

## Research article

# Photophysiological and molecular adaptations of tropical seagrasses to episodic marine heatwaves and tidal fluctuations

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## ABSTRACT

Here, we studied the photo-physiological and molecular responses of intertidal tropical seagrass species to tidal-driven light fluctuations and marine heatwave (MHW) event in India. Intertidal habitats expose seagrasses to extreme irradiance and elevated temperatures during low tide, presenting a unique challenge to their photosynthetic apparatus for resilience under such extreme conditions. Field investigations conducted in seagrass meadows of *C. rotundata*, *E. coroides* and *T. hemprichii* across tidal cycles revealed significant species-specific alterations in chlorophyll fluorescence parameters. These responses included dynamic photo-protective adjustments, characterized by increased effective quantum yield ( $\Delta F/Fm'$ ), enhanced non-photochemical quenching and de-epoxidation state (DPS), accompanied by a reduction in maximum quantum yield (Fv/Fm). Additionally, variations in xanthophyll cycle pigment dynamics between low and high tide conditions highlighted the role of tidal timing in regulating the pigment based photo-protection. Gene expression profiling showed the upregulation of photosynthesis-related genes (psbA, psbD) and stress responsive genes (HSP90 and CAT) during the low tide, reflecting the activation of protein repair and antioxidant defenses under thermal and oxidative stress. In contrast, the expression of mitochondrial respiration-related genes (AOX1A & FES1) remained relatively stable across tidal phases, suggesting the existence of locally adapted seagrass genotypes to contrasting light environments.

## 1. Introduction

Seagrasses are submerged marine angiosperm belong to monocotyledon group, distributed globally in shallow coastal and estuarine environments (Chase et al., 2016). These ecosystems critically serve as a nursery and foraging grounds for a wide array of marine fauna, including finfish, shellfish, herbivores like dugongs and commercially significant fish species (Moussa et al., 2020). Beyond their ecological role, seagrass meadows contribute significantly to nutrient cycling and water quality by facilitating nitrogen burial and denitrification, thereby acting as a natural coastal filters (Thomsen et al., 2023). Being an ecosystem engineers, it provides multiple ecosystem services such as oxygen generation, wave energy attenuation, sediment stabilization, coastal protection and climate change mitigation through carbon sequestration (Zhu et al., 2021; James et al., 2023; Lebrasse et al., 2022). However, the stability and productivity of seagrass habitats (Jung et al.,

2023) are increasingly threatened by climate change, particularly the rising frequency and intensity of marine heatwaves (MHWs). These thermal anomalies have been shown to alter the species distributions, increase mortality rates and cause profound ecological disturbances within seagrass ecosystem (Serrano et al., 2021). Moreover, the cumulative impacts of anthropogenic stressors including salinity fluctuations, hypoxia, desiccation and pollution further exacerbate the vulnerability of seagrass meadows (De Fouw et al., 2024; Johnson et al., 2020; Xu et al., 2020; Hasler-Sheetal, 2023; Deng et al., 2024). Despite their ecological and economic importance, the photo-protective and molecular mechanisms that govern the seagrass responses to thermal and light stress remain poorly understood, specifically in the tropical intertidal environments. In recent years, global warming-induced sea temperature is causing large-scale declines in seagrass cover and function (Litsi-Mizan et al., 2023).

Intertidal seagrass meadows are particularly sensitive to fluctuations

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in temperature and light intensity associated with tidal cycles. In specific, the seasonal and diurnal variations of light conditions in the intertidal regions can be extreme and during the low tide, seagrass beds are often exposed to extreme irradiance of  $>2000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  of photosynthetically active radiation (PAR) and thermal stress which can inhibit photosynthesis and trigger oxidative damage (Deguette et al., 2022). While *in-situ* irradiance level decrease rapidly with incoming tide particularly in the turbid waters (Kohlmeier et al., 2017). The photosynthetic organisms frequently exposed to fluctuating light conditions necessitate shift with effective physiological adjustments through maximizing light capture during low irradiance period while simultaneously safeguarding their photosynthetic machinery against excessive light during high irradiance (Franklin et al., 2003; Bellan et al., 2020). Nonetheless these plants have evolved adaptive photo-acclimatory mechanisms, such as modulating chlorophyll content, altering pigment profiles, and engaging xanthophyll cycle to dissipate excess energy as heat (Minguito-Frutos et al., 2023). These photo protective strategies are essential for mitigating light induced stress, particularly under the dynamic light conditions prevalent in intertidal and shallow marine environments (Lee et al., 2007). While previous research has provided valuable insights into seagrass productivity and physiological responses to environmental stressors, most studies have been confined to temperate ecosystems and controlled laboratory conditions, with limited relevance to tropical intertidal systems. Notably, existing knowledge regarding these mechanisms comes from temperate seagrass species (e.g., *Zostera marina*, *Zostrea capensis*), with studies such as those by Petrou et al. (2013), Kaewsrikhaw et al. (2016), Kohlmeier et al. (2017), Nguyen et al. (2020) and Watson et al. (2024) focusing on physiological plasticity in response to light variability and tidal fluctuations. In contrast, the molecular regulation of photoacclimatory mechanisms remains largely unexplored in tropical Indian seagrasses. This is a significant gap, especially considering that seagrass genus such as *Thalassia*, *Halodule*, *Halophila*, *Syringodium*, *Enhalus*, and *Cymodocea* dominate tidal meadows in the Palk Bay and Gulf of Mannar, where they are frequently exposed to extreme irradiance and heat during emersion (Thangaradjou and Bhatt, 2018).

Although Purvaja et al. (2020) provided valuable insights into the photosynthetic and biogeochemical characteristics of Indian seagrass species in the Palk Bay, their study did not investigate the underlying molecular mechanisms nor the influence of real-time tidal irradiance dynamics on photoprotection. To address this knowledge gap, the present study undertakes a comprehensive, field-based investigation focusing on tide-dependent photoacclimation and molecular photo-protective responses in tropical seagrasses inhabiting the intertidal zone along the Gulf of Mannar. We selected the Kundhukal coastal region as a study site, based on its diverse ecological attributes and seagrass species composition. A detailed diurnal sampling was conducted during two distinct days under MHW conditions, targeting both low and high tide around noon. This approach enabled rigorous assessment of photosynthetic stress physiology, pigment modulation and environmentally regulated gene expression under fluctuating thermal and light stress.

## 2. Materials and methods

### 2.1. Study site

Sampling was conducted in the intertidal seagrass meadow of Kundhukal region (Lat 9.262395°N; Long 79.221676°E), Gulf of Mannar, Rameswaram Island, Tamil Nadu, a biologically rich coastal habitat characterized by dynamic tidal fluctuations and elevated thermal exposure during the summer season. Field observations were carried out on 01<sup>st</sup> May 2024 (low tide) and 07<sup>th</sup> July 2024 (high tide), coinciding with periods of intense solar radiation and spring tidal cycles known to induce episodic thermal maxima across intertidal flats at noon time. *In-situ* surface temperature measurements were recorded using a calibrated HOBO® (water temperature Pro v2 Data Logger) at hourly intervals

during the study period. During extreme low tide conditions, when seagrasses were exposed to direct sunlight and minimal water cover, maximum substrate temperatures reached up to 34.5°C. Conversely, during high tide immersion periods, the water column temperature was recorded upto 31.6°C. These values reflect acute and chronic thermal stressors experienced by intertidal seagrass beds during the late dry season, aligning with previous reports on thermal variability in tropical reef-seagrass ecosystems of Southern India. The study site harbored three dominant seagrass species: *Cymodocea rotundata*, *Enhalus acoroides*, and *Thalassia hemprichii*. Among them, *C. rotundata* formed dense and spatially extensive meadows, while *E. acoroides* and *T. hemprichii* were observed in more fragmented patches.

### 2.2. Experimental setup and sample collection

Seagrass sample collection were strategically timed to coincide with both low tide (LT) and high tide (HT) conditions around midday, allowing comparative assessment of seagrass responses under contrasting tidal exposures. Physiological and morphological observations were recorded *in-situ* during each tidal events. Environmental parameters such as PAR, water temperature, salinity, pH and dissolved oxygen were measured at each sampling time point using calibrated field instruments. To account for natural spatial variability across the intertidal seagrass meadow, eighteen plots were established, six each at upper, middle and lower intertidal zones. The plots were spaced at 5 m intervals within each zone, and the vertical distance between zones was maintained at 30 m.

Morphological assessments were performed during the low tide when the seagrass canopy was fully exposed. Measurements included leaf length and width, internode length, root length, and shoot density. Shoot density was estimated using a 100x100 cm quadrat placed within each transact. No significant variation in morphological or biochemical parameters was observed among the replicate plots confirming uniformity in habitat structure across site. For Chlorophyll fluorescence measurements, a Pulse Amplitude Modulated (PAM; FP110, Czech Republic) Fluorometer was employed to assess the photosynthetic efficiency. PAM measurements and sampling for pigment content were conducted at 3 h intervals on the second youngest leaf of a representative shoot for each species per plot. During high tide, when seagrass canopies were submerged, samples were carefully accessed using transparent plastic sampling pipes to prevent sediment disturbance and water turbidity. The tidal amplitude recorded during the study period peaked at approximately 0.6 m.

