Scientific
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Some Mechanism Seaweeds Employ to Cope with Salinity Stress in the Harsh Euhaline Oceanic Environment

Vincent van Ginneken

Blue Green Technology Foundation, Heelsum, Netherlands
Email: vvanginneken@hotmail.com

Abstract

In order to prevent salt damage because seaweed enzymes can only operate under hypohaline conditions (salinity ≈ 6‰ - 12‰) but also obtain for photosynthesis an in the aquatic environment—due to a 10,000 fold strongly limited carbon source—seaweeds developed several mechanisms to meet these vital demands for survival in the harsh euhaline oceanic environment (salinity range: 32‰ - 35‰), we tested this range of adaptation mechanisms in the euhaline oceanic collected water in combination with the seaweed moisture. We obtained under laboratory conditions at 10 bar mechanical pressure for four seaweed species: *Ulva lactuca*, *Caulerpa sertularioides*, *Caulerpa cf. brachypus* (all three green) and *Undaria pinnatifidia* (brown). Oceanic water and seaweed moisture were measured for salinity, pH and by Inductively Coupled Plasma Spectroscopy (ICP)-techniques concentrations for macro-elements: (Ca, Fe, K, Mg, Mn, Na, P, & S), micro-elements ≈ [HM]: (Al, As, Cd, Co, Cr, Cu, Mo, Ni, Pb & Zn) and nutrients (N-total & P-total). The [seawater compound X]/[oceanic compound X] ration is a reflection of an inward (uptake) or excretion mechanism over the seaweed cellular membrane which is operative. Our observations gave a clear dispersion to salinity stress with on one hand the green seaweed *U. lactuca* and on the other the brown seaweed *U. pinnatifidia*. Both *Caulerpa spp.* took in an intermediate position. Observed in compensatory responses to salinity stress was ranging *Ulva sp.* both *Caulerpa spp.*-*Undaria sp.*: 1) amount pressed seaweed moisture: [ml/g Fresh Weight]; 2) salinity: (in ‰); 3) Na^+ storage vacuole volume; 4) Na^+:K^+ ratio (reflection of K^+ as osmolyticum); 5) Σ[HM] (as osmolyticum); 6) pH (seaweed moisture); 7) Nutrients (N & P); 8) availability of essential metal elements for plants (Cu, Fe, Zn, Mn, Mo, Ni); 9) transport direction of micro- and macro-elements. Finally, the role of brown vs. green seaweeds in the evolutionary eukaryotic tree of life in relation to the ability of the brown seaweeds to produce their own osmolyticum will be discussed.

1. Introduction

At present, terrestrial agriculture is at its limits mainly for available land area and fertilizers (reviewed: [1] [2]). Due to an unfettered population growth estimated at around 9.5 billion people at the midst of the 21st century [3], a looming fertilizer (phosphorus) crisis [4], and lack of available land area to expand terrestrial agriculture [5], we have to go sea-farming [6].

This shift "towards a seaweed-based economy" [2] has tremendous opportunities and challenges because the Earth's surface is for ≈70.8% covered with water [7] while of the remaining terrestrial only 13.31% is arable [8]. In these oceans seaweeds and other marine plants are the primary producers in the marine environment. They form the standing crop and determine the productivity of all communities. Seaweed-based ecosystems are amongst the most productive on Earth [9]. In addition, global seaweed production by aquaculture is boosting as depicted in **Figure 1** based on FAO 2014 data. Recently, we explained in great extent the "seaweed-paradox": which implies that biomass production is severely hampered by a 10,000 fold slower diffusion rate of a Carbon source or Dissolved Inorganic Carbon (DIC) in the biophysical medium water in comparison to terrestrial C3 crops [10]. Despite this negative property, pelagic seaweeds outcompete C3 crops for annual green biomass. However, for global biomass production, seaweeds produce only a small fraction of the global supply of biomass with below 30×10^6 fresh weight (FW) ton of seaweed, in comparison to 16×10^{11} ton of terrestrial crops, grasses and forests [11].

In this research we will focus on the salt extrusion mechanism of the seaweeds, without an efficient aquatic photosynthesis which would be impossible [10]. Salt-damage is very harmful and even life threatening for terrestrial plants as outlined by [12] [13]. However, it might be possibly surprising that our marine seaweeds, inhabiting our oceans, also have a limited salt tolerance [14] and also have to cope with salt stress. Major reason is that the enzymes of seaweeds only can operate under hypohaline conditions [salinity ≈ 6‰ - 12‰], see [15] Material & Methods]. Via several desalination mechanisms they succeed to survive in the harsh euhaline oceanic environment. Consequently, seaweeds in the harsh euhaline oceans must undergo osmotic adjustment involving, in part, the localization of toxic ions (typically Na^+ and Cl^-) into vacuoles away from important metabolic processes located within the cytoplasm, as described for terrestrial plants exposed to salinity stress [12]. Unlike Na^+ which can adversely affect both catabolism and metabolism, K^+ is essential for maintaining osmotic balance, and
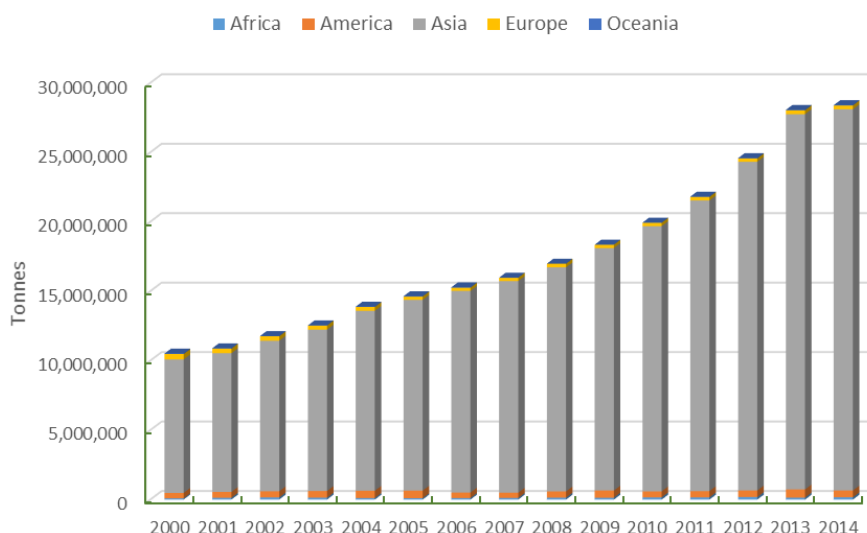
Figure 1. This graph shows the cumulative annual world aquatic plant production (≈mainly seaweeds) (in tonnes) from FAO data (2014) over the past decade. Almost 95% of the global production is currently from Asia (Courtesy: Elisa Capuzzo, gov.uk, Marine Science).

