Research Article

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Effects of increased temperatures during air exposure on the morphology and physiology of *Fucus virsoides* (Fucales, Phaeophyceae)

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The brown alga *Fucus virsoides*, an endangered species endemic to the Adriatic Sea, is subjected to increasingly changing ecological conditions in the intertidal zone, potentially driving its extinction. In this study, temperature fluctuations at one of the last remaining *F. virsoides* sites in the Istrian region (northern Adriatic) during a 1-year period were analysed and found to be only partially associated with water level changes linked to tidal dynamics, suggesting nontidal effects may strongly affect the emersion time and exposure of thalli to dry conditions, especially during spring. Additionally, the effects of increased temperature during air exposure on *F. virsoides* thalli were experimentally assessed under laboratory conditions. The results indicate that acute and repeated exposure to a high air temperature of 33°C can cause long-term damage to the thalli, whereas lower tested temperatures of 20, 25, and 29°C have little to no effect on the morphology or physiology of this algae. This study provides insights into how *F. virsoides*, a species adapted to the northernmost biogeographical region of the Mediterranean, copes with extreme temperature fluctuations during prolonged air exposure, as one of the environmental stressors that are expected to intensify under future climate scenarios.

Keywords: air exposure; *Fucus virsoides*; intertidal; northern Adriatic; physiological effects; thermotolerance

INTRODUCTION

Intertidal macroalgae inherently experience alternating periods of water immersion and air exposure due to tidal fluctuations. The frequency and duration of these emersion periods are influenced by the vertical position on the shore; algae located higher in the intertidal zone are more frequently exposed to terrestrial conditions than those located in lower zones (Bell 1993), although this may not hold in the case of rockpools. The effects of air exposure on intertidal macroalgae have been investigated from various perspectives, such as basic morphology, physiology, survival, growth, and reproduction (Schonbeck and Norton 1980, Maberly and Madsen 1990, Madsen and Maberly 1990, Schagerl and Möstl 2011, Flores-Molina et al. 2014, Du et al. 2022). As a central physiological process, photosynthesis is significantly inhibited under desiccation (Dring and Brown 1982, Lipkin et al. 1993, Blouin et al. 2010). However, many intertidal marine algae are relatively tolerant to desiccation, showing comparatively high photosynthetic rates during air exposure periods. This ability, however, can decrease dramatically as algae lose their water content during extremely long periods of desiccation stress (Johnson et al. 1974, Quadir et al. 1979, Oates and Murray 1983, Bidwell and McLachlan 1985, Madsen and Maberly 1990, Bell 1993). This topic has also been reviewed by Gessner and Hammer (1971)

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and Kremer and Munda (1982) with a particular focus on the intertidal macroalga *Fucus virsoides* J. Agardh. Recently, the effect of water loss was directly related to the physiological state and photosynthetic rate of this species; nonetheless, the results generally confirmed the noteworthy ability of *F. virsoides* to tolerate desiccation (Descourvières et al. 2024*b*).

The intertidal zone is characterized by periodic fluctuations in several abiotic and biological factors, such as atmospheric conditions, hydrodynamics, and grazing intensity (Pignatti 1962, Giaccone and Pignatti 1967, Munda 1972, Battelli 2013). These fluctuations, under certain conditions and in certain areas, can overlap with changes in sea level associated with tidal dynamics, thus creating a highly variable environment with a range of effects on the physiology of affected organisms (Marcos et al. 2009).

While tidal amplitudes in the Adriatic are small compared with those in the open ocean, they increase northwards, and their impact on sea level can be amplified by atmospheric factors such as wind (wind setup, wave action), atmospheric pressure (inverted barometer effect) (Close 1918, Ippen 1966, Wunsch and Stammer 1997, Marcos et al. 2009, Vilibić et al. 2017), and coastal topography. This is typically represented by increased residual values during mareographic measurements but can, in case of extreme atmospheric forcing, also have a very tangible effect on coastal areas, such as flooding (Marcos et al. 2009, Vilibić et al. 2017).

While other fucalean algae commonly inhabiting the shallow subtidal, such as *Gongolaria barbata* (Stackhouse) Kuntze, *Ericaria crinita* (Duby) Molinari & Guiry, *Ericaria amentacea* (C. Agardh) Molinari & Guiry and *Cystoseira compressa* (Esper) Gerloff & Nizamuddin are often heavily affected by environmental stressors when inhabiting the intertidal, *F. virsoides* is a highly specialised intertidal species and is naturally more resistant to such challenges. This resistance is achieved either through physiological mechanisms (Gessner and Hammer 1971, Descourvières et al. 2024*b*) or via the formation of a layered community structure (Munda 1972). Indeed, this species is well adapted to desiccation, being able to withstand significant water loss without long-term damage to the thalli and only temporary reduction in photochemical activity (Gessner and Hammer 1971, Kremer and Munda 1982, Descourvières et al. 2024*b*). Nevertheless, global climate change associated with the increasing intensity and frequency of both terrestrial and marine temperature extremes could generate conditions exceeding the tolerance limits of this species (Frölicher et al. 2018, Rey et al. 2020, Oliver et al. 2021).

F. virsoides is a fucalean species endemic to the Mediterranean, inhabiting only its coldest part, the Adriatic Sea (Linardić 1949, Ribera et al. 1992). It is also the sole species of its genus natively found in the Mediterranean basin (Giaccone and Pignatti 1967, Munda 1972, Rindi and Battelli 2005, Guiry and Guiry 2024). *F. virsoides* is restricted to the intertidal zone of the Adriatic Sea, partly representing "an ecological equivalent" to the larger and more abundant fucalean species that form macroalgal forests in intertidal zones along oceanic coasts and in colder seas (Linardić 1949, Munda 1972). Importantly, the distinct temperature responses of Adriatic *F. virsoides*, which are likely shaped by long-term adaptation to Adriatic conditions, have been linked to its geographic restriction and the absence of Atlantic congeners in the Mediterranean (Linardić 1949, Kremer and Munda 1982, Sancholle 1988, Boero et al. 2008).

The Mediterranean Sea is undergoing significant changes due to warming trends and tropicalization, which could further influence the ecological niche of *F. virsoides* (Bianchi and Morri 2000). Moreover, Mediterranean endemic species are threatened by shifts in biodiversity driven by climate change and invasive species (Bianchi and Morri 2000, Bianchi 2007). In this context, *F. virsoides* was historically distributed along the Adriatic coast extending, between Ancona and Venice (Italy) on the western side of the Adriatic and from the Gulf of Trieste (45.75 N) to Düress-Albania (41.3 N) on the eastern side (Linardić 1949, Zavodnik 1967, Kashta 1996, Rindi et al. 2020, Descourvières et al. 2024*b*). Although historically common and abundant in the Adriatic, especially in the northern part, this species is currently rare and may be considered functionally extinct (Linardić 1949, Munda 1973, Estes et al. 1989, Gljušćić et al. 2023, Descourvières et al. 2024*a*).

