Supplementary Information to Fucoid brown algae inject fucoidan carbon into the ocean

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1. Supplementary Materials and Methods

Study site

We chose a study site in the central Baltic for which long-term monitoring data is available and exhibits abundant populations of key algal and plant species. Rocky shores with abundant bladderwrack, *Fucus vesiculosus*, and vegetated soft sediments with eelgrass, *Zostera marina*, and a variety of aquatic plants with limnic origin dominate the shallow coastal region here (1). The area can be characterized as being microtidal with the salinity commonly ranging between 5-6. The benthic vegetation shows strong seasonal patterns and both *Z. marina* and *F. vesiculosus* tend to have lower biomass in winter and spring with a maximum biomass in late summer/early fall (2, 3). CTD environmental conditions temperature, salinity, oxygen concentration, pH and turbidity in hourly intervals at 4 m depth at Ångbåtsbryggan (DATA AVAILABLE https://www.helsinki.fi/en/ research-stations/tvarminne-zoological-station/research/monicoast/online-data). We used a sparse, monospecific seagrass bed of *Z. marina* in the vicinity of the CTD mooring at 4 meters depth (59°50’30.6”N 23°14’56.4”E) for incubations along with *F. vesiculosus*, lining the shore approx. 50 meters north-westward (59°50'30.8"N 23°14'53.7"E).

Incubations

Field *in situ* incubations aimed at capturing organic molecules released from seagrass and algae under light and dark conditions. In total, six seagrass incubations and six algae incubations were set up. On the day of the incubations, August 5, 2020, seagrass shoots were enclosed in transparent gas impermeable plastic vacuum bags (Packing24, PA/PE 20/70, 90my) with approx. 6 L of the surrounding water. In the case of benthic seagrass incubations, the bags were sealed onto the previously installed PVC tubes (**Fig 1a, top right panel**). In the case of algae incubations on August 18, 2020, transparent gas impermeable plastic vacuum bags with a single *F. vesiculosus* thallus, were closed with rubber bands around PVC end cap fitting of 12 cm diameter and attached to a bottom-anchored line at ~1 m depth (**Fig 1a, bottom right panel**). Thalli of ~50 cm length were detached close to the base by cutting the few millimeters thick, old stipe (**SFig. 8**). The incubations of the algae could not be conducted without removing them from the bedrock, as it was not possible to attach benthic chambers on

the bedrock surface with a gas tight construction. However, the exudation values were within range of other studies where holdfasts were not cut, and we did not find evidence of major cell leakage of intracellular metabolites, such as mannose (4). Half of the incubations were covered with black bags to simulate dark conditions. Immediately prior to starting the incubations, water was sampled from the ambient water to determine initial conditions.

We monitored temperature and irradiation in light-exposed and dark bags as well as in the ambient environment over the course of the incubations (**SFig. 1**). Light loggers (HOBO Pendant® Temperature/Light Data Logger 64 K; Onset Computer Corporation, USA) were programmed to record temperature and irradiation in 5 min intervals. Three loggers were deployed attached to metal rods and stuck into the sediment for monitoring during seagrass incubations or attached to plastic disks during algae incubations. One rod with logger measured inside a light-exposed incubation, a second inside a dark incubation, and a third adjacent to the incubations. The latter logger was complemented with a photosynthetically active radiation (PAR) sensor (RBRsolo Single Channel Logger with a Li-Cor® -192, RBR Ltd., Canada) also recording in 5 min intervals to calibrate the logger data to PAR expressed in Photosynthetic Photon Flux Density (μmol m^{-2} s^{-1}).

At the end of the incubation, all seagrass (above- and belowground) and algal tissue was collected. In the laboratory, the length and width of each seagrass shoot was measured, while the algal thallus was spread out across a cutting mat with a grid for surface area estimation. Epiphytes and epifauna were scraped off shoots and thalli using a razor blade, and collected separately from the plant/algal biomass. All biomass was frozen and subsequently freeze-dried for 24 hours, and weighed to measure dry weight.

Water sample collection

After five hours, we sampled water from the incubations and collected the incubated seagrass, sediment and algae. Water column samples from seagrass incubations were collected into acid washed single use, sterile, 60 mL syringes (Thermo Fisher Scientific, USA) via water column ports on the PVC tubes. Water column samples from algae incubations were collected into acid washed 60 mL syringes after opening incubation bags on board. Water column samples were filtered immediately through a precombusted 45 mm diameter glass microfiber GF/F filter (Whatman, UK). For each sample, 30 mL were pushed through the filter for rinsing before starting collection. To determine the concentration of dissolved organic carbon (DOC), 15 mL sample were filtered into a precombusted 24 mL glass vial containing 60 µL 25% hydrochloric

acid and the vial sealed with an acid washed teflon-lined cap. Another 10 mL of sample were filtered into a precombusted 12 mL glass vial and the vial sealed with an acid washed teflon-lined cap for colored dissolved organic matter (CDOM) and fluorescent dissolved organic matter (FDOM) analysis. Subsamples for DOC were frozen at -20°C and samples for CDOM/FDOM were stored at 4°C until analysis within two weeks. For gas chromatography-mass spectrometry (GC-MS), 2 mL samples were filtered into 2 mL microtubes. About 600 mL of filtered water column samples and 30-60 mL porewater samples were collected in an acid washed PP or HDPE bottle (Thermo Fisher Scientific, USA) for extractions.

The chamber volume of the algal incubations was measured directly with a graduated cylinder and adding the amount of water that was sampled. We attempted to measure the volume of the seagrass incubations by injecting a saline solution and measure the change in salinity. However, the conductivity meter used was not sensitive enough to accurately measure the change, and the benthic chamber volume estimation was not possible.

