PRODUCTIVITY AND CARBON SEQUESTRATION POTENTIAL OF SEAGRASS

ECOSYSTEMS IN THE EASTERN AEGEAN SEA

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Summary

Atmospheric CO_2 levels have been increasing at ever faster rates, fueled by anthropogenic activity. Natural ecosystems, which typically form net autotrophic habitats such as seagrass meadows, could be crucial to counteracting CO_2 emissions.

Increased fragmentation of *Posidonia oceanica* seagrass meadows within the eastern Mediterranean basin, linked to increased sea surface temperature, places these meadows at high risk of loss. Annual metabolism estimates showed patchy shallow water *P. oceanica* within the eastern region of the Aegean Sea to be overall autotrophic. *P. oceanica* net apparent productivity was heterotrophic in Autumn and significantly less than Summer when autotrophic, influenced by relative changes in irradiance and seagrass aboveground biomass. Seagrass biometrics also acted as predictors of carbon sequestration spatially, demonstrating higher productivity in the meadow center compared to the meadow edge. Future forecasts of autochthonous carbon storage must consider seasonal changes in productivity, potentially alongside seasonal changes in irradiance and aboveground biomass. Ultimately shallow patchy *P. oceanica* meadow's contribution to carbon sequestration should not be overlooked.

The non-indigenous seagrass *Halophila Stipulacea* was first recorded in the Mediterranean, within the Aegean Sea. Its tropical origin may enable it to thrive given global climate change predictions for the Mediterranean. However, the *H. stipulacea* community was highly heterotrophic during Autumn. Utilising periods of increased irradiance in Summer may enable the plant to persist at this locality, but it seems to live near its limits for survival. The presence of an uncommon endosymbiotic phytomyxid is documented and its potential influence on *H. stipulacea* metabolism discussed.

Overall, this work demonstrates shallow water *P. oceanica* meadows in the Aegean Sea are annually autotrophic and if able to persist will continue to remove atmospheric carbon. Knowing *H. stipulacea* is near its limits in terms of metabolic balance and survival, indicates Mediterranean autochthonous carbon sequestration may decrease should *H. stipulacea* increase in abundance simultaneous to known *P. oceanica* regression.

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Abbreviations

C_{org} = Organic Carbon

DBL = Diffusion boundary layer

CA = Carbonic anhydrase

SST = Sea surface temperature

NCP = Net community productivity

GPP = Gross primary productivity

DO = Dissolved oxygen

CR = Community respiration

NAP = Net apparent productivity

Introduction

Seagrasses are paraphyletic marine angiosperms, which evolved from terrestrial angiosperms that transitioned into the coastal marine environment. Despite a low global diversity of less than 60 species, they act as the architects of major coastal habitats worldwide. There have long been major concerns about their global and localised decline within regions such as the Mediterranean. There is renewed interest in seagrasses because their large capacity to sequester carbon in long term carbon sinks poses them as a major contributor to the removal of atmospheric carbon. In the Mediterranean the dominant seagrass is *Posidonia oceanica* and within this region it plays an integral role carbon sequestration. In this masters by dissertation, I will be assessing the productivity and carbon sequestration potential of the dominant seagrass *P. oceanica* and the non-native seagrass *Halophila stipulacea*, in the eastern region of the Aegean Seas of Greece.

Climate change and global carbon dioxide emissions

It is now widely accepted that atmospheric concentrations of key greenhouse gases have increased, fuelled by anthropogenic activity. Atmospheric increases in carbon dioxide (CO_2) concentrations are considered to pose the greatest threat, as they may remain irreversible 1000 years after emissions stop (Solomon et al., 2008). It is this longevity of atmospheric carbon that means the discussion surrounding future CO_2 emissions requires a critical understanding now for society to be fully informed and act appropriately. It is a problem that requires a multi-facetted approach. Natural ecosystems play a crucial part in carbon sequestration and act as one means to mitigate against increases in anthropogenic CO_2 emissions. For instance, of the 9.1 Petagrams C yr^{-1} emitted through increased anthropogenic CO_2 emission and land use change between 2000 and 2006, 4.1 Pg C yr^{-1} accumulated in the atmosphere, 2.8 Pg C yr^{-1} was sequestered within the terrestrial biosphere and 2.2 Pg C yr^{-1} consigned to marine sequestration (Battin et al., 2009). This equates to 55% (of those 9.1 Petagrams C yr^{-1} anthropogenic emissions) captured into natural carbons sinks, with a relative contribution of 31 % from the terrestrial biosphere and 24 % from marine sequestration.

Marine carbon sinks therefore pose a major contributor to the removal of global atmospheric CO_2.

The relative ratio of carbon stored within ocean and terrestrial sinks is not constant over time, as these sinks are susceptible to natural fluxes. The ocean CO_2 sink has been shown to be smaller when the El Nino /Southern Oscillation (ENSO) is in a positive state, as it enhances land sinks due to lower temperatures and wetter conditions and reduces the ocean CO_2 sink due to more intense equatorial upwelling of carbon rich waters (Le Quéré et al., 2009). This also highlights how localised carbon sinks can have global impacts. With natural fluctuations changing the relative contributions of different natural carbon sinks in time. It is crucial to understand the changing contributions of natural sinks as the increase in atmospheric CO_2 emissions (1959-2008) was partly due to decreased uptake of CO_2 by carbon sinks, in response to climate change and variability (Le Quéré et al., 2009).Terrestrial and ocean carbon sinks have been described as a 'mosaic' of regions gaining or losing carbon, therefore individual sink regions could have weakened, or individual source regions could have intensified, or sink regions could have transitioned to sources (Canadell et al.,2007). This suggests individual carbon sink or source systems need their current and potential future sequestration dynamics fully assessed.

'Blue Carbon' the Coastal carbon champions

Blue carbon habitats accumulatively occupy 0.2 % of the ocean surface as each of these vegetated marine habitats maintains a specific niche that is limited to coastal waters. Despite this small areal cover, the habitats collectively contribute to 46.9 % of carbon burial in marine sediments (Duarte et al., 2013) and are considered disproportionately important in sequestering CO_2 compared with terrestrial ecosystems (McLeod et al., 2011). Therefore, blue carbon storage rates and the conservation of blue carbon stores pose a significant answer in mitigating climate change.

The ecosystems considered to contribute most to carbon sequestration are mangroves, saltmarsh and seagrass. Carbon stocks suggest conservative estimates of approximately

280 Mg C ha^{-1} for mangroves, 250 Mg C ha^{-1} for tidal marshes and 140 Mg C ha^{-1} for seagrass (Pendelton et al., 2012). These estimates place mangroves, saltmarsh then seagrass in order of importance in terms of the magnitude of their total carbon storage. However, as global areas and trends of seagrass meadows cannot always be retrieved from remote sensing products (Duarte et al., 2013) there is greater uncertainty of their extent and likely underestimation of their services.

Seagrass meadows

The unsung hero of 'Blue Carbon'

Researchers have evaluated the ecosystem service value of mangroves more frequently than either seagrass or saltmarsh ecosystem values (Himes-Cornell et al., 2018). Suggesting a bias in the research implemented across blue carbon systems, potentially because mangroves appear of greater 'value' or perhaps simply the logistical ease of access relative to completing research on submerged seagrass. This bias transitions into the public domain, as seagrass meadows have been shown to attribute lower reports in the media for every scientific paper published, compared to that of mangroves (Duarte et al., 2008) and saltmarsh (Orth et al., 2006). Therefore, the perceived importance of seagrass ecosystems is not yet parallel with other blue carbon ecosystems in the public conscious. Duarte (et al., 2008) suggested more effective communication of scientific knowledge combined with increased understanding could ultimately help inform and motivate effective management of integral ecosystems such as seagrass meadows.

The lack in effective communication on the importance of seagrass ecosystems comes at a time when the loss of seagrass meadows matches that of mangroves and coral reefs (Waycott et al., 2009). Beyond losing the current sequestering potential of seagrass meadows, loss of seagrass habitat could release the large carbon stocks that have already built up over time resulting in a release of 299 Tg C yr^{-1} (Fourqurean et al., 2012). This would instead make seagrass meadows major carbon emitters. As the distribution of seagrass meadows is underestimated, seagrass

meadow loss and predicted estimates of carbon release due to their loss underestimated too. The rate of seagrass loss has increased over time (Waycott et al., 2009) making this issue evermore current. Losses may occur at greater rates in specific regions, because despite a 29 % areal loss globally (Waycott et al., 2009), some localised areas have seen up to 50 % areal loss (Marba et al., 2014). With potentially global scale impacts, global and regional action needs to happen to prevent further loss. Positive indicators of successful restoration show revegetation can restore carbon sequestration ecosystem services (Marba et al., 2015). As such seagrass meadows deserve equal precedence at the global table in terms of understanding, communication and effective conservation strategies. If specific seagrass meadows play an integral role or act as localised regions of importance to carbon sequestration, the research behind this needs to be fully informed and effectively translated to policymakers.

From the sea, to the land and back again

Seagrass is the term given to a functional polyphyletic group of angiosperms that returned 'secondarily' to the marine habitat (Reusch, 2014). The recolonisation of marine habitats likely posed a difficult evolutionary transition for flowering plants, as seagrasses represent only 0.02 % of an estimated 300,000 angiosperm species (Les et al., 1997). Evolving from three separate lineages to produce less than 60 species (Les et al., 1997; Short et al., 2007), it remains a plant group quite unlike any other. The characteristics that make seagrasses different from terrestrial angiosperms, is what enables them to inhabit the marine environment, become the architects of coastal marine ecosystems and subsequently provide numerous ecosystem services. Understanding the changes involved in their transition to the marine environment may answer why they are able to contribute to some ecosystem services, such as carbon sequestration, in a disproportionately important way compared with terrestrial ecosystems (McLeod et al., 2011).

The role of submergence and sedimentation in carbon storage

The submerged nature of seagrass results in different processes attributing to blue carbon storage in comparison to the partially submerged blue carbon coastal ecosystems, mangroves and saltmarshes. Blue carbon in seagrass sediment accumulates from both *in situ* production and sedimentation of particulate carbon from the water column (Greiner et al., 2013). The dense canopy of some seagrass meadows reduces near-bottom velocity by 70 to 90 %, which subsequently promotes the deposition of suspended sediment from the water column (Hansen and Reidenbach, 2012). The longevity of debris progressively buried over time in seagrass meadows, can then remain without alteration for millennia (Mateo et al., 1997; Serrano et al., 2011), this ability enables seagrass meadows to act as long-term carbon sinks.

The carbon sequestered through sedimentation in part relies on allochthonous inputs, which vary dependent on the surrounding connected systems. The inability to model this connectivity between ecosystems has often been criticised as a considerable weakness of many coastal ecosystem service estimations (Barbier et al., 2011). It is the reduced flow within the seagrass canopy that allows initial allochthonous inputs to settle out of the canopy and then prevents sediment resuspension (Gacia and Duarte, 2001). Increased surface area, blade length (Gacia and Duarte, 2001; Hansen and Reidenbach, 2012) and seagrass density (Bos et al., 2007; Hansen and Reidenbach, 2012) have been related to increased sediment accretion, therefore the health and biometrics of a seagrass meadow need to be considered when determining carbon sequestration potential. Seagrass meadows also vary in physiology dependent on the species composition of a meadow. Different seagrass species vary in how they alter flow and accrete sediment (Fonseca and Fisher, 1986). Typically, small pioneer species low structural complexity corresponds to low carbon storage (Samper-Villarreal et al., 2016).

Seagrass presence and sediment accretion impact the sediment type through enhanced presence of fine sediment, which may act as a positive feedback to assist root anchorage and seagrass survival (Bos et al., 2007). The actions of particle sedimentation are therefore

synergistic to creating an environment that increases growth, through reduced flow induced sedimentation. The removal of particles from the water column also enhances light penetration (Hansen and Reidenbach, 2012), which creates a positive feedback to aid *in situ* production of organic carbon (C_{org}), through photosynthesis.

Aquatic adaptations and the consequences for photosynthesis

Genes involved in photosynthesis, metabolism and translation have been shown to strongly diverge after the split of the common ancestor of seagrasses from terrestrial monocots (Wissler et al., 2011). These changes from their terrestrial counterparts enabled them to colonise the marine environment, but also resulted in changes to the processes involved in photosynthesis, a key process in carbon sequestration. However, seagrass adaptation to the marine environment is still not fully understood, with the first fully sequenced seagrass genome of the species *Zostera marina* only completed relatively recently (Olsen et al., 2016).

In terrestrial angiosperms stomata act as the conjunction for gaseous exchange, submerged in the marine environment stomata are redundant (Larkum et al., 2017). Seagrass have an absence of all genes involved in stomatal differentiation (Olsen et al., 2016). The blades of seagrass are not surrounded by atmospheric CO_2 but immersed in seawater where CO_2 concentrations are lower. In addition to low carbon concentrations another obstacle the diffusion boundary layer (DBL) must be overcome before CO_2 reaches the leaf surface. The DBL thickens during low energy events limiting photosynthesis, in comparison high flow reduces the thickness of the DBL which allows for higher carbon availability on the plant surface (Kock, 1994). In high velocity waters increased shoot density also increases the likelihood of 'skimming flow' whereby the seagrass lies flat to the benthos redirecting flow over the meadow, hindering gaseous exchange into the plant and reducing productivity. To combat difficulties in obtaining respiratory carbon in the marine environment the seagrass cellular structure assists diffusion of CO_2 as leaf epidermal cells contain additional polysaccharides compared to terrestrial plants, polysaccharides more similar to those found in the cell walls of macroalgae (Olsen et al., 2016).

Availability of organic carbon –Carbon dioxide and Bicarbonate

The composition of available C_{org} in the marine environment differs to the terrestrial biosphere, because after diffusion of CO_2 at the marine surface interface, CO_2 also reacts with water to form HCO_3^-. HCO_3^- is the most available source of C_{org} in the marine environment before CO_2. For that reason, three systems for the active uptake of HCO_3^- have been described for seagrasses; enzyme carbonic anhydrase (CA) conversion of HCO_3^- to CO_2 at the plasma membrane or chloroplast level; extracellular CA-catalysed dehydration of HCO_3^- to CO_2 within acidified zones created by the extrusion of H^+ across the plasma membrane; and outward pumping of protons (H^+) to aid the cotransport of H^+ and HCO_3^- (Beer et al., 2002; Burnell et al 2014). However, literature is mixed about which mechanisms are definitively supported, most recent debate suggests evidence against a bicarbonate pump (Larkum et al., 2017). Instead focus remains on the evidence for CA conversion outside the outer plasmalemma, together with a proton pump to assist with local acidification and the potential for carbon concentrating mechanisms via CA within the cell in the cytoplasm, chloroplast stroma or inner thylakoid space (Larkum et al., 2017).

Under the predicted CO_2 atmospheric increases, greater availability of CO_2 may mean greater uptake of C_{org} by passive diffusion and decreased use of active uptake of HCO_3^- which requires energetic expenditure (Burnell et al., 2014). Further to this, the fossils of historic seagrass suggest this group of angiosperms moved into the marine environment nearly 90 million years ago during the Cretaceous period, a period when atmospheric CO_2 was higher than it is today (Beer and Koch, 1996). Therefore, the increase in current atmospheric CO_2 may aid increased photosynthetic efficiency, but only if seagrasses can prevail with the simultaneous predicted increase in temperature, pH and sea level rise. Whilst good evidence exists as to how HCO_3^- is involved in plant photosynthesis it has not yet been proved, the varied divergence within the seagrasses may also result in varied adaptations (Larkum et al., 2017). This variation between species needs to be explored as it limits its application in future predictions of productivity within species-specific meadows.

Low carbon availability can also act as a limiting factor in the photosynthetic potential of seagrass, by transitioning form photosynthesis to photorespiration. Photorespiration occurs when the enzyme rubisco reacts with oxygen preferentially over CO_2, favoured by high oxygen levels in comparison to CO_2. Photorespiration can be overcome in seagrasses by flow enhanced removal of oxygen from the plant into the seawater (Mass et al., 2010). But photorespiration may also be induced in the environment due to community changes in the relative concentrations of O_2 and CO_2, as a result of high community photosynthesis, particularly in shallow waters (Buapet et al., 2013). Photorespiration also occurs in seagrasses with increased pH in the surrounding environment (Buapet et al., 2013). However, seagrass photorespiration is considered to be driven by the increase of oxygen saturation rather than the increase of pH (Champenois and Borges, 2012). The lack in understanding about seagrass photorespiration means the true extent of its effect on photosynthetic efficiency cannot be interpreted, but it does waste energy and decreases carbon fixing efficiency compared to photosynthesis. Moving forward into an increasing CO_2 environment, seagrass photorespiration may become reduced allowing for increased carbon fixation efficiency.

