Plant Physiology and Biochemistry 215 (2024) 109000

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Water relations and photosystem II efficiency of the intertidal macroalga *Fucus virsoides*

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ARTICLE INFO

Keywords:
Pressure-volume curves
Photosystem II efficiency
Fucus virsoides
Turgor loss point
Modulus of elasticity
Capacitance

ABSTRACT

Intertidal macroalgae are sessile poikilohydric organisms exposed to desiccation stress during emersion. Water relations parameters are useful tools to evaluate an organism's capacity to withstand water scarcity conditions, but such information on marine intertidal macroalgae is scarce. We assessed the water relations of the intertidal relict *Fucus virsoides*, the unique *Fucus* species endemic to the Mediterranean. We combined measurements of water potential (Ψ) parameters derived from pressure-volume curves and chlorophyll *a* fluorescence (F_v/F_m) in juvenile and adult thalli sampled in three different dates between March and April 2023. *F. virsoides* exhibited remarkable water stress tolerance, as evidenced by the low water potential at turgor loss point (Ψ_{tlp}, -7.0 MPa on average), and the maintenance of high F_v/F_m at low water potentials indicating a prolonged maintenance of healthy physiological status. While no differences were observed between growth stages, Ψ_{tlp}, capacitance (C) and the bulk modulus of elasticity (ε) varied significantly according to the sampling dates, whereas the osmotic potential at full turgor did not significantly change. Ψ measured on thalli collected after a typical prolonged emersion period was markedly lower (−12.3 MPa on average) than the estimated Ψ_{tlp}, suggesting that the population is frequently undergoing turgor loss. Further investigations are required to determine environmental tolerance ranges based on water status characteristics to enhance our understanding of *F. virsoides* responses and vulnerability to climate change, thus providing insight into the possible causes of its widespread decline.

1. Introduction

The midlittoral zone is a dynamic and high-stress environment characterized by rapid and severe fluctuations in environmental conditions, including temperature, salinity, humidity, nutrient concentration, wave action and radiation (Chapman, 1995; Davison and Pearson, 1996; Lobban and Harrison, 1994). Therefore, sessile organisms, such as macroalgae, are subjected to considerable stress during the daily transition from submerged to air-exposed conditions associated with tidal cycles. Tolerance to these stressful conditions is crucial for their survival and reproductive success (da Mota, 2016). The human-induced acceleration of global climate change is expected to have profound impacts on the ecological dynamics of intertidal species (Harley et al., 2006; Kumar et al., 2020; Somero, 2010) leading to shifts in distribution and abundance ranges, simplifying structural habitat complexity and causing a decline in supported biodiversity (Assis et al., 2014; Casado-Amezúa et al., 2019; Whalen et al., 2023).

Macroalgae are integral components of intertidal communities (Davison and Pearson, 1996). They are poikilohydric organisms that lack active mechanisms to regulate water loss (Lüning, 1990; da Mota, 2016). Consequently, they are exposed to desiccation during emersion, which can cause significant physiological and biomechanical stress, including oxidative stress caused by the accumulation of reactive oxygen species (ROS) (e.g., Contreras et al., 2005, 2009; Kumar et al., 2010, 2011; Lee and Shin, 2003), morphological alterations such as a reduction in cortical and medullary cell size, as well as changes in cell ultrastructure with retraction of protoplasts and disorganization of thylakoids (Flores-Molina et al., 2014), and even cell death (Pearson et al., 2009; Tarakhovskaya et al., 2015).

Consequently, macroalgae have developed several morpho-physiological adaptations and strategies to ensure cell viability and to facilitate the recovery of full metabolic activity during tides. These

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https://doi.org/10.1016/j.plaphy.2024.109000
Received 15 April 2024; Received in revised form 11 July 2024; Accepted 30 July 2024

mechanisms include morphological changes such as the reduction of thallus size and surface area (Bergquist, 1959; Dromgoole, 1980; Schagerl and Mostl, 2011; Schonbeck and Norton, 1979), cell wall hardening (McCandless, 1981), and the secretion of mucilage composed of hydrophilic polysaccharides, such as alginic acid and fucoidin (Bérard-Therryault and Cardinal, 1973a; Mariani et al., 1985). Physiological adaptations include reduction of the photosynthetic quantum yield (photoinhibition; Gómez et al., 2004; Contreras-Porcia et al., 2011) with an increase in thermotolerance (Hunt and Denny, 2008), the accumulation of compatible solutes leading to osmotic adjustment (Bäck et al., 1992; Contreras-Porcia et al., 2017; Kirst and Bisson, 1979; Kirst, 1989), the increase in the activity of antioxidant enzymes and compounds to scavenge and neutralize harmful ROS (Burritt et al., 2002; Contreras-Porcia et al., 2017; Guajardo et al., 2016), and the synthesis of desiccation-associated proteins (Contreras-Porcia et al., 2017). Furthermore, ecological strategies such as thriving in clustered canopies have a protective function against desiccation (Bewley, 1979; Clausing et al., 2023; Hunt and Denny, 2008; Migné et al., 2021; Taylor and Hay, 1984).

