Algebraic equilibrium solution of tissue nitrogen quota in algae and the discrepancy between calibrated parameters and physiological properties

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ABSTRACT

Tissue nutrient concentrations are a key factor in determining primary production in a variety of algae, for example the marine macroalga *Ulva*. We present a novel algebraic solution to calculate the equilibrium tissue nitrogen concentration or "quota" Q. The solution is derived from a classical mechanistic description of "luxury uptake" in marine macroalgae using a computer algebra system. Forced by ammonium (NH_4^+) and nitrate plus nitrite (NO_x^-) concentrations, water temperature and irradiance, equilibrium Q can be calculated directly without the need for numerical integration, and the model performs well in reproducing observations of Q in frondose *Ulva* spp. A Sobol' global sensitivity analysis reveals that the degree of uncertainty in physiological parameters has a similar magnitude of influence on model output as the typical environmental range of nutrient forcing data. The environmental forcing variables NH_4^+ and NO_x^- together account for 60% of variance in model output, while the two most influential physiological parameters together account for another 32% of variance. Repeated parameter calibrations with random first guesses and evolutionary adaptations lead to broad and even multimodal distributions for some parameters, as well as values at the extremes of their literature ranges. This shows that although model performance as quantified by statistical measures is high, individual calibrations are not sufficient to give reliable parameter estimates that can be interpreted as physiological system properties.

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1. Introduction

Nuisance blooms of benthic marine macroalgae in coastal and estuarine environments have long been a cause for concern (Cotton, 1911) and lead to ecological, economic and societal problems, such as noxious odours, replacement of seagrass meadows or loss of benthic fauna due to anoxia (Valiela et al., 1997; Teichberg et al., 2010). In many cases, these blooms are dominated by species of the genus *Ulva*, including species previously classified as *Enteromorpha* (Hayden et al., 2003). For management and mitigation that goes beyond symptomatic treatment (e.g., bulk removal of macroalgae accumulations from the shoreline Liu et al., 2013), it is necessary to understand the diverse environmental factors leading to the high standing stocks observed. More recently, commercial harvest of *Ulva* spp. is also being explored as the basis for bioenergy production (Bruhn et al., 2011), where the chemical composition of the

tissue (e.g., the ratio of carbohydrate to protein) determines yield and efficiency of the conversion.

For these and related questions, a number of process-based (or "mechanistic") mathematical models of *Ulva* spp. growth dynamics have been developed and applied for general scenarios (e.g., primary production regime shifts, Zaldívar et al., 2009) as well as specific case studies (e.g., Bendoricchio et al., 1994; Salomonsen et al., 1999; Brush and Nixon, 2010; Ren et al., 2014). For most applications, the model output of primary interest is either net primary production (growth) or absolute biomass (standing stocks). In both temperate and tropical waters ambient nutrient concentrations are often the limiting factor on these variables (Teichberg et al., 2010).

Ulva spp. is capable of "luxury uptake", (Fujita, 1985; Viaroli et al., 2005), an ability frequently found in ephemeral macroalgae (Campbell, 2001) that offers a competitive advantage in habitats with highly variable water column nutrient concentrations (Pedersen and Borum, 1997). For any dynamic model of such macroalgae, it is therefore crucial to describe this two-step process which potentially decouples nutrient uptake and growth in

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time. Droop (1968) first described this process for vitamin kinetics in microphytoplankton, where growth is based on the tissue or cell-internal concentration of a substance ("quota"), which in turn is based on the external concentration in the medium. This concept was further developed first for phytoplankton (Nyholm, 1978) and later for macroalgae, leading to formulations such as the model developed by Solidoro et al. (1997) for *Ulva rigida*. Formulations equivalent or similar to the latter are now commonly implemented in marine macroalgae growth models (e.g., Coffaro and Bocci, 1997; Martins and Marques, 2002; Aldridge and Trimmer, 2009).

The submodel concerned with tissue nutrient concentrations is often parameterized based on controlled experiments in the laboratory. Solidoro et al. (1997) compared their submodel to laboratory data of nutrient concentrations in macroalgae tissue and the surrounding medium, on time scales of hours to days. Brush and Nixon (2010) compared simulation output from a similar submodel to field data with sampling intervals of weeks to months or two consecutive years. Comparisons with field data spanning the wide range of environmental conditions experienced on seasonal to interdecadal timescales, however, is rare, due to the labour- and cost-intensive requirement of collection and analysis of tissue nutrient samples. In addition, model studies on the time scale of years to decades usually explicitly simulate nutrient cycling and are analysed with regard to aggregate output variables such as standing stock biomass rather than tissue nutrient concentrations. Detailed analysis of individual submodels such as that describing tissue nutrient dynamics with long-term field data is therefore less likely to be carried out. Furthermore, the high computational cost of numerical integration schemes of mechanistic simulation models often prohibits extensive sensitivity analysis or repeated calibration procedures. Although individual calibrations may lead to high numerical model performance, the optimised set of parameters may not be sufficiently constrained, leading to "non-unique" calibrations (Janssen and Heuberger, 1995) and unrealistic values for some or all parameters, potentially determined by biases in the input data or deficiencies in model structure (Clark and Vrugt, 2006). Such "sloppy parameter sensitivities" have been found to be almost universal in systems biology models (Gutenkunst et al., 2007) and have recently also been identified in marine biogeochemical models (Ward et al., 2010). To date, they have not been examined in the context of macroalgae growth models, where individual calibrations prevail (e.g., Solidoro et al., 1997; Martins and Marques, 2002; Ren et al., 2014). A better understanding of how these issues influence macroalgae growth and especially tissue nutrient models will benefit both more complex coastal ecosystem models as well as potential stand-alone applications, e.g., examining macroalgae as suitable indicator organisms for environmental nutrient conditions (e.g., Ho, 1975; Barr, 2007) or computational metabolic modelling in the context of biofuel production from macroalgae (Golberg et al., 2014).

