

1 **Growth and physiological features of *Chaetomorpha linum* (Müller) Kütz. in**
2 **high density mats**

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12 **Abstract.** Seasonal changes of high density *Chaetomorpha linum* mats were studied in an eutrophic
13 coastal lagoon. We measured biomass and specific growth rate and analyzed water quality,
14 sediment labile organic matter (LOM), photosynthetic efficiency and pigments in two areas: one
15 subjected to sediment resuspension and algal mass shuffling through specific boats and the other
16 left undisturbed. Low disturbance enhanced algal survival under critical summer conditions, by
17 reducing LOM and promoting growth through thalli fragmentation. *Chaetomorpha linum* grew fast
18 and quickly acclimated its physiology to adverse conditions, although the mat underlayer showed
19 low photosynthetic efficiency. Nevertheless, the ability to respond to the adverse environment was
20 not sufficient to prevent the sudden algal decay, which occurred following a prolonged summer
21 stress. Present results may help to plan the lagoon management through boat operations, to attain a
22 more effective control over the algal growth and a more efficient removal of the mat.

23

24 **Key-words:** *Chaetomorpha linum*, photosynthetic pigments, photosynthetic efficiency,
25 eutrophication, lagoon, macroalgal blooms

26

27 **1. Introduction**

28

29 In the last thirty years, recurrent events of macroalgal bloom of opportunistic species have
30 occurred along the coasts, favored by eutrophication (Raffaelli et al., 1998; Morand and Merceron,
31 2005; Smetacek and Zingone, 2013) and probably also by climate change underway (Leemans and
32 Heickhout, 2004; Lloret et al., 2008). Particularly, many coastal lagoons have been affected by
33 these phenomena, due to the strong human impact and their structural and morphological
34 characteristics (Sfriso et al., 1987; Viaroli et al., 1996; Casabianca et al., 1997; Duck and da Silva,

35 2012). Some macroalgae may grow fast and give rise to high density mats, which results in serious
36 environmental problems. They are a source of labile organic matter (LOM) and are easily attacked
37 by bacteria, whereby their development and persistence, year after year, contributes to the
38 degradation of these coastal environments, leading to increasingly recurrent dystrophic processes,
39 that are fueled by the availability of LOM and the anaerobic activity of sulfate reducing bacteria
40 (Hargrave et al., 2008; Lenzi et al., 2011).

41 Extensive laboratory work has been carried out on the rate of nutrient uptake and on the
42 photosynthetic capacity of opportunistic species capable of producing high density macroalgal mats
43 (McGlathery et al., 1996; Pedersen and Borum, 1996; Lotze and Schramm, 2000; Menendez et al.,
44 2002; Anderson et al., 2002; Lapointe et al., 2005). However, natural environments are extremely
45 heterogeneous and differ for multiple variables, e.g. wind, solar radiation, water flow speed,
46 sediment state, etc.; hence, the responses of a macroalgal mass can vary just as much. High density
47 macroalgal mats may reach a thickness of several tens of centimeters and, in laminar environments,
48 the thalli may almost float at the surface of the water column: under these conditions, stratifications
49 of the physico-chemical variables and of dissolved nutrients can be frequent. Mat density and the
50 morphology of macroalgal thalli can affect the release of nutrients from the bottom and the water
51 quality (Lenzi et al., 2013).

52 *Chaetomorpha linum* (Müller) Kützing is an opportunistic species of Chlorophyceae that
53 frequently gives rise to abundant vegetative blooms (McGlathery et al., 1996; Bischof et al., 2006;
54 Lenzi et al., 2013; Gao et al., 2013). It thrives under eutrophic conditions, in particular with a high
55 availability of orthophosphate (Lavery and McComb, 1991b; Menendez et al., 2002). The mats of
56 this species may be particularly resilient and persistent (Gao et al., 2013; Lenzi et al., 2013, 2017).
57 Since 2005, a high density mat of *C. linum* is affecting an area of about 400 hectares in the
58 Orbetello lagoon (Tuscany, Italy), with a biomass that varies in space and time between 2 and 24 kg
59 m⁻². The mat is self-sustained: the died thalli are decomposed by bacteria and dinoflagellates, sink
60 into the mud and release the nutrients, while the surface of the mat grows almost continuously,
61 owing to the mild winters of these latitudes (Lavery and McComb, 1991a; Krause-Jensen et al.,
62 1996). It is hard to keep under control the overgrowth of the opportunistic macroalgae and to
63 prevent its negative effects in environments such as coastal areas, that are characterized by
64 increasingly crowding, intense production and recreational activities and pollution from industrial
65 and agricultural wastes conveyed by the rivers. A feasible solution to mitigate the consequences of
66 eutrophication is focusing efforts on a downstream management. Whatever the choices made in this
67 regard, like macroalgal harvesting (Lenzi, 1992; Runca et al., 1996; Guyoneaud et al., 1998) or their
68 fragmentation *in situ* and the artificial oxidation of sediments (Lenzi et al., 2015, 2017; Martelloni

69 et al., 2016), it is important to achieve a detailed knowledge of the conditions occurring in high
70 density mats and their specific characteristics. Particularly, it is necessary to study the growth and
71 physiology of the dominant macroalgae in relation to the trend of the main environmental factors.
72 This may help to identify the moment when the macroalgal mat enters a critical state, an
73 information of central importance to take the right decision about the time of intervention and the
74 techniques to be used.

75 In the present study, we performed field measurements in the Orbetello lagoon, in areas where
76 extensive and dense *C. linum* mats develop. The aims were to characterize the mat and some
77 physiological features of this species, to gain more insight into the dynamics of these algal masses
78 and their responses to the environment and to gather information to evaluate the impact of the
79 containment measures that are currently applied. In this regard, the work was carried out both on an
80 undisturbed area and on a lagoon section which underwent recurrent passages of the boats that are
81 presently used for harvesting the algal biomass and for sediment resuspension, within the ordinary
82 management practices of the lagoon.

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84 **2. Materials and methods**

85

86 *2.1. Study area and experimental design*

87 This study was conducted in the western basin of the Orbetello lagoon (Fig. 1), a shallow,
88 eutrophic coastal water body with an area of 25.25 km² in southern Tuscany (Italian west coast
89 42°25' - 42°29'N, 11°10'-11°17'E). It is an eutrophic lagoon due to fish-farm wastewater,
90 intermittent streams contaminated by agricultural run-off and civil effluents, and historical input
91 stored in sediment (Lenzi et al., 2003). This lagoon is subject to abundant macroalgal proliferation,
92 in the last 15 years mainly by *Chaetomorpha linum* (Müll.) Kütz., which can cause dystrophic
93 crises with die-offs.

94 In the period under study, an unexpected extensive dystrophic crisis occurred in the adjacent and
95 connected eastern basin: between summer and autumn there was the spread, in a part of the western
96 basin, of anoxic and reducing water with high turbidity, and then colored of intense green, due to
97 the proliferation of cyanobacteria (Fig. 1).

98 The high density mat of *C. linum* was characterized by analyzing the physiology of this species,
99 by measuring the growth and weight of the whole biomass stand and by evaluating the effect, on
100 these variables, of boat disturbance.

101 Two experimental areas were identified (A, B). Each of them had a high coverage of *C. linum*,
102 an extension of 2500 m² (50 x 50 m), and was marked with bamboo canes, 3 per side. They were

103 distant from each other about 500 m (Fig. 1). The schedule of samplings and measurements of all
104 variables considered. conducted between May 2015 and May 2016, is reported in Table 1. The area
105 B was located within the 400 hectares subjected to sediment resuspension using special boats (Lenzi
106 et al., 2017), while the area A was left undisturbed. Boat passages covered 9 of 15 sectors of about
107 25 hectares each, in which the entire area was divided and were 579 overall, i.e. 64 for each sector.
108 Although the frequency of the passages changed during the 13 months of activity, its average was
109 16.49%, which corresponded to a boat transit every 6 days. A boat passage caused a thorough
110 mixing of the mat, bringing the overlayer to the underlayer and vice versa, i.e. periodically exposing
111 the entire algal mass to sunlight.

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113 2.2. Water quality in macroalgal high density mats.

114 Dissolved oxygen (DO, mg/L), pH and temperature (T, °C) values were obtained by a
115 multiparameter probe (MP) on a fixed station, placed in the western basin inside a dense *C. linum*
116 mat, which sent the readings every 15 min, by radio (Fig. 1). Salinity (S, practical salinity scale)
117 was measured directly *in situ* during the monthly sampling by an ATAGO S/Mill refractometer.

118 Although the MP was close only to area B, the values that it recorded were thought to represent
119 correctly the environmental conditions of the western basin. Seasonal changes of S and T are
120 substantially uniform throughout the basin, excluding the areas close to the sea mouths, or very
121 marginal. Conversely, DO and pH in high density mats are subject to large fluctuations both in time
122 (from one day to another and within the same day) and space (even over short distances), therefore
123 we had planned to disregard these variables. However, the dystrophic phenomenon that occurred in
124 the eastern basin between July and August and the subsequent development of a cyanobacteria
125 bloom between September and December led to the intrusion of anoxic-reducing and highly turbid
126 waters in area A. This changed considerably the conditions between A and B, consequently we
127 recorded in A, between July and August, the values of DO and pH (by OxyGuard Handy Mk III
128 oxymeter and DELTA OHM HD8705 electronic digital pHmeter, respectively) and between July
129 and November, the values of nephelometric turbidity (NTU; by HANNA HI 93703-11 turbidity
130 meter), for two days a week, at 7:00 a.m. and 2:00 p.m.

