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Combined effects of temperature, irradiance and pH on *Teleaulax amphioxeia* (Cryptophyceae) physiology and feeding ratio for its predator *Mesodinium rubrum* (Ciliophora)

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Abstract :

The cryptophyte Teleaulax amphioxeia is a source of plastids for the ciliate Mesodinium rubrum and both organisms are members of the trophic chain of several species of Dinophysis. It is important to better understand the ecology of organisms at the first trophic levels before assessing the impact of principal factors of global change on Dinophysis spp. Therefore, combined effects of temperature, irradiance and pH on growth rate, photosynthetic activity and pigment content of a temperate strain of T. amphioxeia were studied using a full factorial design (central composite design 23*) in 17 individually controlled bioreactors. The derived model predicted an optimal growth rate of T. amphioxeia at a light intensity of 400 µmol photons · m-2 · s-1, more acidic pH (7.6) than the current average and a temperature of 17.6°C. An interaction between temperature and irradiance on growth was also found, while pH did not have any significant effect. Subsequently, to investigate potential impacts of prey quality and quantity on the physiology of the predator, M. rubrum was fed two separate prey: predator ratios with cultures of T. amphioxeia previously acclimated at two different light intensities (100 and 400 µmol photons · m-2 · s-1). M. rubrum growth appeared to be significantly dependant on prey quantity while effect of prey quality was not observed. This multi-parametric study indicated a high potential for a significant increase of T. amphioxeia in future climate conditions but to what extent this would lead to increased occurrences of Mesodinium spp. and Dinophysis spp. should be further investigated.

Keywords : Dinophysis, ecophysiology, full factorial design, global change, Mesodinium rubrum, Teleaulax amphioxeia

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Abbreviations: F_v/F_m, maximum quantum yield of the photosystem II; TChl *a*, total chlorophyll a; TCarotenoids, total carotenoids; μmax, maximum growth rate

INTRODUCTION

The cryptophyte *Teleaulax amphioxeia* (Hill 1992) is observed worldwide and has been reported to forms red tides in coastal waters (Yoo et al. 2017). This organism is a prey and a source of plastids for the mixotrophic ciliate *Mesodinium rubrum* (Lohmann 1908, = *Myrionecta rubra;* Jankowski 1976), which is also known to form red-colored blooms in coastal ecosystems (Lindholm 1985). The ingested plastids and nuclei of *T. amphioxeia* are incorporated in *M. rubrum* (Yih et al. 2004, Johnson and Stoecker 2005, Johnson et al. 2007) and remain photosynthetically and transcriptionally active to sustain growth of the ciliate (Johnson et al. 2007, Kim et al. 2017). Cryptophytes, as *T. amphioxeia*, play an important role in ecosystem dynamics as they are a 'common food organism' (Yih et al. 2004) of several protists (Smith and Hansen 2007, Peterson et al. 2013). Interestingly, the mixotrophic and harmful species of the dinoflagellate genus *Dinophysis* (Ehrenberg 1841) exhibit chloroplasts of cryptophyte origin, obtained by ingestion of *M. rubrum* (Park et al. 2006, Wisecaver and Hackett 2010). A both relationship between *T. amphioxeia* and *M. rubrum* and between *M. rubrum* and occurrence of *Dinophysis* spp. has been suggested in natural environments (Herfort et al. 2011, Peterson et al. 2013, Hamilton et al. 2017). The influence of *M. rubrum* concentration on growth (Park et al. 2006, Kim et al. 2008, Nagai et al. 2011, Tong et al. 2011, Hattenrath-Lehmann and Gobler 2015, Smith et al. 2018) and toxin production (Gao et al. 2017) of *Dinophysis* spp. was even observed in lab experiments. *Mesodinium rubrum* growth depends on cryptophytes including *T. amphioxeia* (Yih et al. 2004, Johnson 2011) but also on abiotic factors, such as light (Moeller et al. 2011), pH (Smith and Hansen 2007) or temperature (Basti et al. 2018). However few studies have focused on the physiology of *T. amphioxeia* and its effects on growth and pigment content of *M. rubrum*. Such studies are thus required to improve knowledge on the bottom of the food chain of *Dinophysis* spp., and consequently on the understanding of environmental dynamics of both *M. rubrum* and *Dinophysis* spp. growth and blooms. It is widely recognized that climate change modifies harmful algal bloom duration and frequency (Glibert et al. 2014, Gobler et al. 2017) and in this context, according to Wells et al. (2015), temperature, light and pH appear to be key variables.

