Structural and functional ecological traits in young and adult thalli of canopy-forming brown macroalga *Gongolaria barbata* (Phaeophyta) from a transitional water system (#94957)

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Structural and functional ecological traits in young and adult thalli of canopy-forming brown macroalga *Gongolaria barbata* (Phaeophyta) from a transitional water system

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Background: *Gongolaria barbata* is a canopy-forming brown macroalgae in intertidal and subtidal habitats of cold-temperate latitudes, largely exposed to current environmental changes due to a combination of global and local factors. Testing whether this species is featured by specific ecological traits allowing efficient use of habitat resources and adaptation to environmental stress, and if this potential might change with population growth, is important to predict the performance of the algae under different environmental settings.**Methods**: Young (juveniles) and adult thalli of *G. barbata* were sampled in the winter season from the Venice Lagoon, Italy, and analyzed for dry matter content (TDMC), photosynthetic activity, photosynthetic pigment content, and antioxidant capacity to assess if thallus age may be considered a significant driver in determining the ecological responses of this species to environmental abiotic factors. **Results**: Our results showed that TDMC was higher in adults than juveniles. At the functional level, rapid light response curves indicated an elevated photosynthetic efficiency in juveniles according to the higher quantum yield of PSII electron transport, electron transport rate, and Rubisco content than in adults, which showed a higher thermal dissipation of light energy and total pigment concentration. No difference in maximum PSII photochemical efficiency and D1 protein content between the two thalli groups was found. Along with better photosynthesis, juveniles also showed an increased content of total polyphenols, flavonoids, and tannins, and consequently displayed a stronger antioxidant capacity compared to adult individuals. **Conclusions:** Our results highlighted remarkable growth-stage differences in the ecological traits of *G. barbata*: younger thalli allocate more energy into photosynthesis and chemical defenses, potentiating antioxidant compounds and being more vulnerable to

abiotic and biotic variables. With advancing age, thalli likely moved toward conservation strategy, reducing photosynthesis, and enhancing structural biomass to limit the risks of damage due to prolonged exposure to environmental stressors (i.e., waves, currents).

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Abstract

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Keywords: Antioxidants, adaptation, brown seaweed, environmental stress, photosynthesis, thallus age.

Introduction

Canopy-forming brown macroalgae (i.e., kelps, fucoids), are well-known for their crucial role in forming habitats in both intertidal and subtidal habitats of cold-temperate latitudes. These habitat-formers increase the three-dimensional complexity and spatial heterogeneity of the habitat they colonize (*Verdura et al., 2018*). Their vertical and branched canopies increase coastal primary production, offer shelter to smaller epiphytic algae and many meiofaunal invertebrates, represent nursery areas for juvenile fish, and protect them from predators and hydrodynamics (*Krumhansl et al., 2016*; *Verdura et al., 2018*; *Orlando-Bonaca et al., 2021*; *Gran et al., 2022*; *Manca et al. 2022*). The conservation status of these long-living species is indicative of habitat loss, environmental degradation (*Orlando-Bonaca et al., 2021*) and quality of Mediterranean coastal waters (*Ballesteros et al., 2007; Orlando-Bonaca et al., 2013*). Finally, they also contribute to many ecosystem services by providing foraging and preserving species of commercial interest, sustaining coastal fisheries, absorbing pollutants and filtering water, reoxygenating sediments and acting as an important sink for carbon through its sequestration to the seafloor; hence, they are known to be one of the most productive ecosystems on Earth (*Gran et al., 2022; Manca et al., 2022*). Regardless of all benefits, these communities are exposed to multiple stressors and threatened by human activities, including eutrophication, pollution, outbreaks of grazers caused by overfishing, invasive species introduction, increasing sediment resuspension and climate change-driven consequences (*Ballesteros et al. 2007; Orlando-Bonaca et al., 2013, 2021*). Species belonging to the genus *Cystoseira s.l.* (*Molinari-Novoa &Guiry, 2020*) are endemic of the Mediterranean, classified as threatened (except for *C. compressa*) under the Barcelona Convention (Annex II of the Barcelona Convention, COM/2009/0585/FIN), and protected by local regulations. Despite the reduction of impacts imposed by legislation, *Cystoseira s.l.* forests experienced regression events at the basin scale that led to habitat loss (*Cebrian et al., 2021*; *Verdura et al., 2023*), and, in some cases, to regime shift to algal turfs, which are less complex and poorly productive communities inhibiting recolonization by canopy-forming species (*Benedetti-Cecchi et al., 2015*). Understanding if the species is featured by different ecological traits according to the growth stage is important to assess its potential ecological adaptation or vulnerability to future changes in environmental settings.

In recent years, several studies have been carried out to assess the ecological strategies of aquatic plants in terms of resource-use strategies by evaluating physiological and structural attributes (*Sakanishi et al., 2023*). Characterizing how organisms' functional traits vary along environmental gradients or in highly disturbed ecosystems can reveal species' capability to face multiple stresses (*Cornwell & Ackerly, 2009*). The thallus age, as plant ontogeny in terrestrial ecosystems (*Rusman et al., 2020*), could represent a valuable trait in assessing growth-defense mechanisms and exploited strategies against multiple environmental stresses (*Pellizzari et al., 2008*). Despite their importance, adaptation patterns of canopy-forming macroalgae of different ages are rarely investigated, neglecting how such studies can provide new insights into species vulnerability and resistance in their habitat.

The objective of this study was to characterize juvenile and adult individuals of *G. barbata* ((Stackhouse) Kuntze 1891) collected from the Venice Lagoon, a transitional water system, for crucial functional and structural attributes, including photosynthetic efficiency in vivo and production of secondary metabolites.

We hypothesize that in the species *G. barbata*, some critical ecological traits, namely photosynthetic performance, antioxidant defenses, and dry matter content, can change with growth, affecting local adaptation mechanisms since this variation is expected to reflect different resource acquisition vs conservation (stress tolerance) strategies in *G. barbata* populations.

Materials & Methods

Sampling and experimental design

Different juveniles and adult individuals (n=10) of the species *G. barbata* were randomly harvested at the beginning of February 2023, at the offshore Ca' Roman natural reserve (45°14'42.2"N 12°17'44.7"E), in the Venice Lagoon (Fig. 1) at a depth of two meters. The permission for sampling was provided by Regione del Veneto (decree number 369, date 04.05.2023). Juveniles and adult thalli were selected in situ on length basis through direct observations and measurements. Thallus age was estimated according to the well-known relationship between age and thallus length (*Khailov & Firsov, 1976*). Therefore, we identified two groups of algae, each of five individuals: the adults, over one year old, with a thallus length in the range 40-50 cm, and the juveniles, less than one year old, with a thallus length within 10-20 cm.

