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This study is part of research aimed at preventing or opposing the effects of eutrophication in shallow lagoons with poor water exchange and subject to intense macroalgal blooms. It belongs to a series of studies to define the consequences of sediment resuspension on high-density algal mats by algae-harvesting boats, irrespective of the quantity of algae harvested.

- 1. Harvest of algae in eutrophic lagoons is costly; biomass disposal is critical
- 2. What are the effects of harvester boat transit in areas with high density mat?
- 3. An experiment showed that disturbance by a boat enhances mats decay in a lagoon
- 4. Boat disturbance may effectively limit algal blooms in eutrophic shallow lagoons

A new approach to macroalgal bloom control in eutrophic coastal areas

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Abstract: In summer 2012, an experiment was conducted in a shallow eutrophic lagoon with poor water exchange to determine the consequences of harvesting algae on the algal mat itself, which was traversed and repeatedly disturbed by large harvester boats. Four areas with high macroalgal density, measuring half a hectare each, were selected. Two were subjected to frequent disturbance of the algal mat and sediment (12 two-hour operations over a 38-day period) and the other two were left undisturbed as control. The following variables were determined: 1) water column physical chemistry and nutrients; 2) redox potential, nutrients and organic load in sediments; 3) C, N and P content of algal thalli; 4) macroalgal biomass.

In 2013, a further experiment was conducted on a larger scale. Biomass was estimated in a highdensity mat measuring 235 ha, where macroalgae were harvested and stirred up by four harvesting boats, and in two high-density mats measuring 120 and 150 ha, left undisturbed as control.

In the first experiment, no significant changes were observed in the water column. In sediment the main variation was a significant reduction in labile organic matter in the disturbed areas and a significant increase mainly in refractory organic matter in the undisturbed areas. Biomass showed a significant drastic reduction in disturbed areas and substantial stability in undisturbed areas. In the large-scale experiment, the biomass of the disturbed mat declined by about 63%, only 6.5% of which was due to harvesting. On the other hand, the undisturbed mat with higher density underwent a natural decline in biomass of about 23% and the other increased by about 50%. These results demonstrate that disturbance of high-density mat in shallow water by boats caused decay of the mat.

Key-words: eutrophication, lagoon, macroalgal bloom, macroalgal harvesting, macroalgal mats, *Chaetomorpha linum*

1. Introduction

In the last 30 years, coastal areas have been subject to increasing eutrophication (Hauxwell and Valiela, 2004), the consequences of which are accentuated by geomorphological conformation and low water turnover in the case of non-tidal lagoons. The increase in nutrient and organic matter loads may induce large increases in primary production with dominance of opportunistic macroalgal and microalgal components.

The development of large dense mats of opportunistic macroalgae is a *facies* of eurihaline, eurithermal lagoon biocoenoses in response to eutrophication (Peres and Picard, 1964). In the last 30 years, major blooms of macroalgae, especially chlorophyceae of the genera *Ulva*, *Chaetomorpha* and *Cladophora*, and rhodophyceae of the genera *Gracilaria*, *Gracilariopsis*, *Spyridia* have occurred in many lagoons and marine coastal areas with low flow conditions. These taxa are widely recognised to resist stress, grow rapidly and reproduce aggressively, being classified as "r-

strategist" opportunistic species (Littler and Littler 1980). Opportunistic species have a thallus with a high surface to volume ratio (SA:V) (Littler and Littler 1980) that increases photosynthesis and nutrient absorption capacity (Rosenberg and Ramus, 1984). They are characterised by a high ratio of maximum uptake to nutrient saturation constant (Vmax/Kmax) which facilitates them in competing for nutrients (Wallentinus, 1984), not only with respect to other macroalgae and phanerogams but also phytoplankton. Reported from coastal areas all over the world (Fletcher, 1996; Morand and Briand, 1996; Hiraoka et al., 2004; Morand and Merceron, 2005; Merceron et al., 2007; Ye et al., 2011), macroalgal blooms are not, however, simply the result of an increase in nutrients but of complex interactions between many variables, such as light, temperature, flow conditions and so forth (Schramm, 1999).

In spring and summer, blooms of opportunistic macroalgae in eutrophic non-tidal lagoons may Geomorphological and hydrological conformation facilitates accumulation of be conspicuous. nutrient, water heating and penetration of light to the bottom. Thus, dense algal mats may form, occupying the whole water column in shallow basins (1-1.5 m) (Bombelli and Lenzi, 1996; Pavoni et al., 1999). Competition for substrate and light-exclusion by macroalgal mats impede the growth of other less tolerant species, reducing phytobenthic biodiversity (Lobban et al., 1985), as well as being a major cause of the disappearance of marine phanerogams (Hauxwell et al., 2001). In highdensity mats, the upper layer prevents light from reaching the lower layers (self-shading) (Gordon and McComb, 1989; Lavery et al. 1991; Peckol and Rivers, 1996). This may lead to stratification of physical and chemical parameters and nutrients in the water column (Krause-Jensen et al., 1996; McGlathery et al., 1997; Lenzi et al., 2013a). Due to prolonged low light conditions, biomass near the bottom begins to die and decompose, becoming itself a source of nutrients (Sfriso et al., 1987; Organic matter forms layers on the bottom, together with the remains of Hanisak, 1993). consumers and saprophytes that have died of natural causes or been killed by environmental conditions (Raffaelli et al., 1998). Build-up of organic matter on the bottom also alters the nature of sediment, transforming sandy/silty sediment into very fine organic mud, with consequent impact on infaunal communities. During decomposition of algal biomass, toxins such as dimethyl sulphide (Bass Becking and MacKay, 1956) and volatile fatty acids (Fusetani et al., 1976) may be released. Indeed, the decomposition of algae leads to impoverishment of dissolved oxygen in the lower layers and a transition to anoxygenic decomposition of organic matter. These anoxygenic processes release ammonium (Marty et al., 1990) and orthophosphates, bound to ferric oxy-hydroxides (Golterman, 2001), making them available for new blooms of vegetation (Rozan et al., 2002). The demolition of organic matter proceeds mediated by sulphate-reducing bacteria which use the sulphate ion as oxidant (Stainer et al., 1988). The reduction of sulphate leads to the formation of hydrogen sulphide (H₂S) which can cause die-off of fauna (Giordani et al., 1996). This phenomenon, defined as dystrophy, occurs especially in warmer seasons under certain meteorological conditions, are accentuated in the basin with reduced hydrodynamic. Though disastrous for fauna and flora, dystrophic crises are dissipative processes: a recovery mechanism for ecosystems that have reached an energetically unsustainable position. The ecosystem gets rid of the excess energy accumulated (Lenzi et al., 2011, 2012).

