

## Changes in the fatty acid composition of *Fucus virsoides* J. Agardh in response to the type of substratum

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The effect of the substratum type (natural, limestone, or artificial, concrete) on the fatty acid composition of *Fucus virsoides* in six habitats around the harbours and beaches of the Istrian coast (northern Adriatic Sea) was investigated during the summer, the period of maximum development. All habitats of *F. virsoides* were regulated by very similar environmental factors and were exposed to moderate anthropogenic influences. *F. virsoides* growing on artificial substrata was characterised by lower concentrations of fatty acids, lower proportions of fatty acids among total lipids, higher C16:0/18:1 ratios (FA:  $6.7 \pm 0.6$  mg g<sup>-1</sup> d.w.; FA/TL: 22%; 16:0/18:1:  $0.57 \pm 0.08$ ) and an overall increase in saturation in comparison to communities growing on natural substrata (FA:  $10.2 \pm 2.9$ – $12.5 \pm 3.4$  mg g<sup>-1</sup> d.w.; FA/TL: 30.8–33.7%; 16:0/18:1:  $0.34 \pm 0.11$ – $0.45 \pm 0.13$ ). These results likely reflect the less favourable conditions for *F. virsoides* development on artificial substrates relative to natural substrates. The physiological processes of the algae growing on artificial substrata were more impaired with increasing age (FA:  $1.9 \pm 0.6$  mg g<sup>-1</sup> d.w.; FA/TL: 18.2%; 16:0/18:1:  $0.68 \pm 0.08$ ).

**Key words:** artificial substrata, environment effects, fatty acids, *Fucus virsoides*, natural substrata

### INTRODUCTION

In general, the building of coastal defences and artificial rocky shores, which have become widespread over the years, results in the loss and fragmentation of the natural habitats of algae and marine animals (BULLERI & CHAPMAN, 2010). Typically, assemblages on artificial substrata are less diverse and less abundant than assemblages on rocky shore communities due to the lower level of structural complexity of the artificial substrata (MOSCHELLA *et al.*, 2005; PERKOL-FINKEL & AIROLDI, 2010). The texture of the substratum has been shown to be an important factor affecting the distribution, abundance and morphology of macroalgae (MCGUINNESS &

UNDERWOOD, 1986) and may be able to modify the physiological condition of these algae as well.

The brown alga *Fucus virsoides* J. Agardh 1868 (family Fucaceae, order Fucales) is an endemic species in the Adriatic Sea and is especially abundant in this sea's northern basin (KREMER & MUNDA, 1982; RINDI & BATTELLI, 2005). The typical habitat of furoid seaweeds in the intertidal zone, an extremely variable environment, is characterised by the periodic fluctuations of several ecophysiological relevant parameters during the tidal cycles. Due to the reduced tidal movement in the Adriatic, *F. virsoides* occupies a relatively narrow belt that is nearly identical to the intertidal zone, and it

colonises moderately exposed rocky sites (KREMER & MUNDA, 1982). The pattern and abundance of *F. virsoides* was shown to be related to substratum configuration, exposure to winds, and wave direction. *Fucus* intertidal habitats were in specific trophic condition, thus the species is suggested to be included as an indicator for water quality in terms of good environmental status (GES) in the northern Adriatic (IVEŠA *et al.*, 2009).

Commonly, seaweed is a very important contributor to the littoral biomass and the ecological distribution of primary production. These organisms are useful sources of food and potential biofuels (PLAZA *et al.*, 2008; GOSCH *et al.*, 2012) and are rich in a wide variety of natural compounds, including n-3 fatty acids, which are used in nutritional and/or pharmaceutical products (SARDESAI, 1992). The specific features of the fatty acid compositions of brown algae are high concentrations of C<sub>18</sub> and C<sub>20</sub> polyunsaturated fatty acids (PUFAs) and oleic acid. Among brown algae, the family Fucaceae contains the highest content of 14:0 and the smallest amounts of n-3 PUFAs and may contain 20:3 non-methylene interrupted (NMI) fatty acid (KHOTIMCHENKO, 1998). In addition, *Fucus* is a good source of polyunsaturated fatty acids with an (n-6)/(n-3) ratio between 1.5 and 3, which is generally accepted as a balanced value for human nutrition (COLOMBO *et al.*, 2006; VAN GINNEKEN *et al.*, 2011). The total lipid content and the fatty acid composition of algae are affected by environmental factors that in turn control the net macroalgal production (HURD, 2000). Under the influence of seasonal variations in or the direct manipulation of any of these factors (e.g., nutrients, salinity and/or light) for aquaculture purposes, the concentrations and compositions of lipids and fatty acids in algae can change considerably (SMITH & HARWOOD, 1984; NAPOLITANO, 1994; KIM *et al.*, 1996; FLORETO *et al.*, 1996; KHOTIMCHENKO, 1998; FLORETO & TESHIMA, 1998; ZHUKOVA & TITLYANOV, 2006; GOSCH *et al.*, 2012). The assessment of the suitability of brown algae as a source of PUFAs requires a detailed evaluation of the fatty acid composition and its dependence on the growth period, population

