Organic aggregates formed by benthopleustophyte brown alga Acinetospora crinita (Acinetosporaceae, Ectocarpales)

Received Date : 03-Dec-2015

Revised Date : 24-Feb-2016

Accepted Date : 07-Mar-2016

Article type : Regular Article

Organic aggregates formed by benthopleustophyte brown alga *Acinetospora crinita* (Acinetosporaceae, Ectocarpales)[1]

Michele Giani

National Institute of Oceanography and Experimental Geophysics (OGS), 34151 Trieste, Italy

Gianfranco Sartoni, Caterina Nuccio

Department of Biology, University of Firenze, 50121 Firenze, Italy

Daniela Berto

Istituto Superiore per la Protezione e la Ricerca Ambientale, 30015 Chioggia, Italy

Carla Rita Ferrari

Agenzia Regionale per la Protezione dell'Ambiente dell'Emilia Romagna, 47042 Cesenatico, Italy

Marjana Najdek

Institut "Ruđer Bošković", Center for Marine Research, 52210 Rovinj, Croatia

Paola Sist, Ranieri Urbani[2]

Department of Life Sciences, University of Trieste, 34127 Trieste, Italy

Editorial Responsibility: M. Posewitz (Associate Editor)

Abstract

This work presents the elemental, polysaccharide, and fatty acid compositions of benthic aggregates formed by the filamentous brown alga *Acinetospora crinita,* which are widely spread on the rocky bottoms of the Mediterranean Sea. The aggregates can be characterized as mineralized centers in which regeneration of nutrients and recycling of dissolved organic matter actively occur and favor the development of an abundant phytoplankton community. Analyses of the stable isotopes of C and N display their marine origin and could provide evidence of the processes that occur inside/outside of the aggregates. The monosaccharide compositions of Adriatic and Tyrrhenian mucilages produced by brown alga *A. crinita* were quite similar. In particular, the Adriatic sample compositions resembled the average composition of the Tyrrhenian high molecular weight exopolymers, and the observed differences could be ascribed to different degradation stages. The fatty acid patterns found for the aggregates were similar to those observed in the isolated *A. crinita* algae with variable contributions from embedded diatom species. The bacterial contribution to the fatty acid pool was quite low, most likely due to the known poor conditions for their heterotrophic growth.

Key index words: *Acinetospora crinita,* aggregates, brown algae, filamentous algae, lipids, polysaccharides.

Abbreviations: CTD probe (Conductivity, Temperature, Depth probe), OM (Organic Matter), TCHO (Total carbohydrates), DCHO (Dissolved carbohydrates), FAMEs (Fatty Acids Methyl Esters), PUFA (Polyunsaturated Fatty Acids), DOC (Dissolved Organic Carbon), DON (Dissolved Organic Nitrogen), DOP (Dissolved Organic Phosphorous).

[1] Received xx December 2015. Accepted xx

[2] Author for correspondence: email: rurbani@units.it, phone +390405588762

The free-living form of *Acinetospora crinita* (Carmichael ex Harvey) Sauvageau, a brown alga belonging to the order Ectocarpales, is widespread in the Mediterranean Sea (Sartoni and Sonni 1992, Welker and Bressan 1994, Antolić et al. 2010, , Tsiamis et al. 2013) and is one of the most frequently reported taxa observed in the benthic mucilaginous aggregates examined in the Tyrrhenian Sea (Sartoni and Sonni 1992, Innamorati 1995, Mistri and Ceccherelli 1996, Hoffmann et al. 2000) and Ligurian Sea (Schiapparelli et al. 2007). Together with other filamentous brown algae, this species is also largely distributed in temperate coastal environments throughout the globe (Sartoni and Sonni 1992, Pedersen and Kristiansen 2001).

Although studies in locally eutrophicated coastal areas all over the Baltic Sea region have shown an increased occurrence of fast-growing filamentous algae and loose-lying algal mats that cause anoxia (Eriksson et al. 1998 and references therein), no evidence exists of eutrophication in the Tyrrhenian and Ligurian areas where the benthic mucilaginous aggregates were observed (Innamorati et al. 2001, Misic et al. 2011).

Mucilaginous benthic aggregates can adversely impact the benthic communities (Calvo et al. 1995) by causing suffocation of gorgonians (Mistri and Ceccherelli 1996, Giuliani et al. 2005) or bleaching of corallinales or scleractinians (Schiaparelli et al. 2007). Moreover, bottom currents can detach the aggregates from the macroalgae to which they are usually attached, allowing them to settle on to the seafloor and causing the formation of anoxic conditions that can adversely impact the benthic communities.

Previous studies on the chemical and biochemical composition of benthic aggregates formed by different filamentous algae (Sartoni et al. 2008) showed that carbohydrates and proteins account for 26.6% to 55.9% of the organic carbon, respectively. Galactose, xylose or mannose and fucose were the main components of the exopolysaccharidic fraction.

The aim of this study was to characterize the benthic aggregates and to assess whether the nature of the organic matrix formed by the filamentous brown alga *A. crinita* is similar to that of the benthic aggregates found in other Mediterranean areas. Knowledge of the chemical and biochemical composition of benthic aggregates formed by different algae is fundamental to distinguishing these aggregates from pelagic aggregates (Giani et al. 2012 and reference therein) that sediment on the bottom, thus allowing a better understanding of their origin and fate in the marine environment.

MATERIALS AND METHODS

Site descriptions. Sampling of benthic aggregates was carried out between 2 and 26 June 2004 in two areas of the Adriatic Sea, i.e., in the Northern sub-basin along the western coast of the Istra peninsula (Croatia) and in the southern sub-basin near the Tremiti Islands (Fig. 1, a and b)

Sampling and physical parameter measurements. The benthic aggregates were collected between 2 and 26 June 2004 by scuba divers using polymethacrilate syringes (0.06 L and 2 L). The sampled aggregates were divided for different analyses. For elemental analyses, the subsamples were immediately frozen at –20°C, successively de-frozen and dialyzed (Cellusep, 3500 MWCO) against ultrapure Milli-Q water, and finally freeze-dried.

Salinity, temperature, and depth profiles were determined using a CTD probe (Conductivity, Temperature, Depth, SEA Bird Electronics - SBE 25).

Microscopy analyses. Light microscope examination of the collected benthic aggregates was performed to describe the macroalgal and microalgal compositions. Macroalgal observations were performed at 250X magnification. For identification of the microalgal components, samples (250 mL) were fixed with neutralized formalin to a final concentration of 4%. Microscopic observations were performed at 400X magnification on subsamples using a Zeiss IM35 invertoscope in sedimentation chambers.

Light microscope examinations of the collected aggregates were used to describe their macroalgal composition and their relative abundance via the method suggested by Jones (1968)

Elemental and isotopic analyses. Water content was determined by gravimetric measurement of weight loss after drying for 48 h at 60°C. The organic matter (OM) was determined gravimetrically by loss on ignition of 100-200 mg of aggregates after combustion for 4 h at 450°C.

Total carbon (C_{tot}), nitrogen (N_{tot}) and sulfur (S) were determined using a CHNSO elemental analyzer Fisons 1108EA, as reported in a previous paper (Sartoni et al. 2008). The reproducibility was ≤3% for carbon, ≤5% for nitrogen and ≤1% for sulfur, and the reference material BCSS (CNRC, Canada) was used to assess the accuracy of the analytical data.

Phosphorus content was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) at 177 nm on 1 mol $\cdot L^{-1}$ HCl extracts of the combusted sample (Aspila 1976). The accuracy and reproducibility were 98.8 ± 4.8% and ≤5%, respectively.

Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes were analyzed using a Finningan Delta Plus Isotope Ratio Mass Spectrometer coupled with a CHNS-O Analyzer Fisons (Italy) model EA1108. The results were expressed as deviation per mil (‰) from the international VDB (Vienna Pee Dee Belemnite) standards. The analytical precision of the measurement was 0.2‰, and the reproducibility was <0.1‰. The $\delta^{13}C$ value was determined after the removal of carbonates via HCl treatment.

The determination of nitrates, nitrites, ammonia, phosphates and silicates were carried out via colorimetric methods with a Bran Luebbe Autoanalyzer Mod. AAIII instrument. Total dissolved nitrogen and total dissolved phosphorus were determined after wet oxidation by persulfate potassium according to Koroleff (1983 a, b).

