

Effects of flooding on the Mediterranean *Cymodocea nodosa* population in relation to environmental degradation

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Abstract: *Cymodocea nodosa* is a common seagrass species of shallow and sheltered Mediterranean waters, where extreme flushing of plumes can occur during excessive rainfall. *Cymodocea nodosa* shoots were sampled from two habitats of Kavala Gulf, one nearly pristine (less stressed, Vrasidas) and another highly stressed (Nea Karvali), to study if flooding might negatively affect seagrass habitats. Photosynthetic performance of shoots from the pristine habitat acclimated better than shoots from highly stressed conditions simulating a flooding event. Indeed, a significant ($p < 0.01$) interaction between habitat and flooding on photochemical energy harvesting ($\Delta F/F_m'$) values was found, with lower $\Delta F/F_m'$ values in the pristine habitat under control conditions. Furthermore, based on relative electron transport rate (rETR) curves reconstructed from fluorescence-versus-irradiance data, shoots from the pristine habitat performed better after 18 days of treatment to flooding. On the other hand, shoots from highly stressed habitats grew faster than pristine ones, but their growth decreased similarly under flooding conditions. The implications for management and conservation priorities for this phenotypically plastic seagrass in the Mediterranean are discussed.

Keywords: Seagrass, chlorophyll *a* fluorescence, growth rate, factorial experiment, North Aegean Sea

1. Introduction

Seagrasses are flowering, rooted plants that form extensive meadows in shallow sandy to muddy coastal waters worldwide (Larkum et al. 2006), receiving about 10–20 % of the incident irradiance at the water surface (Hemminga and Duarte 2000, Green and Short 2003). Their highly productive meadows (Pergent et al. 2014, Holmer 2019) hold a significant role in benthic carbon sequestration (Fourqurean et al. 2012, Trevathan-Tackett et al. 2015, Küpper and Kamenos 2018, Stankovic et al. 2018) and contribute to nutrient cycling and storage (Samper-Villarreal et al. 2016, Kindeberg et al. 2018). They provide a wide range of valuable ecosystem services (ecosystem engineers), such as coastal protection, nursery habitats, and sediment accretion and stabilisation (Boudouresque et al. 2016, Ruiz-Frau et al. 2017, Unsworth et al. 2019). Furthermore, seagrasses can be used as biomarkers (Ferrat et al. 2003, Malea et al. 2021) and bioindicators (Marbà et al. 2013, Malea et al. 2019, Orfanidis et al. 2020) since they can accumulate toxic substances and respond to biotic and abiotic changes in their habitat (Orth et al. 2006, Fernández-Torquemada et al. 2020).

Although seagrasses exhibit a high capacity to alter their physiology, morphology, and behaviour in response to a different environment (Maxwell et al. 2014), known as "phenotypic plasticity" (Sultan 2000, Bertelli et al. 2021), they are declining today due to direct and indirect effects of multiple stressors (Short and Wyllie-Echeverria 1996, Orth et al. 2006, Marbà and Duarte 2010, Stockbridge et al. 2020, Dunic et al. 2021) although a reversal of this trend has been documented in Europe (de los Santos et al. 2019). Indeed, land-based activities such as deforestation, agriculture, and urbanisation contribute to increased erosion, poor inland and coastal water quality, and the eventual loss or deterioration of downstream coastal marine habitats, especially the light availability within the water column (Saunders et al. 2017). Such changes affect seagrasses, which have high irradiance requirements to provide oxygen to roots and rhizomes and support a large amount of non-photosynthetic tissue (Orth et al. 2006, Ralph et al. 2007). Also, nutrient additions from anthropogenic activities (Leoni et al. 2008, Mvungi and Mamboya 2012) may cause loss of seagrass communities in eutrophicated waters (Burkholder et al. 2007, Leoni et al. 2008), where opportunistic algae will grow extensively (Kenworthy and Fonseca 1996, Cloern 2001). High-salinity brine discharges from seawater desalination plants can also negatively impact seagrass meadows . However, despite a better understanding of the causes of this widespread seagrass loss (Preen et al. 1995, Short and Wyllie-Echeverria 1996, Waycott et al. 2009), very few studies have documented post-flood recovery rates of intertidal meadows (Maxwell et al. 2014). Following

major, potentially catastrophic, flooding events, recovery of seagrass meadows may either fail or take many years to recover from the following stress from declining water quality (Short and Wyllie-Echeverria 1996).