### 2.3. Photosynthetically active radiation (PAR)

During the survey, continuous recording of atmospheric incident light was performed as PAR measurements and for the determination of effective quantum yield ( $\Delta Fv/Fm'$ ) and maximum photochemical efficiency ( $Fv/Fm$ ) of PSII, chlorophyll *a* fluorescence measurements was performed using a non-invasive Pulse-Amplitude Modulated (PAM) fluorometer (FP110, Czech Republic) following the method of Jeyapragash et al. (2021). The effective quantum yield ( $\Delta Fv/Fm'$ ) was measured using the sample holder (Universal sample holder, PSII FP110), which also ensured simultaneous measurements of incident light next to the leaf. Following which, the leaf was darkened using a leaf clip and maximum photochemical efficiency ( $Fv/Fm$ ) was measured after 10 min. Non-photochemical quenching was calculated using the formula:  $NPQ = (Fm - Fm')/Fm'$ .

### 2.4. Biochemical leaf C and N content

To estimate carbon (C) and nitrogen (N) content from seagrass biomass collected during high and low tides, aboveground biomass (AGB) was obtained by carefully clipping the seagrass leaves, ensuring minimal disturbance and avoiding epiphyte contamination.

Belowground biomass (BGB), comprising roots and rhizomes, were collected using a sediment core (approximately 10 cm in diameter and 15–20 cm deep), followed by gentle washing over a sieve to remove sediments. After transportation to the laboratory, leaves, roots, and rhizomes were separated and rinsed thoroughly with distilled water to remove salts and debris. The samples were then oven-dried at 60 °C for 48 h or until constant weight is achieved. The dried samples were ground to a fine powder using a mortar and pestle stored in airtight containers with desiccant to prevent moisture absorption. For C and N estimation, a CHNS elemental analyzer (Velp Scientific, Italy) was used and approximately 2–5 mg of the finely powdered dry biomass is weighed and analyzed to determine the percentage of carbon and nitrogen content in each plant component.

## 2.5. Analysis of leaf pigment contents

The leaf pigments (chlorophyll *a*, chlorophyll *b* and total carotenoids) from the collected leaves of three seagrass species were measured by harvesting the second youngest leaves during the low and high tides respectively following the method of Jeyapragash et al. (2025). The harvested leaf samples were washed and kept on ice prior to the fresh weights was measured. The samples were homogenized in liquid nitrogen using mortar and pestle and transferred to 1.5 mL tube containing 1 mL of 80 % acetone (Merck, Germany) and stored in complete darkness for 8 h at 4 °C. Following centrifugation, the supernatant (200 µL) was collected and measured spectrophotometrically at 470, 646 and 663 nm. The concentrations of the carotenoids were calculated from the readings using the equation of Lichtenthaler and Wellburn (1983) respectively.

## 2.6. RNA extraction and cDNA synthesis

For each seagrass species, 3 intermediate leaves (the second youngest leaf in the shoot) were randomly sampled and the epiphytes were washed with native seawater followed by sterile distilled water surface epiphytes were cleaned using a sterile razor blade, and immediately immersed in RNAlater® solution (Transiom™ High quality, India). Subsequently, the samples were transported to the laboratory, stored at 4 °C and stored at –20 °C long term preservation until RNA extraction. For the RNA isolation, approximately 4–6 cm of seagrass leaf blades were excised with a sterile scalpel blade and grinded into a fine powder with pre chilled mortar and pestle under liquid nitrogen. Approximately 100 mg of powdered tissue were used for the RNA extraction following the manufacturers instruction using AurumTM Total RNA Mini Kit (Bio-Rad Laboratories, USA). RNA quantity was quantified spectrophotometric ally using a Nano-Drop (ND-1000 UV-Vis spectrophotometer; NanoDrop Technologies, USA) by measuring the absorbance at 260 nm. Isolated RNA purity was determined at 260/280 nm and 260/230 nm absorbance ratio, both of which consistently exhibited values near 2.0, confirming the high purity of the extracted RNA. All protein contaminants and organic solvents were effectively eliminated from each during RNA extraction process. RNA integrity was evaluated by agarose gel electrophoresis. A total of 500 ng of RNA was reverse transcribed into complementary DNA (cDNA) using the iScript™ cDNA Synthesis Kit (Bio-Rad Laboratories, USA). The amplification was performed on a GeneAmp PCR System 9700 (PerkinElmer). The reverse transcription reaction was set up in a final volume of 20 µl, comprising 4 µl of 5 × iScript Reaction Mix, 1 µl of iScript Reverse Transcriptase, and DNase-free water. The reaction mixture was incubated at 25 °C for 5 min, followed by 30 min at 42 °C for cDNA synthesis, and subsequently heated to 85 °C for 5 min to inactivate enzymes and terminate the reaction. Reverse transcription was performed using a GeneAmp PCR System 9700 thermal cycler (PerkinElmer, USA).

## 2.7. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Expression level analyses were performed for a set of specific genes of interest (GOIs), selected based on their putative roles in key processes namely stress response, photosynthesis, photoprotection, and respiration (Table 1). The genes analyzed included Heat Shock Protein 90 (HSP90), a major molecular chaperone involved in the stabilization and folding of proteins during seagrass cellular stress, Catalase (CAT), an antioxidant enzyme that mitigates oxidative damage, Photosystem II protein D1 (psbA) and Photosystem II protein D2 (psbD), both essential components of the PSII reaction center core involved in light energy conversion, Photosystem II 22 kDa protein, chloroplastic (PSBS), which participates in non-photochemical quenching and photoprotection mechanisms, Zeaxanthin epoxidase, chloroplastic (ZEP), a key enzyme of the xanthophyll cycle regulating photoprotective pigment conversion, Alternative oxidase 1A (AOX1A), associated with the mitochondrial alternative respiratory pathway under stress conditions; and ubiquinol-cytochrome c reductase iron-sulfur subunit (FES1), which plays a major role in electron transport and mitochondrial function. Quantitative real-time PCR (RT-qPCR) assays were employed to determine the relative expression profiles of the selected genes. To ensure reliability and reproducibility of the data each qPCR reaction was conducted in triplicate to evaluate the intra-assay variations. Each primer pair with three no-template controls (NTCs) was included to detect any potential contamination or non-specific amplification. The experimental design incorporated three independent biological replicates per species to capture biological variability. Each biological replicate, three technical replicates were processed to control for technical variation.

## 2.8. Statistical analysis

All experiments were carried out in triplicates and the statistical analysis were performed using R software (v4.2.0; R Core Team, 2022). Results are presented as mean values ± standard error of replicate samples. One-way repeated-measures ANOVA was used to assess the statistical significance of depth (as between-subject factor) on the diel trends of chlorophyll *a* fluorescence parameter (as within-subject factor). Two-Way ANOVAs were used to test main and interactive effects of depth and time at a significance level of  $p < 0.05$ , on pigments, leaf respiration and protein expression.

## 3. Results

### 3.1. Influence of tidal fluctuations on diurnal variations in physico-chemical parameters

Morphometric characteristics of the three seagrass species measured (leaf length, width, etc.) at the study site were given in Table 2. In-situ diurnal variations in key physico-chemical parameters temperature (°C), salinity (psu), pH, and dissolved oxygen (DO, % saturation) were assessed in the seagrass beds of *C. rotundata*, *E. acoroides* and *T. hemprichii* during the LT and HT (Fig. 1A and B) at Kudhukal region, Gulf of Mannar. During LT (Fig. 1A), the surface water temperature showed a sharp increase from  $29.6 \pm 0.3$  °C at 6:00 a.m. to a peak of  $34.5 \pm 0.4$  °C at 12:00 p.m., followed by a decline to  $30.2 \pm 0.3$  °C by 6:00 p.m. Salinity remained relatively stable, varying slightly between 34.4 and 34.9 psu. The pH increased progressively during the forenoon, reaching a maximum of  $8.6 \pm 0.1$  at 12:00 p.m., then decreased toward evening. Dissolved oxygen (DO) concentrations showed a midday peak at  $113.2 \pm 2.5$  %, likely due to elevated photosynthetic activity under strong solar irradiance, and dropped to  $105.6 \pm 2.1$  % by the evening. In case of HT (Fig. 1B), a slightly moderated thermal regime was observed and the temperature raised from  $28.8 \pm 0.4$  °C to a maximum of  $31.6 \pm 0.2$  °C at 12:00 p.m., before declining to  $29.8 \pm 0.4$  °C at 6:00 p.m. Salinity showed a marginal increase, ranging from 34.5 to 35.2 psu,

**Table 1**

Genes of Interest selected for RT-qPCR experiment used in the present study.

