

Article

Revealing Interactions between Temperature and Salinity and Their Effects on the Growth of Freshwater Diatoms by Empirical Modelling

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Abstract: Salinization and warming are of increasing concern for freshwater ecosystems. Interactive effects of stressors are often studied in bifactorial, two-level experimental setups. The shape of environmental reaction norms and the position of the “control” conditions along them, however, can influence the sign and magnitude of individual responses as well as interactive effects. We empirically model binary-stressor effects in the form of three-dimensional reaction norm surfaces. We monitored the growth of clonal cultures of six freshwater diatoms, *Cymbella cf. incurvata*, *Nitzschia linearis*, *Cyclotella meneghiniana*, *Melosira varians*, *Ulnaria acus*, and *Navicula gregaria* at various temperature (up to 28 °C) and salinity (until the growth ceased) shock treatments. Fitting a broad range of models and comparing them using the Akaike information criterion revealed a large heterogeneity of effects. A bell-shaped curve was often observed in the response of the diatoms to temperature changes, while their growth tended to decrease with increasing electrical conductivity. *C. meneghiniana* was more tolerant to temperature, whilst *C. incurvata* and *C. meneghiniana* were the most sensitive to salinity changes. Empirical modelling revealed interactive effects of temperature and salinity on the slope and the breadth of response curves. Contrasting types of interactions indicates uncertainties in the estimation by empirical modelling.

Keywords: modelling; multiple stressors; global changes; primary producers; synergism; tolerance



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1. Introduction

Given their contribution of up to 20% of the global primary production [1] as well as their role in the global cycling of carbon, nitrogen, phosphorus, and silicon [2,3], diatoms are of high importance in the functioning of aquatic ecosystems. Due to the simplicity of sampling, preparation, and preservation of their silicate shells (frustules), freshwater benthic diatoms are widely used as environmental indicators, both in routine water quality assessment and paleolimnology [4,5]. Information on the ecological profiles of individual taxa is often expressed in terms of specific biotic indices, which summarize information on preference and tolerance ranges in a highly compressed form [6]. Alternatively, individual environmental parameters can be quantitatively linked to diatom community composition quantitatively using transfer functions or machine learning approaches [7,8].

One central topic in the assessment of the effects of multiple environmental variables (stressors) is the presence or absence and nature of interactive effects. Such interactions refer to situations when the combined effects of changes in multiple abiotic factors on organismal

viability differ from the summed effects of the individual environmental changes [9,10]. Such interactions can take different forms. For example, at the eco-physiological level, changes in one variable might affect the niche width, the location of the optimum, or the position of the tolerance limits relative to another. In practice, these interactions are often studied in two-level, two-stressor, full-factorial experiments. A classification system has emerged for characterizing multiple stressor effects in this context, differentiating between additive (no-interaction), synergistic, and antagonistic types (and in some cases, more categories are included) [11,12].

One limitation of this approach is that such a stressed-baseline comparison is agnostic of the shape of organismal tolerance profiles, which are nevertheless seen as a fundamental determinant of biological responses [13,14]. In spite of this disadvantage, such a design is still adopted, probably because of the increasing difficulty of statistically modelling ecological profiles in more than one dimension. One aspect of this difficulty is the large number of functional forms proposed to describe ecological reaction norms; for instance, at least a dozen different functional forms have been proposed for temperature [15]. In a more-than-one dimensional setting, different functional forms may apply to the individual abiotic predictors, and possibilities to capture interactive effects contribute further degrees of freedom, leading to an overwhelming variety of possibilities for mathematical-statistical models.

In the present research, we approached this problem by compiling a range of functional forms potentially describing reaction norms, in terms of growth, of freshwater diatoms to salinity and temperature; combining them in additive and interactive forms to depict three-dimensional reaction norm surfaces; and confronting the obtained broad range of possible model forms with data obtained from laboratory experiments. The experiments quantified the growth rate of clonal freshwater diatom cultures under a treatment matrix including several temperature and salinity levels. Compromise between data fit and the number of model parameters was quantified by the Akaike information criterion (AIC). We hypothesized that interactive terms are usually required to capture observed responses to temperature and salinity changes, and that the variability of such interactions would exceed a simple synergism–antagonism dichotomy. Although the effects of temperature [16,17] or salinity [18,19] upon growth were studied repeatedly in marine phytoplankton, such a study has, to our knowledge, never before been published on freshwater phyto-benthic diatoms.

2. Materials and Methods

2.1. Organisms and Culture Conditions

The diatom strains were isolated from a single microphytobenthos sample obtained from a flow-through mesocosm ExStream. The mesocosm was supplied from the small stream Boye (without any modification of the water) at its confluence with the Haarbach and Kischensbach in Gladbeck (51.55° N, 6.95° W, North Rhine-Westphalia, Germany) in the spring of 2021. Using light (oil immersion optics) and scanning electron microscopy of oxidized frustule preparations, the strains were identified as *Cymbella cf. incurvata*, *Nitzschia linearis*, *Cyclotella meneghiniana*, *Melosira varians*, *Ulnaria acus*, and *Navicula gregaria*. The strains were isolated and maintained in SFM + Si medium (Appendix A Table A1) in PK520 WLED climate chambers (polyKlima GmbH, Freising, Germany) with “True Daylight LED” illumination at 15 °C with a light–dark cycle of 10:14 h and a light intensity of 100 µE/cm².

2.2. Temperature and Salinity Experiments

Experiments were performed in 24-well cell culture plates (neoLab Migge GmbH, Heidelberg, Germany) in at least 4 replicates, with 2 mL total volume per well. Salinity was manipulated by adding NaCl (Carl Roth GmbH + Co. KG, Roth, Germany) to the basic SFM + Si media to obtain various concentrations up to 3000 mg/L. In addition, pH was kept at 7.0 (±0.1). The conductivity of the media was measured with a PCE-PHD 1 pH-conductivity meter (Meschede, Germany). After calibrating added salinity

values against measured conductivity in the used media, conductivity could be calculated as $0.432 + 0.00295 \times \text{added salt concentration}$ (the latter expressed as mg/L Cl^-). For consistency, throughout the rest of the paper, total salinity of media will be expressed as electric conductivity. Initial cell densities were separately adjusted to each strain based on a calibration of *in vivo* chlorophyll fluorescence values measured with a Tecan Infinite 200 Pro microplate reader (Tecan Group Ltd., Männedorf, Switzerland), similarly to the method applied by Albrecht et al. [20] with the following specifications: bottom reading mode with an excitation wavelength of 450 nm and detection at 680 nm. The plates were cultured in the climate chambers as described above for one week at different temperatures ranging from 5 to 28 °C. *In vivo* chlorophyll fluorescence was measured with the Tecan Infinite 200 Pro plate reader as described above at the same time of day over the seven days of experiments.

2.3. Calculation of Specific Growth Rate

In cultivation, the growth curve of microalgae usually includes a lag phase [21] at which the growth is delayed because of physiological adjustment and the presence of non-viable cells [22,23]. This was found to be the case in our experiments as well; therefore, the specific growth rate (μ ; 1/d) was calculated for the phase where the fluorescence signal increased exponentially (mostly days 3–7) using the following equation:

$$\frac{dN}{dt} = \mu \times N \quad (1)$$

where N is the fluorescence on the measuring day [24].

2.4. Empirically Modelling Diatom Growth

2.4.1. Multiplicative and Decoupled Models

The growth rate of diatoms as a function of environmental variables has usually been written as a function of growth-affecting factors representing the stressor [25–28]. In this method, each factor ranging from 0 to 1 is a multiplier in the growth rate calculation. Considering the influence of temperature and electrical conductivity:

$$\mu = \mu_{max} \times f_T \times f_S \quad (2)$$

with f_S and f_T (/) being salinity and temperature factors, respectively; and μ_{max} (1/d) being the maximum growth rate. In other words, the effect of each factor is considered multiplicative and decoupled.

As the stress caused by various factors is considered multiplicative and decoupled in this model, the influence of temperature could be integrated as the effects on the maximum growth rate, yielding the temperature-dependent growth rate (μ_T). Accordingly, the above equation could be modified as:

$$\mu = \mu_T \times f_S \quad (3)$$

A variety of equations have been applied previously to describe the temperature-dependent growth rate (Table S1, Supplementary Material), temperature factor (Table S2, Supplementary Material), and salinity factor (Table S3, Supplementary Material). These equations were combined and modified to consider all possible ways that factors in Equations (1) and (2) might be affected by temperature and/or salinity in a simple multiplicative or interactive manner. The latter can include: (1) the temperature-dependent growth rate is influenced by salinity; (2) the temperature factor is impacted by salinity; (3) the salinity factor is affected by temperature; and (4) the optimum, maximum, or the lower/upper level of one environmental factor is affected by another.

2.4.2. Polynomial Regression Equations

Another common approach to modelling diatom growth in various environmental conditions is to use polynomial regression equations [29,30]. Considering the effects of

temperature and salinity, the diatom growth rate can be written as a polynomial equation without (Equation (4)) and with (Equation (5)) interactions:

$$\mu = a + b_1 \times S + c_1 \times T + b_2 \times S^2 + c_2 \times T^2 \quad (4)$$

$$\mu = a + b_1 \times S + c_1 \times T + b_2 \times S^2 + c_2 \times T^2 + d \times S \times T \quad (5)$$

Equations (4) and (5) depict the relationship between the growth rate and two variables (temperature T and salinity S expressed by the electrical conductivity). In these equations, a is the intercept term (i.e., the growth rate at 0 °C and electrical conductivity 0 mS/cm); b_1 and c_1 represent the main effect for each variable; b_2 and c_2 represent square effects; and d stands for the interaction between temperature and electrical conductivity.