supporting biological reactions as co-factors for numerous vital enzymes. Therefore, the uptake and assimilation of K^+ are imperative for overall plant health and growth. However, Na^+ competes with K^+ for intracellular influx, such that many K^+ transport systems tend to also have high affinities for Na^+ and thus function as Na^+/K^+ symporters [16]. Therefore, relatively high environmental Na^+ levels can influence K^+ influx efficiencies in marine plants. A second adaptation mechanism in order to avoid salinity stress is via an ATP-driven Na^+/K^+ pump. This actively Na^+/K^+ pump has two functions: 1) Na^+ sequestering in vacuoles in the seaweed itself in cell-to-cell interaction. 2) In support of Na^+ accumulation in intracellular vacuoles the pump also actively serves to excrete sodium to the oceanic environment in exchange for an osmolyticum acting compound in order to maintain cellular homeostasis. For both mechanisms in seaweeds, H^+ ATPase driven pumps mediate the translocation of H^+ and K^+/Na^+. It is assumed the *milieu interior* of a seaweed with contains clearly visible the vacuole for sequestering abundant sodium ions and/or abundant metallic cations (Heavy Metals) which in some seaweed species can be taken up from the oceanic environment in order to act as "substitute-osmolyticum" to compensate Na^+-extrusion. Based on this model the vacuole is distinctly separated from chloroplast and mitochondrion essential biochemical pathways and vital enzyme systems and also the nucleus with its vulnerable DNA related processes. Third, as compensation mechanism to sodium extrusion seaweeds also can take up remarkably high K^+ levels from the oceanic environment acting in some seaweeds as the major of osmolyticum. Supportive evidence for this statement is given in Table 1 where can see that the mean [$Na^+:K^+$] ration is around 1.25 which suggest that remarkable high amounts of K^+ are taken up from the oceanic

Table 1. Sodium and potassium levels (mg·g^{-1} dry weight) observed in different seaweeds. [#]: Definition according to [15].

Seaweed Species	Na$^+$ (mg·g^{-1})	K$^+$ (mg·g^{-1})	[Na$^+$: K$^+$]	Tissue type	Salinity (euhaline[#])	Reference
Chondrus crispus	42.7	31.8	2.28	Whole plant	Seawater	[18]
Fucus vesiculosus	54.7	43.2	2.15	Whole Plant	Seawater	[18]
Laminaria digitata	38.2	115.8	0.56	Whole plant	Seawater	[18]
Porphyra tenera	36.3	35.0	1.77	Whole plant	Seawater	[18]
Sargassum mangarevense	14.6	70.2	0.35	Whole plant	Seawater	[19]
Turbinaria ornate	19.3	112.2	0.29	Whole plant	Seawater	[19]
Undaria pinnatifida	70.6	86.9	1.38	Whole plant	Seawater	[18]
Mean ± STD	**39.5 ± 7.3**	**70.7 ± 13.4**	**1.25 ± 0.32**			

environment as kind of osmolyticum [Note: the Na$^+$ & K$^+$ composition of seawater is respectively 19.3 and 0.4 mg·ml^{-1} at 35 psu respectively which gives an [Na$^+$:K$^+$] ration of ≈48.25 [17].

In the review article of [20] for terrestrial plants the four ions involved in active ATP-ase dependent plasma membrane transport mechanism are given. These are given H$_3$O$^+$, Na$^+$, K$^+$, and Ca^{2+}. This is the first report that mentions major differences in [Na$^+$: K$^+$] molar ration among seaweed species within their cytoplasma. For seaweeds until now only an ATP-driven [Na$^+$:K$^+$] pump has been described [21] [22]. The major transport proteins/channels are: 1) Sodium-Potassium pump, 2) antiporters, 3) channels, and 4) symporters who described these mechanisms for terrestrial plant [20]. If an organism uses a chemical faster than it can be delivered through this layer, it will become physically limited. Similarly, if an organism excretes a metabolic by-product into this Diffusion Boundary Layer (DBL) faster than it can diffuse out, then an elevated concentration of that bi-product will occur in the water next to the tissue surface. In this research manuscript we described the mechanism(s) of salt extrusion and coping with salt stress of seaweeds and some of the plant physiological mechanisms to remain in ionic homeostasis. Basic principle of this whole Na$^+$ extrusion mechanism of seaweeds is that in order to prevent salt damage at seaweeds -considering the fact that their enzymes can only operate under hypohaline conditions (salinity ≈ 6‰ - 12‰; Table 3 [15]). We hypothesize all kinds of compensation mechanisms needs to be (partly) operative to remain osmotic homeostasis in the seaweed cell. Various intracellular and extracellular compounds might play a role in the Na$^+$ extrusion mechanisms which are of essence so that the seaweed enzymes can operate under brackish intracellular seaweed cell conditions. Theoretically, kinds of mechanisms can serve as counteracting mechanism so that the seaweed cell after sodium extrusion remains osmotically in equilibrium [23]. However, it is not an overall compensation mechanism. Because around 10,000 seaweed species inhabit our oceans we hypothesize differ-

ent mechanisms can be expected, dependent on the seaweed species. In this manuscript we will outline some of these mechanisms.

2. Material & Methods

Seaweeds:

- *Ulva lactuca* (Chlorophyta): Katse Heule, Eastern Scheldt, The Netherlands; approximate coordinates: 51˚32'30 N and 3˚52'E.
- *Caulerpa sertularioides* (Chlorophyta): purchased by Burgers' Zoo, Arnhem, (the Netherlands) Origin: Denpassar, Bali, Indonesia: approximate coordinates: 8˚41'S and 115˚17'E.
- *Caulerpa cf. brachypus* (Chlorophyta): was obtained from "De Jong Marine-life", Spijk, (The Netherlands) Origin: Havanna, Cuba: approximate coordinates: 23˚50'S and 82˚50'W.
- *Undaria pinnatifidia* (Wakame) (Phaeocophycea): Kilcar, West Donegal, Ireland, approximate coordinates: 54˚37'N and 8˚37'W.

While seaweeds were collected, a water sample of the surrounding oceanic water was sampled at the same time stored at −80˚C pending analysis.

Sampling of four seaweeds including surrounding oceanic water was performed during the months June-September in the year 2016.

Dry weight seaweeds:

After collection, the seaweeds were brought as soon as possible to the laboratory. Most epiphytic material was removed; the seaweeds were rinsed quickly with freshwater, air-dried, oven-dried (one night at 60˚C and one night at 105˚C), weighed and the dry matter content calculated.

Experimental set up:

In this experiment we determined for four seaweed species under mechanical pressure until 10 barr (see further) the percentage of moisture weight. Also the dry weight of the seaweeds was determined in an oven overnight (see above) In the freshly collected seaweed moisture we determined directly the salinity (in ‰) and the osmolarity (EC-value) expressed in [mS/cm]. Also the pH of oceanic water and seaweed moisture were determined with a PHH-7011 pH meter with automatic temperature compensation (Omega, the Netherlands) Thereafter the obtained seaweed moisture was immediately stored at −80˚C pending analysis of micro- & macro-elements. Microelements ≈ heavy-metals = [HM], (Al, As, Cd, Co, Cr, Cu, Mo, Ni, Pb & Zn) and macro-elements (Ca, Fe, K, Mg, Mn, Na, P, & S) were measured by Inductively Coupled Plasma Spectroscopy (ICP) techniques at the central Chemical Biological Laboratory for Soil & Water Research at Wageningen University (details see further) The earlier mentioned simultaneously with the seaweeds sampled oceanic water was directly deep frozen but now in conjunction with the samples of the seaweeds in a similar way ICP analyzed. This approach justifies a simultaneously comparison of [micro-] & [macroelements] of both compartments (seaweed moisture *versus* oceanic water). From these laboratory measurements we calculated parameters which

might be important in elucidating the complex sodium extrusion mechanism of the four investigated seaweeds.