The consequences of vegetation blooms have in some cases been opposed by harvesting (Lenzi, 1992; King and Hodgson, 1995; Runca et al., 1996; Lavery et al., 1999; De Leo et al., 2002). Harvesting algae is a burden on the public purse, involves problems of logistics, disposal and legislation, and is often insufficient as sole management criterion, unless undertaken on a massive scale. The material cannot be used easily and competitively recycled in industrial countries (Bastianoni et al., 2008; Migliore et al., 2012) and in Italy it is classified in the same category as solid urban waste.

It may seem evident that by harvesting and disposing of the algal biomass, the impact of bacterial degradation processes on this organic matter is reduced and a quantity of nutrients is removed from nutrient-rich areas. However, though evident, very few studies have evaluated the efficacy of harvesting macroalgal masses in managing eutrophic environments or the impact that

harvesting can have on the ecosystems involved. For example, De Leo et al. (2002) used a mathematical model to evaluate the costs and benefits of harvesting algae in relation to the damage that macroalgal blooms had on small clam production in the Sacca di Goro. Lavery et al. (1999) estimated the time taken for the original zoobenthic population to be restored in a lagoon subject to harvesting of algae. More recently, we evaluated the impact of harvesting in terms of quantity of resuspended sediment and redistribution of macronutrients in the sediment layer (Lenzi et al., 2013b). Nevertheless, there are still many aspects to clarify, such as the minimum quantity of plant biomass to remove to obtain an improvement in environmental quality, the most effective and economic harvesting methods and the best timing during the year to optimise results. The possibility of other solutions has not been investigated and whether these are cost-effective.

On this topic, it is estimated that in Orbetello lagoon, 6000 tons of macroalgae (wet weight) harvested in about 6 months of activity caused resuspension of about 16,500 tons of sediment (Lenzi et al., 2013b). The mere passage of the harvesting vessel over the algae disturbs and stirs up the mat and superficial sediment, as an effect of boat propulsion and propeller rotation (Lenzi et al., 2005). This raised the question of whether sediment resuspension could have more effect than algal removal, considering the quantities involved. Another aspect worth clarifying is what happens to the plant mass stirred up by passage of the boat. Two scenarios and intermediate situations are possible. Stirring up of the algal mat by the boat could expose the lower-layer vegetation to light, reactivating photosynthesis and uptake of nutrients, as suggested by Peckol and Rivers (1995) for Cladophora vagabunda, and stimulating growth by fragmentation. In this way the macroalgal mass may not collapse, causing dystrophy, but may grow to the maximum sterically possible. At the other extreme, resuspension of anoxic sediment under the algal layer and the processes triggered by disturbance could have negative effects on the algae, increasing turbidity of the water column, incrementing the pool of aggressive bacteria and redepositing sediment on algal thalli. The resuspension of superficial anoxic sediment could also modify physical and chemical variables of the water column and alter biogeochemical nutrient cycles, creating nutrient limitation that could promote collapse of the algal mass.

The aim of the present study was to determine the effects of boat movements in lagoon areas with high density macroalgal mats (without removal of plant mass) by assessing mat growth and major chemical and physical variables of the water column. A manipulative field experiment was conducted to answer the following questions: Does frequent passage of boats induce an increase or decrease in macroalgal biomass? Does disturbance by boats exacerbate or mitigate environmental conditions, or has it no appreciable effect?

2. Materials and methods

2.1. Study area

The study was conducted in Orbetello lagoon (mean depth about 1 m) on the Tyrrhenian coast of Tuscany, Italy (42°41'-42°48'N, 11°17'-11°28'E; Fig. 1). The lagoon is 25.25 Km² wide and separated from the sea by two sand bars. It is divided into two basins, western and eastern, by Orbetello isthmus and a dam. Three canals link the lagoon to the sea, two in the west basin and one in the east basin (Fig. 1). Eutrophication of this lagoon ecosystem is mainly anthropogenic, due to past discharge of municipal wastewater (now discharged into the sea), treated and otherwise, which has accumulated in sediments, discharge of fish-farm wastewater, and nutrient input from agriculture in the catchment of the river Albegna, into the estuary of which one of the western canals opens (Lenzi et al., 2003). Mitigation of the effects of eutrophication is conducted by pumping sea-water through the two western canals in summer, and by harvesting macroalgae (Lenzi, 1992; Lenzi et al., 2003). Macroalgae are collected systematically for 5-6 months of the year by four boats that stow up to 2 tons.

2.2. Experimental design

2.2.1. Small-scale experiment

The study was conducted in the west basin of Orbetello lagoon, close to Monte Argentario, in an area between the two underwater channels conveying pumped sea-water into the inner lagoon in summer (Fig. 1). Here depth range is 0.9–1.2 m and substrate grain size is relatively homogeneous: 17.1% coarse sand, >1000 μm; 54.6% fine sand, 250-125 μm; 14.4%, silt and clay <63 μm (Renzi et al., 2007). We identified four homogeneous areas of 5000 m² each (50 x 100 m) (Fig. 1) and marked them out with bamboo canes at ten-metre intervals. The areas contained dense macroalgal mats consisting of the Chlorophyceae Chaetomorpha linum (Müll.) Kütz. and Valonia aegagropila C. Agardh, the first of which was dominant. Two areas were subjected to treatment (Disturbed areas, Ds; D-A1, D-A2), whereas the other two were not treated and used as controls (Undisturbed areas, Us; U-A1, U-A2). Disturbance was created by repeated passages of a boat across the two Ds. It was repeated 12 times at intervals of 48-72 h, over a period of 38 days (22 June - 30 July 2012). The boat (13 x 4.5 m; capacity 13 tonne; draught 0.4 m) stirs up soft surface sediment (3-5 cm) as it navigates and works (Lenzi et al., 2013b). Disturbance was conducted for about 2 hours each time, making sure that all the macroalgal mat in the area was evenly stirred up, without harvesting it. This treatment mimicked what occurs when high density mat is harvested. To harvest a full load, the boat moves tens of tonnes of macroalgae. However, we imposed a much higher frequency of disturbance than necessary for harvesting in a lagoon measuring hundreds of hectares. The distance between the two control areas and the two treated areas was 100 m, while treatment areas were at least 200 m from the closest control. According to Lenzi et al. (2013b), 100 m is the minimum distance to prevent the effects of sediment resuspension in one area from interfering with another.

