



Growth Tests of 5 Native Opportunistic Species Producing Blooms in a Hypertrophic Mediterranean Lagoon

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Abstract

In this study, growth tests are conducted in microcosms of 5 opportunistic macroalgal species (*Gracilaria gracilis*, *Sphaerococcus coronopifolius*, *Chaetomorpha linum*, *Valonia aegagropila*, *Gongolaria barbata*) that produce blooms in the Orbetello Lagoon, a eutrophic non-tidal lagoon in the Mediterranean Sea. The objective was to improve knowledge about algal blooms in the lagoon environment that occur in specific areas and times for each species, and have time-varying dominances. For the purpose, 4 of these species were grown together, competing for substrate by varying salinity and nutrient concentration. The results showed remarkable growth ability of *C. linum* in all microcosms for any environmental condition tested. *S. coronopifolius* grew best in brackish waters and at high nitrate-nitrogen concentrations; *G. gracilis* grew well in brackish waters with high concentrations of both nitrate and ammonia nitrogen; *G. barbata* preferred waters with marine salinity and low nitrogen concentrations and N/P atomic ratio. These results are consistent with the distribution of macroalgal masses in the Orbetello lagoon, from which the test thalli were sourced. Less clear were the results for *V. aegagropila*, which had biomass declines in most of the tests, despite constituting important near-monoxenic blooms in the Orbetello lagoon. For this species, however, there was evidence of a preference for waters with marine salinity and high nitrogen and N/P atomic ratio values.

Keywords: *Gracilaria gracilis*; *Sphaerococcus coronopifolius*; *Chaetomorpha linum*; *Valonia aegagropila*; *Gongolaria barbata*; Microcosms; Orbetello Lagoon; Macroalgal Bloom

Introduction

In the last fifty years, lagoon and estuarine environments worldwide have been subjected to high anthropogenic pressure and high nutrient loads, which have increased eutrophication, changing the lagoon *euryhaline* and *eurytherm biocenosis* towards the dominance of *macroalgae* and *microphyte facies* [1].

Ecosystem responses to eutrophication vary in relation to their ability to buffer the effects [2]. Although lagoon environments structurally have a high resilience capacity, non-tidal lagoons are particularly prone to eutrophication stresses, given the low turnover and strong anthropization of the area, which can trigger a chain of reactions that lead to a shift in vegetation towards the dominance of tolerant and opportunistic species and, eventually, towards a dystrophic state [3,4].

Eutrophication is becoming increasingly important and widespread along coastal areas and not only in lagoons, in fact, under certain conditions, it can lead to macroalgal blooms even in open marine areas [5]. The development of important macroalgal masses accentuates competition with seagrass meadows, accelerating their regression and degradation [6,7]. Although there can certainly be a direct mechanical action of the macroalgae on the seagrasses, resulting in a kind of 'suffocation', other physical and chemical modes of impact have been found to be important, especially the consequences of environmental degradation and the chemical-physical changes in the water column that occur with the formation of vast and dense macroalgal masses and, even more so, the summer decay of the masses themselves that follows.

Eutrophication is not the only factor that can lead to the development of opportunistic macroalgae and the regression of seagrass meadows. Other factors, such as contaminants, water acidification and global warming, must also be considered. Global warming could make lagoon conditions even more extreme [8].

Fast-growing opportunistic macroalgae tolerate, within certain limits, the secondary effects of eutrophication, such as anoxia, high ammonium ion concentrations and releases of hydrogen sulphide from the bottom [9-12]. Macroalgal masses, in dense and thick mats, tend to keep the surface layer of the bottom in constant and strong anoxia, being devoid of roots and rhizomes, and their degradation, consisting mainly of labile organic matter easily attacked by break-down bacteria, can result in periodic dystrophies. Anaerobic decay results in the release of toxic gases, such as ammonia and hydrogen sulphide, and can affect the water column with anoxia and, subsequently, toxic catabolites, which can kill flora and fauna and reduce lagoon biodiversity to only bacterial and a few microalgal species.

In order to manage and counteract the effects of eutrophication, it is necessary to understand the dynamics of macroalgal developments, which manifest the dominance of different opportunistic species in relation to variations in nutrient concentrations, their atomic ratio, temperature, salinity and other environmental variables. Furthermore, the development of one species may be compromised or even facilitated by the development of another.