The objective of this study is to assess whether the ranges of relevant physiological parameters for *Ulva* spp. models are known well enough to allow model applications without site-specific measurements of these parameters, and whether site-specific calibrations will in turn lead to well-constrained estimates of these parameters which can be interpreted as biological or ecological system properties, e.g., physiological adaption. To this end, we examine the tissue nitrogen quota submodel used in the *Ulva* spp. growth model of Solidoro et al. (1997). First we present a novel algebraic equilibrium solution obtained using a computer algebra system, which is valid for short-term (days to weeks) equilibrium conditions under the assumption of constant (laboratory) or averaged (field) water column dissolved inorganic nitrogen concentrations. We then analyse the sensitivity of this algebraic equilibrium solution to the range of uncertainty in physiological parameters as well as the range of variability in environmental conditions. Finally,
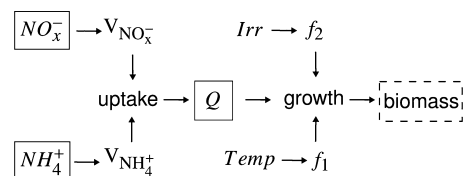

Fig. 1. Conceptual diagram of the model structure, with elements defining the uptake and growth processes as used in the ordinary differential equations taken from Solidoro et al. (1997). Boxes indicate elements which in a dynamic implementation would be considered (nitrogen) mass balance compartments. Under the equilibrium assumption that uptake is equal to growth (Q has reached a steady state), we can derive an algebraic equilibrium solution for Q, termed Q^*. The dashed box around "biomass" indicates that in the formulation used in this study, biomass is not explicitly considered. For symbol definitions see Table 1.

we examine the uncertainty in site-specific parameter calibrations caused by underdetermined model behaviour.

2. Methods

2.1. Model description

Biomass of *Ulva* populations is difficult to estimate due to the advective transport of free-floating thalli and their frequent layered or clumped occurrence, leading to high-frequency variability in both space and time. Especially estimates of intertidal abundance should be interpreted as qualitative or relative rather than absolute values (e.g., as a "biomass index"; Ren et al., 2014). In contrast, the tissue nitrogen quota Q responds more slowly (averaging conditions over space and time), and is easily measured as an absolute concentration. We therefore focus on Q, which is better suited to the long-term but zero-dimensional (box-model) approach taken in this study, and do not explicitly model biomass. Changes in ambient concentrations of ammonium (NH_4^+) and nitrate plus nitrite (NO_x^-) caused by changes in biomass (uptake and decay/remineralisation) are consequently not represented in the model, and NH_4^+ and NO_x^- are considered only as environmental forcing (rather than state) variables. Fig. 1 shows a conceptual diagram of the main model elements used in the present study, and a full list of variables and parameters is given in Table 1.

Solidoro et al. (1997) developed one of the first growth models for species of the genus *Ulva*, and similar or equivalent formulations are commonly used in macroalgae growth models. Following this structure, the evolution over time of Q is determined by the rate of external nutrient uptake for tissue nitrogen quota and incorporation of nitrogen into new tissue biomass. For the simplest case of just one external nutrient, namely NH_4^+, the flux of external nutrients to tissue nutrient quota may then be defined as

$$V_{NH_4^+} = V_{mNH_4^+} \cdot \frac{NH_4^+}{k_{NH_4^+} + NH_4^+} \cdot \frac{Q_{max} - Q}{Q_{max} - Q_{min}} \tag{1}$$

where $V_{mNH_4^+}$ is the maximum uptake rate for NH_4^+, $k_{NH_4^+}$ the half-saturation constant for NH_4^+ uptake, and Q_{min} and Q_{max} the tissue nitrogen quota minimum and maximum concentrations, respectively. $V_{NH_4^+}$ is thus dependent on NH_4^+ via a rectangular hyperbolic (Monod/Michaelis-Menten/Holling type II) function, and on Q by a linear decrease from 1 to zero over the interval Q_{min} to Q_{max}. Ignoring any other potentially limiting factors for now, the flux of nitrogen from tissue quota to new tissue biomass depends on Q through

$$g_1(Q) = \frac{Q - Q_{min}}{Q - k_c} \tag{2}$$