Mechanical pressure procedure: To be able to produce press moisture from the seaweeds, the plant material (300 - 1000 g) was first pulped using a laboratory homogenizer (manufacturer: Foss Tecator, type: Tecator 1094 Homogenizer,), with either a smooth or a serrated knife, at s speed of 1500 rpm or 3000 rpm. Moisture was pressed out of the pulp, approximately 100 g of pulp was used, using a LLOYD INSTRUMENTS (type: LR30K) testing machine that was fitted with a specially constructed unit for pressing pulps at a maximum pressure of 60 bar. Final weight of press cake and press moisture was determined. Afterwards press cake and samples of the obtained seaweed moisture of the four different seaweed species (n = 4 per seaweed species) were immediately stored at −80˚C pending analyses.

Determination of micro- and macro-elements by Inductively Coupled Plasma spectroscopy (ICP-techniques):

1) Al, As, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, S and Zn in seaweed moisture and in the sample of the surrounding waters were measured on an ICP-AES (Thermo Iris) according pretreatment SWV E-3404, measurement SWV E-1304 and conservation SWV E-3404 guide lines at the Chemical Biological Laboratory for Soil & Water Research, Wageningen University, Wageningen (The Netherlands).

2) As, B, Ba, Cd, Co, Cr, Cu, La, Li, Mn, Mo, Ni, Pb, Sb, Se, Sn, and V in seaweed moisture and in the sample of the surrounding waters were measured on an HR-ICP-MS (Thermo Element 2) according pretreatment SWV E-3404, measurement SWV E-1325 and conservation SWV E-3404 guidelines at the same laboratory.

Salinity & pH measurements: Salinity of the seaweed moisture and oceanic water were determined using an EC meter (manufacturer: WTW; type Cond 315i/SET) fitted with a conductivity cell (manufacturer: WTW; type: TetraCon 325, cell constant 0.475 cm^{-1}). The pH of seaweed moisture and oceanic water were determined with a PHH-7011 pH meter with automatic temperature compensation (Omega, the Netherlands).

Classification salinity:

Classification in a certain salt range and terminology will be based on the ([15], Table 2) while classification of water sources to define the term "salinity" and elucidate words like Fresh, Brackish and Sea water salinity with TDS ≈ "total dissolved solids" expressed in milligrams per liter (mg/l) ([24], Table 3).

N-total and P-total measurements: N-total and N-NH_4 measurement were performed on a Segmented Flow Analyzer (SFA) apparatus according to SWV E1417 guide lines at the Chemical Biological Laboratory for Soil Research, Wageningen University, Wageningen (The Netherlands) Determination of P3 was performed on an HR-ICP-MS (Thermo Element-2) according pretreatment SWV E-3404, measurement SWV E-1325 and conservation SWV E-3404 guide lines at the same laboratory.

Table 2. The Venice system gives specific words for salt concentration in linked to the corresponding salinity range (in ‰) [15].

Zone	Salinity range (in ‰)
Limnetic	<0.5
β-Oligohaline	0.5 - 3
α-Oligohaline	3 - 5
β-Mesohaline	5 - 10
α-Mesohaline	10 - 18
Polymixohaline	18 - 30
Euhaline	30 - 40
Hyperhaline	>40

Table 3. Classification of water sources to define the term "salinity" and elucidate words like Fresh, Brackish and Sea water salinity with TDS ≈ "total dissolved solids" expressed in milligrams per liter (mg/l) ([24]).

Salinity content water	mg/l TDS	Definition	Salinity in ‰
Drinking water	500	Consumption water	0.5‰
Fresh water	Less than 1000	Fresh water	=1‰
Slightly saline	1000 to 3000	Brackish water	1‰ - 3‰
Moderately saline	3000 to 10,000	Brackish water	3‰ - 10‰
Highly saline	Over 10,000	Brackish water	>10‰
Oceanic water	35,000	Marine water	35‰

Calculations:

1) $pH = -\log_{10} H^+ \Leftrightarrow \left[H^+ \right] = 10^{-pH} \left[mol/l \right]$ [25].

pH-value and from this value calculated according to 10^{pH} the amount of H^+-ions in (mol/l) was calculated [25].

2) $\text{Accumulation factor} = \dfrac{\text{Heavy-metal} \left(HM \right) \text{ in the seaweed moisture}}{\text{Heavy-metal} \left(HM \right) \text{ in the oceanic water}}$

3) $Na^+ / K^+ \text{ ratio} = Na^+ \left(mg/l \right) / K^+ \left(mg/l \right)$

Weights and Volumes:

The following weights and volumes were obtained from the overnight oven-dried seaweed material and the 10 bar mechanical pressed fresh seaweed biomass:

*Oven-dried:

4) $\text{Dry Weight} \left[g \right] = \dfrac{\text{W-tray oven dried material} \left[g \right] - \text{W-empty tray} \left[g \right]}{\text{W-tray with fresh material} \left[g \right] - \text{W-empty tray} \left[g \right]}$

*Mechanical Pressure:

5) Total Moisture Weight [g] = (Weight pressed moisture [g]) + (Losses moisture in machine [g]).

6) With: Losses moisture in the machine = (W_{pulp} [g] − $W_{press\ moisture}$ [g] − $W_{press\ cake}$

[g]).

7) With: W = Weight in gram [g].

*Vacuole size cell:

Based on the following three assumptions that:

1) The whole seaweed cell vacuole is 100 % filled with seaweed moisture (see Figure 2 left image);

2) At the applied 10 bar mechanical pressure procedure the whole volume of the seaweed cell vacuole will be squeezed empty;

3) 1 ml seaweed moisture will weight 1 gram and has with a corresponding specific gravity of water a volume of one cubic cm.

We will conclude that the calculated "Total Moisture Weight" in (g) will correspond to the volume of the seaweed cell vacuole.

3. Results

In Figure 2 the vacuole size for sequestering Na^+ and to some extent abundant metallic cations (HM) spatially separated from vital biochemical and physiological essential pathways. An impression per seaweed species for its water storage capacity in the vacuoles can be acquired from the [Wet-Weight/Dry-Weight] ration or [WW/DW]-ration. A high [WW/DW]-ration is a reflection of larger vacuoles for storage and the presence of a higher water content in the cell wall. The WW/DW ratio of the four seaweed-species used in this study is given in Table 4. The lowest WW/DW ratios were found for *Ulva lactuca* and *Undaria pinnatifidia* of respectively 4.95 ± 0.11 and 2.36 ± 0.74. The low WW/DW rations for both species are a reflection of a large vacuole capacity and thus of a high seaweed moisture storage capacity. For the two *Caulerpa spp.* the WW/DW ratios were 10 - 20 fold higher in the range 50 - 60.