Carbohydrate analysis. Carbohydrates were sampled by collecting 200 mL of bulk seawater and inner water inside the aggregate, which was divided into two aliquots. One aliquot was used for analysis of total carbohydrates (TCHO), and the remainder was gently filtered (0.7-mm nominal pore size, Whatman GF/F filters) for analysis of extracellular dissolved carbohydrates (DCHO). All samples were stored at -20°C after the addition of 0.02% NaN_3 as an antibiotic preservative until analysis.

Total and dissolved carbohydrates were determined spectrophotometrically using the 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) assay (Johnson and Sieburth 1977) after hydrolysis with 0.1 N HCl at 100°C for 16 h. The carbon content of the carbohydrates was calculated based on the glucose standard.

Isolation and purification of mucilage polysaccharides. The exopolysaccharide extraction and purification methods were described in the 2008 paper (Sartoni et al. 2008). The monosaccharide composition of exopolysaccharides was analyzed via gas chromatography using the derivatization method of Blakeney et al. (1983) for preparation of alditol acetates, as described by Magaletti et al. (2004), after polysaccharide hydrolysis in 2 N trifluoroacetic acid (TFA) and heating at 100°C for 16 h. Inositol was used as an internal standard. The derivatives were analyzed in a capillary gas chromatograph (Perkin Elmer Autosystem XL) equipped with a capillary column SP2330 (Supelco, 30 m×0.25 mm×0.2 μm film thickness) and an FID detector. The RSD of 20 independent analyses was 1.5%.

Fatty acid analysis. Mucilage samples were saponified (1.2 M NaOH in methanol), acidified (6 M HCl), methylated (14% BF3 in methanol), and extracted in dichloromethane as reported by Morrison and Smith (1964). Fatty acid methyl esters (FAMEs) were analyzed by Agilent gas-liquid chromatography (GLC) in a 6890N GC system equipped with a 5973 Network Mass Selective

Detector, capillary column (25 m x 0.3 mm x 0.25 μm; cross-linked 5% phenylmethylsiloxane) and ultra-high purity helium as the carrier gas.

The FAMEs were identified by mass spectral data and a family plot of equivalent chain length data for GC standards for the specific GC column. The bacterial FAME standard mix, FAMEs mix C18-C20, polyunsaturated fatty acids standards (PUFA1 and PUFA3), cod liver oil and various individual pure standards of FAME were also used.

In addition to the native benthic aggregates, *A. crinita* algae were also isolated from the mucilaginous matrix to assess the fatty acid composition solely due to the algae and hence its contribution to the total fatty acid content in all mucilaginous aggregates.

To reveal possible microalgal-bacteria interaction within the benthic mucilage aggregates, as recognized in pelagic aggregates, the conditions of phytoplankton and bacteria growth were inferred from the fatty acid contributions and ratios. The ratios C16:1/16:0 and C16P/C18P were used to determine the growth stage and condition of the aggregated phytoplankton (as a measure of their activity). Diatom ratios C14:0+C16:1+C16P/C16:0 (Leveille et al. 1997) were applied to determine the relative proportion of diatom biomass with respect to other phytoplankton classes in the mucilage.

The relative importance of bacterial activity in the aggregates was estimated from contributions of the principal bacterial markers (C15:0 + C15iso + C15anteiso + C17:0 + C17iso + C17anteiso + C18:1n7) to total fatty acids in the mucilage (Mayzaud et al. 1989, Najdek et al. 2002). The C15:br/C15:0 ratios were used as indicators for bacterial growth within the aggregates; for optimal growth, this ratio is estimated as >2.3 (White et al. 1980).

RESULTS

Thermohaline conditions. The surface and near-bottom temperatures (Table 1) showed a similar increasing trend from February/March to June. At the beginning of May in Istra, the water thermohaline properties were quite homogeneous, whereas in the following period, a strong

stratification was established. At Tremiti Island, the warming was more relevant than at the higher latitude of the Istra peninsula. In June, the surface temperature in the Istrian coastal waters ranges between 22°C and 23°C, and the thermocline is located between -10 m and -20 m; below this line, the temperature ranged from 16.0°C to 16.5°C. At the end of May, a freshened water input decreased the salinity of surface waters along the Istrian coast down to 36.1, with a halocline located between –4 m and –8 m at Secca Porer and Punta Croce and slightly deeper at the Banjole site. At the Tremiti Islands, the salinity was high (38.48-38.77), indicating no significant influence of rivers in the study area. A thermal gradient decreasing from the surface to the bottom was due to the seasonal spring warming.

Macroalgae identification. The observations of the macroalgal community that produce the mucilaginous benthic aggregates as sampled in the two sites of the Adriatic Sea (Fig. 2) have shown that they are similar to those present in various areas of the Tyrrhenian Sea (Sartoni et al. 2008). In both cases, the free-living form of *A. crinita* (Carmichael) Sauvageau plays a fundamental role in their development, as noted also by Welker and Bressan (1994), and this benthopleustophytic species is often mixed with *Nematochrysopsis marina* (Feldmann) Billard, a filamentous chrysophyte usually present in the Tyrrhenian benthic aggregates. Furthermore, in the Adriatic aggregates, both chrysophytes *Chrysonephos lewisii* (W. R. Tylor) W. R. Taylor and *Chrysophaeum taylorii* I. F. Lewis et H. F. Bryan were absent, although they were found in the Tyrrhenian aggregates. This last aspect is not surprising considering that these two allochthonous species were recently introduced into the Mediterranean (Sartoni et al. 1995), and their distribution is actually limited to the Tyrrhenian Sea for *C. lewisii* and to the Tyrrhenian and Aegean Seas (Aktan and Topaloğlu 2011) for *C. taylorii*.

From this point of view, a peculiar aspect of the Adriatic aggregates compared with their Tyrrhenian counterparts is based on the lower percentage of *N. marina.* Most of the examined material appears to be exclusively constructed from the benthopleustophytic form of *A. crinita,* both

in the aggregates collected in Rovinj and in the Tremiti Islands, even if a striking difference exists between the two sites. The Tremiti Island benthic aggregates not only show poor development during the surveys performed from the end of April until the middle of July, but they also exhibit a different temporal evolution because they appear a month later and show remarkably early aging compared with the Rovinj site aggregates.

These differences are not quite understandable even if the benthic aggregates of the free-living form of *A. crinita* are unpredictable in occurrence. Nevertheless, these materials usually show a massive accumulation in the lower infralittoral zone, particularly in the presence of such anchor species as perennial brown algae belonging to the genera *Cystoseira* and *Sargassum*, whereas in the circalittoral zone, the communities of gorgonians represent a suitable support for their development. From this point of view, as noted by Cormaci et al. (2001), the benthic vegetation of the Tremiti Islands appears rather unstructured; the infralittoral photophilic communities with *Cystoseira* spp. and *Sargassum* spp. are not present, and therefore, the sporadic aggregates primarily grow on ephemeral bushy macroalgae. In the circalittoral zone, most of the stations examined in the Tremiti Islands are lacking in gorgonians communities, and the coralligenous assemblages on horizontal and vertical surfaces are largely overgrown by turf-forming species, which represent an unsuitable substrate for the development of benthic mucilaginous aggregates. Among the turf-forming macroalgae, *Womersleyella setacea* (Hollenberg) R.E. Norris appears to be particularly abundant; this material is an allochthonous filamentous Rhodophyta that is widespread in the entire Mediterranean and negatively affects the species number and diversity of invaded communities (Athanasiadis 1997 ,Piazzi et al. 2007, Nikolić et al. 2010).

Microalgal community. Both at Rovinj and the Tremiti Islands, the microalgal community living within the Adriatic benthic aggregates was largely composed of microphytobenthic diatoms, as previously evidenced from previous observations of the Tyrrhenian benthic aggregates (Innamorati et al. 2001, Sartoni et al. 2008). Abundances varied from 10^6 to 10^7 cells $\cdot L^{-1}$, and a time shift in

taxonomic composition of the Rovinj aggregates was revealed as well. In May, *Licmophora* spp. was the dominant diatom genus, ranging from 45% to over 80% of the total abundance, followed by *Striatella unipunctata* (Lyngbye) Agardh and *Cylindrotheca closterium* (Ehrenberg) Lewin et Reimann (Fig.3). From the beginning to the end of May, the dominance of these taxa decreased together with a higher presence of many pennate diatoms (*Synedra, Nitzschia, Navicula, Pleurosigma, Amphora, Grammatophora, Cocconeis, Isthmia*) and specimens of other algal classes (*Dictyocha fibula* Ehrenberg, *Rhabdosphaera claviger* Murray & Blackman, thecate dinoflagellates). In these samples, it is worth noting the presence of *Prorocentrum lima* (Ehrenberg) Dodge, which is a thecate benthic dinoflagellate DSP-producing species. For the aggregates collected during this period, a comparison was performed between the community of the aggregates and the microphytobenthos collected at the same depth on substrates not affected by mucilages. *Licmophora* spp. and *Striatella unipunctata*, which dominated in the aggregates (47% and 41%, respectively), showed notably low abundances on the mucilage-free substrates (4% and 3%, respectively), whereas *Cylindrotheca closterium* reached a higher percentage (31%) with respect to the aggregates (8%), together with nano- and micro- unidentified pennate diatoms.

