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Juan Du, Disney Izquierdo, Hai-Feng Xu, Johann Lavaud, Leanne Ohlund,  
Lekha Sleno, Philippe Juneau

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1 Responses to herbicides of Arctic and temperate microalgae grown under  
2 different light intensities

3 Juan Du<sup>a</sup>, Disney Izquierdo<sup>b</sup>, Hai-Feng Xu<sup>c</sup>, Beatrix Beisner<sup>d</sup>, Johann Lavaud<sup>e,f</sup>,  
4 Leanne Ohlund<sup>g</sup>, Lekha Sleno<sup>g</sup> and Philippe Juneau<sup>b\*</sup>

5 <sup>a</sup> Department of Biological Sciences, Université du Québec à Montréal-GRIL-  
6 TOXEN, Succ Centre-Ville, Montréal, Canada

7 <sup>b</sup> Department of Biological Sciences, Université du Québec à Montréal-GRIL-  
8 EcotoQ-TOXEN, Succ Centre-Ville, Montréal, Canada

9 <sup>c</sup> School of Life Sciences, and Hubei Key Laboratory of Genetic Regulation and  
10 Integrative Biology, Central China Normal University, Wuhan 430079, Hubei,  
11 China

12 <sup>d</sup> Department of Biological Sciences, Groupe de recherche interuniversitaire en  
13 limnologie (GRIL), Université du Québec à Montréal

14 <sup>e</sup> TAKUVIK International Research Laboratory IRL3376, Université Laval (Canada) -  
15 CNRS (France), Pavillon Alexandre-Vachon, 1045 av. de la Médecine, local 2064,  
16 G1V 0A6 Québec, Canada

17 <sup>f</sup> LEMAR-Laboratory of Environmental Marine Sciences, UMR6539, CNRS/Univ  
18 Brest/Ifremer/IRD, Institut Universitaire Européen de la Mer, Technopôle Brest-  
19 Iroise, rue Dumont d'Urville, 29280 Plouzané, France

20 <sup>g</sup> Chemistry Department, Université du Québec à Montréal-EcotoQ-TOXEN, Succ  
21 Centre-Ville, Montreal, Quebec, H3C 3P8, Canada

22

23 **\*Corresponding author:**

24 E-mail addresses: [juneau.philippe@uqam.ca](mailto:juneau.philippe@uqam.ca) (P. Juneau)

25 Full postal address:

26 Department of Biological Sciences

27 Université du Québec à Montréal

28 CP8888, Succ Centre-Ville

29 Montréal (QC)

30 H3C 3P8

31 Canada

32

33 **Highlights**

- 34 • Photoadaptation processes are different between Arctic and temperate microalgae
- 35 • Arctic and temperate microalgae have different sensitivities to two herbicides
- 36 • High light enhances the impacts of herbicides on energy dissipation and  
37 photosynthesis of microalgae
- 38 • Atrazine removal by algae increased under high light
- 39 • These findings have applications for Arctic and temperate microalgae living in  
40 fluctuating light environments

41 **Abstract:**

42 In aquatic ecosystems, microalgae are exposed to light fluctuations at different  
43 frequencies due to daily and seasonal changes. Although concentrations of herbicides  
44 are lower in Arctic than in temperate regions, atrazine and simazine, are increasingly  
45 found in northern aquatic systems because of long-distance aerial dispersal of  
46 widespread applications in the south and antifouling biocides used on ships. The toxic  
47 effects of atrazine on temperate microalgae are well documented, but very little is  
48 known about their effects on Arctic marine microalgae in relation to their temperate  
49 counterparts after light adaptation to variable light intensities. We therefore  
50 investigated the impacts of atrazine and simazine on photosynthetic activity, PSII  
51 energy fluxes, pigment content, photoprotective ability (NPQ), and reactive oxygen  
52 species (ROS) content under three light intensities. The goal was to better understand  
53 differences in physiological responses to light fluctuations between Arctic and  
54 temperate microalgae and to determine how these different characteristics affect their  
55 responses to herbicides. The Arctic diatom *Chaetoceros* showed stronger light  
56 adaptation capacity than the Arctic green algae *Micromonas*. Atrazine and simazine  
57 inhibited the growth and photosynthetic electron transport, affected the pigment  
58 content, and disturbed the energy balance between light absorption and utilization. As  
59 a result, during high light adaptation and in the presence of herbicides,  
60 photoprotective pigments were synthesized and NPQ was highly activated.

61 Nevertheless, these protective responses were insufficient to prevent oxidative  
62 damage caused by herbicides in both species from both regions, but at different extent  
63 depending on the species. Our study demonstrates that light is important in regulating  
64 herbicide toxicity in both Arctic and temperate microalgal strains. Moreover, eco-  
65 physiological differences in light responses are likely to support changes in the algal  
66 community, especially as the Arctic ocean becomes more polluted and bright with  
67 continued human impacts.

68

69 **Keywords:**

70 Marine microalgae, Atrazine, Simazine, Light, Photoadaptation, Ecotoxicology.

71 **Abbreviations:**

72 T-CN: Temperate *Chaetoceros neogracile*; T-MB; temperate *Micromonas bravo*; A-  
73 MP: Arctic *Micromonas polaris*; A-CN: Arctic *Chaetoceros neogracilis*; Car:  
74 carotenoids; Chl *a*: chlorophyll *a*; PSI: photosystem I; PSII: photosystem II; NPQ:  
75 non-photochemical quenching; qE: energy-dependent quenching; Q<sub>A</sub>: primary  
76 electron acceptor of PSII; Q<sub>B</sub>: secondary electron acceptor of PSII; RC: reaction  
77 center; Φ<sub>M</sub>: PSII maximum quantum yield; Φ'<sub>M</sub>: PSII operational quantum yield; ROS:  
78 reactive oxygen species; LL: low light intensity 40 μmol photons m<sup>-2</sup> s<sup>-1</sup>, ML:  
79 medium light intensity 100 μmol photons m<sup>-2</sup> s<sup>-1</sup>, HL: high light intensity 400 μmol  
80 photons m<sup>-2</sup> s<sup>-1</sup>.

81

82 **Introduction**

83 Light intensity is one of the most important environmental factors influencing the  
84 growth of photosynthetic organisms (Edwards et al. 2015). In aquatic environments,  
85 microalgae experience intense light fluctuations due to the daily sunlight exposure  
86 and seasonal variation (Wagner et al. 2006). Meanwhile, turbidity in the water and  
87 refraction of sunlight cause light intensity changes at different depths, with photon

88 flux scarcer in the deeper layers of the water column than at the surface (Dubinsky  
89 and Stambler 2009). To cope with the fluctuating light environments, photosynthetic  
90 organisms have evolved diverse phenotypic adjustments including photoadaptation  
91 processes (Deblois et al. 2013a, Handler 2017). Photoadaptation to low or high light  
92 environments involves changes at the gene level leading to modification of their  
93 physiology, biochemistry, and morphology (Bellacicco et al. 2016, Deblois et al.  
94 2013b). Physiological photoprotection mechanisms of photosynthetic organisms  
95 include the PSII repair cycle, changes in pigment, de novo synthesis of proteins, state  
96 transitions, changes in energy efficiency transferred from the light-harvesting  
97 complex to reaction centers (RCs), and non-photochemical quenching (NPQ) induced  
98 by activation of the xanthophyll cycle (XC) (Deblois et al. 2013a, Dong et al. 2016,  
99 Hopes and Mock 2015). Among them, NPQ is the fastest and most flexible response  
100 to light variation (Goss and Lepetit 2015). In diatoms, the XC (de-epoxidation of  
101 diadinoxanthin to diatoxanthin) is activated by the light-driven acidification of the  
102 thylakoid lumen resulting in the accumulation of diatoxanthin. This process is a  
103 prerequisite for the formation of energy-dependent quenching (qE) that is the major  
104 component of NPQ in diatoms (Lepetit et al. 2017), which is not necessarily the case  
105 in plants and green algae (Allorent et al. 2013, Goss and Lepetit 2015).

106 As primary producers, microalgae constitute the basis of aquatic ecological  
107 trophic networks. The diatom *Chaetoceros sp.* and the small flagellate prasinophyte  
108 *Micromonas sp.* are dominant species in both Arctic and temperate regions (Balzano  
109 et al. 2012, Balzano et al. 2017, Seoane et al. 2019). They are thus among the non-  
110 target aquatic organisms exposed to pesticides (Chen et al. 2016). The effect of  
111 pesticides has been predominantly studied in freshwater ecosystems compared to  
112 marine and estuarine ecosystems (Dar et al. 2021, Dupraz et al. 2016). Herbicides are  
113 the most widely used among the major pesticide classes, and their toxic effects mainly  
114 affect the growth, photosynthesis, morphology, biochemical composition, and lipid  
115 content of microalgae (DeLorenzo 2001, Sun et al. 2020). The deleterious effects of  
116 specific photosystem II (PSII) inhibitor herbicides, such as atrazine and simazine, are

117 primarily to reduce photosynthetic efficiency by inhibiting photosynthetic electron  
118 transfer. It induces the production of reactive oxygen species (ROS) and further  
119 damage the D1 protein of PSII and biomolecules like pigments (Chalifour and Juneau  
120 2011, Zhao et al. 2018). Some studies have shown that short-term light changes can  
121 affect the toxicity of pesticides (Baxter et al. 2016, Dong et al. 2016). However, very  
122 little is known about how photoadaptation to different light intensities influences the  
123 response of marine microalgae to herbicides.

