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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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1 COMBINED EFFECTS OF TEMPERATURE, IRRADIANCE AND PH ON *TELEAULAX*  
2 *AMPHIOXEIA* (CRYPTOPHYCEAE) PHYSIOLOGY AND FEEDING RATIO FOR ITS  
3 PREDATOR *MESODINIUM RUBRUM* (CILIOPHORA)<sup>1</sup>

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25 Running title

26 Factorial design *Teleaulax* and predator feeding

27

28 Abstract

29 The cryptophyte *Teleaulax amphioxeia* is a source of plastids for the ciliate *Mesodinium rubrum*

30 and both organisms are members of the trophic chain of several species of *Dinophysis*. It is

31 important to better understand the ecology of organisms at the first trophic levels before assessing

32 the impact of principal factors of global change on *Dinophysis* spp.. Therefore, combined effects

33 of temperature, irradiance and pH on growth rate, photosynthetic activity and pigment content of

34 a temperate strain of *T. amphioxeia* were studied using a full factorial design (central composite

35 design 2<sup>3\*</sup>) in 17 individually controlled bioreactors. The derived model predicted an optimal

36 growth rate of *T. amphioxeia* at a light intensity of 400  $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ , more acidic pH

37 (7.6) than the current average and a temperature of 17.6 °C. An interaction between temperature

38 and irradiance on growth was also found, while pH did not have any significant effect.

39 Subsequently, to investigate potential impacts of prey quality and quantity on the physiology of

40 the predator, *M. rubrum* was fed two separate prey: predator ratios with cultures of *T. amphioxeia*

41 previously acclimated at two different light intensities (100 and 400  $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ). *M.*

42 *rubrum* growth appeared to be significantly dependant on prey quantity while effect of prey

43 quality was not observed. This multi-parametric study indicated a high potential for a significant

44 increase of *T. amphioxeia* in future climate conditions but to what extent this would lead to

45 increased occurrences of *Mesodinium* spp. and *Dinophysis* spp. should be further investigated.

46

47

48 Key index words

49 *Dinophysis*; ecophysiology; full factorial design; global change; *Mesodinium rubrum*; *Teleaulax*

50 *amphioxeia*

51

52 Abbreviations

53 Chl *c*, chlorophyll *c*;  $F_v/F_m$ , maximum quantum yield of the photosystem II; HL, high light; L: D,

54 light: dark; LL, low light; TChl *a*, total chlorophyll *a*; TCarotenoids, total carotenoids;  $\mu_{max}$ ,

55 maximum growth rate

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72 Introduction

73 The cryptophyte *Teleaulax amphioxeia* (Conrad) Hill (Hill 1992) is observed worldwide and has  
74 been reported to form red tides in coastal waters (Yoo et al. 2017). This organism is a prey and a  
75 source of plastids for the mixotrophic ciliate *Mesodinium rubrum* (Lohmann 1908, = *Myrionecta*  
76 *rubra* Jankowski 1976), which is also known to form red-colored blooms in coastal ecosystems  
77 (Lindholm 1985). The ingested plastids and nuclei of *T. amphioxeia* are incorporated in *M.*  
78 *rubrum* (Yih et al. 2004, Johnson and Stoecker 2005, Johnson et al. 2007) and remain  
79 photosynthetically and transcriptionally active to sustain growth of the ciliate (Johnson et al.  
80 2007, Kim et al. 2017). Cryptophytes, as *T. amphioxeia*, play an important role in ecosystem  
81 dynamics as they are a ‘common food organism’ (Yih et al. 2004) of several protists (Smith and  
82 Hansen 2007, Peterson et al. 2013). Interestingly, the mixotrophic and harmful species of the  
83 dinoflagellate genus *Dinophysis* (Ehrenberg 1841) exhibit chloroplasts of cryptophyte origin,  
84 obtained by ingestion of *M. rubrum* (Park et al. 2006, Wisecaver and Hackett 2010). A both  
85 relationship between *T. amphioxeia* and *M. rubrum* and between *M. rubrum* and occurrence of  
86 *Dinophysis* spp. has been suggested in natural environments (Herfort et al. 2011, Peterson et al.  
87 2013, Hamilton et al. 2017). The influence of *M. rubrum* concentration on growth (Park et al.  
88 2006, Kim et al. 2008, Nagai et al. 2011, Tong et al. 2011, Hattenrath-Lehmann and Gobler 2015,  
89 Smith et al. 2018) and toxin production (Gao et al. 2017) of *Dinophysis* spp. was even observed  
90 in lab experiments. *M. rubrum* growth depends on cryptophytes including *T. amphioxeia* (Yih et  
91 al. 2004, Johnson 2011) but also on abiotic factors, such as light (Moeller et al. 2011), pH (Smith  
92 and Hansen 2007) or temperature (Basti et al. 2018). However few studies have focused on the  
93 physiology of *T. amphioxeia* and its effects on growth and pigment content of *M. rubrum*. Such  
94 studies are thus required to improve knowledge on the bottom of the food chain of *Dinophysis*  
95 spp., and consequently on the understanding of environmental dynamics of both *M. rubrum* and  
96 *Dinophysis* spp. growth and blooms. It is widely recognized that climate change modifies