On both juvenile and adult thalli were conducted measurements of photosynthetic efficiency in vivo. The same thalli were then analyzed for thallus dry matter content (TDMC), photosynthetic pigments, antioxidants, PSII D1 and Rubisco proteins.

Figure 1

Environmental variables at the sampling site

The growth environment of the harvested specimens of *G. barbata* is classified as a transitional water system (TWS), and being part of the Venice Lagoon, is closely related to the Adriatic Sea, meaning that its biochemical conditions are strongly influenced by those of the seawater environment, as well as by human activities.

The analysis of biogeochemical variables at the sampling site, made using the Copernicus Marine Environment Monitoring Service (*CMEMS*) database, during the period 2020-2023, showed that seasonal variations of temperature (Fig. 2a) were stable, with peaks of 25-26 °C during summer and of 8-9 °C during winter. *G. barbata* is a species known for its adaptability to a wide range of temperatures, with recruits developing optimally at 15°C and sufficiently from 10°C to 25°C (*Orfanidis, 1991*). Adult individuals in the vegetative phase can endure high temperatures up to 30-34°C during summer, and freezing temperatures, even below zero, during winter (*Ivesa et al., 2022*).

Regarding pH, reported on the total scale, fluctuations were in line with the general trend of the Adriatic Sea and ranged from 8.045 to 8.175 (Fig. 2b). Studies about the effect of acidification on *Cystoseira* s.l. demonstrated good tolerance of pH decrease and rather an enhancement of growth rate, photosynthetic production, antioxidant activity, and photoprotection (*Celis-Plá et al., 2017*).

Salinity, expressed in Practical Salinity Units (PSU), showed seasonal variations slightly different between spring-summer periods of 2021 and 2022, that may be due to higher loads of freshwater from estuaries and rainfalls, which were more abundant in 2022 than 2021 (*ARPAV Centro Meteorologico di Teolo, Stazione Chioggia - Sant'Anna*) (Fig. 2c). *G. barbata* is a well-known adapted species to both euhaline and polyhaline environments (*Sadogurska et al., 2021*; *Tursi et al., 2023*), and even broad changes in salinity do not affect its growth (*Baghdadli et al., 1990*; *Irving et al., 2009*).

Events such as high nutrient loads discharge, mainly nitrogen and phosphorous, and consequent eutrophication of lagoonal waters occurred steadily in the Venice Lagoon since the 1920s, along with the direct release of heavy metals and organic micropollutants (*Morand & Briand, 1996*; *Caliceti et al., 2002*; *Pastres et al., 2004*). The construction of wastewater treatment plants and the total ban on the use of phosphorous in detergents in 1989, contributed to a fall in nutrient loads, and eutrophication subsists only in proximity of the industrialized shores (*Acri et al., 2020*; *Zirino et al., 2016*). As shown in Fig. 2d, for what concerns the period 2020-2023, nitrogen loads were overall below the threshold of 18 μmol L^{-1} for water bodies with salinity > 30 (PSU), set by the national legislation for the implementation of the Water Framework Directive (MD 260/2010), with peaks exceeding the limit occurring during winter. In contrast, phosphorous levels always exceeded the threshold of 0.48 μmol L^{-1}, except during summer, when usually phytoplankton blooms occur, and primary productivity is at its highest (Fig. 2d).
Although nutrient loads have been reduced and the trophic status of the Venice Lagoon improved significantly (*Çevirgen et al., 2020*), the comparison between data from 2017-2019 (*Regione Veneto*, *ARPAV*, *ISPRA*, *2021*) to 2020-20203 (*Copernicus Marine Environment Monitoring*

Service) suggests a discouraging increase in nitrogen and phosphorous concentrations. However, a good – or sufficient- environmental state was recently recognized, using the Trophic Index (TRIX) assessment (*Vollenweider et al., 1998*), for most of the lagoonal waters (*Çevirgen et al., 2020*; *ARPAV TRIX trophic index for coastal marine waters, 2022*).

Figure 2

Photosynthetic efficiency of thalli

Photosystem II (PSII) chlorophyll-a fluorescence analysis in vivo was performed on the apical part of juvenile and adult thalli of *G. barbata* to assess the photosynthetic performance, by means of pulse amplitude modulated fluorometer (Junior-PAM, Walz Gmbh Effeltrich, Germany). Photosynthetic activity was measured in response to increasing irradiance levels, test the light-use efficiency of thalli in photochemistry. Rapid light response curves (RLCs) were performed on four individuals for each group (n=4) considering 8 actinic light steps at a Photosynthetic Photon Flux Densities (PPFD) of 125, 190, 285, 420, 625, 820, 1150, 1500 µmol photons m^{-2} s^{-1} and lasting 60 s each to allow the steady-state fluorescence in actinic light (*Nielsen & Nielsen, 2008*; *Porzio et al., 2020*). Thalli were disposed at 0.5 mm from an optic fibre of 1 mm diameter inclined at 45° respect to samples immerged in seawater suspension. To measure the PSII maximum photochemical efficiency, F_v/F_m, thalli were 15 min dark-adapted to allow full oxidation of the PSII reaction centres (*Porzio et al., 2017*). Basal fluorescence (F_0) was achieved by applying a weak blue light signal of 1-2 µmol photons m^{-2} s^{-1}, the maximum fluorescence level in the dark (F_m) was obtained by applying a saturating light pulse of 7000 µmol photons m^{-2} s^{-1}. The maximal photochemical efficiency of PSII, F_v/F_m, was calculated according to the formula: $F_v/F_m = (F_m-F_0)/F_m$ (*Beer et al., 2014*). The quantum yield of the PSII electron transport (Φ_{PSII}) was calculated according to *Genty et al.* (*1989*) following the equation: $\Phi_{PSII} = (F_m'–F_t) /F_m'$. The Electron Transport Rate (ETR) was evaluated as: ETR=Φ_{PSII} *PAR*0.5*AF (*Schreiber, 2004*), where AF (absorbing factor) corresponds to AF= (incident PAR–transmitted PAR)/incident PAR and the value of 0.5 represents the energy equally distributed between the two photosystems. Non-photochemical quenching (NPQ) was determined as: $NPQ = (F_m-F_m')/F_m'$ (*Bilger & Björkman, 1990*).