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Therefore, we investigated the effects of these three parameters on the ecophysiology of *Teleaulax amphioxeia*. First, a full factorial design (central composite design 2^{3*}) was applied to assess the direct combined effects of temperature, irradiance and pH as well as their interactions on the maximum growth rate, pigment content and maximum quantum yield of the photosystem II (F_v/F_m). The central composite design 2^{3*} required 15 experimental conditions with triplicate cultures for the central condition (Lundstedt et al. 1998). A culture device composed of 17 photo-bioreactors, previously developed by Marchetti et al. (2012), was used to perform the factorial design. This approach minimizes the number of experiments that need to be carried out to assess the effects of parameters on a specific response. Also, this design easily allows for the development of statistical models of the maximum growth rate, pigment quantity and F_v/F_m. Finally, *Mesodinium rubrum* was fed two photo-acclimated cultures (100 and 400 µmol photons · m^{-2} · s^{-1}) of *T. amphioxeia* displaying different pigment contents and at two different prey: predator ratios to study the effect of prey physiology and quantity on the ciliate.

MATERIALS AND METHODS

Full factorial design experiment on Teleaulax amphioxeia

Culture of *Teleaulax amphioxeia*

The cryptophyte *Teleaulax amphioxeia* (AND-A0710) was cultivated in L1 medium without silicate (L1-Si) at salinity 35 (Guillard and Hargraves 1993). Cultures were maintained at 17.8 ± 0.6°C, a light intensity of ~ 100 µmol photons · m^{-2} · s^{-1} provided by cool-white and pink fluorescent tubes (fluora and cool-white fluorescent light, Osram, Munich, Germany) and a 12: 12 h light: dark cycle (Table S1 in the Supporting Information). *Teleaulax amphioxeia* was maintained in a semi-continuous culture (i.e., bi-weekly dilutions), allowing for constant physiological conditions. Cultures were not axenic.

Factorial design

The direct effects, interactions and optima of temperature, irradiance and pH on the maximum growth rate (µmax), the maximum quantum yield of the photosystem II (F_v/F_m) and the pigment content of

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Teleaulax amphioxeia were studied using a central composite design (2^{3*}; Appendix S1 in the Supporting Information). Five levels were used for each factor to estimate the second order quadratic component of the relationship between a factor and the three parameters. After the determination of a central value, limits and axial points (i.e., star points) for each factor (Table 1), the 17 required measurements (i.e., 15 experimental conditions with a triplicate for the central one) were performed thanks to a culture device consisting of 17 photo-bioreactors placed in a software-controlled incubator. Each photo-bioreactors was thermo-regulated by a heater connected to a temperature sensor, light was supplied by a xenon lamp and pH was measured using a pH electrode (Mettler-Toledo®) and controlled by CO_2 injections (Marchetti et al. 2012). As pH was only controlled by injection of CO_2, it was only possible to limit the increase in pH during the light period; overall variations in pH did not exceed the regulated pH by 0.5 unit.

The day of the experiment, the photo-bioreactors were sterilized with a solution of 0.5% of DEPTIL PA 5 (Hypred SAS, Dinard, France) and thoroughly rinsed with culture medium. The photo-bioreactors were thereafter filled with 150 mL of inoculum at a concentration of 3.5×10^5 cells · mL^{-1} and randomly placed in the culture device with a 12:12 h light:dark cycle. A bi-daily sampling of 1 mL of each culture was used for cell counting. During the exponential growth phase, 10 mL of each photo-bioreactor were sampled for pigment analysis and F_v/F_m measurements.

Effect of the prey on Mesodinium rubrum

Photo-acclimation of *Teleaulax amphioxeia*

Semi-continuous cultures in flasks were established in order to acclimate *Teleaulax amphioxeia* to two light conditions (Wood et al. 2005). The low light (LL; 100 µmol photons · m^{-2} · s^{-1}) and high light (HL; 400 µmol photons · m^{-2} · s^{-1}) conditions were chosen based on the results of the factorial design experiment (Appendix S1 and Table S2 in the Supporting Information) to induce contrasting µmax, F_v/F_m and pigment contents. Temperature was set according to the optimal growth rate conditions (i.e., 17.6°C) and pH was uncontrolled as previous experiments indicated that pH did not significantly influence µmax, F_v/F_m and pigment contents. Growth was monitored every day and cultures were diluted every two days by adding fresh L1-Si medium. The maximum growth rate,

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pigment content and F_v/F_m were measured to monitor the acclimation of the cultures (Wood et al. 2005).

Feeding experiment

The ciliate *Mesodinium rubrum* (AND-A0711) was routinely maintained in sterilized sea water in the same conditions as *Teleaulax amphioxeia* (Table S1) and fed three times a week at a ratio of 1: 1 (prey: predator). The ciliates were starved one week before the experiment to reduce the number of plastids. The day of the experiment, 80 mL *M. rubrum* cultures at a concentration of 5×10^3 cells · mL^{-1} were fed *T. amphioxeia* acclimated at LL or HL conditions and at a prey: predator ratio of 1: 1 (low fed LL or HL) or 10: 1 (high fed LL or HL). In addition, three controls were used, one unfed culture of *M. rubrum* and two cultures of *T. amphioxeia* previously acclimated to LL and HL conditions but maintained in sterilized sea water (i.e., without L1-Si medium enrichment) as for *M. rubrum*. All the cultures were placed in a culture chamber at a temperature of 17.6°C and an irradiance of 100 µmol photons · m^{-2} · s^{-1} (i.e., corresponding to the LL condition). The monitoring of cell growth, pigment content and F_v/F_m was performed during the exponential growth phase of *M. rubrum* and for the control cultures.