124 Most of our knowledge regarding microalgal photoadaptation strategies and  
125 pesticide effects is from temperate species (Gomes and Juneau 2017, Young and  
126 Schmidt 2020). Microalgae have a rich evolutionary history due to their widespread  
127 presence in various habitats, especially marine ecosystems, leading to a wide range of  
128 adaptations, allowing them to thrive in a variety of environmental conditions (Lacour  
129 et al. 2020). Polar microalgae adapted to permanently low temperatures and extreme  
130 variation in irradiance due to seasonally changing ice-cover and day lengths (Handler  
131 2017). Sea-ice of Arctic Ocean is gradually melting because of global warming,  
132 increasing light availability at the sea surface (Osborne et al. 2018). Therefore,  
133 studying the differences in the adaptation processes intrinsic to Arctic and temperate  
134 microalgae can provide insights into how microalgae will cope with the changing  
135 aquatic environment, including light intensity and herbicide presence. It is known that  
136 ice melting may potentially cause an increase in pesticide concentrations in the Arctic  
137 waters (Pućko et al. 2017), although at concentrations that will remain lower than the  
138 ones found in temperate regions. Indeed, the main source of pesticides in Arctic is  
139 from the long-range aerial transport and not the nearby agricultural activities as it is  
140 for temperate regions (Schmale et al. 2018, Vorkamp and Riget 2014). In this study,  
141 we aimed to determine the response to herbicides of Arctic and temperate microalgae  
142 photoadapted to various light intensities and how various photoadaptation strategies  
143 affect herbicide toxicity. Our comparison of eco-physiological responses of Arctic to  
144 their temperate counterparts will facilitate the development of population growth  
145 models for microalgae more generally, as models predicting microalgal biomass

146 changes due to light and contaminants generally use observations from only temperate  
147 algae.

148

## 149 **2. Materials and methods**

### 150 2.1 Algal species and growth conditions

151 Temperate *Chaetoceros neogracile* (T-CN-CCMP1425), temperate *Micromonas*  
152 *bravo* (T-MB-CCMP1646), and Arctic strain of *Micromonas polaris* (A-MP-  
153 CCMP2099) were purchased from NCMA (National Contract Management  
154 Association). The Arctic strain of *Chaetoceros neogracilis* (A-CN-RCC2279) was  
155 obtained from the Roscoff culture collection (France). All species were cultivated in  
156 L1 marine medium (Guillard and Hargraves 1993) with a total volume of 100 mL  
157 medium in 250 mL Erlenmeyer flasks. Microalgae were grown at three different light  
158 intensities under white fluorescent tubes (Philips F72T8/TL841/HO, New York, NY,  
159 USA) (low light intensity-40  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (LL), medium light intensity-100  
160  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (ML), and high light intensity-400  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (HL)),  
161 intensities found at the surface of the Arctic Ocean and in temperate area (Lepetit et al.  
162 2017, Leu et al. 2010), at 14:10 h (light: dark) illumination cycle and shaken  
163 moderately twice a day. Growth temperatures were 18 °C and 4 °C, respectively, for  
164 temperate and Arctic species. Cells were periodically transferred to fresh growth  
165 media to keep them in their exponential growth phase. The cells were cultured for  
166 more than ten generations in these growth conditions. The cell numbers were counted  
167 using Multisizer 3 Coulter Counter (Beckman Coulter Inc., USA). The calculation of  
168 growth rate ( $\mu$ ) is based on the following formula:  $\mu = (\ln N_n) - (\ln N_0) / (t_n - t_0)$ , where  $\mu$   
169 = average specific growth rate,  $N_0, N_n$  indicate cell density ( $\text{cells mL}^{-1}$ ) at respectively  
170  $t_0$  (beginning of the experiment) and  $t_n$  (time, in days, after the beginning of the  
171 experiment).

### 172 2.2 Herbicide preparation and treatment

173 Atrazine and simazine used in this study came from Sigma-Aldrich  
174 (PESTANAL®, analytical standard, Canada). Herbicide stock solutions were made in  
175 pure acetone ( $\geq 99\%$ ) and acetone concentration was 0.01% in the treatments, which  
176 was verified not to be toxic to these microalgae. Six concentrations of atrazine and  
177 simazine were used for the herbicide tests ( $0 \mu\text{g L}^{-1}$ ,  $5 \mu\text{g L}^{-1}$ ,  $25 \mu\text{g L}^{-1}$ ,  $50 \mu\text{g L}^{-1}$ ,  
178  $100 \mu\text{g L}^{-1}$ , and  $250 \mu\text{g L}^{-1}$ ). Cells were collected during their exponential growth  
179 phase and transferred to 1 L Erlenmeyer flasks (with 350 mL growth medium) at a  
180 cell density of  $2.5 \times 10^5$  (*Chaetoceros*) and  $2.5 \times 10^6$  (*Micromonas*) cells  $\text{mL}^{-1}$   
181 respectively, and then exposed to different concentrations of herbicides for 72 h under  
182 the three light conditions. Our experimental design follows standard procedure for  
183 toxicological tests with algae, the pH of the cultures did not change over the exposure  
184 period and all treatments were performed in triplicate. Cell density and cell biovolume  
185 were evaluated at the end of the experiment with a Multisizer 3 Coulter Counter  
186 particle analyzer (Beckman Coulter Inc., USA).

### 187 2.3 Pigment content measurement

188 Algal cultures (25 mL) were collected after 72 h herbicide treatment through a  
189 gentle filtration on  $0.8\mu\text{m}$  filter membranes (Polytetrafluoroethylene; Xingya Purifyin  
190 Company; China). Filters were immediately submerged in liquid nitrogen and placed  
191 in 2 mL Eppendorf tubes, and then stored at  $-80 \text{ }^\circ\text{C}$  until analysis. Each sample was  
192 added to 2 mL of 90% acetone to be extracted overnight at  $-20 \text{ }^\circ\text{C}$  before pigment  
193 analysis. Cells in an ice container were broken for 20 s using an ultrasonic probe to  
194 increase extraction efficiency. The samples were centrifuged ( $10000\times g$ ) at  $4 \text{ }^\circ\text{C}$  for 10  
195 min after the extraction. The supernatants were used to quantify the content of  
196 chlorophyll (Chl *a*) and carotenoids (Car). A Cary 300 UV spectrophotometer (Varian,  
197 USA) was used to determine the absorbance spectra (400–750nm) for each extracted  
198 sample. Based on the equations from (Jeffrey and Humphrey 1975) and Seely et al.  
199 (1972), the contents of Chl *a* and Car were calculated respectively.

### 200 2.4 Variable Chl *a* fluorescence measurement



201 The samples (3 mL) were measured at their growth temperature of 4 °C and  
202 18 °C after dark acclimation for 20 min. Fluorescence light curves performed with a  
203 fluorometer of Water-PAM (Walz, Germany) were used to evaluate the maximum ( $\Phi_M$ )  
204 and operational ( $\Phi'_M$ ) PSII quantum yields, as well as the non-photochemical  
205 quenching (NPQ) (Du et al. 2019). The light curve was obtained by using eight levels  
206 of actinic light intensities (0, 46, 105, 188, 276, 427, 635, 906, and 1207  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ )  
207 with saturation pulses (3000  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ , 800 ms).  $\Phi_M$ ,  $\Phi'_M$   
208 and NPQ were computed according to the following equations:  $\Phi_M = (F_M - F_0)/F_M$ ;  $\Phi'_M$   
209 =  $(F'_M - F_S)/F'_M$  (Genty et al. 1989);  $\text{NPQ} = (F_M - F'_M)/F'_M$  (Bilger and Björkman 1990).  
210 Maximum relative electron transport rates ( $\text{rETR}_{\text{max}}$ ), maximum light efficiency usage  
211 ( $\alpha$ ), and light saturation coefficient ( $E_k$ ) were obtained by fitting the obtained values  
212 according to (Eilers and Peeters 1988, Serodio and Lavaud 2011). To further assess  
213 the PSII energy fluxes, the polyphasic increase in fluorescence transients was also  
214 captured by a PEA fluorometer (Plant Efficiency Analyzer, UK). The OJIP transients  
215 were generated by using a red light pulse of two seconds (3600  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ )  
216 with a maximum emission at 650 nm (Jiang et al. 2008). Table S1 provides a  
217 description of each parameter.

## 218 2.5 Reactive oxygen species (ROS) measurement

219 Intracellular ROS content was determined by using a BD Accuri C6 flow  
220 cytometer (Biosciences, San Jose, CA, USA). The fluorescent dye H<sub>2</sub>DCFDA (2',7'-  
221 dichlorodihydrofluorescein diacetate, Molecular probes, Eugene, OR, USA) was used.  
222 More information about this method was described in (Stachowski-Haberkorn et al.  
223 2013). Samples were analyzed after incubation in complete darkness for 30 min at  
224 room temperature. To prevent possible signal variations due to herbicide influence on  
225 FL1 fluorescence, the mean FL1 values of H<sub>2</sub>DCFDA-stained samples were divided  
226 by the mean FL1 values of the same fresh samples analyzed for morphology. Results  
227 were thus expressed as FL1 ratios.

## 228 2.6 Atrazine determination

229 The concentration of atrazine removed from the growth medium was calculated  
230 by subtracting the concentration found in the sample (atrazine + growth medium +  
231 microalgae) treated for 72 h from the abiotic control (atrazine + growth medium). To  
232 measure atrazine concentration remaining in the growth medium i.e., removal  
233 capacity of microalgae, cultures (in triplicate) were transferred to 250 mL flasks  
234 containing 100 mL of L1 growth media at cell concentrations indicated in section 2.2  
235 for 72 h. Abiotic controls, also in triplicate, were prepared using L1 medium without  
236 cells, and various light intensities did not alter the atrazine concentration. After  
237 inoculation of the microalgae in the medium, 2 mL aliquots of each flask were  
238 sampled and filtered on a 20 mm glass fiber filter (Type A/E, Pall Corporation,  
239 Michigan, USA) and stored in 1.5 mL Eppendorf tubes (polypropylene Safe-Lock  
240 tube, Canada). The filtrate was kept at -80 °C until analysis. After 72 h, the same  
241 procedure was repeated with 2 mL of culture for herbicide analysis. Before analysis,  
242 filtered media containing atrazine were thawed and then filtered again through a 0.22  
243 µm syringe filter (Millex-GV, Millipore, Billerica, USA).

244 A stock solution of atrazine at 1 µg L<sup>-1</sup> was prepared in 50% methanol to perform  
245 a calibration curve ranging from 0.5-20 µg L<sup>-1</sup>. An internal standard (IS) working  
246 solution of isotopically labeled atrazine-d<sub>5</sub> was prepared at 100 µg L<sup>-1</sup>, 20 µL of which  
247 was added to each sample or standard with 180 µL. Quantitative analysis of atrazine  
248 was performed by QTRAP 5500 mass spectrometer (Sciex, Concord, ON, Canada)  
249 with a Turbo-V electrospray ionization source in positive ion multiple reaction  
250 monitoring (MRM) mode (Chalifour et al. 2016). Atrazine and IS were detected using  
251 MRM transitions *m/z* 216-174 and 221-179 for quantitation (at collision-offset voltage  
252 30 and 25 V). Ion source and MS parameters were as follows: ESI voltage, 5000 V;  
253 source temperature, 500 °C; curtain gas, curtain gas, 35 psi; nebulizer and drying  
254 gases both at 50 psi. Peak integration was performed using MultiQuant™ 3.0 (Sciex),  
255 using peak area ratios of analyte/IS and linear regression of calibration curve for  
256 quantitation.