Gene Category	Name of the Protein	Gene	Biological Function	Forward Primer (5'-3')	Reverse Primer (5'-3')
Stress Related Genes	Heat Shock Protein 90	HSP90	Maintaining protein homeostasis	CTCCATCTGCTTCCCTCAG	TCAGTTGGAGGAACCGAAC
Photosynthesis Related Genes	Catalase	CAT	Mitigate oxidative stress	AAGTACCGTCCGTCAAGTGG	CTGGGATACGCTCCCTATCA
	Photosystem II protein D1	psbA	Photosynthesis	GACTGCAATTAGAGAGACGC	CAGAAGTTGCAGTCATAAGGTAG
	Photosystem II Protein D2	psbD	Photosynthesis	CCGCTTTGGTCACAAATCT	AGGATTTCTGCGAAAGCAA
Photoprotection Related Genes	Photosystem II 22 kDa protein, chloroplastic	PSBS	Photosynthesis	CCGCTCTGTGTTCTCAT	GGACCTCCITCCTTGAGACC
	Zeaxanthin epoxidase, chloroplastic	ZEP	Xanthophyll cycle	TGCTCCAGAGAAAGCCAGTT	TGGCATCCCCAAATGTTATA
Respiratory Related Genes	Alternative oxidase 1A	AOX1A	Mitochondrial electron transport chain	TGCTGCATTGCAAGTCTCTAC	GTGTGACACCTCCATGAAGGTC
	Ubiquinol-cytochrome c reductase iron-sulfur subunit	FES1	Ubiquinol-cytochrome c reductase complex	GGTGATCCAAGCAAGAGAGC	CCACGCCACTTGACTGTCA

**Table 2**

Morphological features and other important characters of the studied seagrass species.

Species	Leaf length (cm)	Leaf width (cm)	Root length (cm)	Internode length (cm)	Shoot density (m <sup>-2</sup> )	Other characteristics
<i>Cymodocea rotundata</i>	20 ± 3.1	0.4 ± 0.05	8.6 ± 0.9	2.0 ± 0.2	635 ± 35	Blunt, smooth rounded tips, continuous leaf scars around the upright stem.
<i>Enhalus acoroides</i>	97 ± 5.2	1.3 ± 0.11	20 ± 2.4	0	11 ± 3	Bright to dark green, ribbon like thick leaves, hairy bristles on rhizomes with thick hairless roots.
<i>Thalassia hemprichii</i>	17 ± 2	0.8 ± 0.07	4 ± 0.11	0.6 ± 0.10	20 ± 3	Sickle shaped leaf with thick rhizome and covered with triangular shaped leaf scars.

indicating seawater intrusion. The pH values followed a similar diurnal trend, peaking at  $8.5 \pm 0.1$ , while DO concentrations reached  $110.4 \pm 2.8\%$  at noon and declined further.

### 3.2. Photophysiological response of seagrasses under warming ocean

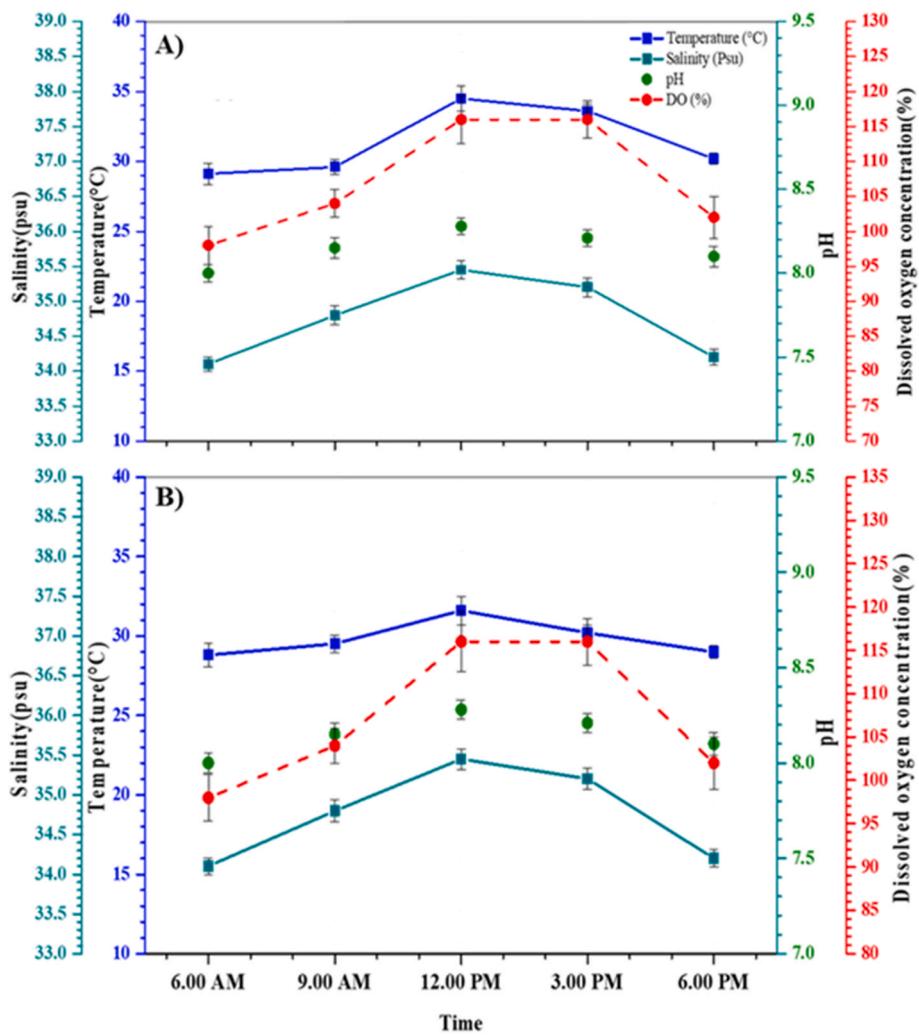
In the present study, three seagrass species *C. rotundata*, *E. acoroides* and *T. hemprichii* exhibited distinct photophysiological strategies to cope up with the fluctuating irradiance and thermal stress associated with tidal and diurnal cycles. Although all species exposed to a general diurnal pattern in response to environmental stressors, their magnitude and their adaptive responses varied significantly. During midday, particularly under low tide conditions when irradiance (PAR) reached peak levels ( $\sim 900 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) and slightly reduced to  $\sim 650 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  during the high tide. PAR levels followed a typical bell-shaped diurnal curve in all tidal conditions and for all the species studied respectively. During low tide, the maximum PAR values were recorded at noon in *T. hemprichii* (921.24  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ), *C. rotundata* (893.11  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) and *E. acoroides* (883.36  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). In contrast, PAR peak values decreased, with maxima at noon measured as 628.91  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  in *C. rotundata* and 613.11  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  in *E. acoroides* (Fig. 2A, B and C).

Effective quantum yield ( $\Delta F/F_m'$ ) showed a decline towards midday with partial recovery in the evening. At low tide,  $\Delta F/F_m'$  exhibited a decline from 0.62 at 9.00 a.m. to 0.29 at 12.00 p.m. in *C. rotundata*, 0.59 to 0.32 in *E. acoroides* and from 0.61 to 0.28 in *T. hemprichii*. Under high tide conditions, the midday reduction was less observed, with minimum  $\Delta F/F_m'$  values at 12.00PM being 0.41, 0.38 and 0.36 in *C. rotundata*, *E. acoroides* and *T. hemprichii* (Fig. 2A, B and C). Maximum quantum yield (Fv/Fm) remained relatively stable across the day, with a minor decline observed at noon during low tide. In *C. rotundata*, Fv/Fm (Fig. 2A) values ranged from 0.74 in the morning to 0.69 at noon, and returned to 0.72 in the evening. *E. acoroides* (Fig. 2B) maintained higher stability with values ranging from 0.75 to 0.73, while *T. hemprichii* (Fig. 2C) showed a dip from 0.76 to 0.70 during the midday low tide, while at high tide fluctuations were minimal in all species.

Non Photochemical Quenching increased sharply towards noon and

declined in the evening. At low tide, peak NPQ values at 12:00 p.m. were 2.78 in *C. rotundata*, 3.21 in *E. acoroides*, and 3.54 in *T. hemprichii* (Fig. 2A, B and C). Under high tide, the corresponding NPQ values were slightly reduced, measured as 2.03, 2.47, and 2.62, respectively, for the three species. DPS values rose steadily to a midday peak before declining. During low tide, DPS peaked at 0.72 in *C. rotundata*, 0.75 in *E. acoroides*, and 0.78 in *T. hemprichii* at noon (Fig. 2A, B and C). At high tide, peak DPS values at the same time point were slightly lower of about 0.58, 0.63, and 0.66, respectively (Fig. 2A, B and C). Dynamic shifts in xanthophyll pigments were observed during the day. Violaxanthin (Vx) content decreased from 1.84  $\mu\text{g/g FW}$  (9:00 a.m.) to 0.86  $\mu\text{g/g FW}$  (12:00 p.m.) in *C. rotundata* (Fig. 2A), from 1.79 to 0.91 in *E. acoroides* (Fig. 2B), and from 1.86 to 0.88 in *T. hemprichii* (Fig. 2C) at low tide, while antheraxanthin (Ax) increased to a maximum of 0.62  $\mu\text{g/g FW}$  in *C. rotundata*, 0.65 in *E. acoroides*, and 0.71 in *T. hemprichii* at noon. The level of Zeaxanthin (Zx) reached peak concentrations of 1.11  $\mu\text{g/g FW}$  in *C. rotundata*, 1.32 in *E. acoroides*, and 1.43 in *T. hemprichii*. At high tide, these pigment changes followed the same trend but were less marked with lower values for Zx and Ax and higher Vx retention (Fig. 2A, B and C). In case of Chlorophyll a content, mild diurnal fluctuations were observed and under low tide, *C. rotundata* showed an increase from 3.42  $\mu\text{g/g FW}$  at 9:00 a.m. to 4.12  $\mu\text{g/g FW}$  at 3:00 p.m. (Fig. 2A). *E. acoroides* had values ranging from 3.86 to 4.08  $\mu\text{g/g FW}$  (Fig. 2B), and *T. hemprichii* from 4.21 to 4.57  $\mu\text{g/g FW}$  (Fig. 2C). During high tide, the chlorophyll a content remained comparatively stable, with marginal increases during afternoon hours.