extension and environmental factors. Considering the farming possibilities for *Fucus* and possible future urbanisation along the coast, the type of substratum, in addition to environmental factors, could be of crucial importance for algal recruitment and development and for the protection and maintenance of biodiversity.

This research was undertaken within the framework of monitoring the development of *F. virsoides* in different habitats along the Istrian coast. To assess the effects of different substrata on the physiological condition of this alga, we evaluated the qualitative and quantitative differences in the fatty acid compositions of *F. virsoides* growing on natural and artificial substrata in six habitats around harbours and beaches during the summer, the period of maximum development for this species.

## MATERIALS AND METHODS

Sampling of the brown alga *Fucus virsoides* was performed in July and August 2010 at six sites including harbours (1H, 2H, 3H) and the adjacent beaches (1B, 2B, 3B) along the Istrian coast of the northern Adriatic Sea (Fig. 1). The observation sites were characterised according to the type of substratum (natural- limestone or artificial- concrete), the species distribution pattern in terms of continuity (continuous or patchy), the position of the settlement (sunny, exposed to full light, or shaded, on artificial stones under a jetty) and the level of anthropogenic influence (determined based on the ammonia concentration and the colony forming units (CFU) of faecal coliforms (FC) and streptococci (FS)).

The salinity and temperature were also measured at each sampling site. The ammonia level was determined using unfiltered water immediately after collection (IVANČIĆ & DEGOBBIS, 1984). Faecal coliforms and faecal streptococci were used to determine the sanitary quality of seawater. The samples were collected in sterile glass bottles and stored in the refrigerator (4°C) until the analysis. From each bottle, 100 mL was removed and filtered through a membrane filter (pore size of 0.45 µm) for each indicator. The filter was then placed on the appropriate medium



Fig. 1. *F. virsoides* sampling sites on natural (●) and artificial (▲) substrata along the Istrian coast (northern Adriatic Sea)

(Mfc agar for the coliforms and Slanetz-Bartley medium for the streptococci) and incubated (for 24 h at 44.5°C and 48 h at 36°C, respectively). After the incubation, the membranes with the faecal streptococci were transferred to bile-esculin agar and incubated for 2 h at 44°C for confirmation. The positive colonies were then counted, and the results were expressed as the number of colonies present in 100 mL of seawater.

At each site, two areas were chosen and within each area, three randomly chosen quadrants (10x10 cm) in the lower part of the intertidal zone were destructively sampled. In the laboratory, the *Fucus* thalli were divided into three growth classes, 0-8 cm (d1), 8-18 cm (d2)

and 18-22 cm (d3), and coverage with epibionts was assessed. After a thorough cleaning and the removal of epiphytes, small invertebrates and sand particles, the algal material and ground were dried (60°C 24 h).

To extract the lipids, 2 g of each algal sample was used. The lipids were extracted by homogenisation and ultrasonication with dichloromethane (DCM)/methanol (MeOH) (2:1). The residue was re-extracted 2-3 times with DCM/MeOH (1:1). The combined extracts were filtered and mixed with DCM and deionised water for phase separation. The DCM layer was collected and evaporated to dryness using rotary evaporation at 30°C (DE ROSA *et al.*, 1988), and the total lipid content was determined gravimetrically.

