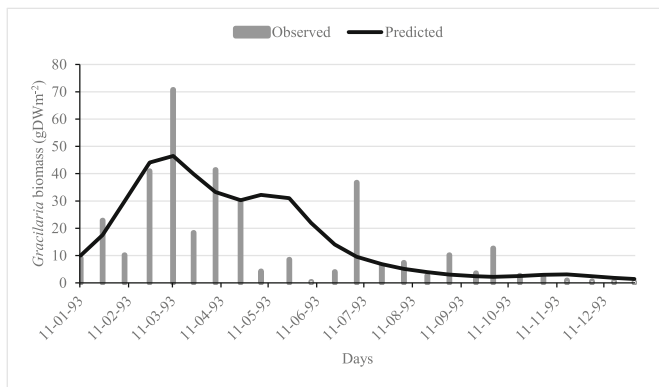


Fig. 5. Predicted *Gracilaria* sp. biomass (gDWm^{-2}) in the base run compared to observed values quantified at the Mondego estuary in 1993 (Root Mean Square Error (RMSE) = 13.183).

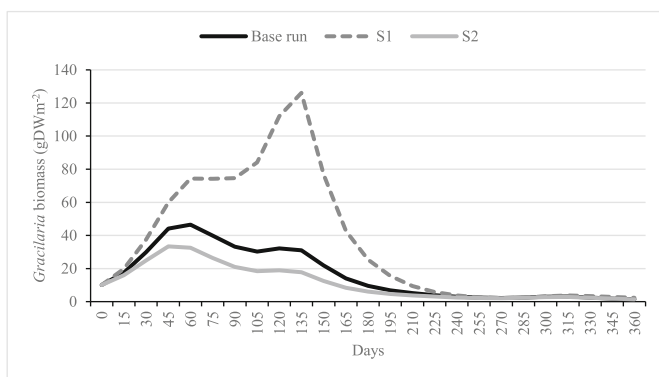


Fig. 6. Range of variation of *Gracilaria* sp. biomass (gDWm^{-2}) ascertained by sensitivity analysis (S1: +10% optimum temperature for grazers; S2: -10% maximum productivity).

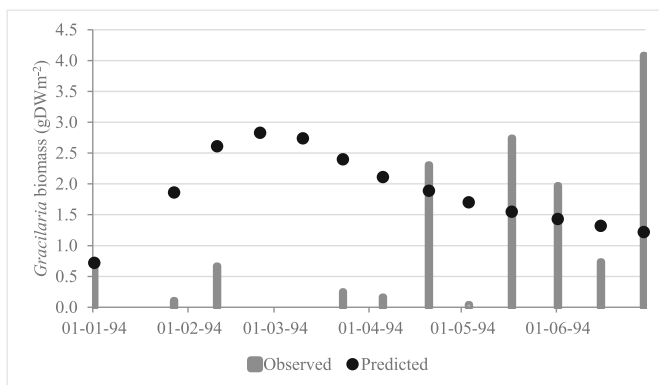


Fig. 7. Validation run comparing observed and predicted *Gracilaria* sp. biomass (gDWm^{-2}) (RMSE = 1.838) during 13 sampling dates between January and June 1994.

their influence on the processes that control macroalgal biomass, namely, respiration, decomposition and grazing. Our results are in line with findings from experimental approaches showing that the net primary production of macroalgae assemblages seem to be minimally affected by natural temperature variation, possibly due to photo-acclimation or temperature acclimation mechanisms, while respiration rates are negatively affected by temperate variations [56].