With the present study, it was sought to make a contribution to the understanding of the behaviour of some opportunistic macroalgae that develop intense blooms in the Orbetello lagoon, a non-tidal Mediterranean lagoon basin, in relation to nutrients and salinity. For this purpose, growth tests were carried out in microcosms of the main macroalgae forming blooms.

Materials and Methods

The Orbetello Lagoon and Macroalgal Blooms

The Orbetello lagoon is a shallow, eutrophic coastal water body of about 25.25 km² in the southern Tuscan coast of Italy (42° 25' -42° 29' N, 11° 10' -11° 17' E), divided into two communicating basins, West and East, with an area 15.25 km² and 10.00 km² respectively, and 1 m mean depth; three artificial sea-lagoon canals (SLC 1, 2, 3), 0.5–3 km long and 10–15 m wide, two in the West and one in the East basin, connect the lagoon with the sea (Figure 1). The lagoon is characterized by intense macroalgal blooms with now one now another species prevailing over the years, with summer decays of biomass that can lead to dystrophic events.

To counter this phenomenon, sea water pumping is carried out (12000 L/s) between May and September to promote water turnover, through an input flow coming from two western pumping stations, at the lagoon-mouth of the two western canals (SLC 1, 2), and an output flow by the eastern canal (SLC 3; Figure 1).

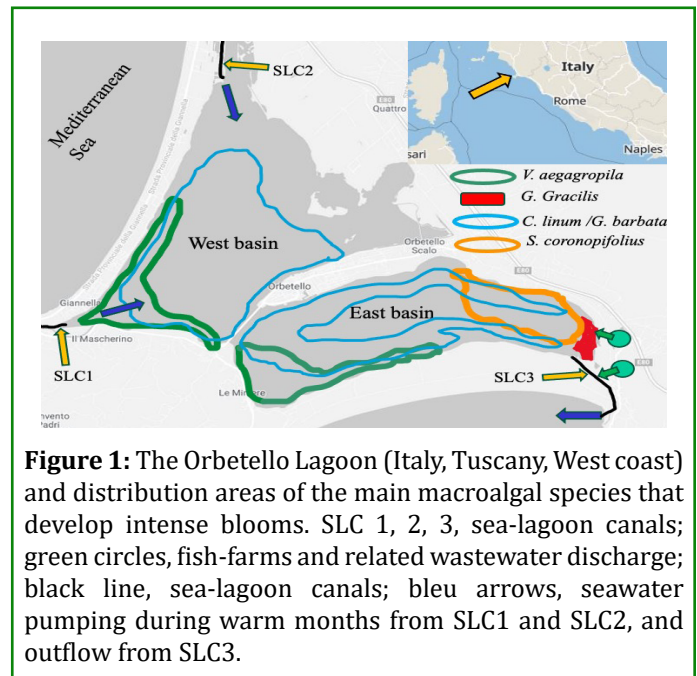


Figure 1: The Orbetello Lagoon (Italy, Tuscany, West coast) and distribution areas of the main macroalgal species that develop intense blooms. SLC 1, 2, 3, sea-lagoon canals; green circles, fish-farms and related wastewater discharge; black line, sea-lagoon canals; blue arrows, seawater pumping during warm months from SLC1 and SLC2, and outflow from SLC3.

Over the last ten years, a spatial distribution of opportunistic macroalgal species is being observed, as shown in Figure 1. Rhodophyta are preferentially distributed in two areas of the East basin, affected by the wastewaters of two land-based fish-farms [13]. In the area of the lagoon affected by the wastewaters, *Gracilaria gracilis* prevails to a relatively small extent, sometimes accompanied by *Gracilariopsis longissima*. A little further away *Alsidium corallinum* and *Sphaerococcus coronopifolius* are distributed [14,15].

The other areas of the lagoon are largely affected by *Chlorophyta*, essentially *Chaetomorpha linum* and *Valonia aegagropila*. In recent years, widespread blooms of the *Ochrophyta Gongolaria barbata* have been added, affecting the two lagoon basins, especially the West basin, where it develops in overlayer to the mats of *C. linum* [16].

During the temperature rise, between late spring and summer, decay of part of the algal standing crops occurs. The species most sensitive to summer criticality are Rhodophyta and *V. aegagropila*. The rapid decay of these species, together with deposits of labile organic matter in the sediment, can support very severe dystrophic processes [17].

Macroalgae Growth Experiences in Microcosms

Between 2023 and 2024, growth experiments were conducted on 5 macroalgal species, of which there is currently the greatest growth in the Orbetello Lagoon, testing behaviour to variation in salinity, temperature, and nitrogen and phosphorus concentration.