Another possible adaptation of macroalgae to prolonged emersions could rely on the ability to adjust their water relation parameters, which have been widely used to assess the capacity of terrestrial organisms (e.g., Beckett, 1995, 1997; Nardini et al., 2013; Petruzzellis et al., 2018; Proctor et al., 1998) and seagrasses (Sandoval-Gil et al., 2015) to withstand water scarcity conditions. Some of the most relevant water relation parameters of vascular plants are derived from pressure-volume (PV) curves (Kikuta et al., 1985; Tyree and Hammel, 1972), which describe the relationship between the water potential and the water content of a leaf. As an example, the water potential at the turgor loss point (Ψ_{tlp}) reflects the cell ability to maintain turgor during dehydration, and terrestrial species with lower Ψ_{tlp} values can extend the time available for gas exchange under drought conditions (Tyree and Jarvis, 1982) and thus for the maintenance of cell functions, structural integrity and overall plant performance (Kramer and Boyer, 1995; McDowell, 2011). Redistributing more water in the apoplast could be also a strategy to reduce Ψ_{tlp} (Bartlett et al., 2012), and a high water storage capacitance (C) may play a functional role in buffering Ψ drops during dehydration (emersion) periods (Sack et al., 2003). Despite their great potential, studies analysing the water relation parameters obtained from the PV curves are less common in marine organisms (Sandoval-Gil et al., 2015; Zimmermann and Hüsken, 1980). Furthermore, previous studies on both vascular plants as well as lichens and terrestrial algae reported that water status, expressed in terms of Ψ, is correlated to photosystem II (PS II) efficiency, which is a proxy of the photosynthetic activity (Barták et al., 2015; Hájek et al., 2006; Petruzzellis et al., 2018). In particular, Petruzzellis et al. (2018) suggested that the initial inactivation of PS II and the subsequent decline in photosynthetic activity are triggered by turgor loss in lichens. However, similar studies focusing on marine organisms, including marine macroalgae, are extremely rare and might help highlighting whether this process also applies to these organisms.

Along the cold-temperate intertidal rocky coast of the Northern hemisphere, macroalgae of the genus *Fucus* (Fucales, Phaeophyceae) are foundation species, *sensu* Dayton (1975)(e.g. Assis et al. (2020); Bringloe et al. (2020); Chapman (1995); Lewis (1964); Lüning (1990); Schiel and Foster (2006); Smale and Wernberg (2013); Steneck et al. (2002)), which are distributed across different vertical zonation (Billard et al., 2010) consistent with their degree of desiccation tolerance (Davison and Pearson, 1996; Dring and Brown, 1982). Canopy-forming *Fucus* spp. Structure complex habitats (Kautsky et al., 1992; Boaden, 1996; Fredriksen et al., 2005), supporting high biodiversity (Aneer et al., 1983; Duffy and Hay, 1991) and providing numerous ecosystem services (Wernberg and Filbee-Dexter, 2019). Their bioactive compounds and nutritional value also make them valuable for cosmetic, pharmaceutical and food industries (Díaz-Rubio et al., 2009; Ale et al., 2011; Patarra et al., 2011; Min et al., 2012; Catarino et al., 2018).

Fucus virsoides J. Agardh is a glacial relict endemic to the Adriatic Sea (Munda, 1972) and the only representative of the genus in the Mediterranean Sea (Ardissone, 1886; Giaccone and Pignatti, 1967; Munda, 1972; Linardić, 1949; Pignatti, 1962; Schiffner and Vatova, 1938). In the past, it was locally ubiquitous, with a distribution range extending from northwestern Italy to southern Albania (Kashta, 1992; Linardić, 1949; Mačić, 2006). However, a sharp decline in its populations has been observed over the last three decades. Currently, only few isolated populations remain (Battelli, 2016; Falace et al., 2010; Gljušćić et al., 2023; Mačić, 2006; Orlando-Bonaca et al., 2013) and the species is now protected under the Barcelona Convention (amended Annex II of the Protocol on Specially Protected Areas and Biological Diversity in the Mediterranean; UNEP, 2019).

The comprehensive assessment of the water relations of this intertidal relict would contribute significantly to the broader knowledge base on the physiology and ecology of the unique *Fucus* species endemic to the Mediterranean. It could also provide valuable insights into the species' specific responses to changing climatic conditions. The decline of *F. virsoides* has already been linked to climate change (Boero and Bonsdorff, 2007; Boero et al., 2008; Munda, 2008), which is expected to increase the severity, duration and frequency of extreme weather events (IPCC, 2019; Oliver et al., 2018), such as drought and heat waves. *Fucus* spp. are sensitive to prolonged emersion, particularly in summer, due to the combination of elevated temperatures and ultraviolet radiation (UVR), which leads to severe desiccation and associated osmotic stress (Hanžek, 2014; Pearson et al., 2009). Moreover, the increased susceptibility of juveniles to air exposure and associated stresses (Henry and Van Alstyne, 2004; Schoenwaelder et al., 2003) intensifies the risk of drastic mortality within the population (Dudgeon and Petraitis, 2005).

The objectives of this study were i) to evaluate the water relation parameters of *F. virsoides* growing in the Gulf of Trieste, ii) to compare the water relation parameters between adult and juvenile individuals, and iii) to describe the relationship between thallus water status and PS II efficiency.

2. Materials and methods

2.1. Sampling site and fronds collection

In late winter and spring 2023, three sampling campaigns were carried out at low tide in the intertidal zone of Marina Julia (NE Adriatic Sea) (45°46′37.7"N 13°32′02.6’’E), which represents the last population of *F. virsoides* in the Gulf of Trieste (Falace et al., 2018). From a phenological point of view, this period is characterized by an intensive vegetative and reproductive development of *F. virsoides,* while in late summer, the fronds are loose and only the basal part remains and begins to regenerate in late autumn (Gessner and Hammer, 1971; Linardić, 1949). For each sampling, we calculated the mean values of the following environmental parameters recorded in the 3 days preceding the sampling dates and on the sampling days (Table 1). The daily mean

Table 1
Average of environmental conditions on the 3 days preceding the sampling days and on the sampling days.