The bell-shaped diurnal PAR pattern, peaking at midday during low tide, is consistent with the exposure of seagrass canopies to intense solar irradiance in shallow waters. This pattern has been documented in intertidal seagrass meadows, where reduced water depth during low tide leads to higher light availability. The midday decline in  $\Delta F/F_m'$  across all species indicates a reduction in the efficiency of photosystem II under high light stress, a common photoprotective response to prevent photodamage. The partial recovery in the evening suggests the reversibility of this downregulation. Similarly, the minor decline in Fv/Fm at noon reflects transient photoinhibition, which is a protective mechanism rather than indicative of permanent damage. The increase in NPQ values at midday, especially during low tide, demonstrates the activation of



**Fig. 1.** | Hourly variation in water quality parameters (temperature, dissolved oxygen, pH, and salinity) for key study species in the Gulf of Mannar during Low Tide (A) and High Tide(B).

energy dissipation mechanisms to protect the photosynthetic apparatus from excess light. Higher NPQ values in tropical seagrasses like *T. hemprichii* and *E. acoroides* have been associated with their capacity to endure high irradiance environments (Fig. 2B and C). The observed decrease in violaxanthin and concurrent increase in antheraxanthin and zeaxanthin during midday align with the operation of the xanthophyll cycle, which facilitates non-photochemical energy dissipation under high light conditions. This pigment conversion is a well-documented response in seagrasses exposed to variable irradiance. The slight diurnal increase in chlorophyll *a* content, particularly under low tide conditions, may reflect short-term adjustments in pigment synthesis or redistribution within the leaf tissues to optimize light harvesting under changing light environments. Such fluctuations have been noted in seagrasses as part of their acclimation to dynamic light regimes.

### 3.3. Biochemical leaf C and N content

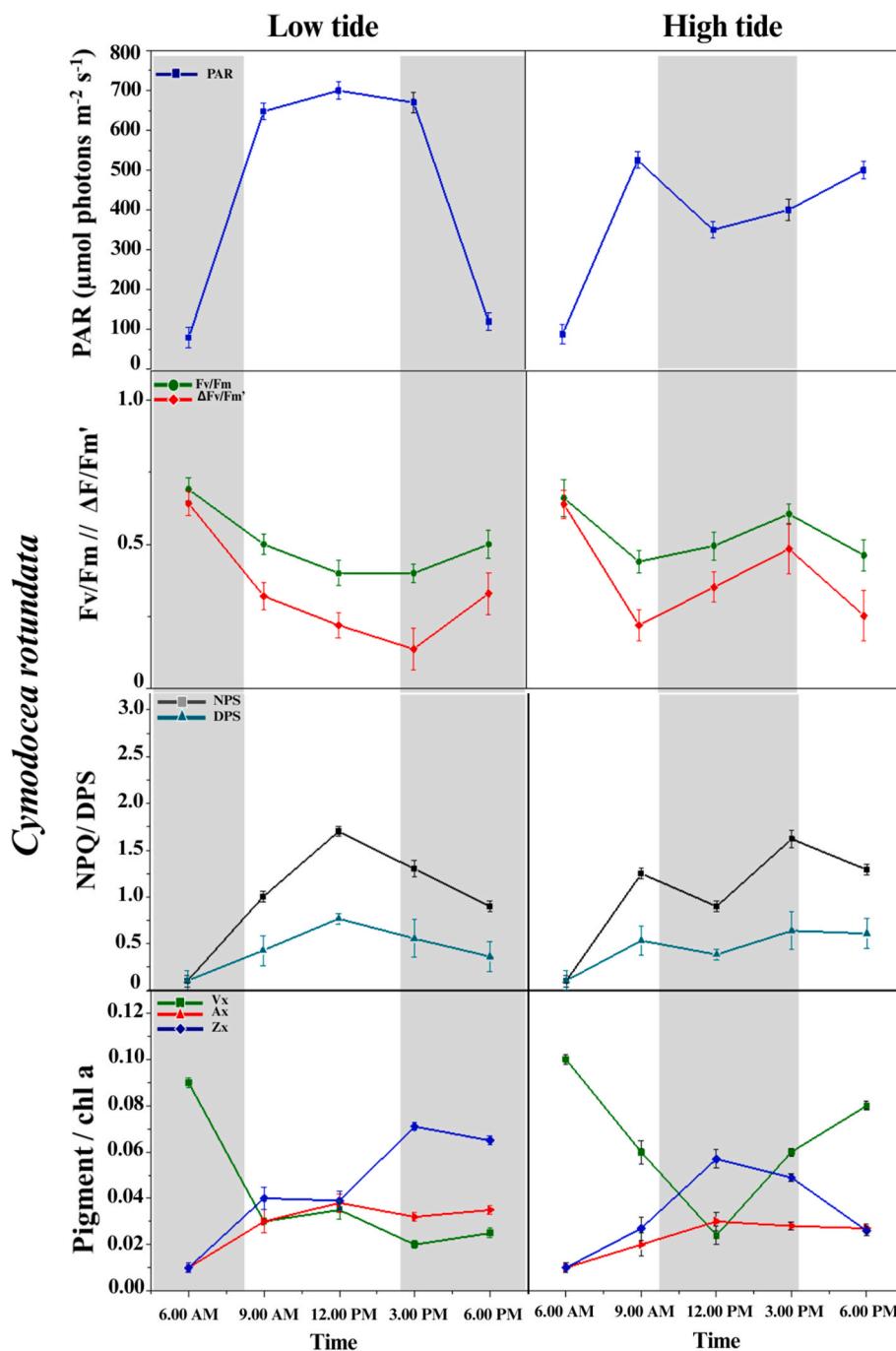
The carbon and nitrogen content in three seagrass species (*C. rotundata*, *E. acoroides*, and *T. hemprichii*) under low tide (LT) and high tide (HT) regimes revealed significant species-specific responses. Above-ground (leaf) carbon content was found increased across all three species under HT conditions, with *C. rotundata* showing the highest relative increase (~30 mg/m<sup>2</sup>) followed by *E. acoroides* (~31 mg/m<sup>2</sup>), and *T. hemprichii* (~30 mg/m<sup>2</sup>) (Fig. 3A). In contrast, the pigment content found relatively low in all three seagrass species exposed under

low tide conditions. On the other hand, above-ground nitrogen content also exhibited notable variation among the species, in which *C. rotundata* showed an increase under HT (~1.6 mg/m<sup>2</sup>) and reduced during LT (~1.3 mg/m<sup>2</sup>), while *E. acoroides* displayed a slight decrease (~1.9 mg/m<sup>2</sup>) during HT with a relative increase under LT and *T. hemprichii* remained relatively stable (~2.1 mg/m<sup>2</sup> in both conditions; Fig. 3C) during both the tidal fluctuations.

In contrast, below-ground (rhizome and roots) carbon and nitrogen dynamics exhibited a distinct response among species studied, out of which *C. rotundata* exhibited a reduction in the below-ground carbon (~31–28 mg/m<sup>2</sup>) with a striking decrease in nitrogen content (~6.5–0.5 mg/m<sup>2</sup>) under LT (Fig. 3B). Meanwhile, *E. acoroides* maintained relatively stable below-ground carbon (~23 mg/m<sup>2</sup>) and the nitrogen content found reduced (~0.15–0.2 mg/m<sup>2</sup>) during both the tides. Interestingly, *T. hemprichii* demonstrated a substantial increase in below-ground carbon (~17–25 mg/m<sup>2</sup>) during HT and LT (Fig. 3B), with a marginal decrease in nitrogen (~0.6–0.7 mg/m<sup>2</sup>) as compared to the other species respectively (Fig. 3D).