Given its historical distribution and abundance, *F. virsoides* has been considered a habitat-forming species capable of supporting diverse assemblages and notable biomass of smaller algae and animal species. Such assemblages have been primarily studied along the western Istrian coast in the mid-20th century and later revisited in 2010, 2014, and 2016, albeit on a smaller scale owing to the already reduced abundances and local extinctions (Zavodnik 1967, Munda 1972, Čelig 2010, Kučinar 2014, Gljušćić 2016). The species was historically abundant and common along the Istrian coastline (northern Adriatic Sea), particularly the western coast, as confirmed by floristic studies and herbarium collections from the 19th and early 20th centuries (Vatova 1928, Linardić 1949, Munda 2000, Battelli and Alberti 2003, Rindi and Battelli 2005, Battelli 2013, Algae Herbarium Portal 2024). However, later assessments revealed a noticeable reduction in biomass (Zavodnik et al. 2002, Kučinar 2014). The current biomass is low but cannot be estimated due to the extremely low abundance and limited availability of the species, which prevents active sample collection (Gljušćić et al. 2023). *F. virsoides* is typically reproductively active during the colder part of the year, namely in spring (Vatova 1948, Linardić 1949); however, during our surveys, we observed significant shifts in reproductive activity, with thalli occasionally being fertile even during the summer months (Gljušćić et al. 2023). The collapse of *F. virsoides* stands in the northern Adriatic, especially along the Istrian coast, has recently become increasingly evident, with most sites lost and others severely degraded (Gljušćić et al. 2023). By July 2024, only two sites remained: one in Camp Lanterna (Tar-Vabriga municipality) and another near Blaz cove (Raša channel). While the distribution along the Istrian coast has been well documented, the remaining Croatian coastline still lacks comprehensive mapping (Descourvières et al. 2024*b*).

This study aimed to investigate how *F. virsoides* responds to elevated temperatures during air exposure, such as those occurring *in-situ* during low water periods, which are expected to become even more extreme in the near future due to climate change. This objective has been pursued: (1) *in-situ*, by measuring the temperature variations at *F. virsoides* site, and (2) *ex-situ*, by estimating and comparing morphological and physiological variables of *F. virsoides* experimentally exposed to increasing temperature levels within simulated emersion and submersion cycles.

MATERIALS AND METHODS

Intertidal conditions

In the northern Adriatic Sea, tides alternate every 15 days between “half-day tides” (i.e., two high tides and two low tides per day) and “day tides” (i.e., one high tide and one low tide per day) (Malačič et al. 2000, Battelli and Catra 2023). The tidal range reaches the largest amplitude (cca. 1 m) in the Gulf of Trieste (Vilibić et al. 2017). The timing of the lowest water levels, which occur around syzygy, shifts seasonally: during winter and spring, they typically occur during the day or evening, whereas in summer and autumn, they occur at night or in the early morning (Supplementary Fig. S1) (Vilibić et al. 2017, Hydrographic Institute of the Republic of Croatia 2025). Springtime conditions (but also occasional summer mornings) combined with low tides may lead to extreme sea and air temperature levels as well as intense irradiation; conditions which are particularly challenging for intertidal flora due to intense water loss, photosystem overload, morphological and physiological damage to the exposed thalli (Schonbeck and Norton 1980, Martone et al. 2010, Gljušćić et al. 2023, Hydrographic Institute of the Republic of Croatia 2025).

Study area

In-situ temperature measurements and *Fucus virsoides* sample collection were both conducted in the vicinity of Blaz cove (45.00001° N, 14.04599° E), which is located in the Raša channel on the eastern Istrian coast in the northern Adriatic Sea (Fig. 1A). This location is characterized by transitional or near-transitional waters due to Raša river inflow (Fig. 1B) and locally present freshwater springs. At the site, *F. virsoides* forms a relatively narrow but long belt on a subvertical substrate, positioned underneath a nearly vertically inclined karstic coastline with a relatively high profile and vegetation, which significantly reduces overall insolation (Fig. 1C).

Temperature measurements

The intertidal nature of *F. virsoides* exposes it to severely and rapidly changing conditions in its habitat, whether periodically or randomly. Hourly temperatures within the *F. virsoides* belt were measured using HOBO Pendant Temperature/Light 64K Data Loggers attached to the rocks among the thalli and were exchanged for readout on a monthly basis. The temperature frequencies measured during low water periods in 2024 were examined via a histogram plot. The recorded temperatures were also matched with the predicted daily low water times (adjusted for an approximate 1-h tidal delay) on the basis of data for the city of Rijeka obtained from the “Asterion” webpage (https://www.asterion.info/).

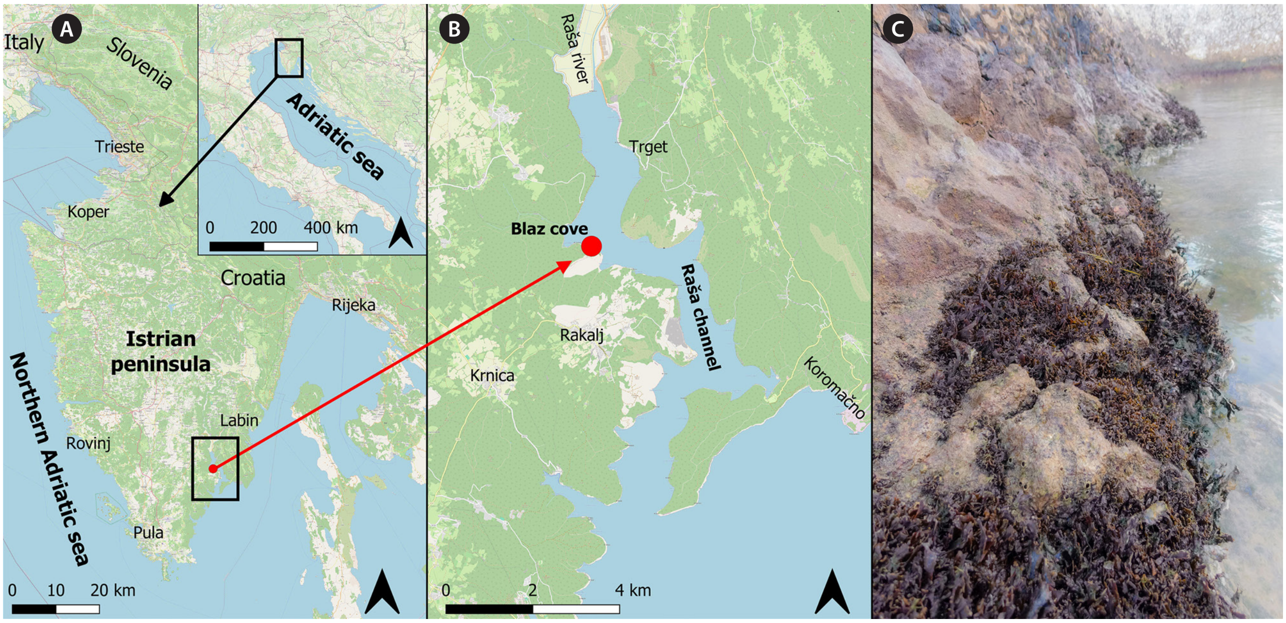

Fig. 1. Location of the study area and sampling site in the northern Adriatic Sea - Istrian Coast (A & B) and image of the study site (C).