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132 2.3. Mat density estimates

133 It is necessary to record the development (density and distribution) of the entire community of
134 species composing the stand, to evaluate the effectiveness of the strategies that are deployed to
135 restrain the build-up of the biomass. If such increase exceeds largely the daily rate of macroalgal
136 mass removal from the water, this management practice will be inadequate; alternately, these data

137 may serve to determine the amount and the frequency of biomass removal. Likewise, the estimation
138 of the whole stand growth is a prerequisite also for assessing the results of alternative management
139 practices, such as the resuspension of sediments and the direct disturbance of the stand. With these
140 goals, it is not mandatory to determine the growth of every single species present within the algal
141 stand, although it may be important to take into account which are the dominant ones. The most
142 relevant feature is that the whole biomass may be the fuel for dystrophic processes.

143 To measure the biomass of the *C. linum* stand, we tested a procedure of weighing on site, to
144 gather the data in a shorter time, for a more efficient management of the environment. Biomass
145 estimates (b) were carried out on 9 samples per area (A, B) and per trial (t1, t3, t5-t8; Table 1);
146 samples were collected using a square metal frame of 3600 cm² (60 x 60 cm) and were drained for
147 3 minutes and weighed directly in the field with a portable electronic balance with sensitivity ± 0.5
148 g. The values were then expressed in kg wet weight per square meter (kg_{ww} m⁻²), using conversion
149 factors, according to equation (I):

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$$151 \text{ kg}_{\text{ww}} 3600 \text{ cm}^{-2} \times 10^{-4} \times 2.778 = \text{kg}_{\text{ww}} \text{ m}^{-2} \text{ (I)}$$

152

153 2.4. Dry yield of *Chaetomorpha linum* samples

154 The masses of macroalgal thalli retain water in different quantities in relation to their
155 morphology. The wide laminar thalli of *Ulva laetevirens* and other associated species hold a large
156 quantity of water between a thallus sheet and the other, adding weight to a collected mass, because
157 of the interstitial water. Filamentous thalli of *C. linum* are stiffer than other filamentous species
158 such as some species of *Ulva* (ex *Enteromorpha*) or *Cladophora* spp. and can lose a lot of water by
159 dripping, once brought to the surface. Other species yet, e.g. *Valonia aegagropila*, can easily lose
160 inter-thallus water (ball pleustophytic thallus), but have high amounts of water inside the thallus,
161 yielding a relatively high weight when harvested. These features related to the morphology have
162 consequences in the estimates of biomass, expressed both as wet and dry weights. Whenever a
163 macroalgal stand is dominated by one opportunistic species, like *C. linum*, the weight/volume ratio
164 and the dry matter yield of the biomass are affected mostly by the features of that species. Dry
165 matter yield is an important parameter to take into account when the harvested biomass can be used
166 profitably, or to estimate the costs for transportation and disposal. These processes operate on an
167 industrial scale, because they involve the handling of thousands of tons of macroalgae: for this
168 reason, it is neither desirable, nor appropriate, to rely on laboratory data (i.e. estimations based on
169 small samples) when we must deal with the processing of very large amounts of biomass.
170 Moreover, managing very widespread algal stands requires fast estimations of the biomass, because

171 the density and distribution of the stands may change quickly: the availability of techniques of
172 measurement directly in the field, without the need for collecting samples and processing them in
173 the laboratory, is functional to the management of the lagoon.

174 In order to normalize the weight estimation conditions, relatively to *C. linum*, we performed
175 weighings in May 2015, on ten samples of about 2 kg each, as follows: 1) directly in the field after
176 dripping for 3 min (WW). The same samples were stored in plastic bags, transported to the lab and
177 subsequently weighed again (points 2, 3 and 4); 2) samples were left on a concrete floor for 2 hours,
178 in the shade, then weighed (SWW; standard wet weight for aquatic plants masses), according to
179 Bellan-Santini (1969); 3) samples were left under the sun until they appeared to be totally dried,
180 then weighed (DW); 4) algal samples were dried in an oven at 75 °C to constant weight (CDW).
181 Ratios between the different weighings were calculated, and also the weight of calcareous
182 concretions and zoobenthos was determined and kept into account.

183

184 2.5. *Sediment labile organic matter*

185 Five sediment samples were collected (at t1, t3 and t5-t8) from the upper 3 cm, both in A and B,
186 after having displaced the algal layer, with a 60 mL syringe, lowered from the boat and guided by
187 an appropriate device (Lenzi and Renzi, 2011). The samples were frozen until analysis. All samples
188 were wet sieved with fresh water (1 mm mesh), dried at 75 °C for 48 h and weighed. Labile organic
189 matter (LOM) was determined as loss of weight after combustion in a muffle furnace for 6 h at 250
190 °C (Loh, 2005).

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192 2.6. *Specific growth rate*

193 The specific growth rate (SGR; $g_{ww}\% d^{-1}$) expresses the daily growth rate of a population or a
194 biomass, here applied for *C. linum*, and is calculated by the equation (II):

195

$$196 SGR = (\ln W_f - \ln W_i) \times 100 \times t^{-1} \text{ (II)},$$

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198 where $\ln W$ is the natural logarithm of the initial (W_i) and final (W_f) macroalgal weight, and t the
199 time in days over which the growth was measured.

200 The operations were carried out at the same time in each area A and B, with six plasticized wire
201 cages 0.5 x 0.5 cm mesh, 50 m distant from each other, within which a weighed quantity of *C. linum*
202 was inserted (range 7.69 - 32.18 g). The cages were placed inside the high density mat, plunged in
203 the first 20 cm of the algal mass. The test was conducted in May 2015, in two periods of 10 days
204 each (May 8-18, and 18-28). For each test, weighings were made at the beginning of the experience

205 (SGR-0), after 5 days (SGR-5) and at the end (SGR-10): at each of these times, the cages were
206 collected and kept immersed in water while transferring them to the lab; the thalli were dried with
207 blotting paper, weighed, and, in the case of SGR-5, brought back under the same conditions to the
208 respective station.

209

210 2.7. *Photosynthetic efficiency and pigments*

211 All macroalgal samples for photosynthetic efficiency and pigments were collected at the same
212 hour (10:00 a.m.) in eight trials (t1- t8, Table 1), within the high density mat of the two areas A and
213 B. Samplings were always done in mats reaching a depth of 70-80 cm, and samples were collected
214 from two different layers of the mat in area A: from 0 to -20 cm, A-20; from -40 cm to -70 cm, A-
215 70. In area B, samples were taken only from the 0-20 cm layer (B-20), because B underwent
216 disturbance by boats. For each mat layer and each sampling time, three replications of about 2 kg of
217 thalli were put into black plastic bags and quickly transferred to the laboratory.

218 Macroalgal samples, collected and quickly transferred in laboratory, were put in plastic trays 50
219 x 30 x 10 cm (length x width x height), with about 5 L of lagoon water, to soak the algal thalli and
220 to spread them to form a thin layer (approximately 1 cm thick). The trays were covered with a black
221 cloth and, within 10 min, they were put in a dark room where the thalli were left to acclimate for 30
222 min at the same temperature as outdoor.

223 Photosynthetic efficiency was evaluated by analysing chlorophyll fluorescence by a portable
224 fluorometer (MINI-PAM Walz, Effeltrich, Germany). Measurements were carried out in the dark
225 room, on the thalli in the plastic trays. The readings were taken while keeping the tip of the optic
226 fiber of the instrument 1 cm above the floating thalli, with the help of a spacer. Twenty records
227 were taken for each replication. The maximum Photosystem II (PSII) quantum yield of dark-
228 adapted samples (F_v/F_m) gives an estimation of the potential photosynthetic efficiency of PSII: it was
229 automatically computed by the instrument and the final value for each thesis was the median of
230 sixty values (twenty readings per tray x three trays).