97 harmful algal bloom duration and frequency (Glibert et al. 2014, Gobler et al. 2017) and in this  
98 context, according to Wells et al. (2015), temperature, light and pH appear to be key variables.  
99 Therefore, we investigated the effects of these three parameters on the ecophysiology of *T.*  
100 *amphioxeia*. First, a full factorial design (central composite design  $2^{3*}$ ) was applied to assess the  
101 direct combined effects of temperature, irradiance and pH as well as their interactions on the  
102 maximum growth rate, pigment content and maximum quantum yield of the photosystem II  
103 ( $F_v/F_m$ ). The central composite design  $2^{3*}$  required 15 experimental conditions with triplicate  
104 cultures for the central condition (Lundstedt et al. 1998). A culture device composed of 17 photo-  
105 bioreactors, previously developed by Marchetti et al. (2012), was used to perform the factorial  
106 design. This approach minimizes the number of experiments that need to be carried out to assess  
107 the effects of parameters on a specific response. Also, this design easily allows for the  
108 development of statistical models of the maximum growth rate, pigment quantity and  $F_v/F_m$ .  
109 Finally, *M. rubrum* was fed two photo-acclimated cultures (100 and 400  $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ )  
110 of *T. amphioxeia* displaying different pigment contents and at two different prey: predator ratios  
111 to study the effect of prey physiology and quantity on the ciliate.

112

## 113 Materials and methods

### 114 *Full factorial design experiment on T. amphioxeia*

#### 115 Culture of *T. amphioxeia*

116 The cryptophyte *Teleaulax amphioxeia* (AND-A0710) was cultivated in L1 medium without  
117 silicate (L1-Si) at salinity 35 (Guillard and Hargraves 1993). Cultures were maintained at  $17.8 \pm$   
118  $0.6$  °C, a light intensity of  $\sim 100$   $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  provided by cool-white and pink  
119 fluorescent tubes (fluora and cool-white fluorescent light, Osram, Munich, Germany) and a 12:  
120 12 light: dark (L: D) cycle (Table S1). *T. amphioxeia* was maintained in a semi-continuous

121 culture (i.e. bi-weekly dilutions), allowing for constant physiological conditions. Cultures were  
122 not axenic.

123

124 Factorial design

125 The direct effects, interactions and optima of temperature, irradiance and pH on the maximum  
126 growth rate ( $\mu_{\max}$ ), the maximum quantum yield of the photosystem II ( $F_v/F_m$ ) and the pigment  
127 content of *T. amphioxeia* were studied using a central composite design ( $2^{3*}$ , Appendix S1). Five  
128 levels were used for each factor to estimate the second order quadratic component of the  
129 relationship between a factor and the three parameters. After the determination of a central value,  
130 limits and axial points (i.e. star points) for each factor (Table 1), the 17 required measurements  
131 (i.e. 15 experimental conditions with a triplicate for the central one) were performed thanks to a  
132 culture device consisting of 17 photo-bioreactors placed in a software-controlled incubator. Each  
133 photo-bioreactors was thermo-regulated by a heater connected to a temperature sensor, light was  
134 supplied by a xenon lamp and pH was measured using a pH electrode (Mettler-Toledo<sup>®</sup>) and  
135 controlled by CO<sub>2</sub> injections (Marchetti et al. 2012). As pH was only controlled by injection of  
136 CO<sub>2</sub>, it was only possible to limit the increase in pH during the light period; overall variations in  
137 pH did not exceed the regulated pH by 0.5 unit.

138 The day of the experiment, the photo-bioreactors were sterilized with a solution of 0.5% of  
139 DEPTIL PA 5 (Hybred SAS, Dinard, France) and thoroughly rinsed with culture medium. The  
140 photo-bioreactors were thereafter filled with 150 mL of inoculum at a concentration of  $3.5 \times 10^5$   
141 cells  $\cdot$  mL<sup>-1</sup> and randomly placed in the culture device with a 12: 12 (L: D) cycle. A bi-daily  
142 sampling of 1 mL of each culture was used for cell counting. During the exponential growth

143 phase, 10 mL of each photo-bioreactor were sampled for pigment analysis and  $F_v/F_m$   
144 measurements.