	1st sampling: 13.03.23	2nd sampling: 20.03.23	3rd sampling: 13.04.23
Mean humidity (%)	67.8	70.5	64.8
Air temperature (°C)	11.7	10.1	12.0
Wind speed (km h^{-1})	14.8	5	13.3
Precipitation (mm)	0.7	0.3	5.2
Radiation (kJ m^{-2})	13526.8	14495.3	11544.5
Superficial sea water temperature (°C)	9.8	10.5	12.5
Salinity (psu)	38.2	38.1	37.8
Tidal coefficient (out of 120)	72	81	52

relative humidity (%), air temperature (°C), wind speed (km h^{-1}), precipitation (mm), and radiation (kJ m^{-2}) were downloaded from the Agenzia Regionale per la Protezione dell'Ambiente of the Region Friuli Venezia Giulia (ARPA FVG). The daily mean superficial sea water temperature (°C) and salinity (psu) were downloaded from the product Global Ocean Physics Reanalysis from Copernicus Marine Service, catalogued as GLOBAL_ANALYSISFORECAST_PHY_001_024 (https://marine.copernicus.eu/). The daily tidal coefficients, which represent the magnitude of the expected tide occurring regardless of meteorological influences, was obtained from the website Meteopesca (https://meteopesca.com/). For each sampling, healthy apical fronds without receptacle, epiphytes and grazing marks of adult thalli (3–4 cm long) and whole juvenile thalli (0.5–2 cm long) of *F. virsoides*, located at a distance of about 1 m from each other in the same vertical zone, were collected and transported to the laboratory of the University of Trieste within 1 h from the sampling.

2.2. Water relations and photosystem II efficiency

PV curves were measured on 7 samples of adult and of juvenile thalli to obtain water relation parameters, namely the osmotic potential at full turgor (π_0, MPa), the water potential at turgor loss point (Ψ_{tlp}, MPa), the modulus of elasticity of cell walls (ε, MPa), the relative water content at turgor loss point (RWC_{tlp}, %) and capacitance (C, mmol MPa^{-1} g^{-1}). At the beginning of the experiment, *F. virsoides* fronds were placed in 50 ml Falcon tubes, containing tissue paper soaked with de-ionized water adherent to vial's walls to ensure complete samples rehydration. The vials were sealed with Parafilm® and stored in the dark at 4 °C for about 36 h. In this way, samples water potential (Ψ, MPa) approached 0 MPa. This procedure was established based on several preliminary tests (Table S1). The protocol used for PV curves measurement was adapted from others used for bryophytes (Beckett, 1997), seagrasses (Sandoval-Gil et al., 2015) and lichens (Petruzzellis et al., 2018). In detail, rehydrated samples were progressively dehydrated at stable humidity (40–45%) and temperature (20–22 °C) and sequential measurements of Ψ, performed using a dew point water potential meter (WP4, Meter Group, Inc., Pullman, WA, USA), were coupled with measurements of fresh weight (FW), obtained through an analytical balance (±0.0001 g), and chlorophyll *a* fluorescence (in terms of F_v/F_m, or "quantum yield", Genty et al., 1989) in dark adapted samples using a portable fluorimeter (Handy PEA, Plant Efficiency Analyser, Hansatech, Norfolk, UK).

After the rehydration procedure, one apical frond for adult samples or 15 thalli for juvenile samples (to ensure sufficient mass for Ψ measurement) were transferred to a sample holder and placed in the dew-point hygrometer for Ψ measurement, which lasted approx. 1 h (i.e. until three Ψ values within the instrument error range were obtained), ensuring samples dark adaptation. Immediately after Ψ measurement, F_v/F_m and FW were measured, carefully maintaining the samples under dark conditions. Generally, F_v/F_m values between 0.6 and 0.8 are considered indicative of healthy samples (Coelho et al., 2001; Falace et al., 2018; Huppertz et al., 1990; Lamote et al., 2007; Martínez et al., 2012; Nielsen et al., 2003; Schagerl and Möstl, 2011). F_v/F_m was measured taking care to always select the same tip area during the whole experiment. This because *F. virsoides* shows a gradient in photosynthetic activity, with the apical frond area identified as the one with highest photosynthetic rates (Kremer and Munda, 1982). For juvenile samples, F_v/F_m was measured on a single thallus. After F_v/F_m measurement, FW was measured to obtain the cumulative water loss (WL, mg):

$$WL_x = FW_0 - FW_x \quad (1)$$

where FW_0 is the turgid weight after the rehydration procedure and FW_x is the FW for the successive measurements. After the first Ψ, F_v/F_m and FW measurements, the sample holder was kept open allowing water loss from the sample. The sample holder was then closed, and samples were allowed equilibrating for at least 45 min before the following set of measurements. This procedure was repeated several times, progressively dehydrating the sample until its F_v/F_m approached 0. Finally, the samples were oven-dried at 70 °C for 48 h and their dry weight (DW) was determined.