In June, *Cylindrotheca closterium* appeared to be the most abundant species and was always accompanied by a rich and diversified diatom community (*Licmophora, Diploneis, Grammatophora, Synedra*); it become the nearly exclusive component of the aggregates in July. The scarce development of the sporadic aggregates at the Tremiti sites did not depict a similar trend but was used only to compare selected samples. At the end of May, the microalgal composition was comparable to that of the Rovinj aggregates (Fig. 3), and in June, the dominance of *Cylindrotheca closterium* was not detected

Elemental composition of the aggregates. The *A crinita* aggregates were highly hydrated (88.5-91.6%; Table 2) with an organic matter content (OM) that ranged from 41% to 63%. The OM/organic carbon ratio showed an average value of 2.26±0.68. The carbonates embedded in the

aggregates constituted a non-negligible fraction of the inorganic constituents of the aggregates (Table 2), likely due to the entrapment of organisms with calcareous skeletons. Nitrogen content ranged from 1.05% to 2.23%, values similar to those reported for pelagic mucilage sampled in the northern Adriatic Sea (Giani et al. 2005). Sulfur content was relatively low (Table 2) with respect to the values reported for benthic aggregates sampled in the Adriatic and Tyrrhenian sea (Sartoni et al. 2008).

The C_{org}/N and C_{org}/P molar ratios of the aggregates (Table 2) were higher than the phytoplankton Redfield ratio (Redfield 1963) and comparable to those found in macroalgae *Ulva rigida* and *Fucus virsoides* (Faganeli et al. 1988). The relatively higher C_{org}/P molar ratios (Fig. 4a) of the Tyrrhenian benthic aggregates compared with those of other types of aggregates suggest a predominance of P-depleted organic compounds as polysaccharides. The high activity of alkaline phosphatase in the aggregates with respect to the surrounding waters could be the cause of depletion of P (Del Negro et al. 2005).

The higher C_{org}/S ratio values observed in the benthic aggregates could be a result of different degradation stages (fresher) if these ratios are compared with those reported for the benthic aggregates sampled in Tyrrhenian sea by Sartoni and coworkers (2008). The $\delta^{13}C$ ratio, which is generally used as indicator of the terrestrial ($\delta^{13}C$ from -30‰ to –25‰) or marine ($\delta^{13}C$ from -23‰ to -15‰) origin of organic matter (Druffel & Williams 1992), evidenced in benthic aggregates (average: -19.7±1.4‰; Fig. 4a), showed a prevalence of marine origins, as also observed in previous works for pelagic aggregates (-19.7±2.2‰, Giani et al. 2006; -19‰, Faganeli et al. 2009) and plankton (–20.7‰, Giani et al. 2009) samples.

The *A. crinita* sampled at Tremiti showed a mean value of $\delta^{13}C$ similar to that reported by Faganeli et al. (1988) for the macroalgae *Ulva rigida* (Fig. 4b), whereas in turf algae on a coastal reef in Florida, less negative values ($\delta^{13}C$: −16.6± 3.1‰, Lamb et al. 2012) have been reported.

The negative relationship between $\delta^{13}C$ found in the *A. crinita* and the C_{org}/N ratios (Fig. 4c),, sampled at the Istra sites in different sampling periods might suggest a change of elemental and isotopic signature with aging and progressive bacterial degradation (Coffin 1989) of these aggregates.

The $\delta^{15}N$ isotopic ratio of the benthic aggregates ranged from 2.68‰ to 5.29‰ (Table 2). This ^{15}N signature falls in the lower range reported for macroalgae by other studies, i.e., 0.5-13.8‰ (Vizzini et al. 2003, Cole et al. 2004) or 4.5-10 ‰ (Moncreiff and Sullivan 2001); however, relatively low values (+2.7±0.9) were reported for turf algae on a coastal reef in Florida (Lamb et al. 2012).

The results of nitrogen isotopic analysis of $\delta^{15}N$ in the benthic aggregates could suggest a low anthropogenic impact in the sampling sites, and in fact, enrichment of ^{15}N values was observed in the seagrasses sampled in the area subject via anthropic input with respect to pristine sites (Lassauque et al. (2010) and authors therein).

Interstitial waters of the benthic aggregates. All of the dissolved nutrients, i.e., phosphates, silicates, nitrates and ammonia, were highly concentrated in the aggregates' interstitial water with respect to the surrounding water (Fig. 5, a and b). Similar behavior was observed for the dissolved organic nitrogen and phosphorus (Fig. 5c).

High concentrations of DOC and DCHO (47.8±10.3 µM) were found in the interstitial water within the aggregates with respect to the outer bulk water (Fig. 5, c and d), as observed for the other nutrients. Moreover, DOC enrichment was up to four-fold higher, whereas the DCHO reached a concentration value that was six times higher. A linear correlation ($n = 5$, $R^2=0.952$, $p<0.01$) between DOC and DCHO was noted in the interstitial water, whereas no correlation was observed in the water outside the aggregates.

Carbohydrate characterization. The purified polysaccharides extracted from the mucilage samples formed by *A. crinita* neutral aldoses (galactose, glucose, mannose, xylose, rhamnose, fucose, ribose and arabinose) were determined. The monosaccharide compositions expressed as relative percentages (w/w) are reported in Table 3**.**

Abbreviations: Gal, galactose; Glc, glucose; Man, mannose; Rha, rhamnose; Fuc, fucose; Rib, ribose; Ara, arabinose; Xyl, xylose.

The mean monosaccharide compositions of the exopolysaccharide fraction were obtained by averaging separately over all samples from the Istra coast and from the Tremiti Islands, and the results are shown in Figure 6. The exopolysaccharides produced by *A. crinita* in these two sites showed similar monosaccharide patterns, with galactose as the main monosaccharide (approximately 30%) followed by xylose and fucose (approximately 20%). The remaining composition included glucose, rhamnose and mannose (approximately 7-11%). In the same figure, the composition of the exopolysaccharide of the aggregates produced by *N. marina* (Istra site) is also shown for comparison. Marked differences can be noted with respect to *A. crinita* samples with higher percentages of glucose (32%) and galactose (41.5%) and lower percentages of mannose, xylose and fucose (Fig. 6).

Fatty acids. The major fatty acids (FA) observed in the *Acinetospora crinita* isolated from benthic mucilage samples were C14:0, C16:0, C18:1(n-9), C18 PUFA (i.e., C18:2(n-6), C18:3(n-3), C18:4(n-3)), and C20 PUFA (i.e., C20:4(n-6) and C20:5(n-3)), as reported in Table 4. These results are in good agreement with data reported for brown algae from other regions (Fleurence et al. 1994, Khotimchenko 1998). The characteristic features of *A. crinita* were recognized in benthic mucilage samples collected along the Istra coast and Tremiti Islands (Punta Ferraio). The ranges for the major fatty acid proportions and ratios were similar to those found in *A. crinita*. One difference in alga (*A.*

crinita) is that its mucilage contained a measurable proportion of C16 PUFA in addition to higher diatom and C16:1/C16:0 ratios (Table 4), thus revealing the contribution of other macroalgal (preferably green) or microalgal (diatoms) species to the total organic matter of aggregates. However, the contribution of bacterial fatty acids and the ratio C15br/C15:0 were quite similar in both the alga and its mucilage. The major FAs in benthic mucilage produced by *Nematochrysopsis marina* (Banjole Island) were C16:0, C18:1, and C18:3(n-3) among the saturated, monounsaturated, and polyunsaturated fatty acids, respectively. The diatom ratio and C16:1/C16:0 and C16P/C18P ratios of *N. marina* mucilage were also similar to the respective ratios obtained for *A. crinita* mucilage. In contrast, *N. marina* mucilage contained a significantly higher proportion of bacterial fatty acids and higher C15br/C15:0 ratios than the mucilage produced by *A. crinita* (Table 4).

