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1 **Herbivory increases on freshwater plants exposed to artificial light at night**

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16

17 **ABSTRACT**

18 Artificial light at night (ALAN) is recognized as one of the major anthropogenic pollutants

19 jeopardizing biodiversity at a global scale. Few studies have focused on the impacts of

20 nocturnal light on freshwater ecosystems despite their increasing exposure to light pollution

21 worldwide due to human activities along rivers or lakes. By modifying metabolism, the

22 disruption of natural light regime could modify the leaf palatability and affect the plant-
23 herbivore interactions which can partly determine the structure of macrophyte communities.
24 By experimentally mimicking light conditions from sub-urban areas, we measured the effects
25 of low-level light at night on the leaf traits of an aquatic plant, *Ceratophyllum demersum*, and
26 herbivory by the snail *Lymnaea stagnalis*, a generalist feeder. We demonstrate that artificial
27 light at night increases the herbivory rate: leaves of *C. demersum* that had been exposed to
28 ALAN for three months were 1.6 times more consumed by *L. stagnalis* than control leaves.
29 The increase in *C. demersum* palatability to the pond snail when plants were exposed to
30 ALAN was not due to a decrease in dry matter content or to a modification of the
31 biomechanical characteristics of the leaves. Because snails have not been exposed to
32 nocturnal light before or during the herbivory experiment, this increase in leave palatability, is
33 possibly linked to modifications of primary and/or secondary metabolism. Our results suggest
34 that ALAN is a potential key factor affecting ecological processes in aquatic ecosystems such
35 as trophic networks.

36

37 **1. Introduction**

38 Artificial light at night (ALAN) has dramatically increased because of the
39 development of urbanized areas and transport infrastructures during the past decades and is
40 emerging as a global threat to biodiversity (Hölker et al., 2010; Gaston et al., 2015). Although
41 less studied than their terrestrial counterparts, freshwater ecosystems are particularly exposed
42 from adjacent urban and sub-urban areas since human activities are frequently located along
43 rivers and lakes and are often exposed to ALAN (Secondi et al., 2017; Jechow and Hölker,
44 2019). ALAN is mainly known to affect the physiology and/or behaviour of a wide diversity
45 of animal groups (e.g., Ouyang et al., 2018; Touzot et al., 2020). Nocturnal light can also
46 affect plants and, more generally, primary producers in many ways, including altering the

47 growth, flowering time and abundance of flowering or the efficiency of photosynthetic
48 processes (e.g., Bennie et al., 2018; Massetti, 2018). These effects result in changes in species
49 abundance and distribution, community structure and consequently the functioning of
50 ecosystems (Hölker et al., 2015; Manfrin et al., 2017; Maggi et al., 2019). At this time, plant-
51 animal interactions have rarely been investigated in the ALAN context. Knop et al. (2017)
52 showed that artificial light at night disrupts nocturnal pollination networks and has negative
53 consequences for plant reproductive success. In a long-term study on experimental outdoor
54 mesocosms allowing interactions between grassland plants, invertebrate herbivores and
55 predators, Bennie et al. (2018) demonstrated that continuous exposure to ALAN within a
56 range typical of roadside vegetation under streetlights modified the population of plants and
57 invertebrates.

58 Several studies have emphasized the importance of herbivory on submerged
59 macrophytes in freshwater ecosystems (Lodge, 1991; Bakker et al., 2016). A recent meta-
60 analysis identified a median change in aquatic plant biomass due to herbivory of - 47%,
61 greater than terrestrial ecosystem (Wood et al., 2017). Hence, herbivory by aquatic
62 herbivores induces and can determine the structure of macrophyte communities (Jacobsen and
63 Sand-Jensen, 1992; Bakker et al., 2016; Wood et al., 2017). More recent works suggested that
64 a synergy between shading and herbivory may drive macrophyte decline and a shift to a turbid
65 state with lower biodiversity in aquatic ecosystems (Hidding et al., 2016). The herbivory rate
66 is partly linked to plant palatability, which itself depends on the mechanical and chemical
67 characteristics of food (e.g., Elger et al., 2005; Hanley et al., 2007; Descombes et al., 2020;
68 Dostálek et al., 2020). The amount and arrangement of cell wall compounds and the presence
69 of strengthening tissues determine leaf toughness, which is a critical mechanical defence
70 against herbivores (Clissold et al., 2006; Sanson, 2006; Ibanez et al., 2013). Onoda et al.
71 (2008) demonstrated a link between the diurnal light level to which plants are exposed and the