From source to shoot, blue carbon fixation

Excess photosynthetic carbon fixation, which occurs when the costs of metabolism are surpassed by photosynthetic activity, allows for growth and increase in biomass. The carbon fixed in non-structural carbohydrate typically gets translocated from the leaves, to the rhizome and roots (Kaldy et al., 2013). As much as two-thirds of global living seagrass biomass exists belowground as rhizome and roots (Fourqurean et al., 2012). This means one-third of the carbon fixed in global living seagrass biomass forms the shoots and blades aboveground (Fourqurean et al., 2012), of which half the leaf production is eventually delivered to the local sediments, (Kennedy et al., 2010). The organic matter, derived from an accumulation of root, rhizome and leaf are progressively buried and if left undisturbed remain unaltered (Mateo et al., 1997; Serrano et al., 2011), acting as carbon sinks. Globally on average half the C_{org} buried in seagrass sediments derives from seagrass tissue, at a total 41 - 6 g C m^{-2} yr^{-1} (Kennedy et al.,

2010). Therefore, the productivity, translocation and burial of carbon fixed by seagrass ultimately contributes to a meadow's blue carbon storage, along with sedimentation.

Seagrasses of the Aegean Sea

The Mediterranean temperate-tropical mix of seagrass species is considered a unique bioregion of seagrass diversity, (Short et al., 2007). The Aegean Sea has prominent presence of two native seagrass species *P. oceanica* and *Cymodocea nodosa.* The most prevalent species being *P*osidonia *oceanica*, the health and status of which can inform the health of the ecosystem (Personnic et al., 2014). The Aegean Sea also hosts two nonindigenous seagrass species, *Halophila stipulacea* since 1894 (Fritsch, 1895) and more recently *Halophila decipiens* (Gerakaris et al., 2019). The eastern region of the Aegean Sea sits at a crossroad for alien species expansion (Pancucci-Papadpooulou et al., 2012), therefore it is more important than ever to understand the current dynamics within these seagrass meadows, as the community dynamics may change in the future. The rise of SST and relative sea level rise has already raised concerns about replacement of these native species for those of lower structural complexity such as *H. stipulacea* (Pergent et al., 2014). Concerns are the native *P. oceanica* will be replaced by *"*warm" affinity species, such as the native *C. nodosa,* and invasive *H. stipulacea* (Pergent et al., 2014).

H. stipulacea is considered invasive primarily because of the rapid nature and extensive distribution it has secured across the Mediterranean. It has been found in some cases to colonise areas previously occupied by native seagrass species, such as *P. oceanica* dead matte or mixed *C. nodosa* meadows (Sghaier et al., 2011). Structural complexity is considered a key factor influencing carbon content in seagrass content (Samper-Villarreal et al., 2016). Pioneer species with low structural complexity, such as *H. stipulacea* can on occasion have relatively high C stocks dependent on other conditions such as water depth and wave height (Samper-Villarreal et al., 2016; Lavery et al., 2013). There are real concerns however, that the replacement of *P. oceanica* or the transition into multi-species seagrass beds including

H. stipulacea could see an overall reduction in the carbon storage potential of seagrass beds in the Aegean Sea and the eastern Mediterranean basin. Ultimately this could have a large impact on global estimates of seagrass carbon stocks given *P. oceanica* meadows are considered to represent the maximum in carbon storage potential among seagrasses (Lavery et al., 2013).

Posidonia oceanica as a global frontrunner

Leading in carbon sequestration potential

The largest pools of C_{org} stored in living seagrasses were found in Mediterranean meadows dominated by *P. oceanica* (Fourqurean et al., 2012). *P. oceanica* is unique, it is the only *Posidonia* species found within the northern Hemisphere and endemic to the Mediterranean. The southern hemisphere has eight recognised *Posidonia* taxa found around Australia (Aires et al., 2011), despite Australian seagrass meadows containing a greater diversity of *Posidonia* species, mean C_{org} content of Australian seagrass habitats was 4 time lower than Mediterranean *P. oceanica* meadows (Lavery et al., 2013). *Posidonia* species have similar morphology but it appears the other *Posidonia* species do not have as great a carbon sequestering capability as *P. oceanica*. Only estuarine *Posidonia australis* meadows accrued stored C_{org} comparable to *P. oceanica* meadows, but this was a comparison of the top 25 cm of the meadow's sediment profile (Lavery et al., 2013). *P. oceanica* meadows typically have deeper organic profiles due to their vertical growth (Mateo et al., 1997). Therefore *P. oceanica* meadows are considered to represent the maximum in carbon storage potential among seagrasses, even in comparison to its closet extant relatives (Lavery et al., 2013). This suggests *P. oceanica* meadows are of global importance in their unique capability for high carbon sequestration and potentially retain unique attributes that allow them to act as a global frontrunner in carbon sequestration, irrespective of other seagrass meadows on Earth.

Is P. oceanica always actively sequestering carbon?

 Though *P. oceanica* may represent a seagrass species of global importance through its ability to sequester large quantities of C_{org}, the plant's *in situ* productivity may not always be high and

sequestering carbon at its maximum potential. Changes in growth rates of *P. oceanica* provide strong evidence to suggest at the plant level, photosynthesis and utilisation of the plant's stored carbon has seasonal variation (Buia et al., 1992). The whole plant carbon balance can show negative carbon balance from fall to Spring and transitions into positive carbon balance only in the Summer months; as such the plant may rely on reserve utilisation, to persist over Winter and for regrowth during Spring (Alcoverro et al., 2001). *P. oceanica* meadows clearly undergo periods when their productivity is low and not actively aiding carbon sequestration within the year. If the periods of high productivity balance or surpass the periods of carbon loss, the meadow should persist and continue to sequester carbon to some extent. A meadow that is heterotrophic on the annual scale, cannot sustain its metabolism requirements, if this continues year-on year, it indicates long-term regression (Alcoverro et al., 2001). Understanding periods of low productivity and high productivity, provide effective estimates on an annual scale as to whether the meadow contributes to net carbon sequestration.

Light availability -driving growth and limits

To adapt to a fully submerged marine existence *P. oceanica* has adapted to lower light availability, as light travels through an additional medium before reaching the plant. The minimum light surface irradiance for *P. oceanica* sits between 16 and 10% of surface irradiance (Ruiz and Romero, 2001), which determines the lower depth limit at which the meadows exist. The minimum surface irradiance values also assist annual carbon budget predictions, as it informs the carbon balance and photosynthetic activity. *P. oceanica* can photoacclimate to lower light levels, but a threshold exists at which deterioration in health and productivity occurs (Ruiz and Romero, 2001). Therefore, seasonal changes in light surface irradiance are within the plant's phenotypic plasticity, to maintain photosynthetic capacity. In addition, dynamic responses to light intensities occur with diel fluctuations in light.

Light varies with spatial scales; across the seagrass blade; within the meadow canopy through self-shading; within the meadow by depth and between sites (Ralph et al., 2007). Within-shoot

variability regarding photosynthetic capacity is important in long-lived species such as *P. oceanica* whose blades can reach up to 300 days age, the ability to cope with changes in environmental conditions will vary across the blades (Alcoverro et al., 1998). *P. oceanica* meadows follow a seasonal growth cycle, with decreased biomass from Autumn to Winter when blades are shed, followed by a major growth spurt in late Winter (Champenois and Borges, 2018), in preparation to maximise plant biomass for photosynthetic gains in the following months. As such the age composition of shoots changes throughout the season (Alcoverro et al., 1998) and interplays with seasonal environmental conditions that control photosynthetic efficiency and productivity.

The seagrass *P. oceanica* is considered to acclimate well to high solar irradiance and shows a high capacity for recovery (Figueroa et al., 2002). However, during periods of high irradiance seagrass can experience photoinhibition which causes damage to the photosystems leading to a period of recovery when photosynthetic capacity and carbon sequestration is reduced. Photoinhibition coincides with maximum light irradiance at the middle of the day, so maximum photosynthetic production occurs in the afternoon when saturating not inhibiting irradiance levels occur (Costa et al., 2015). Photoinhibition may have a more pronounce effect on seagrass meadows during Summer when annual irradiance levels typically peak. This relates to an energetic cost and loss in carbon sequestration as the maximum annual solar irradiance does not coincide with annual maximum GPP (Champenois and Borges, 2018). Shallow meadows are more consistently exposed to high irradiance and irradiance saturation comparatively to those at the lower depth limit, therefore shallow meadows are more susceptible to the energetic repercussions of living in a high light environment.

Mediterranean temperature trends

Sudden increases in temperature such as those driven by heat waves and the gradual increases in the mean annual temperature pose a threat to the current functioning of *P. oceanica* (Marba and Duarte, 2010). The temperature of seawater *in situ* can fluctuate greatly,

as such the impact of temperature on plant tissue mortality near its maximum threshold is dependent on; the extent temperature increases toward or surpasses the maximum temperature threshold; the length of time the seawater temperature stays around the maximum threshold; and the frequency of these events. Based on the distribution of maximum seawater temperature in the relation to presence and absence of *P. oceanica* across the Mediterranean, the maximal thermal tolerance for *P. oceanica* meadows to maintain their presence was 29.21 °C (Chefaoui et al.., 2017). Even if *P. oceanica* meadows have the capability to survive close to the maximum temperature threshold and above optimum temperature conditions 17 - 21 °C (Champenois and Borges, 2018), it is likely that the functioning of the plant is compromised and subsequent the carbon sequestering potential lowered.

Variations within and between P. oceanica populations

Spatial differences in thermal tolerance exist within *P. oceanica* meadows, with shallow ecotypes exhibiting a greater tolerance (Marin-guirao et al., 2016). Shallow ecotypes can acclimate through re-establishing the balance between leaf respiration and photosynthesis, whilst deep plants were unable to stabilise respiration (Marin-guirao et al., 2016). Differences in thermal tolerances may also exist between *P. oceanica* meadows across the Mediterranean given, divergence exists between the *P. oceanica* populations in the eastern and western basins of the Mediterranean (Arnaud-Haond et al., 2007). The differences in environmental conditions between basins may result in varied responses to thermal stress. Predictions suggest the lowest probability of *P. oceanica* meadow occurrence in areas such as the eastern region of the Aegean Sea, where fragmented meadows inhabit marginally high sea surface temperatures (SST) compared to the rest of the Mediterranean (Chefaoui et al., 2017). Yet historically most of the research on *P. oceanica* has occurred on populations in the western Mediterranean; it is important to consider there may be different underlying mechanisms in the eastern basin of the Mediterranean that are specific to predicting the response of *P. oceanica* within these localised areas.

P. oceanica meadows are synonymous with forming large dense continuous meadows, but can form patchy spatial dynamics, due to a variety of factors including hydrodynamic interactions. The differences in architecture between dense and patchy meadows likely change the dynamics that exist within meadows. *P. oceanica* has long strap like blades, within large continuous meadows this creates a constant dense canopy. Patchy meadows have intermittent points of increased light and flow. A dense seagrass canopy may hinder productivity by reducing within canopy irradiance from between 34 – 90 % (Enriquez and Pantoja-Reyes, 2005), resulting in the lower part of the canopy encountering self-shading. The differences in inter-leaf variation between the top of the canopy and the understorey may be greater than the variation between shoots in a meadow (Ralph et al., 2007). The relationship between increased shoot density and decreased light attenuation within the canopy, was used to interpret the relationship between decreased shoot density with depth to maximise solar irradiance (Enriquez and Pantoja-Reyes, 2005). This equally relates to how spatial configuration in shoot density may interact with productivity in meadows. Patchy meadows may have enhanced light availability for photosynthetic activity, whilst dense continuous meadows negatively feedback to reduce carbon sequestration, however continuous meadows have been shown to accumulate greater C_{org} compared to patchy meadows (Ricart et al., 2017).

Continuous *P. oceanica* meadows retain greater autochthonous carbon through the decreased export of blade detritus (Ricart et al., 2017). Whilst the *in situ* productivity may be lowered due to self-shading and reduced gaseous exchange through reduced flow, these decreases in productivity may be balanced at an ecosystem level due to the retention of C_{org} within continuous meadows. Seagrass tissues are considered resistant to degradation back into CO_2, whilst *P. oceanica tissues* are not distinct in this ability to other seagrass taxa, morphologically larger taxa like *P. oceanica* have larger amounts of refractory carbon to contribute (Trevathan-Tackett et al., 2017). Plus *P. oceanica* tissues are more recalcitrant than algae or sestonic detrital inputs (Gacia et al., 2002), if continuous meadows retain a larger amount of

seagrass tissue which are less likely to remineralise, this increases the long-term stored C_{org} in the sediments.

Limitations in estimating seagrass productivity

Mapping Mediterranean meadow distribution

Despite seagrass meadows importance there are serious gaps in the valuation of these habitats most notably involving methodology and coverage (Himes-Cornell et al., 2018). In order to determine the extent of current carbon stocks and their changes over time firstly the distribution of these meadows must be well understood. Large scale mapping typically uses remote sensing techniques; these have their limitations when applied to submerged habitats. Even with large seagrass species such as *P. oceanica* remote sensing may only be accurate for shallow mapping to a depth such as 15m (Fornes et al., 2006). This would result in a major underestimation of meadow distribution, as *P. oceanica* meadow range extends to 50 m depth. Large scale mapping also does not account for the within meadow variation in terms of shoot density it merely presents seagrass meadows in a binary measure of presence or absence. Mapping seagrass presence doesn't inform on the biomass and health status of the meadow present.

It was not until 2018 that the first seagrass coverage maps were produced from satellite images to describe and quantify the spatial distribution of seagrass meadows in Greek waters (Topouzelis et al., 2018), but this study emphasised that accuracy may have been affected by a number of potential errors in the image analysis, reference data and classification of these images. Prior to this only 8 % of Greece's coastline was considered to have been surveyed for *P. oceanica* distribution (Telesca et al., 2015) Therefore despite the considered importance of seagrass habitat, in particular *P. oceanica*, major gaps have long existed in our understanding of its distribution. This lack of understanding in seagrass meadow distribution within the Aegean Sea and the eastern part of the Mediterranean basin, impacts the ability to produce carbon sequestration estimates for these areas of the Mediterranean.

Benthic chambers for assessing seagrass meadow net community metabolism

Methods such as open water optodes (Champenois and Borges, 2012; Champenois and Borges, 2018) and sediment analysis (Kennedy et al., 2010) have been used to determine the contribution of primary productivity to carbon sequestration potential. Yet net community productivity (NCP) by oxygen evolution within *in situ* incubation chambers has widely been used to determine the metabolic balance of gross productivity versus metabolic cost to seagrass meadow carbon sequestration potential (Duarte et al., 2010; Olive et al., 2015). The oxygen evolution within these chambers is measured and converted to carbon production with a photosynthetic quotient ratio, but discrepancies exist in the appropriate quotient for seagrass oxygen to carbon conversion and in the literature range between 1:1 and 1:1.2 (Alcoverro et al., 2001; Apostolaki et al., 2010; Duarte et al., 2010). You might expect to encounter a variety of photosynthetic quotients applied across seagrass species, given seagrasses are only functionally and not phylogenetically grouped. But the application of photosynthetic quotients is not consistent for any given species, with *P. oceanica* productivity measurements as a prime example.

Gas fluxes within benthic chambers have been heavily criticised for underestimating productivity by as much as 10 % (Peduzzi and Vukovic, 1990). Underestimations in productivity and subsequently carbon sequestration are largely because incubation times of 12 and 24 hour are standard practice, despite the flaws to long incubation time (Olive et al., 2015). Water motion has been highlighted as a fundamental factor, equivalent to light and nutrients in determining productivity (Mass et al., 2010), the design of benthic chambers typically undermines factoring natural flow into productivity measurements. Long incubation times create a static environment which increases pH and oxygen, when water currents would replace and mix the water naturally. Under high oxygen saturation and low dissolved inorganic carbon, the enzyme rubisco decreases its carboxylase activity, moving the seagrass from more readily completing photosynthesis to photorespiration. Photorespiration is considered wasteful because it lowers photosynthetic efficiency, as much as 40% in some seagrass species (Buapet et al., 2013).

Photorespiration occurs naturally in shallow water seagrass environments due to the primary productivity of other plants in the system (Buapet et al., 2013). The typical benthic chamber setup imitates these productive shallow water systems, without explicitly accounting for the occurrence of photorespiration in the resultant productivity calculations, thereby underestimating carbon sequestration.

The biomass to chamber volume ratio influences the production and consumption of O_2 and CO_2, when the volume of the chamber is increased relative to the seagrass biomass it should take longer for the chamber to reach oxygen saturation (Olive et al., 2015). Considering the varied morphology between seagrass species in aboveground vegetative canopy biomass the chamber requirements may vary dependent on the studied species. Some long strap leaf seagrass species such as *P. oceanica* are recorded to have maximum blade lengths > 75 cm (Pergent et al., 2008). For benthic chambers to allow for the natural architecture of the seagrass meadow and account for an appropriate seagrass biomass to chamber volume (particularly across long incubation periods), chamber design specifically volume should try to account for this. Yet the volume of benthic chambers used to assess seagrass metabolism can vary greatly from 5 L to 20 L (Dunton and Tomasko, 1994; Santos et al., 2004; Gazeau et al., 2005; Barrón et al., 2006; Champenois and Borges, 2012; Ouisse et al., 2014; Olive et al., 2015), so there is not distinct guidelines on what is most appropriate in each case. Despite the characteristic flaws of using *in situ* benthic chambers to determine net community productivity in seagrass meadows, they remain a valuable, well understood and widely used tool for determining conservative estimates of net community productivity and as such autochthonous carbon sequestration potential.