Cymodocea nodosa (Ucria) Ascherson is a common seagrass species in the Mediterranean Sea and in the Canary Isles (Hemminga and Duarte 2000) that grows relatively fast compared to other seagrass genera (Marbà et al. 2004, Tsioli et al. 2019). Most of the leaf surface is photosynthetically active (Cancemi et al. 2002), contributing to a significant proportion of the total productivity (Hemminga and Duarte 2000), and is often populated by epiphytes (Borowitzka et al. 2006). Temperature but mainly salinity affect multiple biological levels, from molecular to sub-cellular and physiological, decreasing the species' growth and fitness (Tsioli et al. 2022). The evolutionary implications of differential transcriptome genotypes of osmotic and temperature stress (Malandrakis et al. 2017), along with the significant diversity among different geographical areas in the Eastern Mediterranean Sea (Gkafas et al. 2016, Konstantinidis et al. 2022) showed the genotype-specific strategy of the species for colonization and adaptation to habitats. However, in the Mediterranean Sea and the Canary Islands, *Cymodocea nodosa* meadows (Tuya et al. 2014) are declining because of light and nutrient availability and turbidity caused by anthropogenic pressures (Hemminga and Duarte 2000, Short et al. 2001, Papathanasiou et al. 2016, Papathanasiou and Orfanidis 2018, Najdek et al. 2020). Understanding the effects of heavy rain causing reduced salinity and increased nutrients and turbidity (i.e., low irradiance), is vital to predict similar impacts of climate change and to develop effective management measures for seagrass conservation in the area.

Measuring chlorophyll *a* fluorescence is a widely used technique to study photosynthetic performance of plants, including seagrasses, and to monitor the effects of anthropogenic stressors on plant physiology (Beer et al. 1998, Silva and Santos 2003, Ralph and Gademann 2005, Papathanasiou 2013). Effective quantum yield ($\Delta F/F_m'$) measures PSII quantum yield in the light, which requires photosystems to be operational under a certain degree of light pressure. Rapid light curves (RLCs) or photosynthesis-irradiance (P-E) curves are excellent tools for studying relationships between irradiance and photosynthesis. RLCs follow changes in photosynthetic activity related to incremental increases in irradiance.

This study aimed to explore the photosynthetic performance and growth of *C. nodosa* from two different habitats, one near-pristine and another anthropogenically stressed, along

the coastline of the Gulf of Kavala (Greece) under (1) control conditions simulating early summer and (2) treatments simulating hypothetical flood plume events. Two experiments were conducted in the laboratory to test if the following null hypotheses were true: The simulated flooding event does not affect (1) the photosynthetic performance or (2) the growth of randomly chosen pristine versus degraded *C. nodosa* habitats. The response variables were the effective quantum yield ($\Delta F/F_m'$), maximum electron transport rate (ETR_{max}), light-limited photosynthetic efficiency (α), and minimum saturating irradiance (E_k) for photosynthetic performance, and growth rate.

2. Materials & Methods

2.1 Sampling areas and samples

Sampling was conducted at two meadows of known ecological status in the Gulf of Kavala, North Aegean Sea, Greece: Vrasidas (24°19'8.78"E, 40°49'37.53"N) and Nea Karvali (24°31'7.68"E, 40°57'23.62"N; Figure 1). Vrasidas meadow is in the inner part of Cape Vrasidas of Eleutheron Gulf. It is one of the least stressed (called “pristine” from now onwards) areas close to the surrounding mainland of Kavala Gulf and has been included in the European Natura 2000 network (code GR1150009). Main anthropogenic activities include fishing and tourism in the broader region and port activities in the nearby town of Nea Peramos. Nea Karvali is an old agricultural and fishing settlement that, since 1981, has seen increased levels of industrial development, the establishment of a phosphorus fertiliser plant, the Kavala city wastewater treatment facility, and a crude oil de-sulfurisation complex of the Prinos Oil Field, all of which significantly affect the surrounding coastal area. Therefore, the Vrasidas meadow was chosen as a reference site, while the meadow at Nea Karvali was selected as a highly stressed one (see Orfanidis et al. 2011, Papathanasiou et al. 2015, Papathanasiou and Orfanidis 2018).