### 3.4. Pigment analysis

The pigment content, including chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and carotenoids, was quantified in three seagrass species *C. rotundata*, *E. acoroides*, and *T. hemprichii* under High Tide (HT) and Low Tide (LT) conditions (Fig. 4A, B & C). In *C. rotundata*, Chl *a* content



**Fig. 2.** Observed Photosynthetically Active Radiation (PAR), Effective Quantum Yield ( $\Delta F/Fm'$ ), Maximum photochemical efficiency (Fv/Fm), Non-Photochemical Quenching (NPQ) and Chlorophyll Pigment Content of *Cymodocea rotundata* (A), *Enhalus acoroides* (B) and *Thalassia hemprichii* (C) in the Gulf of Mannar. The grey area indicates times when the sites were submerged.

was higher during HT ( $8.13 \pm 0.13 \mu\text{g/g FW}$ ) than LT ( $6.97 \pm 0.24 \mu\text{g/g FW}$ ), while Chl b levels were  $4.16 \pm 0.21 \mu\text{g/g FW}$  in HT and  $3.89 \pm 0.23 \mu\text{g/g FW}$  in LT (Fig. 4A). Carotenoid content was also higher in HT ( $2.72 \pm 0.14 \mu\text{g/g FW}$ ) compared to LT ( $2.45 \pm 0.15 \mu\text{g/g FW}$ ; 4A). In *E. acoroides*, Chl a content during HT was  $7.91 \pm 0.11 \mu\text{g/g FW}$ , which declined to  $7.45 \pm 0.17 \mu\text{g/g FW}$  under LT. Chl b levels were  $4.13 \pm 0.17 \mu\text{g/g FW}$  in HT and  $4.02 \pm 0.20 \mu\text{g/g FW}$  in LT and carotenoids measured  $2.89 \pm 0.11 \mu\text{g/g FW}$  in HT and  $2.76 \pm 0.14 \mu\text{g/g FW}$  in LT (Fig. 4B). In case of *T. hemprichii* showed a higher Chl a content of  $8.05 \pm 0.10 \mu\text{g/g FW}$  during HT, decreasing to  $6.55 \pm 0.15 \mu\text{g/g FW}$  in LT. Chl b levels were  $4.29 \pm 0.15 \mu\text{g/g FW}$  in HT and  $4.01 \pm 0.19 \mu\text{g/g FW}$

in LT (Fig. 4C). Carotenoids decreased from  $2.97 \pm 0.10 \mu\text{g/g FW}$  in HT to  $2.62 \pm 0.13 \mu\text{g/g FW}$  in LT.

### 3.5. Gene expression analysis

Gene expression profiling was conducted for photosynthesis, photoprotection, stress, and respiration-related genes in *C. rotundata* (A), *E. acoroides* (B) and *T. hemprichii* (C) and under marine heatwave (MHW) conditions during low tide (LT) and high tide (HT) at noon. In *C. rotundata*, the expression folds of psbA and psbD at LT (3.4 and 3.1) was higher than at HT (2.6 and 2.4). Photoprotection genes PSBS and

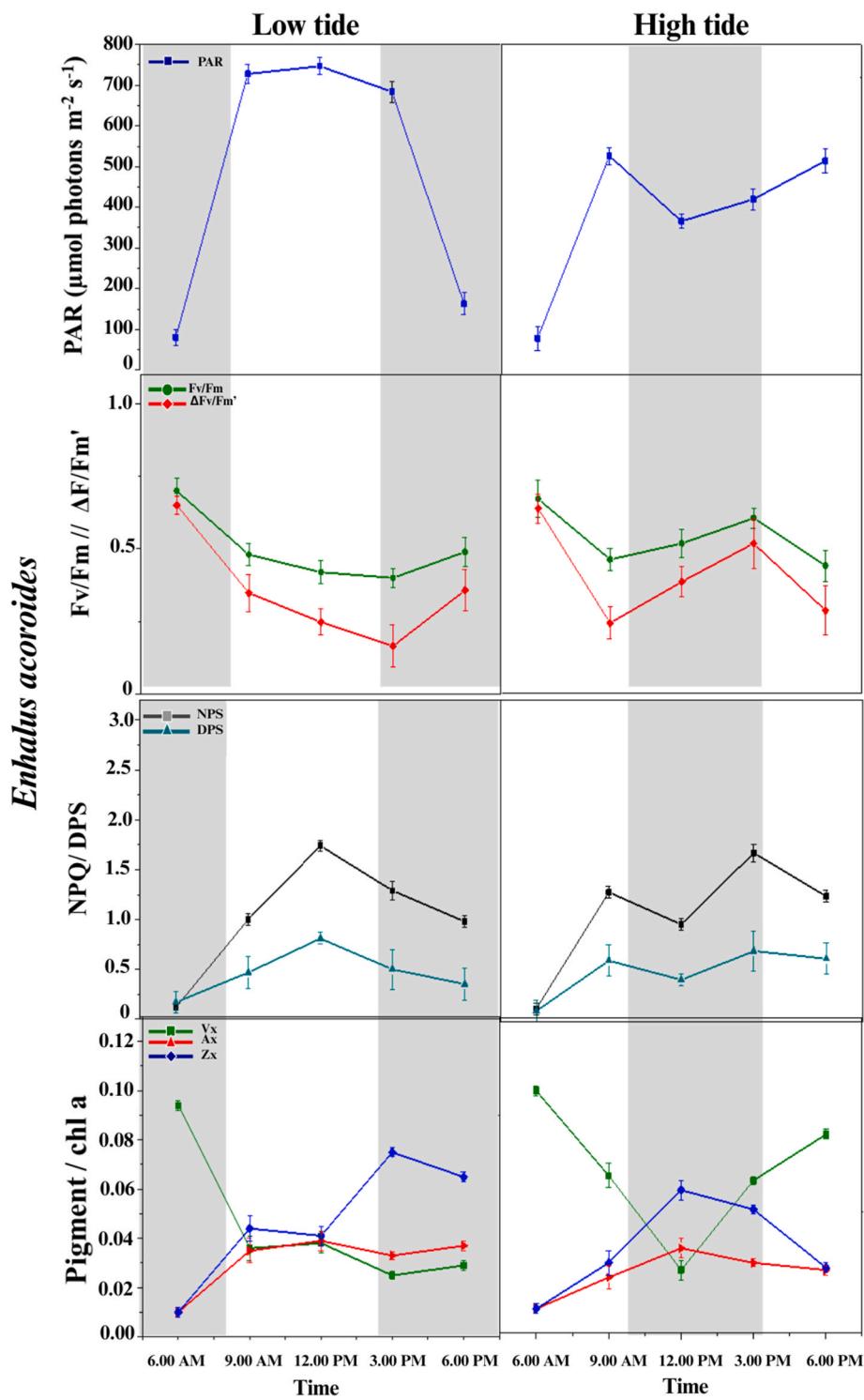


Fig. 2. (continued).

ZEP showed LT fold changes (Fig. 5A) of 1.5 and 2.6, reducing to 1.0 and 0.9 at HT. HSP90 and CAT were upregulated at LT (4.5 and 3.9), while lower fold changes were observed at HT (2.8 and 2.5). AOX1A and FES1 remained relatively stable across tides, with LT values of 2.3 and 2.2, and HT values of 2.1 and 2.0, respectively. In case of *E. acoroides*, photosynthetic genes psbA and psbD showed higher fold expression (Fig. 5B) under LT (3.1 and 2.7 respectively) compared to HT (2.4 and 2.3). Photoprotection-related genes PSBS and ZEP showed fold changes of 1.4 and 2.5 during LT, which declined to 0.9 and 1.0 during HT. Stress-related genes HSP90 and CAT recorded fold changes of 3.0 and

2.9 at LT, respectively, decreasing to 1.5 and 1.9 at HT. Respiration-related genes AOX1A and FES1 exhibited minor differences between LT (2.3 and 2.2) and HT (2.1 and 2.0). While in *T. hemprichii*, psbA and psbD were more expressed (Fig. 5C) during LT (2.9 and 2.6) compared to HT (2.2 and 2.1). PSBS and ZEP showed expression of 1.3 and 2.4 at LT, decreasing to 0.8 and 0.7 at HT. HSP90 and CAT had LT expression levels of 4.4 and 3.6, respectively, which dropped to 2.7 and 2.4 during HT. AOX1A and FES1 expression ranged between 2.1 and 2.2 at LT and 1.8–2.0 at HT respectively.