To understand the growth dynamics of the Rhodophyta *Gracilaria gracilis* (Stackhouse) Steentoft, L.M. Irvine & Farnham, several laboratory growth tests were conducted. In 2023, between April and May, the growth was tested under conditions of variability of salinity and nitrogen and phosphorus concentration.

In July 2024, competing growth was tested between the *Ochrophyta Gongolaria barbata f. aurantia* (Kützing) Falace, Alongi et Kaleb, the Rhodophyta *Sphaerococcus coronopifolius* Stackhouse, and the *Chlorophyta Chaetomorpha linum* (O.F. Muller) Kützing and *Valonia aegagropila* C. Agardh, for changes in salinity, and nitrogen and phosphorus concentrations.

Between July and August 2024, growth of *V. aegagropila* was tested in monoxenic culture for changes in salinity.

For the laboratory tests, conducted at room temperature and with solar light radiation, the chemical-physical variables were measured by DELTA OHM HD8705 for temperature, ATAGO S/Mill refractometer for salinity (practical salinity scale), and HI 97500 Luxmeter for illuminance. The weight of the algal thalli was determined with Sartorius balance B410.

Test 2023. Salinity

G. gracilis thalli were collected in the Orbetello lagoon and housed in the laboratory for 10 days. Subsequently, 4 thalli fragments, dried with adsorbing paper and weighed (1.89-2.95 g), were placed in as many 1 L cylinders (microcosms A-D) into which artificial seawater of different salinities

(10, 20, 28, 35; Table 1) had been added. The test was conducted from May 13 to May 23 and the weighing of the thalli was carried out every 5 days. Nutrients were added using a solution of Walne medium [18] resulting in an N concentration of 1.4 μM and a P concentration of 0.16 μM , and a atomic ratio of about 9. In this experiment, holding the nutrient concentration constant, the behaviour of this species was tested as salinity changes (Table 1).

	s	T °C	kLUX	N μM	P μM	N/P
A	35	28-30	15-43	1.4	0.16	9
B	28	28-30	15-43	1.4	0.16	9
C	20	28-30	15-43	1.4	0.16	9
D	10	28-30	15-43	1.4	0.16	9
E	38	21-28	16-50	0.47	0.12	3.9
F	38	21-28	16-50	0.47	0.2	2.4
G	38	21-28	16-50	0.47	0.34	1.4
H	38	20-28	15-52	0.47	0.05	9
I	38	20-28	15-52	12.27	0.05	236
L	38	20-28	15-52	19.17	0.05	369
M	16	27-32	16-44	2	0.13	15
N	20	27-32	16-44	2	0.13	15
O	30	27-32	16-44	2	0.13	15
P	37	27-32	16-44	2	0.13	15
Q	37	27-32	16-44	8	0.39	21
R	37	27-32	16-44	25	0.77	33
S	37	27-32	16-44	50	0.77	65
T	37	27-32	16-44	100	0.77	130
U	20	29-35	16-53	29.8	0.51	58
V	25	29-35	16-53	29.8	0.51	58
Z	30	29-35	16-53	29.8	0.51	58

Table 1: Macroalgae culture conditions in microcosms A-Z: s, salinity; T, temperature °C; kLUX, sunlight intensity; N, nitrogen in μM ; P, phosphorus in μM ; N/P, atomic ratio.

Test 2023. Nitrogen and phosphorous increases

6 thalli fragments of *G. gracilis* (ranging from 2.62 g to 3.47 g), dried with adsorbing paper and weighed, were placed in as many 1 L cylinders (microcosms E-L) with artificial seawater at salinity 38 and in which varying amounts of nutrients were added (Table 1). The growth test was conducted from April 27 to May 12, weighing every 5 days. Nutrient concentration was obtained using a solution of Walne medium with NaNO_3 ,

NH_4Cl and $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ additions.

In E, F and G, nitrate-nitrogen concentration was $0.47 \mu\text{M}$, while that of P was $0.12 \mu\text{M}$, $0.20 \mu\text{M}$ and $0.34 \mu\text{M}$, respectively, with respective N/P atomic ratios of 3.9, 2.4 and 1.4.