231 Three samples of algal thallus of about 50 g each were taken from each tray, just finished the
232 photosynthetic efficiency measurements, to determine the pigment concentrations. The material was
233 stored at -80 °C for about 18 hours, until analysis. This short period of conservation did not cause
234 any degradation of the pigments, as it was assessed by preliminary analyses on fresh and on frozen
235 samples (storage at at -80 °C for 24 hours; data not shown). Upon examination, the thalli were
236 quickly rinsed under tap water to remove any organisms and foreign matter, and dried on paper
237 towels. Five g of each sample were homogenized (homogenizer Ultra-Turrax IKA, Staufen,
238 Germany) in becher with 40 mL of acetone added with 1 g L⁻¹ CaCO₃. The homogenate was

239 transferred to a 50 mL falcon tube, and stirred at 4 °C for 2 h. The first extraction ended with a
240 centrifugation at 7000 xg at 4 °C for 10 min. The supernatants were stored at 4 °C, while the pellets
241 were extracted again and then centrifuged under the same conditions described above. The
242 supernatants were pooled with those from the previous extraction and the pellets were discarded.
243 The extracts were filtered on Whatman paper (n. 5, Maidstone, UK) and the volume was measured.
244 Using a spectrophotometer UV Mini 1240 (Shimadzu, Kyoto, Japan), the absorption spectrum of an
245 aliquot of 2 mL for each sample was recorded in the range of wavelengths $450 \text{ nm} < \lambda < 700 \text{ nm}$, to
246 verify the presence of impurities and particulates, and to assess if the concentration of the extract
247 was suitable for the subsequent quantitative readings. The latter were performed at the wavelengths
248 specific for each photosynthetic pigment (or pigment group): 661.6 nm for chlorophyll *a* (chl-a),
249 644.8 nm for chlorophyll *b* (chl-b) and 470 nm for total carotenoids (car). Three aliquots 2 mL each
250 for each sample were read and the values averaged. The data for each pigment (chl-a, chl-b, car)
251 and each thesis (A-20, A-70, B-20) were the median (\pm interquartile range) of 9 values (3 trays x 3
252 samples per tray). The concentration of each pigment, expressed in $\mu\text{g mL}^{-1}$, was computed
253 according to Lichtenthaler (1987). Subsequently, the values of concentration in $\mu\text{g mL}^{-1}$ were
254 converted into concentration per sample wet weight unit.

255

256 2.8. Statistical analysis

257 After checking for normality of distribution and homogeneity of variances, the values of the
258 physiological parameters of each sample were expressed as medians \pm interquartile ranges (IQR)
259 and analyzed by the Kruskal-Wallis test, followed by multiple comparisons by the Mann-Whitney
260 U test. The values of SGR of A area were compared with those of B area by a t-test; an analogous
261 comparison was carried out, within each area, between SGR recorded after 5 days and SGR at 10
262 days. For mat density and LOM data, one-way ANOVA followed by Tukey's multiple comparison
263 was performed to highlight significant changes in time of each parameter within each area.
264 Moreover, biomass and LOM data were compared, between A and B areas, at each sampling time
265 by a t-test. The correlations between the physiological parameters and the environment data, and
266 between algal growth and environment data were evaluated through the calculation of Spearman's
267 rank-order correlation coefficient. The environmental parameters that were used to calculate the
268 correlation coefficients were the respective average of the values recorded over 10 days prior to
269 each sampling. The level of significance was 0.05, unless otherwise stated.

270 Statistical analysis was performed using Past software version 3.00 for Windows
271 (<https://folk.uio.no/ohammer/past/>).

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3. Results

3.1. Water quality in macroalgal high density mats.

The daily ranges of environmental variables are reported in Table 2. Values show the establishment of extreme conditions in summer, with a drastic drop of dissolved oxygen (DO). Between early July and mid August, the temperatures of lagoon water were unusually high, with values that for two weeks exceeded 30 °C and reached peaks of 34 °C during the hottest hours. In proximity of B area, DO oscillated widely, between 0 mg L⁻¹, early in the morning and values of oversaturation at maximum sun irradiance. In A, DO values ranged between 0 and 2 mg L⁻¹, for three weeks between July and August. The lowest values of pH, although never below 7, were found in A, in August. Near B, turbidity levels were low, with substantially clear waters, except on a few days of July when there was a marginal intrusion of water from the eastern lagoon, pushed by the wind. Turbidity values were higher in A, initially as a result of the reducing-anoxic water coloration, then due to cyanobacteria development, owing to the intrusion of water from the eastern basin, which was pushed by the wind or displaced by the weak low tide that drew towards the western mouth of the lagoon. Salinity ranged between 35, in May 2015, and 40, in September 2015.

3.2. Mat density estimates

Macroalgal biomass ranged between $9.99 \pm 1.18 \text{ kg m}^{-2}$ in May 2015 and $2.59 \pm 1.29 \text{ kg m}^{-2}$ in November 2015, in A, and between $12.44 \pm 2.46 \text{ kg m}^{-2}$ in May 2016 and $5.57 \pm 1.14 \text{ kg m}^{-2}$ in February 2016, in B (Table 3). As shown in the Table, the two macroalgal mats in A and B displayed a different time course over the study period of one year. Mat A set off with high values and declined markedly following the summer environmental crisis: the biomass recorded in May and June 2015 was significantly more abundant than that of the following months. Mat B was stable throughout the examined period, and increased significantly in spring 2016. The density of the mat was higher in A than in B in spring 2015, but with the onset of summer such difference became weaker. The situation reversed following the summer crisis, with a greater density of the algal mat in B from autumn 2015 up to the end of the period under study.

3.3. Dry yield of *Chaetomorpha linum* samples

The results of the different determinations of wet and dry weights, the yields between the different weighings with respect to the original samples weighed in the field, and the yield for the samples dried at constant weight after cleaning the carbonate concretions are shown in Table 4.

307 From the Table, it may be observed: 1) the mean values recorded in the field (WW) overestimate of
308 about 52% the wet weight measured by the standard mode (wY(1-2) was $51.58 \pm 2.78\%$); 2)
309 industrial weight (DW) was recorded on samples that contained $21.24 \pm 3.83\%$ of residual moisture
310 (as indicated by the value of RM), which was lost after drying to constant weight (CDW); 3) the dry
311 yield (dY) between standard wet weight (SWW) and the respective constant dry weight (CDW) was
312 of $17.56 \pm 0.62\%$; 4) for *C. linum* collected in May in a high density mat, the constant dry weight
313 (CDW) recorded on samples bearing carbonate concretions, was overestimated by $6.08 \pm 0.45\%$
314 with respect to clean samples, as shown by the carbonate concretions (CC) estimates.

315

316 *3.4. Sediment labile organic matter*

317 Labile organic matter (LOM) values of the two areas (mean \pm SD; Table 3), were similar in
318 spring 2015, but then underwent marked fluctuations in A, with higher values than in B in late
319 spring 2015, autumn and winter. In B area the values of LOM were steady and did not differ
320 significantly throughout the period under study. The averages of all records in A ($12.31 \pm 5.06\%$)
321 and in B ($8.05 \pm 1.73\%$) were significantly different ($p < 0.001$).

322

323 *3.5. Specific growth rates*

324 Specific growth rates were calculated (mean \pm SD) in both areas A and B, for the first 5 days
325 (SGR-5), and for the whole period of 10 days (SGR-10), in May 2015, for each set of 6 cages by
326 area (Table 5).

327 The mean values of SGR-5 were substantially similar in A and B, and the same was found for
328 SGR-10. The dynamics of growth in time changed significantly only in A, where the values of
329 SGR-5 were higher than SGR-10, thus showing a slowdown of biomass increase. In 25% of the
330 cages, the SGR-5 values were higher than $7 \text{ g}_{\text{ww}}\% \text{ d}^{-1}$.

331 Taking together the SGR-5 data of the two subsequent experiences, they showed an average of
332 $5.38 \pm 2.43 \text{ g}_{\text{ww}}\% \text{ d}^{-1}$ (range 2.01 – 11.23 $\text{g}_{\text{ww}}\% \text{ d}^{-1}$), while those of SGR-10 showed an average of
333 $4.18 \pm 2.03 \text{ g}_{\text{ww}}\% \text{ d}^{-1}$ (range 1.01 – 7.47 $\text{g}_{\text{ww}}\% \text{ d}^{-1}$).

334

335 *3.6. Photosynthetic efficiency*

336 The heterogeneous dynamics of the algal biomass yielded series of data of different size. The
337 largest data-set was in B-20 (t2-t8), followed by A-20 (t1-t6), while the smallest one was in A-70
338 (t1-t5) (Fig. 2).

339 A-20 showed a relatively high and stable photosynthetic efficiency from t1 to t6, while the A-70
340 displayed lower F_v/F_m values, with a marked rise at the last sampling date. The F_v/F_m of B-20 was

341 not significantly different from that of A-70 at the start on t2, but it increased thereafter, becoming
342 similar to that of A-20, with the exception of the value recorded on t5. The last sampling dates (t7,
343 t8) were characterized by the decay of the algal mat in A site, and the data were recorded only in B-
344 20, which showed a high photosynthetic efficiency.

345

346 3.7. *Photosynthetic pigments*

347 The series of data of photosynthetic pigments corresponded to that of photosynthetic efficiency.
348 The time course of total chlorophyll (chl) concentration is determined by those of chl-a and chl-b;
349 since the latter were extremely similar, only total chlorophyll values are reported (Fig. 3). The A-
350 20 layer showed a low chl concentration in late spring (t1-t3), with an increase starting in early
351 summer (t4). B-20 displayed a relatively high chl concentration in late spring, with a marked
352 decline in summer (t4), followed by a gradual increase (t5-t8). The highest values of chl
353 concentration were found in A-70 layer, with peak on t4, without further remarkable changes.

354 The chl-a/chl-b ratio was slightly greater than 1 for almost all these (Fig. 4). The sole exception
355 was A-20 in spring (t1-t3), with values around 2, which however declined quickly with the onset of
356 summer and did not change notably thereafter.

357 The concentration of total carotenoids (car) showed a time course that was nearly the same as
358 that of chl in all the three layers (Fig. 5), yet the concentration of chl was generally higher than that
359 of car.