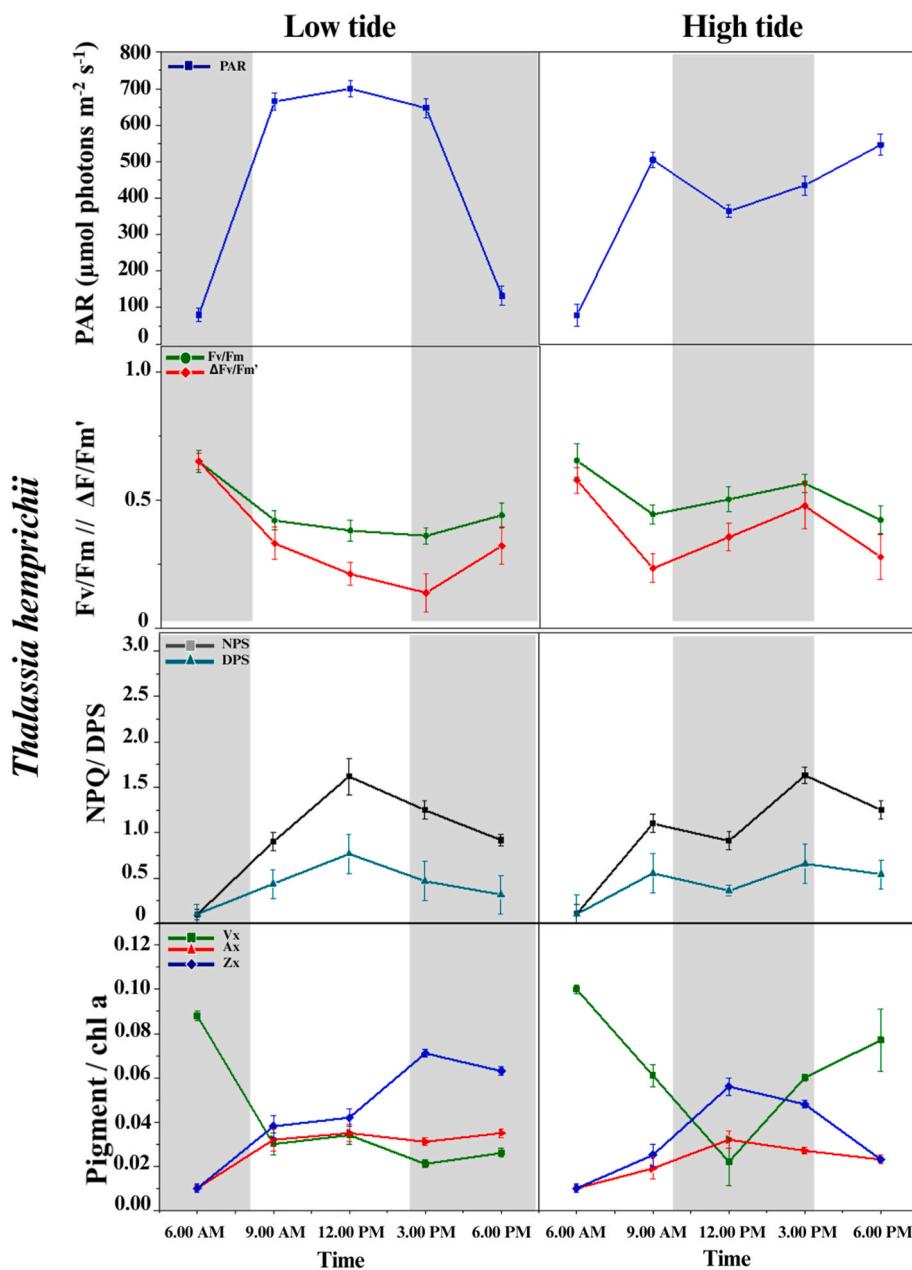


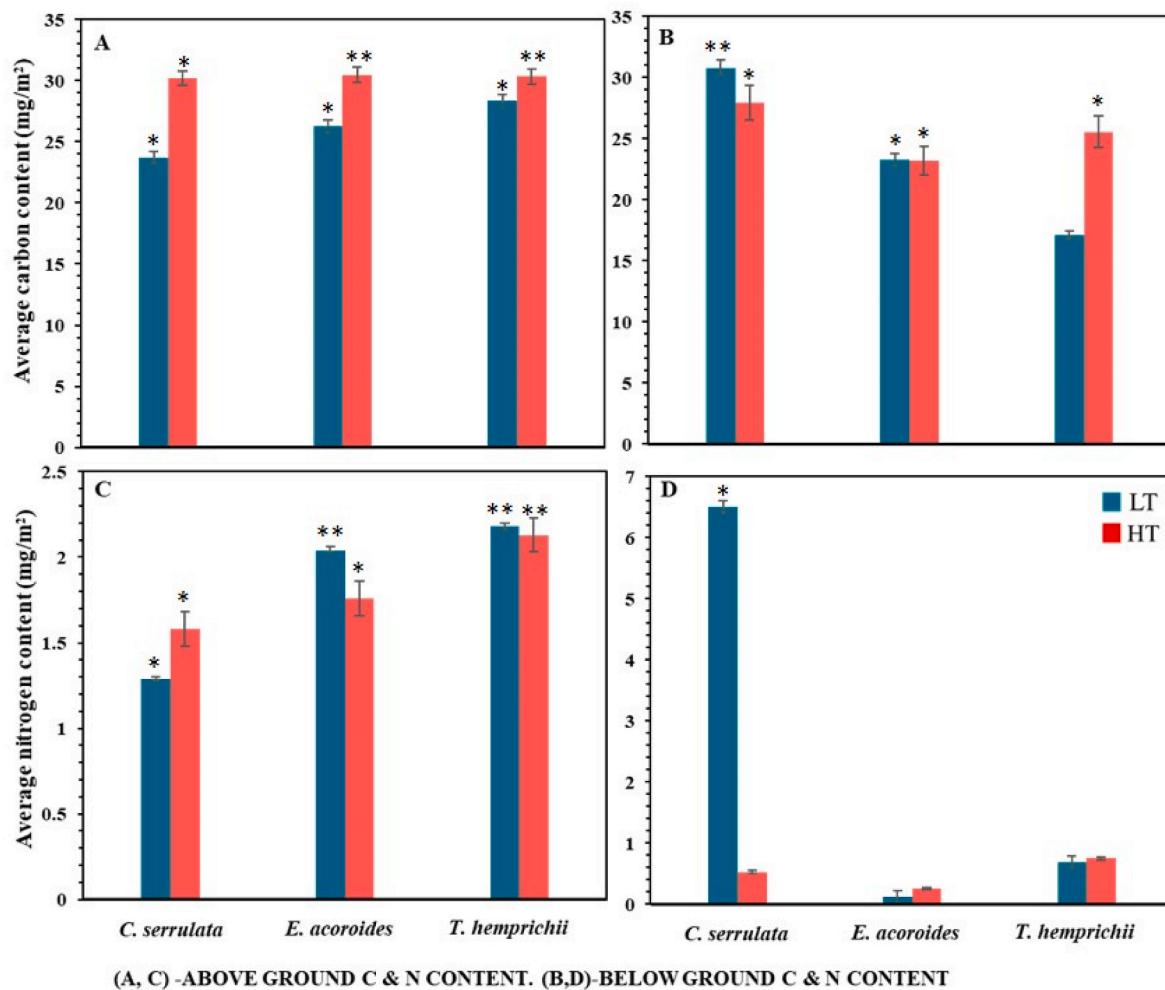
Fig. 2. (continued).

#### 4. Discussion

Seagrasses are the unique marine angiosperm experiencing a global decline in recent years due to various stress factors including anthropogenic pressures and climate change. Understanding how these vital ecosystems respond to environmental stressors such as tidal fluctuations and warming events is crucial especially in the context of climate change. This study provides insights into the physiological, biochemical and molecular responses of three seagrass species, *C. rotundata*, *E. acoroides* and *T. hemprichii* exposed to tidal dynamics and marine heatwave events in the Gulf of Mannar, India. The present study revealed the significant diurnal variations in key water quality parameters including temperature, salinity, pH and dissolved oxygen under tidal fluctuations during the marine heatwave events. These fluctuations reflected the dynamic interplay between tidal stage and thermal stress, exhibit valuable insights into the physicochemical response of intertidal waters under the extreme thermal conditions. During the low tide, the

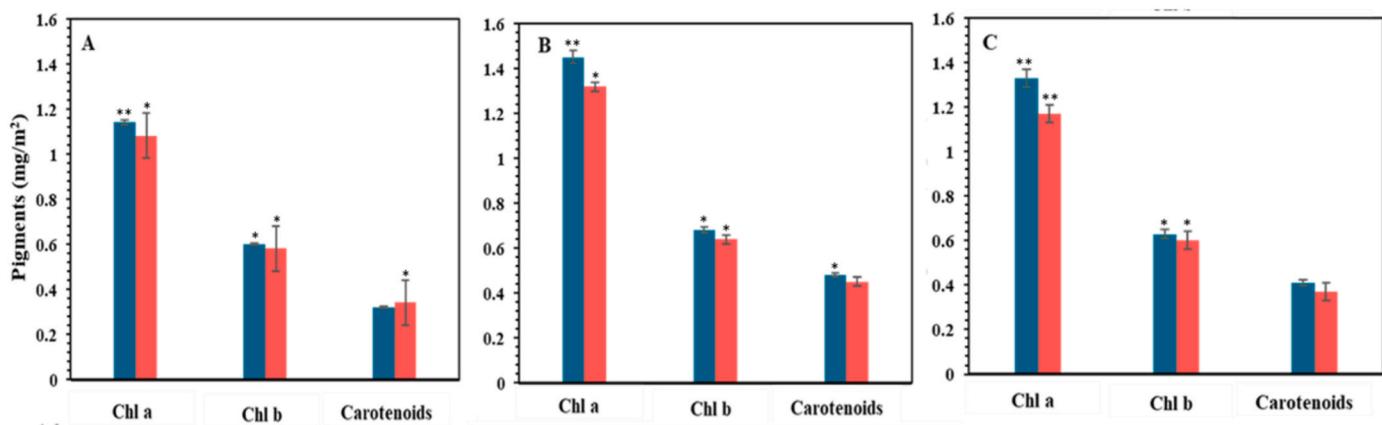
surface water temperature exhibited a marked increase, peaking at  $34.5 \pm 0.4$  °C around noon followed by a decline in the evening. The results obtained were aligned with the previous studies that elevated temperature during low tide might be due to the diminished tidal mixing, thereby promotes heat accumulation in shallow coastal zones. The peak in midday during LT was likely driven by the intense solar irradiance, as reduced water depth enhances solar energy absorption and the subsequent decline in the late afternoon was likely attributable to decreasing solar input and the onset of evening cooling, as the water body began to dissipate the accumulate heat. In contrast, the thermal regime during high tide (Fig. 1B) was comparatively moderated and temperature increase was substantial but less extreme than at low tide, which could be attributed to the greater water column depth and enhanced tidal mixing associated with the HT. These conditions facilitated more effective heat distribution and exchange, thereby mitigating surface warming.