Twenty-four intact shoots were collected twice, on the 8 May (1st experiment) and 13 June (2nd experiment) 2014, of which 12 were from Vrasidas and 12 were from Nea Karvali. All shoots were collected from 2 m depth and consisted of leaves, rhizomes, and roots. Plants were transported in seawater within 1 h to the laboratory, where epiphytic organisms were carefully removed by hand and acclimated in aerated 5-L plastic aquaria filled with seawater under constant irradiance (60–90 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), daylength (14:10 light: dark), and temperature (21–22°C) for 3 days.

Experimental conditions

Two consecutive experiments were conducted: plants from two *C. nodosa* habitats (pristine and stressed) treated under two growth conditions simulating early summer non-flooded (control) and flooded conditions. To study the interaction between habitat (two levels) and flood (two levels), the following treatments were used: a) Control Pristine-group (CP; salinity 33-34, 3 μM N, 0.8 μM P, 80-90 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$), b) Control Degraded-group (CD; salinity 33-34, 3 μM N, 0.8 μM P, 80-90 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$), c) Flooding Pristine-group (FP; salinity 23-24, 30 μM N, 2 μM P, 10-20 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$), d) Flooding Degraded-group (FD; salinity 23-24, 30 μM N, 2 μM P, 10-20 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$).

Twenty-four 3-l plastic aquaria (n=6) filled with 1 l growth medium were prepared by diluting nutrient-free sea salt (Münster Meersalz) in resin-filtered tap water (<15 $\mu\text{S/cm}$). All aquaria were placed in a constant temperature (21-22°C) and irradiance (fluorescent tubes, 14 h light per day) cultivation chamber. They were aerated with continuous water flow and covered with clear glass lids to avoid evaporation. Salinity was fixed using a portable conductivity meter (WTW, LF330), and Photosynthetic Active Radiation (PAR) was measured at the base of aquaria by a quanta-meter (Li-COR Li-250). Different nutrient solutions were prepared by adding NaNO_3 , KH_2PO_4 quantities to the growth media.

2.2 Response variables

Every other day within the 18 experimental days of the 1st experiment, chlorophyll *a* fluorescence was measured using the pulse amplitude modulated (PAM), Diving-PAM underwater fluorometer (Walz, Effeltrich, Germany). All fluorescence measurements were conducted between 10:00 and 12:00 to minimise potential photosynthetic activity variability due to diurnal cycles. The samples were taken from the exposure conditions and transferred to a Haake cryothermostat (regulated to experimental temperatures; ± 0.2 °C), where $\Delta\text{F}/\text{F}_m'$ was measured. All measurements were taken on the second leaves within a shoot (Durako and Kunzelman 2002, Papathanasiou et al. 2015). Effective quantum yield ($\Delta\text{F}/\text{F}_m'$) of photosystem II was computed according to the formula by Genty et al. (1989):

$$\Delta\text{F}/\text{F}_m' = (\text{F}_m' - \text{F}_s)/\text{F}_m'$$

F_m' = maximal fluorescence of a light-adapted plant when all reaction centres are reduced; F_s = fluorescence in the light when the reaction centres are opened (Schreiber and Bilger 1993).

Rapid light curves (RLCs) were recorded for each sample of the 2nd experiment on day 6, 14 and 18 using the Diving-PAM underwater fluorometer (PAM-2000, Walz, Effeltrich, Germany) under the control of preinstalled software using artificial PAR photon flux density. All RLCs were conducted between 10:00 and 12:00 to minimize potential variability of photosynthetic activity due to diurnal cycles. Leaves were connected to a WALZ "dark leaf clip" to maintain a consistent spacing of the PAM fiber optic from the surface of the tissue and to permit fluorescence measurements without ambient light interference. Eight (8) levels of increasing irradiance were applied (LCWIDTH, 30 s each; 53, 115, 189, 263, 358, 510, 654, and 933 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$) to the plants. Each increment was followed by a saturating light pulse, and these data were plotted to create the curve. The fluorescence parameters such as effective quantum yield ($\Delta F/F_m'$), relative electron transport rate (rETR) and quenching factors were calculated automatically by the PAM device. rETR was calculated according to the formula developed by Schreiber et al. (1995) as follows:

$$\text{rETR} = (\Delta F/F_m') \times E \times A \times \text{FII}$$

where E = incident irradiance ($\mu\text{mol photon m}^{-2} \text{s}^{-1}$), $A=0.85$, leaf absorptance, and $\text{FII}=0.5$, fraction of chlorophyll associated with photosystem II.

To quantitatively equate RLC, ETR_{max} , α and E_k variables were assessed by fitting data from each RLC to the Eilers and Peeters (1988) photosynthesis-irradiance model using the solver function in Microsoft Excel (iterative curve fitting).

The growth rates of *C. nodosa* were estimated at the 2nd experiment as leaf elongation rate ($\text{cm shoot}^{-1} \text{day}^{-1}$), which is the length of new leaf tissue per shoot after a fixed period using the *in situ* leaf-marking technique (Short and Duarte 2001, Tsioli et al. 2019).

2.3 Statistical analysis

All data of day 0 were excluded to avoid artificial variance in the mean as the shoots potentially acclimatised during the transfer from a natural to a laboratory environment. To assess the effect of flooding, the variation in the effective quantum yield, $\Delta F/F_m'$ and RLC parameters (ETR_{max} , α , E_k) among both samples from Vrasidas and Nea Karvali in control conditions and flood conditions were determined by the linear mixed-effects model in

the R Environment (R Core Team 2014). Before the analyses, data were tested for normality, homogeneity of variance and sphericity for repeated measures ANOVA. ANOVA was conducted using the R core function `aov()` when these criteria were met. Since the requirements were not met for the RLC data, a non-parametric repeated measures ANOVA was run using the ARTool package (Kay et al. 2021). When data followed the normal distribution, the mean values and standard errors were reported and discussed, while when data were analysed with a non-parametric approach, the median and Inter Quantile Range (Q1-Q3) was used instead. Figures were prepared using the `ggplot2` package (Wickham 2016).

3. Results

3.1 Fluorescence measurements: effective quantum yield ($\Delta F/F_m'$)

A significant interaction between habitat and flooding on $\Delta F/F_m'$ values was found (Table 1; $F_{(1,204)}=40.631$, $p<0.001$). The variation of $\Delta F/F_m'$ values of *C. nodosa* with time under different treatments is shown in Figure 2. A general increase was witnessed in $\Delta F/F_m'$, from $0.766 \pm \text{IQR}(0.749-0.779)$ in the control to $0.774 \pm \text{IQR}(0.765-0.781)$ in the flood treatment. While measurements from the degraded meadow did not vary significantly between the control [median = $0.778 \pm \text{IQR}(0.765-0.783)$] and the flood conditions [median = $0.773 \pm \text{IQR}(0.765-0.779)$], this does not apply for shoots from the pristine meadow [median_{control} = $0.756 \pm \text{IQR}(0.741-0.767)$ & median_{flood} = $0.777 \pm \text{IQR}(0.767-0.783)$].

3.2 Fluorescence measurement: rapid light curve (RLC)

The mean relative electron transport rate (rETR) values of 6, 12 and 18 days vs irradiance (rapid light curves-RLC) from both pristine (Vrasidas) and degraded (Nea Karvali) habitats under two experimental conditions (control, flooding) of cultivation in the laboratory are presented in Figure 3. For samples from the degraded habitat, rETR shows a similar behaviour under both treatments (mean_{control} = $11.48 \mu\text{mol e m}^{-2} \text{ s}^{-1} \pm 0.37$ and mean_{flood} = $10.68 \mu\text{mol e m}^{-2} \text{ s}^{-1} \pm 0.21$), while flooding appears to have a quenching effect on rETR in the samples from the pristine habitat (mean in control = 12.75 ± 0.28 and mean in flood = 11.42 ± 0.24).