Experimental set-up

Counting and growth rate

Counting of *Teleaulax amphioxeia* during the factorial design experiment was directly performed on fresh samples by flow cytometry on a Accuri C6 flow cytometer (Becton Dickinson Accuri™) equipped with blue and red lasers (488 and 640 nm), detectors of forward (FSC) and side (SSC) light scatter, and fluorescence detectors: 585 ± 20 nm (FL2 and 675 ± 12.5 nm (FL4). FL2 vs FL4 channel density plots, corresponding to phycoerythrin and chlorophyll *a*, were used to count *T. amphioxeia*, using Accuri™ C6 software. Counting during the semi-continuous experiment and the feeding experiment were performed on a particle counter equipped with a 100 µm aperture tube (Multisizer 3, Coulter Counter, Beckman, Paris, France).

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The maximum growth rates were calculated from the slope of the linear regression for the natural logarithm-transformed values of population size during the time interval of exponential growth phase (i.e., ranging from 2 to 4 days for both species; Guillard 1973).

The maximum quantum yield of the photosystem II ($F_v/F_{m)}$

F_v/F_m is considered to be a proxy of algal health (Woźniak et al. 2002, Kromkamp and Forster 2003, Moeller et al. 2011) and was assessed with the Pulse Amplitude Modulation (PAM) method (Schreiber et al. 1986) in a Phyto-PAM (Walz GmbH, Effeltrich, Germany).

Pigment analysis

Pigment concentrations were measured by filtering 3 mL of cultures onto 25 mm Whatman GF/F filters (Whatman, Sigma-Aldrich, Maidstone, UK). Filters were immediately frozen in liquid nitrogen and stored in the dark at -80°C (Zapata et al. 2000). The analysis of pigments was performed by using HPLC with UV or fluorescence detection as previously described by Ras et al. (2008). Total chlorophyll *a* (TChl *a*; sum of chlorophyll *a* and chlorophyllid *a*), chlorophyll *c* (Chl *c*) and total carotenoids (TCarotenoids; sum of alloxanthin, crocoxanthin and α-carotene) were expressed on a per cell basis (pg · cell^{-1}) of *Teleaulax amphioxeia* or *Mesodinium rubrum*. The hydrosoluble phycoerythrin, which is a typical pigment of Cryptophyceae (Jeffrey et al. 2011), was not measured in this work.

Statistical analyses

Statgraphics v 18.1.02 was used to analyze the full factorial design experiment and statistical analyses were computed on RStudio v 1.1.463. After checking the assumptions of independence (Durbin-Watson test), homoscedasticity (Bartlett test) and normality (Shapiro-Wilk test) of the residuals, direct effects of temperature, irradiance and pH and their interactions were investigated using two-way ANOVA for the factorial design experiment. For the other experiments, t-test or one-way ANOVA followed by a Tukey post hoc test were performed. Otherwise Mann-Withney U or Kruskal-Wallis

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tests were used, followed by a Conover test. Differences were considered statistically significant when $P < 0.05$, for a significance level of $\alpha = 0.05$. Values are expressed as mean ± SD. Experiments were performed in triplicate.

RESULTS

Direct effects, interactions and optimum of temperature, irradiance and pH on the physiology of Teleaulax amphioxeia

Effect on the maximum growth rate

According to the 2^{3*} experimental design, the model of µmax (µmax $_{th}$) explained 90% of the observed variability (regression coefficients and equation of the model for µmax $_{th}$ are shown Appendix S1 and Table S2). Both significant linear and quadratic effects of temperature (one-way ANOVA, $F_{2,14} = 9.51$, $P = 0.02$) and irradiance (one-way ANOVA, $F_{2,14} = 7.31$, $P = 0.03$) were observed on µmax (Fig. 1A) while pH was not significant across the experimental domain (Fig. 1, A and B). In addition, a significant interaction (two-way ANOVA, $F_{2,14} = 10.93$, $P = 0.01$) between temperature and irradiance (Fig. 1C) was noted, with a positive effect of temperature on µmax at low irradiance and the opposite effect under high irradiance. The predicted value of µmax for *Teleaulax amphioxeia* was 0.88 · d^{-1}, obtained for a temperature of 17.6°C, a pH of 7.6 and an irradiance of 400 µmol photons · m^{-2} · s^{-1} under a circadian cycle 12:12 h light:dark (Fig. 1D). After the experiment, µmax $_{th}$ was checked under the predicted optimal conditions using three photo-bioreactor replicates in the same culture device and the µmax obtained was in very good agreement with the predicted growth rate (0.873 ± 0.003 · d^{-1}).