Herbivore damage has been referred to cause important losses in *Gracilaria* biomass [57,58]. Furthermore, according to the present simulations, grazers will enhance their activity under temperature rise

scenarios, which is also in agreement with results reporting increased feeding rates of invertebrates with temperature rise [6,59–61]. This seems to corroborate the tendency to diminished macroalgal biomass under temperature rise scenarios, in spite primary production being less affected or even increased [62]. Although in the present version of the model, grazing on *Gracilaria* was based on values found in literature followed by calibration, further improvements should consider accounting for more accurate grazing rates. Experimental setups using local *Gracilaria* species and mesograzers such as amphipods, isopods, polychaetes and gastropods that are abundant at the study site [40,63], may be implemented to determine grazing effects on *Gracilaria* sp. growth and production. Further improvements on the model should also include the explicit description of epiphyte's effects on *Gracilaria* production due to the importance of their relationships with the red algae [64,65]. In the present version, losses due to epiphytes were implicitly accounted for in the calibration of *Gracilaria* loss processes.

In addition, decomposition of *Gracilaria* sp. was also enhanced by the simulated warming scenarios which agrees with findings showing increased bacterial abundance and production in warmer condition under non-limiting organic matter resources [66].

Hypersaline conditions (salinity ≥ 40 psu), which may occur in estuaries and other coastal systems in response to high evaporation and/or low freshwater input [67], caused a moderate decrease on *Gracilaria* biomass induced by a decrease of 35% on the algae productivity. Indeed, hypersalinity conditions may be further away from the optimal ranges for *Gracilaria* that usually varies within 20.5–30.5 psu [18].

Besides changes on temperature and salinity, climate change may also increase the potential of eutrophication in coastal systems, accompanied by the increase of N:P:Si supply to estuaries and coasts with a concomitant P limitation [54]. According to the present simulations, the scenario of total N increase had no effect on *Gracilaria* productivity or biomass compared to the control simulation. This may have a two-fold explanation, firstly, under the observed environmental conditions in the Mondego estuary, *Gracilaria* was not N-limited and, secondly, the present model showed a low sensitivity to both N and P related parameters.

The two tested multiple stressor scenarios did not differ among each other and both of them caused a severe decrease on the biomass of *Gracilaria* sp., indicating the occurrence of additive effects between the temperature rise scenarios and the hypersaline conditions scenario.

As in previous works, our results corroborate the idea that stressor's interactions seem to have different impacts at the organism- and population levels when compared to impacts caused by isolated stressors [68–70]. Therefore, the use of models such as the present one, capable of integrating several factors of stress and predict their short- and long-term effects on organisms is crucial to assess the temporal variations of natural populations and support decision-making regarding mitigation measures and the sustainable management of populations and ecosystems. This is particularly relevant under the actual context of climate change. It should be noted that this type of models are capable of integrating other stressors that may reveal paramount to predict variations of macrophytes in coastal areas within the next decades, such as sea level rise, variations in freshwater runoff, anoxic conditions, etc. Furthermore, the present model is easily transferable to algal farms, where given site-specific data, it can be used to predict algal production in cultivation tanks and assess short- and long-term impacts of stressors on algal yield. On the other hand, improvements to primary producer models, such as this one, may be achieved by gathering more accurate data, particularly, related to algal maximum productivity rates, respiration and the effects of grazers.

5. Conclusions

According to the present results, under temperature rise combined with hypersaline conditions, *Gracilaria* sp. will show reduced production that will negatively affect the diverse roles and services played by

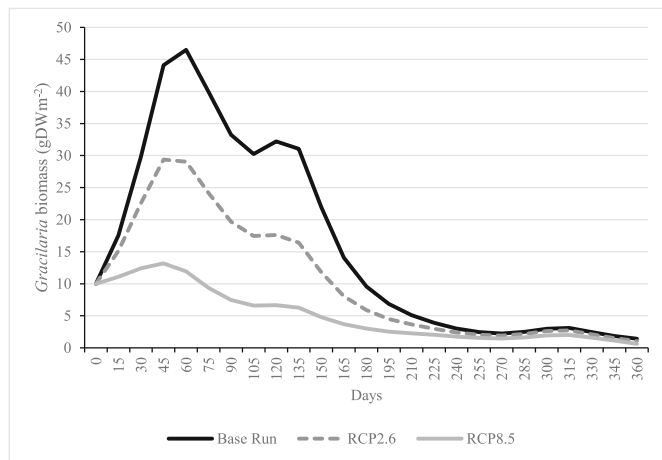


Fig. 8. *Gracilaria* sp. biomass variation (gDWm⁻²) predicted in the temperature rise scenarios (RCP2.6 and RCP8.5) compared to the base run.