DISCUSSION

Algal conenoses. In the first stages of their development, the filaments of *A. crinita* show an evident polarity and well pigmented cells and reproductive structures because they occur in the typical attached form of this species, but during their dimensional growth, the filaments lose their polarity, the reproductive structures are absent, and the aggregates become more compact and display a grayish color caused by the loss of pigments in the algal cells and the large amount of organic and inorganic debris contained inside. As reported in the previous paper (Sartoni et al. 2008), the progressive dimensional growth of the aggregates in their propagation becomes possible through the fragmentation of the filaments of *A. crinita*, which can be caused by senescence, by abscission zones along the filaments and in the apical pseudo-hairs, and by fungal infection; this latter aspect has been frequently observed in the Adriatic aggregates in which the cells of *A. crinita* often are infected by *Eurychasma dicksonii* (Wright) Magnus, a parasite largely spread in various biogeographical sectors that appears to prefer brown algae with filamentous habitus (Konno and Tanaka 1988, Müller et al. 1999).

Although *A. crinita* has been reported in different sub-basins of the Adriatic Sea (Antolić et al. 2010), information on the aggregates formed by these filamentous algae is lacking.

According to Schiaparelli et al. (2007), the spread of macroalgal species involved in benthic mucilage formation, i.e., *A. crinita,* might be favored by the increasing temperatures of the surface of Mediterranean waters. In 2004, no exceptional warming was reported by previous studies; however, the analysis performed by Tedesco et al. (2007) for the surface water temperature during 1986-2005 showed a significant increase in May, June and July in the north Adriatic study sites, which is in agreement with the increase in air temperature and annual heat fluxes observed in the Northern Adriatic Sea as reported by Russo et al. (2002). Therefore, we hypothesize that this temperature trend could favor the spread of *A. crinita* at higher latitudes.

Nutrient regeneration and organic matter cycling. Higher nutrient concentrations and the relevant incidence of dissolved organic matter (DOC and DCHO) have been found in the interstitial waters of the benthic aggregates with respect to that in the surrounding water, which substantiates previous findings reported by Misic et al. (2011) and Giani et al. (2005). This situation could be due to prokaryotic activity inside these aggregates where enzymatic activities can be high (Del Negro et al. 2005, Misic et al. 2011).

The DIN/PO_4 ratio was higher outside of the aggregates and decreased markedly inside, similar to the observations of Misic et al. (2011). The regeneration of P was presumably favored by the higher concentration of DOP, which could be partially hydrolyzed by alkaline phosphatase (Del Negro et al. 2005).

The boundary layer that develops over benthic algae might be a key factor in regulating benthic community metabolism through internal recycling of nutrients and carbon (France 1995 and references therein). The greater turbulence to which planktonic algae are exposed maintains lower diffusion resistances, thereby supplying the cell with fresh C at higher rates and promoting even greater ^{13}C-depletion (France 1995).

The ^{13}C depletion with the C_{org}/N increase observed in benthic algae collected during successive surveys in Istra could be attributed to preferential bacterial degradation during aging of the aggregates of carbohydrates, which tend to be ^{13}C enriched (van Dongen et al. 2002).

This situation can be typical of macroalgae growing in shallow oligotrophic systems that have experienced high irradiance and natural N sources, i.e., nitrogen fixation (Lamb et al. 2012).

The $\delta^{15}N$ values found in the benthic algae could be the result of different fractionation processes of DIN (nitrification/denitrification processes; Cole et al. 2004) that occur inside in the interstitial water and in the surrounding waters of the algae. Further fractionation occurs during assimilation of NO_3 or NH_4 by plants and algae (Raimonet et al. 2013). The increase of $\delta^{15}N$ values could be associated with different mechanisms that occur in the benthic layer, i.e., nutrient regeneration and bacterial elaboration. High concentration of both nutrients inside the aggregates, with more positive $\delta^{15}N$ in algae in particular in *A. crinita* sampled in Tremiti and Istra (sampled in June) and *N. marina* (Table 2), could be the result of bacteria activity that uses preferentially light ^{14}N isotopes and leaves heavier ^{15}N isotopes in the substrate (Dai et al. 2005).

Nutrient recycling in the aggregates can support the development of an abundant phytoplankton population, as previously shown in the studies performed in the Tyrrhenian Sea (De Philippis et al. 2005). Moreover, the enrichment of nutrients and organic matter inside the aggregates supports the hypothesis raised by Misic et al. (2007) that the aggregates can act as a source of nutrients for the water column when they are dispersed by currents induced by wind mixing.

Diatom communities established in the Adriatic aggregates were the microphytobenthic constituent most commonly found in the Tyrrhenian benthic aggregates, as previously evidenced from previous observations (Innamorati et al. 2001, Sartoni et al. 2008), thus confirming the main contribution of diatoms with respect to the other phytoplankton groups to the metabolic pathways occurring in the development of aggregates. From the earlier stages in which microalgae composition partially reproduces the resident microphytobenthic diatoms community, the development and aging of mucilages involve selection from a more diverse community to the

nearly monospecific dominance of *Cylindrotheca closterium.* This species is one of the most recurrent and abundant diatoms in both pelagic (Najdek et al. 2002) and benthic aggregates (Sartoni et al. 2008) and are likely able to exploit the high availability of rapid internal recycling of organic phosphorus (Najdek et al. 2002).

Polysaccharidic matrix. A comparison of the average monosaccharide patterns of Adriatic aggregates (Istra and Tremiti sites) with those obtained from the Tyrrhenian Sea (Sartoni et al. 2008) is shown in Figure 6. The average values from Adriatic samples differ significantly for both galactose and xylose contents with respect to the Tyrrhenian sample produced by *A. crinita* algae with the Tyrrhenian aggregates displaying a higher percentage of galactose (50% vs. 30%) and lower content of xylose (5% vs. 18%). The average composition found for Adriatic samples is notably similar to that found for the high-molecular-weight fraction of *A. crinita* exopolysaccharide after polymer fractionation, as previously reported (Sartoni et al. 2008). Those long-chain polysaccharides formed by approximately 3500-4000 monosaccharide residues connected in the polymer chain (about Mw=800 kDa) exhibited values of approximately 36% w/w of galactose and approximately 11% of xylose (Sartoni et al. 2008), thus suggesting that the Adriatic samples may resemble the average composition of the high-molecular-weight exopolymers, and the difference observed in Fig. 6 might be ascribed to different degradation stages.

Using the sum of deoxysugar (fuc+rha) and galactose contents as an index of the degradation stage of mucilage aggregates, as previously used for both marine and axenic culture samples (Giani et al. 2005, Sartoni et al. 2008, Urbani et al. 2005,), certain other features can be highlighted. As reported, freshly produced exopolymers and aggregates showed the highest sum (fuc+rha) and the lowest galactose contents (Giani et al. 2005). Similar results were found for exopolymers produced in axenic cultures by several diatom species (Magaletti et al. 2004, Myklestad 1974, , Urbani et al. 2005) with a galactose content less than 25% and a sum (fuc+rha) greater than 45%. Conversely, the most heavily degraded samples from both pelagic and benthic mucilage aggregates showed

marked decreases of fucose and rhamnose and an increase of galactose residues (Giani et al. 2005, Sartoni et al. 2008), thus revealing specific and preferential enzymatic degradative processes.

Comparing the Istra and Tremiti samples with the Tyrrhenian *A. crinita* sample reported in a previous paper (Sartoni et al. 2008), it is noteworthy that the galactose and (fuc+rha) percentages of the Adriatic samples (symbols 1 and 2 in Fig. 7) are different from that of the (unfractionated) Tyrrhenian benthic sample (point 4 in Fig. 7) but are quite close to its high molecular weight fraction (point 6 in Fig. 7). This observation suggests fresh algal production for the Adriatic polysaccharides with a lesser extent of depolymerization activity due to the bacteria that colonize the Adriatic aggregates.