2.4.3. Data Fitting

Nonlinear least-square fitting of the growth rate as a function of temperature and electrical conductivity was conducted using SigmaPlot 15.0, providing estimates of the coefficients in the equations as well as statistical parameters (R^2 , p , and AIC). In the data fitting, only the lowest salinity at which the growth did not take place (zero or negative specific growth rate) was considered in order to reduce uncertainties in the analysis. The scenarios with definite confidence intervals for estimated parameters are given in Tables S4–S9, Supplementary Material. For selecting the best model for each species, we used the Akaike Information Criterion [31] as a metric, aiming to balance model complexity (number of parameters) against model fit. The model with the lowest AIC value was considered to provide the best such compromise, i.e., generalizability.

3. Results and Discussion

3.1. Variations in the Response to Temperature and Salinity among Diatom Species

Among the six strains investigated, *C. meneghiniana* (Figure S1C) and *U. acus* (Figure S1E) were least affected by temperature changes (Figure S1, Supplementary material). At the background electrical conductivity level of the culture medium (i.e., NaCl was not added to the culture medium), the growth rate of *C. meneghiniana* stabilized in the temperature range of up to 25 °C (Figure S1C), indicating a high tolerance of this strain to temperature changes in the investigated range. Given conductivity below 2 mS/cm, *U. acus* grew at any temperature (Figure S1E). In contrast, *C. incurvata* was the most strongly affected by temperature changes, with wide variations in its growth rate with varying temperatures (Figure S1A). At 25 °C, the growth of *C. incurvata* ceased, regardless of salinity.

The growth of *C. incurvata*, *M. varians*, *U. acus*, and *N. gregaria* followed a typical bell-shaped curve, i.e., below the optimum temperature, the growth rate increased with increasing temperature; above the optimum temperature, the growth rate decreased with increasing temperature (Figure S1). A different pattern was recorded for *N. linearis* (Figure S1B). At the background electrical conductivity, the growth rate of this strain decreased with increasing temperature up to 20 °C and increased with increasing temperature above 20 °C (Figure S1B). These patterns were altered by the effects of electrical conductivity changes with greater fluctuations in the temperature-growth curve (Figure 1). A bell-shaped temperature-growth curve has been reported previously for diatoms [29,32,33]. Up to the optimum temperature, the increase in the growth rate of phytoplankton with increasing temperature is related to enhanced enzymatic activities, photosynthesis, and nutrient uptake [34]. The sharp decreases in the growth at high temperatures have been ascribed to deactivation of enzymes or modification of proteins [35], which disrupts metabolism and consequently causes mortality of cells [36]. In addition, photosynthetic performance and efficiency of phytoplankton might be inhibited by high temperature due to impairment in the photosynthetic electron transport and carbon fixation [37].

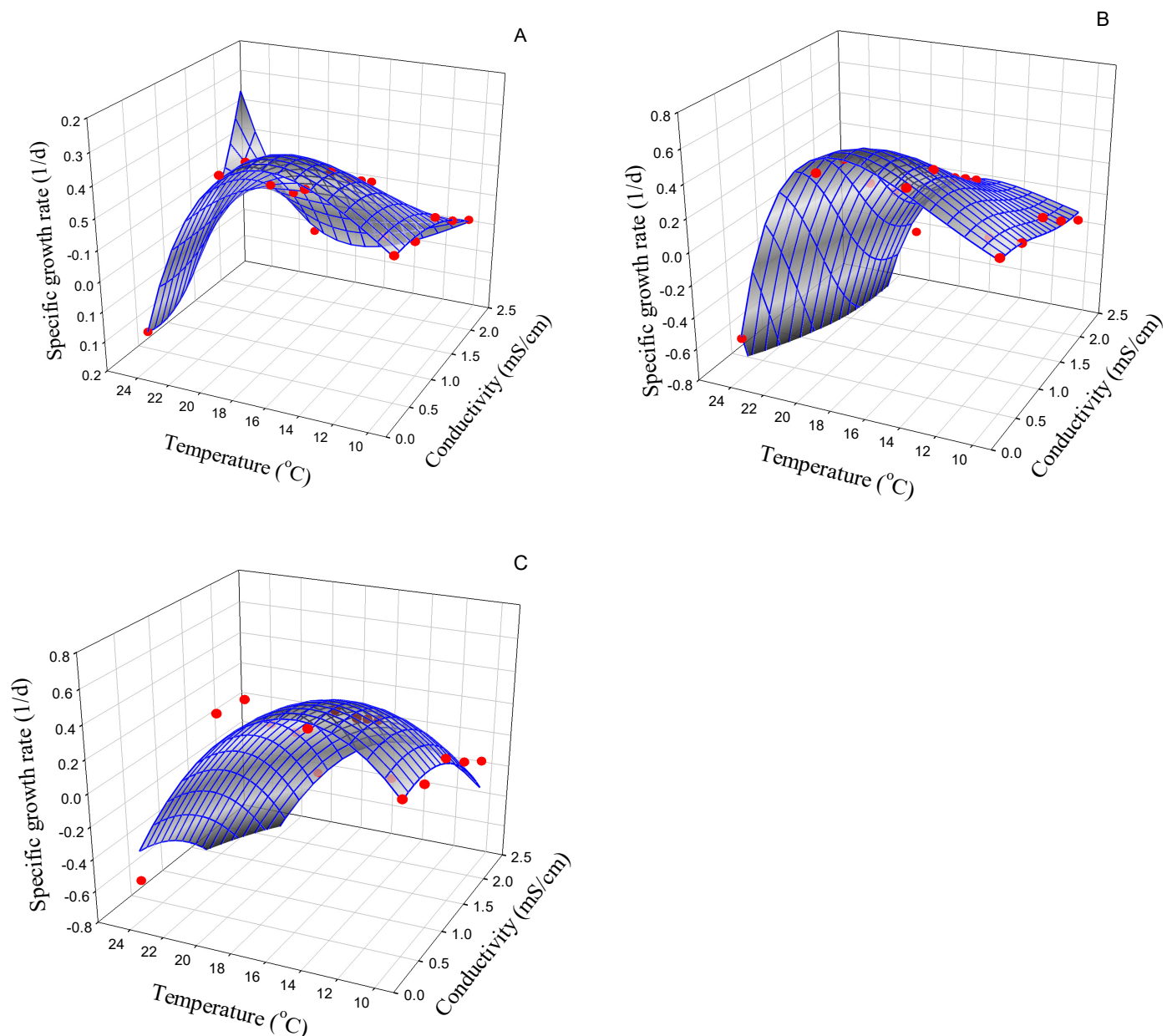


Figure 1. Simulation of the growth of *Cymbella incurvata* based on experimental data according to non-interactive multiplicative Equation (ST13) ((A); $R^2 = 0.97$; AIC = -91.06), interactive multiplicative Equation (ST2) ((B); $R^2 = 0.88$; AIC = -62.08), and polynomial Equation (4) ((C); $R^2 = 0.82$; AIC = -59.42).

The growth rate of the investigated freshwater diatoms commonly declined with increasing electrical conductivity (Figure S1). For example, the growth rate of *C. incurvata* and *C. meneghiniana* decreased with increasing salinity regardless of temperature (Figure S1). For the other strains, their response to salinization was more diverse, sometimes showing a unimodal response (maximal growth rate at intermediate salinity levels). However, an inhibition of the growth rate with increasing electrical conductivity was still dominant (Figure S1). The bell-shaped conductivity-growth curve was only seen at high temperature ($25\text{ }^{\circ}\text{C}$; Figure S1). The growth of *N. gregaria* was least affected by salinity changes (Figure S1F). Decreases in the growth rate of this diatom were only evident at conductivity above 5 mS/cm (Figure S1F). At temperature below $25\text{ }^{\circ}\text{C}$, *N. gregaria* still grew at electrical conductivity above 9.00 mS/cm (Figure S1F). In contrast, *C. meneghiniana* was among the diatoms most affected by salinization (Figure S1C). Different results have been reported for

this diatom. Roubeix and Lancelot [38] revealed a bell-shaped response of *C. meneghiniana* to salinity changes ranging from 0 to 33‰ with a maximum growth rate at 18‰.

According to Kirst [19], organisms might be affected by changes in salinity for several reasons, including: (1) osmotic stress and subsequent effects on the cellular water potential; (2) ion stress resulting from the uptake or loss of ions; and (3) change of the cellular ionic ratios because of the selective ion permeability of the membrane. Salinity exerts profound effects on the growth of algae by influencing the osmotic pressure, nutrient absorption, and suspension of algae [39]. The growth at low salinity levels may be controlled by the availability of certain ions [40]. At high ion concentrations, the decline in water potential can affect metabolism [41]. In addition, energy requirements of osmoregulation can lead to reduced availability of energy and metabolites for cell growth [41]. These factors might contribute to the decline in growth at extreme salinity levels [41].

3.2. Empirical Modelling of Diatom Growth Considering Temperature and Salinity

Based on the value of AIC, various models were considered the best to describe the growth of various strains at different temperature–salinity conditions (Table 1).

3.2.1. *Cymbella cf. incurvata*

Non-interactive multiplicative Equation (ST13) was the best for describing the growth of *C. incurvata* at various temperatures and conductivity levels ($R^2 = 0.97$; AIC = −91.06; Table S4; Figure 1A):

$$\mu = \left(c \times e^{d \times T} \times \left(1 - \left(\frac{T - z}{w/2} \right)^2 \right) \right) \times \left(1 - \left(\frac{S - S_{opt}}{S_{max} - S_{opt}} \right)^2 \right) \quad (\text{ST13})$$

where z (°C) is the optimum temperature of the quadratic portion; when $d = 0$, this value is identical to the optimum temperature of the whole curve (i.e., the growth reaches the maximum rate and the thermal response is symmetric); w (°C) is the thermal breadth (i.e., the range over which diatoms grow); S_{opt} (mS/cm) is the optimum conductivity; and S_{max} (mS/cm) is the maximum conductivity above which the growth ceases. The second and third best models, although quite distant in terms of AIC (Equation (ST12), AIC = −77.93; Equation (ST8), AIC = −69.45), were also non-interactive ones, speaking for a relatively robust conclusion that interactive effects are not necessary to explain the observed pattern in salinity- and temperature-dependent growth of this strain.