The choice of the general area was dictated by homogeneous sedimentary grain-size, the extension and homogeneity of the algal mat, and sufficient water renewal. The latter was necessary to prevent the onset of critical conditions that could lead to summer decay of the mat, which would have made it difficult to determine the influence of the treatment itself. The two canals through which water is pumped in summer determine speeds of $1-2 \text{ cm s}^{-1}$ in the underwater channels (Bucci et al., 1989), which provides sufficient water turnover in the areas affected by the flow, to keep high density mats alive all summer.

In situ measurements of water chemical and physical variables and water sampling were conducted at two depths in the water column (-20 cm from the surface and 20 cm from the bottom), performing 3 replicates per area, yielding 6 measurements and 6 samples for each of the four areas. Five trials were conducted at intervals of about 10 days, for a period of 42 days: 20 June 2012 (before treatment; T_0), 2 July (after the start of treatment; T_1), 13 and 21 July (at advanced stages of treatment; T_2 , T_3) and 31 July (after the end of treatments; T_4).

The chemical-physical variables measured were: salinity (S, practical scale of salinity; refractometer ATAGO S/Mill), temperature (t, °C), pH (electronic digital pHmeter DELTA OHM HD8705) and dissolved oxygen (DO, % saturation; oxymeter OxyGuard Handy Mk III).

Water samples were collected by Niskin bottle, filtered in the field with Whatman GF/F filters (porosity 0.48 μ m, diameter 45 mm), stored in 50 ml Kartell PVC bottles refrigerated during the sampling period and then stored at -20°C until analysis. They were analyzed for: nitric nitrogen (N-NO₃⁻), nitrous nitrogen (N-NO₂⁻), ammonia nitrogen (N-NH₄⁺), soluble reactive phosphorous (SRP), total dissolved nitrogen (TDN) and total dissolved phosphorous (TDP) by BRAN+LUEBBE AA3 Autoanalyzer. All values were expressed in micro-moles (μ M) per litre. Dissolved organic nitrogen (DON) and dissolved organic phosphorous (DOP) were calculated by subtracting the inorganic fraction from the total; the atomic ratio of dissolved inorganic nitrogen (DIN = N-NH₄⁺ + N-NO₂⁻ + N-NO₃⁻) to SRP (DIN : SRP) was then computed. Nitric and nitrous nitrogen values were pooled as N-NO_x.

Sediment samples for redox potential (Eh), nutrient and organic matter (OM) determinations were collected in triplicate, at the same times and in the same stations as water sampling, with a special horizontal sampler equipped with 60-ml syringe, withdrawing the top 3 cm of sediment (Lenzi and Renzi, 2011). Samples immediately underwent Eh measurement using an IDRONAUT portable sensor, transferred to 50 ml plastic tubes, refrigerated and then frozen at -20°C until

analytical determination. Redox potentials were subsequently corrected for the reference electrode potential (+200 mV). Prior to analysis, sediment samples were thawed and oven-dried at 70°C for 48 h; they were subsequently ground finely in an agate ball mill, sieved with a 200-µm mesh and oven-dried at 105°C to constant weight. For nutrients, total organic carbon (TOC) and total nitrogen (TN) were determined with a Thermofinnigan CHN-S EA 1110 element analyzer, and total phosphorus (TP) with a BRAN+LUEBBE AA3 Autoanalyzer. Values were expressed as percentage of material oven-dried at 105°C. C:N, C:P, N:P atomic ratios were then computed. For OM, samples were weighed and incinerated in a muffle furnace at 250°C for 16 h, allowed to cool in a desiccator and weighed, then returned to the muffle furnace at 450°C for 16 h, cooled and weighed. Labile (LOM) and total organic matter (OM) were determined as percentage of weight loss at 250°C and at 450°C, respectively, compared to initial weight at 105°C. Refractory organic matter (ROM) was computed by subtraction: % ROM = % OM - % LOM.

At three dates in July 2012 (4 July, two days after T_1 ; 13 July, T_2 ; 31 July, T_4), three samples of *Chaetomorpha linum* were collected in each area for C, N and P determination. Samples were washed, cleaned of coarse impurities, dried at 75°C and finely ground; determinations proceeded as described for sediment. Relative molar values were used to calculate C:N, C:P and N:P ratios.

For macroalgal biomass (b) determination, four sampling campaigns at intervals of about 20 days, were conducted: 19 June 2012, before treatment; 11 July and 1 August, during the treatment period; and 24 August, 25 days after the last treatment. In each trial, 6 replicate samples of biomass were randomly collected in each area. A sampling area of 3600 cm² (60 * 60 cm) was delineated by a square metal frame. Samples were washed, dried (exposure to air in the shade for 3 h) and weighed (g wet weight, g_{WW}). The data was then expressed per square meter (g_{WW} 3600 cm⁻² * 10,000⁻¹ * 2.778 = g_{WW} m⁻²). Standing crop (SC), i.e. mass of algae present in an area considered at the sampling time, was calculated with the following equation using the biomass values:

 $SC = b * A * CT * 1000^{-1}$

where b is the macroalgal biomass in kg wet weight m^{-2} , A is the surface in m^2 of the area considered, CT is total cover of the substrate by vegetation in the area, expressed as ratio of covered surface to total surface of the area considered, according to Boudouresque (1971), and 1000⁻¹ is the conversion factor for kg into tonnes, as standing crop was expressed in tonnes wet weight (T_{WW}).