257 2.7 Statistical analyses

258 Statistical analyses were performed using Origin® 7.0 (Originlab Corporation,  
259 Northampton, MA, USA). Two-way analysis of variance (ANOVA) was used to  
260 determine the effect of treatments, and Tukey's honestly significant difference  
261 (Tukey's, HSD) test was conducted to test the statistical significance of the  
262 differences between means of various treatments. The assumption of normal  
263 distribution and homogeneity of variances for all t-tests and ANOVAs presented were  
264 respectively tested using Lilliefors' and Levene's tests. Two-way analysis of variance  
265 (ANOVA) to evaluate the interactions between growth light conditions (LL, ML, and  
266 HL), and herbicide concentrations. Contrast analysis was used when there were  
267 significant differences in the studied variables between treatments. The EC<sub>50</sub> (half-  
268 maximum effective concentration) values were calculated from the nonlinear least-  
269 square fits by using the inverse of the regression curve (Juneau et al. 2001).

270

### 271 **3. Results**

#### 272 **3.1 Effects of the growth light intensity on the ecophysiological characteristics of** 273 **the Arctic and temperate microalgae in absence of herbicides**

##### 274 3.1.1 Growth rate, cell biovolume, pigment content, and ROS content

275 Growth rates of Arctic and temperate microalgae (except for T-MB) grown under  
276 LL conditions were drastically lower than those under ML or HL conditions (Tukey's  
277 HSD,  $P < 0.05$ , Table 1, Fig. 1-atrazine concentration = 0  $\mu\text{g L}^{-1}$ ). Moreover, the  
278 growth rates of Arctic microalgae (*C. neogracilis*-A-CN and *M. polaris*-A-MP) were  
279 lower than their temperate counterparts (*C. neogracile*-T-CN, *M. bravo*-T-MB) under  
280 the three different light intensities; the exception being for A-CN under LL condition.  
281 In addition, the increasing trend of growth rate for Arctic microalgae was lower than  
282 that of temperate counterparts when the light intensity was enhanced. Similarly, the  
283 cell biovolume of two Arctic microalgae was smaller under LL condition compared to  
284 ML and HL (Tukey's HSD,  $P < 0.05$ , Table 1). Cell biovolume of the two temperate  
285 microalgae was the largest under HL and the smallest under LL, with an intermediary

286 biovolume for ML grown cultures.

287 The Chl *a* and Car contents of the two Arctic strains were lower than those of  
288 their temperate counterparts under LL, ML, and HL conditions. Furthermore, the Chl  
289 *a* content of the two temperate species significantly decreased under higher light  
290 intensities (ML, HL, Tukey's HSD,  $P < 0.05$ , Fig. 2-atrazine concentration =  $0 \mu\text{g L}^{-1}$ ),  
291 while both Arctic species were unchanged under LL compared to ML and HL  
292 conditions. The Car content of the two temperate species increased significantly under  
293 high light intensity (ML and HL), while the two Arctic microalgae were not affected  
294 (Tukey's HSD,  $P < 0.05$ , Fig. 2). In addition, except for T-MB, we found higher ROS  
295 levels in microalgae grown under HL relative to the other two lower light conditions  
296 (LL and ML) (Fig. 3-atrazine concentration =  $0 \mu\text{g L}^{-1}$ , Tukey's HSD,  $P < 0.05$ ), and  
297 the ROS contents of T-CN, A-CN, and A-MP of HL grown cultures were 1.2, 2.3, and  
298 1.4 times higher than those grown under LL, respectively.

### 299 3.1.2 Photosynthesis and energy dissipation processes

300 The maximum PSII quantum yield ( $\Phi_M$ ) of all studied species significantly  
301 decreased with increasing growth light intensity and  $\Phi_M$  of the two Arctic microalgae  
302 declined more than that of their temperate counterparts (Tukey's HSD,  $P > 0.05$ , Table  
303 1). A similar trend was observed with the operational PSII quantum yield ( $\Phi'_M$ ), but  
304 the amplitudes of the declines were higher than for  $\Phi_M$ ; except for A-MP showing no  
305 alteration from LL to ML (Table 1). Together with the reduction of  $\Phi_M$  and  $\Phi'_M$ , the  
306 maximum light efficiency usage ( $\alpha$ ) showed a decreasing trend with increasing light  
307 intensity (Tukey's HSD,  $P < 0.05$ , Table 1). Furthermore, the maximum electron  
308 transport rates ( $r\text{ETR}_{\text{max}}$ ) and the light saturation coefficient ( $E_k$ ) of Arctic and  
309 temperate diatoms (T-CN and A-CN) increased significantly from LL to ML (except  
310 for  $E_k$  of T-CN) and remarkably decreased from ML to HL (Tukey's HSD,  $P < 0.05$ ,  
311 Table 1). Under the same condition, the  $E_k$  of Arctic and temperate green algae of  
312 *Micromonas* (T-MB and A-MP) demonstrated similar trends, but no significant  
313 difference (Tukey's HSD,  $P > 0.05$ , Table 1). Moreover, the maximal ability for  
314 dissipation of excess energy ( $\text{NPQ}_{\text{max}}$ ) of T-MB and A-MP also significantly increased

315 from LL to ML, and markedly decreased from ML to HL conditions (Tukey's HSD, P  
316 < 0.05, Table 1). However, the  $NPQ_{max}$  of T-CN and A-CN increased significantly  
317 when the growth light intensity was high (LL to ML and HL, Tukey's HSD, P < 0.05,  
318 Table 1). In addition, we observed that the parameters  $\Phi'_M$ ,  $\Phi_M$ ,  $\alpha$ ,  $rETR_{max}$ , and  $E_k$   
319 for the two Arctic species were, to different degrees, lower than those of their  
320 temperate counterparts under the different growth light intensities.

### 321 3.1.3 Photosystem II energy fluxes

322 The energy conservation parameter of  $PI_{ABS}$  significantly decreased for all  
323 species, except for A-CN, with increasing the growth light intensity (Tukey's HSD, P  
324 < 0.05, Fig. 4). The  $PI_{ABS}$  of the two Arctic microalgae was less affected than their  
325 temperate counterparts. The reduction in  $PI_{ABS}$  was also reflected in the decrease of  
326 electron transport per active reaction center ( $ET_0/RC$ ) for all studied species when the  
327 growth light intensity was enhanced. The effective dissipation per active RC ( $DI_0/RC$ )  
328 of the two temperate microalgae (T-CN and T-MB) was increased up to 252% and 224%  
329 from LL to HL (Tukey's HSD, P < 0.05). This was accompanied by an increase in the  
330 absorbed flux per active reaction center ( $ABS/RC$ ) as an indicator of the PSII antenna  
331 size. In contrast,  $DI_0/RC$  and  $ABS/RC$  in two Arctic microalgae did not significantly  
332 change when the algae were grown under different light intensities (Tukey's HSD, P >  
333 0.05, Fig. 4). The maximal rate at which excitons were trapped by the active RCs  
334 ( $TR_0/RC$ ) was not affected by changes in growth light intensity. Furthermore, the PQ  
335 pool size participating in the electron transport ( $qPQ$ ) and non-photochemical  
336 quenching ( $qE_{max}$ ) were significantly increased in the two Arctic microalgae under HL,  
337 compared to ML and LL (Tukey's HSD, P < 0.05, Fig. 4). In contrast, the  $qPQ$  and  
338  $qE_{max}$  of the two temperate species significantly decreased with increasing light  
339 intensity.

## 340 **3.2 Effects of atrazine and simazine on the ecophysiological characteristics of** 341 **Arctic and temperate species**

### 342 3.2.1 Growth rate, cell biovolume, pigment content, and ROS content

343 The growth rates of Arctic species (A-CN and A-MP) and their temperate  
344 counterparts (T-CN and T-MB) significantly decreased after 72 h exposure to atrazine  
345 or simazine independently of the growth light intensity (Tukey's HSD,  $P < 0.05$ , Fig.  
346 1). Interestingly, although that trend was similar among the various light intensities,  
347 the effect was stronger under the lowest light intensity. Moreover, the impact of the  
348 herbicides on the growth rate for Arctic species was stronger than that of their  
349 temperate counterparts when light intensity was enhanced. Although these herbicides  
350 inhibited the growth of microalgae, the cell biovolume of the four studied species did  
351 not significantly change for any tested conditions (Tukey's HSD,  $P > 0.05$ , Fig. S1),  
352 except for T-MB and A-MP at high concentrations (100 and 250  $\mu\text{g/L}$ ) under ML and  
353 HL conditions.

354 Chl *a* and Car contents did not change significantly at low concentrations of  
355 atrazine and simazine ( $\leq 25 \mu\text{g/L}$ , Tukey's HSD,  $P > 0.05$ , Fig. 2), but declined  
356 significantly at high concentrations ( $> 25 \mu\text{g/L}$ ). The maximum reduction was  
357 observed under HL compared to the other two low light intensities (LL and ML).  
358 Despite a decreasing trend in Chl *a* and Car, the ratio of Car/Chl *a* remained  
359 unchanged (Tukey's HSD,  $P > 0.05$ ). ROS levels significantly increased with atrazine  
360 concentrations for each light condition (LL, ML, and HL) (Tukey's HSD,  $P < 0.05$ ).  
361 Although this trend was similar among light intensities, the effect was stronger under  
362 HL (Fig. 3). The ROS content also significantly increased with increasing simazine  
363 concentrations, and its effect was greater for higher light intensities (data not shown).