Effect on the F_v/F_m and the pigment content

Models of maximum quantum yield of the photosystem II ($F_v/F_{m\ th}$), total chlorophyll *a* (TChl *a* $_{th}$), chlorophyll *c* (Chl *c* $_{th}$) and total carotenoids (TCarotenoids $_{th}$) explained 98%, 69%, 69% and 67% of the observed variability, respectively. Briefly, across the experimental domain $F_v/F_{m\ th}$ was influenced by the same factors as µmax (i.e., optimal $F_v/F_{m\ th}$ at intermediate temperature and irradiance) whereas optimum of TChl *a* $_{th}$ and Chl *c* $_{th}$ were obtained at low irradiance (Fig. S1 in the Supporting Information). We further checked independently and in triplicate the accuracy of predicted modeled

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values using the conditions corresponding to μmax $_{th}$. The measured values were in good agreement with predicted ones for $F_v/F_{m\,th}$, TChl *a* $_{th}$ and Chl *c* $_{th}$ but not for TCarotenoids $_{th}$. Regression coefficients and equation of the models for $F_v/F_{m\,th}$, TChl *a* $_{th}$ and Chl *c* $_{th}$ were shown in Appendix S1 and Table S2.

Light acclimation of Teleaulax amphioxeia

After 27-30 generations, μmax of LL and HL-acclimated *Teleaulax amphioxeia* cultures were stable but significantly higher in HL condition (0.85 ± 0.09 vs. 0.77 ± 0.10 · d^{-1}, t-test, $t_{14} = 2.30$, $P = 0.03$; Table 2). However, TChl *a* and Chl *c* contents were significantly (t-test, $t_1 = 9.75$ and 9.27 respectively, $P = 0.001$) ca. twice higher in *T. amphioxeia* grown in LL while similar TCarotenoids contents were observed between the two light conditions (Table 2). F_v/F_m were high for both photo-acclimated *T. amphioxeia* (> 0.6) but significantly higher for the LL-acclimated condition (t-test, $t_1 = 4.53$, $P = 0.01$; Table 2). The maximum growth rate, TChl *a*, Chl *c* and F_v/F_m of the HL-acclimated culture of *T. amphioxeia* were close to the modeled values, whereas for the LL-acclimated culture, μmax was 1.75-fold higher and around 2-fold lower TChl *a* and Chl *c* than modeled values (Table 2).

Feeding experiment

The maximum growth rates, maximum cellular concentrations and pigment contents of *Mesodinium rubrum* were not significantly different when using LL or HL-acclimated *Teleaulax amphioxeia* cultures (Table 3). However, these responses depended significantly on the nutrition ratio applied. Indeed, high-fed condition resulted in 1.5-fold higher μmax (t-test, $t_9 = 4.99$, $P = 0.001$), 2.5 times higher maximum cellular concentrations (t-test, $t_9 = 15.09$, $P < 0.001$) and twice as high TChl *a*, Chl *c* and TCarotenoids (t-test, $t_4 = 8.89$, 9.15 and 7.08, respectively, $P < 0.001$; Table 3). The F_v/F_m ranged from 0.68 ± 0.01 to 0.72 ± 0.02 and were not significantly different among the nutrition conditions. The unfed control of *M. rubrum* did not show a positive growth and had a significantly lower maximum cellular concentration F_v/F_m, TChl *a*, Chl *c* and TCarotenoids (one-way ANOVA, $F_{2,11}$= 19.54, 55.18, 65.67, 70.48 and 37.22, respectively, $P < 0.001$, Table 3). The two *T. amphioxeia* controls maintained in sterilized sea water and previously acclimated to LL and HL conditions had similar μmax, F_v/F_m, TChl *a*, Chl *c* and TCarotenoids contents after three days of growth in LL

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condition. When compared to the semi-continuous cultures, the control LL culture of *T. amphioxeia* possessed 1.5 times less TChl *a* (t-test, $t_4 = 9.40$, $P = 0.001$) and the control HL culture had a 1.2-fold lower μmax (t-test, $t_{16} = 3.59$, $P = 0.002$) whereas its F_v/F_m increased (t-test, $t_4 = 5.71$, $P = 0.005$; Tables 2 and 3).

DISCUSSION

Full factorial design experiment on Teleaulax amphioxeia *and photo-acclimation in semi-continuous culture*

The present study first investigated the effect of temperature, irradiance, pH and their interactions on the growth of *Teleaulax amphioxeia* thanks to a factorial design experiment. Beforehand, we tested a wide range of values for each factor (temperature 13-30°C, irradiance 20-800 μmol photons · m^{-2} · s^{-1}, pH 6-10) and tried to determine the factor levels (Table 1) as conditions allowing for growth in order to increase the robustness of obtained models.