Table 1. Best models selected for the investigated freshwater diatoms based on AIC.

Strain	Best Model	Symbol (Unit)	Model Parameter	AIC
<i>Cymbella cf. incurvata</i>	$\mu = \left(c \times e^{d \times T} \times \left(1 - \left(\frac{T-z}{w/2} \right)^2 \right) \right) \times \left(1 - \left(\frac{S-S_{opt}}{S_{max}-S_{opt}} \right)^2 \right)$	z (°C) w (°C) S_{opt} (mS/cm) S_{max} (mS/cm)	Optimum temperature of the quadratic portion; when $d = 0$, this value is identical to the optimum temperature of the whole curve (i.e., growth reaches the maximum rate, and the thermal response is symmetric) the thermal breadth (i.e., the range over which diatoms grow) Optimum conductivity Maximum conductivity above which growth ceases	−91.06
<i>Nitzschia linearis</i>	$\mu = \mu_{max} \times \left(1 - \left(\frac{T-T_{opt}}{w \times (1+a_{ST} \times S)/2} \right)^2 \right) \times e^{-k_S \times (S-S_{opt})^2}$	μ_{max} (1/d) w (°C) k_S (cm ² /mS ²)	Maximum growth rate reached at the optimum temperature (T_{opt} ; °C) and the optimum conductivity (S_{opt} ; mS/cm) Thermal breadth that can be affected by salinity depending on the interaction coefficient a_{ST} (cm/mS) Salinity effect factor	−133.49
<i>Cyclotella meneghiniana</i>	$\mu = \mu_{max} \times \left(1 - \left(\frac{T-T_{opt}}{w \times (1+a_{ST} \times S)/2} \right)^2 \right) \times e^{-k_S \times (S-S_{opt})^2}$	See above	See above	−43.90
<i>Melosira varians</i>	$\mu = (\mu_{20} \times \theta^{T-20}) \times \left(1 - \left(\frac{S-S_{opt}}{S_{max} \times (1+a_{ST} \times T) - S_{opt}} \right)^2 \right)$	μ_{20} (1/d) S_{opt} (mS/cm) S_{max} (mS/cm)	Growth rate at 20 °C Optimum conductivity Maximum conductivity above which growth ceases	−51.79
<i>Ulnaria acus</i>	$\mu = \mu_{max} \times e^{-k_T \times (T-T_{opt} \times (1+a_{ST} \times S))^2} \times \left(1 - \left(\frac{S-S_{opt}}{S_{max}-S_{opt}} \right)^2 \right)$	k_T (1/°C ²) S_{opt} (mS/cm) S_{max} (mS/cm) a_{ST} (cm/mS)	Temperature effect factor describing the change in the growth rate with increasing temperature below or above the optimum (T_{opt} ; °C) Optimum conductivity for growth Maximum conductivity for growth Represents the interactive effect	−94.70
<i>Navicula gregaria</i>	$\mu = \mu_{max} \times \begin{cases} e^{-k_{T1} \times (T-T_{opt})^2} & \text{for } T \leq T_{opt} \\ e^{-k_{T2} \times (T-T_{opt})^2} & \text{for } T > T_{opt} \end{cases} \times e^{-k_S \times (S-S_{opt})^2}$	μ_{max} (1/d) is k_{T1} and k_{T2} (1/°C ²) k_S (cm ² /mS ²)	Maximum growth rate obtained at the optimum temperature T_{opt} (°C) and the optimum conductivity S_{opt} (mS/cm) Temperature effect factors at temperature below and above the optimum, respectively Salinity effect factor.	−229.82

This equation could explain the growth rate of *C. incurvata* better than interactive multiplicative equations (the best one is Equation (ST2); $R^2 = 0.88$; AIC = -62.08 ; Figure 1B) and the polynomial equation ($R^2 = 0.82$; AIC = -59.42 ; Figure 1C). Noticeably, estimates of all the parameters in Equation (S13) for *C. incurvata* were statistically significant (Table S4). According to Equation (ST13), the growth of *C. incurvata* followed an asymmetric bell-shaped response. The growth rate of this strain increased with increasing temperature, reaching the highest level at the optimum temperature higher than the optimum level of the quadratic portion ($d > 0$) of $15.5\text{ }^{\circ}\text{C}$ ($14.6\text{--}16.3\text{ }^{\circ}\text{C}$). The thermal breadth of this diatom was estimated as $14.8\text{ }^{\circ}\text{C}$ ($12.7\text{--}16.9\text{ }^{\circ}\text{C}$; Table S4; Figure 1A). The temperature response contributes to the difference between Equations (ST13), i.e., asymmetric, and (ST12), i.e., symmetric. Between them, the asymmetric response (ST13) explained the variation in the growth of *C. incurvata* better than the symmetric one (ST12; Table S4). Also, according to Equation (ST13), the conductivity-growth rate followed a bell-shaped response curve. These responses to temperature and conductivity changes produce an evident peak of the growth rate, as displayed in Figure 1A. However, with an optimum conductivity of 0.63 mS/cm , just slightly above the background conductivity (0.43 mS/cm), the growth of *C. incurvata* was mainly inhibited by salinization at the investigated conditions (Table S4). This explains the relatively good fit of Equations (ST8), (ST9), and (ST10), in which the growth is inhibited by the addition of NaCl at any concentration (Table S4). In addition, based on Equation (ST13), the growth of this diatom strain ceased at conductivity of 4.88 mS/cm ($0.88\text{--}8.87\text{ mS/cm}$; Table S4). According to Equation (ST7), which also yields all significant coefficients, the growth rate of this diatom strain was inhibited by 50% at conductivity of 1.81 mS/cm ($1.75\text{--}1.88\text{ mS/cm}$; Table S4). However, the analysis using polynomial equations yielded a different trend (Table S4). In particular, according to these equations, the growth of *C. incurvata* was significantly affected by temperature, but not by salinity (Table S4; Figure 1C); however, the fit of these models was relatively poor when compared to the above-mentioned top candidates (Table S4).

3.2.2. *Nitzschia linearis*

The bell-shaped response of *N. linearis* as observed from the experiment could be described by various equations (Table S5) with a number of them giving statistically significant estimates, i.e., Equations (ST5), (ST2), (ST14), (ST15), and (S19). Among them, the interactive-multiplicative Equation (ST15) gave the lowest AIC value (Table S5; $R^2 = 0.59$; AIC = -133.49 ; Figure 2B):

$$\mu = \mu_{max} \times \left(1 - \left(\frac{T - T_{opt}}{w \times (1 + a_{ST} \times S)/2} \right)^2 \right) \times e^{-k_S \times (S - S_{opt})^2} \quad (\text{ST15})$$

with μ_{max} (1/d) being the maximum growth rate reached at the optimum temperature (T_{opt} ; $^{\circ}\text{C}$) and the optimum conductivity (S_{opt} ; mS/cm); w ($^{\circ}\text{C}$) being the thermal breadth that can be affected by salinity depending on the interaction coefficient a_{ST} (cm/mS); and k_S (cm^2/mS^2) being the salinity effect factor.