Sample collection and treatment preparation

The apical fronds (hereafter referred to as "apices") of *F. virsoides* thalli were collected from the same study site where data loggers were positioned (Blaz cove) and subsequently stored in 18–20°C seawater at the laboratory of the Center for Marine Research, Rovinj (Ruđer Bošković Institute, Croatia), prior to the experiment initiation. Each of the apices was collected from a different individual. For each of the planned temperature treatments, 15 clay tiles were prepared, being previously thoroughly washed and moisturised. The tiles were numbered and marked with coloured cable ties to indicate the corresponding temperature treatment: blue for 20°C, green for 25°C, yellow for 29°C, red for 33°C, and white for 14°C, which represented the control. The temperature treatments were chosen on the basis of previous measurements from the intertidal zone in several *F. virsoides* patches from the Vrsar-Funtana-Poreč area (western Istrian coast, Croatia) during 2023, identified by unusual peaks during springtime (during emersion). The control temperature of 14°C represented not only the springtime (March–April) seawater temperature but also the nighttime/shaded area temperatures measured via data loggers in the intertidal zone.

Fifteen *F. virsoides* apices were selected for each temperature treatment, and initial measurements (T0) were conducted, including wet weight, length, maximum photochemical yield (F_v/F_m) (a proxy for physiological state), as well as assessments of necrosis and regeneration signs (if present).

Each of the 15 apices was gently attached to a clay tile. The tiles were then placed into three separate plastic boxes, with each box containing 5 samples/apices (Fig. 2). The boxes were filled with 1 L of 14°C filtered seawater (5 μm canister filter), closed and left undisturbed until 8 a.m. of the following day (approximately 18 h).

Experimental procedure: air exposure phase and constant immersion phase

The experiment was conducted in two separate phases: first the “air exposure phase” and then the “constant immersion phase.” In the air exposure phase, at 8 a.m., seawater was drained from the boxes, and the apices were gently blotted dry using paper tissues. A 5 g paper-wrapped pack of silica gel was placed inside each box to stimulate dry conditions, with the humidity and temperature levels monitored by a hygro-thermometer. The sealed boxes with apices were subsequently placed inside an incubator (Memmert Compressor Cooling Incubator ICP260; Memmert GmbH + Co.KG, Schwabach, Germany) to be exposed to simulated dry conditions for 6 h at the designated treatment temperature (Fig. 2A). At 2 p.m., after 6 h of simulated emersion, measurements of *F. virsoides* apices, such as wet weight, length and F_v/F_m were ob-

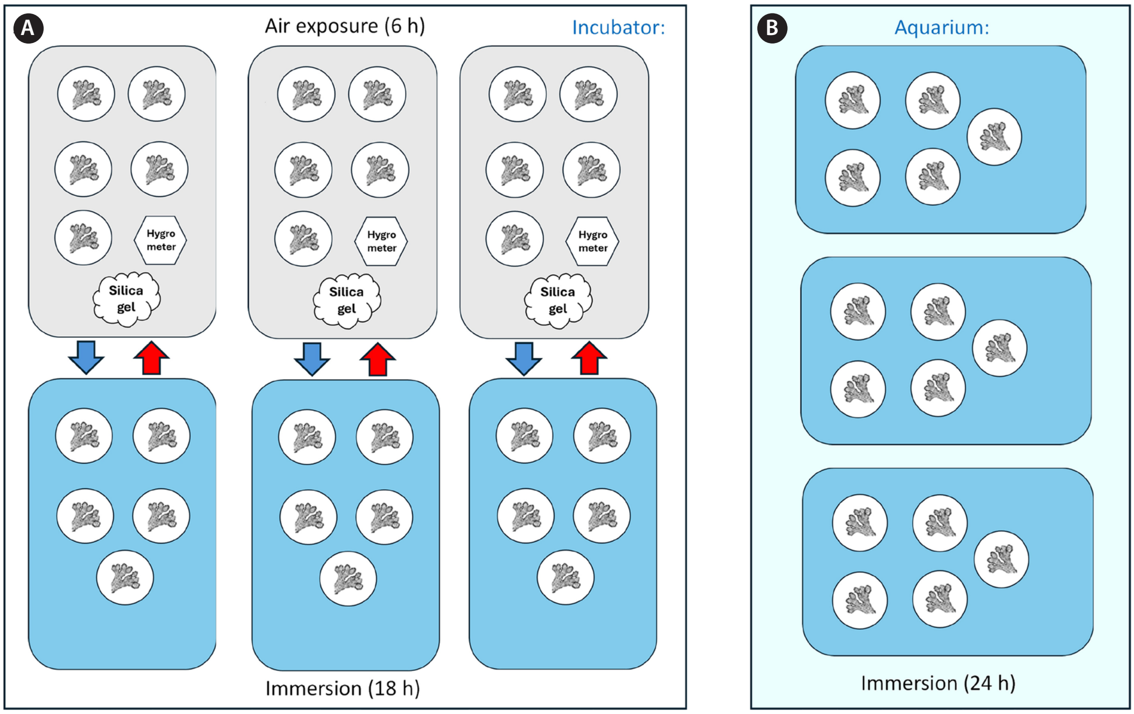

Fig. 2. Schematic of the experiment, consisting of an air exposure phase (A) and a constant immersion phase (B).

tained, and signs of necrosis and regeneration were recorded. Photographs of the apices were taken using a digital camera for documentation and for later precise length measurements. This procedure was repeated for 7 consecutive days at the same time.

Following the 7 days of exposure to specific air temperatures in each treatment, the constant immersion phase was initiated. During this phase, the apices on the clay tiles were kept constantly immersed in filtered seawater at 14°C, simulating neap tide periods under the assumption that *F. virsoides* remains constantly immersed during quarter moon phases (Fig. 2B). The measurements (wet weight, length, and F_v/F_m) along with the assessment of necrosis and/or regeneration were continued. Photographic documentation was conducted daily for 7 additional days. The filtered seawater inside the boxes was changed daily to ensure a clean environment.

This procedure (for both phases) was carried out for each air temperature treatment as follows: the control was maintained at 14°C (C-14), and the experimental treatments were set at 20°C (T-20), 25°C (T-25), 29°C (T-29), and 33°C (T-33). The measurement timepoints were defined as T0 for the initial measurements, T1 to T7 for the air exposure phase, and TR1 to TR7 for the constant immersion phase.