In H, I and L, the concentration of P was $0.052 \mu\text{M}$, that of N was $0.47 \mu\text{M}$, $12.27 \mu\text{M}$ and $19.17 \mu\text{M}$, respectively, with relative N/P molar ratios of 9, 236, 369. In microcosms H and I, the response of *G. gracilis* was tested with a low and higher concentration of nitrate nitrogen, while in L, it was tested for a similar concentration of ammonia nitrogen.

Test 2024. Salinity, Nitrogen and Phosphorous Increases

This experiment tested *G. barbata*, *S. coronopifolius*, *C. linum* and *V. aegagropila* in competition for changes in salinity and nutrients [8]. thalli fragments of each species were dried with adsorbing paper and weighed ($0.11\text{-}0.49 \text{ g}$, Table 2) and were placed in as many 1 L cylinders (microcosms M-T; Table 2). Each cylinder contained one thallus of each species. Artificial seawater of different salinities (16-37; Table 2) had been added in each microcosm. The test was conducted from July 13 to July 23 and the weighing of the thalli was carried out every 5 days.

	G. g.			I/D%				SGR $\text{g}_{\text{ww}} \text{ \% d}^{-1}$			
	I/D%	SGR $\text{g}_{\text{ww}} \text{ \% d}^{-1}$		G.b.	S.c.	CH	V.	G.b.	S.c.	CH	V.
A	3.68	0.36	M	4	30	113.33	-15.1	0.78	5.25	15.15	-3.27
B	5.82	0.57	N	14.81	22.22	178.26	-16.3	2.76	4.01	20.47	-3.56
C	15.74	1.46	O	13.04	24.39	313.04	0	2.45	4.37	28.37	0
D	-8.14	-0.85	P	25	11.54	314.29	0.75	4.46	2.18	28.43	0.15
E	22.51	1.35	Q	16.33	10.2	338.71	-5.13	3.02	1.94	29.57	-1.05
F	13.49	0.84	R	9.09	18.18	300	6.67	1.74	3.34	27.73	1.29
G	18.16	1.11	S	9.09	20.51	279.66	7.32	1.74	3.73	26.68	1.41
H	43.89	2.43	T	0	23.26	292.59	-3.87	0	4.18	27.35	-0.79
I	36.36	2.07	U				-15.33±0.54				-1.19±0.05
L	34.31	1.97	V				-6.13±4.32				-0.46±0.33
			Z				5.43±2.85				0.37±0.19

Table 2: Percentage increase/decrease (I/D %) between the start and end of each test, and specific growth rate (SRG; in % of wet weight per day) for the macroalgae *Gracilaria gracilis* (G.g.), *Gongolaria barbata* (G.b.), *Chaetomorpha linum* (CH), *Valonia aegagropila* (V.), *Sphaerococcus coronopifolius* (S.c.), grown in microcosms A-Z.

For M-P microcosms, nutrients were added using a solution of Walne medium, in the same quantities, for salinity 16, 20, 30, 37, with N/P atomic ratio of 15. For Q-T microcosms, at constant salinity 37, nutrients were increased for increasing N/P between 21 and 130, using Walne medium and appropriate additions of NaNO_3 and $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ (Table 2). Test 2024. Salinity Variation for *V. aegagropila*.

This test aimed to establish the performance of *V. aegagropila* for stable and relatively high N and P conditions (N/P 58), and different salinities, under a framework of relatively high seasonal temperatures (Table 1). Fragments of the thalli, dried with adsorbing paper and weighed ($12.7\text{-}33.8 \text{ g}$), were placed in three cylinders (three thalli in each cylinder; microcosms U-Z), switching from 37 storage salinity to artificial seawater with salinities 20, 25, 30. The test was conducted between 24 July and 7 August with subsequent weighing every 4-6 days.

Growth Measures

The increase or decrease in weight for subsequent weighing to the first was calculated according to the following equation:

$$\text{I/D}\% = (\text{W}_f - \text{W}_i) * 100 * \text{W}_i^{-1}$$

where W_i and W_f are initial and final weight, respectively.

The specific growth rate (SGR; $\text{g}_{\text{ww}} \text{ \% d}^{-1}$) expresses the daily growth rate of a population or a biomass, here applied for macroalgal thalli, and is calculated by the equation:

$$\text{SGR} = (\ln \text{W}_f - \ln \text{W}_i) * 100 * t^{-1}$$

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

The days (t) were: 10 for the microcosms A-D; 15 for E-L; 10 for M-T; 14 for U-Z.

Results and Discussion

In Table 1, the salinity adopted, the temperature and light ranges that occurred during the various experiments, and the N and P concentration and N/P atomic ratio are reported for all tests (A-Z).