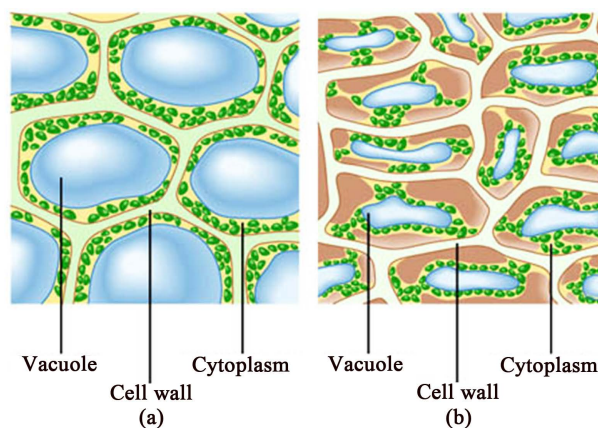

Figure 2. Example how vacuoles within the seaweed cell can contain large amount of (brackish) seaweed moisture. (a): Example of "healthy" cells of fresh seaweed biomass we used in our mechanical 10 bar laboratory pressure in order to obtain seaweed moisture. (b): Theoretical option for a non-salt adapted "unhealthy" seaweed-cells which are partly desiccated. (Source: courtesy Evert de Vries).

Table 4. Some characteristics for the at 10 bar mechanical pressed seaweed moisture for four seaweed species, *Ulva lactuca, Caulerpa sertlatiodes* and *Caulerpa cf. brachypus* and the brown seaweed *Undaria pinnatifidia* found at different locations like: salinity, nutrients like N & P, and pH-value and from this value calculated according to 10^{pH} the amount of H^+-ions in (mol/l) was calculated [25]; mean ± STD of n = 4 samples per seaweed species. B.D.L. = below detection limit ≈ 0.03 mg/l. Definitions for salinity are: marine water: 3500 mg/l TDS; highly saline (brackish): over 10,000 mg/l TDS; moderately saline (brackish): 3000 to 10,000 mg/l TDS; slightly saline (brackish): 1000 To 3000 mg/l TDS; fresh water: less than 1000 mg/l TDS; drinking water 500 mg/l TDS (source: [24]). Σ[HM] Is total sum of metallic cations (≈ Heavy metals) in seaweed moisture.

Seaweed spp.	Lab No	Calculated Dry matter. DW in (g)	Calcul. Total moist. WW in (g)	WW/DW	lab salinity prom.	lab EC mS/cm	lab pH	Lab Na+ mg/l	Seaweed Σ[HM] mg/l	Ocean Σ[HM] mg/l	Lab P-tot mg/l
Ulva	Zs1	9.37	47.19	5.04	9.60	14.77	5.08	40.90	94.20	420.00	88.90
lact.	Zs2	9.56	48.29	5.05	9.40	14.47	5.30	45.80	93.40	432.00	91.40
NL	Zs3	9.36	45.52	4.86	9.40	14.38	4.94	42.70	90.60	360.00	83.10
	Zs4	9.24	44.80	4.85	9.60	13.68	4.93	42.90	87.60	359.00	85.30
	Mean	9.38	46.45	4.95	9.50	14.33	5.06	43.08	91.45	392.75	87.18
	Std.	0.14	1.58	0.11	0.12	0.46	0.17	2.03	2.99	38.71	3.69
Caul.	Cs1	1.39	71.94	51.94	19.10	27.80	4.17	4.81	37.30	362.00	45.00
sert.	Cs2	1.29	70.17	54.40	19.50	28.30	4.20	4.61	38.20	344.00	45.00
Bali	Cs3	1.25	86.86	69.66	19.50	28.30	4.20	4.62	37.60	348.00	44.80
	Cs4	1.21	76.32	62.92	19.30	28.20	4.20	4.71	37.50	351.00	45.20
	Mean	1.28	76.32	59.73	19.35	28.15	4.19	4.69	37.65	351.25	45.00
	Std.	0.07	7.49	8.12	0.19	0.24	0.02	0.09	0.39	7.72	0.16
Caul.	Ci-1	1.19	50.23	42.39	20.50	29.70	4.51	14.40	369.00	922.00	91.40
brach.	Ci-2	1.25	52.05	41.54	20.60	29.70	4.51	14.50	374.00	917.00	92.80
Cuba	Ci-3	1.13	57.47	50.81	20.70	29.90	4.50	14.60	377.00	920.00	92.50
	Ci-4	1.17	53.25	45.40	20.50	29.80	4.51	14.70	374.00	917.00	93.40
	Mean	1.19	53.25	45.03	20.58	29.78	4.51	14.55	373.50	919.00	92.53
	Std.	0.05	3.08	4.19	0.10	0.10	0.00	0.13	3.32	2.45	0.84
Undar	Wa1	28.41	39.88	1.40	9.60	n.d.	6.53	14.60	0.00	123.00	17.80
Pinnat	Wa2	28.74	62.70	2.18	9.80	n.d.	6.44	10.10	0.00	96.60	12.60
IR	Wa3	27.22	83.03	3.05	9.80	n.d.	6.50	8.27	0.00	91.20	11.60
	Wa4	28.15	79.29	2.82	5.90	n.d.	6.57	5.92	0.00	83.10	10.60
	Mean	28.13	66.22	2.36	8.78	n.d.	6.51	9.72	0.00	98.48	13.15
	Std.	0.65	19.66	0.74	1.92	n.d.	0.05	3.67	0.00	17.27	3.21

In **Table 5** is the ability given to excrete sodium to the oceanic environment via active ATP-driven pumps like the Na^+/K^+-pump or solely an active Na^+ extrusion pump.

When considering the composition of Na^+ and K^+ in seawater (19.3 mg·ml^{-1} and 0.4 mg·ml^{-1} at 35 psu, respectively gives in this case an Na^+/K^+ ratio of 48.25 seawater of [17]. In our studies for the Netherlands, Ireland, Cuba and Bali the Na^+/K^+ ratio was in the range 26.1 - 33.3 (Indonesia *vs.* Ireland **Table 5**) It is

Table 5. Sodium and Potassium concentrations determined by Oceanic location. Left: Concentrations of the sodium and potassium ions in the oceanic water at the sampling location [in: (mg/l)] and its ration. Right: Concentrations of the sodium and potassium ions in the four seaweed-species at the sampling location [in: (mg/l)] and its ration. Values given in the Table are the values of one water sample and the mean value of one four seaweed 10 bar pressed subsamples.

Location Ocean	Na^+ (mean)	K^+ (mean)	Na^+/K^+ (mean)	Seaweed species	Na^+ (mean)	K^+ (mean)	Na^+/K^+ (mean)
Netherlands	9983	321	31.1	*Ulva lactuca*	1363.0	1205.0	1.13
Indonesia	10020	384	26.1	*Caulerpa sert.*	6238.8	481.0	12.97
Cuba	10840	361	30.0	*Caulerpa brach.*	6678.5	675.5	9.89
Ireland	18230	547	33.3	*Undaria pinnat.*	3147.0	121.3	25.94

clear that mechanisms must be in place to minimize cytosolic Na^+ accumulation while permitting necessary K^+ uptake. The dissimilarity between Na^+ and K^+ concentrations in seawater at all four oceanic locations is ≈30 with of course $[K^+]$ ≈ 30 fold lower. The $[Na^+:K^+]$ molar ratio of our seaweeds was the lowest in the seaweed *Ulva lactuca* ≈ 1.13 which is indicative that K^+ is in this green seaweed species acting as an important osmolyticum. The two *Caulerpa spp*. Are in the intermediate range of around ≈11.5 (range 9.9 - 13.0), while in the brown seaweed *Undaria pinnatifidia* this value is around 26. For the latter species this implies that K^+ has no important role as major osmolyticum and give strong evidence that this brown seaweed is able to produce biochemically its own osmolyticum. In general, brown seaweeds are able to produce a large variation of economically important osmolyticuma. In general, these by brown seaweeds produced osmolyticuma are or from the carbohydrate fraction or phycocolloid fraction. Although we didn't measure any osmolyticum of one of these fractions in *Undaria pinnatifidia* this extremely high $[Na^+:K^+]$ molar ratio of ≈26 gives strongly evidence that this species is able to produce its own osmolyticum.