During the air exposure phase, the light intensity was maintained at a constant 70 μmol photons s^{-1} m^{-2} inside the incubator by using integrated fluorescent bulbs. The temperature (both intensity levels and exposure times) was controlled with an integrated air conditioning mechanism via prewritten programming for each of the treatments. During the constant immersion phase, the light intensity was maintained at 70 μmol photons s^{-1} m^{-2}, via LED-GNC-Silver Moon Marine aquarium lights (GNC, Perugia, Umbria, Italy), and the temperature was controlled via a connected Teco TK500 chiller (Teco Refrigeration Technologies, Fornace Zarattini, Ravenna, Italy). Wet weight was measured via a laboratory scale (Metler Toledo PB 1502-S; Mettler-Toledo, LLC, Columbus, OH, USA). The F_v/F_m was measured via MINI-PAM-II (Heinz Walz GmbH, Effeltrich, Germany) following 15 min of dark adaptation. Length was measured via ImageJ software (Rasband 2024) after taking daily repeated photographs of apices placed on top of a ruler (for scale) and by comparing them with reference photographs taken at T0 while always using the same 2 points on the apex (Supplementary Fig. S2). All photographs were taken using an Olympus TG-6 camera (Olympus Corporation, Tokyo, Japan).

This thermotolerance experiment was designed on

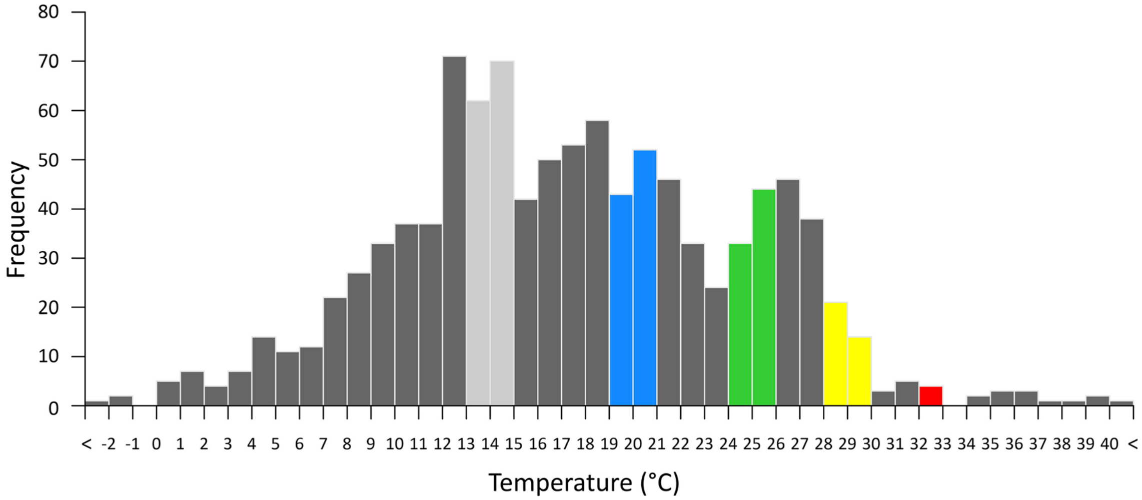

Fig. 3. Frequency of hourly temperatures in the monitored *Fucus virsoides* belt measured in 2024 during low water periods. The grey (13–15°C), blue (19–21°C), green (24–26°C), yellow (28–30°C), and red (32–34°C) columns correspond to the temperatures used in the experimental treatments, +/– 1°C.

the basis of *in-situ* observations and conducted under laboratory conditions on the basis of the following assumptions: (1) *F. virsoides* thalli are exposed to air for up to 6 h during the tidal minimum, (2) *F. virsoides* thalli are immersed for up to 18 h after the tidal minimum, with no re-emersion during the second low water period, (3) *F. virsoides* thalli are not exposed to air during neap tides, and (4) the temperature of algal thalli is equal to the ambient temperature measured in the microhabitat (heating due to sunlight exposure was not excluded).

Statistical analysis

Mixed-effects models were selected to include both fixed and random effects as predictor variables. Furthermore, the use of crossed and nested random effects allowed for the control of the lack of independence among observational units, and supported the use of clustered data and repeated measurements across time in the same model (Bolker et al. 2009, Bates et al. 2015, Harrison et al. 2018). The effects of temperature on wet weight and length were analysed via an linear mixed model (LMM), whereas the effects of temperature on the maximum quantum yield were analysed via a generalized linear mixed model (GLMM) with a Poisson error distribution and a log link function. Measurements of wet weight and length were transformed into percentage changes on the basis of the T0 measurement. Both analyses were performed for the air exposure phase and constant immersion phase of the experiment. Temperature was treated as a fixed factor (five levels: C-14, T-20, T-25, T-29, and T-33), time was treated as a crossed random factor, and the identity of the individual apices (ID) nested within box, was treated as a random factor to take into account that individuals were grouped by five within each box, as well as in order to correct for the non-independence between measurements (Bolker et al. 2009, Bates et al. 2015, Harrison et al. 2018). A type II Wald χ^2 test was applied to each fitted model to determine the effect of each fixed factor. Finally, for each model, a Tukey *post-hoc* test was applied to explore the differences between temperature treatments. The models were fitted using the lme4 (Bates et al. 2015) and MASS packages (Venables and Ripley 2002) in the statistical environment R (R Core Team, 2024; R Foundation for Statistical Computing, Vienna, Austria). p-values were obtained by means of a Wald χ^2 test using the 'ANOVA' function from the CAR package (Fox and Weisberg 2019), and the function 'glht' from the MULTCOMP package (Hothorn et al. 2008) was used to perform *post-hoc* Tukey tests. p-values below 0.05 were considered statistically significant in all analyses.

Additionally, a principal coordinates analysis (PCO), based on the Euclidean distance of untransformed data, was used for both phases of the experiment to visually represent the dependence of the response variables on the factors. For this purpose, PRIMER ver. 7 was used. Other graphical representations and data visualisations were performed with Grapher 24.2.247.