In Figures 2-5, the weight trends obtained by periodic weighings are shown for the various species in the different tests (Figures 2-5).

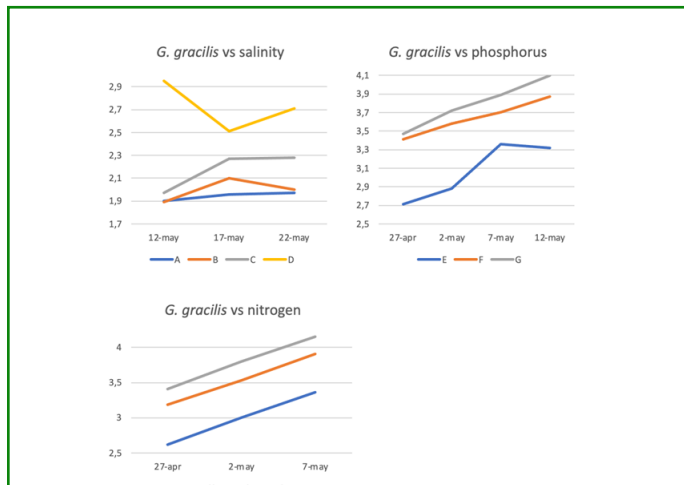


Figure 2: Trend in weights during the growth test of the Rhodophyta *Gracilaria gracilis*, for differences in salinity (microcosms A-D), orthophosphate concentration (microcosms E-G) and nitrate nitrogen (microcosms H, I) and ammonia nitrogen concentration (microcosm L).

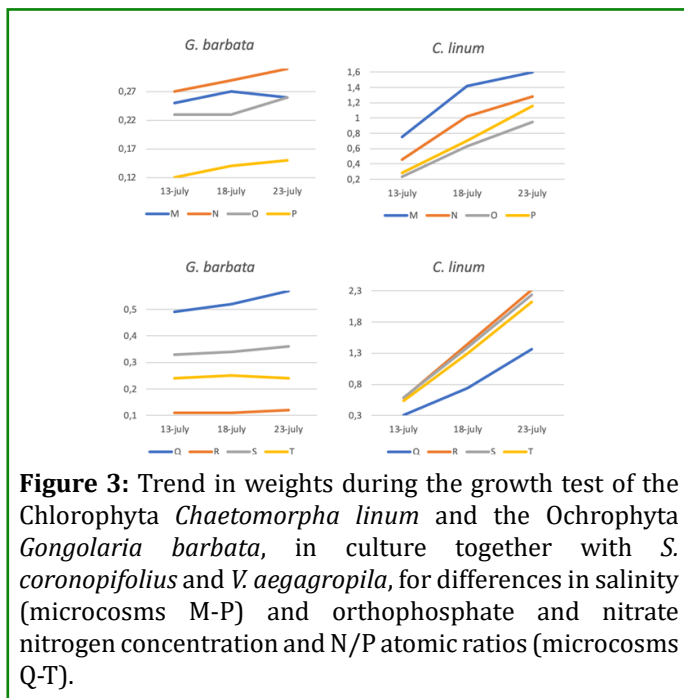


Figure 3: Trend in weights during the growth test of the Chlorophyta *Chaetomorpha linum* and the Ochrophyta *Gongolaria barbata*, in culture together with *S. coronopifolius* and *V. aegagropila*, for differences in salinity (microcosms M-P) and orthophosphate and nitrate nitrogen concentration and N/P atomic ratios (microcosms Q-T).