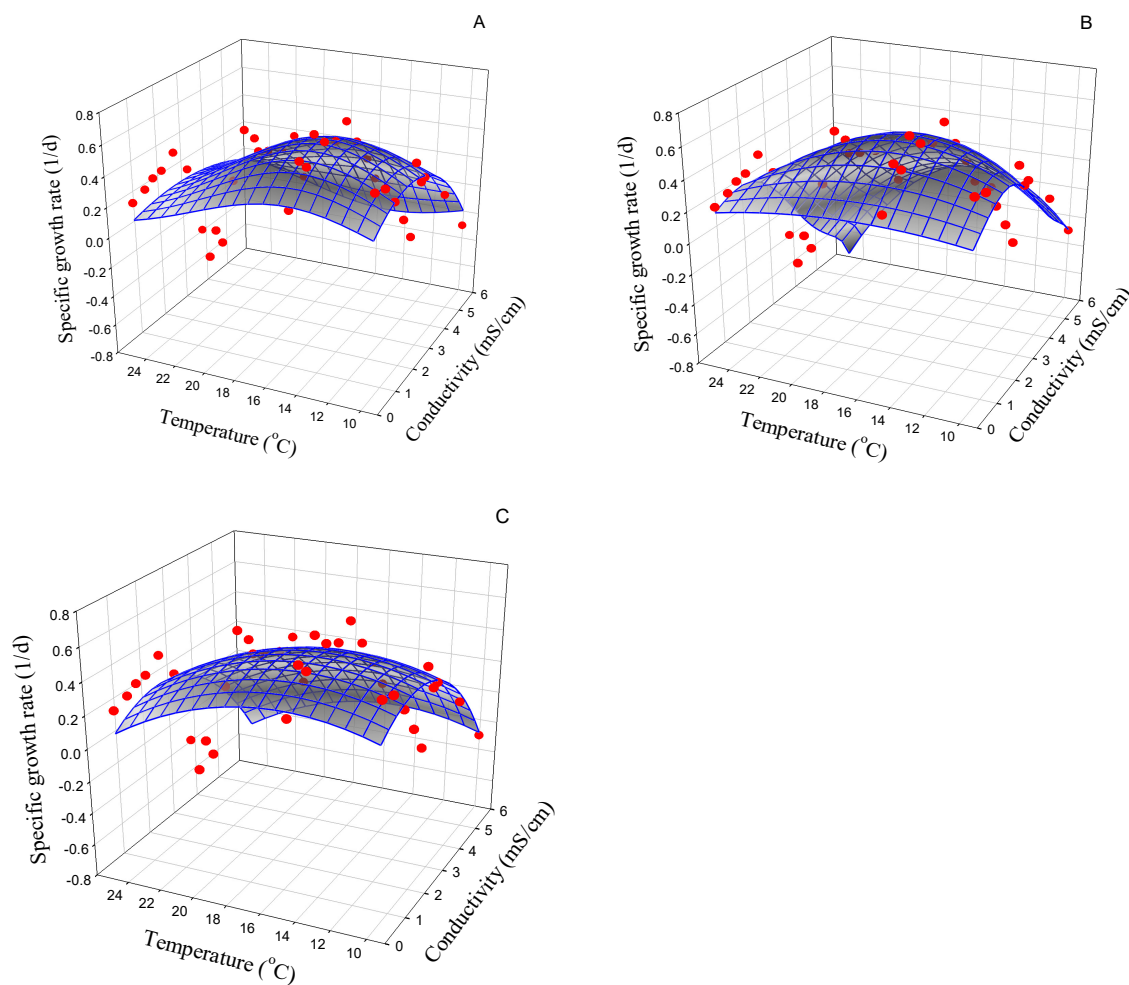


Figure 2. Simulation of the growth of *Nitzschia linearis* based on experimental data according to non-interactive multiplicative Equation (ST14) ((A); $R^2 = 0.53$; $AIC = -130.43$), interactive multiplicative Equation (ST15) ((B); $R^2 = 0.59$; $AIC = -133.49$), and polynomial Equation (4) ((C); $R^2 = 0.51$; $AIC = -128.74$).

Equation (ST15) could describe the variations in the growth of *N. linearis* with varying temperature and conductivity better than non-interactive multiplicative equations (the best of which was Equation (ST14); $R^2 = 0.53$; $AIC = -130.43$; Figure 2A; Table S5) and polynomial equations (Table S5; Figure 2C). Both Equations (ST14) and (ST15) depict a bell-shaped response of growth rate to both temperature and salinity. According to the best Equation (ST15), the thermal breadth for *N. linearis* becomes narrower with increasing salinity (Table S5). Similar interactions were predicted with the second ranking model (Equation (ST17); $AIC = -131.76$). The inclusion of this interactive effect improved the predictive potential. This is shown by the lower AIC of Equation (ST15) compared to Equation (ST5), which uses the same functional forms apart from the interaction (Table S5). With Equation (ST15), the maximum growth rate of 0.59 (1/d) was reached at 15.9 °C (14.7–17.1 °C; Table S5) and 2.44 mS/cm (2.02–2.86 mS/cm; Table S5). The significant influence of temperature and salinity was also found with the non-interactive polynomial equation (Equation (4)) as given in Table S5.

3.2.3. *Cyclotella meneghiniana*

With the limited data from the experiment on the growth of *C. meneghiniana*, high uncertainty is inherent in our empirical modelling (Table S6). No equations with all significant coefficients were found (Table S6). According to polynomial equations (Equations (4) and (5)), the growth rate of *C. meneghiniana* was not significantly affected by temperature or salinity

(Figure 3C; Table S6). This is consistent with the limited variations in the growth rate of this freshwater diatom with temperature and salinity changes described above. However, the optimum temperature and conductivity could be estimated by various multiplicative equations (Table S6). Similar to *N. linearis*, the growth rate of *C. meneghiniana* could be best simulated with interactive multiplicative Equation (ST15) ($R^2 = 0.80$; $AIC = -43.90$; Figure 3B; Table S6). According to this equation, the maximum growth rate of *C. meneghiniana* (0.89; 1/d) was reached at 17.3 °C (13.9–20.8 °C) and 0.69 mS/cm (0.58–0.81 mS/cm). Compared to the estimations for these coefficients, high uncertainty is included in the estimate for the salinity effect factor (k_s) and the thermal breadth (w ; Table S6). Different estimates were obtained by combining the Arrhenius equation for temperature responses with various salinity functions (Table S6). According to the best non-interactive equation (Equation (ST25)) ($R^2 = 0.52$; $AIC = -34.29$; Figure 3A; Table S6), the growth rate of *C. meneghiniana* increased with increasing temperature. An opposite pattern was found with Equation (ST24), which describes the salinity–growth rate with a Michaelis–Menten function (Table S6). Equation (ST24) yielded a maximum conductivity of 1.38 mS/cm (1.06–1.71 mS/cm; Table S6).

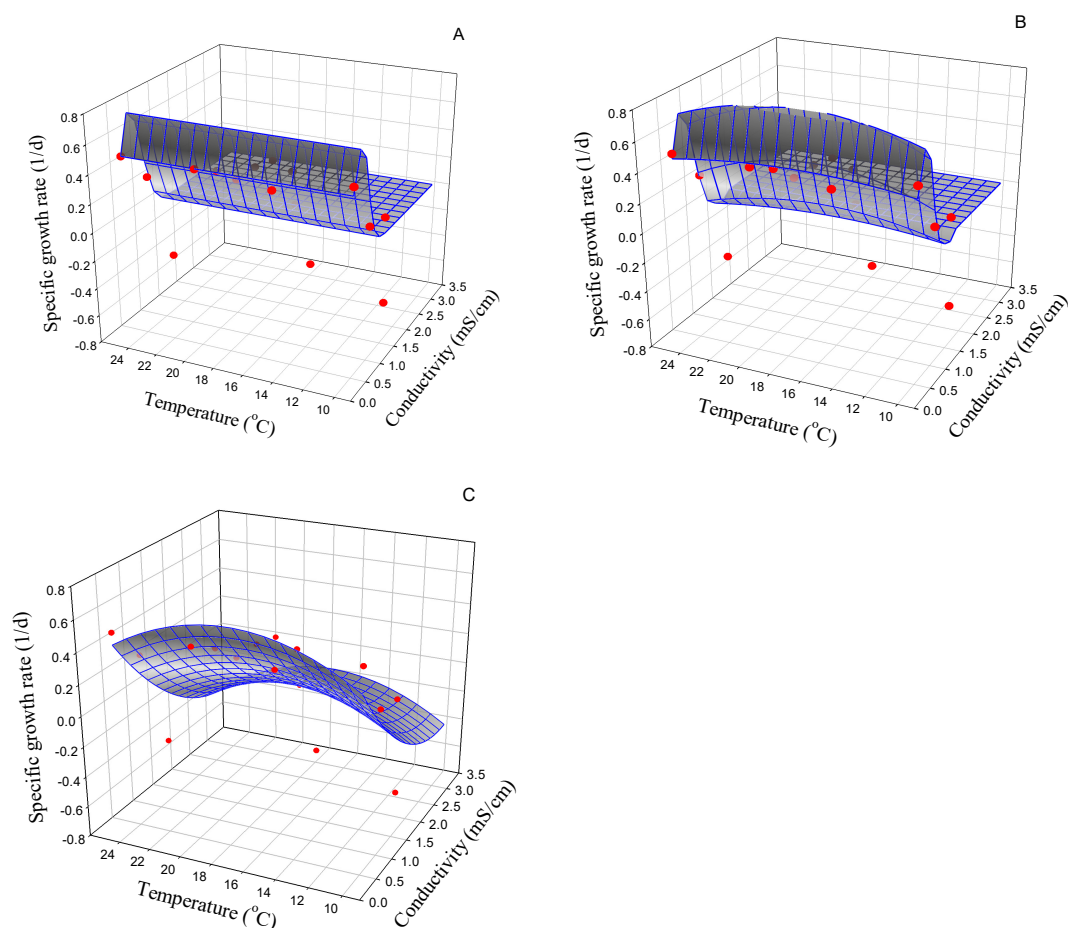


Figure 3. Simulation of the growth of *Cyclotella meneghiniana* based on experimental data according to non-interactive multiplicative Equation (ST25) ((A); $R^2 = 0.52$; $AIC = -34.29$), interactive multiplicative Equation (ST15) ((B); $R^2 = 0.80$; $AIC = -43.90$), and polynomial Equation (4) ((C); $R^2 = 0.58$; $AIC = -34.25$).

3.2.4. *Melosira varians*

The growth of *M. varians* at various temperature and conductivity levels was best simulated with the interactive Equation (ST11) ($R^2 = 0.57$; AIC = -51.79 ; Figure 4B; Table S7):

$$\mu = \left(\mu_{20} \times \theta^{T-20} \right) \times \left(1 - \left(\frac{S - S_{opt}}{S_{max} \times (1 + a_{ST} \times T) - S_{opt}} \right)^2 \right) \quad (\text{ST11})$$

where μ_{20} (1/d) is the growth rate at 20 °C; S_{opt} (mS/cm) is the optimum conductivity; and S_{max} (mS/cm) is the maximum conductivity above which the growth ceases.