The moisture of seaweeds has a rather low salinity (brackish) in order of descending salinity (mean ± std (n = 4) in ‰): *Caulerpa cf. brachypus* ≈ 20.58 ± 0.096 (Polymixohaline); *Caulerpa sertularioides* ≈ 19.30 ± 0.163 (Polymixohaline); *Ulva lactuca* ≈ 9.501 ± 0.115 (*β*-Mesohaline) and *Undaria pinnatifidia* ≈ 9.500 ± 0.115 (*β*-Mesohaline) [15].

Ulva lactuca had the highest P-content (69.7 mg/l), *Undaria pinnatifidia* the lowest (13.2 mg/l) while both *Caulerpa spp*. had an intermediate position. $N-NH_4$ had the highest value in *Ulva lactuca* (34.5 mg/l) and the lowest in *Caulerpa sertlatioides* (4.7 mg/l) Interestingly Nts was the highest in *Caulerpa cf. brachypus* (298.8 mg/l) while was surprisingly zero in *Undaria pinnatifidi* (Tables 6-8).

4. Discussion

Excess salt is toxic for terrestrial plants but also for seaweeds living in the euhalien [15] oceanic environment. Seaweeds can employ a number of mechanisms

Table 6. Macro-elements [MaE]: (cations & anions) in seaweed moisture of four seaweed species [per species mean ± SD, n = 4), in a water sample (n = 1) of the ocean where they were collected and the seaweed/ocean ration for that macro-element. A ratio > 1 for a specific [MaE] is indicative for a higher concentration in the seaweed moisture in comparison to the oceanic water so it can be sequestered (active or passive). Specific details are described in the M & M section.

Sample nr.	Seaweed Species	Ca²⁺ [mg/l]	Fe²⁺ [mg/l]	K⁺ [mg/l]	Mg²⁺ [mg/l]	Mn²⁺ [mg/l]	Na⁺ [mg/l]	SUM MACRO CATIONS	P³⁻ [mg/l]	S²⁻ [mg/l]	SUM MACRO ANIONS
	Threshold	1.20	0.09	0.40	0.15	0.01	0.30		0.10	0.20	
1)	*Ulva lactuca*	552.50	2.74	1205.00	1762.00	3.77	1363.00	4889.00	87.18	5493.25	5580.43
		20.70	0.40	34.29	47.85	0.16	26.26	121.42	3.69	87.55	86.47
2)	*Caulerpa sertlatiodes*	530.00	1.08	481.00	509.25	1.18	6238.75	7761.26	45.00	581.75	626.75
		25.47	0.04	5.35	9.03	0.03	92.33	115.76	0.16	155.56	155.56
3)	*Caulerpa cf. brach.*	440.00	0.90	675.50	776.00	2.05	6678.50	8572.95	92.53	1376.50	1469.03
		3.27	0.14	4.12	12.25	0.07	75.12	84.28	0.84	79.35	80.13
4)	*Undaria pinnatifidia*	110.50	0.25	121.25	327.50	0.02	3147.00	3706.52	13.15	345.50	358.65
		1.00	0.03	5.32	8.66	0.01	32.32	37.79	3.21	7.19	8.67

Sample nr.	Four Oceans	Ca²⁺ [mg/l]	Fe²⁺ [mg/l]	K⁺ [mg/l]	Mg²⁺ [mg/l]	Mn²⁺ [mg/l]	Na⁺ [mg/l]	SUM MACRO CATIONS	P³⁻ [mg/l]	S²⁻ [mg/l]	SUM MACRO ANIONS
	Threshold	1.20	0.09	0.40	0.15	0.01	0.30		0.10	0.20	
1).	Netherlands	291.00	0.09	321.00	1214.00	0.01	9983.00	11809.10	0.10	933.00	933.10
2).	Indonesia	313.00	0.09	384.00	1206.00	0.01	10020.00	11923.10	0.02	866.00	866.02
3).	Cuba	334.00	0.09	361.00	1336.00	0.01	10840.00	12871.10	0.79	983.00	983.79
4).	Ireland	473.00	0.09	547.00	2212.00	0.01	18230.00	21462.10	0.01	1683.00	1683.01
Mean		352.75	0.09	403.25	1492.00	0.01	12268.25	14516.35	0.23	1116.25	1116.48
Std		82.07	0.00	99.31	483.67	0.00	3994.14	4654.91	0.38	380.86	380.75

Sample nr.	Ratio seaweed/ocean	Ca²⁺ [mg/l]	Fe²⁺ [mg/l]	K⁺ [mg/l]	Mg²⁺ [mg/l]	Mn²⁺ [mg/l]	Na⁺ [mg/l]	SUM MACRO CATIONS	P³⁻ [mg/l]	S²⁻ [mg/l]	SUM MACRO ANIONS
	Threshold	1.20	0.09	0.40	0.15	0.01	0.30		0.10	0.20	
1)	Ulva/ocean	1.90	30.39	3.75	1.45	376.75	0.14	414.38	871.75	5.89	877.64
		0.07	4.48	0.11	0.04	16.07	0.00	14.50	36.94	0.09	36.92
2)	Caulerpa sert./ocean	0.35	2.73	0.32	0.27	2.05	0.31	6.04	657.50	0.40	657.90
		0.00	0.35	0.01	0.01	0.58	0.00	0.79	160.29	0.01	160.29
3)	Caulerpa brach./ocean	0.87	1.00	0.89	0.91	1.00	0.92	5.59	0.13	0.95	1.08
		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4)	Undaria/ocean	0.66	1.00	0.70	0.55	1.00	0.55	4.46	2.00	0.51	2.51
		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 7. Microelements [MiE] (cations) in seaweed moisture of four seaweed species [per species mean ± SD, n = 4), in a water sample (n = 1) of the ocean where they were collected and the seaweed/ocean ration for that microelement. A ratio > 1 for a specific [MiE] is indicative for a higher concentration in the seaweed moisture in comparison to the oceanic water so it can be sequestered (active or passive). For more specific details see the M & M section.