2.2.2. Large-scale experiment

To validate the results obtained in 2012 in the small-scale experiment, in summer 2013, *C. linum* and *V. aegagropila* standing crops were estimated in larger areas (representative of the scale of management of the whole west basin), in which macroalgae were or were not harvested. For this purpose, macroalgal harvesting in 2013 concentrated on a high-density mat covering 235 hectares (mat O). Four harvesting boats were used to create significant disturbance. To evaluate the effect of frequent disturbance of the mats by the boats, we considered the macroalgal mass harvested and estimated standing crops in the period 15 August to 15 September 2013. Data was collected from the disturbed and control areas, which contained large dense mats of the two Chlorophyceae. The controls were an area of 120 hectares (mat I), adjacent mat O, and a further area of 150 hectares (mat N), about 1 km from the operative area (Fig. 1).

Biomass sampling took place as previously described and the number of samples was 50, one every 10 hectares, taken at random. SC was computed with the equation:

SC
$$(T_{WW}) = b (kg m^{-2}) * CT * A (m^2) * 1000^{-1}$$

2.3. Statistical analysis

Water chemical-physical variables and nutrient components were analyzed by 3-way ANOVA with treatment (disturbed vs. undisturbed, 2 levels) and time (T_0 - T_4 , 5 levels) as fixed and orthogonal factors, and area (2 levels) as random factor nested in treatment (n = 6). Sediment variables, macroalgal biomass and nutrient content of *C. linum* thalli were analyzed by 3-way ANOVA with the same factors and levels as for water variables (n = 3). Cochran's *C*-test was used

before each analysis to check for homogeneity of variance (Underwood, 1997). The Student Newman Keuls (SNK) test was used for *a posteriori* multiple comparison of means. This analysis was performed with Statistica 7.0 software package.

3. Results

3.1. Small-scale experiment

3.1.1. Water column

Chemical-physical variables are reported in Table 1. Temperature was in the range 26.88–29.75°C, and salinity 35–40, both varying within the typical summer limits of Orbetello lagoon. DO went from oversaturation to undersaturation in Ds and Us between the beginning and end of the experiment, with less extreme values in Ds. Although the variation was not significant, pH fell in Us and rose in Ds in the hottest period. ANOVA showed significant time-treatment interaction for pH and t variables (P = 0.0036, P = 0.0026, respectively), while no significant effects were detected for DO and S. However, the high dispersion of the data (significant *C*-test for all variables) made the ANOVA results unreliable for these variables.

Nutrient variables are reported in Table 2. ANOVA detected significant time-treatment interaction for N-NH₄ and DIN (P = 0.0020). The SNK test showed the highest values of both variables at T_1 in Ds and control areas, and a significant minimum value at T_2 in Us. Values of treated and control areas, compared in parallel at the same sampling date, were significantly different, except at T_2 . N-NO_x, DON, SRP and DOP did not show any significant time-treatment interaction (P = 0.1726, P = 0.0588, P = 0.1173, P = 0.3263, respectively).

DIN/SRP was in the range 20–1069 in treated areas, with an overall average (60 records) of 194.83 \pm 243.48 and a median of 67.25; for control areas, the values were 9–783, 102.60 \pm 128.65 and 62.99, respectively. Means (\pm SD) for each campaign and area are reported in Table 3. Analysis of variance showed significant interaction between treatment and time factors (P = 0.009). 3.1.2. *Sediment*

The range of redox potential was -23 mV to -210 mV (-156.27 \pm 44.47 mV; median -170.50 mV) in Ds, and -96 mV to -212 mV (-187.52 \pm 31.06 mV; median -200.00 mV) in Us. Mean values (\pm SD) at each sampling time are reported in Table 4. Analysis of variance did not show any significant difference between Ds and Us, whereas it detected a significant effect of the time factor (P = 0.003).

Analysis of variance of TOC did not detect any significant effect due to treatment, but it highlighted a significant effect of the time factor (P = 0.019): indeed, values showed a decreasing trend in Ds and Us (Table 5), namely -13.57% and -9.32%, between T₀ and T₄, respectively. Analysis of variance of TN (Table 5) showed a significant effect of the treatment factor (P = 0.046); values remained stable over time and the mean of all records was higher in Us (0.90 \pm 0.13 %) than in Ds (0.63 \pm 0.10 %). Analysis of variance of TP (Table 5) showed a significant effect of the factors treatment (P = 0.033) and time (P = 0.043). The mean of all records was higher in Us (0.062 \pm 0.01%) than in Ds (0.051 \pm 0.01%).

Analysis of variance of the atomic ratio C:N detected significant effects of the factor treatment (P=0.006), and means of all records were 8.73 ± 0.76 for Ds and 7.79 ± 0.62 for Us. C:P and N:P atomic ratios did not show significant effects of the factors considered. The three molar ratios are reported in Table 6.

Figure 2 shows OM, LOM and ROM values obtained in the five sampling trials in Ds and Us. Analysis of variance of OM, LOM and ROM showed a significant interaction between treatment and time factors (P = 0.026, P = 0.035, P = 0.005, respectively). OM was already significantly lower in Ds than in Us at T₀ (post-hoc SNK<0.01), but its decline was faster in treated than in control areas (post-hoc SNK<0.01). Indeed, with respect to initial values, a 38.59% decrease in OM occurred in Ds against 9.97% in Us. LOM decreased in all areas, with greater values in Us than in Ds at T₂ and T₄ (post-hoc SNK<0.01). With respect to initial values, LOM dropped by 74.42% in Ds and by 57.62% in Us. ROM was similar in the four areas at T₀ (post-hoc SNK>0.05) and remained stable in Ds, whereas it increased significantly in Us (post-hoc SNK<0.01). With respect to initial values, ROM showed a decline of 9.32% in Ds and an increase of 35.87% in Us.

3.1.3. Macroalgae

Passage of the boat on the macroalgal mats caused intense mixing, partial fragmentation of thalli and redeposition of resuspended sediment on the algae. This detritus oxidises rapidly and forms a reddish film that is readily resuspended agitating the vegetation and that is visibly different from the anoxic bottom sediment initially resuspended.