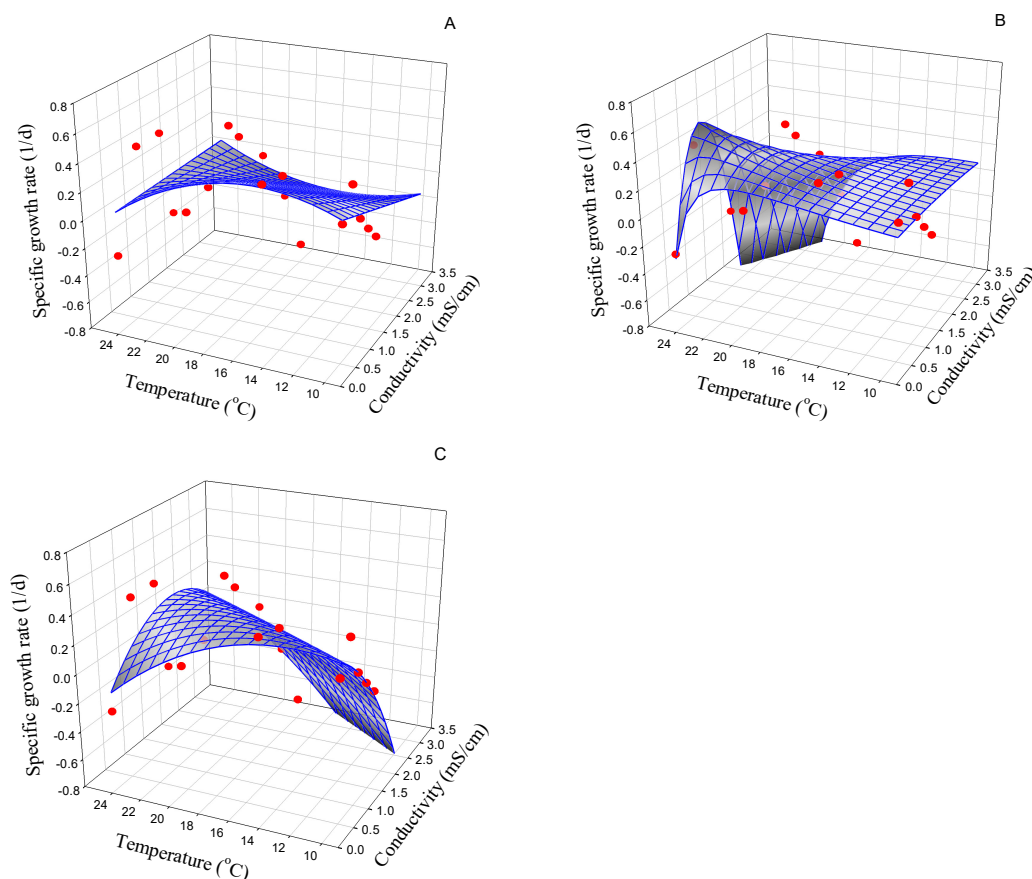


Figure 4. Simulation of the growth of *Melosira varians* based on experimental data according to non-interactive multiplicative Equation (ST9) ((A); $R^2 = 0.21$; AIC = -42.61), interactive multiplicative Equation (ST11) ((B); $R^2 = 0.57$; AIC = -51.79), and polynomial Equation (5) ((C); $R^2 = 0.45$; AIC = -45.37).

Equation (ST11) stood out as it had the lowest AIC and included all significant coefficients (Table S7). According to this equation, the growth rate of *M. varians* exponentially increased with increasing temperature at the investigated range ($\theta = 1.11$; Table S7; Figure 4B). At low salinity levels, the growth rate of *M. varians* increased with increasing conductivity and reached the maximum growth rate at 1.20 mS/cm (1.06–1.34 mS/cm; Table S7). Above this optimum conductivity, the growth rate decreased with increasing conductivity and ceased at the maximum conductivity of 6.41 mS/cm (3.81–8.90 mS/cm; Table S7). Moreover, the maximum conductivity might be lowered by temperature increases. This interaction explains the lower growth rate at 25 °C compared to the rate at lower temperatures at low conductivity (Figure S1D). In other words, the effect of temperature was hidden in the interaction term. Different temperature and salinity responses could be interpreted by the best non-interactive multiplicative equation, which also has all significant coefficients (Equation (ST9); $R^2 = 0.21$; AIC = -42.61 ; Figure 4A; Table S7).

With this equation, the maximum growth rate of *M. varians* (0.44; 1/d) was obtained in the medium without addition of NaCl and at 15.5 °C (9.5–21.5 °C) (Figure 4A). The growth of this diatom was linearly inhibited by NaCl at any concentration. In addition, the growth of *M. varians* ceased at the temperature outside the range of 15.5 ± 9.4 °C (thermal breadth: 18.8 °C; Table S7). In contrast to the influence of temperature and salinity estimated by multiplicative equations, non-significant effects were predicted by polynomial equations (Table S7).

3.2.5. *Ulnaria acus*

With more data on the growth of *U. acus*, more equations could be applied (Table S8). A number of equations yielded statistically significant estimates of all coefficients (Table S8). Among them, Equation (ST35) could describe the growth of this diatom strain best ($R^2 = 0.74$; AIC = −94.70; Figure 5B) with all significant coefficients (Table S8):

$$\mu = \mu_{max} \times e^{-k_T \times (T - T_{opt} \times (1 + a_{ST} \times S))^2} \times \left(1 - \left(\frac{S - S_{opt}}{S_{max} - S_{opt}}\right)^2\right) \quad (\text{ST35})$$

where k_T (1/°C²) is the temperature effect factor describing the change in the growth rate, with increasing temperature below or above the optimum (T_{opt} ; °C); S_{opt} and S_{max} (mS/cm) are the optimum and maximum conductivity for the growth, respectively; and a_{ST} (cm/mS) represents the interactive effect. Also, the second (Equation (ST17), AIC = −90.30) and third (Equation (ST33), AIC = −89.33) ranking model had interactive effects, whilst the best non-interactive model (Equation (ST18)) yielded poor performance (AIC = −88.67; Table S8).

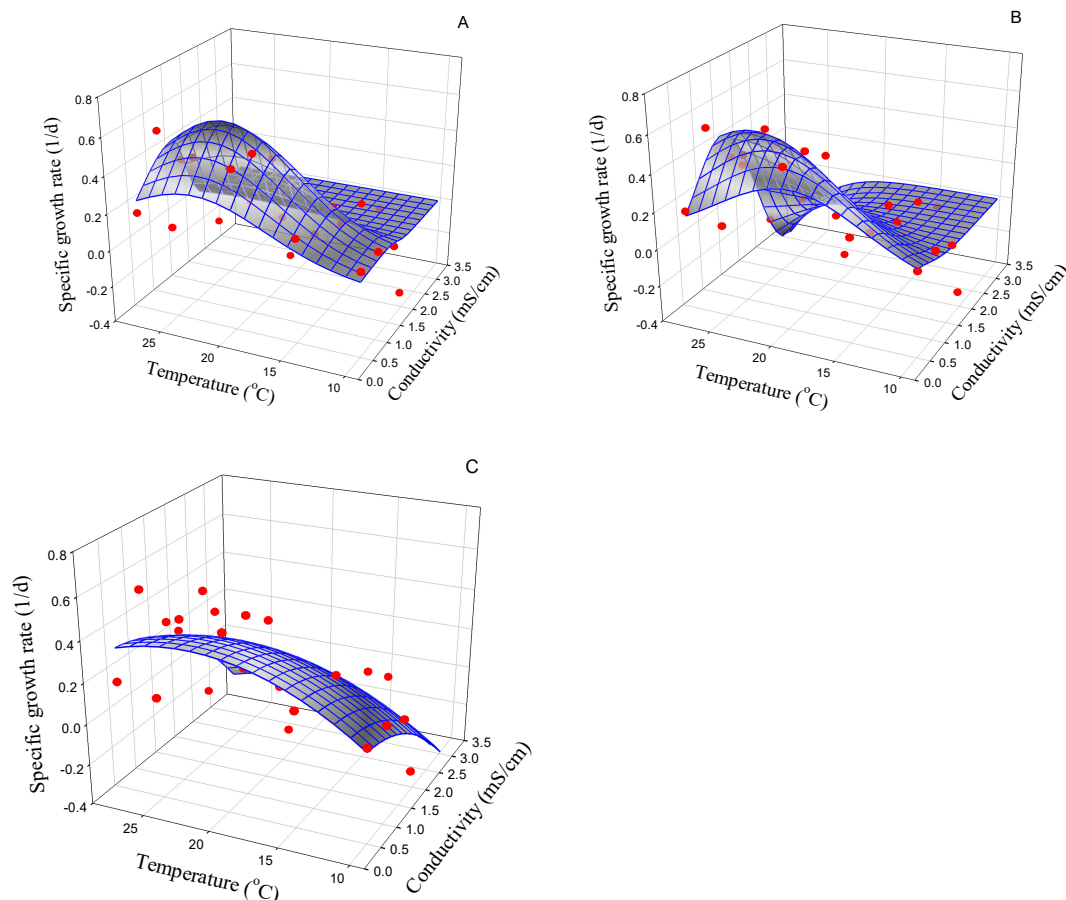


Figure 5. Simulation of the growth of *Ulnaria acus* based on experimental data according to non-interactive multiplicative Equation ST18 ((A); $R^2 = 0.67$; AIC = −88.67), interactive multiplicative Equation ST35 ((B); $R^2 = 0.74$; AIC = −94.70), and polynomial Equation (4) ((C); $R^2 = 0.51$; AIC = −80.87).

According to Equation (ST35), the growth of *U. acus* reached the maximum rate of 0.52 (1/d) at 17.1 °C (12.2–22.0 °C) and 1.08 mS/cm (0.79–1.37 mS/cm) (Figure 6B; Table S8). Remarkably, the optimum of the symmetric temperature–growth curve was swept left by salinization (Table S8). Including such interactive effects improved the predictive potential as shown by better statistical parameters in the estimation with Equation (ST35) (with interactions) compared to Equation (ST34) (without interactions; Table S8). The growth of *U. acus* ceased at conductivity above 2.29 mS/cm (2.02–2.56 mS/cm; Table S8). The response to temperature and salinity in Equation (ST35) could be similarly expressed by Equation (ST33), which yielded a thermal breadth of 28.1 °C (11.7–44.4 °C; Table S8).