Results of the 2^{3*} full factorial design underlined the importance of temperature and irradiance on growth, while pH was not significant on the strain AND-A0710 of *Teleaulax amphioxeia* (direct effects). Interestingly, a significant interaction between the two factors temperature and irradiance was also observed for the maximum growth rate. Generally, the interaction between these two factors results in μmax increasing with temperature under light saturation (Ojala 1993, Edwards et al. 2016, Wirth et al. 2019). However, our results showed that μmax was strongly affected at temperatures higher than the optimal one when irradiance was high. Hence the increase of temperature did not allow the strain to better cope with photoinhibition. The decrease in μmax beyond 400 μmol photons · m^{-2} · s^{-1} may suggest photoinhibition of the photosystem II. It has been shown for microalgae that an increase of temperature can reduce the carboxylase activity of the RuBisCO (i.e., the catalytic enzyme of photosynthesis) while promoting the production of oxygen radicals that lead to oxidation of lipids and reaction centers of photosystem II (Ras et al. 2013, Kale et al. 2017) and ultimately to photoinhibition. We hypothesized that with our conditions of culture and especially because of the wide range of temperatures we applied, the increase of temperature here enhanced the effect of photoinhibition but further work is needed to understand how this interaction impacts *T. amphioxeia*.

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The optimal maximum growth rate for this temperate strain was obtained for intermediate tested irradiance (400 µmol photons · m^{-2} · s^{-1}) and temperature (17.6°C), whereas a deviation towards high temperature and irradiance (>22.6°C and 646 µmol photons · m^{-2} · s^{-1}) led to lower µmax. The maximum growth rate was ca. 0.88 · d^{-1} according to the factorial design and values measured from the independent triplicate verification.

As far as we know, the same strain of *Teleaulax amphioxeia* was used in two other studies with similar culture conditions. In the first one, Rial et al. (2013) found a maximal µmax of 0.98 and 1.6 · d^{-1} at 70 and 200 µmol photons · m^{-2} · s^{-1}, respectively, also suggesting a high impact of light on growth under stress conditions, i.e. without photo-acclimation. In the other study, García-Portela et al. (2018) photo-acclimated the cultures and did not observe a significant difference of µmax between 200 and 650 µmol photons · m^{-2} · s^{-1}. This is similar to what we observed for our photo-acclimated cultures, as µmax at 100 and 400 µmol photons · m^{-2} · s^{-1} were only 10% different (but still significantly different). The differences in µmax between the current study and the previous ones (Rial et al. 2013, García-Portela et al. 2018) are likely explained by a different experimental setup. Nevertheless, our results and those from García-Portela et al. (2018) confirmed the high photo-acclimation ability of *T. amphioxeia*.

Teleaulax amphioxeia can also tolerate or acclimate to other abiotic factors. Indeed, Lee et al. (2019) reported that several strains of *T. amphioxeia* isolated from cold and temperate waters (i.e., 5.4 to 28.9°C) can all be acclimated to the same temperature in the lab (i.e., 20°C). Our results also showed that *T. amphioxeia* was able to grow under stress conditions across all the experimental domain except under high temperature (> 22.6°C), high irradiance (> 646 µmol photons · m^{-2} · s^{-1}) or the combination of both (i.e., n°13, 4, 11 and 16 in Table S3 in the Supporting Information).

In addition, a previous lab study on a Danish strain of *Teleaulax amphioxeia* reported a positive growth at elevated pH 9.4 (Smith and Hansen 2007). Our results suggested a high tolerance to pH of the *T. amphioxeia* strain used, including for values already occasionally found in some coastal waters (e.g., < 8; Feely et al. 2008). According to some predictions, in 2100, average pH in the world ocean would be around 7.7 (Haugan and Drange 1996, Brewer 1997, Orr et al. 2005, Gattuso and Hansson 2011) and would thus not significantly impact the growth of *T. amphioxeia*. Altogether,

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these results suggest an important plasticity of the species which may explain why *T. amphioxeia* is found in diverse ecological niches.

It should be noted that we observed some discrepancies between values predicted by the surface response and values really observed during the factorial design experiment and for the photo-acclimated cultures. For the HL-acclimated condition, the experimental μmax, F_v/F_m and pigment content fitted well with the predicted modeled values. However, for the LL-acclimated condition, observed and predicted values of μmax, TChl *a* and Chl *c* were not in agreement (Table 2). These differences may arise from the fact that this type of factorial design is performed to determine optimal conditions across an experimental domain and not extreme values (Lundstedt et al. 1998). Indeed, while there was almost no difference for the central points between predicted and measured values in the factorial design, we noted for the irradiance star point (Table S3, n°6) a difference of 57%, 5%, 30% and 29% for μmax, F_v/F_m, TChl *a* and Chl *c*, respectively. These levels of difference were reflected in the LL-acclimated culture between predicted and observed values. In addition, experiments were performed in stress condition while the semi-continuous cultures led to acclimation as reflected by the stable μmax, F_v/F_m and pigment contents after more than 27 generations. The presence of bacteria in the cultures of *T. amphioxeia* cannot be excluded. *T. amphioxeia* can feed on bacteria (Yoo et al. 2017), especially in light-limited conditions (Marshall and Laybourn-Parry 2002), thus its mixotrophic ability might also explain the discrepancies observed in LL on growth rate and pigment content