Figure 3 is a histogram of macroalgal biomass calculated for each area during the four trials. The disturbed areas (Ds) showed a progressive decline in biomass to a final reduction of -89.13% with respect to initial values. By contrast, biomass was substantially stable in the undisturbed areas (Us), with fluctuations between growth and decay and a final decline of -7.76% compared to the beginning of the experiment. Analysis of variance showed a significant interaction between treatment and time factors (P = 0.011). Estimates at T₀ did not differ significantly between Ds and Us (post-hoc SNK, P>0.05), whereas at subsequent dates biomass values were always significantly higher in Us than in Ds (post-hoc SNK, P<0.01).

Standing crops (SCs) showed mean decays of algal mass of 19.80 tonnes wet weight (T_{WW}) for Ds and 1.60 T_{WW} for Us between the beginning and the end of the experiment.

C, N and P contents of *Chaetomorpha linum* thalli and the respective atomic ratios C:P, C:N and N:P are reported in Table 7. ANOVA showed significant effects of the treatment factor for C and C:N (P = 0.000) and of treatment-time interaction for N, C:P and N:P (P = 0.050, P = 0.007 and P = 0.006, respectively), whereas there were no interactions of the factors for P. Means of all records of C:N proved higher in Ds than in Us (25.05 ± 3.31 and 15.36 ± 4.94 , respectively). C:P was initially significantly higher in Us thalli than in those of Ds. In Us, there followed a progressive reduction in the C:P ratio to values significantly lower than in Ds (post-hoc SNK <0.01). N:P was initially significantly higher in Us thalli than in those of Ds (post-hoc SNK <0.01), but the subsequent increase in N and to a larger extent P in Us thalli dampened every significant difference with respect to the N:P ratio of Ds thalli.

3.2. Large-scale experiment

The high density macroalgal mat subjected to disturbance and harvesting, called mat O (Fig. 1), consisted almost exclusively of the chlorophycea *C. linum*. Its average biomass (b) in August was $9.15 \pm 0.97 \text{ kg}_{WW} \text{ m}^{-2}$ in an operating area of approximately 235 hectares (A in m²), with 95% cover (CT = 0.95). Standing crop (SC) was therefore calculated to be 20,418 tonnes wet weight (T_{WW}) with the equation SC (T_{WW}) = b (kg m⁻²) x CT x A (m²) x 1000⁻¹. In September this same mat showed a significant reduction in biomass, namely $3.42 \pm 2.97 \text{ kg}_{WW} \text{ m}^{-2}$ with a CT of 0.95 and a SC of 7647 T_{WW}. The mat showed frequent low-density patches and decay of heavily detritus-coated thalli. SC decreased by 62.55% in 30 days. The macroalgal mass harvested in the same period in the operating area (235 hectares) was 1333 T_{WW} (-6.53% of the SC in August).

The undisturbed high density mat N (Fig. 1), measuring approximately 150 hectares, showed the following data in August: biomass = $9.92 \pm 2.10 \text{ kg m}^{-2}$, CT = 0.95, SC = 14,880 T_{WW}. In September this same mat had a biomass of $8.63 \pm 2.20 \text{ kg m}^{-2}$, CT = 0.95 and SC = $11,478 \text{ T}_{WW}$. SC decreased by 22.86% in 30 days.

The other undisturbed high density mat I (Fig. 1), measuring 120 hectares, showed a biomass of 3.68 ± 0.77 kg m⁻², CT = 1 and SC = 4422 T_{WW} in August. In September, biomass was 6.08 ± 0.86 kg m⁻², CT = 0.90 and SC = 6566 T_{WW}. SC increased by 48.48% in 30 days.

The estimates of biomass (b), total cover (CT), standing crop (SC) and harvested macroalgal masses between 15 August and 15 September 2013 and the surface areas (A) of the high density macroalgal mats O, N and I are reported in Table 8. The macroalgal mats O, N and I (Fig. 1) consisted almost exclusively of the chlorophycea *C. linum*.

4. Discussion

4.1. Small-scale experiment

4.1.1. Water column

The chemical and physical variables were similar in the experimental areas, probably due to the proximity of the two underwater channels for water exchange. An exception was the significant difference in certain temperature values and initially pH, higher in Us than in Ds. These differences are of little biological or environmental significance, since differences of a few decimals are negligible compared to the wide range possible in lagoon environments, a range that may even be observed in the space of a few hours, especially when algal mats achieve high density. Dissolved oxygen initially showed conditions of over-saturation, becoming under-saturated during the sampling period as summer advanced. Although the differences were not significant, data from the last sampling showed higher values in Ds than in Us, indicating more intense respiration, also by day, in undisturbed areas with high plant density, probably due to intense bacterial activity.

The increase in ammonium at T_1 , common to Ds and Us, was significantly greater in Us. Being synchronous, it presumably depended on weather conditions, but it is beyond our scope here to explain this finding. Relatively high DON in both areas indicates release of soluble organic matter as a likely consequence of cell lysis and decay of algal masses. In Us this probably mostly occurred in the layer near the bottom, whereas in Ds it involved the whole mat, due to the treatment it had undergone. High values of DON have been reported in other studies on this lagoon (Lenzi et al., 2004, 2005) and are probably a characteristic of eutrophic environments with algal blooms. Dissolved inorganic nutrient values were on the whole relatively low, suggesting high uptake by primary producers. This is in line with the high macroalgal density in Us and initially also in Ds.

In line with Atkinson and Smith (1983) and Wheeler and Bjornsater (1992), the molar ratio DIN:SRP calculated for this experiment expressed a prevalent condition of P-limitation. The wide range of values in Ds expresses a consequent wide ranging variability of nutrient release by sediment. Sediment heterogeneity, which underlies this process, was probably accentuated by disturbance, which evidently did not affect the sediment layer homogeneously. In Us, increasing P-limitation followed an initial equilibrium of nutrients.

4.1.2. Sediment

Redox did not vary significantly during treatment with respect to control, probably because new organic matter continued to be added to the sediment, due to demolition of the macroalgal mat, even though oxidation of resuspended sediment mineralised part of the organic matter in Ds. With regard to the factor time, its significance can be ascribed to the increase in temperature between June and July that shifted conditions towards stable anoxia and opposed the oxidative effects of resuspension. To obtain a greater and significant increase in sediment redox potential in Ds it is probably necessary to resuspend the sediment more frequently, as found by Lenzi and Renzi (2011).