Sample nr.	Seaweed Species	Cu²⁺ [µg/l]	Al³⁺ [µg/l]	Zn²⁺ [µg/l]	Cd²⁺ [µg/l]	Co²⁺ [µg/l]	Cr³⁺ [µg/l]	Mo⁴⁺ [µg/l]	Ni²⁺ [µg/l]	Pb²⁺ [µg/l]	SUM MICRO CATIONS
	Threshold	0.10	0.30	0.30	0.01	0.01	0.02	0.18	0.03	0.04	
1)	*Ulva lactuca*	330.75	153.00	1232.25	2.39	13.88	18.65	14.33	157.50	1.26	1924.00
		74.29	20.99	236.99	0.34	0.33	0.90	1.00	9.68	1.66	271.67
2)	*Caulerpa sertlaiodes*	117.85	582.75	1169.50	5.72	6.99	14.50	6.99	129.75	2.45	2036.49
		13.23	175.55	361.52	0.47	0.21	0.39	4.61	5.32	1.53	341.40
3)	*Caulerpa cf. brach.*	788.25	230.00	1136.00	4.43	22.13	20.23	8.54	295.25	1.33	2506.15
		69.21	46.45	146.50	0.22	0.22	1.25	0.35	9.36	0.95	257.38
4)	*Undaria pinnatifidia*	73.90	156.00	178.75	0.81	1.04	6.16	14.80	20.08	0.09	451.63
		12.78	9.93	18.45	0.13	0.12	0.27	3.00	3.89	0.15	44.40
Sample nr.	Four Oceans	Cu²⁺ [µg/l]	Al³⁺ [µg/l]	Zn²⁺ [µg/l]	Cd²⁺ [µg/l]	Co²⁺ [µg/l]	Cr³⁺ [µg/l]	Mo⁴⁺ [µg/l]	Ni²⁺ [µg/l]	Pb²⁺ [µg/l]	SUM MICRO CATIONS
	Threshold	0.10	0.30	0.30	0.01	0.01	0.02	0.18	0.03	0.04	
1)	Netherlands	0.13	0.30	0.30	0.01	0.01	0.01	2.07	0.03	0.04	2.90
2)	Indonesia	0.02	0.70	0.30	0.00	0.01	0.01	1.14	0.36	0.04	2.58
3)	Cuba	0.10	1.00	0.30	0.02	0.01	0.05	2.49	0.16	0.04	4.17
4)	Ireland	0.10	0.30	0.30	0.00	0.01	0.00	1.11	0.03	0.04	1.89
Mean		0.09	0.58	0.30	0.01	0.01	0.02	1.70	0.15	0.04	2.88
Std		0.05	0.34	0.00	0.01	0.00	0.02	0.69	0.16	0.00	0.95
Sample nr.	Ratio seaweed/ocean	Cu²⁺ [µg/l]	Al³⁺ [µg/l]	Zn²⁺ [µg/l]	Cd²⁺ [µg/l]	Co²⁺ [µg/l]	Cr³⁺ [µg/l]	Mo⁴⁺ [µg/l]	Ni²⁺ [µg/l]	Pb²⁺ [µg/l]	SUM MICRO CATIONS
	Threshold	0.10	0.30	0.30	0.01	0.01	0.02	0.18	0.03	0.04	
1)	Ulva/ocean	2544.23	510.00	4107.50	199.17	1982.14	1865.00	6.92	5250.00	31.38	16496.34
		571.49	69.97	789.95	28.57	47.20	90.37	0.49	322.61	41.40	1281.90
2)	Caulerpa sert./ocean	3695.00	222.86	595.83	403.75	207.50	684.72	12.98	55.76	2.31	5880.72
		639.07	14.19	61.49	65.24	23.69	29.74	2.63	10.81	3.84	826.12
3)	Caulerpa brach./ocean	1.30	0.30	1.00	0.60	1.17	0.20	0.83	0.19	1.00	6.58
		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4)	Undaria/ocean	0.20	2.33	1.00	0.50	1.00	2.25	1.03	12.00	1.00	21.31
		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 8. pH & H⁺-ions in [mol/l] with pH calculated according to [25]; pH= −log [H⁺]; nutrients (N & P) like in seaweed moisture of four seaweed species [per species mean ± SD, n = 4], in a water sample (n = 1) of the local ocean and the seaweed/ocean ration for that microelement. The same remark for a ratio > 1 for a specific element/compound. For more details see M & M section.

Sample nr.	Seaweed Species	H⁺ Ions	pH	P³⁻ [mg/l]	N-NH4 [mg/l]	(NO₃ + NO₂) [mg/l]	N-total [mg/l]	Sum nutrient (N & P)
	Threshold	[mol/l]	X	0.10	0.04	0.03	0.30	
1)	*Ulva lactuca*	0.64	5.06	0.00	0.00	0.01	0.20	0.21
		0.17	0.17	0.00	0.00	0.00	0.00	0.00
2)	*Caulerpa sertlaiodes*	1.66	4.19	0.02	0.02	0.86	1.00	1.90
		0.02	0.02	0.00	0.00	0.00	0.00	0.00
3)	*Caulerpa cf. Brach.*	2.65	4.51	0.79	0.02	14.10	14.70	29.61
		0.00	0.00	0.00	0.00	0.00	0.00	0.00
4)	*Undaria pinnatifidia*	4.12	6.51	0.01	0.04	0.01	0.20	0.26
		0.07	0.05	0.00	0.00	0.00	0.00	0.00
Sample nr.	Four Oceans	H⁺ Ions	pH	P³⁻ [mg/l]	N-NH₄ [mg/l]	(NO₃ + NO₂) [mg/l]	N-total [mg/l]	Sum nutrient (N & P)
	Threshold	[mol/l]	X	0.10	0.04	0.03	0.30	
1)	Netherlands	0.25	8.29	0.02	0.04	0.03	0.20	0.29
2)	Indonesia	0.25	8.12	0.02	0.02	0.86	1.00	1.90
3)	Cuba	0.25	8.11	0.79	0.02	14.10	14.70	29.61
4)	Ireland	0.25	7.96	0.01	0.04	0.01	0.20	0.26
Mean		0.25	8.12	0.21	0.03	3.75	4.03	8.02
Std		0.13	0.13	0.39	0.01	6.91	7.13	14.42
Sample nr.	Ratio seaweed/ocean	H⁺ Ions	pH	P³⁻ [mg/l]	N-NH₄ [mg/l]	(NO₃ + NO₂) [mg/l]	N-total [mg/l]	Sum nutrient (N & P)
	Threshold	[mol/l]	X	0.10	0.04	0.03	0.30	
1)	Ulva/ocean	7.32	0.61	0.05	0.03	0.33	1.00	1.41
		0.02	0.02	0.00	0.00	0.00	0.00	0.00
2)	Caulerpa sert./ocean	2.25	0.80	0.50	2.00	0.01	0.20	2.71
		0.01	0.01	0.00	0.00	0.00	0.00	0.00
3)	Caulerpa brach./ocean	1.34	1.02	0.03	2.00	0.00	0.01	2.04
		0.00	0.00	0.00	0.00	0.00	0.00	0.00
4)	Undaria/ocean	1.35	1.02	2.00	0.50	86.00	5.00	93.50
		0.00	0.00	0.00	0.00	0.00	0.00	0.00

to maintain suitable ion levels by minimizing the influx of Na^+ ions into cells via Na^+-ATPase activity. In principle there are three major mechanisms a seaweed can apply in order to cope with the problem of salt stress in the euhaline oceanic environment [15].