Aims and Objectives

This thesis focuses on assessing the primary productivity of seagrass in the eastern region of the Aegean Sea given there are no current metabolism estimates for seagrass habitat within this area. It considers the limitations associated with benthic chamber metabolism methods by

using chambers that are a relative size to the seagrass biomass within, in particular addressing the high biomass of *P. oceanica* through deploying the largest benthic chamber volume before utilised for this species, with additional internal mixing.

The setup utilised aims to assess seasonal changes in productivity and the relative importance of the concurrent light and temperature conditions. The seasonal *P. oceanica* metabolism measurements will distinguish if and when the meadow is actively sequestering carbon. Moreover, whether the meadow has an overall positive carbon balance on an annual scale. This will represent a conservative estimate of current annual autochthonous carbon sequestration within the Aegean and Eastern Mediterranean, given the representative *P. oceanica* meadow in shallow water. The shallow nature of this seagrass meadow means the seagrass would be subject to high light and temperature thresholds compared to other areas of the meadow and so more likely to exhibit periods of reduced metabolic function. The study location may also mean this would be a conservative representation of shallow *P. oceanica* in the Eastern Mediterranean, given the eastern Aegean Sea in considered to hold particularly fragmented meadows due to the high SST compared to the rest of the Mediterranean (Chefaoui et al., 2017). The influence of patchy and fragmented shallow meadows is explored by gauging the extent of spatial variation in autochthonous carbon sequestration within the meadow between patchy edge habitat and denser central areas. Biometric measures of seagrass patchiness such as percentage cover and shoot density are tested for their effectiveness as indicators of changes in spatial primary productivity.

Finally, the productivity of the non-indigenous seagrass species *H. stipulacea* is assessed within the eastern region of the Aegean Sea, as well as the relative influence of light and temperature. This provides the context to discuss the differences in productivity between these species in the Mediterranean, how future climate change may influence Mediterranean seagrass meadows and the ecosystem services they provide, specifically autochthonous carbon sequestration.

Seasonal and spatial variations in shallow water *Posidonia oceanica* meadow productivity

Introduction

Coastal blue carbon ecosystems are considered disproportionately important to global carbon sequestration as they account for 46.9% of the total carbon burial in marine sediments, despite modestly occupying 0.2 % of the ocean surface (Duarte et al., 2013). Seagrass meadows are one of these coastal blue carbon ecosystems that act as a major global carbon sink. Their ability to capture carbon is two-fold; actively sequestering autochthonous carbon through photosynthesis and passively trapping allochthonous carbon with their architectural structure. Allochthonous carbon is typically considered more labile, therefore deposits of autochthonous carbon are those expected to lead to long-term stable carbon deposits (Mazarrasae et al., 2018). The metabolic rates of global seagrass communities favour net autotrophy, with temperate meadows typically favoured to have a higher net autotrophy than tropical meadows (Duarte et al., 2010), suggesting not all seagrass is equal in its ability to sequester autochthonous carbon.

The temperate seagrass *Posidonia oceanica* forms vast monospecific meadows in the Mediterranean and is unique in its ability to form vertical mattes that can store sedimentary carbon for millennia (Mateo et al., 1997). It is perhaps why some herald these meadows to represent the global maximum in carbon sequestration among seagrasses (Lavery et al., 2013). Unlike terrestrial soils, these *P. oceanica* sediments do not become saturated with carbon over time because they can accrete vertically. If the vertical accretion matches the rate of sea level rise, they potentially have a limitless capacity, which in part demonstrates their suitability for climate mitigation policy efforts (Howard e al., 2017). However, *P. oceanica* meadows have generally undergone severe regression (34%) in the last 50 years, with only localised areas of persistence and growth (Telesca et al., 2015). The causes of decline most prolifically recorded for *P. oceanica* regression are water quality degradation, coastal modification, mechanical damage, extreme events and non-native macroalgae invasion (Santos et al., 2019). Localised

variability in the status of *P. oceanica* meadows is in response to local stressors, rather than to processes at the basin scale (Telesca et al., 2015).

Seagrass metabolism is influenced by multiple variables including light (Champenois and Borges, 2018), nutrient availability (Holmer et al., 2008; Apostolaki et al., 2010), temperature and ocean acidification (Berg et al., 2019); many of which follow seasonal cycles, thus driving seasonal fluctuations in productivity. Primarily seagrass productivity is controlled by the solar cycle, because it determines the relative light availability, but also the water temperature (Alcoverro et al., 1995). Seagrass depth distribution is determined by light availability, because when light conditions are insufficient the plant does not meet the photosynthetic requirements necessary to maintain positive metabolic and carbon balance (Ralph et al., 2007), moving away from a state of net autotrophy and net carbon storage. The annual solar cycle should act as a driver in seasonal changes in *P. oceanica* productivity. However, high light intensity causes photodamage reducing the photosynthetic efficiency and instigating photoinhibition. Subsequently maximum annual solar irradiance does not coincide with annual maximum *P. oceanica* gross primary productivity (GPP) (Champenois and Borges, 2018).

Water temperatures of > 29 °C are lethal to *P. oceanica* (Chefaoui et al., 2017). As shallow water environments are more readily heated by solar irradiance, the metabolism of shallow water *P. oceanica* meadows are more likely influenced by increased water temperature. Given respiration increases faster than the rate of primary production Summer temperatures can move seagrass ecosystems seasonally from autotrophy to heterotrophy, though the likelihood of transition is species specific (Burkholz et al., 2019). Water's high specific heat capacity creates seasonal lags to terrestrial temperatures. The stress induced by high water temperatures may persist beyond the maximum solar irradiance. The optimal thermal conditions for *P. oceanica* are between 17 - 20 °C (Champenois and Borges, 2018), therefore both above and below these conditions its metabolism is not optimal for maximum oxygen production and more likely to become heterotrophic.

At the Mediterranean scale, the *P. oceanica* populations in the Western and Eastern basins have historically been genetically isolated and still maintain genetic differentiation due to present-day dispersal limits (Arnaud-Haond et al., 2007). Genomic and proteomic approaches need to be integrated with ecophysiology and physical approaches in order to determine seagrass productivity and metabolism to different environmental settings (Mazzuca et al., 2013). The genetic differences in *P. oceanica* between basins may influence the underlying plant productivity and metabolism to environmental conditions. There is a greater distribution of *P. oceanica* in the Eastern basin (713,992 ha) compared to the Western basin (510, 715 ha) (Telesca et al., 2015). Despite the genetic variation between basins and the largest population existing in the Eastern Mediterranean basin, the majority of *P. oceanica* metabolism estimates have come from the Western Mediterranean basin (Frankignoulle and Bouquegneau, 1987; Holmer et al., 2004; Gazeau et al., 2005; Barron and Duarte, 2009; Olive et al., 2015; Champenois and Borges, 2018). The only published study on *P. oceanica* metabolism in the Eastern Mediterranean basin comes from a single study, in the western region of the Aegean Sea (Apostolaki et al., 2010). Metabolism and carbon sequestration estimations for *P. oceanica* are considered one of the most well researched amongst seagrass species (Nordlund et al., 2018), yet there are distinct local knowledge gaps and spatial biases, particularly within the Eastern Mediterranean Basin. These spatial biases largely undermine the extensive research on the species when you consider the potential distinctions between Western and Eastern basin populations*.*

In the Eastern Mediterranean basin, the sea surface has warmed by 0.05 ± 0.009 °C yr $^{-1}$ (from 1985 to 2006), more than in the Western Mediterranean (Nykjaer, 2009). It is no surprise then, that future increases in sea surface temperature have been predicted to assist the decline of *P. oceanica* within the eastern basin (Pergent et al., 2014). The Aegean Sea has undergone the greatest change in sea surface temperature within the Mediterranean (Nykjaer, 2009), warming at a rate several times larger than the estimated average global rate (Skliris et al., 2011). The progressive warming of Mediterranean waters since the last glacial maximum had already

caused *P. oceanica* distribution to be most prevalent on the Northern shores of the Eastern Mediterranean basin compared to the Southern (Chefaoui et al., 2017). Considering the physical geography of the Eastern Mediterranean Basin, this means the extensive coastline within the Aegean Sea comprises a large proportion of the available habitat for *P. oceanica* in the Eastern Mediterranean basin. The *P. oceanica* occupying the eastern region of the Aegean Sea exhibits fragmented meadow morphology and this fragmentation is attributed to the marginally higher sea surface temperatures compared to the rest of the Mediterranean (Chefaoui et al., 2017). The *P. oceanica* within the eastern region of the Aegean Sea will likely be the first to respond to future climate change predictions (Pergent et al., 2014) regarding further increases in sea surface temperature. The *P. oceanica* carbon stock within the eastern region of the Aegean Sea is at the highest risk of loss. This study therefore aims to contribute to the knowledge available on the carbon sequestration potential of *P. oceanica* in the Eastern Mediterranean basin, primarily through acquiring *P. oceanica* metabolism rates specifically within the eastern region of the Aegean Sea.

The fragmented meadow morphology within the eastern region of the Aegean Sea (Chefaoui et al., 2017) indicates the patchiness of habitat in this region should be taken into consideration. Seagrass meadows are not always a uniform habitat and whilst *P. oceanica* meadows can form continuous meadows they also exhibit patchy dynamics. The fragmentation of *P. oceanica* meadows creates complex seascapes, a mosaic of patches, that include habitats of sand, *P. oceanica* matte and *P. oceanica* (Abadie et al., 2015). Naturally meadows that experience a wave exposure gradient from low to high energy develop patchier dynamics (Folkard, 2005; Pace et al., 2016). Seagrass habitats are also exposed to anthropogenic factors that increase patchiness including anchoring, impact of WW2 bombs, explosive fishing and fish farming (Montefalcone et al., 2009; Abadie et al., 2015). Often distinct patches are not determined in surface area mapping, as it is the direct underwater observation of the meadow that allows for the discrimination between similar habitat patch types such as living *P. oceanica* and dead matte (Montefalcone et al., 2009). The effects of patchiness in shallow seagrass habitat is

particularly important as patchiness is more of a prominent feature within shallow water beds (Montefalcone et al., 2009) but also because meadow patchiness influences the retention of autochthonous material (Ricart et al., 2017).When a *P. oceanica* meadow is described as patchy it has lower overall cover, more complex patch shapes and reduced within-patch architectural complexity (Pace et al., 2016). Spatial changes in its architecture (seagrass canopy height or blade length) act as describers of patchiness. As the leaf of a plant is this site of photosynthetic activity, they may also act as describers of primary productivity. It is just as important to consider the variation in canopy architecture between areas of *P. oceanica* as it is to consider the discrete patches of seagrass, matte and sand within a meadow.

Herbivory disturbance reduces seagrass canopy, the photosynthetic component of seagrass beds, reducing the carbon fixation (Fourqurean et al., 2010). Other physical disturbance to seagrass meadows such as Autumnal and Winter storms reduce the photosynthetic compartment of seagrass meadows. The loss of seagrass canopy due to storms happens as a result of distinct seasonal patterns whilst other disturbance such as herbivory may remain constant through the year. The seasonal loss of biomass is vast enough that in Autumn part is exported onshore via currents where it forms large banquettes (Gomez-Pujol et al., 2013). Early canopy growth in Spring creates a well-developed canopy during maximum Summer irradiance (Alcoverro et al., 2001). The increase in seagrass canopy in Summer maximises plant productivity. The combination of temporal peaks and troughs in canopy biomass may dictate seasonal primary productivity patterns. This study should therefore check whether changes in seagrass canopy influence seasonal *P. oceanica* productivity patterns.

The Aegean Sea has housed the non-indigenous seagrass species *Halophila stipulacea* the longest, given it was first recorded in the Mediterranean within the Aegean Sea, off Rhodes island (Fritsch, 1895). *H. stipulacea* is a tropical species that may be able to take advantage of the rising global sea surface temperature, when *P. oceanica* is predicted to undergo further regression (Pergent et al., 2014). The eastern region of the Aegean Sea acts as part of the Mediterranean that may best showcase how Mediterranean seagrass dynamics could change.

Assessing whether *P. oceanica* meadows are currently able to maintain a positive annual carbon balance indicates whether under current conditions they are likely to persist, as *P. oceanica* meadows that are heterotrophic on an annual scale are reducing in plant biomass due to the negative carbon balance (Alcoverro et al., 2001).

Annual patterns of *P. oceanica* metabolism comprise of periods that alternate from negative to positive carbon balance (Alcoverro et al., 2001). If carbon estimates were produced only factoring the higher productivity during the Summer months (Champenois and Borges, 2012) it would overestimate the meadow's overall carbon sequestration capacity. To estimate the annual carbon sequestration potential of *P. oceanica* within the eastern Aegean Sea, this study needs to account for the periods of negative carbon balance alongside the periods of positive carbon balance. Notably as stable carbon stocks rely on autochthonous carbon deposits, key environmental parameters that influence photosynthesis such as light and temperature should be assessed to determine whether they drive *P. oceanica* primary productivity in the eastern region of the Aegean Sea. Alongside this changes in seagrass canopy should be assessed to determine if this influences productivity at the meadow scale. Finally, sediment samples should be acquired to determine if variance in spatial primary productivity translates to the relative organic content of the sediment, thus alluding to the relative retention of primary productivity and the gain of autochthonous carbon stocks. The fact remains that seagrass meadows are currently excluded from global carbon budgets, therefore local *P. oceanica* carbon sequestration estimates and their drivers need to be better understood to provide suitable Mediterranean scale carbon budgets.

Methods

Study site

This study took place at Vroulia Bay (37.317460° N, 26.724704° E), NW Lipsi Island, the Dodecanese, in the eastern region of the Aegean Sea, Greece. Data collection took place from Autumn 2018 to Summer 2019; November 3rd – 9th, April 12th – 19th and July 2nd – August 12th.

Vroulia is a sheltered bay with minimal tourist presence. The bay consists of a multispecies seagrass meadow, largely dominated by monospecific patches of *P. oceanica,* but sparsely populated by both *Cymodocea nodosa* and *Halophila stipulacea.* Whilst there is sparse sailing boat activity and disturbance, the study area within the bay was shallow enough (< 2 m) to be unaffected by these activities. The shallow area of the bay consists of several large dense areas of *P. oceanica*, surrounded by numerous small patches.
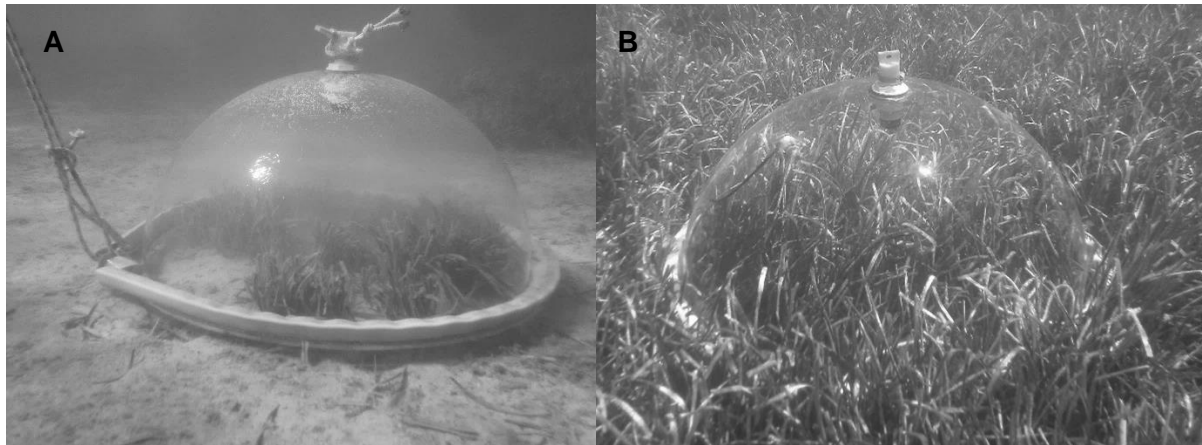
In situ benthic chamber setup

The benthic chambers were dome shaped and made from clear PVC (diameter = 1 m, height = 50 cm, benthic surface area = 0.79 m^2, volume = 576 L) and deployed by free divers between 1.6 – 2 m depth (Fig. 1). PME miniDOT loggers were fitted to the central (highest) part of the dome chamber, to record dissolved oxygen and temperature in 10 minute intervals. HOBO loggers were placed within the chambers on the seafloor to record relative irradiance in 5 minute intervals. HOBO logger irradiance (lux) were converted to PAR (photosynthetic active radiation), according to the conversion factor appropriate to the light source 'daylight' (Thimijan and Heins, 1983). The Instantaneous PAR with the unit µmol s^{-1}m^{-2} is used to calculate Daily Light integral (DLI) given the time interval in seconds (t) between each PAR reading over each 24 hour period:

$$\mathrm{DLI} = \Sigma\Delta\mathrm{t} \times PAR$$

The DLI is presented with the unit mol m^{-2}d^{-1}. The mean PME miniDOT water temperature is calculated across each incubation period.

Figure 1. Large benthic chamber setup **A** Deployed in Autumn over a small *P. oceanica* patch (edge habitat) **B** deployed in Summer over dense central *P. oceanica* meadow area.