#### 145 *Effect of the prey on Mesodinium rubrum*

#### 146 Photo-acclimation of *T. amphioxeia*

147 Semi-continuous cultures in flasks were established in order to acclimate *T. amphioxeia* to two  
148 light conditions (Wood et al. 2005). The low light (LL;  $100 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) and high  
149 light (HL;  $400 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) conditions were chosen based on the results of the  
150 factorial design experiment (Appendix S1 and Table S2) to induce contrasting  $\mu_{\text{max}}$ ,  $F_v/F_m$  and  
151 pigment contents. Temperature was set according to the optimal growth rate conditions (i.e. 17.6  
152 °C) and pH was uncontrolled as previous experiments indicated that pH did not significantly  
153 influence  $\mu_{\text{max}}$ ,  $F_v/F_m$  and pigment contents. Growth was monitored every day and cultures were  
154 diluted every two days by adding fresh L1-Si medium. The maximum growth rate, pigment  
155 content and  $F_v/F_m$  were measured to monitor the acclimation of the cultures (Wood et al. 2005).

156

#### 157 Feeding experiment

158 The ciliate *Mesodinium rubrum* (AND-A0711) was routinely maintained in sterilized sea water in  
159 the same conditions as *T. amphioxeia* (Table S1) and fed three times a week at a ratio of 1: 1  
160 (prey: predator). The ciliates were starved one week before the experiment to reduce the number  
161 of plastids. The day of the experiment, 80 mL *M. rubrum* cultures at a concentration of  $5 \times 10^3$   
162  $\text{cells} \cdot \text{mL}^{-1}$  were fed *T. amphioxeia* acclimated at LL or HL conditions and at a prey: predator  
163 ratio of 1: 1 (low fed LL or HL) or 10: 1 (high fed LL or HL). In addition, three controls were  
164 used, one unfed culture of *M. rubrum* and two cultures of *T. amphioxeia* previously acclimated to  
165 LL and HL conditions but maintained in sterilized sea water (i.e. without L1-Si medium



166 enrichment) as for *M. rubrum*. All the cultures were placed in a culture chamber at a temperature  
167 of 17.6 °C and an irradiance of 100  $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  (i.e. corresponding to the LL  
168 condition). The monitoring of cell growth, pigment content and  $F_v/F_m$  was performed during the  
169 exponential growth phase of *M. rubrum* and for the control cultures.

170

### 171 *Experimental set-up*

#### 172 Counting and growth rate

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

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

184

185 The maximum quantum yield of the photosystem II ( $F_v/F_m$ )

186  $F_v/F_m$  is considered to be a proxy of algal health (Woźniak et al. 2002, Kromkamp and Forster  
187 2003, Moeller et al. 2011) and was assessed with the Pulse Amplitude Modulation (PAM)  
188 method (Schreiber et al. 1986) in a Phyto-PAM (Walz GmbH, Effeltrich, Germany).

189

## 190 Pigment analysis

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

200

## 201 *Statistical analyses*

202 Statgraphics v 18.1.02 was used to analyze the full factorial design experiment and statistical  
203 analyses were computed on RStudio v 1.1.463. After checking the assumptions of independence  
204 (Durbin-Watson test), homoscedasticity (Bartlett test) and normality (Shapiro-Wilk test) of the  
205 residuals, direct effects of temperature, irradiance and pH and their interactions were investigated  
206 using two-way ANOVA for the factorial design experiment. For the other experiments, t-test or  
207 one-way ANOVA followed by a Tukey post hoc test were performed. Otherwise Mann-Whitney  
208 U or Kruskal-Wallis tests were used, followed by a Conover test. Differences were considered  
209 statistically significant when  $P < 0.05$ , for a significance level of  $\alpha = 0.05$ . Values are expressed  
210 as mean  $\pm$  SD. Experiments were performed in triplicate.