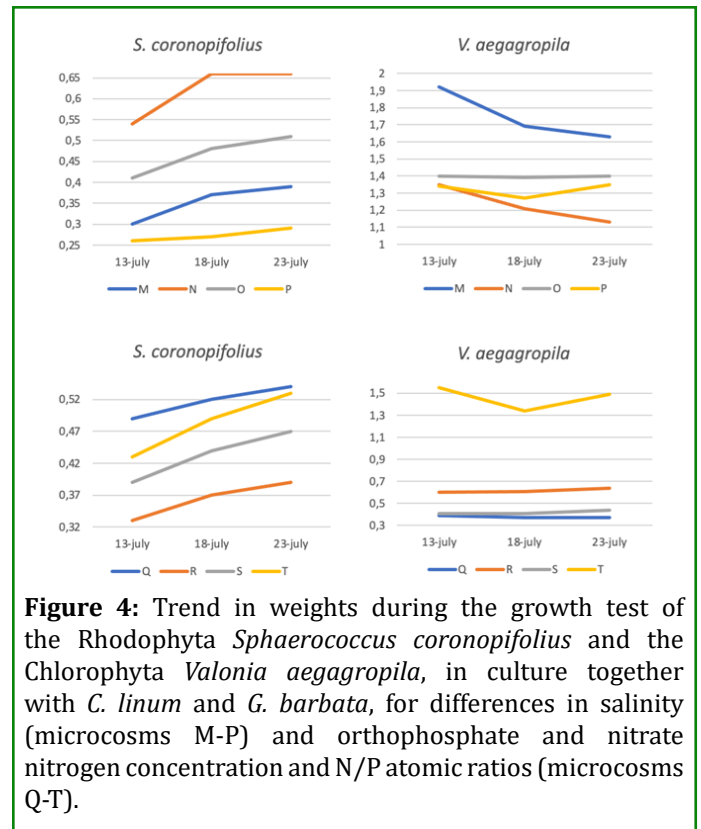


Figure 4: Trend in weights during the growth test of the Rhodophyta *Sphaerococcus coronopifolius* and the Chlorophyta *Valonia aegagropila*, in culture together with *C. linum* and *G. barbata*, for differences in salinity (microcosms M-P) and orthophosphate and nitrate nitrogen concentration and N/P atomic ratios (microcosms Q-T).

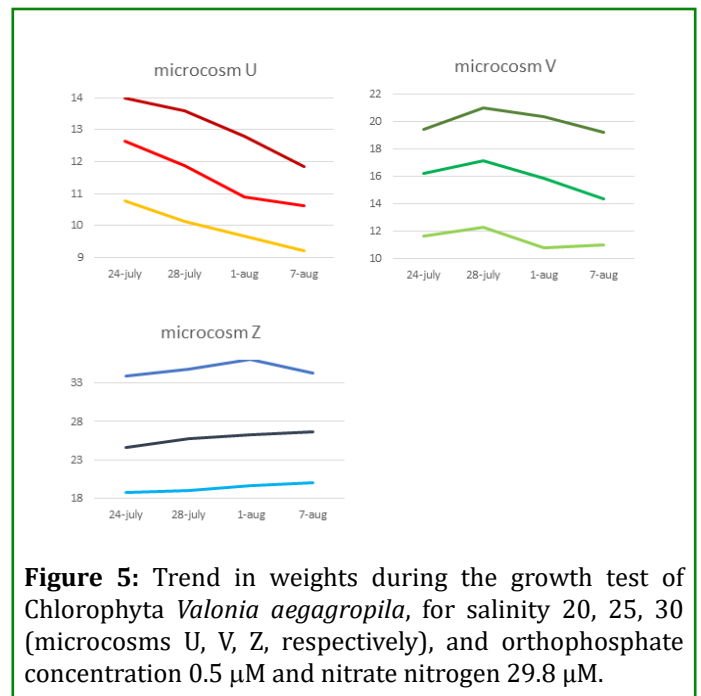


Figure 5: Trend in weights during the growth test of Chlorophyta *Valonia aegagropila*, for salinity 20, 25, 30 (microcosms U, V, Z, respectively), and orthophosphate concentration 0.5 μM and nitrate nitrogen 29.8 μM .

Table 2 shows the percentages of increase/decrease (I/D %) between initial and final weight, and the SGR for *G. gracilis* (microcosms A-L), for *V. aegagropila* (microcosms U-Z), and for *G. barbata*, *S. coronopifolius*, *C. linum*, *V. aegagropila* grown together (microcosms M-T).

Growth was positive in almost all microcosms, whereas a negative response, i.e. a decrease from the original weight, was seen for *G. gracilis* in microcosm D, for *G. barbata* in T, and for *V. aegagropila* in M, N, O, Q, T, U, V. High growth performances were obtained especially for *C. linum* in all microcosms in which it was placed.