For the analysis of fatty acid methyl esters (FAMES), the lipid extracts were saponified following the addition of 1.2 M NaOH in 50% aqueous methanol. The tubes were placed in a boiling water bath for 30 min. After cooling, the saponificate was acidified with 6 M HCL (pH-2), 12% BF<sub>3</sub> in methanol was added, and the sample was heated for 10 min in a boiling water bath. After cooling, FAMES were extracted in DCM. FAMES were analysed by gas-liquid chromatography (GLC) using a 6890N Network GC System (Agilent, Wilmington, DE, USA) equipped with a 5973 Network Mass Selective Detector (Agilent, Wilmington, DE, USA) and a capillary column (30 m x 0.25 mm x 0.25 µm; cross-linked 5% phenyl-methyl-polysiloxane; Agilent). Ultra-high purity helium was used as the carrier gas. The GLC settings were as follows: an initial column temperature of 145°C followed by an increase of 4°C/min up to 270°C and a constant column pressure of 2.17 kPa. The retention times, peak areas and mass spectra were recorded using ChemStation (Palo Alto, CA, USA) software. The mass selective detector operated in the electron ionisation (EI) mode at 70 eV in the scan range of 10-700 m/z. The FAMES in the algae samples were identified based on the mass spectral data and a plot of the equivalent chain length (ECL) data for the GC standards for the GC column used. The FAMES were quantified based on the internal standard heptadecanoic acid (C17:0), which was added prior to extraction. For qualitative analysis, a mixture of C18-C20 fatty acid methyl esters and polyunsaturated fatty acid standards (PUFA1 and PUFA3; Supelco, Bellefonte, PA, USA), cod liver oil and various individual pure standards for the fatty acid methyl esters (Sigma, Steinheim, Germany) were used (IVEŠA *et al.*, 2004).

To test the hypothesis regarding the differences in the fatty acid compositions, ratios and concentrations (14:0, 16:0, 18:4n3, 18:2n6, 18:1n9, 18:3n3, 20:4n6/20:5n3 (ARA/EPA), 16:0/18:1) between *F. virsoides* settlements on different types of substrata (natural vs. artificial), multivariate analysis (PERMANOVA) with three factors was used. The three factors

were type of Substrata (fixed and orthogonal), Site (random, nested under Substrata) and Area (random, nested under Substrata and Site). The same set of variables was analysed by PCA to visualise the information in the multivariate data set and to find key trends and relationships among the variables. The analysis involved the normalisation of all variables due to their different scales. Both analyses were performed in Primer v6.

## RESULTS

The temperature was uniform at all study sites (24.5 - 25°C), whereas somewhat lower salinity values (36.6 - 37.1) suggested localised freshwater input at sites 1H, 1B and 3H (Table 1). The level of anthropogenic influence of urban origin on the study sites was generally low to moderate. The level of anthropogenic influence was less at the beaches than at the harbours (Table 1). The highest ammonia concentration and the highest number of faecal bacteria colony forming units (CFU) were found at harbour 3H. The very similar levels of faecal coliforms (FC) and faecal streptococci (FS) indicated more constant pollution at sites 3H and 3B. The higher level of FS than FC at 1H, 1B, 2H and 2B, due to longer survival of FS, suggested occasional wastewater discharges.

*Fucus virsoides* at harbours 1H and 2H and their adjacent beaches 1B and 2B, respectively, grew in continuous beds on natural substrata (Table 1). The growth class d1 (0-8 cm) was the most frequent class in *Fucus* beds at 1H (92%) and 2H (78%), (Fig. 2). At 2B, about equal frequencies of the d1 and d2 (8-18 cm) growth classes and few of the highest d3 (18-22 cm) growth class were observed. At 1B, d1 (71%) was the most frequent class, and the d2 (29%) class was also present. Coverage of *F. virsoides* thalli with epibionts was negligible at 2H and 2B. In contrast, at 1H (17.2%) and particularly 1B (47.2%), an important percentage of *F. virsoides* thalli were covered with epibionts (Fig. 2). The *F. virsoides* community was distributed in a patchy manner on artificial substrata at sites 3H and 3B (Table 1). At 3B, there were similar

Table 1. Description of *F. virsoides* sampling sites: substrate and bed type, site position, salinity, ammonia concentration and number of faecal coliforms (FC) and streptococci (FS)

Sampling sites		Substrate type	Bed type	Position	Salinity PSU	NH <sub>4</sub> <sup>+</sup> mmolL <sup>-1</sup>	FC/FS CFU/100mL
1H	harbour	natural	continuous	sunny	36,6	0,986	3/12
2H	harbour	natural	continuous	sunny	37,9	1,503	1/2
3H	harbour	artificial	patchy	shaded	37,1	4,669	10/11
1B	beach	natural	continuous	sunny	36,6	0,865	0/4
2B	beach	natural	continuous	sunny	37,9	0,887	0/6
3B	beach	artificial	patchy	sunny	37,9	1,608	2/4

frequencies of the d1 and d2 growth classes and moderate coverage with epibionts (27%). At 3H, the most frequent was d2 (51%), and the d1 (22.5%) and d3 (26.5%) growth classes were present at similar levels. The *Fucus* thalli at this site were completely covered with epibionts (Fig. 2).