Thallus dry matter content

The Thallus Dry Matter Content (TDMC) consists of the proportion of structural compounds and water-filled tissues, which are mainly photosynthetically active. It represents an important functional trait often utilized to assess the adaptability of algae to its environment (*Cappellatti et al., 2019*). For the TDMC determination, single individuals (n=5) were weighed soon after sampling to determine the fresh mass, successively the samples were incubated in an oven at 37°C for 24 hours and weighted again to measure the dry mass. Finally, the ratio between dry mass and fresh mass was determined.

Photosystem II D1 protein and Rubisco determination

Algal samples were fine grounded with liquid nitrogen by a mortar and pestle. Samples were kept on ice in eppendorfs and mechanically homogenized using a pestle and 200 µl of 1x PEB (protein extraction buffer, product no AS08300, Agrisera, Vännäs, Sweeden). Then, samples were centrifuged at 14.000 rpm for 20 min at 4°C and the supernatants transferred into new tubes. Protein extracts were quantified with the Bradford assay (*Bradford, 1976*), using the BioRad Protein Assay Dye Reagent Concentrate (Bio-Rad Laboratories, Milan, Italy) and the bovine serum albumin (BSA) has been used as a protein standard. The SDS-PAGE (10%) was carried out following *Vitale et al*. (*2022*) with slight modifications. Briefly, the western blot procedure started with the blocking solution (100 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.1% Tween20, 5% Milk). To reveal the protein of interest, samples were incubated with the primary antibody (Agrisera, Vännäs, Sweeden) anti-PsbA (rabbit, 1:15000 v/v, AS05 084) for D1 protein of PSII and anti-RbcL (rabbit, 1:10000 v/v, AS03037) for Rubisco. Goat anti-Rabbit IgG (H&L), HRP conjugated (1:6000 v/v, AS09 602) was used as the secondary antibody. Immuno-revelation was performed using the kit for chemiluminescence (Westar supernova, Cyanagen Srl, Bologna, Italy) via ChemiDoc System (Bio-Rad). The software Image Lab version 5.2.1 (Bio-Rad Laboratories, Hercules, CA, USA) was utilized for the densitometric analysis: band signals were quantified, and the background values were subtracted to obtain and adjusted volume in counts for each band. The density value was expressed in arbitrary units and represented as a boxplot.

Photosynthetic pigment content analysis

Photosynthetic pigments content, namely total chlorophylls (a + c) and total carotenoids, was determined on 5 individuals per group, performing 3 replicates each (n=15). The analysis was performed according to (*Jeffrey & Humphrey, 1975*) and (*Lichtenthaler, 1987*) following the procedure reported in *Porzio et al.*, (*2017*). Samples from each thallus (0.040 g of dried powder) were mechanically extracted in 100% acetone inside glass test tubes and left to rest for half an hour in ice and total darkness, to avoid photo-oxidation phenomena. The extracts were centrifuged at 5000 rpm for 5 min in a Labofuge GL (Heraeus Sepatech, Hanau, Germany). The sample absorbance was measured by a spectrophotometer (UV-VIS Cary 100; Agilent Technologies) at wavelengths of 662 nm, 630 nm, and 470nm for chlorophyll a, chlorophyll c and total carotenoids, respectively. Pigment concentration was expressed as µg g^{-1} of dried weight (µg g^{-1} DW).

Soluble antioxidants and antioxidant capacity determination

The polyphenol content was evaluated on 5 individuals per group, performing 3 replicates each (n=15), through the Folin-Ciocalteu method following the procedure reported in *Fabrizzi et al.* (*2023*). Methanolic extracts were made pestering 0.200 g of dried powder in 2 ml of cold methanol and were stored at 4°C for 24 hours to ultimate the extraction. Then, samples were centrifuged at 4°C, 11.000 rpm for 10 minutes in a SL 16R centrifuge (Thermo Fisher

Scientific™, Germany). Then, the supernatant was mixed with 10% Folin–Ciocâlteu solution, 1:1 v/v, and after 3 min, 700 mM Na_2CO_3 solution was added to the resulting mixture (1:5, v/v). Samples were incubated for 45 min in total darkness, and the absorbance was measured at 765 nm by a spectrophotometer (UV-VIS Cary 100; Agilent Technologies). The total polyphenol content was expressed as mg of Gallic Acid Equivalents g^{-1} DW (mg GAE g^{-1} DW) using a gallic acid standard curve. The total flavonoid content was assessed according to the procedure of *Moulehi et al.* (*2012*) and (*Sun et al., 1998*). Methanolic extracts were mixed with a solution of 5% $NaNO_2$ (ratio 3:1 v/v); after 6 minutes, a 10% solution of $AlCl_3$ and a 1M solution of NaOH were added, adjusting the volume with distilled water. Samples were left resting in darkness for 15 minutes to let the colorimetric reaction happen and finally the absorbance was measured at a wavelength of 510 nm. The total flavonoid content was estimated through a standard catechin curve and expressed as mg of catechin equivalent per gram of dried weight (mg CAT g^{-1} DW). Total condensed tannins were estimated by modifying the procedures described by *Sun et al*., (*1998)* and *Moulehi et al*. (*2012*), as reported by *Costanzo et al*. (*2022*). Briefly, 2.5mL of methanol-vanillin solution and 2.5mL of 97% H_2SO_4 were mixed with 1mL of sample methanolic extract. Then, the mixture was incubated for 15 minutes in total darkness, and the absorbance was measured at 500 nm. Tannins were quantified with a catechin standard curve and expressed as mg catechin equivalents per gram of dry weight (mg CAT g^{-1} DW). The antioxidant capacity was measured through the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, where 0.067 mL of methanolic extracts were added to 2mL of $6x10^{-5}$ M DPPH in methanol solution and heated at 37°C for 20 minutes in a dry bath (Benchmark scientific, My block™ Mini Dry Bath). Then, absorbance was measured at a wavelength of 515 nm. Antioxidant capacity was assessed using Trolox as positive control and expressed as percentage of radical inhibition using the formula: % inhibition= ((white Abs- sample Abs)/ white Abs) *100.