### 364 **3.2.2** Photosynthesis and energy dissipation processes

365 The operational PSII quantum yield ( $\Phi'_M$ ) of the four studied species  
366 significantly decreased (Tukey's HSD,  $P < 0.05$ , Fig. 5) with increasing atrazine and  
367 simazine concentrations for each growth light condition. Moreover, both  $\Phi_M$  and  $\Phi'_M$   
368 of A-CN decreased more than that of T-CN. In contrast,  $\Phi_M$  and  $\Phi'_M$  of A-MP  
369 decreased less than that of T-MB except for low atrazine concentrations under LL ( $<$   
370  $25 \mu\text{g/L}$ , Tukey's HSD,  $P < 0.05$ , Fig. 5). The evaluated  $\Phi'_M\text{-EC}_{50}$  of T-CN for  
371 atrazine under LL, ML, and HL conditions were respectively 2.4, 1.7 and 1.8 times

372 higher than those obtained for A-CN (Table 1). Similar results were obtained for  
373 simazine. Both *Chaetoceros* species have the lowest  $\Phi'_{M-EC_{50}}$  (respectively  $90 \mu\text{g L}^{-1}$   
374 and  $28 \mu\text{g L}^{-1}$  for the temperate and Arctic species) for simazine under HL compared  
375 to LL and ML (Table 1).  $\Phi'_{M-EC_{50}}$  of T-MB was equal to or higher than those of A-  
376 MP under LL, ML, and HL conditions for atrazine. The highest simazine  $\Phi'_{M-EC_{50}}$   
377 evaluated for A-MP under HL conditions ( $206 \mu\text{g L}^{-1}$ ) was 4.2 times higher than that  
378 of T-MB (Table 1). The parameter of  $\Phi'_M$  was more sensitive than  $\Phi_M$  in response to  
379 the herbicides. The energy conservation parameter ( $PI_{ABS}$ ) also decreased after  
380 exposure to  $50 \mu\text{g L}^{-1}$  atrazine and simazine under each light condition, but to a  
381 different extent than  $\Phi_M$  and  $\Phi'_M$  depending on the tested species (Table 2). Non-  
382 photochemical quenching ( $qE_{max}$ ) of the four studied species was also significantly  
383 reduced with the addition of  $50 \mu\text{g L}^{-1}$  of atrazine under LL ML, and HL conditions  
384 (Tukey's HSD,  $P < 0.05$ , Table 2).

### 385 **3.3 Effects of growth light intensity on atrazine toxicity**

386 Because the effect of growth light intensity on simazine was similar to that of  
387 atrazine, and because atrazine is detected more frequently than simazine in water  
388 bodies, we mainly focus on atrazine for this section. Atrazine and ML alone  
389 significantly reduced  $PI_{ABS}$  of T-CN by 98% and 95%, respectively, while their  
390 combined action was less effective (71% inhibition) (Tukey's HSD,  $P < 0.05$ ). Similar  
391 results were observed for HL (Table 2). This trend was also observed among the other  
392 studied species (A-CN, T-MB, and A-MP). Both Arctic species increased their non-  
393 photochemical quenching ( $qE_{max}$ ) to varying degrees (18-89%) under ML and HL  
394 growth conditions compared to LL (Table 2). The combined effect of atrazine and ML  
395 decreased  $qE_{max}$  by 60% and 58% respectively in A-CN and A-MP (Table 2).  
396 Furthermore, the combined effect of atrazine and ML on the two temperate species  
397 only reduced  $qE_{max}$  by 14% for T-MB and 63% for T-CN compared to ML exposure  
398 alone (27% and 72% reduction), and the combined effect of atrazine and HL  
399 decreased more  $qE_{max}$  than the combination of atrazine and ML condition (Table 2).  
400 The treatment with atrazine alone significantly induced the production of ROS, but

401 the combined effect of atrazine and ML or HL downregulated the production of ROS  
402 (Table 2). According to Fig. 6, the removal of atrazine was stronger under high light  
403 growth (HL) compared to the other two low light intensities (LL and ML) except for  
404 T-CN (Tukey's HSD,  $P < 0.05$ ). The removal ability of atrazine by A-CN was higher  
405 than that of T-CN under LL, ML and HL conditions. Meanwhile, the A-MP showed  
406 higher removal ability of atrazine compared to T-MB under ML and HL conditions  
407 except for LL condition.

408

## 409 **4 Discussion**

### 410 **4.1 Influence of growth light intensity on Arctic microalgae and their temperate** 411 **counterparts**

#### 412 **4.1.1 Photoadaptation processes**

413 Light adaptation processes developed by microalgae allow them to thrive in low  
414 or high light environments by modulating their eco-physiological properties (Agarwal  
415 et al. 2019, Young and Schmidt 2020). In our experiments, high light induced similar  
416 photophysiological responses among the studied species. Growth under higher (but  
417 not excessive) light intensity led to a significant increase in growth rates, which may  
418 be attributed to the Chl *a*/C evolution with changes in light intensity (Croteau et al.  
419 2022, Lacour et al. 2018). The reduction of Chl *a* in all species after HL  
420 photoadaptation was typically seen as a result of lower light absorption as would be  
421 expected given that light absorption is positively related to cellular Chl *a* content  
422 (MacIntyre et al. 2002). Increase in Car may play a role in protecting the  
423 photosynthetic apparatus under high light intensities. Indeed, either directly or  
424 indirectly, these pigments are involved in scavenging ROS (Sedoud et al. 2014).  
425 Furthermore, the slight but significant increase of the light harvesting antenna size of  
426 all studied species following HL adaptation indicated a relatively low  
427 photochemically effective cross-section to reduce the chance of photons entering the  
428 photosynthetic electron transport chain to avoid photo-damage (Finkel et al. 2010).



429 These modifications observed under high light conditions minimized the excitation  
430 pressure on the photosynthetic apparatus despite the increased light availability  
431 (Agarwal et al. 2019). Non-photochemical quenching (NPQ) (the main component-qE)  
432 is known to be an efficient photoprotective mechanism (Goss and Lepetit 2015). The  
433 higher intrinsic NPQ<sub>max</sub> of the studied Arctic species under HL, as compared to LL  
434 and ML conditions, may indicate that this process is activated to reduce PSII damage  
435 (Kirilovsky 2015). Indeed, light intensity above the photosynthesis saturation point  
436 ( $E_k$  over 400  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , Table 1) can increase ROS production (Metsoviti  
437 et al. 2019), which was consistent with the reduced photosynthetic efficiency ( $\Phi_M$  and  
438  $\Phi'_M$ ) under HL. Therefore, the observed high ROS content inducing inactivation of  
439 PSII under HL as compared to ML and LL, indicates that cellular defense strategies  
440 were not sufficient to deal with photochemical damage and oxidative stress.

#### 441 **4.1.2 Differences between Arctic and temperate microalgae**

442 The observed low Chl *a* could be responsible for the lower photosynthetic  
443 efficiency ( $\Phi_M$  and  $\Phi'_M$ ) in both Arctic species compared to their temperate  
444 counterparts under all light conditions since Chl *a* is proportional to the number of  
445 photosynthetic systems (MacIntyre et al. 2002). Some authors have shown that the A-  
446 MP had only half the content of active PSII reaction centers compared to T-MB (Ni et  
447 al. 2017). This is also confirmed by the low performance index of photons (PI<sub>ABS</sub>),  
448 showing the low photosynthetic efficiency. Furthermore, a small amount of Chl *a* is  
449 usually present in Arctic species growing under light-limiting conditions to avoid  
450 stacking effects between chlorophylls (Yan et al. 2018). As a result, the absence of  
451 markedly reduced Chl *a* content in HL-adapted Arctic cells compared with LL-  
452 adapted cells suggested that they did not possess the same light-modulating ability as  
453 temperate microalgae. Temperate microalgae usually decrease their light absorption at  
454 high irradiance levels by reducing Chl *a* associated with their light-harvesting  
455 complexes, as observed in both temperate species studied here (decreased Chl *a* under  
456 HL, Fig. 2). Interestingly, qPQ, which reflects the plastoquinone (PQ) pool size  
457 participating in the electron transport (Xu et al. 2019), significantly increased in the

458 two Arctic species under HL, indicating that increased electron supply to the PQ pool  
459 was satisfied by increasing the ability to transfer energy away from PSII, and  
460 accompanied by high dissipation capacity (higher  $qE_{\max}$ ) (Fig. 4). In comparison,  
461 temperate species had reduced  $qPQ$  and lower  $qE_{\max}$  under HL. The redox state of the  
462 PQ pool plays an important role in light adaptation of algae and regulation of gene  
463 expression in the chloroplasts and nucleus under varying light conditions (Lepetit et al.  
464 2013, Virtanen et al. 2021). In summary, the photoadaptation strategies between  
465 temperate and Arctic microalgae show different extents. Arctic microalgae appear to  
466 mainly rely on increased PQ pool size and strong dissipation capacity ( $qE_{\max}$ ) to cope  
467 with higher light intensity, whereas their temperate counterparts appear to mainly rely  
468 on the reduction of pigments associated with light-harvesting complex (LHC) to  
469 regulate light absorption. In addition, we observed substantial differences in the  
470 adaptation capacity of the two microalgal classes. The diatoms had higher growth rate  
471 ( $\mu$ ), higher light efficiency use ( $\alpha$ ) and higher photosynthetic electron transfer rate  
472 ( $rETR_{\max}$ ) relative to the green microalgae, exhibiting their high photoadaptation  
473 capacity to change in ambient light intensity (Table 1). This mechanism concords with  
474 the dominance of diatoms in the seasonally changing Arctic Ocean (Croteau et al.  
475 2022, Wolf et al. 2018).