The quantities of C, N and P found in sediment from Us were significantly higher than those in sediment from Ds. This difference, already evident at the start of the experiment, remained substantially unchanged during the study period, indicating that disturbance, at the frequency used, did not affect these variables. The sediment atomic ratio C:N < 12 expressed the dominance of organic matter low in fibre and therefore rapidly mineralised, as in the case of organic matter from decay of macroalgal mats (Covelli et al., 2009). This ratio was higher in Ds than in Us, due to a lower quantity of N, which could be ascribed to more efficient removal of N from the system by denitrification processes, as oxidation accelerates nitrification and leads to pre-dominance of nitrates over the reduced forms (Revsbech et al., 1980), while an increase in nitrate concentration produces an increase in denitrification (Herbert and Nedwell, 1990). The higher molar ratios C:P and N:P in Us than in Ds were due to relatively larger quantities of C and N in sediments of the control areas with respect to disturbed areas. C, N and P were always higher in Us than in Ds, but C and N were relatively more elevated than P.

Organic matter and LOM underwent a greater reduction in Ds than in Us. According to similar experimental (Lenzi et al., 2005, 2010; Lenzi e Renzi, 2011) and laboratory reports (Stahlberg et al., 2006), this can be attributed to the effects of sediment resuspension. In Us, where anaerobic decay of organic matter presumably prevailed, abatement was less and led to greater accumulation of ROM. In Ds, ROM was substantially unchanged, probably because it was partly broken down by direct action of UV and oxygen, to which resuspended organic detritus was exposed in the water column (Hulthe et al., 1998).

Although in this case sediment disturbance was not conducted with sufficient frequency and although it occurred under conditions of continuous intense production of organic detritus following breakdown of abundant macroalgal mat, it seems to be a valid method of opposing accumulation of organic matter in sediment.

4.1.3. Macroalgae

The disturbed areas showed a progressive significant decrease in macroalgal biomass with a final overall reduction of 89.13%, whereas biomass in Us remained substantially unchanged with a final overall reduction of only 7.68%. This result suggests that part of the plant mass in Us decayed, presumably in the lower layers of the mat, whereas a more or less equivalent amount in the upper layer grew. If the "natural" reduction in biomass was that occurring in Us, i.e. 7.68% with respect to the initial estimate, it can be deduced that repeated passage of the boat through high density algal mat under these experimental conditions led to an 81.45% reduction in plant mass.

It is not clear how the algae decayed in Ds, nor was it our aim to investigate this aspect. However, the following explanations are possible. 1) Part of the algal material was broken up by the propellers. This does not necessarily kill the algae and could actually favour their propagation. However, fragmentation in high density mat could have made the plants more susceptible to attack by bacteria during the warm summer months. 2) Redeposition of resuspended sediment on the thalli could reduce penetration of light, hindering gas exchange and absorption of nutrients. It may also lead to an increase in bacteria on the thalli, facilitating bacterial attack. 3) Stirred up plant mass and especially sediment may cause acidic reducing substances to be released from the bottom and these may further stress the algae.

The significant reduction in macroalgal biomass in Ds would not, on the other hand, be due to water turbidity caused by resuspension of sediment. Boat action was limited to 2 h/day every 2-3 days and turbidity persisted for only about an hour after the end of treatment. Shielding out light for about 3 h twice a week could not make such a difference in summer.

The C, N and P content of algal thalli constitutes a "record" of events connected with the bioavailability of nutrients in the environment. The thalli of opportunist species may accumulate large quantities of nutrients, when they are available, and use them as required (Lavery et al., 1991). The C content of *C. linum* was quite stable in all areas; N and P were quite stable in algal samples from Ds but increased in those from Us. This suggests that release of N and P by sediment was more intense in Us, probably due to the more stable anoxygenic conditions produced by the thick uniform layer of algae at the water-sediment interface (Marty et al., 1990; Fenchel and Riedl, 1970).

The values of the three nutrients recorded in *C. linum* from Us (C 19.28 \pm 1.13%; N 1.62 \pm 0.48%; P 0.08 \pm 0.04%) were quite similar to those measured by Lenzi et al. (2012) in dense algal mats in the same lagoon (C 18.23 \pm 5.32%; N 1.65 \pm 1.18%; P 0.05 \pm 0.03%), whereas they were more differentiated, especially C and N, in samples from Ds (23.28 \pm 0.94% and 1.07 \pm 0.09%, respectively).

It is widely recognised that atomic ratios N:P<10 and N:P>30 indicate N- and P-limitation, respectively (Ryther and Dunstan, 1971; Goldman et al., 1979; Atkinson and Smith, 1983; Smith, 1984; Lapointe et al., 1992). In the present experiment, N:P values in *C. linum* were always >30, indicating a condition of P-limitation. This ratio proved to be more stable in samples from Ds than in those from Us, since in the former areas the lower availability of P was matched by an even lower availability of N, with respect to Us. This lower availability is in line with nutrient limitation

phenomena occurring during frequent resuspension of sediment (Lenzi, 2010). The C:P ratio remained quite high and stable in *C. linum* from Ds, whereas in samples from Us this ratio fell sharply from relatively high values, due to the progressive increase in P. The C:N ratio was significantly higher and stable in samples from Ds, due to a lower N content with respect to Us. However, the condition of thalli sampled in Ds cannot be considered critical for *C. linum*, which is not a nitrophilous species and rarely suffers severe N-limitation, except under conditions of prolonged scarcity (Lavery et al., 1991).

4.2. Large-scale experiment

It was evident that the effect of boat disturbance on mat O had a determinant effect on the greater loss of biomass with respect to the undisturbed mat N, even considering the effects of their different locations. For mat I, we found that at relatively lower densities, mat growth is still possible, despite extreme summer conditions.

Since 1333 T_{WW} of macroalgal biomass was removed from mat O in the study period and the reduction in biomass of this mat was about 12,771 T_{WW} (Table 8), the harvested material was only 10.4% of the biomass lost and the other 89.6% was presumably due to "natural" decay and disturbance of the mat by the boats.