In Figure 7, the average data for the monosaccharidic composition of exopolysaccharides produced by *Nematochrysopsis marina* are also reported (point 3 from Adriatic samples and points 5 and 7 from Tyrrhenian samples). As observed for the *A. crinita*, the *N. marina* exopolysaccharides from the Tyrrhenian sea (point 5 in Fig. 7) showed a higher content of galactose with respect to the Adriatic sample (point 3 in Fig. 7) in addition to comparison of the high molecular weight fraction (point 7 in Fig. 7) obtained from sample 5 using size exclusion chromatography (Sartoni et al. 2008). This large polysaccharide fraction again represents the freshly produced exopolysaccharide by *N. marina*, which is quite similar in galactose content to that found in the Adriatic sea (point 3 in Fig. 7).

Fatty acid signature. In the benthic mucilage formed by *A. crinita*, the bacterial fatty acid contribution and C15:br/C15:0 ratios were much lower (0.9-5.8%; 0.0 to 0.98) than in the pelagic mucilaginous aggregates (Najdek et al. 2002) and were either smaller, i.e., flocs (3.1-6.8%; 2.5-3.2) or larger, i.e., clouds (14.2-17.1%; 5.5-6.0), particularly from benthic mucilage formed by *N. marina* (24.9%; 12.6), as shown in Figure 8.

This observation suggested that conditions for heterotrophic bacterial growth were not established within any of the sampled benthic mucilage formed by *A. crinita* with respect to those usually found within pelagic mucilage. In the pelagic mucilaginous aggregates, the bacterial contribution increase parallels the aggregate aging process, i.e., degradation of the mucous matrix. In this process, the bacteria interact with phytoplankton and form the foundation of pelagic aggregate self-sustainability through the production-decomposition cycle (Najdek et al. 2002). In contrast, the bacterial colonization of the benthic mucilage formed by *A. crinita* was negligible. These more unfavorable conditions for bacterial growth might indicate either a highly efficient mechanism in *A. crinita* for extrication from epibionts or that the mucus was continuously exuded and fresh and thus not considerably colonized by bacteria during the time of sampling. Many marine algae have evolved efficient strategies to control epibiosis, either chemically by producing defensive compounds (Nylund and Pavia 2003, Fusetani 2004) and/or physically by sloughing and secreting mucus (Steinberg et al. 1997, Nylund and Pavia 2005). Antifouling metabolites of red and brown algae, i.e., diterpene alcohols (Schmitt et al. 1995), brominated phenols (Phillips and Towers 1982), sesquiterpenoids (de Nys et al. 1998), and halogenated furanones (Steinberg et al. 1997), were found to repel propagules or inhibit growth of microorganisms. In contrast with *A. crinita*, benthic mucilage formed by *N. marina* was heavily colonized by bacteria, thus suggesting that advanced degradation of the mucous matrix took place in these benthic aggregates. Such a high bacterial contribution was observed in aged pelagic aggregates dominated by diatom *Cylindrotheca closterium* (Blažina et al. 2005, Najdek et al. 2005).

In pelagic aggregates, the most prominent contributions of diatoms were documented either as producers of a mucilage polysaccharide matrix (Kaltenböck and Herndl 1992, Degobbis et al. 1995) or as selected species, i.e., *C. closterium*, that can survive such extreme environments and have the ability to reproduce in mucilage (Grossart 1999, Najdek et al. 2005). Therefore, and in contrast with all benthic mucilage investigated in this study (Fig. 9), much higher values of the ratios

C16:1/C16:0 (0.6-1.1) and C16P/C18P (1.3-6.2) as well as the diatom ratio (0.5-1.7) suggested the presence of actively growing diatoms in pelagic mucilage (Najdek et al. 2002). The conditions for phytoplankton growth in all benthic mucilage due to the large variations of C16:1/C16:0 (0.1-1.2) combined with the low C16P/C18 (0.0-0.4) ratios indicated that occasional and different embedding of phytoplankton occurred in the mucilaginous matrix rather than active growth. The relative contribution of diatoms to the fatty acid pool compared with that of other microalgal classes varied from low to dominant contributions (diatom ratio: 0.5-1.7). The similar results obtained for benthic mucilage formed by *N. marina* suggested that diatoms did not considerably contribute to the mucous matrix, as also observed in the microscopic inspection.

CONCLUSIONS

The spread of *A. crinita* in the Adriatic and also in the northern region could be favored by the increasing trend in surface water temperatures. Although the benthic aggregates are colonized by diatoms, the fatty acid and monosaccharide signatures indicated that *A. crinita* was the main constituent and exopolymer-producing species in the bulk aggregate. In contrast with pelagic mucilage, the bacterial contribution formed by *A. crinita* was quite low in benthic mucilage. The recognized unfavorable conditions for bacterial growth in benthic mucilage might be ascribed either to its more recalcitrant nature or to algal defense mechanisms, although more intensive studies are necessary to verify this conclusion. The isotope ratios of $\delta^{13}C$ demonstrated the marine origin of these aggregates, whereas the $\delta^{15}N$ values depended on the fractionation processes that occur inside of the aggregates and in the surrounding waters of the algae.

However, enrichment of nutrients and dissolved organic matter in the interstitial waters of the aggregates suggests that the exoenzymatic activity results in a non-negligible contribution to the degradation of the organic constituents.

Compared with Tyrrhenian benthic aggregates produced by *A. crinita*, the monosaccharide analysis produced the same composition pattern, thus suggesting that these aggregates have the same algal origin irrespective of the geographical site and marine physicochemical features.

Because different benthic algae that form mucilaginous aggregates are spreading in the Mediterranean, the need exists to both understand their ecological role and their impact on the environment.

Aktan, Y. & Topaloğlu, B. 2011. First record of *Chrysophaeum taylorii* Lewis et Brian and their benthic mucilaginous aggregates in the Aegean Sea (Eastern Mediterranean). *Mediter. Environ.* 17:159-170.

Antolić, B., Špan, A., Žuljević, A., Nikolić, V., Grubelić, I., Despalatović, M.& Cvitković, I. 2010. A checklist of the benthic marine macroalgae from the eastern Adriatic coast: II. Heterokontophyta: Phaeophyceae. *Acta Adriat.* 51: 9-33.

Aspila, K.I., Agemian, H. & Chau, A.S.Y. 1976. A semi-automated method for the determination of inorganic, organic and total phosphate in sediments. *Analyst* 101: 187-197.

Athanasiadis, A. 1997. North Aegean marine algae. IV. *Womersleyella setacea* (Hollenberg) R. E. Norris (Rhodophyta, Ceramiales). *Bot. Mar.* 40: 473-478.

Blakeney, A.B., Harris, P.J., Henry, R.J. & Stone, B.A. 1983. A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohyd. Res.* 113: 291- 299.

Bradford, M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein using the principal of protein- dye binding. *Anal. Biochem.* 72: 254-284.

Burney, C.M. & Sieburth, J. 1977. Dissolved carbohydrates in seawater.II, A spectrophotometric procedure for total carbohydrate analysis and polysaccharide estimation. *Mar. Chem.* 5: 15-28.

Calvo, S., Barone, R. & Naselli Flores, L. 1995. Observations on mucus aggregates along Sicilian coasts during 1991-1992. *Sci. Tot. Environ.* 165: 23-31.

Caronni, S., Delaria, M.A., Navone, A., Panzalis, P., Sechi, N. & Ceccherelli, G. 2014. Relevant scales of variability of the benthic allochthonous microalga *Chrysophaeum taylorii.* Mar. Biol. 161: 1787-1798.

Charrier, B., Coelho, S.M., Le Bail, A., Tonon, T., Michel, G., Potin, P., Kloareg, B., Boyen, C., Peters, A.F. & Cock, J.M. 2008. Development and physiology of the brown alga *Ectocarpus siliculosus*: two centuries of research. *New Phytol.* 177: 319–332.

Cole, M.L., Valiela, I., Kroeger, K.D., Tomasky, G.L., Cebrian, J., Wigand, C., McKinney, R.A., Grady, S.P. & Carvalhoda Silva, M.H. 2004. Assessment of a $\delta^{15}N$ isotopic method to indicate anthropogenic eutrophication in aquatic ecosystems. *J. Environ. Qual.* 33: 124-132.

Cormaci, M., Furnari, G., Alongi, G., Catra, M., Pizzuto, F. & Serio, D. 2001. Spring marine vegetation on rocky substrata of the Tremiti Islands (Southern Adriatic) Italy. *In* Faranda, M. F., Guglielmo, L. & Spezie, G. [Eds.] *Structures and processes in the Mediterranean ecosystems*. Springer-Verlag, pp. 245-254.