360 The chl/car ratio (Fig. 6) was low in A-20 in spring (t1-t3), thereafter it started to increase in
361 summer (t4, t5), and decreased slightly in November (t6). In B-20, chl/car ratio maintained values
362 around 4, with the sole exception of July (t4). This ratio remained stable between 4 and 5, in A-70.

363

364 3.8. *Correlations*

365 No significant correlations were found between physiological (Fv/Fm, chl, car, chl a/b, chl/car)
366 and water parameters averaged over 10 days prior to sampling (DO, T, pH, LOM) in A-20 and A-
367 70. In B-20, T was negatively correlated with chl, and DO positively correlated with chl and car
368 (Table 6). A significant negative correlation was found in A between biomass and LOM, while in B
369 macroalgal biomass was positively correlated with T (Table 7). The data of DO and pH collected in
370 A between July and August were not sufficient for a reliable calculation of a correlation coefficient
371 between these parameters and physiological variables and biomass.

372

373 **Discussion**

374

375 In this study we observed that the growth of *C. linum* can be massive and fast, as it was
376 evidenced by SGR values estimated for the top 20 cm of the algal mat. If 25% of *C. linum* in the
377 cages can grow from 7 to over 10 gww% d⁻¹, it means that a 400 hectares mat can produce several
378 hundreds of tons per day. Growth rate did not show the same pace after the first 5 days of the trial,
379 suggesting that the algal mat was probably starting to experience adverse environmental conditions
380 in the cages; otherwise, it may be possible that the values were underestimated and that the overall
381 slowdown of the rate of biomass increase recorded during the last 5 d was owed to the grazing by
382 isopods and gammarids, although they were not detected in the samples carried to the laboratory.
383 Yet, these estimates may yield a picture of the algal stands in the Orbetello lagoon more realistic
384 than the evaluation of the growth potential of the sole *C. linum* under controlled conditions may do.

385 The different procedures of weight determination of the *C. linum* masses allowed to standardize
386 the values in relation to the different uses of the algal material. These data allowed to compare, with
387 a good approximation, the weight recorded in the field with the more accurate values measured in
388 the laboratory. Both these classes of values may in turn be compared with the industrial weight,
389 because the latter can be assimilated to the DW: indeed, the industrial weight is determined on large
390 amounts of harvested algal material that have been left exposed to sun and wind and periodically
391 turned over. The comparison of the weight recorded in the field with the standard wet weight and
392 constant dry weight would allow an easier and faster estimate of vegetation for the determination of
393 the standing crop values, without the need to carry very large quantities of algal material to the
394 laboratory.

395 The algal masses of A and B responded differently to the adverse summer conditions. The mat A
396 was affected by anoxic-reducing water coming from the eastern basin for about one month and by
397 the subsequent intrusion of intensely murky waters due to cyanobacteria overgrowth, thus leading to
398 the progressive decay of *C. linum* mat. The deepest algal layer, i.e. A-70, should have undergone
399 the harshest conditions, which seems to be demonstrated by its early disappearance after September
400 2015. However, a decay of mat A was observed already in late spring, well before the intrusion of
401 water from the eastern basin, concurrently with an increase of LOM. The latter increased in A at
402 different times: its variability in time and space was probably due to the recurrent decay of the
403 underlying algal mat, which caused LOM production and its subsequent anaerobic decomposition.
404 In September there was a transient decrease of LOM in A, likely because the intense anaerobic
405 processes had affected the area, thereafter these processes increased as a consequence of the
406 relentless mat decay, that continued until February.

407 As to the B area, it can be hypothesized that the disturbance caused by the boats, that mixed the
408 mat layers, prevented the establishment of steep light gradients and anoxic conditions within the

409 mat. Boat action might have effectively enhanced LOM resuspension and mineralization,
410 contributing to increase the resistance of *C. linum* to the intense summer stress. Indeed, macroalgal
411 biomass in B was positively correlated with water temperature, although in July peaks of 34 °C
412 were recorded. This suggests that *C. linum* is remarkably tolerant to high temperatures, whereas in
413 Mediterranean eutrophic coastal lagoons other factors may be critical for its survival, such as light
414 and hydrogen sulphide. Therefore, *C. linum* might successfully face the challenge represented by
415 the foreseen rise of average and peak temperatures in the Mediterranean area (European
416 Environment Agency, 2017).

417 Also the steadiness of sediment LOM in B is attributable to the periodic oxidation of the top
418 sediment layer as a consequence of the boat disturbance. However, the boats did not operate on a
419 regular basis, because two out of four boats went damaged. Therefore, the frequency of
420 interventions was considerably reduced between July 2015 and February 2016, and this may have
421 led to LOM increase throughout the treated area of the mat, with the release of nutrients, as
422 observed also by Lenzi et al. (2017). Although it was not statistically significant, there was a rise of
423 LOM in B area following summer, that achieved the maximum value in November: this may have
424 been a consequence of the aforesaid reduced frequency of boat passages, as it appears to be
425 substantiated also by the lowering of LOM from November onward (Lenzi et al., 2017), which
426 coincided with a higher frequency of boat activity. The strong recovery of *C. linum* in Spring 2016
427 in B area may be explained by the marked ability of the alga to accumulate nutrients when they
428 become available (Lavery et al., 1991), especially phosphorus, which may frequently be limiting for
429 this Chlorophyta (Lavery and McComb, 1991a), and to exploit them to sustain growth when
430 conditions become favorable. Moreover, the fragmentation of thalli caused by the boat transit on a
431 dense mat may enhance the decay or the growth of the mat itself, probably depending on the
432 frequency of disturbance that has been imposed. Lenzi et al. (2015) and Martelloni et al. (2016)
433 observed the decay of *C. linum* mats following fragmentation, when the disturbance frequency was
434 31.58% (12 interventions over a period of 38 days) and 22.22% (60 interventions on a period of 270
435 days), respectively. In the present study the disturbance frequency was only 16.49% on average,
436 achieving the minimum during the period of LOM increase; such low disturbance frequency may
437 have favored, through the fragmentation of the thalli, the subsequent growth, as suggested by
438 Peckol and Rivers (1995) for *Cladophora vagabunda*.

439 From a physiological standpoint, A-70 samples showed a low photosynthetic efficiency with the
440 exception of September (t5), when the value increased, probably because of the reduction of the
441 total biomass in A, with the formation of large bare bottom areas, which could have allowed more
442 light to reach the deepest algal layer. *Chaetomorpha linum* showed a considerable ability to

443 acclimate to both low and high irradiance (Gomez et al., 2004), but the low values of F_v/F_m that
444 were detected in A-70 suggest that the thalli growing at this depth were not physiologically able to
445 fully tolerate the stress. Seaweeds may acclimate to different levels of irradiance also through
446 changes in the concentration of photosynthetic pigments (Dring, 1992). Total chlorophyll was high
447 in A-70 and this was a clear response to low irradiance, as already evidenced by Bischof et al.
448 (2006), although these Authors found higher amounts of these pigments in *C. linum*. Accordingly,
449 we also detected low values for chl-a/chl-b ratio and high values for chl/car ratio, that might have
450 contributed to the acclimation process of the alga to the prevailing light regime (Masojídek et al.,
451 2000; Yamazaki et al., 2005). *Chaetomorpha linum* might have acclimated to changing irradiance
452 also through energy spillover between the two photosystems (i.e. by rearranging the antenna
453 complexes), but this could not be detected through our experimental approach. Although the alga in
454 A-70 might have been able, at least partly, to tolerate the low irradiance, it could barely survive to
455 the intrusion of dystrophic water that occurred in summer. High temperatures, hypoxia and
456 sediment LOM increase that were recorded during summer and, likely, the release of acid volatile
457 sulphides, combined to exacerbate the environmental conditions in the deepest layer, leading the
458 biomass to die and decompose.

459 The consequences of the local dystrophic crisis and the flow of water from the eastern basin may
460 have damaged also the thalli in A-20 layer, through the decay of water quality and the increase of
461 turbidity due to the proliferation of cyanobacteria, that was recorded up to December (Lenzi et al.,
462 2016): these changes were probably the cause of the dramatic decline of A-20 layer that occurred
463 two months after that of A-70. The collapse of the macroalgal top layer in A area was not
464 anticipated by any significant decrease of the photosynthetic efficiency, which showed only slight
465 oscillations throughout the period under study.

466 In late spring 2015, the pigment profile of A-20 was characteristic of a sun-exposed vegetation,
467 showing low chl concentration and low chl/car ratio, which indicated that the photosynthetic
468 apparatus was protected from excessive light (Bonente et al., 2012). A further clue of the
469 acclimation to high irradiance is the high value of the chl-a/chl-b ratio, which demonstrates a
470 limited investment of resources in the antenna complexes, since chl-b is present only in light
471 harvesting complexes (Anderson, 1986). The values of such ratio recorded in late spring in A-20
472 were also higher than those generally found in marine green macro-algae (Yamazaki et al., 2015),
473 but with the onset of summer there was a sharp decline, concomitant with the intrusion of highly
474 turbid dystrophic waters from the eastern lagoon basin. Subsequently, the chl-a/chl-b ratio remained
475 low, while total chl and chl/car ratio increased. The response of the A top layer in summer appeared
476 to reverse with respect to spring, further suggesting that a change in the light environment had

477 occurred. This relatively prompt response to the supposed decrease of irradiance might have
478 contributed to sustain photosynthetic efficiency until November 2015. The thalli behavior in A-20
479 was an additional confirmation of the remarkable ability of *C. linum* to cope with the decrease of
480 light availability, although in the following months the alga was overwhelmed by the adverse
481 environmental conditions.