Statistical analysis

To assess the statistically significant differences between the groups (adults and juveniles), the PSII maximal photochemical efficiency (F_v/F_m), electron transport rate (ETR), non-photochemical quenching (NPQ), photosynthetic pigments content, photosynthetic protein amounts and antioxidant concentrations were compared performing t-test using the Sigma-Stat 12.0 software (Jandel Scientific, USA). Differences were considered statistically significant for $P \leq 0.05$. The data reported correspond to average ± standard error. Asterisks indicate statistically significant differences (*** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, ns $P \geq 0.05$). Boxplots report interquartile range, mean line, whiskers, and outliers. For the RLCs, t-tests were performed on the whole data set of RLC-curves and at each PPFD value. Data were plotted and visualized by means of R environment software (version 4.2.2., R Core Team 2022).

Results

Thallus dry matter and photosynthetic pigments content

TDMC showed a significant difference between the groups with higher values (P=0.019) for adult than juvenile thalli. Pigments differed significantly between adults and juveniles, showing higher values in adults for both total chlorophylls (P=0.001) and carotenoids (P=016) compared to juveniles (Table 1).

Table 1

Fluorescence emission fast kinetics curves

The analysis of the quantum yield of PSII electron transport (Fig. 3a) evidenced higher values (P=0.020) in juveniles. Specifically, up to 125 µmol photons m^{-2} s^{-1}, the two groups did not show any statistical differences. Conversely, in the range of PPFD from 190 to 1500 µmol photons m^{-2} s^{-1} juveniles showed higher Φ_{PSII} values (P≤0.01) compared to adults. The PSII electron transport rate statistically differs (p=0.016) between adults and juveniles. In detail, starting from 285 µmol photons m^{-2} s^{-1}, young thalli showed a higher electron transport activity (P≤0.01). (Fig. 3b). Conversely, the non-photochemical quenching (NPQ) of adults and juveniles exhibited statistically significant differences only at the intermediate PPFD levels, namely from 190 to 420 µmol photons m^{-2} s^{-1}, reaching the highest (P≤0.01) values for adult thalli (Fig. 3c). The maximum photochemical efficiency (F_v/F_m) was not statistically different (P≥0.05) between the two groups: the ratio was 0.619 ± 0.041 in adults and 0.612 ± 0.013, in juveniles (Fig. 3d).

Figure 3

Photosystem II D1 protein and Rubisco determination

D1 protein densitometric analysis (Fig. 4a,c) showed similar concentrations for adults and juveniles without statistically significant differences. Conversely, the Rubisco protein was more abundant in juveniles (P=0.001) than in adults, displaying an increase of about 44% (Fig. 3a,b).

Figure 4

Soluble antioxidants content

Total polyphenols content showed significant difference (P=0.0003) between the two groups, with higher values in juveniles (4.948 ± 0.206 mg GAE g^{-1} DW) than adults (3.570 ± 0.102 mg GAE g^{-1} DW) (Fig. 5a). The same behavior was observed for total flavonoids (P<0.00001), with 13.373 ± 0.662 mg CAT g^{-1} DW for juveniles and 6.554 ± 0.279 mg CAT g^{-1} DW for adults (Fig. 5b). Also, tannins were higher (P=0.012) in juveniles (74.880 ± 4.387 mg CAT g^{-1} DW) than adults (55.075 ± 4.345 mg CAT g^{-1} DW) (Fig. 5c). In compliance with these results, radical scavenging activity confirmed to be higher (P<0.00001) in juveniles (% inhibition = 73.463 ± 0.331) than in adults (% inhibition = 61.588 ± 1.164) (Fig. 5d).

Figure 5

Discussion

Our study explores if *G. barbata* is featured by specific ecological traits allowing an efficient use of habitat resources and better adaptation to environmental stress, and if this potential might change with population growth. The results evidenced growth-stage differences in the ecological traits of this species, more specifically in the regulation of photosynthetic light reactions and antioxidant modulation, as well as in biomass partitioning between adult and younger individuals.

The demand for nutrients and photosynthetic activity may change during thalli growth to meet the organism's requirements. This modulation is functional to species adaptability and survival, especially in instable habitats.

The site of our study is characterized by seasonally excursion of environmental variables (i.e. temperature, salinity, nutrients, and pH) which are within the tolerance range for *G. barbata* species (*Orfanidis, 1991*; *Irving et al., 2009*; *Iveša et al., 2022*). However, the ongoing climate change, especially rising level of temperature, could exacerbate the excursions, priming in thalli safety stress-responsive strategies, involving structural and functional mechanisms.

Seasonal variations of morpho-functional attributes are common in Phaeophyceae and other macroalgae in environments characterized by sudden changes in pH, temperature, and nutrients (*Lavaud & Goss, 2014*; *Porzio et al., 2017, 2020*).

One of the most relevant differences found between young and mature individuals was the thallus dry matter content (TDMC), whose modulation during growth is involved in organism survival (*Elger & Willby, 2003*). TDMC indicates the distribution between structural compounds and water-filled, nutrient-rich photosynthetically active tissues (*Cappelatti et al., 2019*). An increase in TDMC is generally associated with high resistance to wave damage and desiccation (*Cappelatti et al., 2019*). *Cystoseira* s.l. is known for seasonal variations in TDMC ratio due to their ontogenetic shift from the period of primary growth (winter-spring) to the period of dormancy (summer-autumn), when individuals shed many secondary branches, resulting in lower water content (*Orfanidis et al., 2017*; *Iveša et al.*, 2022).

In our study, even if TDMC was not measured seasonally, the increase of dry biomass found in adults compared to juveniles during winter, when water circulation was turbulent and temperatures low, likely suggested an investment of carbon in structural compounds with the age to cope with the occurrence of unfavorable environmental conditions such as grazing and water movement.

Notably, the elevated dry matter content, beyond to mechanical resistance, is also used as a proxy to predict variations in macroalgae palatability and resistance against grazers (*Elger & Willby, 2003*).

The different TDMC content between young and adult thalli was also accompanied by a diverse regulation of photosynthetic activity.