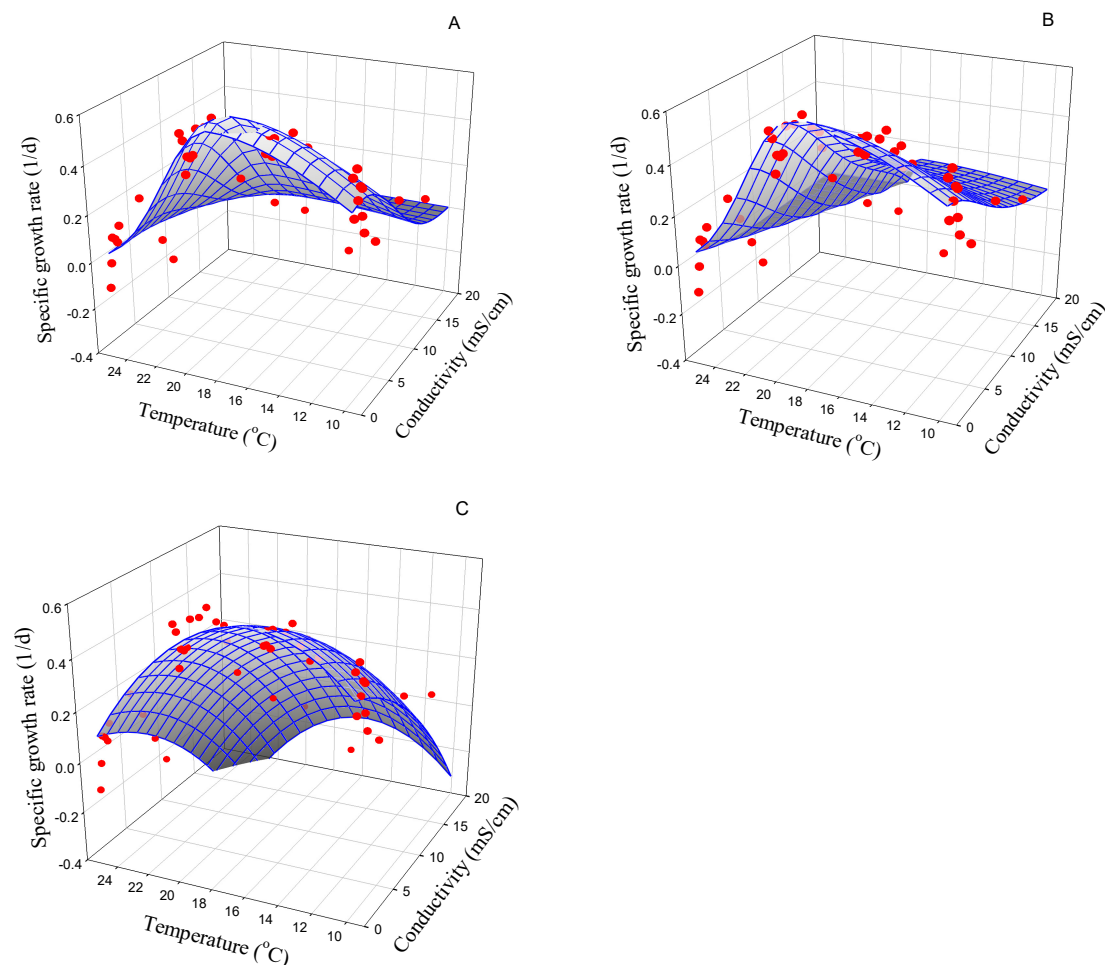


Figure 6. Simulation of the growth of *Navicula gregaria* based on experimental data according to non-interactive multiplicative Equation (ST26) ((A); $R^2 = 0.86$; $AIC = -229.82$), interactive multiplicative Equation (ST52) ((B); $R^2 = 0.83$; $AIC = -219.61$), and polynomial Equation (4) ((C); $R^2 = 0.84$; $AIC = -222.78$).

According to the best non-interactive multiplicative equation (Equation (ST18); $R^2 = 0.67$; $AIC = -88.67$; Table S8; Figure 5A), the conductivity–growth curve exhibits asymmetric responses, i.e., at conductivity above the optimum, the growth rate decreases with a higher slope compared to the change at conductivity below the optimum. Higher uncertainty is included in the estimation with this equation as two effect factors are required to simulate the asymmetric response (Table S8). Statistically significant estimates of all coefficients could be obtained when the number of coefficients was reduced (Equations (ST5), (ST9), (ST10), (ST19), and (ST25); Table S8). However, these equations display different temperature- and conductivity-growth responses. Contrasting with a common bell-shaped response curve (Equations (ST5), (ST10), (ST9), and (ST25)), Equation (ST9) exhibits a

linear inhibition of the growth rate by salinization. Equations (ST10) and (ST25) display an exponential increase in the growth rate with increasing temperature (monotonic response), while the other equations show the opposite trend at temperatures above the optimum (bell-shaped response). In contrast to such influences of temperature and salinity predicted with these equations, the growth rate of *U. acus* was estimated to be not significantly affected based on polynomial equations (Table S8; Figure 5C).

3.2.6. *Navicula gregaria*

Data fitting for the growth of *N. gregaria* with a number of equations yielded statistically significant coefficients, albeit with different interpretations (Table S9). According to Equations (ST5), (ST15), (ST6), (ST9), (ST19), (ST20), and (ST48), this diatom exhibited a symmetric response to temperature changes, contrasting with Equations (ST26) and (ST47). Most of the equations indicate a decrease in the growth rate with decreasing electrical conductivity, while Equations (ST15) and (ST19) represented a bell-shaped response curve. Based on AIC, the growth rate of *N. gregaria* could be best explained by non-interactive Equation (ST26) ($R^2 = 0.86$; AIC = -229.82 ; Figure 6A; Table S9):

$$\mu = \mu_{max} \times \begin{cases} e^{-k_{T1} \times (T - T_{opt})^2} & \text{for } T \leq T_{opt} \\ e^{-k_{T2} \times (T - T_{opt})^2} & \text{for } T > T_{opt} \end{cases} \times e^{-k_S \times (S - S_{opt})^2} \quad (\text{ST36})$$

where μ_{max} (1/d) is the maximum growth rate obtained at the optimum temperature T_{opt} (°C) and the optimum conductivity S_{opt} (mS/cm); k_{T1} and k_{T2} (1/°C²) are the temperature effect factors at temperature below and above the optimum, respectively; and k_S (cm²/mS²) is the salinity effect factor.

According to Equation (ST26), *N. gregaria* responded to temperature changes in an asymmetric norm. In particular, the growth rate decreased more strongly with temperature increases at high temperatures than at low temperatures. This contrasted with the symmetric response to salinity changes. The maximum growth rate (0.6; 1/d) was reached at 18.4 °C (17.3–19.4 °C) and 2.13 mS/cm (0.29–3.96 mS/cm; Table S9). Equation (ST26) could explain the variation in the growth rate of *N. gregaria* better than the interactive multiplicative Equation (ST52) ($R^2 = 0.86$; AIC = -219.61 ; Figure 6B; Table S9) or the non-interactive polynomial Equation (4) ($R^2 = 0.84$; AIC = -222.78 ; Figure 6C; Table S9). Moreover, all the best formulation forms display a non-interactive norm that is consistent with the data fitting with polynomial equations (Table S9).

3.3. Temperature and Salinity Tolerance of Freshwater Diatoms

In the present study, we attempted to estimate the salinity and temperature sensitivity of selected freshwater diatom strains by shock treatments, meaning that the cultures were not acclimated to the treatment conditions before their growth rates were measured. This means that our data might underestimate the niche breadth as well as the severity of non-optimal growth conditions upon growth rates. The lack of a uniform acclimation phase might also contribute to a relatively high noise-to-signal ratio in our growth rate measurements. With these reservations, we summarize the general conclusions from the above observations as relating to possible shapes of the temperature–salinity response surfaces. For further refinement of the modelling approach, pre-acclimated culture experiments will be beneficial.

The temperature tolerance range could be well estimated by empirical modelling described in the previous section for investigated diatom strains, except for *M. varians* and *C. meneghiniana* (Table 2). The growth rate of *M. varians* was expected to increase exponentially with increasing temperature with the best equation, as mentioned above. Our analysis indicates small variations in the optimum temperature among investigated freshwater diatoms (Table 2). In contrast, larger variations were revealed in the thermal breadth (Table 2). Among the diatoms, *C. meneghiniana* had the widest thermal tolerance, followed by *N. linearis* and *U. acus* (Table 2). The variations in the optimum temperature

among the investigated freshwater diatoms (except for *M. varians*) were smaller than those reported in previous studies, probably related to the difference in the isolation approaches. For example, from other previous studies, the optimum temperature range differs among estuarine epipellic diatoms isolated from cohesive sediments sampled from three different sites along the Colne Estuary, UK: 10–20 °C for *Navicula phyllepta*, 10–30 °C for *Navicula perminuta*, and 20–35 °C for *Navicula salinarum* [42]. The diatom *N. gregaria* isolated from the epipelon reached the maximal growth at the lowest temperature (15 °C), compared to *Nitzschia gracilis* and *Nitzschia palea* isolated from the epiphyton, *Navicula minima* v. *atomides* (20 °C) from the episammon, or *Navicula seminulum* (30 °C) from the epipelon [22]. The growth of an estuarine clone of *Nitzschia americana* increased with increasing temperature up to 25 °C, above which the growth rate declined [29].

Table 2. Temperature and salinity tolerance of the freshwater diatoms estimated with the best equation.