3.3 RLC parameters: ETR_{max} , alpha (α), E_k

Median and IQR range for the fitted RLC parameters of all experimental conditions and days are presented in Table S1. ETR_{max} was significantly different between treatment and habitat ($p < 0.001$), while no significant interaction was observed (Table 2). After 18 days, ETR_{max} was higher in the control treatments with the highest values in the pristine habitat [median = $16.715 \mu\text{mol e m}^{-2} \text{ s}^{-1} \pm \text{IQR (15.218-19.158)}$], while lowest values were measured in the degraded habitat under flood conditions [median = $11.305 \mu\text{mol e m}^{-2} \text{ s}^{-1} \pm \text{IQR (11.090 - 12.555)}$] (Figure 4).

No statistical differences were observed at any level for the parameters a and E_k ($p > 0.05$; Table 2). Alpha (a) ranged from $0.262 \pm \text{IQR (0.235 - 0.235)}$ in samples from the degraded habitat under flood to $0.338 \pm \text{IQR (0.311 - 0.311)}$ in samples from the pristine habitat again under flood conditions. E_k had higher values in the control treatments with the highest values in the pristine habitat [$52.050 \pm \text{IQR (39.035 - 65.163)}$] and lowest in the degraded habitat under flood [$40.615 \pm \text{IQR (39.933 - 56.920)}$]. An investigation of the box plot for the three parameters showed that significant differences might exist on the 18th day of the flood treatment (Figure 4). Thus, further data exploration was conducted with a parametric ANOVA (since criteria were met) for the three parameters only for the flood condition after 18 days. The analysis showed that after 18 days, both ETR_{max} and a had significant differences between the two habitats (Table 3). ETR_{max} was significantly higher ($F_{(1,10)} = 6.452$, $p = 0.029$) in the pristine habitat with a mean value of $13.823 \mu\text{mol e m}^{-2} \text{ s}^{-1} \pm 0.560$ than the degraded habitat that had a mean value of $11.863 \mu\text{mol e m}^{-2} \text{ s}^{-1} \pm 0.531$. The same trend was shown for a with samples from the pristine habitat having significantly higher ($F_{(1,10)} = 6.699$, $p = 0.027$) values. Mean a value ranged from 0.262 ± 0.025 in the degraded habitat to 0.365 ± 0.077 in the pristine.

3.3 Growth rate

Analysis of *Cymodocea nodosa* growth rates revealed statistically significant differences between treatments and habitats ($p < 0.05$; Table 4), but there was no statistically significant interaction between the two factors. Growth (Figure 5) was higher in the control treatment (mean = $0.803 \pm 0.070 \text{ cm shoot}^{-1} \text{ day}^{-1}$) than under the flood conditions (mean = $0.612 \pm 0.071 \text{ cm shoot}^{-1} \text{ day}^{-1}$). Shoots from N. Karvali had higher growth (mean = $0.851 \pm 0.075 \text{ cm shoot}^{-1} \text{ day}^{-1}$) than those from Vrasidas (mean = $0.563 \pm 0.047 \text{ cm shoot}^{-1} \text{ day}^{-1}$).

4 Discussion

This study showed that the null hypotheses established should be rejected in favour of the alternative ones: The simulated flooding event affected (1) the photosynthetic performance and (2) the growth of randomly chosen pristine versus degraded *C. nodosa* habitats. While low irradiance and high nutrients affected the growth of *Cymodocea* irrespective of ecological status of a given meadow, photosynthetic performance of shoots from the pristine habitat was better under flooding.

4.1 Effects on photosynthetic performance

Photosynthetic performance of *Cymodocea nodosa* shoots under flood and pristine conditions is highly dependent on habitat (Table 1). This result suggests that flood could have a neutral or even positive influence on photosynthesis in *C. nodosa* shoots. Silva et al. (2013) found that *C. nodosa* responded with a significant increase in photosynthetic efficiency to low irradiance, such as those during a flooding event. It is known that seagrasses maintain high photosynthetic efficiency ($\Delta F/F_m'$) under low irradiance conditions (Lee et al. 2007). Changes at the tissue, subcellular and molecular levels help to increase photosynthetic light capture and efficiency (Durako et al. 2003, Enríquez 2005, Marín-Guirao et al. 2022); carbohydrate reserves can be reallocated from storage organs, such as the rhizome, to reduce respiration and to slow growth pathways demanding biomass through morphological and meadow-scale changes (Fourqurean and Zieman 1991, Olivé et al. 2007, Collier et al. 2009). Leaf, shoot biomass, and growth reduction under low light conditions are typical in plants and can harm carbon fixation but might be a photo-adaptive response to reduce self-shading within the canopy (Lee et al. 2007, O'Brien et al. 2018).