211

## 212 Results

213 *Direct effects, interactions and optimum of temperature, irradiance and pH on the physiology of*

214 *T. amphioxeia*

215 Effect on the maximum growth rate

216 According to the 2<sup>3\*</sup> experimental design, the model of  $\mu_{\max}$  ( $\mu_{\max_{th}}$ ) explained 90% of the

217 observed variability (regression coefficients and equation of the model for  $\mu_{\max_{th}}$  are shown

218 AppendixS1 and Table S2). Both significant linear and quadratic effects of temperature (one-way

219 ANOVA,  $F_{2,14} = 9.51$ ,  $P = 0.02$ ) and irradiance (one-way ANOVA,  $F_{2,14} = 7.31$ ,  $P = 0.03$ ) were

220 observed on  $\mu_{\max}$  (Figure 1 A) while pH was not significant across the experimental domain

221 (Figure 1 A-B). In addition, a significant interaction (two-way ANOVA,  $F_{2,14} = 10.93$ ,  $P = 0.01$ )

222 between temperature and irradiance (Figure 1 C) was noted, with a positive effect of temperature

223 on  $\mu_{\max}$  at low irradiance and the opposite effect under high irradiance. The predicted value of

224  $\mu_{\max}$  for *T. amphioxeia* was 0.88 d<sup>-1</sup>, obtained for a temperature of 17.6 °C, a pH of 7.6 and an

225 irradiance of 400  $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  under a circadian cycle 12: 12 (L: D) (Figure 1 D).

226 After the experiment,  $\mu_{\max_{th}}$  was checked under the predicted optimal conditions using three

227 photo-bioreactor replicates in the same culture device and the  $\mu_{\max}$  obtained was in very good

228 agreement with the predicted growth rate ( $0.873 \pm 0.003 \text{ d}^{-1}$ ).

229

230 Effect on the  $F_v/F_m$  and the pigment content

231 Models of maximum quantum yield of the photosystem II ( $F_v/F_{m_{th}}$ ), total chlorophyll *a* (TChl *a*

232 *th*), chlorophyll *c* (Chl *c\_{th}*) and total carotenoids (TCarotenoids *th*) explained 98%, 69%, 69% and

233 67% of the observed variability, respectively. Briefly, across the experimental domain  $F_v/F_{m_{th}}$

234 was influenced by the same factors as  $\mu_{\max}$  (i.e. optimal  $F_v/F_{m\ th}$  at intermediate temperature and  
235 irradiance) whereas optimum of TChl  $a_{\ th}$  and Chl  $c_{\ th}$  were obtained at low irradiance (Figure  
236 S1). We further checked independently and in triplicate the accuracy of predicted modeled values  
237 using the conditions corresponding to  $\mu_{\max\ th}$ . The measured values were in good agreement  
238 with predicted ones for  $F_v/F_{m\ th}$ , TChl  $a_{\ th}$  and Chl  $c_{\ th}$  but not for TCarotenoids  $_{\ th}$ . Regression  
239 coefficients and equation of the models for  $F_v/F_{m\ th}$ , TChl  $a_{\ th}$  and Chl  $c_{\ th}$  were shown in  
240 Appendix S1 and Table S2.

241  
242 *Light acclimation of T. amphioxeia*  
243 After 27-30 generations,  $\mu_{\max}$  of LL and HL-acclimated *T. amphioxeia* cultures were stable but  
244 significantly higher in HL condition ( $0.85 \pm 0.09$  vs.  $0.77 \pm 0.10\ d^{-1}$ , t-test,  $T_{1,14} = 2.30$ ,  $P = 0.03$ ;  
245 Table 2). However, TChl  $a$  and Chl  $c$  contents were significantly (t-test,  $T_{1,1} = 9.75$  and  $9.27$   
246 respectively,  $P = 0.001$ ) ca. twice higher in *T. amphioxeia* grown in LL while similar  
247 TCarotenoids contents were observed between the two light conditions (Table 2).  $F_v/F_m$  were  
248 high for both photo-acclimated *T. amphioxeia* ( $> 0.6$ ) but significantly higher for the LL-  
249 acclimated condition (t-test,  $T_{1,1} = 4.53$ ,  $P = 0.01$ ; Table 2). The maximum growth rate, TChl  $a$ ,  
250 Chl  $c$  and  $F_v/F_m$  of the HL-acclimated culture of *T. amphioxeia* were close to the modeled values,  
251 whereas for the LL-acclimated culture,  $\mu_{\max}$  was 1.75-fold higher and around 2-fold lower TChl  
252  $a$  and Chl  $c$  than modeled values (Table 2).