Water sample analyses

Optical FireSting O_2 sensors (Pyroscience GmbH, Germany) determined oxygen concentrations in water samples. Calibration of the sensors was achieved using MilliQ water. Oxygen concentrations were measured in 600 mL subsamples from water column within two hours of sample collection. For algae incubations, oxygen concentrations could also be determined in water from incubations without prior filtration.

To determine the total dissolved nitrogen (TDN), the samples were spectrophotometrically analyzed using an autoanalyzer (Aquakem 250). In addition, DOC-concentrations were measured using a Shimadzu TOC-V_{CPH}-analyzer.

Colored DOM (CDOM) absorption was measured using a Shimadzu 2401PC spectrophotometer with 5 cm quartz cuvette over the spectral range from 200 to 800 nm with 1 nm resolution. Ultrapure water was used as the blank for all samples. Excitation-emission matrices (EEMs) of fluorescent DOM (FDOM) were measured with a Varian Cary Eclipse fluorometer (Agilent). Processing of the EEMs was done using the *eemR* package for R software (5). A blank sample of ultrapure water was subtracted from the EEMs, and the Rayleigh and Raman scattering bands were removed from the spectra after calibration. EEMs were calibrated by normalizing to the area under the Raman water scatter peak (excitation wavelength of 350 nm) of an ultrapure water sample run on the same session as the samples, and were corrected for inner filter effects with absorbance spectra (6).

GC-MS analysis

For metabolomics on seawater, 2 mL samples for GC-MS were thawed and 0.5 mL subsamples taken. Processing blanks consisted of artificial seawater (ASW) prepared at a salinity of 3.6 % (w/v) and diluted 1:5 to match Baltic Sea salinity. For a liter of ASW, 26.37 g sodium, 6.8 g magnesium sulfate heptahydrate, 5.67 g magnesium chloride hexahydrate, 1.47 g calcium chloride, 0.6 g potassium chloride and 0.09 g potassium bromide were dissolved in MilliQ-water and autoclaved. Heat-sensitive elements were added to the following final concentrations: 2.5 mM sodium bicarbonate, 150 µM monopotassium phosphate, 500 µM ammonium chloride, 7.55 nM iron sulfate, 4.85 nM boric acid, 0.51 nM manganese chloride, 0.8 nM cobalt chloride, 0.1 nM nickel chloride, 0.06 nM copper chloride, 0.5 nM zinc sulfate, 3.72 nM sodium permanganate, 0.11 nM sodium selenite, 0.02 nM sodium tungstate, 10 nM 4-aminobenzoic acid, 10 nM D-biotin, 100 nM nicotinic acid, 50 nM calcium D-pantothenate, thiamine, 0.8 nM thiamine chloride dihydrochloride, and 36.9 pM cyanocobalamin. For standards, 100 µL of a mixture with 50 metabolites at 0.4 mM concentration each (**STable 5**) were added to diluted ASW. Subsamples, processing blanks and standards were spiked with 100 µL cholestane at 1 mM in ethanol and 40 µL ribitol at 0.2 mg mL^{-1}, dried at 45°C for 6 h in a Concentrator plus speedvac (Eppendorf, Germany) and frozen until further processing. Immediately prior to the derivatization, samples were again dried for 20 min to remove any moisture. After drying, 250 µL anhydrous toluene were added and samples ultrasonicated for 10 min at 100 % intensity in an ultrasonication bath. In a chamber with dry air (humidity <5 %), samples were dried under constant nitrogen gas stream. To each sample, 80 mL of 20 mg mL^{-1} methoxamine in pyridine were added, samples ultrasonicated for 10 min at 100 % intensity and subsequently incubated for 90 min at 37°C shaking at 1350 rpm. Samples were dried again under constant nitrogen gas stream and 100 µL of N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) added. After ultrasonication for 10 min at 100 % intensity and brief vortexing, samples were incubated for 30 min at 37°C shaking at 1350 rpm. Finally, the derivatized samples were ultrasonicated for 10 min at 100% intensity, centrifuged for 2 min at 21 100 x g and 100 µL of clear supernatant transferred to a GC-vial for analysis.

Samples were analyzed within 24 h after derivatization on an Agilent 7890B gas chromatograph coupled to an Agilent 5977A single quadrupole mass selective detector. To this end, an Agilent 7693 autosampler injected 1 µL of sample in splitless mode through the inlet liner onto an Agilent DB-5MS column (30 m length, 0.25 mm diameter, 0.25 µm film thickness) with Agilent DuraGuard guard column (10 m length). Injector temperature was set to 290°C.

Chromatographic separation was achieved with helium as carrier gas at a constant flow of 1 mL min^{-1}. The column oven temperature was increased from 60°C at injection to 325°C over 13.25 min and maintained at 325°C for 2 min. The mass detector acquired spectra from electron ionization at 70eV in a mass range of 50-600 m/z at a scan rate of 3 s^{-1}.

Dialysis

To desalt eluates collected from polysaccharide extractions, we dialyzed eluate subsamples against MilliQ-water. The eluates in 2 M ammonium bicarbonate/carbonate or 5 M NaCl were thawed and vortexed rigorously. Subsamples of 4 up to 10 mL were transferred into prerinsed 1 kDa membrane (SpectraPor® Biotech CE Dialysis Membrane, Carl Roth, Germany) with MilliQ-water controls in separate dialysis tubing to account for processing artifacts. Salt and small molecules were removed by dialyzing three times against 10 L MilliQ-water at 4°C. Dialyzed samples were transferred to 15 mL centrifuge tubes, freeze dried and resuspended in MilliQ-water.

Acid hydrolysis

Dialyzed and freeze dried samples were resuspended in MilliQ-water, of which 200 µL were combined with 200 µL 2 M HCl in a precombusted glass ampule. Ampules were sealed and incubated for 24 h at 100°C to achieve complete acid hydrolysis of polysaccharides in the sample. After acid hydrolysis, samples were transferred from glass ampules to microtubes and dried in an acid proof vacuum concentrator (Martin Christ Gefriertrocknungsanlagen GmbH, Germany). Dried, hydrolyzed samples were reconstituted in MilliQ-water, diluted if necessary and transferred to 100 µL glass inlets in HPLC vials for HPAEC-PAD analysis.