PV curves were considered to start from $\Psi < -0.3$ MPa (Nardini et al., 2013; Petruzzellis et al., 2018) and finished when the relationship between $1/\Psi$ and WL became linear ($r^2 > 0.98$, from 3 to 5 points). PV curves were elaborated following Petruzzellis et al. (2018), thus obtaining π_0, Ψ_{tlp}, ε and RWC_{tlp}. π_0 and Ψ_{tlp} were calculated according to Tyree and Hammel (1972), while ε was calculated considering Ψ values between −1 MPa and Ψ_{tlp}. RWC_{tlp} was calculated as:

$$RWC_{tlp} = \left(FW_{tlp} - DW\right) \Big/ DW \quad (2)$$

where WL_{tlp} is the WL at turgor loss point. We underline that this RWC calculation is usually applied in poikilohydric organisms and is not the one properly used for plants.

Capacitance at full turgor (C) normalized by DW (Nardini et al., 2012) was calculated, as done for ε, for Ψ values between −1 MPa and Ψ_{tlp} as:

$$C = \left(WL_{tlp} - WL_1\right) / \left(\Psi_{tlp} - \Psi_1\right) \Big/ M_{H_2O} \Big/ DW$$

where WL_1 is the WL at $\Psi = -1$ MPa (Ψ_1) and M_{H_2O} is molar mass of water (g mol^{-1}).

2.3. Water potential monitoring and correlated photosystem II efficiency

In order to assess Ψ values reached by *F. virsoides* during a typical prolonged emersion period, five additional adult apical fronds were sampled from the same population used for previous measurements on February 21, 2024. The sampling took place at 16:40 h (solar time) at the end of the low tide (Fig. S1), characterised by a tidal coefficient of 63, on a sunlit day with average radiation intensity of 12211 kJ/m^2 and mean air temperature and relative humidity of 11.7 °C and 68%, respectively. Average vapour pressure deficit (VPD) during the 4 h of emersion prior to sampling was 0.41 kPa. The average sea surface temperature was 9.9 °C and the average salinity was 37.6 psu. The osmotic potential of the sea water collected at the sampling site was measured with the dewpoint water potential meter and was −1.67 MPa. Apical fronds were cut from adult thalli and were immediately enclosed in the same sample holders used for Ψ measurement (see above) to avoid dehydration. Samples were kept in refrigerated bags while transported to the laboratory, where Ψ and F_v/F_m were measured as indicated above.

2.4. Statistical analysis

Differences of each water relation parameter between adult and juvenile samples were evaluated using non-parametric Wilcoxon signed-rank tests ($\alpha = 0.05$), using the *wilcox_test* function in "rstatix" (Kassambara, 2023) R (R Core Team, 2023) package. As no significant differences between adult and juvenile samples were found, we pooled water relation parameters values for each sampling date. Then, the effect of sampling date (explanatory variable with three levels) on each water relation parameter (response variable) was assessed through one-way ANOVA tests (one independent test was run separately for each water relation parameter) using the *lm* function in "stats" R package. For significant tests ($p < 0.05$), pairwise differences between tidal range levels were calculated by means of estimated marginal means using the *emmeans* function in "emmeans" R package Lenth et al., 2023, with p-values adjusted using the Bonferroni-Holm method. Non-linear exponential decay models were fitted to assess the relationship between Ψ and F_v/F_m independently for adult and juvenile samples through the *drm* function in the "drc" R package (Ritz et al., 2015). The differences between growth stages were calculated for each parameter using the *compParm* function in R package "drc". R^2 was calculated using the *R2nls* function in "aomisc" R package (Onofri, 2020). Then, three-parameter log-logistic models were run to examine the relationship between WL and F_v/F_m independently for adult and juvenile samples using the *drm* function, followed by a comparison of the parameters with the *compParm* function. Finally, to assess whether Ψ and the corresponding F_v/F_m values measured in February 2024 were different from the experimental results, we calculated and compared the residuals of both points from the fitted curve using a two-sample *t*-test.

3. Results

3.1. Water relation parameters in F. virsoides: differences among growth stages and sampling dates

Mean values of the water relation parameters measured in both adult and juvenile samples of *F. virsoides* are reported in Table 2. No significant differences were found between the two growth stages (Fig. S2, Table S2). Ψ_{tlp} averaged −6.96 ± 0.91 MPa (±SD), while ε was 2.04 ± 0.54 MPa (±SD). π_0 averaged −1.66 ± 0.38 MPa (±SD), RWC_{tlp} was 85.66 ± 16.33 % (±SD) and C was 13.30 ± 4.32 mmol MPa^{-1} g^{-1} (±SD).

Conversely, statistically significant differences between sampling dates were found for Ψ_{tlp}, ε and RWC_{tlp}, while similar values were recorded for π_0 (Fig. 1, Tables S2, S3, S4). Specifically, the main differences were observed between the individuals from the 2nd and 3rd sampling, with higher Ψ_{tlp}, RWC_{tlp} and C in the individuals from the 2nd sampling (ca. 1.25 MPa, 18 % and 8 mmol MPa^{-1} g^{-1} higher, respectively) and a higher ε of ca. 0.84 MPa in the individuals from the 3rd sampling.

3.2. Relationship between thallus water status and photosystem II efficiency

During samples dehydration, an exponential progressive decrease in F_v/F_m was associated with Ψ decrease (Fig. 2a) for both adult and juvenile samples ($R^2 = 0.85$). Nevertheless, the trends did not differ between the two growth stages (Table S5). F_v/F_m values below 0.6 were recorded at Ψ values of about −7 MPa (i.e. ca. −7.4 MPa for juveniles and ca. −6.7 MPa for adults), which was close to Ψ_{tlp}. F_v/F_m values close to 0 were measured at Ψ values of −39.07 ± 14.14 MPa for juveniles and −38.87 ± 23.92 MPa for adults.