In our study the maximum PSII photochemical efficiency (F_v/F_m) close to values of 0.7 for both juvenile and adult thalli indicated the absence of stress condition for photosynthetic apparatus

(*Baker, 2008*). These results are consistent with studies on other Cystoseira spp. which showed comparable F_v/F_m ratio (*Celis-Plá et al., 2016; Mancuso et al., 2019*), confirming fully functional light transduction reactions in photosynthesis.
However, even if F_v/F_m did not change, the quantum yield of PSII electron rate (Φ_{PSII}), the electron transport rate (ETR), and the non-photochemical quenching (NPQ) were differently modulated in juveniles and adults to guarantee the photosynthetic carbon gain under continuous fluctuations of environmental parameters.
Individuals more than one year old have been exposed to habitat seasonal variability of temperature, pH, salinity, and nutrients for a more extended period than younger individuals, which may have induced the higher investment of carbon in structural compounds and low photosynthesis.
The fluorescence fast light curves analysis evidenced how juveniles and adults differently used harvested light in the photosynthetic process at both unsaturated and saturated irradiances. More specifically, juveniles invest more light energy into photosynthesis, displaying higher quantum yield of PSII electron transport (Φ_{PSII}) and electron transport rate (ETR), along with higher content of Rubisco. In young thalli, the higher activity of the electron transport chain is expected to produce more reducing power in the form of ATP and NADPH molecules, both used in the CO_2 fixation mediated by Rubisco, whose concentration increases with high photochemical activities (*Raven, 1997*; *Gylle et al., 2013*). Our data are consistent with other studies demonstrating that the amount of Rubisco may also be related to the thallus age, showing a decrement over time in adults. Indeed, as often observed in higher plants, photosynthetic capacity, and photosynthetic enzymes, such as Rubisco, decrease as age progresses (*Bertamini & Nedunchezhian, 2002*).
The higher photochemical activity in young thalli decreases the need for thermal dissipation processes within photosystems, explaining the reason for the reduced NPQ values, conversely to adults, where an increase of energy dissipation as heat represents an essential safety mechanism, in the PPFD range from 100 to 500 µmol photons m^{-2} s^{-1}.
In adults, the high NPQ coupled with elevated content of carotenoids, also involved in thermal dissipation of the light energy (*Lavaud & Goss, 2014*), pointed out the demand for photoprotection in longer thalli which are more subjected to unfavorable water surface conditions such as light excess, high or low temperature, higher UV levels, and extreme tide events than younger submerged thalli. Furthermore, beyond to a photoprotective function, the higher photosynthetic pigment content observed in adults may be also a way to compensate for the reduced photochemistry (*Porzio et al., 2017*).
In agreement with no difference in F_v/F_m ratio, the amount of the PSII D1 protein did not show any significant difference between young and adult thalli, highlighting that the reduction of photosynthetic efficiency in adults was due to the regulatory mechanisms of photosynthetic light reactions and not to impairments at photosystems. Being D1 protein the primary target of light-induced oxidative damage (*Mulo et al., 2012*), lower levels of this protein are correlated with photoinhibition and oxidative stress of PSII, which also results in a lower F_v/F_m ratio (*Schofield*

et al., 1998). Our results support the evidence of photosynthetic apparatus's healthy status and the high efficiency in light harvesting and transduction, proving the adaptive solid potential of *G. barbata* thalli in their habitat, regardless of age.

Interestingly, juveniles exhibited elevated levels of antioxidant compounds compared to adults. The increased synthesis of scavengers may be involved in photoprotection and against predation. Indeed, as *Mannino et al. (2016)* reported, young thalli need more protection due to their higher vulnerability to grazing and preferentially allocate resources towards chemical defenses. Indeed, phenolic compounds are well-known secondary metabolites in plants and algae, particularly abundant in brown macroalgae (*Phaeophyceae*) due to their exclusive production of phlorotannins (*Montero et al., 2019*). Polyphenols play structural, antibacterial, photoprotective, and herbivore deterrent roles (*Li et al., 2011*; *Steevensz et al., 2012*; *Stiger et al., 2014*; *Mancuso et al., 2019*).

Likewise, flavonoids are another class of phenolic compounds whose role, even if not thoroughly investigated in algae yet, is primarily to guarantee photoprotection by scavenging reactive oxygen species (ROS) (*Fernando et al., 2022*). The overall content of phenolic compounds in brown seaweeds is species-specific and may change according to environmental factors such as irradiance, UV, temperature, nutrients, and salinity (*Mancuso et al., 2019*).

The antioxidant range found in our study is comparable with values reported by other authors who analyzed specimens of *G.barbata* from natural environments (*Cadar et al., 2019*; *Castillo et al., 2023*). In particular, juveniles displayed higher levels of total phenolic compounds, supporting the hypothesis of an increased allocation of resources into chemical defenses.

To confirm the general trend of antioxidant compounds, radical scavenging activity, measured through the DPPH assay and expressed as % of inhibition of free radicals, was more significant in juveniles than adults. It may be hypothesized that the highest scavenging activity of young thalli has been due mainly to tannins, polyphenols, and flavonoids, as observed in previous studies on other Phaeophyceae (*Ruiz-Medina et al., 2022*).

Conclusions

The overall data suggests that both young and adult thalli of *G. barbata* can thrive in the Venice lagoon transitional water system, characterized by unstable environmental conditions, which are expected to become more exacerbated in the future, approaching temperatures never reached. The adaptability of adult and juvenile thalli to these fluctuations is obtained thanks to different ecological strategies, which allow their survival in changing environments.

More specifically, juvenile thalli, despite low structural biomass, exhibited higher photosynthetic efficiency, which, along with an effective antioxidant defense system, make them fitter to peculiar conditions of the transitional water system. On the contrary, in adults, the highest investment of carbon in structural biomass together with higher photosynthetic pigment content, especially carotenoids, and thermal dissipation processes, despite low photosynthesis, allow to avoid photoinhibition and cope with environmental habitat fluctuation in the long term.

Our data highlight how the flexible regulation of photosynthetic light reactions in *G. barbata*, as age progresses, is a crucial prerequisite to provide sufficient energy flow to downstream metabolism and thalli growth in response to continuous fluctuation of environmental variables, to protect photosynthetic apparatus against photodamage.
Thus, including age as a further distinctive trait for *G. barbata* in future studies may clarify the potential of species adaptation and response to forthcoming environmental changes as predicted by climate change scenarios. However, further studies with more significant sampling efforts are needed to corroborate these results.

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Competing Interests

The authors declare no conflict of interest.

Author Contributions

- Maria Luisa Pica performed the experiments, formal analysis and investigations, used software, analyzed and curated the data, prepared figures and/tables, curated the visualization, authored and reviewed drafts of the article, and approved the final draft.
- Ermenegilda Vitale performed the experiments, formal analysis and investigations, curated and validated data, reviewed drafts of the article, and approved the final draft.
- Rosa Donadio performed the experiments, formal analysis and investigations, reviewed drafts of the article, and approved the final draft.
- Giulia Costanzo performed the experiments, formal analysis and investigations, curated data, reviewed drafts of the article, and approved the final draft.
- Marco Munari curated data, supervised the study, reviewed drafts of the article, and approved the final draft.
- Erika Fabbrizzi curated data reviewed drafts of the article, and approved the final draft.
- Simonetta Fraschetti conceived and designed the experiment, supervised the study, provided founds, reviewed drafts of the article, and approved the final draft.
- Carmen Arena conceived and designed the experiment, supervised the study, curated, and validated data, provided founds, authored and reviewed drafts of the article, and approved the final draft.