72 biomechanical traits of the leaves, such as leaf strength, toughness or stiffness. The
73 biosynthesis of structural compounds (cellulose, hemicellulose, and lignin) is dependent on
74 light (Su et al., 2005; Chen et al., 2002); for instance, experiments on *Arabidopsis thaliana*
75 revealed that several genes encoding enzymes involved in the synthesis of lignin monomers
76 displayed significant changes in transcript abundance over a diurnal cycle (Rogers et al.,
77 2005).

78 Exposure to ALAN could modify leaf mechanical properties such as toughness and
79 hence plant palatability and consumption by herbivores. Naturally, the aquatic environment is
80 dimmer than many terrestrial environments, as light is attenuated owing to surface reflection,
81 back-scattering and absorption by water, by non-biological particles and by dissolved
82 coloured compounds (Sand-Jensen, 1989). As a consequence, we could expect that
83 submerged aquatic plants are sensitive to low light levels and thus would respond
84 significantly to ALAN exposure. The objective of this study was to measure ALAN impacts
85 on morphological and biomechanical properties of freshwater aquatic plant and their possible
86 effects on herbivory. For this purpose, we used *Ceratophyllum demersum* L.
87 (Ceratophyllaceae), a hornwort, which is a submerged rootless macrophyte. It grows rapidly
88 in shallow, muddy, quiescent water bodies at low light intensities (Lauber et al., 2018) and is
89 known to be palatable to macroinvertebrates (Elger and Barrat-Segretain, 2002). Because
90 ALAN increases the illuminance period for *C. demersum* adapted to low illuminance, we
91 predicted that leaves produced under these conditions grow faster and differ from the control
92 leaves in their metabolism, dry matter content and toughness. We expected that these leaves
93 have a higher photosynthetic rate often associated with a lower investment in structural
94 compounds at the leaf level (Reich, 2014). As a consequence, ALAN may induced an increase
95 of the plant palatability and of the herbivory rate by freshwater gastropods.

96

97

98 **2. Materials and Methods**

99 *2.1. Plant sampling and experimental design*

100 Plants of *C. demersum* were sampled in an artificial pond located on the campus of
101 Lyon University in Villeurbanne, France (45°46'54.5"N 4°52'11.1"E) on 25 October 2017.
102 Once brought back to the laboratory, plants were carefully washed and placed in 30 plastic
103 aquaria (30 x 35 x 40 cm) containing 7.5 cm of river sand and 42 L of water. Two millilitres
104 of aquatic fertilizer (Ferropol, JBL GmbH & Co. Germany) was added every month. Aquaria
105 were placed on shelves covered with black synthetic sheets to control light intensity in a
106 climate room kept at $23 \pm 1^\circ\text{C}$ for 3 months.

107 The control group of plants ($n = 55$) did not experience ALAN, and the ALAN group
108 of plants ($n = 55$) was exposed to light at night. The light/dark cycle was set to 12:12 LD.
109 During the day, all plants were exposed to a PAR (photosynthetically active radiation) of
110 $38.86 \pm 7.43 \mu\text{mol m}^{-2} \text{s}^{-1}$ emitted by neon UV used for plants (OSRAM Fluora 77 18 watts,
111 Germany). The irradiance spectrum range was 300 - 700 nm (supplementary material 1A). At
112 night, the control group was left in the dark while the ALAN group was continuously exposed
113 to an average of $0.066 \pm 0.014 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Supplementary material 1B). Light for the ALAN
114 treatment was provided by LED ribbons (LED white hot waterproof Light Plus SC-TWF-
115 WW2) placed above each aquarium at 7.5 cm of the water surface. A light diffuser was added
116 to each LED ribbon to homogenize light emission. PAR was measured using a Jaz
117 spectrometer fitted with a 600 μm fibre, and Spectralon cosine corrector (CC-36UV-S, field
118 of view: 180° , wavelength range: 200 - 2500 nm, diffuser diameter: 3900 μm) (Ocean Optics).
119 The ALAN intensity (3 lux which corresponds to $0.066 \mu\text{mol m}^{-2} \text{s}^{-1}$ with our experimental
120 LED ribbons) mimics the light intensity recorded in urban and suburban areas (Gaston et al.,

121 2013). Light intensity was measured with a T-10A, Konica Minolta lux meter (sensitivity
122 threshold 0.01 lux) and checked every week.