The benthic chambers were setup with a water pump secured to the inside of the chamber roughly 30 cm from the seafloor (Fig. 2). This water pump circulated the water within the dome at a flow rate of roughly 480 L hr^{-1} in 10 minute intervals, as such the overall flow rate within the chamber was considered to be 240L hr^{-1}. A thin cable went between the pump within the benthic chamber to an external submersible battery pack.
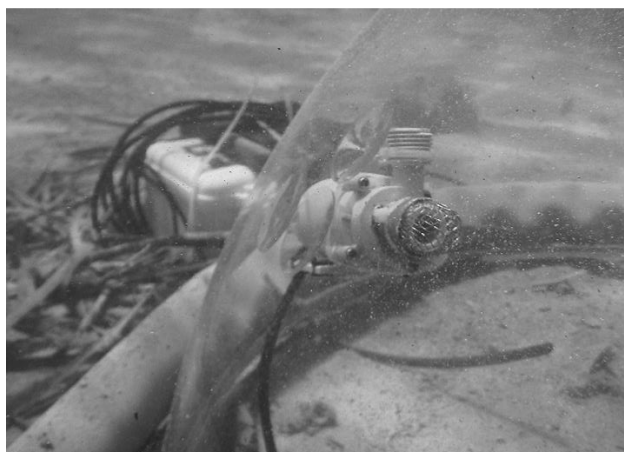
Figure 2. Setup of the internal water pump (foreground). Within a benthic chamber over the control unvegetated habitat. External submersible pump in background.

The benthic chambers were held in place by large chains and sank 5 cm into the sediment to create a seal. The benthic chambers were deployed within the same 2 hr timeframe each morning (10:45 am -12:45 pm) then left *in situ* for; 24 hours (Autumn and Summer) or 23 hours (Spring).

Daily Net Community Productivity

Small *P. oceanica* patches of < 2 m are assumed to consist entirely of edge habitat due to the proximity of the centre to the edge. Measurements of edge habitat are obtained from placing the benthic chambers over the edge of large *P. oceanica* patches or over the edge and centre of small *P. oceanica* patches (< 2 m). The *in situ* benthic chambers were deployed over sampling points of edge habitat in autumn, spring and summer. Edge habitat creates a good seal at the base of the benthic chambers because of the high proportion of sand to low proportion of rhizome. This avoids damage to the rhizome and roots from the chamber. A benthic chamber was simultaneously placed over the adjacent unvegetated sandy control habitat. If it was not possible to simultaneously deploy the control chamber, they were deployed at the next sampling opportunity the day after the first incubation is completed.

There is no need to consider diffusive exchange with the atmosphere as these benthic incubations were carried out in closed systems. The record from the PME oxygen logger is used to calculate net community productivity (NCP) for every 10-minute interval over each 24 hour period (modified from; Cole et al., 2000):

$$\Sigma \Delta DO = NCP_{Daily}$$

where DO is dissolved oxygen. This was applicable to the Autumn and Summer incubations when 24 hour incubations were completed.

The 23 hour incubations were completed missing one hour of daylight incubation. The total hours of daylight (H_d) were determined as:

$$H_d = 24 - H_n$$

where H_n is the total hours of night-time darkness.

$NCP_{Daytime}$ (Champenois and Borges, 2012; Rodriguez et al., 2016) was calculated whereby t is the daylight incubation time :

$$NCP_{Daytime} = \left(\frac{\Sigma\Delta\mathrm{DO}}{t}\right) \times H_d$$

During darkness GPP is 0, therefore the change in dissolved oxygen in any 10-minute interval is assumed to be a result of community respiration. The change that occurred during the night-time dark period were summed to calculate CR_{Night}:

$$\Sigma\Delta DO = CR_{Night}$$

The combination of the net community change during the daytime ($NCP_{Daytime}$) and night-time dark period (CR_{Night}) determine NCP_{Daily} for 23 hour incubations made during Spring:

$$NCP_{Daily} = NCP_{Daytime} + CR_{Night}$$

Daily community respiration and daily gross primary productivity

Since the benthic chamber incubations do not acquire a direct measurement of CR_{Day}, we assume the hourly value of CR_{Night} and CR_{Day} are equal (Cole et al., 2000):

$$CR_{Day} = \left(\frac{CR_{Night}}{H_n}\right) \times H_d$$

This allows daily community respiration (CR_{Daily}) to be found:

$$CR_{Daily} = CR_{Day} + CR_{Night}$$

NCP_{Daily} is then calculated as:

$$NCP_{Daily} = GPP_{Daily} - CR_{Daily}$$

All metabolism measurements (O_2 mg L^{-1} d^{-1}) are then converted into areal based estimates (Olive et al., 2015):

$$O_2 \; mmol \; m^{-2} \; d^{-1} = \frac{\left(\left(\frac{DO}{32}\right) \times V\right)}{A}$$

The corresponding GPP:CR ratios were calculated:

$$\frac{GPP}{CR}$$

Daily Net Apparent Productivity

The net apparent productivity (NAP) of the seagrass and epiphytes, was calculated as the difference between estimates for the NCP of the *P. oceanica* meadow and the average NCP of the sediment and plankton in the unvegetated control chambers (Murray and Wetzel, 1987) from the same corresponding season. This calculation assumes the only difference between the vegetated and unvegetated benthic chambers is the presence of the seagrass and epiphytes. Although the expected contribution of epiphyte productivity is deemed minimal due to the overall high biomass of *P. oceanica* to that of the epiphytes (Cox et al., 2015), therefore the NAP primarily reflects the productivity of the *P. oceanica.*

Daily NAP standardised for seagrass canopy height

After the *in situ* incubations were completed the blade length from 5 – 6 randomly selected blades were taken from the seagrass within the benthic chambers, as a proxy for the average height of the seagrass canopy in the edge habitat. To account for seasonal changes in seagrass canopy the seasonal NAP measurements were standardised by the average blade length with the benthic chamber (O_2 mmol m^{-2} cm^{-1} d^{-1}).

Annual Net Community Productivity and Net Apparent Productivity

Annual NAP and NCP was estimated by scaling up the three average seasonal daily NAP and NCP measurements; the November measurement was chosen to represent the period between October to January (123 days), April represented the period from February to May (120 days) and July represented June to September (122 days):

$$NAP_{Annual} = \left(NAP_{Daily(Nov)} \times 123\right) + \left(NAP_{Daily(Apr)} \times 120\right) + \left(NAP_{Daily(Jul)} \times 122\right)$$

$$NCP_{Annual} = \left(NCP_{Daily(Nov)} \times 123\right) + \left(NCP_{Daily(Apr)} \times 120\right) + \left(NCP_{Daily(Jul)} \times 122\right)$$

The photosynthetic and respiratory quotient of 1 mol of O_2: 1 mol CO_2 is applied to terrestrial plants that use starch and sugars as respiratory substrates, as observed in *P. oceanica* (Alcoverro et al., 2001) and therefore applied to convert all annual metabolism values (O_2 mol m^{-2} d^{-1}) into carbon (C mol m^{-2} d^{-1}).

NAP of central P. oceanica meadow and the meadow edge

In the Summer benthic chambers were placed over *P. oceanica* within the meadow centre in addition to the benthic chambers deployed over the edge habitat. Within the dense areas of the *P. oceanica* meadow the rigid PVC benthic chamber likely cut the rhizomes and roots, however the stress caused is generally considered marginal (Champenois and Borges, 2012). Control chambers were not placed out simultaneously to the central meadow measurements, as the already obtained Summer control measurements (n = 4) were used as the reference. After the incubations were completed several measurements were taken from the seagrass within the benthic chambers; an estimate of *P. oceanica* cover (%); shoot density (Sht_{dens}) (m^{-2}) within three 20 x 20 cm quadrats randomly placed on *P. oceanica*; the number of blades (B_{Number}) ($shoot^{-1}$) from 5 - 6 randomly selected shoots; canopy blade length (B_{length}) and blade width (B_{width}) (cm) from 5 – 6 randomly selected blades.

Plant surface area (m^{-2}) for each chamber was calculated from the seagrass biometrics:

$$Shoot_{Surface\ Area} = 2\left(B_{length} \times B_{width}\right) \times B_{Number}$$

$$Plant\ surface\ Area = Shoot_{Surface\ Area} \times \left(Sht_{Dens} \times \frac{Cover}{100}\right)$$

Sediment analysis of central P. oceanica meadow and the meadow edge

Four sediment samples were collected from within every Summer benthic chamber using a modified (open-ended) PVC syringe inserted to a depth of 10 cm, to collect 10 cm^3 cores. For

each chamber there were already noted several seagrass biometrics e.g. shoot density, percentage cover. All cores taken within seagrass underwent some level of compaction ($\bar{x}$ = 17 %, SD ± 10 %), due to the large rhizome and root network. Cores were resampled if compaction was greater than 45 %.

The PVC syringe was inserted into the sediment without the plunger until 10 cm, then the plunger was inserted to cap the sample at one end. As the sample was removed from the sediment it was capped at the other end, this was directly transferred into 50 ml falcon tubes. The samples were dried to a constant weight at 40 - 70 $^{\circ}$C using a desiccator, then returned to 50 ml falcon tubes for transport from Greece to the UK lab. At the lab the sediment samples were passed through a series of sieves, with the smallest mesh size 250 μm, to remove any seagrass root fragments or fibres. Loss on ignition analysis was conducted to calculate organic content of the sediment (OC) using the organic material lost at 550 $^{\circ}$C to determine sediment organic carbon (%).

$$OC = \left(\frac{(Dry\ weight - Ash\ content\ weight)}{Dry\ weight} \right) \times 100$$

Data analysis

Seasonal P. oceanica metabolism

With three samples from Winter and Spring, and four samples in Summer, finding an appropriate data distribution is challenging, but the distribution of NCP and NAP measurements are approximately normal. ANOVAs were applied to assess if there was a significant difference in NCP and NAP with changes in season. Tukey post hoc tests were used to test the differences between the seasons. An ANOVA was also applied to assess if there was a significant seasonal difference in NAP when standardised by canopy length.

Influence of environmental conditions on P. oceanica NAP

To examine the change in *P. oceanica* NAP relative to the changing light environment, the photosynthesis-irradiance relationship were fit with a hyperbolic tangent function (Jassby and

Platt, 1976) modified to account for respiration (Rheuban et al., 2014) and used prior for the seagrass species in question (Koopmans et al., 2020). The fit was calculated as:

$$Flux = P_{max} tanh \frac{I}{I_k} - R_I$$

where P_{max} is the maximum photosynthetic rate, I_k is the saturation irradiance, and R_I is respiration. The parameters were estimated by non-linear least squares approach (nls function, R version 3.5.1), estimating approximate start values. The irradiance compensation point is the irradiance at which net oxygen production equals zero.

As there was no significant interaction between temperature and seasonal NAP ($F_{1,4}$ = 0.189, p = 0.8345), or significant effect when temperature is assessed as a covariate for change in seasonal NAP by ANCOVA ($F_{1,6}$ = 2.1154, p = 0.1961). The maximal model to assess for significant effect of temperature on NAP was by ANOVA.

Comparison of spatial meadow carbon storage potential

A t- test is applied to determine if the NAP differs between the central meadow area and the meadow edge. Then linear regressions are applied to the Summer NAP to determine if the difference in central meadow and meadow edge relate to changes in plant surface area, percentage cover, shoot density and blade length. Comparison of the R^2 values provides an understanding of which seagrass biometric account for the variation in NAP and are the best spatial predictor for *P. oceanica* NAP.

A t- test is applied to determine the sediment organic carbon content differs between the central meadow area and the meadow edge. Then linear regressions applied to determine in the same seagrass health biometrics plant surface area, percentage cover, shoot density and blade length relate to the sediment organic content within the edge and central areas of the *P. oceanica* meadow.

Results

Seasonal Net Community Productivity and Net Apparent Productivity

The *P. oceanica* meadow NCP is greater than the unvegetated control in every season (Fig. 3A). In Autumn the *P. oceanica* community is overall heterotrophic as CR is greater than GPP (GPP:CR ratio < 1, Fig. 3 B), however the oxygen deficit is greatest in the control community without seagrass (NCP $\bar{x}$ = -6.9, SD ± 16.7 O_2 mmol m^{-2} d^{-1}), than in the *P. oceanica* community (NCP $\bar{x}$ = -4.0, SD ± 6.48 O_2 mmol m^{-2} d^{-1}). The NCP is lowest in Autumn but there is a significant seasonal influence on NCP ($F_{2,7}$ = 10.924, p = 0.007), subsequently NCP is significantly higher NCP in Spring (NCP $\bar{x}$ = 16.7, SD ± 6.25 O_2 mmol m^{-2} d^{-1}, p = 0.0113) and Summer (NCP $\bar{x}$ = 15.6, SD ± 6.05 O_2 mmol m^{-2} d^{-1}, p = 0.0107), compared to Autumn. But not significantly different between the Spring and Summer NCP (Fig. 3A).

During the transition from Autumn to Spring there is a large increase in GPP (GPP $\bar{x}$ = 40.3, SD ± 15.6 O_2 mmol m^{-2} hr^{-1} to GPP $\bar{x}$ = 137.0, SD ± 95.7 O_2 mmol m^{-2} d^{-1}, Fig. 3C). There is also a large increase in CR from Autumn into Spring (CR $\bar{x}$ = 44.4, SD ± 20.2 O_2 mmol m^{-2} d^{-1} to CR $\bar{x}$ = 120.3, SD ± 89.6 O_2 mmol m^{-2} d^{-1}, Fig. 3D), however the increase in GPP is greater than the increase in CR transitioning the *P. oceanica* meadow into an autotrophic ecosystem (GPP:CR ratio > 1, Fig. 3B).

The *P. oceanica* meadow stays in an autotrophic state in the Summer (GPP:CR ratio > 1, Fig. 3B) with its highest GPP (GPP $\bar{x}$ = 188.3, SD ± 148.44 O_2 mmol m^{-2} d^{-1}, Fig. 3C), but also its highest CR (CR $\bar{x}$ = 172.704, SD ± 148.4 O_2 mmol m^{-2} d^{-1}, Fig. 3C). The increase in GPP is not as great as the increase in CR, therefore the GPP to CR ratio and NCP is lower in Summer compared to Spring (Fig. 3B).
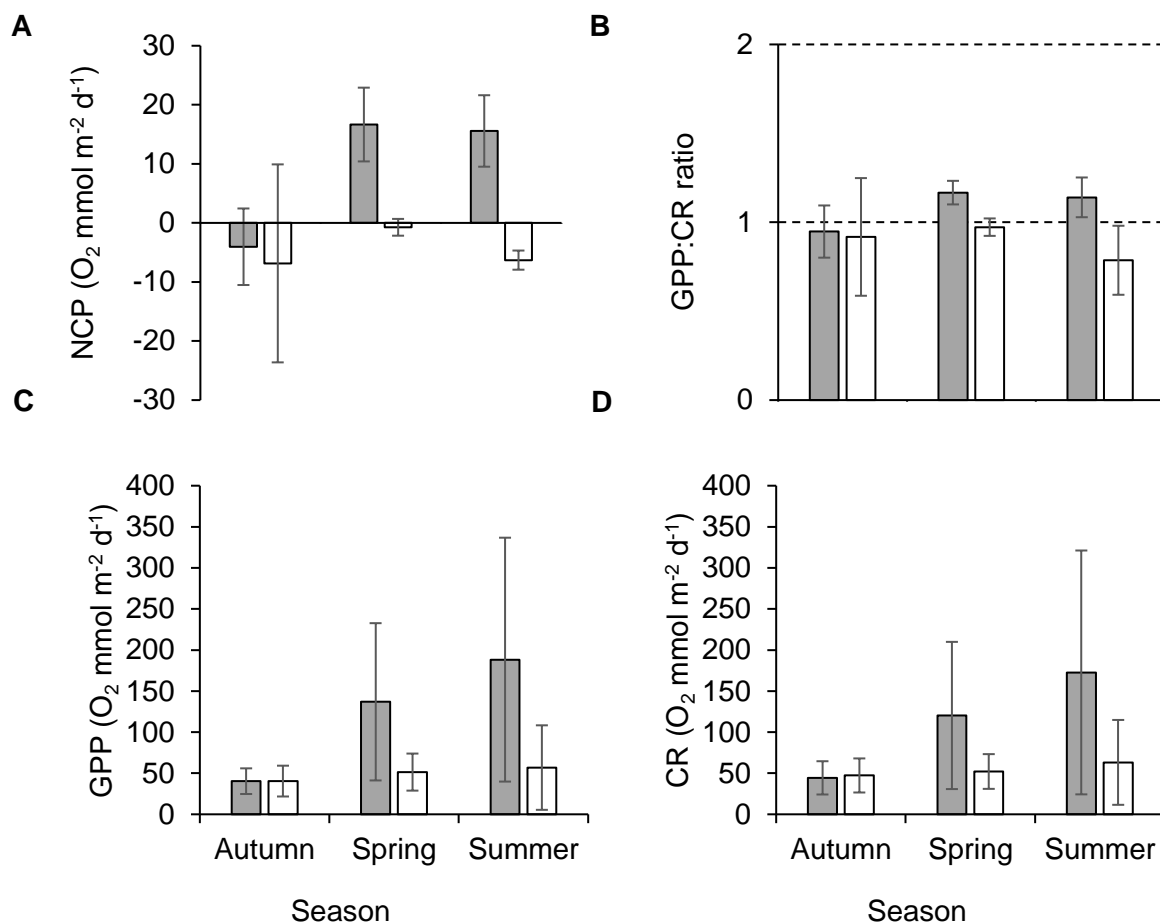
Figure 3. A Net community productivity (NCP), **B** GPP:CR Ratio, **C** Gross primary productivity (GPP) and **D** Community respiration (CR) for the *P. oceanica* meadow edge (grey) and control chamber (White), Autumn (November), Spring (April) and Summer (July). Error bars represent standard deviation (Autumn and Spring n = 3, Summer n = 4).