The factorial design experiment helped to better understand the physiological responses of the temperate strain of *T. amphioxeia* which belongs to the *Teleaulax*/*Plagioselmis*/*Geminifera* clade (Hansen et al. 2012). *Teleaulax amphioxeia* is an important donor of plastids to *Mesodinium rubrum* (Peterson et al. 2013, García-Portela et al. 2018, Hernández-Urcera et al. 2018, Johnson et al. 2018) and thus indirectly to *Dinophysis* spp. (Park et al. 2006). Low light conditions (< 100 μmol photons · m^{-2} · s^{-1}) coupled with intermediate tested temperature resulted in limited growth of *T. amphioxeia* (while maintaining high pigment content and F_v/F_m) and helped prevent the organism "outgrowing" *M. rubrum* and *Dinophysis* spp. in routine laboratory cultures. Furthermore, controlling the pH of *T. amphioxeia* cultures may be not necessary as this species appears to tolerate pH from 6.0 (this study, data not shown) up to 9.4 (Smith and Hansen 2007). Thanks to the factorial design experiment, the

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present study determined the optimal conditions to obtain a large biomass that may be useful to exploit the beneficial role of *T. amphioxeia* as a food enrichment (Peltomaa et al. 2018 and Lee et al. 2019). This approach also provided information on factors driving the bloom initiation of *T. amphioxeia*. In natural environments, *T. amphioxeia* has been found in higher concentration several meters below the surface (Peterson et al. 2013), indicating that the species easily moves in the water column. This field observation coincides with our experimental observation that high light intensity diminishes μmax. We thus hypothesized that *T. amphioxeia* can perform photosynthesis at a water depth of several meters and can thus escape conditions of higher light intensity at surface. Nonetheless, the intraspecific variability of the species should be explored, including strains from polar and temperate regions.

Feeding experiment of Mesodinium rubrum

Mesodinium rubrum is a mixotrophic organism which acquires and maintains photosynthesis by ingestion of cryptophyte plastids (Gustafson et al. 2000, Yih et al. 2004, Johnson et al. 2006) and cryptophyte nuclei (Johnson et al. 2007, Kim et al. 2017). As the contribution of carbon fixation through photosynthesis appears higher than the contribution of prey (Moeller et al. 2011), the aim of the nutrition experiment was to assess the effect of the physiology of the prey on its predator *M. rubrum*.

We first photo-acclimated *Teleaulax amphioxeia* in semi-continuous cultures and obtained a LL-acclimated culture with higher pigment content and F_v/F_m compared to the HL-acclimated culture. The pigment profile of photo-acclimated *T. amphioxeia* was similar to the ones observed by Rial et al. (2013) and García-Portela et al. (2018) with the major lipophilic pigments being Chl *a* and alloxanthin. Alloxanthin to TChl *a* ratio was higher in HL-acclimated culture, as already shown for *Rhodomonas salina* (Schlüter et al. 2000), but in our study it was due to a decrease in TChl *a*, thus not supporting the reported photoprotection role of alloxanthin in *T. amphioxeia* (Laviale and Neveux 2011, Roy et al. 2011). Values of F_v/F_m suggested a good cellular health (Moeller et al. 2011) in both LL and HL acclimations, with a significantly higher value in the LL-acclimated compared to the HL-acclimated culture (0.68 ± 0.01 and 0.61 ± 0.02), as already observed by García-Portela (2018) with the same strain of *T. amphioxeia*. Overall, differences obtained in terms of pigment contents and

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F_v/F_m suggested that the LL-acclimated culture of *T. amphioxeia* may be a better quality food source in term of photosynthetic capacity for *Mesodinium rubrum*

However, feeding with prey of different physiology (i.e., LL or HL-acclimated *Teleaulax amphioxeia*) yielded no significant effect on μmax, F_v/F_m and pigment content of *Mesodinium rubrum*. Unfortunately, the control of *T. amphioxeia* acclimated to the HL condition had already converted its pigment content to a content equivalent to the LL condition after 3 days. Therefore the difference of physiological status of the preys was not maintained during all the feeding experiment and to what extent this influenced the results should be further elucidated. Interestingly, this observation highlights another evidence of the plasticity of *T. amphioxeia*, which can easily acclimate to different light conditions.

This study clearly confirmed the positive effect of prey quantity on *Mesodinium rubrum*, i.e., a high feeding ratio 10: 1 (prey: predator) yielded significantly higher μmax, maximum cellular concentrations, F_v/F_m and pigment contents compared to the low feeding ratio 1: 1. Indeed, prey quantity had been shown to be beneficial for *M. rubrum* growth (Peltomaa and Johnson 2017), with a 75% increase in μmax for a feeding ratio of 44: 1 compared to 1: 1. These experimental studies corroborate the occurrence of *Mesodinium* spp. in situ being quantitatively related to the presence of *Teleaulax amphioxeia* (Herfort et al. 2011, Peterson et al. 2013, Hamilton et al. 2017). However, in the environment, different factors may also contribute to bloom development of *Mesodinium* spp. For instance, in the Columbia River estuary, development and structure of *M. rubrum* blooms may also be explained by changes in abiotic factors (e.g., increases of light intensity and dissolved organic compounds, a decrease of turbulence), or biotic factors (e.g., prey preference and availability), or a combination of those factors (Herfort et al. 2011, Peterson et al. 2013). Nonetheless, the impact of nutrient limitations and ratios on growth and photosynthetic activity of *T. amphioxeia* should be further investigated, as they also appeared to drive *M. rubrum* bloom initiation (Peterson et al. 2013, Hamilton et al. 2017) and directly impact growth of *M. rubrum* (Hattenrath-Lehmann and Gobler 2015).