123

124 2.2. Collection and breeding of the snails

125 We investigated herbivory using a common freshwater generalist feeder aquatic snail.
126 The gastropod snail *Lymnaea stagnalis* (Linnaeus, 1758) was chosen because this freshwater
127 generalist consumes submerged plant tissues, and it was successfully used in similar
128 experiments (Elger and Barrat-Segretain, 2002; Zhang et al., 2018). *L. stagnalis* was collected
129 from a cut-off channel of the Rhône River in October 2017. *L. stagnalis* individuals were
130 grown in the laboratory aquaria filled with tap water and placed in the same climate room as
131 the plants. They were fed an organic lettuce-based diet and allowed to breed. We used 100
132 animals from successive cohorts of the same age (3 months) at the date of the test. A 2-way
133 ANOVA with date, treatment and their interaction as factors was used to test the differences
134 in shell length (log-transformed) between the snails used to test plant palatability for the 2
135 treatments at the 3 dates. The effects of the interaction between date and treatment ($F_{2, 94} =$
136 0.58 , $P = 0.56$) and treatment ($F_{1, 94} = 0.58$, $P = 0.45$) indicated that the shell length of the
137 snails did not differ significantly between the sets of snails assigned to control plants and
138 plants having experienced ALAN. The effect of date was significant ($F_{2, 94} = 12.2$, $P < 0.001$)
139 as the snails used for the third date were slightly smaller (average shell length 32.02 ± 1.41
140 mm) than those used for the first two dates (average shell length 34.33 ± 2.47 mm and $34.37 \pm$
141 2.39 mm).

142

143 2.3. Herbivory experiments

144 The experiment was run on 3 dates in March-April 2018. Snails were individually
145 tested in nonchoice trials with control plants (n = 50) or ALAN-treated plants (n = 50). Each
146 snail was placed in a small glass container (diameter 7.5 x 10 cm) filled with 5.5 cm of
147 breeding water without food for the first 24 h at 18°C. In order to calibrate the herbivory
148 essay, we used only leaves of standard size that had fully grown during the experiment. These
149 leaves were taken on plants without signs of necrosis on the middle part of the plant. Leaves
150 on the basal and apical parts of the plant were discarded. Because snails could have eaten
151 epiphytes in preference to macrophytes (Brönmark, 1989), before herbivory experiment, plant
152 fragments were rinsed, and epiphytic algae were carefully removed with a paintbrush. Leaves
153 were blotted with a paper towel to remove excess water, weighed and placed in each container
154 with a single snail. On average, we put 100 mg (fresh weight) of leaf per container. In all
155 herbivory assays a maximum of 50 % of the plant material was consumed, thus *ad libitum*
156 food supply was guaranteed. The containers were exposed to the natural photoperiod. The
157 snails were removed after 24 h, the plant fragments remaining in each container were
158 collected and their fresh and dry masses (after oven-drying at 65°C for 48 h) were measured (
159 ± 0.0001 g). These data were used to calculate the amount of plant consumed expressed as the
160 difference between initial fresh mass and final fresh mass. The leaf dry matter content
161 (LDMC) was calculated for each sample as the ratio between the dry and fresh masses. We
162 used only undamaged leaves for measuring LDMC because an increase of DMC was often
163 observed after herbivory in plant apices that can be considered as an induced defence against
164 herbivore (Fornoff and Gross, 2014).

165 In an additional test, twenty individual samples were set up with water and control
166 *Ceratophyllum demersum* leaves, but without snail, to control the changes in mass which were
167 not caused by herbivory (e.g., plant growth or necrosis). After 24 hours under the same
168 temperature and photoperiod conditions as for the herbivory tests, the fresh mass of the plants

169 varied on average by -0.4 ± 1.6 mg. As a consequence, in agreement with Peterson and
170 Renaud (1989) concerning the short time feeding trial, we simply compared the initial
171 measures of food mass to final measures to produce quantitative estimates of consumption.