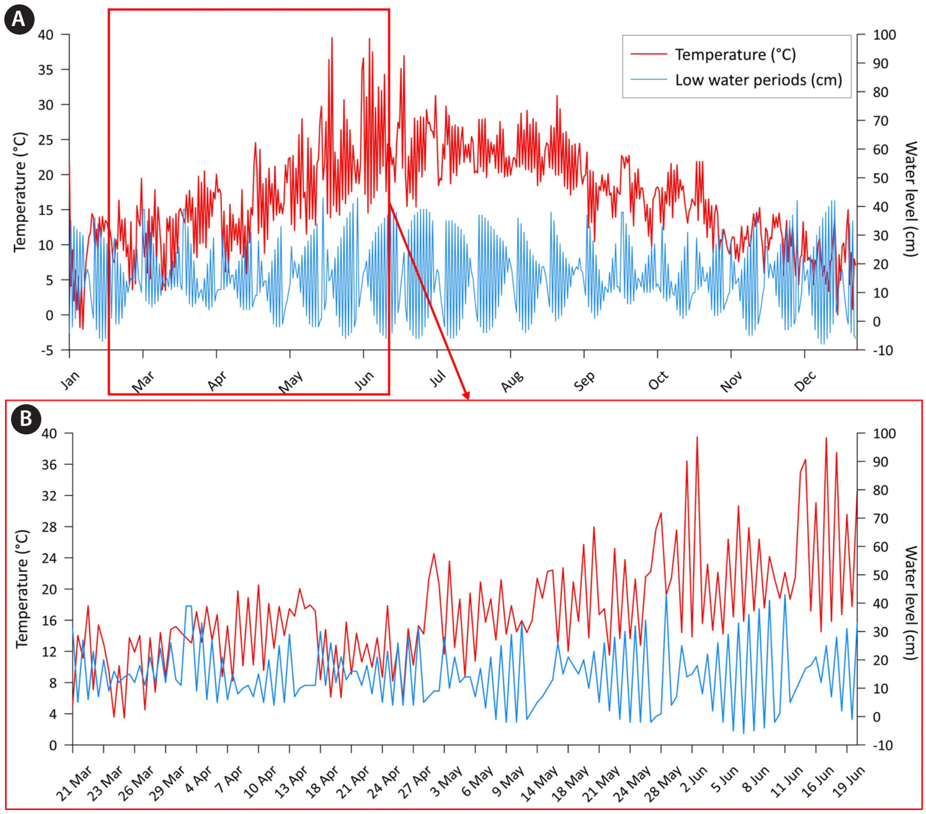

Fig. 4. Temperatures recorded in a *Fucus virsoides* site during daily low water periods over the year 2024 (A) and during the spring of 2024 (B). The predicted water levels are based on mareographic data for Rijeka, adjusted by +1 h to account for distance and local geomorphology.

RESULTS

Temperature data

Analyses of the temperature occurrence frequency revealed that extremely elevated temperatures (>30°C) during low water periods are still relatively rare (Fig. 3) and occur during late spring and summer (Fig. 4A). Moderately elevated temperatures (25–30°C) are more prevalent, although less frequent at the upper end of this range, occurring primarily during the spring and summer months (Figs 3 & 4A). Mildly elevated temperatures (20–25°C) are more frequent and can occur throughout the year, except during winter (Figs 3 & 4A). Lower temperatures (13–15°C), including those close to the assumed control in the *ex-situ* experiment (14°C), are very frequent (Fig. 3) and predominantly occur during autumn, winter, and spring (Fig. 4A).

The yearly temperature overview also revealed that the largest temperature peaks and ebbs, with very high (>35°C) and relatively low (~15°C) levels exchanging rapidly, occur during the late spring (Fig. 4A), causing the exposure of *Fucus virsoides* to stressful conditions.

During the summer months, the generally milder temperatures recorded during low water periods suggest that emersion primarily occurs at night, whereas immersion takes place during the day (Fig. 4A, Supplementary Fig. S1). Furthermore, intervals of lower temperature variability, coinciding with neap tides, indicate more stable environmental conditions. Interestingly, an absence of even moderately increased temperatures (>25°C) is noticeable over the typically warm early autumn period (September), which is expected to resume towards the winter as the temperatures continue to drop regularly (Fig. 4A). During the lowest tide periods in spring (Mar 21–Jun 21, 2024), the temperature occasionally reached very high values (>25°C), but the same values also occurred during neap tides and in between, suggesting more frequent emersion and reimmersion than hypothesised (Fig. 4B).

Visual observations

Except for the loss of naturally present sterile hairs, no signs of necrosis were observed during the experiment for the T-20, T-25, T-29, and C-14 treatments in

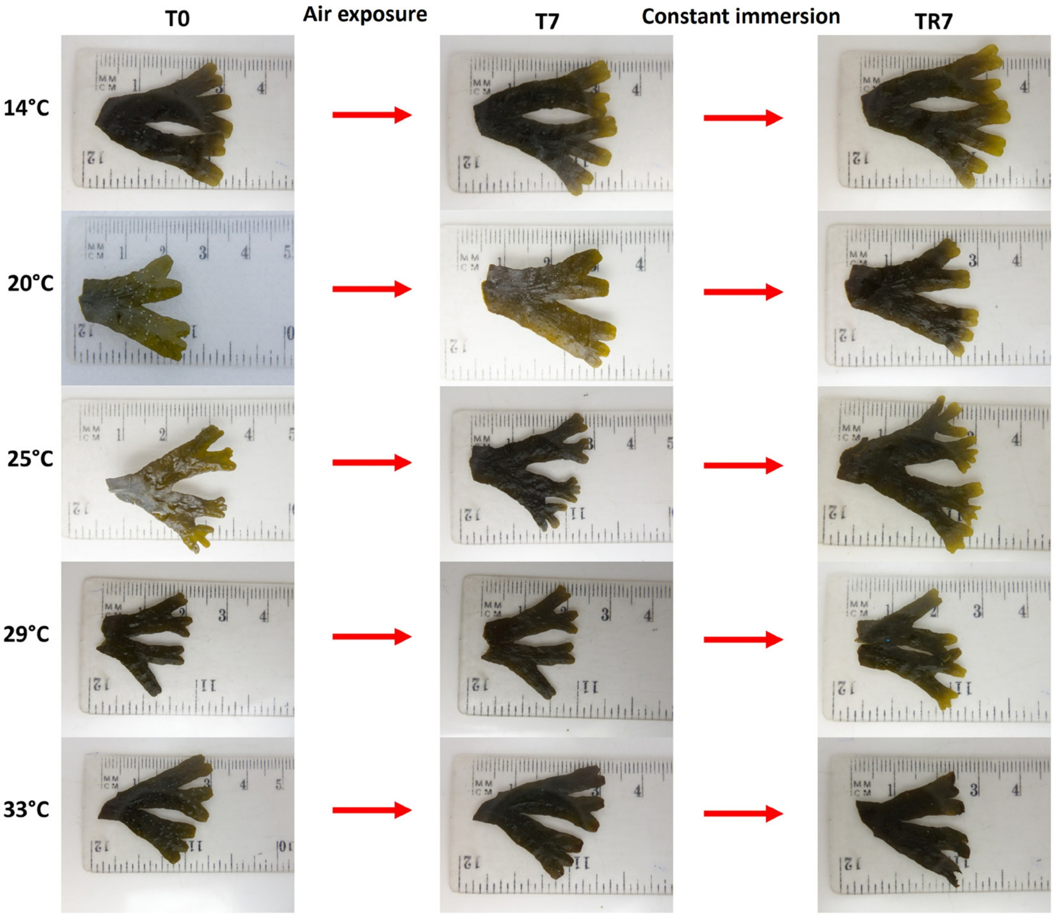

Fig. 5. Examples of physical changes in *Fucus virsoides* apices throughout the experiment, shown at the start of the experiment (T0), at the end of the air exposure phase (T7) and at the end of the constant immersion phase (TR7).

both phases. However, a necrotic smell, characteristic of air-exposed fucalean algae (not quantifiable), was noted in the T-29 and T-33 treatments. In the T-33 treatment, physical thalli necrosis was visible during both phases of the experiment, along with some traces of thalli damage recovery in the constant immersion phase (Fig. 5). An increase in the length of the apices was visible throughout the experiment in both phases and across all the treatments (Fig. 5).