Monosaccharide quantification

Monosaccharides in samples were separated via anionic interaction chromatography and individually quantified using electrochemical detection, as reported previously (7). For analysis, 25 µL of sample were autosampler-injected into a DIONEX ICS 5000+ equipped with a 2 x 250 mm PA10 column with corresponding guard (all Thermo Fisher Scientific, USA). Separation of neutral monosaccharides was achieved during an isocratic phase with 18 mM NaOH, followed by separation of acidic monosaccharides during a gradient up to 200 mM $NaCH_3COO$. Monosaccharides were detected using pulsed amperometric detection. Based on quantitative standards, monosaccharides were identified by retention times and peak areas fitted to the standard series, which consisted of arabinose, fucose, galactosamine, galactose, galacturonic

acid, gluconic acid, glucose, glucuronic acid, iduronic acid, mannose, mannuronic acid, rhamnose, and xylose. Mannose and xylose peaks eluted too close to confidently distinguish between and peak areas are reported combined.

Antibody-based methods

Enzyme-linked immunosorbent assay (ELISA) was used to detect fucoidan epitopes in the dialyzed samples from algae incubations. 50 µL of sample and 50 µL of phosphate buffered saline (PBS, x1) were added in the wells of a microtitre plate (NUNC Maxisorp, Thermo Fisher Scientific). Each sample was added in triplicate and plates were incubated at 4 °C overnight. Plate contents were discarded and wells were washed six times with 200 µL deionized water. Wells were blocked with 200 µL PBS with 5% (w/v) low-fat milk powder (MPBS) for 2 h and washed nine times with 200 µL deionized water. Wells were incubated for 1.5 h with 100 µL of BAM1 or BAM2 (SeaProbes, Roscoff, France) antibody solution diluted 1:10 in MPBS. After antibody probing, wells were washed nine times with 200 µL deionized water. Wells were incubated 1.5 h with 100 µL of secondary antibody conjugated to horseradish peroxidase (A9037, Sigma-Aldrich) diluted 1:1000 in MPBS. Wells were washed nine times with 200 µL deionized water. The plate was developed with 100 µL ELISA tetramethylbenzidine substrate per well. Development reaction was stopped with 100 µL 1 M HCl and absorbance in wells read at 450 nm using a SpectrostarNano absorbance plate reader and MARS software (BMGlabtech).

AEX elutions were analyzed by carbohydrate microarray analysis as described previously (8). In brief, aliquots of the dialyzed 5 M NaCl elutions from AEX were added into wells of 384-microwell plates and the content of the plates printed onto nitrocellulose membrane with a pore size of 0.45 µm (Whatman) using a microarray robot (Sprint, Arrayjet, Roslin, UK). Each sample was printed in duplicates. The printed microarrays were blocked in MPBS for 1 h. Next, each microarray was individually incubated for 2 h with the monoclonal antibodies BAM1, BAM2 or BAM7 (SeaProbes, Roscoff, France) diluted 1:10 in MPBS. After probing, arrays were thoroughly washed in PBS and incubated for 2 h with a secondary antibody conjugated to alkaline phosphatase (A8438, Sigma-Aldrich) diluted 1:5000 in MPBS. Arrays were washed in PBS and deionized water and developed in a solution containing 5-bromo-4-chloro-3-indolylphosphate and nitro blue tetrazolium in alkaline phosphatase buffer (100 mM NaCl, 5 mM $MgCl_2$, 100 mM Tris-HCl, pH 9.5). Developed arrays were analyzed as described previously (8) using Array-Pro Analyzer 6.3 (Media Cybernetics). The highest mean antibody signal intensity detected in the data set (obtained with alginate from brown algae - A1112, Sigma Aldrich -

probed with BAM7, which was included as a positive control) was set to 100 and all other values were normalized accordingly.

Statistical analysis

All statistical analyses and calculations were performed in R version 4.0.3 in RStudio version 1.3.1093. Due to the small sample size and violation of assumptions of parametric tests, we used non-parametric tests to compare dissolved compounds in the benthic chambers experiment. For the bulk parameters (dissolved oxygen, dissolved organic carbon and total nitrogen, humic-like fluorescence and absorption at 254 nm of dissolved compounds), a Kruskal-Wallis rank sum test (*R Base*), followed by a *post hoc* Conover-Iman test (*conover.test* package (9)) for pairwise comparisons, was used to test for significant differences between filtered seawater (n = 6), dark incubations (n = 3) and light incubations (n = 3) (**Fig 1b, c**).

The peak picking of the metabolomics data was performed with the R package *xcms* (10). Each sample was normalized by the intensity of the internal standard, ribitol, quantification ion. A Wilcoxon rank sum test (*rstatix* package (11)) was used for pairwise comparisons between filtered seawater and either dark incubations or light incubations to find five metabolites which differed significantly between the sampling points (**Fig 1d, e**). Comparing with reference spectra and standards from the MassBank of North America (MoNA) and analytical standards (STable 5), one of the five metabolites was identified as mannitol, while the other four were labeled unidentified alcohol-like, unidentified small organic acid-like, unidentified small aromatic-like and unidentified benzoic acid-like compounds.