172

173 *2.4. Plant biomechanical and morphological traits*

174 Shearing tests were used to measure the specific work required to shear leaves (Ibanez
175 et al., 2013). Shearing tests measure the force required to cut the leaf lamina with a razor
176 blade. The test was performed at the end of the experiment (25/04/2018) on 15 mature leaves
177 for each treatment (control and ALAN), randomly chosen on 24 control plants and 25 plants
178 exposed to ALAN. Shearing tests were performed on a universal testing machine (Instron
179 5942, Canton, MA, USA) immediately after plant collection. A single stainless steel blade of
180 a straight razor (Dovo, Solingen, Germany) was mounted on the moving head of the testing
181 machine with an approach angle of 20° and with a constant speed downward of 10 mm/s. The
182 leaf, positioned on two supports (with a 15 mm span), was sheared by the blade moving
183 downward. The total work to shear (W_{shear} , Joules) corresponds to the area under the force–
184 displacement curve.

185 The width and cross-sectional area sheared were measured by making a thin cut
186 adjacent to the shearing plane. Images of the cuts were taken using a binocular microscope
187 (Leica MZ6) and a digital camera (Leica MC170 HD). The images were then analysed with
188 Leica Application Suite 4.3.3 software to calculate leaf width (mm), leaf cross-sectional area
189 (mm^2) and lacunar area (mm^2) (Fig. 1). These measurements were used to calculate the work
190 to shear per unit leaf width W_s (J/m) and the specific work to shear per unit leaf cross section
191 area SW_s (J/m^2). To take into account the high proportion of lacunae in leaves of aquatic
192 plants, SW_s were calculated using the effective cross sectional area (difference between the

193 cross-sectional area and the lacunar area, Fig. 1). This correction made it possible to quantify
194 the effective cross-sectional area of leaf supporting forces in shearing tests (Lamberti-Raverot
195 and Puijalon, 2012).

196

197 *2.5. Statistical analyses*

198 We used ANOVA to test the differences in herbivory rate and plant biomechanical and
199 morphological traits between light treatments for plants (control and ALAN). All variables were
200 tested for normality and heterogeneity of variance using Shapiro–Wilk and Bartlett’s tests,
201 respectively and they were log-transformed before analysis, when necessary, to improve the
202 normality of residuals and the homogeneity of variance. For herbivory rate (log-transformed)
203 and LDMC that were measured on several dates, 2-way ANOVA including light treatment
204 (control, ALAN), date and their interaction (light treatment x date) as factors, was used. Tukey
205 HSD was used as post-hoc test to carry out multiple comparisons between means. For the
206 biomechanical traits (Work to shear and Specific work to shear) and other leaf traits (leaf
207 diameter, leaf cross sectional area and percentage of lacunae) that were measured on only one
208 date, the effect of treatment was tested using one-way ANOVA or Welch ANOVA for variables
209 that did not meet the assumption of homoscedasticity after log-transformation (Work to shear
210 and Specific work to shear). All analyses and graphics were performed using R 3.5.2 software
211 (R Core Team, 2018).

212

213 **3. Results**

214 *3.1. Increase of consumption in plants that experienced ALAN*

215 The light treatment and the interaction between the date of experiment and light
216 treatment had a significant effect on the consumption of *C. demersum* by snail (Fig. 2; light
217 treatment: $F_{1,93} = 53.6$, $P < 0.001$; interaction between the date and the light treatment: $F_{2,93} =$
218 7.55 , $P < 0.001$), whereas the effect of date was not significant ($F_{2,93} = 2.63$, $P = 0.08$). The
219 plants that experienced ALAN during their growth were significantly more consumed by *L.*
220 *stagnalis* than the control plants for the dates 1 and 3 (Tukey HSD, $P < 10^{-3}$ and $P = 0.02$ for
221 the dates 1 and 3, respectively), with a consumption rate on average over the three trials 1.6
222 times higher than that of the control plants (Fig. 2) and did not significantly differ for the date
223 2 (Tukey HSD, $P = 0.4$). The significant effects of the date and the interaction between light
224 treatment and date were due to the lower consumption of the ALAN exposed plants on the
225 second date of the experiment.