Although the comparison cannot have statistical significance, it is however interesting to note that the control mat N, which in August had a similar density to mat O, showed a natural seasonal decline in biomass of 22.9%, whereas mat I, situated between the other two and with a biomass 33.5% and 38.7% lower, respectively, increased by almost 50%, despite seasonal adversity. We can therefore infer that boat disturbance was responsible for at least a 66.7% reduction of biomass in mat O, and that an additional natural decline similar to that of mat N was also possible.

5. Conclusion

These results suggest that disturbance of a high-density mat of macroalgae in a non-tidal eutrophic lagoon contributes to destruction of the mat and its decay in sediment. The small-scale experiment on two disturbed and two control areas showed a slight natural reduction in biomass due to summer conditions, mitigated by the choice of study areas near the underwater channels that convey incoming seawater, and a rapid progressive biomass reduction in the disturbed areas as treatment proceeded. The large-scale experiment in the same lagoon, monitoring an operation conducted for environmental management in an operative area and two control areas, showed substantial natural reduction in biomass in the control area with biomass initially similar to the operative area and an increase in biomass in the control area initially with relatively lower biomass values. This could mean that the natural reduction is not the result of environmental conditions as such but depends mainly on mat density under those conditions. In the disturbed area, biomass fell sharply, not principally due to removal of biomass or environmental conditions, but presumably related to disturbance of the mat by the boats.

These results make it possible to sustain that macroalgal blooms can be treated in the manner described in the experiments, excluding harvesting and removal of algae from the ecosystem. In this way, the formation of sedimentary organic detritus is increased and accelerated. This sedimentation then has to be further treated by resuspension in order to mineralise the recently acquired organic detritus and avoid preparation of a new dystrophic process the following summer.

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Captions

Figure 1 – Orbetello lagoon on the Tyrrhenian coast of Italy. W, N: canals connecting sea to lagoon in western basin; E: canal connecting sea to lagoon in eastern basin; IC1, IC2: inner underwater channels; areas enclose by green dashed/dotted lines: macroalgal mats disturbed (mO) and not disturbed (mI, mN), in 2013; black squares: disturbed areas (Ds: D-A1, D-A2); white squares: undisturbed areas (Us: U-A1, U-A2) in 2012.

Figure 2 – Sediment organic matter trends in samples collected between 20 June and 31 July 2012 $(T_0 - T_4)$ in disturbed (D) and undisturbed areas (U). OM, total organic matter; LOM, labile organic matter; ROM, refractory organic matter. Values are mean (%) ± SD (*n* = 6) dry weight basis.

Figure 3 – Macroalgal biomass production between 19 June and 24 August 2012 in disturbed (D-A1, D-A2) and undisturbed areas (U-A1, U-A2). Values are means \pm SD (n = 6) in kg m⁻² dry weight.

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Biomass kg m⁻²

Table 1 – Chemical and physical water variables measured *in situ* between 20 June and 31 July 2012 ($T_0 - T_4$), in disturbed (Ds) and undisturbed areas (Us). Temperature, t (°C); salinity, S (practical scale of salinity); dissolved oxygen, DO (%); pH. Values are mean \pm SD (n = 30).

	t (°C)		S		DO	(%)	pН	
	Ds	Us	Ds	Us	Ds	Us	Ds	Us
T_0	27.18 ± 0.20	26.98 ±0.53	40.00 ± 0.82	38.75 ± 0.50	114.83 ± 20.20	106.33 ± 13.83	7.68 ± 0.04	8.26 ± 0.19
T_1	29.07 ± 0.21	28.83 ± 0.09	35.25 ± 0.96	36.67 ± 0.52	102.33 ± 9.66	81.10 ± 23.77	8.37 ± 0.14	8.20 ± 1.31
T_2	28.34 ± 0.24	29.75 ± 0.33	36.25 ± 0.96	36.00 ± 1.22	47.61 ± 12.42	54.58 ± 26.66	7.81 ± 0.07	7.72 ± 0.10
T_3	26.88 ± 0.30	27.73 ± 0.32	38.33 ± 0.82	36.33 ± 0.58	67.67 ± 12.19	66.67 ± 7.67	7.83 ± 0.09	7.82 ± 0.04
T_4	27.66 ± 0.35	27.27 ± 0.32	36.17 ±1.33	37.83 ± 0.75	66.30 ± 16.74	38.58 ± 13.14	7.92 ± 0.10	7.77 ± 0.07

Table 2 - Nutrient concentrations in the water column between 20 June and 31 July 2012 ($T_0 - T_4$) in disturbed (Ds) and undisturbed areas (Us). Nitric and nitrous nitrogen, N-NO_x; ammonium nitrogen, N-NH₄; soluble reactive phosphorus, SRP; dissolved organic nitrogen, DON; dissolved organic phosphorus, DOP. Values are mean ± SD (n = 30) in μ M.

	N-NO _x		N-NH ₄		SRP		DON		DOP	
	Ds	Us	Ds	Us	Ds	Us	Ds	Us	Ds	Us
T_0	1.73±0.77	1.75±0.99	$1.04{\pm}1.28$	$1.98{\pm}1.28$	0.042 ± 0.038	0.148±0.113	10.16±2.81	11.32±1.55	0.113 ± 0.059	0.162 ± 0.065
T_1	$2.06{\pm}1.55$	1.73 ± 0.97	2.10 ± 0.58	3.34±1,15	0.025 ± 0.023	0.075 ± 0.030	8.59 ± 2.84	10.66 ± 2.47	$0.209 {\pm} 0.024$	0.160 ± 0.022
T_2	0.68 ± 0.59	0.84 ± 0.57	1.07 ± 0.60	$0.74{\pm}0.61$	0.030 ± 0.020	$0.033 {\pm} 0.016$	6.83±1.72	11.60 ± 3.14	0.144 ± 0.022	$0.207 {\pm} 0.055$
T_3	0.78 ± 0.72	$1.66{\pm}1.09$	0.86±0.43	1.73 ± 0.66	0.030 ± 0.020	0.038 ± 0.022	8.82 ± 3.02	11.29 ± 2.89	$0.154{\pm}0.049$	$0.191 {\pm} 0.057$
T_4	1.13±0.75	2.41±1.89	0.93±0.39	1.41±0.53	0.024 ± 0.024	0.032 ± 0.033	10.48 ± 1.71	12.60±2.64	0.455 ± 0.212	0.480 ± 0.319

Table 3 – Atomic ratio of dissolved inorganic nitrogen (DIN) to soluble reactive phosphorus (SRP) in the water column between 20 June and 31 July 2012 ($T_0 - T_4$) in disturbed (Ds: D A1 and D A2) and undisturbed areas (Us: U A1 and U A2). Values are the mean \pm SD (n = 30) in μ M.