Rapid light curve (RLC) measurements provided an insight into the photosynthetic performance of leaves in different light climates. The results showed that mean rETR values for both habitats differed between the control and the flooding conditions (Figure 3). This result indicates the high capacity of *C. nodosa* to acclimate to both treatment conditions. Shoots from Vrasidas exhibited more sun-adapted behaviour under both conditions than Nea Karvali shoots, which showed more shade-adapted characteristics. Since the treated shoots are derived from genetically identical populations (Vrasidas and Nea Karvali; Konstantinidis et al. 2022), this variation in the shoots' ability to capture light could be explained by phenotypic plastic differences as the plant can modify its physiology, biochemistry, behaviour, and morphology in response to environmental changes (Bulleri et al. 2018).

Light deprivation can increase photosynthetic efficiency (a) and decrease saturating irradiance and $rETR_{max}$ (Ralph and Gademann 2005). These photo-adaptive mechanisms focus on maximising the photosynthetic efficiency under low light conditions. A significant decrease of E_k and $rETR_{max}$ was witnessed after 18 days under flood conditions, showing that these parameters are indeed light stress bioindicators (McMahon et al. 2013). However, a significantly increased in the Vrasidas habitat indicating a higher potential than the stressed habitat to cope with flooding. Variation in plasticity or the acclimation capacity separates populations on the spatiotemporal scale and demonstrates the populations' ability to cope with stress conditions (Ghalambor et al. 2007, Bertelli et al. 2021, Pazzaglia et al. 2021).

Photoacclimation capability can describe the distribution of *C. nodosa*, spanning across a vertical gradient bearing ability to live and survive in the low-light environment of deep waters that extend to 40 m deep (Mazzella et al. 1993, Short et al. 2010). This plasticity towards light limitation associated with depth-dependent variation in photosynthetic-related traits has been described in *C. nodosa* by Olesen et al. (2002). Many studies have also found plasticity for features of other seagrass species. For example, a study by Sharon et al. (2009) concluded that *Halophila stipulacea* in the Gulf of Aqaba exhibits high plasticity concerning irradiance. They suggest fast changes in the photosynthetic response and light absorption features of *H. stipulacea* as an explanation for the abundance of *H. stipulacea* across depth gradients (> 50 m).

4.2 Effects on growth

In the simulated flood conditions, the *C. nodosa* growth rate decreased (Figure 5), showing the physiological acclimation during light scarcity. Leaf senescence is a response in higher plants to strong shading in stressful conditions when photosynthetic acclimation can no longer maintain a positive carbon balance (Brouwer et al. 2012). Based on the correlation between growth rate and Chl-*a* content found by Tsioli et al. (2019), the two meadows (Vrasidas and Nea Karvali) might exhibit differences in pigment content and light absorption capacity, which closely relate to the meadows' morphological characteristics (shoot size), clonal architecture and meadow construction (Olesen et al. 2002). It can be hypothesised that the Nea Karvali meadow might be composed of highly pigmented shoots that build dense canopies, which strongly reduce light availability (Dalla Via et al. 1998). Conversely, shoots at Vrasidas may be less pigmented, forming canopies that expose the photosynthetic tissue to

excess light (Raniello et al. 2004), exhibiting the sun-adapted plant features. Such a hypothesis agrees with the findings by Papathanasiou (2013).