482 *Chaetomorpha linum* in B-20 showed a good photosynthetic efficiency, similar to that of A-20
483 during summer, coinciding with the reduction of the disturbance by boats and only in September it
484 was slightly lower. A lower frequency of mixing of the mat layers may be the reason for the higher
485 photosynthetic efficiency, because the thalli had a longer time to acclimate to the changes of
486 irradiance before the successive boat passage. Total chlorophyll and car significantly declined in B-
487 20 with the rise of T° and the decline of DO, and a transient decline of chl/car ratio was recorded in
488 July (t4); these physiological changes might have been an effective response to the seasonal high
489 irradiance.

490 Despite the thalli in A-20 underwent more critical conditions, their photosynthetic efficiency
491 between late summer and autumn (t5, t6) was higher than or similar to that in B-20. Nevertheless,
492 the algal mat of B showed a higher and longer survival, because the unforeseen intrusion of anoxic-
493 reducing waters negatively affected *C. linum* mostly in A area. It is reasonable that the higher
494 survival and the good physiological performance of B thalli may be attributable, in part, to the more
495 favorable environmental conditions established in this area by the boat transit, particularly in
496 summer, as suggested also by the lower values of LOM.

497 Macroalgal stands dominated by distinct species may exhibit different degrees of resistance and
498 resilience upon exposure to critical environmental conditions. Knowledge of these features is of
499 great importance when planning the management of an eutrophic lagoon for the prevention of
500 severe dystrophic crises, because it provides informations on which species will be more susceptible
501 to decay, thus helping to set the priority of the interventions. The mats of *C. linum* have
502 demonstrated to be highly resistant, probably because the cell walls of this species are rich in
503 cellulose (Kiran et al., 1980; George and Sebapathy, 2015). By contrast, the macroalgal stands
504 dominated by the Rhodophyceae Gracilariaceae or by the Chlorophyceae *Valonia aegagropila* C.
505 Agardh., have shown a low resistance to the elevated summer temperatures when they grow in
506 sedimentary beds rich of LOM (Lenzi, unpublished results); the chemical structure of their cell
507 walls, particularly that of the Gracilariaceae that are rich in sulphated polysaccharides (Balkan et
508 al., 2005), may be responsible for their vulnerability to bacterial attacks.

509

510 **Conclusions**

511

512 Although this work was partly upset by an unexpected event, some conclusions may still be
513 drawn: 1) a relatively low frequency of disturbance of a high density mat by large size boats,
514 capable of mixing the thalli to prevent their stratification and of resuspending in the water column
515 the high organic load sediment, can enhance the survival of the algal mat itself under the critical
516 conditions occurring in summer. Particularly, boat operation may contribute to reducing the LOM
517 and the consequent anaerobic bacteria activity; 2) fragmentation of the thalli due to the boat transit
518 on a dense mat may promote their growth when the disturbance frequency imposed is moderately
519 low; 3) *C. linum* may grow at a high rate and quickly acclimate its physiology to adverse
520 conditions, as observed in A-20 under the influx of turbid water and in B-20 as a result of mat
521 stirring; 4) in spring, the deepest layer (A-70) of the bank, despite the apparent acclimation to low
522 irradiance (suggested by the pigment profile of the thalli), showed a low photosynthetic efficiency,
523 which may have contributed to limit its growth; 5) decay of the mat can occur suddenly, after a
524 prolonged and persistent stress, despite the physiological parameters did not show any clear
525 warning sign; 6) *C. linum* appears to have the potential to respond to the adverse light environment
526 that it experiences whenever it grows in high density mats, in the Orbetello lagoon.

527 The harvest and removal of the algae become effective exclusively when the biomass taken away
528 from the lagoon exceeds a given percentage of the total. Such threshold must be determined
529 carefully, yet it will represent a significant proportion of the primary production and this may
530 seriously restrict the feasibility of these operations, without mentioning the cost of disposal, that
531 must be borne when the harvested biomass are not suitable for industrial processing. Sediment
532 resuspension and mat stirring, which eventually will cause also the fragmentation of the thalli, may
533 be effective in containing the overgrowth of macroalgae, as shown by Lenzi et al. (2011, 2015,
534 2017) and Martelloni et al. (2016). As suggested by the results of the present work and of Lenzi et
535 al. (2017), if these practices are not adequately commensurate to the dynamics of growth, they may
536 be beneficial for the mats, through the enhancement of their survival under unfavorable
537 environmental conditions and of their subsequent growth. Nevertheless, the technique of sediment
538 disturbance appears to be beneficial anyway, because it may hamper the progress of extensive
539 dystrophic processes within the algal stands, that are harmful for several organisms, including fish
540 species.

541

542 **References**

543

544 Anderson, J.M., 1986. Photoregulation of the composition, function and structure of thylakoid
545 membranes. *Ann. Rev. Plant Physiol.* 37, 93-136.

546 Anderson, D.M., Glibert, P.M., Burkholder, J.M., 2002. Harmful algal blooms and eutrophication:
547 nutrient sources, composition, and consequences. *Estuaries* 25, 704–726.

548 Balkan, G., Coban, B., Güven, K.C., 2005. Fractionation of Agarose and *Gracilaria verrucosa*
549 Agar and Comparison of Their IR Spectra with Different Agar. *Acta Pharmaceutica Turcica* 47,
550 93-106.

551 Bellan-Santini, D., 1969. Contribution à l'étude des peuplements infralittoraux sur substrat rocheux
552 (Etude qualitative et-quantitative de la frange supérieure). *Reel. Trav. Stn. Mar. Endoume* 63–47,
553 5–294.

554 Bischof, K., Rautenberger, R., Brey, L., Pérez-Lloréns, J.L., 2006. Physiological acclimation to
555 gradients of solar irradiance within mats of the filamentous green macroalga *Chaetomorpha*
556 *linum* from southern Spain. *Mar. Ecol. Prog. Ser.* 306, 165–175.

557 Bonente, G., Pippa, S., Castellano, S., Bassi, R., Ballottari, M., 2012. Acclimation of
558 *Chlamydomonas reinhardtii* to Different Growth Irradiances. *J. Biol. Chem.* 287, 5833-5847.

559 Casabianca de, M.L., Laugier, T., Marinho-Soriano, E., 1997. Seasonal changes of nutrients in
560 water and sediment in a Mediterranean lagoon with shellfish farming activity (Thau Lagoon,
561 France). *Journal of Marine Science* 54(5), 905-916.

562 Dring, M.J., 1992. *The Biology of Marine Plants*. Chapter 3 (Photosynthesis in the Sea, pp. 43-66).
563 Cambridge University Press, New York, pp 208.

564 Duck, R.W., Figueiredo da Silva, J., 2012. Coastal lagoons and their evolution: A
565 hydromorphological perspective. *Estuarine, Coastal and Shelf Science* 110 (2012), 2-14.

566 European Environment Agency, 2017. Climate change, impacts and vulnerability in Europe 2016.
567 An indicator-based report. Publications Office of the European Union, Luxembourg, pp 419.
568 doi:10.2800/534806

569 Gao, L., Zhanga, L., Houa, J., Weia, Q., Fua, F., Shao, H., 2013. Decomposition of macroalgal
570 blooms influences phosphorus release from the sediments and implications for coastal restoration
571 in Swan Lake, Shandong, China. *Ecological Engineering* 60, 19– 28.

572 George, J., Sebapathy, S.N., 2015. Cellulose nonocrystals: synthesis, functional properties, and
573 applications. *Nanotechnology, Science Applications* 8, 45-54

574 Gómez, I., López-Figueroa, F., Ulloa, N., Morales, V., Lovengreen, C., Houvinen, P., Hess, S.,
575 2004. Patterns of photosynthesis in 18 species of intertidal macroalgae from southern Chile. *Mar.*
576 *Ecol. Prog. Ser.* 270, 103–116.

577 Guyoneaud, R., De Wit, R., Matheron, R., Caumette, P., 1998. Impact of macroalgal dredging on
578 dystrophic crises and phototrophic bacterial blooms (red waters) in a brackish coastal lagoon.
579 *Oceanologica Acta* 21, 551-561.

580 Hargrave, B.T., Holmer, H., Newcombe, C.P., 2008. Towards a classification of organic enrichment
581 in marine sediments based on biogeochemical indicators. *Marine Pollution Bulletin* 56, 810–824.

582 Krause-Jensen, D., McGlattery, K., Rysgaard, S., Christensen, P.B., 1996. Production within dense
583 mats of filamentous macroalga *Chaetomorpha linum* in relation to light and nutrient availability.
584 *Marine Ecology Progress Series* 134, 207-216.