Concentrations of the complete monosaccharide composition in raw samples were compared using multivariate statistics (*vegan* package(12)). Eigenvalues of monosaccharides were computed from a redundancy analysis on all monosaccharide concentrations. A Bray-Curtis dissimilarity analysis (*vegan* package) was used to compare monosaccharide composition between samples. To this end, abundances were normalized to the most abundant monosaccharide in a sample. Relative abundances were used to compute a distance matrix based on the Bray-Curtis method. Samples were clustered hierarchically using the generated distance matrix with complete linkage. Similarities in monosaccharide composition between samples were visualized in a heatmap (**SFig. 4**). Variance structure-corrected generalized linear models were explored to assess significant differences between blanks, seawater and incubation samples for individual monosaccharides with a focus on fucose, the main monomer

of fucoidan. However, the data did not fulfill model assumptions of variance heterogeneity. Log-transformation evened the spread to some extent, but not sufficiently to apply parametric tests.

Linear regression models (*R Base*) were used to quantify the correlations between 1) monosaccharide concentrations from acid hydrolysis with the summed signal intensities of fucoidan antibodies BAM1 and BAM2 (**Fig. 2b**); 2) monosaccharide concentrations from AEX extracts and the signal intensities of fucoidan antibodies BAM1 and BAM2, and the alginate antibody BAM7 (**SFig. 3**); 3) enzyme-produced monosaccharides and standard concentrations of a fucoidan standard (**Fig. 2f**); and 4) acid-produced monosaccharides and standard concentrations of a fucoidan standard (**SFig. 7**).

Linear mixed models (*lme4* package (13)) were used to compare the groups of blanks (n = 9), filtered seawater before incubations (n = 18), dark incubations (n = 9) and light incubations (n = 9) for antibodies BAM1 and BAM2 (**Table 1a**). Analytical triplicates were used for each sample, and thus, a random effect of sample number was used. To compare between fucoidan concentrations the groups quantified by acid hydrolyzed filtered seawater, acid hydrolyzed AEX extracts, and enzyme hydrolyzed filtered seawater, a non-parametric Kruskal-Wallis rank sum test, followed by a *post hoc* Conover-Iman test for pairwise comparisons (**Table 1b**).

2. Supplementary Tables

Supplementary Table 1. Results from Kruskal-Wallis rank sum tests and post-hoc Conover-Iman pairwise comparisons of the concentrations of parameters dissolved oxygen (O2), dissolved organic carbon (DOC), total dissolved nitrogen (TDN), Peak *c* (humic-like fluorescence), and absorption coefficient $a_{(254)}$ between initial seawater, end of light algae incubations, and end of dark algae incubations. Abbreviations: degrees of freedom (df), *p*-value (*p*), T-statistic (T) and chi-square statistic (χ^2).

Parameter	*O_2*			*DOC*			*TDN*			*Peak c*			*$a_{(254)}$*		
Kruskal-Wallis rank sum test															
	χ^2	df	*p*	χ^2	df	*p*	χ^2	df	*p*	χ^2	df	*p*	χ^2	df	*p*
	9.3	2	0.01	7.6	2	0.02	7.6	2	0.02	7.6	2	0.02	9.4	2	0.01
Conover-Iman pairwise comparison															
	T		*p*	T		*p*	T		*p*	T		*p*	T		*p*
initial-dark	4.1		0.001	-3.8		0.004	-3.8		0.004	-1.9		0.044	-4.2		0.002
initial-light	-4.1		0.002	-4.6		0.003	-4.6		0.003	-4.5		0.002	-6.9		<0.001
dark-light	-7.1		<0.001	-0.7		0.258	-0.7		0.258	-2.2		0.040	-2.4		0.020

Supplementary Table 2. Results from Kruskal-Wallis rank sum tests and post-hoc Conover-Iman pairwise comparisons of the concentrations of parameters dissolved oxygen (O2), dissolved organic carbon (DOC), total dissolved nitrogen (TDN), Peak *c* (humic-like fluorescence), and absorption coefficient $a_{(254)}$ between initial seawater, end of light seagrass incubations, and end of dark seagrass incubations. Abbreviations: degrees of freedom (df), *p*-value (*p*), T-statistic (T) and chi-square statistic (χ^2).

Parameter	*O_2*			*DOC*			*TDN*			*Peak c*			*$a_{(254)}$*		
Kruskal-Wallis rank sum test															
	χ^2	df	*p*	χ^2	df	*p*	χ^2	df	*p*	χ^2	df	*p*	χ^2	df	*p*
	7.1	2	0.029	5.3	2	0.07	6.5	2	0.039	2.0	2	0.375	3.2	2	0.197
Conover-Iman pairwise comparison															
	T		*p*	T		*p*	T		*p*	T		*p*	T		*p*
initial-dark	2.2		0.039	ns		ns	-0.7		0.253	ns		ns	ns		ns
initial-light	3.9		0.005	ns		ns	-3.6		0.009	ns		ns	ns		ns
dark-light	1.5		0.088	ns		ns	-2.5		0.026	ns		ns	ns		ns

Supplementary Table 3. Results from Wilcoxon rank sum tests of the signal of metabolites mannitol, alcohol-like, organic acid-like, and aromatic-like metabolites between initial seawater, and end of light and dark algae incubations. Abbreviations: W-statistic (W), *p*-value (*p*)

Parameter	*Mannitol*		*Alcohol-like*		*Organic acid-like*		*Aromatic-like*	
	W	*p*	W	*p*	W	*p*	W	*p*
initial-dark	0	0.024	0	0.024	5	0.381	0	0.024
initial-light	0	0.024	5	0.381	0	0.024	1	0.048

Supplementary Table 4. Results from Wilcoxon rank sum tests of the concentrations of metabolites mannitol, alcohol-like, organic acid-like, and aromatic-like and benzoic-like metabolites between initial seawater, end of light algae incubations, and end of dark algae incubations. Abbreviations: W-statistic (W), *p*-value (*p*).