The relationship between WL and F_v/F_m ($R^2 = 0.67$) exhibited an initial plateau followed by an exponential decline leading to the complete suppression of PS II functionality (i.e. when F_v/F_m approached 0) (Fig. 2b). No significant differences between growth stages were observed (Table S5). The onset of decline, represented by the inflection point of the curve, occurred in juveniles at ca. 43% of WL for F_v/F_m of 0.67, and at ca. 34% of WL for F_v/F_m of 0.73 in adults. The subsequent decrease in PS II efficiency below 0.6 occurred after a WL of about 52% in juveniles and of 53 % in adults.

Table 2
Mean ± SD of osmotic potential at full turgor (π_0), water potential at turgor loss point (Ψ_{tlp}), modulus of elasticity of cell walls (ε), relative water content at turgor loss point (RWC_{tlp}) and capacitance at full turgor (C) obtained through the elaboration of PV curves measured in samples of adult and juvenile individuals of *Fucus virsoides*.

	n	π_0 (MPa)	Ψ_{tlp} (MPa)	ε (MPa)	RWC_{tlp} (%)	C (mmol MPa^{-1} g^{-1})
Adult	7	−1.73 ± 0.44	−7.13 ± 1.07	2.12 ± 0.64	88.19 ± 15.93	13.89 ± 3.66
Juvenile	7	−1.59 ± 0.31	−6.78 ± 0.77	1.95 ± 0.44	83.14 ± 17.57	12.71 ± 5.13
Mean		−1.66 ± 0.38	−6.96 ± 0.91	2.04 ± 0.54	85.66 ± 16.33	13.30 ± 4.32

3.3. Water potential monitoring and correlated photosystem II efficiency

No significant differences were found between the residuals of data points acquired to measure the PV curves and data points collected in the monitoring of February 2024 ($t = -0.78$, $p = 0.48$), indicating that the model calculated in laboratory conditions holds true also when considering field data (Fig. 3). Ψ averaged −12.32 ± 6.62 MPa (±SD), with a corresponding F_v/F_m of 0.54 ± 0.23 (±SD). However, most of the samples fell in a Ψ range between −5.72 and −13.26 MPa, except for one sample that showed a much lower Ψ (−22.65 MPa).

4. Discussion

4.1. Water relation parameters in F. virsoides: differences among growth stages and sampling dates

In this study, we assessed for the first time the water relation parameters of the intertidal macroalga *F. virsoides*. Our analysis underlined the relatively high desiccation tolerance of *F. virsoides*, which has already been observed in other congeneric species such as *Fucus spiralis* (Schagerl and Moestl, 2011), *Fucus serratus*, *Fucus distichus* (Andreev et al., 2012) and *Fucus limitaneus* (Ruiz-Medina et al., 2023). Considering the different stress responses observed in juvenile and adult *Fucus* species (Wahl et al., 2011), we hypothesised that *F. virsoides* might display different water relations depending on the growth stage, possibly resulting in lower π_0 and Ψ_{tlp} in adult than in juvenile samples. However, our analysis revealed no significant differences in water status parameters between growth stages, indicating a similar desiccation tolerance strategy. While *F. virsoides* exhibits a high tolerance to desiccation itself, it is possible that the synergistic interaction of other factors associated with desiccation, such as UVR, could increase its vulnerability during air exposure, as proposed by Lamote et al. (2007).

Since no statistically significant differences were found between adult and juvenile samples (Table 2, Fig. S3, Table S2), we hereafter report the mean values of each water relation parameter pooling the two groups together. We found π_0 in *F. virsoides* fronds to be −1.66 ± 0.38 MPa, a value within the range of other poikilohydric species of angiosperms, ferns, mosses, and lichens (e.g. from −0.38 to −2.79 MPa; see Beckett, 1995, 1997; Nardini et al., 2013; Petruzzellis et al., 2018; Proctor et al., 1998). π_0 is the major driver of Ψ_{tlp} variation for terrestrial plants (Bartlett et al., 2012), and it is considered a good proxy of drought tolerance. Indeed, plants can accumulate intracellular solutes (e.g. K^+, Na^+ and Cl^-) in response to reduced water availability, leading to a reduction in π_0 (Bartlett et al., 2012). This phenomenon, known as osmotic adjustment, allows homoiohydric plants to reduce their Ψ_{tlp} and thus improve their ability to tolerate drought stress (Bartlett et al., 2012; Casolo et al., 2014; Fanjul and Rosher, 1984; Nardini et al., 2003). Similar strategies have been observed under osmotic stress in freshwater algae (Winter and Kirst, 1990, 1992) and infralittoral marine algae (Kirst and Bisson, 1979), but are less common in estuarine and intertidal marine algae, which mostly have a limited ability to regulate their turgor pressure (Bisson and Kirst, 1995). Ψ_{tlp} values of *F. virsoides* fronds averaged −6.96 ± 0.91 MPa with a RWC_{tlp} of 85.66 ± 16.33 %. Generally, species with lower Ψ_{tlp} values have a greater drought tolerance (Lambers et al., 2008), as they can maintain turgor pressure at lower water availability and thus survive under prolonged water shortage periods before wilting or suffering irreversible damage (Nardini et al., 2013). As expected, the capacitance at full turgor (C) was relatively high (ca. 13 mmol MPa^{-1} g^{-1}), as it would buffer the drop in Ψ during dehydration due to prolonged emersion (Sack et al., 2003). With this respect, it is interesting that similar values were measured in the relatively soft leaves of mesophylous trees such as *Acer pseudoplatanus*, in comparison with xerophilous hard-leaved species such as *Quercus ilex*, with C values of about 3 mmol MPa^{-1} g^{-1} (Nardini et al., 2012). Finally, ε is a key indicator of the mechanical properties of cell walls, as flexible