226

227 3.2. ALAN did not modify the morphological and biomechanical traits of *C. demersum*

228 After 3 months of growth, the effects of the date of sampling and of the interaction
229 between date and treatment on the leaf dry matter content were significant (Fig 3A; ANOVA,
230 $F_{2,94} = 18.8$, $P < 0.001$, $F_{2,94} = 12.1$, $P < 0.001$, respectively) and the effect of the treatment
231 was not significant (Fig 3A; $F_{1,94} = 1.3$, $P = 0.3$): the leaf dry matter content was significantly
232 higher for the ALAN plants for the date 3 (Tukey HSD, $P < 10^{-3}$) and did not differ for the 2
233 other dates (Tukey HSD, $P = 0.32$ and $P = 0.99$ for the dates 1 and 2, respectively).

234 Leaf diameter (Fig. 3B), leaf cross sectional area (Fig. 3C) and percentage of lacunae
235 (Fig. 3D) were not significantly changed by light conditions at night (ANOVA, $F_{1,28} = 0.14$, P
236 $= 0.91$ for leaf diameter, $F_{1,28} = 0.05$, $P = 0.82$ for leaf cross sectional area, $F_{1,28} = 0.95$, $P =$
237 0.33 for the percentage of lacunae).

238 ALAN also did not modify the biomechanical traits of plants. Indeed, no differences in
239 work to shear (Fig 4A; ANOVA, $F_{1,19.8} = 1.91$, $P = 0.18$) or specific work to shear (Fig 4B;
240 ANOVA, $F_{1, 18.2} = 1.03$, $P = 0.32$) were observed between control and exposed plants.

241

242 **4. Discussion**

243 This study demonstrates that artificial light at night affects plant quality resulting in an
244 increase of consumption rate by *Lymnaea* gastropods. Indeed, *C. demersum* leaves that had
245 grown under artificial light at night were 1.6 times more consumed by aquatic molluscs than
246 control leaves. Because the snails had neither been exposed to ALAN before nor during the
247 herbivory experiment, which occurred without artificial light at night, the enhancement of the
248 herbivory rate can only be due to changes in plant palatability induced by nocturnal
249 conditions.

250 Firstly, an increase in leaf palatability suggests that the intensity of ALAN used in this
251 experiment is sufficient to induce modification of plant metabolism. In terrestrial urban and
252 sub-urban ecosystems, artificial light at night is present continuously during the nocturnal
253 period (Velez Ramirez, 2011), and its intensity is sufficient to induce photosynthesis and to
254 modify physiological responses affecting productivity and resource allocation (Aubé et al.,
255 2013). However, it is unclear whether the light levels typically measured in ALAN studies in
256 aquatic environments (Secondi et al., 2017; Jechow and Hölker, 2019), which are of low
257 intensity in comparison to sunlight, produce measurable effects on primary producers
258 (Grubisic et al., 2017). Five-month exposure to ALAN emitted by a high-pressure sodium
259 lamp at a light level of $0.08\text{-}0.09 \mu\text{mol m}^{-2} \text{s}^{-1}$ (corresponding to 6.8 - 8.5 lux) increased the
260 abundance of photoautotrophs (diatoms and cyanobacteria) in sediments (Hölker et al., 2015).
261 It has been shown that the ongoing transition from high-pressure sodium light to light

262 emitting diodes (LEDs) for street lighting increases the impact of ALAN on primary
263 producers (Grubisic et al., 2018). The minimum light intensity that supports growth based on
264 aerobic photosynthesis is considered to be between $0.01 \mu\text{mol m}^{-2} \text{s}^{-1}$ (approximately 0.5 lux)
265 and $0.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ (approximately 7.4 lux), slightly above the maximum light of a full moon
266 on a clear night ($0.005 \mu\text{mol m}^{-2} \text{s}^{-1}$, approximately 0.3 lux) (Raven and Cockell, 2006;
267 Grubisic et al., 2017). The intensity applied in our experiment (3 lux , $0.066 \pm 0.025 \mu\text{mol m}^{-2}$
268 s^{-1}) was consistent with these thresholds.