The preponderant effect of feeding ratio was also evident on pigment content, which was twice as concentrated in the high fed *M. rubrum*. Comparing maximal pigment contents between both *M. rubrum* and *T. amphioxeia*, the ciliate had around 8 times more pigments per cell. This observation is

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consistent with the 6 to 36 plastids of *T. amphioxeia* harbored by *M. rubrum* (Hansen and Fenchel 2006). However, there is probably a high intra-specific variability between strains of *M. rubrum* in terms of behavior, size and prey preference, possibly related to different haplotypes (Herfort et al. 2011), thus extrapolation of results should be done with care.

CONCLUSIONS

This study shows the impact of two key variables of global change (temperature and irradiance) on the physiology of the cryptophyte *Teleaulax amphioxeia*, which is one of the first level organism of the trophic chain of *Dinophysis* spp. Also, importantly, pH appeared to not impact on growth of at least the strain in this study. While a slight increase of irradiance and temperature would lead to an increased concentration of *T. amphioxeia*, a negative interaction was observed for high temperature combined with high irradiance. It is not evident whether such a condition is of high environmental relevance for an organism which has been observed to occur at several meter water depth. This study suggests that future climate conditions appear not detrimental to *T. amphioxeia*. An increase of *T. amphioxeia* abundance would favor *Mesodinium rubrum* growth and pigment content, which in turn might lead to increased occurrence of *Dinophysis* spp.

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Conflict of interest

Authors declare no conflicts of interest.

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Figure 1: (A) Standard Pareto chart the model of the maximum growth rate. Linear and quadratic effects of factors on growth are represented by single or double parameters, respectively. (B) Direct effect of T, pH and I on growth rate of *Teleaulax amphioxeia*. (C) Interaction plots of growth rate; + and - correspond to the maximum and minimum values of the second factor. (D) Surface plot of the modeled growth rate. T = temperature (°C) and I = irradiance (µmol photons · m^{-2} · s^{-1}). Significant effects are marked with an asterisk

Table S1: Culture conditions of strains used in this study. [a] Cultures were subjected to light in the PAR domain during a circadian cycle 12:12 h (light: dark)

Table S2: Regression coefficients for the models of maximum growth rate (μmax_{th}), maximum quantum yield of the photosystem II ($F_v/F_{m\,th}$), total chlorophyll *a* (TChl a_{th}) and chlorophyll *c* (Chl c_{th}), where β0 is the model error, $_1$ is for temperature, $_2$ for pH and $_3$ for irradiance

Table S3: Maximum growth rate (µmax), maximum quantum yield of the photosystem II (F_v/F_m), total chlorophyll *a* (TChl *a*), chlorophyll *c* (Chl c_{th}) and total carotenoids (TCarotenoids) for the different conditions in the factorial design experiment

Figure S1: Standard Pareto charts for (A) the model of maximum quantum yield of the photosystem II, (B) total chlorophyll *a* and (C) chlorophyll *c*. Linear and quadratic effects of factors on growth are represented by single or double parameters, respectively. T = temperature (°C) and I = irradiance (µmol photons · m^{-2} · s^{-1})

Appendix S1: The general quadratic model fitted to the data for theoretical maximum growth rate (μmax_{th})

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Table 1: Factor levels in the factorial design experiment, where α is the axial distance between star points and the center of the experimental domain

Factors	- α	- 1	0	+ 1	+ α
Temperature (°C)	13.0	15.4	19.0	22.6	25.0
pH	6.5	6.9	7.6	8.3	8.6
Irradiance (µmol photons· m^{-2} · s^{-1})	40	194	420	645	800

Table 2: Comparison between observed values of maximum growth rate (μmax, d^{-1}), maximum quantum yield of the photosystem 2 (F_v/F_m), total chlorophyll *a* (TChl *a*, pg · $cell^{-1}$), chlorophyll *c* (Chl *c*, pg · $cell^{-1}$) and total carotenoids (TCarotenoids, pg · $cell^{-1}$) of *Teleaulax amphioxeia* acclimated to low light (LL) and high light (HL) conditions, and modeled values (marked with $_{th}$) according to the factorial design experiment. Values are expressed as mean ± SD

	Acclimation conditions	
	LL	HL
Generations	27	30
μmax	0.77 ± 0.10	0.85 ± 0.09
μmax_{th}	0.44	0.88
F_v/F_m	0.68 ± 0.01	0.61 ± 0.02
$F_v/F_{m\,th}$	0.71	0.65
TChl *a*	0.41 ± 0.03	0.24 ± 0.01
TChl a_{th}	0.78	0.24
Chl *c*	0.05 ± 0.01	0.03 ± 0.001
Chl c_{th}	0.12	0.03
TCarotenoids	0.13 ± 0.01	0.10 ± 0.004