#### 476 **4.2 Effects of atrazine and simazine on Arctic microalgae and their temperate** 477 **counterparts under each light condition**

478 Based on the  $EC_{50}$  of  $\Phi_M$  and  $\Phi'_M$  (Table 1), temperate *C. neogracile* was more  
479 tolerant to atrazine and simazine than Arctic *C. neogracilis* under all light conditions,  
480 while Arctic *M. polaris* was less affected by these herbicides than temperate *M. bravo*,  
481 and the sensitivity sequence was T-CN<A-CN<A-MP<T-MP. We found that atrazine  
482 was more toxic than simazine in all studied species under all light conditions even  
483 though they had the same mode of action. This higher toxicity was attributed to the  
484 high  $\log K_{OW}$  of atrazine resulting in a high affinity for the herbicide binding site  
485 (Ronka 2016). Atrazine and simazine, as photosynthetic inhibitor herbicides, can bind  
486 to the  $Q_B$  site of the D1 protein in PSII (Bai et al. 2015). Therefore, the observed

487 inhibition of photosynthetic electron transport chains (decreased  $\Phi'_M$ ) in the Arctic  
488 and temperate species was observed in the presence of these herbicides, which was  
489 also evidenced by lower electron transfer per active RC ( $ET_0/RC$ ). The  
490 transthylakoidal proton gradient required to activate non-photochemical quenching  
491 (qE) (Cao et al. 2013) was also reduced by inhibiting electron transport. As a result,  
492 the microalgae reduced thermal dissipation capacity ( $qE_{max}$ ) under herbicide exposure  
493 preventing the efficient dissipation of excess energy. Therefore, the organisms  
494 underwent higher excitation pressure on PSII due to blocked electron transport,  
495 resulting in the production of large ROS concentrations (Fig. 3), which can eventually  
496 deactivate PSII RC (Gomes and Juneau 2017, Sun et al. 2020). This is in accordance  
497 with the increased effective antenna size of active RC ( $ABS/RC$ ), showing a strong  
498 decrease in the active PSII RC population as previously demonstrated under cadmium  
499 and high light stress (Du et al. 2019). The reduction of active PSII RCs induced by  
500 ROS may explain the decreased photochemistry efficiency of PSII ( $\Phi_M$ ), which was  
501 ultimately reflected in the growth inhibition of all studied species under the different  
502 light conditions. This observation was consistent with previous studies showing that  
503 photosynthesis inhibitor herbicides (diuron and Irgarol) inhibit the growth of  
504 freshwater microalgae (Kottuparambil et al. 2017). In addition, the reduction of Chl *a*  
505 in all species (Fig. 2) was attributed to the continuous accumulation of ROS in the  
506 presence of atrazine or simazine (Almeida et al. 2017). Atrazine and simazine  
507 disturbed the balance between light absorption and energy utilization, since  $PI_{ABS}$ , as  
508 an indicator for energy conservation from photons absorbed by PSII to intersystem  
509 electron acceptors, significantly decreased irrespective of growth light intensity (Sun  
510 et al. 2020).

### 511 **4.3 Combined effects of growth light intensity and atrazine on Arctic microalgae** 512 **and their temperate counterparts**

513 Significant interactions between growth light intensity and atrazine were  
514 observed for growth,  $\Phi_M$ ,  $\Phi'_M$ , and  $PI_{ABS}$  ( $P < 0.0001$ ). Atrazine was chosen to describe  
515 the interaction with light since the obtained results for simazine were similar due to

516 their same mode of action. According to the  $EC_{50}$  of  $\Phi_M$  and  $\Phi'_M$ , all studied species  
517 were more affected by atrazine under HL compared to the other lower light intensities  
518 (LL and ML) (Table 1). Although atrazine and light alone, respectively, decreased the  
519 light energy conversion ( $PI_{ABS}$ ), interestingly it appears that the combination of HL  
520 and herbicides reversed this effect on  $PI_{ABS}$  (Table 2). This indicates that  
521 photoadaptation processes, such as changes in pigments, mitigate the effects of  
522 herbicides on light energy conversion. Indeed, lower Chl *a* in both Arctic species  
523 and higher Car concentration in both temperate species may decrease, respectively,  
524 light absorption and ROS scavenging, which help to protect the photosystem  
525 apparatus. The combined inhibitory effect was small in tolerant species (T-CN and A-  
526 MP) to atrazine compared to the sensitive species (A-CN and T-MB). Changes in PSII  
527 photochemistry are frequently related to the modifications of the energy dissipation  
528 pathway ( $qE_{max}$ ) (Du et al. 2019). The combined effect of HL and atrazine ( $50 \mu\text{g L}^{-1}$ ,  
529 concentration near the  $EC_{50}$  for most species) on the thermal dissipation ability ( $qE_{max}$ )  
530 of Arctic species was greater than the effects of atrazine alone, although high  $qE_{max}$   
531 was already induced after HL adaptation without herbicide. While the effect of  $qE_{max}$   
532 was slight in temperate species under the combined conditions of HL and atrazine, HL  
533 and atrazine alone considerably decreased  $qE_{max}$  (Table 2). Therefore, we can suggest  
534 that other parts of the non-photochemical energy dissipation process ( $qT$  or  $qI$ ) in  
535 temperate species were highly effective under HL adaptation since some authors have  
536 shown that  $qE$  was in general much lower on green microalgae than in diatoms since  
537  $qT$  is more important than  $qE$  in green algae of *Chlamydomonas* (Allorent et al. 2013).  
538 These results showed that  $qE_{max}$ , as an indicator of the thermal dissipation capacity,  
539 was less indicative than  $\Phi'_M$  and  $PI_{ABS}$ , probably because the later integrated the  
540 effects of the herbicide and HL on the photosynthetic electron transport and the  
541 related energy dissipation processes, while  $qE_{max}$  only represents one of the  
542 components of NPQ. This is in accordance with a recent study showing that the  
543 performance index  $PI_{ABS}$  was a more convincing indicator of atrazine toxicity  
544 compared to growth rate and  $\Phi_{E0}$  (Sun et al. 2020). On the other hand, the  
545 combination of HL effect and  $50 \mu\text{g L}^{-1}$  atrazine of microalgae mitigated the ROS

546 burst (Table 2), which was attributed to the increased non-photochemical quenching  
547 (NPQ<sub>max</sub>) after HL adaptation to dissipate excess energy to reduce the excitation  
548 pressure on PSII (Silva et al. 2021). Furthermore, with the exception of T-CN, the  
549 removal of atrazine by microalgae increased with increasing culture light intensity  
550 (LL-ML-HL), which can largely explain the high herbicide toxicity under HL  
551 intensity. We hypothesize that high light condition induces higher uptake of pesticides  
552 as it was previously demonstrated for other contaminants such as metals (Du et al.  
553 2019). As a result, we can assume that even the effective induction of photosynthetic  
554 defense measures (NPQ and Car content) upon HL adaptation was unable to deal with  
555 the combination of higher light intensity and herbicides. Interestingly, the growth  
556 inhibition caused by atrazine of temperate species grown under HL was alleviated  
557 more than the cells grown under LL and ML (Fig. 1). This can be explained that  
558 temperate microalgae can effectively utilize the absorbed light energy and perform  
559 reasonable energy allocation (high  $\alpha$  and PI<sub>ABS</sub>, Table 1) for carbon fixation. However,  
560 Arctic microalgae grown under HL were unable to prevent growth inhibition caused  
561 by atrazine due to the observed inefficient capacity for light absorption and light  
562 energy conversion (Fig. 4).

#### 563 **4.4 Concluding remarks**

564 We demonstrated that the extent of light-adaption responses between Arctic and  
565 temperate microalgae were different, these differences can influence the toxicity of  
566 atrazine, and probably other contaminants. Furthermore, the microalgal protection  
567 measures of NPQ and Car (photoprotective pigment) after HL adaptation were  
568 insufficient to handle the combined impacts of high light intensity and atrazine stress,  
569 as atrazine removal ability was enhanced with increasing growth light intensity. Our  
570 results indicated that light plays a non-negligible role in regulating the toxicity of  
571 herbicide for microalgae. We recommend that variation in light intensities should be  
572 considered in herbicide risk assessments for Arctic and temperate ecosystems,  
573 because of its role in affecting photoprotective strategies and atrazine removal.

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581

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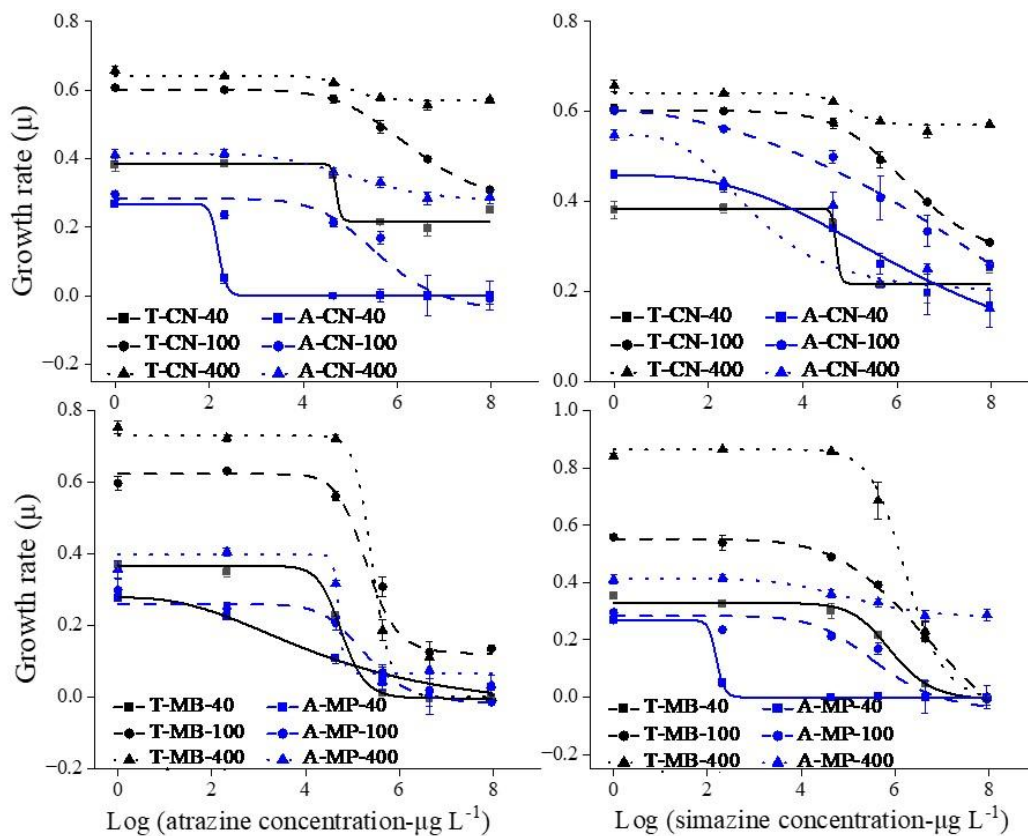
786 Table 1. The effect of light intensity (LL, ML, and HL) on the growth rate, cell  
787 biovolume,  $\Phi_M$ ,  $\Phi'_M$ ,  $\alpha$ ,  $rETR_{max}$ ,  $E_k$ , and  $NPQ_{max}$  of temperate *C. neogracile* (T-CN),  
788 *M. bravo* (T-MB), Arctic *C. neogracilis* (A-CN), and Arctic *M. polaris* (A-MP).  $EC_{50}$   
789 for the operational PSII quantum yield ( $\Phi'_M$ ) after 72 h exposure to atrazine and  
790 simazine under three different light intensities (LL, ML, and HL). Strains in the same  
791 column exposed to the different light intensity with different superscript letters (a-c)  
792 were significantly different (Tukey's HSD,  $P < 0.05$ ). The numbers in parentheses are  
793 the percentages of the control values. Data expressed as means  $\pm$  SD ( $n = 6$ ).