Acclimation strategies include changes in the physical characteristics of the seagrasses. In response to reduced light intensity, seagrasses acclimate by changing their morphological features, such as the number of leaves per shoot (Dennison 1987, Abal et al. 1994), their total shoot biomass and shoot size (Lee and Dunton 1997) and chlorophyll content (Longstaff et al. 1999, Ralph 1999). Reduced light levels may also result in increased seagrass competition for light, thus increasing size distribution asymmetry of the plant canopy, i.e., larger individuals obtain a disproportionate share of light because they shade smaller ones (Orfanidis et al. 2010). Acclimation strategies focus on maximising the exposure of the photosynthetic apparatus to light to increase photosynthetic activity and to reduce respiration (Ruiz and Romero 2001).

During flooding events, seagrasses increase nutrient uptake and assimilation, resulting in increased biomass production (Fabbri et al. 2015). High nutrient levels increase seagrass photosynthesis, inducing growth, canopy height and cover (Leoni et al. 2008). However, internal nitrogen (N) and phosphorus (P) concentrations in *Cymodocea* leaves were significantly higher in Nea Karvali than in Vrasidas in the past (Papathanasiou 2013), and we expected such a photosynthetic effect to be more evident in Vrasidas shoots that showed decreased growth under flood. For Nea Karvali, exposure to high nutrient concentrations was assumed to decrease growth under nutrient-rich flooding conditions. Prolonged exposure to high nutrient concentrations is known to result in meadow deterioration, primarily due to light constraints caused by the growth of phytoplankton and epiphytes and the internal carbon shortage (Papathanasiou et al. 2015). Larger floods high amounts of nutrients and consequently drive excessive primary production (i.e., eutrophication) or modify the mix of primary producer communities, allowing unfavourable species to dominate (Saulnier-Talbot et al. 2018).

The severity and longevity of events such as floods remain a dominant factor affecting the capacity of an ecosystem to adapt or absorb the effect of such events (Barrett et al. 2008, Maxwell et al. 2014). Long-lasting effects of adverse weather will occur when the capacity to buffer the physiological impact is exhausted, causing the ecosystem to shift to other regimes (Allen and Breshears 1998, Weiskopf et al. 2020). As for anthropogenic disturbances, more severe scenarios of seagrass meadow loss are likely to occur as they are also influenced by

the duration, frequency and type of such disturbance (Short and Wyllie-Echeverria 1996, Wu et al. 2018). Over time, recurrence of stress events and ecosystem modification have restrained recovery, which has protracted or not rectifiable (Beisner et al. 2003, O'Brien et al. 2018). Therefore, this study of *C. nodosa* physiology should be expanded to cover a more extended period to better understand the level of response to flood intensities and occurrence in space and time.

The effect of flooding on seagrass biomass needs to be fully understood and incorporated in management plans, as it depends on several factors and can seriously affect the ecosystem's balance. For example, rainfall was shown to enhance seagrass biomass in an Australian seagrass meadow on Mabuiag Island (where the seagrass community includes species from the genera *Cymodocea*, *Halodule*, *Thalassia*, *Halophila*, *Enhalus*, and *Syringodium*) throughout a 30-day trial (Taylor et al. 2013). However, this study also found that 14 days of the same flood conditions decreased seagrass biomass and concluded that short rainfall events are less beneficial than rainfalls over more extended periods. This condition highlights the complexity of the effects during flooding events impacting seagrass meadows and the significance of the temporal structure of such events. Other studies showed that small, rapidly growing species, such as *Cymodocea* or *Halophila* spp., cannot tolerate extended periods of low light, primarily because of limited storage reserves, but that they can recover from such impacts quickly if conditions improve (Longstaff et al. 1999, Waycott et al. 2005).

To date, *C. nodosa* has disappeared in the Nea Karvali seabed (personal observation). This alarming situation underlines the importance of continuous seagrass monitoring in the Kavala Gulf. Brine discharge from nearby phosphorus fertiliser plant, the Kavala city wastewater treatment facility and the crude oil de-sulfurisation complex of the Prinos Oil Field might have significantly caused deterioration of water quality. These activities are harmful to the seagrass communities in the area and other marine species in Kavala Gulf. Flooding events might also add pressure and escalate the disappearance of the seagrass ecosystem from the neighbouring areas. Such disappearance should be documented and brought to attention to make stakeholders focus on such detrimental events and take restoration and management action.