Dai, J., Sun, M.Y., Culp, R.A. & Noakes, J.E. 2005. Changes in chemical and isotopic signatures of plant materials during degradation: Implication for assessing various organic inputs in estuarine systems. *Geophysical Research Letters* 32: L13608, Del Negro, P., Crevatin, E., Larato, C., Ferrari, C.R., Totti, C., Pompei, M., Giani, M., Berto, D. & Fonda Umani, S. 2005. Mucilage microcosms. *Sci. Tot. Environ.* 353: 258-269.

De Philippis, R., Faraloni, C., Sili, C. & Vincenzini, M. 2005. Populations of exopolysaccharide-producing cyanobacteria and diatoms in the mucilaginous benthic aggregates of the Tyrrhenian Sea (Tuscan Archipelago). *Sci. Tot. Environ.* 353: 360-368.

De Nys, R., Dworjanyn, S.A. & Steinberg, P.D. 1998. A new method for determining surface concentrations of marine products on seaweeds. *Mar. Ecol. Prog. Ser.* 162: 79–87.

Druffel, E.R.M. & Williams, P.M. 1992. Importance of isotope measurements in marine organic geochemistry. *Mar. Chem.* 39: 209-215.

Eriksson, B.K.S., Johansson. G. & Snoeijs, P. 1998. Long-term changes in the sublittoral zonation of brown algae in the southern Bothnian Sea. *Eur. J. Phycol.* 33: 241-249.

Faganeli, J., Malej, J., Pezdic, J. & Malacic, V. 1988. C:N:P ratios and stable C isotopic ratios as indicators of sources of organic matter in the gulf of Trieste (northern Adriatic). *Oceanol. Acta* 11 : 377-382.

Faganeli, J., Ogrinc, N., Kovac, N., Kukovec, K., Falnoga, .I, Mozetic, P. & Bajt, O. 2009. Carbon and nitrogen isotope composition of particulate organic matter in relation to mucilage formation in the northern Adriatic Sea. *Mar. Chem.* 114: 102–109.

Fleurence, J., Gutbier, G., Mabeau, S. & Leray, C. 1994. Fatty acids from 11 marine macroalgae of the French Brittany coast. *J. Appl. Phycol.* 6: 527–532.

France, R.L. 1995. Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. *Mar. Ecol. Prog. Ser.* 124: 307-312.

Giani, M., Savelli, F., Berto, D., Zangrando, V., Ćosović, B. & Vojvodić, V. 2005. Temporal dynamic of dissolved and particulate organic carbon in the northern Adriatic Sea in relation to the mucilage events. *Sci. Tot. Environ.* 353: 126-138.

Giani, M., Berto, D., Zangrando, V., Castelli, S., Sist, P. & Urbani, R. 2005 Chemical characterization of different typologies of mucilaginous aggregates in the Northern Adriatic Sea. *Sci. Tot. Environ.* 353: 232-246.

Giani, M., Zangrando, V. & Berto, D. 2006 $^{13}C/^{12}C$ isotope ratio in the organic matter forming the mucilaginous aggregates in the Northern Adriatic Sea. Isotopes in Environmental Studies Aquatic Forum 2004 Proceedings of an International Conference held in Monaco, 25-29 October 2004 *C&S Papers Series No. 26*, IAEA-CN-118, 93-96. ISBN 92-0-111305-X, ISSN 1562-4153

Giani, M., Berto, D., Rampazzo, F., Savelli, F., Alvisi, F., Giordano, P., Ravaioli, M. & Frascari, F. 2009. Origin of sedimentary organic matter in the north-western Adriatic Sea. *Estuar. Coast. Shelf Sci.* 84: 573-583.

Giani, M., Sist, P., Berto, D., Serrazanetti, G.P., Ventrella, V. & Urbani, R. 2012. The organic matrix of pelagic mucilaginous aggregates in the Tyrrhenian Sea (Mediterranean Sea). *Mar. Chem.* 132–133: 83-94.

Giuliani, S., Virno Lamberti, C., Sonni, C. & Pellegrini, D. 2005. Mucilage impact on gorgonians in the Tyrrhenian Sea. *Sci. Tot. Environ.* 353: 340-349.

Hemminga, M.A. & Mateo, M.A. 1996. Stable carbon isotopes in seagrasses: variability in ratios and use in ecological studies. *Mar. Ecol. Prog. Ser.* 140: 285-298.

Hoffmann, L., Billard, C., Janssens, M., Leruth, M.& Demoulin, V. 2000. Mass Development of Marine Benthic Sarcinochrysidales (Chrysophyceae *s.l.*).*Corsica. Bot. Mar.* 43: 223-231.

Innamorati, M. 1995. Hyperproduction of mucilages by micro and macro algae in the Tyrrhenian sea. *Sci. Tot. Environ.* 165: 65-81.

Innamorati, M., Nuccio, C., Massi, L., Mori, G. & Melley, A. 2001. Mucilages and climatic changes in the Tyrrhenian Sea. *Aquatic. Conserv.: Mar. Freshw. Ecosyst.* 11: 289-298.

Jones, R.S. 1968. A suggested method for quantifying gut content in herbivorous fishes. *Micronesica* 2: 369-371.

Johnson, K. & Sieburth, J.M.N. 1977. Dissolved carbohydrates in seawater: a precise spectrophotometric analysis for monosaccharide. *Mar. Chem.* 5: 1-13.

Khotimchenko, S.V. 1998. Fatty acids of brown algae from the Russian Far East. *Phytochemistry* 49: 2363-2369.

Konno, K. & Tanaka, J. 1988. *Eurychasma dicksonii*, a Parasitic Fungus on *Acinetospora crinita* (Brown Alga) in Japan. *Bull. Nat. Sci. Mus. Tokyo Ser. B* 14:119-122.

Koroleff, F. 1983. a. Determination of phosphorus. *In* Grasshoff, K., Ehrhardt, M. & Kremling, K. [Eds.] *Methods of Seawater Analysis*. Verlag Chemie, Weinheim, pp.125-139.

Koroleff, F. 1983. b. Determination of organic nitrogen. *In* Grasshoff, K., Ehrhardt, M. & Kremling, K. [Eds.] *Methods of Seawater Analysis*.Verlag Chemie, Weinheim, pp.162-173.

Lamb, K., Swart, P.K. & Altabet, M.A. 2012. Nitrogen and Carbon isotopic systematic of the Florida reef tract. *Bull. Mar. Sci*. 88: 119-146.

Leveille, J.C., Amblard, C. & Bourdier, G. 1997. Fatty acids as specific algal markers in a natural lacustrian phytoplankton. *J. Plankton. Res.* 19: 469–490.

Littler, M.M., Littler, D.S. & Taylor, P.R. 1983 Evolutionary strategies in a tropical barrier reef. *J. Phycol.* 19: 229-237.

Lubchenco, J. & Gaines, S.D. 1981. A unified approach to marine plant-herbivore interactions: I. Populations and communities. *Ann. Rev. Ecol. Syst.* 12: 405-437.

Magaletti, E., Urbani, R., Sist, P., Ferrari, C.R. & Cicero, A.M. 2004. Abundance and chemical characterization of extracellular carbohydrates released by the marine diatom *Cylindrotheca fusiformis* under N- and P-limitation. *Eur. J. Phycol.* 39: 133–142.

Malej, A. & Harris, R.P. 1993. Inhibition of copepod grazing by diatom exudates: a factor in the development of mucus aggregates? *Mar. Ecol. Prog. Ser.* 96: 33-42.

Mayzaud, P., Chanut, J.P. & Ackman, R.G. 1989. Seasonal changes of the biochemical composition of marine particulate matter with special reference to fatty acids and sterols. *Mar. Ecol. Prog. Ser.* 56: 189–204.

Misic, C., Schiaparelli, S. & Covazzi Harriague, A. 2011. Organic matter recycling during a mucilage event and its influence on the surrounding environment (Ligurain Sea, NW Mediterranean). *Cont. Shelf. Res.* 31: 631-643.

Mistri, M. & Ceccherelli, V.U. 1996. Effects of a mucilage event on the Mediterranean gorgonian *Paramuricea clavata*. I- Short term impacts at the population and colony levels. *Ital. J. Zool.* 63: 221-230.

Moncreiff, C.A. & Sullivan, M. 2001. Trophic importance of epiphytic algae in subtropical seagrasses beds: evidence from multiple stable isotopes analyses. *Mar. Ecol. Prog. Ser.* 215: 93-106.