Graphical representation of the measured variables

No major changes in wet weight were observed in the apices of *F. virsoides* in the T-20, T-25, or T-29 treatment groups compared to the C-14 control group. In these treatments, the wet weight continued to slightly increase over time during both phases of the experiment. However, in the T-33 treatment, the change in wet weight was consistently negative, as the apices progressively deteriorated over time (Fig. 6A).

A similar pattern was observed for average length. While no major change was observed between T-20, T-25, or T-29 and the C-14 control, the average length in all these treatments slightly increased during both phases of the experiment. However, a major decrease in average length was observed in T-33 when compared with the control (Fig. 6B).

The F_v/F_m slightly varied over time in the T-20, T-25, and T-29 treatments, and in the C-14 control during both phases. However, in the T-33 treatment, the F_v/F_m reduction was much more pronounced compared to other treatments, even if a certain improvement was expectedly visible towards the end of the constant immersion phase (Fig. 6C). However, when the F_v/F_m response for the T-20, T-25, and T-29 treatments was compared with that for the C-14 control in both experimental phases, the difference was negligible.

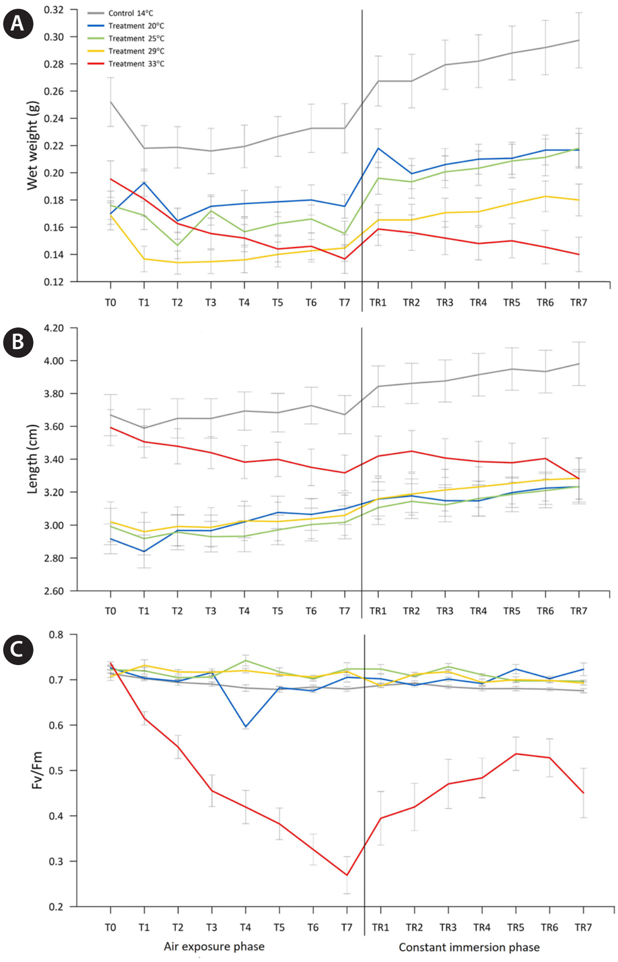

Fig. 6. Changes in the wet weight (A), length (B), and maximum photochemical yield (F_v/F_m) (C) of *Fucus virsoides* apices during the air exposure and constant immersion phases for the 20°C (blue), 25°C (green), 29°C (yellow), and 33°C (red) treatments compared with those for the control treatment at 14°C (grey). The data are presented as the means ± standard error for 15 apices.

LMM & GLMM analysis

Air exposure phase. The LMM analysis of the wet weight percentage change (Ww_c) values revealed strong statistically significant differences between the treatments ($\chi^2 < 0.001$). Tukey *post-hoc* tests revealed that there were strong statistically significant differences between most treatments ($p < 0.001$), except for T-29 vs. C-14 ($p = 0.038$), for which the difference was less pronounced. However, the differences T-25 vs. C-14 and T-33 vs. T-29 were not statistically significant. Similarly, the analysis of the length percentage change (L_c) revealed strong statistically significant differences between the treatments ($\chi^2 < 0.001$). Tukey *post-hoc* tests identified strong statistically significant differences ($p < 0.001$) between all the treatments except T-25 vs. C-14, T-29 vs. C-14 and T-29 vs. T-25, where no significant differences were found. The GLMM analysis of the F_v/F_m values between the treatments also revealed significant differences ($\chi^2 < 0.001$), which stemmed from the strong separation of the T-33 treatment ($p < 0.001$) from all the other treatments. No significant differences were detected between the other treatments.

Constant immersion phase. Similar patterns were observed in the LMM results for the constant immersion phase, where the wet weight percentage change (Ww_C), length percentage change (L_c), and F_v/F_m of *F. virsoides* apices showed strong statistically significant differences between the treatments ($\chi^2 < 0.001$). For the wet weight percentage change (Ww_c), Tukey *post-hoc* tests revealed that there were strong statistically significant differences between most treatments ($p < 0.001$), except for T-29 vs. C-14 ($p = 0.021$), for which the difference was less pronounced. However, no statistically significant differences were found between T-25 and C-14 or between T-25 and T-20. A similar pattern was also observed in the length percentage change (L_c) ($\chi^2 < 0.001$), with strong statistically significant differences ($p < 0.001$) between most treatments except for T-29 vs. C-14 ($p = 0.021$), for which the significance was lower. The results for T-25 vs. C-14 and T-29 vs. T-25, on the other hand, showed no significant differences. The GLMM analysis of F_v/F_m in the constant immersion phase revealed the same patterns of significant differences as those in the air exposure phase ($\chi^2 < 0.001$), with only the results of T-33 treatment significantly differing from the other treatments ($p < 0.001$). The full results of the data analysis are summarized in Supplementary Table S1.

Principal coordinates analysis

PCO analysis of the air exposure phase dataset revealed strong separation of the T-33 data points from those of the other treatments, including the control C-14 (Fig. 7A). No particular separation was observed among the other treatments and the control. Most of the overall separation occurred along the PCO1 axis (92.4%). The overlayed vectors indicate that wet weight (Ww, g) and length (Length, cm) had strong negative associations with PCO1 (−0.557 and −0.999, respectively), emphasizing the roles of both as indicators of stress. The F_v/F_m only slightly positively influenced PCO1 (0.102).