The photo physiological response of seagrass species was studied and among the three species, *E. acoroides* exhibited the highest



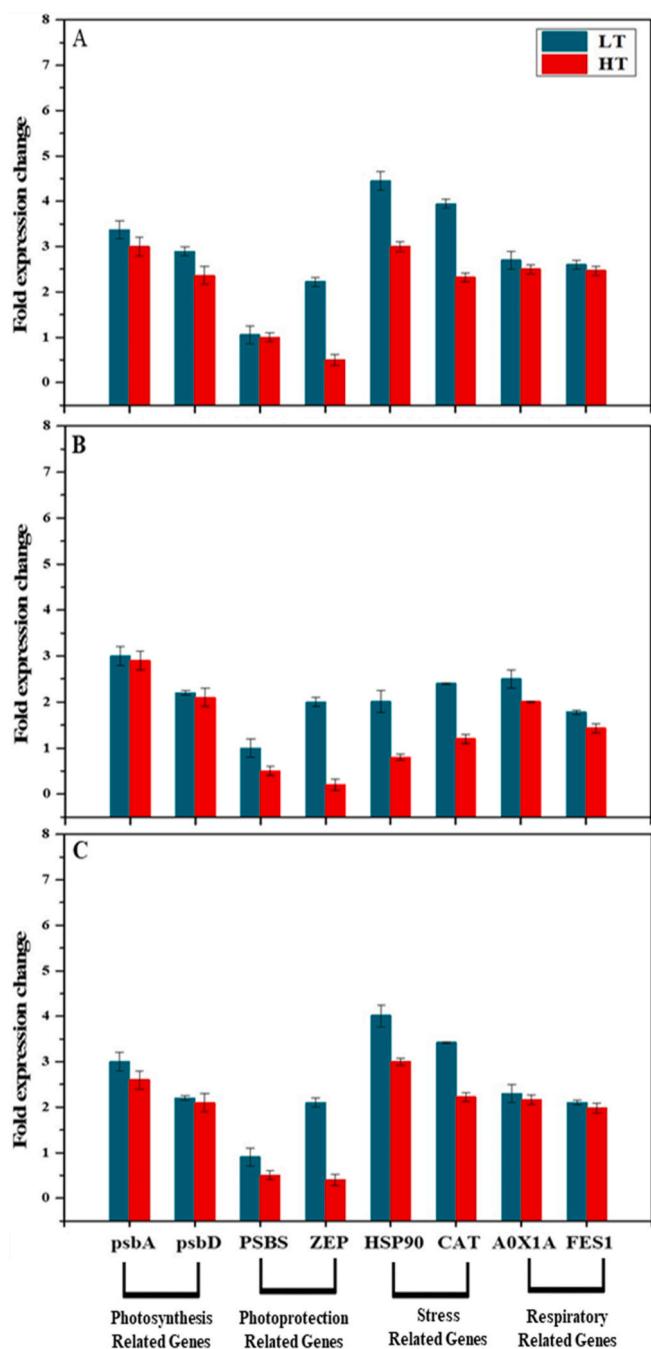
(A, C) - ABOVE GROUND C &amp; N CONTENT. (B,D)-BELOW GROUND C &amp; N CONTENT

Fig. 3. Total Carbon (TC) and Total Nitrogen (TN) Content, measured in key studied seagrass species from the Gulf of Mannar

Fig. 4. | Total pigments (chl a, chl b, carotenoids) content observed in the studied seagrass species during LT (blue) and HT (red) (A - *Cymodocea rotundata*, B - *Enhalus acoroides*, C - *Thalassia hemprichii*)

photosynthetic activity, followed by *T. hemprichii* and *C. rotundata*. These findings are consistent with previous reports (Ralph et al., 2007) that suggest high light intensity can induce reductions in electron transport rate (ETR) and triggered an increase in non-photochemical quenching (NPQ), a photoprotective mechanism to dissipate excess excitation energy as heat. Tidal dynamics play a crucial role in modulating light availability for seagrasses and our field measurements

captured these dynamics, with elevated photosynthetically active radiation (PAR) during low tide likely inducing dynamic photoinhibition. This reversible downregulation of photosystem II activity is a key strategy employed by seagrasses to mitigate oxidative damage caused by excess irradiance (Marín-Guirao et al., 2013). Tidal rhythms play a crucial role in regulating the light environment for intertidal seagrasses. During high tide, the water column buffers incoming irradiance,



**Fig. 5.** | Differential gene expression pattern of photosynthesis-related genes (psbA and psbD), Photoprotection related genes (PSBS and ZEP), Respiratory related genes (AOX1 and FES1) and stress-related (HSP90 and CAT) in *Cymodocea rotundata*(A), *Enhalus acoroides*(B) and *Thalassia hemprichii*(C) exposed to tidal fluctuations.

allowing seagrasses to maintain photosynthetic activity closer to their light compensation point. Studies by Collier et al. (2011) and Longstaff et al. (1999) corroborate these findings, suggesting that tidal immersion reduces the risk of photo damage. In contrast, low tide exposes seagrasses to direct sunlight and thermal stress, necessitating efficient heat dissipation and photo protective strategies (Fang et al., 2020).

All three species followed a characteristic bell-shaped pattern in photosynthetically active radiation (PAR) across the diurnal cycle, with maximum irradiance recorded around midday during low tide and comparatively lower peaks during high tide. These patterns are consistent with the typical exposure of intertidal seagrasses to direct solar

radiation during low tide, when water depth is minimal and irradiance can exceed  $900 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ . This environmental scenario exerts significant stress on the photosynthetic machinery, particularly Photosystem II (PSII), necessitating both physiological and biochemical adjustments to maintain photosynthetic integrity and minimize photo-damage (Kohlmeier et al., 2017). Effective quantum yield ( $\Delta F/Fm'$ ), a sensitive indicator of the operational efficiency of PSII, declined significantly during midday, particularly at low tide. This decline was found high in *T. hemprichii* and *C. rotundata*, reaching values as low as 0.28–0.29, while *E. acoroides* showed a relatively milder reduction (minimum  $\sim 0.32$ ). The partial recovery of  $\Delta F/Fm'$  in the late afternoon underscores the dynamic and reversible nature of photoinhibition, suggesting a downregulation of photochemical efficiency as a protective strategy rather than irreversible damage to the photosystems (Kohlmeier et al., 2017). The smaller midday reduction in  $\Delta F/Fm'$  observed during high tide supports the hypothesis that water column attenuation provides a protective buffer against extreme irradiance. In case of maximum quantum yield (Fv/Fm), the potential efficiency of PSII in the dark-adapted state, remained relatively stable across the day, with only minor noon-time reductions under low tide. These modest changes typically ranging between 0.69 and 0.76 are characteristic of transient photoinhibition and reflect a well-regulated balance between photodamage and repair. Such photophysiological stability suggests an inherent acclimatory capacity in these species, allowing them to maintain robust photosynthetic potential even under high light stress which aligns with the previous study of Jeyapragash et al. (2021). Non-photochemical quenching (NPQ), a key mechanism of energy dissipation via the xanthophyll cycle (Ralph et al., 2002) showed a pronounced midday increase in all three species, with the highest values recorded during low tide. *T. hemprichii* exhibited the greatest NPQ (3.54), followed by *E. acoroides* and *C. rotundata*. This sharp increase in NPQ coincides with the periods of maximum irradiance and is indicative of enhanced thermal dissipation of excess excitation energy to protect PSII reaction centers (Saewong et al., 2024). NPQ values were consistently lower during high tide, reaffirming the role of water depth in modulating light stress and the associated photoprotective responses. Intertidal species have evolved adaptive mechanisms, such as cyclic electron flow and increased NPQ, to survive these challenging conditions (Fang et al., 2020).