Ethical approval

This study did not require ethics approval.

Data availability

The following information was supplied regarding data availability. The raw data are available in the Supplemental Files.

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Figure 1

Figure_1

Fig.1 Sampling location marked with coordinates from satellite perspective; images taken from Google Earth Pro Software.

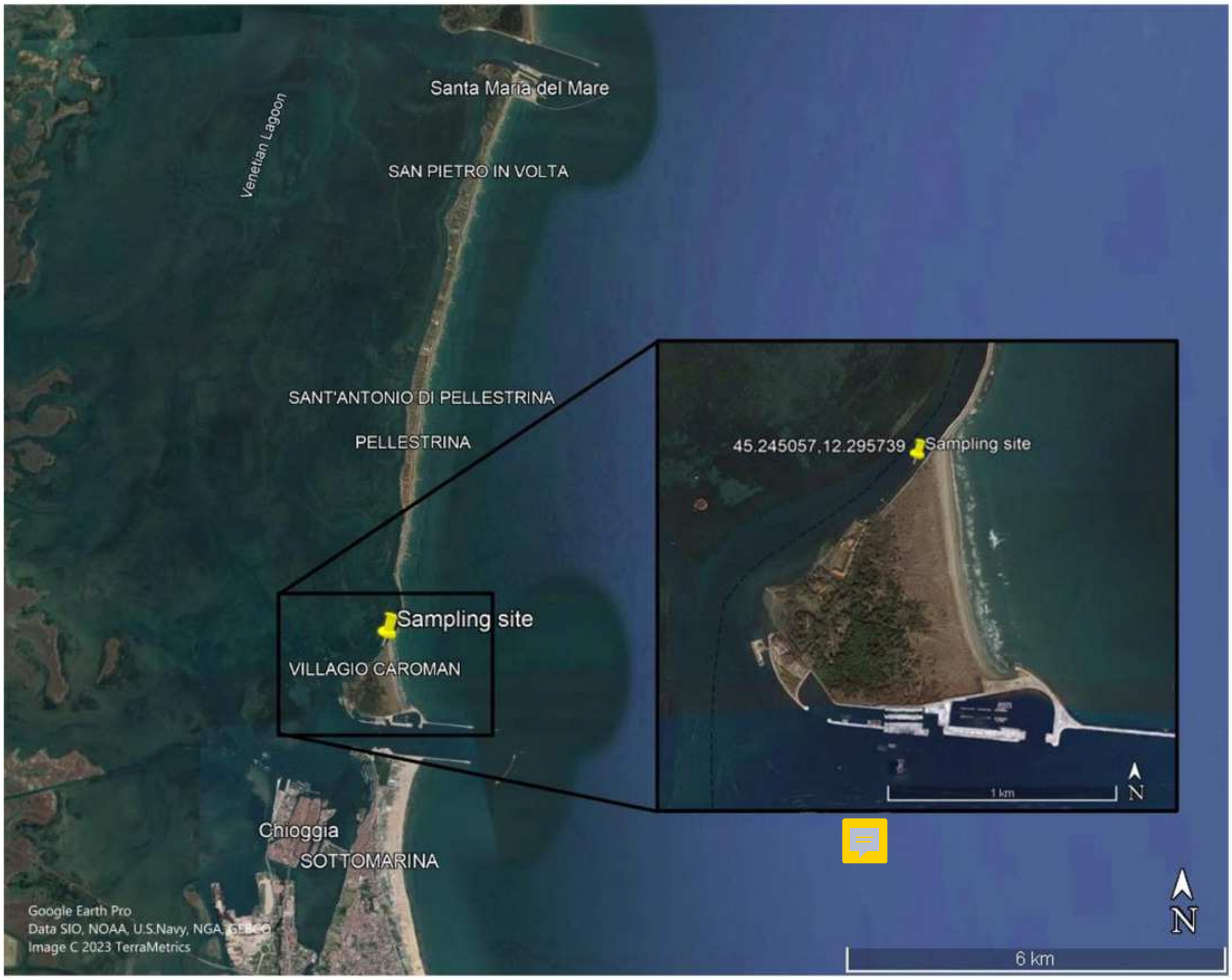
Santa Maria del Mare
Venetian Lagoon
SAN PIETRO IN VOLTA
SANT'ANTONIO DI PELLESTRINA
PELLESTRINA
Sampling site
VILLAGIO CAROMAN
45.245057,12.295739 Sampling site
1 km
N
Chioggia
SOTTOMARINA
Google Earth Pro
Data SIO, NOAA, U.S.Navy, NGA, GEBCO
Image C 2023 TerraMetrics
6 km
N

Figure 2

Figure_2

Fig.2 Analysis of seasonal variations (date YYYY/MM/DD) of environmental conditions before sampling, measured at sampling site. Straight black line marks the approximate start of juveniles' growth. a) Sea Surface Temperature (°C); b) Salinity (PSU) seasonal fluctuations; c) pH seasonal variations reported on total scale; d) Nitrates (NO_3) and Phosphates (PO_4) concentration fluctuations expressed as mmol m^{-3}; all data acquired from Copernicus Marine Environment Monitoring Service (CMEMS) database.