253  
254 *Feeding experiment*

255 The maximum growth rates, maximum cellular concentrations and pigment contents of *M.*  
256 *rubrum* were not significantly different when using LL or HL-acclimated *T. amphioxeia* cultures

257 (Table 3). However, these responses depended significantly on the nutrition ratio applied. Indeed,  
258 high-fed condition resulted in 1.5-fold higher  $\mu_{\max}$  (t-test,  $T_{1,9} = 4.99$ ,  $P = 0.001$ ), 2.5 times  
259 higher maximum cellular concentrations (t-test,  $T_{1,9} = 15.09$ ,  $P < 0.001$ ) and twice as high TChl  
260 *a*, Chl *c* and TCarotenoids (t-test,  $T_{1,4} = 8.89$ ,  $9.15$  and  $7.08$ , respectively,  $P < 0.001$ ; Table 3).  
261 The  $F_v/F_m$  ranged from  $0.68 \pm 0.01$  to  $0.72 \pm 0.02$  and were not significantly different among the  
262 nutrition conditions. The unfed control of *M. rubrum* did not show a positive growth and had a  
263 significantly lower maximum cellular concentration  $F_v/F_m$ , TChl *a*, Chl *c* and TCarotenoids (one-  
264 way ANOVA,  $F_{2,9} = 19.54$ ,  $55.18$ ,  $65.67$ ,  $70.48$  and  $37.22$ , respectively,  $P < 0.001$ , Table 3). The  
265 two *T. amphioxeia* controls maintained in sterilized sea water and previously acclimated to LL  
266 and HL conditions had similar  $\mu_{\max}$ ,  $F_v/F_m$ , TChl *a*, Chl *c* and TCarotenoids contents after three  
267 days of growth in LL condition. When compared to the semi-continuous cultures, the control LL  
268 culture of *T. amphioxeia* possessed 1.5 times less TChl *a* (t-test,  $T_{1,4} = 9.40$ ,  $P = 0.001$ ) and the  
269 control HL culture had a 1.2-fold lower  $\mu_{\max}$  (t-test,  $T_{1,16} = 3.59$ ,  $P = 0.002$ ) whereas its  $F_v/F_m$   
270 increased (t-test,  $T_{1,4} = 5.71$ ,  $P = 0.005$ ) (Tables 2 and 3).

271

## 272 Discussion

### 273 *Full factorial design experiment on T. amphioxeia and photo-acclimation in semi-continuous* 274 *culture*

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

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

296 The optimal maximum growth rate for this temperate strain was obtained for intermediate tested  
297 irradiance ( $400 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) and temperature ( $17.6 \text{ }^{\circ}\text{C}$ ), whereas a deviation towards  
298 high temperature and irradiance ( $>22.6 \text{ }^{\circ}\text{C}$  and  $646 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) led to lower  $\mu_{\max}$ .  
299 The maximum growth rate was ca.  $0.88 \text{ d}^{-1}$  according to the factorial design and values measured  
300 from the independent triplicate verification.

301 As far as we know, the same strain of *T. amphioxeia* was used in two other studies with similar  
302 culture conditions. In the first one, Rial et al. (2013) found a maximal  $\mu_{\max}$  of 0.98 and  $1.6 \text{ d}^{-1}$  at

303 70 and 200  $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ , respectively, also suggesting a high impact of light on growth  
304 under stress conditions, i.e. without photo-acclimation. In the other study, García-Portela et al.  
305 (2018) photo-acclimated the cultures and did not observe a significant difference of  $\mu_{\text{max}}$  between  
306 200 and 650  $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ . This is similar to what we observed for our photo-acclimated  
307 cultures, as  $\mu_{\text{max}}$  at 100 and 400  $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  were only 10% different (but still  
308 significantly different). The differences in  $\mu_{\text{max}}$  between the current study and the previous ones  
309 (Rial et al. 2013, García-Portela et al. 2018) are likely explained by a different experimental setup.  
310 Nevertheless, our results and those from García-Portela et al. (2018) confirmed the high photo-  
311 acclimation ability of *T. amphioxeia*.

312 *T. amphioxeia* can also tolerate or acclimate to other abiotic factors. Indeed, Lee et al. (2019)  
313 reported that several strains of *T. amphioxeia* isolated from cold and temperate waters (i.e. 5.4 to  
314 28.9 °C) can all be acclimated to the same temperature in the lab (i.e. 20 °C). Our results also  
315 showed that *T. amphioxeia* was able to grow under stress conditions across all the experimental  
316 domain except under high temperature ( $> 22.6$  °C), high irradiance ( $> 646 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ )  
317 <sup>1</sup>) or the combination of both (i.e. n°13, 4, 11 and 16 in Table S3).

318 In addition, a previous lab study on a Danish strain of *T. amphioxeia* reported a positive growth at  
319 elevated pH 9.4 (Smith and Hansen 2007). Our results suggested a high tolerance to pH of the *T.*  
320 *amphioxeia* strain used, including for values already occasionally found in some coastal waters  
321 (e.g.  $< 8$ ) (Feely et al. 2008). According to some predictions, in 2100, average pH in the world  
322 ocean would be around 7.7 (Haugan and Drange 1996, Brewer 1997, Orr et al. 2005, Gattuso and  
323 Hansson 2011) and would thus not significantly impact the growth of *T. amphioxeia*. Altogether,  
324 these results suggest an important plasticity of the species which may explain why *T. amphioxeia*  
325 is found in diverse ecological niches.