Parameter	*Mannitol*		*Alcohol-like*		*Organic acid-like*		*Aromatic-like*		*Benzoic acid-like*	
	W	*p*	W	*p*	W	*p*	W	*p*	W	*p*
initial-dark	16	0.095	10	0.905	9	1	6	0.548	0	0.024
initial-light	18	0.024	10	0.905	6	0.548	7	0.714	0	0.024

Supplementary Table 5: Compounds in standard chemical mixture for GC-MS, all at 0.4 mM concentration. Abbreviations: retention time (RT), mass-to-charge ratio (M/Z)

COMPOUND	RT	M/Z	COMPOUND	RT	M/Z
CHOLESTANE	1622	373	**MALTOSE**	NA	NA
FRUCTOSE	1092	307	**MANNITOL**	1129	319
D-GLUCOSE	1119	321	**MYO-INOSITOL**	1224	305
D-MANNOSE	1114	203	**N-ACETYLGLUCOSAMINE**	1216	NA
TREHALOSE	1559	377	**ORNITHINE**	925	NA
D-GALACTOSE	1101	319	**OXALIC ACID**	545	NA
ALANINE	522	218	**PHENYLALANINE**	934	221
ASPARTIC ACID	854	NA	**PROLINE**	683	216
CITRIC ACID	1060	NA	**PROLINE**	860	216
CYSTEINE	882	220	**PYRUVATE**	479	NA
FUMARIC ACID	756	245	**RIBITOL**	992	217
GLUTAMIC ACID	926	177	**RIBITOL**	1419	217
GLUTAMINE	1032	NA	**RIBOSE**	964	204
GLYCEROPHOSPHATE	1023	NA	**SERINE**	731	204
GLYCINE	538	204	**SUCCINIC ACID**	695	262
GLYCINE	688	NA	**SUCROSE**	1500	361
HISTIDINE	1125	NA	**THREONINE**	750	291
ISOLEUCINE	677	232	**THYMINE**	763	255
LACTIC ACID	488	193	**UREA**	638	NA
LEUCINE	659	NA	**UREA**	578	NA
LYSINE	988	174	**VALINE**	614	188
MALEIC ACID	688	NA	**FUCOSE**	1012	277
MALIC ACID	829	233			

Supplementary Table 6. Results from a three-way analysis of variance (ANOVA) of fucoidan signal between blanks, initial seawater, end of light and dark seagrass incubations for the antibody-based technique ELISA (BAM1 and BAM2), and a Kruskal-Wallis rank sum test for fucoidan estimates from acid hydrolysis of filtered seawater, acid hydrolysis of anionic exchange (AEX) extracts, and enzyme digestions. Abbreviations: degrees of freedom (df), *p*-value (*p*), F-statistic (F) and chi-square statistic (χ^2).

Three-way Analysis of Variance

	Sums of squares	*Mean square*	*Numerator df*	*Denominator df*	*F*	*p*
BAM1	1.7	0.6	3	8.2	63.2	<0.001
BAM2	12.4	4.1	3	3.5	751.7	<0.001