Table 3: Comparison between maximum growth rate (μmax, d^{-1}), maximum cellular concentration (cells · mL^{-1}), maximum quantum yield of the photosystem 2 (F_v/F_m), chlorophyll *a* total (TChl *a*, pg · $cell^{-1}$), chlorophyll *c* (Chl *c*, pg · $cell^{-1}$) and carotenoids total (TCarotenoids, pg · $cell^{-1}$) of *Mesodinium rubrum* fed at different prey: predator ratios; high fed and low fed of *Teleaulax amphioxeia* itself acclimated to low light (LL) and high light (HL) conditions, and *M. rubrum* and *T. amphioxeia* controls. Values are expressed as ± SD. No significant differences were found when LL and HL feeding were compared two by two among each nutrition ratio

Nutrition conditions	High fed		Low fed		Control *M. rubrum* not fed	Control *T. amphioxeia*	
	LL	HL	LL	HL		LL	HL
μmax	0.31 ± 0.04	0.29 ± 0.03	0.20 ± 0.03	0.20 ± 0.04	-	0.76 ± 0.02	0.70 ± 0.01
Maximum concentration (× 10^3)	19 ± 1.5	18 ± 1.8	7.6 ± 0.78	7.4 ± 0.42	5.8 ± 0.15	452 ± 10.1	407 ± 17.8
F_v/F_m	0.69 ± 0.01	0.72 ± 0.02	0.68 ± 0.01	0.68 ± 0.01	0.58 ± 0.02	0.68 ± 0.02	0.69 ± 0.01
TChl *a*	3.1 ± 0.45	3 ± 0.5	1.4 ± 0.05	1.4 ± 0.08	1.1 ± 1.2	0.26 ± 0.004	0.26 ± 0.003
Chl *c*	0.38 ± 0.05	0.37 ± 0.06	0.16 ± 0.01	0.16 ± 0.02	0.12 ± 0.02	0.04 ± 0.001	0.04 ± 0.001
TCarotenoids	1.1 ± 0.17	1.1 ± 0.18	0.57 ± 0.02	0.58 ± 0.04	0.51 ± 0.10	0.10 ± 0.002	0.10 ± 0.002

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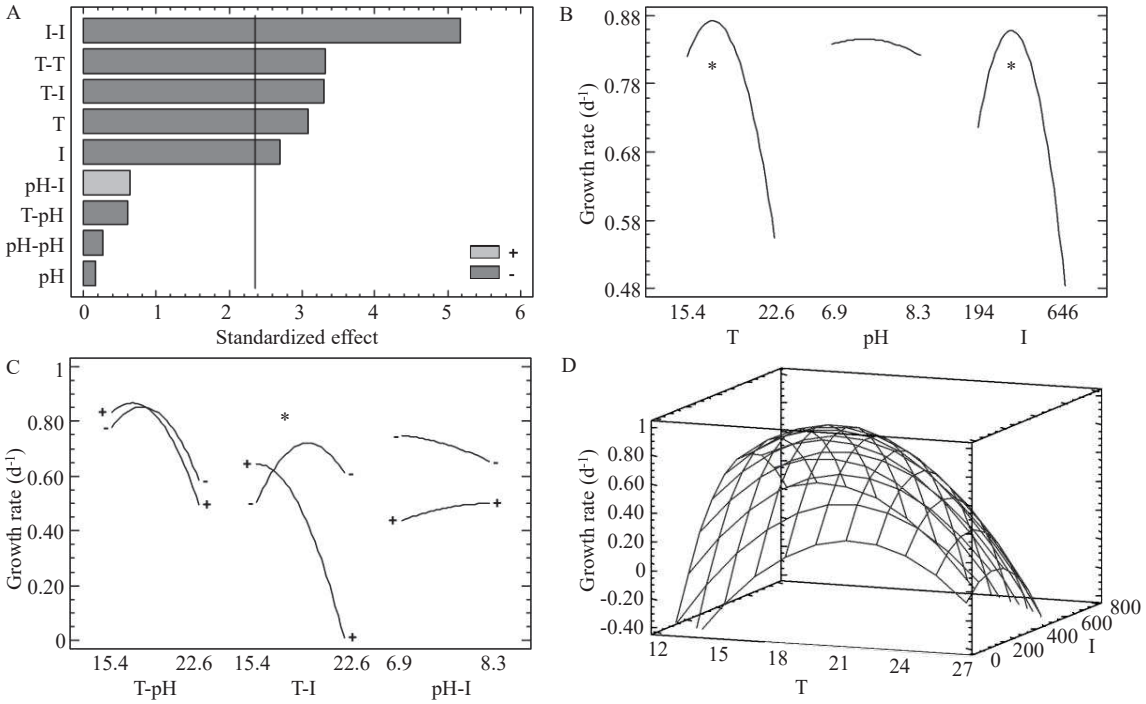

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