The *P. oceanica* NAP is highest in Summer (NAP $\bar{x}$ = 21.87, SD ± 6.05 O_2 mmol m^{-2} d^{-1}). The season of the year also has a significant impact on *P. oceanica* NAP ($F_{2,7}$ = 8.3885, *p* = 0.0139) (Fig. 4A). Tukey post hoc comparisons found NAP is only significantly lower in Autumn (*p* = 0.0123) compared to Summer. There is an observational difference in NAP between the Autumn (NAP $\bar{x}$ = 2.81, SD ± 6.48 O_2 mmol m^{-2} d^{-1}) and Spring season (NAP $\bar{x}$ = 17.40, SD ± 6.25 O_2 mmol m^{-2} d^{-1}), but the post hoc comparisons showed the difference was not significant (*p* = 0.0556), similarly there was no significant difference between the periods of highest NAP in Spring and Summer (*p* = 0.6343).

Despite the highest *P. oceanica* NAP occurring in Summer (Fig. 4), at the meadow's community level net gain is highest in the Spring (Fig. 3A), as the organisms present within the control chambers have a higher metabolic rate in the Summer (NCP $\bar{x}$ = -6.3, SD ± 1.6 O_2 mmol m^{-2} d^{-1}, Fig. 3A) compared to the Spring (NCP $\bar{x}$ = -0.7, SD ± 1.4 O_2 mmol m^{-2} d^{-1}, Fig. 3A). Likewise, despite a net gain by *P. oceanica* in Autumn (NAP $\bar{x}$ = 2.81, SD ± 6.48 O_2 mmol m^{-2} d^{-1}) (Fig. 3), this becomes a net loss at the community level (NCP $\bar{x}$ = -4.0, SD ± 6.5 O_2 mmol m^{-2} d^{-1}, GPP/CR ratio < 1) (Fig. 3A).

Seasonal Net Apparent Productivity standardised for seagrass canopy height

The average canopy blade length within the edge *P. oceanica* habitat is at its lowest during Autumn ($\bar{x}$ = 15.4, SD ± 2.84 cm), this increases into Spring ($\bar{x}$ = 22.3, SD ± 4.77 cm) and peaks during Summer ($\bar{x}$ = 25.1, SD ± 5.90 cm). When the *P. oceanica* NAP is standardised by the blade length of the seagrass canopy in the corresponding season, there is no significant seasonal difference in NAP ($F_{2,7}$ = 3.9782 *p* = 0.070) (Fig. 4B). The NAP standardised for blade length is most similar between Spring (NAP $\tilde{x}$ = 0.85, Range = 0.533 O_2 mmol m^{-2} cm^{-1} d^{-1}) and Summer (NAP $\tilde{x}$ = 0.85, Range = 0.316 O_2 mmol m^{-2} cm^{-1} d^{-1}). Whilst the NAP standardised for blade length is observationally lower in Autumn (NAP $\tilde{x}$ = 0.85, Range = 0.976 O_2 mmol m^{-2} cm^{-1} d^{-1}), it is not significantly different to Spring and Summer.
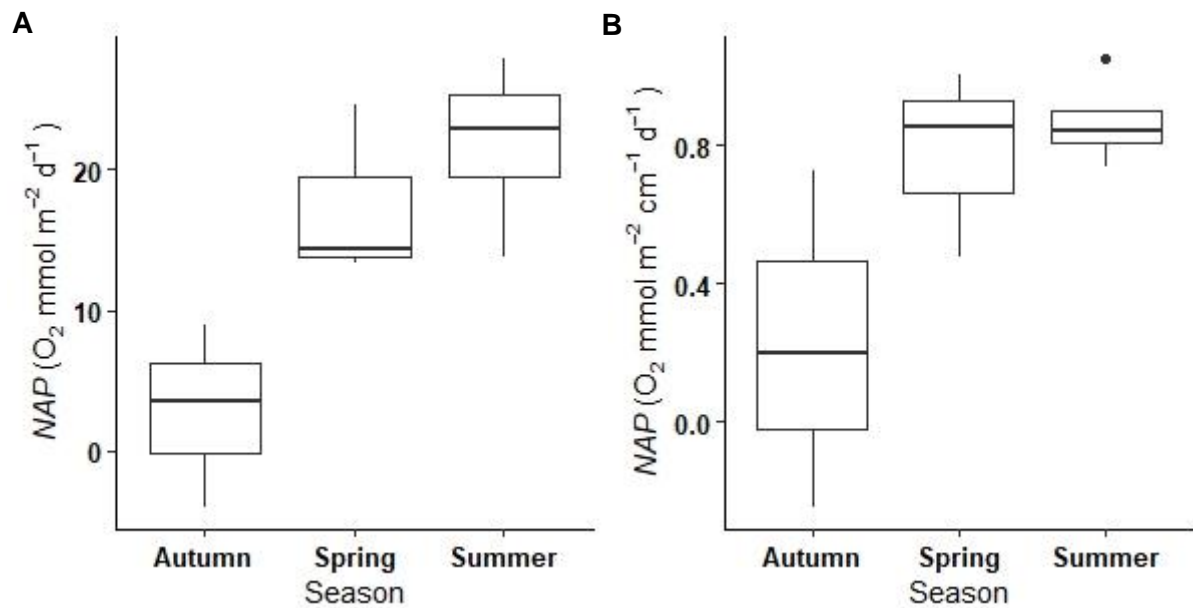
Figure 4. A The *P. oceanica* NAP given the season, **B** The *P. ocenica* NAP when standardised by the blade length of the seagrass canopy in the given season. Autumn and Spring n = 3, Summer n = 4.

Influence of environmental conditions on P. oceanica NAP

The daily light integral ranged from 4.7 mol photons m^{-2} d^{-1} during Autumn to 25.2 mol photons m^{-2} d^{-1} in Summer. Variation in environmental conditionswithin each season allowed for some overlap in the irradiance encountered between seasons. The *P. oceanica* NAP resembled a saturation curve when plotted as a function of irradiance (Fig. 5A), as did the curve for NAP standardised by canopy length (Fig. 5B). Light saturating levels begin to occur in Spring and continue into Summer, when NAP sits near the predicted maximum (20.421 O_2 mmol m^{-2} d^{-1}). Whereas the DLI conditions in Autumn (Min = 4.7 mol photons m^{-2} d^{-1}) sit closer to the predicted light compensation point (I_C= 4.6 mol photons m^{-2} d^{-1}) as such within the Autumnal DLI range there are conditions where the plant may only just or may not maintain metabolic requirements due to low irradiance.
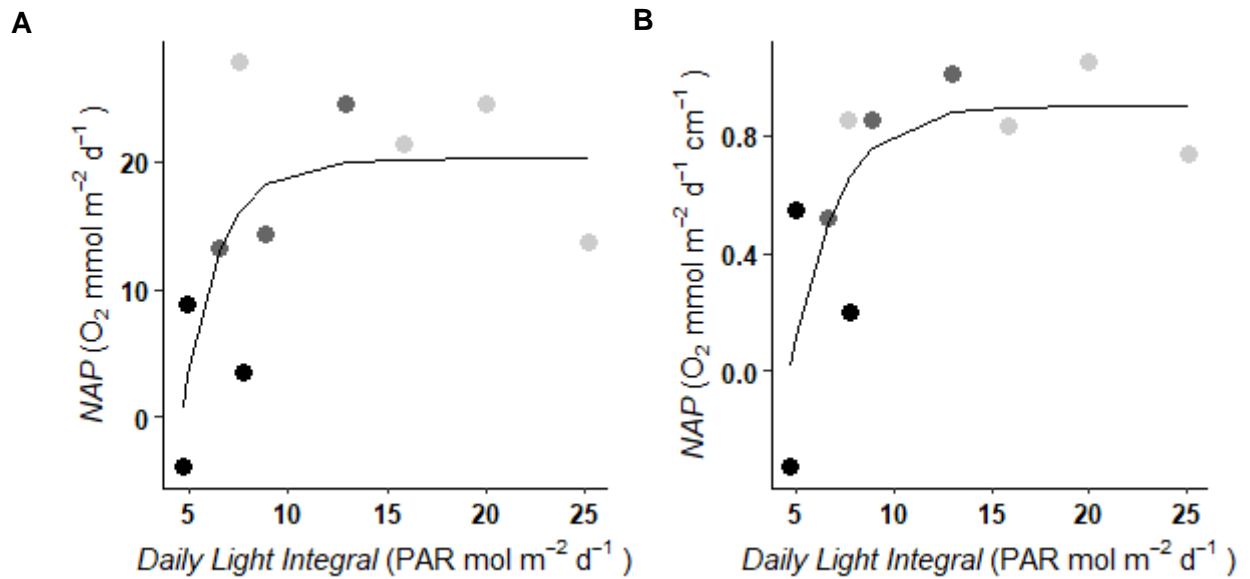
Figure 5 A *P. oceanica* NAP (R^2 = 0.713) and **B** *P. oceanica* NAP standardised by canopy

length (R^2 = 0.711), as a function of PAR given as the Daily Light Integral. The irradiance

compensation point (I_C) was 4.6 mol photons m^{-2} d^{-1}. Replicate season denoted for visual

reference Autumn (●), Spring (●) and Summer (○).

The water temperature was lowest in Spring ($\bar{x}$ = 17.7, SD ± 0.13 °C), increasing to a Summer

high ($\bar{x}$ = 23.6, SD ± 0.40 °C). The Autumn temperatures encountered sat at a relative mid-point

($\bar{x}$ = 20.6, SD ± 0.45 °C). between Spring and Summer, yet the NAP was lowest in Autumn

(NAP $\bar{x}$ = 2.81, SD ± 6.48 O_2 mmol m^{-2} d^{-1}). Therefore the change in NAP was not relative to the

change in water temperature and not a significant factor as main effects, with season (Fig. 6A)

($F_{1,6}$ = 2.1154, p = 0.0.19606) or without season (Fig. 6B) (Temperature $F_{1,8}$ = 0.7265,

p = 0.422).
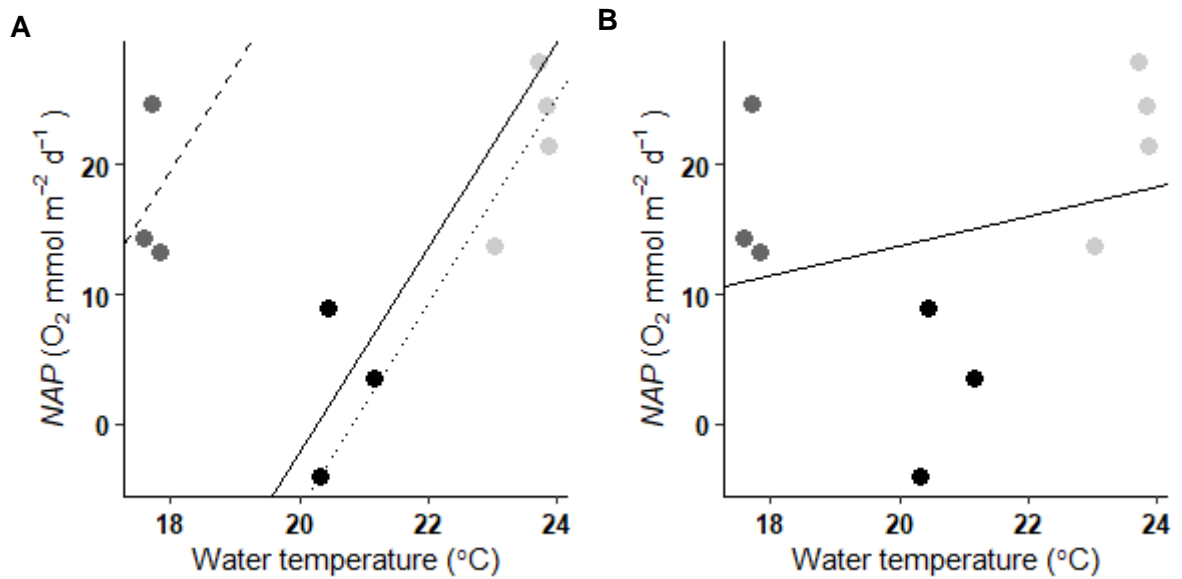
Figure 6. A Realised *P. oceanica* NAP in Autumn (—), Spring (---) and Summer

(·····) **B** Realised NAP irrespective of season (—), given as a function of the average daily water

temperature (°C). Replicate season denoted Autumn (●) and Spring (●) n = 3, Summer (○)

n = 4. The relationships described in this figure are based on predicted temperature

relationships which were of no significance

Annual Net Community Productivity and Net Apparent Productivity

The annual NAP of the *P. oceanica* in this meadow is determined as 5.1 C mol m^{-2} yr^{-1} (SD±

0.02 C mol m^{-2} yr^{-1}). However the organisms present within the *P. oceanica* meadow have their

own metabolic requirements (NCP without seagrass present 1.7 SD± 0.02 C mol m^{-2} yr^{-1}), as

such the net carbon gain at the community level for this *P. oceanica* meadow is less than the

NAP produced annually by the *P. oceanica* (NCP = 3.4 C mol m^{-2} yr^{-1} SD± 0.02 C mol m^{-2} yr^{-1}).

Spatial variance in Net Apparent Productivity and the organic content of sediment

The NAP of the *P. oceanica* in Summer is significantly different between the central areas of the meadow and the meadow edge ($t_{(1,4)}$ = 3.657, *p* = 0.02) (Fig. 7). The *P. oceanica* NAP is highest in the central areas of the meadow (NAP $\bar{x}$ =43.4, SD± 8.4 O_2 mmol m^{-2} d^{-1}), compared to the edge habitat (NAP $\bar{x}$ =21.9, SD± 6.0 O_2 mmol m^{-2} d^{-1}).