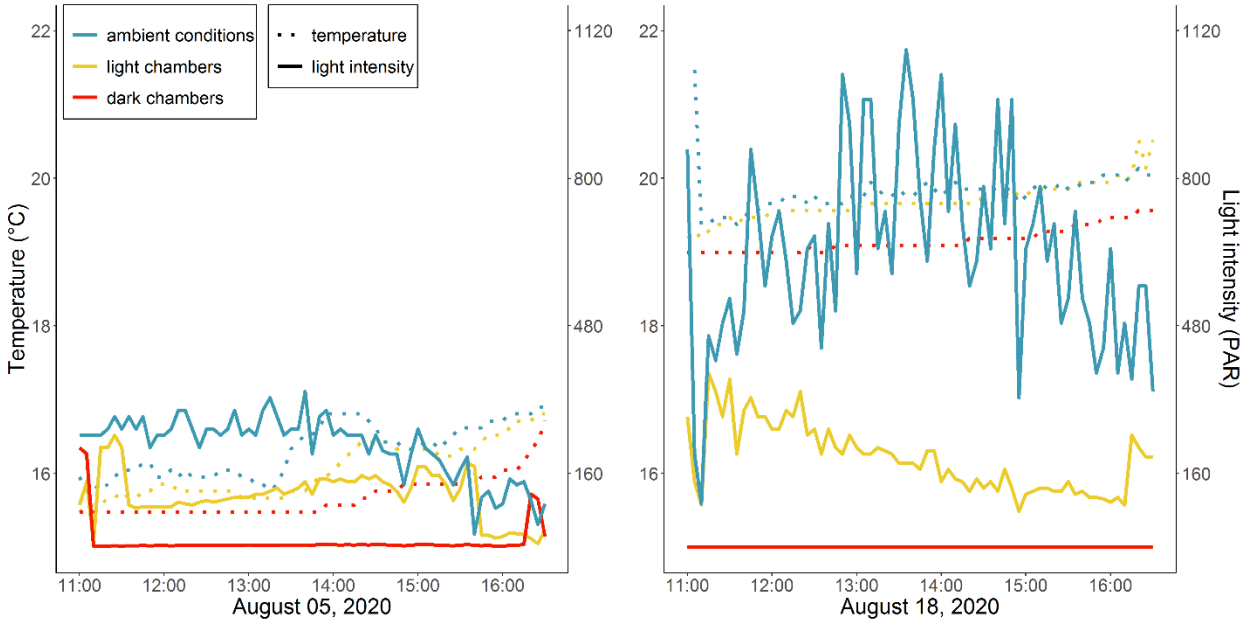
Kruskal-Wallis rank sum test

	df	χ^2	*p*
acid	3	12.6	0.010
AEX	3	10.3	0.016
enzyme	3	11.2	0.011

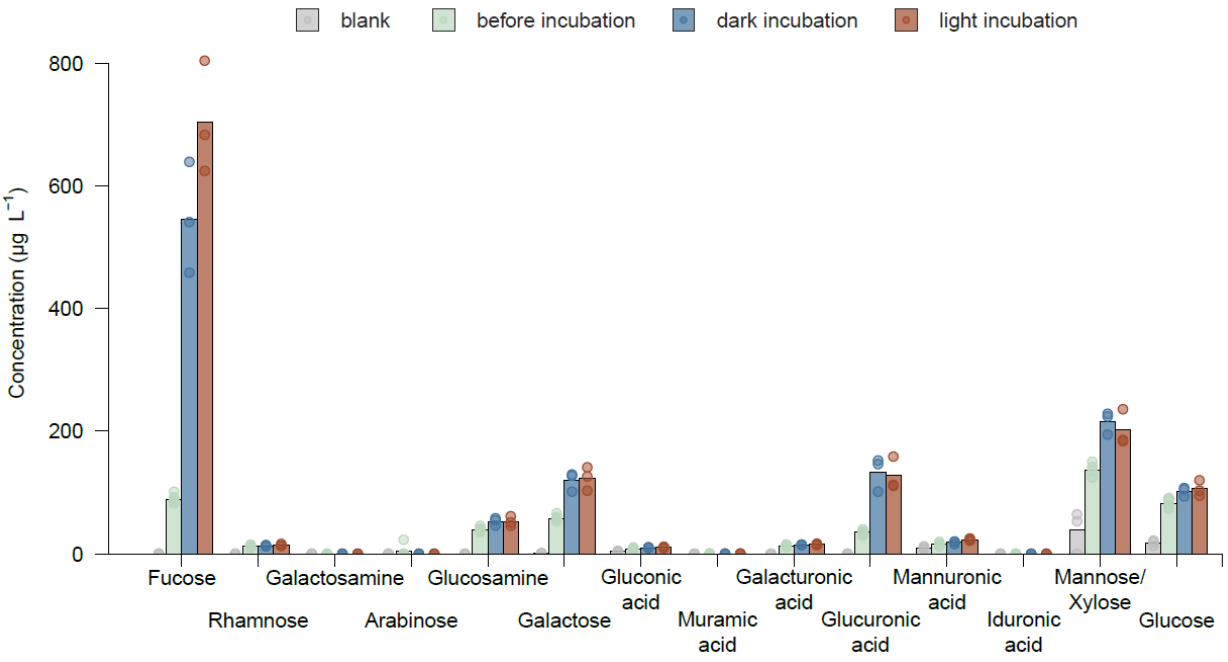
Supplementary Table 7. Results from post-hoc tests Tukey's Honest Significant Difference (HSD) following the three-way ANOVA, and a Conover-Iman pairwise comparison, following the Kruskal-Wallis rank sum test in Supplementary Table 5. Abbreviations: *p*-value (*p*), T-statistic (t-ratio or T).

Analytical technique	*BAM1*		*BAM2*		*acid*		*AEX*		*enzyme*	
	Tukey's HSD test				Conover-Iman pairwise comparison					
	t-ratio	*p*	t-ratio	*p*	T	*p*	T	*p*	T	*p*
blank-dark	12.1	<0.001	29.6	<0.001	-7.1	<0.001	-4.2	0.002	-3.2	0.006
blank-initial	-9.2	<0.001	-1.7	0.463	-3.9	0.001	-1.5	0.086	0	0.500
blank-light	-12.6	<0.001	-28.4	<0.001	-8.8	<0.001	-6.2	<0.001	-4.6	<0.001
initial-dark	5.5	0.002	36.6	<0.001	-4.2	0.001	-3.6	0.005	-3.4	0.005
initial-light	-6.2	<0.001	-35	<0.001	-6.3	<0.001	-6	<0.001	-5	<0.001
dark-light	-0.6	0.934	1.4	0.551	-1.8	0.053	-2.2	0.035	-1.4	0.118