Müller, D.G., Kapp, M. & Knippers, R. 1998. Viruses in marine brown algae. *Adv. Virus Res.* 50: 49-67.

Müller, D.G., Küpper, F.C. & Küpper, H. 1999. Infection experiments reveal broad host range of *Eurychasma dicksonii* (Oomycota) and *Chytridium polysiphoniae* (Chytridiomycota), two eukaryotic parasites in marine brown algae (Phaeophyceae). *Phycol. Res.* 47: 217-223.

Myklestad, S. 1974. Production of carbohydrates by marine planktonic diatoms I Comparison of nine different species in culture. *J. Exp. Mar. Biol. Ecol.* 15: 261–74.

Najdek, M. 1997. Unusual changes of zooplankton fatty acid composition in the northern Adriatic during the 1991 mucilage event. *Mar. Ecol. Prog. Ser.* 159:143-150.

Najdek, M., Degobbis, D., Mioković, D. & Ivančić, I. 2002. Fatty acid and phytoplankton compositions of different types of mucilaginous aggregates in the northern Adriatic. *J. Plankt. Res.* 24: 429–441.

Najdek, M., Blažina, M., Đakovac, T. & Kraus, R. 2005. The role of the diatom *Cylindrotheca closterium* in a mucilage event in the northern Adriatic Sea: coupling with high salinity water intrusions. *J. Plankt. Res.* 27:851–862.

Nikolić, V., Žuljević, A., Antolić, B., Despalatović, M. & Cvitković, I. 2010. Distribution of invasive red alga *Womersleyella setacea* (Hollenberg) R. E. Norris (Rhodophyta, Ceramiales) in the Adriatic Sea. *Acta Adriat.* 51(2):195-202.

Nylund, G.M. & Pavia, H. 2003. Inhibitory effects of algal extracts on larval settlement of the barnacle *Balanus improvisus*. *Mar. Biol.* 143:875–882.

Nylund, G.M., Pavia, H. 2005. Chemical versus mechanical inhibition of fouling in the red alga *Delisea carnosa*. *Mar. Ecol. Prog. Ser*. 299:111–121.

Pedersen, P.M. & Kristiansen, A. 2001 On the enigmatic brown alga *Acinetospora crinita* (Ectocarpales, Fucophyceae). *Cryptogamie* 22:209-218.

Piazzi, L., Balata, D. & Cinelli, F. 2007. Invasions of alien macroalgae in Mediterranean coralligenous assemblage. *Cryptogamie Algol.* 28: 289-301.

Redfield, A.C., Ketchum, B.H. & Richards, F.A. 1963. The influence of organisms on the composition of sea-water. *In* Hill, N. M. [Ed.], *The Sea*. Vol. 2, Wiley, London, pp.27–77.

Russo, A., Rabitti, S. & Bastianini, M. 2002. Decadal Climatic Anomalies in the Northern Adriatic Sea Inferred from a New Oceanographic Data Set. *Mar. Ecol.* 23:340–351.

Sartoni, G. & Sonni, C. 1992. *Tribonema marinum* J. Feldmann e *Acinetospora crinita* (Carmichael) Sauvageau nelle formazioni mucillaginose bentoniche osservate sulle coste toscane nell'estate 1991. *Informatore Botanico Italiano* 23:23-30.

Sartoni, G., Boddi, S. & Hass, .J 1995. *Chrysonephos lewisii* (Sarcinochrysidales, Chrysophyceae), a new record for the Mediterranean algal flora. *Bot. Mar.* 38:121-125.

Sartoni, G., Urbani, R., Sist, P., Berto, D., Nuccio, C. & Giani, M. 2008. Benthic mucilaginous aggregates in the Mediterranean Sea: Origin, chemical composition and polysaccharide characterization. *Mar. Chem.* 111:184–198.

Schiaparelli, S., Castellano, M., Povero, P., Sartoni, G. & Cattaneo-Vietti, R. 2007. A benthic mucilage event in North-Western Mediterranean Sea and its possible relationships with the summer 2003 European heatwave: short term effects on littoral rocky assemblages. *Mar. Ecol.* 28:1-13.

Schmitt, T., Hay, M. & Lindquist, N. 1995. Constraints on chemically-mediated coevolution: Multiple functions for seaweed secondary metabolites. *Ecology* 76:107–123.

Socal, G., Acri, F., Bastianini, M., Bernardi Aubry, F., Bianchi, F., Cassin, D., Coppola, J., De Lazzari, A., Bandelj, V., Cossarini, G. & Solidoro, C. 2008. Hydrological and biogeochemical features of the Northern Adriatic Sea in the period 2003–2006. *Mar. Ecol.* 29:449–468

Steinberg, P., Schneider, R. & Kjelleberg, S. 1997. Chemical defenses of seaweeds against microbial colonization. *Biodegradation* 8:211–220.

Steneck, R.S. & Watling, L. 1982. Feeding capabilities and limitations of herbivorous molluscs: a functional group approach. *Mar. Biol.* 68:299-319.

Tedesco, L., Socal, G., Bianchi, F., Acri, F., Veneri, D. & Vichi, M, 2007. NW Adriatic Sea biogeochemical variability in the last 20 years (1986–2005). *Biogeosciences* 4:673–687.

Tsiamis, K., Panayotidis, P., Economou-Amilli, A. & Katsaros, C. 2013. Seaweeds of the Greek coasts. I. Phaeophyceae. *Mediter. Mar. Sci.* 14:141-147.

Urbani, R., Magaletti, E., Sist, P. & Cicero, A.M. 2005. Extracellular carbohydrates released by the marine diatoms *Cylindrotheca closterium, Thalassiosira pseudonana* and *Skeletonema costatum*. Effect of P-depletion and growth status. *Sci. Total. Environ.* 353:300–306.

van Dongen, B.E., Schouten, S. & Damsté, J.S.S. 2002. Carbon isotope variability in monosaccharides and lipids of aquatic algae and terrestrial plants. *Mar. Ecol. Prog. Ser.* 232:83–92

Vizzini, S. & Mazzola, A. 2003. Seasonal variations in the stable carbon and nitrogen isotope ratios (13C/12C and 15N/14N) of primary producers and consumers in a western Mediterranean coastal lagoon. *Mar. Biol.* 142:1009–1018.

White, D.C., Bobbie, R.J., Nickels, J.S., Fazio, S.D. & Davis, W.M. 1980. Nonselective biochemical methods for the determination of fungal mass and community structure in estuarine detrital microflora. *Bot. Mar.* 23:239–250.

Welker, C. & Bressan, G. 1994. Aggregati mucillaginosi bentonici di *Acinetospora crinita* (Carm. ex Harvey) Sauvageau (Ectocarpales, Phaeophyta) nell'Alto Adriatico. *Giorn. Bot. Ital.* 128:827-829.

Fig. 1: Sampling sites: a) in the northern Adriatic Sea, Istra peninsula; b) in the southern Adriatic Sea, Tremiti Islands.

Fig. 2: a) Aggregate on *Cystoseira corniculata* (Turner) Zanardini (Rovinj, bar=1 cm; b) small aggregates growing on thalii of *Dictyota* sp. (Tremiti Islands); c) filaments of *Acinetospora crinita* (Carmichael) Sauvageau within an aggregate (Tremiti Islands, bar=500 µm); d) apical portions of *Acinetospora crinita* within an aggregate in the early stage of development (Tremiti Islands, bar=500 µm).

Fig. 3: Relative abundances of the dominant microalgae in the benthic aggregates of Rovinj (Secca Croce and Porer shallows, Banjole Island) and Tremiti Island (Ferraio) sampled in May 2004.

Fig. 4: **a**) C_{org}/S vs. C_{org}/P *A. crinita* aggregates from the Adriatic sea and Tyrrhenian sea (*), in pelagic mucilages (**) and phytoplankton (***); **b**) $\delta^{13}C$ and $\delta^{15}N$ in different benthic aggregates of *A. crinita*, phytoplankton (***), and pelagic mucilages (**); **c**) $\delta^{13}C$ vs. C_{org}/N_{tot} in different benthic aggregates of *A. crinita* sampled in the Istra and Tremiti islands. The linear fit is shown for the aggregates sampled in Istra. Data from (*) Sartoni et al. 2008, (**) Giani et al. 2005, (***) Berto, unpublished data, and (****) Giani et al., 2012.