The xanthin cycle pigments violaxanthin (Vx), antheraxanthin (Ax), and zeaxanthin (Zx) exhibited clear dynamic shifts in response to diurnal irradiance patterns. A significant midday decline in Vx, concurrent with an increase in Ax and Zx, particularly under low tide conditions, confirms the operation of the xanthophyll cycle (Kohlmeier et al., 2017). This pigment conversion serves to dissipate excess excitation energy as heat and is a central component of NPQ. *T. hemprichii* consistently showed higher Zx and Ax levels compared to the other species, indicating a greater capacity for photoprotection under intense irradiance. The differential xanthophyll cycle activity among the species likely reflects variation in their ecological niches and light acclimation strategies. De-epoxidation state (DPS), another proxy for xanthophyll cycle activity, peaked at noon in all species, mirroring the NPQ trend (Buapet et al., 2017). The higher DPS values in *T. hemprichii* and *E. acoroides* underscore their robust non-photochemical quenching capacity, which likely underpins their ecological success in high-irradiance intertidal habitats (Heembo et al., 2023a,b). These responses are consistent with previous studies showing that tropical seagrasses exhibit enhanced xanthophyll cycling and NPQ under fluctuating light conditions. Chlorophyll *a* content exhibited mild diurnal fluctuations, with slight increases during the afternoon under low tide conditions. This trend could be attributed to transient increases in pigment synthesis or redistribution within the leaf tissues in response to increased light availability. While such short-term adjustments are subtle, they may contribute to optimizing light harvesting and sustaining photosynthetic performance during periods of elevated irradiance. Among the species, *T. hemprichii* consistently exhibited higher

chlorophyll *a* content, which may reflect its adaptation to shallow, high-light environments (Kohlmeier et al., 2017).

The carbon and nitrogen dynamics in the seagrass species under marine heatwaves during LT and HT were studied and the above-ground carbon (C) content significantly increased across all three species during HT conditions, suggesting enhanced photosynthetic activity due to better hydration, reduced desiccation stress, and improved nutrient uptake (Atkinson and Smith, 1983; Waddell et al., 2024). The most prominent increase was observed in *E. acoroides*, which may reflect its robust photosynthetic capacity and structural adaptations under submerged conditions. This response is consistent with previous findings indicating that tidal submergence facilitates optimal photosynthetic performance and resource acquisition in seagrasses. While nitrogen (N) content in the leaf tissues showed a varied pattern among species and *C. rotundata* demonstrated a notable increase in N during HT, indicating active nitrogen assimilation possibly linked to increased metabolic activity (Berlinghof et al., 2024). Interestingly, *E. acoroides* exhibited higher N levels during LT, which could suggest nitrogen reallocation or conservation mechanisms during suboptimal conditions. In contrast, *T. hemprichii* maintained relatively stable N levels across both tidal regimes, suggesting a more conservative nutrient strategy or physiological buffering capacity. In below-ground tissues, the patterns were more variable among the species out of which *C. rotundata* exhibited a marked reduction in both C and N under LT, possibly due to reduced photosynthate translocation and impaired nutrient uptake under exposure-induced stress. *E. acoroides* maintained stable carbon levels but showed a slight decline in nitrogen content, while *T. hemprichii* interestingly increased its below-ground carbon during HT, possibly as a strategy to support rhizome development or energy storage. The reduced below-ground nitrogen across species during tidal exposure may indicate a decline in nitrogen fixation or microbial-mediated nutrient turnover under stress (Liu et al., 2022).

Pigment analysis revealed that chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and carotenoids were consistently higher during HT across all three species, indicating enhanced light-harvesting potential and photoprotective capacity when fully submerged. The decline in pigment concentrations during LT conditions aligns with reduced photosynthetic efficiency due to aerial exposure, light stress, and potential photo-inhibition. *T. hemprichii* recorded the highest Chl *a* content during HT, which may reflect its adaptive strategy to optimize light capture in clearer waters. *C. rotundata* and *E. acoroides* also showed notable pigment reductions under LT, though to a lesser extent in the latter, which may suggest species-specific resilience to tidal desiccation. Carotenoid levels, essential for photoprotection and ROS scavenging, followed a similar trend with higher rate under HT and reduced under LT further supporting the notion that tidal submergence promotes optimal physiological functioning (Heemboo et al., 2023a,b).

The gene expression profiling of seagrass species under simulated marine heatwave (MHW) conditions across tidal regimes (low tide vs. high tide) reveals clear species-specific transcriptional responses, particularly for genes associated with photosynthesis, photoprotection, stress response, and respiration (Booth et al., 2024). Photosynthetic genes psbA and psbD, encoding essential proteins of the PSII reaction center, were consistently upregulated under low tide conditions in all three species, suggesting enhanced photodamage repair and photosynthetic stress response during periods of greater light and thermal exposure (Robbins and Kelly, 2025; Péter et al., 2008; Jeyapragash et al., 2025). *Cymodocea rotundata* exhibited the highest expression of these genes at LT (3.4 and 3.1 fold), indicating a greater photosynthetic adjustment to cope with environmental stress. Similarly, *E. acoroides* and *T. hemprichii* also showed an increased expression at LT, though with slightly lower fold changes, which suggests that these seagrasses initiate a photoprotective mechanism to compensate for the heat- and light-induced impairment of the photosynthetic machinery (Cai et al., 2022).

Genes related to photoprotection, namely PSBS and ZEP, also

showed heightened expression during LT in all three species, aligning with increased stress from excess irradiance and thermal stress at midday low tides. These genes are central to the xanthophyll cycle and non-photochemical quenching, enabling dissipation of excess excitation energy as heat (Welc et al., 2021). Notably, ZEP was strongly induced in *C. rotundata* (2.6-fold) and *T. hemprichii* (2.4-fold), suggesting a robust mechanism for photoprotection under LT. In contrast, HT conditions showed reduced expression of these genes, indicating comparatively lower stress when the seagrasses were submerged and possibly cooler.

Stress-responsive genes HSP90 and CAT were also significantly upregulated under LT across all species, reflecting their role in cellular homeostasis under thermal and oxidative stress (Song et al., 2025). The elevated expression of HSP90 (4.5-fold in *C. rotundata*, 4.4-fold in *T. hemprichii*) suggests the activation of heat shock response pathways to maintain protein integrity. Likewise, the catalase gene CAT, involved in reactive oxygen species (ROS) scavenging (Zou et al., 2025), showed increased expression during LT, implying elevated oxidative stress levels during these periods. The decrease in their expression during HT suggests reduced environmental stress when tidal water provides thermal buffering (Procaccini et al., 2017). Interestingly, the expression of respiration-related genes AOX1A and FES1 remained relatively stable across tidal conditions in all species. These genes are involved in mitochondrial electron transport and stress recovery, and their relatively uniform expression indicates a sustained basal level of respiratory activity regardless of tidal influence, perhaps to meet ongoing energy demands under MHW conditions (Ruocco et al., 2021).

## 5. Conclusion

To our knowledge, this is the first integrated field based study from India that simultaneously evaluates biochemical, photophysiological, pigmentary, and gene expression responses of multiple tropical seagrass species under tidal and marine heatwave (MHW) conditions. Our results provide compelling evidence for species-specific photoprotective strategies aimed at mitigating photo-oxidative stress induced by fluctuating irradiance and temperature in intertidal environments. Key findings include the coordinated regulation of PSII efficiency, enhanced non-photochemical quenching (NPQ), and dynamic adjustments in chlorophyll and xanthophyll pigments, coupled with upregulation of photosynthetic (psbA, psbD), stress-related (HSP90, CAT), and photoprotective (PSBS, ZEP) genes. These physiological and molecular adjustments reflect a finely tuned balance between energy capture and dissipation under real-time environmental stressors. Importantly, the study revealed interspecific differences in carbon-nitrogen metabolism and stress regulation. *Thalassia hemprichii* exhibited the most stable photochemical and transcriptional response across tidal regimes, suggesting a higher degree of acclimation and resilience. In contrast, *E. acoroides* and *C. rotundata* showed relatively higher variability in physiological performance, highlighting differential capacities for stress tolerance, which may influence their spatial distribution and long-term survival in climate-sensitive habitats. Theoretical implications of this study extend to improving our understanding of functional trait diversity and adaptive capacity among tropical seagrasses, particularly in the context of rising sea surface temperatures and altered tidal patterns. Practically, these findings support the incorporation of physiological and gene-based markers such as pigment dynamics, stress gene expression, and NPQ modulation into early warning systems and restoration frameworks. This can enable the selection of climate-resilient species or primed planting materials for ecosystem-based adaptation strategies. However, the study is constrained by its short temporal scale and lack of long-term monitoring data, which limits extrapolation under future climate projections. Future research should focus on multi-seasonal assessments, epigenetic profiling, and transcriptomic-level investigations across broader geographical gradients to unravel the persistent effects of repeated heat stress and tidal variability.

## CRediT authorship contribution statement

**Jeyapragash Danaraj:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Praveen Kumar Natarajan:** Writing – original draft, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Yosuva Maria-singarayam:** Visualization, Validation, Resources, Formal analysis. **Aiswarya Thomas:** Visualization, Validation, Methodology, Investigation, Formal analysis. **Saranya Unni:** Validation, Methodology, Investigation, Formal analysis, Data curation.

## Declaration of competing interest

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

The authors also declare that we have no conflict of Interest to disclose.

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## Data availability

Data will be made available on request.

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