Regarding the FA composition, the major contributors in all samples were alternately palmitic (C16:0) and myristic (C14:0) acids among saturated fatty acids, oleic (C18:1n-9) among monounsaturated fatty acids and arachidonic (C20:4n-6; ARA) and, to lesser extent C20:5n-3, (EPA) among polyunsaturated fatty acids. The levels of n-6 PUFAs were up to two times higher than the levels of n-3PUFAs in all samples.

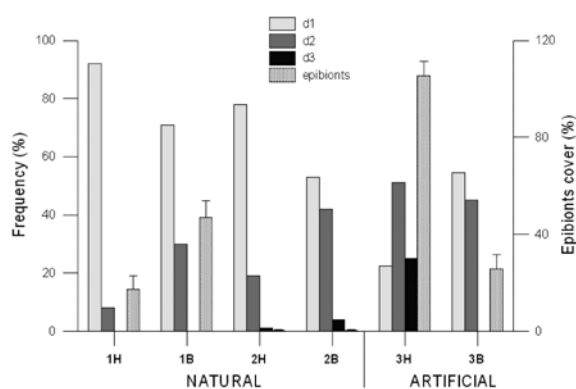


Fig. 2. The frequency of three growth classes—d1 (0-8 cm), d2 (8-18 cm) and d3 (18-22 cm)—and coverage (%) with epibionts of *F. virsoides* growing on natural and artificial substrata

The 16:0/18:1 ratio varied from  $0.34 \pm 0.11$  to  $0.45 \pm 0.13$  and from  $0.57 \pm 0.08$  to  $0.68 \pm 0.08$  in communities on natural and artificial substrata, respectively. On a dry weight basis, total lipids (TLs, mg g<sup>-1</sup>) and fatty acids (FAs, mg g<sup>-1</sup>) were uniformly abundant in *F. virsoides* grown on natural substrata (1H, 1B, 2H, 2B), with concentrations of  $32.1 \pm 5.1$  –  $37.2 \pm 2.5$  and  $10.2 \pm 2.9$  –  $12.5 \pm 3.4$ , respectively (Fig. 3A). The proportion of the TLs made up by FAs was 30.8 – 33.7% (Table 2). In contrast, *F. virsoides* grossing on artificial substrata (3H and 3B) was characterised by lower TL and FA levels and a lower FA/TL ( $10.7 \pm 0.2$  –  $30.1 \pm 2.2$ ,  $1.9 \pm 0.6$  –  $6.7 \pm 0.6$ , 18.2 – 22%, respectively; Fig. 3A, Table 2). The ARA/EPA ratio varied from 1.1 to 2.4 and was the highest at sites 1H and 2H and the lowest at site 3H due to the relatively high proportion of EPA at 3H relative to other sites. In *F. virsoides* at the beaches (1B, 2B and 3B), the ARA/EPA ratios were similar and lower than those determined for *F. virsoides* at 1H and 2H but higher than that for 3H (Fig. 3B, Table 2).

The PERMANOVA results indicated that there is a significant difference in the variability of the *F. virsoides* fatty acid composition between the different substrata (artificial vs natural (P(MC) = 0.049) and also between the sites within each type of substratum. However, among the areas within each site, the variability of the fatty acid composition was not significant (Table 3). The relationship among variables is shown in the PCA plot (Fig. 4). Only PCs with

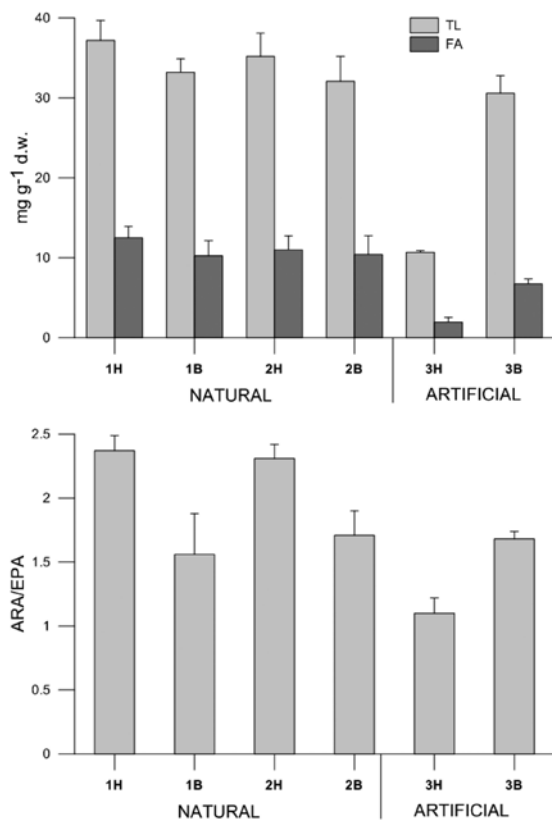


Fig. 3. (A) Mean±SD (mg g<sup>-1</sup> d.w.) of the total lipid and fatty acid contents and (B) the ARA/EPA ratios in *F. virsoides* growing on natural and artificial substrata