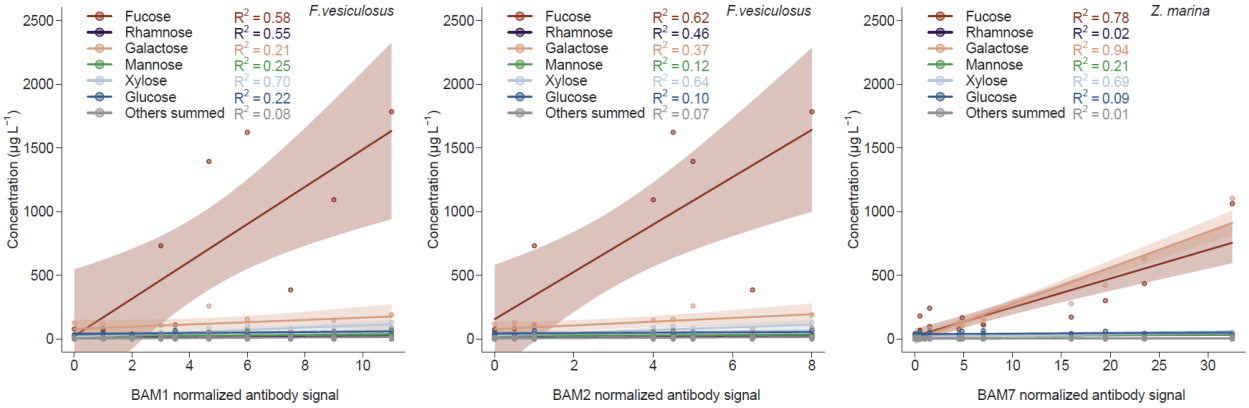
3. Supplementary Figures



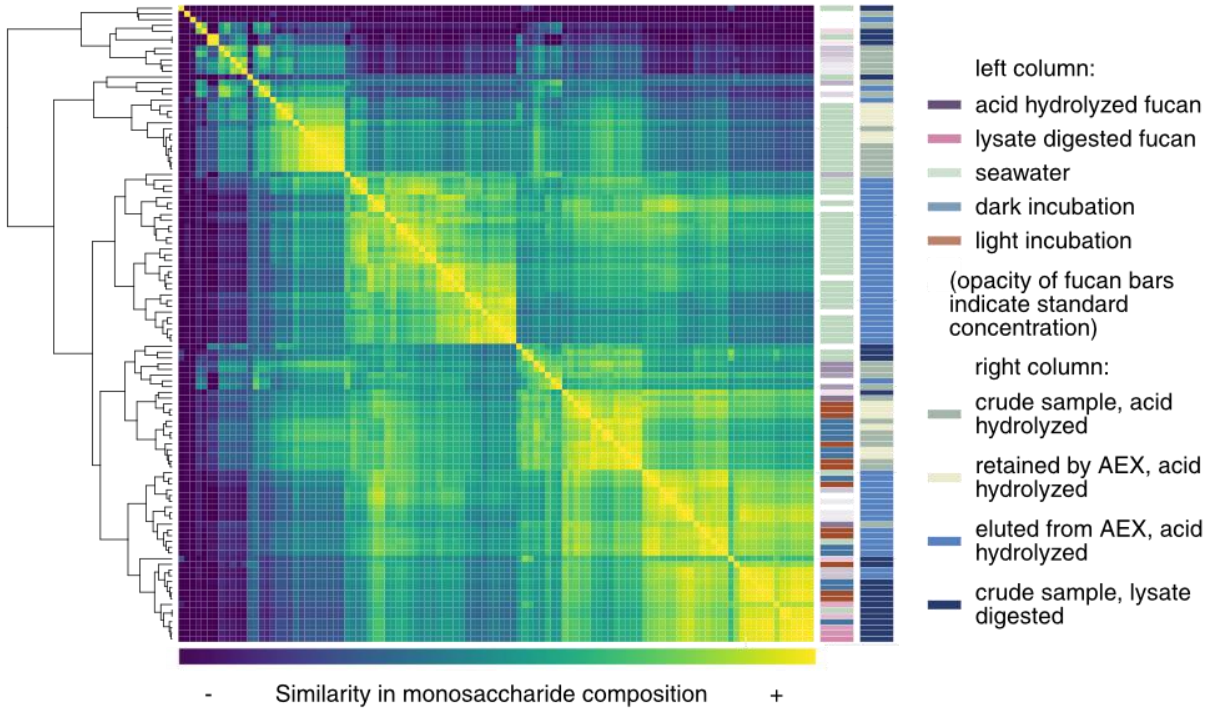
Supplementary figure 1: Similar temperature but contrasting light conditions between light and dark incubations of *Zostera marina* on August 5, 2020 (left panel) and *Fucus vesiculosus* on August 18, 2020 (right panel). Light intensity of PAR is given in µmol photons m^{-2} s^{-1}.



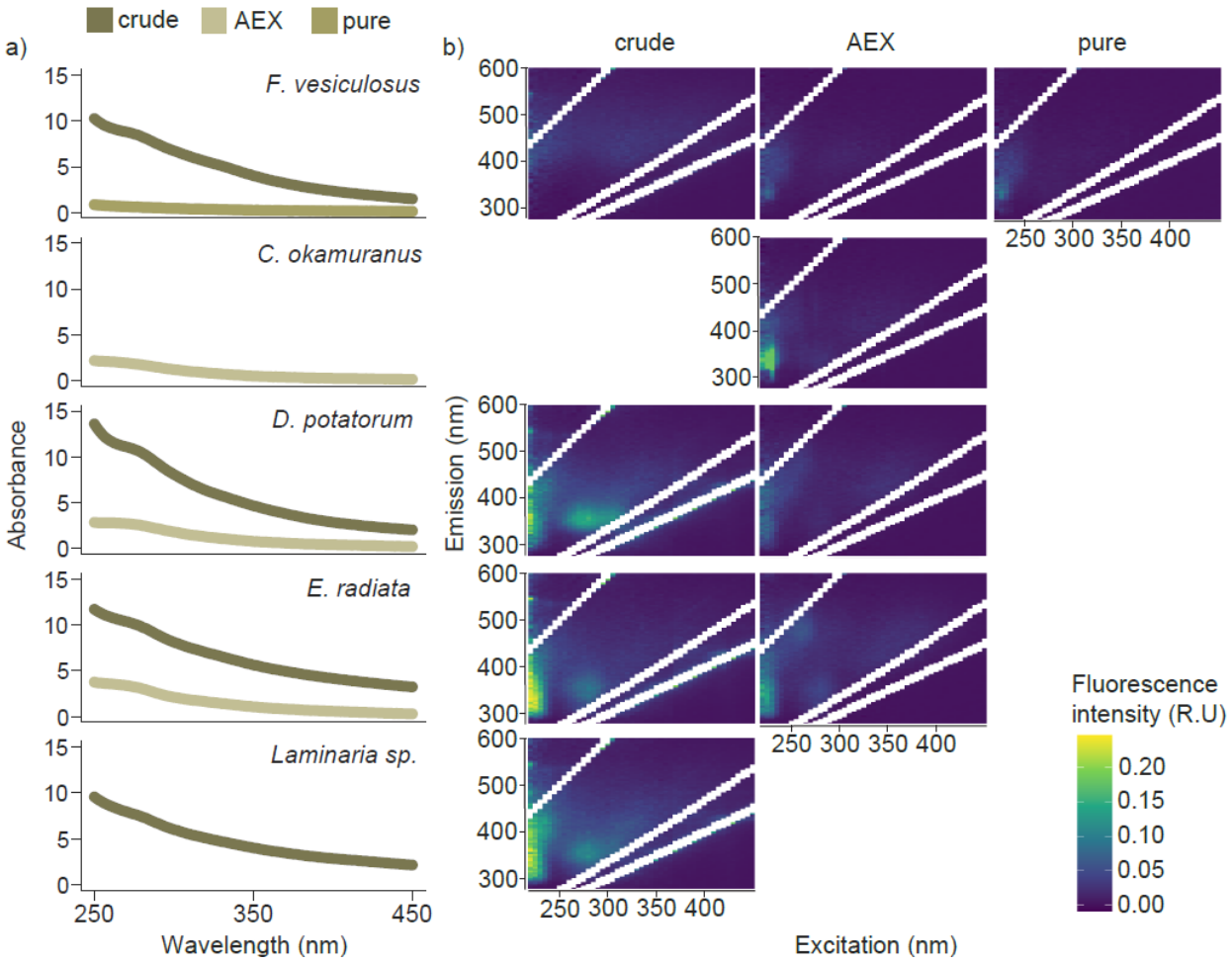
Supplementary figure 2: Mainly fucoidan, but also alginate monosaccharides increase during incubations of *F. vesiculosus*. The figure shows monosaccharide concentrations after acid hydrolysis of filtered, dialyzed seawater samples.