269 Several studies have suggested that plant palatability is linked to plant structural traits
270 (Hanley et al., 2007) and specifically to leaf dry matter content (Elger and Willby, 2003;
271 Elger and Lemoine, 2005). Leaf dry matter content increases in proportion to tissues with
272 thickened cell walls (sclerenchyma and collenchyma) and/or with the presence of heavy
273 elements such as mineral inclusions and is therefore associated with higher leaf toughness and
274 lower palatability (Ibanez et al., 2013; Perez-Harguindeguy et al., 2013). Our results showed
275 that the increase in *C. demersum* palatability to the pond snail when plants were exposed to
276 ALAN was not due to an increased leaf toughness measured with biomechanical parameters.
277 The results of LDMC were less clear. Although we used only leaves newly produced in our
278 experiment, the LDMC differs between dates and we cannot conclude for the impact of
279 ALAN on this trait. Elger and Lemoine (2005) showed that *L. stagnalis* prefers plants with
280 low LDMC. An increase of LDMC in apices of *Myriophyllum spicatum* has been
281 demonstrated as an inducible response to herbivory (Fornoff and Gross, 2014). It seems that
282 the increase of consumption observed in our study is not linked to a decrease of LDMC, but
283 more studies are necessary here.

284 We can hypothesize that the higher rate of herbivory observed in plants exposed to
285 ALAN is due to modifications of primary and/or secondary metabolism. Although the
286 intensity of ALAN is several orders of magnitude lower than that of daylight, it has been

287 suggested that it could support a marginally beneficial amount of additional photosynthesis by
288 the plant (Briggs, 2006). Low intensity of night-time light compared to that of direct sunlight
289 could be interpreted as a shaded environment by plants and this would induce faster growth, a
290 higher leaf extension rate and a higher photosynthesis rate (Briggs, 2006). ALAN may also
291 disrupt the circadian clock in plants which regulates various biological processes throughout
292 their life cycle. Dodd et al. (2005) showed that plants with a clock period matched to the
293 environment photoperiod contain more chlorophyll, assimilate more carbon, grow faster, and
294 survive better than plants with circadian periods differing from their environment
295 photoperiod. Modification of the day/night cycle can disrupt the accumulation of carbon
296 reserves in plants (Graf and Smith, 2011). A difference in free sugar content, or in the form of
297 the reserve, influences plant palatability, as the presence of carbonaceous reserves can be
298 attractive to some species of herbivores (Johnson and Lincoln, 1991). However, it has also
299 been proposed that ALAN could alter the circadian clocks of plants, especially those involved
300 in the repair and recovery of physiological processes that generally occur in the dark period
301 (Gaston et al., 2013). These effects could result in damage to the photosynthetic machinery,
302 leading, for example, to reduce photosynthetic capacities. Further studies are needed to
303 determine which characteristics of leaves involved in their palatability vary with ALAN.

304 Although testing other macrophyte species is required to generalize these results, the
305 increase in herbivory rate observed in our study may have important ecological consequences,
306 especially in urban and peri-urban freshwater ecosystems. Indeed, the change in plant
307 consumption induced by ALAN led to a leaf mass loss 30 % higher for plants exposed to
308 ALAN than control plants, representing possibly severe damage for the plant. In 2017, with a
309 meta-analysis, Wood et al. found that some invertebrate taxa such as molluscs, echinoderms,
310 and crayfish, have impacts on aquatic plants that equal or exceed those of vertebrate taxa. By
311 selectively grazing on palatable macrophyte species, invertebrate herbivores may influence

312 competitive interactions between plants and initiate important changes in the structure of
313 macrophyte communities (Van Donk, 1998; Gross et al. 2001; Wood et al., 2017). Although
314 mature plants are generally only partly damaged by invertebrate herbivores, their competitive
315 ability can be dramatically decreased by grazing. Indeed, herbivory often 1) changes plant
316 growth or morphology, thus changing access to resources, and 2) alters the relative abundance
317 of some species, thereby changing resource availability for competitors (Louda et al., 1990;
318 Gubisic et al., 2017).