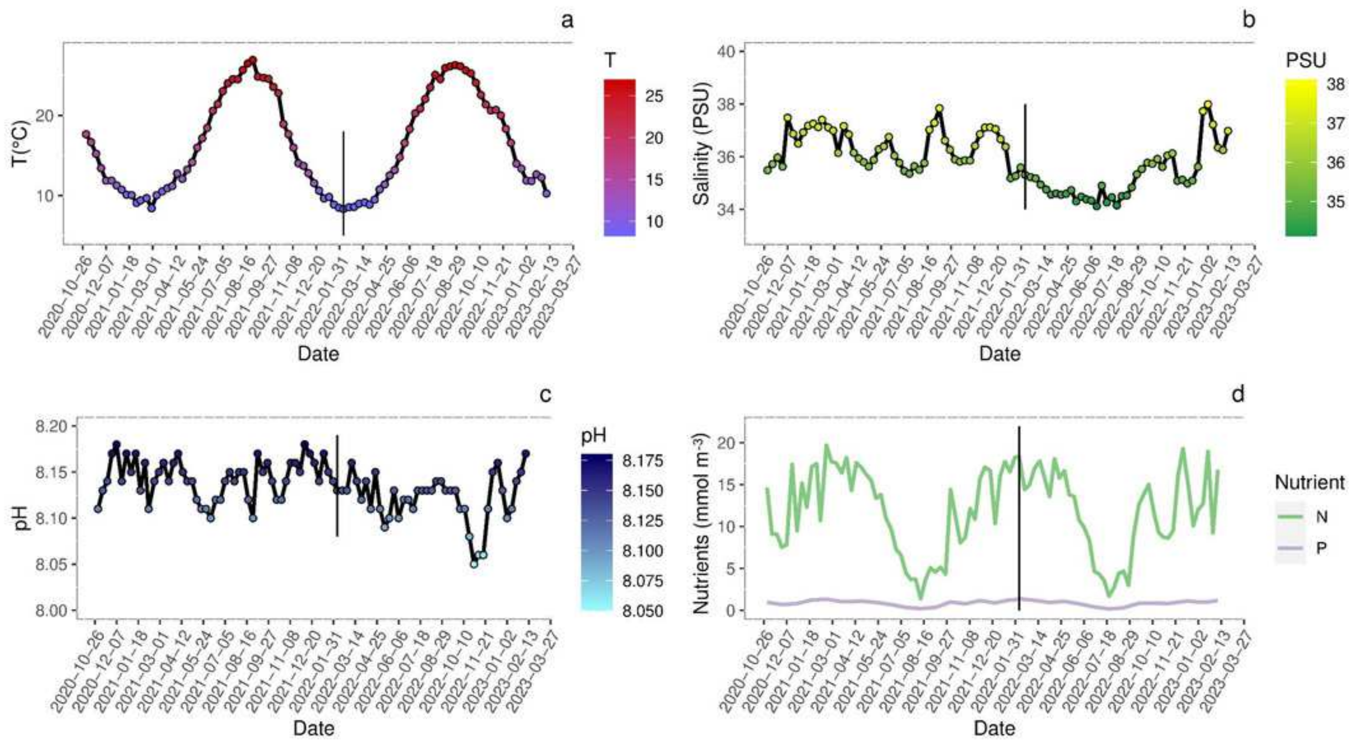

Figure 3

Figure_3

Fig 3. RLCs for the photochemical parameters a) Quantum yield of PSII electron transport, ΦPSII; b) Electron transport rate of PSII (ETR); c) Non-Photochemical Quenching (NPQ); d) maximum PSII photochemical efficiency, F_v/F_m. Data are reported as means ± SE (n =4). Statistically significant differences were checked according to t-test.

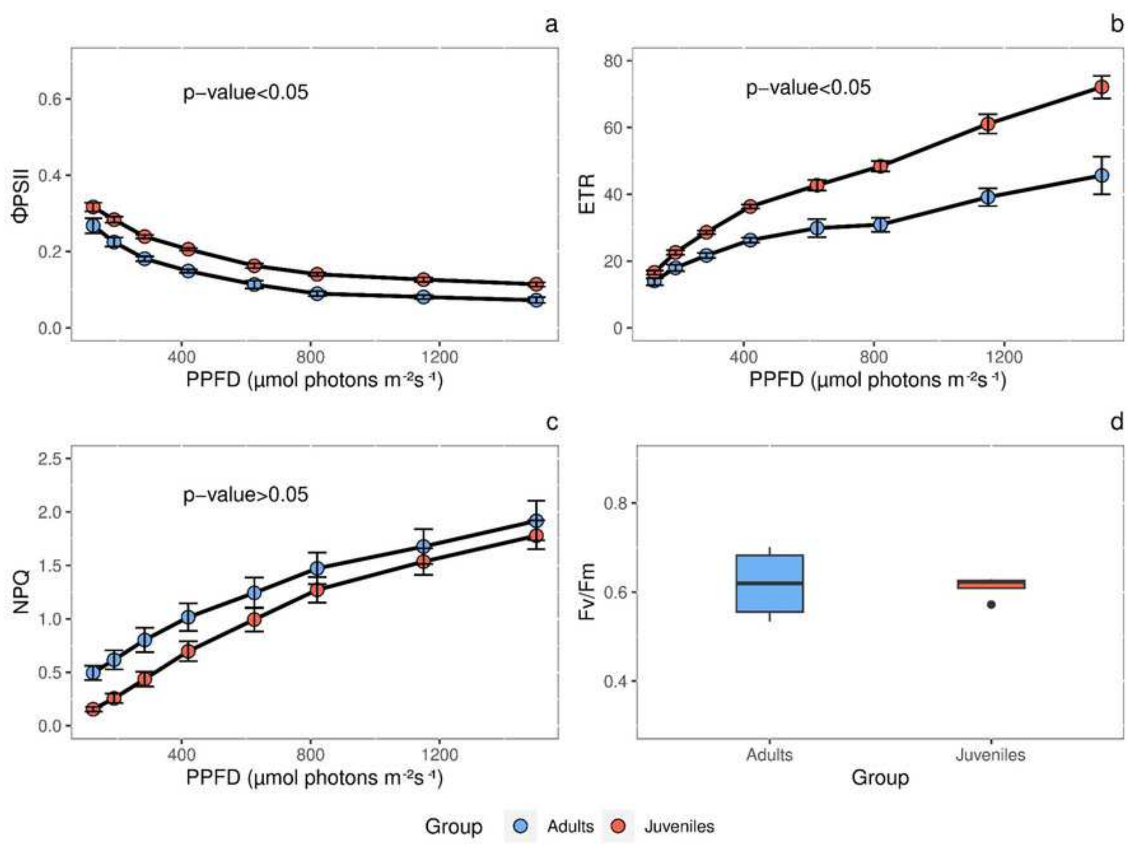

Figure 4

Figure_4

Fig.4 a) Western blot of Rubisco and D1 protein with relative molecular weight; b) densitometric analysis in arbitrary units of Rubisco protein; c) densitometric analysis in arbitrary units of D1 proteins. Asterisks indicate the statistically significant differences (*** P≤0.001, ** P≤0.01, * P≤0.05, ns P ≥0.05) according to t-test.