585 Lapointe, B.E., Barile P.J., Littler M.M., Littler D.S., 2005. Macroalgal blooms on southeast Florida
586 coral reefs II. Cross-shelf discrimination of nitrogen sources indicates widespread assimilation of
587 sewage nitrogen. *Harmful Algae* 4, 1106–1122.

588 Lavery, P.S., Lukatelich, R.J., McComb, A.J., 1991. Changes in the biomass and species
589 composition of macroalgae in a eutrophic estuary. *Estuarine, Coastal and Shelf Science* 33(1), 1-
590 22.

591 Lavery, P.S., McComb, A.J., 1991a. The Nutritional Eco-physiology of *Chaetomorpha linum* and
592 *Ulva rigida* in peel Inlet, Western Australia. *Botanica Marina* 34, 251-260.

593 Lavery, P.S., McComb, A.J., 1991b. Macroalgal-Sediment Nutrient Interactions and their
594 Importance to Macroalgal Nutrition in a Eutrophic Estuary. *Estuarine, Coastal and Shelf Science*
595 32, 281-295.

596 Leemans, R., Eickhout. B., 2004. Another reason for concern: regional and global impacts on
597 ecosystems for different levels of climate change. *Global Environ. Change* 14(3), 219-228.

598 Lenzi, M., 1992. Experiences for management of Orbetello Lagoon : eutrophication and
599 fishing. *Science of the Total Environment* (3 suppl.), 1189-1198.

600 Lenzi, M., Gennaro, P., Mercatali, I., Persia, E., Solari, J., Porrello, S., 2013. Physico-chemical and
601 nutrient variable stratifications in the water column and in macroalgal thalli as a result of high
602 biomass mats in a non-tidal shallow-water lagoon. *Marine Pollution Bulletin* 75, 98-104.

603 Lenzi, M., Leporatti-Persiano, M., Gennaro, P., Rubegni, F., 2016. Wind Mitigation Action on
604 Effects of Eutrophication in Coastal Eutrophic Water Bodies. *International Journal of Marine*
605 *Science and Ocean Technology* 3(2), 14-20.

606 Lenzi M., Leporatti-Persiano M., Gennaro P., Rubegni F., 2017. Artificial top layer sediment
607 resuspension to counteract *Chaetomorpha linum* (Muller) Kutz. blooms in a eutrophic lagoon.
608 Three years full-scale experience. *Journal of Aquaculture and Marine Biology* 5(2), 00114,
609 DOI: 10.15406/jamb.2017.05.00114.

610 Lenzi, M., Renzi, M., 2011. Effects of artificial disturbance on quantity and biochemical

611 composition of organic matter in sediments of a coastal lagoon. Knowledge and Management of
612 Aquatic Ecosystems 402, 08. doi: 10.1051/kmae/2011058

613 Lenzi, M., Renzi, M., Nesti, U., Gennaro, P., Persia, E., Porrello, S., 2011. Vegetation cyclic shift
614 in eutrophic lagoon. Assessment of dystrophic risk indices based on standing crop evaluation.
615 Estuarine, Coastal and Shelf Science. doi:10.1016/j.ecss.2011.10.006

616 Lenzi, M., Salvaterra, G., Gennaro, P., Mercatali, I., Persia, E., Porrello, S., Sorce, C., 2015. A new
617 approach to macroalgal bloom control in eutrophic, shallow-water, coastal areas. Journal of
618 Environmental Management 150, 456-465.

619 Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids: pigments of photosynthetic
620 biomembranes. Methods in enzymology 148, 350-382.

621 Lloret J., Arnaldo Mari´n, La´zaro Mari´n-Guirao. 2008. Is coastal lagoon eutrophication likely to
622 be aggravated by global climate change? Estuarine, Coastal and Shelf Science 78 (2008) 403-
623 412.

624 Loh PS (2005) An assessment of the contribution of terrestrial organic matter to total organic matter
625 in sediments in Scottish sea lochs, Ph.D. thesis, UHI Millenium Institute, 350.

626 Lotze, H.K., Schramm, W., 2000. Ecophysiological traits explain species dominance patterns in
627 macroalgal blooms. J. Phycol. 36, 287-295.

628 Masojídek J, Torzillo G, Kopecký J, Koblížek M, Nidiaci L, Komenda J, Lukavská A, Sacchi A
629 (2000). Changes in chlorophyll fluorescence quenching and pigment composition in the green
630 alga *Chlorococcum* sp. grown under nitrogen deficiency and salinity stress. Journal of Applied
631 Phycology 12: 417–426.

632 Martelloni T., Tomassetti P., Gennaro P., Vani D., Persia E., Persiano M., Falchi R., Porrello S.,
633 Lenzi M. 2016. Artificial soft sediment resuspension and high density opportunistic macroalgal
634 mat fragmentation as method for increasing sediment zoobenthic assemblage diversity in a
635 eutrophic lagoon. Marine Pollution Bulletin. doi: 10.1016/j.marpolbul.2016.06.060

636 Menendez M., Herrera J., Comin FA. 2002. Effects DO nitrogen and phosphorus supply on growth,
637 chlorophyll content and tissue composition of the macroalga *Chaetomorpha linum* (O.F. Mull.)
638 Kutz. in a Mediterranean coastal lagoon. Sci. Mar. 66 (4), 355-364.

639 Morand P, Merceron M (2005) Macroalgal population and sustainability. J. Coast. Res 21(5): 1009–
640 1020.

641 McGlathery KJ, Pedersen MF, Borum J (1996) Changes in Int1-acellular nitrogen pools and
642 feedback controls on nitrogen uptake in *Chaetomorpha linum* (Chlorophyta). J Phycol 32, 393-
643 401.

- 644 Pedersen M.F., Borum, J., 1996. Nutrient control of algal growth in estuarine waters. Nutrient
645 limitation and the importance of nitrogen storage among phytoplankton and species of
646 macroalgae. Mar. Ecol. Prog. Ser. 142, 261-272.
- 647 Peckol, P., Rivers, J.S., 1995. Physiological responses of the opportunistic macroalgae *Cladophora*
648 *vagabunda* (L.) van den Hoek and *Gracilariopsis tikvahiae* McLachlan, to environmental
649 disturbances associated with eutrophication. Journal of Experimental Marine Biology and
650 Ecology 190, 1–16.
- 651 Raffaelli, D.G., Raven, J.A., Poole, L.J., 1998. Ecological impact of green macroalgal blooms.
652 Oceanogr. Mar. Biol. Annu. Rev. 36, 97-125.
- 653 Runca, E., Bernstein, A., Postma, L., Di Silvio, G., 1996. Control of macroalgae blooms in the
654 Lagoon of Venice. Ocean Coast. Manag. 30, 135–143.
- 655 Sfriso, A. Marcomini, B. Pavoni 1987 Relationships between macroalgal biomass and nutrient
656 concentrations in a hypertrophic area of the Venice Lagoon Marine Environmental Research
657 Volume 22, Issue 4, 1987, Pages 297-312.
- 658 Smetacek, V., & Zingone, A. (2013). Green and golden seaweed tides on the rise. Nature, 504, 84–
659 88.
- 660 Viaroli, P., Bartoli, M., Bondavalli, C., Christian, R.R., 1996. Macrophyte communities and their
661 impact on benthic fluxes of oxygen, sulphide and nutrients in shallow eutrophic environments.
662 Hydrobiologia 329, 105–119.
- 663 Yamazaki J, Suzuki T, Maruta E, Kamimura Y (2005) The stoichiometry and antenna size of the
664 two photosystems in marine green algae, *Bryopsis maxima* and *Ulva pertusa*, in relation to the
665 light environment of their natural habitat. Journal of Experimental Botany, 56: 1517–1523.

666

667 Captions

668

669 Figure 1 – Lagoon of Orbetello with the two areas A and B (black squares) in which measures on
670 water, sediment and *Chaetomorpha linum* high density mats were carried out. MP (black dot),
671 position of multiparameter probe control unit. In light grey, the dystrophic area developed during
672 summer 2015; dotted line, the area of the western basin affected by the intrusion of anoxic-reducing
673 water from the eastern basin; in dark grey, the extension of the *Chaetomorpha linum* high density
674 mat.

675

676 Figure 2 - Maximum photosynthetic efficiency of PSII. Each value represents the median of 60
677 measurements \pm interquartile range. t1, 9-May 2015; t2, 30 May; t3, 20 June; t4, 11 July; t5, 8

678 September; t6, 10 November; t7, 4 March 2016; t8, 31 May. Different letters indicate statistically
679 significant differences between theses at each sampling date ($p < 0.05$).

680

681 Figure 3 - Total chlorophyll concentration in *C. linum*. Each value represents the mean of 3
682 replications \pm SD. t1, 9-May 2015; t2, 30 May; t3, 20 June; t4, 11 July; t5, 8 September; t6, 10
683 November; t7, 4 March 2016; t8, 31 May. Different letters indicate statistically significant
684 differences between theses at each sampling date ($p < 0.05$).

685

686 Figure 4 - Chlorophyll a to chlorophyll b ratio in *C. linum*. Each value represents the mean of 3
687 replications \pm SD. t1, 9-May 2015; t2, 30 May; t3, 20 June; t4, 11 July; t5, 8 September; t6, 10
688 November; t7, 4 March 2016; t8, 31 May. Different letters indicate statistically significant
689 differences between theses at each sampling date ($p < 0.05$).