eigenvalues >1 were considered (1<sup>st</sup> and 2<sup>nd</sup> PCs). The variables with the highest loadings in each PC have the greatest influence on the dif-

ferentiation of *Fucus* sampling sites and on the projections for that PC. PC1 explained 77.8% of the variability and PC2 another 12.5% (Fig. 4). For the positive relationships, the highest loadings were for C18:2(n-6) and the C16:0/18:1 ratio onto PC1 (with equal weights) and for C16:0 onto PC2. For the negative relationships, C18:1(n-9) and C18:3(n-3) overlapped due to very similar loadings, ARA/EPA and the fatty acid (FA) concentration loaded uniformly onto PC1, and C18:4(n-3) loaded onto PC2.

The score plots of PC1+PC2 grouped together *F. virsoides* from sites 1H and 2H according to the strong influence of the higher values for the C14:0 concentration, the ARA/EPA ratio and the FA concentration, all of which were negatively related to both PC1 and PC2. *F. virsoides* from 1B and 2B showed low scores on PC1, whereas on PC2, the separation was governed by the relatively higher C16:0 proportions with respect to those at 1H and 2H. The separation of 3B was due to the combined influence of the higher C16:0/18:1 ratio and the higher C16:0 level, both of which were positively related to PC1 and PC2. *F. virsoides* from site 3H was rather different from *F. virsoides* at all other sites, being separated due to the importance of the higher proportions of C18:2(n-6) and C18:4(n-3), which were positively and negatively related to PC1 and PC2, respectively.

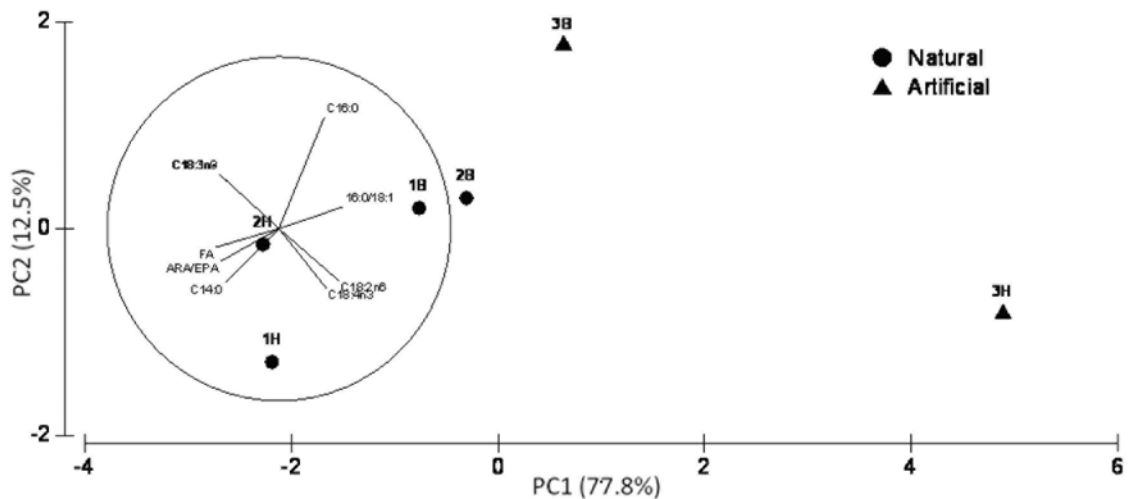


Fig. 4. PC analysis of the *F. virsoides* fatty acid composition. Projections onto PC1 and PC2 are given in the circle

Table 2. Fatty acid composition (FA) of total lipids (TL) in *F. virsoides* on natural and artificial substrata. Values are given as mean  $\pm$  SD ( $N=3$ )