326 It should be noted that we observed some discrepancies between values predicted by the surface  
327 response and values really observed during the factorial design experiment and for the photo-  
328 acclimated cultures. For the HL-acclimated condition, the experimental  $\mu_{max}$ ,  $F_v/F_m$  and pigment  
329 content fitted well with the predicted modeled values. However, for the LL-acclimated condition,  
330 observed and predicted values of  $\mu_{max}$ , TChl *a* and Chl *c* were not in agreement (Table 2). These  
331 differences may arise from the fact that this type of factorial design is performed to determine  
332 optimal conditions across an experimental domain and not extreme values (Lundstedt et al.  
333 1998). Indeed, while there was almost no difference for the central points between predicted and  
334 measured values in the factorial design, we noted for the irradiance star point (Table S3 n°6) a  
335 difference of 57%, 5%, 30% and 29% for  $\mu_{max}$ ,  $F_v/F_m$ , TChl *a* and Chl *c*, respectively. These  
336 levels of difference were reflected in the LL-acclimated culture between predicted and observed  
337 values. In addition, experiments were performed in stress condition while the semi-continuous  
338 cultures led to acclimation as reflected by the stable  $\mu_{max}$ ,  $F_v/F_m$  and pigment contents after  
339 more than 27 generations. **The presence of bacteria in the cultures of *T. amphioxeia* cannot be  
340 excluded. *T. amphioxeia* can feed on bacteria (Yoo et al. 2017), especially in light-limited  
341 conditions (Marshall and Laybourn-Parry 2002), thus its mixotrophic ability might also explain  
342 the discrepancies observed in LL on growth rate and pigment content**  
343 The factorial design experiment helped to better understand the physiological responses of the  
344 temperate strain of *T. amphioxeia* which belongs to the *Teleaulax/Plagioselmis/Geminifera* clade  
345 (Hansen et al. 2012). *T. amphioxeia* is an important donor of plastids to *M. rubrum* (Peterson et  
346 al. 2013, García-Portela et al. 2018, Hernández-Urcera et al. 2018, Johnson et al. 2018) and thus  
347 indirectly to *Dinophysis* spp. (Park et al. 2006). Low light conditions ( $< 100 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot$   
348  $\text{s}^{-1}$ ) coupled with intermediate tested temperature resulted in limited growth of *T. amphioxeia*  
349 (while maintaining high pigment content and  $F_v/F_m$ ) and helped prevent the organism



350 “outgrowing” *M. rubrum* and *Dinophysis* spp. in routine laboratory cultures. Furthermore,  
351 controlling the pH of *T. amphioxeia* cultures may be not necessary as this species appears to  
352 tolerate pH from 6.0 (this study, data not shown) up to 9.4 (Smith and Hansen 2007). Thanks to  
353 the factorial design experiment, the present study determined the optimal conditions to obtain a  
354 large biomass that may be useful to exploit the beneficial role of *T. amphioxeia* as a food  
355 enrichment (Peltomaa et al. 2018 and Lee et al. 2019). This approach also provided information  
356 on factors driving the bloom initiation of *T. amphioxeia*. In natural environments, *T. amphioxeia*  
357 has been found in higher concentration several meters below the surface (Peterson et al. 2013),  
358 indicating that the species easily moves in the water column. This field observation coincides  
359 with our experimental observation that high light intensity diminishes  $\mu_{max}$ . We thus  
360 hypothesized that *T. amphioxeia* can perform photosynthesis at a water depth of several meters  
361 and can thus escape conditions of higher light intensity at surface. Nonetheless, the intraspecific  
362 variability of the species should be explored, including strains from polar and temperate regions.