690

691 Figure 5 - Total carotenoid concentration in *C. linum*. Each value represents the mean of 3
692 replications \pm SD. t1, 9-May 2015; t2, 30 May; t3, 20 June; t4, 11 July; t5, 8 September; t6, 10
693 November; t7, 4 March 2016; t8, 31 May. Different letters indicate statistically significant
694 differences between theses at each sampling date ($p < 0.05$).

695

696 Figure 6 - Total chlorophyll to total carotenoid ratio in *C. linum*. Each value represents the mean of
697 3 replications \pm SD. t1, 9-May 2015; t2, 30 May; t3, 20 June; t4, 11 July; t5, 8 September; t6, 10
698 November; t7, 4 March 2016; t8, 31 May. Different letters indicate statistically significant
699 differences between theses at each sampling date ($p < 0.05$).

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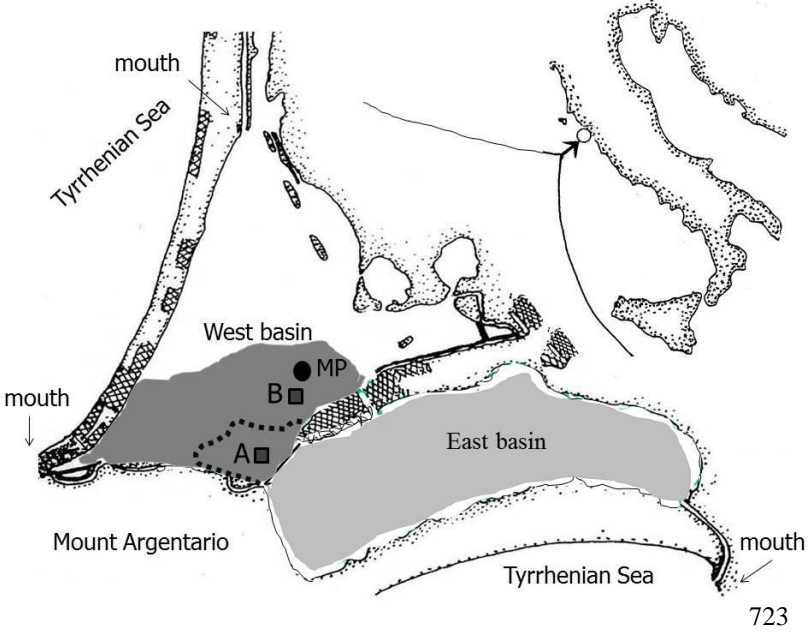
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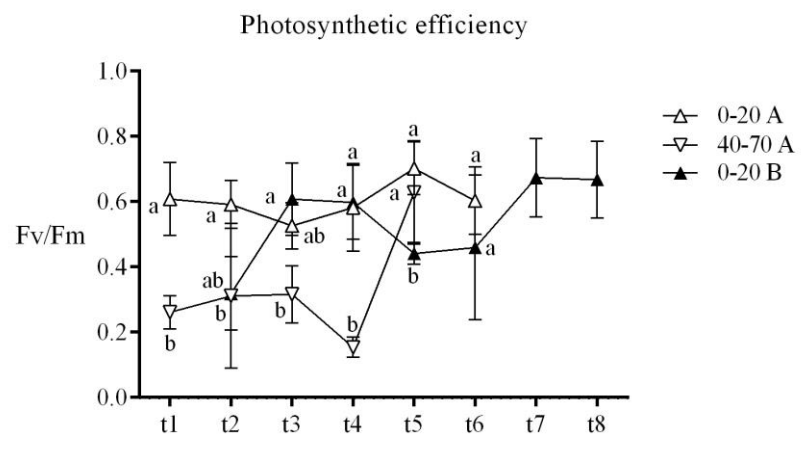
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Fig. 1



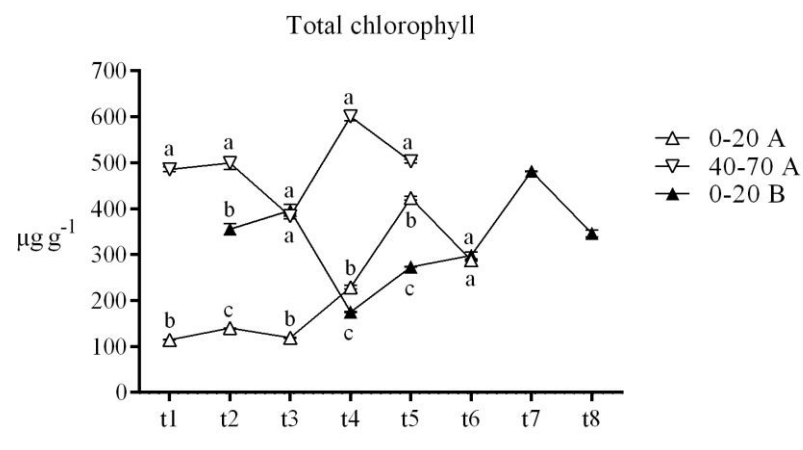
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Fig. 2



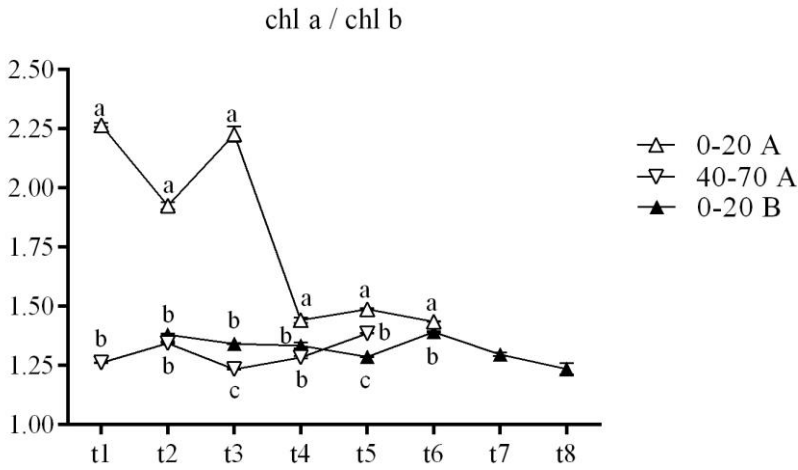
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Fig. 3



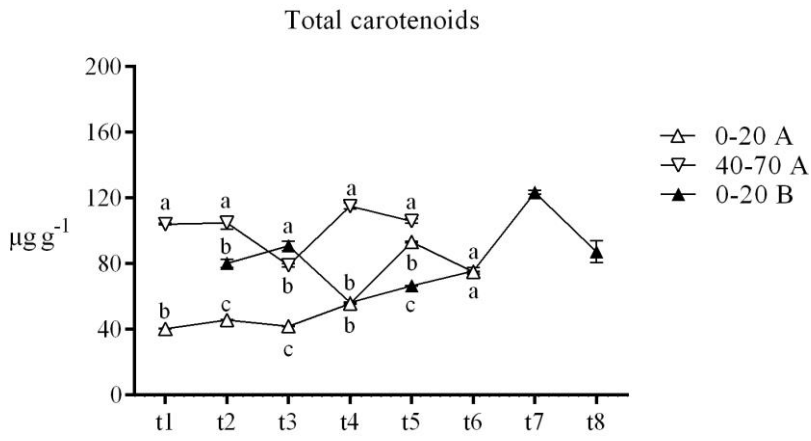
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Fig. 4



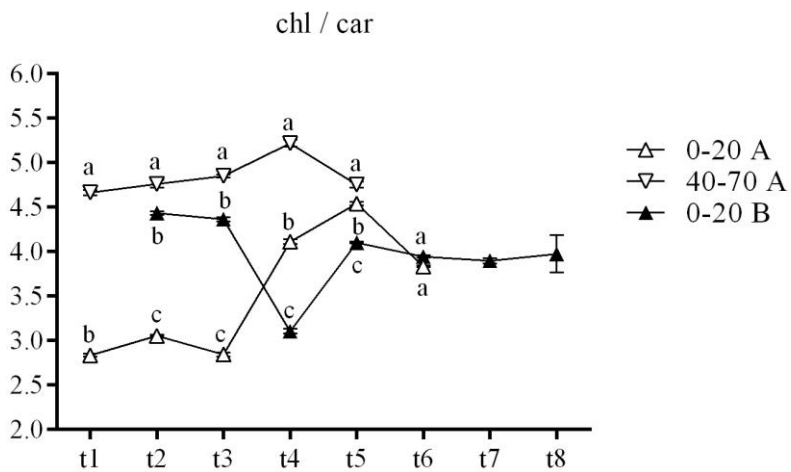
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Fig. 5



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Fig. 6



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780 Table 1 - Schedule of samplings and measurements of all variables considered, during all trials (t1-
 781 t8, and corresponding month), conducted in the areas A and B. Ph.E, photosynthetic efficiency, and
 782 Ph.P, photosynthetic pigments (20cm, 70cm deep); b, biomass; LOM, labile organic matter; w,
 783 different weighing types of *C. linum*; SGR, specific growth rate; pH; DO, dissolved oxygen; T,
 784 temperature; S, salinity; NTU, nephelometric turbidity unit. Unplanned measures were taken in
 785 August.
 786