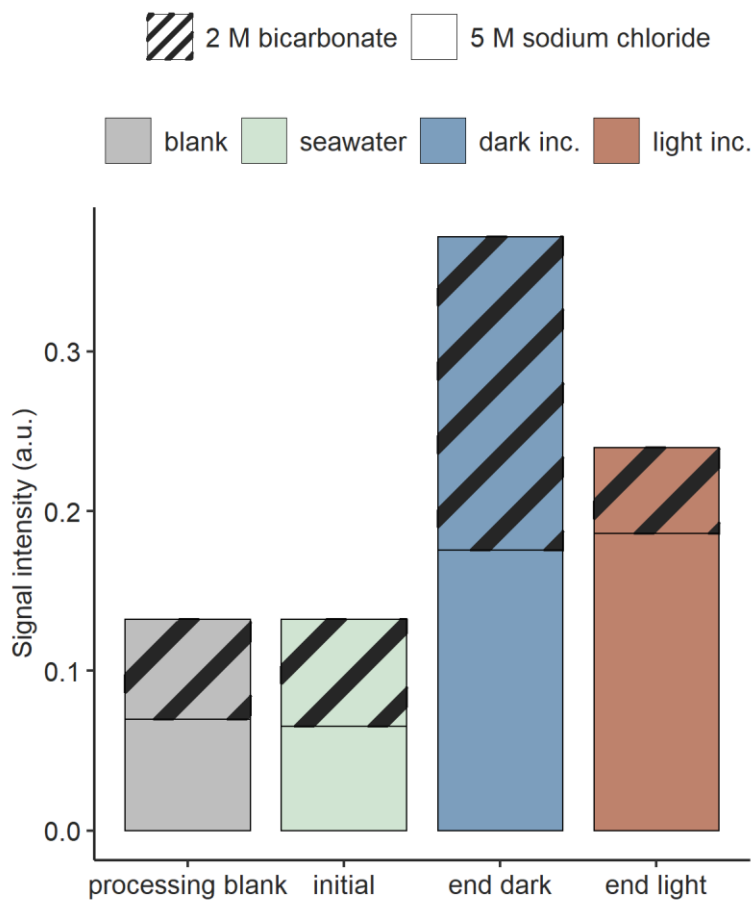
Supplementary figure 3: Monosaccharides in AEX extracts correlate with antibody signal linking glycan composition, charge and structure. Carbohydrate microarrays containing the 5 M NaCl elutions were probed with monoclonal antibodies resulting in significant correlations for BAM1 (left panel) and BAM2 (middle panel) signal intensities in algae samples and BAM7 signal intensities (right panel) in seagrass samples. Fucose concentration in 5 M NaCl elutions correlated with BAM1 and BAM2 signal in algae samples, while fucose and galactose concentrations correlated with BAM7 signal in seagrass samples.



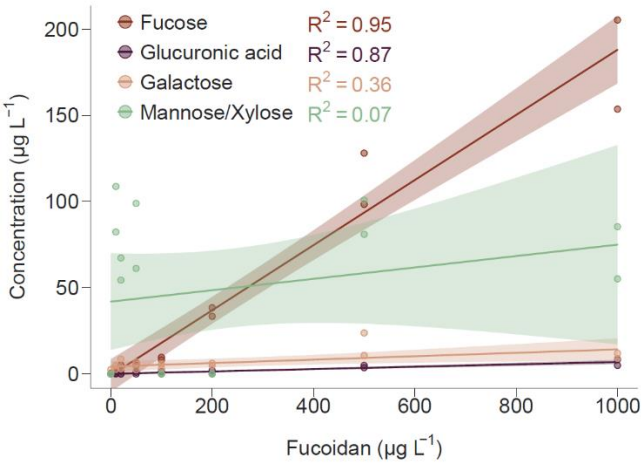
Supplementary figure 4: Enzymes and AEX improve the specificity of fucoidan quantification in a Bray-Curtis dissimilarity analysis on monosaccharide composition of algae incubation samples.



Supplementary figure 5: CDOM absorbance and fluorescence excitation-emission matrices from nine fucoidan standards. Crude fucoidans from various brown algae species systematically show higher absorbance and fluorescence in the 220/350 (Excitation/Emission) nm region, than their purified forms.



Supplementary figure 6: Fucose produced by enzymatic hydrolysis of dialyzed AEX extracts.



Supplementary figure 7: Fucose is the best predictor of dissolved fucoidan concentration in seawater after dialysis and acid hydrolysis. Standards were prepared using >95% pure *F. vesiculosus* fucoidan in 6 psu artificial seawater.

Supplementary figure 8: A *F. vesiculosus* thallus used in the algae incubations. The red circle indicates the location on the stipe that was cut when removing the algae from the bedrock. Every square on the mat is 1 cm^2.

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