A very similar pattern was observed for the constant

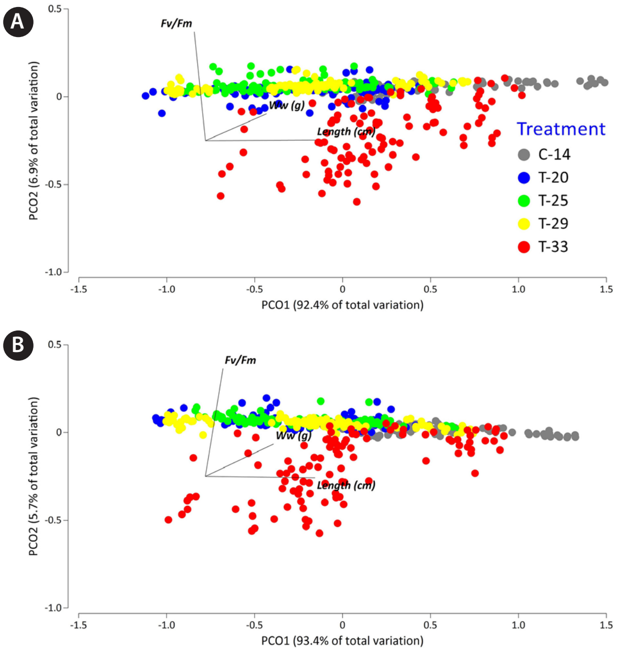

Fig. 7. Principal coordinates analysis (PCO) plot based on the Euclidean distance of wet weight (Ww, g), length (cm), and maximum photochemical yield (F_v/F_m) measurements during the air exposure phase (A) and constant immersion phase (B) of the experiment.

immersion phase, with most of the overall separation occurring along the PCO1 axis (93.4%). The strong separation of T-33 treatment may indicate the inability of severely stressed and damaged apices to recover properly from the induced stress (Fig. 7B). Ww (g) and Length (cm) showed a very strong association with PCO1 (0.621 and 0.999, respectively), indicating their influence in explaining the effects of the treatments. On the other hand, the F_v/F_m was only slightly related to the PCO1 axis (0.161).

DISCUSSION

The findings of this study indicate that moderately increased temperatures during periods of air exposure (20, 25, and 29°C) do not represent a significant threat to *Fucus virsoides*. However, extended exposure to relatively high air temperatures, such as 33°C, can cause long-lasting damage to the thalli of this species. This conclusion was based on analyses of changes in the wet weight, length, and F_v/F_m of *F. virsoides* apices. In addition, statistical analysis, as well as PCO plots, further suggested that a particularly high air temperature (33°C) may have detrimental effects on the morphology and physiology of *F. virsoides* thalli.

Certain factors that could affect algal physiology in the field could not be tested in this study. These include (1) the effects of repeated exposure to direct sunlight (variable intensity and wavelength) on *F. virsoides*' physiological status, (2) the effects of specific moisture levels on *F. virsoides*' physiological status (but see Descourvières et al. 2024*b*), and (3) the effects of specific light intensity levels (laboratory conditions) on *F. virsoides* physiological status. Air temperature, sea temperature, salinity, pH, humidity, wind and wave exposure, wind speed and direction, as well as coastal geomorphology, can strongly influence the presence and persistence of a species in its natural environment (Lipizer et al. 1995, Orlando-Bonaca et al. 2013, Gljušćić et al. 2023). While assessing the effects of individual factors or certain interactions is possible, the combination of multiple involved factors is difficult to reproduce in the laboratory because of the complexity of the required experimental approach, and especially the low availability of *F. virsoides* samples *in-situ* for research activities.

To further contextualise our findings, we compared them with those from a thermal tolerance experiment conducted on *Gongolaria barbata*, a shallow-dwelling (intertidal and shallow subtidal) brown alga from the Istrian coast, in which the effects of marine heatwaves on its thallus integrity and physiological state were examined (Bilajac et al. 2024). Although the two species differ in morphology, life-history traits, and habitat preferences, both experiments identified a recognisable threshold beyond which severe stress and damage occur. However, the strategies by which these species cope with elevated temperatures diverge noticeably as a consequence of their different life-strategies. *G. barbata* displays marked seasonal dynamics; notably the species is vegetatively active in winter and spring, followed by a distinct aestivation phase in summer, during which the thallus is reduced to a persistent cauloid. New growth resumes in autumn from adventive branches (Iveša et al. 2022, Bilajac et al. 2024).

In contrast, *F. virsoides* exhibits more continuous annual growth, with more moderate seasonal variations. Growth is typically more pronounced during cooler months and notably reduced or even fully paused in late summer and autumn, but the species never enters a proper aestivation period (Linardić 1949, Gljušćić et al. 2023). Compared to *G. barbata*, as a true intertidal species, *F. virsoides* is exposed to much more dynamic conditions, regularly alternating between immersion in seawater and exposure to air. These fluctuations also vary seasonally, potentially exposing the thalli to either cooling or heating during emersion. This habitat-specific variability may offer *F. virsoides* some capacity to mitigate thermal extremes through, for instance, submersion during marine heatwaves in summer, or increased light availability and temperatures during winter via emersion (Fig. 4A, Supplementary Fig. S1). These contrasting strategies, such as seasonal dormancy in *G. barbata* and inherent tolerance to environmental fluctuations in *F. virsoides*, reflect different evolutionary pathways for coping with thermal stress. The hypothesis that *F. virsoides* may partially circumvent heatwaves via submersion during summer or utilise more sunlight and higher temperatures via emersion during winter and early spring, should be explored in future experiments.

As briefly noted in the Introduction, *F. virsoides* typically develops receptacles during the colder months, particularly in spring (Vatova 1948, Linardić 1949), a pattern consistent with other cold-water fucalean species. The normally gradual increase in seawater temperature from winter to late spring may trigger the onset of reproductive activity, which could also be replicated under *ex-situ* conditions for cultivation purposes. However, we have observed that receptacle formation occurs year-round, albeit in varying and limited quantities, at several *F. virsoides* sites (unpublished data). The underlying cause of this atypical pattern, while potentially linked to temperature shifts, remains unknown and is currently being studied.

The variations in the measured temperatures in the study area, even when considering the exposure of the logger to sunlight and the high measurement frequency, indicated the frequent emersion/immersion of the data logger and the nearby *F. virsoides* thalli (Fig. 4A), especially in spring (Fig. 4B). These extreme variations are likely due to a combination of tidal dynamics, atmospheric forcing (pressure and wind effects) and variable exposure to sunlight (due to geomorphology and the terrestrial vegetation canopy). While not consistent with the experimental results due to the difference in exposure to specific temperatures during similar timeframes (low or high water level periods), this study provides insight into the instability and complexity of the habitat inhabited by *F. virsoides*, in addition to the actual resilience of the species to acute and extended thermal shocks during both the natural and simulated emersion periods. Moreover, overlaying hourly temperature data with actual hourly water level measurements (either from a data logger or corrected measurements from the nearest mareographic station) viewed over short increments could shed more light on this complex dynamic.