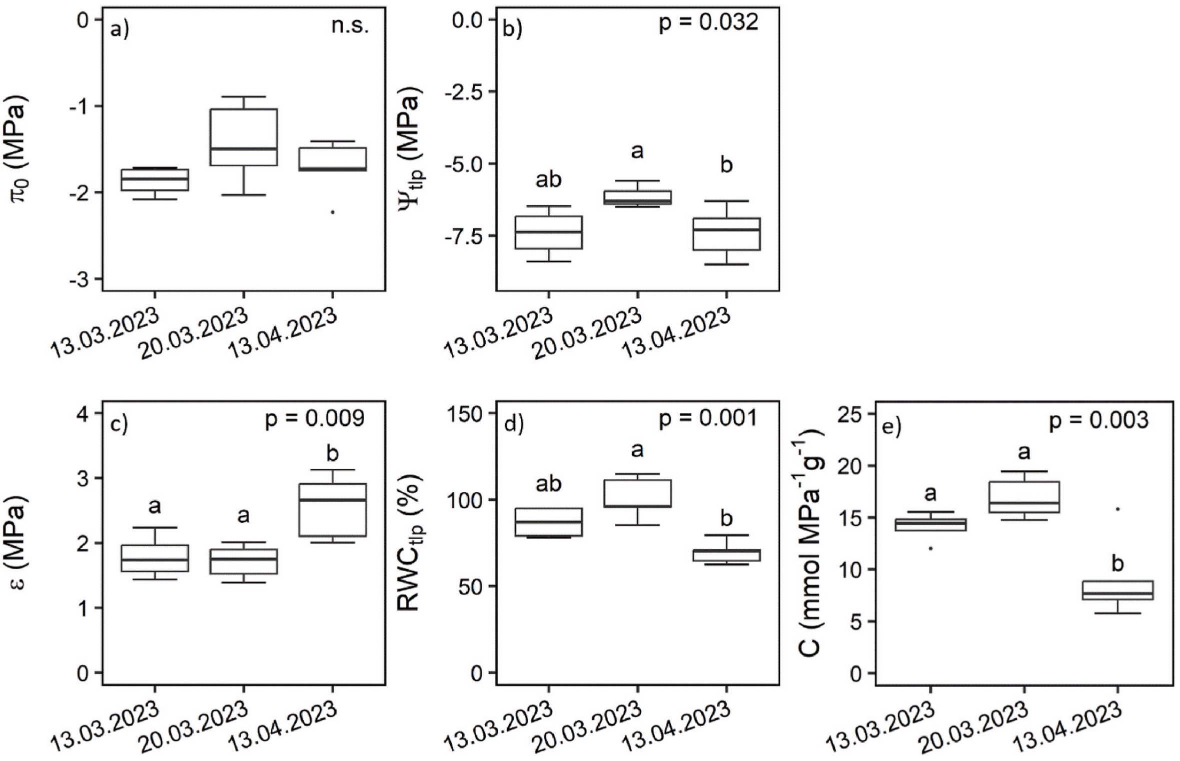

Fig. 1. Median values and 25th and 75th percentiles of osmotic potential at full turgor (π_0, a), water potential at turgor loss point (Ψ_{tlp}, b), modulus of elasticity of cell walls (ε, c), relative water content at turgor loss point (RWC_{tlp}, d) and capacitance at full turgor (C, e) as extrapolated from *Fucus virsoides* individuals according to sampling dates. Different letters indicate statistically significant differences among sampling dates ($p < 0.05$).

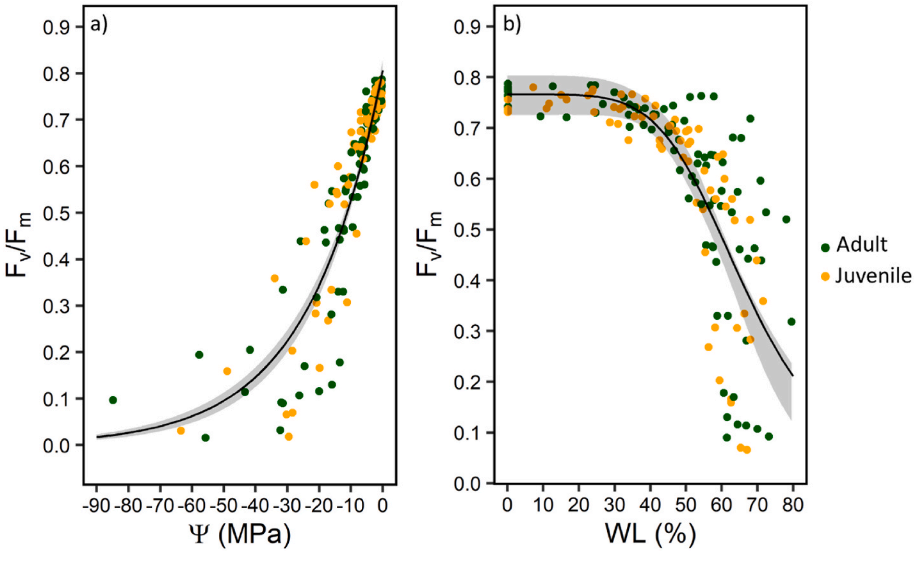

Fig. 2. Optimal photosynthetic quantum yield (F_v/F_m) as a function of (a) water potential (Ψ, MPa) and (b) water loss (WL, %) of juvenile and adult *Fucus virsoides*. The curve is the trend of the values represented by green dots for adult and yellow dots for juvenile individuals, and the shaded zone is the range of variability.