363

#### 364 *Feeding experiment of M. rubrum*

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

371 We first photo-acclimated *T. amphioxeia* in semi-continuous cultures and obtained a LL-  
372 acclimated culture with higher pigment content and  $F_v/F_m$  compared to the HL-acclimated  
373 culture. The pigment profile of photo-acclimated *T. amphioxeia* was similar to the ones observed

374 by Rial et al. (2013) and García-Portela et al. (2018) with the major lipophilic pigments being  
375 Chl *a* and alloxanthin. Alloxanthin to TChl *a* ratio was higher in HL-acclimated culture, as  
376 already shown for *Rhodomonas salina* (Schlüter et al. 2000), but in our study it was due to a  
377 decrease in TChl *a*, thus not supporting the reported photoprotection role of alloxanthin in *T.*  
378 *amphioxeia* (Laviale and Neveux 2011, Roy et al. 2011). Values of  $F_v/F_m$  suggested a good  
379 cellular health (Moeller et al. 2011) in both LL and HL acclimations, with a significantly higher  
380 value in the LL-acclimated compared to the HL-acclimated culture ( $0.68 \pm 0.01$  and  $0.61 \pm 0.02$ ),  
381 as already observed by García-Portela (2018) with the same strain of *T. amphioxeia*. Overall,  
382 differences obtained in terms of pigment contents and  $F_v/F_m$  suggested that the LL-acclimated  
383 culture of *T. amphioxeia* may be a better quality food source in term of photosynthetic capacity  
384 for *M. rubrum*

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

393 This study clearly confirmed the positive effect of prey quantity on *M. rubrum*, i.e. a high feeding  
394 ratio 10: 1 (prey: predator) yielded significantly higher  $\mu_{max}$ , maximum cellular concentrations,  
395  $F_v/F_m$  and pigment contents compared to the low feeding ratio 1: 1. Indeed, prey quantity had  
396 been shown to be beneficial for *M. rubrum* growth (Peltomaa and Johnson (2017), with a 75%  
397 increase in  $\mu_{max}$  for a feeding ratio of 44: 1 compared to 1: 1. These experimental studies

398 corroborate the occurrence of *Mesodinium* spp. *in situ* being quantitatively related to the presence  
399 of *T. amphioxeia* (Herfort et al. 2011, Peterson et al. 2013, Hamilton et al. 2017). However, in the  
400 environment, different factors may also contribute to bloom development of *Mesodinium* spp. For  
401 instance, in the Columbia River estuary, development and structure of *M. rubrum* blooms may  
402 also be explained by changes in abiotic factors (e.g. increases of light intensity and dissolved  
403 organic compounds, a decrease of turbulence), or biotic factors (e.g. prey preference and  
404 availability), or a combination of those factors (Herfort et al. 2011, Peterson et al. 2013).  
405 Nonetheless, the impact of nutrient limitations and ratios on growth and photosynthetic activity  
406 of *T. amphioxeia* should be further investigated, as they also appeared to drive *M. rubrum* bloom  
407 initiation (Peterson et al. 2013, Hamilton et al. 2017) and directly impact growth of *M. rubrum*  
408 (Hattenrath-Lehmann and Gobler 2015).

409 The preponderant effect of feeding ratio was also evident on pigment content, which was twice as  
410 concentrated in the high fed *M. rubrum*. Comparing maximal pigment contents between both *M.*  
411 *rubrum* and *T. amphioxeia*, the ciliate had around 8 times more pigments per cell. This  
412 observation is consistent with the 6 to 36 plastids of *T. amphioxeia* harbored by *M. rubrum*  
413 (Hansen and Fenchel 2006). However, there is probably a high intra-specific variability between  
414 strains of *M. rubrum* in terms of behavior, size and prey preference, possibly related to different  
415 haplotypes (Herfort et al. 2011), thus extrapolation of results should be done with care.

416

## 417 Conclusion

418 This study shows the impact of two key variables of global change (temperature and irradiance)  
419 on the physiology of the cryptophyte *T. amphioxeia*, which is one of the first level organism of  
420 the trophic chain of *Dinophysis* spp.. Also, importantly, pH appeared to not impact on growth of  
421 at least the strain in this study. While a slight increase of irradiance and temperature would lead

422 to an increased concentration of *T. amphioxeia*, a negative interaction was observed for high  
423 temperature combined with high irradiance. It is not evident whether such a condition is of high  
424 environmental relevance for an organism which has been observed to occur at several meter  
425 water depth. This study suggests that future climate conditions appear not detrimental to *T.*  
426 *amphioxeia*. An increase of *T. amphioxeia* abundance would favour *M. rubrum* growth and  
427 pigment content, which in turn might lead to increased occurrence of *Dinophysis* spp..