The *P. oceanica* NAP increased relative to the plant surface area of *P. oceanica* (LM: $F_{1,5}$= 72.15, *p* = 0.0004) (Fig. 7A). The plant surface area was the best predictor of NAP and accounted for more than 92 % of the variation in NAP. As standalone biometrics of seagrass meadow metabolism, shoot density (m^2), blade length (cm) and seagrass cover (%), were all significant predictors of NAP (shoot density, LM: $F_{1,5}$= 19.09, *p* = 0.007; blade length, LM: $F_{1,5}$= 11.93, *p* = 0.018; percentage cover, LM: $F_{1,5}$= 7.359, *p* = 0.04) but they only accounted for 75 %, 64 % and 51 % (Fig. 7B, C and D) of the variation in NAP of the *P. oceanica*.
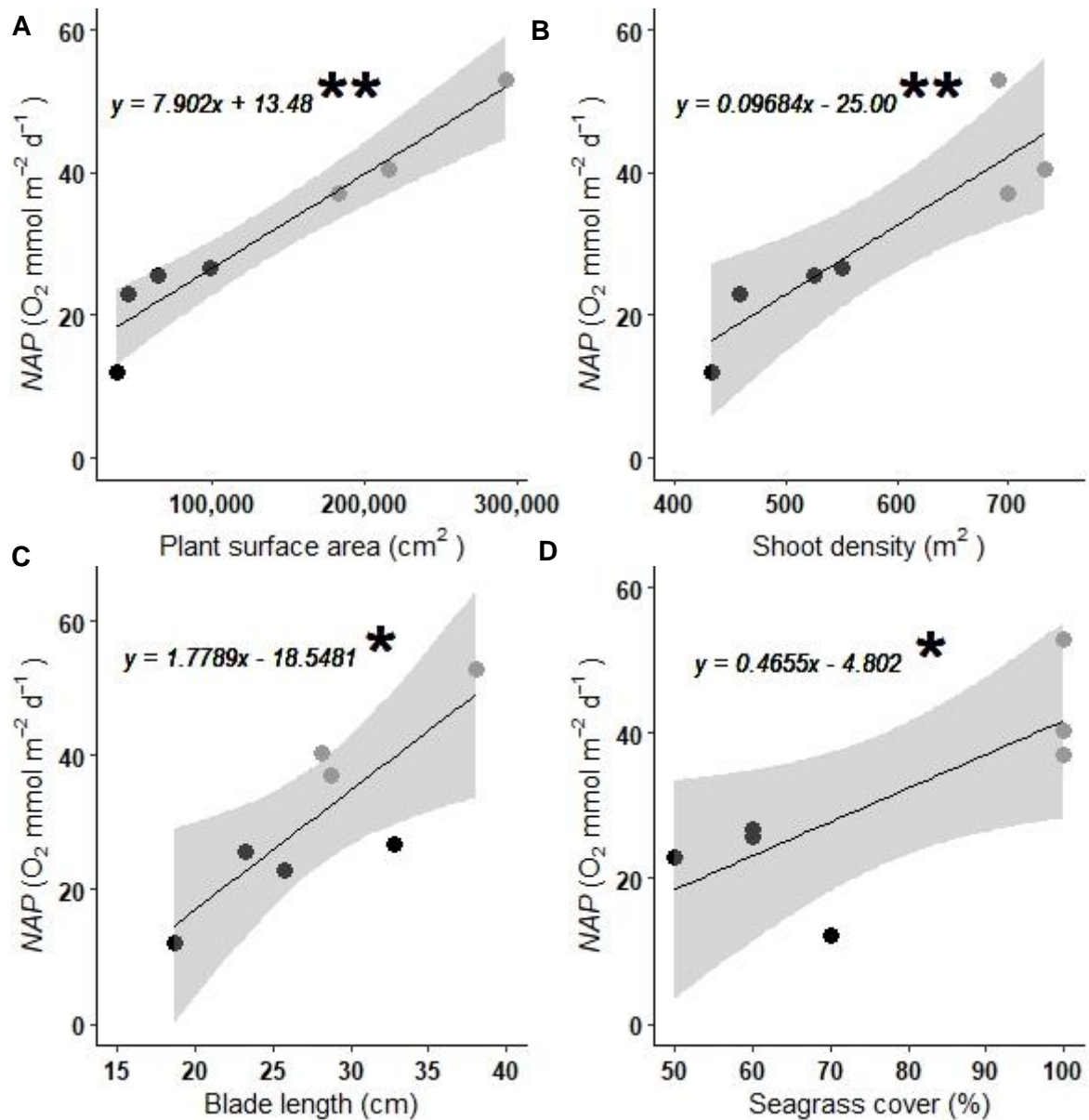
Figure 7. Net apparent productivity (NAP) of the *P. oceanica* in the central meadow (grey) and meadow edge (Black) in relation to **A** *P. oceanica* blade surface area **B** *P. oceanica* shoot density (m^2) **C** *P. oceanica* blade length (cm) and **D** *P. oceanica* cover (%). Central meadow n = 3, meadow edge n = 4. * significant linear regression of $p < 0.05$, ** significant linear regression of $p < 0.01$

The percentage of organic carbon stored within the sediment was observationally higher in the central areas of *P. oceanica* meadow ($\overline{x}$ =5.78 %, SD± 1.01%) than the edge and patchy areas of this *P. oceanica* meadow ($\overline{x}$ =4.89 %, SD± 0.84%), though not significantly higher ($t_{(1,4)}$ = 1.1646 p = 0.31) (Fig. 8). In contrast to the NAP, there was no significant increase in sediment organic with shoot density (p = 0.15), percentage cover (p = 0.21), plant surface area (p = 0.33) and blade length (p = 0.96).
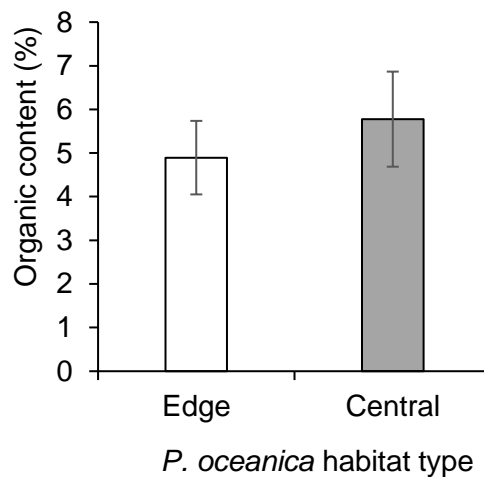
Figure 8. Organic content of sediment for edge and central *P. oceanica* meadow.

Discussion

The strong seasonality in productivity at both the community and plant level, enforces the need for metabolism methodologies to utilise seasonal sampling, thereby ensuring carbon storage estimates are not overscale by only reflecting seasonal peaks. Placing specific emphasis on collecting measurements in opposing seasons, given low heterotrophic metabolism periods occurred in Autumn compared to higher autotrophic periods in Spring and Summer. Irradiance has often been considered one of the main drivers in *P. oceanica* community productivity (Gazeau et al., 2005; Champenois and Borges, 2012). During Autumn the overall irradiance reaching the plant is likely lower as the daylight period is shorter, the likelihood of cloudy conditions increases and increased likelihood of stormy conditions result in greater hydrodynamic activity reducing in-water visibility, the subsequent irradiance would in part promote the distinction in photosynthetic activity between season. Moving through Spring into Summer you typically expect the daylight period to increase and the level of daylight irradiance to intensify resulting in increased productivity. Continued increase in irradiance does not guarantee continued increase in productivity, light saturating levels present throughout Summer, lead to a lower photosynthetic efficiency despite continued increase in irradiance. Long term exposure to saturating irradiance decreases plant productivity and can lead to photoinhibition (Ralph and Burchett, 1995). Though a negative decline as a result of photoinhibition is not modelled in this study, the consecutive days of light saturating irradiance which occur in Summer represent an increasing likelihood that photoinhibition would occur. However, shallow water *P. oceanica* are adapted to high-light light conditions, showing specific photoacclimative and photoprotective responses; significant enrichment of up-regulated transcripts associated to light-dependent reaction of photosynthesis (e.g. light-harvesting proteins); modifications in the structure of photosystems and tocopherol biosynthesis activation in the Summer (Dattolo et al., 2014). Which is potentially why a strong negative photoinhibition response was not seen. The saturation response alone has important considerations for modelling carbon sequestration capacity, re-emphasising the importance of seasonality and the concurrent irradiance levels.

Leaf shedding alongside biomass exported by storm disturbance results in reduced aboveground biomass and photosynthetically active tissue from late Summer to early Winter (Champenois and Borges, 2012; Gomez-Pujol et al., 2013). The strong distinction in *P. oceanica* NAP between late Autumn and Summer, may in part relate to the relative availability of photosynthetic plant surface area due to changing aboveground plant biomass between seasons. Autumnal loss of leaves is followed by new growth during Winter despite this typically being a period of negative carbon balance for the plant (Alcoverro et al., 1995). Growth during a period of negative carbon balance is possible because *P. oceanica* has large long-lived rhizomes which store starch, this combined with the ability to transport photoassimilates further than smaller seagrass species, allows it to use these reserves to support growth patterns that are independent to seasonal environmental conditions (Marba et al., 1996). This *P. oceanica* growth pattern is governed by an internal rhythm (Ott, 1979). which ensures Winter growth is well-timed to coincide with increasing irradiance, allowing for maximum carbon fixation post-Winter (Alcoverro et al., 2001). Therefore, its seasonal metabolism is governed by its asynchronous growth to mis-match irradiance. Reinforcing that light is a key factor in understanding this plant's growth patterns and ultimately determining its carbon acquisition dynamics. Given this internal rhythm can persist for at least two years under constant conditions of irradiance, day length and temperature (Ott, 1979), the predictable growth of *P.* oceanica coupled with anticipated irradiance could be utilised to forecast future carbon sequestration.

P. oceanica meadows within the eastern region of the Aegean Sea have already been noted to exhibit scattered and fragmented meadow morphology in response to the elevated sea surface temperatures (Chefaoui et al., 2017), it is surprising then that temperature did not have a significant effect on the productivity of shallow water *P. oceanica*. Other temperate seagrass species have been shown to transition to negative carbon balance when exposed to high Summer water temperatures (Marsh Jr. et al., 1986). Although our highest average daily water temperature was above optimal thermal conditions (17 - 20 °C) for *P. oceanica* (Champenois and Borges, 2018), this was well below *P. oceanica* lethal temperature limits (> 29 °C) and

perhaps explains why the increased Summer water temperatures did not cause a transition into overall heterotrophy. The *in-situ* nature of this study does not allow for the active control of either the water temperature or the light intensity encountered within our seasonal samples. A variety of conditions may not be encountered, and the predictions not appropriately reflect the response of *P. oceanica* productivity to water temperatures and light intensities outside of what was encountered. Evidence shows shallow water *P. oceanica* has a greater tolerance to increased water temperatures than deep *P. oceanica* (Marin-Guirao et al., 2016). The ability to stabilise respiration, thereby establishing respiratory homeostasis (balanced photosynthetic carbon gains and respiratory carbon losses), was highlighted as the key mechanism for heat acclimation in shallow *P. oceanica* (Marin-Guirao et al., 2016). This suggests that shallow *P. oceanica* may maintain net productivity gains at higher water temperatures for longer, at least in comparison to deeper *P. oceanica*. Given the shallow and deep water ecotypes have been shown to thermally acclimate to local conditions (Marin-Guirao et al., 2016), it is also possible that the *P oceanica* within the eastern Aegean or more broadly the Eastern Mediterranean basin may have a greater existing acclimation to increased water temperature, as the Eastern Mediterranean basin has been exposed to higher sea surface temperatures (since 1985) than the Western Basin (Nykjaer, 2009). Ultimately any temperature effect on *P. oceanica* metabolism in this study was confounded by the strong irradiance effect and thus could not be disentangled.

The planktonic community in the water surrounding the *P. oceanica* meadow tended to be more heterotrophic outside of Spring when blooms typically occur (Gazeau et al., 2005). Mediterranean planktonic and benthic communities show increased respiration rates with warming (Vaquer-Sunyer and Duarte, 2013). The higher water temperatures in Summer and late Autumn increased community utilisation of oxygen produced by *P. oceanica*. Accounting for the metabolism of the planktonic community within a seagrass meadow is important to determine the net gain of carbon at a community level, but as the autotrophy within the community is largely attributed to the primary productivity of the seagrasses (Hemminga and

Duarte, 2000), distinguishing the NAP of the *P. oceanica* enables a clearer determination of the influences driving this metabolic component of the community. Justifying the approach to distinguish between NCP and NAP within the *P. oceanica* meadow ecosystem, as seasonal community metabolism may partially mask the drivers in *P. oceanica* productivity. The calculation of NAP requires the assumption that the only difference between the vegetated and unvegetated control chamber is the presence of *P. oceanica,* the metabolism of the invertebrate community hidden within the seagrass are not accounted for when using the unvegetated control chamber as a proxy for the planktonic and overlying benthic community metabolism, potentially overestimating the NAP of *P. oceanica.* Epifauna community structure, defined by the number of taxonomic groups, doesn't necessarily differ between *P. oceanica* central meadow and sandy bottoms (Sanchez-Jerez et al., 1999), but some species-specific epifaunal differences do occur between these habitat types and could influence the community metabolism (Sanchez-Jerez et al., 1999). Therefore, the use of NCP estimates are widely accepted for benthic chamber metabolism studies within seagrass meadows (Duarte et al., 2010; Olive et al., 2015) because they clearly quantify the extent to which the overall community is a carbon sink or source. Hence this study still presents the results in NCP as it allows for closer comparisons to the existing *P. oceanica* NCP estimates.

Metabolism measurements are typically presented with an area-based unit (Duarte et al., 2010; Olive et al., 2015), but considering productivity has been shown to significantly relate to spatial variations in plant aboveground biomass, carbon sequestration estimates should potentially more away from denoting discrete areal measurements for meadows. Instead acquiring and mapping large scale data on canopy height (shoot size) and shoot density to enable comprehensive habitat wide autochthonous carbon sequestration estimates. Determining annual changes in shoot size would also support the prediction of annual meadow productivity. Acquiring biometric data to support distribution data may require greater effort than simply collating presence-absence or coverage data, but simple plant biometrics of shoot density and blade length have been demonstrated as standalone predictors of carbon sequestration. High

shoot density in shallow areas with a high degree of patchiness creates very specific nest-like patterns, that have their highest shoot density in the centre and decrease in density radially towards the edge of the patches (Zupo et al., 2005). Therefore, this distinction in spatial productivity between edge and central areas of *P. oceanica* may be a specific characteristic to consider for shallow meadow carbon stocks. Because overall shoot densities are highest in the shallow and decrease with depth (Olesen et al., 2002; Zupo et al., 2005). More work would be needed to determine if the same distinctions in shoot density and subsequent primary productivity exist between the edge and central areas of deeper areas of *P. oceanica* meadows.

Patchiness also exists on a small scale less than that of metres, although factors such as physical disturbance, topography and nutrients are thought to operate on this small scale, variation at this small scale in harder to explain (Ballestri et al., 2003). The use of benthic chambers with a large benthic surface area have a greater representation of community-scale metabolism as trialled for heterogenic coral reef habitats (Yates and Halley, 2003). The use of a larger benthic chamber in this study (1m diameter) comprises community metabolism measurements influenced by fine scale variation in shoot density, compared to the previous small chambers (0.18m diameter), which house only a few shoots at similar densities within the benthic chamber (Gazeau et al., 2005; Barron et al., 2006).

The annual net community productivity for this meadow was lower than recorded for the two *P. oceanica* meadows within the western region of the Aegean Sea; one situated 20m away from a fish farm deemed to be negatively impacted by eutrophication (4.83 C mol m^{-2} yr^{-1}) and the second reference meadow (11.97 C mol m^{-2} yr^{-1}) (Apostolaki et al., 2010). This suggests a great variability in NCP exists between meadows in the Aegean Sea, but considering these estimates were acquired from deeper meadow areas it cannot be determined if the variability is due to factors that change with depth such as meadow composition and environmental conditions. The annual NCP in this study was most similar to an NCP measurement in the Western Mediterranean, by open water O_2 mass balance (3.5 ± 0.8 O_2 mol m^{-2} yr^{-1}), however within its dataset it was the lowest annual NCP, with the reduction attributed to accumulation of

leaf litter in the meadow due to lower wintertime wind speeds (Champenois and Borges, 2012). Therefore, the annual NCP of this meadow appears most comparative to *P. oceanica* meadows that are under duress whether that be eutrophication (Apostolaki et al., 2010) or increased decomposition of leaf litter (Champenois and Borges, 2012). The metabolism of shallow water *P. oceanica* habitats potentially does not function at the highest metabolic efficiency, given shallow water *P. oceanica* exists in a light saturating environment for large parts of the year. However, the annual NCP in this study was obtained from patchy *P. oceanica* edge habitat, this underestimates the meadows overall carbon sequestration ability as central areas with high shoot density clearly demonstrated higher levels of productivity. This low annual estimate of NCP reflects that this study focused on shallow water meadows, where patchy edge habitat is a more prominent feature (Montefalcone et al., 2009), therefore it is an appropriate and conservative annual NCP estimate for this habitat. It also highlights the need for future estimates to account for the distinct heterogeneity in carbon sequestration within shallow meadows. Despite a relatively lower annual NCP estimate shallow water *P. oceanica* still represents an autotrophic habitat aiding carbon sequestration. When you consider the potential collective amasses of shallow water *P. oceanica* this represents a considerable carbon sink.

The carbon sequestration potential of seagrass meadows ultimately relates to how much of the organic material produced accumulates as refractory material in the seagrass meadow. Reduced current attenuation should promote significantly higher organic carbon concentration with distance from the edge (Oreska et al., 2017), for both allochthonous and autochthonous carbon. Despite the sediment analysis within this study not determining between allochthonous and autochthonous, the patterns of high productivity should have been retained relating to an increase in the sedimentary organic content of central meadow area. The higher organic content in central areas should perhaps would have been expected to be more distinct given allochthonous carbon would also settle preferentially out in central areas. In shallow patchy meadows the proximity of central areas to the meadow edge may limit organic carbon accumulation, as increased settlement of particles with distance from meadow edge occurs at

concentration gradients over 1km in length (Oreska et al., 2017), within shallow fragmented meadows this spatial scale isn't scalable. Even if the patchiness of shallow meadows may limit the burial of carbon, if the seagrass patches have the long-term ability to persist, they still actively contribute to carbon sequestration. Loss of shallow patchy *P. oceanica* habitat would still represent a loss of current carbon sinks, thus their importance and relative contribution should not be overlooked. The retention of autochthonous material in patchy meadows requires further research and direct relation to the expected primary productivity.

If natural ecosystems such as seagrass meadows are to play a part in mitigating increases in anthropogenic CO_2 emissions variability in primary productivity needs to be accounted for to produce comprehensive carbon stock estimates in the future. Firstly, this means future metabolism studies must account for changes in seasonal productivity, measurements only obtained in peak seasons risk skewing the relative carbon sequestration potential. Utilising predictable seasonal canopy growth patterns in combination with seasonal irradiance levels could be well utilised to fine tune forecasts of annual carbon storage potential. Finally accounting for spatial heterogeneity across a meadow by considering plant surface area and shoot density as predictors of productivity, particularly in shallow meadows, would help to more truly reflect the contribution of these habitats to carbon sequestration. Overall, the autotrophic nature of shallow water *P. oceanica,* even when considering the patchy nature of the habitat, maintains that these areas are valuable in their contribution to the overall carbon sink potential of *P. oceanica* meadows.