5 Conclusions

This study shows that *Cymodocea nodosa* exhibits high phenotypic plasticity across habitats and environmental gradients. Furthermore, the eco-physiological approach of this study provided the first understanding of the impact of a hypothetical flood event on the *Cymodocea* meadows in the Kavala Gulf. It confirmed that low irradiance and salinity, and high nutrients simulated a flooding event as critical parameters for growth irrespective of meadow ecological status. Furthermore, the photosynthetic performance of shoots from the pristine habitat was better under flooding. Additional experiments coupled with long-term mapping surveys with physiological and molecular studies will provide further insight and contribute to defining management priorities for angiosperm conservation in the region.

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Figure legends

Figure 1. Map of the Gulf of Kavala and the sampling sites (habitats), Vrasidas Cape and Nea Karvali.

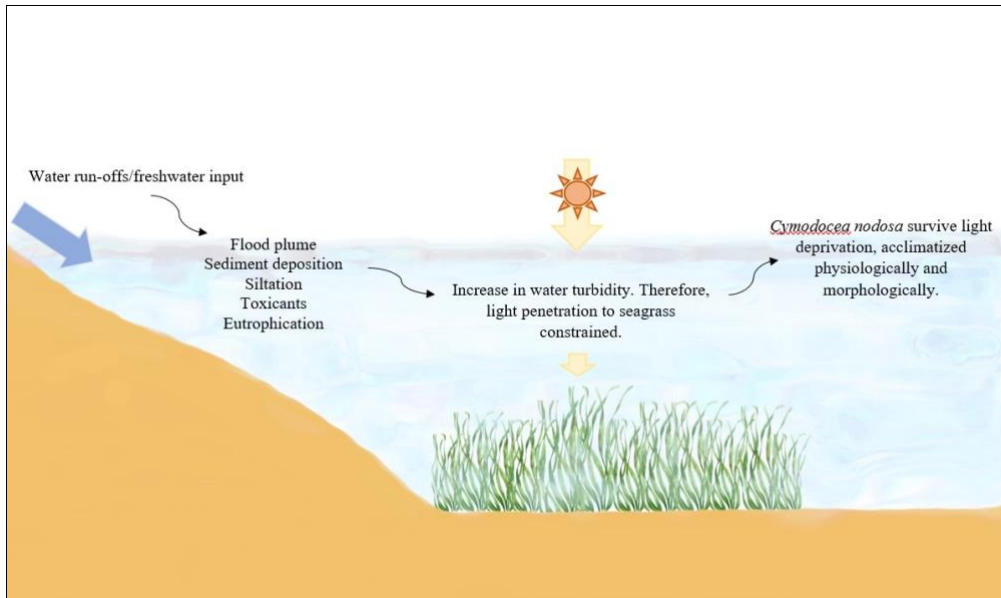
Figure 2. *Cymodocea nodosa*: Median $\Delta F/F_m'$ values (\pm IQR, n=6) collected from pristine (Vrasidas) and degraded (N. Karvali) habitats during treatment of 18 days under two experimental conditions simulating non-flooding (control) and flooding conditions.

Figure 3. Rapid light curves for *Cymodocea nodosa* (mean rETR values \pm SE, n=18) of days 6, 12, and 18 collected from pristine (Vrasidas) and degraded (N. Karvali) habitats under two experimental conditions.

Figure 4. Rapid light curve parameters for *Cymodocea nodosa*: maximum electron transport rate (ETR_{max}), light-limited photosynthetic efficiency (α) and minimum saturating light radiance (E_k) variation based on treatments and habitats throughout 18 days.

Figure 5. Box plots of growth rate under two treatments for *Cymodocea nodosa* shoots from two sites (habitats).

Graphical abstract



- Flood plumes can occur during extreme rainfall, which may devastate *Cymodocea nodosa* habitats.
- The study is about *C. nodosa* photosynthetic and growth performance towards changes in light/turbidity, salinity, and nutrients in the case of flooding due to environmental degradation.
- Low irradiance and salinity, and high nutrients simulated a flooding event as critical parameters for growth irrespective of habitat ecological status. The photosynthetic performance of shoots from the pristine habitat was higher under flooding conditions.

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1.



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Author Contribution

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Soultana Tsioli: Conceptualisation, Writing - review & editing.

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