DIN : SRP									
	D A1	D A2	U A1	U A2					
T_0	78.63±75.07	279.08 ± 299.95	28.48±16.98	20.92±10.25					
T_1	450.95±369.03	300.64±160.03	77.76±24.32	104.92 ± 46.88					
T_2	47.37±16.23	140.51 ± 166.89	61.19±43.31	59.66 ± 25.40					
T_3	202.22±260.54	59.42 ± 53.28	89.53±30.23	120.54±121.13					
T_4	94.65±68.66	294.84±267.14	254.50 ± 268.43	208.51±145.01					

	Eh								
	Ds	Us							
T_0	-102.33 ± 40.64	-131.50 ± 22.76							
T_1	-184.80 ± 9.28	-196.33 ± 3.54							
T_2	-167.50 ± 33.24	-204.00 ± 5.73							
T_3	-179.00 ± 18.28	-207.33 ± 5.28							
T_4	-180.50 ± 25.43	-201.17 ± 7.54							

Table 4 – Redox potential in sediment between 20 June and 31 July 2012 ($T_0 - T_4$) in disturbed (Ds) and undisturbed areas (Us). Values are the mean \pm SD (n = 15) in mV.

Table 5 – Total organic carbon (TOC), total nitrogen (TN) and total phosphorus (TP) in sediment between 20 June and 31 July 2012 ($T_0 - T_4$) in disturbed (Ds) and undisturbed areas (Us). Values are mean (%) ± SD (n = 15) dry weight basis.

	TOC			TN	ТР		
	Ds	Us	Ds	Us	Ds	Us	
T ₀	5.00 ± 0.74	6.299 ± 0.98	0.62 ± 0.13	0.86 ± 0.17	0.054 ± 0.008	0.065 ± 0.005	
T_1	5.21 ± 0.75	6.086 ± 0.61	0.69 ± 0.13	0.97 ± 0.13	0.057 ± 0.009	0.064 ± 0.005	
T_2	4.46 ± 0.53	5.946 ± 0.58	0.60 ± 0.09	0.90 ± 0.11	0.050 ± 0.006	0.062 ± 0.004	
T_3	4.51 ± 0.32	5.824 ± 0.50	0.63 ± 0.07	0.86 ± 0.09	0.048 ± 0.005	0.058 ± 0.005	
T_4	4.32 ± 0.40	5.712 ± 0.71	0.63 ± 0.10	0.93 ± 0.16	0.048 ± 0.007	0.060 ± 0.004	

Table 6 - Atomic ratios in sediment between 20 June and 31 July 2012 ($T_0 - T_4$) in disturbed (Ds) and undisturbed areas (Us). Values are mean \pm SD (n = 15).

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	C:N		C	P	N:P		
	Ds	Us	Ds	Us	Ds	Us	
T_0	9.50 ± 1.00	8.59 ± 0.43	240.88 ± 17.64	248.92 ± 24.52	25.48 ± 1.86	29.08 ± 3.65	
T_1	8.91 ± 4.42	7.35 ± 0.38	$236.10 \pm \! 10.05$	245.12 ± 8.32	26.53 ± 1.62	33.44 ± 1.93	
T_2	8.78 ± 0.52	7.78 ± 0.56	232.33 ± 14.21	249.18 ± 24.99	26.50 ± 1.33	32.08 ± 2.56	
T_3	8.36 ± 0.31	7.95 ± 0.25	243.71 ± 7.33	$258.92 \pm \! 17.64$	29.15 ± 0.40	32.59 ± 2.46	
T_4	8.17 ± 0.54	7.31 ± 0.38	237.36 ± 18.01	241.43 ± 16.37	29.08 ± 1.96	33.19 ± 3.77	

Table 7 – C, N, P contents (% dry weight) of *Chaetomorpha linum* talli and the respective atomic ratios C:P, C:N and N:P on 4 July (two days after T₁), 13 July (T₂) and 31 July (T₄) 2012. Values are mean \pm SD (n = 6).

		С	Ν	Р	C:N	C:P	N:P
Ds	4 July	23.28 ± 0.08	0.95 ± 0.08	0.063 ± 0.001	28.73±2.46	959.70±11.99	33.58±3.05
	13 July	21.63 ± 0.11	1.13 ± 0.01	0.072 ± 0.011	22.39±0.27	788.03±117.81	35.24±5.70
	31 July	$23.03{\pm}1.07$	1.12 ± 0.06	0.066 ± 0.004	24.04±2.32	908.9 ± 87.40	37.81±0.67
Us	4 July	$19.50{\pm}1.95$	1.17 ± 0.32	$0.037{\pm}0.008$	20.11±3.33	1373.50±171.07	68.65±3.44
	13 July	19.45 ± 0.48	1.63 ± 0.44	0.102 ± 0.049	14.82 ± 5.04	623.24±411.82	39.28±12.18
	31 July	19.79±1.00	2.07 ± 0.10	0.110 ± 0.018	11.15±0.29	469.57±57.20	42.17±5.66

Table 8 – Surface area (A; hectares), total cover (CT), biomass (b; Kg_{WW} m⁻²) and standing crop (SC; tonnes wet weight, TPU) of disturbed (O) and undisturbed (N, I) macroalgal mats between 15 August (Aug) and 15 September (Sep) 2013.

-	А	СТ		b)	SC		
		Aug	Sep	Aug	Sep	Aug	Sep	
0	235	0.95	0.95	9.15±0.97	3.42±2.97	20418	7647	
Ν	150	0.95	0.95	9.92±2.10	8.63±2.20	14448	11478	
Ι	120	1.00	0.95	3.68±0.77	6.08±0.86	4422	6566	