Productivity of the 'invasive' seagrass *Halophila stipulacea* and the presence of an uncommon phytomyxid infection

Introduction

The eastern region of the Aegean Sea sits at a crossroad for alien species expansion (Pancucci-Papadpooulou et al., 2012). This includes the marine angiosperm, *Halophila stipulacea* which was introduced to the Mediterranean and first reported in 1894 off the Island of Rhodes in the south-eastern region of the Aegean Sea (Fritsch, 1895). This is not the only new global region this species has spread too, beyond its indigenous distribution in the Red Sea and Indian Ocean (den Hartog, 1970) it has established within the Mediterranean and the Eastern Caribbean (Ruiz and Ballantine, 2004). *H. stipulacea* was listed amongst the '100 Worst Invasive Species' in the Mediterranean (Streftaris and Zenetos, 2006), but there are discrepancies in opinion as to whether it should be considered 'invasive', as no ecological consequences of the introduction and spreading of *H. stipulacea* in the Mediterranean have been reported (Williams, 2007). Many only tentatively chose to include it in the list of invasive macrophytes within the eastern Mediterranean (Boudouresque and Verlaque, 2002).

The term 'invasive' has been inconsistently used to describe *inter alia* in many different *contexts;* as any introduced non-indigenous species; introduced species that spread rapidly in a new region; and introduced species that have harmful impacts particularly on native species (Ricciardi and Cohen, 2007). To many people (especially policy-makers and stakeholders) it implies a species that causes environmental or socio-economic impacts (Ricciardi and Cohen, 2007). An expansive amount of literature exists on the changing and potential range extension of *H. stipulacea* (Fritsch, 1895; van der Velde and den Hartog, 1992; Gambi et al., 2009; Tsiamis et al., 2010; Sghaier et al., 2011; Georgiou et al., 2016). Implying the dominant reason for it to be deemed invasive, is its rapid and vast expansion within the Mediterranean. Less research has focused on its impact or contribution to specific ecosystem services within Mediterranean coastal ecosystems, such as carbon sequestration (Apostolaki et al., 2019). Given *H. stipulacea* is one of the longest monitored nonindigenous species in the

Mediterranean there has been a clear lag from reporting its presence and rate of expansion, to beginning to understand its impact within the communities it has established. Until recently *H. stipulacea* was thought to be the only non-indigenous seagrass species in the Mediterranean but records now demonstrate another species from the *Halophila* genus, *Halophila decipiens* has also entered the Mediterranean (Gerakaris et al., 2019). The unsurprising ease at which these tropical pioneer seagrass species appear to be able to enter the Mediterranean suggest, more than ever, it is crucial to understand, not just where in the Mediterranean they are present but, the impact they may have now and in the future.

Seagrass meadows high productivity means they are typically considered autotrophic ecosystems (Duarte et al., 2010; Champenois and Borges, 2012). This productivity coupled with their canopies' ability to increase particle settlement out of the water column means they can store large quantities of carbon (Duarte et al., 2011) and are considered carbon sinks. This is certainly true of native Mediterranean seagrass meadows, largely dominated by the seagrass *Posidonia oceanica* (Duarte et al., 2010; Champenois and Borges, 2012). Whereas long-term organic carbon accumulation rates in Red Sea seagrass meadows, where *H. stipulacea* is native, have been shown to be lower than estimates for *P. oceanica* meadows in the Mediterranean (Serrano et al., 2016; Serrano et al., 2018). Recent research found sedimentary carbon stocks from Mediterranean *H. stipulacea* meadows to be comparable to native *P. oceanica,* although was attributed to the deposition of allochthonous carbon (Apostolaki et al., 2019). Allochthonous carbon is typically considered more labile, thus habitat that favours the accumulation of autochthonous rather than allochthonous organic carbon lead to stable deposits and carbon sinks (Mazarrasae et al., 2018). To understand to the effect of *H. stipulacea* presence in the Mediterranean on autochthonous carbon sequestration an assessment of *H. stipulacea in situ* productivity in the Mediterranean is integral.

In the Mediterranean *H. stipulacea* can be found in multi-species meadows with the native *Cymodocea nodosa,* in the free spaces between patches of *P. oceanica* or in habitats previously devoid of seagrass (Boudouresque et al., 2009). Whilst there are reports of its

'invasive' behaviour on sandy bottoms when it exists in high abundances, no displacement of native species has yet been reported (Tsiamis et al., 2010). Given that *H. stipulacea* has various contexts in which it can be found in the Mediterranean its contribution to community productivity may differ depending on the context. When *H. stipulacea has* colonised areas previously absent of seagrass its presence has increased the distribution of seagrass habitat in the Mediterranean and is perhaps why it has been highlighted as a new potential blue carbon sink habitat (Apostolaki et al., 2019). But it is important to understand how the community productivity of the habitat devoid of seagrass differs to the 'new' seagrass habitat. Given seagrass habitats are typically considered autotrophic systems (Duarte et al., 2010; Champenois and Borges, 2012) it may suggest *H. stipulacea* has had an additive presence to the community and increased community productivity. This may be dependent on the habitat it has replaced as some algal communities show high community productivity and have traditionally been underestimated as blue carbon systems (Duarte et al., 2013). The positive potential of *H. stipulacea* to increase community productivity and create new carbon sink habitats in the Mediterranean (Apostolaki et al., 2019), is in direct contrast to the negative connotations, implied by the word 'invasive'.

In the Mediterranean *H. stipulacea* has colonised areas previously occupied by native seagrass species, such as *P. oceanica* dead matte or mixed *C. nodosa* meadows (Sghaier et al., 2011). In the Caribbean *H. stipulacea* has been shown to actively displace the dominant native seagrass *Syringodium filiforme* (Willette and Ambrose, 2012). Whilst Sghaier (et al., 2011) does not necessarily describe active displacement of the native species *C. nodosa* and *P. oceanica*, it highlights replacement of native seagrass species and species-specific alteration in Mediterranean seagrass meadows. Morphologically and phylogenetically *P. oceanica, C. nodosa* and *H. stipulacea* are very different seagrasses. Colonisers such as *H. stipulacea* are considered to have lower biomass accumulation, particularly belowground biomass, than larger and long-lived species such as *P. oceanica* (Serrano et al., 2016). The comparatively lower belowground biomass of *H. stipulacea* suggests its accumulation of autochthonous organic carbon is less than native Mediterranean species. If *H. stipulacea* is replacing monospecific

meadows or altering the species composition within mixed meadows, the dynamics and ecosystem services provided by Mediterranean meadows will alter too. The recorded regression of native species like *P. oceanica* (Telesca et al., 2015), may enable further colonisation of *H. stipulacea* and changes in the carbon sequestering potential of Mediterranean meadows.

Within its native range of the Red Sea *H. stipulacea* net productivity has seasonal increases driven by irradiance (Cardini et al., 2018). This suggests that *H. stipulacea* is likely to show decreased productivity in the Mediterranean, due to decreased irradiance compared to its native range in the Red Sea. The minimum expected productivity for *H. stipulacea* should occur during the lower Mediterranean Autumn irradiance, this would provide conservative estimates of Mediterranean *H. stipulacea* community productivity rather than the Summer maximums, which on their own may overestimate annual community productivity. At 10 °C the clonal growth of *H. stipulacea* ceases, although the plant does not die (Georgiou et al., 2016). Low Mediterranean irradiance and water temperatures may both simultaneously influence plant metabolism. Whilst its tropical heritage might suggest it would struggle to expand into the colder regions of the Mediterranean (Sghaier et al., 2011), temperature may not limit its expansion across the entire Mediterranean and further into the Atlantic (Georgioiu et al., 2016).

H. stipulacea productivity may increase with global climate predictions for the Mediterranean in mind. By the end of this century the relative increase in average SST within the Mediterranean is predicted to be 5.8 ºC (Sakalli, 2017). This is in line with predictions that *P. oceanica* will continue to decline especially in the eastern basin where sea surface temperature is expected to rise. Thus "warm" affinity species including *H. stipulacea,* would be well placed to take advantage of the predicted future decline of its competitor *P. oceanica* (Pergent et al., 2014).

Seagrass meadows typically support a high biodiversity of organisms from fish and invertebrates (Heck Jr et al., 1989), to a diverse epiphytic community, largely algae, that use the seagrass leaf as a substrate (Prado et al., 2007). They also support obligate endosymbionts such as phytomyxids. Phytomyxids are a particularly interesting group of organisms as certain species have been considered highly detrimental to seagrass health. Once they have infected

the host, they alter their host's metabolism, changing the metabolic status of their host through either facilitating the diverted transfer of energy derived from the seagrass or indirectly affecting carbon flows by altering seagrass growth (Neuhauser et al., 2011a).

Phytomyxids have a characteristic gall development that in some seagrass species prevents the elongation of the seagrass internodes, thus the seagrass becomes bulbous at the base and the poor root development increases uprooting (den Hartog, 1989). Uprooting of the seagrass host would mean the carbon within the seagrass roots is lost from the seagrass sediment carbon sink (Gleason et al., 2013). The ability to alter metabolic activity may influence the capacity and mechanisms of carbon sequestration in seagrass habitats subsequently impacting global primary productivity (Neuhauser et al., 2011b). Despite their potentially significant role in marine ecosystems, knowledge of many phytomyxean species is limited. As records are limited any new findings are of importance (Vohnik et al., 2017). Documentation of new records are also key because a greater understanding of their impact on seagrass habitats will need to be undertaken in the future.

Currently there are no recorded *in situ* community productivity measurements within the Mediterranean of *H. stipulacea*, in this study we acquire Autumn community productivity measurements from a *H. stipulacea* bed, that has occupied a sandy and rubble coastal benthos situated between *P. oceanica* meadow. This enables an assessment of *H. stipulacea's* contribution to net community productivity (NCP) compared to the seagrass absent habitat. Further to this it enables an *in situ* assessment of *H. stipulacea* productivity relative to Mediterranean light and temperature conditions, which can be compared to its native range. Finally, this study addresses the circumstantial finding an endosymbiotic phytomyxid within *H. stipulacea* and discusses its potential influence on the plant's productivity.

Methods

Study site

This study took place off the southeast of Samos Island, in the eastern Aegean Sea (37.697744° N, 26.970575° E), during the Autumn season between October 22nd to October 31st 2018. With a later seagrass sample collection completed on November 23rd 2018. This took place across a large roughly 50 x 100 m bed of *H. stipulacea* situated on sandy and rubble benthos, between the edge of larger *P. oceanica* meadow.

In situ benthic chamber setup

Two clear dome PVC benthic chambers (diameter = 30 cm, height = 16.5 cm, benthic surface area = 0.28 m^2) were fitted with PME loggers to record oxygen concentration and temperature. Two HOBO loggers were attached to the outside of the benthic chambers to record relative temperature and light intensity. The difference in light attenuation through the chamber was considered negligible, as the light registered on a logger placed outside the chamber whilst higher than the light registered on a logger placed inside the chamber, showed no significant difference in the light intensity across 13 hours of daylight logging ($p > 0.05$). The benthic chambers were deployed by free divers between 5.7 - 6.2 m depth, secured with a small chain and sank into the sediment to create a seal (Fig. 9). The benthic chambers were deployed within the same 1hr 10minute timeframe each morning (10:40 - 11:50 am), to ensure they were setup latest by 12:00 noon (modified from, Cardini et al., 2018). The benthic chambers were left *in situ* for 5 hours, before their retrieval by free divers. When benthic chambers were retrieved those containing *H. stipulacea* had all the leaf matter from within the chambers collected.

Figure 9. Benthic chamber setup deployed over benthos with *H. stipulacea* presence

Daylight Net Community Productivity of adjacent benthic communities

The benthic chambers were placed over areas of vegetated *H. stipulacea* (n = 6) and adjacent areas of sand/rubble benthos without *H. stipulacea* presence (n = 4), to determine NCP for both adjacent benthic communities.

The record from the PME oxygen logger is used to calculate an mean hourly $NCP_{Daylight}$ across the 5 hour incubation time (*t*):

$$NCP_{Daylight} = \frac{\Delta DO}{t}$$

The $NCP_{Daylight}$ value (O_2 mg L^{-1} hr^{-1}) is then converted into areal based estimates (O_2 $mmol^{-1}$ hr^{-1} m^{-2}) (Olive et al., 2015):

$$O_2 \ mmol \ m^{-2} \ hr^{-1} = \frac{\left(\left(\frac{DO}{32} \right) \times V \right)}{A}$$

The difference in mean hourly $NCP_{Daylight}$ between adjacent benthos types were determined by independent t test.

Influence of temperature and light on H. stipulacea NCP

For all *H. stipulacea* chambers (n = 6) there was a comparable four-hour time window from 12:00 to 16:00 at which the chambers were deployed on their respective days. The DO data was split to calculate the change in 4 one-hour NCP (NCP_{Hour}) measurements:

$$NCP_{Hour} = \Delta DO$$

The NCP_{Hour} measurements (O_2 mg L^{-1}) were converted into areal based estimates (O_2 mmol^{-1} m^{-2}).

The HOBO logger fastened to the respective NCP *H. stipulacea* chamber recorded light and temperature at 5 minute intervals. Both the temperature and light data was split across the same hourly time series between 12:00 to 16:00. The first measurement registered on the HOBO logger after 12:00, 13:00, 14:00 and 15:00 and the following 11 measurements recorded within the hour, were averaged to determine the mean light intensity (lux) and seawater temperature (°C), complimentary to the PME logger NCP benthic chamber measurements.

Linear regression analysis was used to determine the influence of light and temperature on the NCP of the benthos with *H. stipulacea* present.

Daily Net Community Productivity

Benthic chambers were blacked out and placed over areas with *H. stipulacea* presence to gauge the average hourly dark community respiration (CR_{Dark}), for the community with *H. stipulacea* presence (n = 4). The chambers were blacked out with a reflective material to prevent heat absorption and aim to maintain a similar temperature inside the *CR* chambers to the NCP chambers. Mean hourly CR_{Dark} was determined across a five hour incubation time (*t*):

$$CR_{Dark} = \frac{\Delta DO}{t}$$

The hourly daytime rate of respiration is assumed to be equal to the hourly night time rate of respiration. Samos Island experienced on average 13 hours 12 minutes (13.2 hours) of night-time darkness during the October sampling period in 2018. CR_{Night} was calculated from CR_{Dark}:

$$CR_{Night} = CR_{Dark} \times 13.2$$

Samos Island experienced on average 10 hours and 48 minutes (10.8 hours) of daylight during the October sampling period in 2018. $NCP_{Daytime}$ was calculated with $NCP_{Daylight}$:

$$NCP_{Daytime} = NCP_{Daylight} \times 10.8$$

The combination of net community change during the daytime ($NCP_{Daytime}$) and night time (CR_{Night}) determine NCP_{Daily}:

$$NCP_{Daily} = NCP_{Daytime} - CR_{Night}$$

The photosynthetic and respiratory quotient of 1 mol of O_2 : 1 mol CO_2 is applied, as it is consistent with estimates reported for seagrass meadows (Duarte et al., 2010), converting the NCP_{Daily} values into carbon (C mmol m^{-2} d^{-1}).

Phytomyxid documentation

From the leaf matter collected within the benthic chambers, photo documentation of a *H. stipulacea* leaf with phytomyxid gall was taken prior to the pressing and drying of leaf samples. The pressed leaf matter was retrospectively assessed for percentage colonisation of blades with the phytomyxid in the late developmental black gall stage (modified from; Katlova et al., 2019), but not accounting for the early white stage. Dried specimens of the *H. stipulacea* infected with the phytomyxid collected from within the benthic chambers were deposited in the Herbarium of the Institute of Botany in Průhonice, Czech Republic under the accession number PRA 15920. Specimens stored in 70% ethanol collected from the same sampling site in November 2018 were deposited under the accession number PRA 15921.

Results

Comparison of habitat NCP *without and with H. stipulacea*

Observationally the benthic community with *H. stipulacea* presence has higher and less variable NCP ($\bar{x}$ = 2.03 SE ± 0.40 O_2 mmol m^{-2} hr^{-1}) compared to the adjacent benthos without seagrass ($\bar{x}$ = 0.79 SE ± 0.64 O_2 mmol m^{-2} hr^{-1}) (Fig. 10). There is no significant difference in NCP between *H. stipulacea* and the adjacent benthic community ($t_{(1,5)}$ = 5.3356, $p > 0.05$).
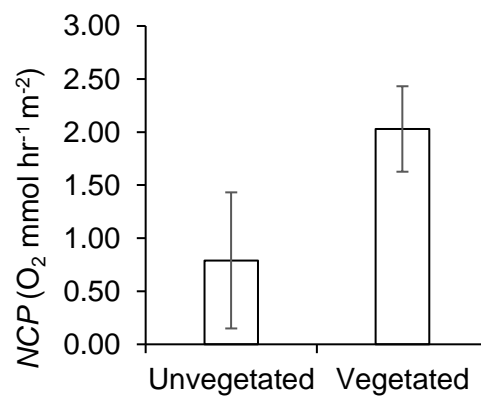
Figure 10. Daylight net community productivity of a vegetated *H. stipulacea* and adjacent unvegetated benthos (errors bars represent SE, unvegetated n= 4 and vegetated n= 6).