Fatty acids %	NATURAL substrate				ARTIFICIAL substrate	
	1H	1B	2H	2B	3H	3B
C14:0	14.8 $\pm$ 0.1	14.9 $\pm$ 0.3	14.6 $\pm$ 0.6	14.2 $\pm$ 1.6	13.5 $\pm$ 0.1	13.7 $\pm$ 1.2
C16:1	1.3 $\pm$ 0.1	1.6 $\pm$ 0.1	1.4 $\pm$ 0.1	1.5 $\pm$ 0.1	3.8 $\pm$ 0.5	1.3 $\pm$ 0.1
C16:0	11.5 $\pm$ 0.2	14.9 $\pm$ 0.3	12.3 $\pm$ 0.5	14.6 $\pm$ 0.6	16.1 $\pm$ 0.9	17.3 $\pm$ 1.3
C18:3(n-6)	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.2 $\pm$ 0.1	0.3 $\pm$ 0.1	0.5 $\pm$ 0.1	0.3 $\pm$ 0.3
C18:4(n-3)	1.2 $\pm$ 0.2	1.1 $\pm$ 0.2	0.8 $\pm$ 0.1	1.3 $\pm$ 0.2	3.3 $\pm$ 0.1	1.1 $\pm$ 0.1
C18:2(n-6)	3.1 $\pm$ 0.1	3.8 $\pm$ 0.1	3.3 $\pm$ 0.3	3.7 $\pm$ 0.4	9.2 $\pm$ 0.9	3.5 $\pm$ 0.2
C18:1(n-9)	33.9 $\pm$ 0.2	32.9 $\pm$ 1.5	32.6 $\pm$ 0.5	32.1 $\pm$ 2.4	23.7 $\pm$ 0.2	30.3 $\pm$ 2.2
C18:3(n-3)	1.7 $\pm$ 0.1	2.0 $\pm$ 0.1	2.1 $\pm$ 1.0	1.5 $\pm$ 0.1	-	1.7 $\pm$ 0.1
C18:0	1.9 $\pm$ 0.1	2.8 $\pm$ 1.1	2.6 $\pm$ 0.5	1.6 $\pm$ 0.1	2.4 $\pm$ 0.4	3.1 $\pm$ 0.2
C20:4(n-6)	15.1 $\pm$ 0.4	9.6 $\pm$ 0.9	13.3 $\pm$ 1.5	12.1 $\pm$ 0.8	10.1 $\pm$ 0.5	9.5 $\pm$ 0.8
C20:5(n-3)	6.4 $\pm$ 0.2	6.1 $\pm$ 1.6	5.7 $\pm$ 0.5	8.1 $\pm$ 0.9	9.2 $\pm$ 0.8	5.7 $\pm$ 0.5
C20:3(n-6)	1.9 $\pm$ 0.2	1.3 $\pm$ 0.2	0.8 $\pm$ 0.9	1.8 $\pm$ 0.1	1.1 $\pm$ 0.2	1.0 $\pm$ 0.2
C20:0	2.1 $\pm$ 0.1	2.8 $\pm$ 0.7	2.9 $\pm$ 0.2	2.5 $\pm$ 0.2	2.1 $\pm$ 0.2	3.0 $\pm$ 0.1
*otherFA	4.9 $\pm$ 0.8	5.9 $\pm$ 1.2	7.5 $\pm$ 1.5	4.2 $\pm$ 0.6	5.2 $\pm$ 1.0	5.6 $\pm$ 1.1
FA/TL (%)	33.7 $\pm$ 5.7	30.8 $\pm$ 4.3	31.3 $\pm$ 1.8	32.5 $\pm$ 5.5	18.2 $\pm$ 3.7	22.0 $\pm$ 2.7
ARA/EPA	2.37 $\pm$ 0.12	1.56 $\pm$ 0.32	2.31 $\pm$ 0.11	1.71 $\pm$ 0.19	1.10 $\pm$ 0.12	1.68 $\pm$ 0.23
n-6/n-3	2.05 $\pm$ 0.06	1.56 $\pm$ 0.16	1.86 $\pm$ 0.09	1.74 $\pm$ 0.07	2.02 $\pm$ 0.08	1.61 $\pm$ 0.06
C16:0/C18:1	0.34 $\pm$ 0.11	0.45 $\pm$ 0.13	0.38 $\pm$ 0.07	0.45 $\pm$ 0.12	0.68 $\pm$ 0.08	0.57 $\pm$ 0.08

\*other FA: 14:1, 15:1, 15:0, 17:0, 17:1, 20:4(n-3), 20:2(n-6), 20:1(n-7), 20:3(n-3), 22:1, 22:0, 24:1, 24:0

Table 3. PERMANOVA analysing differences in fatty acid composition of *F. virsoides* between settlements on artificial (=A) and natural substrata (=N) based on the Bray-Curtis dissimilarities of untransformed data

Source of variation	df	MS	Pseudo-F	<i>P</i>	MS <sub>DEN</sub>
Substrata (A-vs-N)	1	1437.00	5.46	0.0490	St(Su(A-vs-N))
Station (Substrata (A-vs-N))	4	263.25	37.83	0.0001	Si(St(Su(A-vs-N)))
Site (Station (Substrata (A-vs-N)))	6	6.96	0.58	0.8852	Res
Residual	24	12.1			