Fig. 5: Concentration in the interstitial water internal to the benthic aggregates and in the seawater external to the aggregate of: a) phosphates (PO_4), silicates (H_4SiO_4), b) nitrates (NO_3) and

ammonium (NH_4), c) dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP), and d) dissolved organic carbon (DOC) and dissolved carbohydrates (DCHO).

Fig. 6: Relative monosaccharide composition of *A. crinita* aggregates from the Istra and Tremiti Islands. For comparison, values from the aggregates of *A. crinita* sampled in the Tyrrhenian Sea and *Nematochrysopsis marina* sampled at Banjole Island (Istra) and in the Tyrrhenian Sea are also shown.

Fig. 7**:** Galactose percentage on dependence of rhamnose and fucose percentage for 1) Istra, 2) Tremiti, 3) *N. marina*, Istra, 4) *A. crinita*, Tyrrhenian Sea, 5) *N. marina*, Tyrrhenian Sea, 6) and 7) high molecular weight fractions of samples 4) and 5), respectively. Data of Tyrrhenian samples are obtained from the previous paper (Sartoni et al. 2008).

Fig. 8**:** Bacterially derived fatty acids vs. C15:br/C15:0 ratios in the benthic (*A. crinita*, ▲ and *N. marina*, △) and pelagic mucilages (flocs, ◆ and clouds, ◇).

Fig. 9: C16:1/C16:0 *vs* C16P/C18P ratios in the benthic (*A. crinita*, △, and *N. marina*, ▲) and pelagic (flocs and clouds, ◆) mucilages.

Table 1: Surface and near the bottom sampling depths

Site	Date	Depth /m	Depth /m
Istra			
Banjole	05/03/2004	1	15
	29/05/2004	1	15
	25/06/2004	1	15
Porer	05/02/2004	1	16
	30/05/2004	1	16
	26/06/2004	1	16
Punta Croce	05/02/2004	1	16
	29/05/2004	1	16
	25/06/2004	1	16
Tremiti Islands			
Cretaccio	25/05/2004	1	17
Ferraio	26/05/2004	1	22
Secca Vedove	25/05/2004	1	18
Punta Diavolo	26/05/2004	1	24

Table 2: Elemental composition of the benthic aggregates. Averages and standard deviations (in italics) are reported.

Site	date	Depth	H_20	C_{tot}	C_{inorg}	C_{org}	N	S	P
		m	%	%	%	%	%	%	$mg \cdot kg^{-1}$
A. crinita									
Istra	2-3/05/2004	12-16	88.53	32.52	12.87	19.65	2.22	0.68	501.77
			7.7	*4.56*	*4.82*	*5.5*	*0.22*	*0.12*	*80.26*
Tremiti	26/05/2004	17-24	91.55	30.78	4.67	26.11	2.3	nd	778.98
			2.85	*4.11*	*1.56*	*2.56*	*0.49*	nd	*209.28*
Istra	29/05/2004	16-18	90.22	25.71	5.85	19.86	1.7	0.63	768.37
			5.09	*4.17*	*2.35*	*4.97*	*0.23*	*0.04*	*456.74*
Istra	26/06/2004	12-15	89.63	18.75	4.97	13.78	1.05	0.58	571.22
N. marina									
Istra	26/06/2004	12	90.46	22.65	4.38	18.28	0.79	nd	nd

Site	date	Depth	$\Delta^{13}C$	$\Delta^{15}N$	C_{org}/P_{tot}	N_{tot}/P_{tot}	C_{org}/N	C_{org}/S
		m	‰	‰	mole·mole^{-1}	mole·mole^{-1}	mole·mole^{-1}	mole·mole^{-1}
A. crinita								
Istra	2-3/05/2004	12-16	-18.91	2.68	1033	99.3	10.2	83.8
			0.69	1.3	362	13.8	2.2	26.4
Tremiti	26/05/2004	17-24	-18.03	5.17	896	65.9	13.5	nd
			0.73	0.59	164	4.9	2	nd
Istra	29/05/2004	16-18	-20.88	3.06	871	65	13.5	90.7
			0.71	0.44	524	39.9	2.2	16.4
Istra	26/06/2004	12-15	-20.92	5.29	622	40.7	15.3	63.2
N. marina								
Istra	26/06/2004	12	-22.78	4.56	nd	nd	26.95	nd

Date	**Station**	**Algae**	**Depth**	**Gal**	**Glc**	**Man**	**Xyl**	**Rha**	**Fuc**	**Rib**	**Ara**
05/02/2004	Porer	Acinetospora	15m	32.5	3.2	10.3	17	8.4	28.6	0	0
05/02/2004	Punta Croce	Acinetospora		27	4.1	6.9	13.8	7.9	17.9	0	22.3
26/05/2004	Punta Diavolo	Acinetospora		31.8	6.6	11.4	40.4	3.2	4.9	0	2.1
26/05/2004	Ferraio	Acinetospora	22m	32.7	5.7	12.9	21.3	5.4	20.8	0	1.2
29/05/2004	Banjole	Acinetospora	16m	28.7	14.4	10.1	26.1	6.7	11.1	0	2.9
29/05/2004	Banjole	Acinetospora	16m	35	6	9.7	29.2	5.6	11.5	0	2.9
30/05/2004	Porer	Acinetospora	15m	29.3	12.6	14.3	11.5	9.6	20.2	0.7	0
26/06/2004	Banjole	Acinetospora	15m	39.3	14.8	10.9	11	6.9	12.2	2	2.8
26/06/2004	Banjole	Nematochrysopsis	15m	41.5	32	9	7.6	3	4.1	1.5	1.2

Table 3: Monosaccharide composition (% w/w) of exopolysaccharide fractions in the benthic

Adriatic aggregates.

Table 4: Fatty acid composition of the benthic mucilage produced by *A. crinita* and *N. marina* and of the isolated alga *A. crinita*. br – branched fatty acids; bacterial fatty acids – (C15:0 + C15iso + C15anteiso + C17:0 + C17iso + C17anteiso + C18:1n7); diatom ratio – (C14:0 + C16:1 + C16P /C16:0); 16P, 18P – sum of polyunsaturated fatty acids with 16 and 18 C atoms, respectively; tr- traces; na – not applicable

Sample	BENTHIC MUCILAGE		ALGA
Fatty acids	*A. crinita*	*N. marina*	*A. crinita*
C14:0br	-	4.16	-
C14:0	14.82 ± 4.41	3.96	14.50 ± 0.49
C15:0br	0.24 ± 0.33	14.32	0.24 ± 0.32
C15:0	1.31 ± 0.78	1.14	0.92 ± 0.35
C16:4(n-3)	0.77 ± 0.81	tr	tr
C16:3(n-3)	0.49 ± 0.45	4.13	tr
C16:1(n-7)	16.19 ± 9.09	12.59	7.96 ± 0.56
C16:0	34.15 ± 8.71	23.51	41.22 ± 1.16
C17:0br	0.12 ± 0.34	2.26	1.45 ± 0.51
C17:0	0.62 ± 1.19	1.90	0.35 ± 0.50
C18:3(n-6)	0.58 ± 0.50	1.07	0.28 ± 0.39
C18:4(n-3)	3.01 ± 2.49	0.82	2.90 ± 0.08
C18:2(n-6)	4.36 ± 2.33	5.58	5.35 ± 0.19
C18:3(n-3)	5.06 ± 2.89	5.61	4.76 ± 0.23
C18:1(n-9)	7.14 ± 2.86	10.72	9.84 ± 0.61
C18:0	2.76 ± 1.66	5.98	2.28 ± 0.08
C20:4(n-6)	2.26 ± 1.46	0.53	2.48 ± 0.31
C20:5(n-3)	3.49 ± 2.35	1.39	2.62 ± 0.47
C20:0	0.57 ± 0.51	tr	1.08 ± 0.01
C22:6(n-3)	0.04 ± 0.13	0.34	0.18 ± 0.25
C22:1	0.95 ± 0.82	tr	0.40 ± 0.57
C22:0	0.51 ± 0.34	tr	0.93 ± 1.31
BACTERIAL	0.36 ± 0.52	24.87	1.69 ± 0.18
C15:0br/C15:0	0.25 ± 0.36	12.59	0.20 ± 0.28
C16:1/C16:0	0.53 ± 0.35	0.54	0.19 ± 0.02
C16P/C18P	0.13 ± 0.15	0.32	0.00 ± 0.00
DIATOM RATIO	1.02 ± 0.41	0.88	na