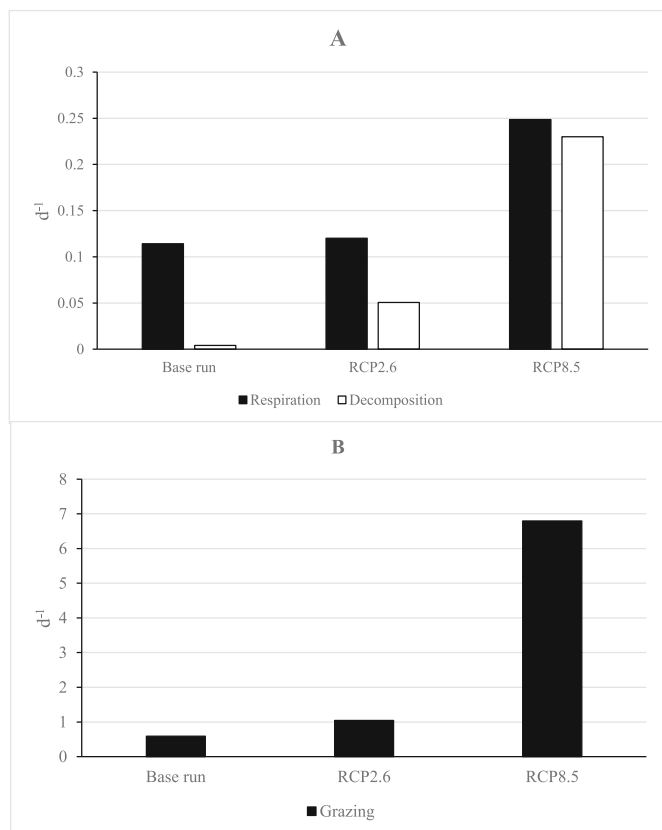


Fig. 9. Comparison between average *Gracilaria* sp. rates in the base run and in the two temperature rise scenarios (RCP2.6 and RCP8.5). A- Respiration (black) and decomposition (white); B- grazing.

Gracilaria, from industry applications to carbon sequestration and nutrient retention (e.g. [1]). Furthermore, temperature rise alone may also pose a problem for *Gracilaria* sp. aquaculture in coastal areas due to the subsequent increase of algal respiration and decomposition, whereas grazing may be less relevant in cultivation tanks.

Ultimately, numerical models are useful tools to predict the growth of organisms in different scenarios, contributing to support cost-effective decisions regarding the choice of natural macroalgae species and conditions for cultivation in aquaculture farms within the following decades.

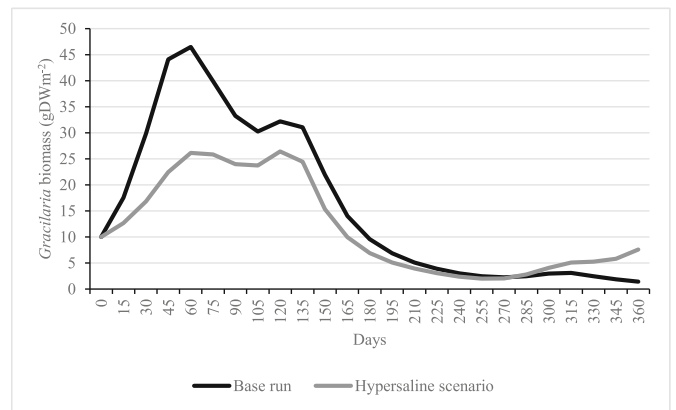


Fig. 10. *Gracilaria* sp. biomass (gDWm⁻²) variation predicted in the hypersaline scenario compared to the base run.