Pair-wise tests for term *Su(A-vs-N)*

A  $\neq$  N

Each test was performed using 9999 permutations of appropriate units. *P*-values were obtained using 9999 Monte Carlo samples from the asymptotic permutation distribution. The term used for the denominator mean square in each case is given in column MS<sub>DEN</sub>. Results of pair-wise tests for the significant term *Su(A-vs-N)* were reported in the Table

## DISCUSSION

The fatty acid composition in *F. virsoides* from all investigated sites showed an invariable pattern of dominating components: C14:0 or C16:0, C18:1(n-9) and C20:4(n-3) among saturated, monounsaturated and polyunsaturated fatty acids, respectively. Furthermore, one specific feature that is taxonomically important to the family Fucaceae and the genus *Fucus* is the prevalence of n-6 PUFAs over n-3 PUFAs. These results are in agreement with previous reports on other *Fucus* species (KHOTICHMENKO, 1998; COLOMBO *et al.*, 2006; VAN GINNEKEN *et al.*, 2011). The major unsaturated FA in *F. virsoides*, oleic acid (up to 33.9%), is commonly the dominant FA in brown algae and is present at the highest relative abundance (up to 38.0% of the total FAs) in the family Fucaceae (KHOTICHMENKO, 1998). Although they varied widely by season, the oleic acid contents *F. serratus* and *F. spiralis* reached the maximum levels during the summer (KIM *et al.*, 1996). The total lipids in *F. virsoides* accounted for 1.1-3.7% of its dry weight (Fig. 3A). This percentage was close to the values reported for *F. serratus* (KIM *et al.*, 1996), *F. vesiculosus*, *F. ceranoides* (MUNDA, 1964) and *F. evanescens* (KHOTIMCHENKO, 1998).

The distribution pattern and abundance of *F. virsoides* along the Istrian coast of the northern Adriatic Sea, which were evaluated in terms of the continuity of its belts, does not seem to be affected by the occasional or more constant inflow of wastewater due to human activities. Our observation of continuous, densely packed belts irrespective of increased nutrient levels or faecal contamination is in agreement with the reports of LIPIZER *et al.* (1995) on the Gulf of Trieste, both of which opposed adopting this alga as an indicator of the environmental trophic level (MUNDA, 1997; IVEŠA *et al.*, 2009). Yet, with respect to the frequency of growth classes, we observed that *F. virsoides* belts at the beaches, irrespective of the substratum type, were steadier and more balanced communities than those found at the harbours. The harbour communities showed either a higher abundance of juvenile algae (1H and 2H) or more mature specimens

(3H). Thus, the overall morphological indices of *F. virsoides* communities pointed to the different ages of the algae between the sites.

The principal component analysis based on the fatty acid composition of *F. virsoides* provided a clear, statistically significant separation of communities attached to artificial substrata (3H, 3B) from those growing on natural substrata (1H, 2H, 1B, 2B). In addition, the harbour communities (1H, 2H, 3H) were separated from those growing at the beaches (1B, 2B, 3B). However, considering the type of substratum, the harbour communities of *F. virsoides* were quite different in terms of both fatty acid composition and morphological indices (1H and 2H vs. 3H). Continuous beds of densely packed juvenile specimens on natural substrata (1H and 2H) were rich in fatty acids and had high relative contents of ARA. In accordance with the finding of a significantly higher ARA/EPA ratio in juvenile specimens relative to adult specimens (NAPOLITANO *et al.*, 1988), the juvenile specimens on natural substrata were also characterised by the highest ARA/EPA ratios among all investigated settlements, whereas with respect to saturated fatty acids, myristic acid prevailed over palmitic acid.

In addition to the temperature and nutrient load, different exposure of the settlement to light can cause conspecific variations in the total lipids and fatty acids (GOSCH *et al.*, 2012). Macroalgae grown in the shade have higher total lipid contents than algae grown in full light (KHOTIMCHENKO, 2002). In contrast, *F. virsoides* harvested from shaded artificial substrata at site 3H showed the lowest total lipid content but the highest relative contents of EPA and stearidonic acid. The increased proportion of these two acids could be the consequent of both, algal age as well as low light conditions according to the shaded position on the study site. The latter effect was most likely intensified by the complete coverage of the algae with epibionts. Algal adaptation to low light is achieved by increasing the surface area of the thylakoid membranes and increasing the amounts of their structural components, i.e., PUFAs, to increase light absorption (SUKENIK *et al.*, 1989; NAPOLITANO, 1994). This process is



related to chlorophyll accumulation, and algae use EPA and stearidonic acid to position the phytol tail of Chla (ZHUKOVA & TITLYANOV, 2006). Chlorophyll accumulates in algal over time, accompanied by a significant decrease in the proportion of fatty acids among total lipids (GERASIMENKO *et al.*, 2010 a, b). Generally, low total lipids are the result of higher relative contents of stipes in the samples because algal stipes usually contain up to three-fold lower levels of lipids than algal fronds (VELIMIROV, 1979).