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Species	Light	Growth rate ( $\mu$ )	Volume ( $\mu\text{m}^3$ )	$\Phi_M$	$\Phi'_M$	$\alpha$	rETR <sub>max</sub>	$E_k$	NPQ <sub>max</sub>	EC <sub>50</sub> - $\Phi'_M$ (Atra) (Sim)	
T-CN	LL	0.37±0.02 (100) <sup>a</sup>	114±8.89(100) <sup>a</sup>	0.692±0.00 (100) <sup>a</sup>	0.682±0.01 (100) <sup>a</sup>	0.713±0.01 (100) <sup>a</sup>	333±36 (100) <sup>a</sup>	467±52 (100) <sup>a</sup>	1.5±1.24 (100) <sup>a</sup>	95±7.4	121±7.9
	ML	0.64±0.03 (170) <sup>b</sup>	148±9.59(130) <sup>b</sup>	0.647±0.00 (93) <sup>b</sup>	0.646±0.00 (95) <sup>b</sup>	0.665±0.01 (93) <sup>b</sup>	421±17 (126) <sup>b</sup>	633±23 (136) <sup>b</sup>	1.3±0.24 (12) <sup>b</sup>	66±1.4	142±6.7
	HL	0.66±0.02 (177) <sup>b</sup>	164±4.88(144) <sup>c</sup>	0.608±0.01 (88) <sup>c</sup>	0.510±0.03 (75) <sup>c</sup>	0.624±0.02 (88) <sup>c</sup>	253±63 (76) <sup>c</sup>	404±91 (87) <sup>a</sup>	10±0.00 (105) <sup>a</sup>	34±1.1	90±12
A-CN	LL	0.43±0.03 (100) <sup>a</sup>	45±3.06 (100) <sup>a</sup>	0.674±0.01 (100) <sup>a</sup>	0.607±0.01 (100) <sup>a</sup>	0.607±0.02 (100) <sup>a</sup>	265±34 (100) <sup>a</sup>	432±55 (100) <sup>a</sup>	1.6±0.62 (100) <sup>a</sup>	39±1.7	52±0.7
	ML	0.57±0.09 (131) <sup>b</sup>	63±3.84 (140) <sup>b</sup>	0.584±0.00 (87) <sup>b</sup>	0.553±0.01 (91) <sup>b</sup>	0.569±0.04 (94) <sup>a</sup>	370±91 (140) <sup>b</sup>	648±158 (150) <sup>b</sup>	1.8±0.54 (113) <sup>a</sup>	37±2.1	62±2.1
	HL	0.57±0.08 (130) <sup>b</sup>	61±7.57 (136) <sup>b</sup>	0.525±0.02 (78) <sup>c</sup>	0.246±0.02 (41) <sup>c</sup>	0.473±0.07 (78) <sup>b</sup>	91±13 (34) <sup>c</sup>	196±46 (45) <sup>c</sup>	3.7±0.92 (231) <sup>b</sup>	12±3.2	28±2.0
T-MB	LL	0.36±0.01 (100) <sup>a</sup>	4±0.09 (100) <sup>a</sup>	0.685±0.01 (100) <sup>a</sup>	0.678±0.02 (100) <sup>a</sup>	0.519±0.05 (100) <sup>ab</sup>	247±61 (100) <sup>a</sup>	369±162 (100) <sup>a</sup>	1.5±0.01 (100) <sup>a</sup>	36±5.9	61±7.0
	ML	0.58±0.03 (160) <sup>b</sup>	5±0.00 (134) <sup>b</sup>	0.647±0.00 (94) <sup>b</sup>	0.562±0.00 (83) <sup>b</sup>	0.597±0.01 (115) <sup>a</sup>	244±25 (99) <sup>a</sup>	430±48 (117) <sup>a</sup>	1.9±0.11 (127) <sup>b</sup>	31±2.3	32±1.8
	HL	0.80±0.05 (220) <sup>c</sup>	5±0.19 (141) <sup>c</sup>	0.598±0.01 (87) <sup>c</sup>	0.357±0.01 (53) <sup>c</sup>	0.499±0.07 (96) <sup>b</sup>	180±31 (73) <sup>a</sup>	368±82 (100) <sup>a</sup>	1.3±0.33 (87) <sup>a</sup>	30±4.8	49±1.4
A-MP	LL	0.27±0.01 (100) <sup>a</sup>	5±0.05 (100) <sup>a</sup>	0.675±0.02 (100) <sup>a</sup>	0.505±0.02 (100) <sup>a</sup>	0.445±0.09 (100) <sup>ab</sup>	114±56 (100) <sup>a</sup>	249±104 (100) <sup>a</sup>	2.2±0.71 (100) <sup>a</sup>	39±1.3	55±3.4
	ML	0.30±0.02 (109) <sup>b</sup>	6±0.26 (102) <sup>a</sup>	0.618±0.02 (92) <sup>b</sup>	0.491±0.02 (97) <sup>a</sup>	0.493±0.04 (111) <sup>a</sup>	156±21 (137) <sup>a</sup>	356±28 (143) <sup>a</sup>	9.8±0.37 (445) <sup>b</sup>	36±1.7	46±1.9
	HL	0.32±0.04 (118) <sup>b</sup>	6±0.10 (114) <sup>b</sup>	0.515±0.00 (76) <sup>c</sup>	0.222±0.01 (44) <sup>b</sup>	0.384±0.04 (86) <sup>b</sup>	106±54 (93) <sup>a</sup>	273±128 (110) <sup>a</sup>	1.6±0.20 (73) <sup>a</sup>	29±0.6	206±39

797 Table 2. Effect of atrazine ( $50 \mu\text{g L}^{-1}$ ), ML & HL exposure and the combined effect (light and atrazine) on  
 798 light energy conservation indicator ( $\text{PI}_{\text{ABS}}$ ), non-photochemical quenching ( $qE_{\text{max}}$ ), and ROS content, in  
 799 percentage from control (control is taken as 100%). Data are means  $\pm$  SD of two independent experiments in  
 800 triplicate. Within treatments, values followed by one asterisk are significantly different from the control, and  
 801 values followed by two asterisks indicate significant differences between the LL and ML or HL exposure,  
 802 while values followed by three asterisks are significantly different from both control and ML and HL  
 803 treatment (Tukey's HSD,  $P < 0.05$ ).

Species	Parameter	Effect of atrazine %				
		of the control (LL)	Effect of ML % of the control ( $\pm$ )	Atrazine+ ML % of the control ( $\pm$ )	Effect of HL % of the control ( $\pm$ )	Atrazine+ HL % of the control ( $\pm$ )
T-CN	$\text{PI}_{\text{ABS}}$	2* (0)	5** (0)	29*** (2)	5** (0)	21*** (1)
A-CN	$\text{PI}_{\text{ABS}}$	37* (2)	75** (5)	16*** (1)	57** (1)	11*** (1)
T-MB	$\text{PI}_{\text{ABS}}$	52* (2)	4** (0)	5*** (0)	3** (0)	8*** (0)
A-MP	$\text{PI}_{\text{ABS}}$	10* (1)	48** (2)	28*** (2)	40** (2)	15 (1)
T-CN	$qE_{\text{max}}$	85* (9)	28** (2)	37*** (1)	26** (2)	24*** (2)
A-CN	$qE_{\text{max}}$	78* (3)	118 (16)	40*** (2)	124** (15)	28*** (3)
T-MB	$qE_{\text{max}}$	97 (5)	73** (6)	86 (5)	66** (3)	70*** (7)
A-MP	$qE_{\text{max}}$	81* (3)	183** (24)	42*** (3)	189** (19)	23*** (2)
T-CN	ROS	171* (12)	101 (10)	131*** (14)	114 (10)	138*** (21)
A-CN	ROS	243* (18)	163** (21)	466*** (42)	227** (32)	286*** (25)
T-MB	ROS	444* (72)	107 (7)	162*** (12)	116 (17)	231*** (18)
A-MP	ROS	176* (12)	105 (8)	113*** (17)	36** (4)	390*** (47)



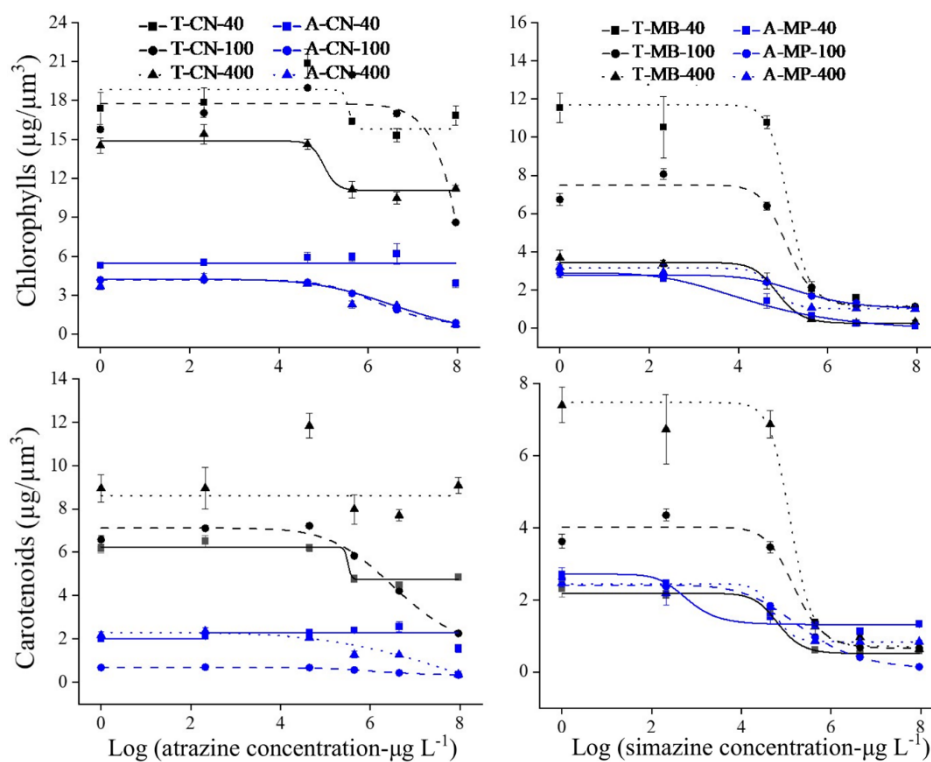
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Figure 1. The effect of atrazine and simazine on the growth rate of four species (temperate *C. neogracile* (T-CN), Arctic *C. neogracilis* (A-CN), temperate *M. bravo* (T-MB), and Arctic *M. polaris* (A-MP)) after 72 h exposure under LL (square), ML (circle) and HL (triangle). Data expressed as means  $\pm$  SD (n = 6)