Direct sunlight as a source of heat for the thalli during emersion (indicated by the data logger records) warrants further investigation, although some related research has been conducted regarding the effects of UV radiation on *F. virsoides* (Hanžek 2014). Field observations in 2023 and 2024 revealed that the remaining *F. virsoides* patches along the Istrian coast persist in shaded areas created by local geomorphology or terrestrial plant canopies. Hourly light exposure intensity measurements were conducted in tandem with the temperature measurements *in-situ* and, although not considered in this study (but see Supplementary Fig. S1), could inspire future research. Moreover, further study into the complexity of the intertidal zone in the northern Adriatic (persistence of *F. virsoides* notwithstanding) is planned.

The atmospheric effects on the sea level in the Adriatic Sea, especially in enclosed bays and channels, should not be ignored (Marcos et al. 2009). These effects can potentially either exacerbate or mitigate those of emersion in intertidal communities, potentially playing a significant role in shaping at least some intertidal areas, such as the habitats of fucalean species, as well as other communities adapted to the unique northern Adriatic conditions. Unfortunately, specific research on these regional dynamics is currently lacking.

Notably, the aerial exposure of 6 h during the experiment did not necessarily represent a typical *in-situ* situation, such as when conditions vary dramatically over a short period of time because of multiple factors (Fig. 4A & B). These include, for instance, changes in humidity and desiccation levels and occasional rainfall, along with the vertical zonation and growth rates, as ascertained in *F. spiralis* (Schagerl and Möstl 2011). Fluctuations in such abiotic factors can imply that stressful periods alternate with favourable conditions for recovery over different time scales (even randomly) than those used in our experiment. Further research is needed to specifically assess how *F. virsoides* responds to such high environmental complexity.

Linardić (1949) hypothesised that the reason *F. virsoides* is the only extant species of its genus in the Mediterranean is that high air temperatures during summer periods prevent Atlantic species' range expansion, although temporary occurrences have been documented (Sancholle 1988). However, at the time, the author did not consider specific tidal dynamics and air exposure periods in relation to day-night cycles as well as season cycles because: (1) the tidal ranges, although still in the microtidal range, are significantly greater in the Adriatic basin (especially the northern part) than in the other parts of the Mediterranean, (2) emersion during the warm period (late spring, summer and early autumn) occurs mostly during the night or early morning, when the relative air temperature is lower, and (3) emersion during colder periods (late autumn, winter and early spring) occurs during the day or evening, when the relative air temperature is higher and direct sunlight is available (data obtained from the "Asterion" webpage https://www.asterion.info/) (Hydrographic Institute of the Republic of Croatia 2025). Importantly, however, *F. virsoides* can be partially or fully emersed during less intense, as well as "quarterly lunar" neap tides, especially in cases with overlapping atmospheric conditions that can exacerbate the reduction in the overall water level (Fig. 4).

Understanding canopy structure is crucial when studying intertidal canopy-forming species. Like those of many congeneric species, the canopy of *F. virsoides* has a layered arrangement, although little is known about the role of its different thalli stages within the canopy structure (Munda 1972). In healthy canopies, upper layers are most exposed to stress due to desiccation, whereas lower layers retain moisture more efficiently, sheltering smaller or younger individuals and associated taxa. Such observations have been noted by Linardić (1949); more densely growing thalli appeared in better physical condition (less necrosis) than the more isolated patches. Today, however, such structural complexity is mostly lost, further reducing the long-term survivability of *F. virsoides.*

Shifts in atmospheric conditions and the frequency of extreme events (e.g., heatwaves and severe storm events) due to climate change or changes in habitat quality (e.g., coastal modification and pollution) have already been linked to the loss of many fucalean assemblages globally (Thibaut et al. 2005, Ling et al. 2015, Iveša et al. 2016, Eger et al. 2022). *F. virsoides* is especially vulnerable to climate change due to its limited geographical distribution, as it is essentially "cornered" in the northern Adriatic (Boero et al. 2008). Its survival is also threatened by ever-expanding tourism infrastructure on the eastern Adriatic coast, severely altering the natural coastal ecosystem and increasing pollution (but see Orlando-Bonaca et al. 2013), as well as by the lack of meaningful monitoring or conservation strategies for the species (Mangialajo et al. 2008, Gianni et al. 2017, Orlando-Bonaca et al. 2022).

There is an urgent need to understand how increased temperature stress affects fucalean algae, especially during earlier stages of development (e.g., recruits and juveniles), notably due to the increased efforts in the restoration of already lost or degraded fucalean assemblages across the Mediterranean (Thibaut et al. 2015, Cebrian et al. 2021, Bilajac et al. 2024, Lokovšek et al. 2024). While mostly concerning *Cystoseira sensu lato*, many of the developed cultivation techniques (Cebrian et al. 2021, Orlando-Bonaca et al. 2021, Eger et al. 2022, Gljušćić et al. 2023) can be applied to the cultivation of other fucalean species, such as *Sargassum* and *Fucus* species. Wild-growing *F. virsoides*, on the other hand, is considered an obligate intertidal species that is closely tied to the tidal dynamics of the area it inhabits (Linardić 1949). While not necessarily required for successful cultivation trials (see Gljušćić et al. 2023), it could be worthwhile to perform tidal simulations during cultivation, including of temperature shifts between air and seawater, to ascertain the corresponding effects on long-term growth and development of algal recruits.

In conclusion, the results of the experiment suggest that *F. virsoides* can be vulnerable to increased air temperatures during warm and dry weather conditions, when these conditions coincide with extended periods of air exposure. In contrast to the assumptions for the conducted experiment, the temperature values, water level variations, and their relationships reflect an unpredictable environment in the study area, with some emerging patterns that warrant further research. While *F. virsoides* is still generally resilient to acute stress, the increasing temperatures, shifting extremes, instabilities resulting from climate change, atmospheric and marine heatwaves, and limited geographical distribution appear to be pushing the tolerance limits of this species.

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CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary Table S1. Results of LMM and GLMM analysis of response variables (change in wet weight, length, and maximum photochemical yield [F_v/F_m]) for both phases of the experiment (https://www.e-algae.org).

Supplementary Fig. S1. Seasonal occurrence of very low water levels (≤10 cm from mareographic zero) in Blaz cove (https://www.e-algae.org).

Supplementary Fig. S2. Example of *Fucus virsoides* apex measurement via ImageJ (A) according to reference pictures using a yellow line as a marker (B) (https://www.e-algae.org).

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