First, Na^+ sequesters in vacuoles within the lumen of the seaweed thallus in such a safe way that this salt is spatially separated from vital plant physiological and biochemical mechanisms. A cell-to-cell channel e.g. in case of the Na^+ sequestering in special vacuoles within the lumen of the seaweed thallus with vacuoles for Na^+ storage spatially separated from vital seaweed organelles like the nucleus, chloroplast with its essential enzymes for photosynthesis and mitochondria for vital plant physiological and biochemical mechanisms. While some seaweeds appear to minimize the influx of ions into cells, ion sequestering within vacuoles is still essential in maintaining osmotic equilibrium, in support of Na^+ accumulation in vacuoles. In these plants, ATPases mediate the translocation of H^+ and K^+/Na^+, and were found to increase in number during salt stress [16] [26]. It is also possible that ATPases with a greater affinity for K^+ may be actively involved in ion influx, while a second type of ATPase transporter, with lower K^+/Na^+ selectivity, is pumping ions in the other direction [12] [16]. As a consequence that seaweeds have to live in a harsh euhaline [15] oceanic environment they must undergo osmotic adjustment involving, in part, the localization of toxic ions (typically Na^+ and Cl^-) into vacuoles and away from important metabolic processes located within the cytoplasm [12] [21]. This spatially separation is in case of the Na^+ ion based on diminishing the deleterious effects of sodium on inner membrane plant physiological parameters.

The [Wet weight/Dry weight] ratio WW/DW is a morphological parameter to make a comparison between seaweeds species possible to estimate the vacuole capacity and thus the water storage capacity. By using this mechanical pressure method until 10 bar we have to consider, we obtained seaweed moisture from two types of vacuoles: first, vacuoles from the epidermal cells with proportionally smaller vacuoles and secondly from the highly vacuolated mesophyll cells [27]. Beyond inner seaweed species differences in total vacuole and water-storage (≈seaweed moisture) capacity, within a seaweed species also the ratio between the two types of vacuoles may determine its water storage capacity. In addition, a high WW/DW ratio is a reflection of larger vacuoles for storage and the presence of a higher water content in the cell wall which may facilitate the accumulation of heavy metals (microelements) into the apparent free space by dilution [28]. Direct indications that seaweeds can cope with salt stress by Na^+ accumulation within vacuoles within the lumen of the seaweed thallus came from field data; the study of [29] was in a brown seaweed based on the salt content of the cell wall components (Dry Weight) constituted up to 40% - 47% of the dry weight of seaweed biomass. This latter observation is indicative that Na^+ ions are spatially separated in the seaweed cell from vital (enzymatic) systems.

Secondly, active or passive extrusion of Na^+ to the oceanic environment followed by finding solutions by absorption (passive or active) of several com-

pounds Heavy Metals [HM] and nutrients like N & P or the ion K^+ from the oceanic environment which can act as osmolyticum so that ionic homeostasis is maintained. Even with the disparity between Na^+ and K^+ concentrations in seawater at all four oceanic locations of ≈ 30 with of course $[K^+] \approx 30$ fold lower) our seaweeds are able to accumulate comparatively high levels of K^+ within their tissues which correspond to the values for seagrasses (Table 5) [16] [30]. These differences may reflect some general variations in physiological processes involved in salt tolerance. For example, while seaweeds accumulate relatively high Na^+ concentrations, they also concentrate remarkably high K^+ levels; resulting in a $[Na^+:K^+]$ molar ratio of 1.25 ([18], [19] see Table 1 introduction). The results of our study indicate that this is not the general case because we found in two *Caulerpa spp.* intermediate values of ≈ 11.5 (range 9.9 - 13.0), while in the brown seaweed *Undaria pinnatifidia* a $[Na^+:K^+]$ molar ratio of ≈ 26.

Third, production by several specific adapted seaweed species of economical important osmolyticum after sodium extrusion is a rather metabolically and energetically expensive method which also from a biochemical point of view much has evolved during course of evolution in some specific seaweed species. It is a much "cheaper" way simply to exchange the extruded sodium ions with an osmolyticum which is already available in the oceanic environment like Heavy Metals and nutrients like N & P. However, during course of evolution some seaweed species "has chosen" for this option, which is nowadays in gratitude explored by industry because these osmolyticum compounds have many purposes and of extremely economic importance. Osmolytica like alginates and carrageenan are mainly produced by brown seaweeds while agar is mainly produced by red seaweeds. From the carbohydrate fraction these alginates are important cell wall component in all brown seaweed spp. constituting up to 40% - 47% of the dry weight of seaweed biomass. The alginates and their oxidation products the sugar-diacides are employed by seaweeds as a sequestering mechanism for heavy metals [HM] in the seaweed moisture. From the phycocoloid fraction carrageenans are a group of biomolecules composed of linear polysaccharide chains with sulphate half-esters attached to the sugar unit. These properties allow carrageenans to dissolve in water, form highly viscous solutions and remain stable over a wide pH range. Especially the brown seaweeds *Chondrus crispus* and *Kappaphycus spp.* can contain up to 71% and 88% of carrageenan, respectively. The other osmolyticum from the phycocoloid fraction -but in contrast to carrageenans extracted from red seaweed such as *Gelidium spp.* and *Gracilaria spp.* is agar. Agar is a mixture of polysaccharides, which can be composed of agarose and agropectin, with similar structural and functional properties as carrageenans. The agar content in *Gracilaria spp.* can reach values up to 31% [31].

Salt extrusion to the oceanic environment in exchange with a certain compound like metallic cations (Heavy metals [HM]) which serve as kind of osmolyticum to maintain cell integrity [18] [19], see also Table 2.

A third adaptation mechanism in some specific seaweeds (mainly red & brown) to salt extrusion is the production of its own osmolyticum. The several

produced osmolytical compounds are in an extensive detailed manner mentioned by [14]. She grouped the several compounds as follows: First, products of the photosynthesis (polyols and amino acids) Secondly, several solutes derived from the quaternary type ammonium compounds and tertiary sulphonium compouds (see further discussion: salt tolerance of *Ulva lactuca* and ROS scavening DMSP) Third, osmolytica from the carbohydrate fraction of seaweeds consisting mainly out of: alginates, laminarine and fucoidine [14]. In addition, osmolytica from the phycocolloids fraction are mentioned with two economical important major groups: carrageenan and agar (reviewed: [31]).

The permeability of biological membranes is highly selective. The flow of molecules and ions between a cell and its environment is regulated by specific transport systems which will be exemplified under **A: Active** and **B: Passive**. These transport systems have several important roles: 1) They regulate cell volume and maintain the intracellular pH and ionic composition within a narrow range to provide a favorable environment for enzyme activity; 2) The molecular mechanism of many transport processes is a very actual research area. With respect to seaweeds and other marine plants it is a nearly unexplored research area [32] [33].

A: Active Of all transport mechanisms over the cell membrane the ATP driven Na^+/K^+ is the best described. For seaweeds in high saline environments it is believed that Na^+ can cross the plasma membrane using the same transport systems developed for K^+ [21] [22] [34] There are a number of ions and channels involved for on transport processes used in plants to achieve osmotic homeostasis. The primary ions involved in osmotic adjustment are (Ca^{2+}, Cl^-, H^+, K^+, and Na^+) These ion levels in the cytoplasm must be must be carefully controlled to prevent metabolic disruptions [16] [30]. The efflux of cytosolic Na^+ (either outside the cell or into a vacuole) may be accomplished through electroneutral Na^+/H^+ antiporters that depend on energy from H^+-ATPases along the plasmalemma or tonoplast [16] [23]. However, because of thermodynamic restrictions, Na^+/H^+ antiporters cannot effectively transport Na^+ outside the cell when the pH of the external medium is relatively high like in seawater [25], while alternative transport systems have been described in other organisms, such as Na^+-ATPase in seaweeds [16].