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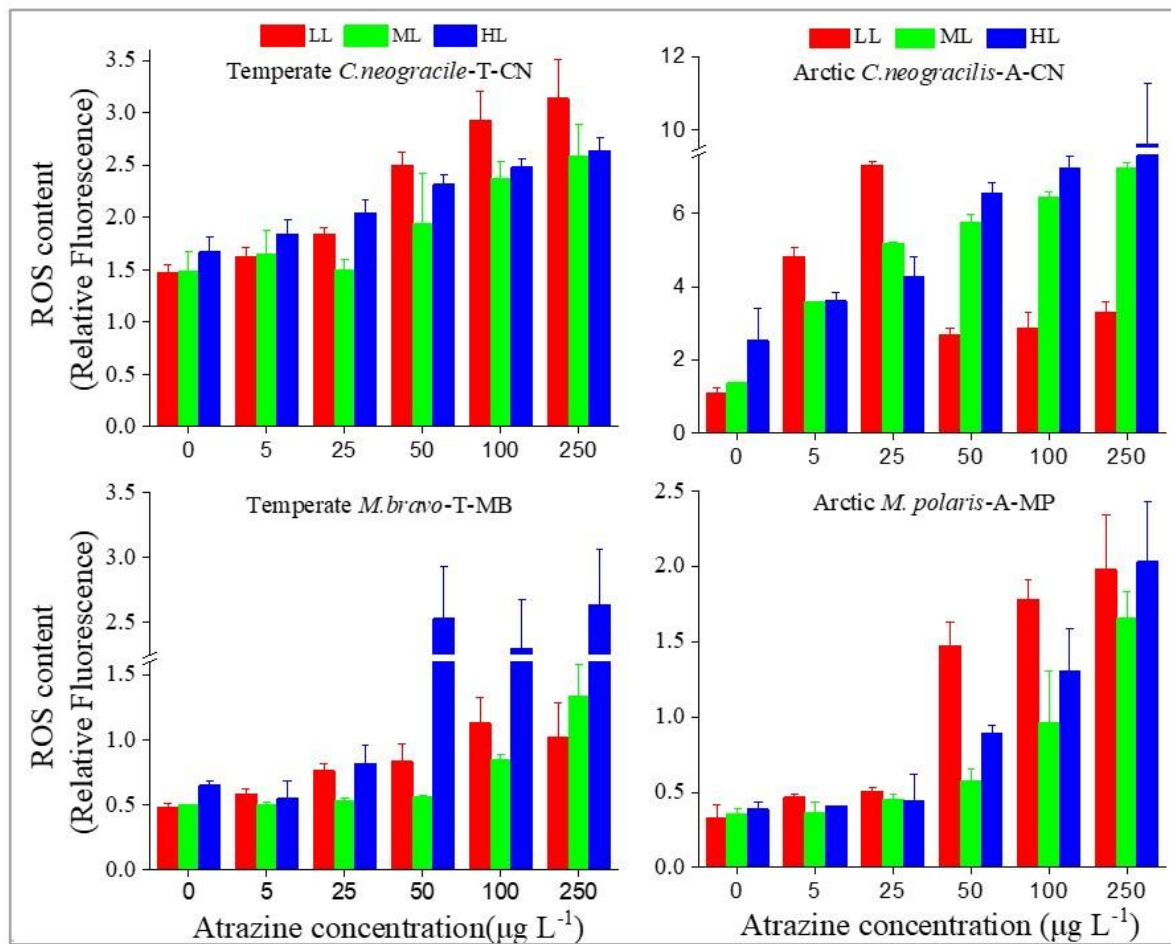
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Figure 2. The effect of atrazine (after 72 h) on the pigment content of four microalgal species (temperate *C. neogratile* (T-CN), Arctic *C. neogracilis* (A-CN), temperate *M. bravo* (T-MB), and Arctic *M. polaris* (A-MP)) under LL (square), ML (circle) and HL (triangle) light intensities. The presented data calculated is relative to cell biovolume ( $\mu\text{m}^3$ ), data expressed as means  $\pm$  SD (n = 6).





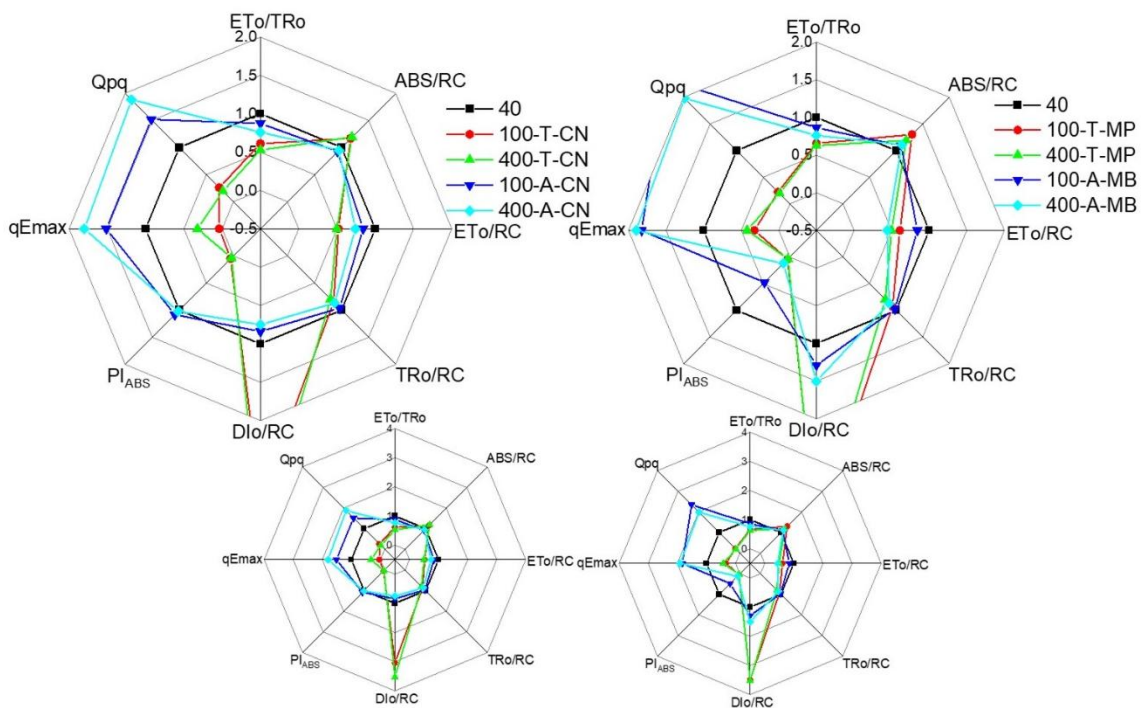
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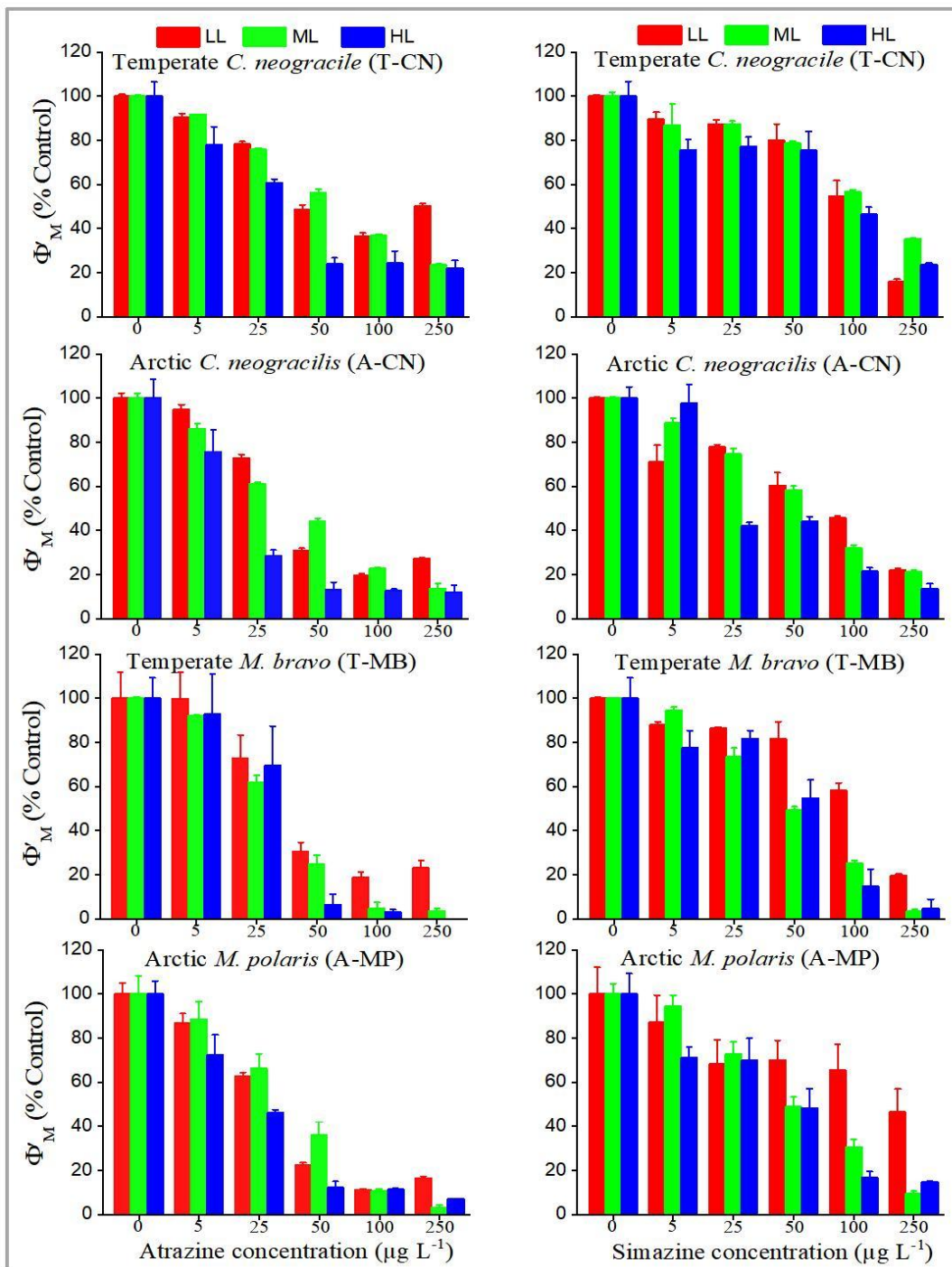
Figure 3. The effect of atrazine on ROS content of the studied species (temperate *C. neogracile* (T-CN), Arctic *C. neogracilis* (A-CN), temperate *M. bravo* (T-MB), and Arctic *M. polaris* (A-MP)) after 72 h exposure under LL, ML and HL. Data expressed as means  $\pm$  SD (n = 6).