319 Plant biodiversity is crucial for the maintenance of ecological balance and stable
320 trophic networks. Even if habitat loss, climate change, pollution, invasive species, and
321 overexploitation negatively affect all ecosystems, ALAN is emerging as another potential
322 threat to biodiversity (Bennie et al., 2015; Davies and Smyth, 2018). To that extent ALAN
323 affects ecological processes in aquatic ecosystems and alters trophic networks in the long-
324 term remains to be investigated.

325

326 **Declaration of Competing Interest**

327 The authors declare no conflict of interest.

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332

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494

495 **Table and Figure legends**

496 Table 1. Traits (leaf diameter, leaf cross sectional area, proportion of lacunae area, work to
497 shear and specific work to shear) measured on control plants (Control, n=15) or on plants
498 having experienced artificial light at night during three months (ALAN, n=15) (mean \pm sd)
499 and results of ANOVA used to test the difference between Control and ALAN plants.

500

501 **Fig. 1.** (A) Leaf cross-section showing the traits measured, (B) leaf width, (C), entire leaf
502 cross-sectional area and (D1-3) lacunar area.

503

504 **Fig. 2.** A) Consumption rates of *Lymnaea stagnalis* (mean \pm sd) and (B) Leaf dry matter
505 content (mg g^{-1}) on control *Ceratophyllum demersum* (Control) or on *C. demersum* exposed to
506 artificial light at night during three months (ALAN). The experiment was run at 3 dates (Date
507 1 n = 40, 2 n = 29 and 3 n = 30). B) Tukey HSD was used as post-hoc test to carry out
508 multiple comparisons between means. NS: non-significant, ***P < 0.001, **P < 0.01, *P <
509 0.05

510

511

512 Table 1.

513

	Control	ALAN	F _{df} ; P
	(mean ± sd)	(mean ± sd)	
Leaf diameter (mm)	1.04 ± 0.21	1.05 ± 0.16	F _{1, 28} = 0.14 ; P = 0.91
Leaf cross sectional area (mm ²)	0.74 ± 0.30	0.72 ± 0.19	F _{1, 28} = 0.05 ; P = 0.82
Proportion of lacunae area (%)	42.1 ± 5.38	44.1 ± 5.51	F _{1, 28} = 0.95 ; P = 0.33
Work to shear (J m ⁻¹)	0.36 ± 0.19	0.28 ± 0.09	F _{1, 19.8} = 1.91 ; P = 0.18
Specific work to shear (J m ⁻²)	968.6 ± 728.2	763.1 ± 287.1	F _{1, 18.2} = 1.03 ; P = 0.32

514

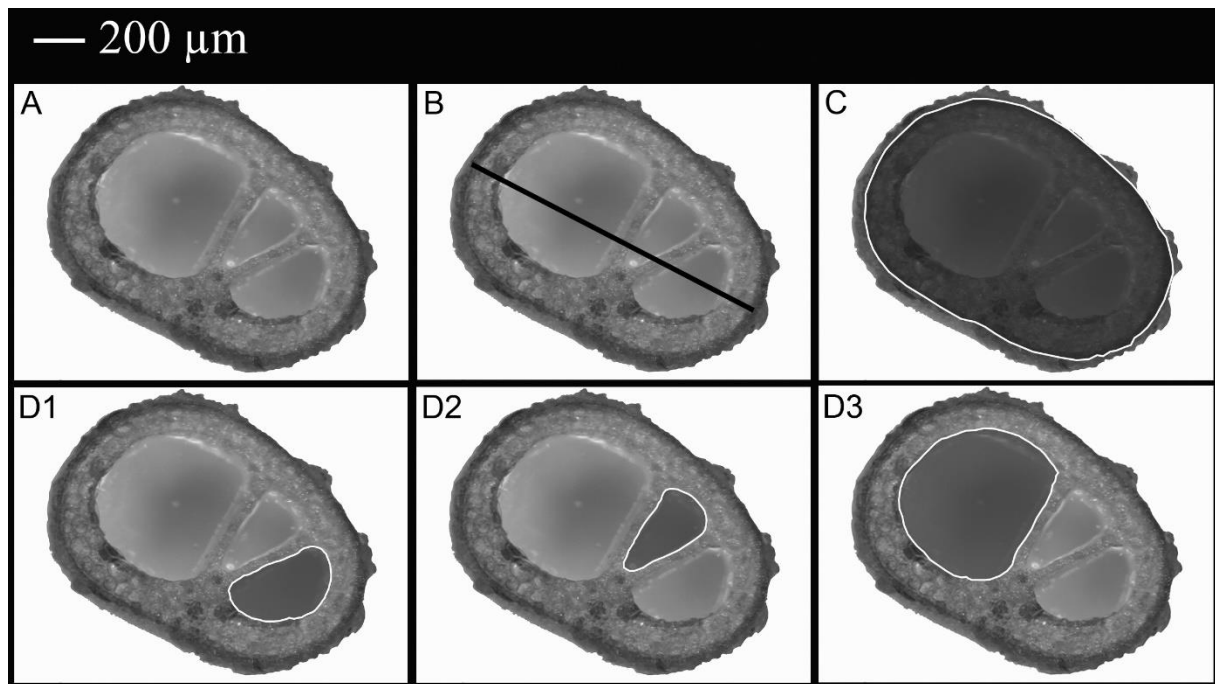
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516 specific work to shear) measured on control plants (Control, n = 15) or on plants having experienced
517 artificial light at night during three months (ALAN, n = 15) (mean ± sd) and results of ANOVA used
518 to test the difference between Control and ALAN plants.