Influence of irradiance and temperature on H. stipulacea productivity

Light intensity is coupled with the NCP of the *H. Stipulacea* benthic community (LM: $t_{1,22}$= 2.191, $p < 0.05$) (Fig. 11A). Lower light intensities occur between 14 - 15:00 and 15 - 16:00, when the NCP values are lowest, representing the time period in the day when light may become limiting (I_C = 186 lux). In contrast no significant relationship exists between seawater temperature and the NCP of the *H. Stipulacea* community (LM: $t_{1,22}$= 0.303, $p > 0.05$) (Fig. 11B).
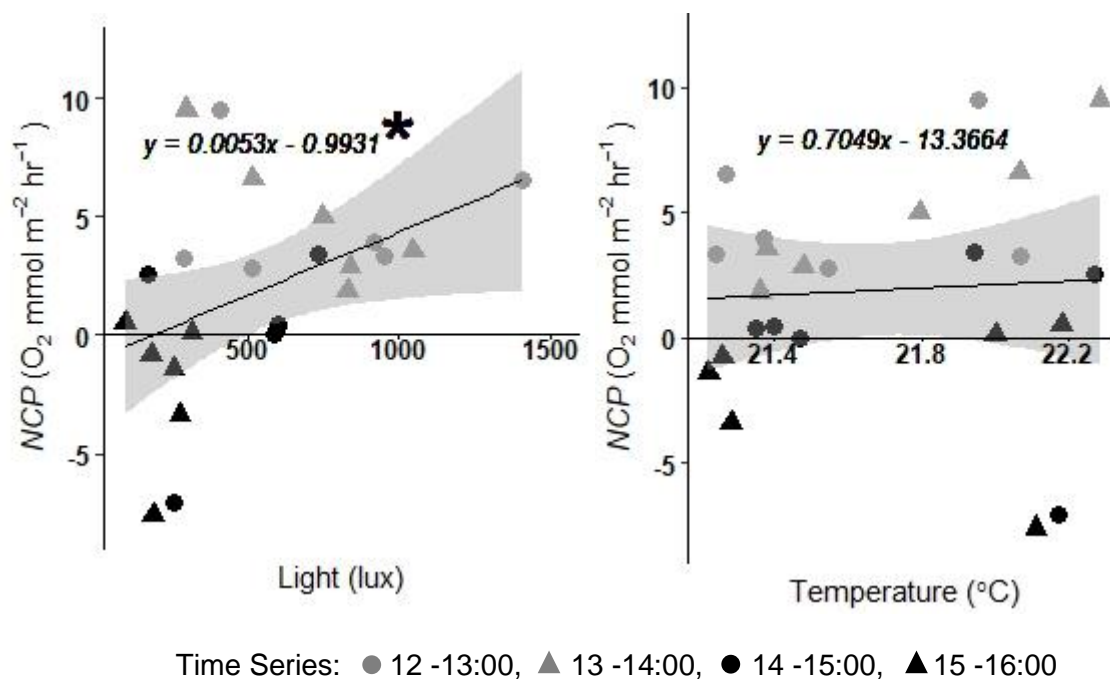
Figure 11. Hourly changes in net community productivity of a benthos vegetated with *H. stipulacea* across comparable time series, given the (**A**) mean light intensity and (**B**) temperature for the hourly time series (n = 24)
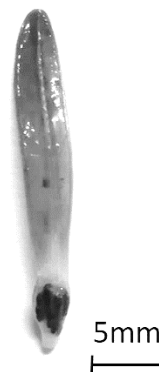
Daily net community productivity

Estimates of daily NCP show the photosynthetic activity doesn't balance the community metabolic demand as the benthic community with *H. stipulacea* present is heterotrophic (NCP $\bar{x}$ = -12.59, SE ± 9.79 C mmol m^{-2} d^{-1}) in Autumn (Table 1).

Table 1. Comparison of reports for daily *H. stipulacea* NCP and CR (C mmol m^{-2} d^{-1}). Includes the study location, date, average light intensity (lux) and water temperature (^{o}C).

Reference	Country	Date completed	Light Intensity	Temperature	NCP	CR
Cardini et al., 2018	Egypt	November 2013	9306 (± 780)	23.0	434	115
This study	Greece	October 2018	545 (± 93)	21.7	-12.6	62.8

Phytomyxid presence

The presence of the phytomyxid reported here as *Plasmodiophora cf. halophilae* was recorded within the *H. stipulacea* collected from this seagrass bed (Fig. 12). This is only the fourth record within the Mediterranean of a phytomyxid symbiosis with the seagrass *H. stipulacea* (Marziano et al., 1995; Vohnik et al., 2017; Katlova et al., 2019) and the first time documented at this locality. The presence of the late developmental black gall stage of the endosymbiont phytomyxid was low ($\overline{x}$ = -2.6 % SE ± 1.1 %) at this site during October 2018.

5mm

Figure 12. Photo documentation of *H. stipulacea* with characteristic phytomyxid black gall.

Discussion

H. stipulacea presence did not increase the community productivity relative to the existing coastal community and therefore suggests it does not assist Mediterranean ecosystem services, such as carbon sequestration. The productivity within the community without seagrass was probably a result of photosynthesis by the algal biofilm on the rubble benthos, sporadic algae and planktonic productivity in the water above the benthos. *H. stipulacea* NCP was less variable than in the community without seagrass, potentially as seagrass rhizome colonisation is more uniform and less variable spatially. Seagrass roots withstand better winter storm erosion than any algal community can provide, better stabilising the sediment (Hendricks et al., 2009). Whilst there was no discernible difference in productivity between habitats, *H. stipulacea* may improve sediment stabilisation and the uniformity of primary production across the habitat. Suggesting it may produce more stable and uniform sedimentary carbon stocks than the existing habitat in this instance, as it colonised a rubble unvegetated benthos.

Irradiance was the main driving factor in *H. stipulacea* productivity within its home range in the gulf of Aquaba (Cardini et al. 2018). Light irradiance was reiterated as the factor driving daily increases in *H. stipulacea* community productivity in the Mediterranean. Maximal growth (Beca-Carretero et al., 2020) and productivity (Cardini et al. 2018) in *H. stipulacea* occurs in the summer within the gulf of Aquaba. The NCP of *H. stipulacea* in the Mediterranean should increase in the Summer with increases in irradiance. *H. stipulacea* is heterotrophic on a daily scale, it must be autotrophic for other periods of the year in order to maintain overall annual carbon balance and persist at this locality. Given the overall extent of *H. stipulacea* distribution within the Mediterranean, its persistence since 1984 and predicted potential to expand (Fritsch, 1895; van der Velde and den Hartog, 1992; Gambi et al., 2009; Tsiamis et al., 2010; Sghaier et al., 2011; Georgiou et al., 2016), its likely to maintain a presence within the Mediterranean. Only direct underwater observation allows for discrimination between *H. stipulacea* and bare substrate, particularly at depth. The persistence and extent of *H. stipulacea* at this locality would need to be monitored routinely to definitively determine if this area of habitat with *H. stipulacea*

is stable, increasing or decreasing. Species of the *Halophila* genus show rapid growth, which allows persistence even under high disturbance (Nakaoka and Aioi, 1999). Given *H. stipulacea* is known to disappear and recolonise areas of the Mediterranean (Gambi et al., 2018), its rapid growth may enable it to utilise small windows of positive carbon balance. Utilisation of high growth in summer months must enable *H. stipulacea* to withstand Autumnal carbon loss .

In this environment *H. stipulacea* lives near its limit in terms of metabolic survival. This introduced species may not act as an 'invasive' species actively competing and displacing native seagrass. It does however have the capacity to opportunistically colonise available space. It has colonised areas previously occupied by native seagrass species, such as *P. oceanica* dead matte or mixed *C. nodosa* meadows (Sghaier et al., 2011). Changes in Mediterranean seagrass species composition through *H. stipulacea* occupying predicted sites of *P. oceanica* regression (Telesca et al., 2015), would change autochthonous carbon sequestration in the Mediterranean.

Sedimentary carbon stocks from Mediterranean *H. stipulacea* meadows are sometimes comparable to native *P. oceanica* (Apostolaki et al., 2019), so its low productivity does not necessarily mean it has low carbon sequestration capabilities. *H. stipulacea* carbon sequestration in the Mediterranean likely depends on deposition of allochthonous material not autochthonous production. High levels of allochthonous organic carbon accumulation compromise the stability of sedimentary organic carbon deposits in the long-term (Mazarrasae et al., 2018). The disappearance and recolonisation of areas of *H. stipulacea* (Gambi et al., 2018), also means any allochthonous carbon accretion is unlikely to be retained in the long-term. The long-term carbon sink potential is then not equal to the carbon stores that can persist for millennia in *P. oceanica* meadows (Mateo et al., 1997)*.*

Increasing the seagrass species richness of Mediterranean meadows makes them more like the multi-species meadows that exist in the tropics. Increased variability of biological traits and life strategies support seagrass bed resistance and recovery during periods of stress (Unsworth et al., 2015). So increased seagrass biodiversity and varied life traits of *P. oceanica, C. nodosa*

and H. stipulacea may enable the overall surface area of Mediterranean seagrass meadows to remain unchanged (Pergent et al., 2014). Maintaining areal cover of Mediterranean seagrass meadows through replacement by a different seagrass species does not mean Mediterranean seagrass meadow ecosystem services will also be maintained.

This assessment primarily focuses on *H. stipulacea* productivity and carbon sequestration, but seagrass ecosystems provide numerous ecosystem services. *H. stipulacea* may also not be equal to native seagrass in assisting the coastal protection of Mediterranean coastlines, *P. oceanica* forms onshore Winter banquettes aiding coastal protection (Gomez-Pujol et al., 2013). The low structural complexity of pioneer species such as *H. Stipulacea* (Pergent el al., 2014), may also create less refuge for juvenile fish and support a lower biodiversity of fish species than compared with the native *P. oceanica* (Guidetti, 2000). The smaller leaf size and leaf life span of small seagrass species like *H. stipulacea* reduces the community diversity of epiphytic species (Mabrouk et al., 2014). Therefore, were the replacement of native meadows by *H. stipulacea* to occur it may reduce community biodiversity at multiple levels. However, the presence of *H. stipulacea* has in this study enabled the endosymbiotic presence of *a* phytomyxid. Phytomyxids can be host species specific and *P. halophilae* is not known to symbiotically occur with the other Mediterranean seagrass species (Vohnik et al., 2017).

The low prevalence of the phytomyxid means no discernible conclusion could be construed directly as to its impact on the plant's metabolic activity and to carbon sequestration in this coastal system. However, given the phytomyxid had altered the hosts cell to form the distinctive late stage galls, its presence did alter the growth and metabolic activity of *H. stipulacea,* as seen in other seagrass species (den Hartog, 1989). If the presence of the phytomyxid causes negative carbon balance to decrease beyond which the plant can recover, it would result in the host seagrass' death. Lost equilibrium between the seagrass host and pathogen resulting in seagrass death, would allow much of the carbon within the plant biomass to be released into the ocean and atmosphere (Gleason et al., 2013).

Leaf shedding has been observed in *H. stipulacea* infected with *P. halophilae* though it was not clear if this was facilitated or triggered by the phytomyxid infection (Kolatkova et al., 2019). It bears distinct resemblance to the swelling and uprooting observed in species of *Zostera* when infected by *Plasmodiophora bicaudate* (den Hartog, 1989). In both instances detachment of the seagrass biomass with the spore would aid the dispersal range and spore protection during dispersal (Gleason et al., 2013; Kolatkova et al., 2019), but mean the carbon within the seagrass biomass is lost from the seagrass sediment carbon sink (Gleason et al., 2013). The formation of galls is also thought to alter the chemical composition of the host tissue, which impacts the diversity of species which feed on the tissues of the infected host (Neuhauser et al., 2011a). The galls may act as energy rich nutrient resources which increases the herbivory pressures on the seagrass and reduces the retention of organic carbon within the seagrass meadow (Neuhauser et al., 2011a). Overall, it may be concluded that the finding of the phytomyxid suggests an altered metabolic activity within *H. stipulacea* and changes in the retention (i.e. herbivory, leaf shedding) of *H. stipulacea* leaf biomass within the meadow, reducing its carbon sequestration potential. However, further research would be needed to conclude if these mechanisms were in place.

The record of the phytomyxid is important as it increases the known distribution of the phytomyxid adding to limited knowledge on its distribution (Marziano et al., 1995; Vohnik et al., 2017; Katlova et al., 2019). Its presence does not detract from fact that during late Autumn this *H. stipulacea* habitat was heterotrophic. Only further estimates of *H. stipulacea* productivity in the Mediterranean will determine if this is true in all cases and without the presence of the phytomyxid*.*

Overall, the introduction of *H. stipulacea* to the Mediterranean may have increased Mediterranean seagrass habitat, but it does not appear to aid autochthonous carbon sequestration. If *H. stipulacea* occupies space previously dominated by *P. oceanica* in the likelihood of further *P. oceanica* regression*,* this replacement could result in an overall decline in the autochthonous carbon sequestration capacity of Mediterranean seagrass ecosystems.

Conclusion

Seagrass metabolism studies in the Eastern Mediterranean and Aegean Sea are few and tend to focus of the dominant species *P. oceanica.* Specifically, within the eastern region of the Aegean Sea the picture is even less clear with seagrass metabolism studies currently non-existent. This thesis contributes conclusions on the metabolic functioning and carbon sequestration of both the dominant seagrass *P. oceanica* and the non-native *H. stipulacea* in the eastern region of the Aegean Sea.

What influences *P. oceanica* carbon sequestration potential in the Eastern Aegean?

Strong seasonality influences the patterns in *P. oceanica* productivity, within the eastern region of the Aegean Sea. The transition from near light limiting to saturating light levels across the seasons, relative to changes in primary productivity indicates that irradiance contributes to this pattern of seasonality in *P. oceanica* productivity. The *P. oceanica* meadow also undergoes seasonal changes in aboveground plant surface area which contributes to the relative quantity of primary production undertaken within the seasons. Projected irradiance levels coupled with estimates in the relative change in *P. oceanica* canopy length could be utilised to forecast future carbon sequestration. Incorporating seagrass biomass into carbon sequestration forecasts could also be applied to better reflect the spatial heterogeneity across *P. oceanica* meadows, especially between the edge and central areas of shallow *P. oceanica* meadows. This is likely of specific importance when considering shallow *P. oceanica* meadows because of their more fragmented nature and higher proportion of edge habitat. Despite the patchy nature of shallow *P. oceanica* habitat in the eastern region of the Aegean Sea it annually sequesters carbon and therefore contributes to the overall carbon sink potential of *P. oceanica* meadows.

What influences *H. stipulacea* carbon storage potential in the Mediterranean and what questions does this raise about Mediterranean seagrass meadows in the future?

H. stipulacea presence did not further Mediterranean ecosystem services, at least relative to the existing rubble and algae coastal community regarding carbon sequestration, Although the colonial nature of the seagrass likely accounted for decreased variability through higher spatial uniformity. This suggests its presence is changing the heterogeneity of these rubble algal habitats. Its presence may therefore result in a cascade of changes to the existing habitat, such as the subsequent species present, including as documented in this thesis an endosymbiotic phytomyxid, *Plasmodiophora cf. halophilae*, not known to symbiotically occur with the other Mediterranean seagrass species (Vohnik et al., 2017).

Light irradiance was driving daily increases in *H. stipulacea* NCP in the Mediterranean, it may utilise windows of high irradiance in Summer, to counteract the heterotrophic Autumn period. Across both seagrass metabolism studies Autumn was a distinct period of heterotrophy, however within this season the *H. stipulacea* habitat was the more heterotrophic (NCP $\bar{x}$ = -12.59, SE ± 9.79 C mmol m^{-2} d^{-1}) of the two seagrass species (*P. oceanica* NCP $\bar{x}$ = -4.0, SD ± 6.5 O$_2$ mmol m^{-2} d^{-1}). Though it must be noted that the benthic chamber methods used to determine these measurements are not directly comparable as derived with differing chamber areal cover, chamber volume, chamber mixing regime, incubation time and island location, to name but a few variables. It does suggest that Mediterranean *H. stipulacea* lives near the edge of its limit in terms of survival and explain whys there is no evidence of it actively competing with native *P. oceanica.*

Even if only opportunistically colonising available space, new *H. stipulacea* habitat can represent an increase in Mediterranean seagrass habitat, but its value in terms of carbon sequestration is not necessarily equal to native Mediterranean seagrass habitat. With regression of *P. oceanica* meadows well documented (Telesca et al., 2015), it implies

autochthonous carbon sequestration by seagrass habitat in the Mediterranean will decrease, as the presence of *P. oceanica* decreases relative to newly forming areas of *H. stipulacea*.

Final remarks

This thesis demonstrates the clear importance of protecting Eastern Mediterranean *P. oceanica* meadows as a nature-based solution to mitigating climate change due to the carbon sequestration services provided, even when shallow and patchy in nature. But it also precautionarily alludes to the potential change in carbon stock afoot as the overall species presence of non-native *H. stipulacea* increases relative to known decline in *P. oceanica* seagrass meadows.

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