cell walls (i.e. low ε) could allow the maintenance of cell turgor even under large water losses (Schulte, 1992), an ability already observed in intertidal algae, such as *Porphyra umbilicalis* (Wiencke and Läuchli, 1980), *Vertebrata lanosa* (Reed, 1983, 1984) and *Codium decorticatum* (Bisson and Gutknecht, 1975). Kirst (1989) found that the osmotic adjustment of macroalgae in estuaries often occurs through changes in both cell volume and osmolyte concentration. Indeed, low ε in algae facilitates the adjustment of internal osmotic pressure via changes in cell volume, leading to moderate responses to external osmotic pressure changes and consequently to smaller changes in turgor compared to cells with high ε (Bisson and Kirst, 1995). The mean ε values of *F. virsoides* fronds was 2.04 ± 0.54 MPa (±SD), indicating relatively low stiffness and consequently high flexibility in response to variations in the cell water content if compared to inter-tidal and subtidal seagrasses (values between ca. 5 and 11 MPa; Sandoval-Gil et al., 2015) as well as to terrestrial plants from different biomes (average values between ca. 5 and ca. 23 MPa; Bartlett et al., 2012).

The marked difference among Ψ_{tlp} and π_0 (ca. 5 MPa) is usually not

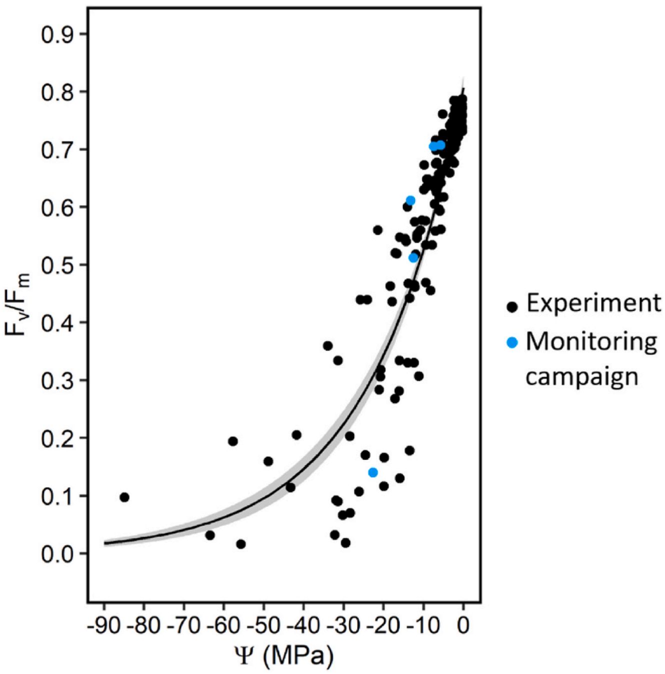

Fig. 3. Photosynthetic quantum yield (F_v/F_m) as a function of water potential (Ψ) of *Fucus virsoides.* The curve is the trend of the values represented by black dots, the shaded zone is the range of variability, and the blue dots are values from the Ψ monitoring campaign.

observed in plants, but a similar result was observed for another poikilohydric organism (Petruzzellis et al., 2018). This finding can be explained by the observed low ε values because for large water content intervals small changes in turgor are accompanied by large volume variations, and this would imply that a high cellular concentration of solutes (i.e. a very low Ψ_{tlp}) would be needed to reach the turgor loss point.

While no differences were found between growth stages, there were significant differences in the pressure-volume parameters between sampling dates, mostly between the 2nd and the 3rd campaign. The main differences in environmental conditions (Table 1) between these two sampling periods were the variations in rainfall and tidal coefficient. Precipitation was recorded prior to the 3rd sampling period, resulting in both direct freshwater input from rainfall and indirect contributions from the Timavo and Isonzo rivers, which flank the sampling area. The individuals at the 3rd sampling showed higher ε value than the previous samplings, indicating an increased cell wall rigidity and a certain plasticity of *F. virsoides* with respect to this trait. Indeed, intertidal algae facing regular salinity fluctuations and lacking effective turgor regulation could use alternative mechanisms of acclimation, such as adjusting their cell wall flexibility (Bisson and Kirst, 1995). For example, Reed et al., 1980 reported an increase in volumetric ε in *Porphyra purpurea* under hyposaline conditions, which hinders additional water influx and cell expansion (Kirst, 1989). In contrast, the 2nd sampling period was characterized by high radiation levels and high tidal coefficients, resulting in prolonged air exposure. In particular, the tidal coefficient reached 103 out of 120 on the day of the 2nd sampling, characterized by both a longer duration and a higher amplitude of the tide, exposing *F. virsoides* to air from ca. 13:00 to 18:00 (https://meteopesca.com/). This condition differs significantly from the 3rd sampling, where the tidal coefficient was 52, indicative of longer immersion time. However, the individuals of the 2nd sampling showed a higher Ψ_{tlp} and RWC_{tlp} despite major air exposure, contrary to our expectations. It is also interesting to note that the increase in ε observed in the last campaign was accompanied by a marked decrease in capacitance at full turgor (C). This result is in agreement with Nadal et al. (2018), which found a coordination among these two traits in vascular plants. Finally, the lack of significant variability in π_0 can be interpreted as the fact that in *F. virsoides* osmoregulatory adjustments are not the major driver for Ψ_{tlp} adjustment.