428

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436

437

#### 438 Conflict of interest

439 Authors declare no conflicts of interest.

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661 Captions

662 Table 1: Factor levels in the factorial design experiment, where  $\alpha$  is the axial distance between star  
663 points and the center of the experimental domain

664  
665 Table 2: Comparison between observed values of maximum growth rate ( $\mu_{max}$ ,  $d^{-1}$ ), maximum  
666 quantum yield of the photosystem 2 ( $F_v/F_m$ ), total chlorophyll *a* (TChl *a*,  $pg \cdot cell^{-1}$ ), chlorophyll *c*  
667 (Chl *c*,  $pg \cdot cell^{-1}$ ) and total carotenoids (TCarotenoids,  $pg \cdot cell^{-1}$ ) of *T. amphioxeia* acclimated to

668 low light (LL) and high light (HL) conditions, and modeled values (marked with  $\theta$ ) according to  
669 the factorial design experiment. Values are expressed as mean  $\pm$  SD

670  
671 Table 3: Comparison between maximum growth rate ( $\mu_{max}$ ,  $d^{-1}$ ), maximum cellular  
672 concentration (cells  $\cdot$  mL $^{-1}$ ), maximum quantum yield of the photosystem 2 ( $F_v/F_m$ ), chlorophyll *a*  
673 total (TChl *a*, pg  $\cdot$  cell $^{-1}$ ), chlorophyll *c* (Chl *c*, pg  $\cdot$  cell $^{-1}$ ) and carotenoids total (TCarotenoids, pg  
674  $\cdot$  cell $^{-1}$ ) of *M. rubrum* fed at different prey: predator ratios; high fed and low fed of *T. amphioxeia*  
675 itself acclimated to low light (LL) and high light (HL) conditions, and *M. rubrum* and *T.*  
676 *amphioxeia* controls. Values are expressed as  $\pm$  SD. No significant differences were found when  
677 LL and HL feeding were compared two by two among each nutrition ratio

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

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686 Table S1: Culture conditions of strains used in this study

687 <sup>a</sup> Cultures were subjected to light in the PAR domain during a circadian cycle 12 h: 12 h (light:  
688 dark)



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690 Table S2: Regression coefficients for the models of maximum growth rate ( $\mu_{\text{max th}}$ ), maximum  
691 quantum yield of the photosystem II ( $F_v/F_{m \text{ th}}$ ), total chlorophyll *a* (TChl *a th*) and chlorophyll *c*  
692 (Chl *c th*), where  $\beta_0$  is the model error,  $\beta_1$  is for temperature,  $\beta_2$  for pH and  $\beta_3$  for irradiance

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694 Table S3: Maximum growth rate ( $\mu_{\text{max}}$ ), maximum quantum yield of the photosystem II ( $F_v/F_m$ ),  
695 total chlorophyll *a* (TChl *a*), chlorophyll *c* (Chl *c th*) and total carotenoids (TCarotenoids) for the  
696 different conditions in the factorial design experiment

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

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703 Appendix S1: The general quadratic model fitted to the data for theoretical maximum growth rate  
704 ( $\mu_{\text{max th}}$ )

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711 Tables & figures

712 Table 1: Factor levels in the factorial design experiment, where  $\alpha$  is the axial distance between star  
713 points and the center of the experimental domain

Factors	- $\alpha$	- 1	0	+ 1	+ $\alpha$
Temperature (°C)	13.0	15.4	19.0	22.6	25.0
pH	6.5	6.9	7.6	8.3	8.6
Irradiance ( $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ )	40	194	420	645	800

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733 Table 2: Comparison between observed values of maximum growth rate ( $\mu_{\max}$ ,  $d^{-1}$ ), maximum  
 734 quantum yield of the photosystem 2 ( $F_v/F_m$ ), total chlorophyll *a* (TChl *a*,  $pg \cdot cell^{-1}$ ), chlorophyll *c*  
 735 (Chl *c*,  $pg \cdot cell^{-1}$ ) and total carotenoids (TCarotenoids,  $pg \cdot cell^{-1}$ ) of *T. amphioxeia* acclimated to  
 736 low light (LL) and high light (HL) conditions, and modeled values (marked with <sub>th</sub>) according to  
 737 the factorial design experiment. Values are expressed as mean  $\pm$  SD

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	Acclimation conditions		
	LL	HL	
Generations	27	30	
$\mu_{\max}$	$0.77 \pm 0.10$	$0.85 \pm 0.09$	
$\mu_{\max_{th}}$	0.44	0.88	
$F_v/F_m$	$0.68 \pm 0.01$	$0.61 \pm 0.02$	
$F_v/F_{m_{th}}$	0.71	0.65	
TChl <i>a</i>	$0.41 \pm 0.03$	$0.24 \pm 0.01$	
TChl <i>a_{th}</i>	0.78	0.24	
Chl <i>c</i>	$0.05 \pm 0.01$	$0.03 \pm 0.001$	
Chl <i>c_{th}</i>	0.12	0.03	
TCarotenoids	$0.13 \pm 0.01$	$0.10 \pm 0.004$	

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

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

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