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821 Figure 4. The effect of growth light intensity (LL, ML, and HL) on the PSII energy fluxes of temperate *C.*  
 822 *neogracile* (T-CN), Arctic *C. neogracilis* (A-CN), temperate *M. bravo* (T-MB), and Arctic *M. polaris* (A-  
 823 MP). Data expressed as means  $\pm$  SD (n = 6).

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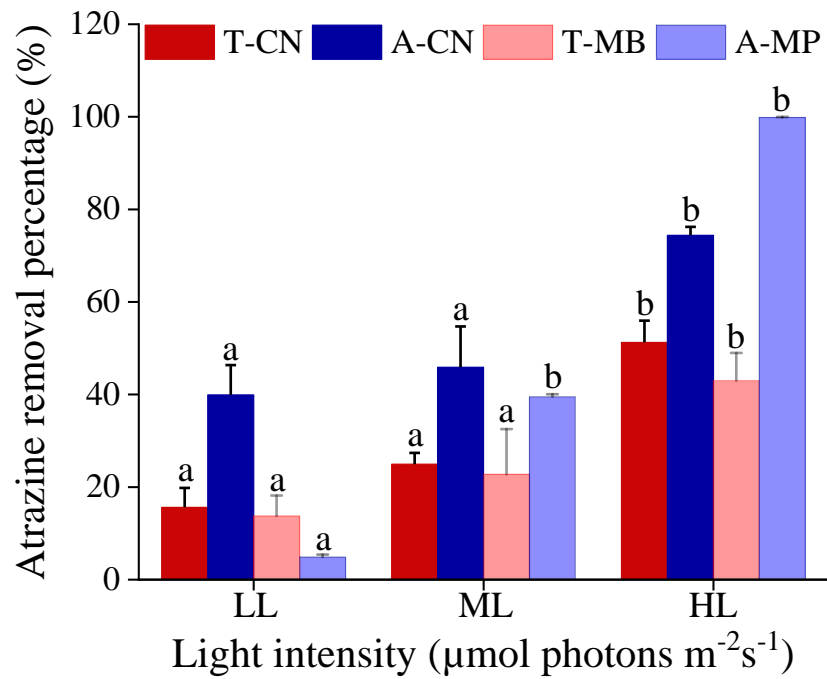
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Figure 5. The effect of atrazine and simazine on the PSII operational quantum yield ( $\Phi'_M$ ) of temperate *C. neogracile* (T-CN), Arctic *C. neogracilis* (A-CN), temperate *M. bravo* (T-MB), and Arctic *M. polaris* (A-MP) after 72 h exposure under LL, ML and HL conditions. Data expressed as means  $\pm$  SD (n = 6).

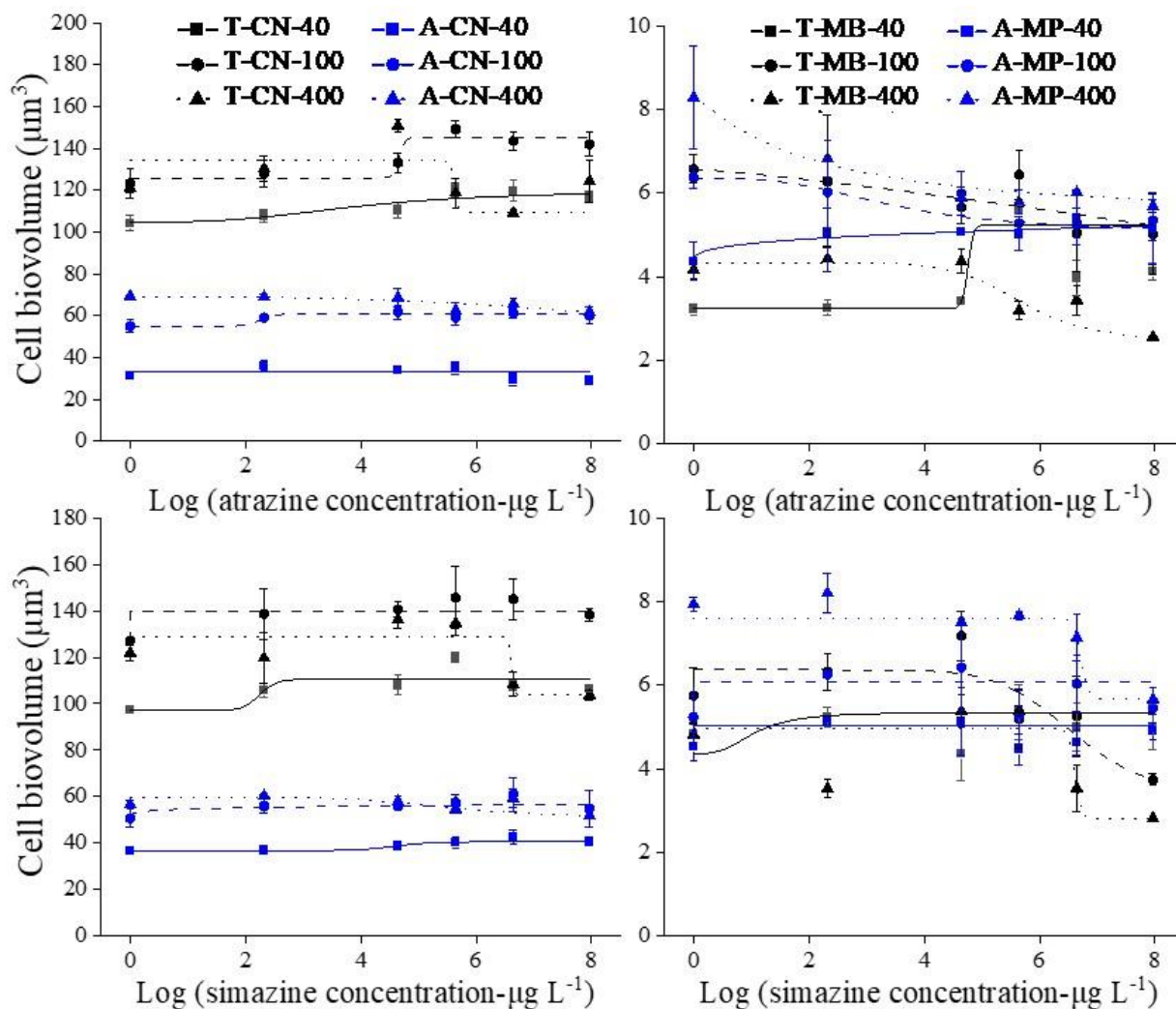


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832 Figure 6. The concentration of atrazine removed from the growth medium for (red color) temperate *C.*  
 833 *neogracile* (T-CN) and temperate *M. bravo* (T-MB), (blue color) Arctic *C. neogracilis* (A-CN) and Arctic *M.*  
 834 *polaris* (A-MP) after 72 h exposure to 50 μg L<sup>-1</sup> under LL, ML and HL conditions. Data expressed as means  
 835 ± SD (n = 6).

836

838 Figure S1. The effect of atrazine and simazine on the cell biovolume of the temperate *C. neogracile* (T-CN),  
 839 Arctic *C. neogracilis* (A-CN), temperate *M. bravo* (T-MB), and Arctic *M. polaris* (A-MP) after 72 h  
 840 exposure under LL, ML, and HL. Data expressed as means  $\pm$  SD (n = 6).



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843 Table S1. In this study, parameters of fluorescence were defined.

<b>Parameters</b>	<b>Definition</b>
$\Phi_M$	Maximal PSII quantum yield
$\Phi'_M$	Operational PSII quantum yield
NPQ	Non-photochemical quenching
NPQmax	Maximum ability for dissipation of excess energy
rETRmax	Maximum relative photosynthetic electron transport rate
$\alpha$	Maximum light efficiency use
$E_K$	Light saturation coefficient
<b>Specific energy fluxes (per <math>Q_A</math> reducing PSII RC)</b>	
ABS/RC	Absorption flux (of antenna Chls) per RC (also a measure of PSII apparent antenna size)
TRo/RC	Trapped energy flux (leading to $Q_A$ reduction) per RC
ETo/RC	Electron transport flux (further than $Q_A^-$ ) per RC
DIo/RC	Dissipated energy flux per RC
<b>Performance index</b>	
$PI_{ABS}=(RC/ABS)$	Performance index (potential) for energy conservation from photons absorbed by PSII to the reduction of intersystem electron acceptors

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