Gracilaria gracilis

G. gracilis is considered to have good resilience to salinity and temperature variations, and optimal growth conditions occur at temperatures between 20°C and 30°C, for salinity between 25 and 42, in shallow water between 0.5 and 2.5 m, for light intensity between 70 and 210 $\mu\text{mol photons m}^{-2}\cdot\text{s}^{-1}$ (~3.5-10.5 kLUX) [19]. This species is a source of compounds of high commercial value, including agar and compounds for pharmaceutical use [19]. It can have a relatively high protein content, over 20 percent on dry weight [20,21], especially if it grows in shallow water [22], and this characteristic could be an important factor in its decay, as it is more susceptible to aggression by anaerobic bacteria of the sediment. *G. gracilis* decays almost completely during the warmer season, in the Orbetello lagoon, and its decay gives rise to dystrophic episodes localized to its range.

In the tests conducted in this study, *G. gracilis* showed the best growth at salinity 20, while the sudden change from 36 (stabling) to 10 produced a drop in weight, from which, however, it subsequently tended to recover (microcosms C and D, respectively; Table 2, Figure 2). With the additions of P, this species always showed growth, although the best growth was observed at the lowest concentration adopted, showing a slight decrease at the end of the experiment (microcosm E; Table 2, Figure 2). For additions of N, *G. gracilis* grew consistently with all concentrations tested, and even for this nutrient, the best growth occurred with the lowest concentration of those tested (microcosm H). In essence, similar growth between the microcosm with nitrate nitrogen and that with ammoniacal nitrogen was observed (microcosms I, L, respectively) (Table 2, Figure 2).

G. gracilis was confirmed to be a nitrophilous species that grows indifferently well with both N-NO₃ and N-NH₄. The fact that it is confined to the extreme area of the East Basin of the Orbetello Lagoon, where wastewater from fish farms, richer in nutrients and with low salinity, is released into the lagoon [13], is consistent with the results obtained showing growth in the microcosm C and microcosms E-L, with high concentrations of N (both nitrate and ammonia nitrogen) and P, as typical of fish-farm wastewater. This species probably dominates in this narrow wastewater-enriched area because it is competitive with other species, such as *S. coronopifolius* and *G. barbata*, which have growth problems in an ammonia-dominated environment and prefer nitrate nitrogen [15, 16].

Gongolaria barbata

G. barbata is a eurytherm species that can tolerate temperatures from very low to >30°C [23]. It is found anchored to rocks, near harbours, on artefacts and populates coastal lagoons where it is predominantly pleustophytic, freely floating on the bottom. It is being looked at as a possible mineral source, rich in macro- and micro-nutrients [24], and for the extraction of fucoxanthin, due to its antioxidant, anti-cancer and anti-diabetic properties [25].

Gongolaria barbata in the 1970s and 1980s, called *Cystoseira barbata* at that time, was present in the Orbetello lagoon and localised near the sea-lagoon communication canal SLC1 (Figure 1), an area most affected by seawater exchange. In the lagoon, it, is essentially in benthic-pleustophytic conditions. Towards the end of the 1990s, this species spread to the innermost areas of the West basin, probably conveyed by the pumping of seawater from stations SCL1 and SCL2 begun in 1996, forming small pleustophytic masses. Starting in 2016, it began to populate with progressively more abundant pleustophytic wisps in both the West and the East basins, mixing with the mats of *C. linum*. Since 2022, *G. barbata* has steadily increased its biomass, now constituting between 5% and 10% of the total macroalgal biomass [16].

In the tests, *G. barbata* grew at all salinities to which it was in competition with the other three species, but the greatest increase occurred at salinity 37 (microcosm P; Table 2; Figure 3). With respect to nutrient increases, this species appears to prefer low nitrogen concentrations, and P values that produce a N/P atomic ratio of around 20. The lowest growth was obtained at high nitrate concentrations and high N/P values. *G. barbata* has been growing in importance in recent years in the Orbetello lagoon, especially in the overlayer on the *C. linum* mats. However, the tests show a modest growth that confirms what was observed in monoxenic culture by Lenzi et al. [16]. This behaviour towards nutrients could explain the over-layer arrangement on the dense, thick mats of *C. linum*, which constitute a filter to the nutrients released from the bottom [16].

Sphaerococcus coronopifolius

There are not many studies on this Rhodophyta reported in floristic lists of the Mediterranean [26] and Atlantic [27]. It has attracted interest for the extraction of antiviral and antibacterial molecules [28], has been reported along the Lazio coast (Italy west coast) and studied for reproductive aspects for its commercial potential by Abdelahad et al. [29], and has been reported for bloom formation in the Mediterranean lagoon environment by Lenzi et al. [15].