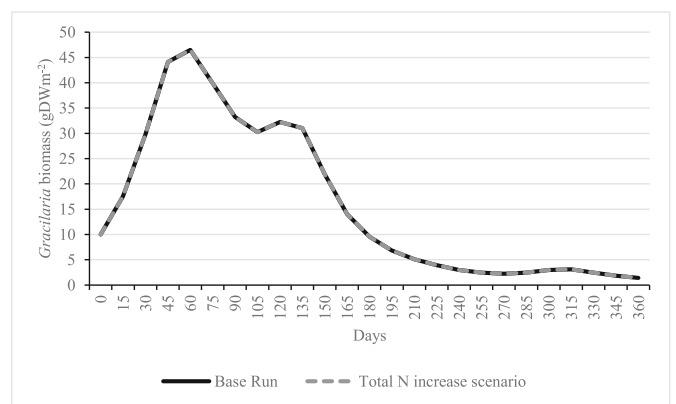


Fig. 11. *Gracilaria* sp. biomass (gDWm⁻²) variation predicted in the Total N increase scenario compared to the base run.

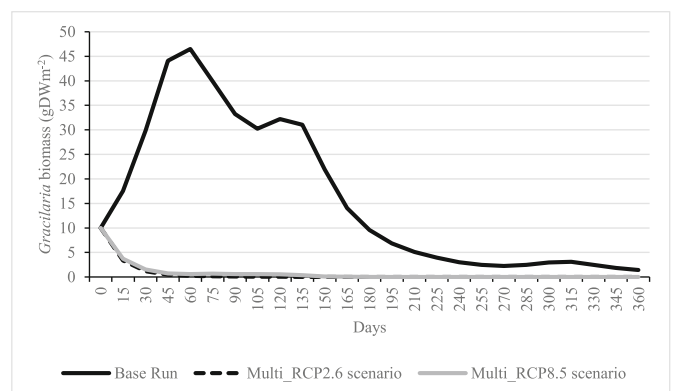


Fig. 12. *Gracilaria* sp. biomass (gDWm⁻²) variation predicted in the two multiple-stressor scenarios (Multi_RCP2.6 and Multi_RCP8.5) compared to the base run.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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CRedit authorship contribution statement

Irene Martins was responsible for the manuscript conception and

Appendix I. Sensitivity analysis of the model parameters to variations of $\pm 10\%$

Parameter	Sensitivity to -10% variation	Sensitivity to $+10\%$ variation
Initial <i>Gracilaria</i> biomass	+0.49	+1.04
Initial Nint	0	0
InitialPint	-0.005	+0.007
Clear sky transmission	+10	-10
Cloud cover	+10	-10
Germination_max	+0.507	+0.899
Grazing rate_max	-4.320	-1.896
KNH4	0	0
KNi	0	0
KNO3	0	0
KPi	-1.375	-0.676
KPO4	-0.207	-0.206
Max_Productivity	+3.405	+9.049
Nimax	0	0
Nimin	0	0
Nreq	0	0.321
PFD_sat	+1.379	+3.015
Pimax	+0.500	+0.728
Pimin	-0.129	-0.122
Preq	-0.674	-0.348
Resp_max	-3.661	-1.488
Sal_crit	-0.016	-0.006
Sal_max	+0.660	+0.212
Sal_min	-0.007	+0.004
Sal_opt	+1.479	+0.273
Temp_max_Gracilaria	+1.279	+1.279
Temp_min_Gracilaria	0	0
Temp_opt_Gracilaria	+1.751	+0.640
Temp_max_Grazer	-0.192	-0.099
Temp_min_Grazer	+1.622	+4.069
Temp_opt_Grazer	+3.247	+7.106
VNH4_max	-0.314	0
VNO3_max	0	0
VPO4_max	+0.424	+0.626

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