This pump has two purposes:

B: Passive Many transport processes are not directly driven by the hydrolysis of ATP. Instead, they are coupled to the flow of an anion down its electrochemical gradient. An example is facilitated diffusion without any ATP costs. Overall, theoretically several mechanisms for transport of ions over cell membranes are possible. These are transporters (or carrier) proteins which can move a single type of molecule in one direction across the cell membrane (a uniporter), several different molecules in one direction (a symporter) or different molecules in opposite directions (an antiporter) [33]. Transporters can allow the movement of molecules down chemical concentration gradients (facilitated diffusion), when the energy required for conformational changes in the transporter protein is

provided by the concentration gradient rather than by metabolic activity [33].

This probably can be ascribed to proton transport across the plasma membrane and tonoplast driven by electrochemical gradients produced thus ATP-driven by H^+ pumps ratio: [H^+ seaweed]/[H^+ ocean] [16] [35] [36] [37]. This secondary transport will assist in the uptake of protons from the oceanic environment inwards the seaweed cell with its vacuole. The development and maintenance of H^+ electrochemical gradients are achieved through H^+-ATPases in the plasma membrane and H^+-ATPases and H^+pyrophosphatases in the tonoplast [23]. While H^+-pyrophosphatases are important in the transport of H^+ into the vacuole, its role in salt tolerance is unclear. It is believed that the primary physiological responsibilities of H^+-pyrophosphatase are to maintain cytosolic pH and to regulate pyrophosphate turnover [16] [23] [35].

From all four investigated seaweeds *Ulva lactuca* has probably the highest tolerance for salt stress. From the study of [38] it became clear that good growth of germlings and young blades of all studies five Ulva sp. in the Netherlands occurred in a wide variety of salinity (mainly 17‰ - 34‰), and didn't show a distinct correlation with differences in salinity the adult plants were exposed to at their natural habitats [38]. In general it can be stated that the *Ulvales* poses an extreme salt tolerance covering a wide range/spectrum 17‰ - 34‰ [39]. *U. lactuca* adapts to salinity with changes in K^+, Na^+ and Cl^-. Hypo-osmotic stress decreased the tissue concentration of K^+, Na^+ and Cl^- while hyper-osmotic stress caused a transient increase in Na^+ and a stable accumulation of K^+ and Cl^- [40]. Also β-dimethylsulphoniopropionate (DMSO)-previously reported in the literature with β-dimethylpropiothetin is important in osmotic adaptation of *U. lactuca* [40]. This sulphonium compound may well cause the characteristic sulphuric smell that accompanies *Ulva* blooms at the beaches in summer time like in Brittany [41]. In addition, because of this salinity tolerance of *Ulva*, with intra-cellular concentrations of K^+ 20-fold higher than in seawater and an intra-cellular ratio between Na^+ and K^+ lower than in human metabolic waste, it has been suggested that *Ulva* sp. is a good candidate species for space agriculture, recycling human material [42]. This extreme salinity tolerance of *Ulva* spp. (*U. fasciata*) can also possibly be ascribed to the highly effective antioxidant defense mechanism against salinity stress [43]. During salinity stress of *Ulva* spp., free oxygen radicals arise during metabolism in the mitochondria. These reactive oxygen species (ROS) are partly neutralized by antioxidant defense mechanisms. During salinity stress, those defense mechanisms are not completely effective, and so a fraction of ROS escape and cause molecular damage. This may be one factor underlying salinity stress. Superoxide Dismutase (SOD) is an interesting enzyme. This enzyme, present in the cytosol (Cu-Zn containing form) and mitochondrial matrix (Mn-containing form) of the cells of aerobic organisms, serves the function of converting superoxide anion radicals $\left(O_2^-\right)$ into H_2O_2 and oxygen [43]. The main assumption of salinity stress is that the normal levels of antioxidant defenses are not fully efficient to neutralize "reactive oxygen species" (ROS) and that a fraction of ROS escapes elimination, causing irreparable

molecular damage. Also catalase and several types of peroxidases are involved in H_2O_2 removal [43]. This mechanism of scavenging ROS and preventing and repairing the damaging effects on macromolecules is critical for salt tolerant plants [43].

On one hand we have seaweed species (genetic) influences which we will elucidate in future studies by determination of species specific characteristics in the biochemical composition of the seaweed cell wall. The involved cell wall constituents for the three different seaweed Phyla (green, brown, red) are mentioned below.

1) For green seaweeds (three of our investigated species) the cell wall contains sulphuric acid polysaccharides, sulphated galactans and xylans,

2) For brown seaweeds like in this study *Undaria pinnatifidia* the cell wall consists of compounds like alginic acid, fucoidan (sulphated fucose), laminarin (β-1,3 glucan) and sargassan,

3) For red seaweeds the cell wall contains agars, carrageenans, xylans, floridean starch (amylopectin-like glucan), water-soluble sulphated galactan.

The cell walls of all these thousands of seaweeds are species-specific. So in our investigation of the topic of salt extrusion of the four selected seaweeds some of the different observation possibly can be explained by seaweed species characteristics. Just like we earlier published that for seaweed lipid compositions (the several Ω-3 and Ω-6 poly-unsaturated acids (PUFA's), as well as its Ω-6/Ω-3 ration), both quantitatively as qualitatively were to a major extent seaweed species (genus) dependent, to a minor extent also an environmental effect [44]. Also another example can exemplify the seaweed species dependents (genetic component) effect. For glucose content in 50 individual plants of *Saccharina sp.*; a combined sample of *Fucus serratus* & *Fucus spiralis* and *Ascophyllum nodusum* its concentration in an autumn sample was respectively 65%, 30% and 20% which is a clear indication for a species effect (reviewed: [31]).

<u>Perspectives:</u> While terrestrial agriculture is presently at its limits [1], seaweed culture has great promises for humanity [45] [46]. In modern agriculture, all seaweed compensation mechanisms employed to cope with oceanic euhaline (32‰) salinity stress can have practical purposes in large seaweed plantations and can be explored to create urgently in order to create large amounts of irrigation water in order to obtain new agricultural area in our tropical deserts like the Sahara. Some of these seaweed adaptation mechanisms are: 1) Produce by mechanical pressure large amounts of brackish seawater ≈ 10‰ available in the seaweed milieu interior which can further be desalinated until fresh (0‰) irrigation water by fermentation of the remaining 20% green biomass "press-cake"; 2) This seaweed moisture contains high concentration of nutrients (N & P) sequestered from the eutrophic oceans; 3) Sequestering of H^+ ions from the oceanic environment to combat ocean acidification [46]; 4) Produce large amounts of new green biomass on planet Earth by exploring our oceans by seaweed plantations to act as sink for CO_2 to combat "Global Earth warming" [47], in combination with "ocean acidification" [25] [46]. These practical applications will be de-

scribed in another manuscript.

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