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522 Figure 1



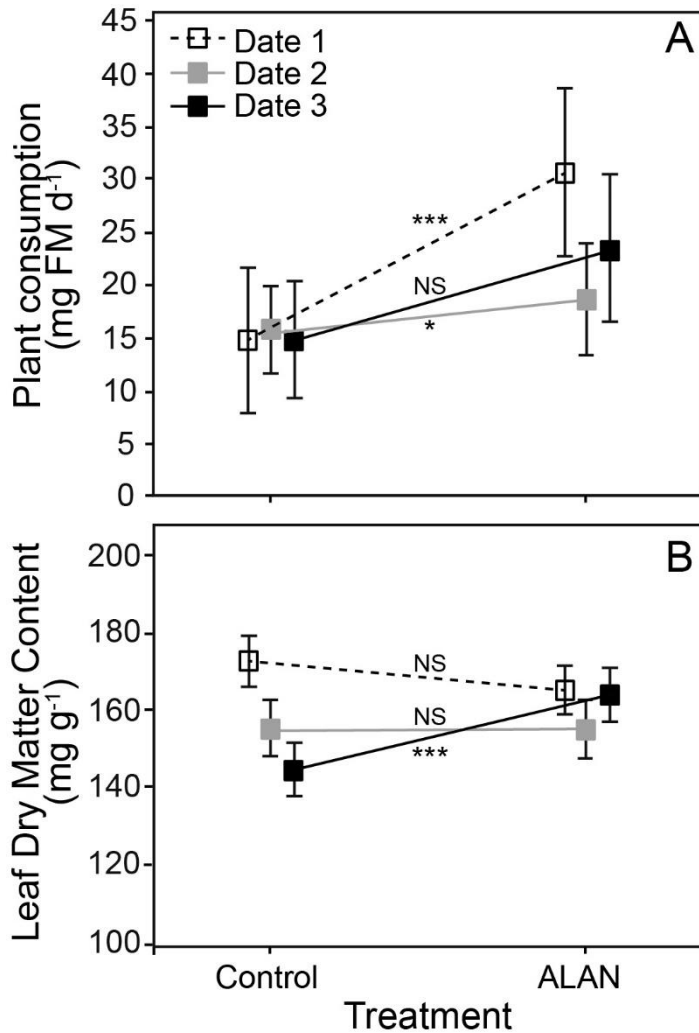
523

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525 cross-sectional area and (D1-3) lacunar area.

526

527

528 Figure 2



529

530

531 **Fig. 2.** A) Consumption rates of *Lymnea stagnalis* (mean \pm sd) and (B) Leaf dry matter
532 content (mg g⁻¹) on control *Ceratophyllum demersum* (Control) or on *C. demersum* exposed to
533 artificial light at night during three months (ALAN). The experiment was run at 3 dates (Date
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535 multiple comparisons between means. NS: non-significant, ***P < 0.001, **P < 0.01, *P <
536 0.05

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