Strain	Temperature Response			Salinity Response		
	Optimum Temperature (°C)	Thermal Breadth (°C)	Temperature Tolerance Range (°C)	Optimum Conductivity (mS/cm)	Maximum Conductivity (mS/cm)	Half-Saturation Conductivity (mS/cm)
<i>Cymbella</i> cf. <i>incurvate</i>	15.8 (15.3–16.3)	14.8 (12.7–16.9)	15.5 ± 7.4	0.63 (0.35–0.90)	1.81 (1.75–1.88)	0.81 (0.01–1.61)
<i>Nitzschia linearis</i>	15.9 (14.7–17.1)	31.8 (24.1–39.4)	15.9 ± 15.9	2.44 (2.02–2.87)	5.29 (4.84–5.74)	4.60 (1.17–8.04)
<i>Cyclotella meneghiniana</i>	17.3 (13.9–20.8)	59.6 (0–121.0)	17.3 ± 29.8	0.69 (0.58–0.81)	1.38 (1.06–1.71)	
<i>Melosira varians</i>				1.20 (1.06–1.34)	6.41 (3.81–8.90)	
<i>Ulnaria acus</i>	17.1 (12.2–22.0)	28.1 (11.7–44.4)	17.1 ± 14.1	1.08 (0.79–1.37)	2.29 (2.02–2.56)	
<i>Navicular gregaria</i>	18.4 (17.3–19.4)	19.3 (17.9–20.6)	18.4 ± 9.7	2.12 (0.29–3.96)		9.01 (6.65–11.37)

The optimum and the maximum conductivity could be estimated for all investigated strains, demonstrating *N. linearis*, *M. varians*, and *N. gregaria* as the most tolerant (Table 2). By contrast, *C. incurvate* and *C. meneghiniana* were the most sensitive to salinization with the lowest optimum and maximum conductivity (Table 2). Except for *N. gregaria*, the growth of the investigated freshwater diatoms ceased at electrical conductivity above 8.90 mS/cm (Table 2). Our results reveal the difference in the tolerance to temperature and salinity changes among the investigated freshwater diatoms. As a result of species-specific tolerance to salinity, the species composition and distribution of diatoms has been considered to be indicative of salinity gradients [43–51]. Therefore, an understanding of the tolerance of diatoms to salinity might provide a better understanding on their distribution in nature and facilitate predicting the species succession. Consequently, diatom-based models have been developed to quantitatively reconstruct salinity changes [47,48,52].

Different mathematical formulations of single-stressor reaction norms can be classified into two groups: (1) formulations that describe a monotonic response to increasing stressor intensity; and (2) formulations that exhibit a bell-shaped (unimodal or hormetic) relationship. The bell-shaped curve can be symmetric around the optimum or asymmetric, i.e., the response decreases to the right more steeply than to the left. In other words, at high stressor intensity, small changes in this environmental variable could significantly inhibit the growth of diatoms. The more profound inhibition of diatom growth by changes at high levels compared to low ranges reported previously [32,53] is clear evidence of the asymmetric response of diatoms. In this regard, our data set generated from four to five different temperature treatments is not sufficiently informative to differentiate between the latter two possibilities for temperature reaction norms. Furthermore, interactive effects between temperature and salinity might lead to or change the asymmetric response to one of these two environmental variables, as discussed below. The asymmetric temperature–growth curve for diatoms is also supported by their different responses between the stressor regime and the recovery phase [54]. In terms of responses to salinity changes, we found both

monotonically decreasing as well as bell-shaped curves, indicating that some freshwater diatoms from the sampled habitat perform better under elevated salt concentrations than at very low salt levels. This is not surprising insofar as the locality sampled being affected by elevated salinity levels (conductivity often reaching, sometimes exceeding, 2 mS/cm).

3.4. Ambiguity in Empirically Modelling Interactive Effects under Various Environmental Conditions

3.4.1. Diversity of Interaction Types

Our assessment above indicates ambiguity in evaluating the effects of temperature and salinity on diatom growth by empirical modelling. Further ambiguity is inherent in the assessment of interactions between these two factors. Interactions between temperature and salinity could be interpreted in different ways (Tables S4–S9, Supplementary material). Of the two polynomial equations used, the one without interaction (Equation (4)) better described the growth of *C. incurvata*, *N. linearis*, *C. meneghiniana*, *M. varians*, *U. acus*, and *N. gregaria* than the one with interaction (Equation (5)), based on AIC (Tables S4–S9). Several further possibilities could be conceived to capture possible interactive effects in polynomial models. However, for the forms applied here, we generally conclude that polynomial formulations were never among the best fitting models tested. Beyond polynomials, a number of multiplicative equations indicated significant interactive effects of temperature and salinity (Tables S4–S9). For four of the six tested strains, interactive models were found to be the best based on AIC values (Tables S4–S9). With respect to ecological reaction norms, interactive effects of two stressors are conceivable in several different types, as illustrated in Tables 3 and 4. The two main basic types of interactive effects can be summarized in several ways: one stressor shifting the location of the optimum to another; one stressor changing the slope of the (monotonic or bell-shaped) response curve, on one or both sides of the optimum; and one stressor affecting through a combination of the above mechanisms. Previous studies have also indicated that temperature and salinity might interact with each other, affecting the growth of diatoms [29,55,56]. The interactions revealed in the present study have been reported previously, mostly for marine diatoms. For example, the optimum temperature for the growth of the diatom *N. americana* depended on the salinity level [29]. The tolerance of *Thalassiosira rotula* to low salinity levels increased with temperatures ranging from 0 to 15 °C [57].

Table 3. Illustration of various types of interactive effects visualized by a comparison of changes in the shape of a reaction norm.

Interaction ID	Type of Interaction	Interactive Effects	Simulation
A	Stressor 1—modulated slope of the response to stressor 2	Salinization increased or decreased the slope of the temperature–growth curve, which is accompanied by the narrowed or broadened thermal breadth, respectively. Similar effects can be exerted by temperature increases on the conductivity–growth curve.	

Table 3. Cont.

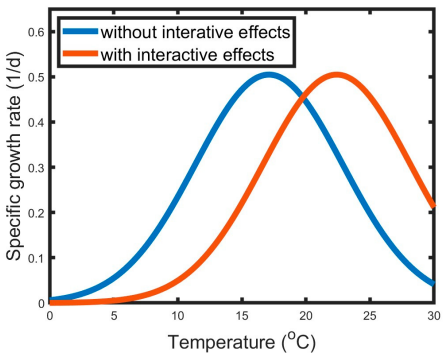
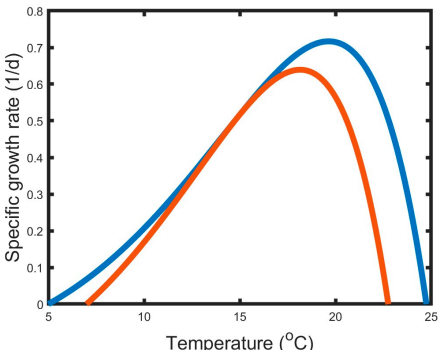
Interaction ID	Type of Interaction	Interactive Effects	Simulation
B	Stressor 1—shifted optimum level of stressor 2	Salinization increases the optimum temperature of the bell-shaped temperature–growth curve	
C	Combination of A and B	The temperature–growth curve is modulated both vertically and horizontally by salinization. Salinization narrows the thermal breadth. Both the optimum temperature and the maximum growth rate are lowered.	

Table 4. Various types of interactions revealed for freshwater diatoms. The best model for each strain with respect to AIC value marked with double asterisk; models within 6 AIC units from the latter (relative likelihood compared to best model < 5%) with a single asterisk.

Equation	Interaction ID	AIC					
		<i>Cymbella incurvata</i>	<i>Nitzschia linearis</i>	<i>Cyclotella meneghiniana</i>	<i>Melosia varians</i>	<i>Ulnaria acus</i>	<i>Navicula gregaria</i>
ST7	A	−52.53		−33.02			
ST15	A		−133.49 **	−43.90 **			−173.43
ST17	A		−131.76 *			−90.30 *	
ST34	A					−88.75 *	
ST11	A	−56.91			−51.79 **		
ST21	A		−120.46	−32.72	−42.93		
ST31	A				−43.25		
ST22	A		−117.83			−78.93	
ST23	A		−118.88			−87.97 *	
ST37	A					−88.43 *	
ST39	A					−88.09 *	
ST27	A				−42.19		
ST29	A				−44.10		
ST28	A				−42.85		
ST30	A				−44.78		
ST32	A				−41.02		
ST36	A					−87.56	
ST38	A					−89.24 *	
ST41	A					−86.34	
ST43	A					−84.32	
ST51	A						−211.67
ST52	A						−219.61 **
ST33	B					−89.72 *	
ST35	B					−94.70 **	
ST2	C	−62.08 **					
ST4	C	−60.63 *					

3.4.2. Ambiguity in Result Interpretation

The most common interactive effect we observed was a change in the slope of the growth curve accompanied by the change in the tolerance breadth (Type A; Tables 3 and 4). Interactive effects frequently increased the slope of the temperature-growth curve and narrowed the thermal breadth (Tables 3 and 4). A narrowing effect of salinization on thermal tolerance was indicated for all investigated diatoms: *C. incurvata* (Equations (ST2), (ST4), and (ST7); Table S4), *N. linearis* (Equations (ST15) and (ST17); Table S5), *C. meneghiniana* (Equations (ST7) and (ST15); Table S6), *U. acus* (Equations (ST17) and (ST34); Table S8), and *N. gregaria* (Equation (ST15); Table S9). However, contrasting patterns could be estimated for the same species. For example, the thermal breadth of *U. acus* could be interpreted to be narrowed (Equations (ST17) and (ST34); Table S8), to be broadened one-sided (Equations (ST23), (ST37), and (ST39); Table S8), or both-sided (Equation (ST22); Table S8) by salinization (Table 4). These effects might change or cause asymmetric temperature-growth curves. An increase in the right-sided slope of the thermal curve (Tables 3 and 4; Equations (ST23), (ST37), and (ST39); Table S8) resulted in asymmetric responses to temperature. This type of effect is more common than the effects on both sides of the curve. Contrasting types of interactions on the temperature-growth curve were also exhibited for *N. linearis* (Table 4). A reduction in an asymmetric thermal breadth could lower not only the maximum growth rate, but also the optimum temperature (Equations (ST2) and (ST4); Tables 3 and 4), as demonstrated for *C. incurvata* (Table S4). By contrast, according to Equation (ST7), another strong candidate model for the same strain, the maximum growth rate of a symmetric response was not affected (Tables 3, 4 and S4).