In the present study, *S. coronopifolius* had the best growth at salinities between 16 and 30 (microcosms M-P), showing that it also tolerated significant salt changes, thus proving

to be a largely euryhaline species. Growth progressively increased as N and N/P increased (microcosms Q-T; Figure 4), proving to be a nitrophilous species, with a preference for nitrate nitrogen according to Lenzi et al. [15].

Chaetomorpha linum

C. linum is a cosmopolitan species that forms blooms in many coastal areas of the world. The mats of *C. linum* effectively sequester nutrient inputs from the sediment, reducing nutrient availability at the upper levels of the mats [30]. Furthermore, this species was found to have rapid growths and rapid ability to acclimatise its physiology to adverse and changing conditions in the mat overlayer, while the underlayer showed a low photosynthetic efficiency [31].

C. linum showed very high growths both at the 4 salinities tested, with a crescendo towards the higher salinity values (microcosms M-P; Figure 3), and at nutrient increases, especially at the lower values of the N/P atomic ratio (microcosms Q-T; Figure 3), confirming that it is a phosphophilous species [32,33]. SGRs between 15 and 28 % $g_{ww} d^{-1}$ were obtained for this species. With these results, considering its high growth potential under eutrophic environmental conditions, it is not surprising that *C. linum* is found almost everywhere in the Orbetello lagoon, with extensive mats at high densities.

Valonia aegagropila

V. aegagropila is a cosmopolitan species distributed in the temperate and tropical belt [34]. When it grows in shallow, wave-exposed areas, it is attached to hard substrate, while in shallow, sheltered areas, it forms aegagropylous thalli (balls) floating on the bottom, that can come to the surface for gas formation. Pleustophytic ball condition is typical of lagoon environments. Recently, it has been studied as a basic element for the production of levulinic acid [35].

V. aegagropila, cultivated with the other three species at relatively low nutrient concentrations with a molar ratio of N/P 15, showed strong declines especially at salinities lower than the maintenance value in lab, and a slight late growth at salinities 37 (microcosms M-P, Table 2, Figure 4). In salinity 37 microcosms with increased nutrients, this species showed a modest increase in growth in N/P atomic ratios 33 and 65, while it decreased in the others (microcosms Q-T, Table 2, Figure 4).

In monoxenic culture, with salinities 20, 25 and 30 and relatively high nutrient concentrations in a constant N/P 58, it showed a strong decrease at lower salinities and an increase at salinities 30 (microcosms U-Z; Figure 5). The U-Z microcosms were those subjected to the highest temperatures, whereby *V. aegagropila* in the Z microcosm

showed that it can tolerate prolonged temperatures of up to 35°C.

Although this species has shown numerous declines in microcosms, both in competition and in monoxenic culture, in the lagoon environment it is often found mixed with *C. linum*, especially in well-lit areas in relatively shallow water (south-east area of the East basin), but also forming extensive mats as a dominant species. The latter are areas of the lagoon that are most affected by marine inputs, such as the area close to the SLC1 (Figure 1), however, areas far from freshwater inputs such as those close to SLC2, which is close to a river, and SLC3, where fish-farm wastewaters bringing low salinity waters are discharged (Figure 1). In tests, the species seems to prefer high salinity and relatively high nutrient concentrations, however, it is likely that other variables also play a role in determining dominant mats of this species, considering that such a hardy, high-growing species like *C. linum* has not supplanted *V. aegagropila*-dominant mats.

In the group of the four competing species, the results show that *C. linum* is the most invasive and environmentally tolerant opportunist species with considerably high growth potential, confirming what has actually been happening in the Orbetello lagoon since 2005, when this *Chlorophyta* became the dominant species in the two basins, West and East [36,17]. At low salinity (microcosm M), the species with the best yield after *C. linum* was *S. coronopifolius* (microcosms N, O), while increasing salinity placed *G. barbata* in second position (microcosmo P). It can be deduced that in low salinity conditions caused by heavy rainfall, *C. linum* tolerates variations very well, while *S. coronopifolius* even seems to benefit. In fact, in the lagoon, the period of greatest growth of this latter species is between late autumn and winter, when there is the greatest rainfall, and the fish-farms, towards which its distribution range prevails, introduce into the lagoon water taken from the subsoil with salinity varying between 15 and 30 (Figure 1) [13]. In contrast, *G. barbata* has only been spreading in the lagoon basins for a few decades, probably conveyed by the artificial flow of seawater, having previously been confined to the area close to the sea mouth (SNC1, Figure 1), so the currently expanding strain comes from a marine salinity area [16].

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