4.2. Relationship between thallus water status and photosystem II efficiency

Our data highlighted that *F. virsoides* can maintain PS II functionality even under severe desiccation, ensuring sustained growth and overall survival. As no statistically significant differences were found between the growth stages (Figs. 2 and 3, Table S4), the mean values of both groups are pooled together in the following discussion.

The relationship between Ψ and PS II efficiency in adult and juvenile individuals showed a similar trend. Under desiccation, F_v/F_m exhibited an exponential decline, in contrast to previous observations of other poikilohydric organisms such as the chlorolichen *Flavoparmelia caperata* in Petruzzellis et al. (2018), where a relationship between the onset of decline in photosynthetic activity and the turgor loss point was observed. The healthy ecophysiological state, which was set at $F_v/F_m >$ 0.6, was exceeded after a Ψ of ca. −7 MPa, a value in the range of Ψ_{tlp}. Below this value the variability increased in both growth stages and the suppression of PS II functionality was recorded at ca. −39 MPa. The reduction in photosynthetic activity when intertidal macroalgae are exposed to low tide acts as a protective measure, preventing potential damage from desiccation stress and excessive UV radiation (Contreras-Porcia et al., 2011; Gómez et al., 2004). Indeed, desiccation-induced photoinactivation of PS II in the thallus acts as a mechanism protecting from photodamage of the photosynthetic apparatus, as suggested by Huppertz et al. (1990).

When examining the relationship between WL and F_v/F_m, a similar sigmoidal curve was observed at both growth stages. An initial maintenance of F_v/F_m of ca. 0.72 until a WL of ca. 38% was observed, which corresponds to the onset of the loss of symplastic water and thus the total loss of apoplastic/extracellular water, a strategy already observed in other poikilohydric species (Banchi et al., 2018; Candotto Carniel et al., 2015; Holzinger and Karsten, 2013; Kirst, 1989; Kramer and Boyer, 1995), delaying the negative effects of desiccation on metabolism. The abundant polysaccharides in the cell walls of Phaeophyceae were previously associated with the retardation of water loss (Bérard-Therriault & Cardinal, 1973a, 1973b; Zaneveld, 1937) because of their hygroscopic properties facilitating water and ion retention in extracellular matrices (Aquino et al., 2011; Torode et al., 2015). Finally, $F_v/F_m > 0$ was detected down to WL of about 70%. Our results are consistent with those of Kremer and Munda (1982), where a rapid decrease in photosynthetic activity of *F. virsoides* was recorded after WL of 35–40% and the signals persisted even at 60% WL.

4.3. Water potential monitoring and related photosystem II efficiency

In this study we measured Ψ in an additional sampling day, to check the difference between Ψ_{tlp} and the minimum Ψ reached by *F. virsoides in situ* after a typical emersion period in the same time of the year (end of February). We found that average thallus Ψ was below Ψ_{tlp} but F_v/F_m values were relatively high (0.54 ± 0.23), suggesting a moderate reduction of PS II efficiency. On one hand, this might imply that *F. virsoides* could maintain a moderately good physiological status even when surpassing turgor loss point, suggesting that this species could recover Ψ and F_v/F_m even after prolonged daily emersion periods. On the other hand, this result could be a warning signal. Indeed, the slope of the relationship between Ψ and F_v/F_m is relatively steep (Fig. 3), especially in the range of Ψ values measured in the monitoring date, so that minimal further decrease of thallus Ψ could result in a large drop towards critical F_v/F_m values. Secondly, in the scenario where thallus Ψ frequently reaches values below Ψ_{tlp} (a condition that is likely to occur especially in hot springs and summers), both primary metabolism and growth of *F. virsoides* could be limited, thus potentially explaining why the distribution of this species has dramatically reduced in the last decades, characterised by prolonged air exposure periods caused by increasing tidal fluctuations. Further analyses conducted both *in situ* and under controlled laboratory conditions are required to test these hypotheses.

5. Conclusion

By combining exhaustive measurements of the water potential components as well as chlorophyll *a* fluorescence with the corresponding thallus water contents, our study emphasises the remarkable water stress tolerance exhibited by both adult and juvenile individuals of *F. virsoides*. This result is supported by the water relation parameters derived from the PV curves, especially by the relatively low water potential at turgor loss point (Ψ_{tlp}), and the maintenance of relatively high PSII efficiency (F_v/F_m) at Ψ values indicating major desiccation. Further investigations are required to determine the environmental tolerance ranges based on water status characteristics, and in particular to assess potential water relations modification as a function of the tidal range. In addition, assessing the relationship between Ψ and PS II efficiency under less favourable conditions, e.g. in summer, can improve our understanding of *F. virsoides* responses and vulnerability in the context of climate change.

Funding

FP was supported by the funding Programma Operativo Nazionale (PON) Ricerca e Innovazione D.M. 1062/21 – Contratti di ricerca, from the Italian Ministry of University and Research (MUR).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2024.109000.

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