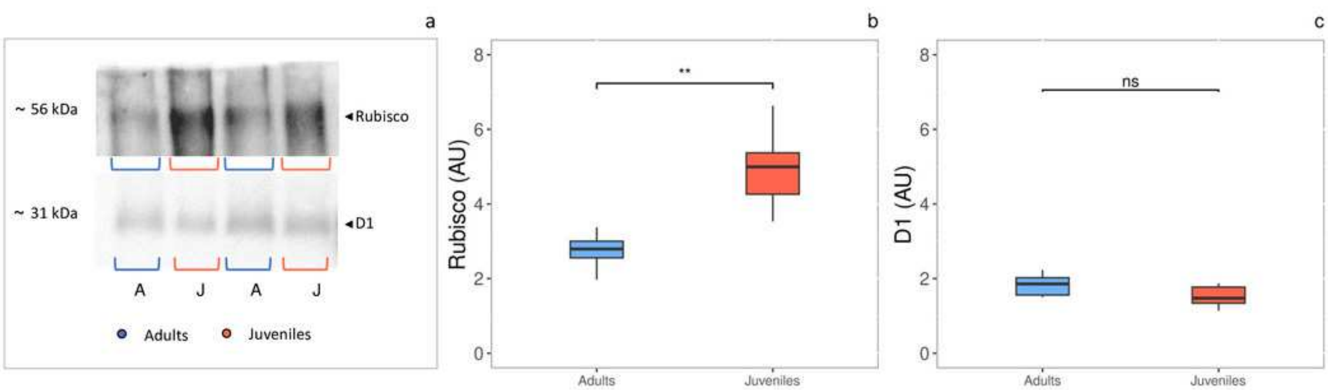

Figure 5

Figure_5

Fig.5 Antioxidant content in adults and juveniles: a) Total Polyphenols; b) Flavonoids; c) Tannins; d) Antioxidant capacity, measures as radical scavenging activity. Data are reported as means ± SE (n = 15). Asterisks indicate the statistically significant differences (*** P≤0.001, ** P≤0.01, * P≤0.05) according to t-test.

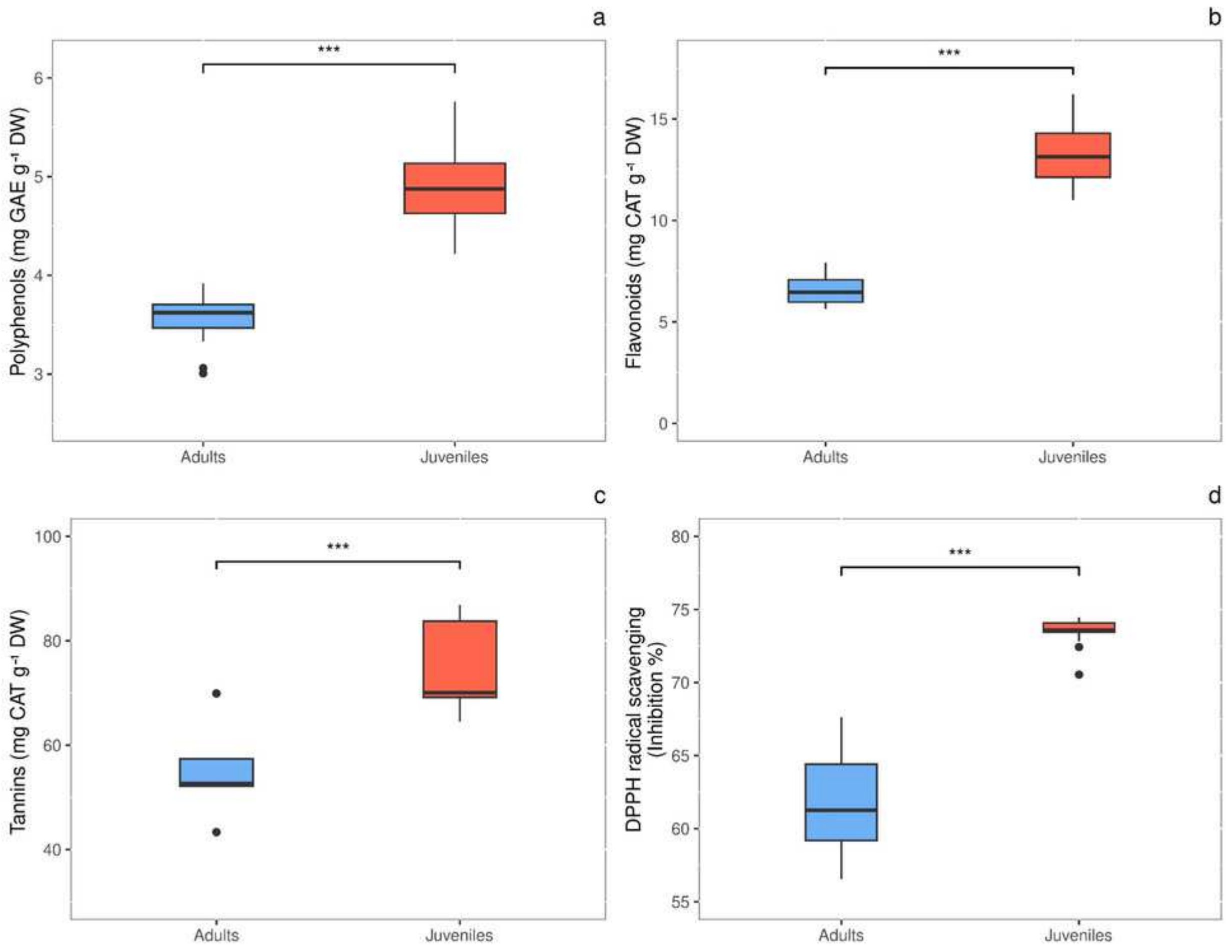

Table 1(on next page)

Table_1

Table 1. Thallus dry matter content (TDMC) and photosynthetic pigments content in adults and juveniles. Data are reported as mean ± SE (TDMC n=5, pigments n=15). Asterisks indicate the statistically significant differences (** P≤0.01, * P≤0.05) according to t-test.

Table 1. Thallus dry matter content (TDMC) and photosynthetic pigments content in adults and juveniles. Data are reported as mean ± SE (TDMC n=5, pigments n=15). Asterisks indicate the statistically significant differences (** P≤0.01, * P≤0.05) according to t-test.

	Adults	**Juveniles**
TDMC		
(g g^{-1} DW)	0.131 ± 0.005 *	0.103 ± 0.008
Total Chlorophylls		
(µg g^{-1} DW)	609.34 ± 41.25**	340.77 ± 36.68
Total Carotenoids		
(µg g^{-1} DW)	166.17 ± 17.51 *	99.72 ± 13.09