This type of interactions (A) was also revealed to express the influence of temperature increases on the conductivity-growth curve (Tables 3 and 4). However, such interactions more frequently resulted in decreased slope and broadened breadth of the conductivity-growth curve (Tables 3 and 4). The slope of a linear conductivity-growth relationship for *N. linearis* (Equation (ST21)), *C. meneghiniana* (Equation (ST21)), and *M. varians* (Equations (ST21) and (ST31)) was reduced by increasing temperature (Tables 3 and 4). Similar effects could exert on exponential response curves. For example, the right-sided slope of the asymmetric conductivity-growth curve of *U. acus* (Equations (ST36), (ST38), (ST41), and (ST43); Tables 3, 4 and S8) was decreased by temperature increases. Similar interactions might also affect the symmetric conductivity-growth response, leading to an asymmetric pattern. This was shown for *M. varians* (Equations (ST28), (ST30), and (ST32); Tables 3, 4 and S7). Alternatively, both sides of the symmetric response to conductivity changes could be affected as was also displayed for *M. varians* (Equations (ST27) and (ST29); Tables 3, 4 and S7). However, this type of interaction is less common than the effects on the right side of the exponential response curve (Table 3). Only for *C. incurvata* was an increase in temperature expected to reduce the maximum conductivity (Equation (ST11); Tables 3, 4 and S4).

Compared to such common interactions, the change in the location of the optimum of the response curve was expected for two strains only. The optimum temperature of *U. acus* could be shifted to the right due to salinization (type B; Equations (ST33) and (ST34); Tables 3, 4 and S8). Its optimum temperature was shifted to the right by salinization (Tables 3 and 4). In addition, the temperature-growth curve for *C. incurvata* might be affected by a combination of the above interactions (type C; Equations (ST2) and (ST4); Tables 3, 4 and S4). Accordingly, the temperature-growth curve could be altered horizontally and vertically by salinization. Both the thermal breadth and the optimum temperature could be lowered by increasing conductivity.

The interpretation in the present study is solid evidence of the diversity in the interaction types that can be deduced from empirical modelling. The question is which one reflects the nature of the interactions. With our limited physiological understanding of the interactions between various environmental variables, statistical parameters such as AIC might be considered. However, the validity of this method is of concern because of the limited variation in the AIC in the empirical modelling with these equations (Table 3). In other words, substantial uncertainties are inherent in the analysis of interaction types with

empirical modelling. Among the five models that were found to be the best at describing the growth of the six investigated strains, interactive factors were included in four (Table 1). This result supports our hypothesis that interactive factors are commonly required to explain the variations in the responses of freshwater diatoms to temperature–salinity changes. Moreover, the simulation of various types of temperature–salinity interactions as given in Table 3 clearly demonstrates that the variability in the interactions could not be interpreted simply by synergism–antagonism dichotomy.

4. Conclusions

The response of organisms to a single environmental variable (stressor) could be expressed by a broad variety of functional forms, based on theoretical, mechanistic, or pragmatic grounds. These forms could be categorized into monotonic, symmetric bell-shaped, and asymmetric bell-shaped. In the context of exposure to binary stressors, stressor 1 might affect the response to stressor 2 by altering: (a) the slope, and consequently the tolerance breadth, of the response curve to stressor 2; (b) the location of the optimum for the response to stressor 2; and (c) both of these factors. Such a diversity of both the response to a single environmental variable and interaction effects leads to a large number of combinatorial possibilities for possible functional forms describing 3D reaction norm surfaces. While the fundamental niche breadth could be well estimated by empirical modelling, capturing interactions between temperature and salinity proved challenging in this study. Presence/absence or type of interaction could not be inferred unambiguously for most strains. Because of our limited understanding of the underlying mechanisms, our interpretation is purely based on statistical parameters, leading to ambiguity in such an assessment. Interactions between environmental variables, such as temperature and salinity, might enlarge the uncertainties in empirical modelling. When contrasting the vast diversity of possible functional forms, empirical data is usually not fully conclusive, raising the question of what approaches can improve the estimation. First, more treatments covering a large number of temperature and salinity levels should be included in the experiment. The present study indicates high uncertainties in empirical modelling with limited data. Second, background knowledge should be considered to pre-select functional forms. And third, the effects of environmental variables on growth could be mechanistically delineated via the effects on the processes that control growth, for example, photosynthesis, respiration, and energy dynamics. A more reliable estimate of algal growth that considers interactions between various environmental variables might improve our estimates of water quality models, food chain models, and biomass growth models for aquatic ecosystems as they are based on the growth rate of primary producers.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/phycolgy3040028/s1>, Figure S1: The salinity response curve describing the specific growth rate (1/d) of *Cymbella cf. incurvata*, *Nitzschia linearis*, *Cyclotella meneghiniana*, *Melosira varians*, *Ulnaria acus*, and *Navicula gregaria* with changing salinity represented by the electrical conductivity (mS/cm) at the temperature range of 10–28 °C. *Cymbella incurvate*: 25 °C not included as the diatom did not grow regardless of the conductivity level; Table S1: Temperature-dependent maximum growth rate [58–67]; Table S2: Temperature factor [25,26,28,32,62,68–83]; Table S3: Salinity factor [26–28,70,72]; Table S4: Results of empirical modelling of the growth rate of *Cymbella cf. incurvate* at various temperatures and electrical conductivities; Table S5: Results of empirical modelling of the growth rate of *Nitzschia linearis* at various temperatures and electrical conductivities; Table S6: Results of empirical modelling of the growth rate of *Cyclotella meneghiniana* at various temperatures and electrical conductivities; Table S7: Results of empirical modelling of the growth rate of *Melosira varians* at various temperatures and electrical conductivities; Table S8: Results of empirical modelling of the growth rate of *Ulnaria acus* at various temperatures and electrical conductivities; Table S9: Results of empirical modelling of the growth rate of *Navicula gregaria* at various temperatures and electrical conductivities.

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curation, A.B., J.K., S.B., B.B.; writing—original draft preparation, T.T.Y.L.; writing—review and editing, T.T.Y.L., L.F., N.A.S.M., S.B. and B.B.; visualization, T.T.Y.L.; supervision, T.T.Y.L., S.B. and B.B.; project administration, T.T.Y.L. and B.B.; funding acquisition, T.T.Y.L. and B.B. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Recipe for the SFM + Si Medium.

Components and Final Concentration in Culture Medium	Stock Solution	Addition Per Litre of Culture Medium
1. HEPES (1.00 mM)	238.10 g/L dH ₂ O	1.0 mL
2. Ca(NO ₃) ₂ × 4 H ₂ O (0.21 mM)	100.00 g/L dH ₂ O	0.5 mL
3. MgSO ₄ × 7 H ₂ O (0.203 mM)	20.00 g/L dH ₂ O	2.5 mL
4. K ₂ HPO ₄ × 3 H ₂ O (13.20 µM)	5.00 g/L dH ₂ O	
+ NaNO ₃ (0.35 mM)	50.00 g/L dH ₂ O	0.6 mL
+ Na ₂ CO ₃ (0.19 mM)	32.00 g/L dH ₂ O	
5. H ₃ BO ₃ (16 µm)	1.00 g/L dH ₂ O	1 mL
6. Vitamin Solution		1 mL
Vitamin B12 (0.15 nM)	0.20 mg/L dH ₂ O	
Biotin (4.10 nM)	1.00 mg/L dH ₂ O	
Thiamine-HCl (0.30 µM)	100.00 mg/L dH ₂ O	
Niacinamide (0.80 nM)	0.10 mg/L dH ₂ O	
pH of the Vitamin Solution should be around pH 7		
7. Trace Metals		1 mL
7.1. Preparation of Trace Metal Solution		
Na ₂ EDTA × 2 H ₂ O: 4.36 g		
FeCl ₃ × 6 H ₂ O: 3.15 g		
Dissolve in 1000 mL dH ₂ O, then add 1 mL of Primary Trace Metals each (see below).		
Primary Trace Metals are stored frozen as 1 mL aliquots.		
7.2. Primary Trace Metals		
7.2.1. K ₂ CrO ₄	0.194 g/100 mL dH ₂ O	
7.2.2. CoCl ₂ × 6 H ₂ O	1.00 g/100 mL dH ₂ O	
7.2.3. CuSO ₄ × 5 H ₂ O	0.25 g/100 mL dH ₂ O	
7.2.4. MnCl ₂ × 4 H ₂ O	18.00 g/100 mL dH ₂ O	
7.2.5. Na ₂ MoO ₄ × 2 H ₂ O	1.89 g/100 mL dH ₂ O	
7.2.6. NiSO ₄ × 6 H ₂ O	0.27 g/100 mL dH ₂ O	
7.2.7. H ₂ SeO ₃	0.13 g /100 mL dH ₂ O	
7.2.8. Na ₃ VO ₄	0.184 g /100 mL dH ₂ O	
7.2.9. ZnSO ₄ × 7 H ₂ O	2.20 g/100 mL dH ₂ O	
8. Na ₂ SiO ₃ × 9 H ₂ O (0.50 mM)—optional	28.42 g/L dH ₂ O	5 mL

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