*F. virsoides* collected at the beaches were more similar in age than those collected from the harbour communities. These settlements were similarly exposed to light. Therefore, the comparison of the physiological characteristics related to the type of substratum was more reliable. For algae growing on natural substrata (1B and 2B), those algae attached to the artificial substrata (3B) exhibited significantly lower concentrations of all fatty acids, lower percentages of FAs among TLs and higher values for the 16:0/18:1 ratio. The minimum values of the 16:0/18:1 ratio during the summer indicate the favourable conditions for the elongation of fatty acids (KIM *et al.*, 1996). For this reason, the increased value of the 16:0/18:1 ratio at 3B indicated that artificial substrata offer limited conditions for algal development in comparison to natural substrata (1B and 2B). This insufficiency might be related to the available nutrients, as suggested by the significantly lower TFA/TL ratio (HAYAKAWA *et al.*, 2002). Because settlements on artificial substrata (3B and 3H) received additional nutrients from anthropogenic sources, the lower availability of nutrients to *F. virsoides* was most likely caused by low

capacity of artificial substrata to retain and create nutrient pools. Nutrient scarcity most likely caused the formation of a significantly larger holdfast of *F. virsoides* attached to artificial substrata (Iveša, unpubl. data) in contrast to *F. virsoides* attached to natural substrata (site 2H) similarly exposed to excess nutrients. The algal physiological response worsens with increased algal age, as occurred for *F. virsoides* at site 3H.

In conclusion, this study showed that there are differences in the total lipid content and the fatty acid composition between *F. virsoides* grown on natural and artificial substrata. Although our results strongly indicate that algal growth on the artificial substrata was less favourable than growth on the natural substrate, the clear cause-effect relationship is not obvious due to the possible synergism between the substratum type, age of the thalli and shading of position of the settlement at site 3H. Considering the increasing demand for coastal infrastructure, to sustain residential and tourist activities as well as biodiversity along the Adriatic coast, more extensive studies focused on algae of similar ages growing under similar environmental conditions on a larger spatial scale are necessary to verify the conclusions presented herein.

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## Promjene sastava masnih kiselina u algi *Fucus virsoides* J. Agardh na različitim podlogama

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### SAŽETAK

U proljeće tijekom maksimuma vegetacijskog razvoja smeđe alge *Fucus virsoides* istražen je učinak podloge (prirodna, vapnenac ili umjetna, beton) na sastav masnih kiselina u algi. Uzorkovano je na šest staništa, uključujući luke i plaže, duž istarske obale (sjeverni Jadran). Sva staništa su bila pod utjecajem vrlo sličnih okolišnih čimbenika, i izložena umjerenom antropogenom utjecaju. Za vrstu *F. virsoides* na umjetnoj podlozi karakteristična je niža koncentracija masnih kiselina (MK), njihov niži udio u ukupnim lipidima (UL), povišeni C16:0/18:1 omjeri (MK:  $6.7 \pm 0.6$  mg g<sup>-1</sup> s.t.; MK/UL: 22%; 16:0/18:1:  $0.57 \pm 0.08$ ) te veća zasićenost u usporedbi sa zajednicama na prirodnoj podlozi (MK:  $10.2 \pm 2.9$ – $12.5 \pm 3.4$  mg g<sup>-1</sup> s.t.; MK/UL: 30.8–33.7%; 16:0/18:1:  $0.34 \pm 0.11$ – $0.45 \pm 0.13$ ). Dobiveni rezultati odražavaju nepovoljne uvjete za rast i razvoj vrste *F. virsoides* na umjetnoj podlozi u odnosu na prirodnu. Na umjetnoj podlozi je utvrđeno lošije fiziološko stanje u starijim primjercima alge (MK:  $1.9 \pm 0.6$  mg g<sup>-1</sup> s.t.; MK/UL: 18.2%; 16:0/18:1:  $0.68 \pm 0.08$ ).

**Ključne riječi:** umjetna podloga, utjecaj okoliša, masne kiseline, *Fucus virsoides*, prirodna podloga