		Ph.E	Ph.P	b	LOM	w	SGR	pH		DO		T	S	NTU	
		A	B	A,B	A,B	A, B	A,B	A	B	A	B			A	B
9 May 2015	t1	20, 70		x	x	x	x			x	x	x	x		
30 May	t2	20, 70	20			x	x			x	x	x	x		
June	t3	20, 70	20	x	x					x	x	x	x		
July	t4	20, 70	20					x	x	x	x	x	x	x	x
August								x	x	x	x	x	x	x	x
September	t5	20, 70	20	x	x					x	x	x	x	x	x
November	t6	20	20	x	x					x	x	x	x	x	x
March 2016	t7		20	x	x					x	x	x	x		
May	t8		20	x	x					x	x	x	x		

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790 Table 2 – Environmental variables measured inside a dense mat of *C. linum* in the western basin of
 791 the lagoon in the study period. DO, dissolved oxygen; S, salinity; NTU, nephelometric turbidity.
 792 DO, T and pH were recorded by a multiparameter probe (MP) located near B area; the values
 793 reported are the average of the recordings taken over 10 days prior to each sampling date. S was
 794 measured near B area. Extra measurements of DO and pH were carried out within an algal mat in A
 795 area during the dystrophic crisis that occurred in summer 2015 (t4 and August).
 796

	DO, mg L ⁻¹				T °C		pH		S	NTU	
	A		MP		MP		A	MP		A, B	A
	range	mean ± SD	range	mean ± SD	range	mean ± SD	range	range			
t1			3.8 - 7.0	8.4 ± 0.9	20 - 23	21.7 ± 0.9		8.4 - 8.7	35		
t2			0.9 - 7.8	8.0 ± 0.9	19 - 25	21.9 ± 1.7		8.5 - 9.0	35		
t3			0.8 - 7.7	5.5 ± 1.4	24 - 29	26.1 ± 0.6		8.0 - 8.6	37		
t4	0.0 - 6.2	1.8 ± 2.4	0.0 - 8.0	4.5 ± 3.4	29 - 34	31.2 ± 0.5	7.1 - 8.2	8.0 - 8.4	39	2 - 73	2 - 22
Aug.	0.0 - 7.4	3.2 ± 2.7	0.0 - 8.5	4.2 ± 4.2	26 - 32	30.8 ± 1.1	7.0 - 8.1	7.7 - 8.2	39	10 - 17	1 - 5
t5			1.5 - 7.4	5.0 ± 1.2	23 - 28	27.5 ± 1.5		7.6 - 8.1	40	15 - 17	1 - 3
t6			4.8 - 10.0	8.8 ± 1.2	17 - 18	17.4 ± 0.7		8.0 - 8.4	37	11 - 17	1 - 2
t7			8.8 - 13.1	9.4 ± 1.4	14 - 17	13.2 ± 1.2		8.1 - 8.5	36		
t8			1.0 - 15.0	8.8 ± 1.4	23 - 27	24.0 ± 1.5		8.7 - 9.3	36		

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800 Table 3 - Macroalgal biomass (b, kg m⁻²) and sediment labile organic matter (%) in the
 801 experimental areas. Each value is the mean of 9 measurements ± SD for b and 5 measurements ±
 802 SD for LOM. Different letters indicate significant differences within each time series of a single
 803 area. Asterisks indicate significant differences between the two areas at each sampling date, for b
 804 and LOM (**p* < 0.05; ***p* < 0.01; ****p* < 0.001).
 805

	A	B	A	B
	b, kg m ⁻²	b, kg m ⁻²	LOM %	LOM %
t1	9.99 ± 1.18 a***	5,66 ± 1.91 b	8.96 ± 0.53 c	8.50 ± 1.08 a
t2	8.12 ± 3.37 ab	6,68 ± 2.45 b	13.41 ± 4.12 abc*	7.48 ± 1.30 a
t5	5.89 ± 0.16 bc	7,63 ± 4.38 b	8.06 ± 0.28 c	8.84 ± 1.45 a
t6	2.59 ± 1.29 d***	7,04 ± 0.77 b	15.40 ± 3.43 ab*	9.05 ± 2.57 a
t7	2.70 ± 0.99 d***	5,57 ± 1.14 b	18.25 ± 5.52 a**	7.66 ± 0.83 a
t8	3.70 ± 1.03 cd***	12,44 ± 2.46 a	9.77 ± 3.47 bc	6.45 ± 0.97 a

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810 Table 4 – WW (1), wet weight (g ± SD) of samples weighed directly in the field after dripping for 3
 811 min; SWW (2), standard wet weight, wet weight of the same samples left exposed to free air, in the
 812 shade, on a concrete floor for 2 hours; DW (3), dry weight of the same samples dried in the sun
 813 until total apparent drying; CDW (4), samples dried-up in the sun and then dried again in the oven
 814 to constant weight at 75°C; wY (1-2), wet yield (% ± SD) between WW and SWW; idY (1-3),
 815 industrial dry yield between WW and DW; dY (2-4), dry yield between SWW and CDW; RM (3-4),
 816 residual moisture between industrial dry weight and constant weight; dY (1-4), dry yield between
 817 WW and CDW; CC, percentage (± SD) of carbonate concretions on CDW.
 818

g	1	WW	1297.70 ± 89.94
	2	SWW	666.70 ± 23.17
	3	DW	148.70 ± 1.57
	4	CDW	117.10 ± 5.20
%	1-2	wY	51.58 ± 2.78
	1-3	idY	11.52 ± 0.83
	2-4	dY	17.56 ± 0.62
	3-4	RM	21.24 ± 3.82
	1-4	dY	9.04 ± 0.22
		CC	6.08 ± 0.45

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824 Table 5 - Specific growth rate ($g_{ww}\%$ d^{-1}) calculated in May 2015 in two 10-day trials each, with
 825 measurements every 5 days (SGR-5, SGR-10). Each value is the mean of 6 measurements \pm SD.
 826 Asterisks indicate significant differences between columns ($p < 0.05$).
 827

		SGR-5	SGR-10
A	8-18 May	4.52 \pm 1.49	2.86 \pm 1.37
	18-28 May	6.62 \pm 0.45	5.16 \pm 0.24
B	8-18 May	5.27 \pm 3.25	4.37 \pm 2.97
	18-28 May	5.43 \pm 2.78	4.35 \pm 1.70
A	all records	5.57 \pm 1.51*	4.01 \pm 1.51
B	all records	5.35 \pm 3.02	4.36 \pm 2.42

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860 Table 6 - Correlation between physiological (Fv/Fm, chl, car, chl a/b, chl/car) and water parameters
 861 averaged over 10 days prior to sampling (dissolved oxygen, mean temperature, pH, labile organic
 862 matter), expressed as Spearman's rank-order coefficient and the related *p* value. Dissolved oxygen
 863 and pH data collected in A between July and August were not sufficient for a reliable calculation of
 864 the correlation coefficient. Significant correlations in bold.
 865

	A-20		A-70		B-20	
	rho	<i>p</i> value	rho	<i>p</i> value	rho	<i>p</i> value
Fv/Fm vs DO					0.486	0.271
Fv/Fm vs T°	-0.281	0.756	-0.032	0.959	-0.250	0.594
Fv/Fm vs pH					0.214	0.594
Fv/Fm vs LOM	-0.673	0.213	-0.632	0.502	-0.580	0.233
chl vs DO					0.667	0.041
chl vs T°	0.285	0.669	0.677	0.343	-0.679	0.038
chl vs pH					0.321	0.444
chl vs LOM	-0.051	0.962	-0.051	0.924	-0.221	0.673
car vs DO					0.721	0.039
car vs T°	0.325	0.489	0.653	0.373	-0.642	0.109
car vs pH					0.357	0.445
car vs LOM	-0.066	0.862	-0.105	0.786	-0.145	0.778
chl a/b vs DO					0.054	0.922
chl a/b vs T°	-0.118	0.780	0.366	0.634	-0.286	0.498
chl a/b vs pH					-0.107	0.782
chl a/b vs LOM	-0.616	0.354	-0.632	0.561	0.464	0.372
chl/car vs DO					-0.091	0.859
chl/car vs T°	0.429	0.325	0.695	0.183	0.045	0.923
chl/car vs pH					0.321	0.441
chl/car vs LOM	-0.205	0.767	0.737	0.334	-0.058	0.931

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878 Table 7 - Correlation between fresh biomass and water parameters averaged over 10 days prior to
 879 biomass estimation (dissolved oxygen, mean temperature, pH, labile organic matter), expressed as
 880 Spearman's rank-order coefficient and the related *p* value. Dissolved oxygen and pH data collected
 881 in A between July and August were not sufficient for a reliable calculation of the correlation
 882 coefficient. Significant correlations in bold.
 883

	A		B	
	rho	<i>p</i> value	rho	<i>p</i> value
b vs DO			-0.012	0.981
b vs T°	0.429	0.356	0.771	0.028
b vs pH			0.086	0.802
b vs LOM	-0.754	0.021	0.058	0.934

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